Physical Properties of Water-Borne Polyurethane blended with Chitosan

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ABSTRACT: Water-borne polyurethanes based on 4,4-diphenylmethane diisocyanate, poly(butylene adipate), and chain extender *N*-methyldiethanolamine (MDEA) that provided tertiary amine groups were synthesized. The polyurethane–chitosan (PU/CS) blends can be dissolved in the acetic acid and cast into films. The mechanical properties including tensile strength and elongation, as well as the water absorption and thermal properties of the PU/CS films were evaluated. The tensile strength increased with the increased amount of chitosan, but the elongation decreased accordingly. The chitosan in the blends promoted the water absorption. Chitosan was more thermally-stable than PU, as shown in the thermal gravity analysis. Chitosan also had higher crystallinity, as demonstrated by differential scanning calorimetry. The blends were partial compatible mixtures, based on the data obtained from a dynamic mechanical analysis. Biocompatibility test was conducted utilizing immortalized rat chondrocytes (IRC). After IRC were seeded onto the PU/CS films for 1.5 and 120 h, the number of cells was counted and the morphology of cells was observed by light microscopy and scanning electron microscopy. Blends containing 30% chitosan had more cells attached initially. However, the blends containing more than 70% chitosan appeared to promote the cell proliferation. IRC were round on PU/CS films with more PU, but spread when the chitosan content in blends was higher. Overall, PU/CS films with more chitosan had better mechanical properties as well as biocompatibility. J Appl Polym Sci 104: 2683–2689, 2007

Key words: compatibility; biocompatibility; water-soluble polyurethane; chitosan; thermal properties

INTRODUCTION

Human cartilage can be injured by causes such as exercise or disease of arthritis. The cartilage, once damaged, never heals by itself.[1] Many literature were focused on developing biomaterials,[2–8] serving as artificial carriers for chondrocytes, to fill defects and to regenerate neotissues.

The main structure of segmented polyurethane contained di- or trifunctional isocyanate groups which can react with polyol under condensation polymerization with chain extender diols or diamines. So the properties of polyurethane can be manipulated by using different polyols, isocyanates, and chain extenders. The properties of good elasticity, tensile strength, elongation, and blood compatibility of polyurethane are extensively appreciated for using polyurethanes as biomaterials,[9–11] especially in cardiovascular applications.[12–14] After long contact with blood, the polyurethane calcified and became harden, which could cause the loss of mechanical and other properties.[12,13] Some strategies were employed to improve properties of polyurethane, including grafting functional groups of sulfonate or carboxylic acid to improve its biocompatibility,[15–17] and coating biomimic materials on the surface to reduce protein adsorption.[14] Chitosan owns antibacterial properties, and has been applied in making as artificial skin[18] and wound dressing.[19–21] It has been used as a wound healing accelerator, a health food to reduce blood cholesterol level, and an immune system stimulant.[22–24] In some investigations, chitosan was used to improve the properties of collagen, polyvinylpyrrolidone, cellulose, and viscose rayon.[15–17,25–30] Giusti studied a series of blends of synthetic polymers [e.g., poly(acrylic acid), poly(vinyl acid)], and some natural materials (e.g., collagen, hyaluronan, and gelatin) for their possible applications in dialysis membranes, artificial skin, wound dressings, heart and graft materials, and drug release carrier.[31–34] Convenient

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manufacture of a variety of medical devices with good biocompatibility was achieved with the blends of synthetic polymers and natural polymers as mentioned above. Scotchford also studied the blend of poly(vinyl alcohol) and collagen in a similar investigation, and found that the blended films can promote the growth of osteoblast cells.[35] The combined advantages of polyurethane and chitosan were examined in the current investigation by using a series of blends of the two materials. Polyurethane is generally soluble in organic solvents, while chitosan is more hydrophilic and soluble in acetic acid solution. To increase the compatibility of these two polymers, hydrophilic polyurethane was synthesized with a chain extender containing tertiary amine, which provides cationic water-borne characteristics in polyurethane chain. Blends of the polyurethane and chitosan in different weight ratios were obtained by first homogeneously mixing the two compounds in acetic acid solutions before casting into films. Then physical properties including tensile strength, elongation, water absorption, thermal properties, and biocompatibility of these blends were evaluated.

