Photoinitiating Polymerization to Prepare Biocompatible Chitosan Hydrogels

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ABSTRACT: Chitosan hydrogels were prepared from water soluble chitosan derivatives (chitosan-MA-LA, CML) by photoinitiating polymerization under the existence of Irgacure2959 and the irradiation of UV light. The CML was obtained by amidation of the amine groups of chitosan with lactic acid and methacrylic acid. Gelation time of the hydrogel could be adjusted within a range of 5–50 min, and controlled by factors such as the degree of MA substitution, initiator concentration, existence of oxygen, and salt. The dry hydrogel adsorbed tens to hundred times of water, forming a highly hydrated gel. The swelling ratio was smaller at the higher degree of MA substitution, higher pH, and higher salt concentration. Rheological test showed that the hydrogel is elastomeric in the measuring frequency range, with a storage modulus and loss modulus of 0.8–7 kPa and 10–100 Pa, respectively. *In vitro* culture of chondrocytes demonstrated that the cells could normally proliferate in the extractant of the hydrogels, showing no cytotoxicity at lower initiator concentration. By contrast, the extractant of the hydrogel made by the redox initiating system, i.e., ammonium persulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), showed apparent cytotoxicity. Thus, the chitosan hydrogels initiated by the Irgacure2959 have better comprehensive properties, in particular better biocompatibility, and are more suitable for biomedical applications. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 110: 1059–1067, 2008

Key words: chitosan; hydrogels; injectable; photo crosslinking

INTRODUCTION

Injectable biomaterials are of great importance in recent years in the field of tissue engineering and orthopaedics, as they can perform clinical implantation in a minimally invasive way. Many natural and synthetic hydrogels such as collagen,[1] chitosan,[2] gelatin,[3] alginate,[4,5] poly(propylene fumarate) (PPF),[6,7] and PEG are widely applied as the injectable scaffolds.[8,9] Drugs, cells, and other bioactive components such as cell growth factors can be integrated into the polymer sol, which can be then injected into the wanted sites and gelled *in situ*. The water content in the hydrogels can be as high as hundreds times of the polymer weight, leading to the ease of exchange of gas, nutrients and metabolite substances. In this sense, the hydrated polymer network is more similar to the native extracellular matrix (ECM), which is generally composed of proteins and polysaccharides. Moreover, functioned as injectable scaffolds, the hydrogels and their soluble counterparts should possess good biocompatibility and biodegradability. For this context, the gelation conditions should be as mild as possible.[10]

Chitosan has been widely used in the fields of drug delivery and tissue engineering because of its biodegradability and good biocompatibility.[11,12] Chitosan hydrogels can be obtained by pH change and covalently or ionically crosslinking.[13] However, unmodified chitosan is only soluble in acidic solution, e.g., below pH 5. The acidic solubility and the available gelation methods largely limit its application as an injectable hydrogel for tissue regeneration *in vivo*. Therefore, enhancing the solubility of chitosan at neutral pH and ease of gelation is of both practical and technological significance. In our previous study, the chitosan molecule was modified with methacrylic acid (MA) and lactic acid (LA), endowing the chitosan with crosslinkable and water-soluble features.[14] The modified chitosan has good solubility at neutral pH. Chitosan hydrogel was made by a redox initiated polymerization at body temperature.[14] However, cell culture tests found that

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the redox system, i.e., ammonium persulfate (APS) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) has a certain degree of cytotoxicity.

To eliminate as possible as the cytotoxicity, photoinitiated polymerization shall be adopted, using 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (Irgacure2959) as the initiator. When the photoinitiators are exposed to specific wavelength of light, free radicals are produced to initiate the polymerization of carbon double bonds.[15] However, the high-energy radicals have a potential of oxidative damage to the cell populations.[16–18] Researches show that the oxidative damage depends on cell type and proliferation rate,[19,20] suggesting that the photoinitiators may have different effects on different cell types.[21] Among the photoinitiators used so far, Irgacure2959 causes minimal cytotoxicity (cell death) over a broad range of mammalian cell types and species.[20] It is worth to mention that the photopolymerization has been widely adopted in commercial and biological applications such as printing, dentistry, optical materials,[15] encapsulating pancreatic islet cells,[22,23] and blood vessel adhesives.[24] It can rapidly polymerize liquid monomer or macromer solution into a crosslinked network under physiological conditions, thus has been widely applied in the biomedical field.[15] Moreover, as previously indicated by Williams et al., it has also the advantages of powerful spatial and temporal control of reaction's kinetics, minimal heat production, ability to uniformly encapsulating cells, and significant adaptability for clinical applications.[20]

Although the efficacy and biocompatibility of Irgacure2959 has been demonstrated in other systems,[25–29] work still needs to be done to assess its applicability in the CML system and effect on the properties of the resulted hydrogel. Moreover, its cytotoxicity in our system, if any, should be determined too. The swelling performance of the hydrogel in response to pH variation, ionic type, ionic concentration is not clear so far. These results are of great importance for any application of the photo-initiated CML hydrogel. Since our previous work has demonstrated that the hydrogel itself is nontoxic and possess good biocompatibility,[14] it is not our purpose to assess the long term biocompatibility of the hydrogel in this work.

