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Synthesis and properties of carboxymethylchitosan hydrogels modified with poly(ester-urethane)

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ABSTRACT

Preparation and properties of carboxymethylchitosan (CMC) modified with polyurethane (PU) containing poly(ethylene adipate) (PEA) as a soft segment is described. Urethane prepolymer was first synthesized by the reaction of PEA with an excess of 1,6-hexamethylene diisocyanate (HDI) to terminate its ends with isocyanate functional groups, followed by chain extension reaction using ethylene glycol as a chain extender. Its chemical structure was characterized by ^{1}H NMR and FTIR, molecular weight by GPC, and thermal behavior by DSC. To prepare PU-modified CMC (CMC-PU), 1–60 wt% of PU were introduced into the CMC solution of THF:H_2O mixture (50:50 v/v) in the presence of 10 wt% of hexamethylene-1,6-di-(aminocarboxysulfonate) (HDA) to increase network density. Formation of the network structure was confirmed by investigating percent crosslinking and water swelling properties of CMC-PU compared to CMC network without PU. When percent of PU increased from 1 to 60 wt%, percent crosslinking of CMC-PU gradually increased up to 82%, whereas equilibrium water content (EWC) dropped and retained at 1000%. SEM showed microphase separation of PU (10–50 μm) thoroughly dispersed in CMC surface and in the bulk. In addition, CMC-PU exhibited a slight enhancement in toughness properties. Cytotoxicity and biocompatibility tests indicated that CMC-PU was non-toxic.

1. Introduction

Chitosan, prepared by alkaline deacetylation of chitin, is a biodegradable, biocompatible and inexpensive natural polymer comprising of β-(1,4)-2-amino-2-deoxy-D-glucopyranose units (Mao et al., 2005; Wu et al., 2005; Zeng, Fang, & Xu, 2004). It has gained marked attraction in recent years owing to its potential applications in medicine, cosmetics, agriculture and biomaterials. In particular, excellent activity as a wound healing accelerator (Ishihara et al., 2002; Khan, Peh, & Ch'ng, 2000; Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004) combined with antibacterial capability and good tissue compatibility of chitosan made it become an excellent wound dressing materials (Mi, 2005; Mi, Shyu, Lin, Wu, & Tsai, 2003a; Mi, Sung, Shyu, Su, & Peng, 2003b; Mi, Tan, Liang, & Sung, 2002; Mi et al., 2003c). However, limited solubility of chitosan in water and common organic solvents inhibited its extensive studies and utilization (Morita, Sugahara, Ibonai, & Takahashi, 1999).

Carboxymethylation reaction of chitosan with monochloroacetic acid in alkali solutions results in a water soluble chitin derivative, so-called carboxymethylchitosan (CMC). Precedents demonstrated potential use of CMC especially in wound healing application due to its antibacterial activity, non-cytotoxicity, biocompatibility and excellent water swellability (Fan et al., 2006; Zhao, Wang, & Wang, 2003). In addition, it was also non-thrombogenicity and thus was suitable for use as blood-contacting materials (Hagiwara et al., 1999). However, the wound healing mechanism of CMC has not been fully understood (Chen, Wang, Liu, & Park, 2002). Chemical modification of CMC (Sun, Du, Fan, Chen, & Yang, 2006), or physical blending of another polymer into CMC (Fan et al., 2006) has been widely studied to obtain resulting materials with novel properties. Due to its solubility in water, formation of network structure of CMC is necessary when it is intentionally used as a wound dressing material. Only limited numbers of studies reported the investigation of the formation of CMC network (Chen, Tian, & Du, 2004; Yin, Fei, Cui, Tang, & Yin, 2007; Yu et al., 2006). Glutaldehyde was mostly used as a crosslinking agent to form CMC-based interpenetrating networks (IPN). In addition, formation of network structure can also promote miscibility of CMC with another type of polymer (Yu et al., 2006).

Polyurethane (PU) elastomers are versatile engineering and medical materials used in a wide variety of products due to their excellent flexibility and elasticity (Mackey, 2002). The major advantage in preparation of PU is that they can be synthesized from various types of polymers that contain different mechanical

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and physical properties (Barrere & Landfester, 2003; Gong & Zhang, 2003; Guan, Fujimoto, Sacks, & Wagner, 2005; Guan, Sacks, Beckman, & Wagner, 2004; Krol & Pilch-Pitera, 2003). In particular, poly(ester-urethane) elastomers are of our interest because they can be synthetically designed to have hydroxyl functional groups at their chain terminals, which readily react with diisocyanate groups to form urethane linkages (Guelcher et al., 2005). Another advantage especially in medical application is that they exhibit relatively good antithrombogenicity and hemocompatibility as compared to other synthetic polymers (Kang, Kwon, Kim, Lee, & Sung, 1997, 1999; Larsson, Jannasch, & Wesslen, 2000; Lee et al., 2001). Chemical modification (Gibson, Walls, Kennedy, & Welsh, 2003; Lin, Yu, & Yang, 2005; Silva, Menezes, & Garcia, 2003; Welsh, Schauer, Qadri, & Price, 2002; Zeng, Zhang, & Kennedy, 2005) of chitosan with PU elastomers has been previously studied to improve mechanical properties and blood compatibility of the materials. However, the studies and exploitation of CMC modified with PU elastomer have been very limited (Yu et al., 2006).

In the present studies, preparation of CMC networks modified with poly(ethylene adipate) (PEA)-containing PU was reported. It is believed that the combination of biocompatible CMC and elastomeric PU can produce biomaterials with novel properties. Percent crosslinking and water swelling behavior of the networks were investigated to confirm the formation of network structure. The objective of this work was to investigate the influence of PU concentrations in CMC on water swelling properties and toughness properties of the modified CMC. The effect of PU concentrations on microphase separation of the modified CMC, which is in relation to water wettability of its surface, was determined. In addition, cytotoxicity and biocompatibility tests were also reported.

2. Experimental

2.1. Materials

Carboxymethylchitosan (CMC) was prepared from the reaction between chitosan with monochloroacetic acid. Briefly, 85% DD chitosan from shrimp was swollen in isopropanol for 12 h, reacted with sodium hydroxide solution at room temperature for 75 min, and followed by the reaction with monochloroacetic acid at 60 °C for 5 h. pH of the solution was then adjusted to neutral with 6 N HCl solution. CMC was precipitated in an excess of methanol, followed by washing with methanol:H_2O solution (70:30 v/v) to remove salts. It was filtered and dried at 40 °C under vacuum for 6 h. Dihydroxyl-terminated poly(ethylene adipate) (PEA) having the molecular weight of 1000 g/mol (Acros) and 1,6-hexamethylene diisocyanate (HDI), 99% (Acros) were used as received. Sodium metabisulphite, $Na_2S_2O_5$ (Carlo Erba reagent) were used as received. Ethylene glycol (Mallinckrodt) were fractionally distilled and stored under N_2 until used.

