

KEISHENI GANESON

DOCTOR OF PHILOSOPHY

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**DEVELOPMENT OF A MICRONEEDLE
DELIVERY SYSTEM USING P(3HB-*co*-
4HB)-DOCETAXEL NANOPARTICLES FOR
ENHANCED BREAST CANCER
TREATMENT**

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KEISHENI GANESON

**Thesis Submitted in Fulfilment of the Requirement for the
Doctor of Philosophy of Science
in the Institute of Climate Adaptation and Marine Biotechnology
Universiti Malaysia Terengganu**

JULY 2025

DEDICATION

To every woman who has fought the unseen battles,

who has turned pain into power,

and who continues to rise despite the weight of expectations.

This is for your resilience, your voice, and your unbreakable spirit

This is dedicated to you, to us, and the limitless power of womanhood

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfilment of the requirements for the degree of Doctor of Philosophy

**DEVELOPMENT OF A MICRONEEDLE DELIVERY SYSTEM USING
P(3HB-*co*-4HB)-DOCETAXEL NANOPARTICLES FOR ENHANCED
BREAST CANCER TREATMENT**

KEISHENI GANESON

JULY 2025

Main Supervisor : Associate Professor Vigneswari Sevakumaran, Ph.D

Co-Supervisor 1 : Professor Amirul Al-Ashraf Abdullah, Ph.D

Co-Supervisor 2 : Thaigarajan Parumasivam, Ph.D

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Breast cancer (BC) significantly contributes to the rise in mortality rate among women across the globe. However, current treatments have many limitations. Docetaxel (DTX), a commonly used chemotherapy drug, has poor solubility, requires intravenous (IV) administration, and causes severe side effects, reducing patient comfort and treatment adherence. This study developed a more precise, personalized and targeted therapy for BC treatment using dissolving microneedles (DMNs) loaded with docetaxel-incorporated poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) [P(3HB-*co*-4HB)] nanoparticles (DTX-NP). The biopolymer P(3HB-*co*-4HB) was produced using *Cupriavidus malaysiensis* USMAA1020 in a 15L bioreactor through a single-stage fermentation process. Optimized conditions resulted in a Polyhydroxyalkanoate (PHA) content of $95.04\% \pm 9.05\%$ in crude polymer with an equimolar monomer composition of 49.83% 3HB and 50.17% 4HB, ensuring good mechanical strength and biodegradability. DTX-NPs were fabricated through solvent evaporation. Optimization showed that a 40% drug-to-polymer ratio, 4-hour solvent evaporation with D- α -Tocopheryl Polyethylene Glycol 1000 Succinate (TPGS), and 2-minute sonication produced the most stable nanoparticles (NPs). The optimized NPs had a

size below 100 nm, a polydispersity index (PDI) of 0.238 ± 0.025 , and a Zeta potential (ZP) of -19.13 ± 0.404 mV, indicating good stability. Encapsulation efficiency (EE) was $87.07 \pm 0.043\%$, and drug loading (DL) capacity was $13.71 \pm 0.82\%$, ensuring effective delivery. The drug release study of NPs showed sustained release over 72 hours, following Korsmeyer-Peppas. Free DTX was released 100% in 24 hours, while the NPs were released 90% in 48 hours, with more controlled release. This extended-release can reduce dosing frequency and minimise side effects. The optimised NPs were further incorporated into DMNs made from polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP) polymers using a stepwise centrifugation method. The microneedles (MNs) were about 976 ± 1.90 μm in height, had good mechanical strength, and effectively penetrated agarose gels mimicking breast tissues. The NP-loaded DMNs exhibited a higher fracture force of 73.67N and maintained structural integrity under forces up to 200g, ensuring effective skin penetration. Drug release from DMNs followed a similar model to NPs, with a sustained release of 97.86% of the drug over 72 hours. Compared to traditional chemotherapy, this MN system offers pain-free delivery, lower toxicity, and better patient compliance. This study could aid in revolutionising BC treatment, particularly for long-term management and self-administration.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu
sebagai memenuhi keperluan untuk Doktor Falsafah

**PEMBANGUNAN SISTEM PENGHANTARAN JARUM MIKRO
MENGUNAKAN P(3HB-co-4HB)-DOCETAXEL NANOZARAH UNTUK
RAWATAN KANSER PAYUDARA TERTINGKAT**

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Salah satu faktor utama yang menyumbang kepada kadar kematian wanita di peringkat global ialah kanser payudara. Walau bagaimanapun, rawatan semasa masih mempunyai banyak keterbatasan. Docetaxel (DTX), ubat kemoterapi yang sering digunakan, mempunyai keterlarutan rendah, memerlukan pengurusan intravena (IV), dan menyebabkan kesan sampingan yang teruk, mengurangkan keselesaan pesakit serta pematuhan terhadap rawatan. Kajian ini membangunkan terapi yang lebih tepat, bersifat peribadi, dan disasarkan untuk rawatan kanser payudara menggunakan mikrojarum boleh-larut (DMNs) yang dimuatkan dengan DTX dalam poli(3-hidroksibutirat-co-4-hidroksibutirat) [P(3HB-co-4HB)]. Biopolimer P(3HB-co-4HB) dihasilkan menggunakan *Cupriavidus malaysiensis* USMAA1020 dalam bioreaktor 15L melalui proses fermentasi satu peringkat. Pengoptimuman menghasilkan kandungan Polihidroksialkanoat (PHA) sebanyak $95.04\% \pm 9.05\%$, dengan komposisi 49.83% 3HB dan 50.17% 4HB, memastikan kekuatan mekanikal dan biodegradasi yang baik. Nanozarah P(3HB-co-4HB) dimuatkan dengan DTX (DTX-NP) menggunakan kaedah penyejatan pelarut, menghasilkan saiz <100 nm, PDI 0.238 ± 0.025 , dan potensi Zeta (ZP) -19.13 ± 0.404 mV, menunjukkan kestabilan yang baik. Kecekapan pengkapsulan ialah 87.07%, dan kapasiti pemuatan ubat ialah 13.71%,

bagi memastikan penghantaran ubat berkesan. Kajian pelepasan ubat menunjukkan pelepasan berterusan selama 72 jam mengikut model Korsmeyer-Peppas. DTX bebas dilepaskan sepenuhnya dalam 24 jam, manakala 90% daripada DTX dalam nanozarah (NP) dilepaskan dalam 48 jam, memastikan pelepasan lebih terkawal. NP yang dioptimumkan dimasukkan ke dalam DMNs diperbuat daripada polimer polivinil alkohol (PVA) dan polivinil pyrrolidone (PVP) menggunakan kaedah sentrifugasi bertahap. Mikrojarum (MN) ini mempunyai ketinggian $976 \pm 1.90 \mu\text{m}$, kekuatan mekanikal tinggi, dan mampu menembusi gel agarosa yang meniru tisu normal dan tumor payudara. MN yang dimuatkan dengan NP mempunyai kekuatan patah 73.67N, mengekalkan integriti struktur sehingga 200g, memastikan penembusan kulit berkesan. Pelepasan ubat daripada DMNs menunjukkan 97.86% pelepasan dalam 72 jam. Berbanding kemoterapi konvensional, sistem ini menawarkan penghantaran tanpa rasa sakit, ketoksikan lebih rendah, dan pematuhan lebih baik. Kajian ini berpotensi merevolusikan rawatan kanser payudara, khususnya untuk pengurusan jangka panjang dan pengurusan sendiri.

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APPROVAL

I certify that an Examination Committee has met on the 25th June 2025 to conduct the final examination of Keisheni Ganeson, on her Doctor of Philosophy thesis entitled "Development of A Microneedle Delivery System Using P(3HB-*co*-4HB)-Docetaxel Nanoparticles for Enhanced Breast Cancer Treatment" in accordance with the regulations approved by the Senate of Universiti Malaysia Terengganu. The Committee recommends that the candidate be awarded the relevant degree. The members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Terengganu or other institutions.

KEISHENI GANESON

Date:

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LIST OF ABBREVIATIONS

ACN	Acetonitrile
ACOSOG	American College of Surgeons Oncology Group
adh	Alcohol/Aldehyde dehydrogenase
AJCC	American Joint Committee on Cancer
ASD	Amorphous Solid Dispersion
ALND	Axillary Lymph Node Dissection
ANOVA	Analysis of variance
ATP	Adenosine Triphosphate
ATR	Attenuated Total Reflectance
BCS	Biopharmaceutics Classification System
BRCA	Breast Cancer Gene
CDK	Cyclin-Dependent Kinase
CME	Caprylic methyl ester
CoA	Coenzyme A
COVID-19	Coronavirus Disease 2019
d-spacing	Spacing between diffraction lattice places; interlayer space
EPR	Enhanced Permeability and Retention
DCIS	Ductal Carcinoma in Situ
DMSO	Dimethyl Sulfoxide
DL	Drug Loading
DSC	Differential scanning calorimetry
DLS	Dynamic Light Scattering
EE	Encapsulation Efficiency

ER	Estrogen Receptor
FDA	Food and Drug Administration
FTIR	Fourier transform infrared spectroscopy
GC-FID	Gas chromatography-flame ionisation detection
GPC	Gel Permeation Chromatography
HMWBP	High Molecular Weight Biodegradable Polymer
HPLC	High-performance liquid chromatography
HPMC	Hydroxypropyl Methylcellulose
HR	Hormone Receptor
ICH	International Council for Harmonisation
IDC	Invasive Ductal Carcinoma
ILC	Invasive Lobular Carcinoma
IMRT	Intensity-Modulated Radiation Therapy
IS	Internal Standard
IV	intravenous (IV) administration
KeTTHA	Kementerian Tenaga, Teknologi Hijau dan Air – Ministry of Energy, Green Technology and Water (Malaysia)
LAL	Limulus Amebocyte Lysate
LMWBP	Low Molecular Weight Biodegradable Polymer
MBC	Metastatic Breast Cancer
MDR	Multidrug Resistance
MRI	Magnetic Resonance Imaging
MSM	Mineral salts medium
MOH	Ministry of Health (Malaysia)
n	Freundlich sorption nonlinearity factor; sample size
NA	Nutrient agar

NAC	Neoadjuvant Chemotherapy
NCD	Non-Communicable Diseases
NR	Nutrient-rich
NSABP	National Surgical Adjuvant Breast and Bowel Project
NSPCC	National Strategic Plan for Cancer Control
NST	No Special Type
OCT	Optical Coherence Tomography
OrfZ	4-hydroxybutyrate-CoA transferase
P(3HB)	P(3-hydroxybutyrate)
P(3HB- <i>co</i> -3HV)	P(3-hydroxybutyrate- <i>co</i> -3-hydroxyvalerate)
P(3HB- <i>co</i> -4HB)	P(3-hydroxybutyrate- <i>co</i> -4-hydroxybutyrate)
P(3HO)	P(3-hydroxyoctanoate)
PAH	Polycyclic aromatic hydrocarbon
PBS	Phosphate Buffer Solution
PCB	Polychlorinated biphenyl
PCL	Polycaprolactone
PDI	Polydispersity Index
P-gp	P-glycoprotein
PHA	Polyhydroxyalkanoate
PhaA	β -ketothiolase enzyme
PhaB	Acetoacetyl-CoA reductase enzyme
PhaC	Polyhydroxyalkanoate synthase enzyme
PhaG	3-hydroxyacyl-ACP-CoA transferase enzyme
PhaJ	Enoyl-CoA hydratase enzyme
PLA	Polylactic acid
PLGA	Poly (lactic- <i>co</i> -glycolic acid)

PR	Progesterone Receptor
PTFE	Polytetrafluoroethylene
PVA	Polyvinyl alcohol
PVP	Polyvinylpyrrolidone
rpm	Revolutions per minute
RPT	Rabbit Pyrogen Test
RT	Radiation Therapy
scl	Short-chain-length
SDG	Sustainable Development Goals
SEM	Scanning electron microscope
SLNB	Sentinel Lymph Node Biopsy
SNEDDS	Self-Nanoemulsifying Drug Delivery System
sucD	Succinic semialdehyde dehydrogenase gene
T _c	Crystallisation temperature
TCA	Tricarboxylic acid
TE	Trace element
TEM	Transmission Electron Microscopy
T _g	Glass transition temperature
TGA	Thermogravimetric Analysis
T _m	Melting temperature
TNBC	Triple-Negative Breast Cancer
TNM	Tumour, Node, Metastasis (Staging System)
TPGS	D- α -tocopheryl Polyethylene Glycol Succinate
USD	United States dollars
WHO	World Health Organisation
XRD	X-ray diffraction

3HB	3-hydroxybutyrate
4HB	4-hydroxybutyrate
4hbD	4-Hydroxybutyrate dehydrogenase gene
orfZ	4-Hydroxybutyryl-CoA transferase gene

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Breast cancer (BC) remains the most prevalent form of cancer diagnosed globally, with women being the most affected group (Arnold et al., 2022; Momenimovahed and Salehiniya, 2019). It is also the second leading cause of cancer-related deaths among women. Recent global reports indicated approximately 2.3 million new cases of BC in 2022, with an estimated 670,000 deaths recorded during the same year (Bray et al., 2024; Kim et al., 2025). Alarmingly, future projections suggest a continuous rise in BC cases worldwide, with mortality rates expected to increase by nearly 50% by the year 2040 (Arnold et al., 2022). In Malaysia, it has been reported that one out of every nineteen women is at risk of developing BC, emphasising the urgent need to strengthen preventive strategies and improve available treatment options (Azizah et al., 2019).

Despite advancements in early detection techniques and therapeutic innovations, the journey from diagnosis to treatment remains physically and emotionally taxing for both patients and their families. Currently, the treatments for BC, which include radiation therapy (RT), surgery, targeted therapy, immunotherapy, hormonal therapy, and chemotherapy, face various challenges (Cardoso et al., 2019; Chaudhari et al., 2024; Hunis, 2024). Among these, chemotherapy is commonly used before and after surgery to shrink tumours (Imran et al., 2020). Taxanes, specifically Paclitaxel and Docetaxel (DTX), are widely utilised chemotherapeutic agents,

accounting for approximately 3.5 billion USD in sales, the highest among current anticancer drugs, due to their effectiveness (Sohail et al., 2018; Imran et al., 2020). These drugs work by inhibiting microtubule activity during cell division, leading to apoptosis and cell death.

Despite their effectiveness, these drugs possess hydrophobic characteristics that necessitate intravenous (IV) administration, which is associated with several drawbacks. These include systemic toxicity, limited patient tolerance, the potential development of resistance by tumour cells, suboptimal targeting, and challenges in sustaining therapeutic concentrations at the tumour site (Panthi et al., 2024; Chaudhari et al., 2024). Furthermore, patients may experience adverse effects such as nausea, fatigue, and neutropenia as a result of these treatments (Altun and Sonkaya, 2018; Mustapha et al., 2021).

Moreover, commercial formulations of these drugs use solubilizers or surfactants such as Tween 80 and ethanol, which exacerbate side effects, reducing patient compliance and quality of life. The non-specific targeting of these drugs and their route of administration can harm healthy cells, leading to poor treatment outcomes (Eslami et al., 2024). This causes an increased risk of cancer recurrence, necessitating repeated dosing or an increased dosage (Eslami et al., 2024). The high cost and lack of personalized treatment options further complicate effective management.

To address these challenges, nanotechnology and microtechnology have emerged as promising alternatives. Nanoparticles (NPs) offer a potential solution by enhancing drug solubility, stability, and targeted delivery, but NP-based therapies are still primarily administered intravenously. This leads to systemic distribution and degradation of drugs before reaching tumour cells. This inefficiency underscores the need for more localised and effective delivery systems.

Microneedle (MN) technology is a more significant advancement in transdermal drug delivery. MNs, consisting of tiny patches with needles, are self-

administrative, minimally invasive, and painless (Yang et al., 2019; Singh and Kesharwani, 2021). MNs have been explored extensively for vaccines, drug molecules, proteins, genes, antibodies, and NPs (Rajput et al., 2021; Ganeson et al., 2023). However, their potential in cancer treatment, particularly BC, remains an under-explored area. Among various MNs, dissolving microneedles (DMNs) offer unique advantages by eliminating the risk of biohazardous waste and enabling the sustained release of encapsulated drugs as they dissolve within the skin.

Therefore, this study encapsulates DTX within a microbial polymer, poly-3-hydroxyalkanoate-*co*-4-hydroxyalkanoate P(3HB-*co*-4HB), for more sustained drug delivery. P(3HB-*co*-4HB) is a microbial polymer under the polyhydroxyalkanoate (PHA) class and is well-known for its notable mechanical strength, biodegradability, and biocompatibility. This polymer is equally competent, perhaps even more than conventional polymers such as Poly (lactic-*co*-glycolic acid), PLGA, and Poly (lactic acid), PLA, which are often used in drug delivery. P(3HB-*co*-4HB) has been used more for scaffolds and hydrogels, and recent studies show they are also being used for NPs, but their application in transdermal drug delivery and cancer is still limited.

These drug-loaded NPs were then further encapsulated within DMNs fabricated using polyvinyl alcohol and polyvinylpyrrolidone (PVA and PVP) for a more localised, targeted, and sustained delivery. The NP formulation and DMNs were optimised and further characterised using advanced analytical techniques, focusing on mechanical strength, thermal stability, drug release, and *in vitro* skin permeation. The novelty of this study lies in its dual encapsulation technique, which focuses on a dual drug delivery approach where the MN dissolves to release NPs locally, which subsequently degrade to release the encapsulated drug in a controlled manner.

There are also very few studies conducted on the impact of the diagnosis and treatments of cancer on the psychological aspects of patients, especially women. Thus, incorporating such patient-centred innovations into BC care reflects a commitment to empowering women and honouring their roles as mothers, caregivers, and equal members of society. This study is critical and can potentially create a substantial

breakthrough in the medical sector of Malaysia and globally by offering a more targeted delivery system and reducing the need for repeated dosing. Additionally, it aims to provide a more patient-compliant, self-administrative, personalized treatment option for BC patients.

1.2 Problem Statement

Chemotherapy remains a primary treatment strategy for BC; however, it is associated with systemic toxicity and unintended damage to healthy cells (Eslami et al., 2024; Chaudhari et al., 2024). Administering chemotherapeutic drugs through oral administration is generally not preferred due to their poor bioavailability, enzymatic degradation in the gastrointestinal system, and extensive first-pass metabolism in the liver (Yang et al., 2019; Bilal et al., 2021; García-Alfonso, 2021). Consequently, IV administration is commonly employed despite its invasive approach, longer treatment time, and the requirement for professional medical supervision (Ganeson et al., 2023).

The emergence of drug resistance in BC cells poses a significant challenge in chemotherapy, often leading to treatment failure and disease relapse (Eslami et al., 2024). Traditional drug delivery approaches struggle to address multidrug resistance (MDR), emphasising the importance of developing more accurate and targeted delivery techniques. Conventional chemotherapy lacks specificity, affecting both cancerous and healthy cells, which lowers therapeutic effectiveness and increases unintended toxicity. Therefore, there is an urgent need to improve the precision and localisation of drug delivery methods.

DTX, a standard chemotherapy drug used for BC, presents an additional challenge due to its hydrophobicity, which results in rapid degradation in systemic circulation and reduces therapeutic efficacy (Sohail et al., 2018; Imran et al., 2020). Maintaining adequate drug concentrations necessitates higher dosages, further increasing the risk of toxicity and burdening the patient with frequent hospital visits.

Using solubilizers like Tween 80 to enhance DTX's solubility further contributes to intense side effects and reduced patient compliance. Despite being promising in enhancing drug solubility and targeting, clinical application of NP-based systems is limited by rapid systemic clearance and insufficient accumulation at tumour sites. This limitation highlights the need for improved delivery strategies to sustain therapeutic drug concentrations at the target site.

The financial burden of BC treatment is substantial, particularly in Malaysia, where the average cost reaches RM395,000, excluding additional expenses for managing side effects and related complications (FWD Takafu, 2023; Yee et al., 2023). This cost significantly strains low and middle-income patients and their families. The rise in BC incidence also stresses healthcare infrastructure, especially during pandemic situations, leading to escalated healthcare costs and a substantial economic burden for both patients and the healthcare system.

The side effects of chemotherapy, including nausea, fatigue, and neutropenia, significantly impact patients' physical, emotional, and mental well-being, leading to reduced self-confidence and diminished quality of life (Altun and Sonkaya, 2018). A recent systematic review reported that 32.2% of the global occurrence of depression is high among women diagnosed with or under treatment for BC, highlighting the urgent need for more patient-centred therapeutic approaches (Pilevarzadeh et al., 2019). Additionally, the burden extends to caregivers, who often need to take time off work or adjust their schedules to provide support, further amplifying the socioeconomic impact.

Another major dilemma associated with conventional chemotherapy is the substantial generation of medical and plastic waste, which poses significant environmental risks (WHO, 2018; Greene et al., 2022). The production and disposal of syringes, intravenous bags, and other medical devices contribute to environmental pollution and increase the risk of disease transmission due to improper waste management (Joseph et al., 2021; Greene et al., 2022). Reports indicate that over

100,000 needlestick injuries occur in hospitals annually, underscoring the need for safer and more sustainable drug delivery methods (Permana et al., 2021).

Current BC treatments present several limitations, along with financial, emotional, and environmental burdens, highlighting the pressing need for advanced drug delivery solutions. MN and NP-based delivery systems have emerged as promising alternatives, offering improved targeting ability, reducing systemic toxicity, and providing a less invasive and more patient-friendly treatment approach for individuals with BC.

1.3 Significance of Study

This research, which centres on P(3HB-*co*-4HB) polymer-based DTX NPs administered via DMNs for BC therapy, is significant in addressing the complex challenges associated with current BC treatment approaches. It also supports global and local healthcare objectives, particularly in the Malaysian healthcare landscape.

MNs present an advantageous alternative to IV chemotherapy by enabling the targeted delivery of chemotherapeutic agents to specific sites, such as the breast area in BC patients. This targeted delivery approach promotes drug accumulation within the tumour microenvironment while reducing exposure to healthy tissues, lowering off-target effects and improving treatment effectiveness. In contrast to IV methods, where drugs are distributed systemically and may degrade before reaching the intended site, MNs allow for direct and controlled drug administration. This not only reduces the frequency of dosing but also enhances treatment efficiency.

Furthermore, enabling MNs for self-administration could significantly reduce healthcare costs by lowering the dependence on hospital facilities, medical personnel, and transportation expenses. This approach can also help address healthcare disparities in low-income regions and provide better access for marginalized groups, including

the elderly, disabled, and underprivileged communities while easing the financial burden on patients and their families.

Incorporating MN technology into Malaysia's healthcare system may also lower national healthcare spending on BC treatment and support economic sustainability. Such an initiative aligns with Sustainable Development Goal 3 (SDG 3), which aims to decrease premature deaths from non-communicable diseases (NCDs), including cancer, by one-third by the year 2030 (Morgan et al., 2018; DOS, 2019).

Malaysia's healthcare sector faces the dual challenges of minimising costs and environmental impacts while maintaining high-quality care. Using biodegradable MN devices in cancer therapy is consistent with Malaysia's green economy ambitions and dedication to sustainable development. This is because DMNs eliminate the risks associated with sharp waste and reduce medical waste and energy consumption in healthcare facilities. This technology supports Malaysia's Green Technology Master Plan 2030 (KeTTHA, 2017). Malaysia's National Strategic Plan for Cancer Control (NSPCC) also emphasises the need for accessible and innovative cancer treatments (MOH, 2021).

Integrating biodegradable P(3HB-*co*-4HB) NPs and DMNs supports a circular healthcare system by reducing waste. Employing microbial polymer-based DTX NPs aligns with green nanotechnology efforts to develop safer and more environmentally sustainable drug delivery platforms. These NPs are biodegradable, breaking down into lactic and glycolic acids that are naturally processed by the body, thereby reducing toxicity and environmental risks (Chai et al., 2021).

Additionally, MN-based delivery offers a painless and patient-friendly method that improves the overall well-being and quality of life of women receiving BC treatment. This approach addresses the physical challenges of cancer therapy and considers patients' emotional and social well-being, promoting a more comprehensive healing experience.

Overall, DMNs incorporated with P(3HB-*co*-4HB) encapsulated DTX-NPs present a promising strategy to transform BC treatment in Malaysia. This approach significantly improves cancer therapy and the Malaysian healthcare system.

1.4 Objectives of the Study

The primary objectives of this study include:

1. To synthesize and characterize P(3HB-*co*-4HB) copolymers for the fabrication of polymeric nanoparticles intended for breast cancer treatment.
2. To measure the drug loading capacity, entrapment efficiency, and release mechanism of docetaxel-loaded P(3HB-*co*-4HB) nanoparticles.
3. To fabricate and optimise dissolvable microneedle patches for localised and efficient transdermal drug delivery.
4. To incorporate the polymeric nanoparticles into dissolvable microneedle patches and evaluate the system's performance in terms of drug release efficiency, physicochemical stability, and therapeutic efficacy.

CHAPTER 2

LITERATURE REVIEW

2.1 Breast Cancer (BC)

2.1.1 Anatomy of the Breast

All individuals, regardless of gender, are born with breast or mammary glands (Camarillo et al., 2021). In females, the primary role of the breast is to produce milk for nurturing offspring while also contributing to secondary sexual characteristics (Bazira et al., 2022; Franjić, 2023; Semakane et al., 2024). Structurally, the breast comprises a variety of tissues, including glandular lobules, ductal tissues, adipose tissue, and fibrous components, along with nerves, blood vessels, and lymphatic vessels, as shown in Figure 2.1A, which provides a general anatomical overview of the breast's lobular and ductal systems (Semakane et al., 2024). Combining these tissues gives the breast its distinctive rounded appearance (Haider, 2023). The two primary types of breast tissue are the glandular tissue, comprising the milk-producing lobules and ducts and the stromal tissue, comprising fatty and fibrous connective tissues (Figure 2.1A) (Camarillo et al., 2021). From an anatomical perspective, the breast is considered a modified sweat gland with several layers: the skin, pre-mammary and mammary layers, retro-mammary layer, muscle, and the chest wall, as depicted in Figure 2.1B, which highlights these layered microstructures in greater detail (Waldman et al., 2019).

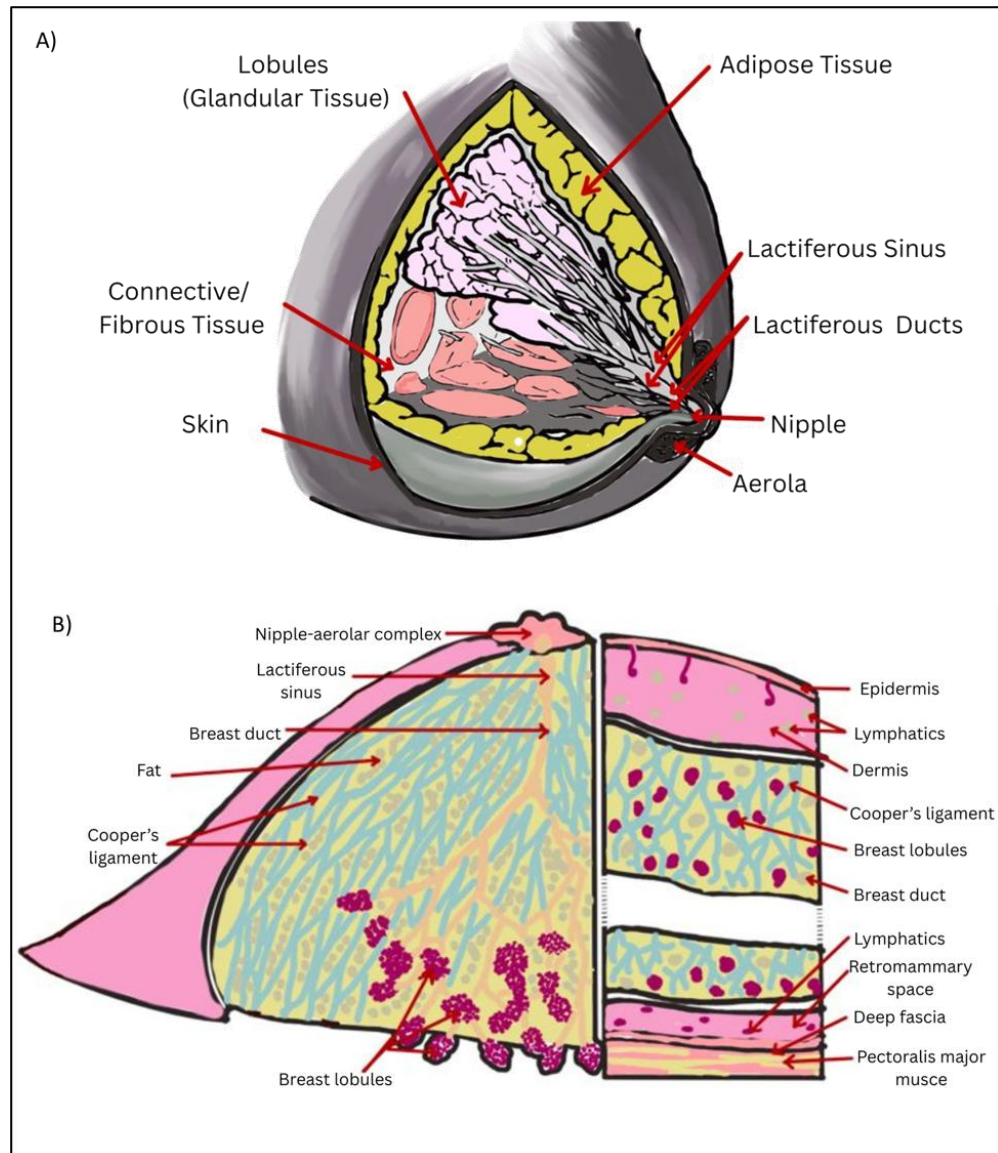


Figure 2.1: Anatomical cross-sections of the human breast. (A) Realistic illustration highlighting lobules, adipose tissue, ducts, sinus, nipple, and areola. (B) Schematic representation of breast microanatomy including skin, fat, Cooper's ligament, ducts, lobules, lymphatics, and deep muscular structures. Adapted and modified from Teixeira and Martins, 2023; Lockwood and Collings, 2025; drawn using Procreate.

The pre-mammary layer primarily comprises subcutaneous fat and connective tissues, supporting the glandular breast tissue (Figure 2.1A) (Waldman et al., 2019). This layer also forms the primary anatomical plane dissected during skin-sparing mastectomy, a standard surgical procedure associated with cancer recurrence

(Waldman et al., 2019). The breast spans across the chest wall, stretching from the clavicle to the midline of the sternum and extending laterally into the axilla (the root of the breast), even reaching the latissimus dorsi muscle that links to the upper arm's humerus bone (Semakane et al., 2024). This anatomical coverage, including the axillary extension and lymphatic pathways, is visually mapped in Figure 2.2, which also shows how breast cancer (BC) may spread through these lymphatic channels. This lymphatic network serves as a critical route for metastatic spread, especially to the axillary and internal mammary lymph nodes, which are commonly involved in the early dissemination of BC cells. The adult female breast has a broad base, ranging from the second to the sixth ribs, lying between the medial edge of the sternum and the midaxillary line laterally (Bazira et al., 2022; Franjić, 2023). A tongue-shaped extension of breast tissue, referred to as the axillary tail, extends upwards and into the axilla (Figure 2.2) (Bazira et al., 2022). The upper outer quadrant is reported as the most prevalent site for both benign and malignant tumours (Bazira et al., 2022). Around two-thirds of the breast tissue lies atop the pectoralis major muscle, whereas the lower third is supported medially by the serratus anterior muscle and laterally by the upper rectus sheath (Bland 2020; Czajka and Pfeifer, 2023).

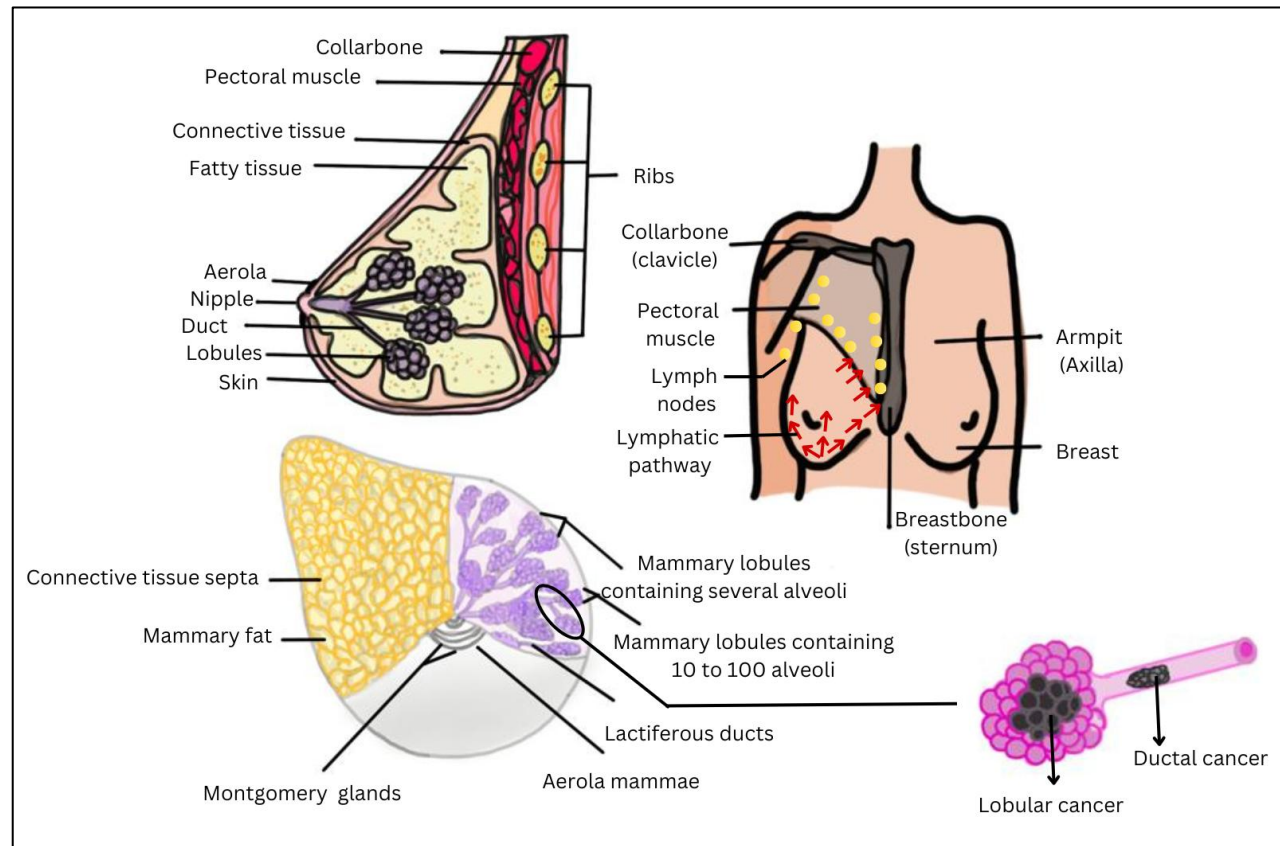


Figure 2.2: Schematic representation of breast anatomy showing major structural components, including lobules, ducts, connective tissues, and lymphatic pathways that facilitate metastatic spread. The lymphatic routes leading to the axillary and internal mammary nodes are highlighted, along with the sites commonly affected by ductal and lobular cancers. Adapted and modified from Darkwah et al., 2021; Lawrence, 2022; drawn using Procreate.

The breast is covered by superficial fascia, with the outer layer below the dermis. This anatomic position makes it easier to separate the glandular mass without leaving tissue behind. In contrast, the deeper fascia layer is continuous with the chest wall fascia (Figure 2.1B) (Bazira et al., 2022). At its peak, the breast has a nipple surrounded by a pigmented areola containing large sebaceous glands known as areolar glands (of Montgomery), which are indicated in Figure 2.2 (Bazira et al., 2022).

Before puberty, the development of male and female breasts is identical (Önder et al., 2020; Camarillo et al., 2021). However, after puberty, hormones like estrogen and progesterone trigger the maturation of female breast tissue, particularly the ductal and connective tissue (Camarillo et al., 2021; Paavolainen et al., 2024). The structure of the human breast undergoes considerable changes during reproductive events such as pregnancy, lactation, and menopause, adapting to functional needs (Paavolainen et al., 2024). These hormonal effects and the resulting structural alterations can influence BC risk, as extended exposure to estrogen has been linked to a higher likelihood of developing BC (Hong and Xu, 2022). Male BC only makes up about 1% of all BC cases and is associated with genetic factors, hormonal imbalances, and radiation exposure (Hong and Xu, 2022). Men are often diagnosed at more advanced stages of BC due to a lack of awareness and delayed detection, highlighting the importance of education regarding male breast anatomy and associated risks (Hong and Xu, 2022).

Since BC often originates in the epithelial cells of ductal and lobular tissues, monitoring structural changes in these areas can facilitate early detection (Kothari et al., 2020). Common anatomical warning signs include the presence of stiff, immobile, irregular, or fixed breast and/or axillary lumps, changes in breast size, shape, or symmetry, and skin abnormalities such as erythema, dimpling, ulceration, or peau d'orange (Katsura et al., 2022). Nipple changes, including inversion, discharge, or skin irregularities, may also indicate malignancy, while breast pain alone is rarely a primary indicator but should not be ignored if accompanied by other symptoms (Katsura et al., 2022).

Moreover, modifiable and non-modifiable factors can significantly influence breast anatomy and tissue structure. Modifiable factors include obesity, sedentary lifestyle, alcohol consumption, exposure to external hormones, and radiation exposure, all of which can alter breast density and hormonal balance, thereby elevating the risk of cancer (Katsura et al., 2022; Hong and Xu, 2022; Franjic, 2023). On the other hand, non-modifiable factors include age, genetic predispositions such as Breast Cancer gene 1 (BRCA1), BRCA2, and Partner and localiser of BRCA2 (PALB2) mutations, as well as endogenous hormone exposure related to early menarche, late menopause, and nulliparity, which prolong estrogen activity and influence breast tissue composition (Katsura et al., 2022; Hong and Xu, 2022). Protective factors such as lactation, regular physical exercise, reduced alcohol intake, and consumption of aspirin or non-steroidal anti-inflammatory drugs can help maintain breast tissue health and lower the likelihood of carcinogenic alterations (Katsura et al., 2022). Therefore, awareness of breast anatomy could assist individuals in detecting changes in the skin and breast during self-examination and facilitate the early identification of possible malignancies (Bazira et al., 2022; Haider, 2023; Semakane et al., 2024; Paavolainen et al., 2024). Such early detection plays a crucial role in improving prognosis and treatment success, emphasising the relevance of anatomical knowledge in BC prevention, early diagnosis, and therapeutic intervention.

2.1.2 Types and Stages of Breast Cancer

BC is a heterogeneous disease with multiple classifications based on molecular and histopathological characteristics, influencing prognosis and treatment outcomes (Tsang and Gary, 2020; Rakha et al., 2023; Fisusi and Akala, 2019). Generally, BC classification relies on tumour typing, grading, and staging (Tsang and Gary, 2020). Tumour typing and grading assess aggressiveness based on cellular structure and growth patterns (Tsang and Gary, 2020). Despite advances in classification, certain molecular overlaps and tumour heterogeneity pose challenges in precise subtyping, impacting targeted therapy decisions (Fisusi and Akala, 2019).

BC is broadly categorised histologically into non-invasive and invasive types. Figure 2.3 below provides a summary of the histological and molecular subtypes of BC. Ductal Carcinoma in Situ (DCIS) is the most common non-invasive form, remaining confined to the milk ducts without invading surrounding tissues (Zubair et al., 2021). DCIS is frequently detected through mammography due to its association with microcalcifications (Fahad Ullah, 2019). It has subtypes such as comedo, cribriform, micropapillary, and papillary, with a generally high survival rate (Trayes and Cokenakes, 2021). However, up to 40% of DCIS cases may progress to invasive BC if untreated, highlighting the need for precise management strategies (Lukong, 2017).

Invasive BC spreads beyond the ducts into the surrounding tissue. The most prevalent type is Invasive Ductal Carcinoma (IDC), accounting for 50–80% of cases (Zubair et al., 2021). IDC is further classified into No Special Type (NST) and special types (Trayes and Cokenakes, 2021). The second most common invasive type is Invasive Lobular Carcinoma (ILC), comprising 5–15% of cases and spreading in a single-file pattern through the stroma (Zubair et al., 2021). Other rare invasive types include Mucinous Carcinoma, Tubular Carcinoma, and Metaplastic Carcinoma (do Nascimento and Otoni, 2020). Metaplastic Carcinoma is particularly aggressive, constituting about 1% of cases and containing various cell types (do Nascimento and Otoni, 2020). Mucinous Carcinoma, typically found in older women, has a favourable prognosis due to its well-differentiated nature (do Nascimento and Otoni, 2020). Medullary Carcinoma, associated with BRCA1 mutations, appears aggressive but often has a relatively good prognosis (do Nascimento and Otoni, 2020).

Molecular classification of BC, based on genetic profiling, categorizes it into five subtypes: Basal-like, Luminal A, Luminal B, human epidermal growth factor receptor 2 (HER2)-positive/HER2-enriched, and Normal-like tumours (Figure 2.3) (Fisusi and Akala, 2019; Abu Samaan et al., 2019). While this subtyping system has greatly enhanced the ability to predict prognosis and select treatments, tumours displaying characteristics of multiple subtypes can complicate classification, resulting in variable treatment responses (Fisusi and Akala, 2019). Luminal A cancers are oestrogen receptor (ER)-positive, progesterone receptor (PR)-positive, and human

epidermal growth factor receptor 2 (HER2)-negative, with a low Ki-67 index, making them less aggressive and associated with a favourable prognosis (Barrios, 2022). In contrast, Luminal B cancers are ER-positive, PR-positive, and either HER2-positive or negative but have a higher proliferation rate and poorer prognosis than Luminal A tumours (Barrios, 2022). HER2-enriched BC are HER2-positive and ER- and PR-negative, making them more aggressive, although they respond well to HER2-targeted therapies (Barrios, 2022). Triple-negative breast cancer (TNBC), which is ER-negative, PR-negative, and HER2-negative, is highly aggressive and has limited treatment options (Barrios, 2022). TNBC is further divided into Basal-like, Mesenchymal, and Immunomodulatory subtypes, each exhibiting distinct biological behaviours and treatment responses (Trayes and Cokenakes, 2021).

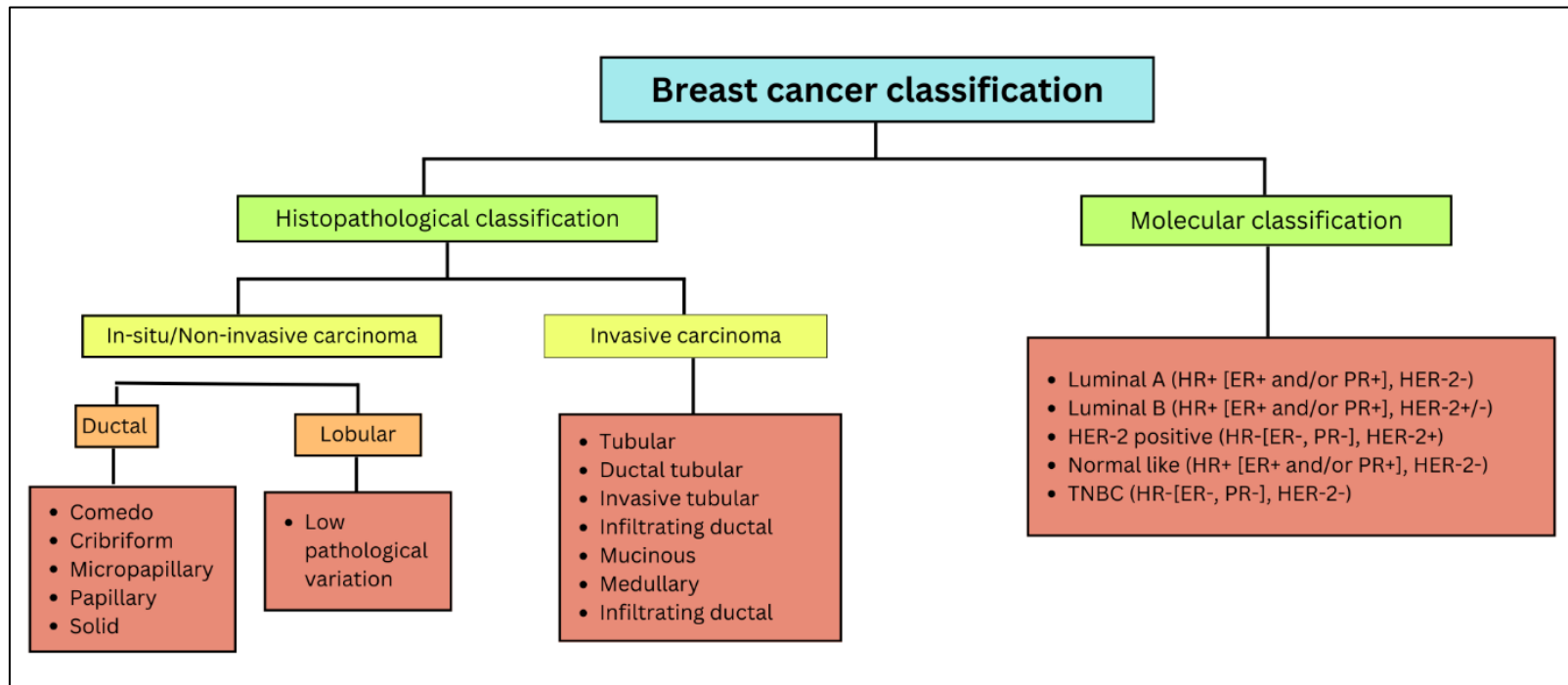


Figure 2.3: Breast cancer classification based on histopathological and molecular subtypes, including in-situ/non-invasive, invasive, and receptor-based categories (Created in <https://BioRender.com>).

Tumour staging evaluates the extent of cancer spread, guiding treatment decisions. The TNM system, developed by the American Joint Committee on Cancer (AJCC), is widely used to classify BC stages based on tumour size (T), lymph node involvement (N), and metastasis (M) (Koh and Kim, 2019; Teichgraeber et al., 2021). Figure 2.4 below provides an overview of the TNM staging system for BC, including tumour stage and size, lymph node involvement, and prognosis. Despite the advancements in BC diagnostics, TNM staging alone may not capture the complexity of tumour biology, necessitating additional prognostic markers (Teichgraeber et al., 2021). The AJCC 8th edition of this system integrates additional prognostic factors, including ER, PR, HER2, and multigene panels, refining treatment strategies (Zhou et al., 2017; Kim et al., 2020). The two main staging categories include an anatomic stage, which is based on TNM criteria and determined through clinical and/or pathological assessment, and a prognostic stage, which incorporates biomarkers such as ER, PR, HER2, and genomic profiling for a more comprehensive prognosis (Cserni et al., 2018; Teichgraeber et al., 2021). Each TNM component plays a crucial role in staging. The T category, which assesses tumour size and invasion, is determined by measuring the largest invasive component of the tumour (Lim et al., 2018). The N category evaluates nodal involvement, determining whether metastases have reached regional lymph nodes, significantly influencing treatment decisions (Teichgraeber et al., 2021). The M category differentiates between M0 (no metastases) and M1 (presence of distant metastases), with the most common metastatic sites being the bones, lungs, liver, and brain (Park and Moon, 2018).

Stage 0 represents non-invasive cancer, such as DCIS, where abnormal cells remain confined to the ducts without spreading (Koh and Kim, 2019). Stages I and II are early-stage BCs with limited spread, typically associated with more favourable outcomes (Duggan et al., 2021). Stage III is divided into subcategories IIIA, IIIB, and IIIC based on the extent of local tissue spread and lymph node involvement (Koh and Kim, 2019). When cancer has spread to nearby lymph nodes without affecting distant organs, it is classified as locally advanced (Koh and Kim, 2019). Stage IV is the most advanced, characterized by cancer that has metastasized to distant organs such as the liver, lungs, brain or bones, and is associated with the poorest prognosis (Duggan et al., 2021).

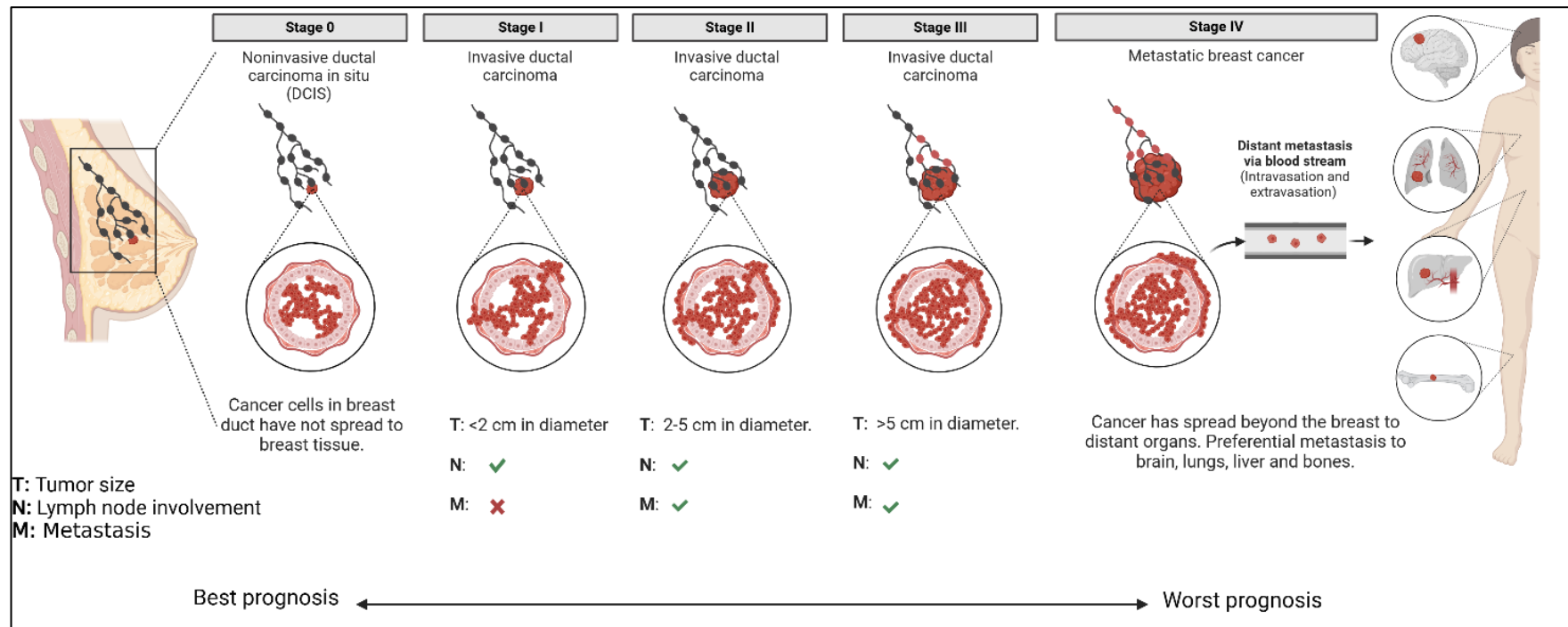


Figure 2.4: Stages of breast cancer based on the TNM system, illustrating tumour size (T), lymph node involvement (N), and metastasis (M) status. The figure begins with Stage 0 (non-invasive ductal carcinoma in situ) and progresses through Stage I to III (invasive ductal carcinoma with increasing tumour size and lymph node involvement), ending with Stage IV (metastatic breast cancer spreading to distant organs such as the brain, lungs, liver, and bones via the bloodstream). Adapted and modified from Li et al., 2021; created in <https://BioRender.com>.

Early detection of BC substantially increases survival chances, with several countries reporting significant reductions in mortality rates when early-stage detection reaches around 60% (Duggan et al., 2021). In response to the need for improved screening, the World Health Organization (WHO) initiated the Global Breast Cancer Initiative (GBCI) in 2021, aiming to strengthen early detection and improve access to treatment, particularly in economically disadvantaged regions (Duggan et al., 2021). Although screening programs such as mammography and clinical breast examinations play a vital role, disparities in healthcare resources continue to hinder timely diagnosis and treatment in underserved areas (Duggan et al., 2021). Despite advancements in molecular classification and staging, challenges remain due to overlapping tumour characteristics and variability in biomarkers. Therefore, future research incorporating artificial intelligence (AI) and genomic profiling in staging could further support precision medicine, enabling more accurate risk stratification and personalized treatment strategies (Teichgraber et al., 2021). Ultimately, a deeper understanding of the different BC types and stages is essential to develop targeted therapeutic options, ensuring optimal outcomes. Additionally, such efforts would provide a foundation for more innovative, individualized, and patient-centered treatment strategies.

2.1.3 Current Treatments and Limitations of Breast Cancer

BC management typically involves multiple treatment strategies, incorporating surgery, RT, chemotherapy, hormonal therapy, and targeted treatments (Fisusi and Akala, 2019; Gupta et al., 2020). Recent technological progress, early diagnosis, and advanced imaging methods have improved survival outcomes, particularly for patients diagnosed early (Gupta et al., 2020). Nevertheless, each treatment method is associated with its own set of drawbacks, including adverse effects and limited effectiveness, especially in cases of advanced-stage cancer.

Surgical intervention remains the primary treatment choice for localised and initial-stage BC (Czajka and Pfeifer, 2023). Typically, surgical procedures include two key options, lumpectomy and mastectomy, selected based on the tumour size, location,

and patient's clinical condition (Fisusi and Akala, 2019; Trayes and Cokenakes, 2021). Mastectomy is often performed for tumours larger than 5 cm, multiple tumour sites, inflammatory BC, Paget's disease of the nipple, or cases where cancer returns after previous treatment (Czajka and Pfeifer, 2023). Some women at high genetic risk, such as BRCA mutation carriers, may also choose preventive mastectomy to reduce their risk (Czajka and Pfeifer, 2023). Figure 2.5 illustrates the types of BC surgeries based on the tumour stage, showing the extent of tissue removal.

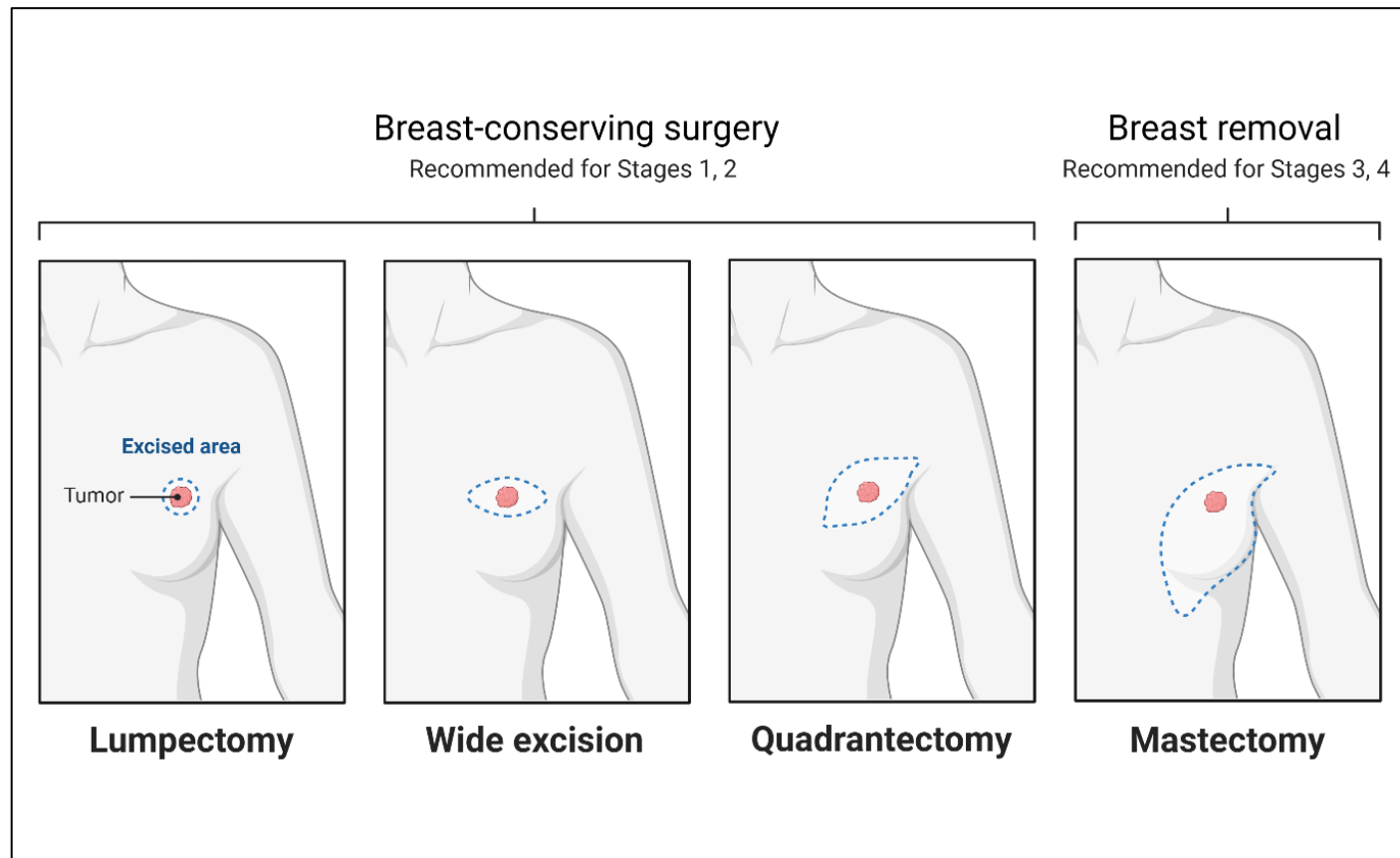


Figure 2.5: Types of breast cancer surgeries by tumour stage, highlighting tissue removal extent and recommendations for early vs. advanced stages (Created in <https://BioRender.com>).

Lymph node surgery remains an essential component of BC management, as it helps determine whether the cancer has spread to surrounding tissues (Burguin et al., 2021). Previously, axillary lymph node dissection (ALND) was the conventional technique used to assess lymph node involvement; however, this method often led to complications such as lymphedema (swelling of the arm) and restricted shoulder movement (Burguin et al., 2021). Sentinel lymph node biopsy (SLNB) is preferred because it achieves comparable survival results while minimising adverse effects (Burguin et al., 2021; Shen and Liu, 2023).

Nevertheless, ALND is still recommended in certain situations, particularly in patients diagnosed with advanced or inflammatory BC, when there are more than two positive sentinel lymph nodes, or when SLNB is unable to detect affected lymph nodes (Czajka and Pfeifer, 2023). Evidence from studies such as the NSABP B-32 trial and the ACOSOG Z0011 trial indicates that ALND can be omitted in patients with T1-T2 tumours, absence of clinically detectable axillary lymph nodes, no more than two positive sentinel nodes, and those receiving systemic therapy and RT for the entire breast (Czajka and Pfeifer, 2023). Despite these findings, ALND remains essential in cases involving locally advanced or inflammatory BC, more than two positive sentinel lymph nodes, or failed sentinel lymph node mapping, as these conditions indicate a higher likelihood of lymphatic spread and necessitate more extensive surgical intervention (Czajka and Pfeifer, 2023).

Breast reconstruction is considered a treatment option for patients who have undergone mastectomy, typically involving the use of implants or tissue harvested from the patient's own body (Riis, 2020). Although reconstruction aims to restore physical appearance and improve self-esteem, not all patients are content with the results (Riis, 2020). Research indicates that approximately 40% of women are dissatisfied with the outcome of their reconstruction, and receiving RT after mastectomy may further complicate the healing process and implant placement (Riis, 2020). The NSABP B-06 trial compared mastectomy and lumpectomy, followed by RT (Czajka and Pfeifer, 2023). The study concluded that survival outcomes were similar among the groups; however, the rate of cancer recurrence was notably higher

among patients who underwent lumpectomy without subsequent RT, with a recurrence rate of 39.2% after 20 years (Czajka and Pfeifer, 2023).

The existing recommended treatment protocol for early-stage BC includes lumpectomy combined with RT (Czajka and Pfeifer, 2023). The recommended standard treatment protocol for managing early-stage BC now involves lumpectomy followed by RT (Czajka and Pfeifer, 2023). Years of research have shifted treatment preferences from radical mastectomy toward breast-conserving strategies (Czajka and Pfeifer, 2023). Generally, surgical intervention combined with systemic therapy continues to be the preferred approach in BC treatment (Chen et al., 2023). Additional treatments following surgery, known as adjuvant therapy, may involve RT, chemotherapy, targeted therapy, or a combination of these modalities. These treatments are intended to eradicate any remaining cancer cells, reduce the likelihood of recurrence, and improve the survival rate in patients with early-stage BC (Fisusi and Akala, 2019).

RT is considered a primary therapeutic approach for treating malignant conditions, applied in both curative and palliative contexts for solid tumours (Wang and Tepper, 2021a). In BC management, RT is commonly recommended after lumpectomy to minimise the likelihood of recurrence and is also utilised following mastectomy, especially when there is evidence of cancer dissemination to the lymphatic system (Trayes and Cokenakes, 2021; Burguin et al., 2021; Wang and Tepper, 2021a). Nonetheless, RT is associated with various adverse effects, including skin inflammation, pneumonia, lymphedema, fibrosis, cardiovascular issues, and secondary lung cancers (Taylor et al., 2017; Burguin et al., 2021; Waks and Winer, 2019; Wang and Tepper, 2021a). Strategies like prone positioning and respiratory control techniques have been explored to lessen these side effects (Burguin et al., 2021). Despite these challenges, RT remains a critical element in BC therapy, contributing to reduced recurrence risk (Fisusi and Akala, 2019; Wang and Tepper, 2021a). Technological advancements, such as intensity-modulated RT (IMRT) and proton therapy, further aim to enhance treatment effectiveness while minimising toxicity, ensuring improved outcomes and reducing long-term complications among BC survivors (Wang and Tepper, 2021a).

Chemotherapy plays a vital role in the management of aggressive BC, aiming either to reduce tumour size before surgery (neoadjuvant chemotherapy, NAC) or to remove any remaining malignant cells following surgery (adjuvant chemotherapy) (Smolarz et al., 2022; Chen et al., 2023). NAC is particularly beneficial for cases of advanced-stage or non-operable BC, as it helps to decrease tumour volume, improve the possibility of breast-conserving surgery, and enhance tumour responsiveness to treatment, thereby increasing the feasibility of surgery (Fisusi and Akala, 2019; Faruk, 2021; Chen et al., 2023). It is commonly prescribed for individuals diagnosed with hormone receptor-negative BC and those with HER2-positive BC (Fisusi and Akala, 2019). In addition, for patients identified with estrogen receptor (ER)-positive and HER2-negative BC, systemic chemotherapy is recommended to lower the chances of disease recurrence. This decision is guided by various factors, including tumour grade, size, lymph node involvement, and hormone receptor status (Hong and Xu, 2022). Chemotherapy is also a critical component in the treatment strategy for TNBC, which lacks specific targeted therapeutic options (Abu Samaan et al., 2019). For stage II and III TNBC, NAC, mainly dose-dense anthracycline and taxane-based regimens are considered the preferred treatment approach (Hong and Xu, 2022).

First-line chemotherapy regimens vary, with common drugs including anthracyclines, taxanes (such as paclitaxel and docetaxel (DTX)), and platinum-based agents (Abu Samaan et al., 2019; Fisusi and Akala, 2019; Łukasiewicz et al., 2021; Hong and Xu, 2022). While these treatments are effective, chemotherapy can lead to both short-term and long-term side effects, including nausea, immune suppression, nerve damage, and cardiovascular issues (Waks and Winer, 2019; Shen and Liu, 2023). Traditional chemotherapy agents face challenges like poor solubility, low bioavailability, and non-targeted distribution, which often result in significant side effects such as nausea, fatigue, hair loss, immune suppression, peripheral neuropathy, and cardiotoxicity (Tran et al., 2020).

Treatment resistance remains another major challenge, as some cancer cells develop insensitivity to chemotherapy, impacting long-term survival (Ji et al., 2019; Abu Samaan et al., 2019; Faruk, 2021). Resistance can be intrinsic, where tumours are inherently unresponsive to treatment, or acquired, developing throughout therapy (Ji

et al., 2019). Additionally, it can be classified as primary resistance, where treatment fails from the outset, or multidrug resistance (MDR), where cancer cells become resistant to multiple chemotherapy agents (Ji et al., 2019).

Several factors contribute to drug resistance, affecting drug absorption, transport, metabolism, and cancer cell response (Ji et al., 2019). Mechanisms include changes in membrane proteins that pump drugs out of cells, enzyme-mediated drug inactivation, altered hormone receptor levels, and the activation of resistance-related genes (Ji et al., 2019). The tumour microenvironment, DNA repair mechanisms, and cancer stem cells also play crucial roles in resistance (Ji et al., 2019). Strategies such as P-glycoprotein (P-gp) inhibitors, glutathione (GSH) inhibitors, and targeted therapies have been explored to counteract these challenges (Ji et al., 2019). Targeted therapies focus on specific molecules that help cancer grow. For HER2-positive BC, drugs like trastuzumab and pertuzumab have greatly improved survival rates (Smolarz et al., 2022). Similarly, cyclin-dependent kinase 4/6 (CDK4/6) helps in treating Luminal B BC by slowing down cancer cell growth (Smolarz et al., 2022). However, targeted therapies are often costly and unavailable to all patients, leading to inequality in treatment access. Also, many cancers eventually become resistant to these treatments, showing the need for new approaches to bypass resistance mechanisms.

Immunotherapy demonstrated potential in overcoming resistance, particularly when combined with targeted therapies (Ji et al., 2019). Commonly used immune checkpoint inhibitors are nivolumab and ipilimumab (Ji et al., 2019). Gene therapy using MDR1 antisense oligonucleotides and ribonucleic acid (RNA) interference may increase chemosensitivity by reducing resistance gene expression (Ji et al., 2019). Immunotherapy aids the body's immune system in combating cancer. Checkpoint inhibitors such as pembrolizumab and atezolizumab have shown potential in treating TNBC (Tokumaru et al., 2020). However, immunotherapy only works for a small group of patients, usually those whose tumours have a marker called programmed death-ligand 1 (PD-L1) (Tokumaru et al., 2020). The high cost and side effects, such as fatigue, skin rash, diarrhoea, fever, and inflammation of organs like the lungs or liver, also limit its use.

Despite advancements in improving drug sensitivity, clinical outcomes remain unsatisfactory, emphasising the need for further research on resistance mechanisms and improved treatment strategies (Ji et al., 2019). NAC regimens have evolved from single-drug chemotherapy to combination therapies based on tumour subtypes. For example, HER2-targeted therapy combined with chemotherapy and neoadjuvant hormone therapy is now a standard approach (Chen et al., 2023). Although NAC has improved surgical outcomes, not all patients respond equally, making it difficult to predict treatment success (Chen et al., 2023). Advances in chemotherapy regimens, including new cytotoxic agents and neoadjuvant strategies, have improved overall and disease-free survival (Fisusi and Akala, 2019).

Hormonal therapy remains an essential treatment option for patients with hormone receptor-positive BC. It involves tamoxifen and aromatase inhibitors that block hormones that stimulate tumour growth (Smolarz et al., 2022). These drugs inhibit hormonal activity, contributing to cancer progression (Smolarz et al., 2022). Nevertheless, hormonal therapy may cause adverse effects such as reduced bone density and increased risk of blood clots, and its efficacy may decline over time as resistance develops in certain patients (Waks and Winer, 2019). Endocrine therapy plays a crucial role in treating tumours that are positive for hormone receptors. Ovarian suppression therapy is recommended for premenopausal women under the age of 35 or those undergoing chemotherapy (Shen and Liu, 2023). The typical duration of hormone therapy has been prolonged from five years to a range of five to ten years, and CDK4/6 inhibitors like abemaciclib are now prescribed for patients at high risk (Shen and Liu, 2023). Furthermore, advancements in anti-HER2 adjuvant therapy have evolved from trastuzumab monotherapy to dual-HER2 blockade (pertuzumab + trastuzumab) and more intensive options like trastuzumab emtansine or extended therapy using neratinib (Shen and Liu, 2023).

For metastatic breast cancer (MBC), treatment focuses on symptom control and survival extension rather than achieving a cure (Fisusi and Akala, 2019). While significant advancements have been made, MBC remains incurable mainly, though some patients achieve long-term remission (Fisusi and Akala, 2019). Each treatment approach has strengths, but side effects, resistance, and accessibility remain significant

challenges. Research now focuses on combining multimodal strategies to improve treatment effectiveness while ensuring patient safety and comfort. Nanotechnology also offers a promising alternative by enhancing drug release, improving stability, and increasing efficacy (Tran et al., 2020). NPs and liposomes improve drug retention and reduce systemic toxicity (Ji et al., 2019). NPs can co-encapsulate multiple drugs, improving biodistribution, solubility, and pharmacokinetics while reducing toxicity (Fisusi and Akala, 2019). Studies have shown that polymer-lipid hybrid NPs loaded with doxorubicin and mitomycin C exhibit efficacy in drug-resistant BC models (Fisusi and Akala, 2019). NP micellar formulations of paclitaxel, rapamycin, and 17-AAG have demonstrated superior anti-tumour effects in xenograft models (Fisusi and Akala, 2019). Trastuzumab-functionalized nanoparticles have also been developed for HER2-targeted drug delivery (Fisusi and Akala, 2019). Unlike traditional chemotherapeutic drugs that affect both normal and cancerous cells, NP-coated drugs accumulate in tumours through the enhanced permeability and retention (EPR) effect (Singh et al., 2017). NPs can deliver chemotherapy drugs directly to tumour sites, reducing side effects by improving solubility, extending circulation time, and targeting tumour tissues via the EPR effect. Furthermore, advanced drug delivery systems, such as MNs, can improve tumour targeting while minimizing damage to healthy cells (Ji et al., 2019; Abu Samaan et al., 2019). Table 2.1 below outlines the current treatment options for BC and their associated limitations.

Table 2.1: Summary of breast cancer treatment modalities, their advantages, and limitations.

Treatment modality	Advantages	Limitations	References
Surgery (Lumpectomy and/or mastectomy)	• Effective for localised tumours • Options for breast reconstruction	• High dissatisfaction rate • Radiation post-mastectomy can cause complications with implants • Risk of lymphedema • Loss of mobility, loss of quality of life, pain, infections • Skin necrosis, hematoma, or infections	• Fisusi and Akala, 2019 • Burguin et al., 2021 • Czajka and Pfeifer, 2023
Radiation Therapy (intensity-modulated RT (IMRT), X-rays, gamma rays, or proton beam)	• Lowers risk of recurrence	• Side effects include pneumonitis, lymphedema, fibrosis, and heart problems.	• Wang and Tepper, 2021 • Trayes and Cokenakes, 2021 • Burguin et al., 2021

Chemotherapy (anthracyclines, taxanes (e.g., paclitaxel and docetaxel), and platinum- based agents)	• Effective for aggressive cancers • Can shrink tumours pre-surgery	• Severe side effects like immune suppression, nerve damage, and heart problems • Non-specific targeting damages healthy cells. • Drug resistance	• Faruk, 2021 • Łukasiewicz et al., 2021 • Smolarz et al., 2022 • Chen et al., 2023
Hormonal therapy (tamoxifen and aromatase inhibitors)	• Useful for hormone receptor-positive cancers • Slows or stops cancer growth	• Side effects like bone thinning and blood clots • Resistance development limits the effectiveness	• Waks and Winer, 2019 • Smolarz et al., 2022 • Shen and Liu, 2023
Targeted therapy (trastuzumab and pertuzumab, CDK4/6 inhibitors)	• Improved survival for HER-2 positive and Luminal B cancers • Less damage to healthy cells	• High cost and limited access • Resistance develops over time.	• Singh et al., 2017 • Fisusi and Akala, 2019

Immunotherapy (Checkpoint inhibitors like pembrolizumab and atezolizumab) nivolumab and ipilimumab	• Effective for a subset of patients, especially TNBC with PD-L1 marker	• Works for a small patient group • High cost • Significant side effects • Weak immune response	• Tokumaru et al., 2020
Nanomedicine (Polymer-lipid hybrid NPs or polymeric micelles loaded with chemotherapeutic drugs such as doxorubicin and mitomycin C, paclitaxel, rapamycin, and 17-AAG)	• Reduces side effects by targeting tumours directly • Promising for overcoming drug resistance	• High cost and limited availability • Requires further clinical trials for widespread use	• Singh et al., 2017 • Tran et al., 2020

Recently, combination therapy has been a key strategy in BC treatment (Fisusi and Akala, 2019). Historically, combination chemotherapy involved alkylating agents (e.g., cyclophosphamide) and antimetabolites (e.g., methotrexate, 5-fluorouracil) (Fisusi and Akala, 2019). The CMF regimen (cyclophosphamide, methotrexate, and 5-fluorouracil) was one of the first adjuvant chemotherapy protocols to significantly reduce recurrence rates (Fisusi and Akala, 2019). Polychemotherapy generally results in higher response rates than single-agent regimens, although its impact on overall survival varies (Fisusi and Akala, 2019). Taxane-based combination therapies have demonstrated significant efficacy (Fisusi and Akala, 2019). The combination of DTX and doxorubicin (AT) has shown superior response rates compared to doxorubicin and cyclophosphamide (AC) in MBC treatment (Fisusi and Akala, 2019). Other combinations, such as DTX plus cisplatin, have been effective in anthracycline-resistant MBC patients (Fisusi and Akala, 2019). Additionally, taxanes combined with HER2-targeted therapies have revolutionised HER2-positive BC treatment (Fisusi and Akala, 2019). The CLEOPATRA trial, a Phase III study evaluating first-line (1L) PERJETA and Herceptin® (trastuzumab)-based therapy for HER2-positive MBC, demonstrated that adding pertuzumab to trastuzumab and DTX significantly improved progression-free and overall survival in these patients (Fisusi and Akala, 2019). Advancements in targeted therapy, immunotherapy, cancer vaccines and nanomedicine continue to reshape BC treatment. Emerging nanomedicine-based treatments would require more research to improve drug delivery precision, reduce toxicity, and enhance patient outcomes.

2.1.4 Pain and Quality of Life of Breast Cancer Patients

BC and its treatment can take a serious toll on both the body and mind. Many patients go through pain, fatigue, and emotional stress, especially if they need aggressive treatments like chemotherapy or RT (Zubair et al., 2021). The side effects of surgery, chemotherapy, and RT can also affect their daily lives and how they feel about their bodies, making the emotional burden even heavier (Zubair et al., 2021). As more people survive BC, it is becoming clear that it is not just about living longer but

also about living well. This is why there is more focus now on quality of life and long-term outcomes, like whether the cancer returns or spreads (Arnold et al., 2022).

Survival outcomes for BC are generally favourable in most high-income countries, often exceeding 90% (Arnold et al., 2022). However, these survival outcomes differ across regions, with five-year overall survival (OS) rates reported at 94.6% in Korea, 90.5% in Hong Kong, and 74% in Vietnam (Youn and Han, 2020). In contrast, survival rates can drop as low as 12% in lower-income regions such as sub-Saharan Africa, as seen in Uganda (Arnold et al., 2022). This disparity is primarily due to delayed diagnoses and limited access to treatment facilities in poorer regions (Arnold et al., 2022).