MATERIALS AND METHODS

Materials

4,4-Diphenylmethane diisocyanate (MDI) was purchased from Tokyo Chemical Industry Company (Tokyo, Japan). Poly(butylene adipate) (PBA, molecular weight = 1000) was supplied by Taigen Chemical Industry Company (Kaoshan, Republic of China). The chain extender *N*-methyldiethanolamine (MDEA) was obtained from Acros (USA). Dimethylformamide was purchased from Merck Company (Germany). Di-*n*-butylamine was obtained from TEDIA (USA). Chitosan with 86.2% deacetylated and molecular weight of 300 kDa was also purchased from Tokyo Chemical Industry Company. The fetal bovine serum, trypsin, Dulbecco's Modified Eagle's Medium, streptomycin/penicillin, and trypan blue were all purchased from Life Technologies (Grand Island, NY).

Methods

Polymerization of water-soluble polyurethane

The water-soluble polyurethane was polymerized by a two-stage synthetic method. At first, the prepolymer was synthesized in bulk reaction. MDI and PBA were added into a four-neat bottle under stirring speed 200 rpm, 70°C, and purged with nitrogen gas. The molar ratio of MDI to PBA was 2 to 1. After 1.5 h, dimethyl formamide (50% wt of the prepolymer) was added followed by the chain extender MDEA. The molar ratio of MDI in prepolymer to MDEA was 2 to 1. The reaction took 2 h under 200 rpm, 75°C, and purged with nitrogen.

Preparation of the blends[36,37]

The PU films were cut to small pieces and dissolved in 17% acetic acid to obtain 10% PU solution. 1% chitosan (CS) solution was prepared in 2% acetic acid. The PU/CS blends of different weight ratios were prepared by mixing two solutions. The mixtures were cast into films in the molds at room temperature. The PU/CS films were placed in a oven under 95°C for 3 days to remove the acetic acid.

Water absorption

The samples were cut from films with a diameter of 15 mm, and dried under 60°C for 6 h. After weighting the dry mass (W_1), the samples were immersed into water and the wet mass (W_2) at room temperature was measured for 24 h until the weight of the sample became constant then the sample was dried again and weighted as W_3. The water absorption is defined by $(W_2 - W_3)/W_3 \times 100\%$, the sol fraction is defined by $(W_1 - W_2)/W_1 \times 100\%$.

Water contact angle

Water contact angles were measured on smooth films of 1 mm thickness. The films were placed in a vacuum oven at 60°C overnight, to remove any volatile impurities, and rinse with methanol immediately prior to contact angle measurement. The contact angles were measured by contact-angle analyzer (Olympus Model SZ-ST) using the sessile drop method with water as the test fluid. A minimum of 10 angles were measured for each blends.

Mechanical properties

Films were cut into $45 \times 6 \times 1$ mm^3 sample (ASTM-D412). The tensile strength and elongation were determined with a extension rate of 10 mm/min (sample number = 5) by the universal tensile testing instrument (RTM-1T, Yashima Works). Samples were tested either in dry state or in wet state at (25 ± 2)°C. Prior to testing in wet condition, all samples were neutralized in 1*N* NaOH for 15 min and rinsed thoroughly under tap water and immersed in phosphate buffer saline (PBS, pH = 7.4) for 30 min.[38,39]

Thermal properties

The thermal properties were investigated using a thermal gravity analyzer (Perkin–Elmer TGA-7,

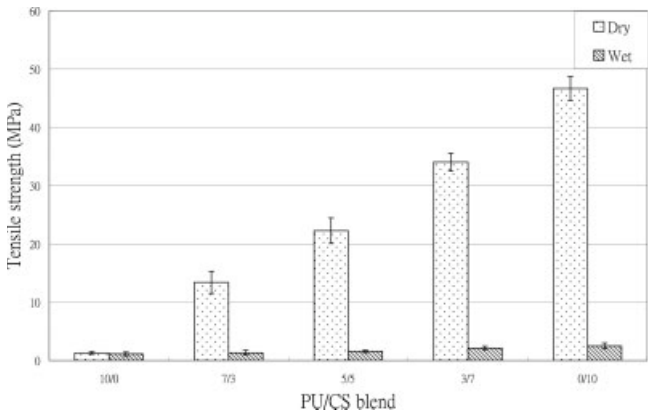

Figure 1 The tensile strength for the polyurethane and chitosan blends.

dynamic nitrogen atmosphere, 10°C/min heating rate), a differential scanning calorimeter (TA Instruments DSC 2010, dynamic nitrogen atmosphere, 10°C/min heating rate from −120°C to 280°C) and a dynamic mechanical analyzer (TA Instruments DMA-7, heating rate at 5°C/min).

