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Semi-interpenetrating polymer network hydrogels based on water-soluble *N*-carboxylethyl chitosan and photopolymerized poly (2-hydroxyethyl methacrylate)

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ABSTRACT

Semi-interpenetrating polymer network (semi-IPN) hydrogels were prepared by UV irradiation of water-soluble *N*-carboxylethyl chitosan (CECS) and 2-hydroxyethyl methacrylate (HEMA) aqueous solutions in the presence of D-2959 as photoinitiator. Hydrogels were characterized by using scanning electron microscopy (SEM), thermal gravimetric analysis (TGA) and X-ray diffractometry (XRD). SEM showed that semi-IPN hydrogels displayed porous surface and therefore had high surface area. XRD indicated that CECS/poly (HEMA) semi-IPN hydrogels had amorphous structure. The thermal stability and equilibrium degree of swelling improved obviously with increase of CECS content. Differential scanning calorimetry (DSC) indicated that free water content increased with increase of CECS content while bonded water content decreased. Cytotoxicity results suggested that semi-IPN hydrogels had good biocompatibility. From these preliminary evaluations, it is possible to conclude that these materials have potential applications in the biomedical field.

1. Introduction

Chitosan (CS) is a partially N-deacetylated derivative of chitin, which is the second most abundant natural polysaccharide in the world. Due to its unique properties such as biocompatibility (Kumar, 2000), biodegradability (Mi, Tan, Liang, & Sung, 2002), renewability, antimicrobial activity (Lim & Hudson, 2004; Zheng & Zhu, 2003) and wound-healing property, chitosan has its potential applications in wound dressings (Knill et al., 2004), wound-healing (Aiedeh, Gianasi, Orienti, & Zecchi, 1997), drug delivery systems (Berger, Reist, Mayer, Felt, & Gurny, 2004) and tissue engineering (Yagi et al., 1997; Zhang & Zhang, 2001). However, a limiting factor in the processing and application of chitosan is the low solubility in most solvents. (Kurita, 2001; Rathke & Hudson, 1994; Uragami, Ohsumi, & Sugihara, 1981).

Recently, the hydrogels based on chitosan and synthetic polymers were investigated extensively due to their special properties and thus potential utilization in the field of biomedicine and pharmacy (Kaewpirom & Boonsang, 2006; Ng & Swami, 2005; Verestiuc et al., 2006).

However, during the preparation of these hydrogels, the aqueous acid was usually used to dissolve chitosan or chitosan derivatives, which inevitably led the presence of small quantity of residual acid. These residuals, even very small quantity, may be harmful when it is applied to wounded human skin or tissue.

To overcome these problems, in this research, a water-soluble N-carboxyethyl chitosan was synthesized by Michael addition reaction, and then CECS/poly (HEMA) hydrogels were prepared by photopolymerization technique which has many merits such as sufficiently mild to be carried out in the presence of biological materials, spatial and temporal control of the polymerization, etc. (Lu & Anseth, 1999). A series of properties of the hydrogels including swelling property, thermal stability, mechanical property and cytotoxicity were investigated. The CECS/poly (HEMA) hydrogels could be potentially used as transdermal drug delivery matrix or wound dressing materials.

2. Experimental

2.1. Materials

The chitosan (CS) with an average molecular weight of 1.2×10^5 and degree of deacetylation of 84.4% was purchased from Zhejiang Golden-Shell Biochemical Co., Ltd., China. 2-Hydroxyethyl methacrylate (HEMA) and acrylic acid were obtained from Beijing

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Chemical Reagents Company (China), distilled under reduced pressure in the presence of hydroquinone, and stored at 4 °C until use. UV photoinitiator Darocur 2959 (D-2959, 2-hydroxy-1-[4-(hydroxyethoxy) phenyl]-2-methyl-1-propanone) was obtained from Ciba-Geigy Chemical Co. (Tom River, NJ). Tetra (ethylene glycol) dimethacrylate (TEGDMA, Sartomer Company, Inc., USA) was used as a crosslinking agent without further purification. Mouse fibroblast (L929) was obtained from Department of Microbiology, Peking University Health Science Center.