In Malaysia, the five-year relative survival rate for BC is 66.8%, which is notably lower than in high-income countries (Hans et al., 2021; Mohan et al., 2021). The survival rates decrease drastically with advancing cancer stage: 85.8% for Stage I, 76.0% for Stage II, 53.3% for Stage III, and only 23.3% for Stage IV (Hans et al., 2021; Sung et al., 2021; Mohan et al., 2021). Furthermore, there are disparities between sexes, with women having a survival rate of 67.6%, while men exhibit a lower rate at 48.5% (Hans et al., 2021; Mohan et al., 2021). Ethnic variations are also evident; the survival rate is 69.0% for Chinese, 65.8% for Malays, and 59.6% for Indians, indicating potential socio-economic and access-related factors at play (Hans et al., 2021; Mohan et al., 2021).

Late-stage diagnosis remains a critical challenge in Malaysia. A national review of cancer registry data found that over 47.5% of BC cases were diagnosed at late stages (Stage III and IV), which drastically reduces the likelihood of successful treatment and long-term survival (Cheng et al., 2015; Sung et al., 2021). This late presentation is attributed to poor awareness, delayed health-seeking behaviour, and limited accessibility to early detection services in certain regions (Cheng et al., 2015; Sung et al., 2021; Mohan et al., 2021). These findings emphasise the urgent need for strengthened public awareness campaigns, improved access to screening, and timely treatment to improve BC outcomes in Malaysia.

This highlights the importance of increasing public understanding of BC and promoting early detection initiatives (Arnold et al., 2022). Public awareness campaigns and reducing the stigma surrounding BC can contribute to better detection rates and the overall well-being of affected individuals (Arnold et al., 2022).

Additionally, the battle faced by BC survivors continues even after their treatment concludes. Many survivors struggle with ongoing physical discomfort, fatigue, and emotional challenges (Arnold et al., 2022). Proper follow-up care is vital to help them cope with these long-term effects. Persistent pain, for instance, is reported in approximately 25% to 60% of BC survivors (Wang et al., 2018). This chronic pain may worsen anxiety, depression, and emotional distress, leading to a complex cycle that is difficult to break.

In some cases, pain after surgery can last for more than three months, affecting around 40% of those who have surgery (Wang et al., 2018). Even less invasive surgeries like breast-conserving therapy can cause numbness, limited arm movement, and swelling due to lymphedema (Wang et al., 2018). RT and chemotherapy can also cause lasting pain, mainly because of nerve damage and skin problems (Wang et al., 2018).

The emotional impact of a mastectomy can be difficult for some women. Losing a breast can make them feel less feminine or less attractive, which can cause severe emotional stress (Türk and Yılmaz, 2018). Women who see their breasts as a big part of their identity tend to feel this loss more strongly, which can lead to anxiety, depression, and a fear that the cancer might come back (Türk and Yılmaz, 2018). Almost half of all cancer patients deal with some mental health issue, with anxiety and depression being the most common (Villar et al., 2017). BC patients, in particular, are more likely to face these challenges, especially if they are younger, unmarried, or have a history of mental health problems (Villar et al., 2017). Treatments like chemotherapy, RT, and hormone therapy can also add to the emotional strain because of their side effects (Villar et al., 2017).

Needle fear is another common issue that prevents many BC patients from starting or going to their treatments (Duncanson et al., 2021). The fear could range from mild discomfort or anxiety to severe phobia, especially when they have to go for treatments continuously (Duncanson et al., 2021). In the Netherlands, about 16.1% of adults have injection fear, while needle phobia is less common but still present, with rates of 1.1% in the Netherlands, 0.5% in South Korea, 1.6% in Sweden, and 2.1% in the USA (Duncanson et al., 2021).

In Malaysia, studies have similarly reported the prevalence of needle-related fear, particularly in clinical and hospital settings. Sulaiman et al. (2018) highlighted that even among healthcare professionals, apprehension and negative perceptions toward procedures such as lumbar puncture were common. Dalawi et al. (2022) observed that fear and anxiety related to injections contributed to procedural hesitancy, potentially affecting cancer patients who require regular IV therapies. Additionally, Hassali et al. (2012) discussed how concerns surrounding pain and injection safety can negatively impact patient adherence, particularly in long-term treatments involving frequent needle administration. Although specific data on needle phobia among Malaysian BC patients remain scarce, these findings point to a wider cultural and psychological barrier that may similarly delay diagnosis, treatment initiation, or follow-up care in oncology settings.

Unfortunately, many patients do not report their needle distress because they see it as an unavoidable part of staying alive (Duncanson et al., 2021). Moreover, this issue is not often measured in clinical care and tends to go unnoticed and untreated (Duncanson et al., 2021). Figure 2.6 below shows the schematic representation of an overview of pain and quality of life challenges faced by BC patients across different stages of diagnosis, treatment, and post-treatment.

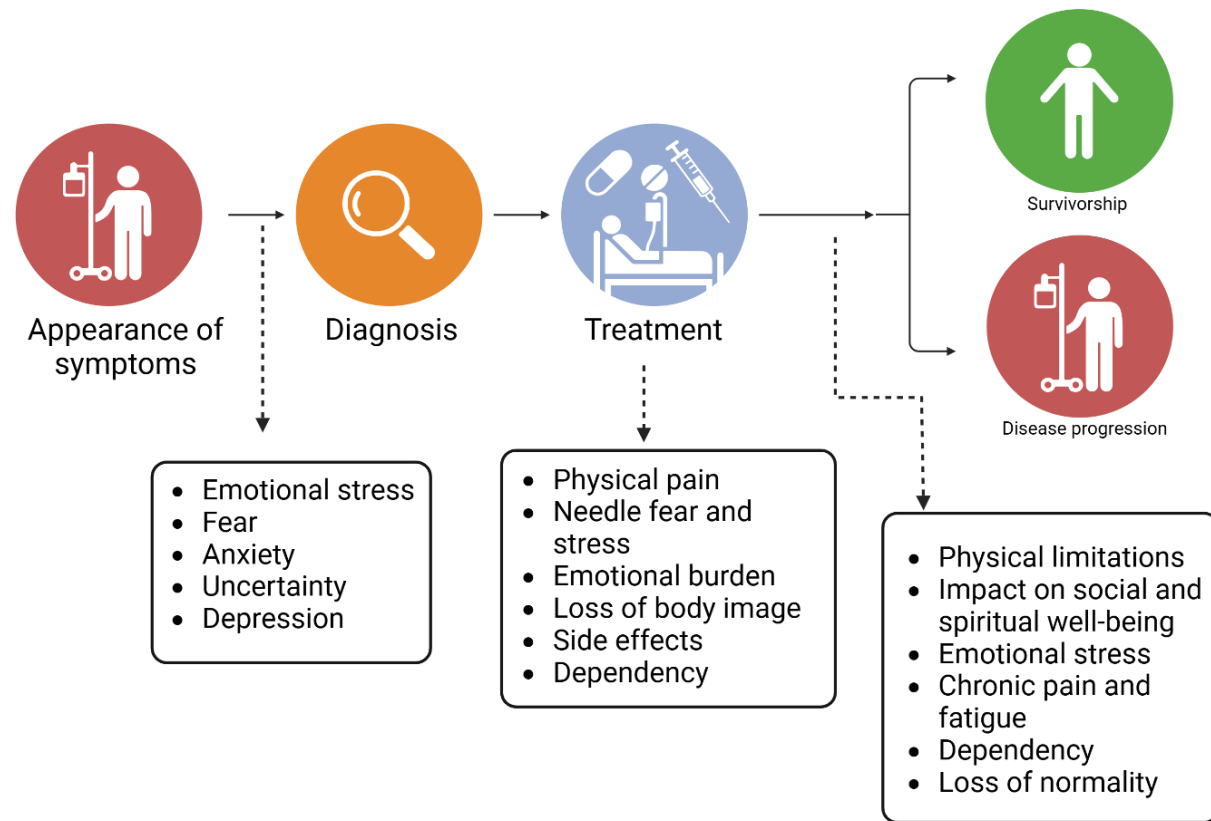


Figure 2.6: Schematic overview of pain and quality of life challenges for breast cancer patients at different stages of diagnosis, treatment, and post-treatment (Created in <https://BioRender.com>).

Additionally, the COVID-19 pandemic made things even harder for BC patients. Many hospitals delayed cancer treatments to focus on COVID-19 cases, which led to more advanced cancer diagnoses and higher anxiety among patients (İlgün and Özmen, 2021). During this time, hormone therapy was given to patients with HR-positive BC to control the tumour until surgery could be done safely (İlgün and Özmen, 2021). Reconstructive surgeries were also postponed to free up emergency healthcare resources (İlgün and Özmen, 2021). Many patients were scared to go to hospitals because they did not want to contract the virus, which worsened the delays and left them feeling more anxious and hopeless (İlgün and Özmen, 2021).

Focusing on quality of life means dealing with both the physical pain and the emotional struggles that come with BC. Pain management, emotional support through therapy or support groups, and ensuring patients are part of the decision-making process can all help. However, researchers and medical professionals should consider developing more patient-compliant treatment approaches to tackle these issues more effectively. Additionally, there are very few studies on the impact of treatment modalities on the physiological and psychological well-being of BC patients. This gap in research is concerning, especially since women, as core members of society handling roles like motherhood, often face the unfair expectation of enduring pain due to stereotypes associated with menstruation and childbirth. The dismissal of these issues is problematic and needs to be seriously addressed. Moreover, in the upcoming years, we could also be faced with more serious cases like COVID-19, which would have an even more significant impact on BC patients. Thus, there is a dire need for a more personalised treatment approach for patients.

2.2 Docetaxel (DTX)

2.2.1 Pharmacology and Mechanism of Action of Docetaxel

Taxanes, such as paclitaxel and DTX, are commonly used as antineoplastic agents in both adjuvant and neoadjuvant chemotherapy for BC (National Comprehensive Cancer Network, 2015; Chan, 2019). DTX was initially extracted from the bark of the Pacific Yew tree and the needles of *Taxus baccata* L, as illustrated in Figure 2.7 (A), and was first developed by Pierre Potier (Andrigueti et al., 2017; Zhang et al., 2019).

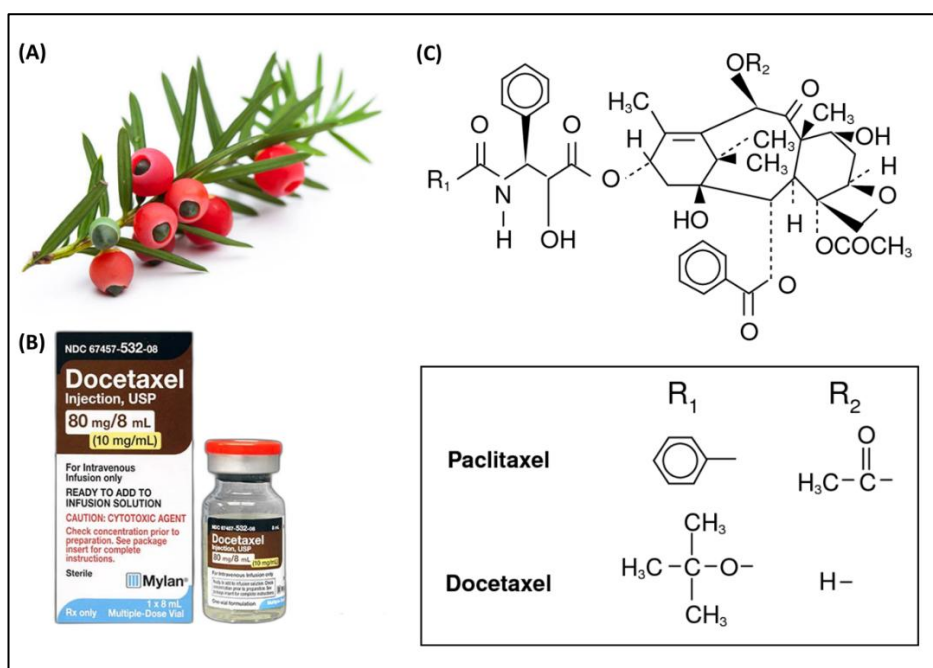


Figure 2.7: The natural source of docetaxel from *Taxus* species is shown in (A) (stock image from Kovalenko, 2019). The commercial formulation of docetaxel is depicted in (B) (image sourced from Fvasconcellos, Wikimedia Commons, accessed March 20, 2025). The chemical structure of docetaxel is illustrated in (C), and (D) highlights the key structural differences between paclitaxel and docetaxel (adapted from Zhang et al., 2023 and created in <https://BioRender.com>).

DTX is chemically similar to paclitaxel but has been modified to enhance its properties, as shown in Figure 2.7 (C). Key modifications include the addition of a tert-butyl carbamate ester in its side chain and a hydroxyl group at position C-10, which increases DTX's water solubility and potency compared to paclitaxel (Ashrafizadeh et al., 2021; Sharma et al., 2017; Imran et al., 2020). These structural alterations also contribute to reduced toxicity and improved drug absorption and efficacy in the body (Imran et al., 2020; Sekino and Teishima, 2020). Compared to paclitaxel, another taxane, DTX is more soluble, potent, and easier for the body to absorb (Sousa-Pimenta et al., 2023).

The Food and Drug Administration (FDA) of the United States approved DTX for BC treatment in 1996 after it demonstrated strong efficacy in clinical trials (Ebrahimi Fart et al., 2017; da Silva et al., 2018). Since then, DTX has been successfully used to treat various other cancers, including lung, ovarian, and gastric (Imran et al., 2020; Sekino and Teishima, 2020). Due to its high lipophilicity, DTX dissolves well in fats, efficiently targeting and penetrating cancer cells (da Silva et al., 2018; Imran et al., 2020). Its ability to treat multiple cancer types underscores its versatility and significance in cancer therapy.

DTX works by stabilising microtubules, which are structures that help cells divide. It binds to a part of tubulin, a protein that makes up microtubules, and prevents them from breaking down during cell division (Imran et al., 2020; Sekino and Teishima, 2020; Perez et al., 2024). DTX disrupts the normal dynamics of microtubules necessary for cells to divide correctly. As a result, cancer cells get stuck at the G2/M phase of the cell cycle, which eventually leads them to self-destruct through a process called apoptosis (Sohail et al., 2018; Tian et al., 2021). The working mechanism of DTX in cancer is shown in Figure 2.8.

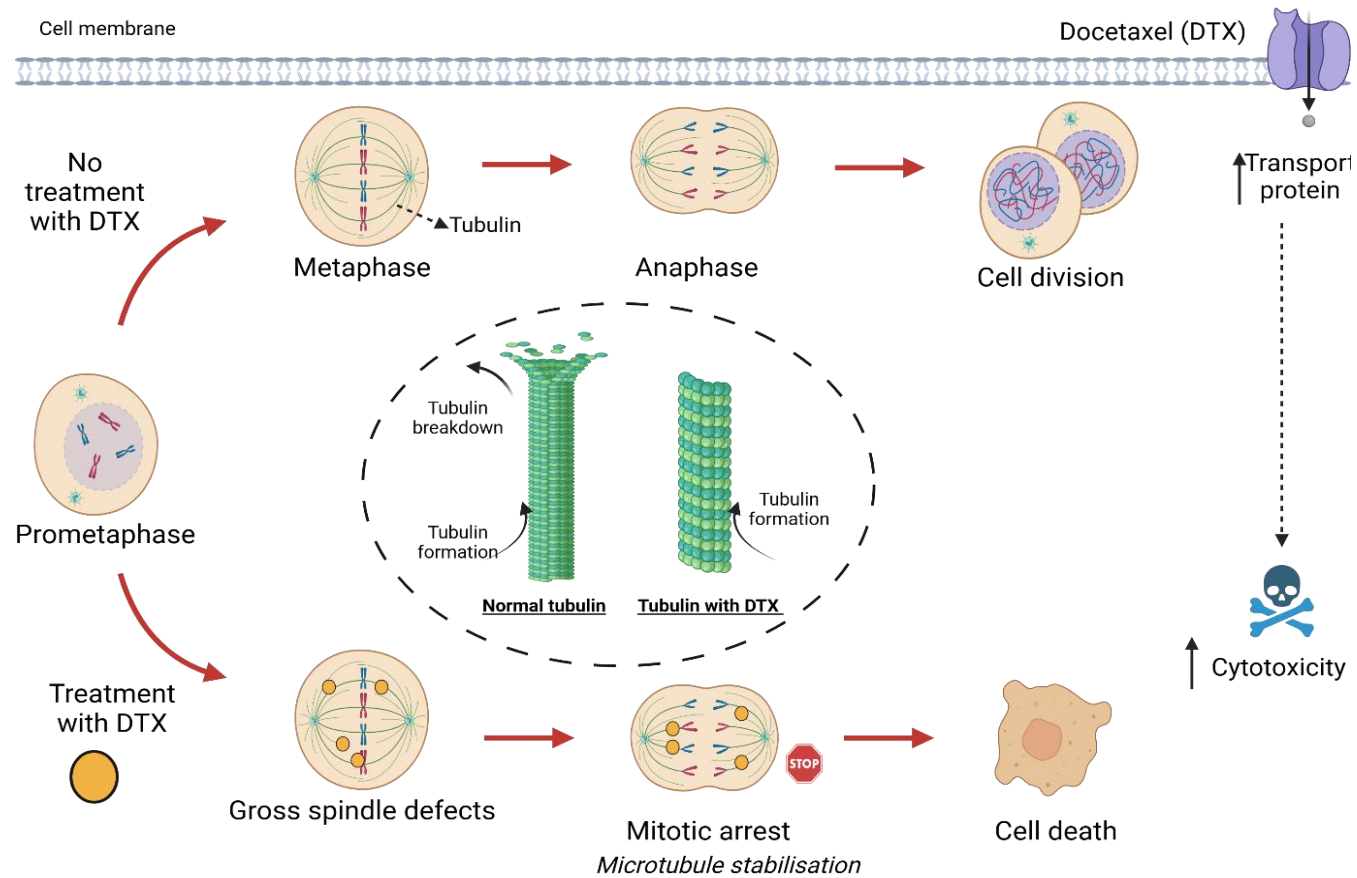


Figure 2.8: Schematic illustration of the mechanism of action of docetaxel in inhibiting cancer cell division. Docetaxel stabilises microtubules, arrests the cell cycle at the G2/M phase, and induces apoptosis in tumour cells. Created in <https://BioRender.com>.

Besides that, DTX has a higher affinity for microtubules, and studies have shown that DTX is about five times better at stabilising microtubules than paclitaxel, binding more strongly than paclitaxel (Zhang et al., 2019). This stronger binding makes DTX more effective at stopping cancer cells from dividing and allows for lower doses, which can help reduce side effects.

Beyond microtubule stabilisation, DTX influences multiple molecular pathways in cancer cell survival. It has been found to promote apoptosis by increasing the accumulation of cleaved poly (ADP-ribose) polymerase (PARP), a key marker of programmed cell death (Scherbakov et al., 2023). Additionally, it downregulates B-cell lymphoma 2 (Bcl-2), an anti-apoptotic protein, thereby increasing the susceptibility of cancer cells to apoptosis (Scherbakov et al., 2023). DTX also interferes with critical signalling pathways such as the protein kinase B/mammalian target of rapamycin (Akt/mTOR) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) pathways, both of which play essential roles in regulating cancer cell proliferation and survival (Sousa-Pimenta et al., 2023). Furthermore, it induces extracellular signal-regulated kinase (ERK) activation in a dose-dependent manner, suggesting its influence on multiple cellular regulatory mechanisms beyond microtubule stabilisation (Scherbakov et al., 2023).

The role of ribosomal protein S6 kinase (S6K) in docetaxel resistance has also been explored, as overexpression of S6K has been correlated with poor prognosis in several cancers. However, in BC cells, DTX did not significantly alter S6K expression or activity (Scherbakov et al., 2023). Another significant effect of DTX treatment is its impact on cell cycle regulation. It has been shown to induce a notable decrease in cyclin D1, a key regulator of cell cycle progression, but only in hormone-resistant BC cells, indicating its potential role in disrupting the regulatory pathways that control cell cycle progression (Scherbakov et al., 2023). These findings suggest that DTX exerts a more profound effect on cancer cells with pre-existing resistance to hormone therapies, further reinforcing its importance as a chemotherapeutic agent.

DTX is primarily metabolised in the liver by cytochrome P450 (CYP) enzymes, particularly CYP3A4 and CYP3A5 (Imran et al., 2020; Steinberg et al., 2020). These enzymes facilitate the breakdown and elimination of the drug from the body. However, variations in liver function can significantly affect DTX clearance, leading to potential toxicity. Patients with liver dysfunction often require dose adjustments to minimise adverse effects. Understanding the metabolic pathways of DTX is essential for optimising dosage regimens and ensuring its safe and effective use in clinical settings.

Overall, the pharmacology of DTX underscores its importance in cancer treatment, particularly its ability to target microtubules, disrupt key cancer pathways, and enhance apoptosis. The ongoing exploration of its mechanisms of action continues to contribute to improved strategies for enhancing its efficacy, overcoming resistance, and reducing toxic side effects.

2.2.2 Docetaxel in Breast Cancer

DTX is commonly used in both neoadjuvant (before surgery) and adjuvant (after surgery) settings in the treatment of early-stage BC to improve the chances of a full recovery (Tian et al., 2021; Perez et al., 2024; Panthi et al., 2024). Neoadjuvant therapy helps to shrink tumours before surgery, making it easier to remove them altogether, and adjuvant treatment is used to target any remaining cancer cells to prevent reoccurrence (Tian et al., 2021; Perez et al., 2024; Panthi et al., 2024).

Generally, DTX is given to BC patients through an IV because it does not dissolve well in water and is often mixed with solvents to navigate it through the body more effectively (Steinberg et al., 2020). DTX is usually given at doses between 60 and 100 mg/m², with the exact dose depending on factors like the treatment setting and the patient's condition (Gupta et al., 2023). The most common schedule is a one-hour infusion every three weeks, as this regimen has shown reliable effectiveness (Gupta et al., 2023). Weekly dosing schedules have also been studied, using doses of

30 to 40 mg/m² over a six-week cycle or three weeks in a four-week cycle (van Eijk et al., 2022).

While weekly dosing is linked to a lower risk of febrile neutropenia, it may cause other chronic side effects (van Eijk et al., 2022). In Western countries, the standard dose is usually 75–100 mg/m² every three weeks. However, in some Asian populations, a lower dose of 60 mg/m² every three weeks is often used due to increased sensitivity to blood-related toxicities (Kusaka et al., 2022; van Eijk et al., 2022). Thus, DTX is also often used in combination therapies to enhance treatment efficacy, especially in metastatic and recurrent BC (rMBC) (Li et al., 2019a).

DTX is frequently combined with other cancer therapies to enhance its effectiveness against BC (Sim et al., 2018; Sousa-Pimenta, 2023). In advanced BC, it is often used alongside targeted agents that block key molecular pathways involved in tumour progression and survival. These combinations have been shown to improve tumour response rates and reduce the likelihood of treatment resistance (Sousa-Pimenta, 2023). Due to its established efficacy, DTX remains a cornerstone therapy in early-stage and metastatic BC, with clinical trials continuously evaluating its long-term efficacy, either as a monotherapy or in combination regimens (Sousa-Pimenta, 2023).

A meta-analysis by Gherzi et al. (2015) demonstrated that a three-week DTX regimen significantly improved overall survival and disease progression time in advanced BC, leading to its recommendation as a first-line treatment for rMBC (Li et al., 2019a). However, combination therapies have shown even greater efficacy and tolerability. The DTX-gemcitabine (DG) regimen enhanced treatment outcomes with milder side effects, offering a viable alternative for advanced-stage disease (Li et al., 2019a). Additionally, the DTX-lobaplatin (DL) regimen demonstrated manageable toxicity profiles with no treatment-related mortality, further supporting its role in combination therapy (Li et al., 2019a).

Several clinical trials have evaluated DTX-based combination regimens in BC treatment. A phase II neoadjuvant trial investigating a DTX-epirubicin combination in patients with large operable, locally advanced, or inflammatory BC (Stage III) observed a 76.7% response rate (Fisusi and Akala, 2019). Despite high rates of grade 4 neutropenia (80%) and febrile neutropenia, the administration of prophylactic hematopoietic growth factor support allowed for the successful completion of therapy, confirming the feasibility and efficacy of this regimen (Fisusi and Akala, 2019).

The National Surgical Adjuvant Breast and Bowel Project Protocol B27 further demonstrated that adding preoperative DTX to doxorubicin and cyclophosphamide significantly improved clinical and pathological response rates in operable BC (Fisusi and Akala, 2019). Similarly, a phase II trial assessing a DTX-carboplatin combination in stage II/III BC reported high response rates, particularly in TNBC patients (Fisusi and Akala, 2019).

A phase III multicenter trial compared DTX-doxorubicin (AT) to doxorubicin-cyclophosphamide (AC) in MBC patients. Among 429 patients, those receiving AT had a significantly higher overall response rate (59%) compared to AC (47%) ($P = 0.009$), with complete response rates of 10% and 7%, respectively (Fisusi and Akala, 2019). Furthermore, a prospective randomised trial found that carboplatin plus DTX with granulocyte colony-stimulating factor (G-CSF) support demonstrated significant anti-tumour activity in stage I–III TNBC with manageable toxicity (Sharma et al., 2017). These findings suggest that anthracycline-free regimens incorporating DTX should be explored further compared with standard taxane-anthracycline protocols in future neoadjuvant studies for TNBC (Sharma et al., 2017).

Overall, DTX's broad-spectrum activity and its synergistic potential in combination therapies make it a valuable option in BC management. However, treatment-related toxicities remain a concern, necessitating continued research to optimise therapeutic approaches and improve patient outcomes.

2.2.3 Challenges, Limitations, and Side Effects of Docetaxel

DTX is an effective chemotherapy drug, but it has several serious side effects that depend on the dose (Sohail et al., 2018; Imran et al., 2020; Sekino and Teishima, 2020). One of the most dangerous side effects is neutropenia, where the body has too few white blood cells, making patients more likely to get life-threatening infections (Sohail et al., 2018). Many neutropenia patients must be hospitalised and treated with IV antibiotics to prevent infections (Sohail et al., 2018; Steinberg et al., 2020).

In addition to neutropenia, DTX can cause myelosuppression (reduced bone marrow activity), peripheral neuropathy (nerve damage that leads to tingling or numbness), and gastrointestinal problems like nausea and diarrhoea (Hashemi et al., 2023). Other serious side effects include hypersensitivity reactions, swelling in the arms or legs (peripheral oedema), weight gain, and fluid buildup around the heart (pericardial effusion) (Sohail et al., 2018). Figure 2.9 summarises the significant barriers of DTX associated with IV delivery and the side effects.

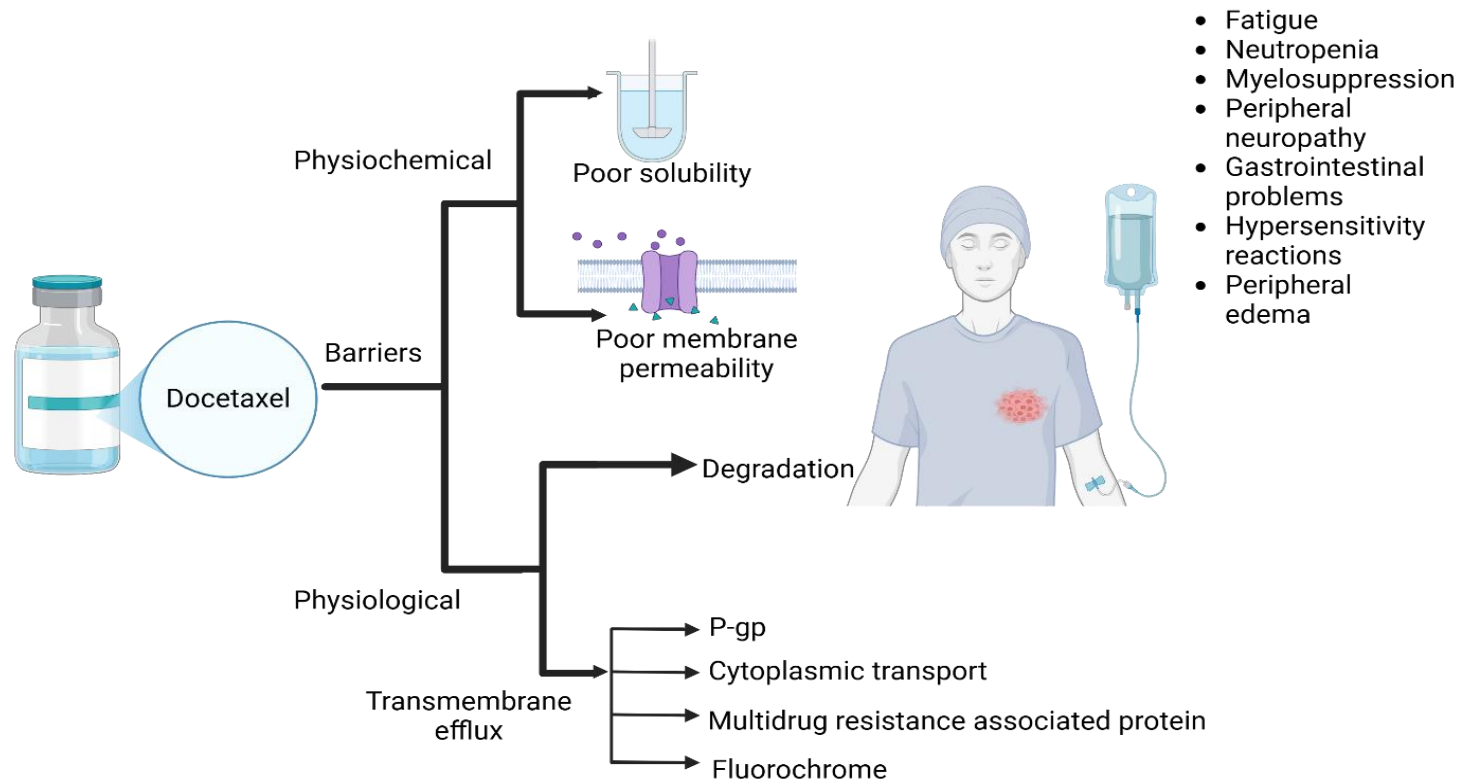


Figure 2.9: Challenges and limitations of intravenous (IV) administration of docetaxel in breast cancer treatment (adapted and modified from Panthi et al., 2024; created in <https://BioRender.com>).

These side effects are caused by DTX and the additional substances used in its commercial formulation to help dissolve the drug, such as Tween 80 (polysorbate 80) or ethanol (Sohail et al., 2018). Table 2.2 below presents the concentrations of DTX, polysorbate 80, and ethanol, along with other excipients used in various DTX formulations, including Taxotere® (original and other variants) and generic versions such as Accord, Actavis, Hospira, Sandoz, and Teva.

Table 2.2: Excipients contained in the different docetaxel products used in the study

Formulation	Docetaxel (mg/ml)	Polysorbate 80 (mg/100mg)	Ethanol (mg/100mg)	Other excipients	References
Taxotere® (Original)	100	2600	925	Citric acid (NR)	Garrido-Siles et al., 2015; González-Haba- Peña et al., 2020
Taxotere®	100	2000	1975	NR	Garrido-Siles et al., 2015; González-Haba- Peña et al., 2020
Docetaxel Accord	100	2600	1975	Citric acid (NR)	Cheng et al., 2019; González-Haba-Peña et al., 2020
Docetaxel Actavis	100	2120	2000	Citric acid (NR), povidone (NR)	Cheng et al., 2019; González-Haba-Peña et al., 2020
Docetaxel Hospira	100	2600	1820	Citric acid (~40 mg/100 mg), PEG 300 (NR)	Cheng et al., 2019; Garrido-Siles et al., 2015; González-Haba- Peña et al., 2020

Docetaxel Sandoz	100	800	2562	Citric acid (NR), Macrogol 300 (NR)	Cheng et al., 2019; Garrido-Siles et al., 2015; González-Haba- Peña et al., 2020
Docetaxel Teva	100	NR	<500	-	González-Haba-Peña et al., 2020

*Note: NR= Not reported in the cited sources.

Tween 80 has been linked to allergic reactions and can make cell membranes more permeable, possibly leading to heart-related problems (Sohail et al., 2018; Tian et al., 2021). It also interacts with proteins in the blood, which can change how DTX is absorbed and processed by the body (Sohail et al., 2018; Tian et al., 2021). This interaction can increase the free DTX in the blood, worsening blood-related side effects like neutropenia (Sohail et al., 2018; Ashrafizadeh et al., 2021). The way DTX is given can also influence its side effects. Studies show that a weekly schedule of DTX can be just as effective as the standard three-weekly schedule but with a lower risk of severe neutropenia (Stoicescu et al., 2021; van Eijk et al., 2022; Gupta et al., 2023).

However, the weekly schedule tends to cause more chronic side effects like fatigue, fluid retention, and nail disorders (Gupta et al., 2023). Fatigue is one of the most common side effects reported by patients receiving DTX. This kind of fatigue differs from regular tiredness because it does not go away with rest and can affect a person's physical, emotional, and mental energy (Bergin et al., 2017; Steinberg et al., 2020). This means that doctors often have to weigh the risks and benefits of each schedule based on the patient's tolerance of the treatment (Gupta et al., 2023).

Overall, while DTX is a powerful tool for treating BC, its side effects can be severe and limit its use in some patients. Most of the current formulations use Tween 80, ethanol, or other excipients, which could cause more intense and severe side effects in BC patients. This is why improving DTX delivery through carriers such as NPs or developing better formulations without Tween 80 or ethanol could be a promising way to reduce side effects while maintaining or enhancing its effectiveness.

2.3 Nanocarriers in Cancer Therapy

Nanotechnology has become a crucial tool in cancer treatment, particularly for enhancing the effectiveness of drug delivery (Wang, 2024). Nanocarriers, which typically measure between 1 and 100 nm in size, possess unique physical and chemical characteristics that facilitate site-specific drug delivery, improve bioavailability, and enable controlled drug release, thereby minimizing systemic toxicity (Aghebati-Maleki et al., 2020).

Nanocarriers are classified into organic and inorganic NPs (Aghebati-Maleki et al., 2020). Organic nanocarriers encompass micelles, dendrimers, solid lipid NPs (SLNs), liposomes, and polymer-based NPs. In contrast, inorganic nanocarriers comprise gold NPs (AuNPs), silica-based NPs, quantum dots, and magnetic NPs (Singh et al., 2017; Aghebati-Maleki et al., 2020). These nanocarriers facilitate chemotherapy drug delivery by improving solubility, extending their systemic circulation time, and increasing tumour accumulation through the enhanced permeability and retention (EPR) effect (Singh et al., 2017).

Liposomes, spherical vesicles made of phospholipid bilayers enclosing an aqueous core, deliver hydrophilic and hydrophobic drugs effectively (Tran et al., 2020). Their biocompatibility and tendency to accumulate in tumour tissues due to the enhanced permeability and retention (EPR) effect have contributed to their widespread application in cancer therapy (Tran et al., 2020). An example is Doxil®, a PEGylated liposomal formulation of doxorubicin, which has received FDA approval for BC treatment (Tran et al., 2020). Additionally, paclitaxel-loaded liposomes have demonstrated increased cytotoxic activity against MCF-7 BC cells compared to the free drug formulations (Tran et al., 2020).

Solid lipid NPs (SLNs) comprise a solid lipid core that improves drug stability, controlled release, and biocompatibility (Aghebati-Maleki et al., 2020). SLNs have been employed to encapsulate tamoxifen, paclitaxel, and doxorubicin, demonstrating improved cellular uptake and overcoming drug resistance by enhancing intracellular

drug accumulation (Aghebati-Maleki et al., 2020). Tamoxifen-loaded SLNs have been found to reverse resistance in tamoxifen-resistant BC cells by inducing apoptosis, while DTX-incorporated SLNs exhibited prolonged circulation time and higher tumour accumulation than conventional formulations (Tran et al., 2020).

Dendrimers are highly branched polymers possessing numerous reactive sites that enable attachment of anticancer agents, targeting ligands, and imaging molecules (Aghebati-Maleki et al., 2020). Their high drug-loading capacity and ability to minimize systemic toxicity and improve tumour targeting make them promising nanocarriers for BC therapy (Aghebati-Maleki et al., 2020).

Gold NPs (AuNPs) possess distinctive optical and thermal features, making them suitable candidates for photothermal therapy and site-specific drug delivery (Aghebati-Maleki et al., 2020; Wang et al., 2021b). Functionalised AuNPs conjugated with DTX or doxorubicin have demonstrated improved drug stability, targeted accumulation in BC cells, and enhanced cytotoxicity (Tran et al., 2020). Additionally, antibody-conjugated AuNPs have been explored for selective tumour imaging and therapy (Tran et al., 2020).

Quantum dots (QDs) are semiconductor nanoparticles that exhibit fluorescence, making them useful for imaging and tracking drug delivery (Aghebati-Maleki et al., 2020). However, concerns regarding their potential toxicity limit their widespread clinical application, though they hold promise for early BC detection and theranostic applications (Aghebati-Maleki et al., 2020).

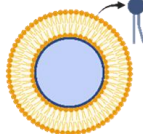
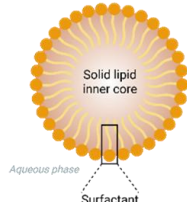
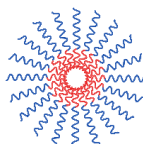
Magnetic NPs, particularly iron oxide-based, have been applied in magnetic resonance imaging (MRI) and site-specific drug transport (Aghebati-Maleki et al., 2020). These NPs may be guided magnetically towards tumour sites, ensuring accurate drug placement while lowering systemic toxicity (Aghebati-Maleki et al., 2020).

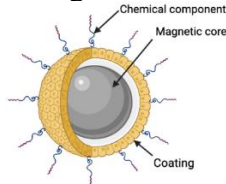
Polymeric micelles are self-assembled amphiphilic molecules that form a hydrophobic core surrounded by a hydrophilic shell, which facilitates the

solubilization of poorly water-soluble drugs such as paclitaxel and cisplatin (Aghebati-Maleki et al., 2020). Pluronic block copolymers, a typical micelle formulation, have been shown to sensitize multidrug-resistant (MDR) BC cells by lowering intracellular ATP levels (Tran et al., 2020). Studies have shown that teniposide-loaded micelles demonstrated cytotoxicity and improved cellular uptake in BC cells compared to unformulated drug solutions (Tran et al., 2020). Furthermore, co-loaded micelles containing paclitaxel and cisplatin have demonstrated synergistic effects in preventing the proliferation of BC cells (Tran et al., 2020).

Polymeric NPs, made from biodegradable and biocompatible polymers, have been extensively studied for controlled and targeted drug delivery (Aghebati-Maleki et al., 2020). NPs based on PLGA and chitosan have shown significant promise in BC therapy by improving drug solubility, extending circulation time, and minimizing premature drug degradation (Aghebati-Maleki et al., 2020). Table 2.3 outlines the various types of nanocarriers discussed in this section, highlighting their structures, properties, and typical applications in drug delivery, especially in cancer treatment.

Table 2.3: Summary of nanocarriers for cancer therapy (illustrations created with BioRender.com).

Type of nanocarrier	Type of nanoparticles	Description	Common Applications
Organic	Liposomes	Spherical vesicles with a phospholipid bilayer encapsulating hydrophilic and hydrophobic drugs.	FDA-approved for BC therapy (e.g., Doxil®). Used in paclitaxel delivery.
			
	Solid Lipid NPs	Solid lipid core improves drug stability, controlled release, and biocompatibility.	Encapsulation of tamoxifen, paclitaxel, and doxorubicin. Enhances tumour accumulation.
			
	Dendrimers	Branched macromolecules with multiple attachment sites for drug molecules and targeting ligands.	Doxorubicin and methotrexate delivery for improved tumour targeting and reduced systemic toxicity.
			
	Polymeric Micelles	Self-assembling amphiphilic molecules form a hydrophobic core and hydrophilic shell for drug solubilisation.	Used for solubilising hydrophobic anticancer drugs like paclitaxel and cisplatin. Enhances tumour retention.
			

Inorganic	Polymeric NPs 	Biodegradable and biocompatible carriers encapsulate drugs for controlled and targeted release.	PLGA and chitosan-based NPs for BC therapy. Prolongs circulation and reduces degradation.
	Gold NPs 	NPs with unique optical and thermal properties, suitable for photothermal therapy and drug delivery.	Targeted drug delivery (DTX, doxorubicin). Can be conjugated with antibodies for precision targeting.
	Quantum Dots 	Semiconductor nanoparticles exhibiting fluorescence are valid for imaging and tracking drug delivery.	Primarily used for imaging and early cancer detection due to fluorescence properties.
	Magnetic NPs 	Iron oxide NPs are used for MRI and targeted drug delivery, directed via an external magnetic field.	Used in MRI-guided drug delivery, ensuring precise drug localisation to tumours.

Silica NPs



Mesoporous silica particles with tunable pore sizes are used for controlled drug release and imaging applications.

Used in controlled drug delivery systems and bio-imaging for real-time tracking of drug release.

2.3.1 Polymeric Nanoparticles for Cancer Therapy

Polymeric nanoparticles (PNPs) have gained significant interest in drug delivery systems in recent years because of their small dimensions, which offer distinct properties suitable for diverse biomedical uses, including drug delivery and diagnostics (Crucho and Barros, 2017; Wang et al., 2024). Typically, these NPs are under 1000 nm in diameter and can encapsulate a broad spectrum of therapeutic compounds, such as chemotherapy agents, proteins, and nucleotides (Wang et al., 2024). Their advantages as carriers include an improved therapeutic index, protection of drug molecules, and their capacity to perform both therapeutic and imaging roles (theranostics) (Crucho and Barros, 2017).

Various materials are used to fabricate NPs, comprising natural and artificial polymers. While naturally derived polymers provide inherent biocompatibility, they may vary and pose immunogenic risks. Conversely, synthetic polymers offer a well-defined structure and predictable properties (Crucho and Barros, 2017). Inorganic NPs, like gold and iron oxide, are also applied in imaging and therapy; however, their ability to carry adequate drug amounts is limited (Karlsson et al., 2018).

A variety of natural and synthetic polymers, including proteins, sugars, biodegradable macromolecules, and pharmaceutically acceptable non-biodegradable polymers, are incorporated into PNP formulations to enhance drug stability and release efficiency (Crucho and Barros, 2017). Additionally, PNPs can be synthesized from biodegradable polymers such as PLGA, polycaprolactone (PCL), and polyhydroxyalkanoates (PHAs), making them particularly advantageous for cancer therapy (Mishra and Dey, 2018; Tran et al., 2020).

The reduced size and extensive surface area of PNPs contribute to prolonged circulation time in the bloodstream, enhanced accumulation of the drug in tumour tissues, and minimal systemic toxicity (Wang et al., 2024). Previous studies have indicated that tamoxifen-loaded PNPs exhibit enhanced accumulation in breast

tumours compared to free tamoxifen (Tran et al., 2020). Similarly, DTX-encapsulated in PLGA and polyethylene glycol 2000 (DSPE-PEG2000)-NH₂ PNPs have shown superior cytotoxicity and cellular uptake in MDA-MB-231 BC cells, reducing tumour burden more effectively than free drugs (Jadon and Sharma 2019; Tran et al., 2020).

Recent advancements in nanotheranostics, which combine therapy and diagnostics, have further expanded the potential of PNPs in cancer treatment. These NPs can deliver therapeutic agents while tracking treatment response, paving the way for personalised cancer therapy (Indoria, Singh, and Hsieh, 2020). Additionally, these nanocarriers enhance combination therapies by encapsulating multiple drugs within a single NP (Afsharzadeh et al., 2018). Research efforts focus on improving NP synthesis methods and meeting regulatory standards for clinical application (Panda et al., 2019).

A fundamental factor influencing the success of polymeric drug delivery is NP uptake at the cellular level. NPs primarily enter cells through endocytotic mechanisms, which are influenced by particle size and surface properties (Crucho and Barros, 2017). Despite these advantages, challenges remain in achieving scalable production, reproducible particle characteristics, and regulatory compliance for clinical applications (Crucho and Barros, 2017). Recent advancements in nanotechnology have focused on modifying NP surfaces by targeting ligands to improve drug accumulation in tumour sites (Tran et al., 2020). For example, trastuzumab-conjugated liposomes and PLGA nanoparticles have been developed to selectively target HER2-positive BC cells, enhancing cellular uptake and cytotoxicity (Tran et al., 2020).

There are still hurdles to overcome in NP-based drug delivery. Concerns about NP toxicity, large-scale production challenges, and variability in patient responses require further investigation (Tran et al., 2020). Additionally, PNPs must achieve an optimal balance between stability and controlled degradation to ensure sustained drug release (Tran et al., 2020). Future research should focus on developing multifunctional NPs capable of simultaneous imaging, drug delivery, and therapy to improve treatment outcomes for BC patients (Tran et al., 2020).

2.3.2 Mechanism of Drug Release from Polymeric Nanoparticles

The release mechanism of drugs from PNPs is crucial for ensuring their effectiveness in cancer treatment (Sartaj et al., 2021). Once administered, NPs must overcome several biological barriers, such as enzymatic degradation, immune system clearance, and kidney filtration, to successfully reach tumour sites (Karlsson et al., 2018). Additionally, they must avoid aggregation with blood proteins, which could shorten their circulation time (Karlsson et al., 2018). After reaching the tumour, NPs should release the drug outside cancer cells or facilitate intracellular drug delivery to enhance therapeutic outcomes (Karlsson et al., 2018). As the polymer matrix undergoes hydrolysis, ester bonds break down gradually, facilitating a controlled and sustained drug release (Korde and Kandasubramanian, 2020).

Additionally, NP characteristics such as particle dimension, crystallinity, and hydrophilic nature can affect the rate of drug diffusion and polymer degradation (Masood, 2016; Rafiei and Haddadi, 2019). Drug release from PNPs is influenced by multiple elements, including polymer breakdown, diffusion rate, and external environmental factors (Crucho and Barros, 2017). Generally, the release process is classified into passive and active mechanisms (Karlsson et al., 2018; Aghebati-Maleki et al., 2020; Wang et al., 2024).

In passive drug release, the drug gradually escapes from the polymer matrix, a process primarily driven by the diffusion of the drug and the breakdown of the polymer, leading to a consistent release over time (Karlsson et al., 2018). Additionally, passive targeting utilizes the increased permeability and retention (EPR) phenomenon, which enables NPs to build up in tumour tissues because of the irregular architecture of blood vessels and inadequate lymphatic clearance in these regions (Wang et al., 2024; Aghebati-Maleki et al., 2020). However, a significant challenge of passive drug release is the burst release, during which a substantial amount of the drug is rapidly discharged from the NP surface at the start of treatment (Tran et al., 2021). This is caused by loosely bound drug molecules that detach rapidly. After the initial burst, the remaining drug is released more slowly through diffusion and polymer degradation

(Juan et al., 2020). Factors related to the polymer type, molecular mass, hydrophobicity, and cross-linking density influence the passive drug release rate (Juan et al., 2020).

On the other hand, active drug release involves the use of specific internal or external triggers to ensure that drug release occurs precisely at the tumour site (Karlsson et al., 2018). These triggers include pH changes, temperature variations, enzymes, redox potential, light, ultrasound, or magnetic fields (Karlsson et al., 2018). By designing NPs to respond to these triggers, drug release can be optimised for improved efficacy (Karlsson et al., 2018). For example, pH-sensitive NPs respond to the acidic microenvironment by swelling or degrading faster, accelerating drug release at the tumour site (Juan et al., 2020; Karlsson et al., 2018).

Enzyme-responsive NPs react to cancer-associated enzymes that break down the polymer, triggering drug release at the tumour site (Masood, 2016). Redox-sensitive NPs release drugs in response to high levels of glutathione, a compound in more significant amounts inside tumour cells (Karlsson et al., 2018). Thermoresponsive NPs alter drug release rates in response to temperature changes, which is helpful because tumour sites are often warmer than normal tissues (Karlsson et al., 2018). Additionally, light-triggered NPs use external light sources to activate drug release, enhancing treatment precision (Karlsson et al., 2018).

Active targeting is possible by altering the NP surface using specific ligands, including antibodies, peptides, folic acid, and environmental stimuli. These ligands help improve NP specificity by recognizing receptors that are highly expressed in cancer cells, such as HER2 or folate receptors (Aghebati-Maleki et al., 2020; Wang et al., 2024). Furthermore, functionalizing NPs with targeting ligands has shown promise in enhancing tumour-specific drug delivery (Tran et al., 2020). For instance, trastuzumab-linked liposomes and PLGA NPs specifically target HER2-positive BC cells, promoting cellular internalization and boosting cytotoxic activity (Tran et al., 2020). Figure 2.10 illustrates the key differences between passive and active targeting mechanisms of PNPs in cancer treatment.

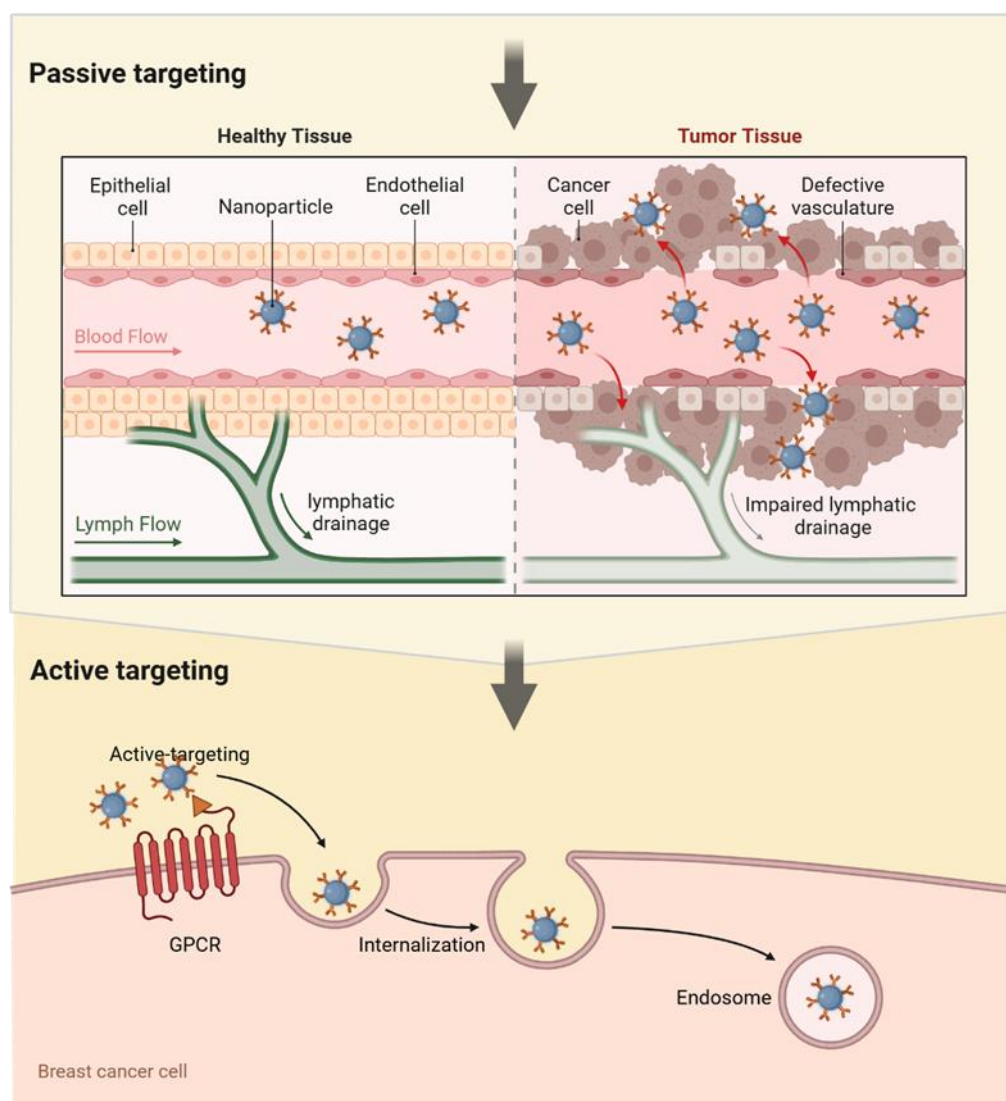


Figure 2.10: Mechanisms of passive and active targeting of polymeric nanoparticles for cancer treatment, highlighting how nanoparticles accumulate in tumour tissues and interact with cancer cells for controlled drug release (Adapted from Rahman et al., 2025).

Sustained release of drugs using PNPs helps lower administration frequency, minimize adverse effects, and enhance patient adherence (Juan et al., 2020; Tran et al., 2021). Several investigators have also explored co-encapsulation strategies, where multiple drugs are loaded into a single NP system (Juan et al., 2020). This approach enables controlled sequential drug release, improving the efficacy of combination therapies (Juan et al., 2020). Additionally, encapsulating NPs within microneedles

(MNs) or layered polymer matrices allows for further modification of drug release profiles, ensuring optimal therapeutic delivery (Juan et al., 2020).

Despite the promising advantages of PNPs, several challenges remain in achieving clinical translation. Stability concerns, batch-to-batch variations, and rapid initial drug release must be carefully addressed (Crucho and Barros, 2017). Emerging strategies such as host-guest chemistry for controlled drug release and glycosylated NPs for targeted drug delivery are being explored to improve NP stability and drug release precision (Crucho and Barros, 2017).

Despite advancements, challenges related to NP toxicity, large-scale production, and variability in patient response remain and require further research (Tran et al., 2020). Moreover, identifying an optimal balance between NP stability and their controlled biodegradation is essential to achieve effective and sustained drug release (Tran et al., 2020). Future studies should aim to design multifunctional NPs capable of combining imaging, drug delivery, and therapeutic applications to improve treatment accuracy in cancer patients (Tran et al., 2020). Drug release from PNPs typically occurs through several mechanisms, each playing a role in determining the overall effectiveness of cancer therapy. By utilizing passive diffusion, targeted delivery, polymer degradation, and stimulus-responsive systems, drug release profiles can be adjusted to enhance precision and therapeutic outcomes. PNPs provide flexibility in drug delivery, making it possible to control when and where drugs are released, ultimately improving the safety and effectiveness of cancer therapy.

2.3.3 Docetaxel-Loaded Polymeric Nanoparticles for Cancer Treatment

Recent studies have looked into using PNPs to deliver DTX more effectively for cancer treatment (He et al., 2017). Table 2.4 summarises various PNPs used for DTX delivery, their associated limitations, and observed therapeutic outcomes in BC treatment.

Table 2.4: Overview of polymeric nanoparticles used for delivering docetaxel (DTX) in breast cancer treatment, highlighting the associated limitations, types of polymers used, and therapeutic outcomes.

Polymer used	Limitations	Outcome	References
Amphiphilic polymer integrated with fluorescent markers.	• Limited targeting ability of the drug • Low tissue penetration of fluorescence signal	• Improved drug incorporation • Enhanced cytotoxic effect, reducing IC₅₀ dosage by 3–4 times • Elevated MRI imaging clarity	• Abbasi et al., 2015 • Sartaj et al., 2021
PAMAM-based poly (6-benzyl-L-glutamate)-b-TPGS (PAM-PBLG-b-TPGS)	• Reduced solubility in water • Exhibits MDR	• Increased cytotoxicity against cancer cells • Greater efficiency in cellular internalization • Strong antitumour response • Superior DTX delivery to the tumour region	• Wang et al., 2019 • Sartaj et al., 2021

PLGA/TPGS	• Presence of MDR • Efflux of DTX by P-gp • Notable adverse reactions linked to Poloxamer 235	• Improved intracellular accumulation of DTX in PLGA-TPGS/Poloxamer 235 NPs compared to standard PLGA-TPGS NPs • Poloxamer-conjugated NPs demonstrated greater cytotoxic activity • Effective reversal of MDR • Elevated drug concentration within tumour cells	• Tang et al., 2015 • Sartaj et al., 2021
PLGA	• Toxicity that limits dosage levels	• NPs loaded with DTX exhibited a 376-fold rise in plasma drug concentration compared to standard formulations. • Tumour uptake of DTX was increased twofold. • Effective against TNBC that is resistant to taxanes	• Bowerman et al., 2017 • Sartaj et al., 2021

PHBV	• Harmful reactions and cytotoxicity affecting healthy cells	• Improved tumour suppression by PHBV-based NPs, accompanied by strong apoptotic responses	• Vardhan et al., 2017a • Sartaj et al., 2021
PEG-PLGA functionalised by E2	• Adverse effects due to non-selective distribution of the drug	• Prolonged drug circulation time by approximately 12-fold • Improved drug permeability and retention • Enhanced cellular absorption of docetaxel	• Jain et al., 2015 • Sartaj et al., 2021
Chitosan and sodium tripolyphosphate (TPP)	• Poor solubility in water • High uptake by immune cells • Fast elimination • Non-targeted delivery	• Cell viability dropped by 85%, indicating high cytotoxicity • Targeting ability improved due to TPP cross-linking	• Jain et al., 2015 • Sartaj et al., 2021
PLGA	• Resistance and adverse effects linked with Herceptin • Ligand binding complexities	• Improved therapeutic performance • Minimized burst release with extended-release profile	• Yang et al., 2015 • Sartaj et al., 2021

		• Demonstrated synergistic enhancement	
Chitosan	• Limited solubility in aqueous environments • Subject to P-gp mediated efflux • Side effects like hypersensitivity and high blood pressure	• Remarkably enhanced cytotoxicity against TNBC • Elevated anti-angiogenic activity • Improved intracellular uptake and endosomal escape • Strengthened anti-angiogenic impact	• Zafar et al., 2020 • Sartaj et al., 2021
PLGA and HA	• Rapid elimination of drug compounds • Lack of tissue-specific targeting	• Inhibited autonomous behaviour of breast cancer stem cells (BCSCs) • Enhanced anti-cancer efficacy of the combined treatment • Demonstrated cooperative therapeutic effects	• Huang et al., 2016 • Sartaj et al., 2021
PEG	• Non-targeted accumulation in tissues	• Boosted nanoparticle uptake through clathrin-assisted endocytosis	• Kushwah et al., 2018 • Sartaj et al., 2021

	• Uncontrolled pharmacokinetics of DTX and Gemcitabine (GEM) when administered separately • Accelerated breakdown of GEM	• More significant inhibition of tumour progression • Lower toxicity was observed in the liver and kidneys • 8.4-fold improvement in Area under curve (AUC) of GEM compared to the commercial formulation	
PLGA/TPGS	• Limited solubility • Poor oral absorption	• Exhibited synergistic enhancement • Boosted cytotoxic potency • Prolonged drug circulation • Improved targeting performance	• Gao et al., 2019 • Sartaj et al., 2021
Chitosan and Hyaluronic acid	• Weak targeting capacity • Limited water solubility • Toxic effects depend on the dose • Potential for allergic response	• Reduced cell viability • Marked downregulation of gene expression in treated cells	• Mirzaie et al., 2016 • Sartaj et al., 2021

DTX is commonly used to treat BC, but it has some limitations, like poor water solubility and serious side effects (Misra et al., 2020). NP-based systems have been developed to improve the dissolution rate of DTX and to reduce its toxicity to healthy cells (Imran et al., 2020).

PNPs made from materials like PLGA and PCL are often used to encapsulate DTX (Juan et al., 2020). These NPs help control the body's release of DTX, making the treatment more effective. The release of DTX depends on several factors, including diffusion, hydrolysis, enzymatic breakdown, and polymer erosion (Masood, 2016). Factors like the polymer's composition and molecular weight also affect the release rate of DTX from the NPs (Korde and Kandasubramanian, 2020).

These NPs can be modified with targeting ligands to ensure DTX reaches cancer cells directly (He et al., 2017; Sakhi et al., 2022). These ligands help the NPs bind specifically to cancer cells, making the treatment more precise (He et al., 2017; Sakhi et al., 2022). The *in vitro* studies have shown that DTX-NPs can kill more cancer cells than free DTX, suggesting that this delivery method could make the drug more powerful (Sartaj et al., 2021).

Using DTX-NPs in combination with other treatments, like small interfering RNA (siRNA) or GEM, can help overcome drug resistance in cancer. This strategy targets multiple cancer pathways simultaneously, making the treatment more effective (Masood, 2016; Misra et al., 2020). Researchers have also developed HER2-targeted DTX-NPs designed to be absorbed more easily by HER2-positive BC cells (Masood, 2016). Some NPs have pH-responsive properties, which means they release DTX more effectively in the acidic environment of tumour tissues (Juan et al., 2020). Others are designed to be enzyme-sensitive, allowing for a more targeted drug release directly to cancer cells (Masood, 2016). The development of multifunctional NPs that can carry chemotherapy drugs, gene therapy, and targeting ligands simultaneously is being explored to make cancer treatments even more effective (Katiyar et al., 2016; Juan et al., 2020).

DTX-NPs also show improved pharmacokinetics, including a longer plasma half-life and better tumour accumulation (Gupta et al., 2021). Their biocompatibility and biodegradability make them suitable candidates for sustained drug delivery (Xu et al., 2021). These NPs can also help protect DTX from degrading too quickly while circulating in the body (Indoria et al., 2020).

Pharmacodynamic studies show that DTX-NPs can increase the AUC for DTX in plasma, which means the drug stays active in the bloodstream longer (Sakhi et al., 2022). Combining NPs with anti-angiogenic agents, which help stop the growth of new blood vessels that feed tumours, could lead to new, more effective cancer treatments (Juan et al., 2020).

Some researchers are also working on adding imaging agents to DTX-NPs. This would allow doctors to track the treatment's work in real-time (Indoria et al., 2020). Ongoing research focuses on creating "smart" NPs that respond to the tumour environment to deliver drugs more precisely (He et al., 2017; Indoria et al., 2020). Overall, using DTX-NPs in targeted cancer treatment seems promising. These systems can help reduce side effects and make DTX more effective by ensuring it reaches cancer cells directly and stays active for longer.

2.3.4 Clinical Trials and Future Directions of Docetaxel Nanoparticles

The clinical translation of DTX-NPs remains a significant challenge (Kolluru et al., 2020). While various magnetic-based nano-drug delivery platforms have been explored, a standardized formulation specifically for MBC treatment has not yet been approved for clinical application (Jadon and Sharma, 2019). One of the primary hurdles is the need for comprehensive pharmacokinetic (PK) and pharmacodynamic (PD) studies to confirm the safety, therapeutic performance, and stability of such NP-based formulations in human subjects (Panda et al., 2019).

Preclinical studies on PLGA-IONP-based formulations have shown improved PK parameters, including increased AUC under the plasma concentration-time curve, prolonged drug retention, and enhanced tumour accumulation (Kolluru et al., 2020). These formulations also exhibit a high plasma half-life (Kolluru et al., 2020). Building on these findings, several DTX-NP formulations have been developed for clinical applications. Among them, BIND-014, a PLGA-based NP designed for prostate-specific membrane antigen (PSMA)-targeted delivery, has shown promising outcomes (Karlsson et al., 2018). Table 2.5 summarises various DTX-NP formulations, their developers, and clinical trial status.

Table 2.5: Summary of docetaxel-loaded nanoparticle formulations in clinical trials, including their types, developers, and clinical status

Trial identifier	Formulation	Cancer	Phase	Status	Refs
NCT01300553	BIND-014, a DTX-containing nanoparticle targeting PSMA	Metastatic cancer, solid tumours	1	Completed	Von Hoff et al., 2016
NCT02049718	DTX-PNP	Urothelial carcinoma, cholangiocarcinoma, cervical cancer, squamous cell carcinoma of the head and neck, non-small cell lung carcinoma	2	Terminated	Beheshtizadeh et al., 2024
NCT02283320	Liposome-encapsulated DTX (LE-DT)	Solid tumours, metastatic	2	Completed	Beheshtizadeh et al., 2024

NCT01929479	DTX-PNP	Non-small cell lung cancer	2	Completed	Masuda et al., 2022
NCT01812746	89 Zirconium CirpEc DTX PET	Cancer, ovarian cancer	2	Completed	Sharma et al., 2016
NCT01513542	DTX-containing CirpEc nanoparticles	Cancer, ovarian cancer	2	Completed	Beheshtizadeh et al., 2024
NCT01089371	CirpEc DTX (DTX- containing nanoparticles)	Solid tumours, metastatic cancer	2	Completed	Beheshtizadeh et al., 2024
NCT03712423	ATI-1123 as a liposomal formulation of DTX	Solid tumours, metastatic cancer	2	Completed	Miedema et al., 2022

NCT03742713	Nanosomal DTX Lipid Suspension	Triple-negative breast cancer	3	Recruiting	Atrafi et al., 2019
NCT04245311	Nanosomal DTX Lipid Suspension	Triple-negative breast cancer	3	Recruiting	Subramanian et al., 2020
NCT03671041	Nanosomal DTX Lipid Suspension	Triple-negative breast cancer	3	Recruiting	Beheshtizadeh et al., 2024

Notably, BIND-014 (NCT01300553) successfully completed its trial phase with positive results, including reduced circulating tumour cells and manageable toxicity, demonstrating the clinical potential of targeted NP-based drug delivery. In contrast, DTX-PNP (NCT02049718) was terminated during Phase 2, likely due to limited efficacy or translation issues from preclinical studies. Liposome-encapsulated DTX (LE-DT) also reached completion, showing reduced systemic toxicity and improved delivery. These outcomes underline that while completed trials validate the promise of nanocarriers for safe and effective chemotherapy, terminated trials highlight the need for optimising formulation stability, dosing strategies, and tumour selectivity to ensure successful clinical translation.

Building on this, integrating dual-drug delivery systems and combination therapies holds significant potential for overcoming drug resistance and improving patient outcomes (Juan et al., 2020; Sartaj et al., 2021). However, only a few PNP-based formulations have received FDA approval for clinical use (Korde and Kandasubramanian, 2020). Future research should focus on optimising the PK/PD of DTX-NPs (Imran et al., 2020) and streamlining regulatory approval processes (He et al., 2017; Imran et al., 2020). Personalised approaches that leverage genetic and molecular markers could further refine these therapies (Imran et al., 2020).

While there are still many challenges, the future looks promising for DTX-NPs. Researchers are more focused on improving their effectiveness, making them safer, and finding ways to get them approved faster. If these challenges can be overcome, these treatments could become an essential part of how cancer is treated in the future.

2.4 Biodegradable Polymers in Nanoparticles

Biodegradable polymers have become increasingly significant in nanomedicine due to their ability to overcome challenges related to drug delivery (Ahlawat et al., 2018; Prajapati et al., 2019; Idrees et al., 2020). Previously, non-biodegradable PNPs such as polymethyl methacrylate, polyacrylamide, and polystyrene were widely utilised in drug delivery systems (Banik et al., 2016; Prajapati et al., 2019; Idrees et al., 2020). However, their accumulation in the body has led to notable toxicity and adverse health effects (Ahlawat et al., 2018; Idrees et al., 2020). This has prompted a shift toward biocompatible and biodegradable polymers, which offer safer and more effective options for diagnostic and therapeutic use in both *in vivo* and *in vitro* settings (George et al., 2019; Hassan and Zhang, 2019; Idrees et al., 2020).

These polymers degrade enzymatically or non-enzymatically within the body, producing non-toxic or biocompatible by-products (Prajapati et al., 2019). Their role in improving drug PK and reducing side effects has advanced the development of sustained and controlled drug delivery systems, vaccine platforms, nucleic acid and protein-based therapeutics, cancer treatments, tissue engineering, and regenerative medicine (Prajapati et al., 2019). They are valued for their non-toxicity, minimal inflammatory degradation products, permeability, and suitability for biomedical applications. Many are abundant and cost-effective, making them ideal for processing and modification (Prajapati et al., 2019).

Biodegradable polymers used in nanocarriers are commonly classified into natural and synthetic types (Prajapati et al., 2019). Natural biodegradable polymers, or biopolymers, are derived from animal, plant, or microbial sources (Gagliardi et al., 2021; Prajapati et al., 2019). Examples include chitosan, hyaluronic acid, collagen, gelatin, albumin, elastin, and PHAs, and are used in cancer therapy, wound healing, and gene delivery because of their biodegradability, biocompatibility, and low toxicity (Gagliardi et al., 2021; Prajapati et al., 2019). These polymers also break down into non-toxic by-products within the body, making them safe carriers for therapeutic agents (Chai et al., 2021). Although PHAs such as P(3HB-*co*-4HB) are biosynthesised

naturally by microorganisms and thus fall under natural biopolymers, they are often grouped under synthetic biodegradable polymers in nanomedicine due to their engineered processing, tunability, and thermoplastic properties (Korde and Kandasubramanian, 2020; Gagliardi et al., 2021)

Synthetic biodegradable polymers include PLA, polyglycolic acid (PGA), PLGA, PCL, polyanhydrides, polyurethanes, and poly(trimethyl carbonates) (Ahlawat et al., 2018; Idrees et al., 2020). Although some biopolymers, such as PHAs, are biologically derived, they are often classified under synthetic polymers in nanomedicine due to their engineered properties and processing methods (Gagliardi et al., 2021). Synthetic biopolymers like poly(α -hydroxy acids), poly(alkyl cyanoacrylate) (PACA), and PHAs are widely used, with poly(α -hydroxy acids) being particularly common in NP production (Gagliardi et al., 2021). These degrade through non-enzymatic hydrolysis into lactic and glycolic acid, and their degradation profile can be tailored based on molecular weight, structure, and hydrophobicity (Doppalapudi et al., 2016; Gagliardi et al., 2021). Figure 2.11 shows the classification of biodegradable polymers often used in drug delivery.

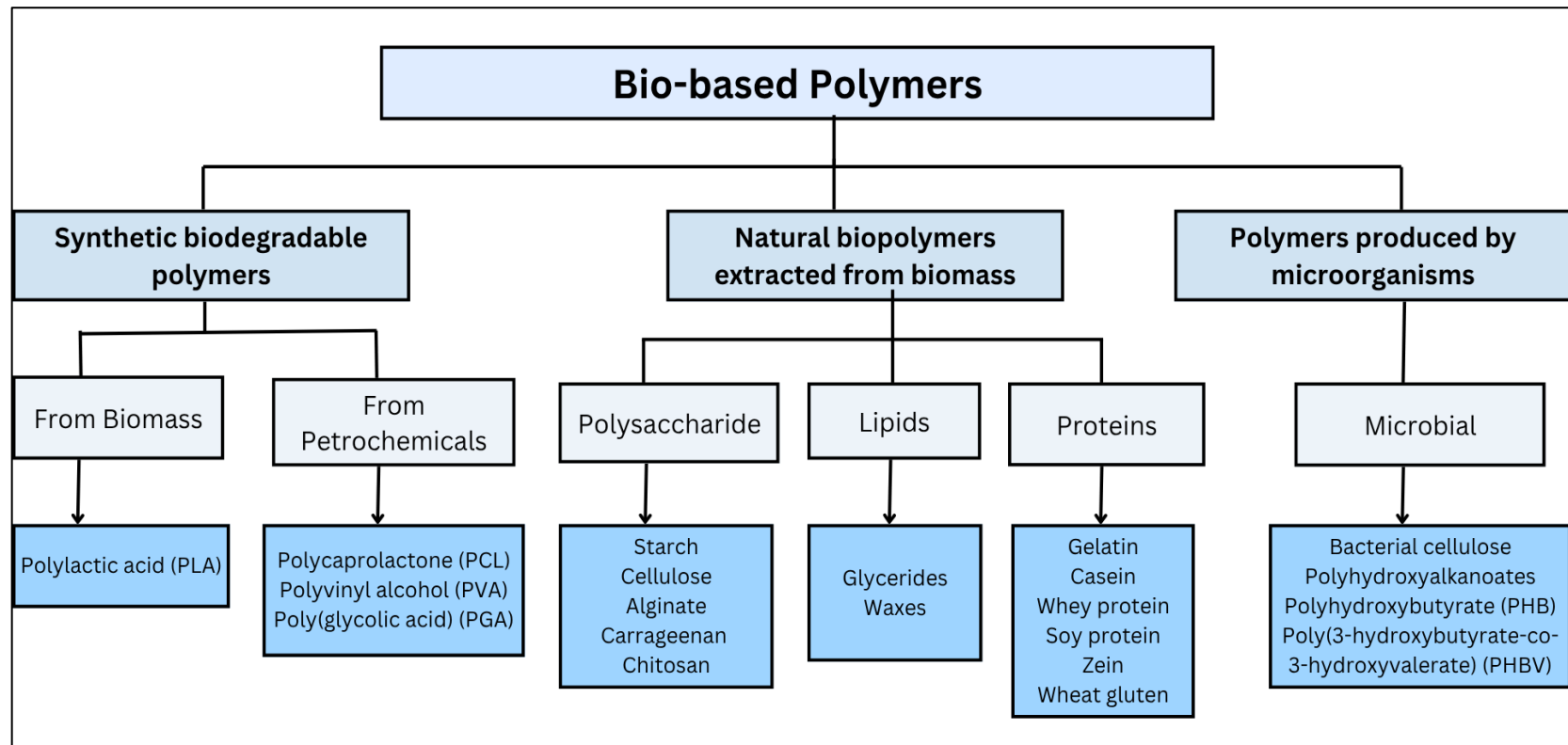


Figure 2.11: Classification of biodegradable polymers often used in drug delivery (adapted and modified from Baghi et al., 2022; created with BioRender.com).

Unlike synthetic polymers produced through chemical synthesis, biopolymers can undergo natural recycling via biological processes (Gheorghita et al., 2021). This enhances their appeal as the industry shifts towards sustainable and eco-friendly materials (Gheorghita et al., 2021). Moreover, they facilitate accurate targeting and reduce side effects by minimising the exposure of healthy tissues to drugs (Fazal et al., 2023) while allowing physicians to control drug release profiles (Ahlawat et al., 2018; Talebian et al., 2018). Nonetheless, natural biopolymers often exhibit limitations in mechanical and thermal stability (Fazal et al., 2023). These challenges are typically addressed through polymer blending or chemical modifications to enhance mechanical properties and flexibility (Li et al., 2016).

The design of these biopolymers at the nano and microscale levels is essential for precise targeting and improved therapeutic outcomes (Starbird-Perez et al., 2021). Their ability to mimic the extracellular matrix makes them ideal for tissue engineering and regenerative medicine applications (Biswas et al., 2022). As sustainable materials become increasingly important, biopolymers are gaining traction for their non-toxic nature, low cost, ease of fabrication, and biological fluid stability (Prajapati et al., 2019).

Among all of these, PHAs have garnered particular interest, especially in recent times. Although naturally synthesised by bacteria and classified as natural in origin, their tunability and thermoplasticity have led to their classification under synthetic polymers in many drug delivery contexts due to their processing methods (Korde and Kandasubramanian, 2020; Gagliardi et al., 2021). These polymers are made from hydroxyalkanoate monomers and can be produced through microbial biosynthesis or chemically via ring-opening polymerisation of β -lactones (Li et al., 2016; Gagliardi et al., 2021). Their microbial origin, biocompatibility, biodegradability, and adaptability make PHAs a promising material in developing nanocarriers.

The performance of polymers in nanocarriers is influenced by factors such as in situ administration, on-demand release, and compatibility with radiotherapy and

immunotherapy. Additionally, using FDA-approved materials adds to their clinical appeal (Srivastava et al., 2015; Prajapati et al., 2019; Idrees et al., 2020). Drug-polymer interactions, including hydrogen bonding, electrostatic attractions, van der Waals forces, and hydrophobic interactions, help ensure that drugs remain stable until they reach the target site (Fazal et al., 2023). Natural and synthetic biodegradable polymers contribute significantly to developing advanced, safe, and effective nanocarrier drug delivery systems.

In addition to standalone biodegradable polymers, hybrid systems that combine these polymers with other carrier matrices such as polymeric hydrogels are increasingly being explored to enhance nanoparticle-based delivery. Hydrogels, owing to their three-dimensional hydrophilic networks, can encapsulate biodegradable polymer nanoparticles, provide a biomimetic environment, and enable sustained or stimuli-responsive drug release (Chai et al., 2017; Ganeson et al., 2023).