Biocompatibility test

Samples were clipped from PU/CS films and placed into the bottom of the 24-well cell culture plate (Costar®). Culture glass surface was used as control. Five hundred microliters of culture medium was added into each well. The immortalized rat chondrocytes (IRC) with a concentration of 3.52×10^5/mL or 6×10^5/mL were prepared and seeded to each well to an amount of 1.27×10^5 IRC for cell proliferation. The cell attachment was quantified in about 1.5 h after deposition of IRC. The proliferated cells were counted again after 120 h. The adherent cells were trypsinized, centrifuged, and resuspended cell counting with a hemacytometer in combination with an inverted phase contrast microscopy (Nikon TE300). The morphology of cells was observed by a scanning electronic microscopy (SEM, Hitachi S-800). The statistic analysis was performed with the SAS computer program.

RESULTS

Physical properties

Mechanical properties

The membranes were first tested in the dry state. The chitosan membrane was very brittle and the PU membrane was elastic. For pure chitosan, the tensile strength was 46.7 MPa and the elongation was 19.6%. Chitosan has higher tensile strength and lower elongation than those of PU. When the content of chitosan decreased to 0%, the tensile strength decreased to 1.3 MPa and the elongation increased to 138.9%. The tensile strength increased linearly with the increase in amount of chitosan (Fig. 1). However, the elongation decreased relatively sharply when the amount of chitosan increased to a amount over 30% (Fig. 2). Next, the membranes were tested in the wet state. These results showed a significant difference in the mechanical properties relative to the dry state. The chitosan membrane was swelling and elastic. The break stresses of the membranes with chitosan were lower by an order-of-magnitude from the dry samples. However, samples had a longer elongation (55–153%) at break. The tensile strength of the blends was about 2 MPa. The elongation increased linearly with the in amount of PU.

Water absorption

The water absorption of pure chitosan was 275 wt %, which was much higher than PU (5%). Water absorption of all blends tested fell between those of PU and those of chitosan. The higher water absorption rate for the blends with higher chitosan content (Fig. 3). Chitosan appears to improve the water absorption of the blends tested.

Water contact angle

The water contact angle of pure chitosan was 98.05°. Chitosan has higher water contact angle than those of blends. The contact angles for the blends are observed to increase with increasing chitosan (Fig. 4). However, water-borne polyurethane appears to improve the water contact angle of the blends and the surface of blends show the higher hydrophilicity compared with those of water-borne polyurethanes. The hydrophilicity of PBA containing ester groups contributes to rise to a smaller contact angle.

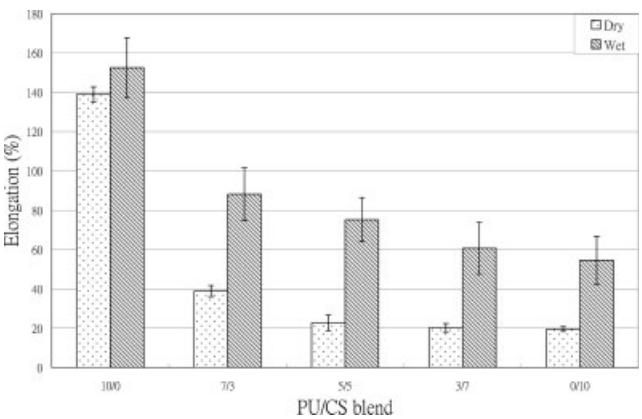

Figure 2 The elongation of the polyurethane and chitosan blends.

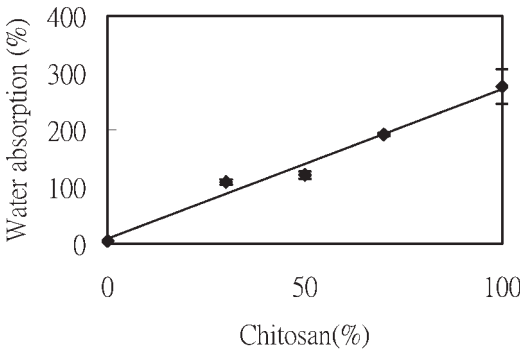

Figure 3 The water absorption for the polyurethane and chitosan blends.