2.2. Synthesis of water-soluble CECS

N-Carboxyethyl chitosan was synthesized according to the method established by Sashiwa, Yamamori, Ichinose, Sunamoto, and Aiba (2003). Briefly, 2.0 g of chitosan (corresponding to 10 mmol NH_2), 100 mL of water, and 1.40 mL of acrylic acid was put into a round bottom flask and reacted at 50 °C under constant stirring for 2 day. Thereafter, 1 N aqueous NaOH was added to the reaction mixture to adjust the pH to 10–12 to convert the carboxylic acid to its sodium salt. The mixture was then dialyzed using a dialysis membrane (membrane molecular weight cut-off 12,000 g mol^{-1}) to remove salt against water for two days and lyophilized to obtain pure CECS. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AMX 600 M NMR instrument. The intrinsic viscosity ($[\eta]$) of the *N*-carboxyethyl chitosan was measured by using a modified Ubbelohde viscometer and the viscosity average molecule weight (M_v) was calculated from the Mark–Houwink equation: $[\eta] = K \cdot M_v^{\alpha}$. Where K is 1.81×10^{-3} mL/g, α is 0.93 (Roberts & Domszy, 1982).

2.3. Preparation of photopolymerized hydrogel

The hydrogel was achieved by mixing a 1.0 wt.% CECS aqueous solution with HEMA monomer containing 1 wt.% TEGDMA and 0.5 wt.% D-2959 (relative to amount of HEMA). The composition of hydrogel was listed in Table 1. The solution was transferred into a disk-shaped mold consisting of two glass microslides separated by a spacer, then irradiated with UV light source (15 mW/cm^2, EFOS Lite, 50 W miniature arc lamp, with 5 mm crystal optical fiber, Canada) for 10–30 min at ambient temperature. After polymerization, the resultant hydrogel was immersed into distilled water, and then cut into circular pieces (diameter = 1.25 cm, and thickness = 2 mm) with a perforator and dried in vacuum at 60 °C for 48 h.

2.4. Scanning electron microscopy

To visually examine surface morphology of the hydrogel, a Hitachi Model S-450 SEM was used to analyze the pore structure. The freeze-dried samples were loaded on the surface of an aluminium SEM specimen holder and sputter coated with gold before observation. The accelerating voltage was 20 kV.

2.5. Cytotoxicity measurement

Cytotoxicity of the hydrogel was evaluated based on a procedure adapted from the ISO10993-5 standard test method. The prepared membrane or hydrogel was sterilized with highly compressed steam for 15 min and placed in 1 mL RPMI1640 medium (10.0% bovine serum, 1.0% penicillin–streptomycin and 1.2% glutamine) respectively, and steeped at 37 °C for 48 h. Then the membrane or the hydrogel was removed and the extracts were stored for evaluation of cytotoxicity.

Mouse fibroblasts (L929) were cultured in RPMI1640 medium with or without the extracts for 24 h, respectively. The viabilities of cells were determined by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Thiazolyl blue) assay. The MTT assay is based on the reduction of yellow tetrazolium salt to purple formazan crystals by dehydeogenase enzymes secreted from the mitochondria of metabolically active cells. The amount of purple formazan crystals formed is proportional to the number of viable cells.

L929 cells were seeded in wells of a 96-well plate at a density of 4×10^3 cells per well. After incubation for 24 h, the culture medium was removed and replaced with the as-prepared extraction media. After incubation for 24, 48, 72 h, each well was taken out, and 100 μL MTT solution was added to each well. After 4 h incubation at 37 °C, 150 μL of dimethyl sulfoxide was added to dissolve the formazan crystals. The dissolved solution was swirled homogeneously about 10 min by the shaker. The optical density of the formazan solution was detected by an ELISA reader (Multiscan MK3, Labsystem Co. Finland) at 570 nm.

For the reference purpose, cells were seeded to medium containing 0.64% phenol(positive control) and without the extracts (negative control) at same condition, respectively. Each assay was performed six times in triplicate.

2.6. Water state

Differential scanning calorimetry (DSC 204 F1, Netzsch, Germany) was employed to examine the crystallinities of the hydrogels by observing the melting endotherms. The state of water in the swollen hydrogels with different water content was also evaluated from DSC analysis. Samples sealed in aluminium pans were cooled down to −30 °C and then heated to 220 °C at a heating rate of 10 °C/min under 50 mL/min of nitrogen flow.

