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Palm kernel oil-based polyester polyurethane composites incorporated with multi-walled carbon nanotubes for biomedical application

Nurul Nabilah bt Zulkifli¹, Khairiah bt Hj Badri² and Khairul Anuar Mat Amin^{1,3*}

Abstract

Background: In this study, polyurethane (PU) films from palm kernel oil-based polyester (PKO-p) incorporated multi-walled carbon nanotubes (MWNTs) were prepared via evaporative casting method. Nanoparticle fluid dispersions containing 0.01, 0.04 and 0.08 % wt. of MWNTs were added into PKO-p-based resin and mixed by digital probe sonicator for 20 min followed by mixing with isocyanate to produce PU-MWNTs composite films. The mechanical properties, water resistance, water vapor transmission rates (WVTR), biocompatibility, and antibacterial activities of the PU-MWNTs composite films were examined.

Results: Results show that PU containing 0.01 wt. % of MWNTs demonstrated optimum mechanical properties as it possessed high tensile strength, modulus, and good flexibility compared to PU film and other PU-MWNTs composite films. There are no significant difference in swelling values as well as water vapor transmission rates for PU film and PU-MWNTs composite films. All the films showed low swelling values (17–23 %) and WVTR values in the range 181–269 g m⁻² d⁻¹. Cell studies revealed that PU and PU-MWNTs composite films are non-cytotoxic to human skin fibroblast cells (CRL2522) and the cell proliferation was increased after incubation of 72 h. The in vitro qualitative antibacterial results showed both PU and PU-MWNTs composite films exhibited bactericidal effect against Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*).

Conclusions: In summary, incorporation of MWNTs improved the mechanical properties of the polyurethane films with no cytotoxic effect against normal human skin fibroblast cells.

Keywords: Polyurethane, Palm kernel oil-based polyester, MWNTs, Biocompatibility, Antibacterial activities, Composites

Background

Polyurethane (PU) has been widely used in many areas such as automotive, medical, and chemical industries due to their wide range of properties. Polyurethanes are highly preferred materials in the constructions of biomedical applications due to their physiochemical, biochemical, and mechanical design criteria (Campos et al. 2011). These criteria's include, but are not limited to,

blood compatibility, biocompatibility, biodegradability, and high mechanical performances. In biomedical application, it has been used since 1980 in producing such as the artificial heart, pacemaker leads, heart valves, and catheters (Kütting et al. 2011; Alves et al. 2014). In the recent years, polyurethane has been showing a promising result as a wound dressing material and bio-scaffolds for biomedical applications (Yari et al. 2012). Not limited to that, a few studies have reported successfully in producing the PU materials using the polyol-based polyester from vegetable oils such as oil palm, soybean, and linseed oil (Badri et al. 2001; Petrović 2008). These PU materials

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are expected to minimize the toxicity from synthetic petrochemical-based polyester and enhanced the biocompatibility of the PU products.

Polyurethane nanocomposites using carbon nanotubes and graphite have gained a great attention because they offer a great potential to enhance mechanical properties and increased electrical conductivity due to their large specific surface area. These fillers are considered as an ideal reinforcing agent for high-strength nanocomposites. Recent studies show that single-walled carbon nanotubes (SWNTs) and multi-walled carbon nanotubes (MWNTs) are among attracting nanofillers to produce advance polyurethane (PU) materials with high mechanical properties and conducting materials (Kim et al. 2010). These nanocomposites need only a small amount of nanofillers to give a tremendous improvement in mechanical properties than pure PU composites. For example, incorporation of SWNTs into polyurethane [polyester-based (polycaprolactone (PCL))] improved the Young's modulus compared to pristine PU (Xia and Song 2006). The Young's modulus of 0.7 wt. % SWNT-grafted-PU increased by ~278 % compared to the pure PU. Other study reported that the incorporation of MWNTs in waterborne polyurethane exhibits a huge increase in the tensile properties to 370 % due to a strong covalent bonding in the system (Kuan et al. 2005).

For biocompatibility of polyurethane nanocomposites, some recent studies indicated that carbon nanotubes may have promising potentials to be used at molecular and cellular levels (Smart et al. 2006; Lee et al. 2011). For example, Meng et al. (Meng et al. 2009) reported that polyurethane (polyether-based polyol) containing multi-walled carbon nanotubes (PU-MWNT) films are non-cytotoxic to the mouse fibroblast cell (3T3). In other study, Verdejo et al. reported that the addition of MWNTs (0.01, 0.05, and 0.10 %, wt. %) into PU (polyether-based, Bayer Group, UK) did not significantly affect the cytotoxicity of human osteosarcoma cell line (SaOs-2) (Verdejo et al. 2009). The blood compatibility of PU (polyether-based polyol)-MWNT composites also reported as promising than pure PU composites with the MWNT significantly improved the anticoagulant function as well as the platelets activation (Meng et al. 2005).

