Preparation and characterization of pH sensitive sugar mediated (polyethylene glycol/chitosan) membrane

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Novel biodegradable membrane based on chitosan matrix was prepared and characterized by Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA) and swelling test. Native sugar, which is commonly used in human life, was utilized to prepare the crosslinked hydrophilic chitosan-polyethylene glycol (PEG) polyblend. According to TGA and FTIR results, the chemical reaction occurred in imine bonds (C=N) between sugar and amino groups in chitosan. Chitosan blending with high swelling capacity of PEG increased the water affinity and reacted with sugar decreased the water affinity. The equilibrium water content (EWC) value of the sugar mediated membrane is in the sequence of sucrose > D-fructose > glucose and they all present much lower water uptake ability than polyblend. The chitosan was not degraded by lysozyme, but all of the sugar-mediated membranes were susceptible to lysozyme. Scanning electron microscope (SEM) morphology shows that the degradation rate not only was controlled by the chemical complexation between sugar and polyblends, but the surface morphology of membranes also has great influence. Sucrose-mediated membrane supports the attachment and growth of NIH 3T3 fibroblasts. The pH-sensitive and well degradable property of the sucrose-mediated membrane can be applied for biomedical application.

1. Introduction

Chitosan, a natural biodegradable polymer, is a low acetyl substituted forms of chitin named (1 → 4)-2-amino-2-deoxy-(D-glucose), has been demonstrated to be biodegradable, homeostatic active, non-toxic, non-antigenic, and biocompatible [1]. In the past few years, chitosan and some of its modification types have been reported for use in biomedical applications, such as artificial skin and suture, drug carrier, and dietary fibers. Recently, the use of chitosan and its derivatives as temporary scaffolds to activate the promotion of mineralization or stimuli endochondral ossification has received much attention [2–4].

Although chitosan and its derivatives seem to have very excellent properties as biomaterial, but the quite low solubility due to highly crystallized and rigid structure presents problems to its application. To obtain good solubility in body fluid, chemical modification of chitosan and incorporated hydrophilic polymer is frequently used. Poly (ethylene glycols)(PEGs) is water-soluble amphipathic polymer used in pharmacy as galenic excipient for suppositories, ointments, tablets and capsules [5]. On the other hand, PEG can avoid the strong interaction between the constituents since PEG is an uncharged polymer [6]. The PEG molecular length and doped weight have a great influence on the hydrophilic of polyblend. Amiji [7] in studying the chitosan-PEG blend membrane found that with increasing PEG molecular weight to 10 000, the maximum equilibrium water uptake value was obtained. From this point of view, choosing PEG molecular length in appropriate range to blend with chitosan can not only improve the water affinity but also accelerate the degradation rate of polyblend.

However, chitosan is easily dissolved in weak acid, crosslinking of chitosan to form a network is necessary. Conventional chitosan crosslinkers including bifunctional reagents, such as formaldehyde, glutaraldehyde or epichlorohydrin, cycloheptamylose are chemically synthesized [8–10]. Chitosan crosslinked by glutaraldehyde swelled under acidic conditions but remained in shrunken state under neutral conditions. By utilizing this property, chitosan films or other dosage form have been exploited widely for oral sustained drug delivery in the stomach [11]. However, the chemical crosslinker may lead to toxic effect in physicalogical environment, due to the presence of residual crosslinker [12]. From this point of view, the search for naturally occurring crosslinker to

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obtain partially soluble devices is preferable. Reaction of amino group of chitosan with carbonyl compounds leading to the formation of Schiff bases (e.g. glutaraldehyde and glyoxal) has been reported [13]. Low molecular weight sugars such as native mono- (i.e. glucose, fructose) and disaccharides (i.e. sucrose), which are commonly used in human life and could be biocompatible crosslinkers. It has been reported that the aldehyde or keto group of native sugar can react with the free amino groups of gelatin molecule resulting in the crosslinking structure [14]. Otherwise, conformational ordering and intermolecular relationship of oxidized starch also can be stabilized by introduction of sugars as cosolute [15]. The possibility of using sugar to prepare chitosan-PEG membrane was taken evaluated.

The purpose of this study is to prepare a membrane with hydrophilic, degradable and reinforced polymer-based structure by introducing PEG polymer to improve the water affinity, and their structure in biological environment was stabilized by three native sugars. The influence of different sugars on the solubility was investigated by enzymatic degradation experiment.

2. Materials and methods

2.1. Materials

Chitosan (ch) (degree of deacetylation was 85% and $M_\gamma \sim 1.5 \times 10^5$) was obtained in the flake form from Fluka, PEG $M_n = 6000$ was purchased from Ridel-dehaen. Sucrose (crystal), dextrose anhydrous (glucose) and D-fuctose were supplied by J. T. Baker Inc. All other reagents were extra pure grade and used as received.

2.2. Preparation of polyblend membrane

Chitosan (3 g) was dissolved in 100 ml of 2% aqueous acetic acid. The mixture was stirred for 24 h to obtain a perfectly transparent solution. Chitosan-PEG blend was prepared by mechanical stirring the filtered chitosan and PEG flakes in a percentage of 70 : 30 at room temperature. The appropriate amount of sugar (0, 3, 5, 10, 20, 30 wt %) was then added, with continued stirring until the solution was clear again at 120 °C. After drying for 24 h in ambient temperature, the reaction mixture solution was poured into glass petri dishes and was spread on a membrane. After curing for 2 days at 68 °C, membrane was neutralized with 10%(w/v) sodium hydroxide followed by rinsing with deionized distilled water and dried at 30 °C for 60 min. The thickness of the polyblend membrane varied from 50 to 60 μm.

2.3. TGA and FTIR analyses for sugar-mediated membrane

The thermal properties of sugar-mediated membranes were analyzed by using TGA, a Hi-Res TGA 2950 TA Instruments. Samples (5–10 mg) were heated at 10 °C/min from 25 °C to 400 °C in a flowing nitrogen atmosphere.

FTIR spectra of sugar mediated membrane was measured by KBr disk method using a Jacob FTIR 8100 spectrometer in transmission mode. The sample chamber was purged with dry nitrogen gas.