2.7. Swelling behavior of the semi-IPN hydrogel

Dried semi-IPN hydrogels were left to swell in distilled water at 25 °C. Swollen gels removed from the solution at regular intervals were dried superficially with filter paper and weighted. The measurements were continued until a constant weight was reached for each sample. The degree of swelling, *Q*, is expressed as the amount of absorbed water per gram of dry polymer during a regular time interval.

$$Q = (W_s - W_0)/W_0$$

where, W_S and W_0 are the weights of the samples in the swollen and dry state, respectively.

2.8. XRD

The XRD patterns of the dry CECS and CECS/poly (HEMA) hydrogels were performed via X-ray diffractometer (Rigaku, Damax2500) with CuKa characteristic radiation (wavelength λ = 0.154 nm at 40 kV, 50 mA, and scan speed of 1°/min in the 2θ range of 5–90°).

2.9. TGA

Thermal gravimetric analysis (TGA) was performed with a NETZSCH TG 209 F1 analyzer (Germany) between 20 and 500 °C with a 10 °C/min heating rate under a nitrogen flow rate of 20.0 mL/min.

Table 1
Formulations of semi-IPN hydrogels

	CECS solution (%)	HEMA (%)	TEGDMA (%)	D-2959 (%)
Semi-IPN-1	33.3	65.7	0.67	0.33
Semi-IPN-2	50.0	49.3	0.50	0.25
Semi-IPN-3	66.7	32.5	0.33	0.17

3. Results and discussion

3.1. Synthesis of CECS

A water-soluble N-carboxyethyl chitosan was synthesized by reaction of chitosan with acrylic acid. The obtained CECS was characterized by NMR in D_2O, the ^1H NMR data was as following. ^1H NMR (D_2O): δ = 2.01(NHAc), 2.61 (—CH_2—CO_2Na), 3.12 (H-2 of glucosamine(GlcN)), 3.42 (H-2 of *N*-alkylated GlcN), 3.67-4.10 (N—CH_{2a}- of N-alkyl group, H-2 of GlcNAc, H-3,4,5,6 of GlcN and GlcNAc), 4.81(H-1 of GlcNAc residue), 4.93(H-1 of *N*-alkylated GlcN residue). The degree of substitution (DS) was calculated by comparison of the peak area at δ = 2.61 of the —CH_2— proton and that of δ = 2.01 of the NHAc proton. The DS of synthesized CECS was 0.18, which meant that 21% of the NH_2 reacted with acrylic acid.

^{13}C-NMR was also performed on CECS to confirm the Michael addition reaction (Fig. 1). The carbonyl carbon of the ester bond in the substitute was found at 180 ppm while the methyl carbons, adjacent to carboxyl group and imine group in the substitute were seen at 45 and 37 ppm, respectively. Other peaks of CECS were assigned in the region between 0 and 200 ppm by comparison with the spectrum recorded for the CS precursor: 21.9 (–CH_3 of acetyl), 56.5 (C2), 60.1 (C6), 68.5–80.6(C3, C5, and C4), 102.2 (C1), 174.5 (C=O of acetyl).

The viscosity average molecule weight (M_v) of CECS calculated from the Mark–Houwink equation was 5.9×10^4 mol^{-1} g, lower than that of CS (1.2×10^5). It meant that Michael addition reaction of CS with acrylic acid would cause degradability of the CS backbone.

3.2. TGA analysis

Fig. 2 shows the TGA traces of CECS, poly (HEMA) and CECS/poly (HEMA) semi-IPN hydrogels with various compositions. The CECS showed obvious loss of weight starting from 220 to 350 °C, which could be attributed to a complex process including dehydration of the saccharide rings, depolymerization, decomposition of the acetylated and deacetylated units of the polymer (Peniche-Covas, Argüelles-Monal, & San Román, 1993), and the elimination of carboxyl ethyl groups. The pure poly (HEMA) began its first decomposition at relatively higher temperature (~300 °C) than of CECS (~220 °C). For CECS/poly (HEMA) semi-IPN hydrogels, the start temperature of weight loss showed a shift towards high temperature region from 346 to 371 °C with an increase of CECS content. The results demonstrated the improvement of the thermal stability for semi-IPN hydrogel with increase of CECS, which was probably due to more intermolecular interactions between CECS and poly (HEMA) caused by hydrogen bonds between —OH in poly (HEMA) and —NH_2 or —OH groups in CECS.

