Antibacterial Oil-Based Polyurethane Films for Wound Dressing Applications

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ABSTRACT: As an alternative to petroleum-based polyol, hydroxyl containing material was prepared from linseed oil for polyurethane synthesis. Hexamethylene di-isocyanate (HMDI) and/or 4, 4′-methylene diphenyl di-isocyanate (MDI) were used as isocyanate source. The polymerization reaction was carried out without catalyst. Polymer films were prepared by casting-evaporation technique. The MDI/HMDI-based polyurethane and its films had higher T_g and better thermal property than that of the HMDI-based one because of the existence of benzene ring in the polymer chain. Static water contact angle was determined to be 74° and 77.5° for HMDI and MDI/HMDI-based films, respectively. Water adsorption was found to be around 2.6–3.6% for both films. *In vitro* degradation of polyurethanes in phosphate buffered saline at 37°C was investigated by gravimetric method. Fourier transform infrared spectroscopy and scanning electron microscopy were used for confirmation of degradation on the polymer surface. The degradation rate of the HMDI-based polyurethane film was found higher than that of the MDI/HMDI-based film. Both the direct contact method and the MMT test were applied for determination of cytotoxicity of polymer films, and the polyurethane films investigated here was not cytotoxic. Silver-containing films were prepared using Biocera A® as filler and were screened for their antibacterial performance against *Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus*, and/or *Bacillus subtilis*. The films prepared with and without Biocera A® exhibited antibacterial activity.
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INTRODUCTION

Polyurethanes (PUs) are an important member of the group of thermoplastic elastomers. They can be prepared by the step-growth polymerization of di-isocyanates with diols. They consist of hard and soft segments. The hard segments are derived from the di-isocyanates and the chain extender. The soft segment consists of the polyol. PUs exhibit a broad range of physical properties. It is possible to obtain very brittle and hard materials or soft, tacky, and viscous materials, depending on the properties of raw materials used in their synthesis.

Since PUs possess excellent mechanical, physical properties, and good blood and tissue compatibility, they are widely used as biomaterials.[1] Wound dressing is one of the most important applications of PUs.[1–4] The ideal wound dressing should maintain a moist environment at the wound interface, remove excess exudates to the surface of the dressing, provide thermal insulation and mechanical protection, act as a barrier to micro-organisms, allow gaseous exchange, be not adherent and easily removed without trauma, leave no foreign particles in the wound, and be nontoxic, nonallergenic, and nonsensitizing.[5,6]

To reduce the microbial load in the wound, antimicrobial agents are widely used in wound healing.[7–9] The antibacterial property of silver has been well known for a long time. It is assumed that silver ions bind strongly to electron donor groups on biological molecules containing sulfur, oxygen, or nitrogen resulting in damage to their membrane. In addition to its antibacterial property, silver is known for improving healing rate in wound care.[10]

To improve end-product properties, triglyceride oils and fatty acids have been widely used in the preparation of polymers for special purposes.[11–24] The oil/fatty acid-based polymers are especially important for biomedical applications due to their high biocompatibility properties. In a previous study, transparent and flexible linoleic acid-based polyurethane films were successfully prepared in the absence of any catalyst for use as wound dressing

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material.[25] We also prepared polyurethane films from linseed oil-based hydroxyl containing material and hexamethylene di-isocyanate (HMDI) and/or diphenyl methylene di-isocyanate (MDI) for wound dressing applications.[4] Notably, films were flexible and permitted flow of oxygen and carbon dioxide.

In this study, triglyceride oil-based hydroxyl containing raw material (HCC) was used as the polyol component in preparation of polyurethane. The use of oil-based hydroxyl containing material is expected to yield products with enhanced degradability in synthetic body fluid due to the ester bonds in the polymer chain. Breaking of ester bonds results in hydroxyl groups on the film surface, and hence hydrophilicity of the product increases. It is accepted that surfaces with sufficient hydrophilicity (water contact angle of $\sim 70^\circ$) are able to adsorb a proper amount of protein without changing native protein conformation, hence resulting in cell adhesion and growth.[26]

To investigate the effect of the structure of the di-isocyanate components on polymer properties, an aliphatic diisocyanate, hexamethylene di-isocyanate (HMDI), and an aromatic di-isocyanate, 4,4′-methylene diphenyl di-isocyanate (MDI), were used. In addition to structural, thermal, and viscoelastic characterization, biodegradability and cytotoxicity of polymer films were determined. Antibacterial properties of polymer films with and without antibacterial agent were also investigated.

EXPERIMENTAL

Materials

Commercially purchased linseed oil was supplied from Polisan Boya Sanayi Tic. A.S., Kocaeli, Turkey. The main characteristics of the oil were: refractive index (n_D^{20}), 1.4813; acid value, 1.3; saponification value, 196; iodine value, 165.6. Hexamethylene diisocyanate (HMDI, Merck), 4,4′-methylene diphenyl di-isocyanate (MDI, Merck), and glycerol anhydrous (Merck) were used in the synthesis. Xylene (Merck) was used as a solvent both in polymer synthesis and film preparation. Calcium hydroxide was used as a catalyst for the preparation of hydroxyl containing component from linseed oil. Biocera A® (particle size 3–4 μm), was obtained from Biocera Co., Ltd. and was composed of silver, zinc, magnesium, calcium phosphate, alumina, and silica.

Polymer synthesis

Polymer synthesis was achieved in two steps as described in the literature[27]: (1) preparation of hydroxyl containing component (HCC) from linseed oil and glycerol, and (2) synthesis of polyurethane from HCC and di-isocyanate.

Preparation of hydroxyl containing component

Oil and glycerol (8.5 wt % of the oil) were placed into the reaction flask and heated. When the temperature reached 218°C, calcium hydroxide was added as a catalyst in the amount of 0.1 wt % of the oil portion. The temperature was then raised to 232°C and maintained at this temperature for 45 minutes. The reaction was carried out under nitrogen atmosphere. After cooling the reaction mixture, a sufficient amount of diethyl ether was added to dilute the mixture and dissolve the organic phase. The mixture is then washed first with dilute sulphuric acid solution and then with distilled water to remove the catalyst and free glycerol. The ethereal solution was dried over Na_2SO_4, and then the solvent was evaporated. The hydroxyl and acid values of hydroxyl containing component were 112.1 mg KOH/g and 2.5 mg KOH/g, respectively. The molecular weight (M_n) was determined to be 1090 g/mol.