2.4. Measurement of swelling properties

The water uptake contents for membranes were determined as follows. Pre-dried samples were soaked in pH1, pH7 and pH10 buffer solution, respectively, and kept 37 °C for 2 days. After a given time interval, the excess surface solution was blotted out with filter paper. The weight of swollen sample was then placed in a vacuum oven at 30 °C for 24 h. The swelling procedure was repeated until there was no further weight increase. Equilibrium water content was calculated by the following equation:

$$\text{Equilibrium water content) (\%)} = [(\text{swollen sample weight} - \text{dry sample weight})/(\text{dry sample weight})] \times 100\%$$

2.5. Enzymatic degradation of sugar mediated membrane

Three times recrystallized chicken egg-white lysozyme were obtained from Sigma Chemical Co. (USA) and used without further purification. The membranes were cut into small pieces (0.5 cm × 0.5 cm) and immersed in 4 mg ml^{-1} lysozyme solution in 0.01 M PBS at pH 7.4 and 37 °C. After incubation for determined intervals of time, the membrane was rinsed with ethanol and dried at 60 °C. Weight reduction compared to the initial weight of membrane was recorded. For investigating the molecular weights of the material liberated from membranes, an aliquot of the reaction mixture were assayed by Gel permeation chromatography (GPC) (a Water liquid chromatograph (Waters Associates, Milford, MA, USA) with pullulan standard (column, Waters ultrahydrogel linear, 30 cm × 2; eluent, 0.15 M CH_3COOH and 0.5 M CH_3COONa; flow rate, 0.8 ml/min; temperature, 30 °C)). For investigating morphology of membranes for different time intervals, the degraded and partially degraded samples were observed by using JEOL 5120 scanning electron microscope (SEM) conducted at 25 kV on platinum-coated surface of the membrane under an argon atmosphere.

2.6. Cytocompatibility test

NIH 3T3 fibroblasts were cultured in Dulbecco's modified Eagle's Medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, USA) and 1% antibiotic–antimycotic (Gibco, USA). The cells were subcultured for about 3 day interval with trypsin-Ethylenediaminetetraacetic acid (EDTA) and maintained at 37 °C in a water-jacketed incubator with a humidified 5% CO_2 atmosphere.

To evaluate the direct cytotoxicity of the materials toward fibroblasts, the prepared membrane was deposited on cell cultures. All of the membranes with $4 \times 4\ mm^2$ in area, sterilized in 75% alcohol overnight and rinsed with Phosphate buffer solution (PBS) solution were placed in a six-welled tissue culture polystyrene plates (Costar, USA). After aspiration of PBS, 2 cc medium of cell suspension at a density of 1×10^5 cells/ml, DMEM was placed on each well and maintained in an incubator with 5% CO_2 at 37 °C. For

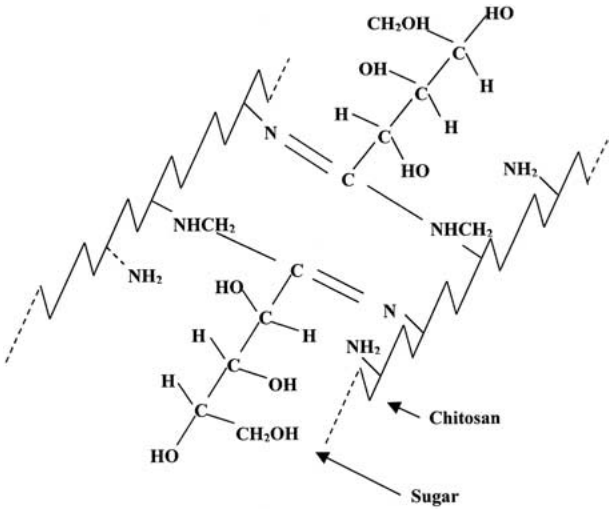

Figure 1 Hypothetical structure of final product for sugar-mediated chitosan.

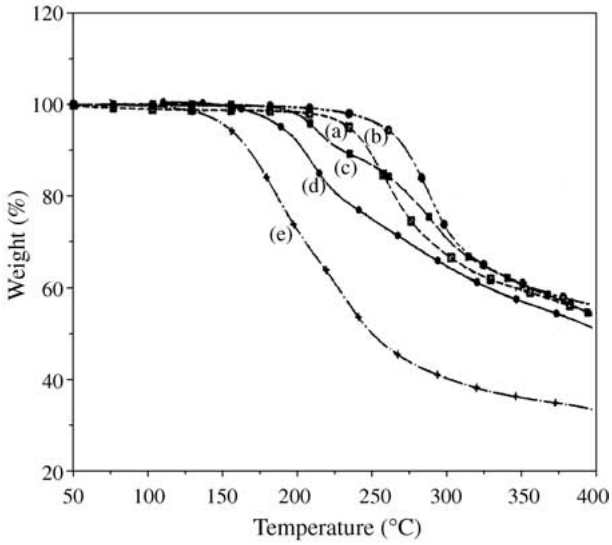

Figure 2 TGA thermograms of chitosan and other membranes: (a)□, chitosan; (b)○, ch/6k; (c)■, ch/6k/su10; (d)●, ch/6k/glu10; (e), ch/6k/d–f10.

morphological observation, the cells adhering to the membrane were washed with phosphate buffered saline (PBS) after 2 days of incubation, and then fixed with 4% formaldehyde in PBS for 1 h. After thorough washing with PBS, the cells were dehydrated by graded ethanol process. The membranes were then observed by object microscopic (OM) observation. It was compared to a blank constituted of the same culture in the same 2 cm diameter Petri dishes (polystyrene) without biomaterial deposit.

2.7. Cell proliferation

After cell culturing for 1, 2 and 4 days, the prepared membranes were washed twice with a sterile PBS solution to eliminate dead cells. Then, a 3-(4,5-dimethylthiazol-2-yl)-2, 5-dip henyl tetrazolium bromide (MTT) test, according to Mossman [16] and Hansen *et al.* [17] was carried out to quantity the viability of the fibroblasts. It was compared to the plastic (polystyrene) Petri dish used as a reference. All experiments were repeated four times, and results are expressed as mean $\pm$ standard deviation of mean.

