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Research paper

Preparation and properties of an injectable scaffold of poly(lactic-co-glycolic acid) microparticles/chitosan hydrogel

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ABSTRACT

Hydrogels are more and more attractive in biomedical fields, since they can be used as injectable scaffolds, drugs and gene carriers and smart sensors. The highly hydrated hydrogels, however, generally have low mechanical strength. In this work, a composite chitosan hydrogel was prepared by blending water soluble and crosslinkable chitosan derivative (CML) with poly(lactic-co-glycolic acid) (PLGA) particles whose surfaces were grafted with double carbon bonds containing gelatin (GM), following gelation under UV irradiation. The as-prepared composite hydrogel showed lower swelling ratio than that of the CML hydrogel, and higher elastic stiffness (i.e. storage modulus) than that of the CML hydrogel and the hydrogel filled with the same amount of PLGA particles or gelatin modified PLGA particles. Moreover, the storage modulus of the composite hydrogel was increased with the amount of GM modified PLGA particles. In vitro chondrocyte culture revealed that viability of the cells co-cultured with the GM modified PLGA particles was higher than that of the cells co-cultured with the unmodified PLGA particles. The composite hydrogel blended with the GM modified PLGA particles also showed higher cytoviability than that of the original CML hydrogel after 9d culture.

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1. Introduction

Injectable biomaterials are increasingly attractive in fields of drug delivery and tissue engineering, because they have the advantages of a minimally invasive procedure of injection (Tememoff and Mikos, 2000) and of filling irregular defects of tissues such as cartilage (Kretlow et al., 2007). Requirements for the injectable biomaterials include biocompatibility, biodegradability, mild solidification conditions such as neutral pH, body temperature and a nontoxic initiating system, and formation of suitable pores for tissue ingrowth. Their mechanical properties such as elasticity and bending strength are required to match the specific applications, for example, restoration of cartilage, too (Kretlow et al., 2007).

Among the injectable biomaterials investigated so far, hydrogels are more and more attractive in the field of tissue engineering (Chenite et al., 2000; Ibusuki et al., 2003; Lee et al., 2003; Balakrishnan and Jayakrishnan, 2005). In body fluid they form a highly hydrated structure (Lee and Mooney, 2001; Drury and Mooney, 2003; Hoffman, 2002) which mimics the natural extracellular matrix (ECM) such as cartilage

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(Hoffman, 2002). Many natural and synthetic polymers such as collagen, gelatin, chitosan, alginate, chondroitin sulphate (CS), poly(ethylene glycol) and poly(propylene fumarate) (PPF) are fabricated into injectable hydrogels (Emily et al., 2006; Dimpy et al., 2006). In our previous work, covalently crosslinkable and water soluble chitosan (CML) was prepared by grafting methacrylic acid (MA) and lactic acid (LA) onto the chitosan chains (Hong et al., 2006). The modified chitosan has good solubility at neutral pH, and can form a chemical hydrogel at body temperature under the initiation of a redox system (ammonium persulfate /N, N, N', N'-tetramethylethylenediamine) (Hong et al., 2006). However, the redox system showed some extent of cytotoxicity. More recently, gelation of the CML was also achieved by a photoinitiating system using Irgacure2959, a photoinitiator which showed very small cytotoxicity (Williams et al., 2005; Wang et al., 2007, 2003; Li et al., 2004).

Particles with a size ranging from nanometer to micrometer are another kind of injectable materials. Considerable numbers of works and reviews have described the processing and use of some microcarrier materials made of poly(lactic-*co*-glycolic acid) (PLGA) (Jain, 2000; Jiang et al., 2005), gelatin (Young et al., 2005) etc. It is known that PLGA has no cytotoxicity (Hollinger and Battistone, 1986), good mechanical properties and controllable biodegradability (Yoon et al., 2004). PLGA micro- or nanoparticles containing drugs can be embedded into the hydrogel to reduce the release rate as well (Chen et al., 2007; Chung, 2007; Zhang et al., 2005).

However, compounding of the hydrogel with the PLGA particles has hardly ever been reported for aiming at improving the hydrogel mechanical strength, which is a critical issue for scaffolding materials. In this work, a biocompatible and crosslinkable gelatin derivative shall be immobilized onto the PLGA particles, which are further suspended in the CML solution. Under the initiation of Irgacure2959, the CML hydrogel containing the PLGA particles was finally obtained that showed improved mechanical strength and cytocompatibility. To our best knowledge, design of injectable materials in this way has not been reported previously, especially the integration of polymer microparticles having a polymerizable hairy layer. Since the reaction is very common, this concept is not restricted to the present system. Other hydrogels and other types of particles can be similarly integrated to obtain various composite structures, which may find extensive applications in the biomedical field.