Thermal analysis

PU (for PU/CS = 100/0) showed an initial thermodegradation temperature at about 220°C, chitosan had an initial thermodegradation temperature at about 300°C, chitosan (PU/CS = 0/100) had an initial thermodegradation temperature at about 200°C with negligible weight loss from about 400 to 900°C. In all blends, PU component degraded first before about 400°C, and the remaining chitosan component contributed to the near constant residual weight of the samples above 400°C. The degradation patterns of two components appeared to be independent. Chitosan appeared to be more thermal stable than the PU at high temperature higher than 350°C, as demonstrated by TGA data (Fig. 5). In DSC diagrams (Fig. 6), the area under the peak caused by the crystallinity was found to increase with the increasing amount of chitosan in blends. Chitosan had higher crystallinity, in contrast to the amorphous PU. The glass transition temperature (T_g) of polymers were usually determined from the DMA data. The glass transition peak became broader when the polymer has a wide distribution of molecular weight. In Figure 7, the T_g peak of PU was narrower than that of chitosan, because the latter only had a 86.2% of composition is chitosan with wide molecular weight distribution. The E'' peaks of the blends were the broad peaks for PU/CS blends and little T_g shifting under the blending with various PU/CS ratios, comparing the neat PU with a shape peak of a low T_g. These indicated the blends of the polyurethane–chitosan are semicompatible with heterogeneous morphology.

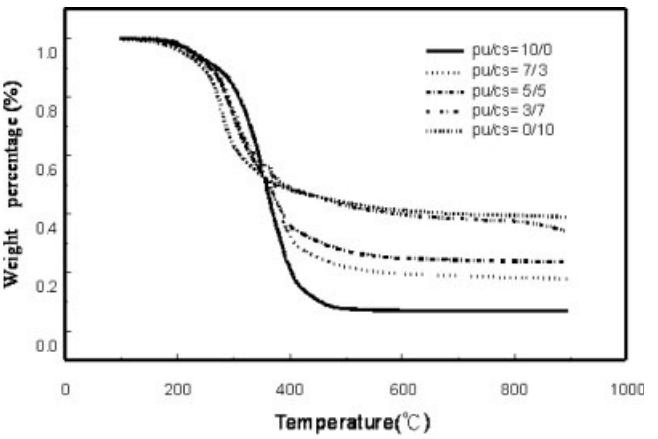

Figure 5 The thermal gravimetric analysis (TGA) for the polyurethane and chitosan blends.

Biocompatibility test

Cell attachment. The highest amount of cell attachment appeared at 1.5 h after cell were seeding onto the films [Figs. 8(a,b)]. The average number of cells attached on different materials showed the same trend for different seeding densities at 1.5 h. The statistical analysis was performed by the SAS computer program, using ANOVA analysis and Duncan's multiple range test. Pure PU (PU/CS = 10/0) showed

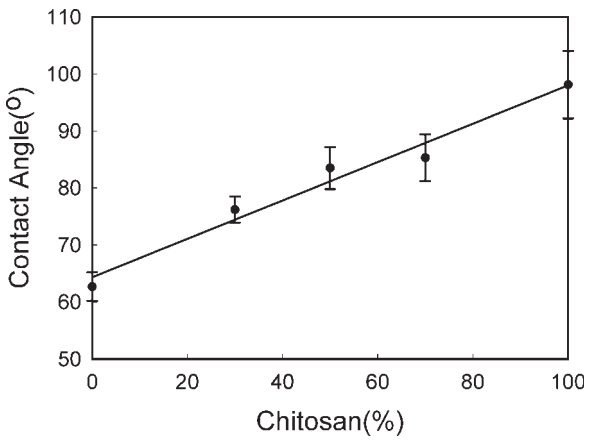

Figure 4 The contact angle for the polyurethane and chitosan blends.