For antibacterial activities, a limited study has been reported on the polyurethane-carbon nanotubes (CNTs). Yadav et al. (Yadav et al. 2012) observed the hyperbranched polyurethane nanocomposites [polyether-based (polytetramethylglycol)] incorporated with 0.04 % wt of multi-walled carbon nanotubes (MWNT) which inhibited the growth of *Escherichia coli* (*E. coli*) about 50 % after 21 h. These nanocomposites inhibit the growth of *E. coli* even at low concentration (0.01 % wt) and the inhibition become stronger as the concentration of

MWNT is increased. This could be due to specific physicochemical and structural characteristics such as surface area, functional group, length, and diameter of CNTs.

In our review, there has been limited studies using polyols-based polyester from vegetable oils in producing PU-CNTs film (Ali et al. 2014; Liu et al. 2016). Most of the studies produced PU-CNTs nanocomposites using polyols-based polyether and polyester derived from petroleum based and shows a promising result in mechanical characteristics and electrical conductivity (Cho et al. 2005; Jell et al. 2008; Khan et al. 2010, 2011; Pokharel 2014). Therefore, in this study, we fabricated and characterized the polyurethane films from palm kernel oil (PKO)-based polyester incorporating multi-walled carbon nanotubes. The mechanical characteristics, water vapor transmission rates, and swelling of the polyurethane multi-walled carbon nanotubes (PU-MWNTs) composite films were carried out. The cell viability and cell proliferation through in vitro study of the PU nanocomposites were assessed against human fibroblast skin cell (CRL-2522, American Type Tissue Collection). The antibacterial activities were examined via in vitro qualitative studies against two bacterial strains, i.e., Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*) bacteria.

Methods

Materials

The palm kernel oil-based monoester polyol (PKO-p) was prepared as reported by (Badri et al. 2001). 2, 4-diphenylmethane diisocyanate (MDI) was obtained from Cosmopolyurethane (M) Sdn. Bhd., Klang, Malaysia. Acetone and polyethylene glycol (PEG 400) were purchased from Merck Sdn. Bhd., Malaysia. A multi-walled carbon nanotube (MWNT, product number 773840 and lot number MK13N5858 V, diameter = 10 ± 1 nm and length = 3–6 μ m) was purchased from Sigma Aldrich, Malaysia. Penicillin disks (product number CT0043B, lot number 523987) were obtained from Oxoid, England. All materials were used as received without further purification.

Preparation of polyurethane (PU) films

The polyurethane films were prepared from palm kernel oil-based monoester polyol (PKO-p) via evaporation casting method with the ratio of PKO-p, PEG400, and MDI at [100:70:150]. Initially, the PKO-p was mixed with PEG400 and agitated at 100 rpm in a round-bottomed flask under nitrogen gas atmosphere for 10 min to form urethane pre-polymer. Prior to mixing the said mixture (PKO-p and PEG) with MDI, acetone (35 %) was added into the mixture followed by stirring at 100 rpm for

5 min. The acetone (35 %) was also added into MDI to accommodate the evaporation process. The mixture and MDI were then stirred at 100 rpm for 5 min at room temperature and then casted onto a Teflon plate and dried in humidity chamber (37 °C, 50 % \pm 5 relative humidity) for 24 h to remove the solvent. The PU films were then pre-conditioned at the same temperature for next 24 h prior to any characterizations.

Preparation of PU-MWNT composite films

The nanoparticle fluid dispersions containing PKO-p, PEG400, and acetone (35 %) with 0.01, 0.04, and 0.08 % (w/w) of multi-walled carbon nanotubes (MWNTs) were prepared using a digital sonicator horn (Qsonica-Q500, USA) with a probe diameter of 6 mm, in a pulse mode (0.5 s on/off) with an amplitude set at 40 %. Different intervals of sonication were carried out to determine the optimum sonication time (min). During sonication, the sample vials were placed inside a water bath to keep the dispersion temperature constant. The dispersions were then mixed with MDI (containing 35 % acetone) and stirred for 5 min. The mixtures were casted onto a Teflon plate and dried in a humidity chamber (37 °C, 50 % \pm 5 relative humidity) for 24 h to remove the solvent. The PU composite films containing 0.01, 0.04, and 0.08 % (w/w) of MWNTs hereafter known as PU-MWNT01, PU-MWNT04, and PU-MWNT08, respectively. The PU-MWNT composite films were then pre-conditioned at the same temperature for next 24 h prior to any characterizations.

UV-visible spectroscopy

UV-visible absorption spectra of homogenous PU-MWNTs dispersions were obtained with a dual-beam UV-vis spectrophotometer (Shimadzu UV-1800) using quartz cuvettes (path length = 10 mm). All samples were measured after a 10-fold dilution with Triton X-102. The transmittance spectra of PU films and PU-MWNTs composite films were measured in the range of 200–700 nm wavelengths light.