Polyurethane synthesis

Two types of oil-based polyurethanes were synthesized from the reaction of HCC with HMDI and/or MDI using the solvent method: HMDI-based and HMDI/MDI-based. Equivalent amounts of diisocyanate components and HCC were used in the synthesis of both polyurethanes. HMDI/MDI-based polyurethane was prepared in two steps. In the first step, HCC and HMDI were reacted in the molar ratio of 1 : 2 (isocyanate : hydroxyl) to obtain prepolymer. After the isocyanate groups were consumed, MDI was added to the reaction medium in the same mole amount as HMDI, and, therefore, the total amount of the di-isocyanate group was equivalent to the amount of the hydroxyl group. The reaction was carried out in xylene medium at 90–95°C under inert gas atmosphere and was monitored by FTIR spectroscopy. The disappearance of the absorption peak at 2250 cm^{-1}, assigned to the N=C=O group, was sought to confirm that all the di-isocyanate were consumed in the reaction. This was usually achieved in about 16 h.

Film preparation

Polyurethane films (100 μm thick) with and without Biocera A® were prepared by casting-evaporation technique. Some of the films were prepared with the addition of metal-organic components such as cobalt octoate and lead naphthenate as catalyst to facilitate drying.

For preparation of Biocera A®-containing polymer films (0.5–10 wt %), Biocera A® was first dispersed in xylene by using ultrasonic bath and magnetic stirrer, and then polyurethane solution (with or without catalyst) was added into the Biocera A® dispersed phase. Films with controlled thickness were solution

cast on Teflon-coated glass plate into a steel ring. Cast films were kept at room temperature for 48 h and under vacuum at 45°C for 24 h for solvent removal. As the solvent was being removed from the film, oxidative polymerization of double bonds on fatty acid chain was also achieved, and hence the polymer was cured.

Characterization of polymers and polymer films

FTIR spectroscopy analysis was carried out on a Perkin Elmer1 spectrometer by using the ATR mode. ^{1}H-NMR spectra were obtained in $CDCl_3$ using a 250 MHz Bruker spectrometer. Molecular weight of the polymers was determined by gel permeation chromatography (GPC) (Agilent 1100) equipped with a differential refractometer and polystyrene columns, at a flow rate of 1 mL/min, using tetrahydrofuran (THF) as the solvent. Calibration of GPC was performed by using polystyrene standards. The TGA studies were carried out using a TA Q50 analyzer, by heating from room temperature to 600°C, under nitrogen atmosphere at a heating rate of 20°C/min. The DSC measurements were carried out on a Perkin Elmer Diamond DSC between −100 and 200°C with a heating rate of 20°C/min. The viscoelastic properties of polymer films were determined by Perkin Elmer Diamond DMA operating in tensile mode. The relaxation spectrum was scanned from −60 to 100°C with a frequency of 1 Hz and heating rate of 2°C/min. The morphological characterization of the polymer films was carried out by examining the surface of the membranes by SEM (JEOL JSM-5410). SEM samples were prepared by breaking them in liquid nitrogen and then coating with gold using a sputter coater.

Surface hydrophilicity, water absorption and biodegradation

Static contact angle measurements were made using KSV CAM200 goniometer by placing 5 μL of distilled water on the polymer surface. Water absorption properties were determined by immersing polymer films (2 × 1 cm) into distilled water at 25°C until equilibrium was reached. Percent water absorption was determined gravimetrically by weighing the water saturated films after removing excess water on the film surfaces with a paper towel. Hydrolytic degradation was determined by immersing dry polymer films (2 × 1 cm) in phosphate buffered saline (BPS) at pH 7.4 and 37°C. Test was continued for 8 weeks, and eight specimens were used for each polymer film. Every week one of the specimens were removed from the solution and weighed after drying in vacuum for 2 days. The weight loss was determined by using the measurements before and after phosphate buffered saline incubation. The films were also examined by ATR-FTIR and SEM to determine the surface degradation of polymers.

Antibacterial performance

Microbial growth and antibacterial performance of the films was studied using a gram-negative bacterium, *Escherichia coli* or *Pseudomonas aeruginosa*, and a gram-positive bacterium, *Staphylococcus aureus* or *Bacillus subtilis*. The antibacterial performance was evaluated using both agar diffusion and bacterial colony counting methods.

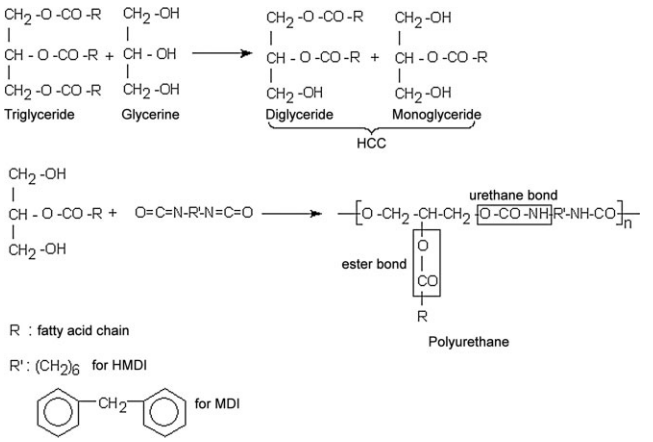
In the agar diffusion method, tests were carried out in solid media to observe a zone of inhibition around polymer film disks. For this purpose, polymer disks (1 cm diameter) were placed on an agar plate seeded with 0.1 mL of inoculums containing $\sim 10^6$ CFU/mL of *Pseudomonas aeruginosa* or *Staphylococcus aureus* or *Bacillus subtilis* and left in an incubator at 37°C for 24 h. At the end of the incubation period, the diameter of the zone of inhibition around polymer disk was measured. To determine whether the film is bactericidal (kills bacteria) or bacteriostatic (inhibits bacteria), the incubation was continued without polymer films at 37°C for 24 h.

In the bacterial colony counting method, the polymer films were cut into 1 cm diameter discs, placed on a well of 24-well plates and then incubated with a calibrated bacterial suspension (10^3 CFU/mL) of *Staphylococcus aureus* or *Escherichia coli* in Brain Heart Broth at pH 7.4. The samples were incubated at 35–37°C under rotational agitation for 24 h. At the end of the incubation period, possible bacterial adhesion on the polymers was examined by microscope. The samples were removed from the cultures growing at 37°C, serially diluted using sterile BPS and viable counts were carried out in triplicate on nutrient agar media (BD). Viable colonies were counted 24 h later. Growth medium with bacteria, but without polymer, were used as negative control. All experiments were carried out in duplicate on at least two separate occasions, and mean values were reported.