3. Results and discussion

3.1. Thermo gravimetric analysis

The cross-linking reaction of the polyblend with different sugar types was carried out at 100 °C. The aldehyde or keto group of reducing sugars can react with the amino groups of the chitosan leading to form Schiff base complex which then transformed to aminoglycoside complex by a tautomerization process and further reacted with another amino group of chitosan again [18]. The polyblends, consisting of colored and plastid, whose inner structure is schematically depicted in Fig. 1, were extensively washed with sonicator and distilled water.

Fig. 2 shows the TGA curves of chitosan and all the polyblends added with 10 wt % sugar. The chitosan degrade stage started at 235 °C and reached a maximum at 260 °C with weight loss of 17%. Table I represents the TG and DTG data for chitosan and Schiff base polyblends. Except the chitosan-PEG blend, the TG thermograms indicate that the sugar-crosslinked samples had low overall degradation temperatures (T_{max} = 211–243 °C) in comparison with that of polyblend and chitosan. It means that all the sugar-crosslinked polyblends were less stable than chitosan. This is similar to the results suggested by Tirkistani [19]. It seems that the formation of C = N in the crosslinked structure was more unstable than chitosan which has the amino group. The DTG data shown in Table I indicate that the crosslinked sample had a degradation stage except the glucose-crosslinked one. The peak split in glucose-crosslinked may come from the uncrosslinked sugar residue [20]. Because of the stable chair type conformation of reduced sugar can dissolve over 1% of

TABLE I Data evaluated from TG and DTG thermograms of chitosan and sugar mediated polyblends

Polyblends	Tst[a] (°C)	Tmax1 (°C)	Tmax2 (°C)	Wt Loss (%)[b]	Remaining after 400 °C
Chitosan	235	260	—	17	52
Ch/6k[c]	258	289	—	21	58
Ch/6k/su10	223	243	—	18	54
Ch/6k/glu10	148	187	234	23	37
Ch/6k/d–f10	187	211	—	15	52

[a]Determined at 5% weight loss.
[b]Analyzed by Universal V 2.5 TH instrument software.
[c]Chitosan, PEG6K, sucrose, glucose and d–f fructose represented as ch, 6k, su, glu and d–f in short. The 10 represents sugar addition weight percentage.

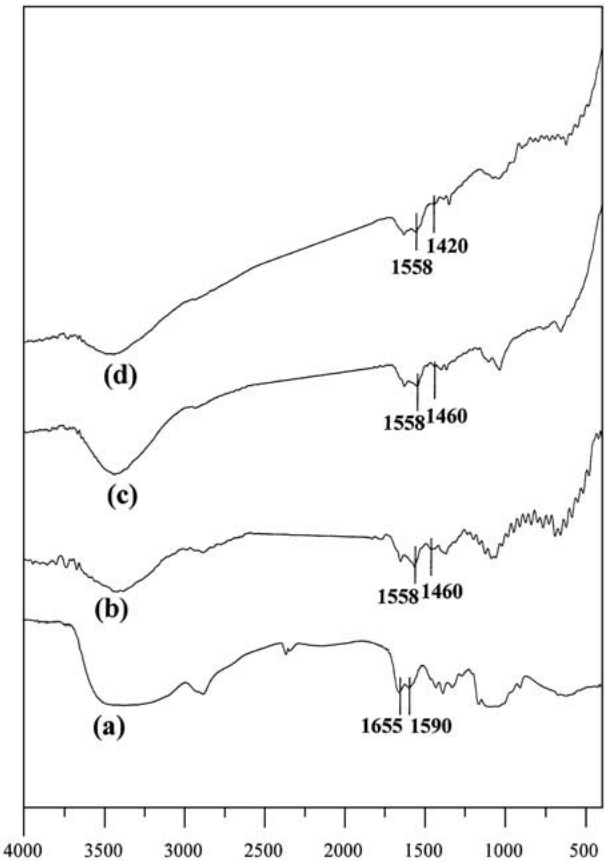

Figure 3 FTIR spectra of (a) chitosan and (b) sucrose, (c) glucose, (d) fructose treated chitosan/PEG polyblend.

molecular form in an open ring structure as reaction temperature increase [21] and supply more aldehyde groups for chitosan to react, then the caramelized glucose will be left in the membrane.

3.2. FTIR analysis

Fig. 3 shows the FT-IR spectra of sugar-crosslinked polyblends and chitosan. The spectrum of the chitosan film exhibits an absorption around 1655 and 1590 cm^{-1}, which represent the amide I and amide II bands, respectively. There are bands at 1420, 1380 and 1320 cm^{-1}, in addition to the usual C–H aliphatic band at 2880 cm^{-1}. The OH and NH_2 overlapping bands are found around 3310–3450 cm^{-1}. The chitosan films with sucrose exhibit a broad deformation peak at approximately 1460 cm^{-1}. Both of the glucose and D-fructose mediated polyblends also show a peak around 1460 cm^{-1} and slight peaks around 1538, 1634 and 1645 cm^{-1}. It is proposed that the mechanism of crosslinking between sugar and amino groups in chitosan follows a Schiff base reaction resulting in imine bonds (C=N) vibrations. Lee and co-workers [22] reported that PEGM/chitosan crosslinked with glutaraldehyde, the C=N peak was strong at 1563 cm^{-1} and Knaul and co-workers [13] indicated that some actual type bondings occurred in Schiff base reaction and the morphology of the film may cause a large shift in C=N peak range. From this point of view, it is reasonable to believe that the crosslinking reaction was occurred between polyblends and sugar.

3.3. Swelling behavior

By immersing the chitosan membrane in PBS buffer solution, an equilibrium swelling was reached after soaking for a period of time. Fig. 4 shows the equilibrium water content of sugar-mediated membranes as a function of the sugar feed. All of the membranes have the same trends: as the sugar content increase from 3 to 10 wt %, the water content decrease. However, as the sugar content over 10 wt %, the water contents increase. Arvanitoyannis and co-workers [23] reported that the sucrose was as a plasticizer to promote the chain flexibility in chitosan/PVA blends. In the sugar-mediated system, the physical property of membranes with sugar feeding weight higher than 10 wt % was more flexible. It means that the reaction between the amino groups and aldehyde group have been reach the maximum value when sugar feed in weight was 10 wt %, and the unreacted sugar, which was entrapped in the membrane, would have an influence on water content. Also Tomihata *et al*. [24] indicated that hydration of chitin derivatives depends on not only degree of deacetylation but others factors such as the OH group in branch of crosslinker.