EXPERIMENTAL SECTION

Materials

Chitosan ($M_\eta \approx 600$ kDa) was obtained from Haidebei Marine Bioengineering Company, Ji'nan, China. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and (3-(4,5-dimethyl) thiazol-2-yl-2,5-dimethyl tetrazolium bromide (MTT) were purchased from Sigma. 2-Hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (Irgacure2959) was obtained from Ciba Specialty Chemicals. Methacrylic acid (MA) and ammonium persulfate (APS) were purified via distillation under reduced pressure and recrystallization, respectively. Lactic acid (LA) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED, >98%) were used as received.

Synthesis of CML

Modification of the chitosan chain with MA and LA to obtain chitosan-MA-LA (CML) was conducted according to Ref. 14. Briefly, 800 mg chitosan (containing 3.3 mmol NH_2) was dissolved in 100 mL water containing 100 μL (1.09 mmol), 200 μL (2.18 mmol), 400 μL (4.35 mmol) or 800 μL (8.7 mmol) of MA. Then EDC with a weight ratio to MA of 0.5 was added. The reaction lasted for 24 h at room temperature under magnetic agitation. The pH value of the mixture solution increased from 4 to ~ 7 during this process owing to the alkaline nature of the resultant urea. To remove the unreacted MA and other small molecular weight products, the resultant mixture was sealed in a membrane with a cut off molecular weight of 10 kDa and dialyzed in a lager amount of triple-distilled water for 3 days. Finally, chitosan-MA (CM) was obtained by freeze-drying. Seven hundred milligram CM with a degree of MA substitution of 5–46% was dissolved in water containing 400 μL (4.8 mmol) LA overnight. Then EDC with a weight ratio to LA of 0.5 was added. The mixture was stirred for 24 h at room temperature. Following the purification steps described earlier, the water-soluble and crosslinkable chitosan (CML) was obtained. The degree of substitution of the CML was determined by elemental analysis.[14] The final product is designated as CM_nL_m, where n and m represent the 100× the degree of MA and LA substitution, respectively. In this work, the degree of LA substitution (i.e., the substitution degree of $-NH_2$ group) is kept as 33%, while the degree of MA substitution is varied from 5, 18, 34 to 46%.

Gelation of the CML

Two hundred microliter of 2% CML solution with variable concentrations was put in 1-mL polypropylene test tube, into which different amount of 5% (w/v) Irgacure2959 (photoinitiator) in 75% ethanol solution was added. After incubation at 37°C for 8 h, the mixture was irradiated by 365 nm UV light with a power of ~ 10 mW/cm^2, which was determined by a radiometer. For each data point, 3–5 samples were simultaneously put under a UV lamp. The samples were taken out at desired time interval to determine the gelation time, judging from the loss of fluidity.

Swelling ratio of the hydrogel

The hydrogels were freeze-dried and weighted (W_0). The dry hydrogels were submerged in water at 37°C for t time and weighed (W_1). The swelling ratio of the hydrogel is defined as $\frac{W_1}{W_0}$. To measure the equilibrium swelling ratios, the dry hydrogels were submerged in different kinds of solutions at 37°C for 24 h and weighed (W_2). The equilibrium swelling ratio of the hydrogel is defined as $\frac{W_2}{W_0}$.

Weight loss

A 200-μL hydrogel made from a 2% CML solution was incubated in 4 mL of 1 mg/mL lysozyme/PBS at 37°C. The weight of the hydrogel at different time was measured. Weight loss of the hydrogel is defined as $\frac{W_0-W_t}{W_0} \times 100\%$, where W_0 and W_t represent the initial weight and the weight of the hydrogel at t time.

Rheological test

CML with different degree of MA substitution and concentration was dissolved in PBS, into which Irgacure2959 with a final concentration of 0.05% (w/v) was added. The obtained solution was injected into a circular and transparent glass mold (25 mm diameter, 0.22 mm height), which was successively exposed to UV light. Ten minutes later, the formed hydrogel was harvested, which was then immersed into 5 mL PBS to allow the hydrogel swelling.

The sample was then placed in a parallel plate mode for the rheological measurement by a strain-controlled ARES rheometer (Advanced Rheometric Expansion System). Dynamic oscillatory mode (compression mode) was used to measure the storage and loss modulii. All tests were performed at 37°C, and the strain was set at 1%.