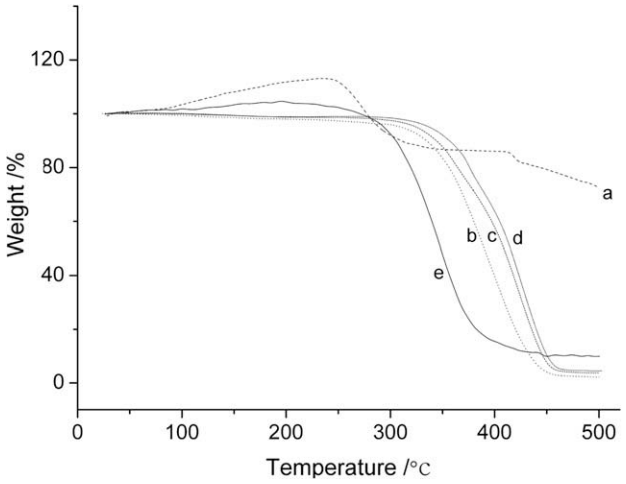

Fig. 2. TGA analysis of CECS, poly (HEMA) and CECS/poly (HEMA) semi-IPN hydrogels with various compositions. (a) CECS; (b) semi-IPN1, (c) semi-IPN2; (d) semi-IPN3, (e) poly(HEMA).

3.3. Swelling behavior

Fig. 3 shows the water content of semi-IPN hydrogels measured at various time intervals. It could be seen that all semi-IPN hydrogels had similar swelling behavior and reached equilibrium within 25 h. As shown in Fig. 3, as amount of CECS increased, the semi-IPN hydrogels exhibited a faster swelling rate and larger swelling ratios. This behavior might be mainly due to the formation of more loosely crosslinked network with increase of CECS content. With increasing amount of CECS, crosslinking density of poly (HEMA) decreased and molecular entanglement between CECS and poly (HEMA) was weakened, which resulted in improvement of its water absorbing ability.

The state of water in the swollen hydrogels with different water content was evaluated from DSC analysis, as shown in Fig. 4A. Free water and bonded water contents were measured from the DSC melting thermograms of swollen hydrogels and calculated according to Sawhney' paper (Sawhney, Pathak, & Hubbell, 1993). The thermogram showed an endothermic peak between 0 and 7 °C. This peak was assigned to free water associated with the CECS/poly

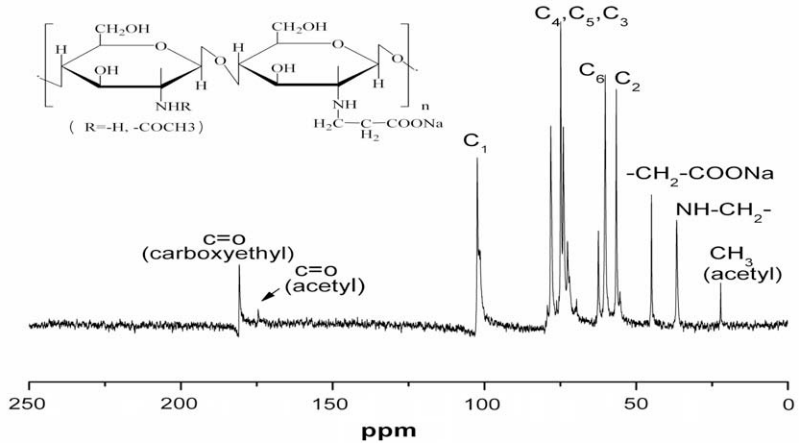

Fig. 1. ^{13}C-NMR spectrum of CECS in D_2O.