Fig. 5 shows the equilibrium water content of chitosan, polyblend and 10 wt % sugar mediated membrane at different pH environment. These samples exhibit a rapid increase in water content and reached equilibrium within 30 min after soaking 2 days. For chitosan and chitosan/PEG membranes, they easily dissolve in acidic environment and shrinkage in basic environment. The blend system without sugar addition increased the hydrophilic ability of chitosan from 46 to 60% water content in neural environment, which agreed with the results of Amiji and co-workers [7]. The factors determining the water absorption were the hydrogen bonding and crystallinity. For chitosan structure, the loss of some interchain hydrogen bonds in their crystal structure or the amino groups induced in can increase the water solubility, but the intromolecular hydrogen bonds across each β (1 → 4)-glycosidic linkage result in a low-energy, rigid and linear polymer backbone. The high

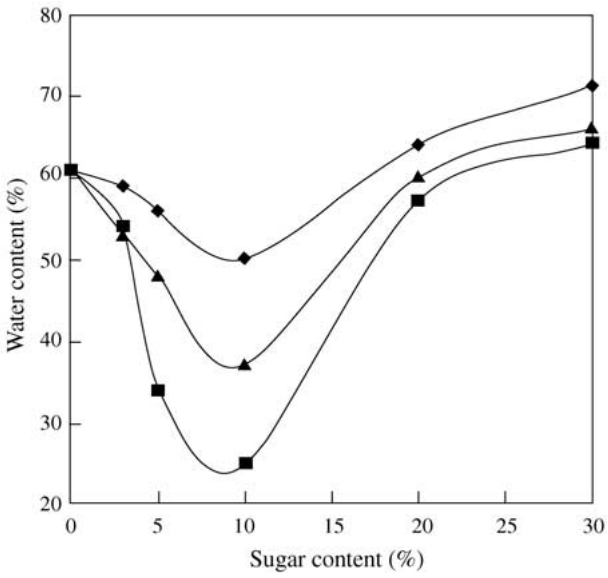

Figure 4 Water content of sugar-mediated membrane as a function of sugar feed in weight for: ◆, ch/6k/su;, ■ ch/6k/glu; ▲, ch/6k/d–f.

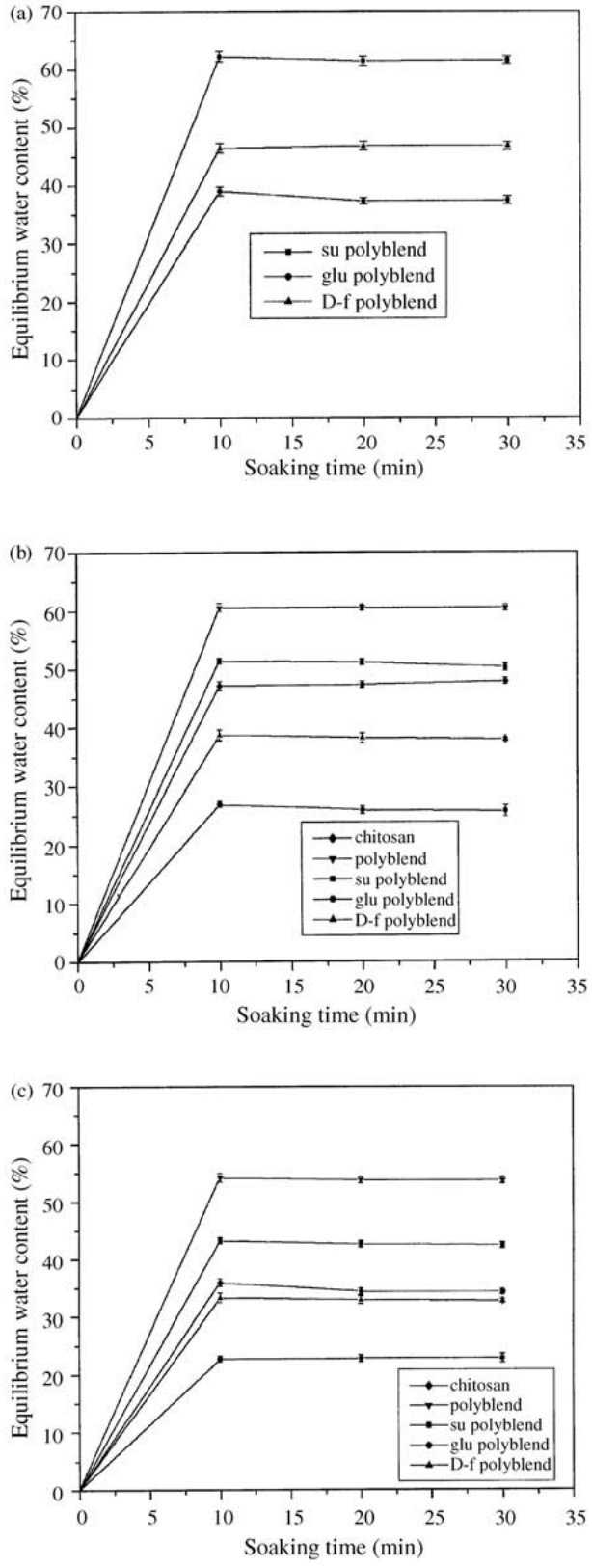

Figure 5 The equilibrium water content of chitosan, polyblend and 10 wt % sugar mediated membrane at different pH environment for 2 days (a) in pH 1, (b) in pH 7 and (c) in pH 10.

equilibrium water content observed in chitosan-PEG membranes was probably owing to the intermolecular hydrogen bonding between chitosan and PEG chains or the amorphous region in polyether induced small molecular water to penetrate in the blend. In comparison with high equilibrium water content of chitosan-PEG blend, the sugar-mediated membranes all present much lower water content in the range of 25–61% at different pH environment. It is noted that for the sugar mediated membrane, the water content increases as the pH value decreases. It has been demonstrated that the intermolecular hydrogen bonds were dissociated inducing the polymer network loosen and leading to the water content increased in acid environment. However, the intermolecular hydrogen bonds were reassociated, and causing the water content decreased in alkali environment [25]. Otherwise, the water content of the sugar mediated membrane is in the sequence of sucrose > D-fructose > glucose in this system. It inferred that the Schiff base products were easier formed between reduced sugar and NH_2 groups than unreduced ones.