2.2. Synthesis

Poly(ester-urethane)s were prepared following a two-step synthesis (Fig. 1): (1) preparation of poly(ethylene adipate) (PEA) terminated with isocyanate functional groups (2) preparation of polyurethane containing PEA soft segments (PU). To prepare PU-modified CMC, 1–60 wt% of PU was incorporated into CMC in the presence of hexamethylene-1,6-di-(aminocarboxysulfonate) (HDA) crosslinking agent. Synthesis of HDA crosslinking agent has also been provided herein.

2.2.1. Synthesis of PEA-based PU

PEA and HDI (1:2.5 molar ratio) were charged into a round-bottomed flask filled with N_2. The temperature was increased to 105 °C for 2 h to obtain PU prepolymers with isocyanate terminals. Then, ethylene glycol chain extender (0.5 M equiv.) was added into the prepolymer to continue the chain extending reaction for another 2 h. M_n of the polymers was determined using GPC technique. Its chemical structure was elucidated using ^{1}H NMR and functional groups were investigated by FTIR.

2.2.2. Synthesis of hexamethylene-1,6-di-(aminocarboxysulfonate) (HDA)

HDA was prepared following the procedure previously described (Gibson et al., 2003; Welsh et al., 2002). Briefly, HDI (10 g, 0.06 mol) was added to a $Na_2S_2O_5$ aqueous solution (12 g, 0.06 mol in 25 ml

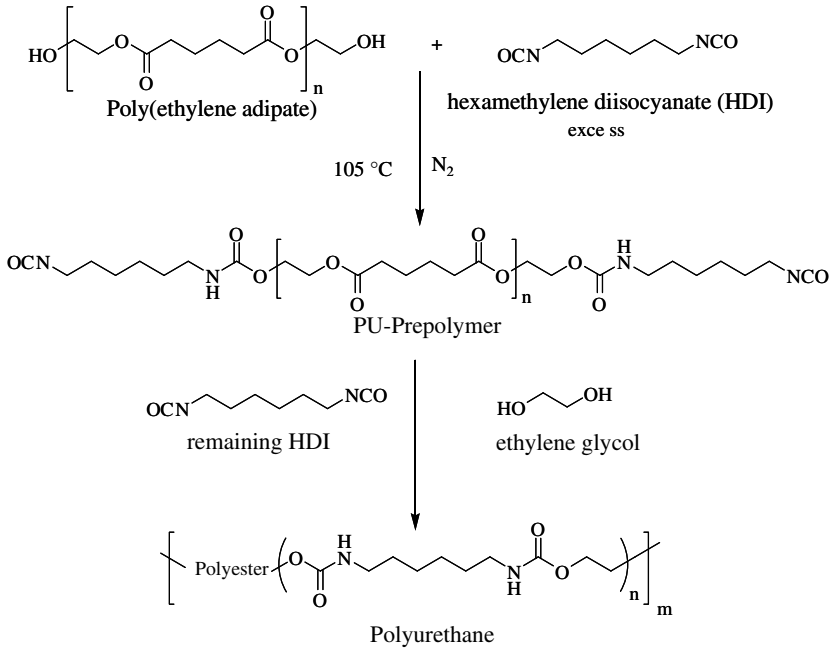

Fig. 1. Synthetic scheme of PEA-based PU.

H_2O) and stirred for 20 h at room temperature. The product was then precipitated in an excess of acetone, filtered and dried in vacuum. Water soluble HDA was dissolved in water and insoluble byproducts were removed by filtration. The product was repeatedly precipitated in an excess of acetone and dried to obtain a white powder of HDA.

2.2.3. Preparation of poly(ester-urethane)-modified carboxymethylchitosan (CMC- PU)

CMC modified with PU was prepared via a solvent casting method using hexamethylene-1,6-di-(aminocarboxysulfonate) (HDA) as a crosslinking agent. In two separated aliquots, PU was dissolved in 100 ml of THF and HDA (0.5 g) was dissolved in 20 ml of distilled water. These two solutions were introduced into a CMC solution (5.0 g in 100 ml water, 10 wt% based on CMC) with continuous stirring for 2 h. The solution was then poured into a glass mold to obtain a square sample film with $10 \times 10\ cm^2$ and kept at 60 °C for 24 h. PU-modified CMC (CMC-PU) was somewhat opaque as compared to the unmodified CMC networks (CMC-HDA network without PU).

2.3. Characterization

2.3.1. Characterization of PEA-based PU

^1H NMR was performed on a 400 MHz Bruker NMR spectrometer using $CDCl_3$ or D_2O as a solvent. FTIR was performed on a Perkin-Elmer Model 1600 Series FTIR Spectrophotometer. The neat samples were directly cast onto potassium chloride plates. GPC data was conducted on PLgel 10 μm mixed B2 columns and a refractive index detector. THF was used as a solvent with a flow rate of 1 ml/min at 30 °C. Thermal analyses were conducted on a Perkin-Elmer Pyris-1 differential scanning calorimeter (DSC). The samples were heated from −150 to 200 °C with a 10 °C/min heating rate. DSC thermograms of the second scan were reported.

2.3.2. Determination of percent crosslinking

To determine percent crosslinking, sample films with the dimension of $2 \times 2\ cm^2$ were submerged into distilled water at room temperature for 48 h to dissolve uncrosslinked CMC and HDA. The undissolvable CMC was filtered and washed with distilled water, followed by acetone to remove uncrosslinked PU. It was then dried at 60 °C in a vacuum oven for 24 h. Percent crosslinking was calculated as following:

$$\text{Percent crosslinking } (\%) = (W_2/W_1) \times 100$$

where W_1 and W_2 are the dried weights of the samples before and after the dissolution, respectively. The reported values are the average of three different measurements.

2.3.3. Water swelling measurements

Water swelling behavior was determined from equilibrium water content (EWC) values. After curing reactions, the samples were cut into $2 \times 2\ cm^2$ dimension and submerged into aliquots containing an excess of water to obtain fully hydrated samples. After 24 h, the hydrated samples were removed from water, wiped

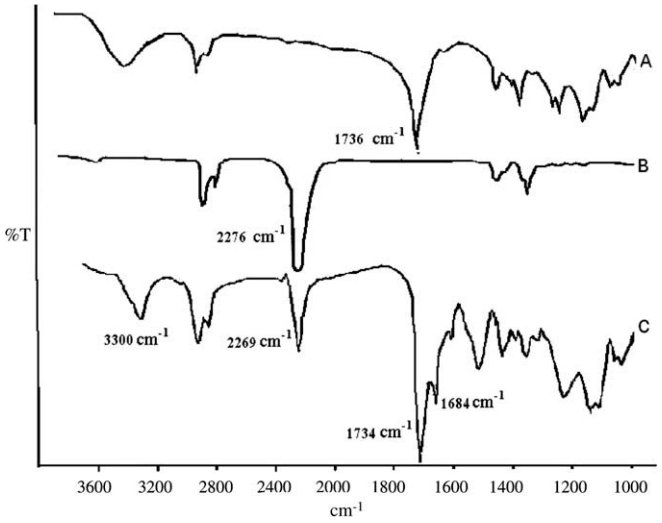

Fig. 3. FTIR spectra of (A) PEA homopolymer, (B) HDI, and (C) PEA-based PU.