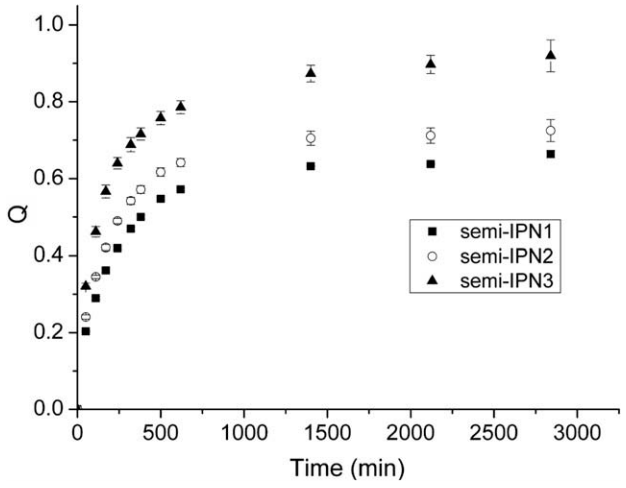

Fig. 3. Swelling kinetics of semi-IPN hydrogels.

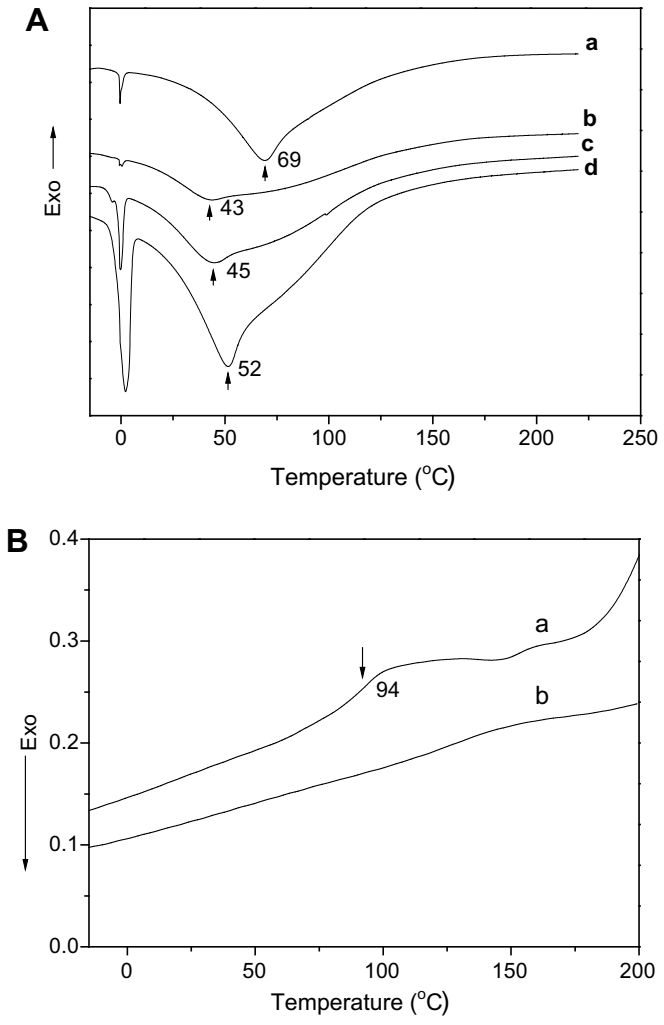

Fig. 4. (A) DSC thermogram first run curves of wet CECS/poly (HEMA) hydrogel. (a) poly (HEMA), (b) semi-IPN1, (c) semi-IPN2, (d) semi-IPN3. (B) DSC thermogram curves of dry samples. (a) poly (HEMA), (b) CECS.

(HEMA) hydrogel and was used to calculate the amount of free water present in these gels. The fraction of free water in total water was calculated as the ratio of endothermic peak area for water-swollen hydrogel to melting endothermic heat of fusion (334 J/g) for pure water. Bonded water due to hydrogen bonding with poly (HEMA) or CECS chains was expressed as the difference between the total water and the free water. The results from calculation showed that free water content increased from 0.5% to 42.4% with increase on CECS content while bonded water content decreased from 65.5% to 49.6%.