2.4.1 Polymeric Hydrogels in Biodegradable Nanoparticle Systems

Polymeric hydrogels are hydrophilic three-dimensional (3D) polymer networks capable of retaining large amounts of water or biological fluids (Chai et al., 2017). Their high-water content, tunable mechanical properties, and biocompatibility make them ideal for biomedical applications such as drug delivery, wound healing, and tissue engineering (Spicer, 2020). Crosslinking can be achieved physically (e.g., hydrogen bonding, ionic interaction) or chemically (covalent bonding), with chemically crosslinked hydrogels generally offering greater mechanical stability and resistance to degradation *in vivo* (Parhi, 2017; George et al., 2020).

In nanoparticle-based drug delivery, hydrogels can act as supportive matrices that encapsulate biodegradable polymer nanoparticles, enabling localised delivery, sustained release, and stimuli-responsive behaviour (Desai and Lee, 2015; Abascal and Regan, 2018). The porous architecture of hydrogels allows for controlled diffusion of therapeutic agents, while their soft, tissue-like consistency minimises irritation at the site of application (Sahajpal et al., 2022). Self-healing hydrogels, in particular,

restore their structure after mechanical disruption, improving durability and maintaining drug release performance in physiological environments (Sosnik and Seremeta, 2017; Talebian et al., 2019). As shown in Figure 2.12, self-healing hydrogels can autonomously rejoin after being cut into separate pieces, forming an integral structure without external intervention.

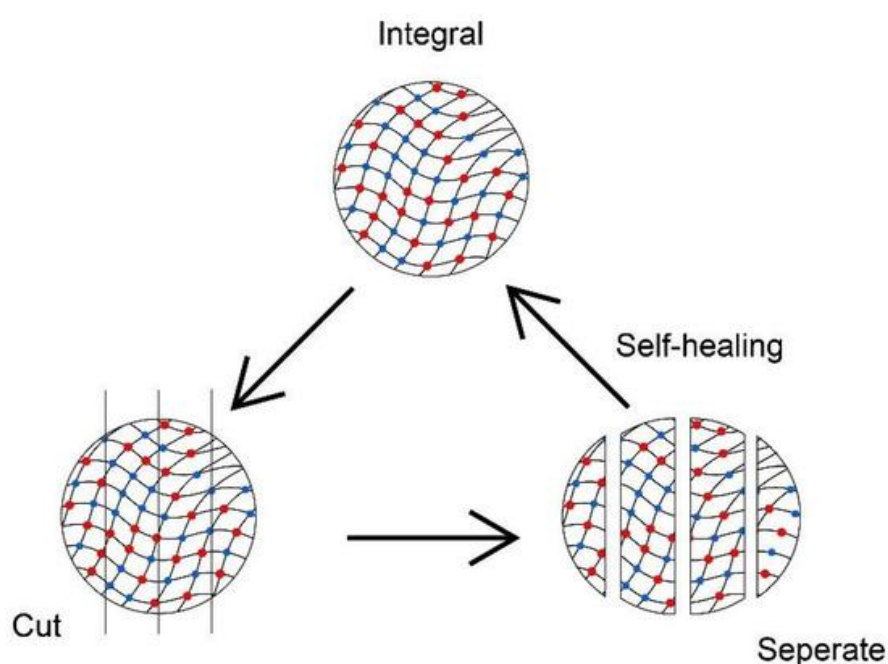


Figure 2.12: Schematic illustration of a schematic of the self-healing process, where cut hydrogel segments autonomously rejoin to form an integral structure.

Hydrogels can be derived from natural polymers such as alginate, chitosan, and gelatin, or from synthetic biodegradable polymers such as poly(vinyl alcohol) (PVA) and polyethylene glycol (PEG) (Han et al., 2012; Yu et al., 2015; Nayak and Das, 2018). When combined with biodegradable nanoparticles, for instance, poly(lactic-co-glycolic acid) (PLGA) or polyhydroxyalkanoates (PHAs), these hybrid systems offer synergistic benefits: the hydrogel provides mechanical stability and environmental responsiveness, while the nanoparticles contribute precise drug loading and targeted delivery (Miyamoto et al., 2017). Such hybrid systems have been explored for cancer therapy, transdermal delivery, and biosensing applications, with

promising results in enhancing drug bioavailability and reducing off-target effects (Xie et al., 2021; Li et al., 2023).

In the context of transdermal microneedle systems, hydrogel-forming matrices can swell upon insertion into the skin, facilitating both drug release from encapsulated nanoparticles and the uptake of interstitial fluid for diagnostic purposes (Kasiński et al., 2020). This property is particularly relevant to cancer therapy strategies aiming for sustained, localised release at tumour sites while enabling potential biosensing capabilities. Although various biodegradable nanoparticles have been integrated into hydrogels, the combination of self-healing hydrogels with PHA-based nanoparticles remains relatively underexplored, offering an opportunity for novel therapeutic platforms in breast cancer treatment.

2.4.2 Polyhydroxyalkanoates (PHAs)

PHAs are gaining recognition as promising alternatives to traditional commercial polymers due to their environmentally friendly nature (Vodicka et al., 2022). Unlike petrochemical-based polymers, PHAs are fully biodegradable and biocompatible (Utsunomia et al., 2020; Vodicka et al., 2022). The first PHA, poly(3-hydroxybutyrate), P(3HB), was discovered in 1926 by French scientist Maurice Lemoigne while studying the bacterium *Bacillus megaterium* (Utsunomia et al., 2020; Zhou et al., 2023). Industrial interest in PHAs grew in the late 1950s, with companies like W. R. Grace exploring their potential applications (Utsunomia et al., 2020). In the past five decades, research has progressed substantially, identifying over 140 PHA monomers, including unsaturated and aromatic types (Alves et al., 2023; Costa et al., 2024; de Macedo et al., 2024).

PHAs are a versatile family of biodegradable and biocompatible polyesters produced by various microorganisms as intracellular storage materials for carbon and energy (Utsunomia et al., 2020; Alves et al., 2023; Costa et al., 2024; de Macedo et al., 2024). Their production occurs when microorganisms are exposed to excess

carbon sources while facing limitations in essential nutrients such as nitrogen, phosphorus, or iron (Zhou et al., 2023; Huang et al., 2024). Under nutrient-limited conditions, these natural polymers accumulate as water-insoluble granules, typically ranging from 0.2 to 0.5 μm in diameter (Zhou et al., 2023).

PHAs are composed of (R)-hydroxyalkanoic acid (HA) monomers, with the alkyl side chain (R) influencing the diversity of PHA types (Zhou et al., 2023). PHAs are hydrophobic, enantiomerically pure due to the stereospecificity of *phaC* towards (R)-3-hydroxyacids, biocompatible, and capable of being synthesized into high molecular weights with low polydispersity (Utsunomia et al., 2020). The general structure of PHAs, as illustrated in Figure 2.13, can vary based on the number of carbon atoms in the monomer units, affecting their physicochemical properties (Utsunomia et al., 2020; Vodicka et al., 2022; Zhou et al., 2023).

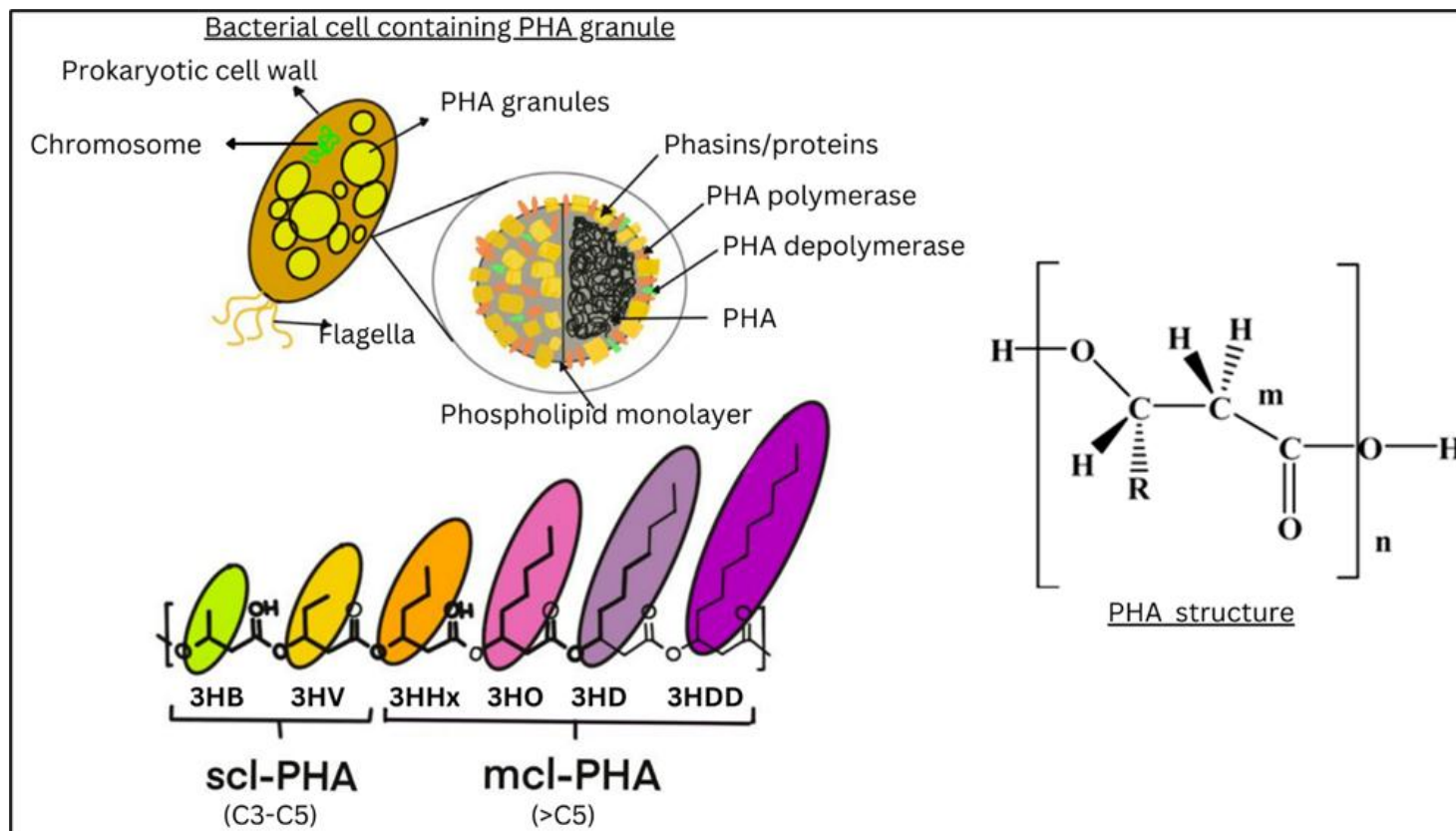


Figure 2.13: The schematic overview of a PHA granule accumulated intracellularly in the bacterial cells and the general chemical structure of PHA (Adapted and modified from Koller, 2018; Chai et al., 2020, and Alves et al., 2023; drawn using Procreate).

PHAs are classified into three primary categories according to their monomer composition: short-chain-length (SCL) PHAs, medium-chain-length (MCL) PHAs, and long-chain-length (LCL) PHAs (Utsunomia et al., 2020; Zhou et al., 2023; Jang et al., 2024). These polymers display different physicochemical properties based on their monomer structure, influencing mechanical properties, degradation behaviour, and suitability for healthcare applications (Vodicka et al., 2022; Kalia et al., 2023). SCL-PHAs, such as P3HB and P(3HB-*co*-4HB), tend to be rigid and brittle due to their high crystallinity, whereas MCL-PHAs, like poly(3-hydroxyhexanoate) (PHHx) and poly(3-hydroxydecanoate) (PHD), are more flexible and elastomeric (Jang et al., 2024).

The composition of PHAs depends on factors like the bacterial strain producing them, the type of PHA synthase *phaC*, and the carbon substrates used (Utsunomia et al., 2020; Kalia et al., 2023; Jang et al., 2024). When extracted, PHAs show thermoplastic and elastomeric characteristics, with SCL-PHAs being similar to polyethylene or polypropylene and MCL-PHAs resembling rubbers and elastomers (Utsunomia et al., 2020; Jang et al., 2024). This broad range of physical properties makes PHAs suitable for various uses, including pharmaceuticals and medical devices (Utsunomia et al., 2020).

PHAs can also be classified as homopolymers or copolymers based on their monomer composition (Zhou et al., 2023). Homopolymers, such as P(3HB), exhibit a highly crystalline structure, whereas copolymers, including poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), possess improved mechanical flexibility (Kalia et al., 2023). Notable copolymers such as poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (PHBHHX), and P(3HB-*co*-4HB) are frequently applied in various fields because of their excellent flexibility and mechanical characteristics (Zhou et al., 2023).

2.4.3 Polyhydroxyalkanoates (PHA)-based Nanoparticles

The global PHA market was estimated to be worth around USD 73.6 million in 2021 and is expected to grow significantly due to its applications in biomedical and industrial fields (Kalia et al., 2023). PHA-based materials have demonstrated high biocompatibility, supporting cell adhesion, proliferation, and differentiation (Kalia et al., 2023). PHAs serve as excellent materials for drug carriers and delivery systems due to their ability to encapsulate and release therapeutic agents in a controlled manner (Kalia et al., 2023).

PHAs are a promising drug delivery choice due to their biocompatibility, biodegradability, and controlled drug release properties (Huang et al., 2024). Compared to other biodegradable polyesters like PLA and PCL, PHAs have a similar backbone structure but feature unique side chains that impact their mechanical properties and degradation behaviour (Huang et al., 2024). There are six commercially available types of PHAs: PHBV, poly(4-hydroxybutyrate) (P4HB), PHBHHx, P3HB-*co*-4HB, P(3HB), and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate-*co*-3-hydroxyhexanoate) (PHBVHHx or PBVHx) (Huang et al., 2024). These materials have been extensively studied for applications in drug carriers, biomedical implants, and tissue engineering (Huang et al., 2024).

PHAs have also been investigated for their potential in transdermal drug delivery systems, with specific copolymers reported to enhance drug permeability across the skin barrier (Kalia et al., 2023). Microspheres composed of PHB have been employed to facilitate the transport and controlled release of antibiotics, such as rifamycin, ensuring localised therapeutic delivery (Kalia et al., 2023). Additionally, copolymers like PHBV and P(3HB-*co*-4HB) have been incorporated into implant systems for antibiotic-release applications (Kalia et al., 2023). The mechanical strength and biological activity of PHAs have been further improved by blending them with bioceramics such as hydroxyapatite (Kalia et al., 2023). Moreover, PHB, a subtype of PHA, has shown potential for targeted drug delivery owing to its slower degradation rate and reduced effect on tissue pH when compared to polylactides

(Korde and Kandasubramanian, 2020; Gagliardi et al., 2021). According to ISO 10993 standards, PHB NPs are considered biocompatible and safe for *in vivo* use (Masood, 2016; Gagliardi et al., 2021).

PHAs offer several advantages as drug nanocarriers (Huang et al., 2024). Their hydrophobic nature improves the encapsulation of hydrophobic chemotherapeutic agents such as paclitaxel, doxorubicin, and rapamycin (Huang et al., 2024). PHA nanoparticles, particularly PHB, PHBHHx, PHBV, and P(3HB-*co*-4HB), have been used to deliver anticancer drugs, including DTX, doxorubicin, sorafenib, ellipticine, and 5-fluorouracil (5-FU), enhancing drug bioavailability and therapeutic efficacy (Faisalina et al., 2020; Kalia et al., 2023; Paul et al., 2024).

Furthermore, PHAs undergo enzymatic degradation, where ester bonds are cleaved by lipases, resulting in non-toxic byproducts such as 3HB and 4HB. These degradation products are naturally occurring metabolites in the human body (Huang et al., 2024). This controlled degradation mechanism supports sustained drug release, helping to lower the frequency of administration and reduce systemic toxicity (Huang et al., 2024). For instance, polyethyleneimine (PEI)-functionalized PHB NPs have been employed to deliver microRNA-124, successfully inhibiting tumour progression in prostate cancer cells. Additionally, PEGylation has been reported to prolong NP circulation time and decrease immune system clearance (Huang et al., 2024).

Researchers have also developed receptor-mediated PHA drug delivery systems by modifying NPs with ligands such as human epidermal growth factor (hEGF) and folic acid, enabling selective tumour targeting (Huang et al., 2024). Another promising use of PHA-based nanocarriers is in photodynamic therapy (PDT). PHBV NPs have been employed to encapsulate photosensitizers like 5,10,15,20-tetra(4-hydroxyphenyl) porphyrin (pTHPP), allowing for light-triggered drug release and the selective destruction of tumour cells (Huang et al., 2024). Due to their tunable mechanical properties and degradation rates, PHAs are emerging as a highly promising biodegradable polymer in nanomedicine, particularly in cancer treatment.

2.4.4 Challenges and Limitations of PHA

Despite their promising applications, PHAs face several challenges that must be addressed for broader commercialization. One key issue is the clinical translation of PHA-based nanocarriers, which still require improvements (Huang et al., 2024). The mechanical properties of PHAs, particularly their brittleness and low elasticity, must be enhanced through copolymerization and blending with other biopolymers to improve their performance (Kalia et al., 2023). Additionally, molecular weight and monomer composition variations can influence drug loading capacity and release profiles, which need to be optimized for more efficient drug delivery (Huang et al., 2024).

Globally, the large-scale production of PHAs is still constrained by high production costs, inconsistencies in raw materials, and processing challenges (Utsunomia et al., 2020). The cost of PHAs is estimated to be three to four times higher than that of conventional synthetic plastics like polypropylene and polyethylene, with nearly half of the production expenses attributed to the use of pure sugars and fatty acids as precursor materials (Utsunomia et al., 2020). Furthermore, strict patent regulations have hindered research progress and financial investment in this area (Utsunomia et al., 2020). Despite these obstacles, PHAs continue to show significant potential in medical applications due to their biodegradable and biocompatible nature (Utsunomia et al., 2020). Alternative and more cost-effective feedstocks, including agricultural and industrial biowaste, have been suggested to lower production expenses (Kalia et al., 2023). Additionally, improvements in genetic engineering techniques and microbial fermentation processes could enhance the efficiency of PHA production for biomedical purposes (Kalia et al., 2023). The complexity of microbial fermentation and subsequent purification steps remains one of the key contributors to the overall high production cost of PHAs (Huang et al., 2024).

Further research is necessary to enhance the functional characteristics of PHAs to broaden their application in drug delivery systems, regenerative therapies, and medical implants (Kalia et al., 2023). Additional efforts should also aim to optimise

large-scale production, improve carrier stability, and develop new functionalized PHAs for targeted cancer treatment (Huang et al., 2024). With continuous progress in synthetic biology and nanomedicine, PHA-based nanocarriers are considered promising candidates to become next-generation platforms for cancer therapy (Huang et al., 2024).

2.4.5 Poly (3-hydroxybutyrate-*co*-4-hydroxybutyrate) [P(3HB-*co*-4HB)]

Among PHA copolymers, those containing 4HB have attracted considerable attention due to their suitability for medical applications (Vodicka et al., 2022). Unlike other biodegradable polyesters like PLA, PGA, and PCL, P(4HB) is synthesized exclusively through microbial fermentation (Utsunomia et al., 2020). P(4HB) stands out among PHAs for its unique characteristics, including high elasticity that rivals ultrahigh molecular weight polyethylene, making it a promising material for medical use (Utsunomia et al., 2020). The first PHA to receive FDA approval was P(3HB-*co*-4HB), underscoring its potential in medical applications (Utsunomia et al., 2020; de Macedo et al., 2024).

Copolymers like PHBV and P(3HB-*co*-4HB) create fibrous meshes and scaffolds for regenerating bone, cartilage, and liver (Kalia et al., 2023). P(3HB-*co*-4HB) has gained attention among different PHA polymers due to its distinct physical and physicochemical properties (de Macedo et al., 2024). Research has highlighted the significance of P(3HB-*co*-4HB) in terms of its mechanical properties and potential for large-scale industrial production (de Macedo et al., 2024).

P(3HB-*co*-4HB) demonstrates superior mechanical and thermal properties to other PHA copolymers, making it a promising candidate for drug delivery NP formulations (Li et al., 2016; Biswas et al., 2022). Notably, P(3HB-*co*-4HB) exhibits better elasticity and a lower melting temperature than P(3HB) (Li et al., 2016; Utsunomia et al., 2020). The incorporation of 4HB into the P(3HB) chain enhances

flexibility and reduces crystallinity, overcoming the brittleness of P(3HB) (Kumar et al., 2020; Mitra et al., 2021). The mechanical properties of P(3HB-*co*-4HB) can be fine-tuned by adjusting the 4HB content, offering greater versatility compared to more rigid polymers (Chai et al., 2020). Studies have shown that P(3HB-*co*-4HB) NPs effectively release antibiotics and anticancer drugs (Ramachandran et al., 2018). Compared to conventional synthetic polymers, P(3HB-*co*-4HB) offers the advantages of biodegradability and biocompatibility, making it ideal for biomedical applications (Alves et al., 2023; Costa et al., 2024). The presence of 4HB in the copolymer also contributes to reduced stiffness and enhanced degradation rates, which are beneficial compared to more rigid polymers (Singh et al., 2015).

Although synthetic biodegradable polymers like PGA, poly(L-lactic acid) (PLLA), and PLGA have been used in medical applications, they face challenges due to their long-term biostability and acidic degradation products (Kaur and Roy, 2015). These degradation byproducts can lower the local pH, affect drug delivery and absorption, and lead to severe inflammatory responses and tissue necrosis at implant sites (Kaur and Roy, 2015). P(3HB-*co*-4HB) presents a lower risk of altering local pH in biological environments, making it a safer alternative (Kaur and Roy, 2015; Ali and Jamil, 2016; Mitra, Xiang, and Han, 2021). Unlike synthetic biopolymers that degrade into lactic and glycolic acids, which may cause tissue inflammation, PHAs degrade into non-toxic metabolites, enhancing their suitability for applications in tissue engineering, wound healing, and controlled drug release (Chai et al., 2021).

The thermal properties of P(3HB-*co*-4HB), such as its melting and glass transition temperatures, can be optimized to suit specific application requirements, unlike PLA, which has relatively fixed thermal properties (Norhafini et al., 2017; Utsunomia et al., 2020). P(3HB-*co*-4HB) exhibits exceptional mechanical strength and elongation properties, making it ideal for various biomedical applications (Huong et al., 2018; Norhafini et al., 2019).

P(3HB-*co*-4HB) can also be effectively used in targeted drug delivery systems, utilizing ligand attachment technologies similar to those used with PLGA (Zhang et

al., 2018). The development of blend strategies further enhances P(3HB-*co*-4HB) properties, allowing it to be used in various composite materials (Fazal et al., 2023). The microbial fermentation process used to synthesize P(3HB-*co*-4HB) offers a more environmentally friendly production method than PLA and PLGA, which rely on petrochemical sources (Zhang et al., 2018; Alves et al., 2023). However, the raw materials for P(3HB-*co*-4HB) are more expensive than those used for synthetic polymers, which could affect its industrial applications. Nevertheless, this limitation is offset by the absence of recycling and waste disposal costs, making it a more sustainable option in the long term (Norhafini et al., 2019; Gheorghita et al., 2021; Costa et al., 2024).

2.4.6 Biosynthesis of P(3HB-*co*-4HB)

The biosynthesis of P(3HB-*co*-4HB) involves microbial fermentation, primarily using *Cupriavidus necator* and other bacterial strains that can accumulate PHAs (Koller and Mukherjee, 2022; Koller and Obruča, 2022). This biosynthesis process consists of two key steps: first, the synthesis of 3-hydroxybutyryl-CoA (3HB-CoA) and 4-hydroxybutyryl-CoA (4HB-CoA), followed by their polymerization by *phaC* (Huong et al., 2021). Figure 2.14 illustrates the metabolic pathways that play a role in the biosynthesis of P(3HB-*co*-4HB).

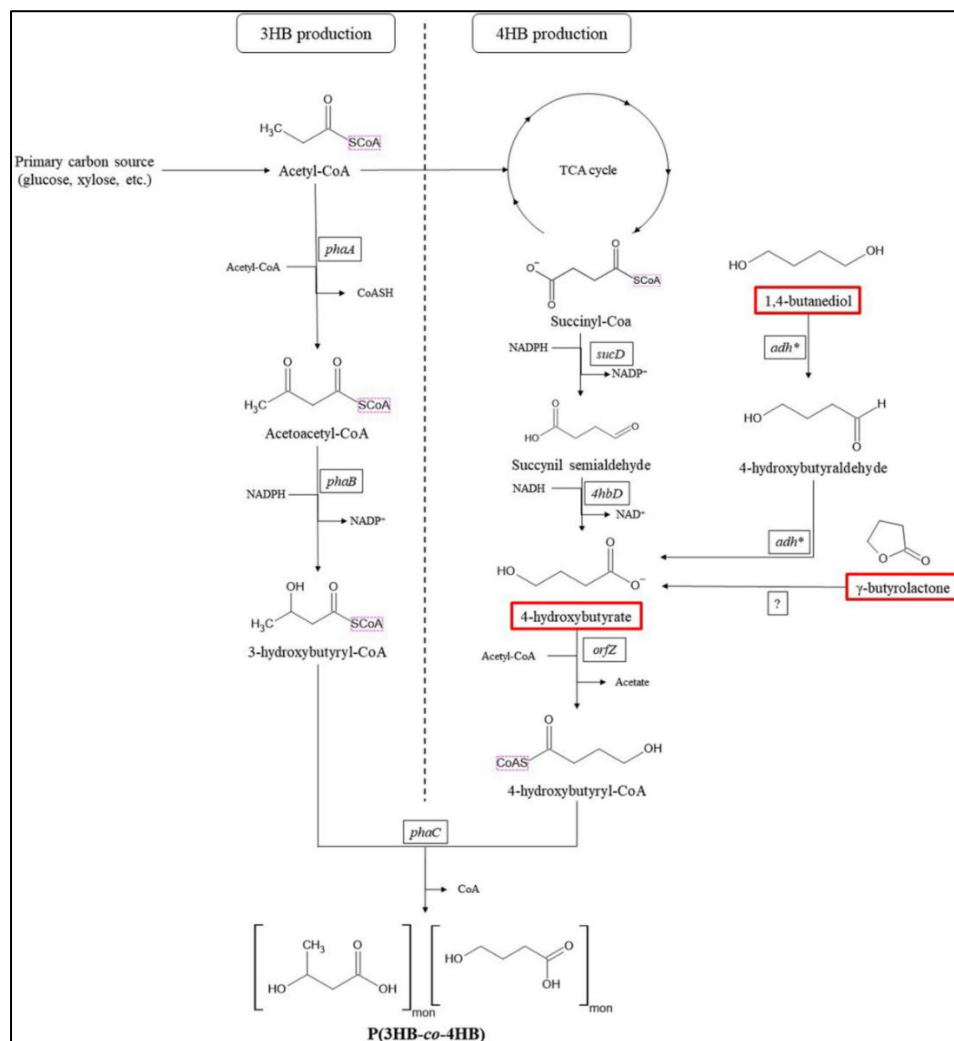


Figure 2.14: Metabolic pathways involved in the biosynthesis of P(3HB-co-4HB) (Adapted from de Macedo et al. (2024)). The diagram illustrates the microbial conversion of primary carbon sources into the monomers 3-hydroxybutyryl-CoA (3HB-CoA) and 4-hydroxybutyryl-CoA (4HB-CoA), which are subsequently polymerized into P(3HB-co-4HB). Carbon substrates structurally related to 4HB are highlighted in red boxes. Key genes involved in the pathway are shown in black boxes: *phaA* (3-ketothiolase), *phaB* (acetoacetyl-CoA reductase), *phaC* (PHA synthase), *sucD* (succinic semialdehyde dehydrogenase), *4hbD* (4-hydroxybutyrate dehydrogenase), *orfZ* (4HB-CoA transferase), and *adh** (putative alcohol/aldehyde dehydrogenase). The symbol “?” indicates an unidentified 1,4-lactonase enzyme potentially involved in γ -butyrolactone metabolism. The final copolymer, P(3HB-co-4HB), is synthesized by polymerising 3HB-CoA and 4HB-CoA monomers by PHA synthase.

In the acetoacetyl-CoA pathway, acetyl-CoA is condensed through the action of β -ketothiolase (PhaA) to form acetoacetyl-CoA. This is then reduced to 3HB-CoA by acetoacetyl-CoA reductase (PhaB) (Mierzati and Tsuge, 2020; Zhou et al., 2023; Fook et al., 2024). The precursor for 4HB is derived from 1,4-butanediol, γ -butyrolactone, or 4-hydroxybutyric acid, which undergoes conversion to succinate or 4HB through enzymatic pathways (Utsunomia et al., 2020; Zhou et al., 2023). The activation of 4HB-CoA is facilitated by 4-hydroxybutyrate dehydrogenase and 4-hydroxybutyryl-CoA transferase, which leads to its incorporation into the copolymer (Utsunomia et al., 2020; Mierzati and Tsuge, 2020). *PhaC* catalyzes the polymerization of 3HB-CoA and 4HB-CoA monomers, forming P(3HB-*co*-4HB) with tunable mechanical properties based on the 4HB content (Utsunomia et al., 2020; Mierzati and Tsuge, 2020).

The production of P(3HB-*co*-4HB) is mainly dependent on the type of carbon source utilized during microbial fermentation (Huong et al., 2021; Utsunomia et al., 2020). Various carbon sources have been identified for PHA synthesis, including gaseous compounds such as methane and carbon dioxide, alcohols like methanol and ethanol, and carbohydrates such as glucose, fructose, and cellulose. N-alkanoic acids (for example, acetic and butyric acids) and n-alkanes like hexane and octane have also been used (Jiang et al., 2016; Ray et al., 2023). The capacity of microorganisms to metabolize a wide range of carbon substrates highlights the potential of PHAs as sustainable materials for bioplastic production and waste management (Jiang et al., 2016; Ray et al., 2023).

The proportion of 3HB and 4HB monomers within the copolymer can be accurately controlled by modifying the type and concentration of carbon sources during fermentation (Zhou et al., 2023). For example, incorporating 1,4-butanediol in a mixed-substrate system can increase 4HB content, improving the copolymer's flexibility and elasticity (Ramachandran et al., 2025). Conversely, a higher 3HB content tends to produce polymers that are more crystalline and brittle (Zhou et al., 2023). Therefore, adjusting the precursor ratio is essential to fine-tune the final material's mechanical strength and degradation characteristics, particularly for drug delivery applications.

Additionally, fermentation parameters play a significant role in determining the copolymer synthesis. Variables such as fermentation mode (batch, fed-batch, or continuous), pH level, temperature, oxygen availability, and the carbon-to-nitrogen (C: N) ratio all influence bacterial growth and the monomer composition of the resulting polymer (Anjum et al., 2016). Specifically, fed-batch fermentation offers better control over precursor feeding, increasing biomass and copolymer production while minimizing the buildup of inhibitory substrates like 1,4-butanediol (Norhafini et al., 2019).

In recent years, the genetic engineering of microbial strains has been investigated to improve production efficiency and provide better control over monomer ratios (Zhang et al., 2020; Paduvari et al., 2025). Recombinant strains of *E. coli*, *Halomonas*, and *Cupriavidus*, which have been engineered to overexpress PHA biosynthesis genes and optimize metabolic pathways, have shown improved yields and expanded substrate versatility (Zhang et al., 2020; Paduvari et al., 2025).

In conclusion, the biosynthesis of P(3HB-co-4HB) can be precisely controlled by selecting microbial strains, optimising substrate use, and controlling fermentation processes. These factors influence polymer yield and allow customization of its mechanical properties, elasticity, and degradation rate, making it ideal for biomedical applications like controlled drug release.

2.4.7 Biocompatibility and Degradation of P(3HB-*co*-4HB) *in Vitro*

The biocompatibility and degradation behaviour of P(3HB-*co*-4HB) are vital factors for its use in drug delivery systems and medical implants. This polymer does not provoke significant immune responses, which helps minimize inflammation risk during implantation (Ali and Jamil, 2016). P(3HB-*co*-4HB) degrades through hydrolytic and enzymatic processes, producing non-toxic byproducts during its breakdown (Utsunomia et al., 2020; Vodicka et al., 2022). Hydrolytic degradation, which impacts the stability of the polymer's ester bonds, is influenced by factors such as pH and temperature (Utsunomia et al., 2020). Enzymatic degradation occurs with the help of enzymes like lipases and depolymerases, which promote polymer breakdown in biological systems (Utsunomia et al., 2020).

Unlike synthetic biopolymers that can release harmful byproducts, P(3HB-*co*-4HB) degrades into harmless metabolites that are integrated into the tricarboxylic acid (TCA) cycle for safe metabolism (Chai et al., 2021). Animal studies have confirmed that P(3HB-*co*-4HB) demonstrates excellent biocompatibility *in vivo*, further supporting its use in biomedical applications (Ali and Jamil, 2016; Utsunomia et al., 2020; Vodicka et al., 2022). The degradation rate can be tailored by adjusting the copolymer ratio, providing flexibility for different therapeutic applications (Chai et al., 2021). For instance, high molecular weight P(3HB-*co*-4HB) degrades more slowly, making it suitable for extended drug release systems (Norhafini et al., 2019).

In comparison, the biodegradation of PLGA is generally faster than that of P(3HB-*co*-4HB), which could benefit applications requiring a quick drug release (Chai et al., 2021). However, the controlled degradation of P(3HB-*co*-4HB) can enable programmed release profiles, offering advantages for therapeutic applications (Alves et al., 2023). Regarding biocompatibility, P(3HB-*co*-4HB) exhibits minimal cytotoxicity and inflammatory response, similar to PLA and PLGA (Kaur and Roy, 2015; Ali and Jamil, 2016; Vodicka et al., 2022).

The degradation rate of PHA is affected by its crystal morphology; more crystalline regions are more resistant to hydrolysis, while amorphous regions degrade

faster (Vodicka et al., 2022). Since 3HB homopolymers have high crystallinity, PHA copolymers, such as P(3HB-*co*-4HB), tend to be more amorphous, which makes them more prone to hydrolytic degradation (Vodicka et al., 2022). P(3HB-*co*-4HB) stands out as a promising copolymer with enhanced mechanical properties, demonstrating a potent combination of *in vivo* biodegradability and biocompatibility, making it an excellent candidate for medical applications (Vodicka et al., 2022).

2.5 Microneedles in Cancer Therapy

MNs are generally classified into four major categories according to their structure and method of drug delivery: dissolvable microneedles (DMNs), hollow MNs, drug-coated MNs, and solid MNs (Fan et al., 2023). Solid MNs do not contain any active ingredients but function to form small channels in the skin (Fan et al., 2023). These channels enable drug molecules on the skin to permeate through the stratum corneum, the skin's outer barrier (Fan et al., 2023).

On the other hand, hollow MNs contain a tiny channel that permits liquid drugs to be delivered into the deeper layers of the skin (Fan et al., 2023). Drug-coated MNs are coated with a fine layer of medication that quickly dissolves when inserted into the skin (Fan et al., 2023). DMNs consist of polymers that are both biocompatible and biodegradable, and these MNs disintegrate after insertion, gradually releasing the encapsulated drug (Fan et al., 2023). Additionally, hydrogel MNs, which absorb fluids from the skin, help achieve sustained drug release (Avcil and Çelik, 2021). Figure 2.15 below provides a schematic representation of the various types of MNs and their drug delivery mechanisms.

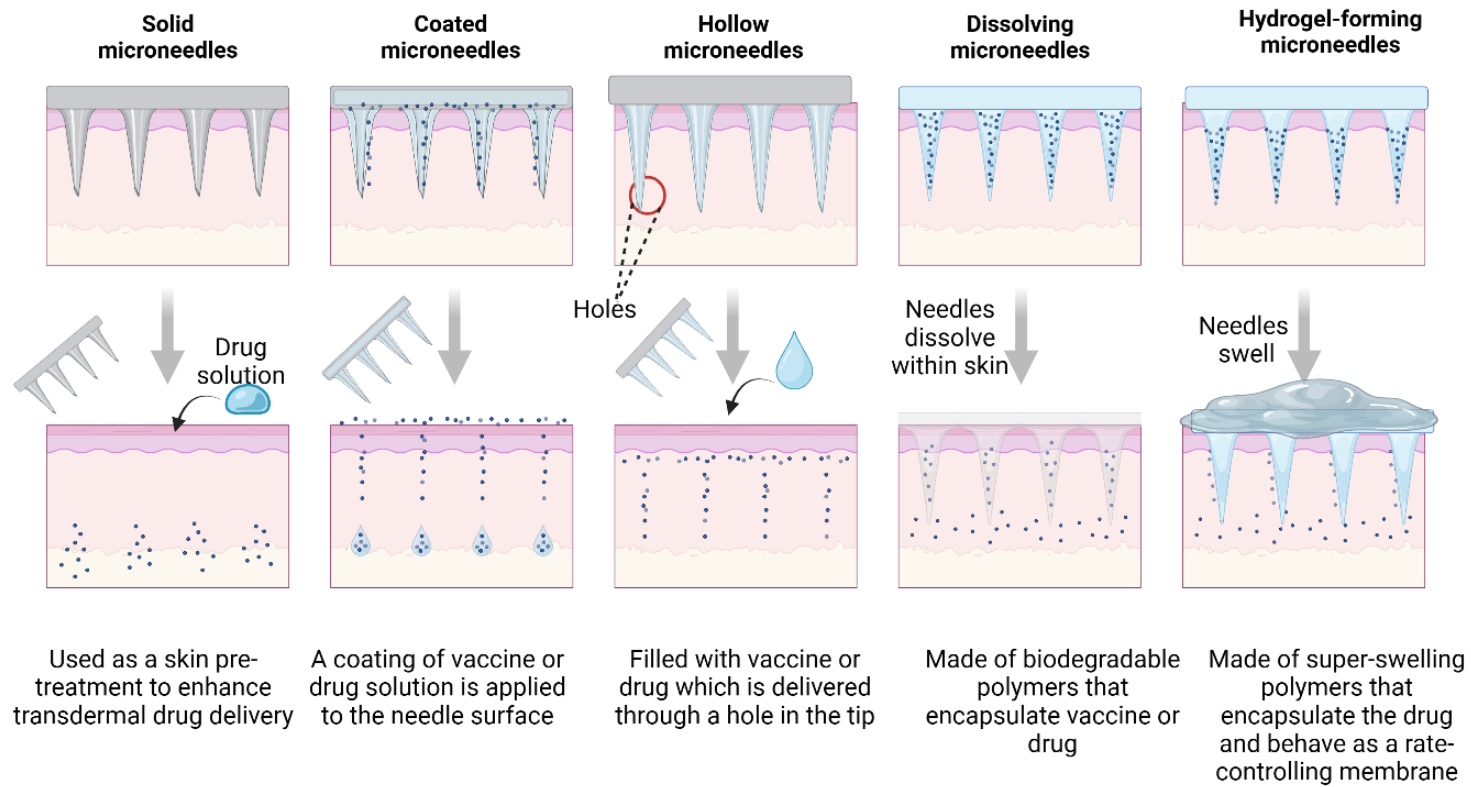


Figure 2.15: Schematic illustration of the different types of microneedles and their delivery mechanism (Created at <https://BioRender.com>).

Each type of MN has its advantages and is chosen based on the type of drug and the treatment needed. However, making sure that the drug is evenly distributed inside the MNs is still a big challenge for achieving consistent treatment results. DMNs release their drug payload as the polymer matrix gradually breaks down in the skin, allowing for controlled drug release. Meanwhile, hollow MNs allow for a steady infusion of drugs through the tiny channels they create, ensuring accurate dosing and a stable flow rate. Drug-coated MNs rely on the fast dissolution of the drug coating for an immediate effect, while solid MNs help with passive drug absorption by making it easier for drugs to penetrate through the skin barrier.

Among these types, DMNs seem to hold the most promise for BC treatment because they can deliver drugs directly to the tumour site with less pain and fewer side effects. Solid MNs offer strong mechanical properties, good physical stability, and adequate drug loading, but they come with the risk of infection due to reuse, require rapid healing post-application, have low biocompatibility, and can lead to inaccurate dosing (Aldawood et al., 2021; Jung and Jin, 2021; Tariq et al., 2022; Ganeson et al., 2024). Coated MNs also offer strong mechanical properties but can suffer from changes in formulation stability during storage, peeling during insertion, low biocompatibility, and limited dosing capacity (Kapoor et al., 2020; Aldawood et al., 2021; Jung and Jin, 2021; Ganeson et al., 2024).

Hollow MNs, while allowing for accurate dosing and stable drug delivery, face limitations such as the risk of clogging, infection from reuse, low biocompatibility, the need for a prefilled syringe, poor mechanical durability, and potential body fluid ingress (Aldawood et al., 2021; Cárcamo-Martínez et al., 2021; Jung and Jin, 2021; Ganeson et al., 2024). On the other hand, DMNs are simple to apply, cost-effective, and easy to produce, but have weak mechanical properties, stability, and biocompatibility concerns, as well as limited drug dosage (Aldawood et al., 2021; Jung and Jin, 2021; Zhang et al., 2021; Ganeson et al., 2024). Hydrogel-forming MNs have the advantage of not leaving behind any remaining excipient traces within the skin after their removal, offering controlled release and adequate drug loading, but suffer from weak mechanical and physical stability, along with body fluid ingress (Aldawood et al., 2021; Jung and Jin, 2021; Turner et al., 2021; Ganeson et al., 2024).

Recently, certain MNs have been engineered to be sensitive to external stimuli, meaning they can release their drug content in response to particular triggers, such as variations in pH, temperature, or the presence of specific enzymes within the body. This helps ensure the drug is released only where needed, making the treatment more targeted and effective. Overall, the different types of MNs each have their strengths and weaknesses, and choosing the right type depends significantly on the drug delivered and the specific treatment needs.

2.5.1 Skin Structure and Pathways of Transdermal Drug Transport

The core concept of an MN-based therapeutic system lies in its ability to deliver drugs through the skin in a targeted and controlled manner (Ganeson et al., 2023). To fully appreciate this, it is important to understand the skin's structure, which acts as a significant barrier to protect the body from rapid substance absorption (Yu et al., 2021). The skin has three primary layers: the epidermis, dermis, and subcutaneous tissue (Lyu et al., 2023). Figure 2.16 illustrates human skin anatomy and compares MNs with traditional injectable delivery methods.

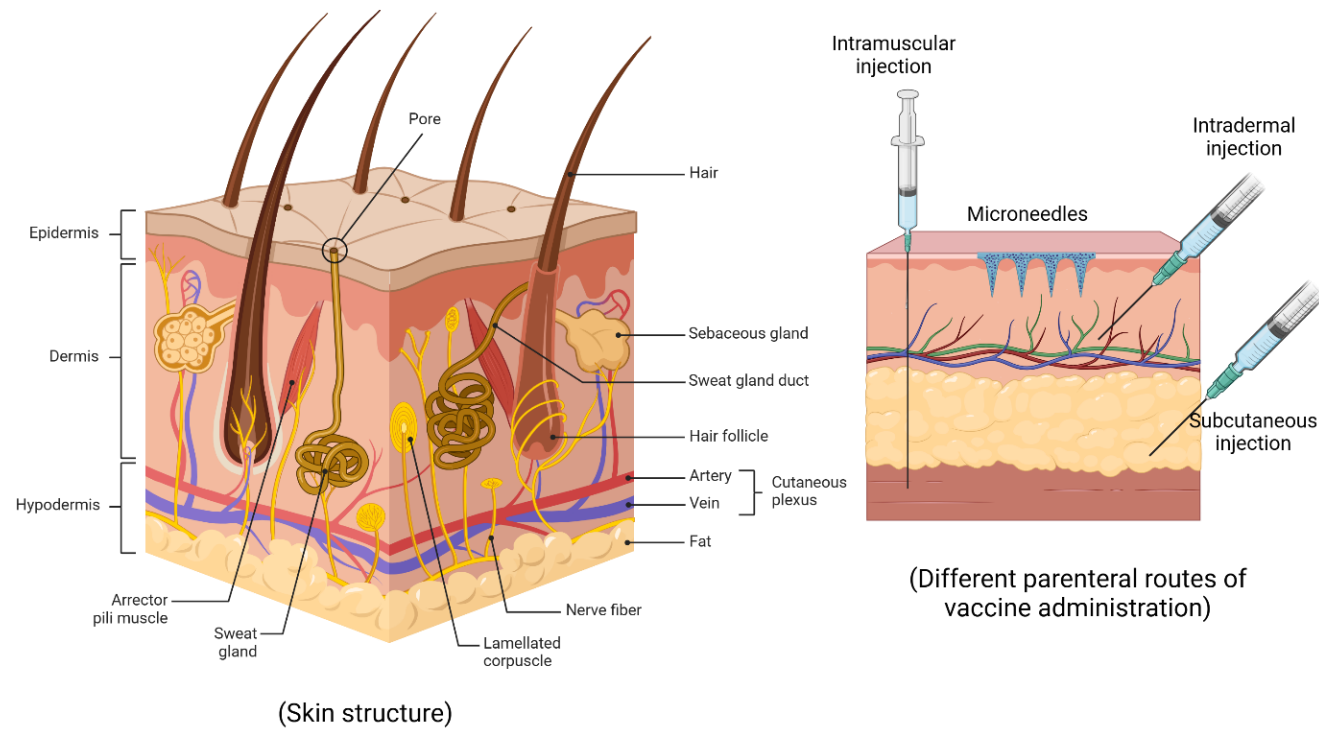


Figure 2.16: Human skin anatomy and comparison of skin penetration by microneedles and traditional needles, highlighting the ability of microneedles to deliver drugs effectively without causing pain (Created in <https://BioRender.com>).

The outermost part of the skin, also known as the stratum corneum (SC), primarily protects our body from potentially harmful external substances (Chatterjee et al., 2022). It comprises tightly bound, rigid keratinocytes containing keratin filaments and cross-linked proteins, forming a brick-mortar structure where intracellular multilamellar lipids act as the mortar (Chatterjee et al., 2022). It is a substantial barrier to most drugs because its thick, keratinized structure limits drug absorption through the skin (Khan et al., 2021; Chatterjee et al., 2022; Fan et al., 2023). Since drugs with high molecular weight and extreme hydrophilicity or lipophilicity are generally considered barriers to skin permeation following transdermal application, modified formulations such as nanoemulsions, microemulsions, solid lipid NPs, nano lipid carriers, transfersomes, and ethosomes are often used to enhance drug transport across the skin (Hirakawa et al., 2019; Chatterjee et al., 2022).

The epidermis, situated above the dermis, consists of keratinocytes that progressively mature and eventually form the skin's outer barrier (Fan et al., 2023). Beneath the epidermis, the dermis is a supportive layer characterized by collagen and elastin fibres, crucial in maintaining the skin's strength and elasticity (Khan et al., 2021). The thickness of the epidermis and dermis varies across anatomical regions, influencing MNs' appropriate design and length for effective drug delivery (Zhou et al., 2023). MNs are particularly advantageous as they penetrate the skin's outermost barrier without causing significant pain, making them suitable for transdermal drug administration (Chen et al., 2020; Muresan et al., 2023). Typically, the epidermal thickness ranges between 50 and 150 μm , while the dermal thickness may vary from 1 to 4 mm, depending on the body area, impacting MN design considerations (Ganeson et al., 2023).

MN arrays consist of tiny needles, typically with a height less than 2000 μm and a width between 10 and 50 μm . The MNs are usually painless as it is designed to penetrate only the outermost layer of the skin (Yadav and Pattanayek, 2020; Zhou et al., 2023; Muresan et al., 2023). By creating microscopic channels in the skin, MNs enable drugs to bypass the outermost skin barrier and deliver them directly into the inner layers of the epidermis and dermis (Hirakawa et al., 2019; Zhou et al., 2023). In transdermal drug delivery, the skin serves as a depot, facilitating drug diffusion into

the lower epidermal and dermal regions while reducing drug accumulation on the surface (Ganeson et al., 2023). This method significantly improves drug absorption and enables more precise and efficient drug delivery than conventional transdermal patches (Naik et al., 2023). The ability of MNs to penetrate the skin with minimal discomfort or injury makes them a suitable strategy for administering various drugs, including anticancer agents used in BC therapy. DMNs are fabricated from biodegradable materials that safely dissolve or break down within the skin, eliminating the requirement for needle disposal, reducing infection risks, and increasing convenience for patients (Chen et al., 2020; Muresan et al., 2023; Zhou et al., 2023).

Transdermal drug delivery occurs through several pathways, each influencing the drug's ability to permeate the skin (Chatterjee et al., 2022). These pathways include the transcellular route (direct penetration through the SC), the paracellular or intercellular route (traversing the tight junctions and lipid matrix between cells), and the transappendageal route (through hair follicles and sweat ducts) (Chatterjee et al., 2022). Sometimes, microneedling or skin-stripping techniques create micro-sized pores that enhance penetration (Chatterjee et al., 2022). Although the transappendageal route involves naturally occurring skin structures, these appendages represent only about 0.1% of the total skin surface, limiting their effectiveness in drug absorption (Chatterjee et al., 2022). Therefore, most transdermal drug delivery strategies remain the SC's primary focus. Various enhancement methods, including iontophoresis, electroporation, chemical enhancers, and nanocarriers, aim to overcome this barrier and improve delivery efficiency (Chatterjee et al., 2022). Understanding the skin's structural and functional characteristics is essential in designing effective MN systems to overcome physiological barriers and maximize drug delivery outcomes.

2.5.2 Comparative Effectiveness of Microneedles and Traditional Delivery Methods in Breast Cancer Treatment

Chemotherapy drugs are primarily delivered through IV injections or oral administration, both of which lead to widespread systemic exposure and often result in severe side effects (Bathara et al., 2020). Conventional oral administration presents several challenges, as oral drugs are subject to first-pass metabolism in the liver, reducing their overall effectiveness (Seetharam et al., 2020). Additionally, oral drugs often experience inconsistent absorption and poor solubility, making their effectiveness less reliable (Seetharam et al., 2020). Parenteral delivery, such as injections, is more effective but can be painful and carries a risk of infection. Figure 2.17 below compares oral, hypodermal, and transdermal drug delivery methods.

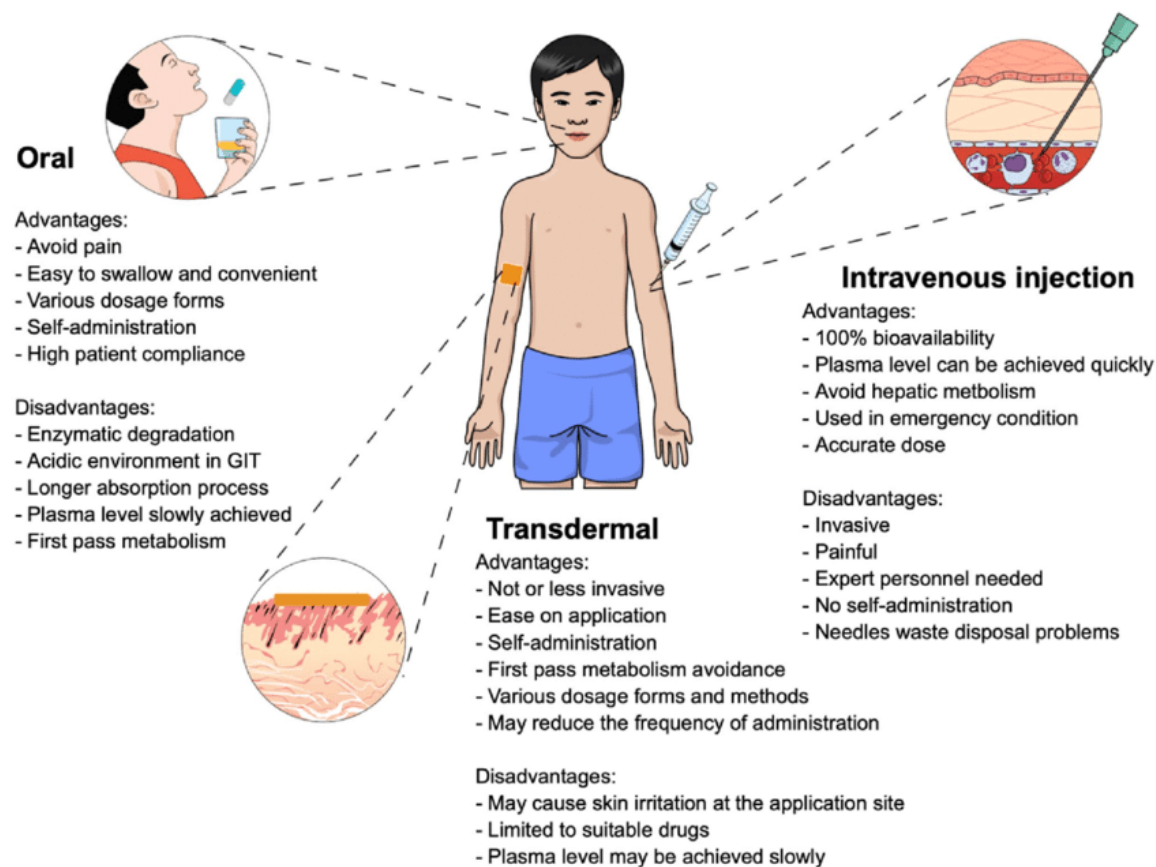


Figure 2.17: Illustration of comparisons among various routes of administrations (oral and hypodermal) commonly involved in conventional drug delivery methods with a transdermal microneedle (Adapted from Ramadon et al., 2022).

Systemic drug delivery can also reduce drug concentration reaching the breast tissue because of barriers such as the milk duct structures and the distance required to reach systemic circulation (Bathara et al., 2020). This results in excessive systemic drug exposure, with relatively lower concentrations in the targeted areas, leading to side effects and potential damage to vital organs (Bathara et al., 2020). Evidence from preclinical and clinical investigations has demonstrated that direct application of drugs to breast tissue can lead to preferential drug accumulation in the region, provided the skin is permeable (Bathara et al., 2020). In contrast, drugs applied to other body areas often enter systemic circulation (Bathara et al., 2020). This observation is linked to the developmental origin of breast skin, which possesses an extensive internal lymphatic and venous network and subcutaneous and retro-mammary fat deposits (Bathara et al., 2020). The fat pad of the breast and the lymphatic channels within the breast region aid in the retention of drugs in the targeted breast tissue (Bathara et al., 2020).

Localised transdermal drug delivery can effectively deliver anticancer drugs based on this unique anatomical feature for BC treatment while minimising systemic side effects (Bathara et al., 2020; Avcil and Çelik, 2021; Khan et al., 2021). In addition to bypassing first-pass metabolism, transdermal delivery offers several benefits, including simple and painless application, controlled drug release, the potential for zero-order drug release, and the advantage of avoiding the harsh gastric environment (Chatterjee et al., 2022). MNs have shown better penetration into tumour tissues (Apolinário et al., 2024). Bioimaging studies confirmed that MNs helped achieve higher drug accumulation within tumours, suggesting they can deliver drugs more effectively without spreading them to other body parts (Bathara et al., 2020).

Previous studies showed that MNs managed to shrink tumours as much as IV-administered DTX, proving they can be just as effective in killing cancer cells (Bathara et al., 2020). In preclinical studies with animal models, MNs loaded with FENR-based ethosomes for chemoprevention of BC displayed a unique PK profile that allowed for sustained drug release over a more extended period (Apolinário et al., 2024). Unlike oral drugs that reach a peak concentration quickly, MNs provide a more controlled release, avoiding sudden spikes in drug levels (Apolinário et al., 2024). Another example is HPV-incorporated MNs, which could release drugs steadily for up to 48

hours, allowing for more prolonged drug exposure at the tumour site compared to the rapid clearance seen with IV methods (Bathara et al., 2020).

DMNs have been used to deliver immune boosters and checkpoint inhibitors directly to tumours, helping to activate T cells and shrink tumours more effectively in TNBC treatments (Huang et al., 2024). Unlike traditional IV injections, which require high doses that cause severe side effects, DMNs can provide a steady drug release directly into the tumour, ensuring a more effective treatment with less toxicity (Huang et al., 2024).

MNs also can reduce side effects, unlike IV chemotherapy, which often leads to weight loss and organ damage (Bathara et al., 2020). Patients treated with MNs had stable body weight and lower levels of liver and kidney damage markers (Bathara et al., 2020). This is mainly because MNs can localise the drug delivery directly to the tumour, reducing systemic exposure (Bathara et al., 2020). MNs offer a promising alternative by combining the benefits of transdermal patches and hypodermic injections without drawbacks (Seetharam et al., 2020; Khan et al., 2021). They enable targeted drug delivery with controlled release, which reduces toxic side effects and improves treatment results (Seetharam et al., 2020).

MNs can deliver drugs directly to breast tumours, helping to minimize systemic side effects like bone marrow suppression and heart toxicity (Seetharam et al., 2020). In summary, MNs provide a more comfortable and patient-friendly option and ensure better drug delivery, lower side effects, and improved treatment outcomes compared to traditional methods. However, there are still some challenges to overcome, like cost and scaling up production, before they can become a standard option for cancer treatment.

DMNs are also being explored for vaccine delivery to boost the immune system's response to BC antigens (Avcil and Çelik, 2021; Khan et al., 2021). Some advanced MN systems are being combined with photothermal and photodynamic therapies. Light-activated compounds help enhance drug release and kill cancer cells

more effectively (Khan et al., 2021). For example, hollow MNs can be prefilled with drug solutions to allow controlled release once inserted into the skin (Seetharam et al., 2020). These systems work like traditional injections but are designed to cause less pain and tissue damage (Seetharam et al., 2020).

The customizable nature of DMNs is another significant advantage. They can be designed and adjusted for different types of drugs and treatment plans (Zhou et al., 2023). For example, MNs help drugs bypass the SC, which can increase drug absorption by making microchannels in the skin (Alshammari et al., 2023). This kind of localised drug delivery can also reduce the frequency of hospital visits and make treatment less stressful for patients, which can help improve their quality of life (Sabri et al., 2020). This is especially helpful for patients afraid of needles, as MNs are generally less painful than regular injections (Seetharam et al., 2020; Fan et al., 2023). Some research is even looking at ways to combine MNs with biosensors. This could make it possible to monitor the treatment outcomes of BC patients in real-time, right at the administration site (Avcil and Çelik, 2021). Doctors could adjust drug dosages more accurately by tracking the treatment's effectiveness. Overall, MNs, specifically DMNs, seem to be a promising option for BC treatment. They offer targeted drug delivery with fewer side effects, can be combined with other technologies, and make treatments more convenient and comfortable for patients.

2.5.3 Advantages of Microneedles

MNs are a promising, minimally invasive method for drug delivery, bypassing the SC and offering an effective alternative to traditional injection-based systems (Apolinário et al., 2024). This characteristic makes MNs particularly beneficial for long-term treatments such as BC chemoprevention, which requires regular drug administration (Apolinário et al., 2024). By directly delivering drugs into the dermal layers, MNs minimize systemic toxicity and allow for controlled drug release (Muresan et al., 2023; Alshammari et al., 2023). Various brands and manufacturers

have designed MN systems for different medical applications. For instance, TheraJect Inc.'s DrugMAT and VatMAT DMNs have been used for transdermal delivery of large molecules like proteins, vaccines, and genetic materials (Ganeson et al., 2023). On the other hand, Becton Dickinson's Onvax MN array patch offers plastic micro-projections with an MN size of approximately 200 μm , suited for vaccines and other therapies (Nguyen et al., 2021; Ganeson et al., 2023).

DMNs offer a significant advantage in that they release drugs in a controlled manner and do not require disposal like traditional needles, which reduces the risks of infections and environmental contamination (Apolinário et al., 2024). These MNs are typically made from biocompatible materials such as PVA and PVP, ensuring long-term safety without causing skin irritation or other adverse reactions (Apolinário et al., 2024). The ease of use and minimal invasiveness contribute to improved patient compliance. For example, the Nanoject® dissolvable peptide MN patch from Debiotech is applied in both intradermal and hypodermic drug delivery, offering a non-invasive solution for delivering peptides (Halder et al., 2021; Ganeson et al., 2023).

Moreover, MNs can be precisely customized to control the amount and timing of drug release (Seetharam et al., 2020). This precision, combined with their painless nature, makes them an attractive alternative to traditional injection methods, enhancing patient adherence to treatment plans (Sabri et al., 2020). Research has shown that patients experience less pain with MNs than with conventional injections, which could improve patient outcomes (Seetharam et al., 2020).

MNs, particularly the dissolving varieties, are also highly versatile in application. For instance, Zosano Pharma's Macroflux metal MN patch is designed for peptides and vaccines, offering an intracutaneous system that allows for rapid drug release (Fierce Biotech, n.d.; Ganeson et al., 2023). Their easy self-application also reduces hospital visits, offering a more convenient option for patients (Sabri et al., 2020). Their capacity to accommodate a wide range of drug formulations with a single patch makes MNs suitable for different treatment strategies (Zhou et al., 2023).

Additionally, MNs improve drug absorption and bioavailability, especially for poorly soluble drugs (Seetharam et al., 2020). These patches are also effective for vaccine delivery, providing immune responses comparable to traditional injections but with the added benefit of stability at room temperature. This could reduce storage costs and enhance vaccine distribution, making it more accessible globally (Reinke et al., 2024). Using MN patches for flu vaccines in the U.S. could save billions annually due to lower storage costs and better patient compliance (Reinke et al., 2024).

Beyond treatment, MNs have potential applications in diagnostic fields. They can detect cancer markers and deliver drugs directly to breast tissue (Khan et al., 2021). Emerging research also explores the combination of MNs with biosensors, enabling real-time monitoring of treatment efficacy. This integration could allow for more precise dosing adjustments, improving overall treatment outcomes (Avcil and Çelik, 2021).

In conclusion, DMNs represent a valuable strategy for cancer treatment and other medical applications. Their ability to offer controlled, targeted drug delivery with fewer side effects and their convenience and versatility make them an increasingly attractive option for patients and healthcare providers.

2.5.4 Limitations of Microneedles

MNs do have some disadvantages. It can be challenging to ensure that the drug is evenly distributed within the MNs, which can affect the efficacy of the treatment (Apolinário et al., 2024). Incorporating drugs or NPs into MN formulations could be challenging, affecting their strength (Apolinário et al., 2024). Ensuring that enough drugs can be loaded into the MNs while keeping them mechanically strong is a big concern (Apolinário et al., 2024). The amount of drug that MNs can deliver is a limiting factor. Some MNs might not be able to hold enough of the drug to be effective, especially for treatments requiring high doses (Apolinário et al., 2024). The rate at

which MNs dissolve also needs to be carefully controlled. If they dissolve too quickly, the drug might be released immediately instead of gradually. On the other hand, if they dissolve too slowly, the drug might not be absorbed effectively (Sabri et al., 2020).

Mechanical strength is another challenge. MNs must be strong enough to penetrate the skin effectively and flexible enough not to break during application (Alshammari et al., 2023). MNs might fail to penetrate the skin or even break off during use if not appropriately designed (Seetharam et al., 2020). Even though MNs are great for targeted drug delivery, getting the release profile just right is still a big challenge (Apolinário et al., 2024). This means ensuring the drug is released steadily and reaches the right concentration at the target site without causing side effects.

The cost of producing MNs is also higher than traditional drug delivery methods, which could limit their use (Apolinário et al., 2024). Additionally, some patients may worry about using MNs, even though studies have shown them to be safe and effective (Apolinário et al., 2024).

Another problem is the risk of infections (Seetharam et al., 2020). Since MNs pierce the skin, they can potentially let in bacteria and other microbes (Seetharam et al., 2020). This means it is essential to apply them in sterile conditions and, in some cases, to use MNs with antimicrobial coatings (Seetharam et al., 2020). Some patients have reported skin irritation and mild inflammation at the site where MNs were applied, but these effects are usually temporary and not severe (Seetharam et al., 2020). However, the thickness and composition of the skin can vary between different people, which can affect how well the MNs penetrate the skin and deliver the drug (Khan et al., 2021).

Injection accuracy is also an issue for some types of MNs. In some cases, MNs may not deliver drugs as accurately as traditional hypodermic needles (Khan et al., 2021). Polymer-based DMNs can also be fragile, making them less reliable for consistent drug delivery (Khan et al., 2021). There is also a risk that small fragments of MNs could get stuck in the skin if they break off, which is a concern for their

widespread clinical use (Avcil and Çelik, 2021). Making sure that MNs dissolve entirely and safely is crucial to avoid complications.

Scaling up production is also a challenge. Even though advanced techniques can make high-quality MNs, making them in large quantities without losing quality is still difficult and expensive (Sabri et al., 2020). Combining drugs and biodegradable polymers with MNs requires many optimizations. Ensuring the drug stays stable and is released quickly (Alshammari et al., 2023). There are still some challenges to overcome, like cost and production complexity, before they can fully replace traditional drug delivery methods.

2.5.5 Challenges in Microneedle Fabrication and Drug Incorporation

There are several challenges in fabricating MNs and incorporating drugs into them. One major issue is adding drugs or NPs into MNs without losing their stability during the making process (Apolinário et al., 2024). Ethosomes, for example, have a high amount of ethanol, and keeping their structure during MN production can be challenging (Apolinário et al., 2024). Another problem is incorporating sufficient drugs into the MNs without making them too weak to pierce the skin (Apolinário et al., 2024).

Ensuring MNs are uniform in size and strength is also a challenge. Methods like micro-moulding and 3D printing can make precise MNs, but they are expensive and difficult to scale up for mass production (Seetharam et al., 2020). The materials used for MNs must be both strong enough to pierce the skin and biodegradable, so they dissolve properly after delivering the drug (Sabri et al., 2020). Polymers like PVA and PVP are often used because they are safe for the body, but achieving the right balance between strength and dissolution rate is quite tricky (Sabri et al., 2020).

Incorporating drugs into MNs is not always easy because some drugs can break down during the manufacturing process, and others might not spread evenly through the MNs, which can lead to uneven drug delivery (Reinke et al., 2024). Drugs like DTX are not easily dissolved in water, so additional steps like using chemical enhancers or nanocarriers are needed to help them get through the skin (Bathara et al., 2020). Besides that, incorporating drugs or NPs may alter the structural integrity of MNs, necessitating optimized formulations to maintain adequate mechanical strength for skin penetration (Chen et al., 2020). Figure 2.18 below highlights the key challenges in MN fabrication and drug incorporation, including mechanical strength, regulatory matters, and manufacturing scalability.

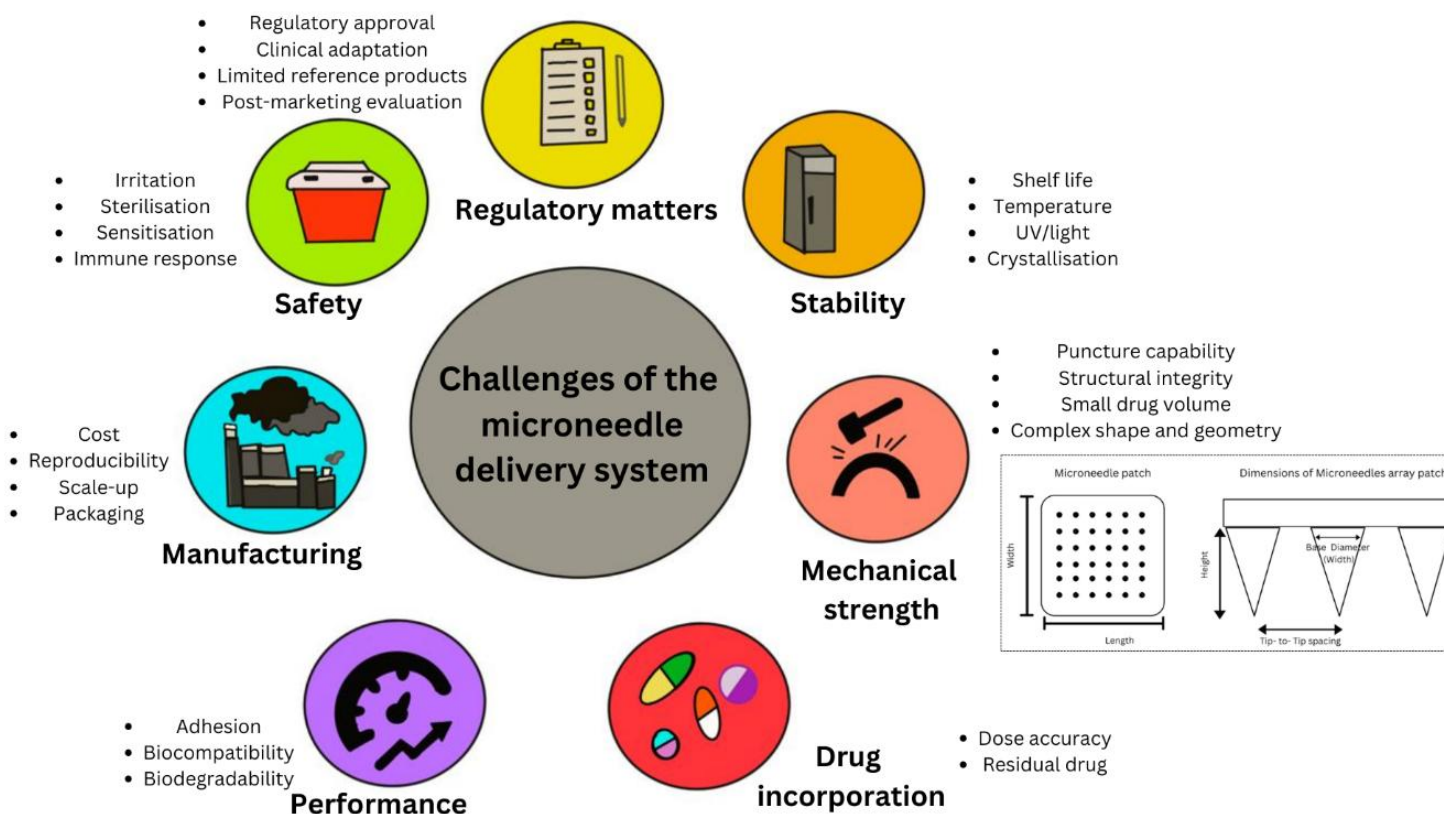


Figure 2.18: Challenges in microneedle fabrication and drug incorporation, highlighting key issues such as mechanical strength, stability, regulatory matters, safety, manufacturing scalability, and performance (Created in <https://BioRender.com>).

The way MNs dissolve can also be problematic because high molecular weight polymers tend to dissolve slowly, which is suitable for long-term treatment. Still, they might not release the drug fast enough when immediate action is needed. On the other hand, low molecular weight polymers dissolve quickly but might not hold enough drugs for a long-term effect (Sabri et al., 2020). Finding the right polymer to balance these issues is a key challenge.

Another problem is ensuring MNs are strong enough to penetrate the skin without breaking, because if they fail, it can lead to uneven drug delivery or even cause tiny pieces to get stuck in the skin, which can lead to infections (Seetharam et al., 2020). Storage stability is another issue, as some MN formulations might lose their effectiveness over time if not stored properly (Fan et al., 2023).

Additionally, large-scale production of NP-MNs with consistent drug loading and release profiles remains challenging (Chen et al., 2020). Ensuring the prolonged safety of NPs within MNs demands in-depth research, especially concerning possible toxicity, immune system reactions, and systemic distribution (Chen et al., 2020). Despite promising advancements, the clinical application of NPs remains limited by several factors, including aggregation in solution, immune system interactions, and accumulation in biological tissues (Sully et al., 2021). Aggregation occurs due to interparticle collisions, contributing to the development of clusters or agglomerates, which compromise drug efficacy (Sully et al., 2021). Standardization in nanomedicine is necessary, particularly in assessing drug loading/release, immune system interactions, and biodistribution (Sully et al., 2021).

Aggregation occurs due to interparticle collisions, leading to the formation of aggregates or agglomerates, which compromise drug efficacy (Sully et al., 2021). Standardization in nanomedicine is necessary, particularly in assessing drug loading/release, immune system interactions, and biodistribution (Sully et al., 2021). The safe-by-design (SbD) approach has emerged as a framework to assess potential hazards early in the innovation process, promoting the development of safer nanomedicines (Sully et al., 2021). While NP-MN systems hold great promise,

challenges remain in standardizing manufacturing processes, ensuring consistent drug loading, and addressing variability in skin penetration (Sully et al., 2021). Patient compliance and regulatory approval are critical factors for clinical translation. Further research is needed to optimize MN geometries, enhance mechanical strength, and establish guidelines for large-scale production (Sully et al., 2021).

Designing advanced MN systems that adjust to physiological stimuli is essential to improve therapeutic accuracy (Chen et al., 2020). In short, even though MNs have huge potential, many challenges still exist before they can be used widely in clinics. Overcoming these challenges will require better materials, more efficient manufacturing methods, and safer ways to incorporate and release drugs.

2.5.6 Clinical Trials and Regulatory Considerations of Microneedles in Cancer Therapy

Clinical trials increasingly focus on using MNs for cancer treatment, showing promising results. For example, the University of Pittsburgh is currently running a trial to test an MN applicator loaded with doxorubicin, a standard chemotherapy drug (Seetharam et al., 2020). The goal is to find the correct dose to kill tumour cells effectively while keeping side effects as low as possible (Seetharam et al., 2020). Another study by Skinject Inc. tests DMNs loaded with doxorubicin for treating basal cell carcinoma, a common type of skin cancer (Seetharam et al., 2020). This trial looks at how well the MNs work and their safety for patients (Seetharam et al., 2020).

Other trials have started but are not recruiting new patients, which shows great interest in this area (Seetharam et al., 2020). One notable development is Pulse Biosciences, which has created a technology called Nano-Pulse Stimulation™ (Seetharam et al., 2020). This uses MNs that can carry electric pulses to the target and destroy tumour cells without harming nearby healthy tissue (Seetharam et al., 2020).

This method aims to make cancer treatments more precise and reduce unwanted side effects (Seetharam et al., 2020).

The U.S. FDA has strict rules for getting these new treatments approved to ensure they are safe, effective, and reliable (Seetharam et al., 2020). This is to prove that MNs can deliver drugs at the correct dose every time, are sterile, and can be easily used by both healthcare providers and patients (Seetharam et al., 2020). Regulatory approval also requires comprehensive testing to show that MNs do not cause infections or other complications (Seetharam et al., 2020). This systematic approach aligns with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and World Health Organization (WHO) guidelines, as highlighted in Figure 2.19, which underscores the importance of maintaining MN's physical and chemical stability throughout the regulatory approval process. By addressing these comprehensive quality requirements, regulatory bodies aim to facilitate the safe and effective use of MNs in clinical settings for cancer treatment.