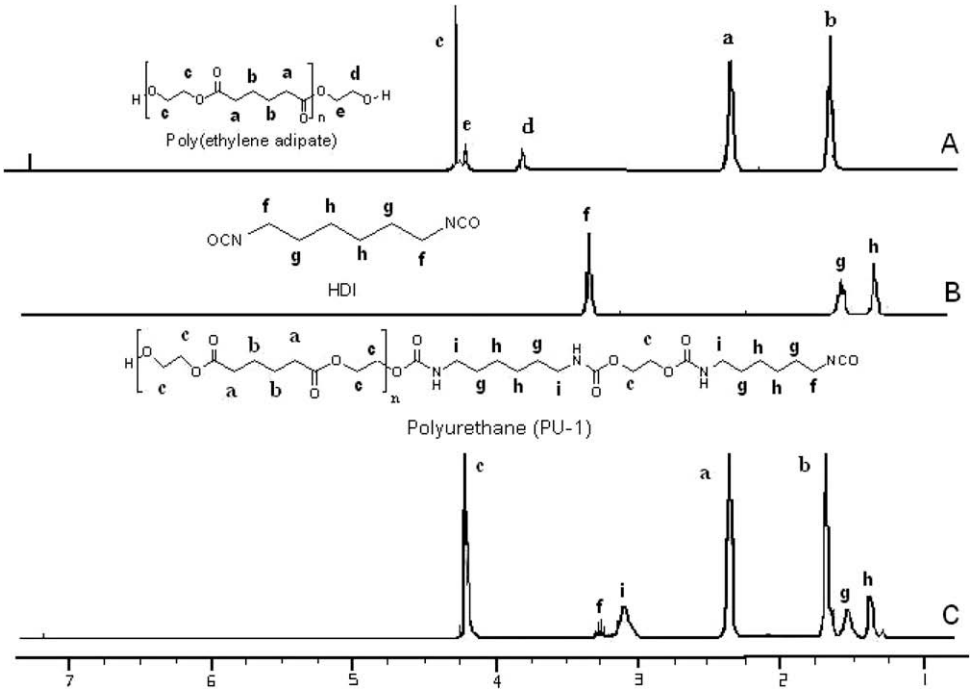

Fig. 2. ^1H NMR spectra of (A) PEA homopolymer, (B) HDI, and (C) PEA-based PU.

off excess surface moisture and precisely weighed (W_{sw1}). The samples were then dried for 48 h at 50 °C under vacuum. The dried weights (W_d) were subsequently determined. EWC1 values were calculated as follows:

$$\text{EWC1 } (\%) = (W_{sw1} - W_d) \times 100/W_d$$

To determine water swellability of the pre-dried samples, the same samples used to determine EWC1, were again immersed into an excess of water at room temperature for another 48 h. The swollen samples were removed from water, wiped off excess water on the surface and weighed (W_{sw2}). EWC2 values were calculated as follows:

$$\text{EWC2 } (\%) = (W_{sw2} - W_d) \times 100/W_d$$

The reported values are the average of at least three measurements.

2.3.4. Morphological studies by scanning electron microscopy (SEM)

Morphological studies of sample surface and in the bulk were carried out through LEO 1455 VP scanning electron microscopy (SEM) with an accelerating voltage of 20 kV. Sample films were dried in a vacuum oven at 50 °C for 2 days. They were cut into 1×1 cm^2 in size and adhered onto an aluminum stub.

2.3.5. Water contact angle measurement

Contact angles (θ) between water and sample films were measured using the sessile method (Rutnakornpituk & Ngamdee, 2006) on a Krüss DSA 10 Contact Angle Meter at room temperature. Water was carefully dropped on sample films and contact angles were quickly determined before the films commenced to swell. The reported values are the average of five different measurements.

2.3.6. Determination of tensile strength and percent elongation

Tensile strength and elongation properties were performed on a Universal Testing Machine (Instron Model 55R4502). The samples were cut into a rectangular shape with 1.0 cm width (ASTM D882). The samples were performed at the 30 mm gage length with the crosshead rate of 10 mm/min. Tensile strength and percent elongation at break were calculated as follows:

$$\text{Tensile strength } (\text{N/mm}^2) = \frac{\text{Breaking force (N)}}{\text{Cross-sectional area of the sample (mm}^2)}$$

$$\text{Percent elongation at break } (\%) = \frac{\text{The increase in length at breaking point (mm)} \times 100}{\text{Original length (mm)}}$$

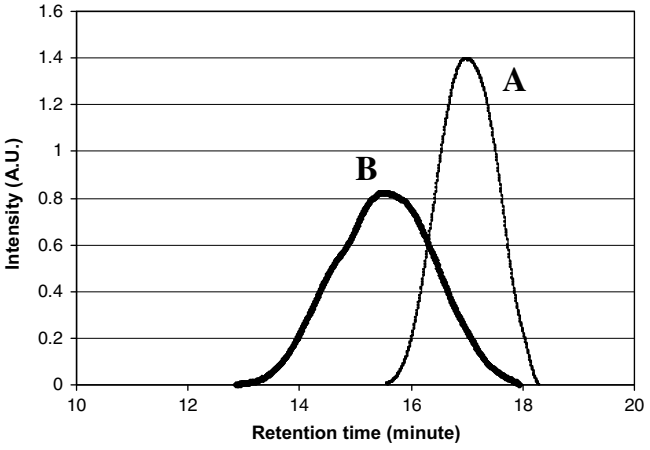

Fig. 4. GPC chromatograms showing the increment of the molecular weight and molecular weight distribution of (B) PEA-based PU when compared to (A) PEA homopolymer.

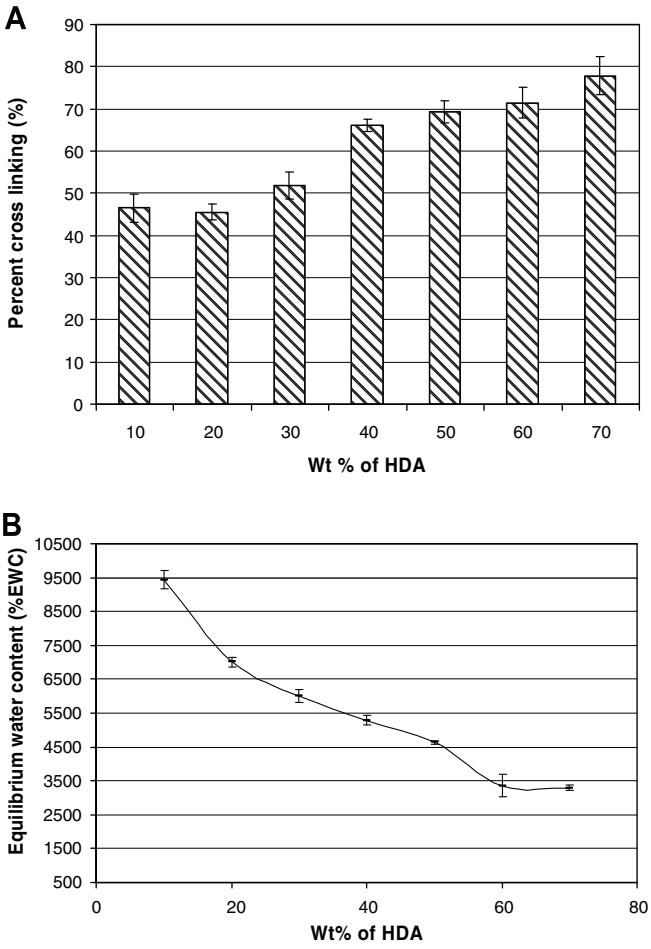

Fig. 5. (A) Percent crosslinking and (B) equilibrium water content (%EWC) of unmodified CMC crosslinked with 10–70 wt% of HDA crosslinker (without PU).