Cytotoxicity test

Cytotoxicity of the hydrogels was assessed by *in vitro* chondrocyte culture by supplementing with extractant from the hydrogels. For this purpose, the CML with the highest degree of MA substitution, CM46L33, was used, since this hydrogel has the best mechanical performance investigated so far. Dulbecco's minimum essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS) was used as a negative control. Redox initiator, APS and TEMED, was used as a positive control. Both compounds were dissolved in PBS to form 1 *M* solutions, which were sterilized by a membrane filtration with a pore size of 0.22 μm, respectively. Dry CM46L33 was sterilized under UV radiation for 1 h and then dissolved in PBS with a final concentration of 2%. Hydrogels were fabricated with different amount of APS/TEMED or Irgacure2959 at 37°C, keeping the gelation time constant for the two types of initiating systems. The obtained hydrogels were treated with DMEM supplemented with 10% FBS at a ratio of 100 mg hydrogel/mL DMEM/FBS at 37°C for 24 h. The solutions extracted from the hydrogels made by 5 and 10 m*M* APS/TEMED were denoted as RI6 and RI4, respectively, while the solutions extracted from the hydrogels made by 2.5 and 5 m*M* Irgacure2959 were denoted as PI6 and PI4, respectively.

Each well of a 96-well culture plate was seeded with 1×10^4 chondrocytes. For the testing groups, the cells were cultured in 200 μL solution extracted from the hydrogels at 37°C in 5% carbon dioxide atmosphere, while the negative control group was fed with DMEM/FBS. The cytoviability was quantitatively measured by MTT assay at different culture time. Briefly, 20 μL of 5mg/mL MTT in PBS was added into each well. The cells were continually cultured for another 4 h. During this period, live cells can reduce the MTT to formazan pigment, which was dissolved by μL dimethyl sulfoxide after removal of the culture medium. The absorbance at 570 nm was recorded under a microplate reader (Bio-Rad 550). Viable cells were also measured at day 3 by fluorescence microscopy (Zeiss Axovert 200). The cells were incubated in 5 μg/mL fluorescein diacetate (FDA)/PBS solution for 10 min. During this process, FDA (no fluorescence) can penetrate through the cell membranes and was hydrolyzed into fluorescein by viable cells, which enables observation of the viable cells by excitation at 488 nm.

Statistical analysis

Data were analyzed using one-way ANOVA with *post hoc* Tukey testing for differences. Results are reported as mean ± standard deviation. The significant level was set as $P < 0.05$.

RESULTS AND DISCUSSION

Synthesis of CML

The chitosan can be successfully modified with MA and LA molecules through reaction between NH_2 and COOH under the existence of EDC.[14] As the feeding ratio of MA to NH_2 of chitosan increased from 0.33, 0.65, 1.31, to 2.61, the degree of MA substitution increased from 5, 18, 34, to 46% with a corresponding MA reaction efficiency of 15, 27, 26, and 18% respectively. When the feeding ratio of LA to NH_2 was in a range of1.52–2.67, the degree of LA substitution kept constant at ~ 33% with a reaction efficiency of 22%.

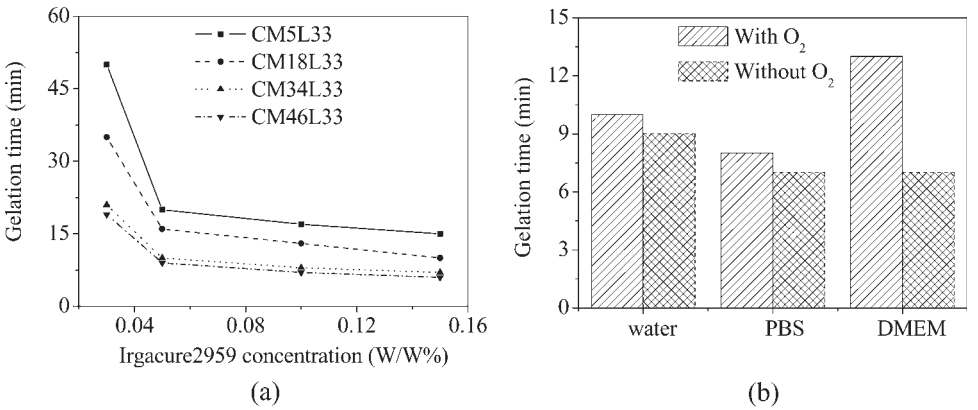
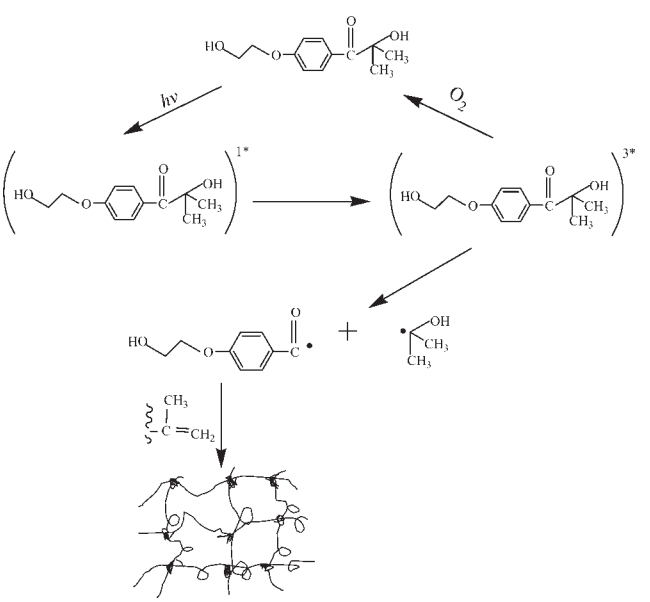