Optical microscopy

Dispersions of PU films containing multi-walled carbon nanotubes (PU-MWNTs) were imaged using an optical microscope (Olympus IX73) with cell Sens Dimension software.

Mechanical properties

Stress-strain measurements were obtained using an Instron Universal Testing machine (model 3366) with \pm 10 kN grips and the cross speed set at 20 mm/min. Film thickness (2.0 \times 6.0 cm) was measured by a hand-held micrometer (Mitutoyo). Young's modulus (E), tensile

strength (TS), and toughness (T) were calculated from the slope of the linear part of the stress-strain curve and maximum stress and through integration of the area under the curve, respectively. Strain-at-break (γ) was also recorded.

Swelling ratio

Water uptake was measured by weighing the dried films (W_{dry}) prior to immersion into solutions of pH 7 in water bath (37 \pm 0.5 °C). The temperature was set at 37 °C to mimic the condition of wound which similar to body temperature. The films were removed after 24 h, wiped gently with a filter paper to expel surface solution, and then weighed (W_{wet}). Water uptake was then determined from the equilibrium-swelling ratio $[(W_{\text{wet}} - W_{\text{dry}}) / W_{\text{dry}}] \times 100$.

Water vapor transmission rates

The water vapor transmission rates (WVTR) of the samples were measured by following a modified ASTM E96 standard method (Wu et al. 1995). Each membrane was fixed on the circular opening of a permeation bottle (diameter = 1.5 cm, height = 5.0 cm) with the effective transfer area (A) of 1.33 cm². The permeation bottle was placed in the temperature humidity chamber at 37 °C and 50 \pm 5 % relative humidity. The WVTR was then determined by the rate of change of mass (m) in these water-filled permeation bottles at 24 h exposure time as follows:

$$\text{WVTR} = m / A \Delta t,$$

where $m/\Delta t$ is the water gain per unit time of transfer and A is the area exposed to water transfer (m²).

Cell studies

Normal human skin fibroblast cells (CRL2522-ATCC) were cultured in Eagle's minimal essential medium (EMEM, Sigma Aldrich) supplemented with 10 % (v/v) fetal bovine serum (FBS, ATCC) and 1 % antibiotic (Penicillin/Streptomycin, ScienCell) at 37 °C in a humidified atmosphere of 5 % CO₂. Cells were sub-cultured every 3 days using established protocols and then were harvested at 60–80 % confluence.

To investigate the cell viability, PU and PU-MWNTs composite films were placed into a 96-well culture plates and sterilized in a laminar air flow chamber using UV radiation for 20 min. Prior to cell seeding, PU and PU-MWNTs composite films were soaked in medium (EMEM only) for 24 h and the supernatant removed. Suspensions of normal human skin fibroblast cells (5000 cells/well) were seeded into wells containing PU and PU-MWNTs composites and cultured at 37 °C in humidified atmosphere of 5 % CO₂ for 24, 48 and 72 h. Tissue culture polystyrene plates (TCPP) used as control contained

the same number of cells. Cell viability of PU and PU-MWNTs composite films were examined through a staining procedure of calcein-AM (Life Technologies, USA) and observed by light microscope (Olympus TH4-200) equipped with fluorescence filter (Olympus U-RFLT UV with blue light excitation).

Cell proliferations were quantified using a CellTiter 96 aqueous one solution assay (Promega, USA) which contained tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphonyl)-2H tetrazolium, inner salts; MTS(a)] with electron coupling reagent (phenazine ethosulfate). Prior to addition of assay solution (20 μ L in each wells), the media in all wells that contained film samples, except for the negative control, were replaced with fresh media and later incubated for 4 h at 37 $^{\circ}$ C in humidified atmosphere of 5 % CO₂. Then, 100 μ L of the inoculants was transferred to a new well and the absorbance was then measured at 492 nm with a microplate reader (TECAN, Infinite M200, USA). The absorbance was then related to cell numbers using calibration curves.

Antibacterial studies

Gram-positive (*Staphylococcus aureus*-*S. Aureus*, *Bacillus cereus*-*B. Cereus*) and Gram-negative (*Escherichia coli*-*E. coli*, *Klebsiella pneumoniae*-*K. pneumoniae*) microbes were used for antibacterial assays. Mueller–Hinton (MH, Difco, Malaysia) agar was used for the growth of both bacterial types. It was prepared by suspending 38 g of the MH powder in 1 L purified water, heated with frequent agitation, and boiled for 1 min to dissolve the powder. The prepared agar was autoclaved at 121 $^{\circ}$ C for 15 min with the final pH at 7.3 ± 0.1 . (*S. Aureus*, *B. Cereus*) and (*E. coli*, *K. Pneumoniae*) were grown on Mueller–Hinton (MH) agar and incubated aerobically at 37 $^{\circ}$ C in an incubator. (*S. Aureus*, *B. Cereus*) and (*E. coli*, *K. Pneumoniae*) suspensions were evenly spread on the solid MH agar and dried in a laminar air flow chamber. The polyurethane (PU) and polyurethane multi-walled carbon nanotubes (PU-MWNTs) composite films (diameter \approx 6 mm) were then placed on the MH agar and incubated at 37 $^{\circ}$ C for 24 h. The presence of any clear zone around the disks on the MH agar was recorded as an indication of inhibition against (*S. Aureus*, *B. Cereus*) and (*E. coli*, *K. Pneumoniae*).