The presented results were the average numbers of three independent measurements.

2.3.7. Cytotoxicity and biocompatibility tests

2.3.7.1. Material preparation. Tested materials, CMC-HDA and CMC-PU 30%, were sterilized with ethylene oxide gas prior to the biological property assessment. The cell line used in the assay was L929 (ECACC No. 85011425), mouse connective tissue, fibroblast-like

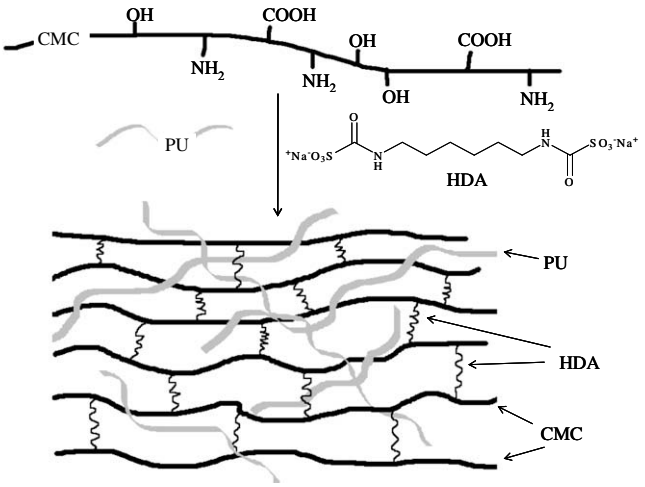

Fig. 6. Preparation of CMC hydrogels modified with poly(ester-urethane).

cells. The growth medium used was Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), together with penicillin (100 U/ml) and streptomycin (100 μg/ml).

2.3.7.2. Cytotoxicity test. The test specimens were cut into small circular 5-mm diameter discs and saturated with growth medium. The discs were then placed in the middle of a 35-mm dish. L929 cells were seeded onto the dish at a density of 6×10^4 cells/dish and incubated for 48 h. Cell morphology and the toxic zone were evaluated by inverted phase contrast light microscopy after a 48-h exposure to the cells. The cells were stained with 0.01% neutral red in phosphate buffer saline (PBS) for membrane integrity. High-density polyethylene (HDPE) and natural rubber containing carbon black were used as negative and positive controls, respectively. Each sample was tested in triplicate, and the test was repeated twice.

2.3.7.3. Biocompatibility test. The test specimens were cut into square pieces and saturated with growth medium before being placed onto a 35-mm dish. L929 cell suspension (1×10^6 cells/dish) was directly seeded onto the surfaces of the specimens. At 7-day and 14-day incubation periods, the samples with the attached cells were fixed with 2% (v/v) glutaraldehyde in 0.1 M phosphate buffer (PB) pH 7.2 for 4 h at 4 °C. The samples were subsequently washed with 0.1 M phosphate buffer (PB), dehydrated by graded ethanol series, and dried using a critical point CO_2 method. The samples were eventually gold sputtered in vacuum and examined by SEM (Jeol JSM-5410) (Jeol, Japan) to observe the cellular morphology and behavior of the L929 cells on the samples. The samples were tested in triplicate.

3. Results and discussion

3.1. Synthesis of PEA-based PU

PU elastomers are usually prepared via a two-step reaction; (1) synthesis of PU prepolymers by endcapping polymeric glycol with diisocyanate functional groups, and (2) chain extension of the PU prepolymers with diisocyanate and diol monomers to form soft and hard segments (Fig. 1). High molecular weight and content of polymeric glycol presenting in PU structure are desirable to obtain elastomeric PU. However, incorporation of high molecular weight of hydrophobic polyester enhanced its immiscibility with hydrophilic CMC. Therefore, in the current work, low molecular weight hydroxyl-terminated PEA (1000 g/mol) was used as polymeric glycol due to its commercial availability. Molar ratio

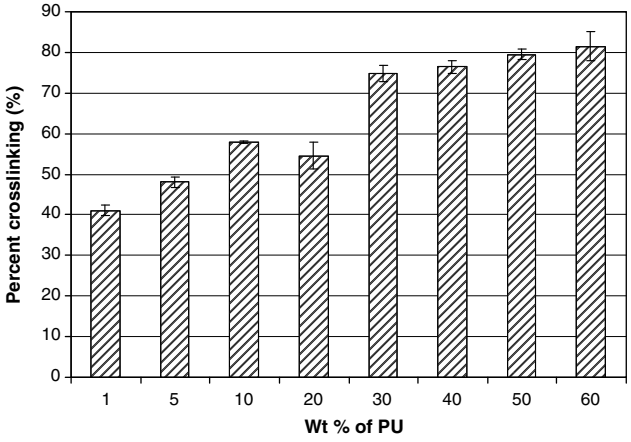

Fig. 7. Percent crosslinking of CMC-PU as a function of weight percent of PU.

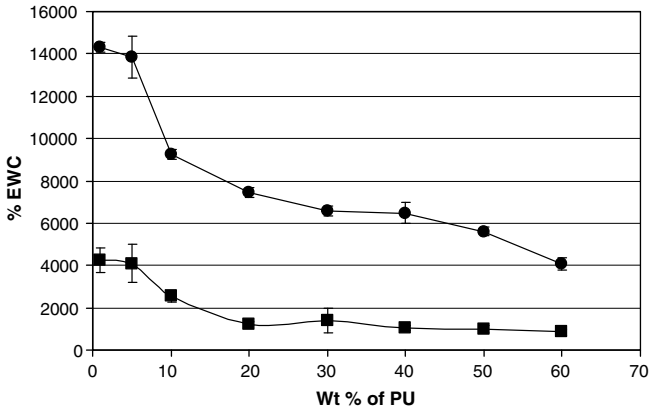

Fig. 8. EWC1 (●) and EWC2 (■) of CMC-PU networks as a function of weight percent of PU.

(1:2.5) of PEA to HDI was used in the first step reaction to terminate PEA polymeric glycol with diisocyanate functional groups. Dropwise adding PEA into HDI solution combined with using an excess of HDI was thought to endcap PEA with isocyanate groups at both PEA terminals. One mole of PEA can hypothetically react with two moles of HDI and allowed 0.5 M equiv. of HDI remaining in the reaction. Ethylene glycol chain extender (0.5 M equiv.) was then added to the PU prepolymer to further react with the remaining HDI to form PU hard segments and resulted in chain extension reaction. The slight excess of HDI in the first step (0.5 M equiv.) was intended to limit the formation of PU hard segment.