Figure 1 (a) Gelation time of 2% CML with different degree of MA substitution in water solution as a function of initiator concentration. (b) Gelation time of 2% CM34L33 in different medium solution with an initiator concentration of 0.05%.

Gelation time

Since the water soluble CML contains polymerizable C=C bonds, gelation is inspired by photoinitiation to obtain covalent linkage between the polymer chains, forming transparent chitosan hydrogel. Here Irgacure2959 is chosen as the photoinitiator, since it has the least cytotoxicity as reported before.[20,26–29] It is recognized that one of the general requirements for an injectable biomaterial is that the polymer sol should have long enough fluidity for the convenience of handling, but can rapidly form a gel upon required. Since both the C=C double bond content and the Irgacure2959 concentration may have major influence on the gelation time, these factors were investigated first and the results are shown in Figure 1(a). The gelation time decreased rapidly along with the increase of Irgacure2959 concentration initially, but varied minimally when the concentration of Irgacure2959 reached 0.05%. No significant difference was found until 0.15%. At a given initiator concentration, the gelation time is shorter for the CML with a higher degree of MA substitution except for CM46L33, which has no significant difference with CM34L33. At an initiator concentration of 0.05%, both CMLs have a gelation time of $\sim$ 10 min. Under the irradiation of UV light, Irgacure2959 is excited to split into benzoyl and 2-hydroxypropyl radicals as shown in Scheme 1. The former is primarily responsible for initiation.[30] Since the polymerization was performed in water with a macromonomer concentration not larger than 4%, the autoacceleration of polymerization speed, which usually encounters in bulk polymerization, has not appear until complete gelation. With a higher concentration of initiator more radicals are produced at a given period. Consequently, the double bonds have more chance to be crosslinked. At still higher concentration, Senich and Florin regarded that the initiator will aggravate the light penetration through the sample by light scattering, leading to decrease of the initiator's efficiency.[31] However, we found that the absorbance of the CML solution with different concentration of Irgacure2959 had no significant difference. In our opinion, the available C=C bonds within a definite volume may take a more important role. In fact, formation of the covalent linkage requires that the C=C bonds are spatially approachable. In case that the approachable number of C=C bonds is limited, the reaction between the C=C bonds, hence the gelation time should be constant regardless of high concentration of the free radicals. That the CML with higher degree of MA substitution (hence higher C=C bond concentration within the same spatial volume) has shorter gelation time confirms partially this explanation.



Scheme 1 Reaction scheme of Irgacure 2959 to initiate the polymerization, forming crosslinked hydrogel. 1* and 3* denote the excited photoinitiator and triplet excited photoinitiator, respectively. O2 can quench the excited initiator from excited state to its ground state.

Other factors such as oxygen and ions are known to influence the radical polymerization. Figure 1(b) shows that for the same CML and initiator concentration, the gelation time was slightly shortened in PBS, while largely increased to 13 min in DMEM. After the CML solution was purged with N_2 to remove the O_2, the gelation times in all the mediums were shortened, in particular in DMEM. It is known that oxygen can inhibit radical photopolymerization by quenching the triplet excited photoinitiator and by reaction with the radicals.[30] Influence of ions is rather complicated. Generally, higher ionic strength screens the charge repulsion between the CML molecules, and increases the hydrophobicity of the polymers. Consequently, the hydrophobic C=C bonds have large chance to spatially approach, thereby leads to shorter gelation time. The DMEM used in this research contains various amino acids, Riboflavin (0.4 mg/L), Thiamine hydrochloride (4 mg/L) and pyridoxine hydrochloride (4 mg/L) etc. It is known that the Riboflavin is free radical scavenging agent,[32] thereby the gelation time in DMEM is longer than that in PBS.

Hydrogel swelling

Hydrogel swelling is related with the hydrogel network and mass transport characteristic.[33,34] Figure 2 presents the dynamic swelling of the hydrogels made from CML with different degree of MA substitution. Along with the prolongation of incubation time, the dry hydrogels increased their weights rapidly within the first 2 h, then increased slowly till the longest detecting time of 24 h. No significant difference in the swelling dynamics was found for all the hydrogels, although the swelling ratio of the hydrogel with a larger degree of MA substitution, for example CM46L33, was smaller. In the next study, the incubation time is set as 24 h to compare the influence of other factors.