2. Experimental section

2.1. Materials

Poly(lactic-*co*-glycolic acid) (PLGA, 85/15 lactide/glycolide ratio, Mn = 108 kDa, Mw = 203 kDa) was purchased from China Textile Academy, China. Poly(vinyl alcohol) 124 (PVA 124, Mw 85 kDa–124 kDa, 98%–99% hydrolyzed) was purchased from Shanghai Medicine and Chemical Company, China. Chitosan (average $M_\eta \approx 600$ kDa) was obtained from Haidebei Marine Bioengineering Company, Ji'nan, China. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), 2-morpholinoethane sulfonic acid (MES), trypsin, collagenase type II, Dulbecco's minimum essential medium (DMEM) and 3-(4,5-dimethyl)thiazol-2-yl-2,5-dimethyl tetrazolium bromide (MTT) were purchased from Sigma. BCA protein assay kit was purchased from Beyotime Institute of Biotechnology, China. Fetal bovine serum (FBS) was purchased from Sijiqing biotech. Co., China. 2-Hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (Irgacure2959) was obtained from Ciba Specialty Chemicals. Methacrylic acid (MA) was purified via distillation under reduced pressure. All other chemicals and reagents were used as received.

2.2. Preparation of PLGA particles

PLGA particles were prepared by an emulsion-solvent evaporation technique (Bodmeier and McGinity, 1986). Briefly, 4 mL 2% PLGA/CH_2Cl_2 solution was rapidly poured into 20 mL 7% (w/v) PVA solution under magnetic agitation. The agitation was maintained for 4 h at 25 °C to evaporate the organic solvent. The produced particles were collected by centrifugation, washed with triple-distilled water 5 times. Finally, the PLGA particles were obtained by freeze-drying at −60 °C with a pressure of 7–8 Pa. The average diameter of the PLGA particles was 820 ± 121 nm measured by scanning electron microscopy (SEM, SIRION 100, FEI).

2.3. Synthesis of gelatin-g-MA

Gelatin-g-MA (GM) was obtained by grafting methacrylic acid (MA) onto the gelatin chains. 400 mg EDC (2.1 mmol) was added into 100 mL 1% gelatin solution containing 800 μL MA (9.4 mol) under magnetic agitation. The reaction was maintained for 8 h at room temperature. In order to remove the unreacted MA and other small molecular weight products, the resulted 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 d. Finally, gelatin-g-MA was obtained by freeze-drying as mentioned above. The C and N contents were determined by elemental analysis (Eager 300) (Hong et al., 2006).

2.4. Grafting of GM or gelatin on the PLGA particles

The PLGA particles were covalently immobilized with gelatin or GM by a reaction between the –COOH groups on the chain termini of the PLGA and the –NH_2 groups of gelatin. Briefly, 5 mg PLGA particles were dispersed into 2.5 mL 1% MES buffer solution. Then 15 mg (0.079 mmol), 35 mg (0.183 mmol), 50 mg (0.262 mmol) or 100 mg (0.524 mmol) EDC and NHS with a mole ratio to EDC of 0.5 were added. The reaction was performed for 10 min under agitation at 1000 rpm. Then 2.5 ml 2% gelatin solution or GM solution containing 2.5 mg (0.012 mmol) MES was added. The reaction was maintained for 4 h under agitation at 1000 rpm. The modified PLGA particles were purified by centrifugation and washing with triple-distilled water 5 times. Finally, the modified PLGA particles were obtained by freeze-drying as mentioned above. The gelatin and GM content on the PLGA particles was determined by BCA protein assay kit (Zheng et al., 2004).

2.5. Synthesis of CML

Chitosan-g-MA-LA (CML) was obtained by grafting MA and LA onto the chitosan chains by see (Hong et al., 2006). Briefly, 200 mg (1.05 mmol) EDC was added into 100 mL 0.8% (w/v) chitosan solution containing 400 μL (4.7 mmol) MA under magnetic agitation. The reaction was maintained for 24 h at room temperature. Following the purification steps described in the synthesis of GM, CM was obtained. 200 mg (1.05 mmol) EDC was added into 100 mL 0.7% (w/v) CM solution containing 400 μL (4.7 mmol) LA. The mixture was stirred for 24 h at room temperature. Following the purification steps described in synthesis of GM, CML was obtained. The grafting ratios of MA and LA were determined by elemental analysis (Hong et al., 2006).