The formation of PU was evidenced by ^{1}H NMR (Fig. 2C). Signal *i* (3.10 ppm) corresponding to methylene protons adjacent to urethane functional groups indicated the formation of urethane linkages. In addition, the absence of signals *d* and *e* (3.82 and 4.20 ppm, respectively) (Fig. 2A and C) indicated the disappearance of hydroxyl groups in the reaction because they were completely reacted with HDI. The presence of signal *f* (3.25 ppm) in PU (Fig. 2C) indicated that there were isocyanate functional groups remaining at the chain terminals of PU. This might be important in the reaction of PU with CMC in such a way that PU can covalently bond to CMC network structure.

FTIR was used to observe the existence of C═O (1684 cm^{-1}) and N—H (3300 cm^{-1}) stretching of urethane functional groups (Fig. 3C). In good agreement with ^{1}H NMR result, isocyanate functional groups still remained at 2269 cm^{-1} and its intensity

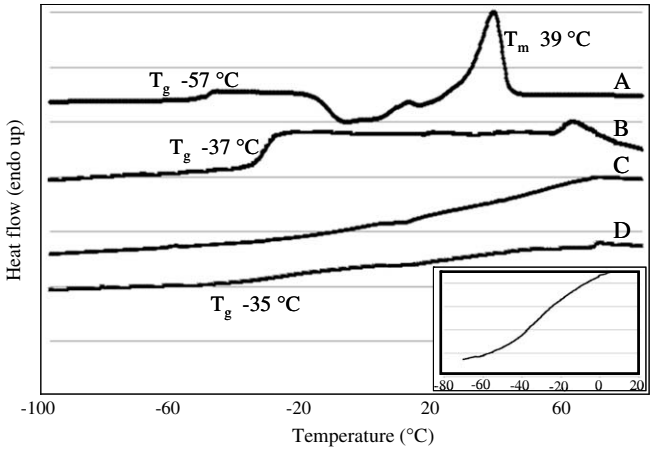

Fig. 9. The second scan DSC thermograms of (A) PEA homopolymer, (B) PU, (C) unmodified CMC, and (D) CMC-PU 30 wt%. The inset is the expansion of −80 to 20 °C region of the thermogram D.

decreased due to the reaction with hydroxyl groups of PEA and ethylene glycol chain extender. GPC results showed that the molecular weight of PU was approximately 9700 g/mol with molecular weight distribution (MWD) of 2.95, indicating that the molecular weight and MWD of PU significantly increased when compared to PEA homopolymer (M_n 2200 g/mol, MWD 1.42) (Fig. 4).

3.2. Properties of PU-modified CMC (CMC-PU)

3.2.1. Percent crosslinking and water swelling behavior

Because CMC is water soluble polymer, it is necessary to incorporate crosslinkers to form CMC networks. Hexamethylene-1,6-di-(aminocarboxysulfonate) (HDA) (10 wt%), water soluble derivative

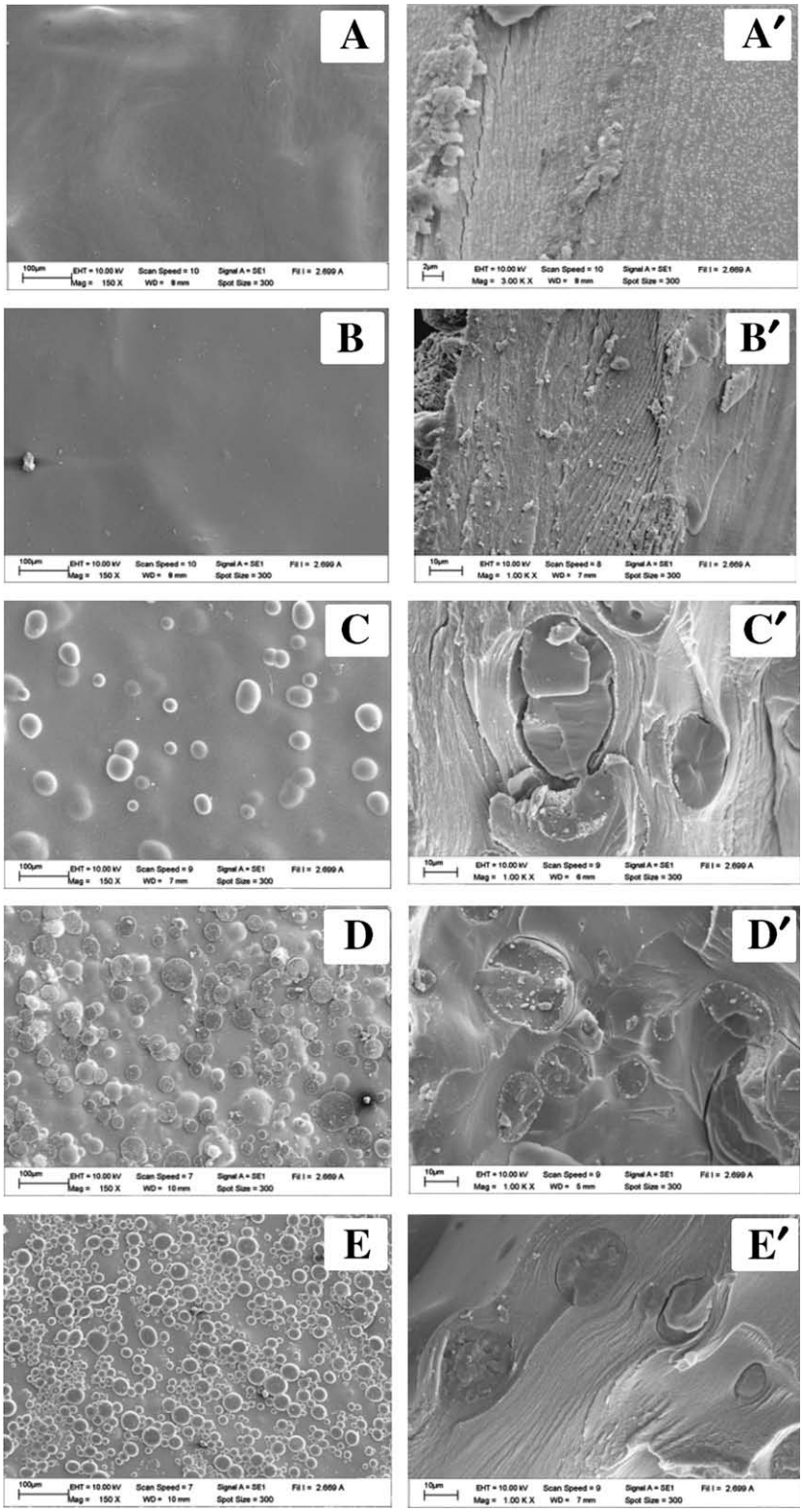

Fig. 10. Surface (A–E) and cross-sectional (A′–E′) morphologies of (A) CMC, (B) CMCHDA, (C) CMC-PU 10 wt%, (D) CMC-PU 30 wt%, and (E) CMC-PU 50 wt%.