The swelling ratio decreased along with the degree of MA substitution as shown in Figure 3(a). At a higher degree of MA substitution, the crosslinking density of the resultant hydrogel is larger, leading to the smaller swelling ratio. Figure 3(a) shows also that the swelling ratio decreased at higher CML concentration. This is understandable since the crosslinking density per volume is improved, which enables the hydrogel less swelling. Since the CML has $—NH_2$ groups in its molecular chain, pH mediated swelling of the hydrogel can be thus expected. Figure 3(b) shows that along with the increase of the medium pH value, the swelling ratio decreased almost linearly until pH 9, after which more rapid decrease ($P < 0.05$) was observed. The p*K*a of the amine group of chitosan is about 6.5.[35] Therefore when pH is lower than 7, the $—NH_2$ groups gain extra H^+ ions to form $—NH_3^+$, which bring charge repulsion between the molecular chains. Therefore, the chitosan network is in an expansion state. By contrast, when pH is higher than 7, deprotonation of the $—NH_3^+$ groups occurs. Consequently, the chitosan molecular chains tend to aggregate with each other, leading to the shrinkage of the network.

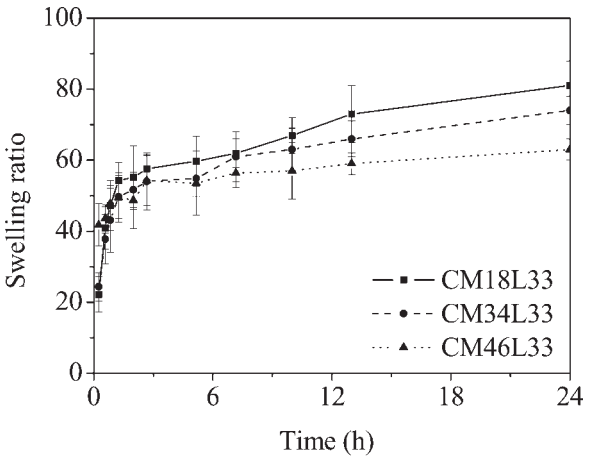


Figure 2 Swelling ratio of dry chitosan hydrogel formed by CML33 with different degree of MA substitution as a function of balancing time at 37°C.

In vivo application of the hydrogel will inevitably encounter the question of salt influence. Figure 3(c) shows that the swelling ratio in PBS and DMEM is significantly smaller than that in water. This is understandable as a result of charge screening brought by the ions in the salt solution. Detail influence of the salt concentration and salt types is shown in Figure 3(d). Again the swelling ratio of the hydrogel in all kinds of salts decreased along with the increase of salt concentration. Compared with the mono valent Cl^- ion, the bi-valent SO_4^{2-} and HPO_4^{2-} ions have stronger ability to reduce the swelling ratio of the hydrogel at the same salt concentration. On the other hand, no significant difference could be found for the cations such as Na^+, K^+, and Ca^{2+}. This is reasonable since the chitosan hydrogel has excess positive charge, thus attracts the anions which in turn cause the hydrogel deswelling. Besides the charge screening effect, the bi-valent anions may further bind with two amino groups, leading to higher efficiency of deswelling at the same salt concentration.

Rheological properties

It is known that the mechanical strength is one of the critical factors controlling the quality of the regenerated tissues including the amount and compositions of the ECM. For the polymerizable hydrogels, their mechanical strength can be easily tailored by many factors such as concentration of the