Results and discussion

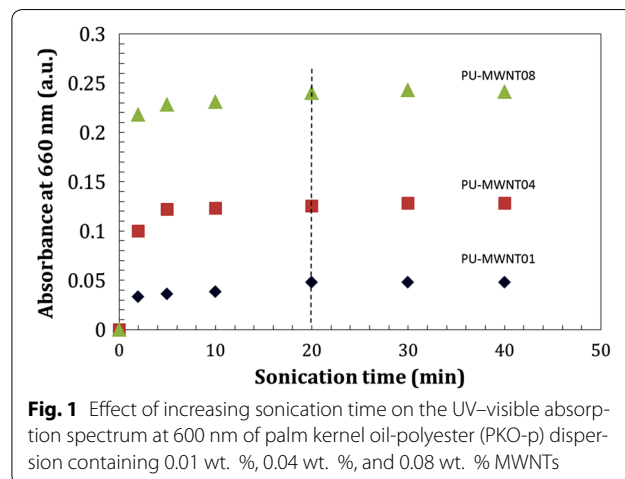
Dispersing multi-walled carbon nanotubes

To prepare the PU-MWNTs composite films, the optimum sonication time is required to successfully disperse the multi-walled carbon nanotubes (MWNTs) in the PKO-p solution. According to Aldalbahi and co-workers, the optimum sonication time was defined as the

minimum amount of time required to effectively disperse the multi-walled carbon nanotubes (MWNTs) in the dispersion (Aldalbahi 2011), which an excess sonication can shorten or cause defects to the tubes and thereby diminish their properties (O'connell MJ, Bachilo SM, Huffman CB, Moore VC, Strano MS, Haroz EH, Rialon KL, Boul PJ, Noon WH, Kittrell C 2002; Benedict et al. 2005; Vaisman et al. 2006). The optimization process was determined by monitoring the level of the UV–Vis absorption intensity at different sonication times and disappearance of visible aggregates. Figure 1 shows that the UV–Vis absorbance intensity ($\lambda = 660$ nm) increases with sonication time, reflecting to an increasing amount of MWNTs [0.01, 0.04, and 0.08 % (w/w)] which became dispersed over time. The absorbance of the dispersions at 660 nm was plotted as a function of sonication time and showed that the sonication becomes independent after 20 min for all dispersions. This particular wavelength was selected as it correlated with the maxima of an absorption band arising from the Van Hove singularities for single-walled carbon nanotubes (SWNTs) (Ryabenko et al. 2004; Grosiord et al. 2005; Attal et al. 2006).

Physical appearance of PU-MWNTs composites

Polyurethane films and polyurethane multi-walled carbon nanotubes (PU-MWNTs) composite films were successfully prepared via solution casting (Fig. 2). The polyurethane films exhibit smooth surface, void-free and transparent (Fig. 2a). However, the additions of multi-walled carbon nanotubes (MWNTs) have decreased the transparency of the films (Fig. 2e). The polyurethane films with 0.01 wt. % of multi-walled carbon nanotubes (PU-MWNT01) were still clear at transmittance ($\lambda = 700$ nm), $T = 97$ %, but became opaque when the amount of MWNTs increased (Fig. 2c, d). The T values



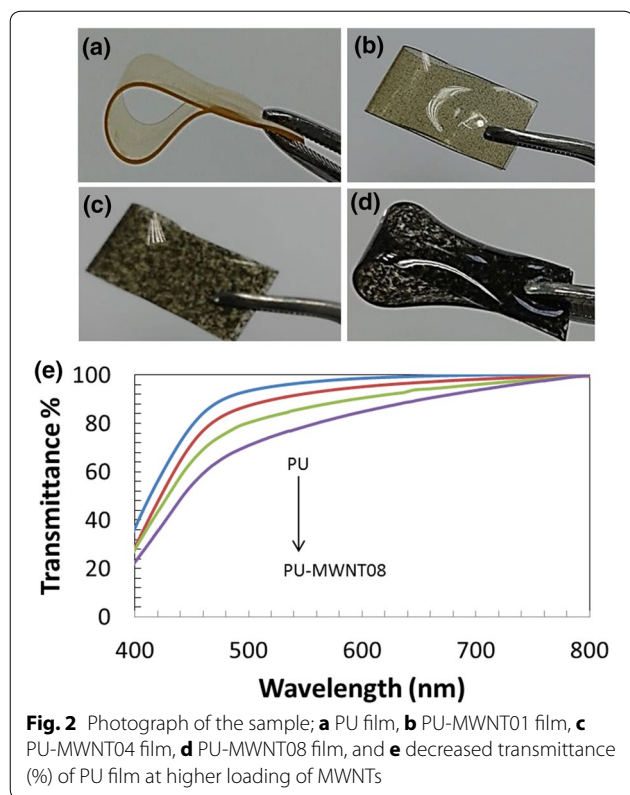


Fig. 2 Photograph of the sample; **a** PU film, **b** PU-MWNT01 film, **c** PU-MWNT04 film, **d** PU-MWNT08 film, and **e** decreased transmittance (%) of PU film at higher loading of MWNTs