of HDI, was effectively used to crosslink CMC at room temperature. Precedent reported that water soluble HDA reacted with amine functional groups faster than hydroxyl functional groups of chitosan (Gibson et al., 2003; Welsh et al., 2002). In the current studies, amine functional groups remaining in CMC readily reacted with aminocarboxysulfonate functional groups of HDA to form urea linkages. HDA in the mixed solvent of water and THF (50:50 v/v) as indicated by formation of swollen hydrogel after leaving the solutions at room temperature. Formation of CMC networks without sacrificing its excellent water swelling properties was desirable. Thus, a minimal amount of HDA just enough to form CMC networks was determined to avoid formation dense network structure. According to the results in Fig. 5A, addition of 10–70 wt% of HDA gradually increased percent crosslinking from 47% up to 77%. The increase in percent crosslinking of the CMC networks as increasing amount of HDA crosslinker was attributed to the formation of dense network structure. However, when less than 10 wt% of HDA was introduced into CMC, the networks were not fully cured as indicated by the presence of detached debris after submerging the networks into an aqueous solution for 1 day. The effect of percent HDA crosslinker on water swelling properties was investigated by incorporating 10–70 wt% HDA crosslinker into CMC and equilibrium water content (EWC) of the networks was determined (Fig. 5B). It is apparent that 9500% EWC (95 times of the original dried weight) was obtained as only 10 wt% of HDA incorporated. Increasing percent of HDA crosslinker resulted in a decrease in %EWC due to the formation of dense network structure.

PU (1–60 wt%) was incorporated into CMC containing 10 wt% HDA crosslinker, then called CMC-PU. PU chains were thought to interpenetrate in CMC networks having HDA crosslinker as shown in Fig 6. Percent crosslinking and water swelling properties of the networks as a function of percent of PU were then investigated. Percent crosslinking of CMC-PU increased from 42% to 82% as percent of PU increased from 1 to 60 wt% (Fig. 7). It was reasoned that increasing percent of CMC-PU increased amount of isocyanate active functional groups as evidenced by ^{1}H NMR and FTIR, resulting in enhancing network density.

Water swelling behavior was studied by determining EWC1 and EWC2 values. EWC1 is the percent of water at equilibrium determined from the fully swollen CMC networks to the dried samples, whereas EWC2 was determined from the same dried sample to the fully re-swollen network. CMC containing 1 wt% PU showed %EWC1 as high as approximately 14,000%, indicating its excellent water swellability (Fig. 8). EWC1 rapidly decreased to 7500% when less than 20 wt% PU was added and gradually decreased to 4000% as percent of PU increased up to 60%. The decreases of EWC1 were attributed to hydrophobic nature of PEA in PU structure combined with the formation of dense network structure when PU concentration was increased.

According to the results in Fig. 8, it was observed that %EWC2 was significantly lower than %EWC1 in every case. This was probably due to additional network formation upon drying process of CMC-PU preparation, resulting in additional curing reaction and deteriorating its water swellability. It is worth to mention that %EWC2 retained at about 1000% at high percent of PU in CMC networks and these correspond to about 70–80% crosslinking of the networks (Fig. 7). This implied that there was no or only a slight additional network reaction taking place when more than 30% PU was added.

3.2.2. Thermal behavior

Thermal behavior of PEA homopolymer, PU and CMC containing 30 wt% PU (CMC-PU 30 wt%) was investigated using DSC to observe the change of T_g of PEA soft segment in PU structure and also in CMC-PU network when compared to PEA homopolymer (Fig. 9). The observed T_g at −37 °C of PU (Fig. 9B) was attributed to the glass transition of PEA soft segments. This value is 20 °C higher than T_g of the corresponding PEA homopolymer (T_g −57 °C in Fig. 9A). The formation of hard segments in PU might inhibit mobility of PEA soft segments, resulting in the increase of its T_g. Crystallization of PEA in PU structure was also hindered as observed by the disappearance of its melting temperature. It should be noted that T_g of unmodified CMC was not observed when determined by DSC technique (Fig. 9C). Therefore, when 30 wt% PU was incorporated into CMC, the observed T_g at −35 °C (Fig. 9D and the inset) was attributed to those of PU phase in CMC.

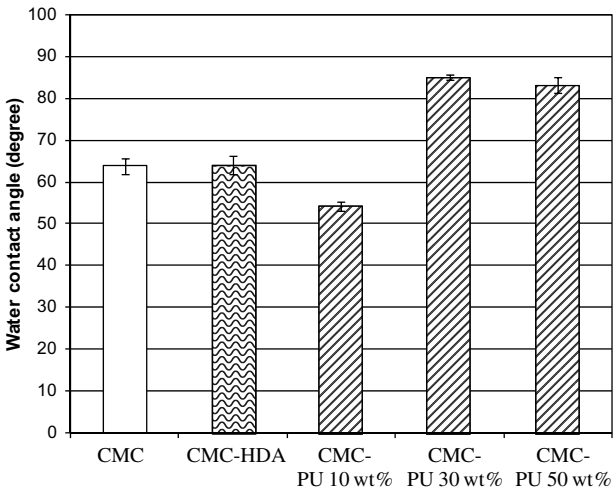

Fig. 11. Water contact angles of unmodified CMC, CMC-HDA and CMC containing 10–50 wt% PU.

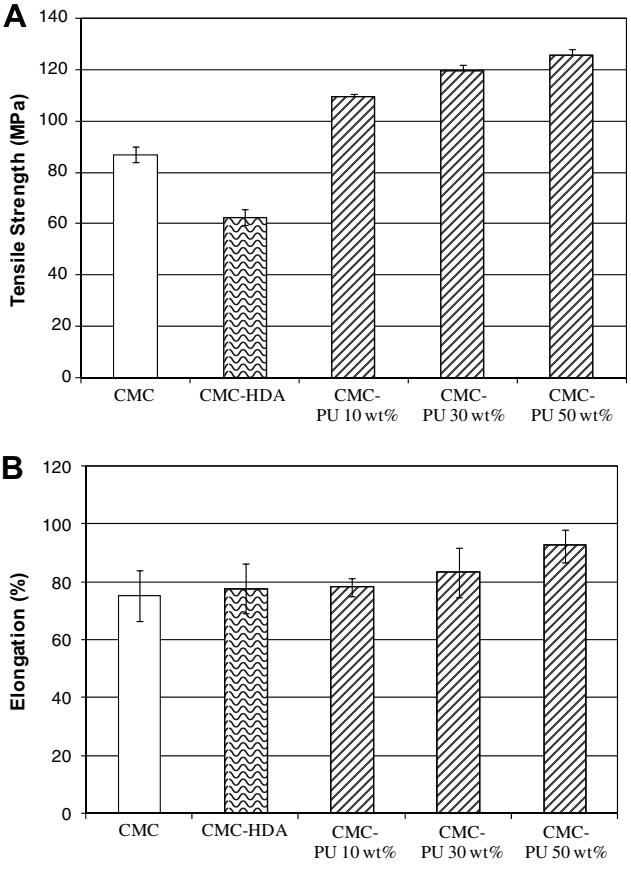

Fig. 12. (A) Tensile strength and (B) percent elongation of unmodified CMC, CMC-HDA, and CMC containing 10–50 wt% PU.