2.6. PLGA particles suspension in the CML solution

No obvious sedimentation was observed within 8min when 5 mg PLGA particles was mixed with 2% CML solution at room temperature.

2.7. Gelation of the CML

10 mL 2% CML solution containing 0.05% Irgacure2959 (photoinitiator) was incubated at 37 °C for 8 h. Then 50 mg control or GM (25 mg also) or gelatin modified PLGA particles were dispersed in the CML solution. 200 μL of the mixtures was injected into a 1 mL polypropylene test tube. Then the tube was irradiated by 365 nm UV light with a power of $\sim 10\ mW/cm^2$. The gelation time was recorded at which the solution lost its fluidity.

2.8. Swelling ratio of the hydrogel

200 μL of the mixtures described above was injected into a 1 mL polypropylene test tube. The hydrogel was obtained by irradiation with UV light for 15 min. The hydrogel was freeze-dried as mentioned above and weighed (W_0). The dry hydrogel was hydrated in water at 37 °C. 24 h later the hydrogel was taken out and blotted with filter papers to remove the excess water on the outside of the hydrogel, and then weighed (W_1). The swelling ratio of the hydrogel is defined as W_1/W_0.

2.9. Rheological test

2.5 mL of the mixtures described above was injected into a circular and transparent glass mold (25 mm diameter, 0.22 mm height), which was exposed to UV light for 15 min to obtain the hydrogel. The sample was then placed in a parallel plate mode for the rheological measurement by a strain-controlled ARES rheometer (Advanced Rheometric Expansion System, Rheometric). The dynamic oscillatory mode (compression mode) was used to measure the storage and loss modulii. All tests were performed at 37 °C with a fixed strain of 1%.

2.10. Chondrocyte culture

Chondrocytes were isolated from the cartilage tissue of rabbit ears (Japanese big ear white) under the institutional guideline (Hong et al., 2007). Briefly, the cartilage tissue was cut into small pieces. The chondrocytes were isolated by incubating the cartilage pieces in DMEM containing 0.1% collagenase type II at 37 °C for 4 h. The isolated chondrocytes were centrifuged, resuspended in DMEM/10% FBS containing 100 U/mL penicillin and 100 μg/mL streptomycin. The suspension was seeded into an 11 cm plastic tissue culture dish (Falcon) and incubated in humidified atmosphere of 95% air and 5% CO_2 at 37 °C. After a confluent cell layer was formed, the cells were detached using 0.25% trypsin in PBS, and then were used for the experiment.

4 mg control or modified PLGA particles were sterilized by 75% alcohol. After washing by PBS, they were dispersed in 2 mL DMEM/10% FBS in a polypropylene test tube. Then 100 μL of the particle suspension was placed in each well of a 96 well culture plate. After 24 h, 100 μL DMEM/10% FBS solution containing 10,000 chondrocytes was slowly injected into each well along the wall. Cytoviability was assayed after the cells were cultured for 1d, 3d, 5d, and 7d. After supplementing with 20 μL MTT, the cells were continually cultured for another 4 h. During this period, viable cells reduced the MTT to formazan pigment, which was dissolved by 200 μL dimethyl sulphoxide (DMSO) after removal of the culture medium. The absorbance of 100 μL solution at 570 nm was recorded by a microplate reader (Bio-Rad 550) (Hong et al., 2007).

160 mg CML was sterilized under UV irradiation for 1h and then dissolved in 8 mL sterilized PBS to obtain a 2% CML/PBS solution. Then 80 μL 5% Irgacure2959 in 75% alcohol was mixed with the CML/PBS solution, which was incubated at 37 °C for 8 h before use. 20 mg GM modified PLGA particles were dispersed into 4 mL of the above solution. The mixture with or without GM modified PLGA particles was added into a centrifugal tube containing 16,000,000 cells. After sufficient mixing, 200 μL of the cell containing solution was injected into a 1 mL polypropylene test tube, which was then irradiated by UV light for 10 min to form a hydrogel. The hydrogel was removed from the tube and placed in a well of a 24 well culture plate. Then 1 mL DMEM/10% FBS solution was added into each well. Cytoviability was measured after the cells were cultured for 1d, 3d, 6d, and 9d. After supplementing with 100 μL MTT, the cells were continually cultured for another 4 h. The hydrogel was smashed and centrifuged at 11,000 rpm to ensure the complete extraction of the formazan pigment by DMSO.