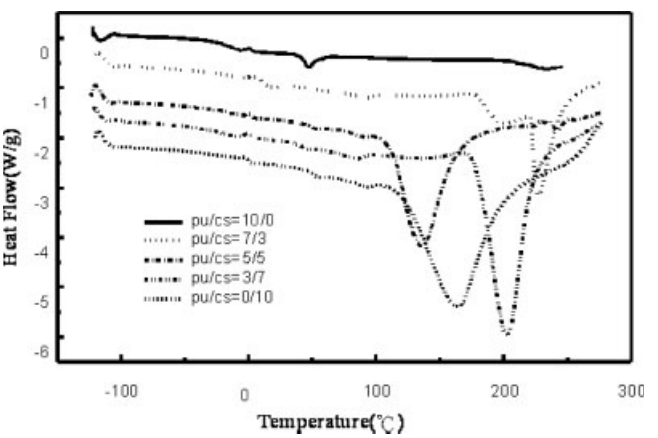

Figure 6 The differential scanning calorimetry (DSC) for the polyurethane and chitosan blends.

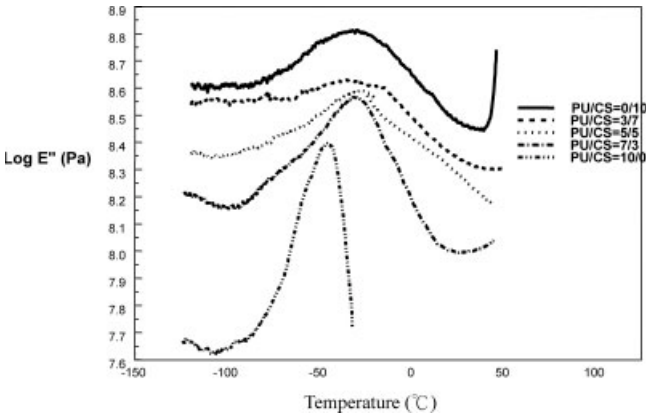

Figure 7 The compatibility of polyurethane and chitosan blends determined by dynamic mechanical analyzer (DMA).

the lowest amount of cells. PU/CS = 7/3 showed the highest amount of cells. There was no significant difference among PU/CS = 0/10, PU/CS = 3/7. and PU/CS = 5/5. Species of materials affected cell attachment quite significantly ($P < 0.0001$). The percentage of attachment was significantly higher for cells seeded at a lower (3.52×10^5 cells) rather than a higher (6×10^5 cells) density ($P = 0.0004$). There was no interaction of sympathy between materials and cell number seeded ($P = 0.2867$).

Cell proliferation. If the materials provide a suitable environment, the cells will start proliferating after attachment. As shown in Figure 9, the cell number on PU/CS = 10/0 only slightly increased after 120 h. The cell numbers on PU/CS = 7/3 and PU/CS = 5/5 were both significantly less than those initially seeded number. Cells on PU/CS = 10/0 and PU/CS = 3/7 did proliferate and increased to about twice of the initial number. By using ANOVA analysis and Duncan's multiple range test, there were more cells on PU/CS = 10/0 and PU/CS = 3/7 surfaces than on PU/CS = 0/10, PU/CS = 7/3 or PU/CS = 5/5 surfaces.

Cell morphology. In this investigation, the PU films adhered tightly, and became fragmental when picked off from culture wells. The morphology of IRC on five other films after 72 h was examined by scanning electron microscopy (Fig. 10). Normal attachment and growth of IRC was observed on the control group of glass [Fig. 10(a)], where IRC spread like fibroblasts.

Interesting morphology was found on PU/CS = 7/3. There were material extrusions shown as broken round beads on these films [Fig. 10(b)], which could be a result of high water absorption typical of the samples. PU component formed continued phase with only 5% water absorption, while chitosan component was enchased among the PU polymer chains. When chitosan absorbed abundant water from the