Fig. 4B shows the DSC curves of dry CECS and poly (HEMA). It could be observed that the glass transition temperature (T_g) of poly (HEMA) network was at about 94 °C, while CECS did not present T_g due to the reason that being a natural polymer, some properties such as crystallinity, molecular weight and deacetylation degree, could present wide variations according to the source and/or method of extraction (Neto et al., 2005). All wet semi-IPN hydrogels presented apparently endothermic events (>10 °C). For pure poly (HEMA) the peak was centerd at 69 °C which was different from dry poly (HEMA) (Fig. 5 a and b). The peak centerd at 69 °C disappeared, confirming that this peak was related to water molecules bound to poly (HEMA) molecular chain and the peak of water evaporation could cover the glass transition of poly (HEMA) network. However for CECS/poly (HEMA) semi-IPN hydrogels, there was an obvious overlapped band in the range from 40 to 100 °C. The overlapped band could be divided into two peaks using the Lorentzian curve-fitting procedure, referring to Liu (Liu, Chen, Lin, & Liu, 2006). The obvious peak in the range of 40–55 °C was attributed to endothermic peak of the water bonded to CECS molecular chain by comparing wet poly (HEMA) hydrogel and various CECS/poly (HEMA) hydrogels. The position of the endothermic peak at 43 °C was shifted to higher temperature (52 °C) and the ratio of the endothermic peak area at 43 °C to that at 69 °C increased from 209/83 (semi-IPN1) to 768/3 (semi-IPN3) with increasing CECS content. The differences in the peak area and position of endotherm indicated that macromolecules differed in their water holding capacity and strength of water–polymer interaction. With addition of CECS, new hydrophilic centers are formed, i.e. the hydroxyl groups, carboxyl and amino groups of CECS bind more and more number of water molecules, confirming that CECS is a more hydrophilic polymer than poly (HEMA).

3.4. SEM

Microstructures of the networks surface investigated by scanning electron microscopy are presented in Fig. 5. It could be seen that the semi-IPN membrane displayed a channel like and porous surface. The distribution of porosity became more uniform and dense with increasing concentration of CECS. When CECS solution content rose, from semi-IPN1 to semi-IPN3, the equilibrium water content of semi-IPNs increased, which led to more pores in semi-IPN hydrogels obtained from lyophilization. The microporous surface structure of the semi-IPNs could lead to high surface areas with low diffusion resistance in the matrix. These observations implied that HEMA might have used the CECS macromolecule as a scaffold for the formation of the final product (Bayramoglu & Arica, 2003).

3.5. XRD

Fig. 6 presents XRD patterns of CS, CECS and dry semi-IPN hydrogels. It could be seen that CS powder exhibited three typical peaks at $2\theta = 10.3°$, 15.2° and 19.9°, agree to Samuels' observation (Samuels, 1981). According to Samuels, the reflection falling at $2\theta = 10.3°$ was assigned to crystal form I. The strongest falling at $2\theta = 19.9°$ corresponded to crystal form II. However, for CECS, the crystalline peak at $2\theta = 10.3°$ and 15.2° disappeared and strong peak at $2\theta = 19.9°$ became a relative obtuse. It suggested that the large number of hydrogen bonding in the CS powder was de-

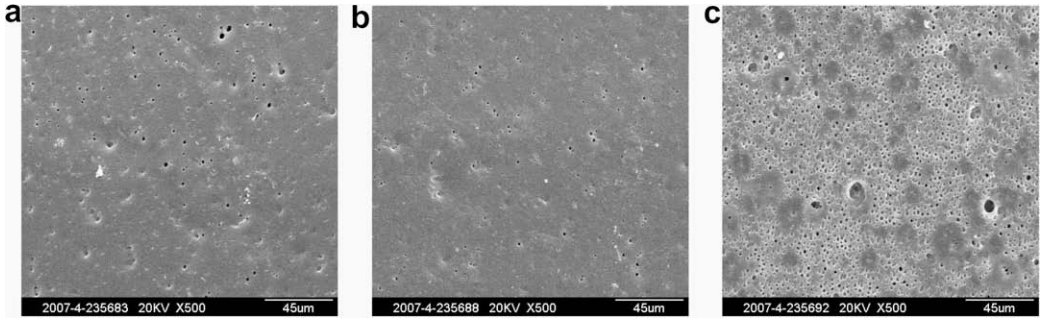

Fig. 5. SEM photograph of surface of the semi-IPNs. (a) semi-IPN-1, (b) semi-IPN-2, (c) semi-IPN-3.