2.11. Statistical analysis

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

Table 1 – The weight percentage of C and N elements, the C/N mole ratio and the derived grafting ratio for gelatin, GM, chitosan, CM and CML

Sample	C content (w/w, %)	N content (w/w, %)	C/N mole ratio	Grafting ratio (%)
Gelatin	42.36	14.17	3.30	/
GM	43.51	15.06	3.37	54%
Chitosan	40.26	7.36	6.38	/
CM	39.46	5.93	7.76	34% (MA)
CML	40.51	5.42	8.72	32% (LA)

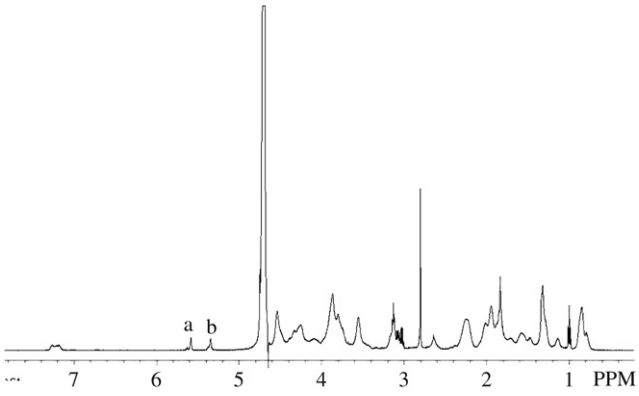

Fig. 1 – ^{1}HNMR spectrum of GM measured in D_2O.

3. Results

3.1. *Synthesis of gelatin-g-MA and CML*

MA was grafted onto gelatin chains to obtain the gelatin-g-MA (GM). The ^{1}HNMR spectrum of GM (Fig. 1) confirms the existence of MA, since the typical $\delta = 5.64$ (a) and $\delta = 5.36$ (b) are assigned to the protons of $\underline{H}_2C{=}C(CH_3){-}$, which were not found in the spectrum of original gelatin (not shown). The grafting ratio of MA is defined as the substitution degree of available $-NH_2$ groups on the gelatin chain, which was determined by elemental analysis in this work (Table 1). The mole ratio of C/N of the GM was increased from 3.30 (the original gelatin) to 3.37. The net increase value of 0.07 is contributed by MA (4C in one molecule), which corresponds to a MA/N mole ratio of 0.07/4. Since the $-NH_2$/N mole ratio of gelatin is 31 (Marc et al., 2007), the MA grafting ratio was finally found to be $31 \times 0.07/4 = 54\%$. Spectroscopic verification of MA and LA grafting onto the chitosan chains has been shown previously (Hong et al., 2006). By a similar calculation using the C/N ratios shown in Table 1, the grafting ratios of MA and LA were found to be 34% and 32% respectively.

3.2. *Modification of PLGA particles*

The PLGA particles with a spherical morphology (Fig. 2(a)) were prepared by an oil-in-water (O/W) single emulsion method. Immobilization of GM onto the PLGA particles was accomplished by surface grafting under EDC catalyzation. The GM content on the particle surface as a function of EDC concentration is depicted in Fig. 3. The GM content increased with an increase of the EDC concentration when the EDC concentration was lower than 10 mg/mL, then leveled off. No significant difference was found between 10 mg/mL and 20 mg/mL of EDC. The surface modification did not bring apparent variation on the particle morphology (Fig. 2(b)), except that the effective particle diameter was increased from 884 nm to 1.1 μm measured by DLS.

3.3. *Gelation time*

In the next step, the control or the modified PLGA particles were mixed with the CML to obtain a composite hydrogel. The CML was fixed at 2% (w/v), into which 0.05% (w/v) Irgacure2959 was added. Here 3 types of PLGA particles were compared: GM modified (the GM content was 11.4 ± 0.8 μg/mg PLGA particles), gelatin modified and the blank control. Firstly, gelation time of the composite hydrogel was studied. The gelation time for all the samples was $\sim$ 8 min, regardless of existence of the PLGA microspheres as well as their amount and surface chemistry. Morphology of a typical freeze-dried hydrogel was shown in Fig. 2(c). No apparent difference between all the samples was found.