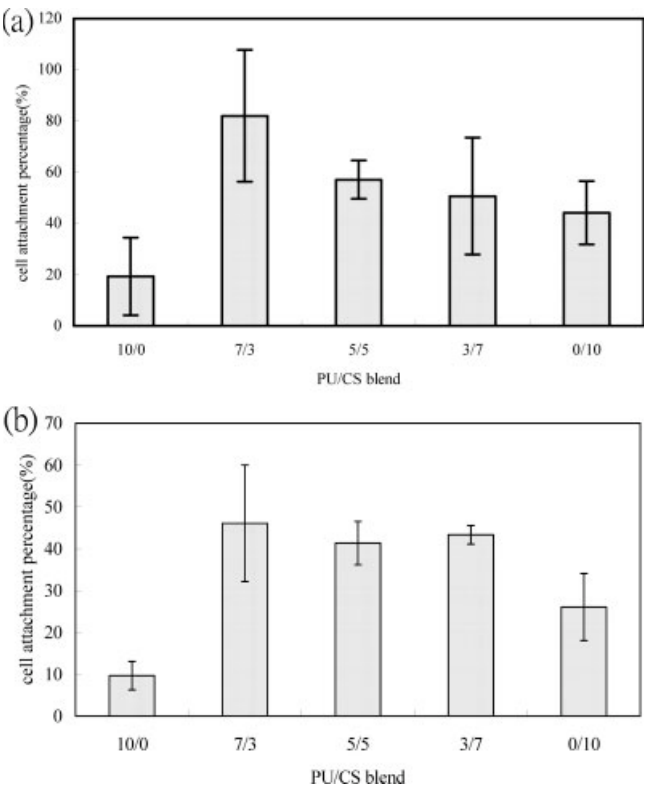

Figure 8 The cell attachment for polyurethane/chitosan blends after IRC seeded for 1.5 h. The initial cell number was 3.52×10^5 (a) and 6×10^5 (b) cells.

culture medium, it could be extruded from films as round, broken beads. On the other hand, nonspread and round IRC were found on films of PU/CS = 7/3 [Fig. 10(c)]. These cells were probably dead, probably become they could not adhere on the material surface in time. Round IRC and beads were also found on the films of PU/CS = 5/5 [Fig. 10(d)]. Spread IRC were shown on the films of PU/CS

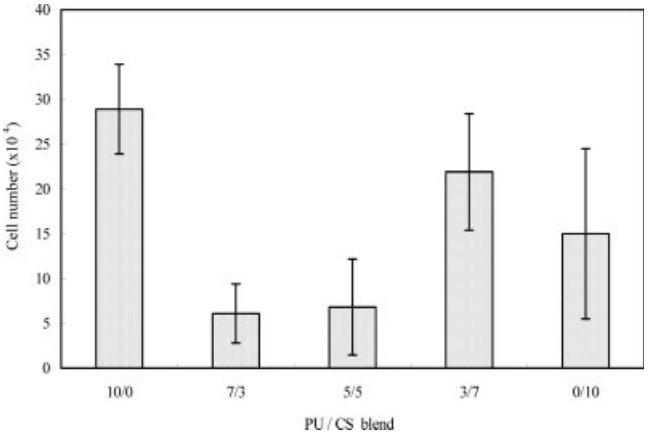

Figure 9 The cell proliferation for polyurethane and chitosan blends, 120 h after seeding. The initial cell density was 1.27×10^5 cells/mL.