Cytotoxicity of polyurethane films

Polymer films were sterilized by ethylene oxide before cytotoxicity tests. Two methods were employed for determination of cytotoxicity, direct contact and MMT tests.

In the direct contact test, Murine fibroblast NIH 3T3 cell line (seeding density 1.5×10^4 cells per well) were precultured for 18 h in Dulbecco's modified essential medium supplemented with bovine serum (10%) in 96-well plates and exposed for 24 h to the polymers placed in the center of each well. A



Scheme 1 The reactions for polymer preparation.

growth medium, containing cells but no polymer, was tested for negative control. The morphological changes indicating cytotoxicity and cell growth characteristics were recorded using a Nikon-TMS microscope equipped with a Canon PC1049 camera.

In MTT test, after 24 h of cell culturing in the presence of individual polymer, the medium was removed and 100 μL of growth medium with MTT (5 mg/mL in PBS) was added to the cultures. Cells were incubated at 37°C in humidified atmosphere for 3 h. Then the growth medium was removed, 100 μL of lysis solution including 99.4% dimethyl sulfoxide (DMSO), 0.6% acetic acid, 10% sodium dodecyl sulphate (SDS) was added to each well to dissolve purple crystals of formazan. Dissolved formazan of each well were transferred to another 96-well plate, and the absorbance was measured in a spectrophotometer at a wavelength of 570 nm. Reported values are the means of three replicates and are expressed as percentages of the control values.

RESULTS AND DISCUSSION

Polymer characterization

The reactions for polyurethane synthesis are shown in Scheme 1. Two different polyurethane samples were prepared: one of them was synthesized using HMDI (encoded PU/HMDI) and the other was from both HMDI and MDI (encoded PU/HMDI-MDI). It is speculated that when both HMDI and MDI are used they are distributed to the polyurethane chain in a random manner. The linseed oil used in the preparation of polyurethane is an ester product of glycerol and fatty acids, and, therefore, the resulting polymer chains include ester bonds in addition to urethane bonds as seen in Scheme 1.

The polyurethane structure was confirmed with FTIR studies. The polymer spectra are given in Figures 1(b) and 2(d) together with the spectra of the prepolymer [Fig. 2(b)], the reaction mixtures for the synthesis of prepolymer and polymer [Figs. 1(a) and 2(a,c)]. The disappearance of the absorption peaks at around 2270 cm^{-1} (assigned to the N=C=O group) and 3456 cm^{-1} (assigned to the OH group), and the appearance of the absorption peak at around 3330–3350 cm^{-1} (assigned to the NH group) are an important evidence for consumption of both HMDI and MDI and production of polyurethane. The peak around 1740 cm^{-1} is attributed to the carbonyl stretching of the ester group. A typical FTIR spectrum of polyurethane shows characteristic absorption band for C=O stretching of urethane group at 1710 cm^{-1} and C—N—H bending at 1526 cm^{-1}. For PU synthesized from both HMDI and MDI, the peak at around 1598 cm^{-1} has been assigned to the aromatic rings in the polymer structure.

The ^{1}H-NMR spectra of polymers are presented in Figures 3 and 4. ^{1}H-NMR analysis also confirms the

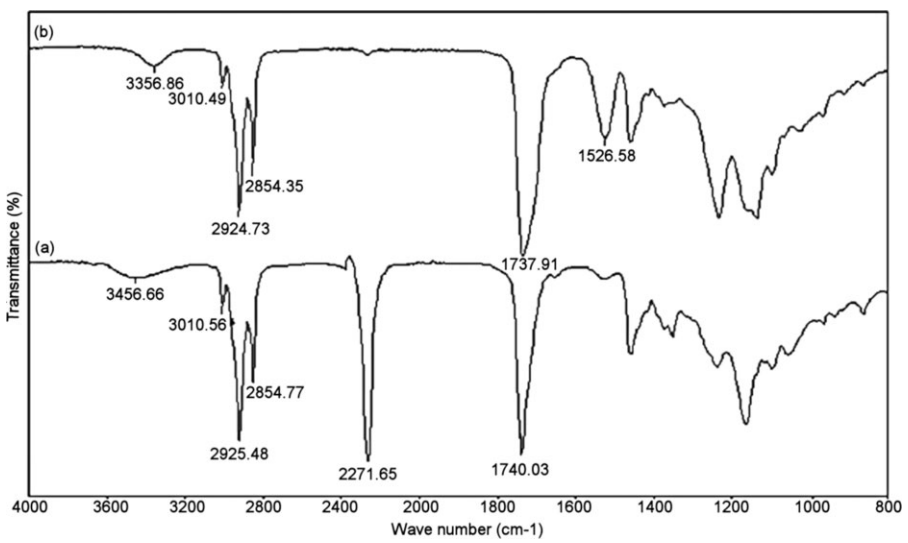


Figure 1 FTIR spectra of: (a) the reaction mixture for the synthesis of PU/HMDI, and (b) PU/HMDI.

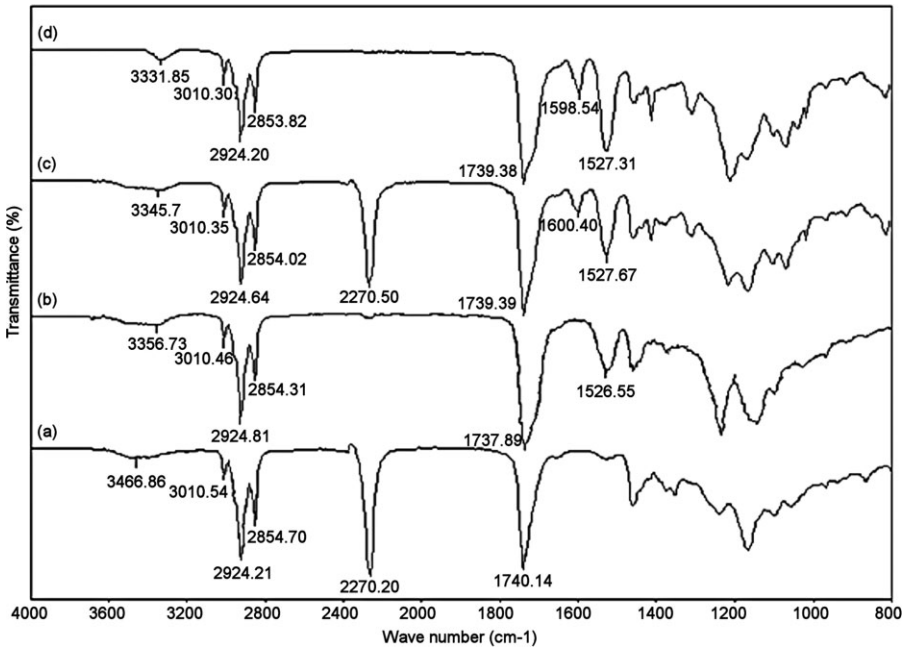


Figure 2 FTIR spectra of: (a) the reaction mixture for prepolymer synthesis, (b) prepolymer, (c) the reaction mixture for the synthesis of PU/HMDI-MDI, and (d) PU/HMDI-MDI.