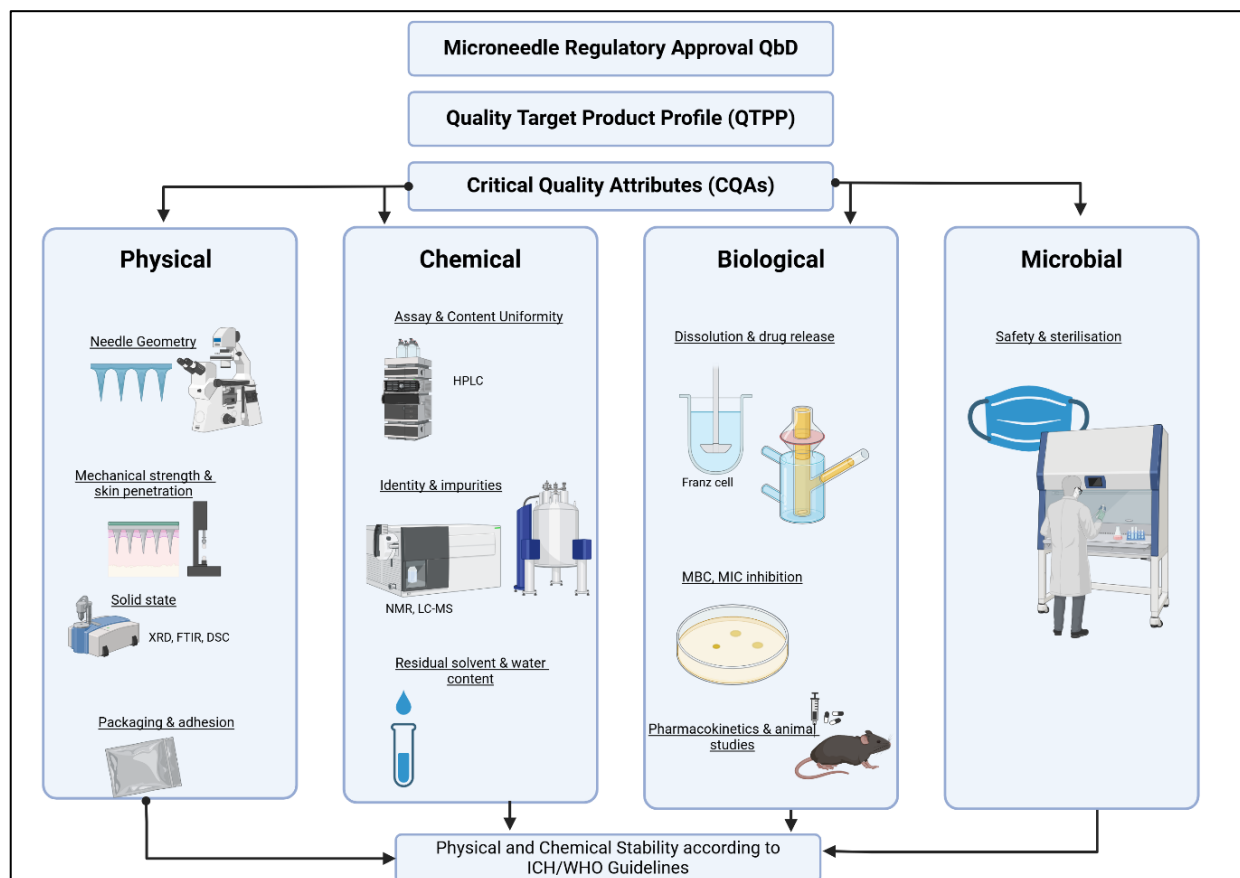


Figure 2.19: Overview of regulatory considerations for microneedles in cancer therapy, highlighting the physical, chemical, biological, and microbial quality attributes required for approval according to ICH/WHO guidelines (Adapted with slight modifications from Sartawi et al., 2023; Created using <https://biorender.com>).

Another challenge is scaling up the production of MNs while keeping costs reasonable and quality high. To get regulatory approval, companies must show that they can make large quantities of MNs without any issues in quality or performance (Seetharam et al., 2020).

Despite the growing interest in MNs for cancer therapy, clinical trials focusing specifically on this application are still limited. Most existing clinical trials for MNs primarily address conditions such as diabetes, influenza, and local anaesthesia (Jamaledin et al., 2020; Avcil and Çelik, 2021). While preclinical studies demonstrate promising results for cancer treatment, including targeted drug delivery and reduced systemic toxicity, few studies have progressed to clinical trials. This gap emphasises the need for comprehensive clinical trials to assess MN's safety, efficacy, and regulatory feasibility in oncology. Addressing these challenges could significantly enhance the quality of life for cancer patients by offering less invasive and more targeted therapeutic options.

Further research and improvements in MN design and manufacturing processes are essential. If these hurdles can be overcome, MNs could become a common way to deliver cancer treatments, offering a less painful and more targeted option compared to traditional methods like IV infusions or oral drugs (Seetharam et al., 2020).

CHAPTER 3

METHODOLOGY

3.1 Overall Flowchart

The experimental design of this study involved four key phases: (1) biosynthesis of Poly (3-hydroxybutyrate-*co*-4-hydroxybutyrate) [P(3HB-*co*-4HB)], (2) fabrication and characterization of docetaxel-loaded nanoparticles (DTX-NPs), (3) optimization and fabrication of dissolving microneedles (DMNs), and (4) evaluation of the microneedles (MNs) incorporated with docetaxel-loaded nanoparticles (NP-MNs) as illustrated in Figure 3.1.

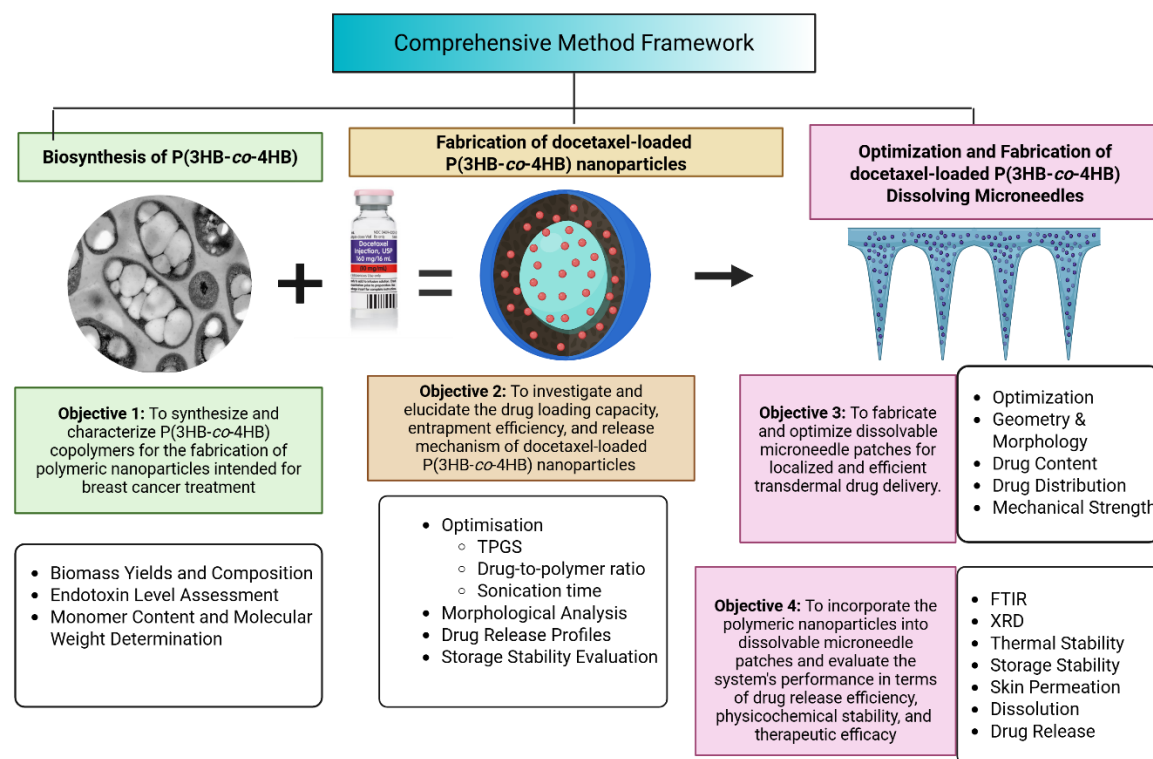


Figure 3.1: Comprehensive Method Framework of this study (Created in <https://BioRender.com>).

In the first phase, P(3HB-*co*-4HB) was biosynthesized using a modified single-stage cultivation process using recombinant strain of *Cupriavidus malaysiensis* USMAA1020, which possessed an excess copy of the PHA synthase (*phaC*) gene from *C. malaysiensis* USMAA2-4. The polymer was then characterized for its purity, molecular composition, thermal properties, and endotoxin levels to ensure its suitability for biomedical applications. The second phase involved fabricating and characterising DTX-NPs through solvent evaporation. Various parameters, including drug-to-polymer ratio, emulsification conditions, and stabilizers, were optimized to achieve stable nanoparticles (NPs) with high encapsulation efficiency, controlled drug release, and favourable physicochemical properties. Key parameters such as particle size, polydispersity index (PDI), Zeta potential (ZP), and drug loading (DL) capacity were analyzed.

Additionally, the drug release profile was evaluated under physiological conditions to determine the sustained release behaviour of the NPs. In the third phase, dissolving microneedles (DMNs) were fabricated and optimized using a mould-based stepwise centrifugation method. The mechanical properties of the MNs were assessed by evaluating fracture force, insertion capability, and penetration efficiency in agarose gel, ensuring they could effectively pierce the skin and deliver the encapsulated drug. The final phase focused on the evaluation of the microneedle-nanoparticle system (NP-MNs). The stability of the combined system was analysed by monitoring particle integrity, mechanical strength, and drug release kinetics over time. Drug release efficiency and therapeutic potential were assessed to determine the suitability of the MN system for localised transdermal chemotherapy in breast cancer (BC) treatment.

3.2 Materials

The chemicals, solvents, consumables, instruments, kits, standards, and microorganisms used throughout this study are summarised in Appendix 1-5.

3.3 Formulation of Media and Buffer for P(3HB-co-4HB) Synthesis

3.3.1 Kanamycin Solution Preparation for Plasmid Stability

To ensure plasmid stability during the cultivation of recombinant *C.malaysiensis*, kanamycin was incorporated into the medium to inhibit non-transformed cells selectively. A kanamycin solution (50 mg/mL) was prepared by dissolving 50 mg of kanamycin powder in 1 mL of sterile distilled water (Amirul et al., 2008; Rozina, 2022). The solution was then filtered through a 0.22 µm nylon filter for sterilization and stored at 4°C for future use.

3.3.2 Preparation of Nutrient-Rich (NR) Broth and Nutrient Agar (NA)

In this study, the NR broth served as the cultivation medium for growing and propagating the recombinant *C.malaysiensis*, which was then used for seed culture and the preparation of glycerol stocks (Amirul et al., 2008; Rozina, 2022). Meanwhile, Nutrient Agar (NA) provided essential nutrients for microorganism growth and was used to isolate single, pure colonies of the *C.malaysiensis* (Amirul et al., 2008; Rozina, 2022). For NA preparation, 2 g/L of yeast extract, 5 g/L of bacteriological peptone, 1 g/L of beef extract (Lab-Lemco powder), 5 g/L of sodium chloride (NaCl), and 15 g/L of agar were added, sterilized, and aseptically poured into Petri dishes. After the agar solidified, the Petri dishes were sealed with parafilm and kept for later use. The NR broth was prepared by dissolving 10 g/L of yeast extract, 2 g/L of bacteriological

peptone, and 10 g/L of beef extract (Lab-Lemco powder) in 1 litre of distilled water. This solution was then transferred to a 250 mL conical flask, filled to 50 mL, and autoclaved (TOMY SX-500, Japan) at 121°C for 20 minutes. After cooling, a filter-sterilized kanamycin solution was added to the NR broth and NA medium to achieve a final 50 µg/mL concentration in each medium.

3.3.3 Preparation of Mineral Salts Medium (MSM) Agar

The Mineral Salts Medium (MSM) agar used for selective growth of the recombinant strain *C.malaysiensis* was prepared by dissolving 3.7 g/L of monopotassium phosphate (KH_2PO_4), 5.8 g/L of dipotassium hydrogen phosphate (K_2HPO_4), and 1.1 g/L of ammonium acetate ($\text{CH}_3\text{COONH}_4$) in distilled water. To this mixture, 10.0 mL of 0.1M magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) and 1.0 mL of a separately prepared trace element solution were added, and the volume was adjusted to 1 L. The solution was then autoclaved for sterilization. After sterilization, 15 g/L of agar was incorporated to solidify the MSM agar. The solution was cooled to approximately 50°C before the trace element solution was aseptically added and thoroughly mixed to prevent degradation of the trace elements. Finally, the prepared MSM agar was poured into sterile Petri dishes and stored at 4°C once solidified until further use. The trace element solution was prepared as outlined in Table 3.1 by dissolving various components in 1 L of 0.1M hydrochloric acid (HCl).

Table 3.1 List of materials required for the preparation of trace elements (Amirul et al., 2008).

Materials	Amount (g/L)
$\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$	2.78
$\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$	2.81
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	1.98
$\text{CaCl}_2 \cdot 4\text{H}_2\text{O}$	1.67
$\text{CuCl}_2 \cdot 4\text{H}_2\text{O}$	0.17
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.29

3.4 Bacterial Strain Culturing, Activation, and Maintenance

Cupriavidus malaysiensis cultures were grown in NR broth and mixed with 40% (v/v) glycerol to create glycerol stocks for long-term storage (Amirul et al., 2008; Rozina, 2022). The mixture was stored in sterile cryovials at -20°C for future use. The bacterium was streaked onto NA plates for culture maintenance and incubated at 30°C for 24 to 48 hours using an incubator (Mettler IN110, Germany). The glycerol stock was prepared by combining the bacterium during its log phase with 40% glycerol, with 1 mL of this mixture stored in a sterile 1.5 mL tube at -20°C for long-term preservation.

The bacterial strain was streaked from the glycerol stock onto MSM agar and incubated at 30°C for 48 hours to promote selective growth. A single colony was then transferred to a nutrient agar plate to provide a more enriched environment for bacterial recovery.

3.5 Seed Culture Preparation

Seed culture uses actively growing bacterial cells in the exponential (log) phase to initiate the main culture. Following incubation, a single colony from the NA plate was picked using a sterile inoculation loop and transferred into 30 mL of NR broth. The culture was then incubated at 30°C with continuous shaking on an orbital shaker (New Brunswick Scientific, USA) at 200 rpm for 12-18 hours, ensuring that the culture reached the exponential growth phase (Amirul et al., 2008; Rozina, 2022). This step ensured the bacteria were actively growing and ready for further experiments.

Once the seed culture reached the exponential growth phase with a biomass concentration of approximately 0.1 g/L (measured by absorbance at 540 nm, OD₅₄₀),

2% of the culture (4 mL from the 30 mL bacterial culture in NR broth) was aseptically transferred into 200 mL of fresh NR broth containing kanamycin. This procedure was repeated across four separate inoculations (i.e., four flasks containing 200 mL each), resulting in a total inoculum volume of 800 mL. The NR broths were incubated for 12 hours at 30°C on an orbital shaker set to 200 rpm. Afterwards, the cultures continued to incubate in an orbital shaker at 30°C, 200 rpm for sustained growth of the bacteria.

Bacterial growth was monitored by measuring the optical density (OD) at 540 nm using a spectrophotometer (Thermo Fisher Scientific, USA) (Amirul et al., 2008; Rozina, 2022). If the OD exceeded 0.5, the culture was diluted with distilled water to bring the reading within the desired range.

3.6 Carbon Source Calculation and Medium Preparation

The total carbon concentration in the biosynthesis medium was adjusted to 0.75 wt%. The carbon sources used were 1,4-butanediol (H1,4) and 1,6-hexanediol (L1,6), combined in a 5:1 ratio (Huong et al., 2018). This specific ratio has been identified as an effective combination for copolymer production during batch cultivation by the transformant strain (Norhafini et al., 2017; Norhafini et al., 2019). The mass of each carbon source was determined using the following formula and calculations.

$$\text{Formula: } \frac{\text{No. of carbon} \times \text{molar mass carbon}}{\text{Molar mass carbon source}} \times xg = \text{wt}\%C \quad (1)$$

$$H_{1,4} + L_{1,6} = 0.75\text{wt}\%C$$

$$Ratio = 5:1$$

$$H_{1,4} \left(\frac{5}{6} \right) \times 0.75 = 0.625wt\%C$$

$$\frac{4 \times 12}{90.122} \times xg = 0.625wt\%C$$

$$xg = 1.1735 \text{ in } 100mL$$

$$\sim 93.88g \text{ in } 8L$$

$$Density(p) = \frac{m}{V}$$

$$1.02gcm^{-3} = \frac{93.88}{V}$$

$$V = 92.04mL$$

$$L_{1,6} \left(\frac{1}{6} \right) \times 0.75 = 0.125 \text{wt}\%C$$

$$\frac{6 \times 12}{118.17} \times xg = 0.125 \text{wt}\%C$$

$$0.6093xg = 0.125 \text{wt}\%C$$

$$xg = 0.2052 \text{ in } 100 \text{mL}$$

$$\sim 16.416g \text{ in } 8L$$

P(3HB-*co*-4HB) copolymer was synthesized using a mineral medium (MM) that excludes a nitrogen source. The components required to prepare 1 L of MM are divided into two groups: A and B. Table 3.2 below presents the quantities of each component, calculated and prepared for introduction into the bioreactor (INFORS, Switzerland). All components in group A were autoclaved separately at 121°C for 20 minutes, except for kanamycin, which was aseptically added after sterilization. Component B was prepared and sterilized directly within the bioreactor.

Table 3.2: Materials and amount in components A and B used in the biosynthesis of P(3HB-*co*-4HB) copolymer (Amirul *et al.*, 2008).

Components		Quantity /Amount (per L)
Component A	Magnesium sulphate heptahydrate, MgSO ₄ ·7H ₂ O (0.1M)	8 mL
	Trace elements	8 mL
	Kanamycin	8 mL
	1,4-butanediol	92 mL
	Dipotassium phosphate (K ₂ HPO ₄)	46.4 g
Component B	Monopotassium phosphate (KH ₂ PO ₄)	29.6 g
	Ammonium sulphate (NH ₄) ₂ SO ₄	8.8 g
	1,6- Hexanediol	16 g

3.7 Biosynthesis of P(3HB-*co*-4HB) Copolymer

The biosynthesis of P(3HB-*co*-4HB) was performed following the method described by Vigneswari et al. (2012). A 2% (v/v) of the *C. malaysiensis* seed culture was cultivated in 1 L of NR broth within a 15L fermenter. The mineral medium, carbon sources, trace elements, and 0.1M MgSO₄·7H₂O were added to achieve a total volume of 8 L. The bioreactor was sterilized following standard procedures, ensuring proper calibration and securing probes, ports, and components. The bioreactor pressure was maintained below 1.5 bar during sterilization and below 2 bar during fermentation, with the condenser water turned on only during fermentation. The stirring speed was set to 100 rpm throughout the process. The PO₂ probe was calibrated at 0% during and 100% after sterilization. Inoculation was done using a transfer bottle and a peristaltic pump (Watson-Marlow 323, UK), with continuous stirring and aeration maintained throughout the inoculation phase.

Bacterial growth was monitored by sampling every 24 hours and measuring the OD at 540 nm. If the OD exceeded 0.5, the culture was diluted with distilled water to maintain the desired optical range. A standard curve for bacterial growth was previously established to correlate OD with cell dry weight. This allows for continuous monitoring of bacterial growth during the fermentation process.

Following 96 hours of fermentation, the culture was collected by transferring it into sterile 50 mL Falcon tubes. The cells were then centrifuged (Eppendorf, Germany) at 10,000 rpm for 10 minutes at 4°C. After centrifugation, the supernatant was discarded, and the resulting cell pellets were kept at -20°C or lyophilized using a freeze dryer (Labconco Corporation, Kansas City, Missouri, US) for further extraction. For gas chromatography (GC) analysis (Agilent Technologies, USA), the pellets were washed in sterile distilled water and centrifuged repeatedly to remove any remaining media components.

To extract the P(3HB-*co*-4HB) polymer, 1.0 g of freeze-dried cells were stirred in 200 mL of chloroform at room temperature for 24 to 72 hours. The mixture was then filtered to eliminate cell debris, and the chloroform phase was concentrated using

rotary evaporation (EYELA, Japan) at 58°C until the volume was reduced to approximately 10–15 mL. The concentrated solution was gradually added, dropwise, into 150 mL of chilled methanol while stirring continuously. The polymer was allowed to precipitate and subsequently filtered through a 0.45 µm PTFE membrane. Finally, the recovered polymer was air-dried at room temperature until it reached a constant weight. A schematic of the extraction process is shown in Figure 3.2.

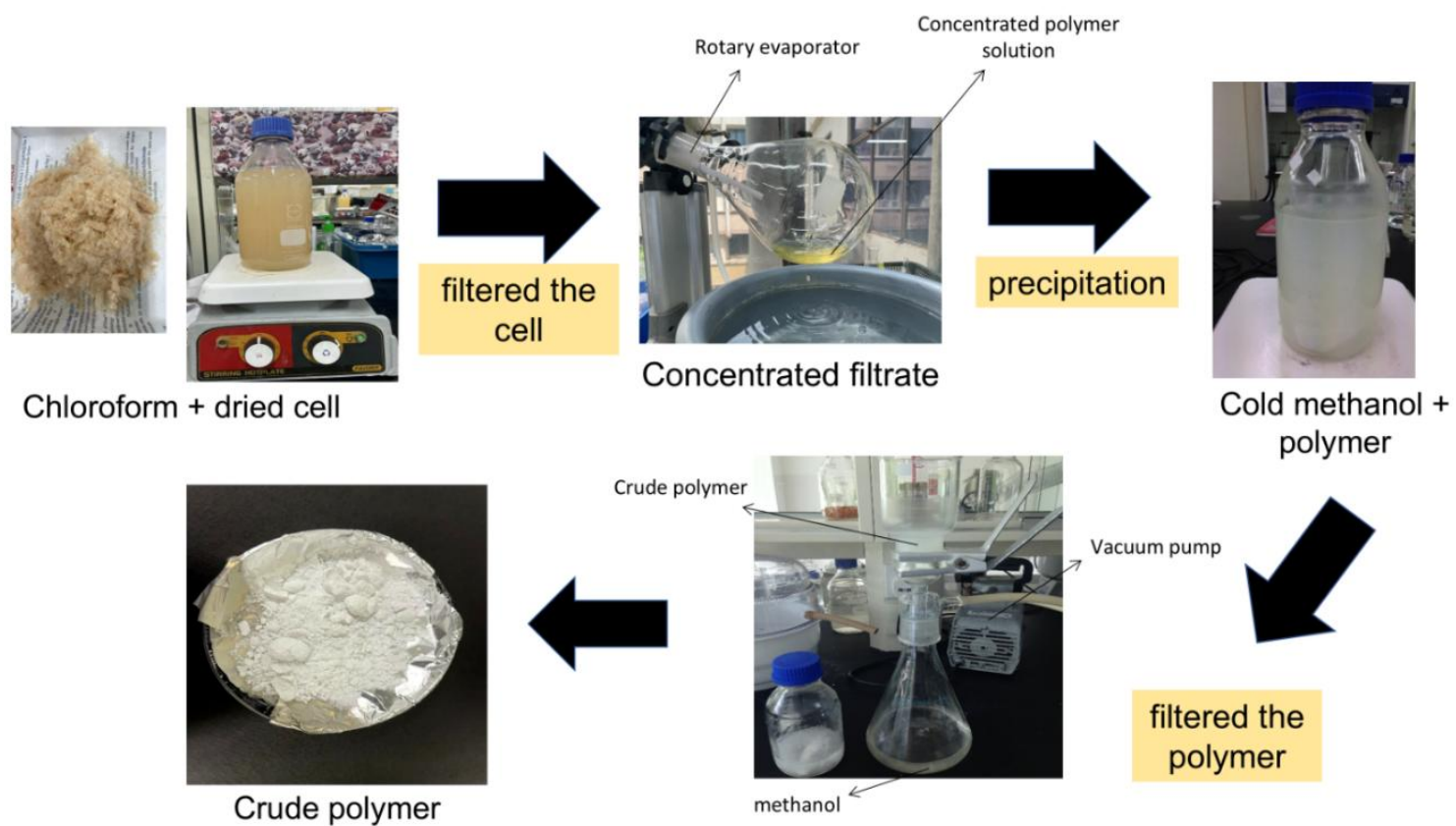


Figure 3.2: Visual workflow showing the steps involved in the extraction of crude P(3HB-*co*-4HB) polymer from the lyophilised cell.

3.8 Endotoxin Removal and Quantification

Endotoxin removal is essential for the safe application of polymers in biomedical fields. The procedure was carried out as described by Rao et al. (2010) and Vigneswari et al. (2015). Briefly, 2 g of crude polymer was dissolved in 100 mL of 2% (w/v) chloroform and heated to 30–40°C. Hydrogen peroxide (H₂O₂) was added in three separate aliquots (110 µL/g) at 20-minute intervals. The solution was then allowed to cool, and chloroform was evaporated. Methanol was added, stirring the mixture overnight to precipitate the polymer. The resulting product was filtered through a 0.45 µm PTFE membrane, dried, weighed, and stored in a dark, airtight container to prevent degradation.

Endotoxin quantification was performed using the Limulus Amebocyte Lysate (LAL) assay (E-TOXATE™, Sigma-Aldrich). All glassware and plasticware were rinsed thoroughly, autoclaved at 121°C for 1 hour, and heated at 175°C for 3 hours using a dry heat oven (Mettler UFB400, Germany) to eliminate endotoxins. The LAL reagent was freshly reconstituted with endotoxin-free water and kept on ice. Endotoxin standards ranging from 4000 to 3.125 EU/mL were prepared through serial dilutions.

For the assay, 0.1 mL of each test sample or standard was added to sterile test tubes with 0.1 mL of LAL reagent. Tubes were incubated at 37°C for 1 hour, and gel formation was assessed by gently inverting the tubes. Positive results were identified by a firm gel, and endotoxin levels were determined by comparing samples to the lowest positive standard dilution. The assay sensitivity allowed the detection of endotoxin concentrations as low as 3.125 EU/mL.

3.9 Characterization of P(3HB-co-4HB) Polymer

3.9.1 Polyhydroxyalkanoate (PHA) Content and Monomer Composition

Methanolysis was conducted as a preparatory step before GC analysis to analyze the composition of PHA. The methanolysis solution was prepared based on the method outlined by Amirul et al. (2008) and Rozina (2022). The solution was formulated by combining methanol with concentrated sulfuric acid in an 85:15 (v/v) ratio. For the preparation, 85 mL of methanol was poured into a 100 mL Schott Duran bottle and then cooled in an ice bath. Subsequently, 15 mL of concentrated sulfuric acid was added drop by drop into the methanol. The prepared solution was kept at 4°C until further use.

A sample containing 15 mg of freeze-dried cells and 8 mg of crude polymer was mixed with 2 mL of chloroform and 2 mL of methanolysis solution. The mixture was heated at 100°C for 2 hours and 20 minutes using a dry heating block (Digisystem Laboratory Instruments Inc., Taiwan). The tube was gently tapped at 20-minute intervals throughout heating to promote phase separation and maintain homogeneity.

After the heating process, the mixture was brought back to room temperature. Then, 1 mL of distilled water was introduced, and the tube was agitated for 20 to 30 seconds. The mixture was then left undisturbed overnight, forming two distinct layers: an upper aqueous layer and a lower organic layer containing the methyl ester form of the polymer. The organic phase was carefully transferred into Bijoux bottles containing anhydrous sodium sulfate (Na_2SO_4) to eliminate any remaining water from the chloroform layer. Subsequently, 0.5 mL of the lower layer was carefully transferred into clean GC vials containing 0.5 mL of caprylic methyl ester (CME) solution.

The prepared samples were injected into a GC system and carried through a separation column by nitrogen gas. The separation was monitored, and the 3HB and

4HB monomers were detected as they exited the column. A reference sample with a known concentration was used as a blank to ensure the accuracy of the analysis.

The composition of the P(3HB-*co*-4HB) copolymer produced by the bacterial strain was quantified by GC analysis, as per the conditions outlined in Table 3.3. Approximately 10 μ L sample was injected into the GC injector port using a micro syringe. The PHA content, concentration, and molar fractions of 3HB and 4HB were then calculated from the chromatographic data.

The GC system used in this analysis was a Shimadzu GC-2014, equipped with an SBP-1 column (30 m x 0.25 mm x 0.25 μ m film thickness), an AOC-20I auto-injector, and a Flame Ionization Detector (FID). Nitrogen was used as the carrier gas, and both the injection and analysis were automated to ensure precision and consistency in the results. This approach allowed for the accurate quantification of the PHA content and the determination of the monomer composition of the copolymer, providing insights into the P(3HB-*co*-4HB) biosynthesis process.

Table 3.3: Parameters for PHA analysis in GC

Operating Parameters	Settings
Injector temperature	260 °C
Detector temperature	260 °C
Initial column temperature	50 °C
Final column temperature	250 °C
Pressure	92.0 kPa
Total flow	27.8 mL/minute
Hydrogen flow	40 mL/minute
Airflow	400 mL/minute
Total analysis time	14.33 minutes

Calculations:

$$PHA \text{ content (wt\%C)}: \frac{K[(Area \text{ of } 3HB) + k(Area \text{ of } 4HB)]}{(Area \text{ of } IS)/(Weight \text{ of freeze dried cell (mg)})} \times 100 \quad (2)$$

PHA concentration $\left(\frac{g}{L}\right)$:

$$\frac{Cell \text{ dry weight } \frac{g}{L}}{[K(Area \text{ of } 3HB) + k(Area \text{ of } 4HB)] / [(Area \text{ of } IS) / (Weight \text{ of freeze dried cell (mg)})]}$$

$$4HB \text{ molar fraction (\%)}: \frac{k(Area \text{ of } 4HB)}{Area \text{ of } 3HB}$$

$$3HB \text{ molar fraction (\%)}: 100 - (4HB\%)$$

K: Partition coefficient

k: Retention factor or capacity factor

IS: Internal standard (CME)

The retention times of the peaks identified in the chromatogram were as follows:

3HB methyl ester: 4.1 minutes

4HB methyl ester: 5.5 minutes

Caprylic methyl ester (CME):10.05 minutes

3.9.2 Molecular Weight Determination

The Gel Permeation Chromatography (GPC) (Agilent Technologies, USA) method, used to determine the molecular weight of the polymer, followed the procedure described by Syafiq et al. (2017) with some modifications. Initially, scintillation bottles and GC vials were meticulously cleaned by soaking them in chloroform of high-performance liquid chromatography (HPLC) grade and then rinsing and drying them in an oven to remove contaminants. Approximately 5 mg of crude polymer was dissolved in 1 mL of chloroform in the cleaned scintillation bottles to prepare the samples. The bottles were tightly sealed and covered with aluminium foil to prevent light degradation. The polymer solution was placed on an orbital shaker overnight to ensure complete dissolution.

Before injecting the solution into the GPC system, glass syringes were rinsed with HPLC-grade chloroform to ensure cleanliness. The polymer solution was drawn into the syringe, and the needle was removed and replaced with a 0.45 μm nylon filter. After reattaching the needle, 1 mL of the filtered solution was transferred into clean GC vials for analysis. The GPC system used a refractive index detector (RID) and a PLgel 5 μm MIXED-C column, with HPLC-grade chloroform as the mobile phase at a 1.0 mL/min flow rate. The column temperature was maintained at 35°C throughout the analysis. The sample (1 mL) was injected, and the number-average molecular weight (M_n), weight-average molecular weight (M_w), and polydispersity index (PDI, M_w/M_n) were calculated using Clarity chromatography software (Version 2.4). A

calibration curve was constructed using polystyrene standards with low PDI to ensure accuracy.

3.10 Fabrication and Optimization of Docetaxel-Loaded P(3HB-*co*-4HB) Nanoparticles

NPs were synthesized via the solvent evaporation technique, as illustrated in Figure 3.3. A solution containing 50 mg of P(3HB-*co*-4HB) and docetaxel (DTX) was prepared by dissolving in 10 mL of dichloromethane at a concentration of 0.5% (w/v) (Faisalina et al., 2020; Fisol et al., 2022). This organic phase was gradually introduced into the aqueous phase, which contained 1.0% (w/v) polyvinyl alcohol (PVA) and 0.03% (w/v) D- α -tocopheryl polyethylene glycol succinate (TPGS). Sonication was performed for 1 minute at 50–60 Hz using a probe sonicator (Qsonica, United States), and the mixture was kept in an ice bath to avoid overheating the polymer. The solution was magnetically stirred for 4 hours to facilitate the evaporation of the organic solvent. Blank NPs were produced following the same procedure, except that the drug was omitted. The NP suspension was centrifuged at 10,000 rpm and washed three times with distilled water to remove excess PVA. The purified NPs were then frozen and lyophilized. Finally, the lyophilized NPs were stored in a desiccator for future use.

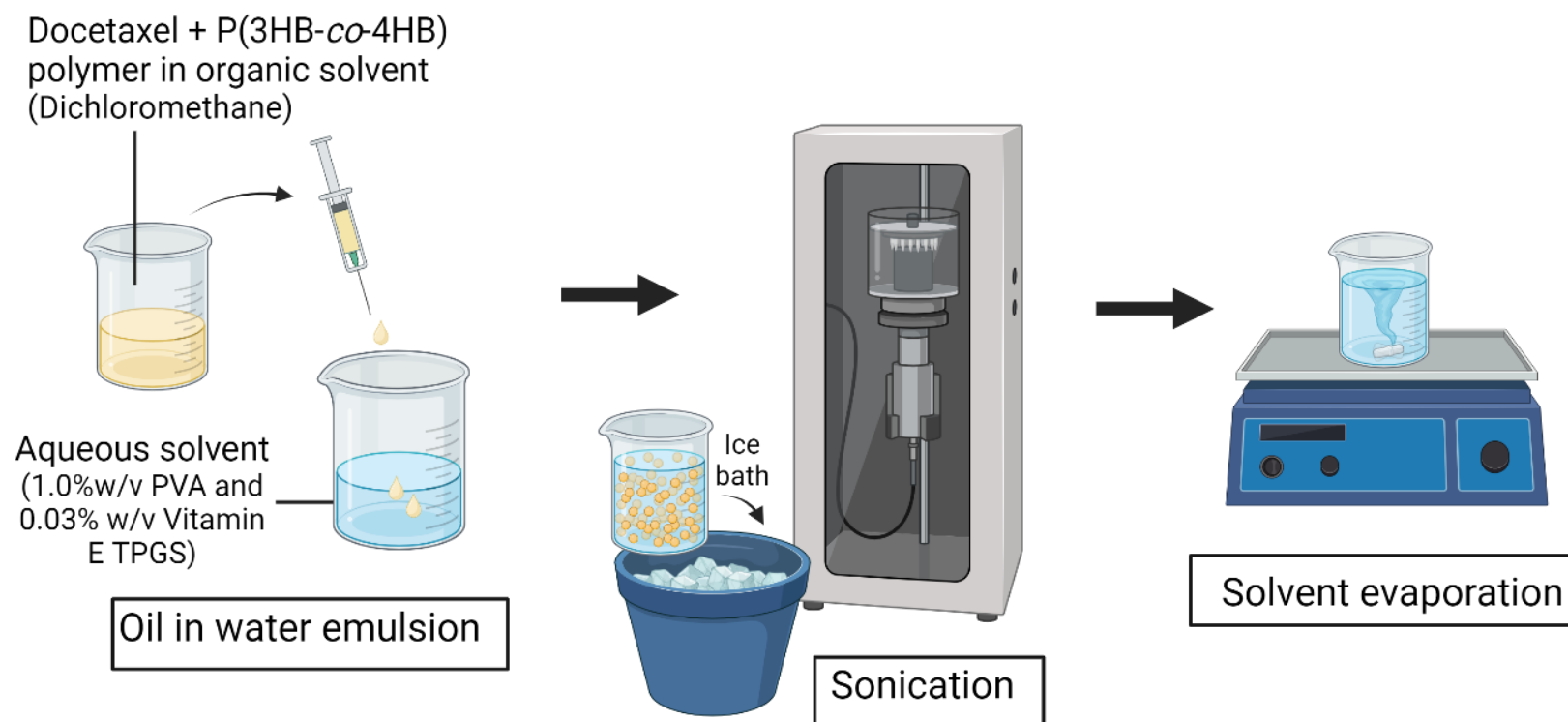


Figure 3.3: Schematic illustration of the step-by-step process of fabricating docetaxel-loaded P(3HB-co-4HB) nanoparticles (Created in <https://BioRender.com>).

Table 3.4 below summarises the parameters tested during the formulation process, including TPGS, evaporation time, drug-to-polymer (D:P, % w/w), and sonication time. The drug-to-polymer ratio is expressed as a percentage, representing the weight of the drug to the polymer (e.g., 40% indicates 20 mg of the drug in 50 mg of polymer). These parameters were systematically varied to assess their impact on NP stability, particle size, and overall formulation quality.

Table 3.4: Summary of the different parameters tested during the formulation and optimisation process of P(3HB-*co*-4HB) nanoparticles.

Parameters	Tested conditions	Details
Presence of TPGS	TPGS included, and TPGS excluded	Formulations were compared to evaluate the effect of surfactant on stability and particle size.
Evaporation time	4 hours and 12 hours	The effect of evaporation time on particle stability was tested for both with and without TPGS.
Drug-to-polymer ratio	5 different ranges (0%, 5%, 10%, 20%, 30%,40%)	Various drug-to-polymer ratios were tested to optimise the formulation.
Sonication time	1 and 2 minutes	Different sonication times were applied to determine the impact on particle size and distribution.

3.11 Characterization of Nanoparticles

3.11.1 Particle Size and Polydispersity Index (PDI) Measurement

Particle size and polydispersity index (PDI) were assessed using dynamic light scattering (DLS), utilizing a 633 nm laser at a 90-degree angle, performed using a Zetasizer (Malvern Instruments, United Kingdom) (Faisalina et al., 2020; Fisol, 2022). This technique analyzes the Brownian motion of particles, converting the data into size and distribution values using the Stokes-Einstein equation as shown below:

$$D = \frac{kBT}{6\pi\eta r} \quad (3)$$

Where:

D = diffusion coefficient (m²/s)

kB = Boltzmann constant (1.380649×10⁻²³ J/K)

T = absolute temperature (Kelvin)

η = dynamic viscosity of the fluid (Pa·s)

r = hydrodynamic radius of the particle (m)

π = mathematical constant (~3.1416)

Around 1 mL sample of the NP solution was transferred into a sterile microcentrifuge tube. From this, 1-2 drops were carefully pipetted into a clean cuvette, with distilled water added to adjust the volume for analysis. Cumulant analysis was used to calculate the Z-average size, which provided two key parameters: the mean particle size and the PDI. The intensity-weighted mean size was obtained from the signal intensity. PDI values range from 0 to 1, with lower values (0-0.2) indicating a narrow size distribution and higher values (above 0.2) suggesting a wider distribution or distinct particle populations. All measurements were repeated in triplicate to ensure consistency and accuracy.

3.11.2 Zeta Potential (ZP) Analysis

The ZP of the NPs was measured to assess their stability in the colloidal state using a Litesizer (Anton Paar, Austria) (Faisalina et al., 2020; Fisol, 2022). This was accomplished by evaluating the electrophoretic mobility of the particles, which was then combined with the Henry equation as shown below:

$$P = \frac{C}{k_H} \quad (4)$$

Where:

C = concentration of the gas in the liquid (mol/L or mol/m³)

k_H = Henry's Law constant (depends on gas-solvent pair and temperature; units vary, e.g., mol/(L·atm) or mol/(m³·Pa)

P = partial pressure of the gas above the liquid (atm or Pa)

Electrophoresis was performed to measure the particle velocities using Laser Doppler Velocimetry (LDV). The velocity of particles with a ZP moves toward the oppositely charged electrode, and this movement is directly proportional to their ZP. The experimental setup included a dispersant refractive index of 1.330, viscosity of 0.8871, dielectric constant of 78.5, and a temperature of 25°C. Twelve replicate measurements were taken. The samples were suspended in deionized water and injected carefully into a folded capillary cell using a syringe for analysis with the Zetasizer. The cell was filled slowly to avoid air bubbles. After filling the cell, it was placed in a holder, and the lid was closed to start the measurement. Each measurement was repeated three times to ensure the consistency and reliability of the results.

3.11.3 Surface Morphology Analysis Using Scanning Electron Microscopy and Transmission Electron Microscopy

The surface morphology of the NPs was analyzed using a Scanning Electron Microscope (SEM) (JEOL model JSM-360LA (JEOL Malaysia Sdn Bhd, Model No: JSM-360LA, Serial No: MP18700061), Japan) coupled with an energy-dispersive X-ray (EDX) detector, operating at an acceleration voltage of 2500 volts (Fisol, 2022). Before scanning, the freeze-dried NP powders were mounted onto carbon tape and coated with 15 nm platinum using a sputter coater (Novaclic Scientific, Malaysia). Additionally, Transmission Electron Microscopy (TEM) analysis was conducted (JEOL JEM-2100, JEOL Ltd., Tokyo, Japan). For TEM preparation, a drop of the NP suspension was placed on a copper grid and allowed to dry under ambient conditions. The grid was then examined under the TEM to capture high-resolution images for detailed analysis.

3.11.4 Drug Loading Efficiency

The drug loading (DL) of DTX-NPs was determined through HPLC (LC-20A HT, Shimadzu Corporation, Japan). The UV detector was set to 230 nm, with the mobile phase consisting of a 50:50 mixture of acetonitrile and phosphate buffer (0.02M, pH 2) and a flow rate of 0.5 mL/min (Fisol, 2022). The column was maintained at 30°C, and the injection volume was 10 µL. Known concentrations of DTX were utilized as external standards, and the total analysis time was 10 minutes. The DTX retention time was recorded as 6.3 minutes, and the prepared solutions were stored in acetonitrile under refrigerated conditions. Calibration curves were established within a concentration range of 1–200 µg/mL ($r^2 > 0.999$).

DL was determined using both direct and indirect techniques. For the direct technique, the sample was initially processed by centrifugation at 15,000 rpm for 30 minutes at 4°C to collect the drug-loaded polymer as a pellet. The supernatant was

discarded, and the pellet was washed three times with distilled water to remove any residual free drug. In each washing step, the pellet was resuspended in 1 mL of distilled water and centrifuged under identical conditions. After the final wash, the pellet was subjected to freeze-drying. Approximately 1 mg of the freeze-dried NPs was dissolved in 0.5 mL of acetonitrile and 0.5 mL of distilled water to disrupt the polymer structure and release the encapsulated drug. The mixture was briefly vortexed (IKA Works, Germany) and centrifuged at 10,000 rpm for 15 minutes at 4°C. The resulting supernatant containing the released DTX was collected, filtered through a 0.22 µm syringe filter, and analysed via HPLC. Each evaluation was performed in triplicate. The DL and encapsulation efficiency (EE) of DTX were calculated using the specified formula.

In the indirect approach, the amount of unencapsulated drug was quantified. After the evaporation step in NP preparation, 1 mL of the NP suspension was collected and centrifuged at 10,000 rpm for 15 minutes. Subsequently, 0.5 mL of the supernatant was combined with 0.5 mL of acetonitrile and centrifuged again to precipitate the PVA. The supernatant containing the dissolved DTX was collected and analysed via HPLC.

$$\begin{aligned} \text{Drug loading, DL (\%)} & \quad (5) \\ &= \frac{\text{Total amount of drug} - \text{Free amount of drug}}{\text{Weight of the nanoparticles}} \\ & \times 100 \end{aligned}$$

$$\begin{aligned} \text{Free drug (FD)} & \\ &= \frac{\text{Concentration} \times \text{Total volume} \times \text{Dilution factor}}{1000} \end{aligned}$$

Concentration is the x value from the $Y = mx + C$

$$Y = \text{Area under curve value}$$

m and C are taken from trendline equation in standard curve

Encapsulation efficiency, EE (%)

$$= \frac{\text{Initial amount of drug} - \text{Free drug}}{\text{Initial amount of drug}} \times 100$$

3.11.5 Drug Release Studies

An initial solubility assessment of DTX was conducted, considering its limited solubility in water-based solvents to determine the optimal conditions for the release experiments (Fisol, 2022). Around 1 mg of DTX was dissolved in 1 mL of phosphate buffer solution (PBS) as the release medium. The prepared solution was vortexed briefly to achieve a uniform suspension and subsequently placed in an orbital shaker at 37°C and 100 rpm for 48 hours. Upon completion of the incubation period, the mixture underwent centrifugation at 10,000 rpm for 1 hour at ambient temperature. The supernatant obtained was analyzed by HPLC to quantify the solubility of DTX in the release medium. The procedure was repeated in triplicate to ensure consistency in the results.

The release of the drug *in vitro* was evaluated using a dialysis technique based on the procedure described by Fisol (2022). A total of 2.16 mg of DTX-NPs, corresponding to a drug concentration of 5 µg/mL, were introduced into a Regenerated Cellulose Dialysis Membrane containing 15 mL of the nanoparticle suspension. The dialysis tubing was immersed in 50.0 mL of PBS at pH 7.4 to maintain sink conditions and incubated in a shaker at 100 rpm and 37°C. Samples

were collected at specific intervals (0.5, 1, 2, 4, 8, 12, 24, 48, 72, and 96 hours), with 1.0 mL withdrawn from the release medium and replenished with fresh PBS. The withdrawn samples were analyzed using HPLC. A separate experiment was performed to evaluate the release profile of the free drug, using an equivalent dose of DTX as that loaded in the P(3HB-co-4HB) NPs. Each experiment was conducted in triplicate to ensure accuracy and reproducibility.

3.11.6 Stability Studies of Nanoparticles

The stability of the NPs was assessed according to International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines, with the formulations stored at both 4°C and 37°C for 90 days. Standard stability studies typically involve six months of testing at 40°C ± 2°C / 75% RH ± 5% RH for regulatory submissions (González-González et al., 2022). However, since Malaysia is classified under climate zone IVb ("hot and very humid"), long-term stability is often tested at 30°C ± 2°C / 75% RH ± 5% RH (González-González et al., 2022). During the study, particle size, PDI, ZP, and drug content were evaluated at 0, 30, and 60 days. Any significant changes in these parameters were monitored to determine the physical and chemical stability of the formulations.

3.12 Microneedle Optimization and Fabrication

Dissolving microneedles (DMNs) were fabricated using a modified mold-based stepwise centrifugation technique adapted from Peng et al. (2021), as shown in Figure 3.4. The silicone-based MN mould had pyramidal needles measuring 1000 µm in length, arranged in a 6 × 6 array. Each needle featured a base width of 250 µm and a pitch distance of 1500 µm, ensuring consistent spacing for efficient skin penetration. A polymer solution was prepared by dissolving polyvinyl alcohol (PVA) and

polyvinylpyrrolidone (PVP) to achieve final concentrations of 32 mg/mL for PVA and 48 mg/mL for PVP. A 200 μ L volume of this polymer solution was pipetted into the MN mould and processed by centrifugation at 3000 rpm for 10 minutes at 4°C, promoting uniform distribution within the mould cavities.

Before each centrifugation step, the moulds were kept in a desiccator for 30 minutes to ensure proper filling of the cavities and prevent overflow. After the initial centrifugation, any residual polymer solution was removed, and the MNs underwent a second centrifugation at 4000 rpm for 30 minutes to compress the solution at the tips and eliminate excess water. 150 μ L of a PVA (150 mg/mL) and PVP (250 mg/mL) mixture was added to form the MN matrix. The mould was vacuum-degassed for 10 minutes to remove trapped air bubbles, followed by centrifugation at 3000 rpm for 5 minutes. Any remaining solution on the mould surface was discarded, and 250 μ L of PVP solution was added to form the backing layer of the DMNs, followed by final centrifugation at 4000 rpm for 5 minutes.

The mould was dried in a desiccator at ambient temperature for 36 hours. After drying, the DMNs were gently detached from the mould using tweezers and stored inside the desiccator for subsequent use and analysis. The optimization process involved testing different centrifugation speeds and drying times, with images captured after each step to assess the MNs' quality. The optimal MNs exhibited uniform height, base width, and smooth tips, which were confirmed through optical microscope and SEM.

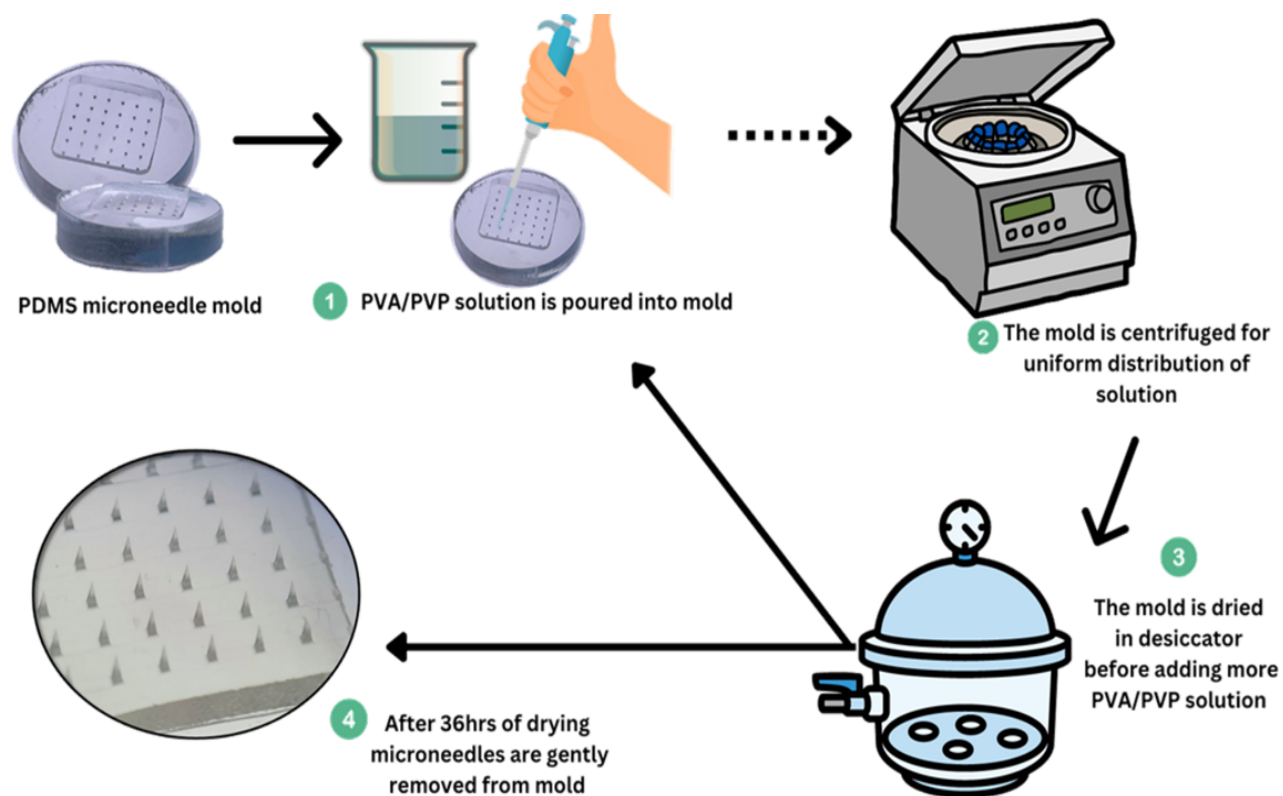


Figure 3.4: Schematic diagram of the steps involved in the fabrication of dissolving microneedles using the stepwise centrifugation method (Created in <https://BioRender.com>).

3.13 Characterization of Microneedles

3.13.1 Optical Microscopy and Scanning Electron Microscopy

The DMN's geometry and surface morphology were analysed using a stereomicroscope (Olympus, Japan) and SEM according to Bhatnagar et al. (2019). The SEM images were captured after sputter-coating the samples with a thin layer of gold to ensure conductivity during imaging. The SEM was operated at an accelerating voltage of 10 kV. MNs were imaged at various magnifications to measure key dimensions, including needle height, base width, and tip sharpness. Additionally, the SEM analysis helped verify the uniformity and smoothness of the MNs' surface, ensuring there were no structural defects.

3.13.2 Drug Content Analysis

The amount of DTX incorporated into the MNs was determined by referring to a previously published study by Bhatnagar et al. (2019), which reported a maximum of 227 ± 23 μg of DTX per MN patch. This study set the total DTX content per patch to deliver approximately 227 μg of the drug across the MN array. The DL and encapsulation efficiency were calculated to adjust the total weight of NPs in each MN patch, ensuring the amount of DTX per patch did not exceed the established dose of 227 μg . The amount of NP powder was calculated using the following equations:

$$\text{Amount of nanoparticle (mg) to be incorporated into microneedle (6)} \\ = \frac{\text{Desired Drug Amount}}{\text{Drug loading (\%)} \times \text{Encapsulation efficiency (\%)}} \quad)$$

After fabricating the DTX-incorporated MN patches, the drug content was determined using HPLC. The MN patches were dissolved in a solvent mixture containing 10 mL of distilled water and 10 mL of acetonitrile to completely dissolve the polymer matrix, facilitating the release of DTX. The solution was gently mixed and centrifuged at 10,000 rpm for 15 minutes to remove undissolved polymer residue. The supernatant containing the dissolved DTX was subsequently analysed to measure the drug content.

The total amount of DTX present in the MN patch was determined using the concentration values obtained through HPLC measurement and the applied dilution factor during dissolution. Given the hydrophobic nature of the NPs, they do not readily dissolve in aqueous solutions. Therefore, various solvents were tested to improve solubility before incorporating them into the MNs. Solvents such as Tween 80, distilled water, PVA/PVP solutions, ethanol, and dimethyl sulfoxide (DMSO) were evaluated. DMSO was the optimal solvent because it produced a clear, homogeneous solution. This DMSO solution was subsequently combined with the PVA/PVP mixture and used to prepare the MN moulds for fabrication.

3.13.3 Drug Distribution Evaluation

The P(3HB-*co*-4HB) NPs encapsulated with coumarin-6 were prepared by replacing DTX with coumarin-6, following the same fabrication procedure, to address the drug's non-fluorescent properties (Xu et al., 2021). These NPs were then incorporated into the DMNs patches using the identical fabrication method. A fluorescent microscope (Leica Microsystems, Germany) was used to visualize the distribution of the NPs within the MNs. The MN patch was carefully cut into smaller sections with a scalpel and arranged in a row-by-row layout on a glass slide to facilitate clear observation.

3.13.4 Mechanical Strength Testing

The mechanical strength of the DMNs was assessed through static force testing and texture analyser, as illustrated in Figure 3.5. In the static force test, weights ranging from 50 g to 1000 g were gently placed on the MN tips for 5 minutes (Dawud and Ammar, 2023). After the designated time, the weights were removed, and the MNs' ability to resist bending or fracture was evaluated. The morphological alterations were examined under a Scanning Electron Microscope, and the acquired images underwent analysis using ImageJ software. The height reduction was quantified by calculating the MN height (μm) change, which was then expressed as the percentage decrease in height, as shown by the equation below (Dawud and Ammar, 2023).

$$\begin{aligned} & \text{Percentage change in microneedle height(\%)} \\ &= \frac{\text{Initial height} - \text{Final height}}{\text{Initial height}} \times 100 \end{aligned} \quad (7)$$

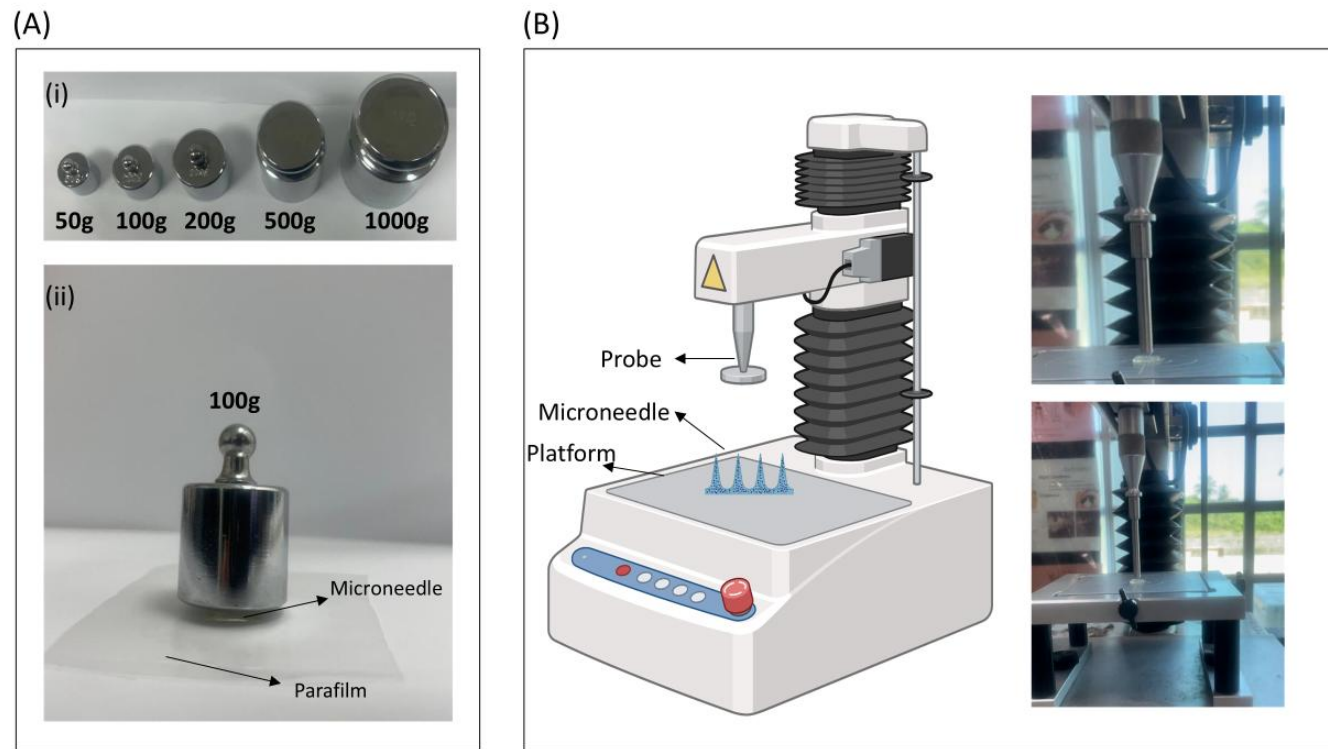


Figure 3.5: (A)(i) Image shows the static forces used for mechanical strength analysis of microneedles in this study, and (ii) shows the static force of 100g placed on a microneedle patch. Image (B) shows the texture analyser used for the mechanical strength analysis of the microneedles.

A texture analyser (Stable Micro Systems, United Kingdom) was utilized to exert a compression force on the DMNs, based on the method described by Yan et al. (2021), with slight modifications. The fracture force of the MNs was carefully measured. For this test, the prepared DMN patches were positioned on an aluminium plate of the texture analyser to prevent direct contact between the probe and the base. A P/5 probe was employed, and the compression mode was selected. The probe was set to move at a speed of 0.1 mm/s with displacement levels of 100, 200, 300, 400, and 500 μm . A trigger force of 0.049 N was used, and the data acquisition rate was fixed at 50. Throughout the compression process, both the probe and the DMNs were maintained in parallel alignment, and the analyser recorded the pressure applied by the probe.

3.13.5 Fourier Transform Infrared (FTIR) Spectroscopy of Nanoparticle-Loaded Microneedles

FTIR analysis was conducted using a spectrometer (Shimadzu Corporation, Japan) equipped with a Diamond ATR holder to examine the chemical structures and potential bonding interactions within the P(3HB-co-4HB), NPs, and NP-MNs (Castilla-Casadio et al., 2022). All samples were scanned across the 4000–400 cm^{-1} wavenumber range, and each spectrum was obtained by averaging 32 scans to enhance the signal-to-noise ratio. The spectra were baseline-corrected and normalised prior to interpretation. Shifts in peak positions, intensity variations, or the appearance of new peaks were assessed to determine whether NP incorporation altered the polymer structure or induced new interactions within the MNs. Comparative evaluation of the spectra enabled a systematic assessment of structural changes and bonding interactions throughout the different formulation stages.

3.13.6 Diffraction (XRD) Analysis of Nanoparticle-Loaded Microneedles

X-ray diffraction (XRD) analysis was performed based on Arshad et al. (2024) with slight modifications to evaluate the crystallinity of the P(3HB-*co*-4HB), DTX-NPs, and NP-MNs. All samples were freeze-dried, finely ground, and mounted onto a glass sample holder for analysis. The measurements were carried out using an XRD (Bruker Corporation, Germany) equipped with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) at an operating voltage of 40 kV and a current of 30 mA. Data were collected over a 2θ range of 5° to 100° , with a scanning speed of 1° per minute. The interplanar spacing (d-spacing) for prominent peaks was calculated using Bragg's Law as shown below:

$$n\lambda = 2d \sin\theta \quad (8)$$

Where:

n is the order of diffraction (taken as 1),

λ is the X-ray wavelength, and

θ is the Bragg angle corresponding to each 2θ value.

The resulting diffractograms were analysed to assess changes in crystallinity and to determine whether NP incorporation influenced the polymer framework. The presence, absence, or shifting of characteristic peaks was used to distinguish between crystalline and amorphous regions and to evaluate structural modifications across the different formulation stages.

3.13.7 Thermal Stability Assessment of Nanoparticle-Loaded Microneedles

Thermal analysis was conducted to evaluate the thermal stability and phase transitions of P(3HB-*co*-4HB), DTX-NPs, and NP-MNs using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC), based on the method by Arshad et al. (2019) with slight modifications. TGA (TA Instruments, USA) was used to assess thermal degradation by heating approximately 5–10 mg of each sample under inert conditions. The P(3HB-*co*-4HB) and DTX-NP samples were analysed under a combined nitrogen and airflow atmosphere, with heating up to 800°C at a rate of 10°C per minute. For the NP-MN samples, heating was performed from 25°C to 600°C at the same rate in an argon atmosphere to minimise oxidation. The resulting thermograms were used to determine thermal decomposition behaviour, including the onset and major stages of weight loss.

For DSC (TA Instruments, USA), 5–6 mg of each sample was sealed in aluminium pans and subjected to programmed heating. The P(3HB-*co*-4HB) samples underwent two heating cycles: first from –50°C to 200°C, followed by cooling to –50°C and reheating to 200°C, enabling the identification of glass transition temperature (T_g), crystallisation temperature (T_c), and melting temperature (T_m). In contrast, the DTX-NP and NP-MN samples were heated from 25°C to 300°C at 10°C per minute under a nitrogen atmosphere. The DSC thermograms were used to observe thermal transitions such as T_g and T_m . Together, these analyses provided a comprehensive understanding of the thermal behaviour and stability of the materials across different formulation stages.

3.13.8 *In Vitro* Skin Permeation Studies

The capability of the MNs to pierce the skin was evaluated using an eight-layer parafilm model, which acted as a skin simulant, following the procedure outlined by Dawud and Ammar (2023). The parafilm sheets were folded into eight layers, each with an approximate thickness of $130 \pm 10 \mu\text{m}$, and arranged flat on a surface. MN patches were manually pressed onto the parafilm layers using consistent pressure applied by thumb for 30 seconds to mimic clinical application conditions (Dawud and Ammar, 2023). After removal, the parafilm was examined under a microscope to evaluate the number of pores created per layer. Additionally, the height of the MNs was measured before and after application and evaluated using SEM, and the collected data were processed with ImageJ software. To further assess the penetration depth and visualize the structural interaction with the skin simulant, Optical Coherence Tomography (OCT) (CIRRUS HD-OCT system, USA) imaging was performed. The OCT analysis provided cross-sectional images to confirm MN insertion depth.

3.13.9 *In Vitro* Solubility Studies

The solubility of the MNs was evaluated *in vitro* using a tumour tissue-mimicking agarose gel, adopting the procedure described by Moreira et al. (2020), as illustrated in Figure 3.6. Agarose was reconstituted in two different concentrations (2% w/v and 6% w/v) with double-distilled water to mimic the texture of normal breast tissue and tumour-like breast tissue, respectively. The prepared agarose solution was heated to 90°C and poured onto six-well plates. MNs were pressed into the agarose gels, and at specific time intervals (5s, 15s, 45s, 120s, and 300s), the MNs were removed and dried in a desiccator. The dried MNs were examined under a SEM for further analysis.

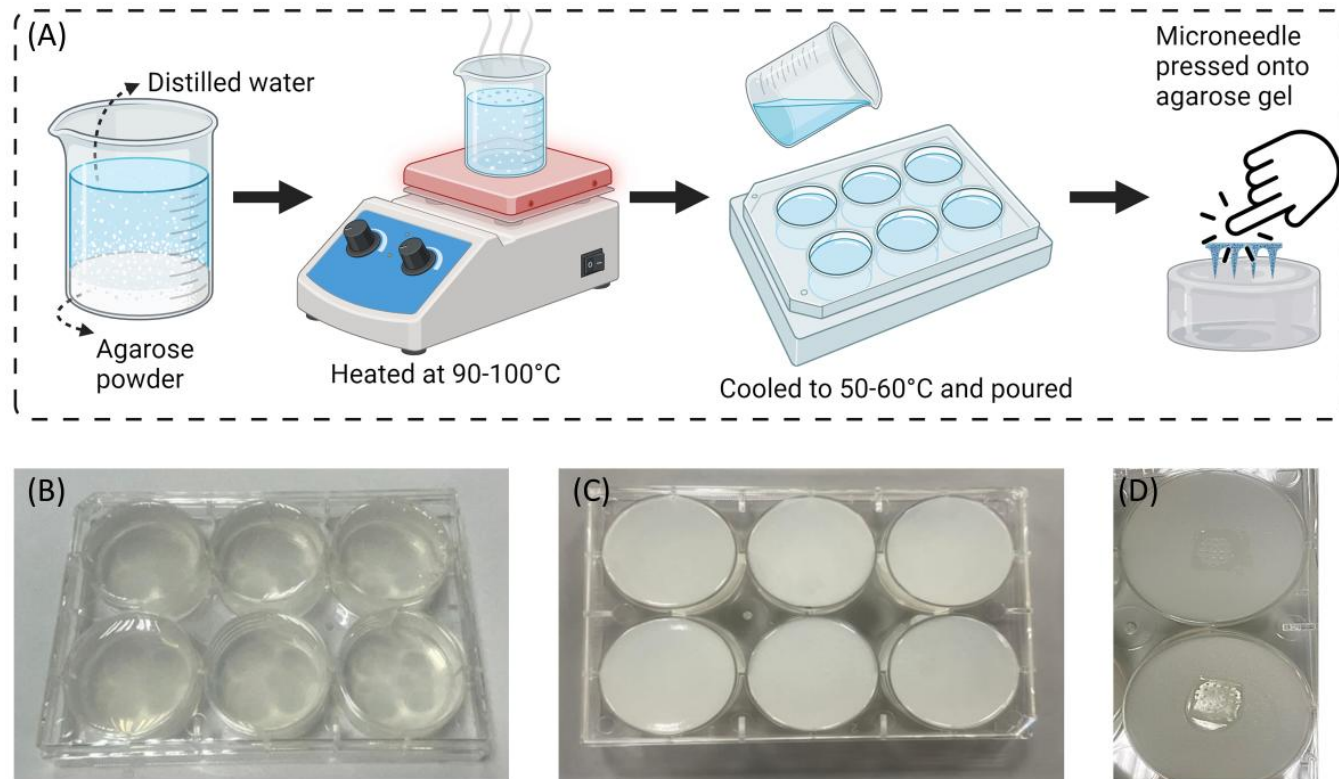


Figure 3.6: Image showing (A) schematic illustration of the steps involved in casting agarose gel involving the solubility analysis of microneedles, (B) 2% agarose gel, (B) 6% agarose gel, and (C) the solubility of penetrated microneedles in 6% agarose gel (Created in <https://BioRender.com>)

3.13.10 Drug Release from Nanoparticle-Loaded Microneedles

The release experiments were performed using a method modified from Larrañeta et al. (2014) and Donnelly et al. (2012a), with minor adjustments to suit DMNs. The MN arrays were pressed into a single Parafilm M® (PF) sheet. The MNs, still embedded in the parafilm, were placed in the release medium, consisting of 30 mL of phosphate buffer solution (PBS, pH 7.4), and maintained at 37°C in an orbital shaker set to 100 rpm. In this setup, only the MN tips penetrated the parafilm layer, while the backing layer remained outside the release medium. At predetermined times, 1 mL of the release medium was withdrawn and replenished with an equivalent volume of PBS to ensure a consistent total volume. DTX-MNs were also included in the study for comparison. All experiments were performed in triplicate.

3.13.11 Storage Stability Evaluation of Nanoparticle-Loaded Microneedles

The stability of the MN patches during storage was assessed by placing them in a desiccator containing silica gel at ambient room temperature (approximately 25–30°C) with 0% relative humidity (RH) for 1 month, based on a modified method by Mistilis et al. (2017). The use of a desiccator helped minimise moisture exposure, which is essential for maintaining the structural and chemical integrity of MNs, particularly under Malaysia's climate, which falls under ICH Climatic Zone IVB (hot and very humid) (ICH, 2022). Although the ICH-recommended long-term storage conditions for Zone IVB are 30°C ± 2°C with 75% ± 5% RH, the desiccator in this study provided a controlled low-humidity environment to prevent moisture-induced degradation. After the storage period, the MNs were analysed for drug content and thermal stability using previously described procedures to evaluate any degradation or changes in physical integrity.

3.14 Statistical Analysis

Statistical analyses were performed using mean $\pm$ standard deviation. GraphPad Prism Version 10 was used for one-way ANOVA, two-way ANOVA, and unpaired t-tests where appropriate. A *p*-value of less than 0.05 was considered statistically significant.

CHAPTER 4

RESULTS AND DISCUSSION

2.5 Biosynthesis and Characterization of P(3HB-*co*-4HB)

4.1.1 Biomass Yields and Composition Analysis

During the cultivation process of Poly (3-hydroxybutyrate-*co*-4-hydroxybutyrate) [P(3HB-*co*-4HB)], the fermentation process in the bioreactor was monitored by measuring the dry cell weight (DCW) every 24 hours over a 110-hour cultivation period. Figure 4.1 shows that the bacterial culture exhibited a typical sigmoidal growth profile, which aligns with the expected microbial growth dynamics under controlled bioreactor conditions. The graph is divided into three distinct phases: lag, exponential, and stationary, with dashed vertical lines separating each phase.

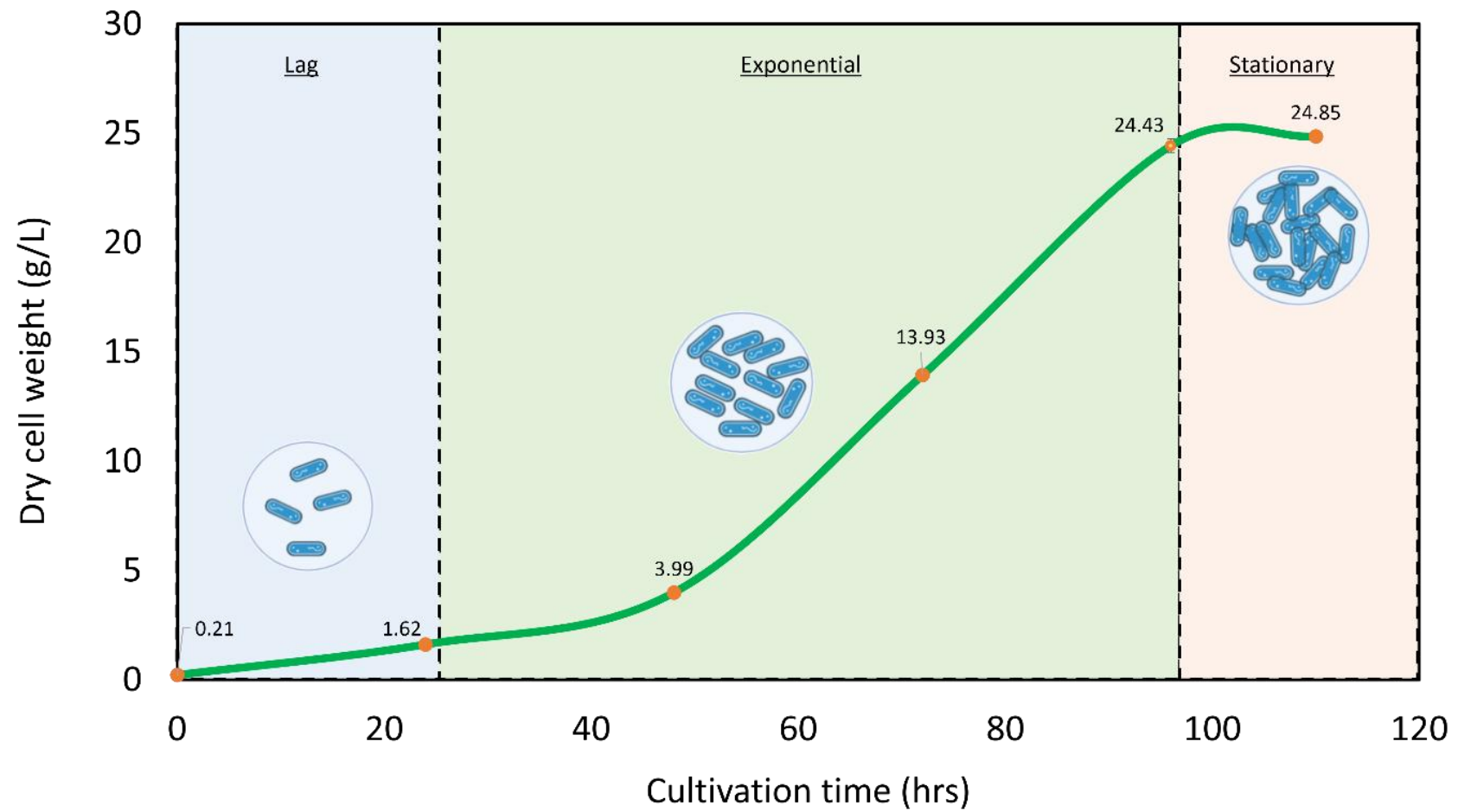


Figure 4.1: Graph of dry cell weight (g/L) of *Cupriavidus malaysiensis* USMAA1020 at different time intervals of 0 hours to 110 hours.

The bacterial growth during each phase is also illustrated in the graph insets. The lag phase is observed from 0 to 24 hours, the exponential growth phase occurs between 24 to 96 hours, and the stationary growth phase spans from 96 to 110 hours. The harvest time falls within the stationary phase at 110 hours. During the initial lag phase, the DCW increased slightly, ranging from 0.21 g/L to 1.62 g/L. The inset image in this phase shows a small number of bacterial cells, reflecting the slow growth. The exponential phase is marked by a rapid increase in DCW from 3.99 g/L, achieving a DCW of 24.43 g/L. The curve in this section is steep, showing a rapid and continuous increase in bacterial growth due to the high division rate of bacterial cells. The inset image in this phase shows a dense population of bacteria, indicating an actively growing culture. Subsequently, a stationary phase started, and the curve flattened, suggesting that growth has slowed, marking the cessation of significant growth at 110 hours, with a final DCW of 24.85 g/L.

Generally, microorganisms accumulate polyhydroxyalkanoate (PHA) as insoluble granules within the cytoplasm, acting as carbon and energy storage under adverse conditions (Utsunomia et al., 2020; de Macedo et al., 2024). These granules comprise a hydrophobic core surrounded by a protective membrane within the bacterial cell (Vigneswari et al., 2020; Ramachandran et al., 2025). Stressful conditions arise when crucial nutrients like phosphorus, nitrogen, and oxygen become deficient while carbon remains abundant (Utsunomia et al., 2020; de Macedo et al., 2024). So, during the adaptation phase, there is only minimal bacterial growth due to bacterial adaptation to the new medium. This phase reflects the period required for *C. malaysiensis* to acclimatize to the surplus availability of supplied carbon substrates, 1,4-butanediol (0.625 wt% C) and 1,6-hexanediol (0.125 wt% C), and initiate the biosynthetic processes for P(3HB-co-4HB) production.