3.4. Enzymatic degradation

The lysozyme reaction on chitosan, synthetic membrane was examined in membrane system. Fig. 6 shows the weight loss compared with the initial weight of the membrane. In chitosan membranes, they still maintained its physical form without obvious weight loss after 48 h soaking, but chitosan-PEG polyblend degraded 8% after 2 h and reached 25% weight loss for 48 h incubation. The lysozyme seems cannot catalyse hydrolysis of chitosan, which is free acetamide groups [26]. From this point of view, the chitosan-PEG degradation may owe to the hydrolysis of PEG segment or physical structure of the membrane. For sugar-mediated membrane, they show slow degrading within 8 h, but the degradation rate was increased after 8 h soaking. The degradation process was dependent not only on the lysozyme susceptibility of membrane but also on its solubility to lysozyme solution [27]. It is believed that for the first 8 h, the chemical reaction in membrane was not susceptible to lysozyme, but the cleavage of glycosidic linkages in a pentamer of β-(1 → 4)-linked N-acetyl glucosamine occurred after 8 h.

The degradation rate for the samples added with different types of sugar was in the sequence of

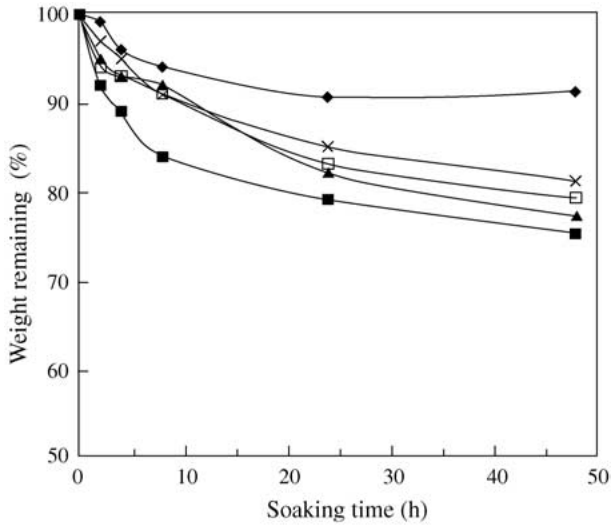

Figure 6 In vitro degradation of membranes of chitosan, polyblend, sugar 10 wt % mediated membrane in 4 mg ml^{-1} lysozyme solution of pH 7.4 at 37 °C: for ◆, chitosan; ■, ch/6K; ▲, ch/6k/su10; × , ch/6k/glul0; □, ch/6k/d-f10.

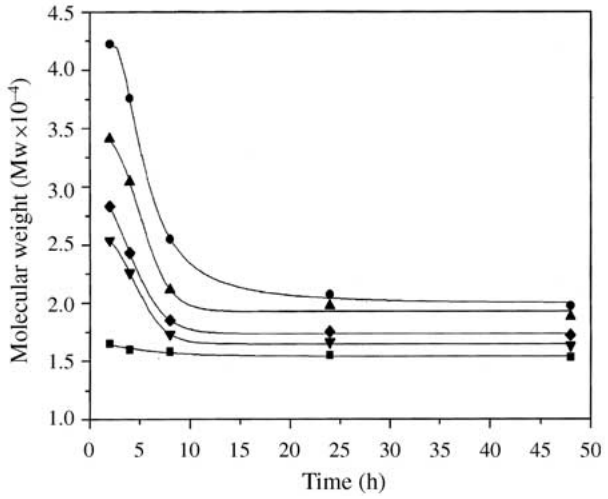

Figure 7 Molecular weight of soluble chitosan degradation product liberated from membranes by lysozyme: ■, chitosan; ●, ch/6K; ▲, ch/6k/su10; ▼, ch/6k/glu10; ◆, ch/6k/d-f10.

sucrose > D-fructose > glucose as shown in Fig. 6. It implies that the different sugar types have influence on materials performance. Sucrose, an unreduced disaccharide, was formed by glucose and fructose molecules, although can dissolve into two mono saccharide molecules but most of them still keep in closed six carbon rings at a temperature over 100 °C [21]. Therefore, the sucrose will not easily react with amino groups of chitosan and transform to Schiff base complex. For low molecular weight glucose and fructose, lots of closed chain formed molecules will be changed to open chain linear form as the reaction temperature increase. The high ratio of aldehyde and keto groups in glucose and fructose bringing about higher crosslinking degree in membrane. It also may be the reason why the flexible property of sugar mediated membrane is in the sequence of sucrose > D-fructose > glucose in this system.

GPC was performed to examine the low molecular weight of chitosan liberated from the membrane. As Fig. 7 shows, the low molecular weight chitosan liberated from the chitosan, chitosan/PEG polyblend and sugar mediated membrane was in the range of 1.5×10^4 to 4×10^4 and it seems that even after liberation from the membrane, enzymatic degradation still continued. This result was agreed with the study of Lee *et al*. [27]. In the case of chitosan and polyblend, the higher lysozyme susceptibility was achieved for polyblend having less chitosan feeding weight. For sugar-mediated membrane, the progress of the hydrolytic reaction is slower than polyblend. It may be due to the lysozyme were more difficult to access the cross-linked carbohydrate chains and the Schiff base product delay the degradation process obviously. Morphological examination of chitosan and others membranes is shown in Fig. 8. The micrograph clearly shows that the chitosan membranes (Fig. 8(a)) were smooth and nonporous. On the contrary, the chitosan-PEG (Fig. 8(b)) membrane surface was rough and porous. The small round pores were distributed uniformly throughout the membrane surface. The small circular shape pores in chitosan-PEG membrane possibly formed by leaching out the low molecular weight PEG oligomer during the membrane rinsing process [7]. The effect of 10 wt % sugar addition on the morphology of membrane was distinct. As shown in Fig. 8(c), the D-fructose mediated membrane had a rough surface and formed a mountain ridge structure. In comparison with D-fructose mediated membrane, the sucrose and glucose mediated membrane (Fig. 8(d) and (e)) have a rough and smooth surface, respectively. Fig. 9 shows the morphology of polyblend and sugar mediated membrane after soaking in PBS with lysozyme addition for 48 h. There was no obvious morphology changed in chitosan sample (Fig. 9(a)), but lots of round pores presented on the chitosan/PEG surface (Fig. 9(b)). In comparison with the surface of the chitosan/PEG membrane, the sucrose- and glucose-mediated membrane was changed to porous and sliced structure (Fig. 9(c) and (d)), respectively. The D-fructose mediated membrane changed to a smaller mountain ridge domain size and also had some degraded fragment attached on their surface (Fig. 9(e)) after enzymatic degradation. According to the results of weight loss, GPC and SEM observation, it may infer that the degradation rate not only was controlled by the chemical complexation between sugar and polyblends, but the surface morphology of membranes also has great influence.