of PU-MWNT04 and PU-MWNT08 films were at 93 and 90 %, respectively.

Stress–strain analysis

The stress–strain properties of PU-MWNTs composite films are shown in Fig. 3 and summarized in Table 1.

The tensile strength of PU-MWNT01, PU-MWNT04, and PU-MWNT08 composite films were higher than that of PU film, indicating that the modulus (E) as well as the toughness (T) of the composites were improved by the addition of the multi-walled carbon nanotubes (MWNTs). For example, the T values of the PU-MWNT01 and PU-MWNT04 films were increased about twofold and fourfold, respectively, than PU films. The increment of strain-at-break (γ) of the PU films also can be observed after the inclusion of MWNTs. PU-MWNT01 films recorded highest γ value at 132 ± 11 % followed by PU-MWNT04 films at 113 ± 7 %. The addition of higher amount of MWNTs (0.08 % wt. %) into PU composite films (PU-MWNT08) caused the γ value to decrease 95 ± 3 %, which was below than PU films at 102 ± 9 %. The decreasing value of the γ may be caused by uneven dispersion of multi-walled carbon nanotubes (MWNTs) in polymer matrix (Xiong et al. 2006). Optical microscopy revealed that there were large aggregates present in PU-MWNT08 composite films followed by PU-MWNT04 composite films (Fig. 3d, e). This aggregation resulted to the uneven dispersion of multi-walled carbon nanotubes (MWNTs) in the matrix, which contributed to the lower value of the strain-at-break of the films (Xiong et al. 2006). Interfacial stress and aspect ratio between the MWNTs and PU as well as orientation of the MWNTs are among important factors to obtain the effective load transfer and could improve the mechanical performances of the composites (Tijing et al. 2013).

Swelling ratio and water vapor transmittance rates (WVTR)

Swelling ratio and water vapor transmittance rate (WVTR) values of the PU-MWNTs composite films are

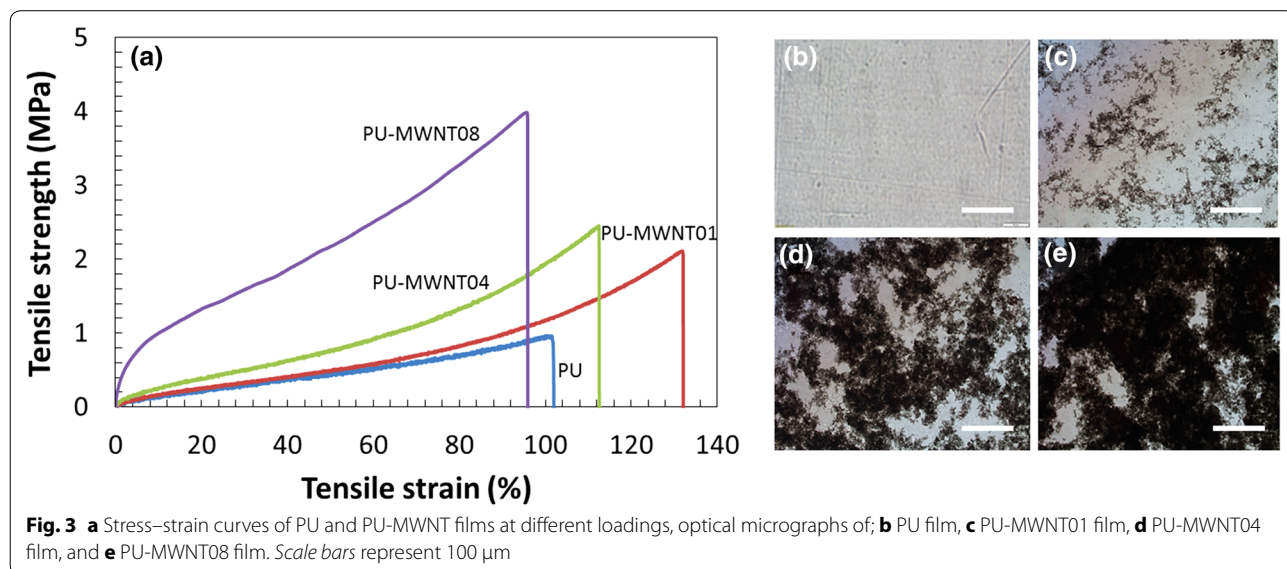


Fig. 3 **a** Stress–strain curves of PU and PU-MWNT films at different loadings, optical micrographs of; **b** PU film, **c** PU-MWNT01 film, **d** PU-MWNT04 film, and **e** PU-MWNT08 film. Scale bars represent 100 μm