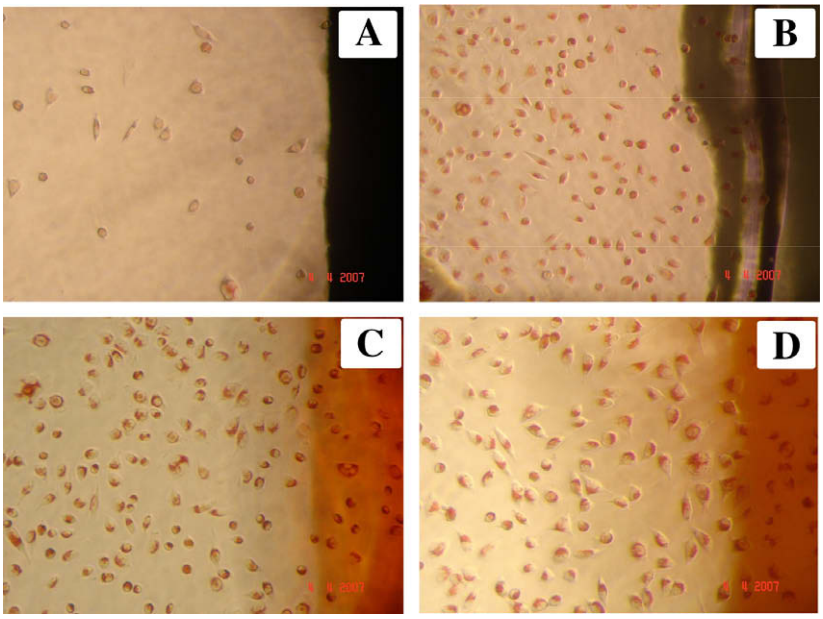

Fig. 13. Optical micrographs of L929 cells in contact with (A) natural rubber containing carbon black (positive control), (B) HDPE (negative control), (C) CMC-HDA, and (D) CMC-PU 30 wt%.

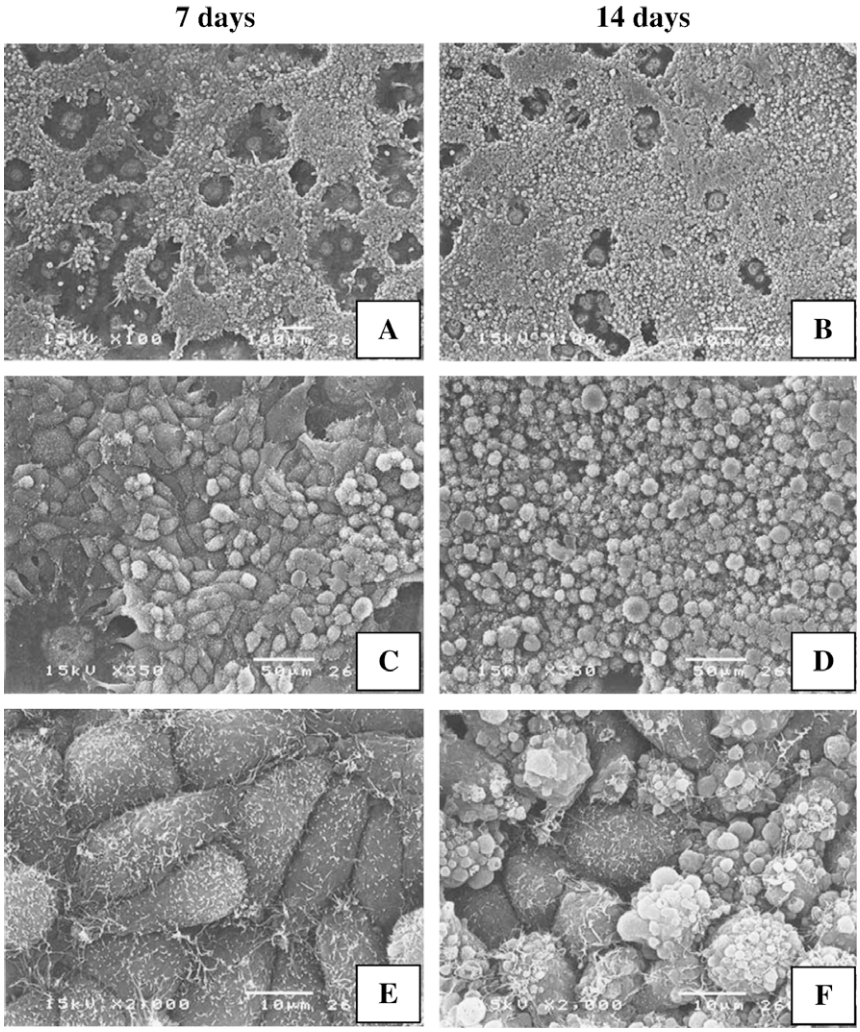

Fig. 14. SEM micrographs of L929 cells cultured on CMC-HDA for 7 (A, C, and E) and 14 days (B, D, and F) at different magnifications, 100× (A and B), 350× (C and D), and 2000× (E and F).

3.2.3. Morphological studies

The formation of PU microphases was confirmed by SEM by observing surface (Fig. 10C–E) and cross-sectional (Fig. 10C′–E′) morphologies of CMC containing 10–50 wt% of PU and compared with those of unmodified CMC (Fig. 10A and A') and CMC networks crosslinked with 10 wt% HDA (CMC-HDA) (Fig. 10B and B'). The unmodified CMC and CMC-HDA networks showed dense and homogeneous morphology without any microphase observed. When 10 wt% PU was introduced into CMC, microphases with approximately 10–50 μm in diameter were thoroughly dispersed in the CMC continuous phase. It is thought that these microphases were PU which was initially soluble in CMC aqueous solution and exhibited microphase separation during film drying process. The driving force of this separation was attributed to hydrophobic characteristics of PU dispersing in hydrophilic CMC continuous phase. A number of these PU microphases were increased as the concentration of PU in CMC increased from 10 to 50 wt%.