3.5. Cytocompatibility tests

Large differences in cell morphology were observed on the various surfaces. On the chitosan and chitosan/PEG surfaces (Fig. 10(b) and (c)), both cell types exhibited a clear stellate appearance comparable to the control polystyrene (Fig. 10(a)). The sucrose-mediated membrane appeared to be extending several pseudopodal structures from a spread central cell body (Fig. 10(d)) and fibroblasts were able to spread enough to establish a cellular network. On the glucose and D-fructose mediated membrane surfaces, fibroblasts exhibited a rounded morphology, though cells were attached (Fig. 10(e) and (f)). According to Brand's report [28], ketose and aldose sugar show a high mutagenic effect on food safety, but the involvement of maillard reaction in the formation and elimination of mutagens is still unknown. In this study, the C=N occurring in glucose and fructose mediated polyblends seems to have some demutagens effect on cell behavior. Fig. 11 demonstrates the proliferation level (% of control) to various membranes after 4 days incubation period. Control group is the tissue culture polystyrene dish. The surface of polystyrene dish has been known to have good cellular attachment and show rapid cellular confluency in incubation period [29]. The number of the cells on glucose and fructose mediated membrane is significantly lower than on the chitosan, chitosan/PEG and sucrose-mediated membrane over a 4 day period. Approximately 110–120% of fibroblasts adhered on the chitosan, chitosan/PEG and sucrose-mediated membranes relative to the control, but only about 50–60% fibroblasts adhered on the glucose- and fructose-mediated membranes relative to the control. The MTT data correlate with the morphological differences of the fibroblasts on various membranes by OM observation. The extent in cellular proliferation implies that chitosan, chitosan/PEG and sucrose-mediated membrane have good cellular adaptability.

Several studies have demonstrated greater cell

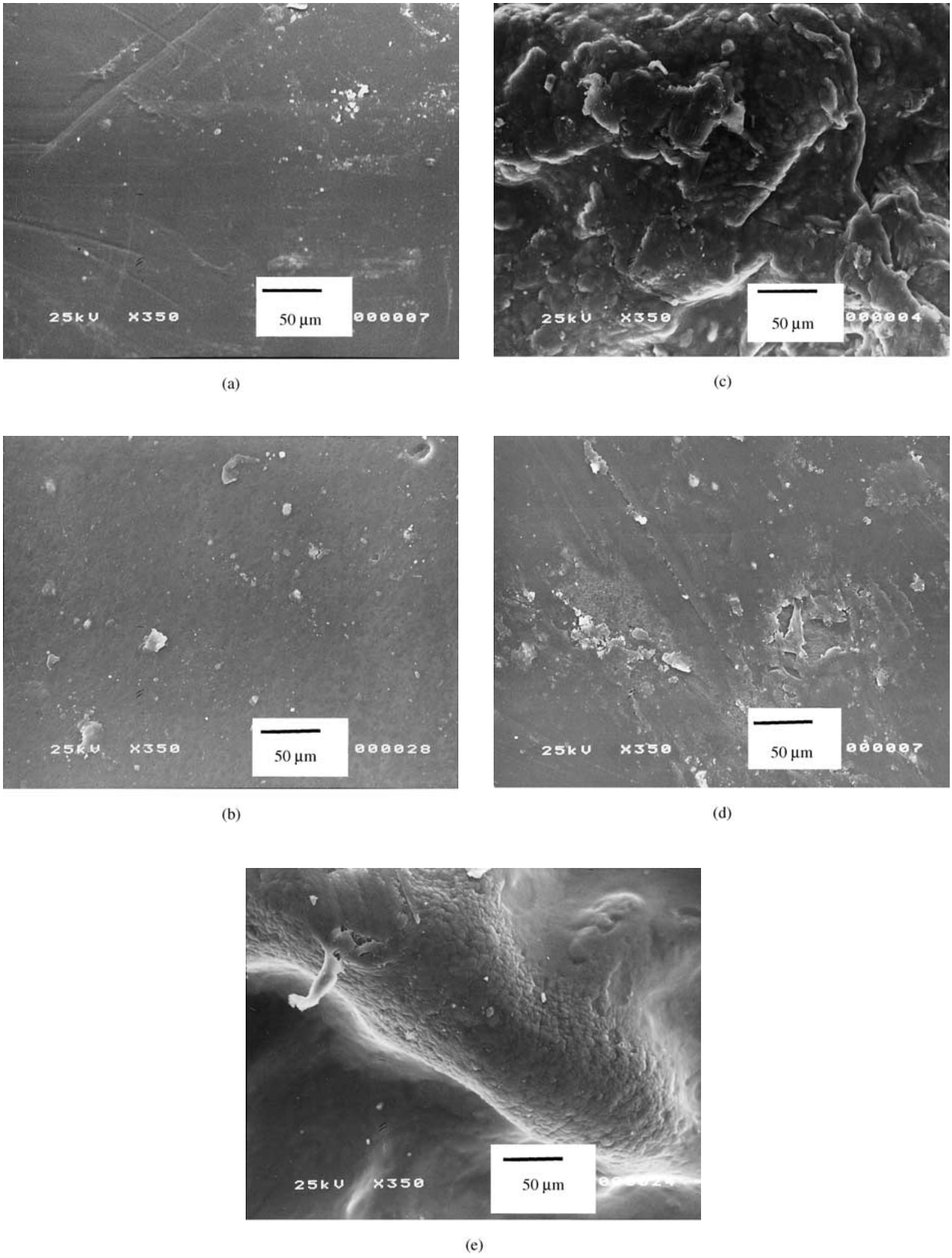

Figure 8 SEM photos of chitosan-based membranes: for (a) chitosan; (b) chitosan/6K; (c) sucrose; (d) glucose; (e) D-fructose mediated membrane.