polyurethane structure for both polymers. The peak at 2.8 ppm is due to the —CH_2 group attached to —CH=CH in the fatty acid chain of HCC. The peak at 3.15 ppm is attributed to the methylene group attached to —NH of HMDI. The signal around 5.35 ppm is due to the —CH=CH group of the fatty acid chain. The aromatic protons show peaks around 7.23–7.08 ppm and the peak at 3.69 ppm is due to the methylene groups attached to aromatic rings for PU/HMDI-MDI.[28] The urethane N—H proton in MDI-based polyurethane is expected to show peaks between 9.5 and 8.0 ppm, whereas the urethane N—H proton in HMDI-based polyurethane is expected to appear around 6.7–6.3 ppm. Both peaks do not appear in the NMR spectra of PU/HMDI-MDI and PU/HMDI. The absence of these peaks

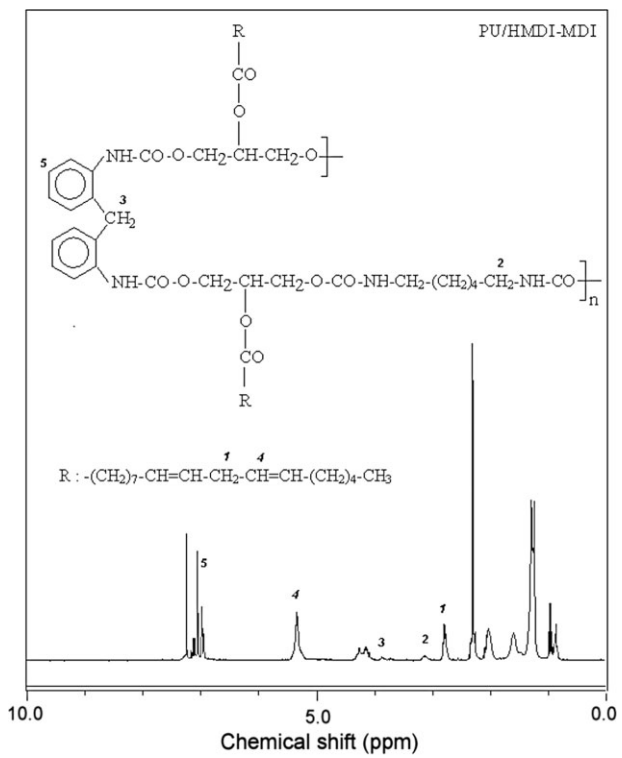


Figure 3 NMR spectra of PU/HMDI-MDI.

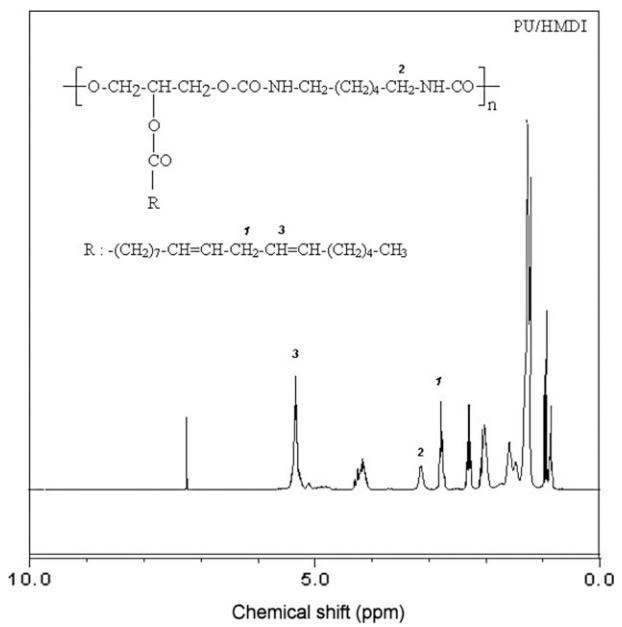


Figure 4 NMR spectra of PU/HMDI.

TABLE I
Molecular Weight and Thermal Analysis Date of Polymers and Polymer Films

Code	Molecular weight (M_w)	Polydispersity index (M_w/M_n)	Temp. at 50% weight loss (°C)	Glass transition temp. (T_g,°C) by: DSC	DMA
PU/HMDI	4692	2.0	–	−2.6	–
PU/HMDI(f)	–	–	360.2	7.5	16.7
PU/HMDI-MDI	13730	5.0	–	13.0	–
PU/HMDI-MDI (f)	–	–	392.6	30.5	34.7

may be due to the existence of hydrogen bonding between C=O and N—H groups in the polymer chain.[29] The integration of peaks 2 and 3 for PU/HMDI-MDI sample is $\sim 2:1$. This shows that the numbers of the mers coming from MDI and HMDI in polymer structure are equal to each other.

Table I presents the weight average molecular weight (M_w) and polydispersity index of polymers prepared. Since the molecular weight of HCC prepared from linseed oil was relativity low (as compared with the commercial petroleum-based polyols), and a chain extender was not used in polymer synthesis, polymers of $M_w > 15{,}000$ Daltons could not be synthesized. As shown in Table I, the M_w of PU/HMDI-MDI is higher than that of PU/HMDI. On the other hand, the number average molecular weights (M_n) calculated from M_w and polydispersity index, are close to each other for both polymers (2346 for PU/HMDI and 2746 for PU/HMDI-MDI). The difference between M_n of polymers is due to the difference between molecular weights of monomers used. Since the polydispersity index for both polymers are not close to each other, the difference between the M_w of PU/HMDI and PU/HMDI-MDI is much higher than the difference between M_n. The polydispersity index for both polymers is relatively high. This result can be explained with the structure of HCC. As discussed in the experimental section, HCC was prepared by transesterification reaction of linseed oil and glycerol. The reaction product is a mixture of monoglyceride, diglyceride, and unreacted triglyceride, which has no hydroxyl groups (Scheme 1). Monoglyceride and diglyceride have two and one hydroxyl group(s), respectively. Polymer is formed when the monoglyceride molecules react with di-isocyanate molecules. Diglyceride molecules cause formation of short polymer chains. The reactivity of hydroxyl groups attached to α- and β-position of di- and monoglyceride molecules are different, however, it is not possible to select the hydroxyl group to react with isocyanate during the polymerization reaction. Thus, the composition of HCC is an important effect on the polydispersity of the final polymer. In addition, since two different types of diisocyanates having different molecular weights react with HCC, and the preparation procedure (two-step polymerization) is different for PU/HMDI-MDI synthesis, the polydispersity index was higher for PU/HMDI-MDI polymer.