3.2.4. Water contact angle measurement

Investigation of water contact angle of CMC containing 10–50 wt% PU was carried out in air using the sessile method (Rutnakornpituk & Ngamdee, 2006). Those of unmodified CMC and CMC-HDA network were also measured to compare their surface wettability to the PU-modified CMC. Low degrees of water contact angle indicate high water wettability of the sample surface. According the results shown in Fig. 11, addition of 10 wt% PU into CMC lowered water contact angle as compared to those of unmodified CMC and CMC-HDA networks (both are 64 degrees). This indicated the enhancement of water wettability of its surface upon adding only 10 wt% PU. This was probably due to the presence of polar urethane linkages when small amount of PU was added. However, increasing percent of PU from 10 to 30 or 50 wt% lowered water wettability of its surface as shown by the increase of water contact angles. It was rationalized that hydrophobic character of PEA soft segment existing in PU structure dominated its surface properties when high percent of PU was incorporated. These results are in good agreement with SEM observation. Namely, in CMC-PU 10 wt%, PU microphases loosely dispersed in CMC continuous phase and allowed water drops to be in contact with hydrophilic CMC phase (Fig. 10C). In CMC-PU 30 wt% and CMC-PU 50 wt%, PU microphases densely dispersed and covered most of CMC continuous phase, resulting in lowering its water wettability (Fig. 10D and E).

3.2.5. Toughness properties

PU containing polyester soft segment is a well-known elastomer that is commercially produced and used in various applications. Incorporation of PU elastomer containing PEA soft segments into CMC networks is thought to promote flexibility of the materials. Fig. 12 shows tensile strength and percent elonga-

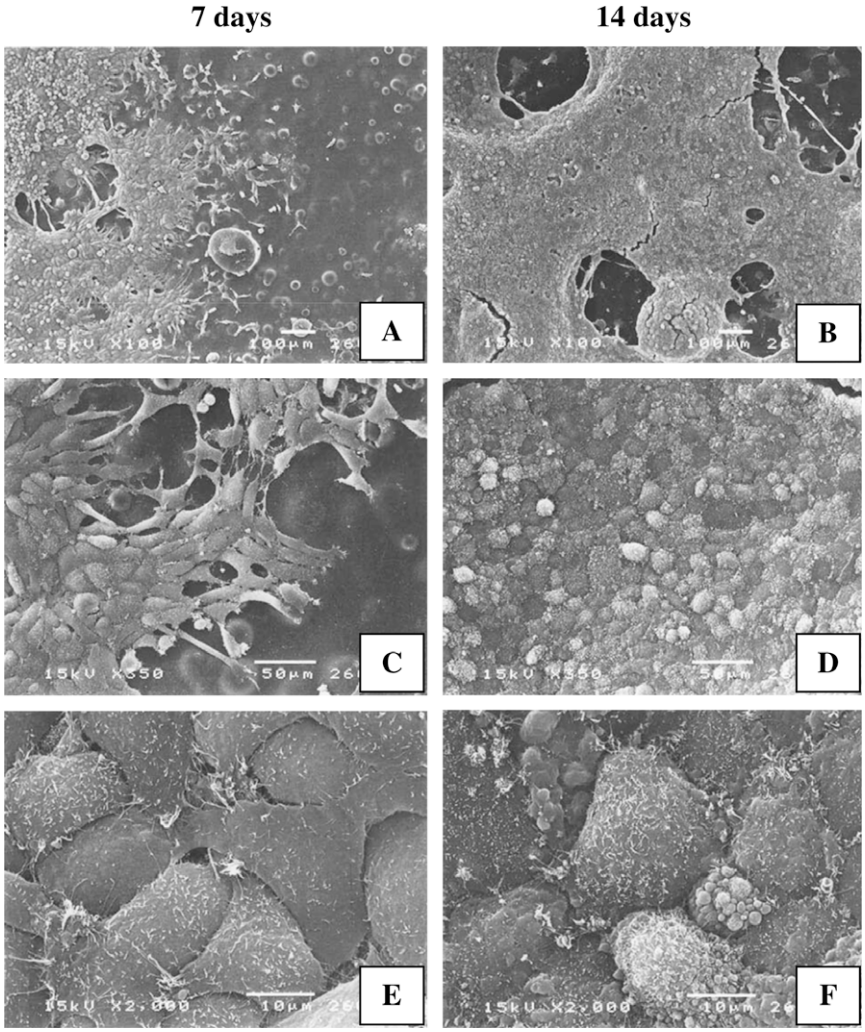

Fig. 15. Biocompatibility testing of CMC-PU 30 wt% at 7 (A, C, and E) and 14 days (B, D, and F) at different magnifications, 100× (A and B), 350× (C and D), and 2000× (E and F).

tion of CMC containing 10–50 wt% PU and compared with those of unmodified CMC and CMC-HDA network. The results indicated a decrease in tensile strength of CMC-HDA compared to the unmodified CMC. It is rationalized that hydrogen bonding in the unmodified CMC due to the existence of hydroxyl, amine and carboxylic acid functional groups caused its structure to have physical crosslinking throughout the materials, resulting in rather high in tensile strength when extended. In addition to loosely covalent crosslinking, incorporation of small amounts of HDA into CMC might possibly deteriorate this hydrogen bonding in CMC structure, resulting in less resistant to extension and lower tensile strength than the unmodified one. Addition of 10–50 wt% PU into CMC resulted in an enhancement of tensile properties due to the presence of polyester soft segments in PU structure. In addition, increasing percent of PU in CMC tended to increase percent elongation, indicating the enhancement of toughness properties of the materials.

3.2.6. Cytotoxicity

Fig. 13 reveals the optical micrographs of the L929 cells in contact with the test specimens. The results clearly demonstrated that both CMC-HDA and CMC-PU 30% were non-cytotoxic. The cells possessed normal morphology after 48-h incubation and were well stained with neutral red, indicating that they were alive.

3.2.7. Biocompatibility (cell-materials response)

In general, once cells are seeded on a substrate, they secrete cell-binding serum protein essential for the cell attachment (Verrier, Bareille, Rovira, Dard, & Amedee, 1996). Adequate adhesion yields proper cell functions. Figs. 14 and 15 illustrated the L929 morphology on the surfaces of CMC-HDA and CMC-PU 30%, respectively. At 7-day incubation, a large number of L929 cells covering the surfaces of the materials were observed; they responded to the materials quite compatibly. The cells appeared flat, spreading, and proliferating. A larger number of the cells were detected when the incubation time was prolonged to 14 days. The cellular morphology observed on CMC-HDA and CMC-PU networks was slightly different in that, in the CMC-HDA sample at a prolonged incubation (14 days), some blebs were observed when the cells became crowded. Overall, both materials were compatible with the mouse fibroblasts.

4. Conclusions

CMC networks modified with PEA-containing PU were successfully prepared. PU (1–60 wt%) was incorporated into CMC in the presence of 10 wt% HDA crosslinker. Investigation of percent crosslinking and water swelling behavior of CMC-PU indicated the formation of network structure. As percent of PU in CMC increased, EWC values tended to decrease due to the hydrophobic character of PU combined with the formation of dense network structure as indicated by the increase of percent crosslinking. SEM showed microphase separation of PU with approximately 10–50 μm in diameter thoroughly dispersed in CMC surface and in the bulk. Water wettability of the surface decreased when high concentration of PU incorporated (30–50 wt%). According to tensile strength and elongation measurements, CMC-PU showed a slight enhancement in toughness properties. Most importantly, cytotoxicity and biocompatibility tests indicated that CMC modified with 30 wt% PU was non-toxic and could be safely used as a biomaterial.

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