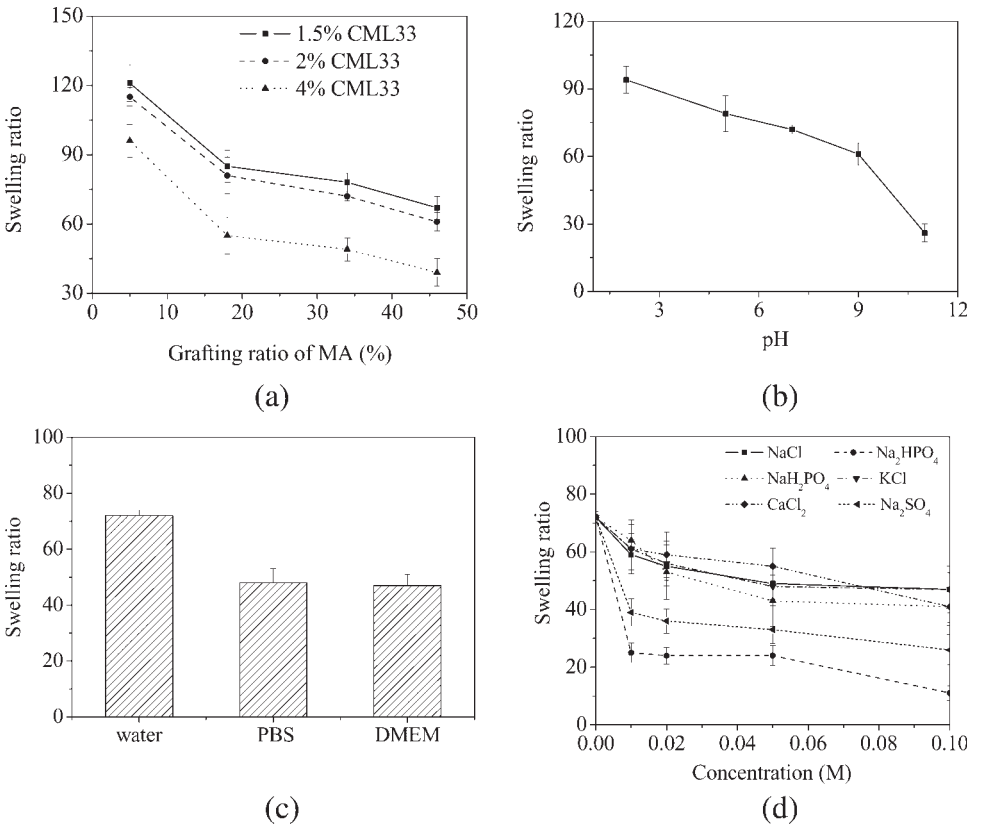


Figure 3 (a) Swelling ratio of dry chitosan hydrogel formed by different CML33 concentration as a function of degree of MA substitution. Swelling ratio of dry chitosan hydrogel formed by 2% CM34L33 as a function of (b) pH value, (c) different medium at neutral pH and (d) salt concentration of different kinds of salts. The balancing temperature and time were 37°C and 24 h, respectively.

precursory polymers and crosslinking degree of the final hydrogels.[36,37] Since the modulus of most hydrogels including the present one is rather small, dynamic force measurement is often used to determine the viscoelastic behavior. As shown in Figure 4, the storage modulii [Fig. 4(a,c)] are nearly 10 times higher than the loss modulii [Fig. 4(b,d)] over a frequency range of 10^{-1}–10^{2} rad/s, indicating that the hydrogels are elastomeric. Along with increase of the frequency, both kinds of modulii increase slightly, with no sign of hydrogel breakage in the measuring range. This is apparently different with the uncrosslinked polymer system such as pure chitosan[38] and polypropylene-poly(styrene-*co*-acrylonitrile) blend,[39] in which the storage modulus increases rapidly along with the frequency increase. Generally, at lower frequency the polymer chains have longer time to slip, resulting in a smaller modulus, whereas at higher frequency the physically entangled chains have less chance to slip, yielding a larger modulus.[40] Since the hydrogels are already crosslinked, which inhibits the relative slippery of chitosan chains, the storage and loss modulii are less influenced by the frequency change.

The storage modulii are less sensitive to the hydrogel concentration than the loss modulii, as shown in Figure 4(a,b) respectively. The reason for the insensitivity of the storage modulus to the CML concentration is not quite clear at present. One possible explanation can be that the variable range of the concentration is too narrow to produce significant difference. In contrast to the concentration, degree of MA substitution has significant influence on both kinds of modulii [Fig. 4(c,d)], especially when it increases from 5 (CM5L33) to 34% (CM34L33). Higher degree of MA substitution can obtain a hydrogel with a higher crosslinking density with a stronger ability to resist deformation.

Weight loss

Chitosan degrades slowly *in vitro*. In the existence of lysozyme, however, the degradation speed can be intensely accelerated. Figure 5 shows that in lysozyme solution the chitosan hydrogels lost their weights almost linearly during the initial stage until the weight loss reached $\sim$ 90%. With a higher crosslinking degree, the time for the 90% weight loss is