attachment and cell spreading on hydrophilic, positively charged amine-modified surfaces relative to hydrophobic surfaces [30, 31]. In our previous study [32], the more Schiff base product formed in sugar-mediated membrane, the less hydrophilic amino group was presented on the membrane. According to the results of swelling test in this study, the chitosan/PEG, chitosan, and sucrose-mediated membrane with equilibrium water content in the range of 46–60% were considered hydrophilic while the fructose and glucose mediated membrane were considered hydrophobic. The surfaces of the various membranes also have different effects on the fibroblasts. It appears that cells on the glucose and fructose mediated membranes were able to attach but unable to follow this

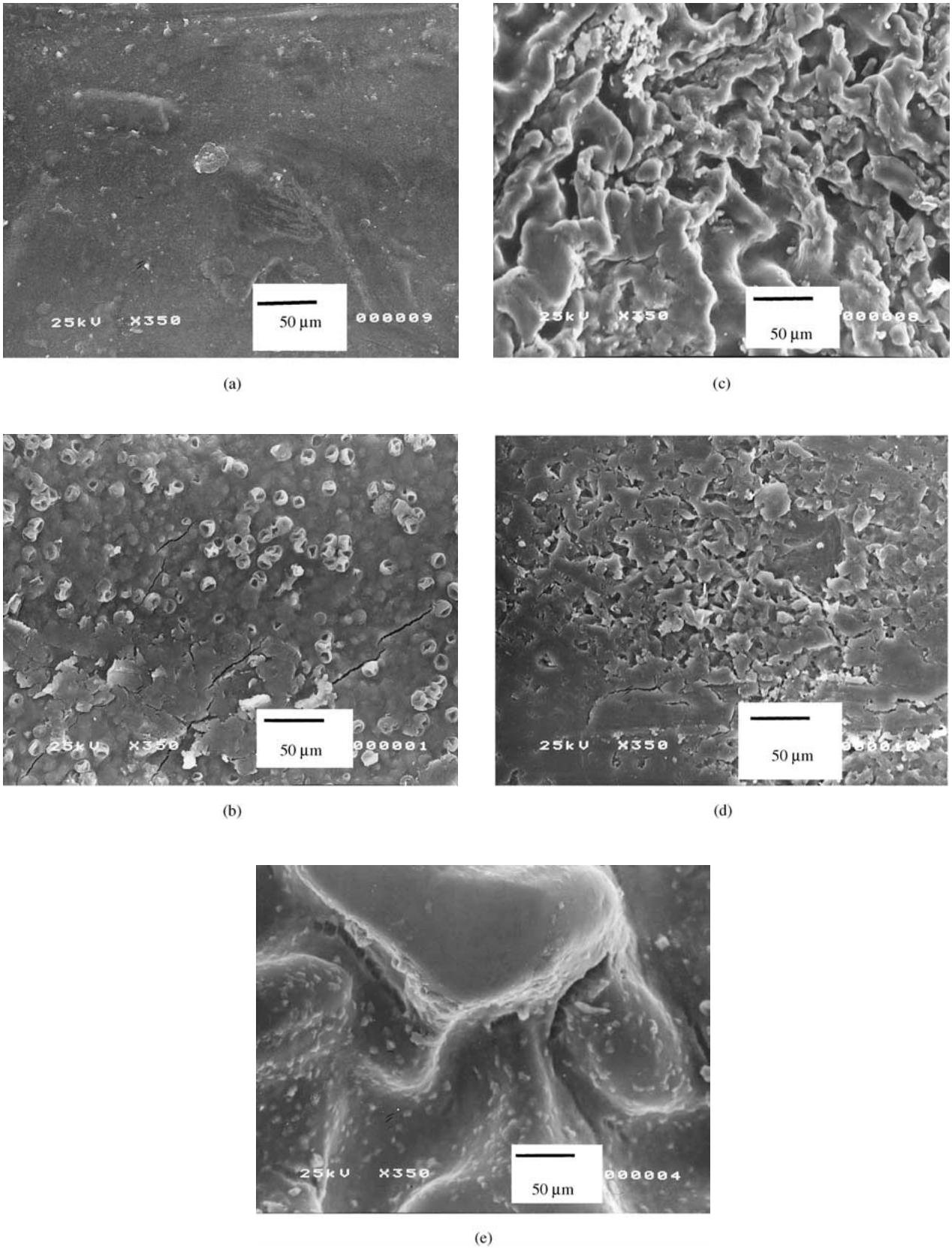

Figure 9 SEM micrographs of surface of (a) chitosan; (b) chitosan/6K; (c) sucrose; (d) glucose; (e) D-fructose mediated membrane after soaked in lysozyme solution for 48 h.

attachment with spreading. It has been demonstrated that some proteins secreted by cells have great influence on adhesion and spreading of cells on substrate [33]. An explanation for the cell responses observed on glucose- and fructose-mediated membranes may be that protein binding sites on the chitosan are being blocked or distorted by the Schiff base product. In contrast, the cells attached and spread on the sucrose-mediated membrane without apparent impairment of cell morphology was due to the low content of Schiff base product occurred during the Schiff base reaction. Therefore, it is both physical and chemical modifications that make the sucrose-mediated membrane and ideal candidate for a potential bio-material.