Characterization of polyurethane films

Thermal and viscoelastic behavior, surface hydrophilicity, water absorption, biodegradation, antibacterial performance, and cytotoxicity of the polymer films prepared with drying catalyst were determined. The films prepared without catalyst were only tested for antibacterial performance and cytotoxicity.

Thermal Characterization and Viscoelastic Properties

The initial decomposition temperature of polymers was determined to be between 225 and 250°C by TGA. It is well known that thermal decomposition of conventional polyurethanes takes place above 200°C.[30] As shown in Table I, the temperature at 50% weight loss is higher for PU/HMDI-MDI film because of the existence of benzene rings in the polymer structure.

Dynamic mechanical response of polyurethane films (PU/HMDI(f) and PU/HMDI-MDI(f)) is shown in Figure 5. The T_g of polyurethanes was identified from the peak of the tan δ curve [Fig. 5(a)]. A comparison of the T_g values determined by DSC and DMA methods (Table I) indicates that the latter method gave higher T_g values, however, the trend was the same; the lower T_g value was determined for PU/HMDI(f).

T_g is strongly influenced with the chemical structure and molecular weight of polymers. In general, T_g increases with increasing molecular weight (at low molecular weights), and with decreasing flexibility of the polymer chain. Aromatic groups in the polymer backbone hinder the flexibility of the polymer chain. Similar trends were observed for the polymers and polymer films prepared in this study (Table I); that is, the T_g of HMDI-MDI-based polymer, and the film is higher than that of HMDI-based ones.

Crosslink density is another important parameter affecting T_g. The value of T_g increases with increasing number of crosslinks.[31] In this study, crosslinked polyurethane films were obtained during the curing

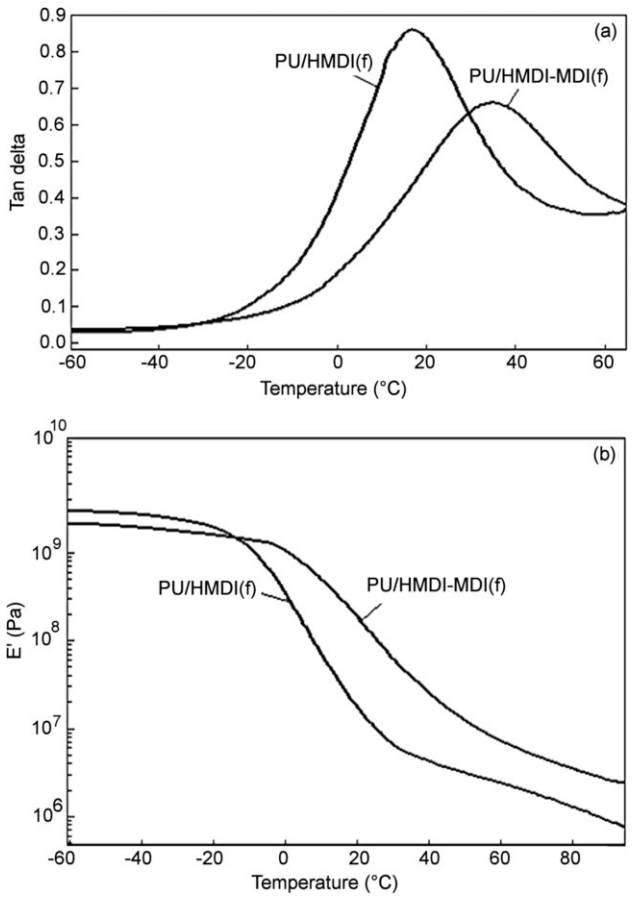
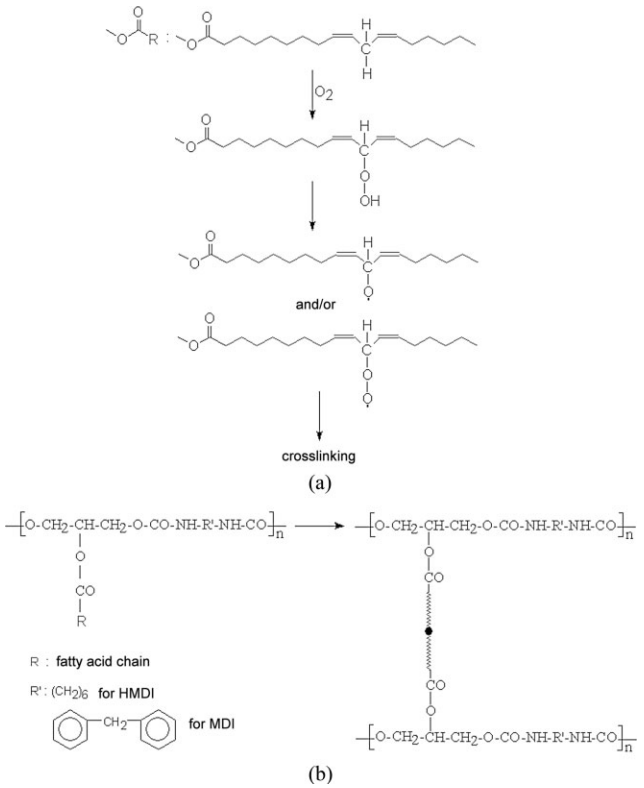


Figure 5 Viscoelastic properties of polyurethane films; (a) Tan δ, (b) Storage modules.



Scheme 2 Polymer film by: (a) formation of crosslinked-polyurethane, (b) oxidative polymerization reaction of fatty acid chain.

process. (Scheme 2a). As expected, the T_g value of the films was higher than that of the corresponding bulk polymers (Table I) due to the crosslink formation. The crosslink mechanism occurred through the fatty acid chain is shown in Scheme 2b.