3.4. *Swelling*

The swelling property of the as-prepared hydrogels is crucial for substance exchange when they are used as injectable scaffolds (Lin and Metters, 2006). Fig. 4 shows that the swelling ratio of the composite hydrogel was gradually decreased along with an increase of the GM modified PLGA particles, whereas no difference was found between different kinds of particles.

3.5. *Viscoelasticity*

Mechanical properties of the hydrogels were studied by a dynamic mechanical analysis method (Fig. 5). Along with the increase of the GM modified particles (the GM content was 11.4 ± 0.8 μg/mg PLGA particles), the storage modulus of the composite hydrogels were improved correspondingly, whereas the loss modulus was not altered significantly. Moreover, the composite hydrogel filled with 5 mg/mL GM modified PLGA particles had a larger storage modulus than those filled with the same amount of gelatin modified and blank control PLGA particles.

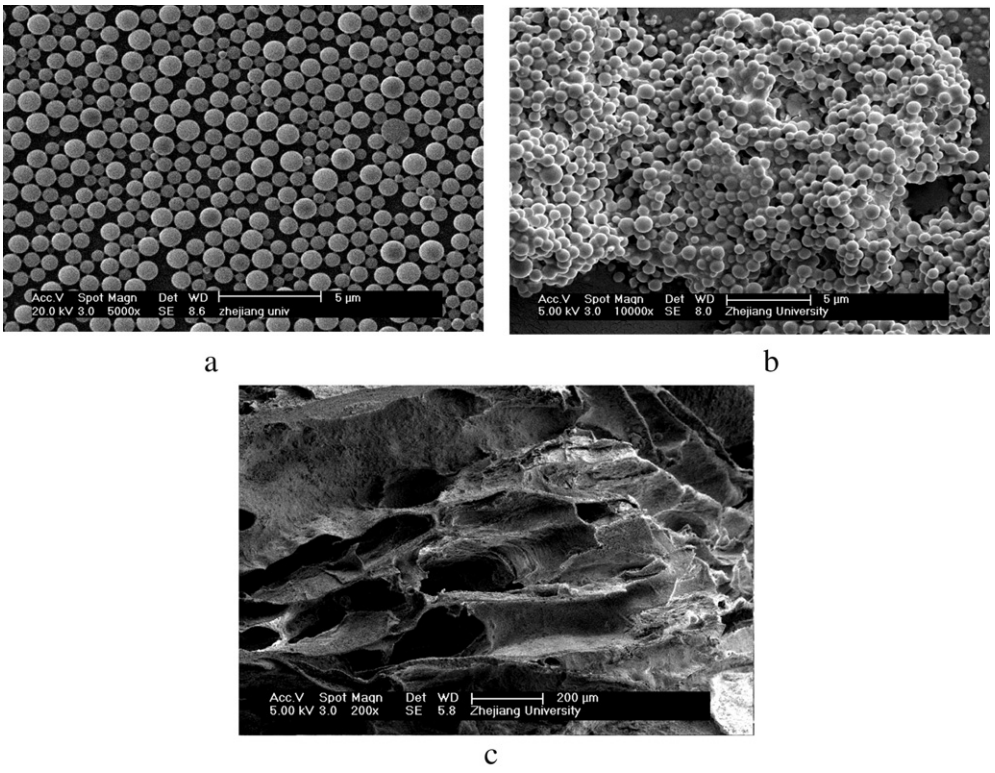

Fig. 2 – SEM images of PLGA particles before (a) and after (b) immobilization of polymerizable gelatin on their surfaces. (c) A typical SEM image showing the freeze-dried composite hydrogel containing 5 mg/mL GM modified PLGA particles.

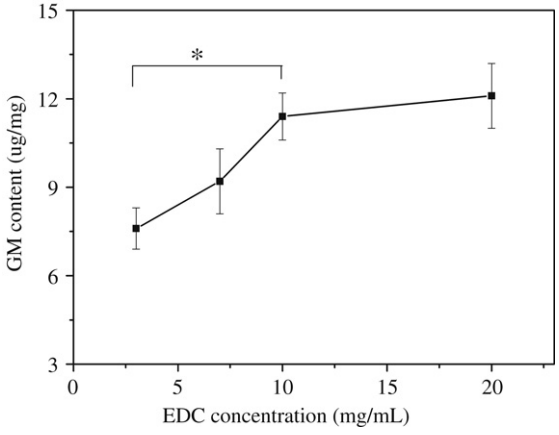

Fig. 3 – GM content on the PLGA particles as a function of EDC concentration. Sample was prepared by incubation of 5 mg PLGA particles in 5 mL 1% GM solution. NHS:EDC molar ratio was kept as 0.5. * $p < 0.05$.