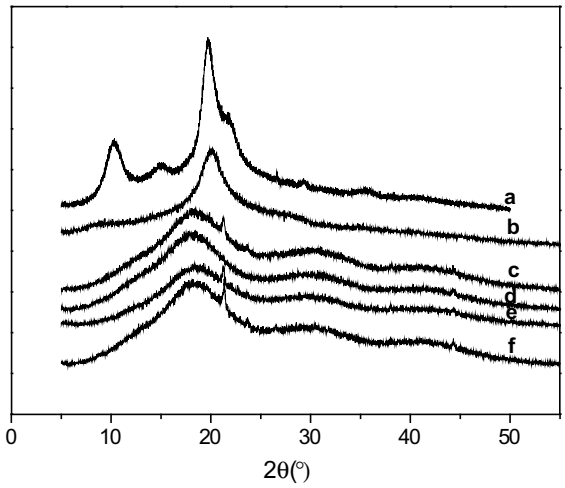

Fig. 6. The XRD patterns of (a) CS, (b) CECS, (c) semi-IPN1, (d) semi-IPN2, (e) semi-IPN3, (f) poly (HEMA).

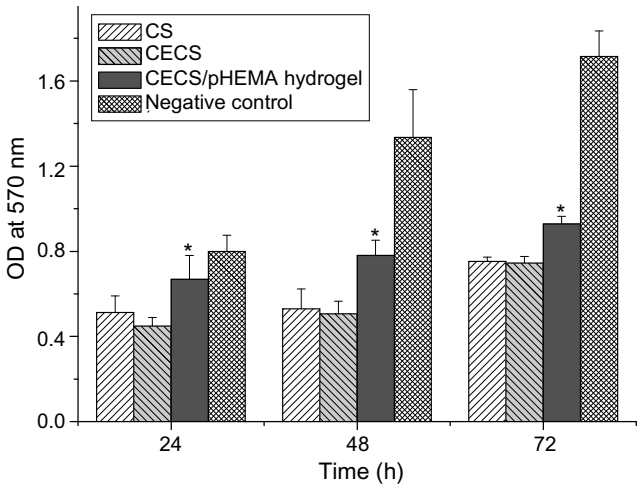

Fig. 7. Cytotoxicity test of CS membrane, CECS membrane, CECS/poly (HEMA) semi-IPN hydrogels and negative controls ($p < 0.05$). The data represented mean and standard deviations of six samples.

stroyed through the *N*-acylation, thus forming a smaller fraction of crystalline phase and a larger fraction of amorphous phase. The CECS/poly (HEMA) semi-IPN had one broad peak at around $2\theta = 18.2°$, which could attribute to the weakening of hydrogen bonding between the amino groups and hydroxyl groups in the CECS molecules.

3.6. Cytotoxicity

Fig. 7 shows the absorbance obtained from an MTT assay of L929 cells which were cultured with the extraction media in comparison with control. It could be seen that the absorbance intensity of all samples was lower than that of control during 72 h seeding time and statistically significant differences were observed ($p < 0.05$) in the cell activity compared with negative control for all samples, which meant all samples showed cytotoxicity to L929 cell. The reason might be high concentration of extraction medium. However, For CECS/poly (HEMA) semi-IPN hydrogel, statistically significant differences ($p < 0.05$) were observed in the cell activity in comparison with CS membrane. Because chitosan has been shown to be relatively non-toxic (Corsi, Chellat, Yahia, & Fernandes, 2003; Lee et al., 2001), these hydrogels were considered as non-toxic. The obtained results clearly suggested that hydrogels have better biocompatibility than that of CS.

4. Conclusion

Biocompatible hydrogels based on HEMA and water-soluble *N*-carboxyethyl chitosan were prepared by UV irradiation. Hydrogels obtained were characterized by using TGA, XRD, DSC and SEM, respectively. The TGA results indicated that the thermal stability of semi-IPN hydrogel improved with increase of CECS. XRD results showed there was amorphous phase in the CECS/poly (HEMA) semi-IPN hydrogel. And the results of cytotoxicity revealed that hydrogels have good biocompatibility. The CECS/poly (HEMA) hydrogels could be potentially used as drug delivery matrix or wound dressing materials.

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