The E' values obtained in DMA can be used to obtain information regarding the stiffness of the polymer, degree of cure and crosslink density.[32,33] As discussed above, both polymer films were crosslinked due to the presence of double bonds on fatty acid chain. Since the same hydroxyl-containing material was used for preparation of both polymers, they had the same degree of crosslink density. Hence, the difference between the storage moduli in glassy and rubbery plateaus ($\Delta E'$) can be used to provide information about the stiffness of polymer films. A greater $\Delta E'$ is associated with flexible polymer film. The $\Delta E'$ value of PU/HMDI(f) was determined to be higher than that of PU/HMDI-MDI(f) [Fig. 5(b)]. This can be attributed to its flexible polymer chain due to the aliphatic structure of HMDI. It is clear that the flexible polymer chain resulted in a low T_g and a high damping capacity. On the contrary, MDI causes to increase the stiffness of the polymer due to the presence of the aromatic ring in its structure.

Surface hydrophilicity, water absorption, and biodegradation

The surface hydrophilicity of polyurethane films can be characterized from the static water contact angle measurements reported in Table II. There is no significant difference between the contact angle values for both films prepared. Both of them can be considered hydrophilic because their contact angle values are smaller than 90°. The water absorption capacity for both polymer films is also presented in the Table II. Both films did not absorb water, i.e., less than 4%.

The polyester-based polyurethanes have generally high oxidative and low hydrolytic stabilities. The ester bonds are hydrolytically unstable, whereas urethane bonds are not considered susceptible to hydrolysis in the aqueous environment of the body. The results of *in vitro* degradation studies for polyurethane films prepared in this study are presented as mass loss as a function of time in Figure 6.

TABLE II
Contact Angle and Water Adsorption

Code	Contact angle (°)	Water absorption (%)
PU/HMDI(f)	74 ± 0.12	3.6 ± 0.19
PU/HMDI-MDI(f)	77 ± 0.11	2.6 ± 0.21

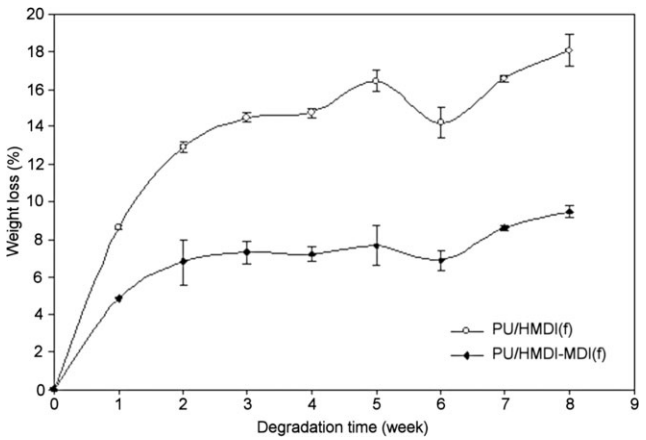


Figure 6 Weight loss of polymer films during biodegradation tests.

Biodegradation rate of PU/HMDI(f) was found higher than that of PU/HMDI-MDI(f). The difference between biodegradation rates may be due to both the molecular size and the structure of the polymers. The aromatic rings in the structure of PU/HMDI-MDI may be protecting the ester bonds against the attack of water molecules. Although the molecular weight of polymer does not directly affect the degradation rate, increasing molecular weight causes to a decrease in the free volume. Hence, the diffusion rate of water into polymer decreases resulting in a decrease in the biodegradation rate. This result was corroborated by SEM study (Fig. 7). Physical damage for PU/HMDI film was observed to be higher than that of PU/HMDI-MDI film. A punched-hole-like morphology is observed for both films starting at week 1. There are examples of polyurethane films in the literature developing a large number of pits even after 24 h of incubation.[34] In another case, big holes were observed for a poly (carbonate urethane) sample after 15 months.[35] The biodegradation rate depends on the polymer structure and degradation media.

FTIR was used to examine the surface degradation of polymer films. Characteristic spectra of polymers before and after biodegradation are given in Figures 8 and 9. The peaks at 2854–3010 cm^{-1} assigned to aliphatic CH_2 were used as an internal reference peak for normalization since they remained unchanged during biodegradation. The significant loss of around 1738 cm^{-1} carbonyl peak for both films was attributed to hydrolytic degradation of ester bonds. After 2 weeks of biodegradation in BPS, a new peak appeared in the spectrum of the PU/HMDI film at 978 cm^{-1} assigned to the O—H out-of plane band.[36] The intensity of this peak increased with increasing biodegradation time. This reveals that free acid groups were released due to the breaking of ester bonds during biodegradation. On the other hand, there was no systematic change in the peak at around 975 cm^{-1} in the spectrum of PU/HMDI-MDI film. FTIR analysis were repeatedly carried out at different locations of the film surface but the peak at around 900–1100 cm^{-1} was not reproducible. No correlation was found between the degradation time and the intensity of the peak at 975 cm^{-1}. For both polyurethane films there is no significant change at around 1535 cm^{-1} assigned to C—N—H bending suggesting that urethane bonds did not degrade during biodegradation of PU/HMDI film.

Antibacterial performance

The antibacterial performance of the polymer films containing 0.5-10 wt % Biocera A®, and the film without Biocera A® was determined both agar diffusion and bacterial colony counting methods.

At the end of the incubation period the zone of inhibition was not observed in the agar diffusion method for *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and