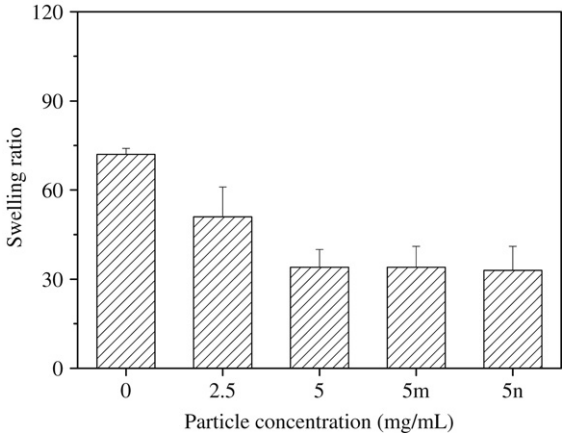

Fig. 4 – Swelling ratio of composite CML hydrogels containing different amount of GM modified PLGA particles. 5m: a hydrogel containing gelatin modified PLGA particles; 5n: a hydrogel containing control PLGA particles.

3.6. *Cytoviability of the microparticles*

Fig. 6 compares the OD values recorded from the chondrocytes co-cultured with different types of PLGA particles. From day 1 to day 3, all the OD values were increased, illustrating that the cells could proliferate in the existence of the PLGA particles. At day 5 and day 7, the OD values dropped again. At the same culture time, for example at day 3 and day 1, significantly higher OD values were recorded from the chondrocytes co-cultured with gelatin (the gelatin content was 12.4 ± 1.8 μg/mg PLGA particles) and GM (the GM content was 11.4 ± 0.8 μg/mg PLGA particles) modified PLGA particles than that from the control PLGA particles ($p < 0.05$), whereas no significant difference was found between the gelatin and the GM modified particles.

3.7. *Cytoviability of the composite hydrogels*

As mentioned above existence of the PLGA particles did not bring significant influence on the gelation time, i.e. the

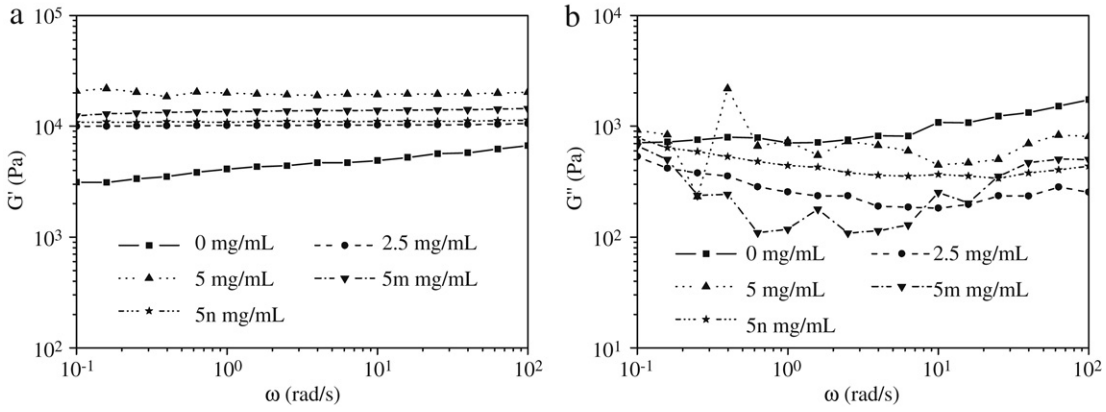

Fig. 5 – (a) Storage modulus and (b) loss modulus of the composite hydrogels containing different amount of GM modified PLGA particles as a function of compressing frequency (ω). 5m: a hydrogel containing gelatin modified PLGA particles; 5n: a hydrogel containing control PLGA particles.