In the log or exponential phase, microorganisms actively utilize the nutrients for substrate assimilation, and cellular proliferation occurs, significantly increasing the DCW. The rapid growth during this phase indicates efficient utilization of the mixed carbon substrate and rapid biomass accumulation, a strategy known to enhance

substrate-to-product conversion during biosynthesis. PHA accumulation peaks at the end of the log phase before declining when the cell enters the stationary phase (Sangkharak et al., 2021). The polymerization of hydroxy fatty acids derived from these carbon substrates is catalyzed by PHA synthases, leading to PHA production (Ramachandran et al., 2025).

In the stationary phase, nutrient depletion and potential accumulation of inhibitory byproducts occur, limiting further biomass production. Harvesting at this stage is critical to prevent intracellular degradation of the accumulated polymer (PHA mobilization) and the accumulation of toxic byproducts. This is because once the external carbon sources are depleted, bacterial metabolism shifts, triggering the mobilization and degradation of stored PHA for survival (Ramachandran et al., 2025). This process is initiated by intracellular depolymerase enzymes, which hydrolyze PHA into 3-hydroxybutyric acid (3HB) and 4-hydroxybutyric acid (4HB), allowing the bacteria to use these monomers as an alternative energy and carbon source (Ramachandran et al., 2025). The 4HB monomers in P(3HB-*co*-4HB) are synthesized from 1,4-butanediol, which is metabolized into 4-hydroxybutyryl-CoA. This intermediate is then polymerized by PHA synthase (*phaC*) to form P(3HB-*co*-4HB). Thus, the harvesting should be done at the optimal time, or PHA degradation will increase and significantly reduce the final polymer yield.

Once harvested, extracellular degradation that causes PHA to exit the bacterial cells most often occurs due to cell lysis, bacterial death, or solvent extraction (Ramachandran et al., 2025). Various environmental microbes secrete PHA depolymerase and esterases, which further break down the polymer into smaller components, eventually degrading into carbon dioxide and water (Kalia et al., 2023; Ramachandran et al., 2025). *In vitro* depolymerization occurs when PHA granules are extracted intact from bacterial cells, retaining their protein coating and flexible, amorphous nature (Ramachandran et al., 2025). This allows PHA depolymerase activity to persist in a cell-free system, enabling controlled degradation of PHA into monomers, dimers, or oligomers, which can serve as valuable starting materials for various applications (Ramachandran et al., 2025).

Overall, the stationary phase harvesting strategy minimised polymer degradation, ensuring a high-quality final product suitable for downstream applications (Huong et al., 2017). The single-stage fermentation method provides several benefits compared to the conventional two-stage cultivation process for producing P(3HB-*co*-4HB). In contrast to two-stage cultivation, which involves centrifugation and transferring the inoculum from the bacterial growth vessel to the PHA accumulation vessels, where steps are time-consuming and require considerable effort, the single-stage process streamlines the procedure (Huong et al., 2017). This simplifies the handling process and helps reduce the likelihood of contamination. Thus, this result indicates successful synthesis of P(3HB-*co*-4HB) copolymer. Additionally, the use of a mixed substrate strategy, combining 1,4-butanediol and 1,6-hexanediol, likely contributed to the observed high dry cell weight (Huong et al., 2017; Norhafini et al., 2017; Vigneswari et al., 2020). The observed bacterial growth profile, high dry cell weight, and efficient substrate utilisation highlight this process's suitability for large-scale polymer biosynthesis.

4.1.2 Endotoxin Level Assessment

Endotoxin contamination is a major concern when using PHAs in biomedical applications due to their bacterial origin. In this study, the P(3HB-*co*-4HB) copolymer was biosynthesised using the Gram-negative strain *C. malaysiensis*, which naturally contains lipopolysaccharides (LPS) in its outer membrane (Wang et al., 2021c). These LPS function as a protective barrier, selectively controlling the exchange of substances and inhibiting the movement of substrates and metabolites across the cell membranes. However, they are classified as endotoxins that can cause inflammatory and immune responses, and must be removed or reduced to ensure safety in medical use (Bolden et al., 2016; Wang et al., 2021c; García-Chumillas et al., 2024). Endotoxin contamination seriously threatens the safety of pharmaceuticals and parenteral products (Bolden et al., 2016). Solvent extraction, commonly employed for PHA recovery, does not fully eliminate endotoxins, necessitating additional treatment steps. Generally, PHAs extracted using the solvent-extraction method (for example, chloroform extraction

used in this study) have low contents of endotoxin (Bedade et al., 2021). The endotoxin levels of the extracted P(3HB-*co*-4HB) copolymer were assessed using the E-TOXATE™ Limulus Amebocyte Lysate (LAL) assay kit.

A summary of the LAL test results is presented in Table 4.1. Prior to treatment, the endotoxin concentration in the polymer was measured at 30.15 EU/mL. After hydrogen peroxide (H₂O₂) treatment, the concentration was significantly reduced to 0.27 EU/mL. The results were interpreted based on gel formation in the tubes, where the presence of a hard gel indicated a positive reaction for endotoxins.

Table 4.1: Observations and Endotoxin Levels for P(3HB-*co*-4HB) Polymer

Sample	Tube	Observed Gel	Dilution Factor	Endotoxin Concentration (EU/ml)
Untreated P(3HB- <i>co</i> -4HB)	Tube A	Hard gel	1:4	30.15
H ₂ O ₂ Treated P(3HB- <i>co</i> -4HB)	Tube A	Hard gel	1:64	0.27
Inhibitor test	Tube B	Hard gel	-	No inhibitors
Negative Control	Tube C	Soft gel	-	No contamination
Positive Control	Tube D	Hard gel	4 EU/mL	Assay validated
	Tube E	Hard gel	0.5 EU/mL	Assay validated
	Tube F	Hard gel	0.25 EU/mL	Assay validated
	Tube G	Soft gel	0.125 EU/mL	Below the detection limit
	Tube H	Soft gel	0.03 EU/mL	Below the detection limit
	Tube I	Soft gel	-	Blank (no endotoxin)

After pyrogen removal, a rapid decrease in endotoxin levels was observed, reaching 0.27 EU/g, compared to the endotoxin content of distilled water, which is approximately 20 EU/mL (Zalewska-Piątek, 2023). According to the US FDA guideline, the permitted level of endotoxins for PHA in medical applications is 4-5 EU/g (Vigneswari et al., 2015). The polymer produced in this study falls well within these safety limits, making it suitable for *in vivo* applications. The European Pharmacopeia has established a threshold limit for intravenous applications, setting the maximum allowable endotoxin level at 5 EU/kg/h (Zalewska-Piątek, 2023). Therefore, it should be noted that once endotoxins are removed, the P(3HB-*co*-4HB) copolymers produced in this study are suitable for *in vivo* applications.

However, removing endotoxins makes the downstream process (DSP) more expensive (García-Chumillas et al., 2024; Tebaldi et al., 2019; Kumar et al., 2020; Diankristanti et al., 2024). For *in vivo* applications, efficient endotoxin removal is essential (Sanhueza et al., 2019; Prakash et al., 2022). Standard methods to remove endotoxins include benzoyl peroxide, H₂O₂, and sodium hydroxide (Sanhueza et al., 2019; Prakash et al., 2022).

H₂O₂ was selected in this study due to its strong oxidative properties, low endotoxin content, minor degradation of the polymer, and applicability to small-scale (Prajapati et al., 2021). However, for large-scale applications, the use of H₂O₂ may not be feasible due to environmental concerns and scalability limitations (Sanhueza et al., 2019; Prakash et al., 2022). Safer and more sustainable alternatives such as ultrafiltration, endotoxin-binding resins, or enzymatic detoxification should be considered to ensure industrial viability and regulatory compliance (Sanhueza et al., 2019; Prakash et al., 2022).

Different approaches are now utilized to measure endotoxins. In 1973, Levin and Bang developed the LAL assay to replace the FDA-approved rabbit pyrogen test (RPT), demonstrating its equivalency, speed, and greater sensitivity (Li et al., 2015; Bolden et al., 2016). The LAL test, also called the Bacterial Endotoxin Test (BET), relies on the coagulation response triggered by endotoxin binding to factor C in LAL (Dullah and Ongkudon, 2017). Except for endotoxin-free water used for dilution, all

other components of the sample may interfere with the assay, potentially affecting the endotoxin measurement (Li et al., 2015).

4.1.3 Monomer Content and Molecular Weight Determination

The PHA content and composition of the extracted pure/crude P(3HB-*co*-4HB) polymer and the dry bacterial cells were analyzed using gas chromatography (GC). Additionally, the molecular weight distribution of the crude P(3HB-*co*-4HB) polymer was determined using gel permeation chromatography (GPC). The results are summarized in Table 4.2.

Table 4.2: PHA concentration, 3HB, 4HB monomer composition and molecular weight of [P(3HB-*co*-4HB)].

Samples	PHA content	PHA composition (mol%)		PHA yield (g/L)	Molecular weight		PDI
	%PHA	3HB	4HB		M _w (kDa)	M _n (kDa)	M _w / M _n
Dry cells	51.98 ± 1.26	51.30 ± 0.32	48.70 ± 0.32	7.17 ± 0.23	-	-	
Crude polymer	95.04 ± 9.05	49.83 ± 1.63	50.17 ± 1.63	13.09 ± 0.89	44.8 ± 2.2	25.3 ± 0.7	1.77

The percentage of PHA content in dry cells was $51.98 \pm 1.26\%$, with a composition of 51.30 ± 0.32 mol% 3HB and 48.70 ± 0.32 mol% 4HB. For the crude polymer, the PHA content increased to $95.04 \pm 9.05\%$, with the 3HB and 4HB compositions being 49.83 ± 1.63 mol% and 50.17 ± 1.63 mol%, respectively. The PHA yield from dry cells was 7.17 ± 0.23 g/L, further increased to 13.09 ± 0.89 g/L in the purified crude polymer. The molecular weight of the crude polymer, determined by GPC, showed a weight-average molecular weight (M_w) of 44.8 ± 2.2 kDa and a number-average molecular weight (M_n) of 25.3 ± 0.7 kDa, resulting in a polydispersity index (PDI) of approximately 1.77.

The 49.83% and 50.17% monomer composition values demonstrate a near-equimolar distribution of 3HB and 4HB monomers. This reflects a balanced substrate utilization and incorporation of the two monomers into the copolymer. Mixed substrates likely played a crucial role in achieving this balance. The substrate mixture used in this study consisted of 1,6-hexanediol and 1,4-butanediol. When used in a 1:5 ratio, this combination was the most effective in yielding the highest composition of 4HB monomer and PHA, which aligns with the findings of a previous optimization study by Norhafini et al. (2017). The polymer composition is mainly determined by several factors, including the type and metabolic pathway of the carbon source and the specificity of PHA synthase (Norhafini et al., 2017).

The increase in PHA content from 52% in dry cells to 95% in crude polymer shows the high efficiency of the solvent extraction process used for purifying PHA. The PHA content of $51.98 \pm 1.26\%$ in dry cells also demonstrates the ability of *C. malaysiensis* to efficiently convert mixed carbon substrates (1,4-butanediol and 1,6-hexanediol) into PHA. Besides that, the M_w of the crude polymer of 44.8 kDa and an M_n of 25.3 kDa, falls within the lower to mid-range of the reported values for P(3HB-co-4HB), which span approximately 20–1060 kDa (Kovalcik et al., 2021). This suggests that the copolymer produced in this study falls within the expected range for microbial PHA production.

Including specific carbon sources in the PHA production medium triggers the production of 4HB monomers (Huong et al., 2017). However, these carbon sources do

not exclusively result in the formation of the homopolymer P(4HB), as 4-hydroxybutyric acid can also be converted into 3-hydroxybutyryl-CoA (Huong et al., 2017). This conversion facilitates the integration of 3HB co-monomers, synthesising the P(3HB-*co*-4HB) copolymer (Huong et al., 2017). Copolymers with a higher content of 4HB monomers are often preferred in healthcare applications because they exhibit superior biocompatibility and faster biodegradability (Aziz et al., 2017; Norhafini et al., 2019; Vigneswari et al., 2020).

Various factors, including carbon source composition, influence molecular weight in PHA production. This can be attributed to chain transfer events, bacterial metabolic activity, and fermentation parameters, including aeration and agitation rates, which are crucial in determining polymer chain length. Additionally, the hydroxyl (OH) groups present in 1,4-butanediol and 1,6-hexanediol act as chain transfer (CT) agents, terminating the growing PHA chain and thereby reducing M_w (Huong et al., 2017). A compound can act as a CT agent if it contains an OH group that interacts with the carboxyl group of the elongating PHA chain through a transesterification reaction (Huong et al., 2017). Since both carbon sources used in this study contain OH groups, a M_w reduction was expected when used together (Huong et al., 2017).

Beyond CT agents, the M_w can also be influenced by variations in the 4HB monomer composition and active *phaC* enzyme expression levels in bacterial cells (Syafiq et al., 2017). High PHA synthase activity promotes chain initiation, forming shorter polymer chains and lowering the M_w (Syafiq et al., 2017; Vidili, 2023). Other key parameters affecting M_w include the selected bacterial strain, the nature of the production media, fermentation conditions, copolymer composition, and isolation techniques (Kovalcik et al., 2021; Vidili, 2023).

M_w significantly influences the drug release behaviour of nanoparticle (NP)-based drug delivery systems (DDSs) and dissolving microneedles (DMNs). The key objective is to achieve a balance where the polymer does not degrade too rapidly, leading to premature drug release, nor too slowly, which could hinder drug absorption at the intended site. Previous research has shown that lowering the M_w of Poly (lactic-

co-glycolic acid) (PLGA) NPs from approximately 100 kDa to 10 kDa accelerates drug release kinetics from around 30% to 90% within 24 hours, preventing premature degradation of therapeutics while ensuring sustained delivery (Narmani et al., 2023). While no studies have directly investigated the impact of P(3HB-*co*-4HB) M_w on drug release, lower M_w PHA-based systems have been shown to reduce crystallinity, crystal size, and melting temperature, thereby enhancing flexibility and biodegradation (Martínez-Herrera et al., 2023). These properties are crucial for overcoming brittleness issues and developing nanocomposites with tailored mechanical performance (Martínez-Herrera et al., 2023). However, high M_w PHAs result in increased entanglement of polymer chains, leading to higher viscosity, which can complicate processing and extraction methods (Werker et al., 2023).

Additionally, higher M_w polymers dissolve more slowly in solvents, impacting their formulation into nanocarriers or films (Flórez et al., 2023). In contrast, lower M_w polymers exhibit improved solubility, enhanced flexibility, and better degradation behaviour, making them particularly favourable for biomedical use (Narmani et al., 2023). Another critical aspect is the balance between biocompatibility and toxicity. Higher M_w synthetic polymers such as polyethylenimines (PEIs) exhibit intense biological activity but are often linked to elevated cytotoxicity (Ewe et al., 2019). Conversely, low M_w PEIs display greater biocompatibility, though they may require optimization to achieve the desired transfection efficiency (Ewe et al., 2019).

P(3HB) with low M_w (LMWPHB) showed improved thermal stability compared to neat P(3HB). Additionally, incorporating LMWPHB reduced crystallinity, crystallization rate, and melting point while enhancing flexibility and degradation capacity (Dhanial et al., 2022). High M_w biodegradable polymers (HMWBP), such as pullulan, heparin, dextran, and hyaluronic acid, have been reported in earlier studies as potential NP carriers aimed at the delivery of drugs, proteins, and genetic materials (George et al., 2019). Despite their effectiveness, NPs made from HMWBPs degrade more slowly *in vivo*, which could lead to a higher likelihood of tissue buildup (George et al., 2019). As a result, LMWBP has gained significant interest as a promising delivery system due to their better permeability,

solubility, and encapsulation efficiency, which are related to their relatively smaller particle size (George et al., 2019).

LMW polymers have also been explored for stabilizing supersaturated amorphous drug formulations, a strategy known as co-amorphous formulation, where a drug is combined with a low M_w stabilizer to prevent recrystallization and maintain a homogeneous single-phase amorphous state (Chavan et al., 2016; Laitinen et al., 2017). This property is particularly advantageous for drug-polymer systems, as low M_w components have been found to inhibit drug recrystallization, thereby enhancing drug stability in a dissolved state (Ojarinta et al., 2017; Hirakawa et al., 2019; Wang et al., 2021a). Given that P(3HB-*co*-4HB) in this study has a low M_w , it may similarly contribute to improved drug stabilization in NP-based delivery systems.

Thus, the M_w of the polymer in this study is optimal for forming NPs suitable for drug encapsulation and breast cancer (BC) treatment via microneedles (MNs). Despite the demonstrated influence of M_w on polymer properties, limited studies have explored its direct impact on drug delivery efficacy in PHA-based systems. Future studies should optimise M_w control strategies to refine PHA properties for targeted applications (Werker et al., 2023). While studies on PEG and PLGA have established M_w -drug release relationships focusing on circulation and immune clearance (Shi et al., 2021), fewer reports exist for PHA-based systems. Further research is needed to determine whether M_w is comparable in PHA NPs, enabling their future development to compete with synthetic polymers.

The M_n of a polymer plays a vital role in defining its mechanical strength and capacity to be shaped or moulded (Vigneswari et al., 2012). Earlier investigations reported that an increase in 4HB monomer composition resulted in a decrease in M_n . Besides, different combinations of carbon sources and feeding strategies have also been reported to affect polymer M_n (Vigneswari et al., 2012). Polymers' M_w distributions (MWDs) impact their properties, including their processability, mechanical strength, and morphological phase behaviour (Gentekos et al., 2019). Dispersity ($\bar{D}$) is the most widely used molecular parameter for characterizing polymer

M_w distributions (MWDs) (Gentekos et al., 2019). It represents the normalized standard deviation of chain sizes within a polymer sample and is expressed as the ratio of the weight-average molar mass (M_w) to the number-average molar mass (M_n) (Gentekos et al., 2019). The PDI of P(3HB-*co*-4HB) produced in this study is 1.77, and a PDI that is close to 1, which is similar to that of chemically synthesized polymers, is highly favoured (Phong et al., 2016). Therefore, the P(3HB-*co*-4HB) produced in this study has optimum properties suitable for the fabrication of NPs to be integrated into DMNs.

4.2 Fabrication and Optimization of Docetaxel- Incorporated P(3HB-*co*-4HB) Nanoparticles

This nanoparticle (NP) fabrication process was conducted using a focused optimization approach as it is built upon the work of a previous study, which extensively optimized the synthesis of docetaxel (DTX)-incorporated P(3HB-*co*-70%4HB) nanoparticles (DTX-NPs) for the potential lung cancer treatment (Faisalina et al., 2020). The previously reported study consists of 70% 4HB monomer composition. However, in this study, the copolymer used contained a 50% 4HB composition, which differs significantly in its physical, mechanical, and physicochemical properties (Norhafini et al., 2017). Additionally, it is well known that both microbial polymer synthesis and NP fabrication outcomes can vary due to batch-specific differences, processing conditions, and environmental factors.

The prior study systematically evaluated multiple critical parameters, including the selection and concentration of tensioactives, different M_w of PVA, and a wide range of drug-to-polymer ratios, to ensure optimal NP properties for lung cancer treatment (Faisalina et al., 2020). Since this study focuses on utilizing a previously synthesized NP for BC treatment through transdermal delivery and most of the significant groundwork had already been established, this study prioritized the validating and adapting the most relevant parameters for the transdermal application

through MNs, specifically the incorporation of D- α -tocopheryl polyethylene glycol succinate (TPGS), solvent-evaporation time, drug-to-polymer ratios, and sonication time specific to the NP formulation. By leveraging the prior study's findings, the current work avoided redundant optimization steps and focused on parameters critical to the specific application and formulation. Moreover, the adaptations made to the formulation (e.g., polymer composition and delivery route) necessitated re-evaluating key parameters to align with the unique requirements of this study, ensuring both scientific rigour and practical relevance. This targeted approach ensures that the NPs are optimized for the intended goal.

4.2.1 Effects of TPGS Incorporation on Nanoparticle Properties

TPGS is frequently used to develop a drug delivery system. It is synthetically derived from natural α -tocopherol through esterification of α -TOS and polyethylene glycol (PEG) 1000 (Neophytou et al., 2019). The optimization of NPs with and without TPGS was evaluated based on particle size (nm), drug loading (DL%), and encapsulation efficiency (EE%) across evaporation time of 4 hours and 12 hours, as shown in Figure 4.2A-C.

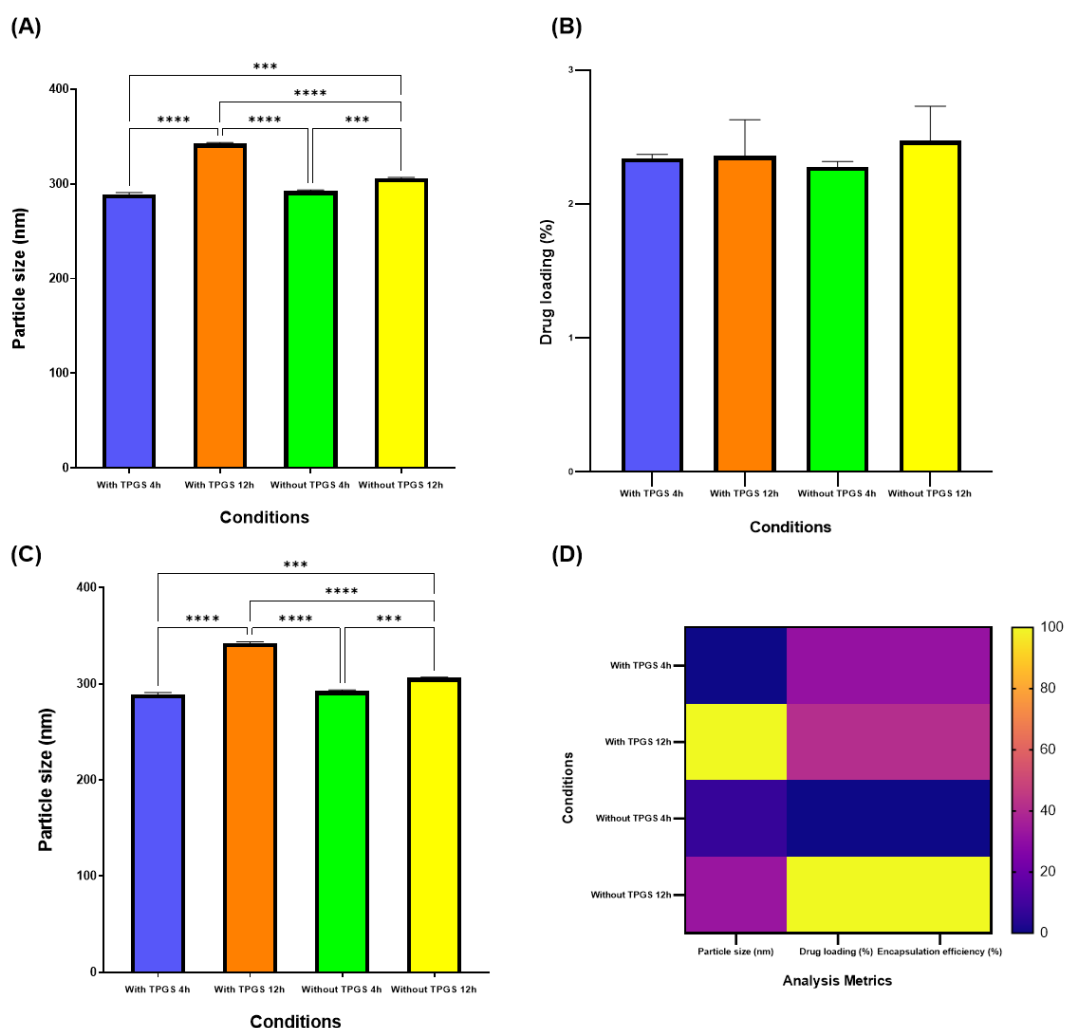


Figure 4.2: The effects of TPGS and evaporation time (4 hours vs. 12 hours) on (A) particle size, (B) drug loading (DL), (C) encapsulation efficiency (EE), and (D) a heatmap summarizing the combined optimization. Data are presented as mean $\pm$ standard deviation (SD) of three independent experiments. Asterisks indicate significant differences between groups: $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

Figure 4.2A illustrates that NPs formulated with TPGS during a 4-hour solvent evaporation time demonstrated significantly smaller particle sizes than a 12-hour evaporation time. In contrast, formulations without TPGS exhibited smaller particle sizes during a 12-hour stirring time. Figure 4.2B shows that DL percentages showed slight differences across conditions. With TPGS, the 4-hour condition yielded slightly higher DL, while without TPGS, the 12-hour stirring condition showed slightly higher

values. These differences, however, were not statistically significant ($p > 0.05$). Figure 4.2C presents EE%, where formulations with TPGS under the 4-hour evaporation condition showed slightly higher EE than those without TPGS. In the 12-hour stirring condition, NPs without TPGS displayed slightly higher encapsulation efficiency, but the differences were not significant. The heatmap (Figure 4.2D) further visualises the comparative trends across all tested conditions for particle size, DL, and EE.

Based on these results, the formulation with TPGS and a 4-hour solvent evaporation time was selected as the optimal condition. This choice was driven primarily by the benefits of including TPGS in the formulation: improved cellular uptake, P-glycoprotein (P-gp) inhibition, and cytotoxicity enhancement against tumour cells (Tan et al., 2017; Choudhury et al., 2017). TPGS is also commonly used to improve the DL of polymeric nanoparticles (PNPs) (Liu et al., 2020). Besides that, TPGS can solubilise many poorly water-soluble drugs, including taxanes. TPGS is utilized in various nanomedicine formulations, enhancing the bioavailability of drugs like DTX and paclitaxel, which are known for their poor solubility (Jasim and Jawad, 2021; Mehata et al., 2023). In previous studies, doxorubicin-loaded nanocarrier was observed to have increased therapeutic efficacy due to the addition of TPGS, while DTX with TPGS synergistically reduced the viability of MCF-7 cells (Neophytou et al., 2019).

Moreover, the drug release profiles *in vitro* of TPGS-chol-liposomes provided a sustained release of DTX, and further experiments on cellular uptake, cell apoptosis, and cytotoxicity demonstrated that TPGS-chol-liposomes significantly concentrated DTX within cancer cells, effectively suppressing cell growth and overcoming multidrug resistance (MDR) in BC cells (Li et al., 2019b). This strongly proves that the addition of TPGS enhances the effects of DTX. TPGS is also an approved pharmaceutical excipient by the United States Food and Drug Administration (FDA) and the China Food and Drug Administration (CFDA), which could be a promising alternative compared to Tween 80, which causes many toxic reactions in most commercially formulated DTX (Taxotere®) (Tan et al., 2017; Sohail et al., 2018). A significant improvement in bioavailability and *in vivo* pharmacokinetics was also

observed in the co-administration of TPGS with anti-cancer drugs (Neophytou et al., 2019).

Additionally, the organic solvent used in this study was found to have evaporated in just 4 hours based on the reduction in volume of the total solvent. The 12-hour evaporation time also increased the particle size (Figure 4.2A), and this could be due to the aggregation of particles, which is typically observed when the stirring time is very long. Additionally, dichloromethane used as the organic solvent in this study has a boiling point of 39.6°C. Previous studies on nanospheres of polylactic acid (PLA) with acetone and dichloromethane as the solvent system also used almost the same time range of 1–5 hours to evaporate the solvent (Crucho and Barros, 2017). It was also noted that the rate at which the organic solvent evaporated and the increased volume of the aqueous phase did not affect the size of the nanospheres, as observed in previous studies (Crucho and Barros, 2017). Thus, TPGS and 4-hour stirring time for solvent evaporation were selected for the subsequent NP fabrication conditions.

The heatmap in Figure 4.2D highlights that the formulation incorporating TPGS with 4-hour evaporation achieved consistently favourable values across all three parameters, shown by the dominant warmer colour tones (e.g., orange to purple). In contrast, conditions without TPGS or with longer evaporation times showed more variability and relatively cooler tones, reflecting comparatively lower performance. This visual matrix supports the selection of the TPGS + 4 hours condition as optimal. Overall, adding TPGS is beneficial because it is an efficient drug carrier element that can considerably lower the carrier material required when combined with DTX in this BC therapy research.

4.2.2 Influence of Drug-to-Polymer Ratios on Nanoparticle Properties

Various drug-to-polymer (D:P, % w/w) ratios (0%-40%) were tested to optimise the NP formulation and assess their impact on particle size, EE, DL, and stability. The drug-to-polymer ratio is expressed as a percentage, representing the weight of the drug in relation to the polymer (e.g., 40% indicates 20 mg of the drug in

50 mg of polymer). The aim was to identify a formulation that maximizes DL while maintaining stable NP characteristics suitable for transdermal delivery. Figure 4.3 shows the impact of different drug-to-polymer ratios on NP characteristics.

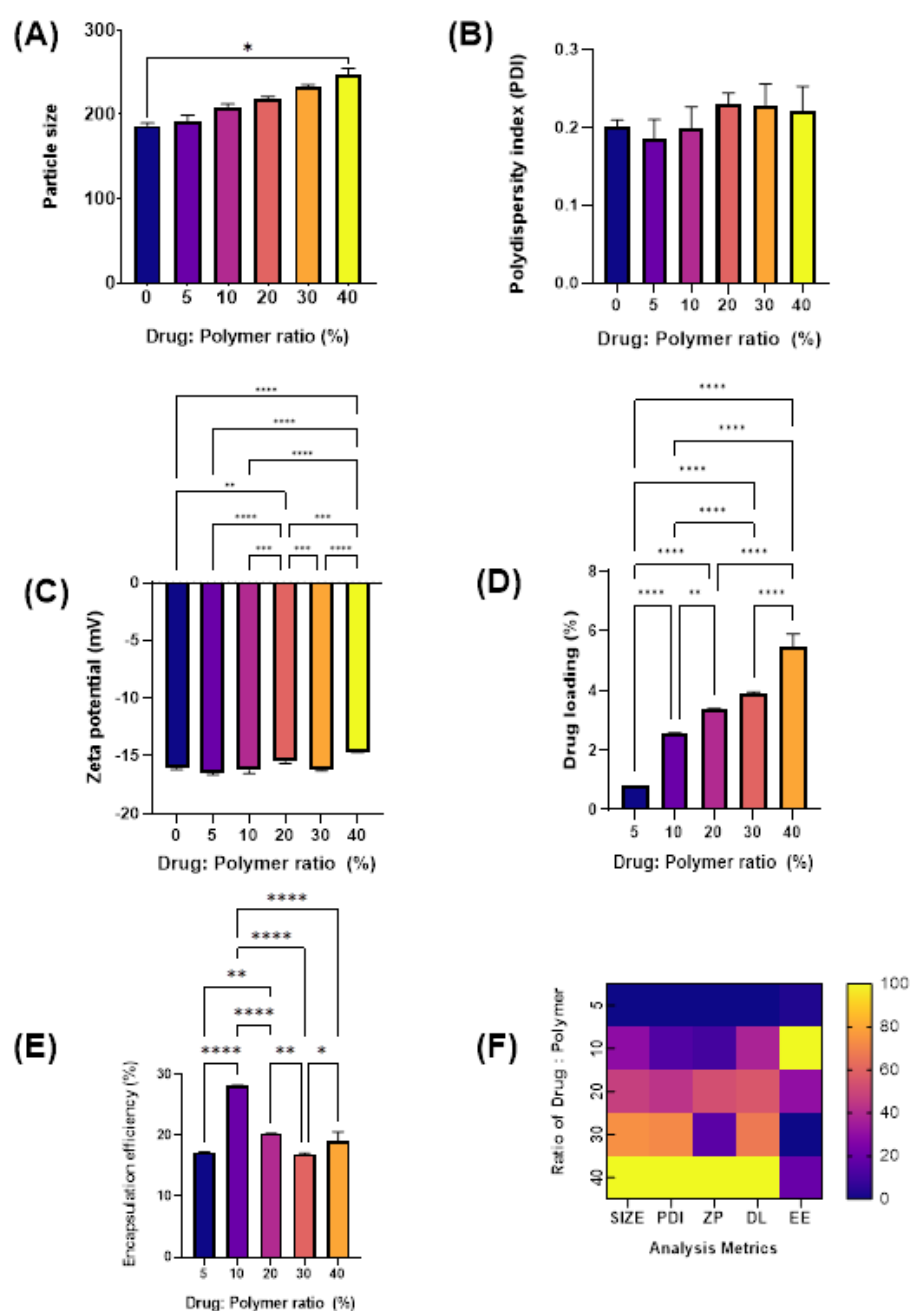


Figure 4.3: The effects of different drug-to-polymer ratios on (A) particle size, (B) polydispersity index, (C) Zeta potential, (D) drug loading (DL%), (E) encapsulation efficiency (EE%), and (F) a heatmap summarizing the combined optimization. Data are presented as mean $\pm$ standard deviation (SD) of three independent experiments. Asterisks indicate significant differences between groups: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Particle size increased as the drug-to-polymer ratio increased (Figure 4.3A). The PDI remained below 0.3 across all formulations, indicating uniform particle size distribution (Figure 4.3B). Zeta potential (ZP) was negative for all samples but showed a trend of increasing negativity with higher drug content (Figure 4.3C). DL increased with the drug-to-polymer ratio, with 40% showing the highest value (Figure 4.3D). However, EE peaked at a 10% drug-to-polymer ratio before declining at higher concentrations (Figure 4.3E). The heatmap (Figure 4.3F) provides a visual summary of all evaluated parameters, such as particle size, PDI, ZP, DL, and EE, across the tested drug-to-polymer ratios.

Among the tested ratios, 40% drug-to-polymer was the most appropriate condition, balancing particle size, DL, and EE. The 40% drug-to-polymer ratio led to larger particle sizes than the lower ratios; however, the differences were minimal, except for the blank NPs (0%). The increase in particle size corresponded to the higher amount of drug within the polymer. This trend was also reported in an earlier study, which showed that particle size increased with the drug concentration ranging from 0.25% to 1% (w/v), while the EE decreased within this concentration range (Tran et al., 2021). All the drug-to-polymer ratios produced NPs with sizes under 300 nm, suitable for effective skin penetration (Leong et al., 2023).

There were no significant differences in the PDI among the drug-to-polymer ratios, which were all lower than 0.3. Previous studies have demonstrated that PDIs < 0.3 indicate a narrow distribution profile among particle populations (Rafiei and Haddadi, 2019).

Besides that, ZP is considered one of the key factors affecting NP behaviour and the stability of dispersions (Muruvea et al., 2023). Surface charges of NPs depend on the material properties and fabrication methods used (Shao et al., 2015). The ZP measurement of DTX-NPs showed a negative value, likely due to the presence of terminal carboxyl groups in the P(3HB-co-4HB) polymer chains (Faisalina et al., 2020). A notable variation in ZP was observed among NPs prepared using different drug-to-polymer ratios; however, they were all within -30 to +30 mV. NPs with positive ZP or large similar charges were more toxic and had higher cytotoxicity than

NPs with negative ZP (Shao et al., 2015). Previous research has also shown that ZP exceeding ± 30 mV is associated with good stability, whereas particles with ZP values ranging from -5 mV to $+5$ mV are likely to undergo rapid agglomeration (Murueva et al., 2023). Moreover, a negative ZP is advantageous for prolonged systemic circulation and targeted tumour accumulation. This aligns with previous findings demonstrating that aggregates with negative or neutral charges can block the uptake of serum proteins and macrophages *in vivo*, leading to slower clearance and improved retention of NPs in the bloodstream (Li et al., 2019a). Furthermore, due to the improved permeability and retention (EPR) effect, these properties encourage the selective build-up of NPs within tumour tissues, making them highly suitable for BC therapy (Li et al., 2019a).

As for the DL, the 40% drug-to-polymer ratio showed a significantly greater amount compared to other ratios. High DL is a critical factor in PNPs for transdermal delivery. Usually, most hydrophobic drugs tend to cluster when in aqueous environments, causing very minimal DL below 5 wt% (Chen et al., 2018). Higher DL is preferable because higher DL means less dosage frequency, but it can also influence the stability and release of the drug. However, each drug is different, and the carriers for the drug have to be customized to maximize the therapeutic efficiency. (Chen et al., 2018). The EE was not very proportional to the results of DL, as it shows that 10% drug: polymer ratios have the highest EE (Figure 4.3E). This suggests that at lower drug content, the available polymer is more effectively utilised to encapsulate the drug, resulting in higher efficiency. However, at higher DLs, the polymer may become saturated or insufficient to fully entrap the additional drug, leading to a drop in EE despite the increased drug content.

In this study, the 40% drug-to-polymer ratio was selected despite the 10% ratio showing higher EE, as DL was prioritised to ensure a higher drug payload per NP. This approach aligns with previous findings that emphasise the importance of high DL in formulations, particularly for drugs with poor water solubility, as seen in Biopharmaceutical Classification System (BCS) Class II and IV drugs (Yao et al., 2021). High DL also reduces the polymer required, preventing excessively large formulations that could compromise patient compliance (Wilson et al., 2020).

Furthermore, transdermal delivery via MNs benefits from higher DL, allowing for sustained drug release while maintaining therapeutic efficacy with a lower dosage frequency. Since the increase in drug concentration resulted in a rise in particle size, further optimization was performed by increasing the sonication time. This successfully decreased the particle size and enhanced the EE, ensuring that the NPs remained within an optimal size range for effective skin penetration.

The colour intensity indicates performance for each parameter, with brighter regions representing more favourable outcomes. Notably, the 40% drug-to-polymer ratio exhibited strong scores across key criteria such as DL and acceptable particle size, supporting its selection as the optimal formulation for further development.

Overall, in this optimization part, the 40% drug-to-polymer ratio was chosen for further optimization due to its higher drug loading and lower ZP, which equates to higher stability. The PDI was also within the optimum range. Since the particle size was large, the sonication time of the 40% drug: polymer ratio was further optimised.

4.2.3 Impact of Sonication Time on Nanoparticle Formation

Since the previously selected 40% drug: polymer ratio with TPGS in an evaporation rate of 4 hours has larger particle sizes, the sonication time was increased from 1 minute to 2 minutes. This modification aimed to improve particle size, DL, and EE while maintaining stability. Figure 4.4 shows the impact of sonication time on NP characteristics.

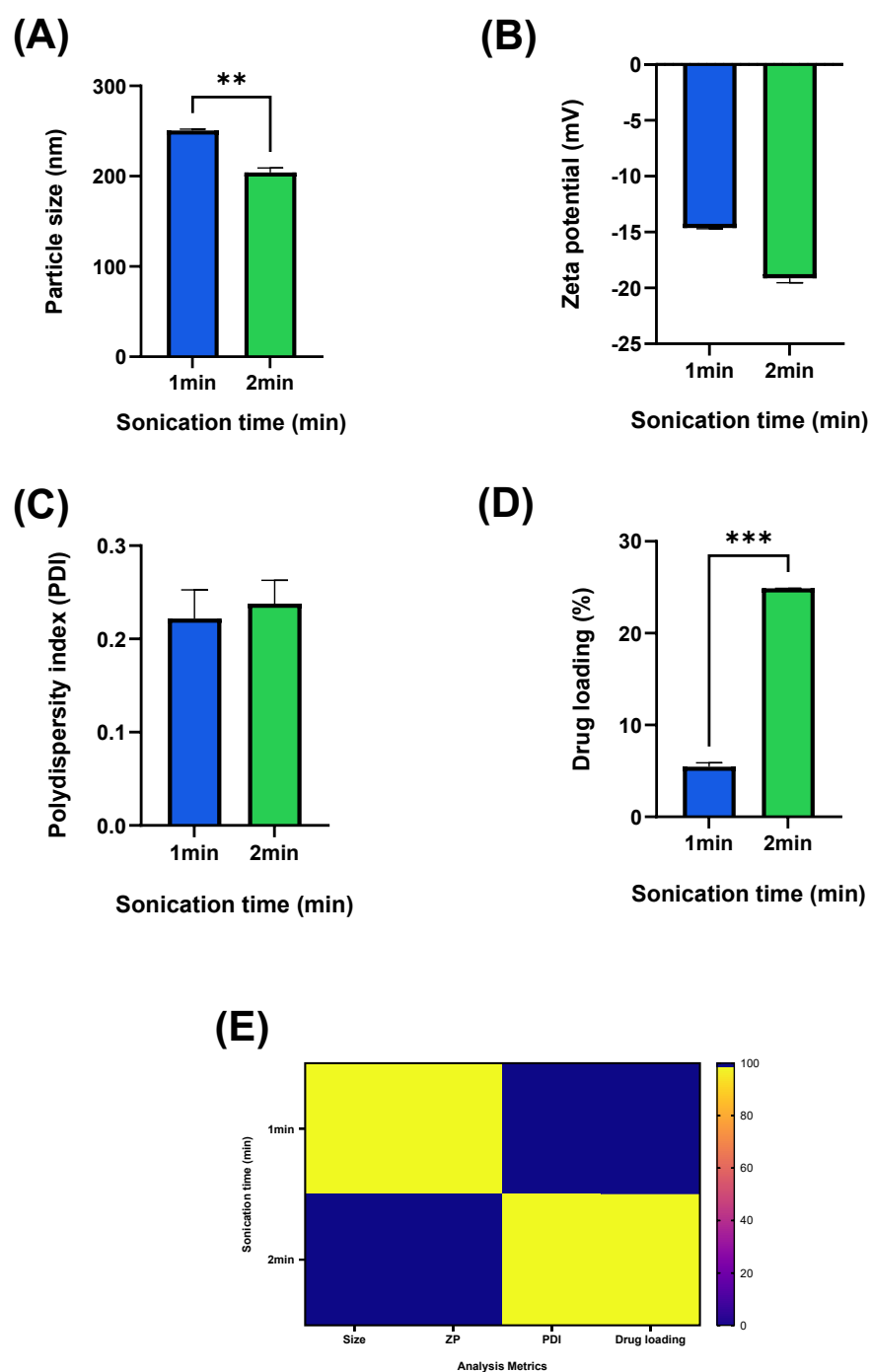


Figure 4.4: The effects of sonication time on (A) particle size, (B) Zeta potential, (C) polydispersity index, (D) drug loading (DL%), and (E) a heatmap summarizing the combined optimization. Data are presented as mean $\pm$ standard deviation (SD) of three independent experiments. Asterisks indicate significant differences between groups: $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

As shown in Figure 4.4A, increasing the sonication time from 1 minute to 2 minutes resulted in a noticeable decrease in particle size. The ZP (Figure 4.4B) became more negative, but the change was not significant. Similarly, the PDI (Figure 4.4C) remained stable across both sonication times, indicating that the particle size distribution did not vary drastically. The amount of drug entrapped increased with longer sonication time as shown in Figure 4.4D. The overall optimization results are summarized in a heatmap (Figure 4.4E).

Since the previously selected 40% drug-to-polymer ratio (D:P, %w/w) (calculated as the percentage of the drug with respect to polymer weight) with TPGS in an evaporation rate of 4 hours has larger particle sizes, we increased the sonication time from 1 minute to 2 minutes. The increase in sonication time significantly reduced the particle size of NPs while enhancing DL and EE. The observed results may be due to the application of higher shear stress, which prevents the emulsion droplets from merging (Haggag et al., 2018; Haggag et al., 2021). This prevention of droplet coalescence reduces their size, ultimately resulting in the generation of smaller NPs (Haggag et al., 2018; Haggag et al., 2021). It could also potentially cause an increase in DL as smaller particles offer more internal volume to accommodate the drug, thereby improving the loading efficiency (Lestari et al., 2022). Ultrasonication time is also inversely proportional to NPs' particle size (Vardhan et al., 2017a). Though the PDI and ZP show an increase, it was not significant, as observed in an earlier study (Haggag et al., 2021) and was still within the optimum range.

However, the sonication time should not be excessively prolonged, as the elevated temperature increases the kinetic energy of the particles. This increases van der Waals forces between particles, promoting NP aggregation to minimise surface energy (Takma et al., 2023). Besides, too much heat generated during sonication might also degrade the properties of the drugs or the polymer. In this study, the emulsion was placed in an ice bath, and the sonication was performed in 30-second intervals, with pauses in between, to prevent excessive heating and ensure stability throughout the process. Thus, the 2-minute sonication time was sufficient to show the optimum result for particle size, DL and EE.

The heatmap (Figure 4.4F) further consolidates the optimisation outcomes by visually comparing all key parameters under the different sonication times. This heatmap clearly illustrates that the 2-minute sonication condition produced improved values across multiple parameters, indicated by warmer colours such as orange and yellow. In contrast, the 1-minute sonication condition appears in cooler colours (blue and purple), suggesting lower performance. The visual summary reinforces the selection of the 2-minute sonication time as optimal, offering a quick yet comprehensive overview of how this parameter influenced the NP properties.

The final optimized NPs based on the selected parameters were centrifuged and washed with distilled water three times to remove the free drugs and PVA solution. The NPs were then freeze-dried before being analyzed using the direct method in HPLC. The final results of the NPs are shown in Table 4.3 below.

Table 4.3: Physicochemical Characteristics of optimized nanoparticles to be incorporated in microneedles.

Parameters	Values
Particle size (nm)	204.17 $\pm$ 4.891
Zeta potential (mV)	-19.13 $\pm$ 0.404
Polydispersity index (PDI)	0.238 $\pm$ 0.025
Drug loading (%)	13.71 $\pm$ 0.820
Encapsulation efficiency (%)	87.07 $\pm$ 0.043

*Parameters include particle size, polydispersity index (PDI), Zeta potential (ZP), drug loading (DL%), and encapsulation efficiency (EE%). Particle size was determined using Dynamic Light Scattering (DLS). Drug loading and EE% were determined using the direct method in HPLC. All values represent mean $\pm$ standard deviation (SD) of three independent experiments.

The final optimized NPs in this study demonstrated an average particle size of 204.17nm, a ZP of -19.13 mV, and a PDI of 0.24 and DL of 13.71%. The DL from the optimizations was calculated using an indirect method, where the supernatant of the NP emulsion after solvent evaporation was analyzed to determine the free drug content, which was then subtracted to calculate DL.

In conclusion, the 40% drug-to-polymer ratio with TPGS fabricated using a 2-minute sonication time and with a solvent evaporation rate at 4 hours stirring time was selected as the optimal formulation in this study. It demonstrated an optimum particle size range, EE, and DL without the drawbacks observed in other ratios. This optimization is particularly beneficial for transdermal delivery via MNs as smaller particle sizes facilitate deeper penetration through the skin layers, ensuring efficient drug absorption. The enhanced DL also improves therapeutic efficacy by allowing sustained drug release at the breast tumour areas.

4.3 Characterization of Docetaxel-Loaded P(3HB-co-4HB) Nanoparticles

4.3.2 Morphological Analysis of Nanoparticles via Scanning Electron Microscopy and Transmission Electron Microscopy

The morphology, particle size, and structural characteristics of the synthesised DTX-NPs were evaluated using electron microscopy and size distribution analysis to confirm their suitability for drug delivery applications. Figure 4.5 presents the morphological analysis of DTX-NPs observed through Scanning Electron Microscopy (SEM) (Figure 4.5A) and Transmission Electron Microscopy (TEM) (Figure 4.5B). The particle size distribution graph (Figure 4.5C) illustrates that the NPs predominantly have sizes centred around 70 nm, with a Gaussian distribution, confirming uniformity in size.

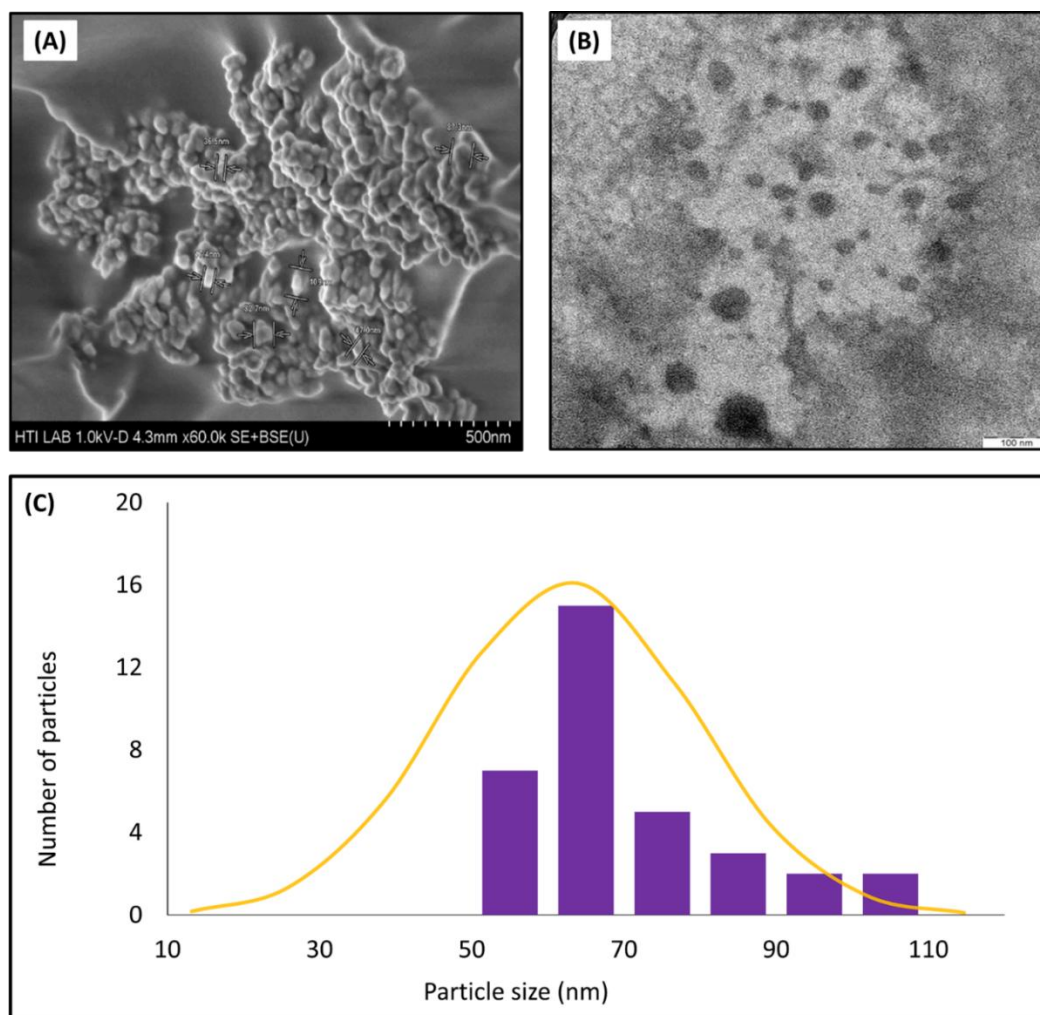


Figure 4.5: Morphological analysis of docetaxel-loaded P(3HB-co-4HB) nanoparticles by (A) Scanning Electron Microscopy (SEM), (B) Transmission Electron Microscopy (TEM), and (C) Particle size distribution calculated from SEM images.

The SEM in the image (A) reveals that the NPs exhibit a generally spherical shape with smooth surfaces, suggesting uniformity in particle morphology. No visible drug crystals were detected in the SEM image. As observed in the micrograph, the NPs' average size appeared smaller than the size recorded using the particle size analyser, as in Table 4.3. This is because the DLS technique evaluates changes in scattered light intensity due to Brownian motion. This information is subsequently used to determine the particles' diffusion coefficient and hydrodynamic diameter (de la Calle et al., 2019).

The spherical structure and relatively homogeneous surface indicate that the fabrication resulted in consistently shaped NPs, favouring controlled drug release and interaction with biological systems. Meanwhile, SEM shows a more accurate measurement of NP sizes. In (B), the TEM image provides a closer look at the morphology of the NPs, confirming their spherical shape and size consistency at the nanoscale level. The TEM analysis reveals a well-defined core structure, indicating successful drug encapsulation within the polymer matrix.

The SEM and TEM images confirm the successful synthesis of PNPs with desirable morphological and structural properties, indicating that the fabrication process effectively produced NPs suitable for drug delivery applications. Nanovesicles with diameters of 70 nm or smaller are more efficient in penetrating the outer and inner skin layers, in contrast to particles with diameters of approximately 300 nm, demonstrating a clear relationship between particle size and skin permeability (Leong et al., 2023).

Better skin penetration is achieved with smaller particle sizes (Zhou et al., 2018). A decrease in particle size results in a larger specific surface area, contributing to an enhanced occlusive effect that facilitates more effective transdermal drug delivery (Zhou et al., 2018). This particle dimension range aligns with polymer-based particles currently under clinical development (Caster et al., 2017). NPs between 10 and 150 nm are ideal for improved EPR effects during systemic intravenous (IV) administration (Liu et al., 2020). Moreover, smaller-sized NPs could penetrate deeper, thus achieving better anti-tumour effects (Liu et al., 2020). Previous studies on PEG nanocarriers show that tumour tissues selectively absorb nanocarriers less than 200 nm through the EPR effect (Li et al., 2019a).

4.3.2 Drug Release Profiles of Nanoparticles

Modulating drug release kinetics is often the most important goal when developing a drug delivery system (Vardhan et al., 2018). Figure 4.6 below illustrates the cumulative drug release over a 96-hour interval of free DTX and DTX-NPs (Figure 4.6A) and the graph of the best-fit models (Figure 4.6B).

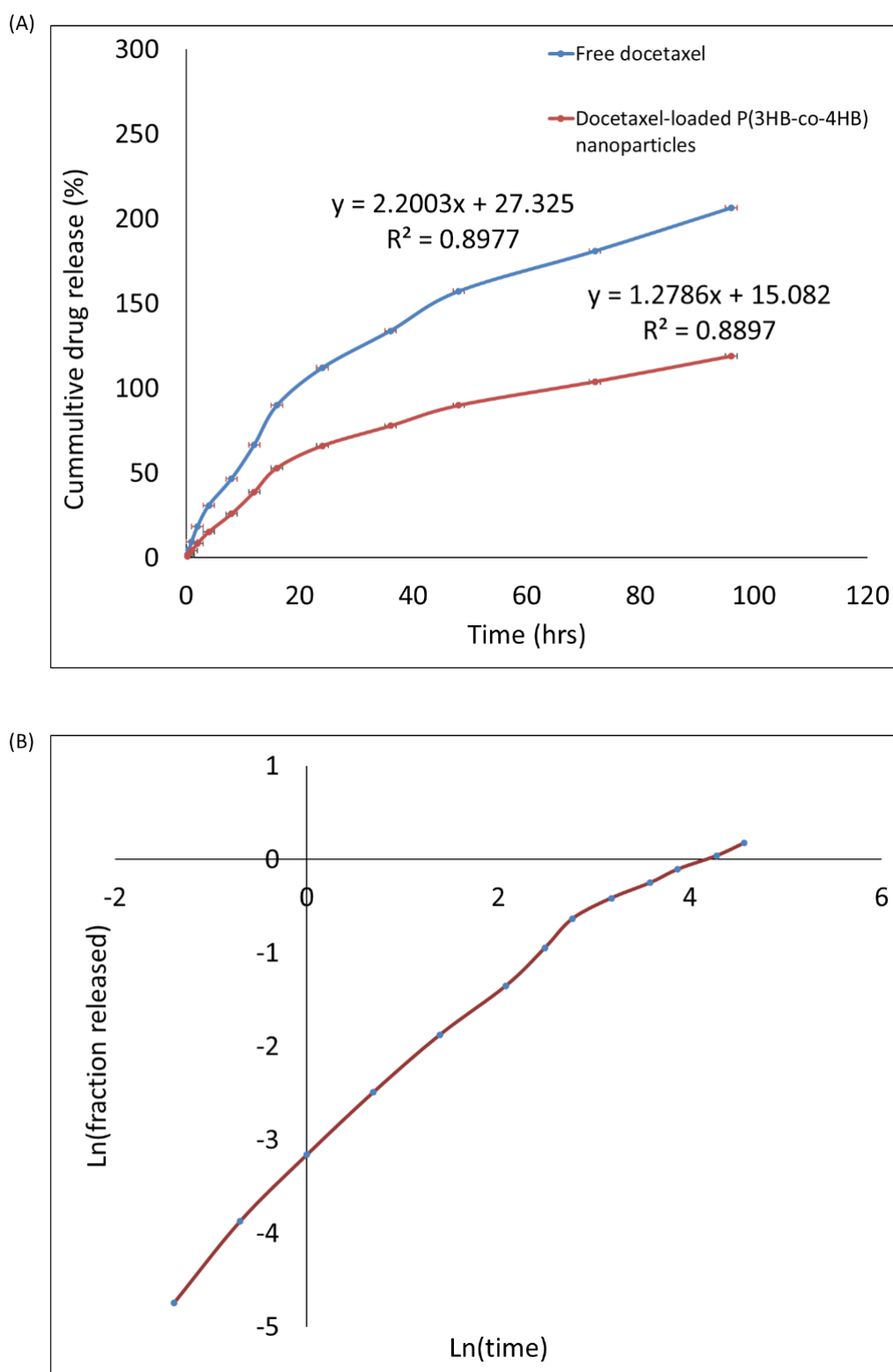


Figure 4.6: (A) Comparative cumulative drug release profiles of pure docetaxel and docetaxel-loaded P(3HB-co-4HB) nanoparticles and (B) the Korsmeyer-Peppas graph as the best-fit model of docetaxel-loaded P(3HB-co-4HB) nanoparticles.

Based on Figure 4.6A, free DTX exhibited rapid release, achieving 100% drug release within just 24 hours, while the DTX-NPs demonstrated a more controlled release profile, requiring approximately 72 hours to reach complete release. Almost 90% of drugs are released within 48 hours. Various factors can affect the release of drugs from NPs, such as the solubility of the drug, its diffusion rate, the degradation of the polymer, particle size, and the amount of drug loaded (Lappe et al., 2017).

Most previous studies that exhibit free DTX released within less than 24 hours were added with either Tween 80 or ethanol in PBS to increase their release (Badran et al., 2018; Haseeb et al., 2019). Taxotere is a commercially available DTX formulation that contains a 50:50 mixture of Tween 80 and dehydrated ethanol as the solubilizing vehicle (Shaw et al., 2017). Tween 80 is associated with certain adverse effects, including severe hypersensitivity reactions, hyperlipidemia, anaphylaxis, erythrocyte aggregation, and abnormal lipoprotein patterns (Shaw et al., 2017). Since TPGS was already added during the NP fabrication in this study, further addition of Tween 80 will complicate the process and increase toxicity or cost. Moreover, excluding Tween 80 from PBS better mimics real-world conditions and ensures the findings directly apply to practical scenarios.

P(3HB-*co*-4HB) and DTX are both hydrophobic, which makes the release from the NPs slow. A previous study by Faisalina et al. (2020) observed that more than 80% of DTX was released from NPs within 24 hours at a drug/polymer ratio of 30, and 40% of the total release was achieved after 48 hours. This study also used a 40% of the drug: polymer ratio, but the release was observed to be more than 48 hours. This could be due to the lower amount of 4HB monomer in this study (50%) compared to the 70% in previous studies, as it has been proven that a higher 4HB monomer concentration causes faster polymer degradation. Table 4.4 below summarizes the best-fit release models for the pure DTX drug and NP formulations, determined based on R^2 values calculated from the release graphs as shown in Figure 4.6B.

Table 4.4: Correlation coefficients (R^2) of different drug release models for pure docetaxel and docetaxel-loaded P(3HB-*co*-4HB) nanoparticles.

Samples	Best-fit release models			
	Zero-order	First order	Higuchi	Korsmeyer Peppas
Pure DTX	$R^2 = 0.8977$	$R^2 = 0.9225$ (not a straight line)	$R^2 = 0.9913$	$R^2 = 0.9773$
DTX-NPs	$R^2 = 0.8897$	$R^2 = 0.9676$ (not a straight line)	$R^2 = 0.9039$	$R^2 = 0.9796$

Free DTX followed Higuchi's model, indicating that its release process was mainly controlled by Fickian diffusion across the medium. The Higuchi release model describes drug diffusion from an insoluble matrix as a time-dependent phenomenon, which follows a square root pattern and is driven by Fickian diffusion (Jose et al., 2019). Fickian diffusion is the conventional diffusion mechanism influenced by concentration differences or gradients (Jose et al., 2019). The higher R^2 value (diffusion-controlled, Higuchi model) indicated that the drug release was diffusion-controlled.

NPs exhibited a slower release than free DTX, best fitting the Korsmeyer–Peppas model. According to this model, the typical indication of Fickian diffusion is reflected by an n value of 0.43 for spherical monodisperse particles. In the case of polydisperse particles, their n -value is generally lower (Jose et al., 2019). The n value of 0.829 obtained in this study, which falls between 0.45 and 0.89, indicates that the drug release mechanism is governed by non-Fickian diffusion. Non-Fickian diffusion involves polymer relaxation and diffusion mechanisms contributing to the drug's release. The Korsmeyer–Peppas kinetics reflect anomalous diffusion, where multiple parameters control the release (Shaw et al., 2017). A similar outcome was observed in a prior study using Transferrin-conjugated DTX–PLGA NPs (Jose et al., 2019).

The amount of drug loaded into NPs significantly influences both the initial burst release and the subsequent speed at which the drug is discharged (Lappe et al., 2017). The DTX-NPs show an initial burst release of 6.97% within the first 2 hours, and this might be attributed to the free drugs attached to the surface of the NPs or the drug encapsulated close to the NP surface, which is usually observed in NPs.

The initial burst release is critical to ensure the drug quickly reaches the therapeutic window (Laracuenta et al., 2020). Hydrophobic drugs typically exhibit slower and less pronounced burst release due to their tendency to stay inside the delivery system, reducing contact with the surrounding aqueous phase (Laracuenta et al., 2020). In contrast, hydrophilic drugs can diffuse into the surrounding media once a sufficient pore network forms, linking them to the aqueous phase. Strongly

hydrophobic drugs, however, are only released after the surrounding polymeric matrix undergoes degradation sufficiently (Laracuenta et al., 2020).

Therefore, the findings from this study confirm that DTX-NPs exhibit a prolonged release pattern with regulated delivery over 72 hours, in contrast to free DTX, which releases within 24 hours. The sustained drug release profile achieved with these NPs could enhance drug retention in the skin, reduce systemic toxicity, and improve therapeutic outcomes, making NP-MNs delivery an effective strategy for localised BC therapy.

4.3.3 Storage Stability Evaluation of Nanoparticles

Stability studies are crucial for assessing and ensuring the quality of Active Pharmaceutical Ingredients (APIs) and Finished Pharmaceutical Products (FPPs) under different conditions, including temperature, humidity, and light (González-González et al., 2022). Storage stability is a critical parameter for evaluating the long-term performance and shelf life of nanoparticle formulations intended for therapeutic use. Figure 4.7 shows the effect of storage time and humidity on the particle size (Figure 4.7A), zeta potential (ZP) (Figure 4.7B), polydispersity index (PDI) (Figure 4.7C), and drug loading (DL) (Figure 4.7D) of DTX-NPs over 6 months under controlled conditions.

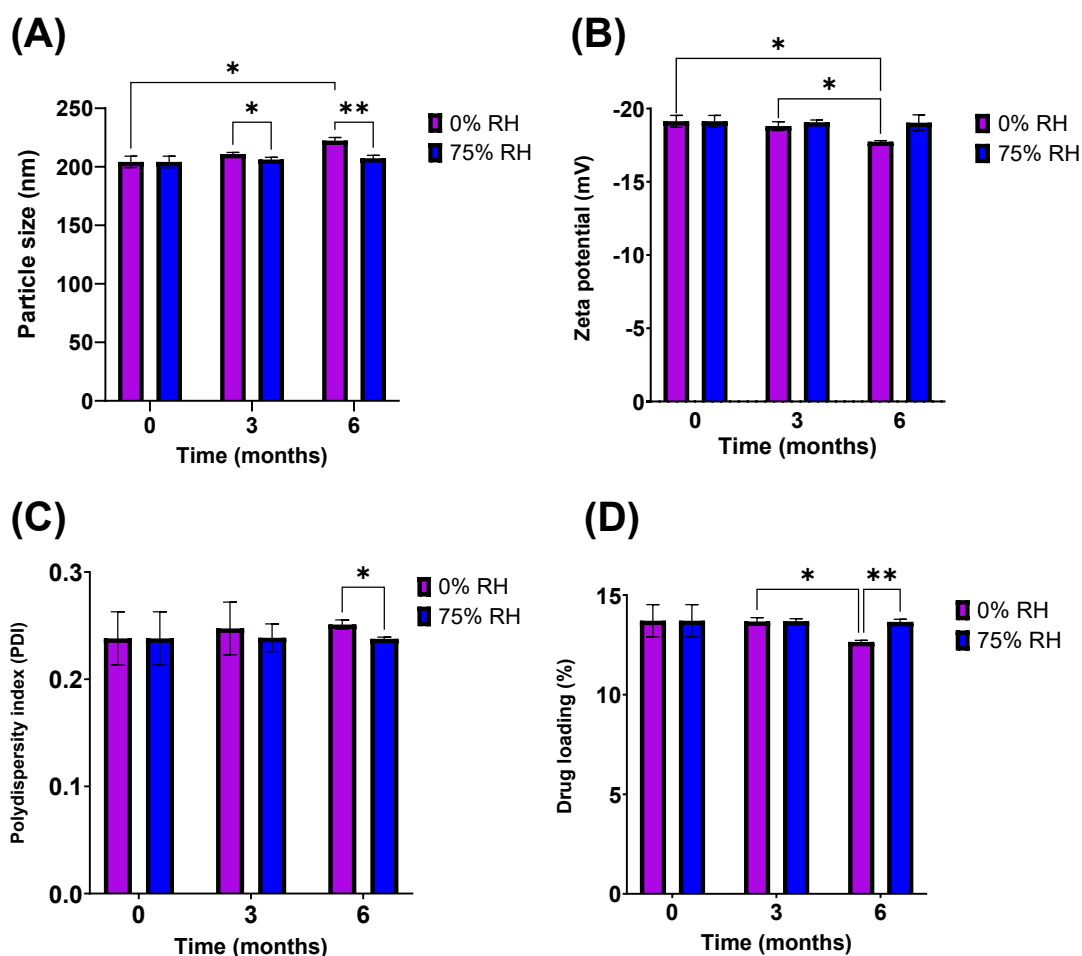


Figure 4.7: Stability analysis of nanoparticles stored at 0% RH and 75% RH over 0, 3, and 6 months: (A) particle size, (B) Zeta potential, (C) polydispersity index (PDI), and (D) drug loading. Data are presented as mean $\pm$ SD. Asterisks indicate significant differences between groups: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

The particle size increased significantly over time under both storage conditions. At 0% RH, the size increased from 204.17 $\pm$ 4.89 nm at 0 months to 222.42 $\pm$ 2.64 nm at 6 months ($p < 0.05$). Similarly, at 75% RH, the size increased significantly from 204.17 $\pm$ 4.86 nm to 207.29 $\pm$ 2.67 nm ($p < 0.05$). A comparison between the two humidity levels showed significant differences at 3 and 6 months, with larger particle sizes at 75% RH ($p < 0.05$). The ZP became less negative at both humidity levels over time, indicating potential changes in NP surface stability. At 0% RH, ZP changed from -19.1 mV to -17.7 mV over 6 months ($p < 0.05$). At 75% RH, a similar trend was observed, with significant differences between storage times. No

significant differences in ZP were detected between the two humidity conditions at any specific time. PDI values remained below 0.3, indicating good particle uniformity. However, significant increases were observed at 6 months for both humidity levels ($p < 0.05$). At 75% RH, PDI values were slightly higher compared to 0% RH, but the difference was not statistically significant. DL exhibited significant reductions over time under both storage conditions. At 0% RH, DL decreased from $13.71 \pm 0.81\%$ to $12.7 \pm 0.08\%$ over 6 months ($p < 0.05$). At 75% RH, DL also declined significantly from $13.71 \pm 0.81\%$ to $13.65 \pm 0.14\%$ ($p < 0.05$). Significant differences in DL were observed between the two humidity conditions at 6 months, with lower values at 75% RH ($p < 0.05$). These findings indicate that both time and humidity significantly influence the stability of NPs. Increased humidity (75% RH) exacerbates changes in particle size and DL, highlighting the need for optimized storage conditions to maintain NP stability. The results observed are similar to previous studies of PLGA NPs (Aldawsari et al., 2020).

NPs are commonly synthesized as a water-based suspension but tend to be physically unstable, leading to aggregation and clumping occurring over extended periods of storage (Fonte et al., 2016). Hydrolytic action occurs when water comes in contact with the polymer matrix, leading to drug leakage, reduced or eliminated sustained release properties, and forming undesirable degradation products (Fonte, Reis, and Sarmiento, 2016). NPs in liquid suspension are also susceptible to microbial growth during storage (Fonte, Reis, and Sarmiento, 2016). Moisture is a potent driver of molecular mobility, potentially accelerating the crystallization of amorphous drugs, particularly under elevated temperatures (Yao et al., 2021). Thus, the apparent solution is transforming it into a solid dosage form through a dehydration process, including freeze-drying or lyophilization, which has been proven to reduce moisture (Fonte et al., 2016).

Since amorphous drug crystallization is exacerbated by heat and humidity, the study supports that NPs should be stored below their glass transition temperature (T_g) and at low humidity for long-term stability (Fonte, Reis, and Sarmiento, 2016). Overall, the combination of 40 °C and 75% RH (termed the “G condition”) represents the most stringent environment for pharmaceutical stability assessment and sets the highest

benchmark for evaluating the stability of amorphous drugs (Yao et al., 2021). Thus, this study's 6-month stability at 0% RH highlights the feasibility of MN-mediated NP delivery, supporting its potential as a clinically relevant and scalable drug delivery system. The study findings prove that the NPs can be successfully incorporated within the MNs for transdermal therapeutic delivery in BC management.

4.4 Fabrication and Characterization of Dissolving Microneedles Loaded with Nanoparticles

4.4.1 Optimization of Microneedle (MNs) Fabrication

Dissolving microneedles (DMNs) are typically produced using methods like drawing lithography, droplet-born air blowing, micro moulding, casting using solvents, and micro-casting (Dalvi et al., 2021; Rad et al., 2021; Yang et al., 2021; Kang et al., 2022; Liu et al., 2022). Among these, the solvent casting method is the simplest, where a polymer or drug-loaded solution is poured into a silicon mould, left to dry, and subsequently removed for use (Ahmad et al., 2023). Several benefits are associated with this method, such as low cost, ease of scaling up, and the application of biodegradable and biocompatible synthetic polymers (Ahmad et al., 2023).

The DMNs in this study were fabricated using a mould-based centrifugation method, as shown in Figure 4.8, which is optimised to ensure homogeneity and maintain optimal mechanical integrity for skin penetration, NP incorporation, and drug release. This fabrication follows a two-step method in which the first solution, containing the drug, was used to cast the needle layer, while the base layer was prepared without the drug (Wang et al., 2015).

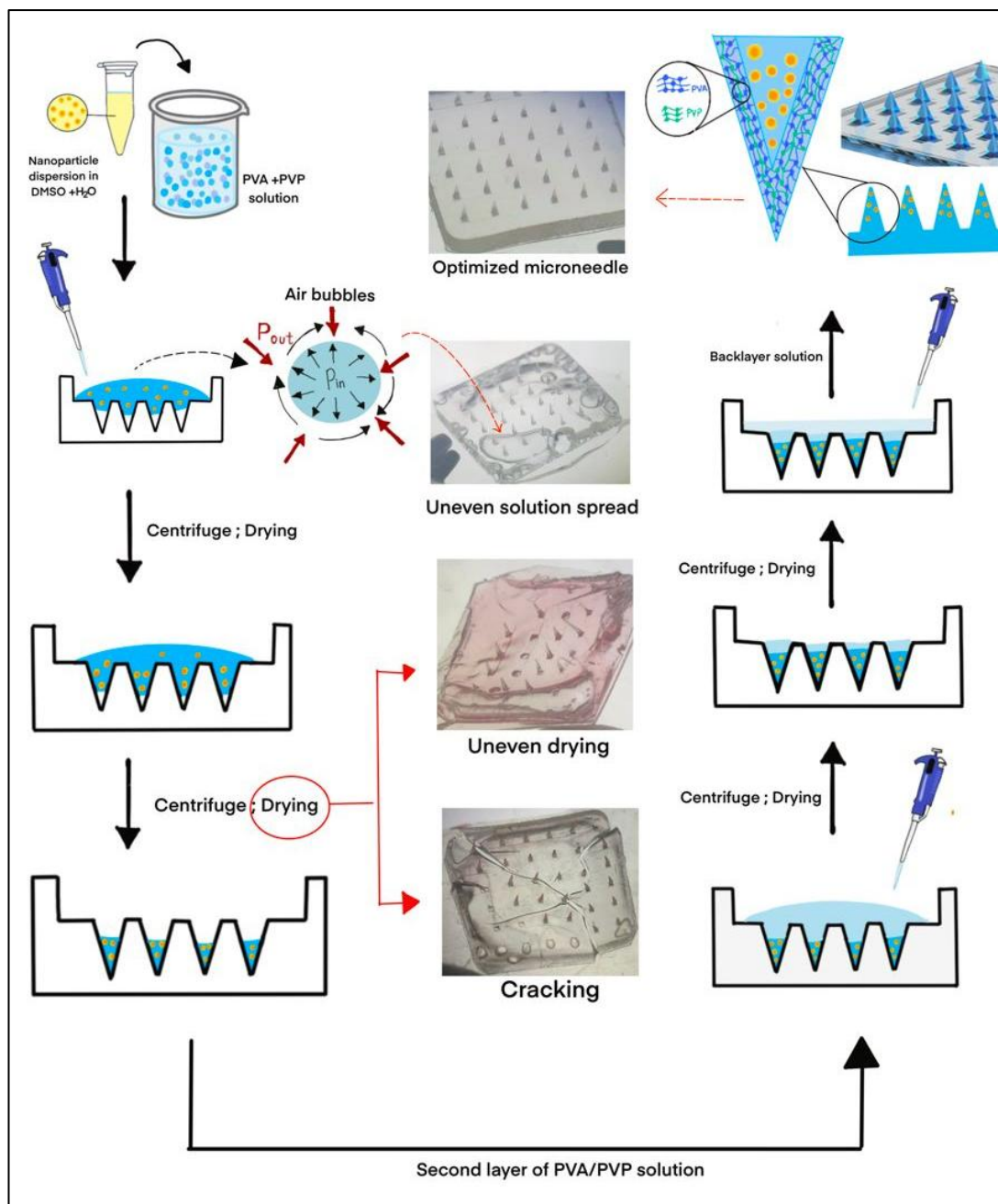


Figure 4.8: Schematic illustration depicting the flow of steps involved in the microneedle fabrication, incorporation of nanoparticles in microneedles, and the key factors influencing the quality of microneedles.

Figure 4.8 clearly explains how each factor, such as air bubble formation, drying temperature, solvent volume and concentration, affects the physical and chemical interactions within the PVA/PVP MN matrix, affecting the quality of the end product. The schematic illustration shows the stepwise fabrication process of DTX-MNs. The process begins with preparing DTX-NPs dispersed in a PVA/PVP polymer solution to form a homogeneous suspension. This drug-polymer mixture is then pipetted onto an MN mould, as shown in the first step of the image.

The mould is placed in a centrifuge, as depicted in the next step, which applies centrifugal force to ensure the thorough filling of the MN mould cavities. After centrifugation, the MN mould is dried to allow solidification, ensuring that the polymer matrix develops a stable structure. A second polymer layer is then applied over the mould to form the backing layer, which is essential in ensuring the mechanical strength of the MN patch. The mould is placed in a vacuum drying chamber, as shown in the next step of Figure 4.8, to enhance the integrity of the MNs further. This vacuum drying step ensures the removal of residual moisture and prevents deformation. After drying, the fully formed MN patch is carefully removed from the mould, revealing a structured array of MNs. The final section of the image magnifies the MN composition, showing that the polymer matrix encapsulates the NPs within each MN tip. The microscopic images on the right side of the schematic provide real-life visuals of the fabricated MNs, highlighting their sharp and well-defined structures.