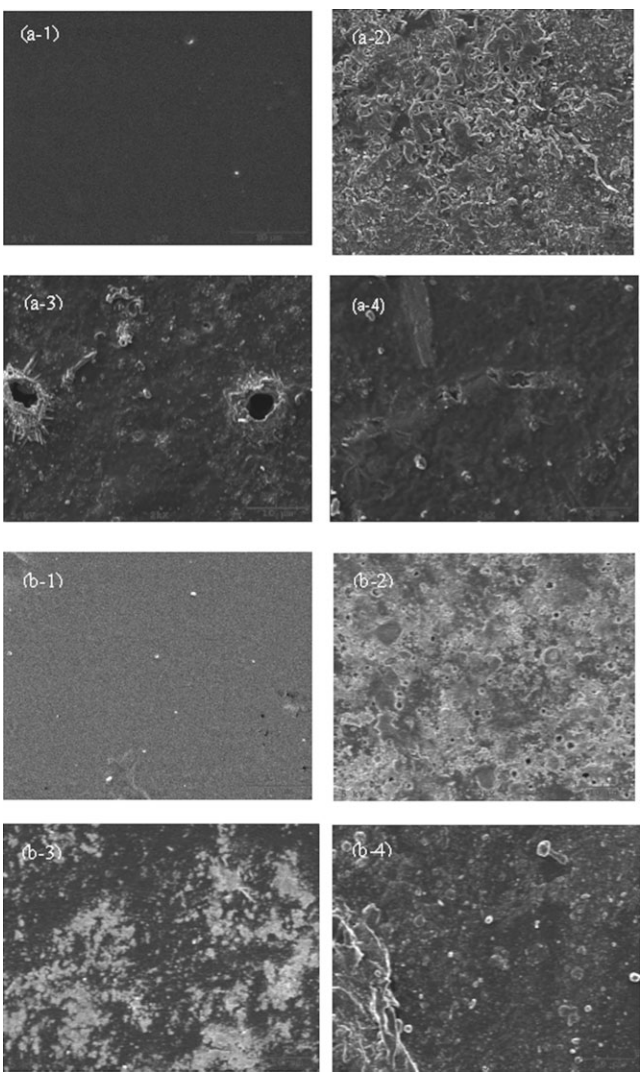


Figure 7 SEM image of the films for; (a) PU/HMDI and (b) PU/HMDI-MDI, (1) before degradation, (2) after 1 week, (3) after 4 weeks, and (4) after 8 weeks of biodegradation.

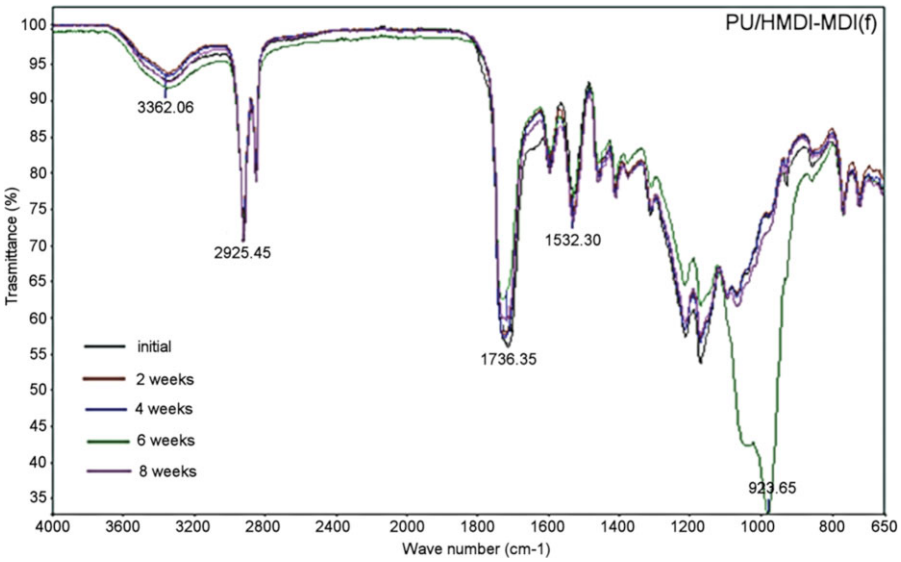


Figure 8 FTIR spectra of the film of PU/HMDI-MDI before and after biodegradation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Bacillus subtilis for all polymer films. However, when the polymer films were removed from the agar plate, and the incubation was continued, the bacteria growth was not observed on the open areas once occupied by the films (Fig. 10). This observation indicates that the films may have bactericidal effect against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. There are no significant differences among the agar plates seeded with all three bacteria for all Biocera A® concentrations. The film prepared without Biocera A® also showed the same antibacterial effect with the Biocera A® added films.

In the bacterial colony counting method, bacterial growth was not determined in the medium of the films prepared with and without silver ions, whereas the number of bacterial colonies for control sample was determined to be 1.25×10^6 bacteria/mL. The results are in agreement with that of agar diffusion method and reveal that all films exhibited antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. This is an expected result for the films containing silver ions. The antibacterial effect of the polyurethane films without silver ions may be due to the isocyanate groups it contains. A similar result for metal containing polyurethanes has been reported by Jayakumar et al.[37]

The films prepared with the addition of the drying catalyst (PU/HMDI-MDI(c) and PU/HMDI(c)) exhibited a similar antibacterial effect as the film prepared without catalyst (PU/HMDI-MDI and PU/HMDI).

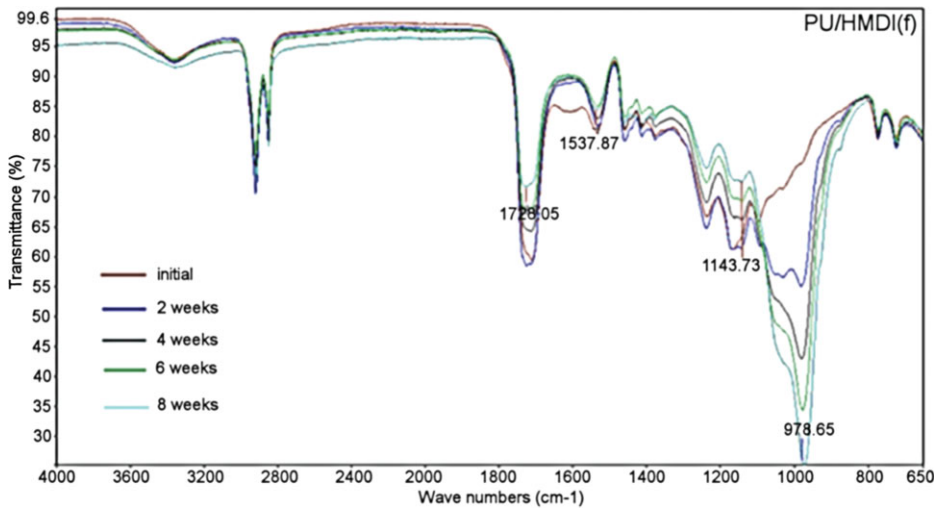


Figure 9 FTIR spectra of the film of PU/HMDI before and after biodegradation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