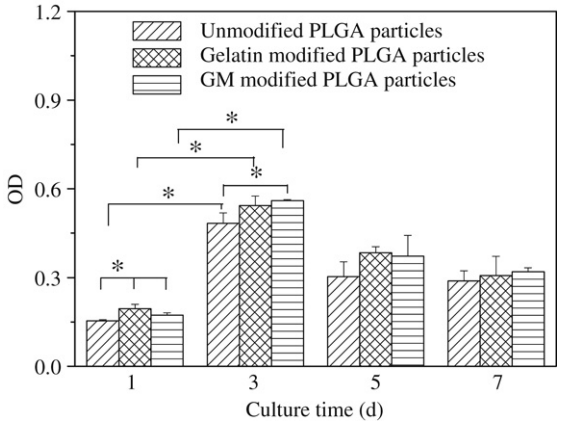

Fig. 6 – Optical density measured from different samples as a function of culture time. Cell seeding density 1×10^4/well.

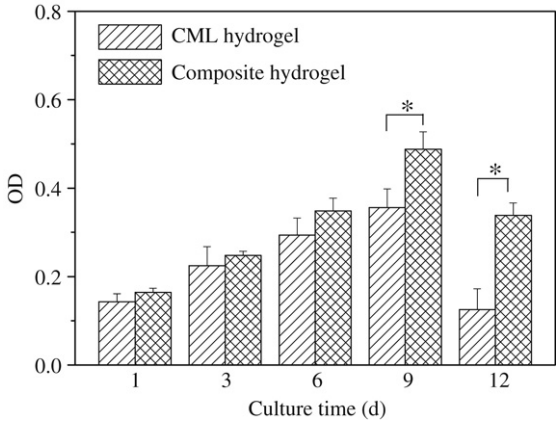

Fig. 7 – Optical density measured from CML hydrogel with or without GM modified PLGA particles as a function of culture time. Cell seeding density 4×10^6/mL; particles density 5 mg/mL. * $p < 0.05$.

gelation time is all around 8 min. In our experiment we found, however, that the gelation time was shorter when the cells were incorporated. Therefore, in order to maximally reduce the potential impairment of the UV light on the live cells, if any, the hydrogels containing the cells were all made by 10 min irradiation. The composite hydrogel containing 5 mg/mL GM modified particles and the control CML hydrogel were then similarly assessed in terms of cytoviability (Fig. 7). The OD values recorded from both samples increased steadily with prolongation of the culture time until 9d, and then dropped again. The inhibition time was postponed pronouncedly because unlike the 2D culture as shown in Fig. 6 the 3D hydrogels can provide more space for the cell growth. Significant higher OD values were recorded for the composite hydrogel only after 9d culture.

4. Discussion

It is known that the hydrogels have generally low mechanical strength, which retards many of their applications in practice. In this study, two types of injectable materials were integrated to obtain a composite structure with better comprehensive properties, especially improved mechanical strength. For a composite material, the interfacial action between the components is one of the critical factors influencing the final properties (Hamed, 2000). For this context, the PLGA particles were decorated with a hairy layer of gelatin (GM) molecules, which contain polymerizable double carbon bonds. The results show that these GM molecules could copolymerize with the CML to chemically embed the PLGA particles into the crosslinked CML networks. Since gelatin is a denatured product of collagen, such a modification can improve the cytocompatibility as well (Malafaya et al., 2007).

By spectroscopic and elemental analyses, grafting of MA and LA onto the chitosan chains to obtain the CML was confirmed. This grafting can largely reduce the inter-chain hydrogen bonding interaction. Consequently, unlike its precursory chitosan, the obtained CML is readily soluble in neutral water, which is substantial for usage as an injectable material. Since each sugar unit has a $-NH_2$ group, the 34% grafting ratio of MA implies that on average 1/3 of the $-NH_2$ groups were transformed into amides of MA, which ensures a

sufficient crosslinking between the chitosan chains. Grafting of MA onto the gelatin molecules was confirmed by ^{1}HNMR spectrum (Fig. 1). Elemental analysis further proved that the grafting ratio of MA is 54%, implying that half of the $-NH_2$ groups were transformed into amides of MA. Grafting of GM and gelatin molecules onto the PLGA particles was confirmed by a BCA method. The grafting amount was quickly leveled off at a given concentration of GM, which should be attributed to a definite number of available –COOH groups with which the $-NH_2$ groups of gelatin react. The results show that 10 mg/mL EDC is high enough for amidation of the –COOH groups, at which condition the GM content was 11.4 ± 0.8 μg/mg PLGA particles. Grafting of the GM or gelatin did not bring significant influence on the particle morphology, although the size of the particles became slightly larger. We believe that the size increase is mainly attributed to the hairy layer of the gelatin or GM, which is highly hydrated in water and contribute greatly to the hydrodynamic radius.