Incorporating NPs within the MNs was also optimised to retain the required amount of drugs and maintain the MNs' mechanical integrity. The ratio of PVA to PVP here plays a very crucial role. The first layer is composed of PVA: PVP solution of 4:1. PVP is more viscous compared to PVA, and the layer that makes up the tips has to be less viscous for the polymer solution to flow inwards and reach the very end of the tips, the subsequent layers will have a higher ratio of PVP to make sure it is more viscous and ensure or prevent the solution in the tips to pour out during centrifugation, it is also notable that the tips will have the NPs so it has to be less viscous so that NPs can flow smoothly inwards towards the tips of the MNs and not stay at the back layer. A higher molecular weight of PVP is used for the base layer because the longer molecular chains of PVP result in a highly entangled network,

increasing the viscosity of the solution and limiting the mobility of drug molecules (Wang et al., 2015). PVA and PVP were selected for these DMNs due to their beneficial properties. PVA is recognised for its excellent biocompatibility, quick swellability, non-toxicity, and efficient water absorption, making it a popular choice in controlled drug delivery systems (Bajpai et al., 2019). The tips of the DMNs were prepared by blending PVP, a material known for its brittleness, with PVA, which provides flexibility (Li et al., 2024). Literature shows that combining the two polymers (PVA/PVP) enhances the formulation's mechanical strength, the polymer matrix's DL capacity, and the drug release (Ahmad et al., 2023).

Another issue in optimising the DMNs is that air bubbles are easily formed within the MN mould during fabrication due to inadequate pouring techniques, quick solvent evaporation, or insufficient degassing of the mould and solvent (Tucak et al., 2020). The air bubbles create regions of varying pressure, affecting the surface tension and causing uneven distribution of the solution poured into the MN mould. This causes incomplete filling or needles unattached to the back layer, which results in weak or incomplete MN arrays (Tucak et al., 2020).

Temperature or humidity is also another essential criterion that should be maintained. Higher temperature ensures fast drying, but when the solvent on the top layer of the mould (back layer) evaporates faster than the inner needles portion, it causes uneven drying. This difference in the evaporation rate causes the top layer to sink in as the needles dry and compact, causing the top layer to crack, as noted in a previous study (McGrath et al., 2014). Lowering the temperature is preferable for slower and even drying but might increase the risk of contamination or solvent retention, affecting MNs' mechanical strength and biocompatibility (Yadav and Pattanayek, 2020).

Besides that, the solvent volume and concentration determine the viscosity and flow properties of the polymer solution. A higher solvent concentration lowers the viscosity but increases the chances of air bubble formation (Bauleth-Ramos et al., 2023). Conversely, a low solvent concentration increases the viscosity but causes

uneven solution spread (McGrath et al., 2014). Thus, the polymer solution's volume and concentration should be optimised to avoid defects like incomplete filling or shrinkage during drying. Insufficient volume of polymer solution in the MN mould also causes an incomplete MN patch. Techniques such as sonication, vacuum degassing, and centrifugation are often employed to improve mould filling by eliminating air bubbles, ensuring a more complete and uniform MN formation, as highlighted in McGrath et al. (2014).

Optimising the drying process, including adjusting the temperature, drying time, and humidity, is essential for creating mechanically robust MNs. In this study, MNs were dried using a desiccator for 36 hours to minimise the formation of defects such as cracks and voids. The interplay between air bubble formation, solvent concentration, surface tension, and temperature during MN fabrication is complex.

Optimisation of the parameters involved in MN fabrication, including temperature, humidity, solvent concentration, surface tension, and polymer interactions, is essential for achieving mechanically robust MNs with uniform DL and efficient drug delivery properties. The hydrogen bonding between PVA and PVP creates a stable matrix for the MNs, but disruptions in the drying process or the presence of air bubbles can weaken these bonds, resulting in reduced mechanical strength. Careful control of all these factors ensures the production of high-quality MNs suitable for various drug delivery applications, particularly those involving NP formulations.

The NPs to be incorporated into the MN polymer matrix also had their limitations, as both the DTX and the polymer, P(3HB-co-4HB) used in this study, are hydrophobic, and no prior study was done using DTX-NPs for transdermal drug delivery. Thus, the key challenge in this study was achieving a stable and homogeneous dispersion of hydrophobic NPs within a hydrophilic matrix composed of PVA and PVP. This required optimising solvent selection and processing conditions to prevent phase separation and aggregation and to ensure uniform NP distribution throughout the MN matrix.

Thus, we had to ensure the NPs were mixed well with the PVA/PVP matrix to avoid agglomeration and to ensure the drugs were also localised at the tips of the needles. So, optimisations were done using different solvents to ensure the NPs were homogenous in the PVA/PVP matrix. Solvents such as distilled water, Tween 80, ethanol, and dimethyl sulfoxide (DMSO) were used to attempt to form a clear suspension or dispersion of the NPs. However, based on the observations shown in Table 4.5 below, only the NPs pre-dissolved in DMSO and then added to the PVA/PVP solution had clear and uniform dispersion within the PVA/PVP polymer solution.

Table 4.5: Various solvents used for dispersion of docetaxel-loaded P(3HB-co-4HB) nanoparticles within PVA/PVP matrix for incorporation into microneedles

Solvent	Observation
Distilled water	Lyophilised nanoparticle sediment
PVA/PVP solution	Lyophilised nanoparticle sediment
Tween 80 (0.1-1%v/v)	Lyophilised nanoparticle sediment
Water + Tween 80	Phase separation and sedimentation
Ethanol (0.1-10%v/v)	Phase separation and sedimentation
Water + Ethanol	Initial dispersion improved but resulted in sedimentation over time.
DMSO (0.1-1% v/v)	Clear suspension
Water+ DMSO	Clear suspension

Initial attempts with direct mixing, ethanol, Tween 80, and water-based mixtures were unsuccessful due to aggregation or phase separation, leading to visible suspensions. The process was repeated with varying concentrations and other techniques, such as vortexing and water bath sonication. Using a minimal concentration of DMSO with distilled water successfully dissolved the NPs and facilitated their homogeneous incorporation into the polymer matrix.

Similar issues regarding incompatible characteristics between drugs and polymer-based materials for directly loading drug molecules into DMNs were observed in previous studies (Zhao et al., 2021). This also highlights a gap that has to be addressed to establish a robust, innovative, and multifunctional drug delivery system. Achieving stable NP dispersion ensures drug efficacy and uniformity in polymer nanocomposite systems. Achieving stable NP dispersion ensures drug efficacy and uniformity in polymer nanocomposite systems. As Kim et al. (2016) outlined, controlling the interparticle interaction through solvent quality significantly contributes to the dispersion state of NPs in polymer films.

Studies report that DMSO concentrations up to 10% w/v are biocompatible and do not compromise cell viability or skin integrity (Lönqvist et al., 2020). This study had a final DMSO concentration of 0.4–0.67% v/v in the entire MN formulation, which was less than 2%, to ensure safety while maintaining efficient drug incorporation. The effectiveness of DMSO in maintaining a stable dispersion while preserving drug activity has been corroborated by studies such as Nguyen et al. (2018), which demonstrated superior NP performance when DMSO was used as the dispersion medium.

The final optimised DMNs have all the needles attached to the back layer with sharp tips and consistent geometry, and it was easy to remove from the mould, not brittle, not sticky, or slimy. Thus, they continued to be used for the subsequent evaluations. The accurate drug positioning in DMNs is fundamental to quantifying precisely the drug and for effective utilisation of the drug. Still, it is minimal because of the complexity involved in drug placement during the fabrication process (Wang et al., 2015). Considering safety and optimisation, only a limited number of studies use varied solvents to fabricate the needle and base segments together in one process. To our knowledge, this remains the sole study to incorporate P(3HB-co-4HB) NPs, and thus, there are not many previous studies regarding the dispersion of this particular NP within PVA/PVP polymeric matrix or MNs.

4.5 Characterization of Docetaxel-Loaded P(3HB-*co*-4HB) Microneedles

4.5.1 Geometry and Morphology of Microneedles

The geometry and morphology of MNs have a crucial impact on their functional capabilities, including insertion efficiency, penetration depth, and drug delivery potential. The design parameters of MN arrays, such as the distance between individual needles, their shape, tip diameter, and base diameter, must be thoughtfully optimised to achieve effective skin penetration with minimal discomfort (Makvandi et al., 2021). Figure 4.9 depicts the geometry and morphology related to the MN patch, which consists of multiple MNs aligned in an array of 36 needles arranged in a 6×6 manner.

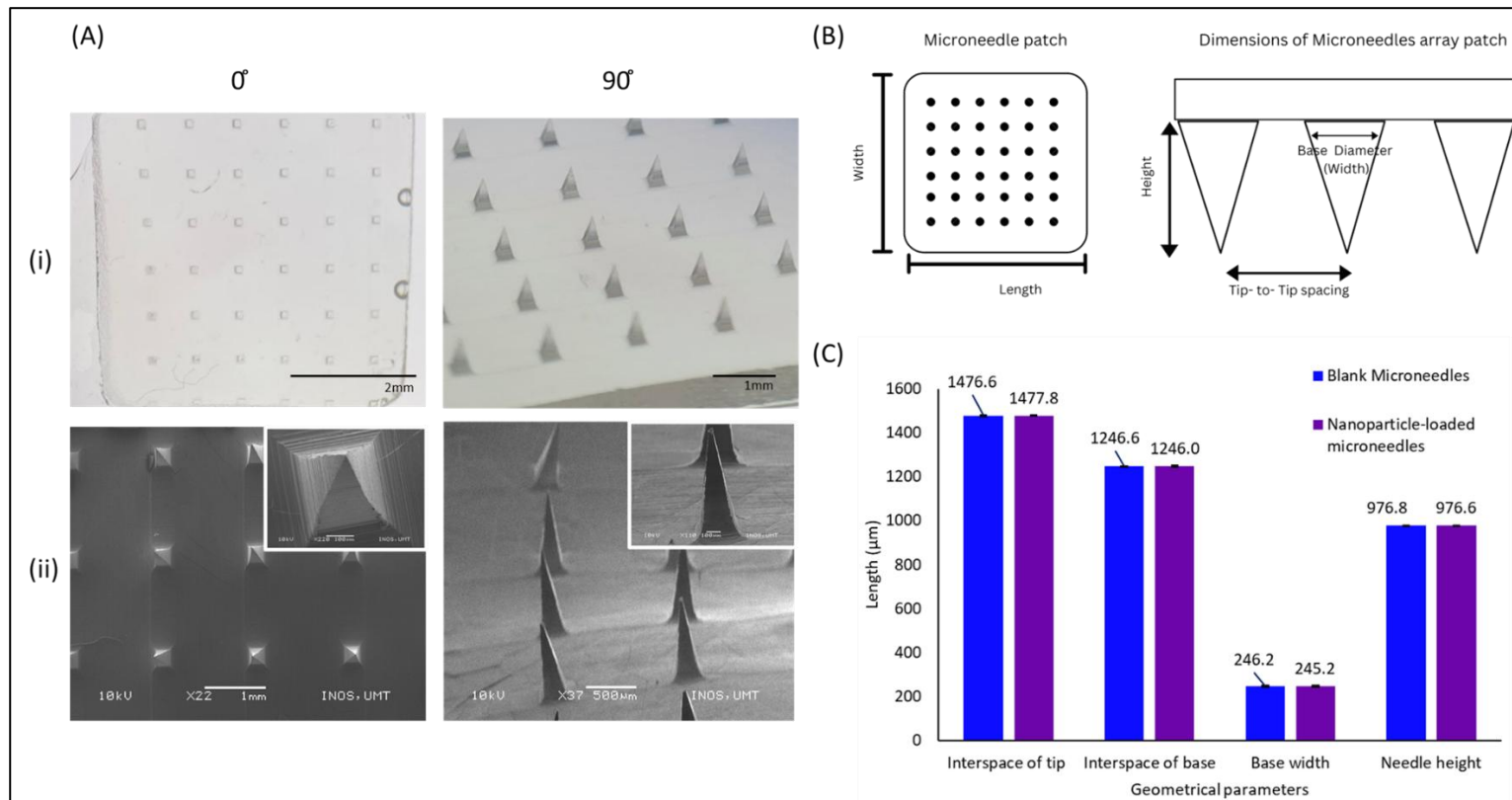


Figure 4.9: (A) Stereomicroscopic images (i) and scanning electron microscopic images (ii) of dissolving microneedles at 0° and 90° views. (B) Schematic representation of a microneedle array and its key geometrical parameters. (C) Comparison of geometrical specifications of dissolving microneedles, including tip-to-tip spacing, base spacing, base width, and needle height.

The schematic (A) shows the stereomicroscopic images, (B) highlights the tip-to-tip spacing, base width, and needle height, which are critical factors influencing penetration efficiency and mechanical strength. The design emphasises consistent inter-needle spacing to avoid the more densely packed needles "bed-of-nails" effect, ensuring successful skin insertion (Makvandi et al., 2021). The optical images at 0° and 90° views reveal a uniform MN array with well-defined tips, showcasing the precision of the fabrication process. The alignment of MNs appears consistent across the patch, minimising variability during insertion. SEM images provide a detailed view of the MN tips, confirming sharp apices essential for efficient penetration. The close-up view further verifies the absence of defects, such as cracks or irregularities, which could compromise the mechanical performance.

The graph in Figure 4.9 (C) shows that the tip-to-tip spacing of the MNs is $1476.6 \pm 3.47 \mu\text{m}$ for blank MNs and $1477.8 \pm 2.74 \mu\text{m}$ for NP-MNs. This ensures sufficient spacing for effective skin penetration while maintaining skin integrity. The base-to-base spacing is $1246.6 \pm 2.19 \mu\text{m}$ for blank MNs and $1246.0 \pm 3.08 \mu\text{m}$ for NP-MNs, contributing to the mechanical stability of the patch. It has also been reported that the highest activation of antigen-presenting cells (APCs) in the epidermis and dermis was observed when the spacing of individual MNs ranged between 1 mm and 1.5 mm (Xiu et al., 2022). This finding supports the suitability of the MN design in this study, as the observed tip-to-tip spacing falls within this optimal range, which may improve immunogenic or therapeutic outcomes. The base width is $246.2 \pm 2.17 \mu\text{m}$ for blank MNs and $245.2 \pm 2.77 \mu\text{m}$ for NP-MNs, which influences the structural strength and ability to withstand compressive forces during insertion. The MNs fabricated in this study are pyramidal, with dimensions optimised to ensure effective skin penetration while minimising pain. The height of the MNs is $976.8 \pm 0.84 \mu\text{m}$ for blank MNs and $976 \pm 1.90 \mu\text{m}$ for NP-MNs.

The measurements between blank and NP-MNs showed no significant differences because both were fabricated using the same solution, method, and mould volume. Using mould produces a uniform length of MNs. However, according to the mould measurement, the MN height should be 1000 μm . Some reduction in height is observed due to shrinkage. Shrinkage due to the drying process is another critical

factor influencing MN morphology. DMNs, such as those made of PVA and PVP, often experience a dimension reduction due to polymer dehydration, which may affect their tip sharpness and penetration efficiency. Previous studies have observed this phenomenon, indicating a 5-10% reduction in dimensions post-drying (Ando et al., 2024). The manufacturing process, including factors like polymer concentration, viscosity, drying temperature, and duration, can influence the extent of shrinkage observed in DMNs (Ando et al., 2024).

Additionally, studies have demonstrated that MNs longer than 600 μm can considerably improve transdermal drug delivery compared to untreated skin (Xiu et al., 2022). One investigation involving 10 healthy participants revealed that pain intensity was directly related to the MN length (Xiu et al., 2022). Nevertheless, another recent investigation highlighted the importance of adequate MN length ($>600 \mu\text{m}$) to overcome skin viscoelasticity and enhance transdermal drug delivery effectively (Ando et al., 2024). In addition, the proportion of APCs located within the epidermis and dermis reduces as the MN length increases (Xiu et al., 2022). APCs such as Langerhans cells in the outer and inner skin layers trigger adaptive immune responses (Pan et al., 2025). Higher APC activation would benefit drug delivery applications, drug permeation, and retention, particularly for transdermal chemotherapy (e.g., DTX-NPs) (Suek et al., 2019).

The aspect ratio is another important aspect, which refers to the comparison between the MN's height and its base diameter. It was calculated to be 3.97 ± 0.37 and can influence mechanical integrity and insertion capability. Higher aspect ratios provide sharper tips, leading to easier skin penetration, but they may compromise structural stability, increasing the chances of breakage during application. In this study, the pyramidal MNs demonstrated a well-balanced aspect ratio that ensured effective penetration while maintaining mechanical robustness. Usually, the tip sharpness of MNs is determined based on their aspect ratio, especially for a standard conical and pyramidal MN (Xiu et al., 2022).

This is because MNs with elevated aspect ratios concentrate stress at their tips, require lower insertion forces, exhibit reduced skin contact areas, and produce minimal skin compression, all of which facilitate effective skin penetration (Xiu et al., 2022). The resistance of polymer MNs to deformation tends to decline with an increase in aspect ratio (Xiu et al., 2022). Moreover, the aspect ratio influences MN volume, consequently impacting their DL capacity (Xiu et al., 2022). The base width has the least effect on the MN efficiency (Xiu et al., 2022). This suggests that, while base width contributes to mechanical strength, other factors such as height, tip sharpness, and inter-needle spacing play a more decisive role in insertion and delivery performance.

In conclusion, optimising the penetration depth and enhancing the drug delivery performance of MNs is attainable by carefully adjusting their length, tip diameter, base diameter, needle geometry, tip configuration, and distance between them (Xiu et al., 2022). Previous studies stated that for a good penetration depth, it has been recommended that the MN's dimensions range from 300–1450 μm in length, with a base diameter of 150 μm and a tip diameter between 10–15 μm (Xiu et al., 2022). The findings of this study are within the optimum range and, thus, promising for good penetration, mechanical strength, and drug delivery.

4.5.2 Drug Content Determination in Microneedles

The amount of drug to be incorporated within the DMNs is essential to ensure an efficient quantity of the drug delivered to the intended site to produce the expected therapeutic outcome and reduce adverse effects. Since DTX-NPs delivered through MNs for BC is a novel study, we had to ensure the precise amount of drug was incorporated. This study also had to make sure the drugs were not underdosed or overdosed, as both had their complications. Figure 4.10 shows the amount of DTX successfully incorporated into the MN patches and compares the DL between the DTX-NPs and the NP-MNs.

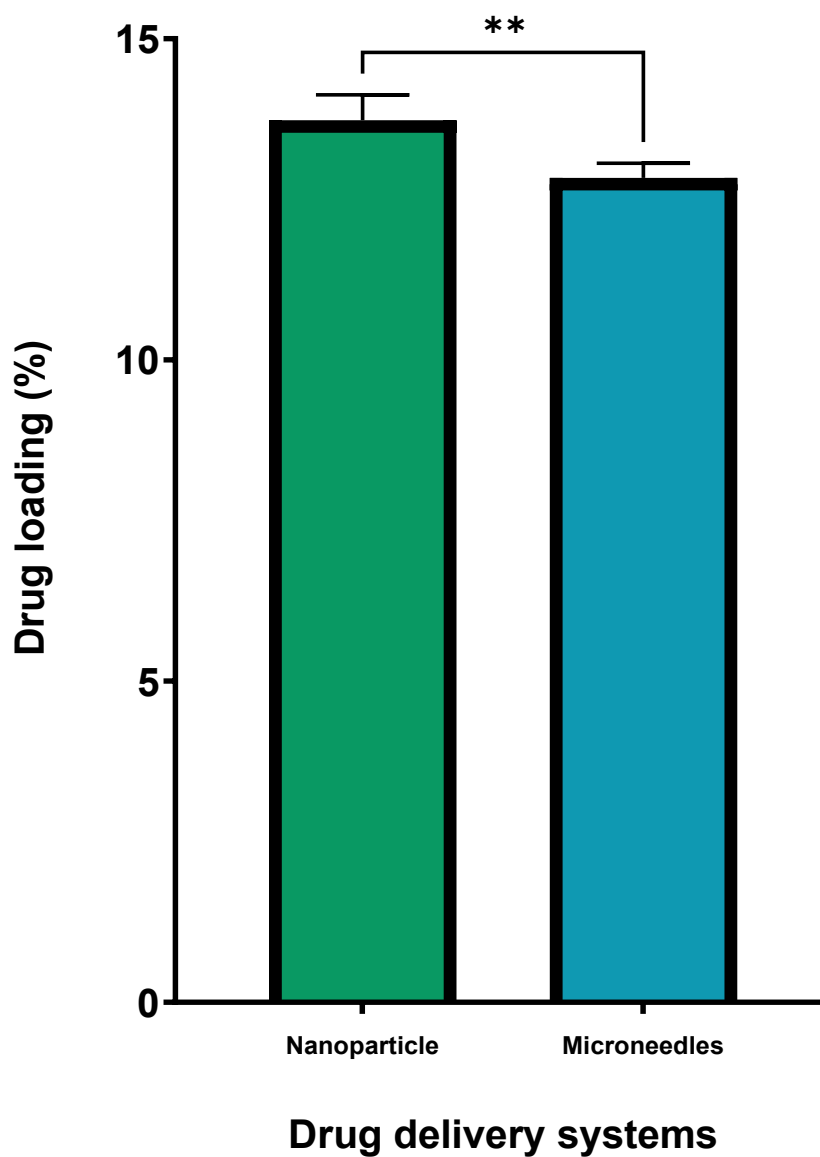


Figure 4.10: Graph of comparison between nanoparticle and microneedles drug loading (%). ($p < 0.01$), as indicated by the paired t-test. Error bars represent the standard error, $n=4$. Asterisks indicate significant differences between groups: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.

The DTX-NPs exhibited a DL of 13.71% and an EE of 87.07%. Thus, based on the calculation, 1.66 mg of lyophilised NPs has to be added to have 227 µg of DTX drug within the MNs. The NPs were incorporated into MNs to target a total drug content of 227 µg per patch based on a previous study (Bhatnagar et al., 2019). The dosage of 227 µg was chosen because the previous study demonstrated the effectiveness of 227 µg of DTX in combination with other drugs for tumour reduction in a 4T1 BC model (Bhatnagar et al., 2019). Using a similar drug dose delivered in pure form but reformulated into an NP-based system addresses a research gap identified in the previous work.

BC patients are typically administered doses ranging from 60–100 mg/m² of DTX (Bhatnagar et al., 2019). Therefore, developing MNs capable of carrying comparable payloads would be ideal (Bhatnagar et al., 2019). Nevertheless, the combined weight of the 36 MNs forming the patch amounts to approximately 2.5 mg, while the drugs should be more concentrated at the tips for efficient delivery (Bhatnagar et al., 2019). Earlier investigations have also consistently indicated that micro-dosing would be a better strategy for delivering chemotherapeutic drugs (Nguyen et al., 2018; Bhatnagar et al., 2019). Based on previous studies, the MNs were formulated at a polymer-to-drug ratio of 8:2, and exceeding this weight ratio compromises the formation and strength of the PVA/PVP MNs. (Bhatnagar et al., 2019). The layer thickness used in coating and the size attributes of the needles significantly affect the DL capacity (Yang et al., 2021).

The fabricated MNs revealed a slight reduction in drug content, with an average measured value of 212.93 µg/patch (representing a loss of 6.2%). This discrepancy may be attributed to drug loss during fabrication, such as adsorption to the mould surface, diffusion during solvent evaporation, or degradation due to process conditions (Koenitz et al., 2024). Studies by Peng et al. (2021) highlight similar challenges, noting drug losses of up to 10% in MN systems. Drug loss observed during MN fabrication is a common challenge as it involves manual work, which invites human error in loading the drug within the moulds and involves a series of steps. Optimisation of mould coatings or alternative drying techniques could help mitigate these losses in future work.

4.5.3 Drug Distribution Assessment

The distribution of DTX-NPs within the DMNs was analysed using a fluorescence microscope. Since DTX does not naturally have a fluorescence property, DTX was replaced with coumarin-6 as a model drug, as it is also hydrophobic and can mimic its characteristics (Tran et al., 2021; Zhukova et al., 2021). The coumarin-6 incorporated P(3HB-*co*-4HB) NPs were synthesised using the same method as DTX-NP fabrication and incorporated within the MN using the same fabrication method. The fluorescence analysis in Figure 4.11 shows how coumarin-6-incorporated P(3HB-*co*-4HB) NPs distribute throughout the MN matrix. The results illustrate that NP deposition is non-uniform, with notable differences in distribution along the MN length. The intensity profile (A) and quantified bar metrics (B) confirm higher accumulation at the tip and middle regions compared to the base. The schematic illustration in (C) contextualises this distribution, depicting how concentration gradients can enhance drug diffusion and transdermal delivery efficiency. The fluorescence images in (D) visually validate NP presence, particularly at the tip and shaft, correlating well with the quantitative findings.

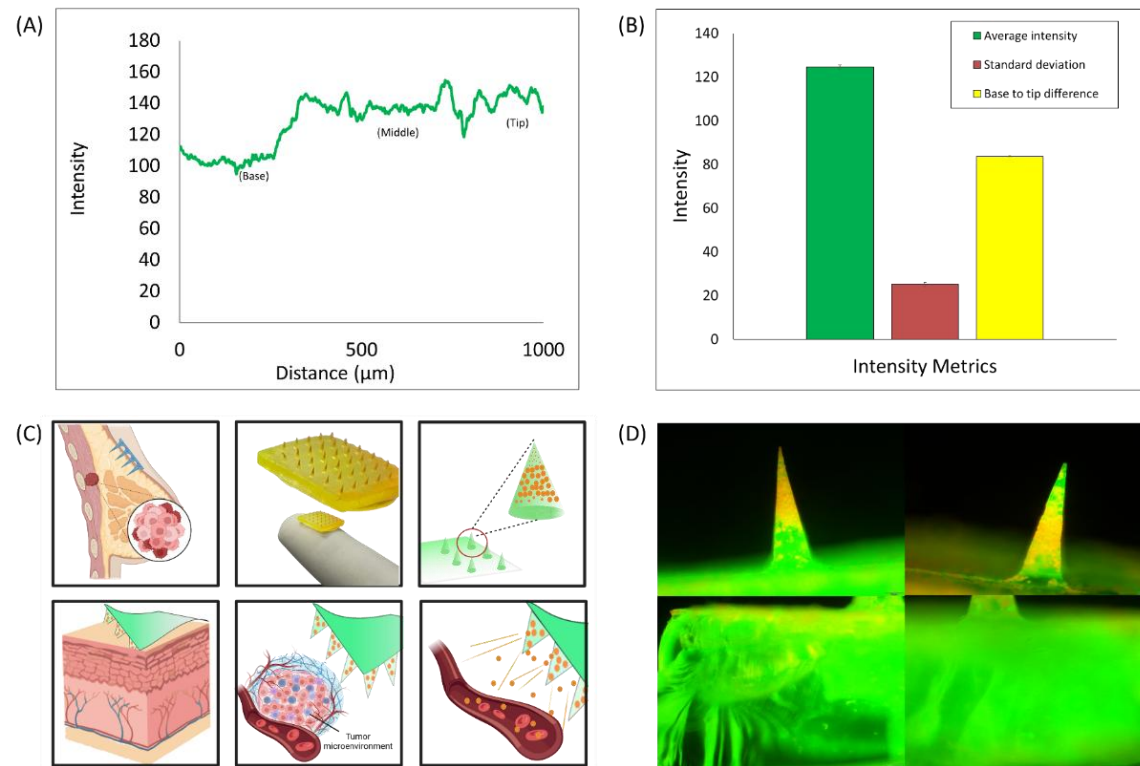


Figure 4.11: Drug distribution and intensity profile of microneedles. (A) The fluorescence intensity graph of nanoparticle distribution within microneedles, measured from base (0 μm) to tip (1000 μm). (B) Intensity metrics (average intensity, standard deviation, and the base-to-tip difference) of nanoparticle distribution within microneedles. (C) Schematic representation of nanoparticle distribution and its effects on drug delivery. (D) Fluorescence images of microneedles. Data represent mean values ($n=3$), with error bars indicating a standard error.

The graph in Figure 4.11A shows the higher NP distribution at the needle's tip and middle part, proving an effective localisation of the drug. It is deemed desirable for MN-based drug delivery as it can ensure efficient penetration of the drug at the tips. It will ensure less wastage of drug residues on the skin's surface (Peng et al., 2021). The bar chart in Figure 4.11B summarises the intensity metrics: average, standard deviation, and base-to-tip intensity differences. The high average intensity represents the overall drug distribution within the MNs, while the low standard deviation shows the consistency of the uniform NP distribution across the needle length. The high base-to-tip differences (83.73%) indicate that the concentration gradient between the tip and base is high, meaning NPs are more concentrated at the tip than at the base.

Figure 4.11(C) clearly illustrates the concept of NP-MNs penetrating the skin, with arrows indicating the diffusion gradient from the tip (highest concentration) toward the base (lowest concentration). This schematic complements the quantified results by visually representing how the deposition at the tip enhances initial drug release, followed by gradual release from the middle and base regions as the MN dissolves through the skin layers.

In the microscopic images of Figure 4.11D, the yellow spheres are the NPs, and the back layer does not contain NPs but is also green fluorescent due to the staining of the coumarin-6 in the PVA/PVP polymer solution during casting. This allows for more effective targeting. Since the NPs are more concentrated in the middle of the needles and then only the tips and the lowest at the base, this also will provide a more sustained release of the NPs as the needle tips will dissolve fast, so the NPs at the middle with high concentration will release slower than the tips and the fewer NPs at the base will release even slower.

This study's findings confirm that the MN's fabrication method ensures that most of the NPs are effectively deposited at the tip of the MNs. Centrifugation in the fabrication process is crucial to achieve this outcome, and the rotation speed (rpm) and duration must be optimised to prevent NPs from spilling out of the mould, which

would result in wastage (Mönkäre et al., 2018). This observation is confirmed by previous research indicating that centrifugation drives the dense polymer solution into the MN cavities through centrifugal force (Zhu et al., 2016). The application of a highly viscous polymer mixture during the second casting step, followed by an appropriate drying process, is vital to ensure that the drugs or NPs are compacted and concentrated towards the needle shafts, thereby minimising diffusion (Zhu et al., 2016), which was implemented in this study. Overall, the outcomes validate that the NP-MNs fabricated in this study effectively achieve uniform drug distribution with enhanced concentration at the tip, aligning with the intended design for transdermal drug delivery systems.

4.5.4 Mechanical Strength Evaluation

The mechanical strength of DMNs is a critical factor for various clinical uses, as it directly influences their capability to penetrate the skin effectively (Yan et al., 2021; Xiu et al., 2022), as the mechanical strength significantly impacts how well the MNs can pierce the skin barrier (Zhang et al., 2024). In this study, the mechanical strength of the MNs was evaluated using two methods: compression mode using a texture analyser (Figure 4.12) and static force application with a range of weights from 50 g to 1000 g (Figure 4.13 and Figure 4.14). The reason for using both methods was to comprehensively assess the durability and mechanical behaviour of the MNs under varying types of stress that simulate real-world application scenarios. The results revealed critical insights into the performance differences between blank MNs and NP-MNs.

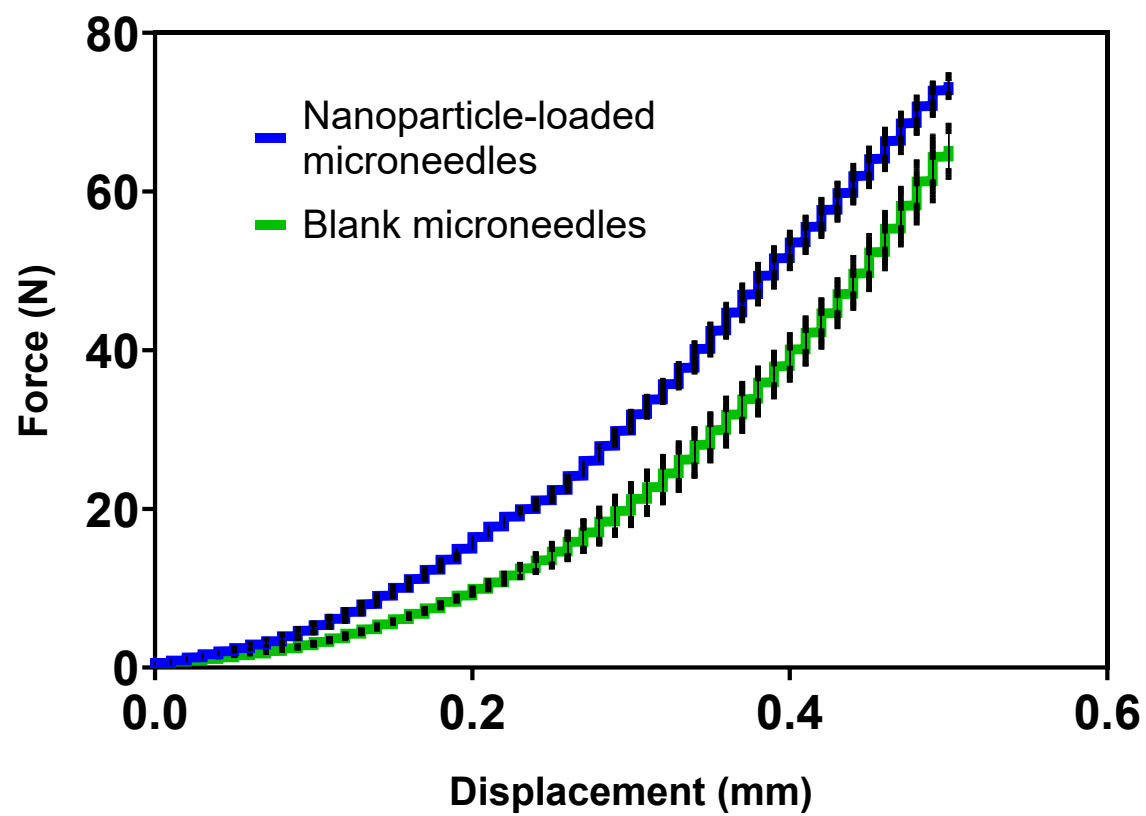


Figure 4.12: Force–displacement profile of nanoparticle-loaded microneedles and blank microneedles obtained using a texture analyser in compression mode. Error bars represent the standard error of the mean from $n = 3$.

It is evident from the graph that the NP-MNs exhibit a higher force-displacement curve compared to the blank MNs, indicating enhanced mechanical properties. At each displacement increment (100–500 μm), the NP-MNs demonstrated greater resistance to compression, requiring higher forces for deformation. This suggests that incorporating NPs into the DMNs significantly increased their stiffness and structural integrity. The force required to achieve a specific displacement was consistently higher for the NP-MNs, with a clear separation between the curves. These force-displacement values were obtained using a texture analyser operated in compression mode. The fracture point for the NP-MNs was 73.67 N, which is 2.05 N per needle, and 66.72 with 1.85 N per needle. The results from the mechanical force testing system indicated that incorporating NPs enhanced the mechanical strength of the DMNs, increasing it from 0.26 N to 1.05 N at a displacement depth of 600 μm (Xiu et al., 2022), which is similar to the results obtained in this study. Typically, dissolvable or degradable polymer MN arrays require a static or quasi-static insertion force ranging from 0.022 N to 0.16 N per individual needle, which depends on both the material used for MN fabrication and the characteristics of the targeted skin (Xiu et al., 2022).

The MNs, composed of dissolvable polymers, exhibit viscoelastic behaviour influenced by the strain rate applied during mechanical testing. This viscoelasticity can result in non-linear or step-like features in the force-displacement curves, reflecting the material's time-dependent response to loading (Zhang et al., 2023).

Besides that, based on the images in Figure 4.13, the static forces ranging from 50 to 1000 g were placed on the MNs. The height changes of the needle before and after were calculated, and the percentage of height reduction was plotted, as seen in Figure 4.14.

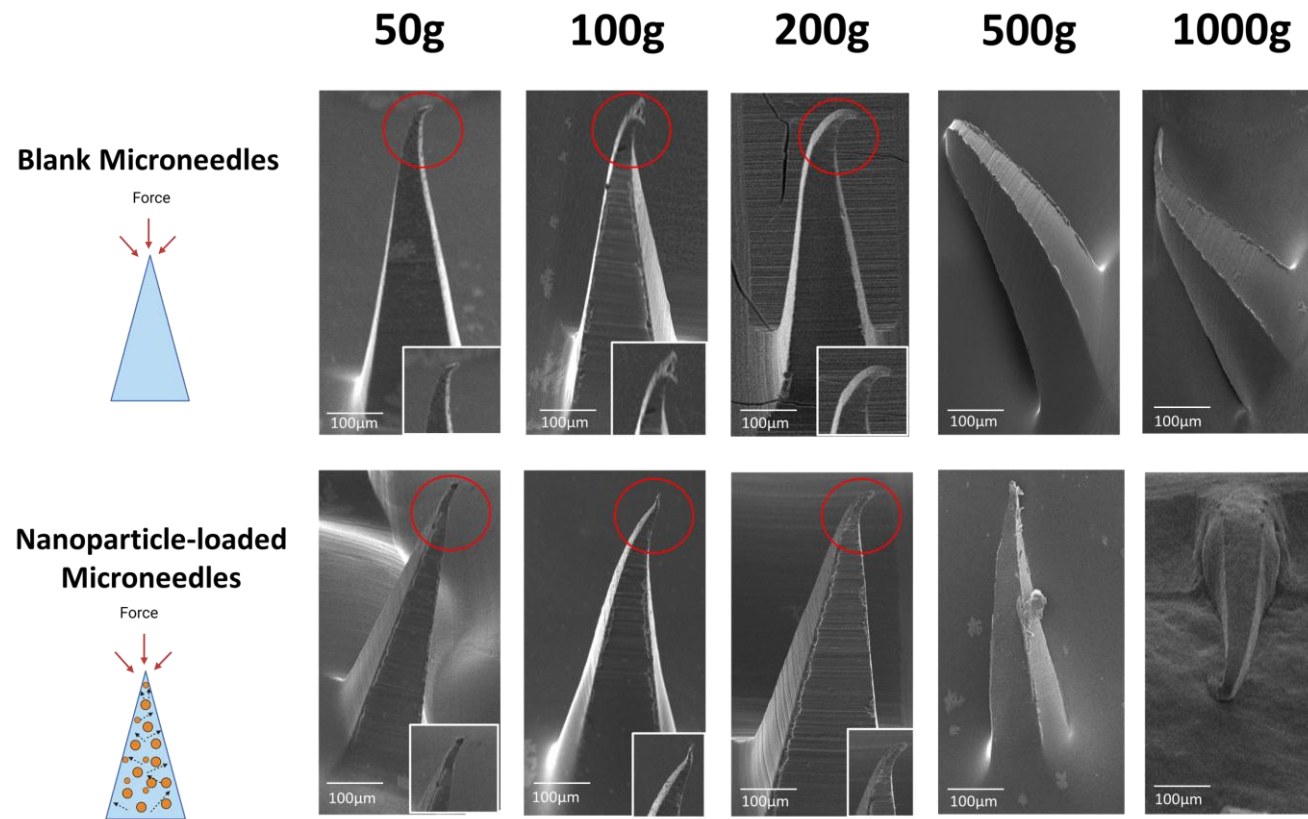


Figure 4.13: SEM images of blank microneedles (top row) and nanoparticle-loaded microneedles (bottom row) subjected to increasing static weights (50 g, 100 g, 200 g, 500 g, and 1000 g from left to right). The insets highlight the deformation and fracture of the microneedle tips (circled in red) under applied forces. All images were captured at the same magnification, and the scale bar represents 100 μm .

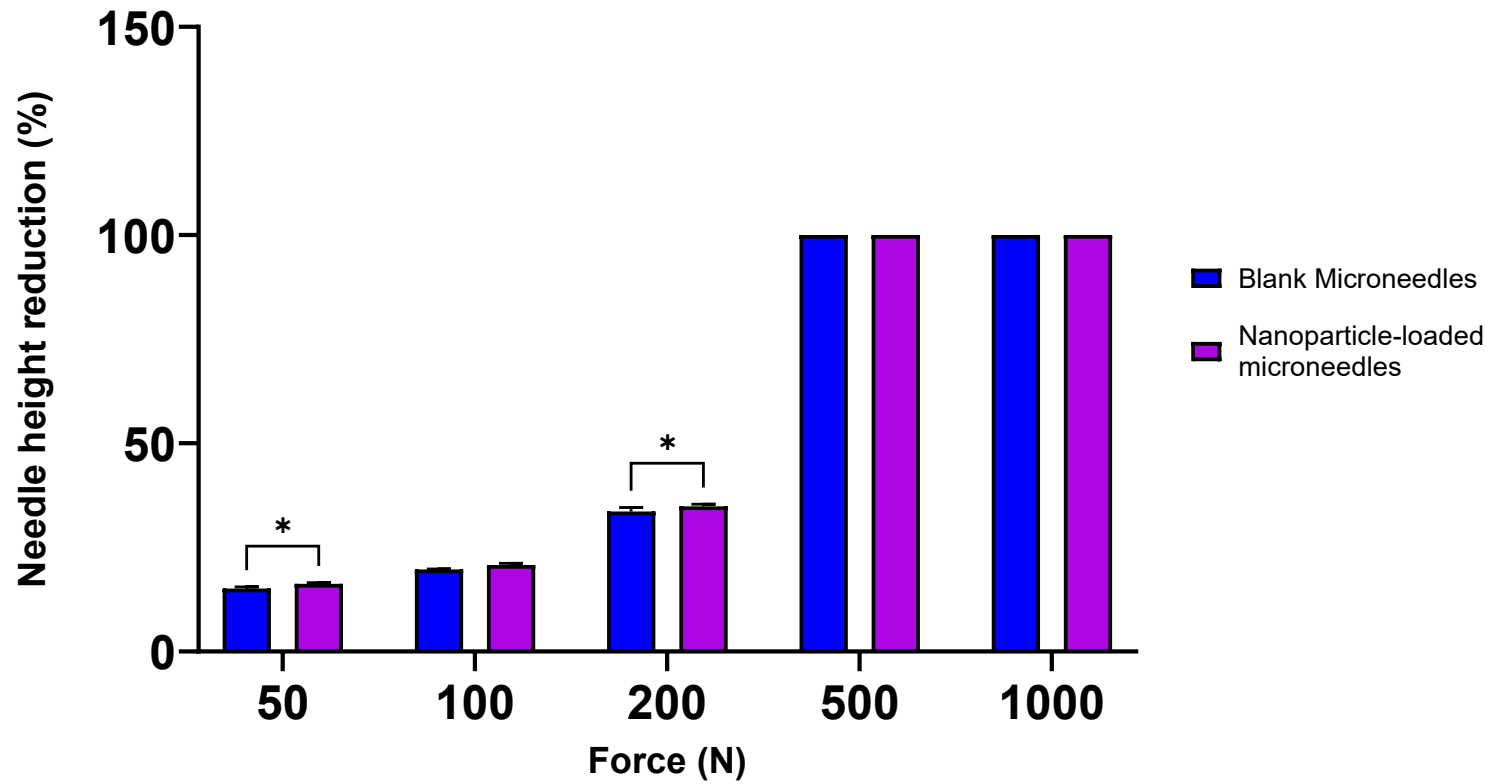


Figure 4.14: Needle height reduction (%) of microneedles under static forces (50 N, 100 N, 200 N, 500 N, and 1000 N). Error bars represent the standard error of the mean ($n = 3$). Asterisks indicate significant differences between groups: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.

After placing the weights, the SEM images of the needles also provided critical insights into the MN's surface morphology and structural changes post-testing. The results demonstrate a progressive increase in MN height reduction with increasing applied load. The MNs exhibited minimal deformation at a compressive force of 50 g, with a height reduction of approximately 15-16%. This indicates sufficient structural integrity to withstand lower forces. At 100 g and 200 g, the MN height reduction increased to 20%. Under higher loads of 500 g and 1000 g, the MN height reduction reached 100%, indicating significant structural deformation.

The MNs were pressed against the back layer. A significant difference was observed between blank and NP-MNs at 50 g and 200 g. The SEM images associated with each load visually confirm the extent of deformation, with the MNs maintaining sharp tips under lower forces but exhibiting severe bending or fracture at higher forces. These results indicate that the MNs can maintain their structural integrity at forces up to 200 g, within the range required for skin penetration, as reported in the literature. Most of the needles did not break the tips but were somewhat bent because MNs with less mechanical strength may buckle, break, or bend when inserted into the stratum corneum (Makvandi et al., 2021; Zhang et al., 2024).

The distribution of NPs within the needles also creates an outward force, balancing the force from the weight placed on top and reducing the proneness of tips to breakage or deformation, as illustrated in the figure above. MNs fabricated using substances with high mechanical durability and decreased brittleness, like PVA, typically experience substantial plastic deformation prior to fracturing, making them more susceptible to bending or buckling when penetrating the skin (Zhang et al., 2022). However, in this study, we have added PVP to the PVA, so the link between these two polymers would only further enhance the mechanical robustness of the MNs. Prior reports revealed that when MNs were subjected to a pressure of 100 N for 5 seconds, the remaining height of the MNs exhibited a ranking pattern, with pyramidal MNs exhibiting greater mechanical strength than conical MNs (Zhang et al., 2024).

The mechanical features of MNs are influenced by multiple factors, including the inherent characteristics of the selected polymer, the composition, formulation of the materials, and the specific structural design of the MNs (Makvandi et al., 2021; Zhang et al., 2024). The force necessary for MN penetration into the skin ranges between 3.183 MPa and 1.6 MPa (Zhang et al., 2024). The stratum corneum, having a dense brick-wall-like structure, increases the resistance of the MN face during insertion (Zhang et al., 2024). Therefore, it is crucial to ensure adequate mechanical strength of MNs. MNs will remain mechanically stable, effectively resisting stress and preventing breakage during skin insertion if the applied force or insertion pressure is insufficient compared to the two required critical forces.

Recently, detailed theoretical investigations have been carried out to better comprehend how these factors influence the mechanical properties of MNs (Ando et al., 2024; Zhang et al., 2024). Nevertheless, an extensive review that discusses systematic strategies for improving the mechanical durability of MNs is still lacking (Zhang et al., 2024). Consequently, existing approaches rely heavily on extensive yet unsystematic optimisation of material formulations and geometric designs (Zhang et al., 2024). Overall, the results of this analysis prove that the DMNs incorporated with DTX-NPs in this study have the optimal and adequate mechanical integrity to pierce through the skin tissues and to avoid tip breakage as well as deliver the NPs to the targeted area efficiently.

4.5.5 FTIR Spectroscopy Interpretation of Microneedles

FTIR spectroscopy was employed to analyse the chemical structure, functional groups, and molecular interactions within the NP-MNs, with supporting comparisons to the DTX-NPs, the P(3HB-*co*-4HB) polymer, and the free DTX drug itself. Figure 4.15(A) presents the FTIR spectra of blank and NP-MNs, while Figure 4.15(B) includes the comparative spectra of DTX, P(3HB-*co*-4HB), DTX-NPs, and both types of MNs. The characteristic peaks of all components are summarised in Table 4.6.

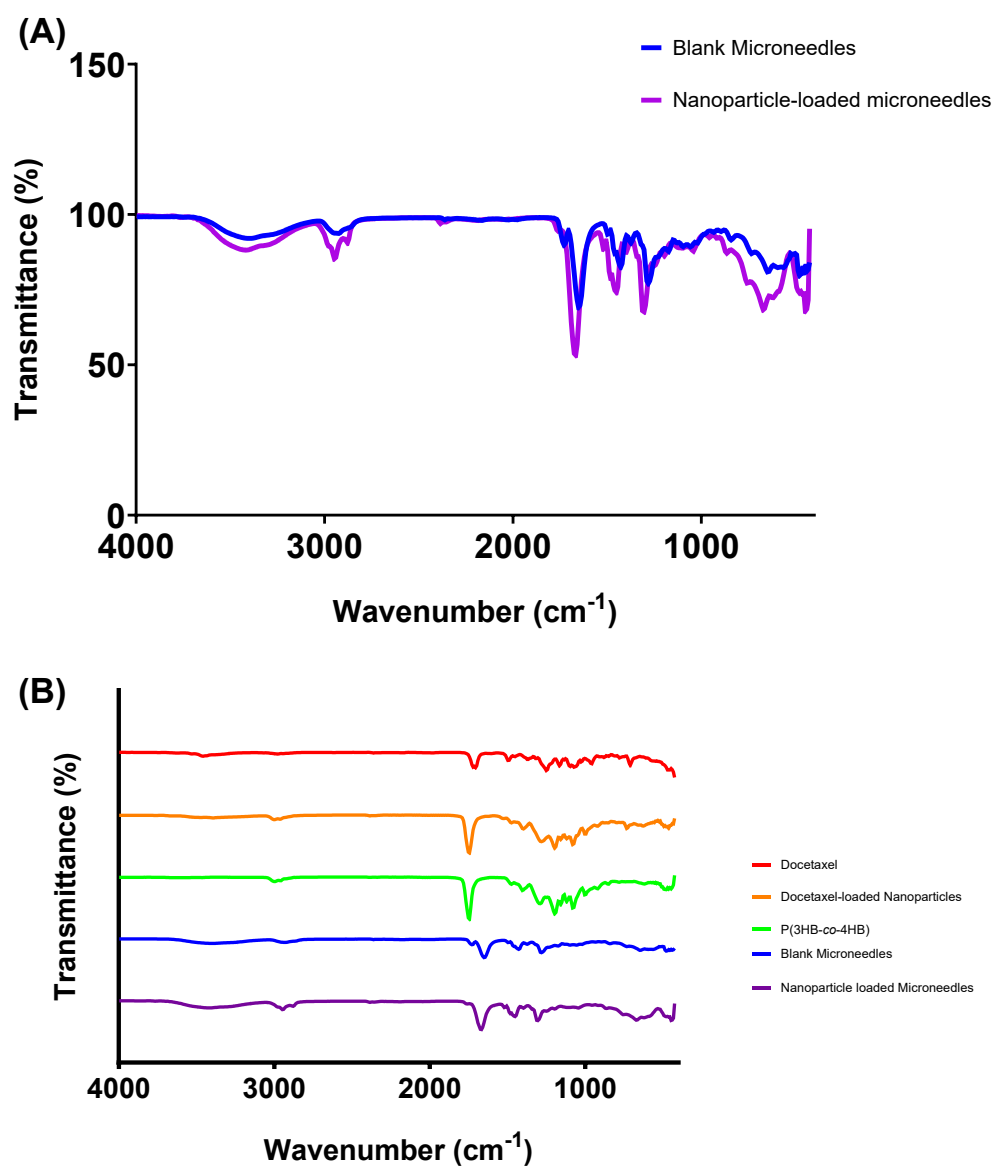


Figure 4.15: Fourier transform infrared (FTIR) spectra of (A) blank and nanoparticle-loaded microneedles and (B) comparative analysis of FTIR spectra for docetaxel, docetaxel-loaded nanoparticles, P(3HB-co-4HB), blank and nanoparticle-loaded microneedles.

Table 4.6: Main characteristic FTIR peaks of dissolving microneedles (DMNs with PVA/PVP blend), docetaxel (DTX), docetaxel-loaded nanoparticles (DTX-NPs), and P(3HB-*co*-4HB), along with their corresponding functional groups and bond assignments.

Peaks (cm ⁻¹)	Bonds
DMNs (PVA/PVP blend)	
3250-3600	O–H stretching (hydroxyl group)
2800-2950	Symmetric and asymmetric C–H stretching (CH ₂ and CH ₃)
1550-1650	C=O stretching (pyrrolidone ring in PVP)
1322-1550	CH ₂ vibration wagging
1300-1425	C-N bending movements.
1290- 1450	CH group deformation
1048-1265	C–C and C=O stretching vibrations (PVA backbone)
DTX	
3466.49- 3294.44	(N–H and O–H stretching)

	2978.09	(C–H stretching)
	2360.87	Asymmetric and symmetric C–H vibrations (aromatic rings)
	1705.07-1496.76	C=O vibration
		C=C stretching vibration (aromatic rings)
	1496.76-1072.42	C=C stretching
	1165.00-709.80	C–H bond out of plane (outside benzene ring)
DTX-NP		
	3533.49-3560.44	(N–H and O–H stretching)
	2978.07	Asymmetric and symmetric C–H vibrations
	1720.50	C=O group (carbonyl ester)
	1615.19	C=O vibration
		C=C stretching vibration (aromatic rings)
	1373.32 -1056.99	C=C asymmetric stretch

1200 -700	C-H bonding
P(3HB- <i>co</i> -4HB)	
2980.51	CH ₃ asymmetric stretching
2938.44	C-H ₂ stretching
1725.72	C=O group (carbonyl ester)
1456.91-1049.59	C-O-C stretching
978-826	C-H bending

In the MN spectrum shown in Figure 4.15(A), the NP-MNs displayed visible shifts and variations in peak intensity and sharpness when compared to the blank MNs. These changes suggest chemical interactions between the incorporated NPs and the MN matrix. Specifically, a peak within the 2938.44–2980.51 cm^{-1} range, corresponding to CH_3 and CH_2 stretching vibrations in P(3HB-*co*-4HB), was absent in the blank MNs but appeared clearly in both the NPs and NP-MNs. This indicates that the characteristic backbone of the polymer remained detectable even after encapsulation and integration into the MN system. Similarly, the $\text{C}=\text{O}$ stretching vibration around 1725–1720 cm^{-1} , found in both DTX and the polymer, underwent shifts and intensity reductions across the DTX-NPs and NP-MNs, reflecting molecular-level interactions during drug loading and incorporation.

These findings are supported by the presence of characteristic functional groups of both PVA and PVP in the MN spectrum. For PVA, peaks include O–H stretching (3250–3600 cm^{-1}), C–H symmetric stretching (2867–2943 cm^{-1}), CH_2 vibration wagging (1440–1550 cm^{-1}), and C–C/ $\text{C}=\text{O}$ backbone stretching (1048–1265 cm^{-1}). For PVP, relevant bands are observed for $\text{C}=\text{O}$ stretching of the pyrrolidone group (1600–1650 cm^{-1}), asymmetric CH_2 stretching (2800–2950 cm^{-1}), C–N bending (1300–1425 cm^{-1}), and CH deformation (1290–1450 cm^{-1}). Notably, the broad band found at 1643 cm^{-1} in the NP-MNs, which corresponds to the $\text{C}=\text{O}$ group of PVP, was slightly shifted from 1632 cm^{-1} in the blank MNs. This shift is indicative of hydrogen bonding between the $\text{C}=\text{O}$ of PVP and the OH group of PVA, and also suggests additional interaction with the NP matrix. Such shifts have been widely reported in previous studies as evidence of intermolecular interactions and plasticising effects within polymeric MN systems (Tekko et al., 2020; Zafar et al., 2022).

To further confirm these interactions, the FTIR spectra of the DTX-NPs provide important intermediate insight. These spectra display combined features of both the drug and the polymer, with several clear peak shifts indicating molecular-level interactions. The strong ester carbonyl peak ($\sim 1720.50 \text{ cm}^{-1}$) in the NPs, consistent with the P(3HB-*co*-4HB) polymer (1725.72 cm^{-1}), confirms the retention of the polymer backbone during NP formulation. This ester peak remained identifiable in the MNs, albeit slightly shifted and reduced in intensity, which further supports the

successful incorporation of NPs into the MN matrix. Additionally, peaks around 1049.59 cm^{-1} in the NP spectrum, attributed to C–O–C stretching, were also observed in the final MNs, indicating the persistence of the polymer's amorphous regions.

DTX itself displayed characteristic FTIR peaks between $3466\text{--}3294\text{ cm}^{-1}$ (N–H and O–H stretching), $1705\text{--}1496\text{ cm}^{-1}$ (C=O vibration and C=C aromatic ring stretching), and $1165\text{--}709\text{ cm}^{-1}$ (C–H out-of-plane vibrations). Although reduced in intensity, these peaks remained visible in the NP spectrum, confirming the drug's presence and physical encapsulation within the polymer. Key aromatic and CH stretching peaks also persisted throughout the formulation process (Albano et al., 2019; Al Saqr et al., 2021; Tariq et al., 2024).

Lastly, the FTIR spectrum of the pure P(3HB-*co*-4HB) polymer (Figure 4.15) served as a benchmark for interpreting the molecular structure of the drug delivery system. A strong peak at 1725.72 cm^{-1} confirmed the presence of ester bonds, which are characteristic of short-chain-length PHAs. Peaks at 2980.51 and 2938.44 cm^{-1} corresponded to CH_3 and CH_2 stretching, while bands at $1456.91\text{--}1049.59\text{ cm}^{-1}$ and $978\text{--}826\text{ cm}^{-1}$ represented C–O–C stretching and C–H bending, respectively. Additional peaks at 1269.15 and 1175.75 cm^{-1} associated with ester C–O stretching further confirmed the copolymer's structure. These peaks were retained in both the NP and MN spectra, albeit with some shifts and changes in intensity, supporting the conclusion that the polymer's chemical backbone remained intact while interacting with the drug and MN matrix.

Overall, the comparative FTIR analysis reveals that the major functional peaks of PVA, PVP, P(3HB-*co*-4HB), and DTX are preserved across the formulation process, although notable shifts and broadening of certain bands occurred due to intermolecular interactions. These spectral modifications indicate successful encapsulation of DTX within the P(3HB-*co*-4HB) NPs and confirm their subsequent integration into the MN matrix. The preserved peak positions along with intensity reductions and band shifts provide strong evidence of physical entrapment and structural compatibility among the hydrophilic and hydrophobic components,

validating the functional and chemical integration of the complete drug delivery system.

4.5.6 XRD Structural Analysis of Microneedles

X-ray diffraction (XRD) analysis was carried out to evaluate the structural characteristics of the NP-MNs, with detailed comparisons to the DTX-NPs, the P(3HB-*co*-4HB) copolymer, and the free DTX drug. Figure 4.16 displays the overlaid diffractograms, while Table 4.7 summarises the major peak positions and corresponding d-spacing values. The NP-MNs exhibited two broad diffraction humps, one ranging from 9° to 16° and another from 19° to 25° 2θ , suggesting a semicrystalline to predominantly amorphous structure. These broad halos indicate disrupted crystallinity and molecular dispersion, likely due to the presence of both the hydrophilic PVA/PVP matrix and the embedded NPs.

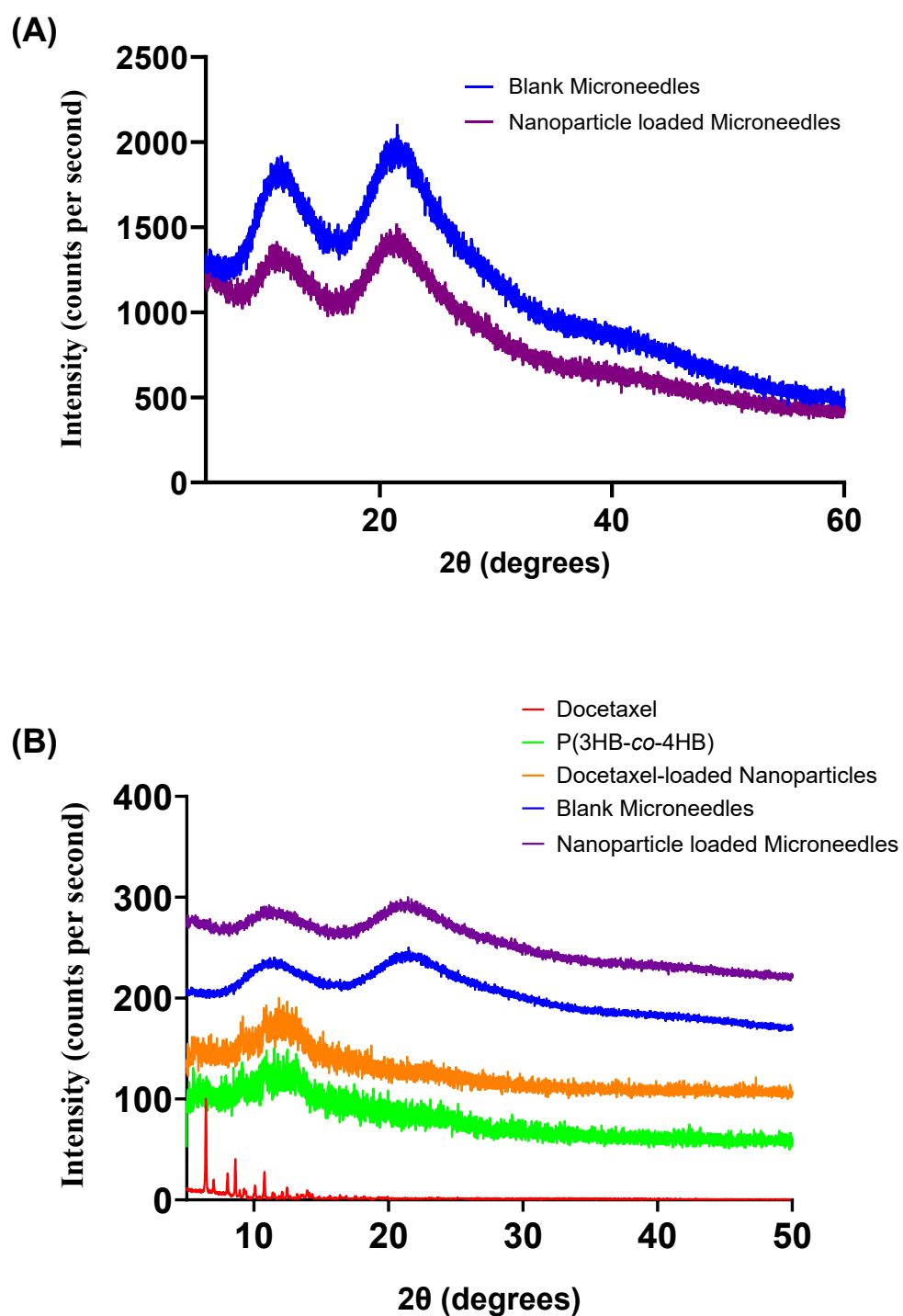


Figure 4.16: X-ray diffraction (XRD) patterns of (A) blank and nanoparticle-loaded microneedles and (B) a comparative analysis of docetaxel, P(3HB-co-4HB), docetaxel-loaded nanoparticles, blank microneedles, and nanoparticle-loaded microneedles.

Table 4.7: Peak intensity and d-spacing values of blank and nanoparticle-loaded microneedles, docetaxel, docetaxel-loaded P(3HB-*co*-4HB) nanoparticles, and P(3HB-*co*-4HB)

Peak intensity	d-spacing
Blank MNs	
11.06	7.99
12.01	7.36
13.04	6.78
19.01	4.66
19.06	4.65
21.01	4.22
22.04	4.03
25.90	3.44
NP-MNs	

	11.11	7.96
	12.13	7.29
	13.04	6.78
	19.2	4.62
	21.01	4.22
	22.07	4.02
	25.93	3.43
DTX		
	6.57	13.45
	8.22	10.75
	9.06	9.75
	11.42	7.74
	12.47	7.09

	13.06	6.77
	14.08	6.28
	15.47	5.72
	17.6	5.04
	19.23	4.61
DTX-NP		
	6.56	13.46
	8.23	10.74
	9.05	9.76
	11.41	7.75
	12.46	7.09
	13.05	6.78
	14.07	6.29
	15.46	5.73

	17.59	5.04
	19.22	4.61
P(3HB- <i>co</i> -4HB)		
	13.05	6.79
	17.02	5.20
	25.22	3.55
	27.09	3.23
	28.4	3.14

Compared to the blank MNs, the NP-MNs demonstrated subtle but important changes in peak intensity and position, particularly within the 9° - 16° region. This region overlaps with known reflections of both P(3HB-*co*-4HB) and PVA, suggesting composite interactions. The broadening and smoothing of peaks in the MN formulation relative to the sharper diffraction profiles of the raw polymer and NPs indicate intermolecular interactions and partial disruption of crystalline domains. Specifically, PVA typically shows characteristic peaks at $2\theta \approx 11.7^{\circ}$, 13° , and 19° - 21° , which were visible in both blank and NP-MNs. Meanwhile, PVP exhibits weaker, broader peaks around $2\theta \approx 10^{\circ}$ and 20° , consistent with its amorphous nature (Teodorescu et al., 2019; Li et al., 2024). The presence of fused and broadened peaks in the MN spectra, especially in regions corresponding to crystalline PVA and P(3HB-*co*-4HB), reflects successful mixing and possible molecular-level dispersion of NPs within the matrix.

These observations are supported by the XRD pattern of the DTX-NPs, which demonstrates broader and less intense peaks compared to the sharp reflections seen in pure DTX. Pure DTX exhibited intense diffraction peaks at 2θ values between 6° and 20° , including strong reflections at 6.46° , 9.06° , 13.06° , and 17.51° , confirming its highly crystalline orthorhombic structure (Thambiraj et al., 2019; Albano et al., 2019; Al Saqr et al., 2021). Upon encapsulation into P(3HB-*co*-4HB), these peaks were significantly reduced in intensity and broadened, although the corresponding d -spacing values remained similar. This confirms that while short-range order was partially retained, the long-range crystalline order was disrupted, suggesting the formation of a metastable amorphous state. The polymer matrix interferes with the drug's regular lattice, allowing molecular dispersion while stabilising the drug in an amorphous solid dispersion (ASD)-like form (Shaikh et al., 2016; Al-Nemrwai et al., 2024).

Furthermore, the diffraction pattern of the P(3HB-*co*-4HB) copolymer alone showed a broad halo centred at 20° 2θ with no prominent sharp peaks, indicating a predominantly amorphous structure. However, minor peaks at 13.03° , 17.00° , 25.04° , and 27.04° were detected, corresponding to the (020), (110), (121), and (040) planes of the orthorhombic lattice of P(3HB), respectively (Rennukka et al., 2014; Vishnu

Chandar et al., 2017). The partial crystallinity observed in the polymer arises from its semi-crystalline nature, which varies depending on the copolymer composition and synthesis conditions. As reported in prior studies, the crystallinity of P(3HB) can be as high as 70–80%, but the incorporation of 4HB monomers significantly reduces crystallisation, favouring the formation of amorphous domains (Lakshmanan et al., 2019). This increased amorphousness not only enhances mechanical flexibility but also promotes hydrolytic degradation, drug diffusion, and molecular dispersion properties highly beneficial for biomedical applications such as transdermal drug delivery (Vodicka et al., 2022; Wilson et al., 2020).

The semicrystalline nature of the PVA/PVP MN matrix, when combined with the amorphous characteristics of the NPs, led to a fused diffraction pattern with diminished sharp peaks and broadened humps in the NP-MNs. These spectral features confirm the homogeneous integration of the NPs and their structural compatibility with the MN matrix. The disappearance of sharp drug peaks, the shift of polymer peaks, and the transformation of distinct NP reflections into broader regions are all indicative of molecular-level interactions and dispersion. Moreover, the incorporation of the drug in an amorphous state is expected to enhance solubility and bioavailability, especially for class IV compounds like DTX, which suffer from poor solubility and permeability (Sohail et al., 2018; Nora et al., 2022). The amorphous state achieved within the MN system mimics that of ASDs, offering improved drug release and stability over time while preventing recrystallisation through matrix entrapment (Narula et al., 2025; Shi et al., 2019).

In conclusion, the XRD analysis confirms that the NP-MNs possess a semicrystalline to amorphous nature, resulting from the structural integration of DTX-NPs into a hydrophilic PVA/PVP matrix. The progressive reduction in peak intensity from pure DTX to NPs to MNs reflects successful encapsulation, dispersion, and polymer–drug interactions. These findings validate the effectiveness of the MN system as a delivery platform and support the role of P(3HB-*co*-4HB) as a suitable carrier for poorly soluble drugs like DTX, providing structural and functional advantages in transdermal drug delivery applications.

4.5.7 Thermal Stability of Microneedles

Thermal analysis using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) was conducted to evaluate the thermal stability, transition behaviour, and structural integrity of the NP-MNs, with comparative reference to the P(3HB-*co*-4HB) copolymer, DTX-NPs, and blank MNs. These results, summarised in Table 4.8, provide insight into the thermal transitions and degradation behaviour across each formulation stage.

The NP-MNs exhibited multiple thermal events in the DSC thermogram, with melting transitions (T_m) at 271.72°C, 376.61°C, and 467.04°C. In contrast, the blank MNs displayed a single broad endothermic peak at 406.68°C. The presence of multiple transitions in the NP-loaded formulation suggests interactions between the NPs and the MN matrix, possibly due to structural rearrangements and differences in thermal response between the NP core and the hydrophilic polymer matrix. The decomposition temperature (T_d) for NP-MNs was recorded at approximately 376°C, slightly lower than that of blank MNs (400°C). This decrease, along with a higher total weight loss (87.05% vs 72.83%), reflects the inclusion of thermally less stable components, namely, the NPs and drug, which influence the overall degradation profile of the formulation.

Table 4.8: Thermal properties of blank and nanoparticle-loaded microneedles, docetaxel, docetaxel-loaded P(3HB-*co*-4HB) nanoparticles and P(3HB-*co*-4HB)

Thermal properties (°C)							
	T _g (°C)	T _m (°C)	T _c (°C)	T _d (°C)	Weight loss (%)	Residual mass (%)	ΔH (J/g)
Blank MNs	ND	406.68	ND	467.04	72.83	11.83	779.93
NP-MNs	ND	271.72	ND	376.61	87.05	1.93	157.93
DTX-NPs	-22.5	46.0	ND	271.76	98.25	1.72%	0.28
P(3HB- <i>co</i> -4HB)	-19.2	50.4	ND	282.74	97.31	2.67%	2.68

Note: Thermal analysis of the P(3HB-*co*-4HB) and DTX-NPs was conducted using two heating cycles to better capture thermal transitions due to their semi-crystalline characteristics. In contrast, a single heating cycle was applied for both blank and NP-MNs to preserve their original thermal profile post-fabrication.

^a Glass transition, crystallisation, and melting temperatures were determined using DSC

^b Decomposition temperature determined using TGA

ND means not detected

Values are means of two replications

Compared to the MN system, the DTX-NPs alone showed a distinct thermal pattern, with a glass transition temperature (T_g) of -22.5°C , a T_m of 46°C , and a decomposition onset (T_d) at 271.76°C . These values indicate that DTX incorporation reduced both the T_g and T_d of the P(3HB-*co*-4HB) polymer. The reduction in T_g compared to the neat polymer (-19.2°C) suggests that the drug disrupts chain packing and increases polymer mobility, thereby enhancing the amorphous character. The lower T_d of the NPs relative to the raw polymer (271.76°C vs 282.74°C) further supports the hypothesis that drug encapsulation compromises some thermal stability, likely due to increased chain flexibility and reduced crystallinity.

The pure P(3HB-*co*-4HB) polymer exhibited a T_g of -19.2°C , T_m of 50.4°C , and T_d of 282.74°C with 97.31% weight loss and 2.67% residual mass. These values are consistent with literature reports, which show that increasing the 4HB content reduces polymer crystallinity and thermal resistance due to enhanced chain flexibility (Vidili, 2023; Chai et al., 2020). The absence of a crystallisation temperature (T_c) and the relatively low ΔH (2.68 J/g) indicate limited crystallisation ability, supporting the amorphous profile confirmed by XRD. The polymer's degradation mechanism, initiated via β -elimination and inhibited by the presence of 4HB, explains its single-step thermal decomposition (Omura et al., 2021; Jo et al., 2022). This structure is thermally stable up to around 280°C , which allows for NP and MN fabrication below this range without triggering early degradation.

The shift in thermal parameters between the P(3HB-*co*-4HB), NPs, and MN system demonstrates the influence of drug encapsulation and polymer blending on thermal behaviour. The absence of a detectable T_g or T_c in the DSC of both blank and NP-MNs can be attributed to the amorphous nature of PVA and PVP, which dominate the matrix. Rapid solidification during MN moulding likely suppresses crystallisation, as also observed in previous studies where PVP addition eliminated T_g peaks in hydrogel-based systems (Aung et al., 2020; Anjani et al., 2021). The thermogram of the MNs lacked any sharp thermal transitions associated with the original drug or polymer, indicating a homogenous dispersion of components in an amorphous phase.

The reduction in melting enthalpy and the presence of overlapping peaks further confirm that the physical mixing and NP integration disrupted the crystalline domains within the MN matrix.

Notably, the absence of a drug-related endothermic peak in both NP and MN DSC profiles confirms that DTX exists in an amorphous state throughout the system. This amorphous form enhances solubility and prevents premature crystallisation, which is especially valuable for poorly water-soluble class IV drugs like DTX (Sohail et al., 2018; Nora et al., 2022). Moreover, storage below T_g , particularly for NPs (-22.5°C), contributes to the stabilisation of this metastable state and minimises the risk of recrystallisation (Chatterjee et al., 2022; Shi et al., 2019).

In summary, thermal analysis demonstrated that the NP-MNs are thermally stable, with modified degradation and melting behaviour due to the presence of NPs and the amorphous blend of PVA/PVP. The transition from crystalline DTX and semi-crystalline polymer to an amorphous, structurally integrated MN system supports improved drug dispersion and controlled release. These findings validate the formulation's suitability for transdermal drug delivery, offering both thermal robustness and functional efficiency for therapeutic applications.

4.5.8 Skin Permeation Studies of Microneedles

The fabricated MNs were evaluated for their skin permeation performance using Parafilm®M, a widely accepted skin simulant due to its mechanical and barrier properties resembling mammalian skin, including that of humans (Abdelghany et al., 2022). The graph in Figure 4.17(A) presents the total number of holes formed in each parafilm layer and the corresponding insertion depth. The blank and NP-MNs created 36 square-shaped pores in the first three layers. Blank MNs created 28 and 10 pores for the fourth and fifth layers. In comparison, NP-MNs created 28 and 7 pores, respectively, but the pores were less prominent with reduced density and clarity than

the first three layers. No significant differences were observed between the insertion depths caused by both MNs. However, slight differences could be due to the density of NPs within the MNs.