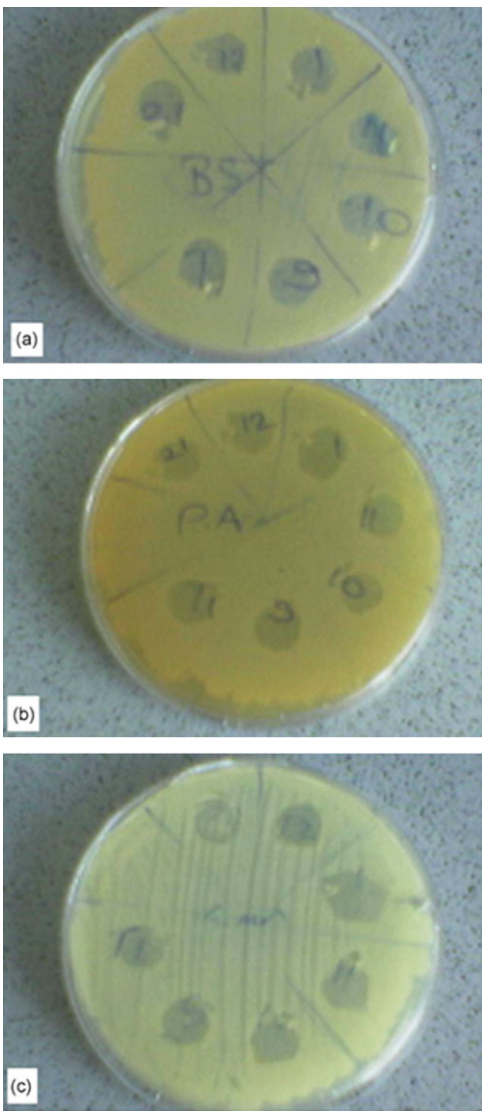


Figure 10 Determination of antibacterial activity for the films by agar diffusion method against; (a) *Bacillus subtilis*, (b) *Pseudomonas aeruginosa*, (c) *Staphylococcus aureus*. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Cytotoxicity

The percentage of cell proliferation on polyurethane films after 24 h of incubation is given in Figure 11. The cell proliferation on PU/HMDI-MDI film is relatively close to the control sample and commercial product. It should be noted here that PU/HMDI-MDI(c) and PU/HMDI(c) did not show acceptable noncytotoxicity, whereas the films prepared without catalyst were not cytotoxic. The effect of the catalyst type on cytotoxicity in the synthesis of copolymers for biomedical use was also studied by Tanzi et al.[38] Although at different extent, all catalysts used in their study, including stannous octoate, which has the similar chemical structure with the drying catalysts used in our study, proved to be cytotoxic.

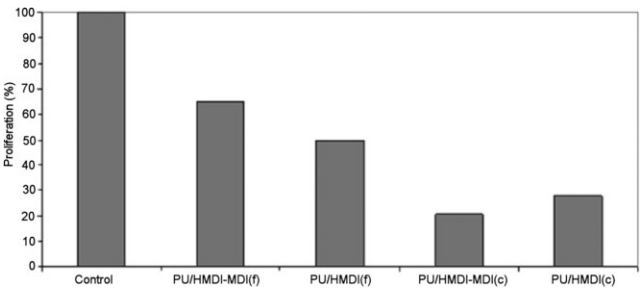


Figure 11 The cell proliferation on the surface of polymer films.

The cytotoxicity results were also supported by the phase contrast micrographs (Figure 12). The cells were successfully attached to the surface of PU/

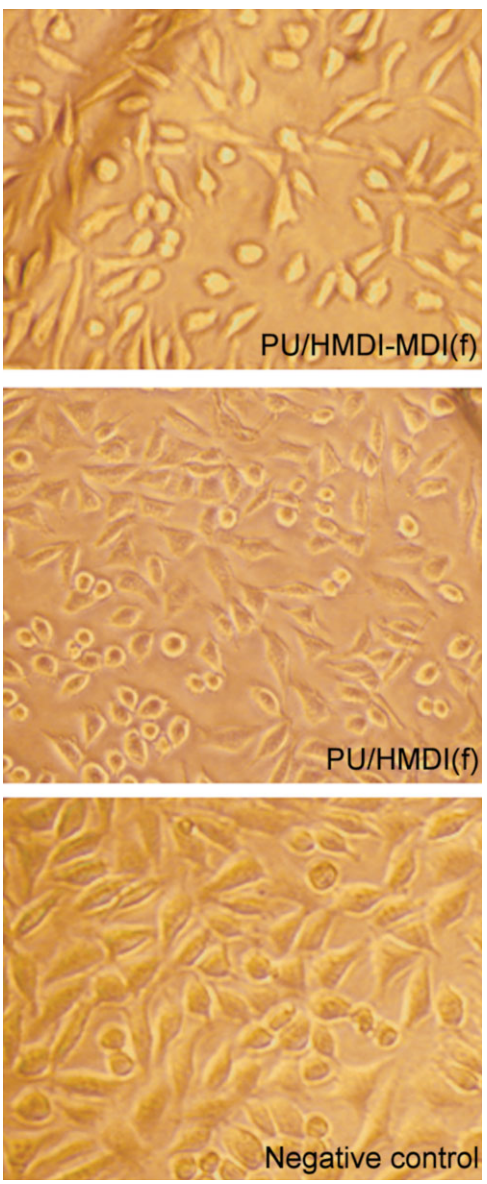


Figure 12 Phase contrast micrographs. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

HMDI-MDI and PU/HMDI films. The amount and morphology of the cells on the polymer surfaces are significantly similar to that observed on the control. These results suggest that the polyurethane films prepared allow the adhesion of cells and may be suitable for use as wound dressing material. A more conclusive result can be obtained from the *in vivo* studies currently in progress.

CONCLUSIONS

The polyurethane films were prepared successfully from linseed oil in the absence of any catalyst and characterized for use as wound dressing material. HMDI or HMDI-MDI-based films showed different thermal and viscoelastic properties. MDI containing film had higher T_g and better thermal property because of the existence of benzene ring in the polymer chain. Both films were hydrophilic and showed almost the same surface hydrophilicity. Water absorption of both polymer films was found to be around 2.6–3.6%. *In vitro* degradation of the films was determined to be around 9 and 18% for MDI-HMDI- and HMDI-based films, respectively. FTIR and SEM studies confirmed the degradation of the polymer surfaces. Cell proliferation studies indicated that both films were not cytotoxic.

Silver-containing films prepared using Biocera A® as filler and films without filler both exhibited bactericidal effect against *Bacillus subtilis, Pseudomonas aeruginosa*, and *Staphylococcus aureus*. In addition, all films showed antibacterial effect against *Staphylococcus aureus* and *Escherichia coli* in bacterial colony counting experiments.

Triglyceride oils are renewable, nontoxic, and cheaper than petroleum-based materials and therefore are very attractive raw materials for polyurethane synthesis. The noncytotoxic and antibacterial nature of the linseed oil-based polyurethane film is encouraging for their consideration as wound dressing material.

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