Table 1 Summary of the tensile strength (TS), Young's modulus (E), toughness (T), strain-at-break (γ), swelling and water vapor transmission rates (WVTR) of polyurethane (PU) films, PU film containing 0.01 % wt % MWNT (PU-MWNT01), PU film containing 0.04 % wt % MWNT (PU-MWNT04), and PU film containing 0.08 % wt % MWNT (PU-MWNT08)

Sample	TS (MPa)	E (MPa)	T (J g ⁻¹)	γ (%)	Swelling (%)	WVTR (g m ⁻² d ⁻¹)
PU	0.96 ± 0.3	1.28 ± 0.4	0.46 ± 0.2	102 ± 9	23 ± 1.5	269 ± 16
PU-MWNT01	2.11 ± 0.3	3.25 ± 0.4	0.89 ± 0.2	132 ± 11	22 ± 0.5	254 ± 14
PU-MWNT04	2.45 ± 0.2	3.92 ± 0.3	0.95 ± 0.2	113 ± 7	21 ± 0.8	229 ± 14
PU-MWNT08	4.00 ± 0.2	4.35 ± 0.1	1.73 ± 0.1	95 ± 3	17 ± 0.2	181 ± 12

shown in Table 1. PU films recorded highest swelling ratio at 23 ± 1.5 %, and the values were decreased upon addition of MWNTs. These low swelling ratio values of PU-MWNT composite films were considered as hydrophobic as they showed less water absorption (Yücedag et al. 2010) and consistent with the previous study (Badri 2012).

The water vapor transmission rate (WVTR) values of PU films and PU-MWNTs composite films are in the range of 181–269 g m⁻² d⁻¹ (Table 1). Addition of multi-walled carbon nanotubes (MWNTs) slightly decreased the WVTR values which could be due to dispersion of MWNTs in the polyurethane matrix and blocked the water vapor transmission (Kavoosi et al. 2014). However, these values still remain within the range of WVTR of commercial wound dressings values at 90–2893 g m⁻² d⁻¹ (Wu et al. 1995).

Biocompatibility of films

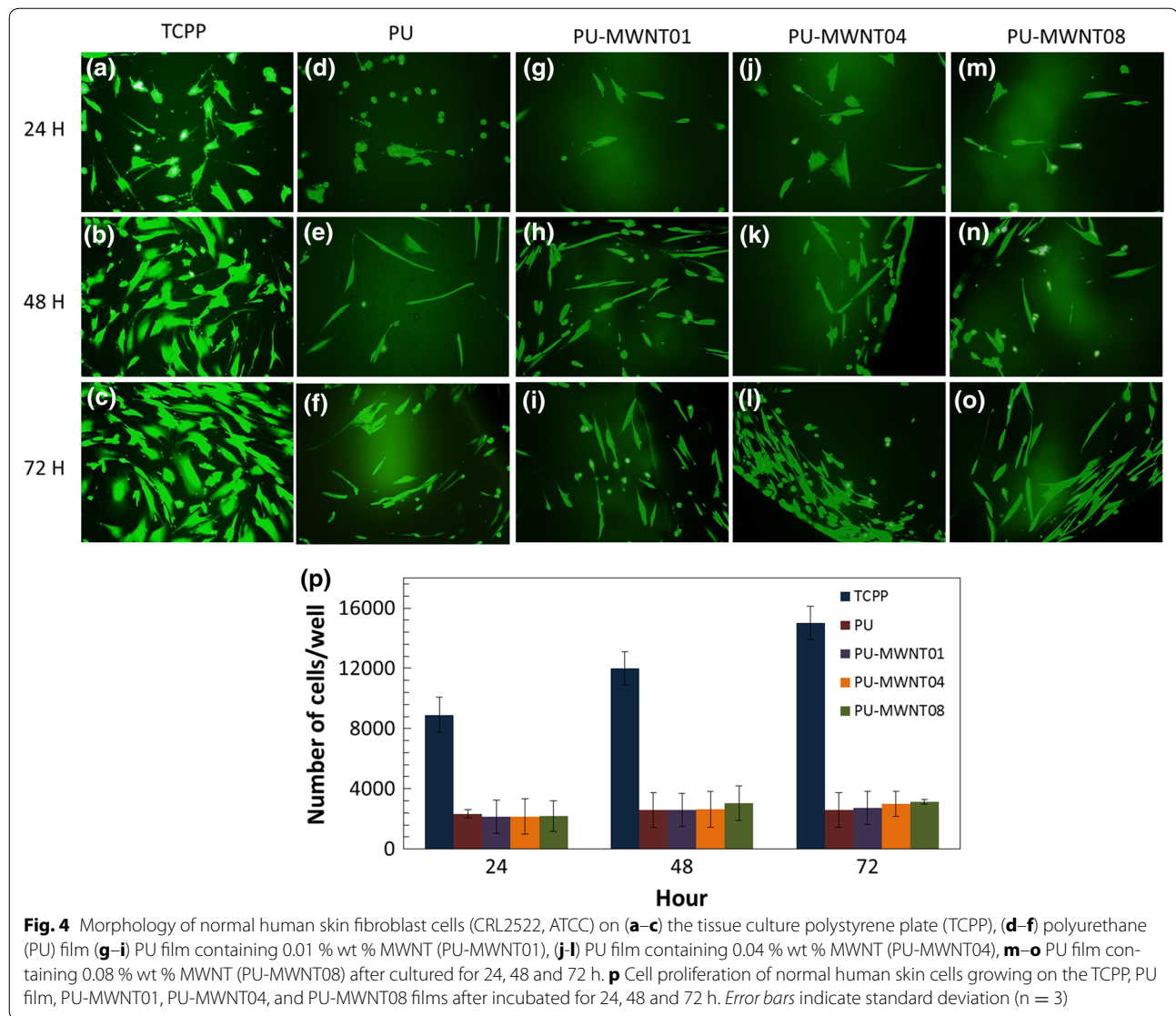
Biocompatibility of PU and PU-MWNTs composite films was determined by examining the cell viability and cell proliferation that involved human skin fibroblast cells (CRL2522-ATCC). The cell morphological attached onto the surface of PU film were changed from rounded shape (24 h incubation) to elongated/spindle-like shape after being incubated for 72 h (Fig. 4a–o). Even though the cell viability on the PU films and PU-MWNTs composite films was low compared to control (TCPP), it showed that the cell growth increased over time on PU film as well as on PU-MWNTs composite films from 24 to 72 h. In addition, it also showed that the PU and MWNTs were unlikely to be cytotoxic to the CRL2522 cell and consistent with the prior reports (Shim et al. 2002; Meng et al. 2005; Lee et al. 2011).