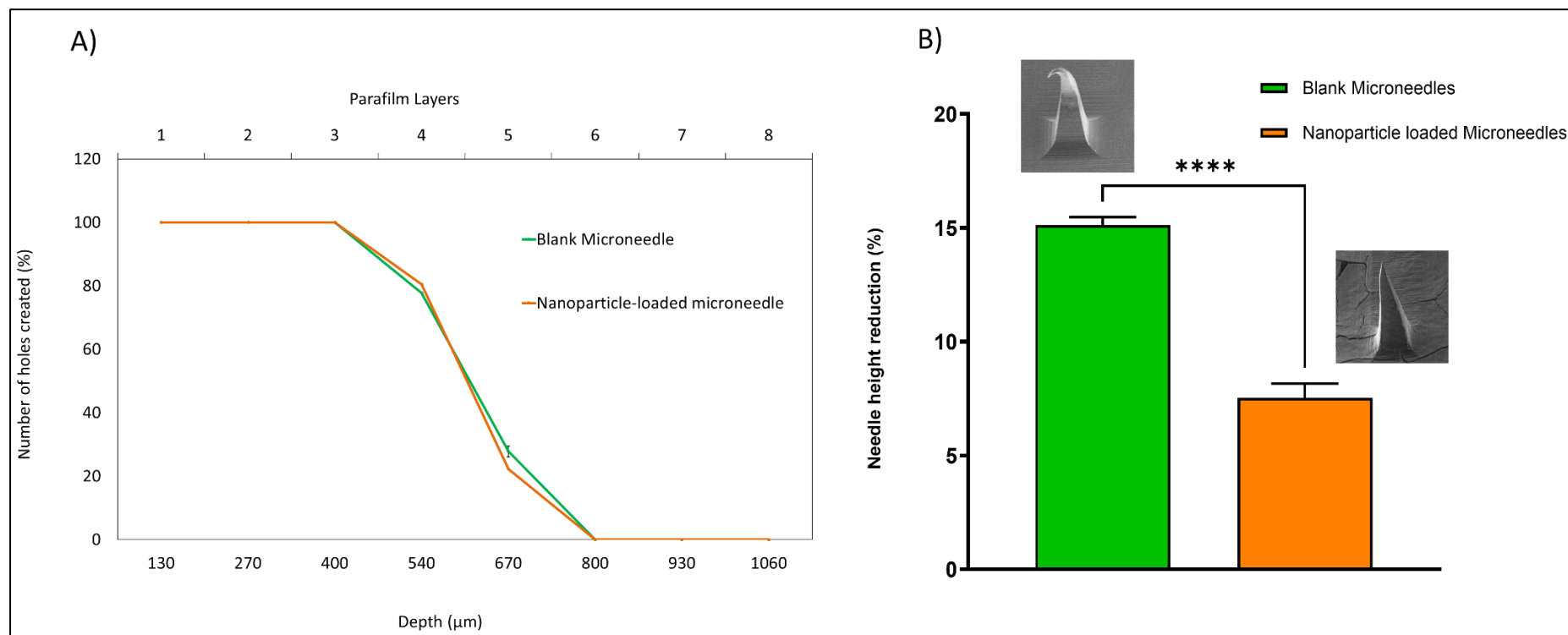


Figure 4.17: Skin permeation analysis of dissolving microneedles penetration into 8-layer Parafilm®M stacks (A) Number of holes created and depth of insertion percentage across layers (B) Percentage of needle height reduction after insertion into parafilm layers

Based on Figure 4.17 (A), visible disruptions of depths correspond to needle bending or deformation points. The MNs' height prior to and after their insertion through the parafilm was further evaluated using SEM, and the percentage of needle height reduction is plotted in Figure 4.17 (B). Blank MNs have a 15% reduction, whereas NP-MNs have a 7.5% needle height reduction. This also means that around 85% of the needle integrity of the blank MNs and 93% of NP-MNs are still maintained. No breakage of needle tips was observed, but there was some bending and deformation of the needles in both blank and NP-MNs. Besides that, some cracking of the back layer can be observed for the NP-MNs in SEM images after insertion. This also indicates that the increased strength of the needles requires more force for penetration.

While both MN types pierced the same number of layers and formed similar pores, variations in colour intensity in the OCT images and the stereomicroscopic images of parafilm layers in Figure 4.18 imply differences in penetration force, likely due to NP incorporation (Teodorescu et al., 2019). Optical coherence tomography (OCT), shown in Figure 4.18A, indicates that MNs were observed to have penetrated through the fifth and sixth layers, as there is visible disruption at the fifth and sixth layers, which could be where the MNs bent or deformed. Although both MNs pierced through the same layers and created similar holes, the needle's strength differs based on the colours in OCT images, where darker colours often indicate higher force. This could be due to the forces and rigidity of the NPs distributed within the needles.

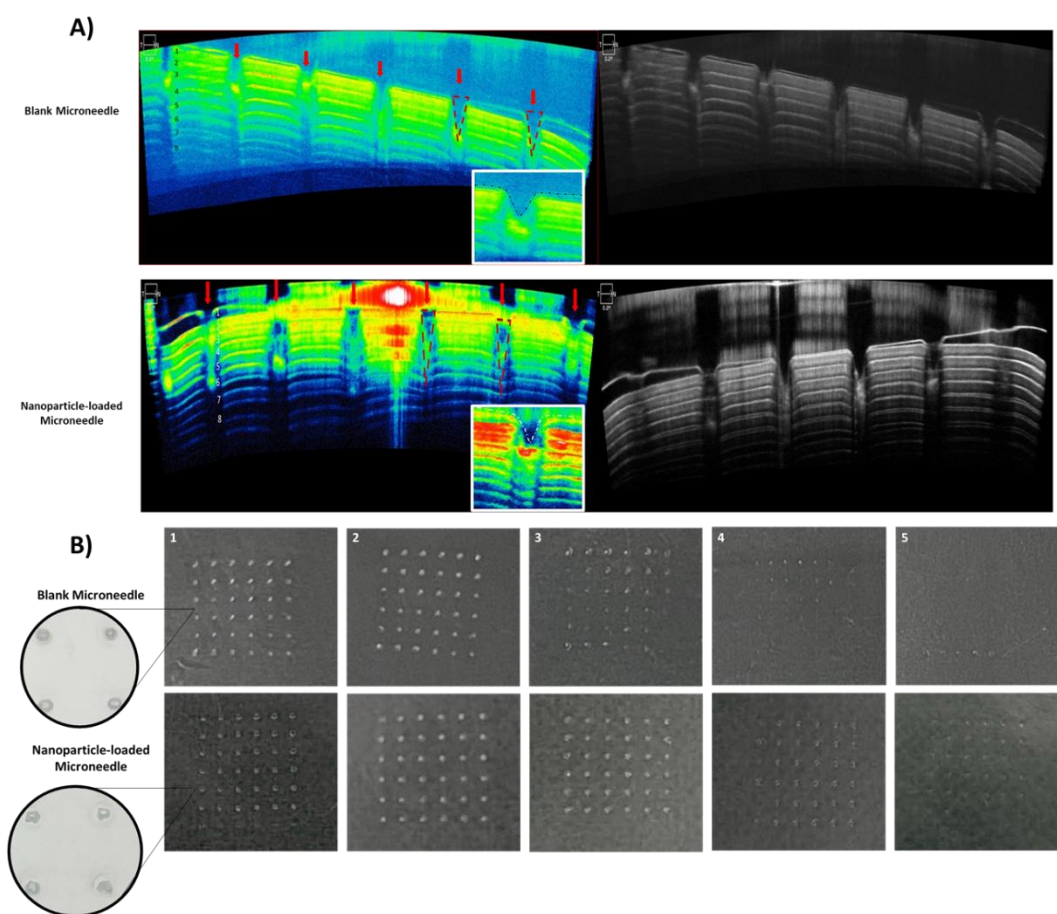


Figure 4.18: Skin permeation analysis of dissolving microneedles penetration into 8-layer Parafilm®M stacks (A) Optical coherence tomography (OCT) visualisation of blank microneedles and nanoparticle-loaded microneedles and (B) stereomicroscopic images of parafilm layers.

These results are promising, as most previous MNs in previous studies enter only the first and second layers of parafilm. One study reported that MNs made from hydrogel could achieve full penetration in the first parafilm layer and less than half in the next layer (Teodorescu et al., 2019). The fourth and fifth layers of the MN are around 540-670 μm (Teodorescu et al., 2019). This demonstrates that MNs can pierce through the skin's outer barrier, specifically the stratum corneum (approximately 50 μm thick), thereby supporting their insertion into the epidermis (Dawud and Ammar, 2023) but would still not touch the nerve endings and would not cause pain, as that is also one of the most significant factors of using MNs.

The skin in the breast for women is the thinnest, especially in the lateral and upper quadrants, which is around 1.38 ± 0.24 mm to 1.45 ± 0.30 mm, and thickest in the medial and lower areas (1.97 ± 0.26 mm) (McGhee and Steele, 2020; Zadehrafai, 2021). A subcutaneous fat layer (~ 0.5 – 2.5 cm thick) lies beneath the breast (McGhee and Steele, 2020). At the same time, the breast tumour usually causes the skin tissues to be thicker, which can cause the needles to penetrate deep enough to reach the tumour microenvironment. Thus, the MNs in this study can successfully breach the stratum corneum layer of the breast but still do not reach the nerves of the breast to cause pain or irritation.

These findings also confirm that the MNs' optimal needle length of 977 μm provides the ideal penetration depth, ensuring minimal pain while enabling effective drug delivery (Ahmad et al., 2023). The MN fabrication process is also verified, as the needles all had the same impact and maintained the same integrity (Ahmad et al., 2023). Evaluating insertion depth can also serve as a validation method for MN production, confirming that the MNs consistently meet the desired specifications. The variations in depth of insertion indicate problems during the fabrication of MNs.

Furthermore, this study was also important for effectively studying MNs' insertion behaviour, length uniformity, and mechanical uniformity because how MNs are inserted is influenced by factors such as their density, shape, material type, needle dimensions, sharpness, and base size (Teodorescu et al., 2019).

This aligns with our hypothesis that incorporating DTX-NPs into DMNs improves their penetration performance. This was also observed in previous studies using PLGA NPs incorporated in DMNs (Dawud and Ammar, 2023), which is due to the increased density of NPs (Dawud and Ammar, 2023). Thus, this proves that the DMNs incorporated with DTX-NPs could optimally penetrate and transdermally deliver the drugs effectively for BC patients.

4.5.9 Dissolution Properties of Microneedles

Dissolution plays an essential role in determining the performance of DMNs. An optimal MN should promptly dissolve upon contact with biological fluids, releasing the encapsulated drug or active compound (Chanabodeechalermrung et al., 2024). The time taken for MNs to dissolve is critical to ensure an efficient drug delivery, particularly when a fast and sustained release is required to achieve therapeutic effects. The dissolution behaviour of the fabricated MNs was evaluated through direct observation of morphological changes and quantification of height reduction over time. SEM micrographs in Figure 4.18 illustrate the progressive deformation of MNs in 2% and 6% agarose gels at defined time points, while Figure 4.19 presents the corresponding needle height reduction percentages.

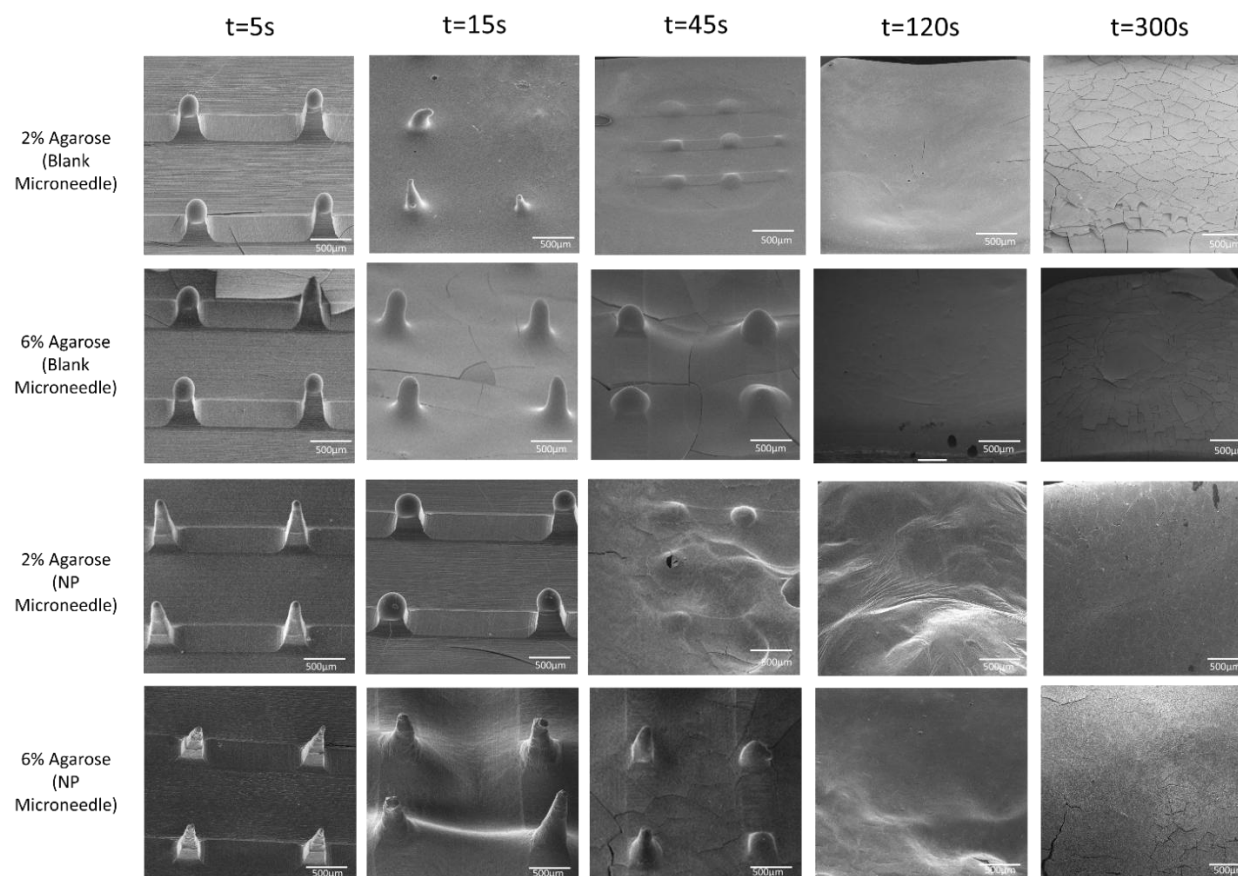


Figure 4.19: SEM micrographs showing microneedles' morphology and deformation patterns before and after insertion into 2% and 6% agarose gels. All images include a 500 µm scale bar for reference.

Based on Figure 4.20, some dissolution of the needle tips was evident at 5 seconds, indicating initial interactions with the agarose gel's hydrated surface. There was some swelling at the tip of the needles, resulting in a rounded tip. This is due to the dissolution of the water-soluble PVA tip upon exposure to the moist tissue environment (Ziesmer et al., 2021). PVA and PVP are both naturally highly soluble in water and biocompatible, which makes them quickly dissolve in aqueous environments without compromising the mechanical integrity of the MNs during skin insertion (Bajpai et al., 2019). At 15 to 45 seconds, further degradation was observed, with the needle structures beginning to collapse and dissolve into the agarose gel. The needle tips appeared blunted, and the overall shape of the array was less distinct. At 2 minutes, most of the MN structure had dissolved. At 5 minutes, the back layer starts dissolving and shows some cracking. The dissolution time was significantly prolonged, with blank MNs and NP-MNs in the 6% agarose gel.

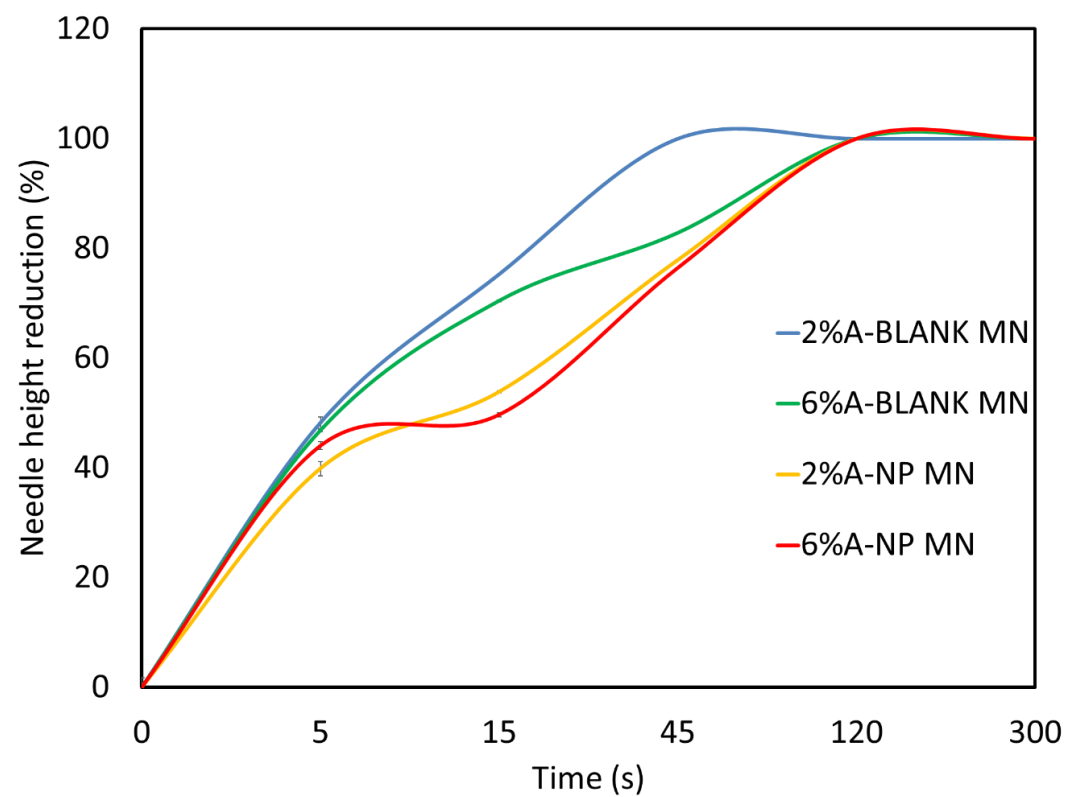


Figure 4.20: Graph of percentage reduction in microneedle height after insertion into 2% and 6% agarose gels over a 300-second interval. Error bars represent the mean (SEM) standard error for n=3.

The trend in the graph in Figure 4.19 shows that MNs inserted into the 2% agarose gel (blank and NP-MNs) demonstrate higher needle height reduction than those inserted into the 6% agarose gel. At 5s, the blank MNs and NP-MNs significantly differ when inserted into 2% agarose gel. The MNs inserted into 6% agarose gel also had significantly higher needle reduction than NP-MNs in 2% agarose gel. At 15s and 45s, there are no significant differences, only among the 2% and 6% agarose gel for NP-MNs. This indicates that the gel's stiffness or density influences the MN's deformation or height reduction during insertion.

The findings from this study suggest that the NP-MNs could be suitable for clinical use in situations that require rapid drug action or immediate drug delivery. This is attributed to the fact that microparticles or NPs provide a double-encapsulation mechanism. The results imply that the recommended usage duration for the MN patch may exceed 45 seconds. Once the MNs dissolve upon insertion and subsequently release the NP-loaded drug, the remaining base can be easily removed with water, assisting in the healing of the skin and restoring its protective function (Dawud and Ammar, 2023). This is also crucial in minimising the risk of skin infections arising from the microchannels formed during MN insertion (Dawud and Ammar, 2023).

So, the MNs would act as an initial protective barrier or a vehicle, allowing for quick insertion and minimising the patient's need to spend time in clinical settings. This aligns with the core advantage of MNs, providing comfort and convenience for patients. This combination of rapid MN dissolution and controlled NP delivery strikes an optimal balance between fast-acting treatment and sustained drug protection, making it ideal for conditions that require targeted, long-lasting drug release.

However, it is crucial to recognise some limitations, such as the lack of specific pharmacopeial guidelines tailored for MN-based formulations, along with the unavailability of validated and standardised *in vitro* procedures to assess the dissolution and release characteristics of therapeutics embedded in MNs containing nanosystems (Dawud and Ammar, 2023). Additionally, as pointed out in prior studies, discrepancies in the structural composition and moisture levels between actual human

skin and the skin simulants applied in this research may influence the accuracy and relevance of the results (Dawud and Ammar, 2023). However, skin-simulant models, such as agarose gel, were chosen for this study due to their ease of preparation, reproducibility, and ethical considerations. While actual human or animal skin would offer greater physiological relevance, agarose gel has been widely validated for simulating skin's mechanical and hydration properties in MNs studies.

Fresh animal skin is commonly utilised and readily available for skin-related research, making it a preferred choice over human skin. However, international regulations and ethical frameworks concerning the use of animals in research have become increasingly stringent in recent years (Khiao-in, 2022). Consequently, various alternative models have emerged to mimic or replace human skin for *in vitro* experiments, such as artificial and reconstructed skin models (Khiao-in, 2022). The Principles of Humane Experimental Technique, introduced by Russell and Burch in 1959, emphasise the concept of the 3Rs, which stands for Reduction, Refinement, and Replacement in animal testing (Khiao-in, 2022). These principles advocate for minimising animal use by replacing them with non-sentient alternatives when possible, limiting the number of animals used, and enhancing experimental procedures to lessen any potential pain or discomfort experienced by the animals (Russell and Burch, 1959; Khiao-in, 2022).

4.5.10 Drug Release from Nanoparticle-Loaded Microneedles

It is essential for DMNs that the needle tips dissolve within a specific timescale to ensure efficient drug release (Larraneta et al., 2014). *In vitro* drug release testing is critical for developing drug delivery systems and quality control. Traditional transdermal patches are usually tested using methods such as the "Paddle over Disk" described by the US Pharmacopeia (Larraneta et al., 2014), but these tests do not accurately reflect the mechanism of DMNs. This is because DMNs differ from transdermal patches, as they deliver drugs by penetrating the outermost skin barrier

(stratum corneum) with the MN tips, followed by the breakdown of the polymer matrix within the viable skin layers. This study used a modified drug release method based on Larraneta et al. (2014), where the MNs were pierced into a Parafilm layer and floated on PBS solution.

Figure 4.21 (A) shows the cumulative drug release of free drug DTX-loaded MNs and NP-MNs in a 168-hour interval, and (B) the overall comparison of free DTX, NPs, MNs loaded with free DTX, and NP-MNs in 72 72-hour interval. Graphs (C) and (D) illustrate the best-fit models for free drug DTX-loaded MNs and NP-MNs, respectively. Table 4.8 summarises the best-fit release models for the free DTX drug, NP formulations, free drug DTX-loaded MNs, and NP-MNs determined based on R^2 values calculated from the release graphs.

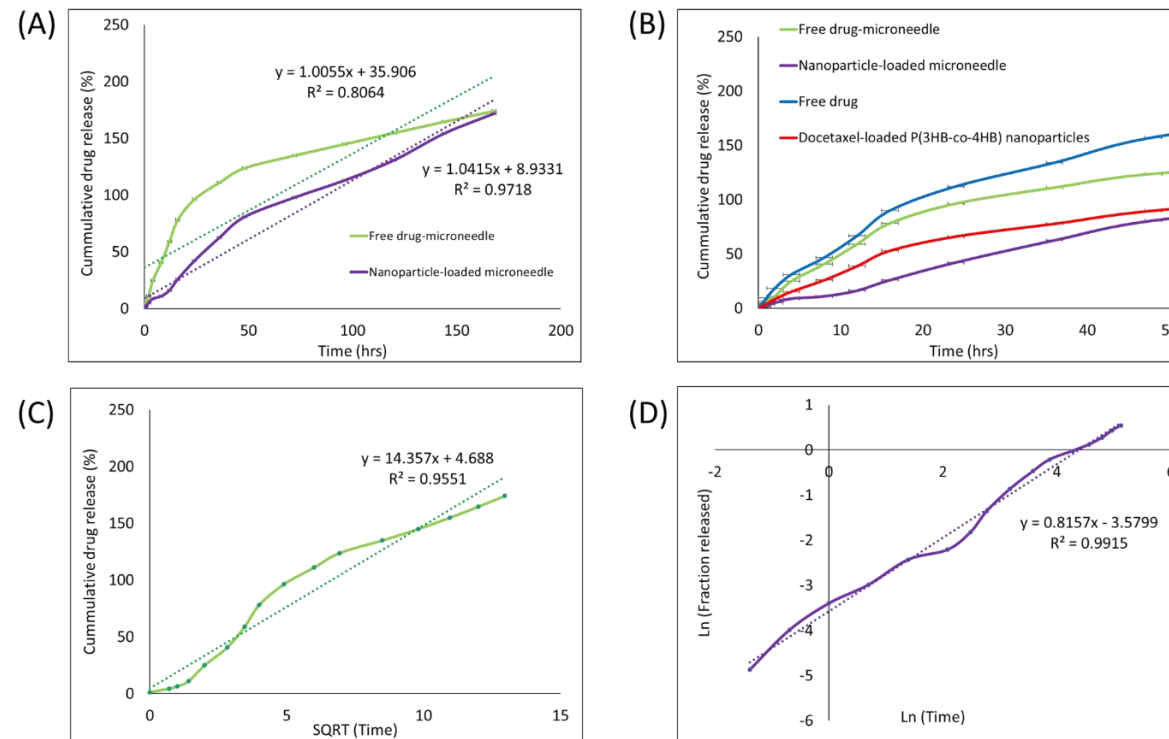


Figure 4.21: Cumulative drug release profiles over time. (A) Free drug-loaded microneedles and nanoparticle-loaded microneedles over 168 hours (B) Comparison of the cumulative release profile of free drug, nanoparticles, free drug-loaded microneedles, and nanoparticle-loaded microneedles. (C) the Higuchi graph as the best-fit model of free drug-loaded microneedles, and (D) the Korsmeyer-Peppas graph as the best-fit model of nanoparticle-loaded microneedles. Error bars represent the standard deviation (n=3).

Table 4.9: Correlation coefficients (R^2) of different drug release models for free docetaxel drug, nanoparticle formulations, free drug-loaded microneedles and nanoparticle-loaded microneedles.

Samples	Best-fit release models			
	Zero-order	First order	Higuchi	Korsmeyer Peppas
Free DTX	$R^2 = 0.8977$	$R^2 = 0.9225$ (not a straight line)	$R^2 = 0.9913$	$R^2 = 0.9773$
DTX-NPs	$R^2 = 0.8897$	$R^2 = 0.9676$ (not a straight line)	$R^2 = 0.9039$	$R^2 = 0.9796$
DTX-MN	$R^2 = 0.8064$	$R^2 = 0.9283$	$R^2 = 0.9551$	$R^2 = 0.949$
NP-MNs	$R^2 = 0.9718$	$R^2 = 0.9094$	$R^2 = 0.9812$	$R^2 = 0.9915$

Based on the results above, free DTX-MNs adhered to the Higuchi model, showing a release profile similar to that of the free DTX. However, the rate of drug release from MNs was slower compared to the free DTX alone. This variation is likely due to the specific experimental setup, as the free DTX release was assessed using a dialysis tubing method, which offered a larger contact area between the drug and the release medium (PBS). On the other hand, the MN-based release demonstrated a slower rate due to extra physical barriers that limited the contact area. Specifically, the parafilm layer acted as a barrier, restricting the contact to only the tips of the MNs that touched the PBS surface and dissolution medium.

NP-MNs exhibited a slower drug release profile than free DTX-MNs, with the Korsmeyer-Peppas model providing the best fit, similar to the NP release pattern. This could be attributed to the dual-layer encapsulation of the drug, involving both the NP's polymer shell and the MN matrix. Another contributing factor is the spatial distribution of the DTX drug within the MN structure. The NPs positioned at the tip regions will likely release the DTX first, followed by those in the middle segment. Additionally, the presence of the parafilm layer serves as a physical barrier, potentially delaying the dissolution of the upper portion of the MN tips in the PBS medium. For NP-MNs, nearly 97.86% of the drug was released at 72 hours, whereas NPs alone showed 89.86% release at 48 hours. No notable differences were identified between NPs and NP-MNs, except at specific intervals, namely at 30 minutes and at 96 hours. In contrast, free DTX-MNs exhibited a release of 96.36% at 24 hours, while free DTX achieved complete release within 24 hours.

The best-fit models have no significant differences; however, the findings demonstrate that the MN system minimised the biphasic release pattern typically observed with free DTX drugs and NPs. Although an initial burst release is evident, it is notably lower, and the cumulative release profile for NP-MNs tends to follow a more linear trend. The calculated burst release for NP-MNs was 4.5% within the first two hours, which is lower than the 6.97% recorded for NP release. In this study, MNs exhibited a prolonged and regulated drug release profile. This outcome is likely due to the compact architecture of the double-layer structure, which acts as a physical

barrier, thereby slowing drug diffusion over a more extended period (Jadon and Sharma, 2019).

Earlier research involving doxorubicin and DTX in MNs reported complete drug release within 5 to 15 minutes, reinforcing the efficacy of NPs in maintaining sustained drug release profiles. Another study indicated that DTX release from Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) NPs in PBS (pH 7.4, 25°C) was gradual and extended over three to four days, consistent with the present study's findings (Vardhan et al., 2017b). A slower release profile contributes to a favourable protective timeframe by reducing toxicity during systemic circulation. It also improves the selective accumulation of NPs in tumour tissues by utilising the EPR effect within the tumour vasculature (Vardhan et al., 2017b).

Researchers are consistently working to improve drug delivery systems to address the pharmacokinetic (PK) drawbacks of conventional drug release techniques. The method adopted for drug release in this study addresses various limitations associated with the variability of biological tissues, which is a common challenge in conventional research (Larraneta et al., 2014). The Parafilm layer is an effective skin simulant, ensuring consistent MN penetration and dissolution patterns while preserving structural integrity during release. This strategy is especially beneficial in the initial phases of formulation development, where only limited active ingredients are available. The setup allows for precise monitoring of drug release patterns, which, according to previous reports (Larraneta et al., 2014), are mainly controlled by the dissolution of the MN matrix. Furthermore, it requires fewer resources and avoids the complexity associated with Franz cell studies.

These advancements aim to achieve controlled and prolonged drug release over varying durations, from a few hours to several months (Laracuate et al., 2020). Various systems have been developed, including degradable polymer-based particles, implants, and diffusion-driven platforms like passive microchips, hydrogels, and MNs (Laracuate et al., 2020). In this study, the MN system can potentially optimise drug administration by reducing the immediate release of the drug into the bloodstream.

This helps to slow its metabolism and elimination (Laracuenta et al., 2020). Consequently, the drug remains in circulation for an extended period with lower peak concentrations, helping to maintain therapeutic levels over a longer duration, thereby ensuring its safe and effective use. This approach could benefit chemotherapeutic drugs (Laracuenta et al., 2020).

Additionally, the rapid dissolution of MNs within 2 minutes reduces patient discomfort, while therapeutic effects are initiated within the first 15 minutes (Bhatnagar et al., 2019). Thus, combining DMNs with nano-or microparticle technology is an efficient strategy to regulate controlled drug release (Ramadon et al., 2022).

4.5.11 Storage Stability of Nanoparticle-Loaded Microneedles

The storage stability analysis in this study was conducted for the NP-MN patches to determine if there are any differences in the drug content of the MNs. Storage stability is essential as the MNs are aimed at self-administration for patients, and thus, the drug within the MNs must be ensured that it does not disintegrate or degrade in a specific period. Visual inspection of the patches indicated no differences. The changes in drug content of the NP-MNs after a month are shown in Figure 4.22 below.

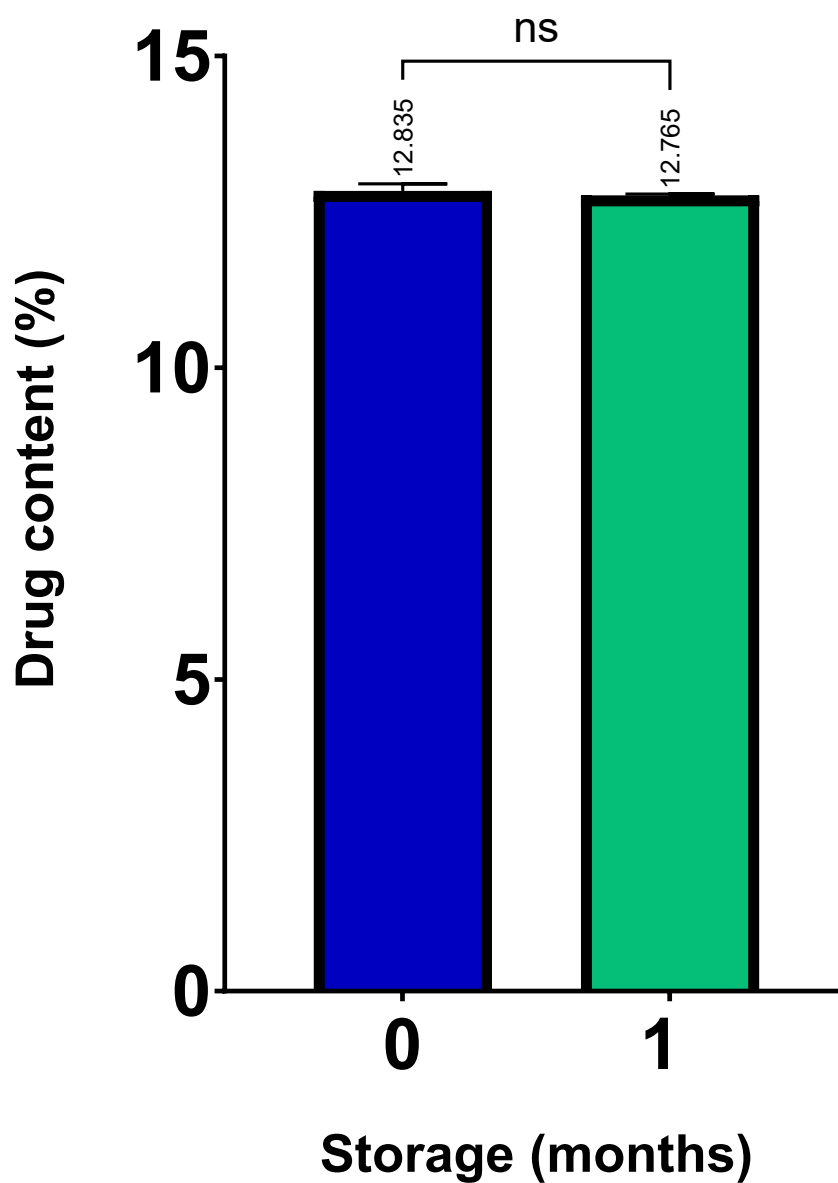


Figure 4.22: Drug content (%) of dissolving microneedles immediately after fabrication (0 months) and after 1 month of storage under specified conditions. Data are presented as mean $\pm$ SE ($n = 3$). No significant difference was observed between the two time points ($p > 0.05$). ns: not significant.

Comparing the DL stability of NPs alone and NPs within MNs over one month, the NP-MNs showed no significant drug loss, confirming their stability. In contrast, lyophilised NPs alone exhibited gradual drug loss over six months, decreasing from 14.5% to 12.8% (0% RH) and 14.3% to 12.2% (75% RH) as shown in Figure 4.11. Assuming a linear degradation trend, this corresponds to an estimated 0.28–0.35% drug loss per month for NPs alone, which is relatively small.

However, despite the low drug loss in NPs alone, MNs provide an additional layer of protection, likely due to the polymer matrix shielding NPs from environmental exposure. This suggests that MN encapsulation further enhances drug stability, ensuring better retention and controlled release. While MNs demonstrated strong one-month stability, additional studies over an extended period (e.g., 6 months, 1 year) are required to validate their sustained performance and suitability for long-term storage.

An essential requirement for formulations containing sensitive biotherapeutics is maintaining their structural and functional stability throughout the preparation and storage processes (Zhu et al., 2014). High storage stability could help lower the expenses associated with cold chain storage while enhancing practicality (Zhu et al., 2014). This study used a gentle fabrication process that generates or requires less heat to encapsulate the fragile drug in a biocompatible polymer matrix. The drug formulation in a dried solid state of lyophilised NPs was more stable in retaining sensitive biomolecules than the liquid formulation (Zhu et al., 2014). The storage stability results show no substantial degradation of the MN patches after storage. However, long-term stability with adding sugars (e.g., sucrose and trehalose) or dry atmospheres with nitrogen gas should be conducted in the future (Zhu et al., 2014).

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

This study successfully developed a dissolving microneedle (DMN) system incorporating docetaxel-incorporated Poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) nanoparticles (DTX-NPs) as a novel transdermal drug delivery method for breast cancer (BC) treatment. This study achieved all its objectives, demonstrating an effective, minimally invasive, and sustained drug delivery system.

The first objective, synthesising and characterising P(3HB-*co*-4HB) copolymers, was achieved through single-stage fermentation using *Cupriavidus malaysiensis* USMAA1020. The polymer exhibited high purity (95.04% $\pm$ 9.05% Polyhydroxyalkanoate, PHA content), an equimolar monomer composition (49.83% 3HB and 50.17% 4HB), a molecular weight of 44.8 ± 2.2 kDa, and a glass transition temperature (T_g) of -19.2°C . Additionally, it had a low endotoxin level (0.27 EU/g), ensuring its suitability for biomedical applications. These properties proved that P(3HB-*co*-4HB) is biodegradable and has an optimal balance of mechanical strength and flexibility, making it a promising potential candidate for nanoparticle (NP) fabrication and drug delivery.

The second objective, fabricating and characterising DTX-NPs, was achieved by optimising solvent evaporation using D- α -tocopheryl polyethylene glycol succinate (TPGS), drug-to-polymer ratios, and sonication time. The optimised NPs were spherical and less than 100 nm, highly stable, and had a high encapsulation efficiency (EE) of 87.07% with a drug loading (DL) of 13.71%. The drug release profile showed that 90% of the drug was released within 48 hours, ensuring sustained and controlled

drug delivery. Although the tested dose may not precisely reflect clinical concentrations, it can be tailored in real-world settings by adjusting the number of applied microneedle (MN) patches. Furthermore, due to the localised and sustained delivery mechanism, therapeutic efficacy is expected to be higher at the target site compared to systemic administration, potentially reducing the required dose. These NPs protected DTX from premature degradation, extended its therapeutic effect, and improved drug retention, making them suitable for transdermal applications. Additionally, this NP system addresses the limitations of current DTX formulations, such as Taxotere®, which relies on Tween 80 and is associated with severe side effects.

The third objective, fabricating and optimising polyvinyl alcohol and polyvinyl pyrrolidone (PVA/PVP)-based dissolving MN patches, was achieved using a stepwise centrifugation method. The fabricated MNs demonstrated sufficient mechanical strength, effectively penetrating agarose gels mimicking normal and tumour breast tissues. The nanoparticle-loaded microneedles (NP-MN) exhibited a fracture force of 73.67 N and maintained structural integrity under forces up to 200 g, ensuring successful skin penetration and drug delivery. Additionally, the MNs had a drug content of 212.93 µg per patch, further supporting their effectiveness in delivering the encapsulated drug, which falls within the preclinical range explored for BC treatment, suggesting real-world applicability. Moreover, in the rare case of MN breakage, the use of biodegradable components such as P(3HB-co-4HB), PVA, and PVP ensures safe degradation within the epidermis, thereby minimising safety concerns.

The final objective, incorporating NPs into DMNs and evaluating their performance, was achieved, with results confirming that DMNs efficiently delivered DTX with a sustained release of 97.86% over 72 hours, following a diffusion-controlled release mechanism (Korsmeyer-Peppas model). The combination of MNs and polymeric NPs improved drug retention, enabled localised delivery, and reduced systemic toxicity compared to free DTX, making this system a better approach than intravenous (IV) administration. This study presents a feasible alternative to conventional chemotherapy, which relies on IV administration and is associated with pain, hospital dependency, systemic side effects, and high financial costs. This study

addresses key gaps in chemotherapy treatment for BC patients by offering a self-administerable, localised, painless, and less invasive alternative. The MN system developed in this research can enhance patients' well-being by minimising discomfort, reducing the need for hospitalisation, and lowering the financial impact of IV chemotherapy.

The impact of this novel MN-based drug delivery system extends beyond cancer treatment, aligning with green healthcare, sustainable nanotechnology, and global developmental goals. This study promotes eco-friendly drug delivery solutions by utilising biodegradable P(3HB-co-4HB) NPs and DMNs, reducing reliance on petroleum-based excipients and minimising environmental and medical waste. Integrating biopolymers in pharmaceuticals supports the principles of green nanotechnology, contributing to sustainable material innovation in drug delivery systems. Developing biodegradable MNs reduces medical waste associated with conventional syringes and IV drug administration, supporting waste reduction initiatives in healthcare. Furthermore, this research aligns with the United Nations Sustainable Development Goals (SDGs), particularly SDG 3 (Good Health and Well-being) and SDG 9 (Industry, Innovation, and Infrastructure).

This study also fosters advancements in industrial biotechnology by demonstrating the feasibility of microbial biopolymers in biomedical applications, aligning with the Fourth Industrial Revolution (Industry 4.0), where sustainable materials and innovative healthcare solutions are prioritised. By integrating biodegradable polymers into transdermal drug delivery, this research contributes to the circular bioeconomy, paving the way for scalable, cost-effective, and environmentally responsible pharmaceutical manufacturing.

However, as this is a novel study, the primary focus was on the fundamental aspects of fabricating MNs and incorporating NPs to evaluate their structural and mechanical properties before starting biological testing. Additionally, ethical considerations prevent direct *in vivo* studies without confirming safety and effectiveness *in vitro*. This study has some limitations that could be overcome in future

studies. With the completion of this thesis, several future research directions are proposed, as outlined below:

1. Evaluation of biocompatibility and local skin responses – Future research should investigate the potential for skin irritation, inflammation, swelling, or allergic reactions following MN insertion. This includes histological analysis of skin tissue post-application and assessment of any immunological responses to ensure the safety and comfort of patients in long-term or repeated use.
2. *In vitro* cytotoxicity and *in vivo* studies: Upcoming research should prioritize evaluating the anti-cancer cytotoxic effects *in vitro* and conducting animal studies to assess the MN system's pharmacokinetics, biodistribution, and treatment effectiveness. These investigations would contribute towards improving the knowledge of its biological behaviour and safety characteristics, facilitating the progression to clinical trials and regulatory endorsement.
3. Exploring different polymers and alternative drugs: Combining PVA/PVP with other biocompatible polymers may improve mechanical strength, flexibility, and drug-release properties. This study focused on DTX, but future research could explore other anticancer drugs or combine DTX with immune-modulating agents to enhance treatment efficacy.
4. Commercial production and clinical translation: Large-scale manufacturing techniques should be explored to ensure cost-effectiveness, reproducibility, and regulatory compliance.
5. Application to other diseases: While this study focused on BC, the NP-MN platform could be adapted for other cancers or chronic diseases requiring controlled drug release.

With further optimization, *in vivo* testing, and clinical validation, this technology can positively transform breast cancer treatment approaches, offering a safer, more efficient, and patient-friendly alternative to traditional chemotherapy.

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APPENDICES

Appendix 1: List of chemicals and solvents used throughout the analyses

Name	Source
1,4-butanediol	Acros Organics, Belgium
1,6-Hexanediol	Merck KGaA, Darmstadt, Germany
Acetonitrile, HPLC grade	Elite Advanced Materials Sdn Bhd, Malaysia
Agar	Sigma-Aldrich, USA
Agarose gel ((HyAgarose™, 500 g, CAS No. 9012-36-6)	Repfon Glamor Sdn Bhd, Malaysia)
Ammonium acetate (CH ₃ COONH ₄)	Sigma-Aldrich, USA
Ammonium sulphate ((NH ₄) ₂ SO ₄)	QRëC, Malaysia
Anhydrous sodium sulphate (Na ₂ SO ₄)	PC Laboratory Reagent, Malaysia
Bacteriological peptone (LP0037)	Oxoid Ltd., Basingstoke, Hampshire, England
Calcium chloride tetrahydrate (CaCl ₂ ·4H ₂ O)	Sigma-Aldrich, USA
Caprylic methyl ester (CME)	Acros Organics, Belgium
Chloroform, HPLC grade	Elite Advanced Materials Sdn Bhd, Malaysia
Cobalt (II) sulphate heptahydrate (COSO ₄ ·7H ₂ O)	Sigma-Aldrich, USA
Copper (II) chloride tetrahydrate (CuCl ₂ ·4H ₂ O)	Sigma-Aldrich, USA
Coumarin-6	Sigma-Aldrich (Product number: C8726, USA)

D-Alpha-Tocopherol Polyethylene Glycol 1000 Succinate (TPGS)	Sigma Aldrich, USA
Dichloromethane, HPLC grade	Elite Advanced Materials Sdn Bhd, Malaysia
Dimethylsulfoxide (DMSO)	Invitrogen, Thermo Fisher Scientific, USA
Dipotassium hydrogen phosphate (K_2HPO_4)	R&M Chemicals, UK
Distilled water (for solvent and chemical mixing)	Water Purification System (Arium Comfort), Sartorius, Germany
Docetaxel, 98%, 250 mg (Cat. No. FSA45626-2500)	Acros Organics, Belgium
Ethanol, HPLC grade	Elite Advanced Materials Sdn Bhd, Malaysia
Ferrous sulphate heptahydrate ($Fe_2SO_4 \cdot 7H_2O$)	Sigma-Aldrich, USA
Glycerol	Bendosen Laboratory, Malaysia
Hydrochloric acid (HCL)	Supelco, Merck KGaA, Germany
Hydrochloric acid (HCL) [1.0 M]	Merck KGaA, Darmstadt, Germany
Hydrogen peroxide (H_2O_2)	Merck KGaA, Darmstadt, Germany
Kanamycin powder	Sigma-Aldrich, St. Louis, MO, USA
Lab-lemco powder (LP0029)	Oxoid Ltd., Basingstoke, Hampshire, England
Magnesium sulphate heptahydrate ($MgSO_4 \cdot 7H_2O$) (0.1M)	R&M Chemicals, UK
Manganese (II) chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$)	Sigma-Aldrich, USA

Methanol, HPLC grade	Elite Advanced Materials Sdn Bhd, Malaysia
Monopotassium phosphate (KH_2PO_4)	QR&C, Malaysia
Phosphate Buffered Saline (PBS tablets, pH 7.4)	Bio Basic Canada Inc., Canada
Phosphoric acid	Merck KGaA, Darmstadt, Germany
Polyvinyl alcohol (PVA) (Molecular weight: 89,000 - 98,000, 80% hydrolyzed)	Sigma-Aldrich (Product number: 360627, Germany)
Polyvinylpyrrolidone (PVP) (Molecular weight: ~1,300,000)	Chemiz (Malaysia)
Potassium Dihydrogen Orthophosphate	Unilab, Ajax Chemicals Pty Limited, Australia
Sodium chloride	Bendosen Laboratory, Malaysia
Sodium hydroxide (NaOH) [1.0 M]	Fisher Scientific, USA
Sodium phosphate monobasic	Sigma-Aldrich, USA
Sterile water	Baxter Healthcare, USA
Sulphuric acid (H_2SO_4)	Merck KGaA, Darmstadt, Germany
Yeast extract (LP0021)	Oxoid Ltd., Basingstoke, Hampshire, England
Zinc sulphate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$)	Sigma-Aldrich, USA

Appendix 2: List of kits used throughout the analyses

Name	Source
E-TOXATE™ Kit (Cat. No. ET0300-1KT)	Sigma-Aldrich, Germany

Appendix 3: List of microorganisms used throughout the analyses

Name	Source
Transformant <i>Cupriavidus malaysiensis</i> USMAA1020 containing the kanamycin-resistant plasmid pBBR1-PC2-4	Obtained from Lab 308, Biological Sciences, Universiti Sains Malaysia

Appendix 4: List of Consumables used throughout the analyses

Name	Source
0.45 μ m PTFE membrane	Sartorius Stedim Biotech, Germany
Aluminium foil	Kumpulan Saintifik F.E. Sdn. Bhd., Selangor Malaysia
Carbon tape	Ted Pella, Inc., USA
Cellulose dialysis tube (Spectrum Spectra/Por 4, RC) (MWCO: 12,000-14,000 Dalton, 45mm x 30m) (Cat. No. FISH08- 867E),	Fisher Scientific, Waltham, MA, USA
Column with Cartridge Holder Set (Inert sustain C18, 5 μ m, 3.0 x 10 mm) (Catalog No: 5020-19190)	GL Sciences, Japan
Cuvettes	BrandTech Scientific, Germany BRANDON, Malaysia
Disposable Petri dishes Eppendorf microcentrifuge tubes (1000 and 2000 μ L)	Eppendorf AG, Hamburg, Germany
Eppendorf tips (0.1, 20, 200, 100, 2000 μ L)	Eppendorf AG, Hamburg, Germany
Falcon Centrifuge Tubes (10, 50 mL)	Fisher Scientific, USA
Falcon tubes (15ml and 50ml) Filter paper (Type: Qualitative No.1; Sizes: 90 and 240 mm)	Corning Inc., USA Whatman, England

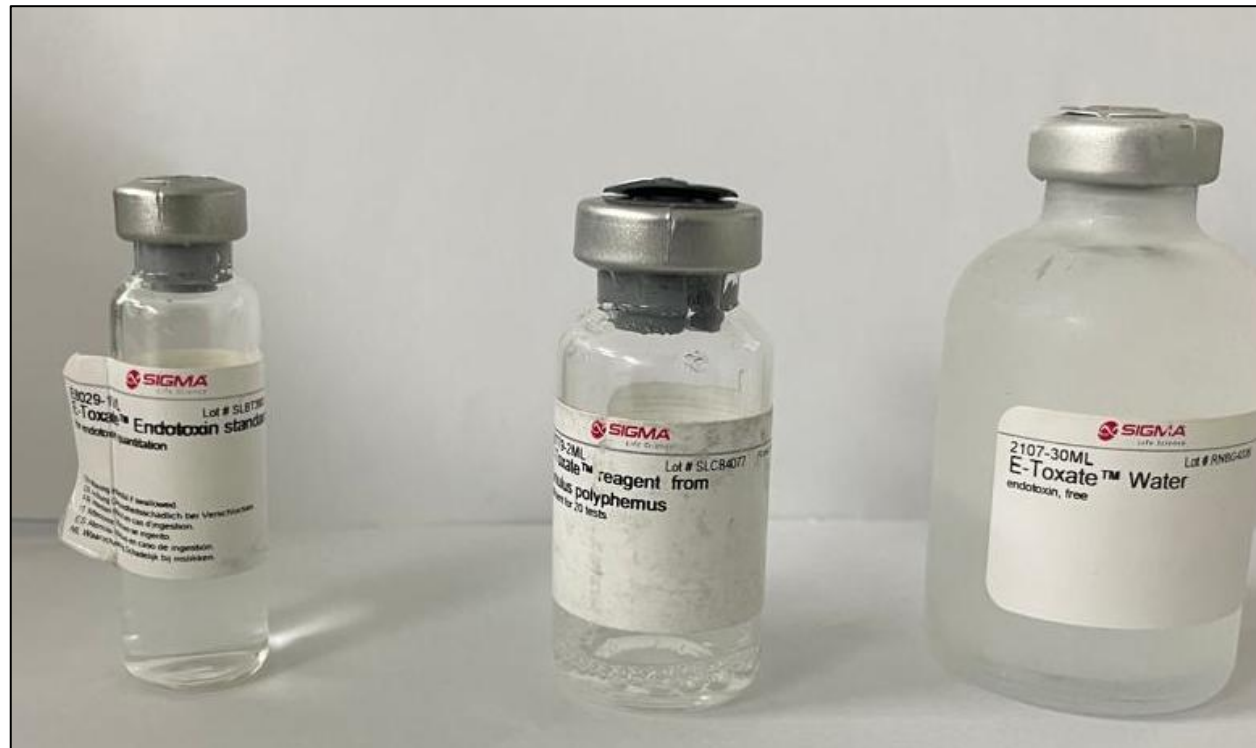
Gas chromatography vials	Agilent Technologies, USA
Gloves	Top Gloves, Malaysia
Methanolysis tubes	Nunc™, Thermo Fisher Scientific, USA
Microcentrifuge tubes	Eppendorf AG, Hamburg, Germany
Microneedle Mold template (Model: ST-II)	Micropoint Technologies Pte Ltd, Singapore
Nylon filters (0.22 and 0.45 µm)	Fisher Scientific, USA
Parafilm	Bemis, United States
Pasteur pipette	Brand GmbH, Germany
PTFE Syringe Filters (Millipore)	Merck, Darmstadt, Germany
Schott Duran bottles	Schott Duran, Germany
Scintillation bottles	PerkinElmer, USA
Serological Pipette (1, 3, 5, and 10 mL)	Thermo Fisher Scientific, Korea
Sterile 6-well plates	Nunc™, Thermo Fisher Scientific, USA
Sterile cryovials	Nunc™, Thermo Fisher Scientific, USA
Surgical Blade	Kumpulan Saintifik, F.E. Sdn. Bhd., Malaysia
Syringe (1, 3, 5 and 10 cc) (Sterile and non-sterile)	Big Pharmacy, Pulau Pinang, Malaysia

Appendix 5: List of Instruments used throughout the analyses

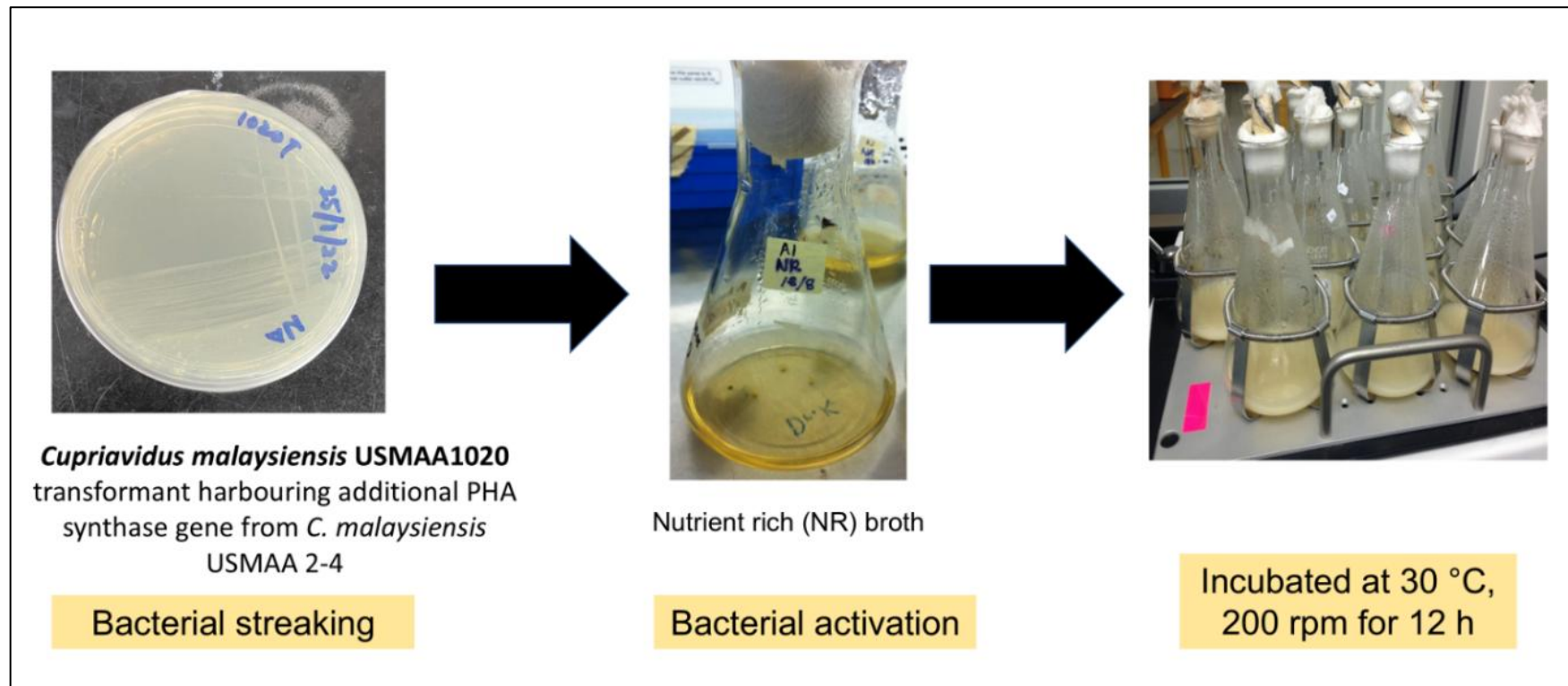
Name	Source
Autoclave	TOMY (SX-500), Japan
Bioreactor	INFORS HT, INFORS AG, Switzerland
Differential Scanning Calorimeter (DSC)	TA Instruments, New Castle, DE, USA
Drying Oven	Memmert, Schwabach, Germany
Freeze dryer	Labconco Corporation, Kansas City, Missouri, USA
FTIR	Shimadzu IRTracer-100 spectrometer (Shimadzu Corporation, Japan)
Gas chromatography	Agilent Technologies, USA
Gel permeation chromatography	Agilent Technologies, USA
Heating block (Lab Dry Bath, DSD-200E)	Digisystem Laboratory Instruments Inc., Taiwan
High-Performance Liquid Chromatography, HPLC	LC-20A HT, Shimadzu Corporation, Japan
Hot plate and stirrer	AREX Digital, VELP Scientifica, Italy
HPLC System	Shimadzu Corporation, Kyoto, Japan
Incubator shaker	New Brunswick Scientific, USA
Laminar Flow Hood	Nextech Engineering, Malaysia
Litesizer	Anton Paar, Austria
Micro balance	Sartorius Microbalance, Goettingen, Germany
Microinjector Manipulator and Research Fluorescence Microscope	Eppendorf FemtoJet, Germany Leica Microsystems, Germany

Mini Centrifuge	Eppendorf, Germany
Optical Coherence Tomography (OCT)	CIRRUS HD-OCT system (Model 5000, Carl Zeiss Meditec, Dublin, CA, USA)
pH meter	Hanna Instruments, USA
Probe sonicator	Qsonica, United States
Rotary evaporator (Model: OSB-2000)	EYELA, Japan
Scanning electron microscope	JEOL model JSM-36OLA (JEOL Malaysia Sdn Bhd, Model No: JSM-36OLA, Serial No: MP18700061), Japan
Sputter coater	Novacoliq Scientific, Malaysia
Stereo microscope	Olympus, Japan
Tabletop refrigerated centrifuge with a swing bucket rotor.	Eppendorf 5810 R, Eppendorf AG, Hamburg, Germany
Texture Analyzer	TA.XTplus, Stable Micro Systems, United Kingdom
Thermogravimetric Analyzer (TGA)	TA Instruments, New Castle, DE, USA
Transmission electron microscope	JEOL JEM-2100, JEOL Ltd., Tokyo, Japan
UV-vis spectrophotometer (SP-UV 300)	GENESYS 20, Thermo Fisher Scientific, USA
Vacuum pump filter	Millipore, USA
Vortex	MS3 Control, IKA Works, Germany
Water bath	Memmert, Schwabach, Germany

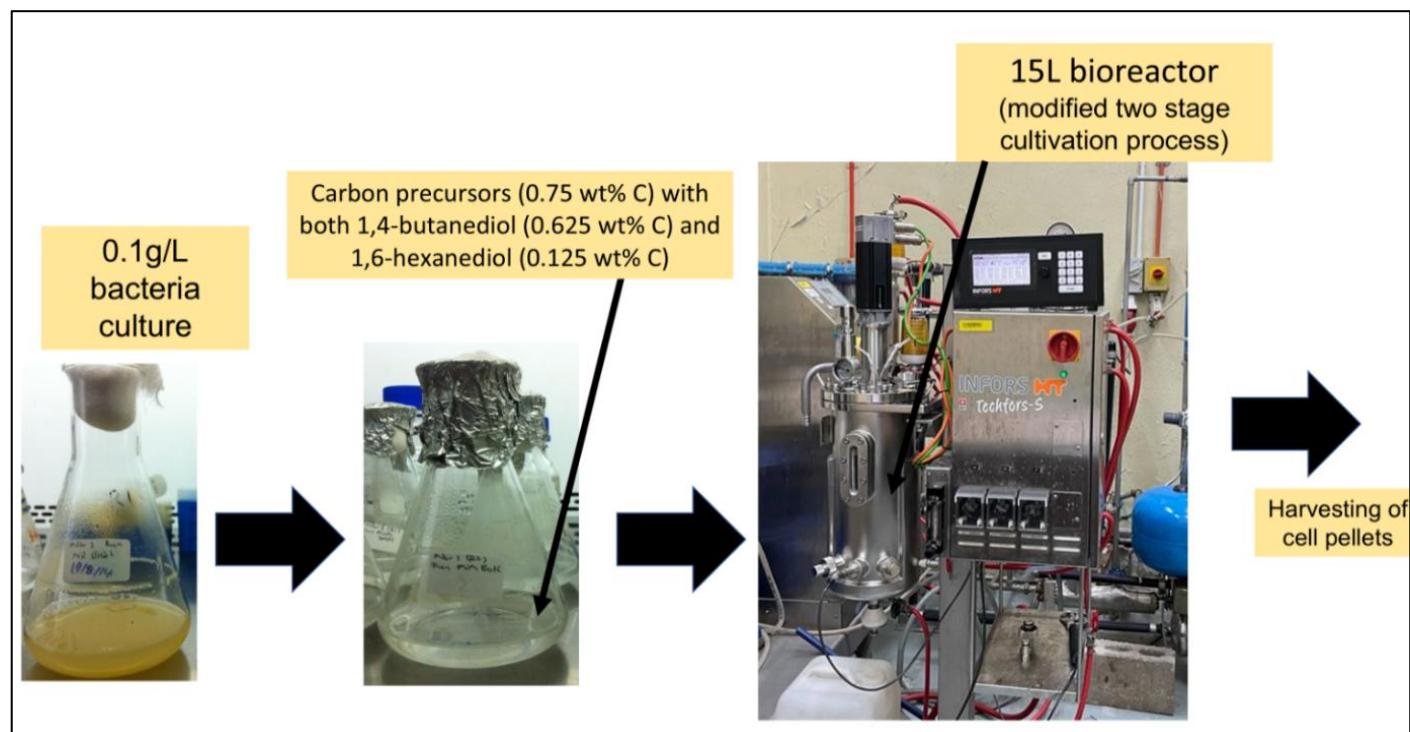
Water bath sonicator	Power-Sonic 405, Hwashin Technology, South Korea
Weighing balance	Ohaus Corporation, NJ, USA
X-ray Diffractometer (XRD)	Bruker D8 Advance, Bruker Corporation, Germany
Zetasizer	Malvern Instruments, United Kingdom



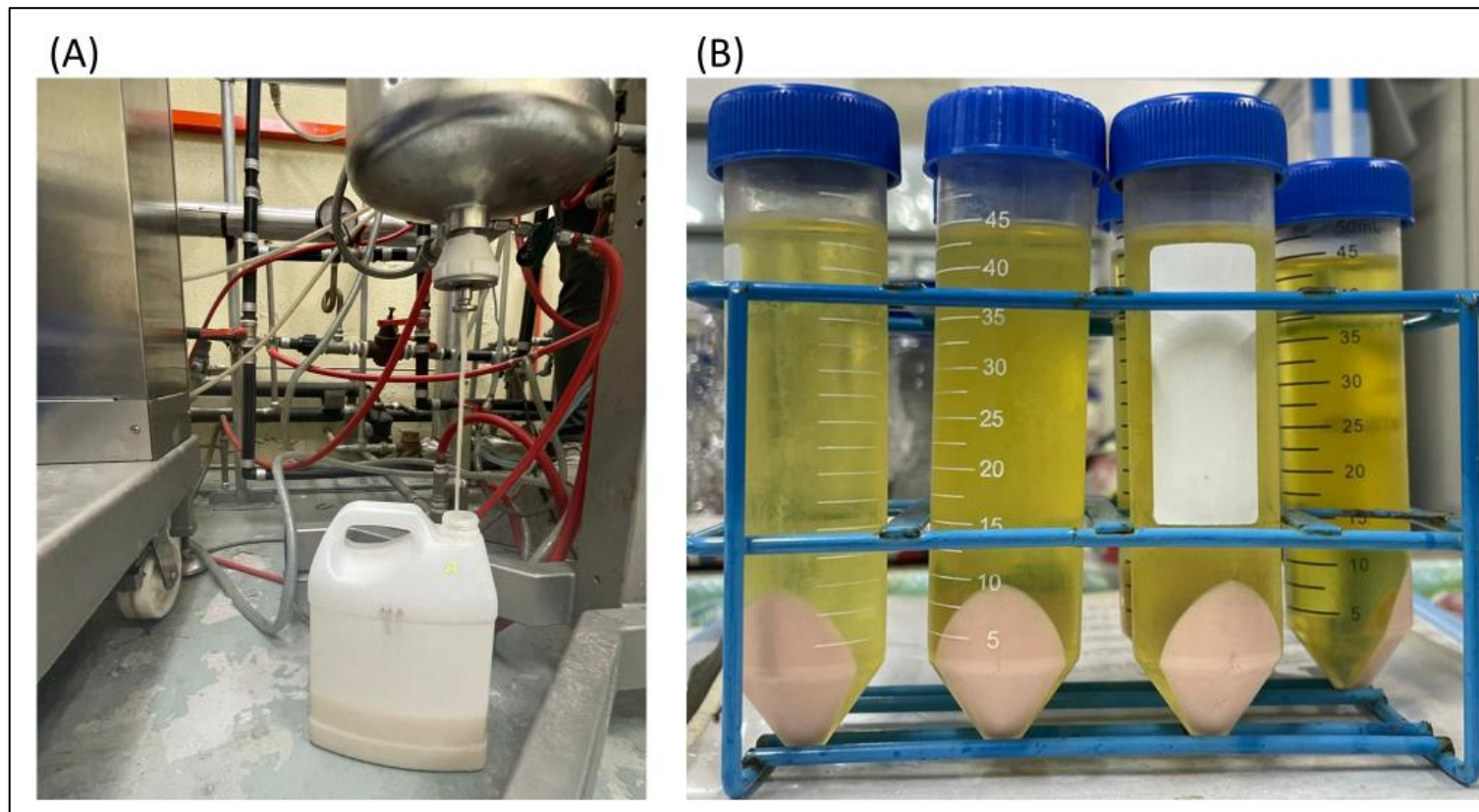
Appendix 6: Image shows the reagents, including lyophilized LAL reagent, endotoxin standards, and endotoxin-free water from Sigma-Aldrich's E-TOXATE™ Kit used for the detection and quantification of endotoxins in this study.



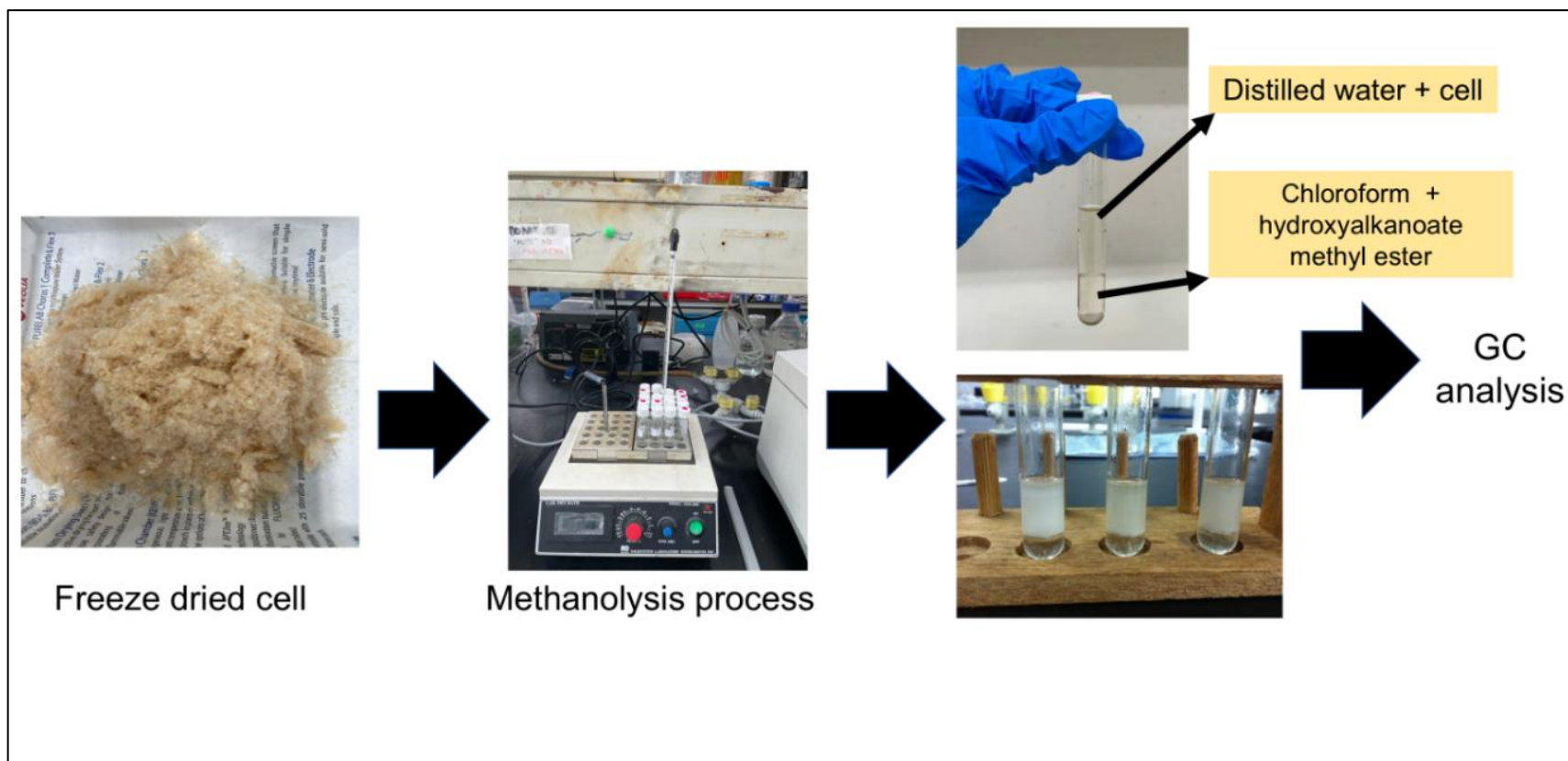
Appendix 7: Schematic illustration of steps involved prior to and during the bacterial activation and seed culture preparation method



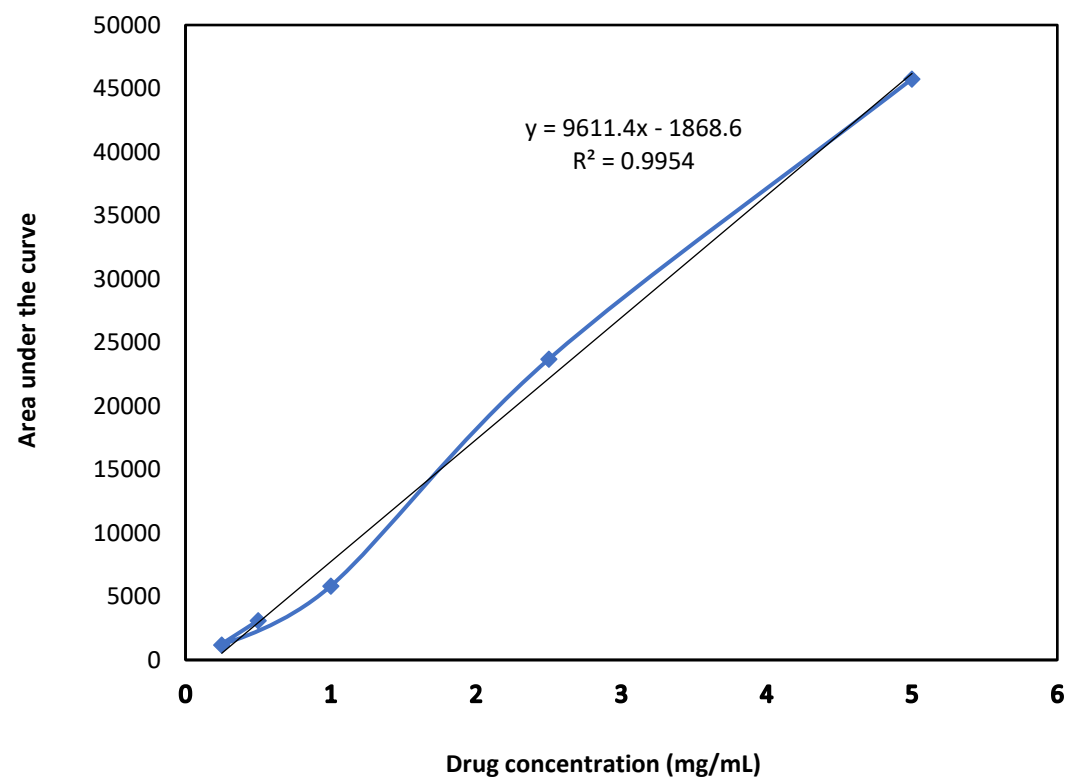
Appendix 8: Schematic illustration of steps involved in the biosynthesis of P(3HB-co-4HB) copolymer by *Cupriavidus sp.* USMAA1020 using 15L fermenter



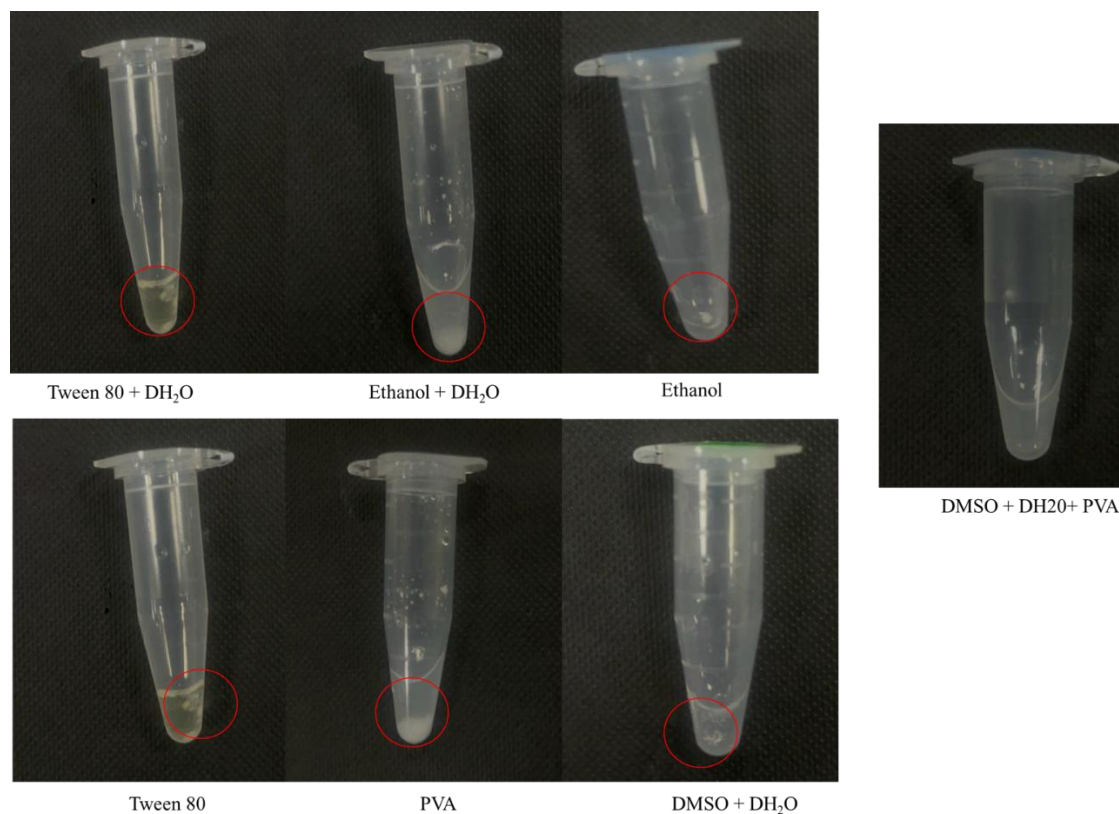
Appendix 9: Image in (A) shows the harvesting process of collecting the sample through the sampling port. The image in (B) shows the pellet and supernatant of the collected sample after centrifugation.