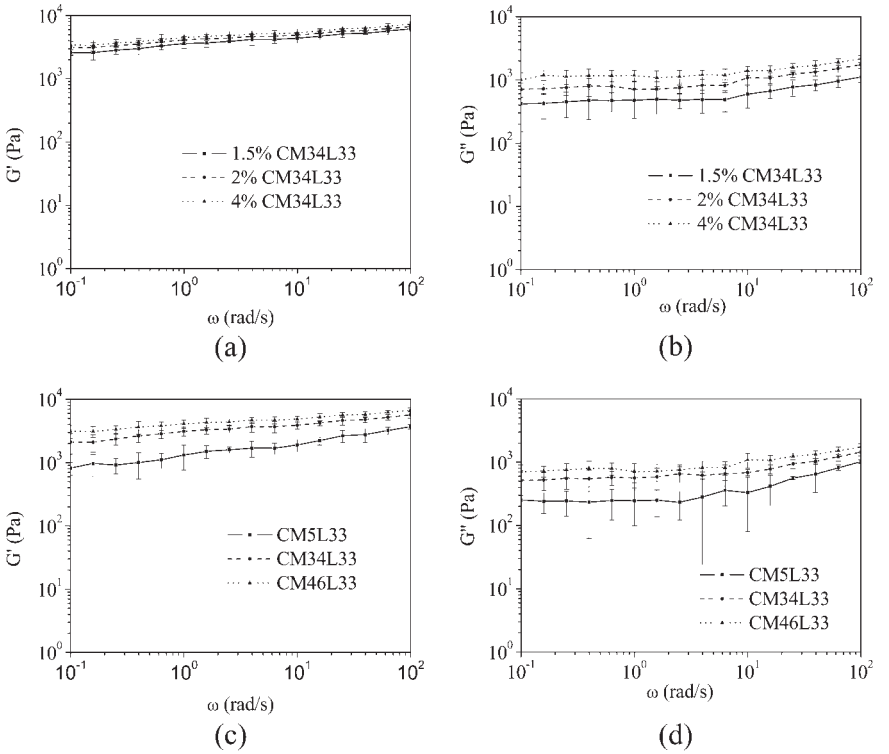


Figure 4 (a,c) Storage modulus and (b,d) loss modulus of chitosan hydrogels as a function of compressing frequency (ω). (a,b) Influence of CM34L33 concentration; (c,d) Influence of degree of MA substitution. Strain and temperature were set as 1% and 37°C, respectively.

also longer, e.g., 6 days for the CM5L33 and CM34L33 hydrogels, and 10 days for the CM46L33 hydrogel. This result implies that although in many other aspects the CM34L33 and CM46L33 are similar, the degree of the crosslinking networks should be different, at least in terms of the ability to resist degradation. It is known that the *in vivo* degradation speed of biomaterials is generally faster than that *in vitro* because of the existence of various enzymes. Therefore, in the cell culture study CM46L33 hydrogel was chosen for the sake of longer handling time.

Cytotoxicity

To assess the influence of initiating systems on the cell viability, *in vitro* culture of chondrocytes were performed by feeding with the extractant from the hydrogels (without cells) made by the redox initiators and the photoinitiator. Here two groups of samples were compared: (1) 2.5 m*M* Irgacure2959 (PI6) and 5 m*M* APS/TEMED (RI6); (2) 5 m*M* Irgacure2959 (PI4) and 10 m*M* APS/TEMED (RI4). With a chondrocyte concentration of 5×10^4/mL the gelation time for group (1) and group (2) is 6 and 4 min, respectively. Figure 6 compares the cell viability of the two groups. The chondrocytes of the PI6 group can normally proliferate along with the culture time, with no significant difference with the DMEM/FBS control group, demonstrating the nontoxicity of PI6. Even at a doubled photoinitiator' concentration (PI4), the chondrocytes could still proliferate along with the culture time, but the cell viability was largely decreased. In contrast to the PI group, the RI6 already showed half cell viability of the DMEM/FBS control in each detecting time, though the cell viability could still increase. At a still higher

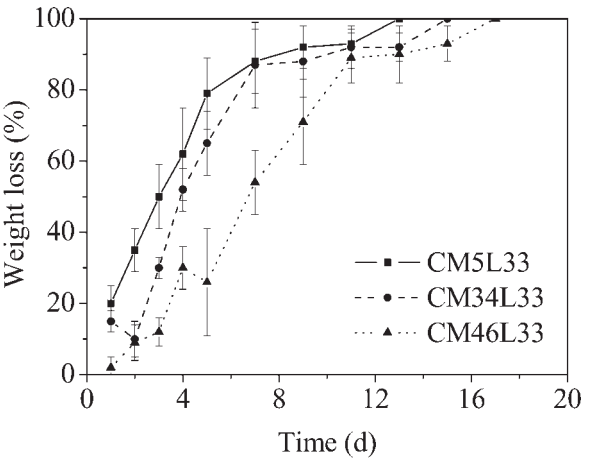


Figure 5 Weight loss of different chitosan hydrogels in 1 mg/mL lysozyme/PBS at 37°C as a function of incubation time.

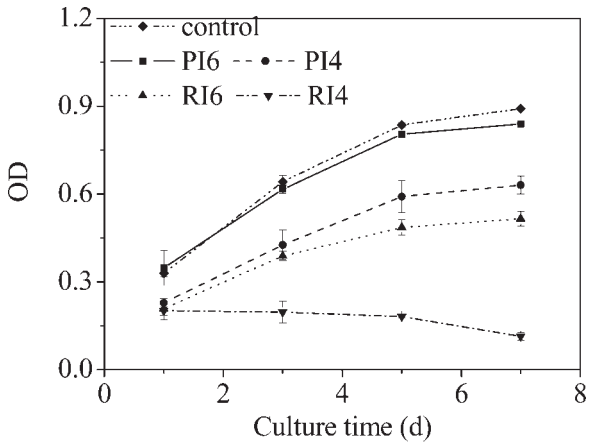


Figure 6 Optical density of chondrocytes after incubated with MTT as a function of culture time. The chondrocytes were cultured in 10% FBS/DMEM, or in 10% FBS/DMEM supplemented with extractant of 2% CM34L33 hydrogels, which were made by photo-initiated (PI) polymerization (10 min under UV) with Irgacure2959 concentration of 2.5 m*M* or 5 m*M*, or redox-initiated (RI) polymerization (10 min at 37°C) with 5 m*M* or 10 m*M* APS/TEMED. Cell seeding density was 1×10^4/well.