To enumerate the cell growth on the PU films and PU-MWNT composite films, cell proliferations were carried out using cellTiter 96 aqueous one solution assay after being cultured for 24, 48 and 72 h (Fig. 4p). The cell proliferation observed was comparable with the cell viability results, in which the cell numbers were increased for PU films and PU-MWNTs composite

films after being incubated for 24 to 72 h. PU films containing MWNTs showed higher cell proliferation than PU films. For example, the cell numbers cultured on PU-MWNT01, PU-MWNT04, and PU-MWNT08 composite films after incubation of 72 h were 2740, 3000, and 3200 cell/well, respectively (Fig. 4p). Compared to PU films, the cell number was recorded at 2600 cell/well after being incubated for 72 h. PU-MWNT08 composite films showed the highest cell increment at 45 % from 24 to 72 h of incubation. Higher cell growth enumerated on PU-MWNT08 composite films than the other PU films could be due to the influence of the material's surface such as roughness, hardness, hydrophobicity, and hydrophilicity of the substrate (Hu et al. 2011). An addition of high amount of MWNTs in PU composite films (PU-MWNT08) contributed to the lowest water sorption (17 ± 0.2 %), or in another word improved the hydrophobicity and led to a positive biological response of viable cells on the substrate (Zhou et al. 2007; Tanodekaew et al. 2004; Muzzarelli et al. 2005). Incorporation of nanoparticles also has been reported to increase the surface area-to-volume ratio of nanocomposites for the cell growth, and therefore increased cell proliferation on the substrate (Azad et al. 2004). For that reason, this factor also could explain the higher cell proliferation obtained on the PU-MWNT08 composite films compared to other samples. Not limited to that, incorporation of MWNTs also has been reported to change surface topology of the composites which could affect cell proliferation (Sharma and Elisseff 2004).

Antibacterial performances

The antibacterial performance of PU films and PU-MWNTs composite films were studied through qualitative method (disk diffusion) against Gram-positive, i.e., *Staphylococcus aureus* (*S. Aureus*), *Bacillus cereus* (*B. Cereus*) and Gram-negative, i.e., *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. Pneumoniae*). The zone of inhibition was not observed on all films against all bacteria strains, i.e., *S. Aureus*, *B. Cereus*, *E. coli* and



K. pneumoniae after being incubated for 24 h. Figure 5 shows the representative images of the bacteria strains cultured on penicillin disks and PU-MWNT08 composite films. To examine the bactericidal effect of the sample indirectly, we have removed the sample on the MH agar and further incubated the agar for 24 h to observe if there was any bacteria growth on the open area once occupied by the samples. The results showed that there is no bacteria growth on the samples' area after further incubated (24 h) and could support that the PU films containing MWNTs have the antibacterial activities. Our result was in agreement with the study conducted by Yücedag and co-workers on the bactericidal effect of MWNTs samples (Yücedag et al. 2010).