Gelation of the CML, regardless of the existence of PLGA and modified PLGA particles, was initiated by Irgacure2959, which can produce free radicals under UV irradiation. For all the systems studied so far, the gelation time is all around 8min. Gelation of the CML and CML composite system is triggered mainly by crosslinking between the CML chains, which makes the solution lose its fluidity upon gelation. The PLGA particles have little chance to deter the photo-initiated crosslinking reaction, thus do not influence the gelation time. The free-dried hydrogels showed a porous structure, with no significant difference between all the systems.

All the hydrogels could be highly swollen in water, which is a result of water absorption and maintenance of the crosslinked chitosan networks. Swelling of the PLGA particles is neglectable compared with that of the hydrogel. Hence, the added particles contribute only to the dried weight of the composites, leading to decrease of the "apparent" swelling ratio. However, the lowest swelling ratio is still as high as 30. Therefore, inclusion of the PLGA particles does not affect the ability of transporting nutrients as well as metabolites.

The CML hydrogel blended with the GM modified PLGA particles has a larger storage modulus than those blended with the same amount of gelatin or blank control PLGA particles. This enhancement can be explained by the energy dissipation mechanism, which relieves local stress concentration and results in a more uniform distribution of load among the whole network (Hamed, 2000). When the force is applied to the composite hydrogels, the CML chains are deformed, dissipating energy to the particles. With more particles the dissipation effect is also stronger, resulting in a larger storage modulus. The crosslinkable particles can be integrated into the CML networks to a larger extent owing to the stronger interfacial action, thus can dissipate energy more efficiently and yield a larger storage modulus than others. That the loss modulus had no significant difference between all the samples implies that their viscosity is almost same. It is reasonable since the viscosity is mainly decided by the continuous phase, namely the chitosan hydrogel, while the contribution of the PLGA particles is negligible since their volume is less than 0.4%. These tests were repeated twice, giving a variability of 2–3 kPa for the storage modulus and several hundreds Pa for the loss modulus. It is known that cartilage tissue has a complex modulus (root-mean-square value of the storage modulus and the loss modulus) of 2×10^6 to 2.5×10^7 Pa with a frequency ranging from 10^{-1} to 10^2 rad/s under small shear/torsion amplitudes (Mow et al., 1992). Therefore, measures must be taken in the future to further improve the mechanical strength of the present systems if they are used as the injectable scaffold for cartilage restoration, although many types of the hydrogels with similar strength have been diversely used for the same purpose (Li et al., 2004).

The cytoviability of the particles firstly increased and then dropped after in vitro culture for 5d, which might be caused by the so-called contact inhibition effect. Nonetheless, the results still show that the gelatin modified PLGA particles have better biocompatibility, and the double bond containing gelatin has the same effect as the original gelatin.

A steady increase of the OD values was recorded for both the CML hydrogel and the composite hydrogel, with no significant difference before 6d. However, significant higher OD values were recorded for the composite hydrogel after 9d culture. Apart from the contribution of the biocompatible PLGA particles, another reason could be that the composite hydrogel has a smaller degree of collapse, an often observed phenomenon for a construct of cells/soft scaffold. Consequently, the cells may have more space for proliferation. This is rather promising since rather long culture time of the construct is usually required, although the in vivo performance may be more or less different.

5. Conclusions

In this work a composite chitosan gel was fabricated by blending with GM (gelatin grafted with methacrylic acid) modified PLGA particles. Some of its physical and biological properties were studied. The GM grafted PLGA particles were obtained by covalent immobilization of GM under the catalyzation of EDC. The GM content on the PLGA particles increased with EDC concentration until 10 mg/ml. When the concentration of the PLGA particles was below 5 mg/ml, the gelation time was not influenced, regardless of existence of gelatin or GM on the particle surfaces. Along with increase of the particle concentration, the swelling ratio of the composite hydrogel decreased, whereas the storage modulus increased. The CML hydrogel blended with the GM modified PLGA particles had a larger storage modulus than those blended with the same amount of gelatin or blank control PLGA particles. Larger cytoviability was recorded from the chondrocytes co-cultured with the gelatin or GM modified PLGA particles than that co-cultured with the blank PLGA particles. Blending the GM modified PLGA particles into the CML hydrogel also yielded higher cytoviability than that of the pure CML hydrogel after a longer culture time. In conclusion, mechanical property and cytoviability of the CML hydrogel can be improved by blending with GM modified PLGA particles. The composite hydrogel might have greater opportunity to be used in chondrogenesis.

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