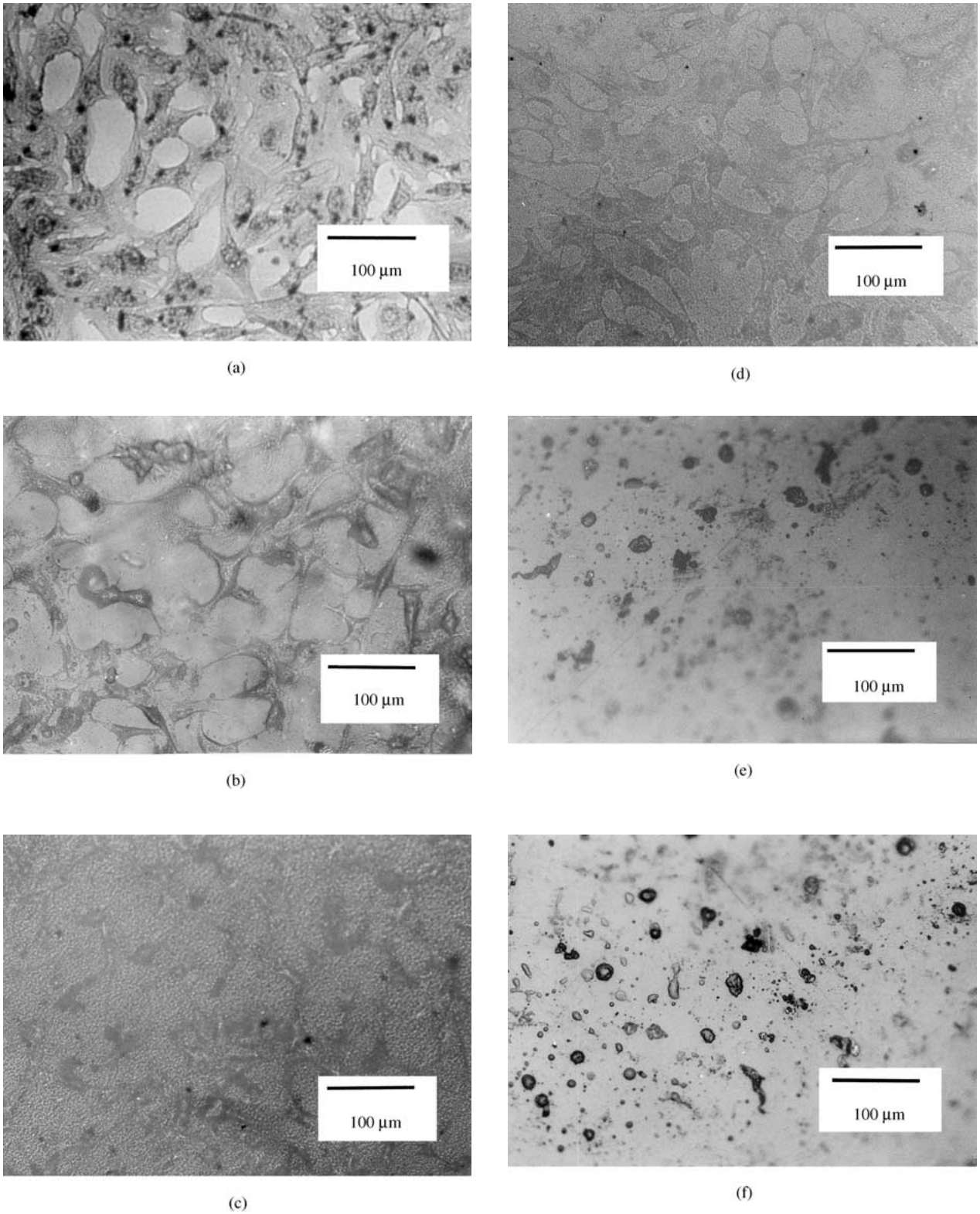

Figure 10 OM photographs of 3T3 fibroblast cells on membrane surfaces after 2 days culture for (a) culture dish as control (polystyrene); (b) chitosan; (c) chitosan/PEG6K; (d) sucrose-mediated membrane; (e) glucose-mediated membrane; (f) D-fructose-mediated membrane.

4. Conclusion

With the aim of finding hydrophilic and degradable membrane based on chitosan matrix, a novel membrane was prepared by using native sugars, hydrophilic polymer PEG to improve the chitosan property. According to TGA and FTIR analyses, the cross-linking reaction occurred and resulted in imine bonds (C=N) between sugar and the amino groups in chitosan. The results indicate that the use of native mono- or di-saccharides could be a feasible way to cross-link chitosan.

Blending introduced a high swelling capacity of PEG increased the water affinity, and reacted with reducing sugars decreased the water affinity in the polyblend. The EWC value of the sugar mediated membrane is in the sequence of sucrose > D-fructose > sucrose and they all present much lower water uptake ability than polyblend in the range of 25–61% at different pH environment. It is

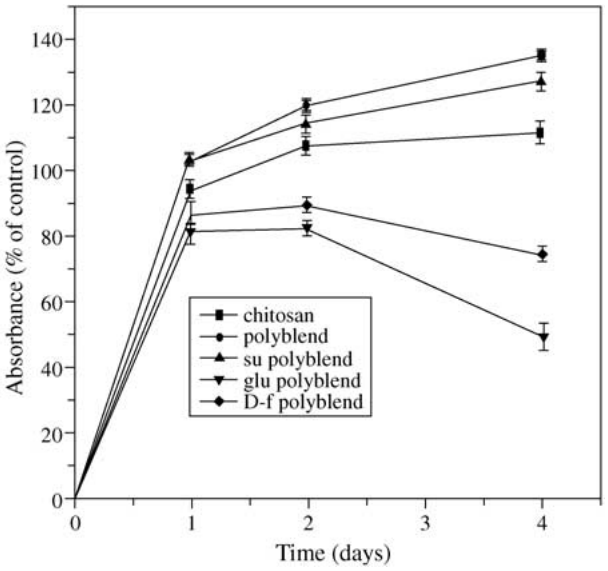

Figure 11 Proliferation profile of NIH 3T3 fibroblasts on chitosan, chitosan/PEG polyblend and sugar mediated membrane (% of control) for 4 days incubation period.

noted that for the sugar-mediated membrane under different pH environment, water content increases with the decrease of pH. Thus, it can be demonstrated that by blending PEG polymer and reacting with sugar, the chitosan have different pH dependant property.

The chitosan cannot be degraded by lysozyme due to the high degree of deacetylation of chitosan. However, all of the sugar-mediated membranes obtained a well degradable behavior. It indicates that they were susceptible to lysozyme. SEM morphology shows that the degradation rate not only was controlled by the chemical complexation between sugar and polyblends, but the surface morphology of membranes also has great influence. Chitosan, polyblend and sucrose-mediated polyblend show sufficient cellular adaptability. However, glucose and D-fructose mediated membranes have low cell viability implies that their surface chemistry and structure inhibit the cell growth. The pH-sensitive and well degradable property of the sucrose-mediated membrane may be applied for drug delivery system.

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