In contrast, the control, i.e., penicillin disk, showed a strong antibacterial activity toward gram-positive bacteria (*S. Aureus*) and less activity against gram-negative bacteria (*E. coli*) which are consistent with the previous studies (Reinthal et al. 2003; Shahverdi et al. 2007). Strong antibacterial activity of *S. Aureus* than *E. coli* on penicillin disk was attributed to the difference of bacteria cell walls. The cell wall of the Gram-positive is made up of networks with plenty of pores which allow foreign molecules to come into the cell without difficulty and allow more rapid absorption of ions into the cells. However, the cell wall of the Gram-negative is made up of complicated bilayer in which the outer membrane constituted of lipopolysaccharide, lipoprotein, and phospholipids which

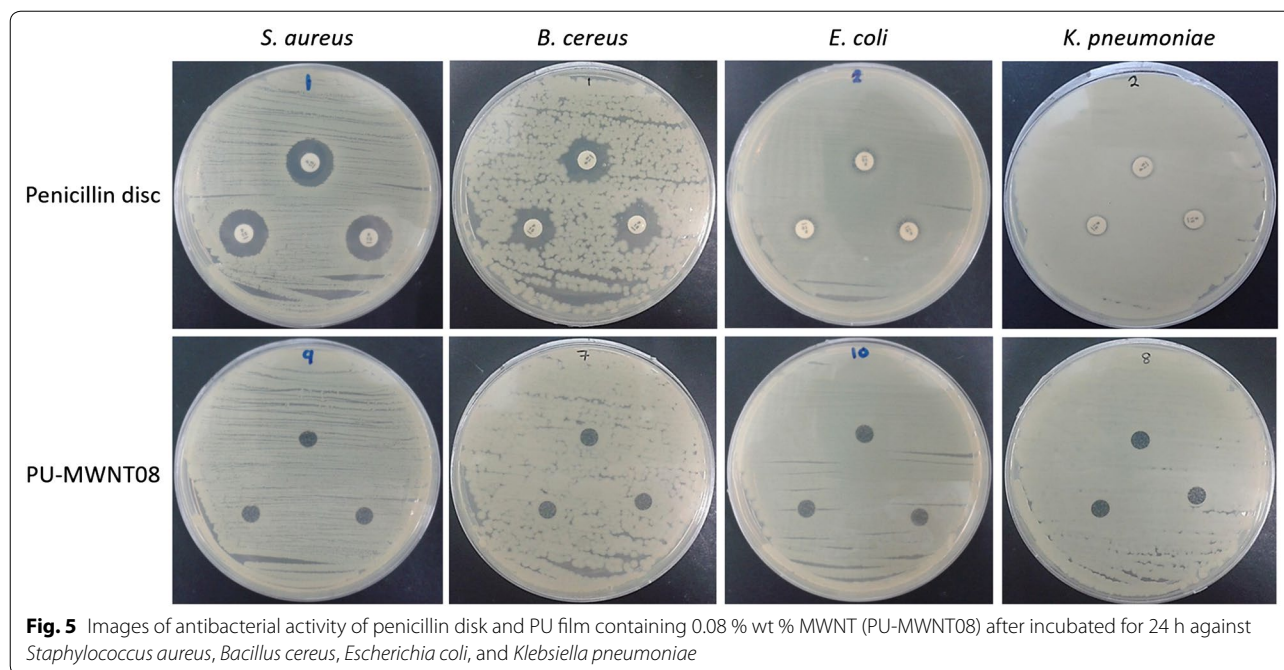


Fig. 5 Images of antibacterial activity of penicillin disk and PU film containing 0.08 % wt % MWNT (PU-MWNT08) after incubated for 24 h against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Klebsiella pneumoniae*

act as a potential barrier against foreign molecules with high molecular weight (Jung et al. 2007).

Conclusions

The polyurethane (PU) films and polyurethane (PU) composite films with 0.01 wt. %, 0.04 wt. %, and 0.08 wt. % of multi-walled carbon nanotubes (PU-MWNT) were successfully prepared using palm kernel oil-based polyester. The effect of multi-walled carbon nanotubes (MWNTs) on the mechanical properties of PU films was investigated. It showed that incorporation of MWNTs gave significant improvement in mechanical properties of PU films. Biocompatibility study revealed that PU-MWNTs composite films exhibited enhancement to both the cell viability and cell proliferation than PU film. Moreover, this study also concluded that both samples, PU films and PU-MWNTs composite films, exhibited bactericidal effect against Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*) and Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria.

Authors' contributions

NNZ performed the experiment and analysis, interpreted the results, and helped in preparing the manuscript. KHB helped in synthesis and preparation of palm kernel oil-polyester polyol to be used throughout the experiment. KAMA planned, supervised the whole study, and wrote the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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