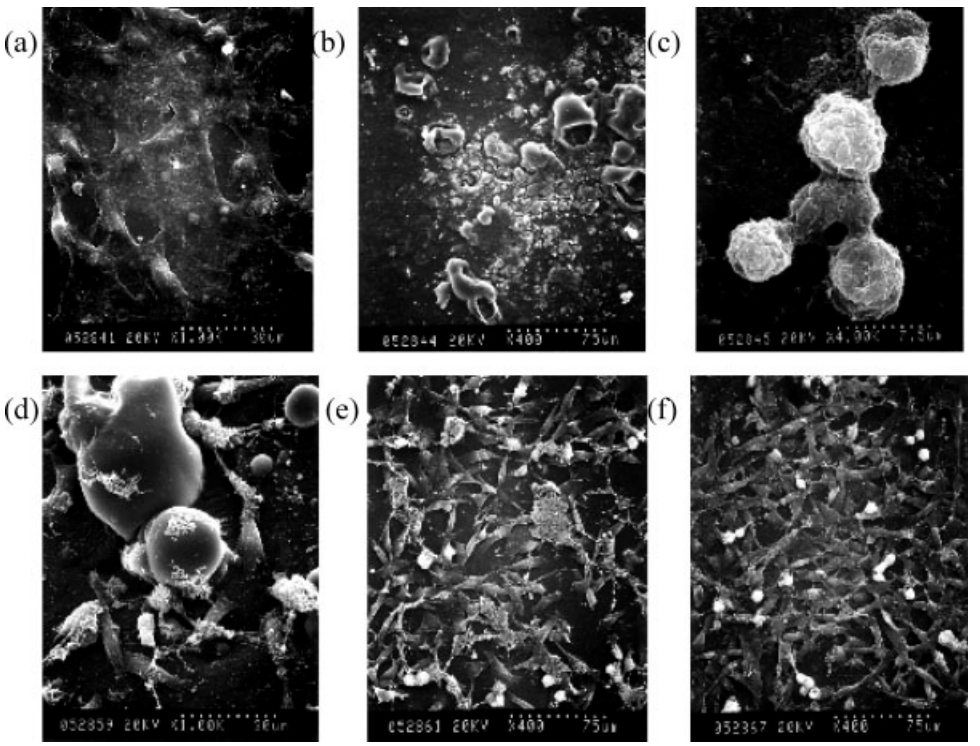

Figure 10 The SEM cell morphology for polyurethane and chitosan blends, 72 h after seeding: (a) Culture glass (×1000); (b) PU/CS = 7/3 (×400); (c) PU/CS = 7/3 (×4000); (d) PU/CS = 5/5 (×1000); (e) PU/CS = 3/7 (×400); (f) PU/CS = 0/10 (×400).

= 3/7 [Fig. 10(e)] and PU/CS = 0/10 [Fig. 10(f)], where cells grew to confluency.

DISCUSSION

Meyer et al. investigated the attachment kinetics of osteoblasts on different biomaterials and found that materials of higher wettability had increased the cell attachment, while the media for osteoblast attachment onto materials were ions.[40]

Eugene on the other hand indicated that the superfluity of water reduced cell attachment, because the anchoring sites for cells became less.[41] Maroudas indicated that for cell attachment and growth on the surface of materials, a hard footing in molecular order is required so that the cells would anchor to the surface.[42] These might explain initial addition of chitosan in PU increased wettability and cell attachment; whereas further addition of chitosan decreased cell attachment.

Meyer et al. also indicated that the materials with more cells attached may not be conducive to cell growth.[40] In our investigation, the highest number of attached cells was found on the film of PU/CS = 7/3 and the confluent cell growth appeared on the films of PU/CS = 0/10.

Downe et al. observed the growth of chondrocytes on polymer and proposed that the materials have to absorb fluid and proteins from tissue, including cytokines and growth factors, to obtain bio-activity similar to normal tissue or cellular environment to promote the growth of chondrocytes.[43] Leonard also presumed that the interactions between cells and materials depended on chemical affinity of polymer surface.[44] The above literatures showed high particularity of different material surfaces for components absorbed from medium. The water absorption of chitosan could promote the adsorption of proteins that were either secreted from cells or existed in the medium, onto the material surface. This may also contribute to the higher amount of cells on the films of PU/CS = 0/10 and 3/7 after 120 h.

The amount of cells on the films of PU/CS = 7/3 and 5/5 were the lowest. Based on the sample morphology, chitosan was extruded from the surface as round beads probably due to distinct wettability between PU and chitosan. Since cells tend to adhere on flat surface, no cells were found on these two scabrous surfaces in the study.

Among all blends, good biocompatibility was founded on the films of PU/CS = 3/7 and 0/10. On these films, cells grew to confluency and spread in fibroblast-like morphology. In addition to biocompatibility, they also showed good mechanical strength. However, it was noted that wet chitosan films (PU/CS = 0/10) lost their mechanical strength and integrity, probably due to high water absorptivity. From

such aspect, the blend PU/CS = 3/7 would be the best material among PU/CS blends for further exploration of its application in cartilage repair.

CONCLUSIONS

In this study, chitosan was blended successfully with water-borne polyurethane using the acetic acid solution. The thermal stability of the blends was attributed to their chitosan components. The DMA results indicated the blends of the polyurethane-chitosan are semicompatible with heterogeneous morphology. The blends exhibited superior elongation as the water-borne polyurethane was added. Both of the better SEM and the cell proliferation results indicated the samples of PU/CS = 3/7 and 0/10 had the better biocompatibility. The blend of PU/CS = 3/7 is the best candidate for further exploration due to its good biocompatibility and reasonable mechanical strength.

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