concentration of redox initiators (RI4), the cell proliferation was completely inhibited, i.e., the cell viability decreased along with the culture time. Fluorescence microscopy images shown in Figure 7 confirm the cell viability results, where the DMEM/FBS group [Fig. 7(a)] has the largest number of viable cells, whereas only few cell clusters were found for the RI4 group [Fig. 7(e)]. All these results confirm that the photoinitiator adopted in this work has much smaller cell toxicity to chondrocytes, endowing the obtained chitosan hydrogel more suitable for tissue regeneration.

CONCLUSIONS

Gelation of the water-soluble CML is conveniently performed by a photoinitiator, Irgacure2959, under mild conditions. The gelation time is mediated by factors such as degree of MA substitution, initiator concentration, existence of salt and oxygen. A higher initiator concentration, larger degree of MA substitution and absence of oxygen can shorten the gelation time. The dry hydrogels can adsorb water by a factor of tens to hundred. The swelling ratio is smaller at larger crosslinking density, higher pH value, and in salt solution, especially in Na_2SO_4 and Na_2HPO_4 solution. Rheological measurement records the storage modulus and loss modulus of 0.8–7 kPa and 10–100 Pa, respectively. With a higher degree of MA substitution, the resultant hydrogel has a larger modulus too. *In vitro* chondrocyte culture shows that the cytotoxicity of the hydrogel made by Irgacure2959 is much smaller than that of the hydrogel made by APS/TEMED at a same gelation time (with the presence of cells). In conclusion, the chitosan hydrogel made by photoinitiator has good comprehensive performance and less cytotoxicity, thus is more suitable for biomedical applications.

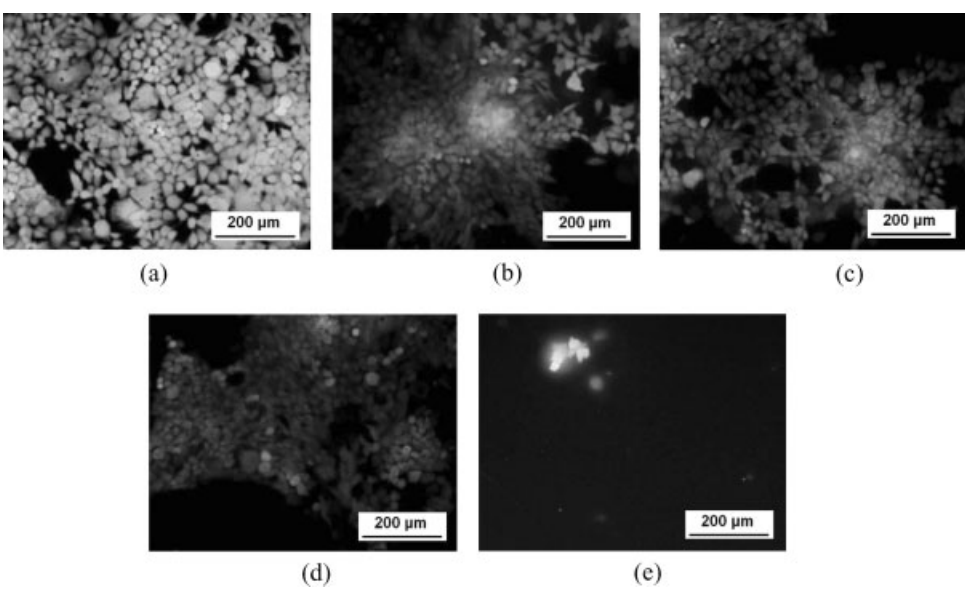


Figure 7 Chondrocytes observed by fluorescence microscopy after the cells were stained with fluorescein diacetate. The cells were cultured for 3 days in (a) 10% FBS/DMEM only, and (b–e) 10% FBS/DMEM supplemented with extractant of 2% CM34L33 hydrogels, which were made by photo-initiated (PI) polymerization (10 min under UV) with Irgacure2959 concentration of (b) 2.5 m*M* and (c) 5 m*M*, and redox-initiated (RI) polymerization (10 min at 37°C) with (d) 5 m*M* and (e) 10 m*M* APS/TEMED. Cell seeding density was 1×10^4/well.

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