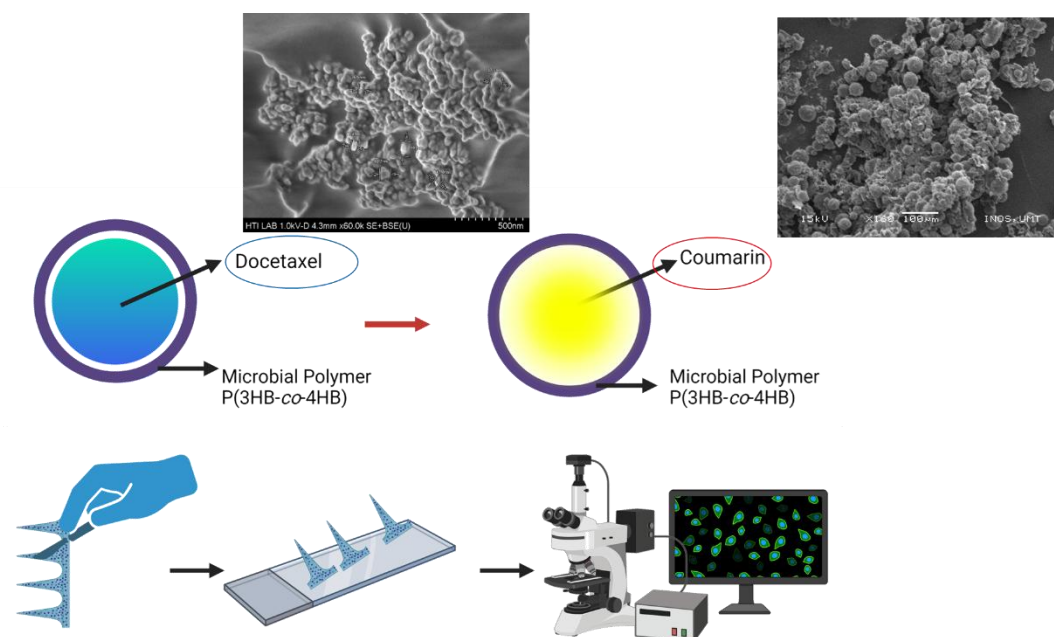
Appendix 10: Schematic illustration of the steps involved in the methanolysis process before Gas chromatography analysis



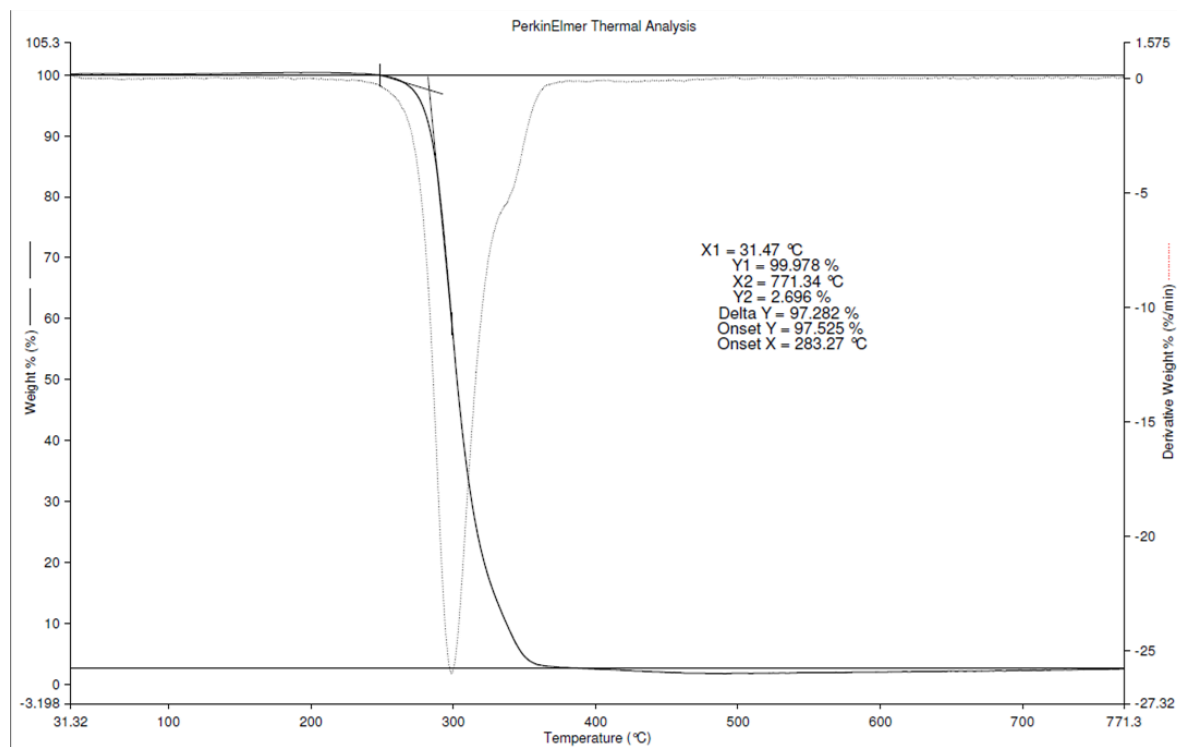
Appendix 11: Standard calibration curve of docetaxel. The linear regression equation and correlation coefficient ($R^2 = 0.9954$) indicate a strong linear relationship between drug concentration and area under the curve.



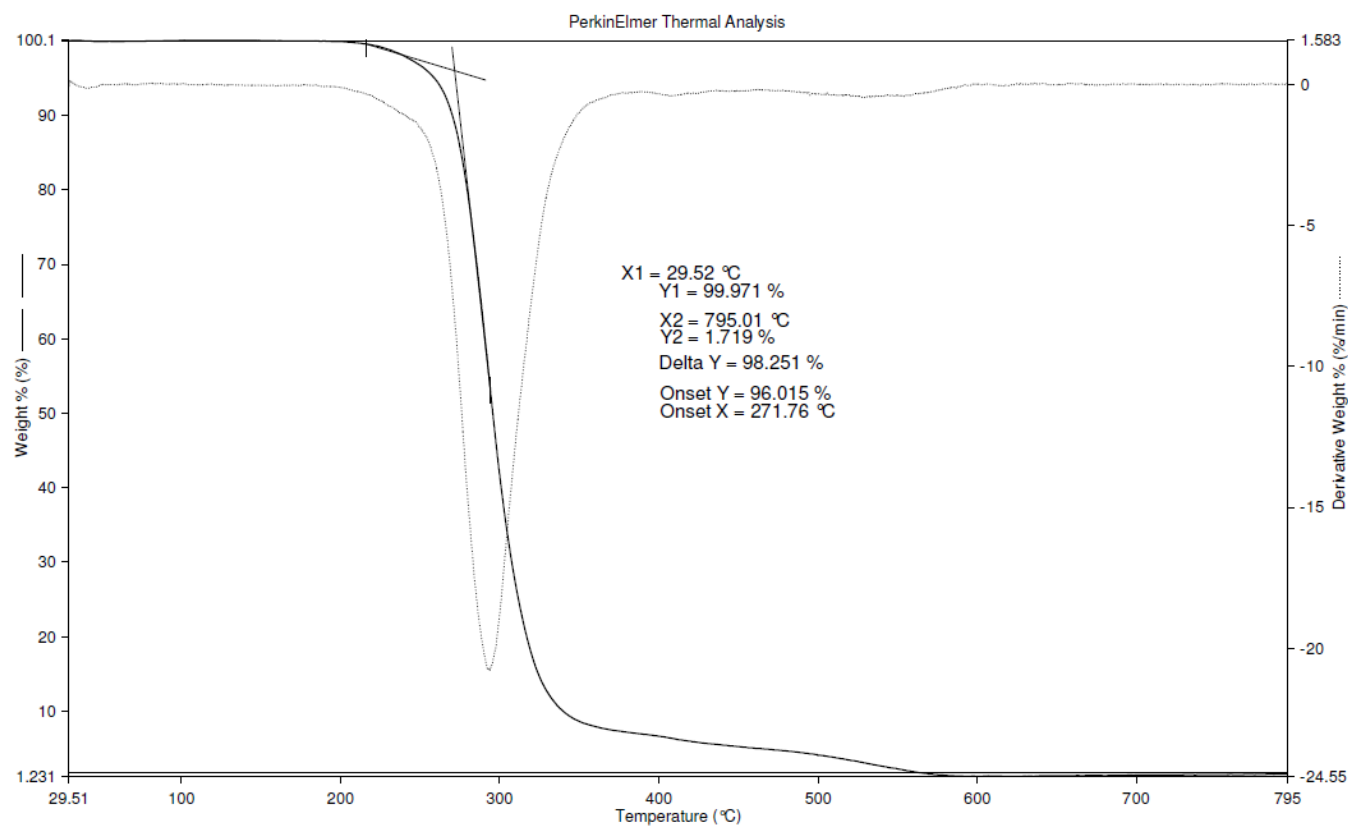
Appendix 12: Dispersion of docetaxel-loaded polymeric nanoparticles in various solvent systems during microneedle formulation optimization. Different solvents and solvent combinations were tested to evaluate nanoparticle dispersion efficiency. The red circles highlight visible nanoparticle aggregation, floating, or sedimentation. Based on visual clarity and stability, a combination of DMSO, distilled water, and PVA was selected as the optimal dispersion medium for microneedle fabrication.



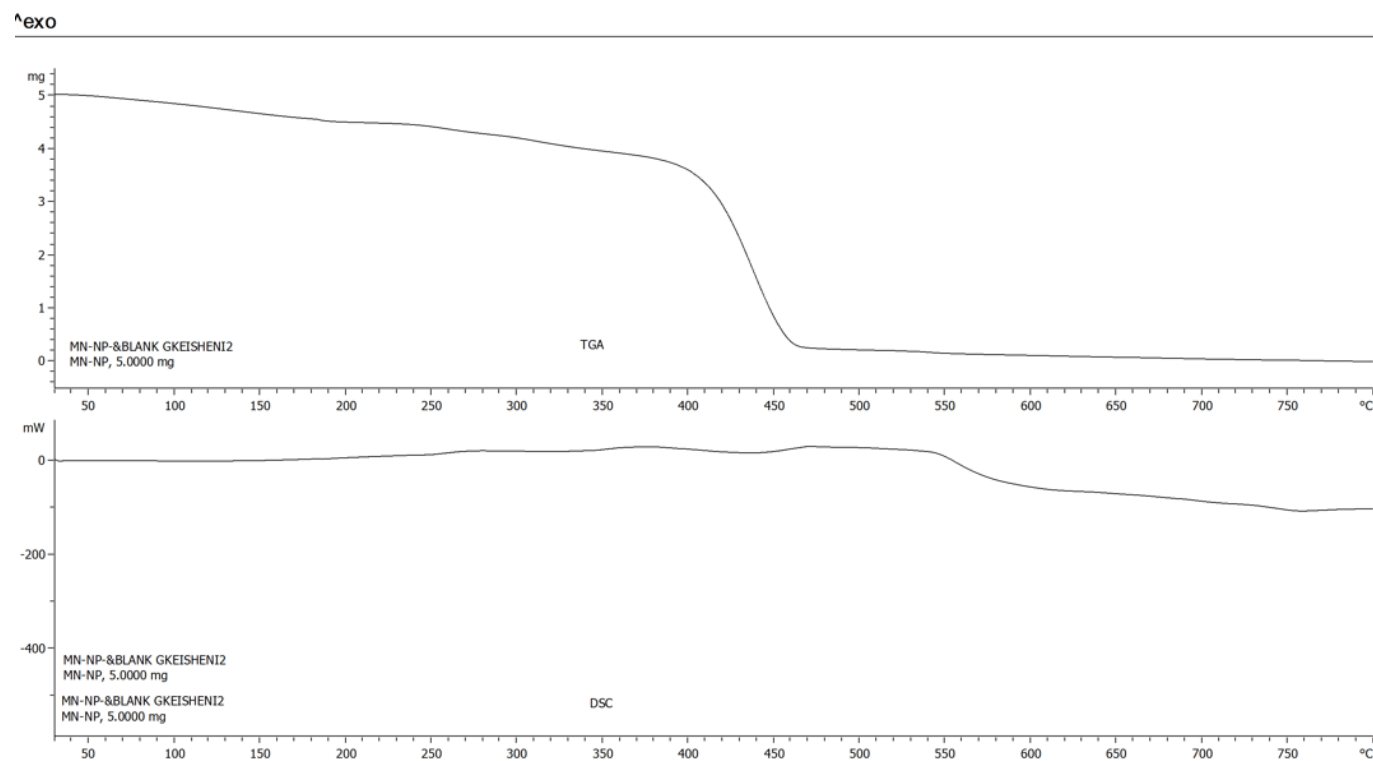
Appendix 13: Fluorescent model drug (coumarin)-loaded P(3HB-co-4HB) nanoparticles were used to study drug distribution in microneedles under a fluorescence microscope. Coumarin was used as a substitute for docetaxel to enable visualization, as both nanoparticles exhibited similar morphology, size, and shape under SEM imaging (top panel). The bottom panel illustrates the method of preparing microneedle samples for imaging, including cutting the microneedles and placing them on a glass slide for observation under a fluorescence microscope.



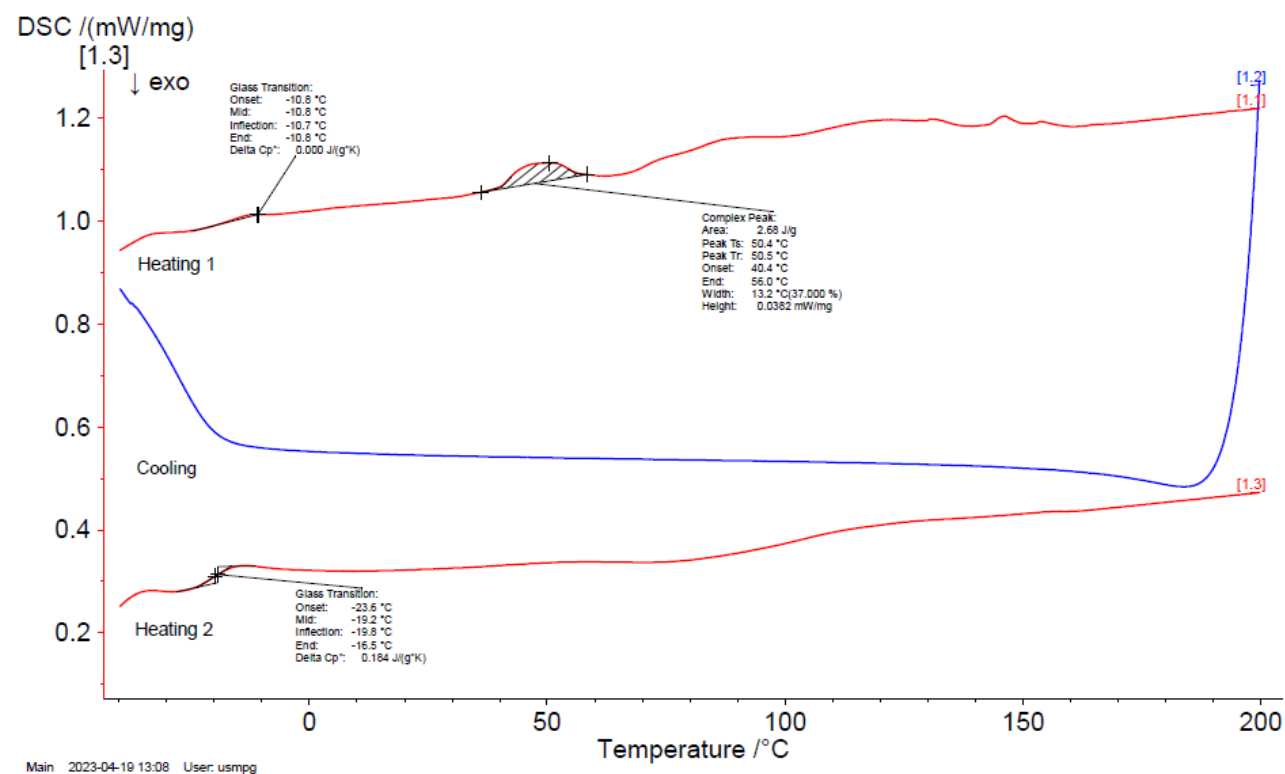
Appendix 14: Thermogravimetric Analysis (TGA) curve of P(3HB-co-4HB), showing the weight loss profile as a function of temperature.



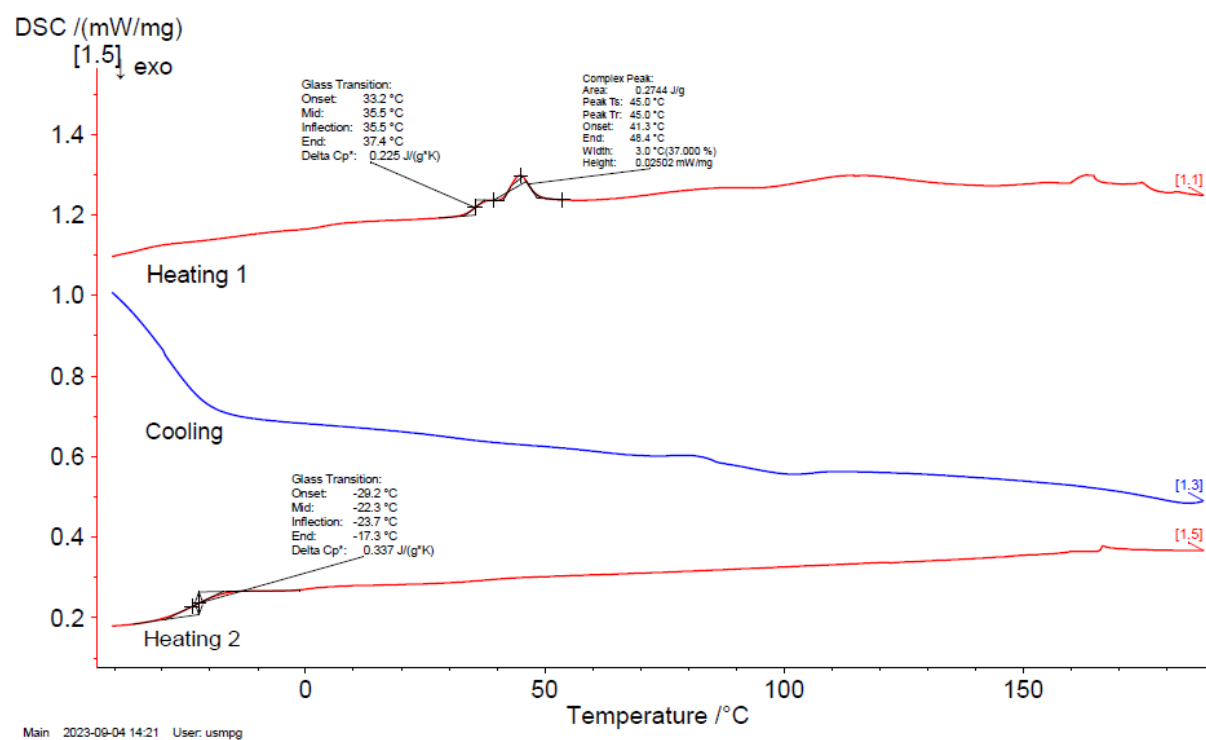
Appendix 15: Thermogravimetric Analysis (TGA) curve of the docetaxel-loaded nanoparticles, showing the weight loss profile as a function of temperature.



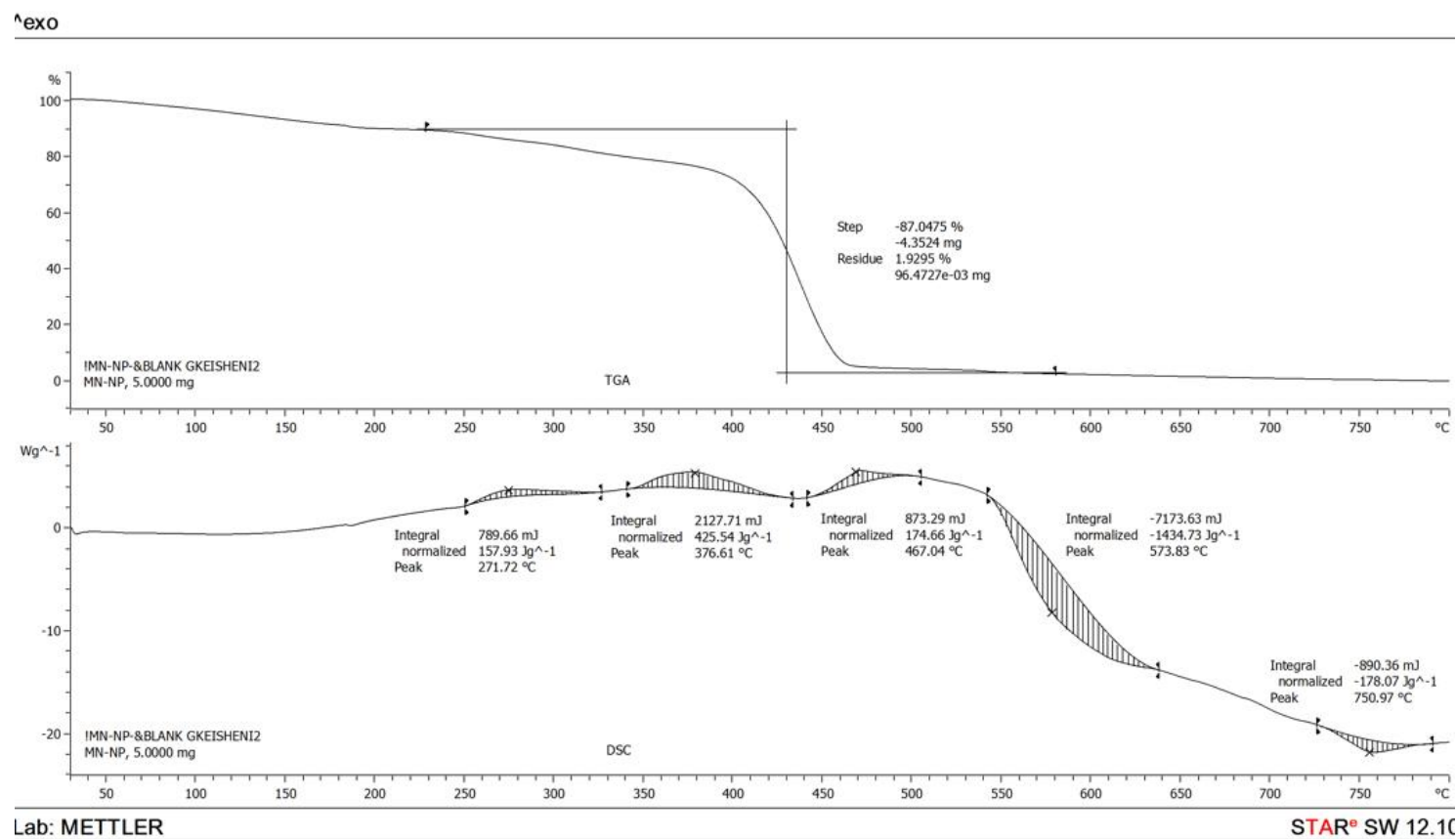
Appendix 16: Thermogravimetric Analysis (TGA) curve of the microneedles incorporated with docetaxel-loaded nanoparticles, showing the weight loss profile as a function of temperature.



Appendix 17: Differential Scanning Calorimetry (DSC) curve of the P(3HB-co-4HB), showing the weight loss profile as a function of temperature.

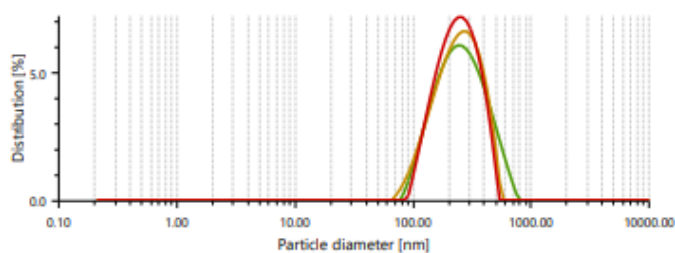


Appendix 18: Differential Scanning Calorimetry (DSC) curve of the docetaxel-loaded nanoparticles, showing the weight loss profile as a function of temperature.



Appendix 19: Differential Scanning Calorimetry (DSC) curve of the microneedles incorporated with docetaxel-loaded nanoparticles, showing the weight loss profile as a function of temperature.

Particle size distribution (intensity)



Statistics table

Name	Hydrodynamic diameter [nm]	Polydispersity index [%]	Peak 1 [nm]	Peak 2 [nm]	Peak 3 [nm]
Mean value	247.3	22.2	261.9 (Intensity)	- (Intensity)	- (Intensity)
Standard deviation	7.11	3.1	13.90 (Intensity)	- (Intensity)	- (Intensity)
Rel. standard deviation	2.88	13.76	5.31 (Intensity)	- (Intensity)	- (Intensity)

Measurements (intensity)

Name	Color	Hydrodyn. diam. [nm]	Polydispersity index [%]	Peak 1 [nm]	Peak 2 [nm]	Peak 3 [nm]	Transmittance [%]
Untitled 351 1	Green	251.4	19.2	277.7 (Intensity)	- (Intensity)	- (Intensity)	80.02
Untitled 351 2	Yellow	251.5	25.3	256.4 (Intensity)	- (Intensity)	- (Intensity)	81.59
Untitled 351 3	Red	239.1	22.1	251.5 (Intensity)	- (Intensity)	- (Intensity)	81.62

Appendix 20: Particle size distribution of nanoparticles (NPs) formulated with a 40% drug-to-polymer ratio, as determined by dynamic light scattering (DLS). The mean hydrodynamic diameter was 247.3 ± 7.11 nm with a polydispersity index (PDI) of 0.222 ± 0.031 , indicating a narrow size distribution suitable for drug delivery applications.

Appendix 21: Tukey's multiple comparisons test results following a two-way ANOVA for needle height reduction (%) under static forces (50 N, 100 N, 200 N, 500 N, and 1000 N). Rows represent five levels (Rows 1–5), while columns compare Blank Microneedles vs. Nanoparticle-loaded microneedles. This table details mean differences, 95% confidence intervals, significance levels, adjusted *p* values, and test statistics for each pairwise comparison.

Compare cell means with others in its row and its column					
Number of families	7				
Number of comparisons per row family	1				
Number of comparisons per column family	10				
Alpha	0.05				
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of dif f.	Below threshold ?	Summary	Adjusted P Value
Row 1					
Blank Microneedles vs. Group B	-1.160	-2.316 to -0.004092	Yes	*	0.0493
Row 2					

Blank Microneedles vs. Group		-			
B	-1.092	2.248 to 0.06414	No	ns	0.0628
Row 3					
Blank Microneedles vs. Group		-2.384 to -			
B	-1.228	0.07233	Yes	*	0.0384
Row 4					
Blank Microneedles vs. Group					
B	0.000	-1.156 to 1.156	No	ns	>0.9999
Row 5					
Blank Microneedles vs. Group					
B	0.000	-1.156 to 1.156	No	ns	>0.9999
Blank Microneedles					
Row 1 vs. Row 2	-4.606	-6.264 to -2.948	Yes	****	<0.0001
Row 1 vs. Row 3	-18.53	-20.18 to -16.87	Yes	****	<0.0001
Row 1 vs. Row 4	-84.89	-86.54 to -83.23	Yes	****	<0.0001
Row 1 vs. Row 5	-84.89	-86.54 to -83.23	Yes	****	<0.0001
Row 2 vs. Row 3	-13.92	-15.58 to -12.26	Yes	****	<0.0001

Row 2 vs. Row 4	-80.28	-81.94 to -78.62	Yes	****	<0.0001
Row 2 vs. Row 5	-80.28	-81.94 to -78.62	Yes	****	<0.0001
Row 3 vs. Row 4	-66.36	-68.02 to -64.70	Yes	****	<0.0001
Row 3 vs. Row 5	-66.36	-68.02 to -64.70	Yes	****	<0.0001
Row 4 vs. Row 5	0.000	-1.658 to 1.658	No	ns	>0.9999
Group B					
Row 1 vs. Row 2	-4.538	-6.196 to -2.879	Yes	****	<0.0001
Row 1 vs. Row 3	-18.59	-20.25 to -16.94	Yes	****	<0.0001
Row 1 vs. Row 4	-83.73	-85.38 to -82.07	Yes	****	<0.0001
Row 1 vs. Row 5	-83.73	-85.38 to -82.07	Yes	****	<0.0001
Row 2 vs. Row 3	-14.06	-15.71 to -12.40	Yes	****	<0.0001
Row 2 vs. Row 4	-79.19	-80.85 to -77.53	Yes	****	<0.0001
Row 2 vs. Row 5	-79.19	-80.85 to -77.53	Yes	****	<0.0001
Row 3 vs. Row 4	-65.13	-66.79 to -63.47	Yes	****	<0.0001
Row 3 vs. Row 5	-65.13	-66.79 to -63.47	Yes	****	<0.0001

Row 4 vs. Row 5	0.000	-1.658 to 1.658	No	ns	>0.9999			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF
Row 1								
Blank Microneedles vs. Group								
B	15.11	16.27	-1.160	0.5541	3	3	2.960	20.00
Row 2								
Blank Microneedles vs. Group								
B	19.72	20.81	-1.092	0.5541	3	3	2.786	20.00
Row 3								
Blank Microneedles vs. Group								
B	33.64	34.87	-1.228	0.5541	3	3	3.135	20.00
Row 4								
Blank Microneedles vs. Group								
B	100.0	100.0	0.000	0.5541	3	3	0.000	20.00
Row 5								
Blank Microneedles vs. Group								
B	100.0	100.0	0.000	0.5541	3	3	0.000	20.00

Blank Microneedles								
Row 1 vs. Row 2	15.11	19.72	-4.606	0.5541	3	3	11.75	20.00
Row 1 vs. Row 3	15.11	33.64	-18.53	0.5541	3	3	47.28	20.00
Row 1 vs. Row 4	15.11	100.0	-84.89	0.5541	3	3	216.6	20.00
Row 1 vs. Row 5	15.11	100.0	-84.89	0.5541	3	3	216.6	20.00
Row 2 vs. Row 3	19.72	33.64	-13.92	0.5541	3	3	35.53	20.00
Row 2 vs. Row 4	19.72	100.0	-80.28	0.5541	3	3	204.9	20.00
Row 2 vs. Row 5	19.72	100.0	-80.28	0.5541	3	3	204.9	20.00
Row 3 vs. Row 4	33.64	100.0	-66.36	0.5541	3	3	169.4	20.00
Row 3 vs. Row 5	33.64	100.0	-66.36	0.5541	3	3	169.4	20.00
Row 4 vs. Row 5	100.0	100.0	0.000	0.5541	3	3	0.000	20.00
Group B								
Row 1 vs. Row 2	16.27	20.81	-4.538	0.5541	3	3	11.58	20.00
Row 1 vs. Row 3	16.27	34.87	-18.59	0.5541	3	3	47.45	20.00
Row 1 vs. Row 4	16.27	100.0	-83.73	0.5541	3	3	213.7	20.00

Row 1 vs. Row 5	16.27	100.0	-83.73	0.5541	3	3	213.7	20.00
Row 2 vs. Row 3	20.81	34.87	-14.06	0.5541	3	3	35.87	20.00
Row 2 vs. Row 4	20.81	100.0	-79.19	0.5541	3	3	202.1	20.00
Row 2 vs. Row 5	20.81	100.0	-79.19	0.5541	3	3	202.1	20.00
Row 3 vs. Row 4	34.87	100.0	-65.13	0.5541	3	3	166.2	20.00
Row 3 vs. Row 5	34.87	100.0	-65.13	0.5541	3	3	166.2	20.00
Row 4 vs. Row 5	100.0	100.0	0.000	0.5541	3	3	0.000	20.00

Appendix 22: Tukey's multiple comparisons test results following a two-way ANOVA among four treatments (Free Drug, Drug-Loaded Nanoparticles, FD-Microneedles, and NP-Loaded Microneedles) in a drug delivery analysis. The table presents mean differences, 95% confidence intervals, statistical significance (p values), test statistics (q , DF), and a compact letter display summarizing pairwise comparisons.

Number of families	1							
Number of comparisons per family	6							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff. 95.00% CI of diff. Below threshold? Summary Adjusted P Value							
Free drug vs. Nanoparticles	6.882	-0.002291 to 13.77		No	ns	0.0501	A-B	
Free drug vs. FD-Microneedles	4.879	-2.005 to 11.76		No	ns	0.2486	A-C	
Free drug vs. NP-loaded microneedles	7.347	0.4626 to 14.23		Yes	*	0.0322	A-D	
Nanoparticles vs. FD-Microneedles	-2.002	-8.886 to 4.882		No	ns	0.8667	B-C	
Nanoparticles vs. NP-loaded microneedles	0.4649	-6.419 to 7.349		No	ns	0.9979	B-D	
FD-Microneedles vs. NP-loaded microneedles	2.467	-4.417 to 9.351		No	ns	0.7774	C-D	
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Free drug vs. Nanoparticles	16.03	9.147	6.882	2.594	14	14	3.752	52
Free drug vs. FD-Microneedles	16.03	11.15	4.879	2.594	14	14	2.660	52

Free drug vs. NP-loaded microneedles	16.03	8.682	7.347	2.594	14	14	4.006	52
Nanoparticles vs. FD-Microneedles	9.147	11.15	-2.002	2.594	14	14	1.092	52
Nanoparticles vs. NP-loaded microneedles	9.147	8.682	0.4649	2.594	14	14	0.2535	52
FD-Microneedles vs. NP-loaded microneedles	11.15	8.682	2.467	2.594	14	14	1.345	52
Compact letter display								
Free drug	A							
FD-Microneedles	A B							
Nanoparticles	A B							
NP-loaded microneedles	B							

Appendix 23: Tukey's multiple comparisons test results following a two-way ANOVA for the optimization of nanoparticle fabrication, comparing "With TPGS" vs. "Without TPGS" at two time points (4 hours, 12 hours). The table shows mean differences, 95% confidence intervals, significance levels, adjusted *p* values, test statistics, and a compact letter display summarizing all pairwise comparisons.

Number of families	1							
Number of comparisons per family	6							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
With TPGS 4h vs. With TPGS 12h	-53.82	-60.41 to -47.23	Yes	****	<0.0001		A-B	
With TPGS 4h vs. Without TPGS 4h	-3.787	-10.38 to 2.808	No	ns	0.3238		A-C	
With TPGS 4h vs. Without TPGS 12h	-17.39	-23.98 to -10.79	Yes	***	0.0001		A-D	
With TPGS 12h vs. Without TPGS 4h	50.03	43.44 to 56.63	Yes	****	<0.0001		B-C	
With TPGS 12h vs. Without TPGS 12h	36.43	29.84 to 43.03	Yes	****	<0.0001		B-D	
Without TPGS 4h vs. Without TPGS 12h	-13.60	-20.19 to -7.006	Yes	***	0.0008		C-D	
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
With TPGS 4h vs. With TPGS 12h	288.7	342.5	-53.82	2.059	3	3	36.96	8
With TPGS 4h vs. Without TPGS 4h	288.7	292.5	-3.787	2.059	3	3	2.601	8

Appendix 24: Tukey's multiple comparisons test results following a two-way ANOVA on microneedle geometry (interspace of tip, interspace of base, base width, needle height, tip diameter, tip angle/apex angle, and aspect ratio) in MN BLANK and MN NP groups (Rows 1–5). This table shows pairwise mean differences, 95% confidence intervals, significance levels, adjusted *p* values, and test statistics for each comparison within columns.

Within each column, compare rows (simple effects within columns)						
Number of families	2					
Number of comparisons per family	21					
Alpha	0.05					
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff	Below threshold?	Summary	Adjusted P Value	
MN BLANK						
Interspace of tip vs. Interspace of base	230.0	226.0 to 233.9	Yes	****	<0.0001	
Interspace of tip vs. Base width	1230	1226 to 1234	Yes	****	<0.0001	
Interspace of tip vs. Needle height	499.8	495.8 to 503.7	Yes	****	<0.0001	
Interspace of tip vs. Tip diameter	1464	1460 to 1468	Yes	****	<0.0001	

Interspace of tip vs. Tip angle/apex angle	1457	1453 to 1461	Yes	****	<0.0001
Interspace of tip vs. Aspect ratio	1473	1469 to 1477	Yes	****	<0.0001
Interspace of base vs. Base width	1000	996.5 to 1004	Yes	****	<0.0001
Interspace of base vs. Needle height	269.8	265.9 to 273.7	Yes	****	<0.0001
Interspace of base vs. Tip diameter	1234	1230 to 1238	Yes	****	<0.0001
Interspace of base vs. Tip angle/apex angle	1227	1223 to 1231	Yes	****	<0.0001
Interspace of base vs. Aspect ratio	1243	1239 to 1247	Yes	****	<0.0001
Base width vs. Needle height	-730.6	-734.5 to -726.7	Yes	****	<0.0001
Base width vs. Tip diameter	234.0	230.1 to 238.0	Yes	****	<0.0001
Base width vs. Tip angle/apex angle	227.0	223.0 to 230.9	Yes	****	<0.0001
Base width vs. Aspect ratio	242.2	238.3 to 246.2	Yes	****	<0.0001
Needle height vs. Tip diameter	964.6	960.7 to 968.6	Yes	****	<0.0001
Needle height vs. Tip angle/apex angle	957.6	953.6 to 961.5	Yes	****	<0.0001
Needle height vs. Aspect ratio	972.8	968.9 to 976.8	Yes	****	<0.0001

Tip diameter vs. Tip angle/apex angle	-7.069	-11.02 to -3.122	Yes	****	<0.0001
Tip diameter vs. Aspect ratio	8.185	4.238 to 12.13	Yes	****	<0.0001
Tip angle/apex angle vs. Aspect ratio	15.25	11.31 to 19.20	Yes	****	<0.0001
MN NP					
Interspace of tip vs. Interspace of base	231.8	227.8 to 235.7	Yes	****	<0.0001
Interspace of tip vs. Base width	1233	1229 to 1236	Yes	****	<0.0001
Interspace of tip vs. Needle height	501.2	497.2 to 505.1	Yes	****	<0.0001
Interspace of tip vs. Tip diameter	1466	1462 to 1470	Yes	****	<0.0001
Interspace of tip vs. Tip angle/apex angle	1458	1454 to 1462	Yes	****	<0.0001
Interspace of tip vs. Aspect ratio	1474	1470 to 1478	Yes	****	<0.0001
Interspace of base vs. Base width	1001	996.9 to 1005	Yes	****	<0.0001
Interspace of base vs. Needle height	269.4	265.5 to 273.3	Yes	****	<0.0001
Interspace of base vs. Tip diameter	1234	1230 to 1238	Yes	****	<0.0001
Interspace of base vs. Tip angle/apex angle	1227	1223 to 1230	Yes	****	<0.0001

Interspace of base vs. Aspect ratio	1242	1238 to 1246	Yes	****	<0.0001			
Base width vs. Needle height	-731.4	-735.3 to -727.5	Yes	****	<0.0001			
Base width vs. Tip diameter	233.2	229.3 to 237.2	Yes	****	<0.0001			
Base width vs. Tip angle/apex angle	225.7	221.8 to 229.7	Yes	****	<0.0001			
Base width vs. Aspect ratio	241.2	237.3 to 245.2	Yes	****	<0.0001			
Needle height vs. Tip diameter	964.6	960.7 to 968.6	Yes	****	<0.0001			
Needle height vs. Tip angle/apex angle	957.1	953.2 to 961.1	Yes	****	<0.0001			
Needle height vs. Aspect ratio	972.6	968.7 to 976.6	Yes	****	<0.0001			
Tip diameter vs. Tip angle/apex angle	-7.492	-11.44 to -3.545	Yes	****	<0.0001			
Tip diameter vs. Aspect ratio	7.989	4.041 to 11.94	Yes	****	<0.0001			
Tip angle/apex angle vs. Aspect ratio	15.48	11.53 to 19.43	Yes	****	<0.0001			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff. N1	N2	q	DF	
MN BLANK								
Interspace of tip vs. Interspace of base	1477	1247	230.0	1.291	5	5	251.9	56.00
Interspace of tip vs. Base width	1477	246.2	1230	1.291	5	5	1348	56.00

Interspace of tip vs. Needle height	1477	976.8	499.8	1.291	5	5	547.5	56.00
Interspace of tip vs. Tip diameter	1477	12.15	1464	1.291	5	5	1604	56.00
Interspace of tip vs. Tip angle/apex angle	1477	19.22	1457	1.291	5	5	1597	56.00
Interspace of tip vs. Aspect ratio	1477	3.968	1473	1.291	5	5	1613	56.00
Interspace of base vs. Base width	1247	246.2	1000	1.291	5	5	1096	56.00
Interspace of base vs. Needle height	1247	976.8	269.8	1.291	5	5	295.6	56.00
Interspace of base vs. Tip diameter	1247	12.15	1234	1.291	5	5	1352	56.00
Interspace of base vs. Tip angle/apex angle	1247	19.22	1227	1.291	5	5	1345	56.00
Interspace of base vs. Aspect ratio	1247	3.968	1243	1.291	5	5	1361	56.00
Base width vs. Needle height	246.2	976.8	-730.6	1.291	5	5	800.4	56.00
Base width vs. Tip diameter	246.2	12.15	234.0	1.291	5	5	256.4	56.00
Base width vs. Tip angle/apex angle	246.2	19.22	227.0	1.291	5	5	248.7	56.00
Base width vs. Aspect ratio	246.2	3.968	242.2	1.291	5	5	265.4	56.00
Needle height vs. Tip diameter	976.8	12.15	964.6	1.291	5	5	1057	56.00

Needle height vs. Tip angle/apex angle	976.8	19.22	957.6	1.291	5	5	1049	56.00
Needle height vs. Aspect ratio	976.8	3.968	972.8	1.291	5	5	1066	56.00
Tip diameter vs. Tip angle/apex angle	12.15	19.22	-7.069	1.291	5	5	7.745	56.00
Tip diameter vs. Aspect ratio	12.15	3.968	8.185	1.291	5	5	8.967	56.00
Tip angle/apex angle vs. Aspect ratio	19.22	3.968	15.25	1.291	5	5	16.71	56.00
MN NP								
Interspace of tip vs. Interspace of base	1478	1246	231.8	1.291	5	5	253.9	56.00
Interspace of tip vs. Base width	1478	245.2	1233	1.291	5	5	1350	56.00
Interspace of tip vs. Needle height	1478	976.6	501.2	1.291	5	5	549.0	56.00
Interspace of tip vs. Tip diameter	1478	11.97	1466	1.291	5	5	1606	56.00
Interspace of tip vs. Tip angle/apex angle	1478	19.46	1458	1.291	5	5	1598	56.00
Interspace of tip vs. Aspect ratio	1478	3.983	1474	1.291	5	5	1615	56.00
Interspace of base vs. Base width	1246	245.2	1001	1.291	5	5	1096	56.00
Interspace of base vs. Needle height	1246	976.6	269.4	1.291	5	5	295.1	56.00
Interspace of base vs. Tip diameter	1246	11.97	1234	1.291	5	5	1352	56.00

e	Interspace of base vs. Tip angle/apex angl	1246	19.46	1227	1.291	5	5	1344	56.00
	Interspace of base vs. Aspect ratio	1246	3.983	1242	1.291	5	5	1361	56.00
	Base width vs. Needle height	245.2	976.6	-731.4	1.291	5	5	801.3	56.00
	Base width vs. Tip diameter	245.2	11.97	233.2	1.291	5	5	255.5	56.00
	Base width vs. Tip angle/apex angle	245.2	19.46	225.7	1.291	5	5	247.3	56.00
	Base width vs. Aspect ratio	245.2	3.983	241.2	1.291	5	5	264.3	56.00
	Needle height vs. Tip diameter	976.6	11.97	964.6	1.291	5	5	1057	56.00
	Needle height vs. Tip angle/apex angle	976.6	19.46	957.1	1.291	5	5	1049	56.00
	Needle height vs. Aspect ratio	976.6	3.983	972.6	1.291	5	5	1066	56.00
	Tip diameter vs. Tip angle/apex angle	11.97	19.46	-7.492	1.291	5	5	8.208	56.00
	Tip diameter vs. Aspect ratio	11.97	3.983	7.989	1.291	5	5	8.752	56.00
	Tip angle/apex angle vs. Aspect ratio	19.46	3.983	15.48	1.291	5	5	16.96	56.00

Appendix 25: Paired *t* test results comparing Blank Microneedles and Nanoparticle-loaded Microneedles for drug content determination. The table displays *p* values, mean difference, 95% confidence interval, and additional test statistics (including correlation of pairing) to illustrate the effectiveness of the pairing and the significance of the observed differences.

Table Analyzed	Data 8
Column B	Microneedles
vs.	vs.
Column A	Nanoparticle
Paired <i>t</i> test	
P value	0.0018
P value summary	**
Significantly different ($P < 0.05$)?	Yes
One- or two-tailed P value?	Two-tailed
<i>t</i> , <i>df</i>	$t=10.61$, $df=3$
Number of pairs	4
How big is the difference?	

Mean of differences (B - A)	-0.8975
SD of differences	0.1692
SEM of differences	0.08459
95% confidence interval	-1.167 to -0.6283
R squared (partial eta squared)	0.9740
How effective was the pairing?	
Correlation coefficient (r)	0.9954
P value (one tailed)	0.0023
P value summary	**
Was the pairing significantly effective?	Yes

Appendix 26: Tukey's multiple comparisons test results following a two-way ANOVA examining Blank Microneedles vs. Nanoparticle-Loaded Microneedles in 2% and 6% agarose gels at five time points (Rows 1–5: 0, 15, 45, 120, 300). This table details the simple effects within each row, showing mean differences, 95% confidence intervals, significance levels, adjusted *p* values, and test statistics for each pairwise comparison.

Within each row, compare columns

(simple effects within rows)

Number of families	5
Number of comparisons per family	6
Alpha	0.05

			Below	Adjusted	
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	threshold?	Summary	P Value
0					
2%Blank vs. 6%Blank	1.376	-5.596 to 8.348	No	ns	0.8783
2%Blank vs. 2%NP	8.336	1.131 to 15.54	Yes	*	0.0308
2%Blank vs. 6%NP	4.188	-3.957 to 12.33	No	ns	0.4076
6%Blank vs. 2%NP	6.960	4.643 to 9.277	Yes	***	0.0007
6%Blank vs. 6%NP	2.812	-4.358 to 9.981	No	ns	0.5169

2%NP vs. 6%NP	-4.148	-11.55 to 3.253	No	ns	0.2466
15					
2%Blank vs. 6%Blank	4.831	0.4790 to 9.183	Yes	*	0.0350
2%Blank vs. 2%NP	21.43	15.97 to 26.90	Yes	****	<0.0001
2%Blank vs. 6%NP	25.60	21.27 to 29.93	Yes	****	<0.0001
6%Blank vs. 2%NP	16.60	11.37 to 21.83	Yes	***	0.0006
6%Blank vs. 6%NP	20.77	17.89 to 23.64	Yes	****	<0.0001
2%NP vs. 6%NP	4.166	-0.9191 to 9.251	No	ns	0.1061
45					
2%Blank vs. 6%Blank	17.15	16.44 to 17.87	Yes	****	<0.0001
2%Blank vs. 2%NP	22.13	21.36 to 22.90	Yes	****	<0.0001
2%Blank vs. 6%NP	23.54	22.06 to 25.03	Yes	****	<0.0001
6%Blank vs. 2%NP	4.974	4.144 to 5.805	Yes	****	<0.0001
6%Blank vs. 6%NP	6.387	4.967 to 7.807	Yes	****	<0.0001
		-			
2%NP vs. 6%NP	1.412	0.009576 to 2.835	No	ns	0.0514

120								
2%Blank vs. 6%Blank	0.000							
2%Blank vs. 2%NP	0.000							
2%Blank vs. 6%NP	0.000							
6%Blank vs. 2%NP	0.000							
6%Blank vs. 6%NP	0.000							
2%NP vs. 6%NP	0.000							
300								
2%Blank vs. 6%Blank	0.000							
2%Blank vs. 2%NP	0.000							
2%Blank vs. 6%NP	0.000							
6%Blank vs. 2%NP	0.000							
6%Blank vs. 6%NP	0.000							
2%NP vs. 6%NP	0.000							
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1	N2	q	DF

0

2%Blank vs. 6%Blank	48.21	46.84	1.376	1.866	5	5	1.043	4.844
2%Blank vs. 2%NP	48.21	39.88	8.336	1.778	5	5	6.631	4.036
2%Blank vs. 6%NP	48.21	44.03	4.188	2.543	5	5	2.329	7.994
6%Blank vs. 2%NP	46.84	39.88	6.960	0.5917	5	5	16.63	4.339
6%Blank vs. 6%NP	46.84	44.03	2.812	1.912	5	5	2.080	4.801
2%NP vs. 6%NP	39.88	44.03	-4.148	1.826	5	5	3.213	4.034

15

2%Blank vs. 6%Blank	75.29	70.46	4.831	1.105	5	5	6.181	4.288
2%Blank vs. 2%NP	75.29	53.86	21.43	1.694	5	5	17.89	7.754
2%Blank vs. 6%NP	75.29	49.69	25.60	1.307	5	5	27.70	6.988
6%Blank vs. 2%NP	70.46	53.86	16.60	1.316	5	5	17.84	4.201
6%Blank vs. 6%NP	70.46	49.69	20.77	0.7561	5	5	38.84	4.639
2%NP vs. 6%NP	53.86	49.69	4.166	1.490	5	5	3.955	6.281

45

2%Blank vs. 6%Blank	100.0	82.85	17.15	0.1761	5	5	137.8	4.000
2%Blank vs. 2%NP	100.0	77.87	22.13	0.1898	5	5	164.8	4.000
2%Blank vs. 6%NP	100.0	76.46	23.54	0.3648	5	5	91.27	4.000
6%Blank vs. 2%NP	82.85	77.87	4.974	0.2589	5	5	27.17	7.955
6%Blank vs. 6%NP	82.85	76.46	6.387	0.4050	5	5	22.30	5.768
2%NP vs. 6%NP	77.87	76.46	1.412	0.4112	5	5	4.858	6.019
120								
2%Blank vs. 6%Blank	100.0	100.0	0.000	0.000	5	5		
2%Blank vs. 2%NP	100.0	100.0	0.000	0.000	5	5		
2%Blank vs. 6%NP	100.0	100.0	0.000	0.000	5	5		
6%Blank vs. 2%NP	100.0	100.0	0.000	0.000	5	5		
6%Blank vs. 6%NP	100.0	100.0	0.000	0.000	5	5		
2%NP vs. 6%NP	100.0	100.0	0.000	0.000	5	5		
300								
2%Blank vs. 6%Blank	100.0	100.0	0.000	0.000	5	5		

2%Blank vs. 2%NP	100.0	100.0	0.000	0.000	5	5
2%Blank vs. 6%NP	100.0	100.0	0.000	0.000	5	5
6%Blank vs. 2%NP	100.0	100.0	0.000	0.000	5	5
6%Blank vs. 6%NP	100.0	100.0	0.000	0.000	5	5
2%NP vs. 6%NP	100.0	100.0	0.000	0.000	5	5

Appendix 27: Tukey's multiple comparisons test results following a two-way ANOVA examining how time (Rows 0, 3, 6, etc.) and humidity (0% RH vs. 65% RH) affect nanoparticle characteristics (particle size, polydispersity index, zeta potential, and drug loading). Each cell is compared within its row and column, with the table providing mean differences, 95% confidence intervals, significance levels, adjusted *p* values, and test statistics for every pairwise comparison.

Compare cell means with others in its row and its column					
Number of families	5				
Number of comparisons per row family	1				
Number of comparisons per column family	3				
Alpha	0.05				
	Mean Diff	95.00% CI of diff	Below threshold	Adjusted P	
Tukey's multiple comparisons test	.	.	?	Summary	Value
0					
0% RH vs. 65% RH	0.000	-11.07 to 11.07	No	ns	>0.9999
3					
0% RH vs. 65% RH	4.743	0.8438 to 8.643	Yes	*	0.0289

6								
0% RH vs. 65% RH	15.13	9.280 to 20.98	Yes	**	0.0020			
0% RH								
0 vs. 3	-6.767	-23.43 to 9.896	No	ns	0.2417			
0 vs. 6	-18.25	-34.58 to -1.922	Yes	*	0.0404			
3 vs. 6	-11.48	-24.75 to 1.783	No	ns	0.0656			
65% RH								
0 vs. 3	-2.023	-23.22 to 19.17	No	ns	0.8517			
0 vs. 6	-3.120	-11.42 to 5.183	No	ns	0.2712			
3 vs. 6	-1.097	-14.08 to 11.88	No	ns	0.8800			
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff	N1	N2	q	DF
0								
0% RH vs. 65% RH	204.2	204.2	0.000	3.989	3	3	0.000	4.000
3								

0% RH vs. 65% RH	210.9	206.2	4.743	1.337	3	3	5.016	3.563
6								
0% RH vs. 65% RH	222.4	207.3	15.13	2.103	3	3	10.17	3.981
0% RH								
0 vs. 3	204.2	210.9	-6.767	2.829	3	3	3.383	2.000
0 vs. 6	204.2	222.4	-18.25	2.772	3	3	9.312	2.000
3 vs. 6	210.9	222.4	-11.48	2.252	3	3	7.211	2.000
65% RH								
0 vs. 3	204.2	206.2	-2.023	3.598	3	3	0.7952	2.000
0 vs. 6	204.2	207.3	-3.120	1.410	3	3	3.130	2.000
3 vs. 6	206.2	207.3	-1.097	2.204	3	3	0.7038	2.000

LIST OF PUBLICATIONS

Journal Publications

Vigneswari, S., Kee, S. H., Hazwan, M. H., **Ganeson, K.**, Tamilselvan, K., Bhubalan, K., ... & Ramakrishna, S. (2024). Turning Agricultural Waste Streams into Biodegradable Plastic: A Step Forward into Adopting Sustainable Carbon Neutrality. *Journal of Environmental Chemical Engineering*, 112135. (WOS indexed, Q1; 9.402 SJR impact factor)

Ganeson, K., Alias, A. H., Murugaiyah, V., Amirul, A. A. A., Ramakrishna, S., & Vigneswari, S. (2023). Microneedles for Efficient and Precise Drug Delivery in CancerTherapy. *Pharmaceutics*, 15(3), 744. (WOS indexed, Q1; 0.8 SJR impact factor)

Ganeson, K., Mouriya, G. K., Bhubalan, K., Razifah, M. R., Jasmine, R., Sowmiya, S., ... & Ramakrishna, S. (2023). Smart packaging– A pragmatic solution to approach sustainable food waste management. *Food Packaging and Shelf Life*, 36, 101044. (WOS indexed, Q1; 1.34 SJR impact factor)

Ganeson, K., Tan Xue May, C., Abdullah, A. A. A., Ramakrishna, S., & Vigneswari, S. (2023). Advantages and prospective implications of smart materials in tissue engineering: Piezoelectric, shape memory, and hydrogels. *Pharmaceutics*, 15(9), 2356. (WOS indexed, Q1; 0.8 SJR impact factor)

Ganeson, K., Razifah, M.R., Mubarak, A., Vigneswari, S., Ramakrishna, S. (2022) Improved functionality of cinnamon oil emulsionbased gelatin films as potential edible packaging film for wax apple Food Bioscience, 47, 101638 <https://doi.org/10.1016/j.envres.2023.115988> (WOS indexed, Q1; 4.240 SJR impact factor)

Seng Hon Kee; **Keisheni Ganeson**; Noor Fazielawanie Binti Mohd Rashid; Ain Farhana Mohd Yatim; Sevakumaran Vigneswari, Al-Ashraf Abdullah Amirul; Seeram Ramakrishna; Kesaven Bhubalan (2022) A review on biorefining of oil palm and sugar case agro-industrial residues by bacteria into commercially viable bioplastics and biosurfactants. *Fuel*, 321. (WOS indexed, Q1; 6.609 SJR impact factor)

Bhubalan, K., Tamothran, A.M., Kee, S.H., Foong, S.Y., Lam, S.S., **Ganeson, K.**, Vigneswari, S., Amirul, A.A., Ramakrishna, S. (2021). Adopting blockchain concepts as watermarkers of plastics for sustainable waste management in progressing circular economy model. *Environmental Research*. (WOS indexed, Q1; 6.405 SJR impact factor)

Razifah, M.R., Kadir, N.H.A., Ghazaly, M.M., **Ganeson, K.**, Manikam, R.V.S., Jamaludin, N.H. (2020). Toxicity determination of cinnamon oil infused aloe vera edible coating matrix on normal human lung fibroblast MRC-5 cells. *Malaysian Applied Biology*, 49(4),267–271. (Non indexed, Q4; 0.16 SJR impact factor)

Razifah, M.R., **Keisheni, G.**, Aidilla, M. (2017). Preparation and characterization of edible aloe vera films incorporated with cinnamon oil for fruits and vegetables coating. *Malaysian Applied Biology*, 46(4), 183–190. (Non indexed, Q4; 0.16 SJR impact factor)

Proceedings

Keisheni, G., Razifah, M.R., Amin, KAM., Aidilla, M. (19-21 July 2019). Edible film incorporated with ternary blend cinnamon oil: a natural source for fruit preservation. *Trends in Natural Product Research – PSE Young Scientists' Meeting*, Budapest.

Conferences/Proceedings/Oral presentations/Poster presentations

Keisheni, G., Abdullah, A. A. A & Vigneswari, S. “Biosynthesis of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) bioplastics through microbial fermentation using locally isolated *Cupriavidus malaysiensis* USMAA1020”. Oral presented at the 4th Seminar on Biological Security and Sustainability (BIOSES), Universiti Malaysia Terengganu, UMT (28th -29th June 2022), Terengganu, Malaysia.

Keisheni, G., Abdullah, A. A. A., Thaigar, P., Vigneswari, S. “Fabrication of docetaxel-encapsulated nanoparticles for potential biomedical application with poly(3-hydroxybutyrate-co-4-hydroxybutyrate) biopolymers synthesized through microbial fermentation using locally isolated *Cupriavidus malaysiensis* USMAA1020”. Oral presented at the International Conference on Sustainable Environment and Energy 2022 (ICoSEE 2022) “CALL TO THE ERA OF SUSTAINABLE MOBILITY” (5th - 6th November 2022), Songkhla, Thailand.

Keisheni, G., Razifah, M.R., Amin, KAM & Aidilla, M. (2020, August). “Effect of cinnamon oil incorporated edible aloe vera films on shelf life of wax Apples (*Syzygium samarangense* L.)”. Oral presented at the International Virtual Conference on Fundamental and Applied Sciences 2020, Kasetsart University, Thailand.

Keisheni, G., Razifah, M.R., Amin, KAM & Aidilla, M. (2019, July). “Effect of cinnamon oil incorporated edible aloe vera films on shelf life of wax Apples (*Syzygium samarangense* L.)”. Oral presented at the 14th UMT International Annual Symposium (UMTAS-2019), Terengganu, Malaysia.

Keisheni, G., Razifah, M.R., Amin, KAM & Aidilla, M. (2018, November). “Physico mechanical properties of cinnamon oil incorporated edible aloe vera films”. Oral presented at the Challenges in Environmental Science and Engineering (CESE-2018) conference, Bangkok, Thailand.

Keisheni, G., Razifah, M.R., Amin, KAM & Aidilla, M. (2018, June). “Characterization of aloe vera edible films incorporated with cinnamon oil”. Poster presented at the International Fundamentum Science Symposium (iFunS-2018), Terengganu, Malaysia.

CURRICULUM VITAE

Name : Keisheni Ganeson
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 Bandar Baru Farlim,
 11500, Pulau Pinang



PhD candidate in Biotechnology with a strong background in biomaterials, biodegradable polymers, nanotechnology, and sustainable materials for applications in drug delivery, food technology, and environmental solutions. Experienced in leading interdisciplinary research projects, guiding undergraduate and postgraduate students in lab and presentation work, and publishing in Q1 scientific journals. Proven ability to connect material science with real-world industry needs, especially in developing eco-friendly, bio-based platforms using Malaysia's natural resources. Awarded multiple research grants and recognised for excellence in scientific innovation and communication. Currently, a semi-finalist for the Merdeka Award Grant 2025 (International Attachment Track), reflecting national-level recognition for impactful research.

Research Interests

- Functional biopolymers and biodegradable materials for materials engineering, structural applications, and sustainable product design
- Smart microneedle and sensor-integrated platforms for diagnostics, environmental monitoring, and data-driven materials research
- Green synthesis of nanomaterials for scalable industrial, environmental, and healthcare applications
- Analytical and mechanical characterisation of polymeric materials using advanced instrumentation (e.g., SEM, XRD, FTIR, mechanical testing)
- Application of bio-based polymers in engineered packaging and medical devices
- Integration of biopolymer composites in next-generation functional and responsive materials
- Sustainable alternatives to petrochemical-derived materials through natural polymer design and engineering



EDUCATION

PhD	Universiti Malaysia Terengganu (UMT), Biotechnology	2022-2025
Thesis	: Development and characterisation of docetaxel-incorporated P(3HB-co-4HB) nanoparticle-embedded in dissolvable microneedles for transdermal drug delivery	
Advisor	: Assoc Prof Ts Dr Vigneswari Sevakumaran	
Co-advisor(s)	: Prof Dato Dr Amirul Al-Asyraf Dr Thaigarajan Parumasivam	
Attachment	: 2 years at Universiti Sains Malaysia, School of Biological Science and School of Pharmacy	
Collaboration	: National University of Singapore	
MSc	Universiti Malaysia Terengganu (UMT), Biotechnology	2018-2020
Thesis	: Edible film based on Aloe vera (<i>Aloe barbadensis</i> Miller) incorporated with cinnamon essential oil to maintain the quality and safety of wax apples (<i>Syzygium samarangense</i> L.)	
Advisor	: Dr Razifah Mohd Razali	
Co-advisor(s)	: Prof Dr Khairul Anuar Mat Amin Dr Aidilla Mubarak	
BSc	Universiti Malaysia Terengganu (UMT), Biological Science	2014-2017
Thesis	: Increasing tomatoes (<i>Solanum lycopersicum</i>) shelf life with characterised edible aloe vera film incorporated with cinnamon oil	
Advisor	: Dr Razifah Razali Dr Aidilla Mubarak	
Grade	: 3.26 CGPA (2 semesters of Dean's List)	

TECHNICAL SKILLS

Polymer Synthesis & Characterisation

- Production and downstream purification of P(3HB-co-4HB) using single-stage fermentation in a 15L bioreactor
- Endotoxin removal (LAL assay), GC-FID and GPC-based molecular weight analysis
- Thermal and structural characterisation via FTIR, DSC, and XRD

Nanoparticle Engineering

- Solvent evaporation and emulsification techniques for polymeric nanoparticle fabrication
- Optimisation of particle size, PDI, and zeta potential using DLS

- Quantification of drug loading and encapsulation efficiency
- Long-term stability testing under various humidity conditions

Microneedle Fabrication & Evaluation

- Stepwise centrifugation casting of dissolvable microneedles (PVA/PVP-based)
- Mechanical strength testing (fracture force, texture analysis)
- In vitro penetration studies using Parafilm® M and agarose gels

Analytical Instrumentation & Characterisation

- Scanning/Transmission Electron Microscopy (SEM, TEM) for morphological analysis
- Fourier Transform Infrared (FTIR) Spectroscopy, X-ray Diffraction (XRD), Differential Scanning Calorimetry (DSC)
- High-performance Liquid Chromatography (HPLC) for drug quantification
- Drug release kinetics modelling (Korsmeyer-Peppas, Higuchi)

Drug Delivery & Pharmaceutical Science

- Transdermal delivery system design and performance evaluation
- Knowledge of breast cancer pharmacology (docetaxel mechanism, toxicity profile)
- Familiarity with regulatory and quality considerations (combination product guidelines)

Quality Assurance & Good Laboratory Practice (GLP)

- Safe handling of cytotoxic compounds and lab waste disposal protocols
- Statistical data analysis (ANOVA, Tukey's test) and reproducibility verification

SOFT SKILLS

Project Management & Leadership

- Coordinated multi-phase research (polymer production, nanoparticle optimisation, microneedle fabrication) under deadlines
- Managed lab timelines, resources, and cross-functional team coordination

Problem-Solving & Critical Thinking

- Adapted protocols to troubleshoot experimental issues (contamination, batch variability)
- Analysed complex data sets to refine research hypotheses and optimise formulations.

Technical Writing & Communication

- Authored a PhD thesis with rigorous method descriptions and clear data interpretation
- Prepared technical reports, manuscripts, and presentation slides for academic conferences

Collaboration & Interdisciplinary Work

- Worked across microbiology, materials science, and pharmaceutical engineering domains
- Coordinated with researchers from multiple institutions and disciplines

Leadership & Mentoring

- Supervised and trained interns, undergraduates and postgraduates in lab methods (polymer extraction, nanoparticle formulation and microneedle fabrication)
- Facilitated group discussions and peer feedback on experimental design

Adaptability & Resilience

- Handled concurrent experiments with shifting priorities
- Maintained well-documented lab notebooks and digital data tracking systems

Time Management & Organization

- Handled multiple concurrent experiments (fermentation, polymer processing, mechanical testing)
- Kept detailed laboratory records and version-controlled data sets

Ethical & Regulatory Awareness

- Followed cytotoxic handling protocols and recognised the need for ethical approvals in future in vivo studies
- Aware of combination product classification (drug + device) and relevant regulatory guidelines

LANGUAGES

- **English:** Fluent in all areas (IELTS score: 7.5)
- **Malay:** Fluent in all areas
- **Tamil:** Fluent in all areas

WORK EXPERIENCE

Research Assistant (Universiti Malaysia Terengganu)

Jul 2020 – February 2025

- Supervised and mentored Master's students, junior PhD candidates, and undergraduate research assistants, providing guidance on experimental design, data analysis, and academic writing.
- Developed research materials, including literature reviews, proposals, and manuscripts, in collaboration with the principal investigator and multidisciplinary teams.
- Collected, analysed, and interpreted both quantitative and qualitative data using relevant statistical software, ensuring accurate results and precise documentation.

- Coordinated weekly lab meetings and progress reports to keep team members informed of milestones, troubleshooting issues, and sharing best practices.
- Maintained laboratory equipment, managed supplies, and enforced safety protocols to create a secure and efficient research environment.

Lab demonstrator (Universiti Malaysia Terengganu)

Jul 2022– July 2024

- Led practical demonstrations for undergraduate lab sessions, guiding students in proper experimental procedures and adherence to safety standards.
- Prepared and delivered instructional materials such as lecture slides, handouts, and lab manuals to clarify complex concepts and support hands-on learning.
- Provided individualised support during lab sessions, troubleshooting experiments, clarifying methodologies, and ensuring students gained practical skills.
- Assessed student performance by grading lab assignments, quizzes, and practical exams, offering constructive feedback for improvement.
- Collaborated with faculty on course planning and laboratory schedules to optimise resource allocation and enhance the overall learning experience.

Freelance Tutor

Jan 2021 – present

- Provide online tutoring in mathematics, biology, chemistry, and general science to a wide range of students (e.g., secondary school, pre-university).
- Develop personalised lesson plans and teaching materials to strengthen students' subject understanding and academic performance.
- Utilise interactive methods (e.g., problem-solving exercises, real-life examples) to boost engagement and critical thinking skills.
- Track student progress through regular assessments, offering detailed feedback and strategies for improvement.

Proofreader (Proofwise Legacy)

Dec 2024 – present

- Review and edit academic, business, and scientific articles for grammar, punctuation, style consistency, and clarity.
- Ensure each project meets specified style guides, formatting requirements, and publication deadlines.
- Provide constructive editorial feedback to authors, maintaining clear communication to uphold high-quality standards.

Intern (Penang Science Cluster)

Jun - Sep 2014

- Organised and facilitated hands-on workshops in AI, coding, Arduino, and broader STEM topics for students and teachers.
- Developed training materials to help school teachers integrate coding and robotics into their lesson plans, enhancing their capacity to guide students in STEM activities.

- Mentored student groups on programming and robotics projects, fostering problem-solving skills and innovative thinking.
- Participated in “Coding n Wheels,” travelling to local schools to teach students essential coding concepts and spark their interest in technology.
- Assisted in planning and executing the Penang International Science Fair, providing logistical support and ensuring an engaging, educational experience for participants.

PUBLICATIONS

Journal Publications

Vigneswari, S., Kee, S. H., Hazwan, M. H., **Ganeson, K.**, Tamilselvan, K., Bhubalan, K., ... & Ramakrishna, S. (2024). Turning Agricultural Waste Streams into Biodegradable Plastic: A Step Forward into Adopting Sustainable Carbon Neutrality. *Journal of Environmental Chemical Engineering*, 112135. (WOS indexed, Q1; 9.402 SJR impact factor)

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Ganeson, K., Razifah, M.R., Mubarak, A., Vigneswari, S., Ramakrishna, S. (2022) Improved functionality of cinnamon oil emulsionbased gelatin films as potential edible packaging film for wax apple Food Bioscience, 47, 101638 <https://doi.org/10.1016/j.envres.2023.115988> (WOS indexed, Q1; 4.240 SJR impact factor)

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Bhubalan, K., Tamothran, A.M., Kee, S.H., Foong, S.Y., Lam, S.S., **Ganeson, K.**, Vigneswari, S., Amirul, A.A., Ramakrishna, S. (2021). Adopting blockchain concepts as watermarkers of plastics for sustainable waste management in progressing circular economy model. *Environmental Research*. (WOS indexed, Q1; 6.405 SJR impact factor)

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matrix on normal human lung fibroblast MRC-5 cells. *Malaysian Applied Biology*, 49(4),267–271. (Non indexed, Q4; 0.16 SJR impact factor)

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Total Citations	h-index	i10-index
330	7	7

(Source: Google Scholar)

Conferences/Proceedings/Oral presentations/Poster presentations

Keisheni, G., Abdullah, A. A. A & Vigneswari, S. “Biosynthesis of poly(3hydroxybutyrate- co-4-hydroxybutyrate) bioplastics through microbial fermentation using locally isolated *Cupriavidus malaysiensis* USMAA1020”. Oral presented at the 4th Seminar on Biological Security and Sustainability (BIOSES), Universiti Malaysia Terengganu, UMT (28th -29th June 2022), Terengganu, Malaysia.

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Keisheni, G., Razifah, M.R., Amin, KAM & Aidilla,M. (2020, August). “Effect of cinnamon oil incorporated edible aloe vera films on shelf life of wax Apples (*Syzygium samarangense* L.)”. Oral presented at the International Virtual Conference on Fundamental and Applied Sciences 2020, Kasetsart University, Thailand.

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Keisheni, G., Razifah, M.R., Amin, KAM & Aidilla, M. (2018, June). “Characterisation of aloe vera edible films incorporated with cinnamon oil”. Poster presented at the International Fundamentum Science Symposium (iFunS-2018), Terengganu, Malaysia.

Intellectual property

- Dissolving Microneedle Patch Comprising of Polymeric Nanoparticles for Transdermal Drug Delivery – (Patent filing in progress, 2025)
- Registered copyrights under MyIPO: *AveraTics* (LY2019000716), 2019, Universiti Malaysia Terengganu
- Registered copyrights under MyIPO *AloeWraps* (LY2019000706), 2019, Universiti Malaysia Terengganu.

Research Grants

Malaysia Toray Science Foundation (Science & Technology Research Grant). Project Title: Smart Microneedle Patches Combined with Biosensors and Feedback-Induced Mechanisms as Transdermal Delivery System for Breast Cancer Therapy. Main researcher. January-December, **2023**), Total amount: Rm 20,000.

MERCK Malaysia and Young Scientists Network – Academy of Sciences (YSN-ASM). Project title: Transdermal drug delivery based on smart microneedle system combined with biosensors and feedback-induced mechanisms for effective immunisation and vaccination Main researcher. February **2021**, Total amount: RM 20, 000.

Exhibitions/Research Innovations

Keisheni, G., Abdullah, A. A. A., Vigneswari, S. “Micro-X-Patch: Dissolvable Microneedle Patch with Polymeric Nanoparticles for Breast Cancer Therapy” Minggu Penyelidikan Inovasi (MPI), May 28-29, 2024

Keisheni, G., Abdullah, A. A. A., Thaigar, P., Vigneswari, S. “BioXel: Bio-inspired polymeric nanoparticles for targeted breast cancer treatment” Minggu Penyelidikan Inovasi (MPI), May 28-29, 2023

Keisheni, G., Razifah, M.R., Amin, KAM & Aidilla, M. “AveraTics-A Quality Solution for Plastic Pollution,” Minggu Penyelidikan Inovasi (MPI), Mar 10-11, 2020.

Keisheni, G., Razifah, M.R., Amin, KAM & Aidilla, M. “Aloe Wrap For Lifelong Fruits and Vegetables,” Minggu Penyelidikan Inovasi (MPI), Apr 7-9, 2018.

HONOURS AND AWARDS

Good Clinical Practice 2024

Description : Organised by Clinical Research Centre
Ministry of Health, Malaysia

Date/Venue : 5 Sept 2024

3 Minute Thesis Competition 2023

Award : 1st runner up

Description : Organised by
UMT

Date/Venue : 25 Apr 2023, UMT Convention Centre

Graduate Technologist 2022

Certified by : MBOT (Malaysia Board of
Technologists) Date : 26 Aug 2022

Best Oral Presenter

Description : The 4th Seminar on Biological Security and
Sustainability (BIOSES 2022)

Date/Venue : 28-29th June 2022, Universiti Malaysia Terengganu, Malaysia

Best Oral Presenter

Description : 14th UMT International Annual Symposium (UMTAS
2019) Date/Venue : 23-25th July 2019, Primula Beach Hotel, Terengganu,
Malaysia

Innovation and Research Week

Award : Gold medal

Title Awarded : Micro-X-Patch: Dissolvable Microneedle Patch with
Polymeric Nanoparticles for Breast Cancer Therapy

Description : Organised by Universiti Malaysia

Terengganu Date/Venue : 28-29 May 2024, UMT

Award : Silver medal

Title Awarded : BioXel: Bio-inspired polymeric nanoparticles for targeted breast
cancer treatment

Description : Organised by Universiti Malaysia Terengganu,
Universiti Sultan Zainal Abidin, University College

TATI Date/Venue : 10-11 Mar 2020, University College TATI

Award : Silver medal

Title Awarded : AveraTics-A Quality Solution for Plastic
pollution

Description : Organised by Universiti Malaysia Terengganu,
Universiti Sultan Zainal Abidin, University College

TATI Date/Venue : 10-11 Mar 2020, University College TATI

Biasiswa Universiti Malaysia Terengganu Scholarship 2022

Organisation : Universiti Malaysia
Terengganu Amount: MYR64,800

Most Creative Participant 2020

Organisation : iTRoSco, Universiti Diponegoro, Indonesia
Description : Participated in the 2020 International Tropical Summer Course (ITRoSCo) at Universitas Diponegoro, Indonesia, which focused on tropical marine and coastal ecosystems, conservation efforts, and Indonesian culture. Collaborated with international faculty and peers through interactive workshops, virtual field trips, and group discussions, enhancing cross-cultural communication skills and environmental awareness.

Top Achiever Award 2018

Organisation : Universiti Malaysia Terengganu
Description : Awarded at the faculty level in recognition of outstanding academic performance during Master's studies

Dean's List Award 2017

Semesters : 2 semesters

PROFESSIONAL SERVICE

Conference/Forum Co-Organiser

- Universiti Malaysia Terengganu-National University of Singapore Research Advances Forum, 2020
Role : Committee of Photography
- Universiti Malaysia Terengganu International Annual Symposium, 2019 Role: Session Chairperson

ACTIVITIES/INVOLVEMENT

- President of the postgraduate club of faculty (Sci-Met) 2022
- Participated in Famelab (International Science Communication) Competition 2020
- Participated in iTRoSco (Summer camp, Universiti Diponegoro, Indonesia) 2020
- Participated in Falling Walls (International Science Communication) competition 2019
- Program Director of Postgraduate Family Day (Faculty of Science and Marine Environment, FSSM, Universiti Malaysia Terengganu) 2019
- Lab demonstrator for Foundation in Science Program 2019
- Secretariat and bureau member in STEM Carnival 2019

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