A Composite Scaffold of PLGA Microspheres/Fibrin Gel for Cartilage Tissue Engineering: Fabrication, Physical Properties, and Cell Responsiveness

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Abstract: A composite scaffold of poly(l-lactic-co-glycolic acid) (PLGA) microspheres and fibrin gel was fabricated by blending fibrinogen-immobilized PLGA microspheres with fibrinogen and thrombin solution. The PLGA microspheres with a size of 70 ~ 100 μm were aminolyzed in a hexanediamine/n-propanol solution to introduce free amino groups on their surface. The fibrinogen immobilization was achieved by glutaraldehyde coupling. When the ─NH₂ content on the microsphere surface was increased from ~2 × 10⁻⁷ mol/mg to ~4 × 10⁻⁸ mol/mg, the fibrinogen amount was correspondingly increased from ~35 μg/mg to ~70 μg/mg. Measured by UV-VIS spectroscopy, the clotting time of the composite was less influenced by the microsphere amount, but mainly controlled by the thrombin concentration. When the thrombin concentration was higher than 15 U/mL, the gelation could be finished within 1 min and yielded a composite with evenly suspended and distributed PLGA microspheres. Blending with the microspheres could significantly improve the elastic modulus of the hydrogel as well, whereas less influence on the chondrocyte proliferation and extracellular matrix production.

Keywords: fibrin; hydrogel; microcarriers; poly(l-lactic-co-glycolic acid); chondrocytes

INTRODUCTION

Nowadays tissue engineering and regenerative medicine has appeared as a new approach in treatment of damaged or disabled tissues/organisms such as bone, cartilage, and skin. Among this study, injectable scaffolds especially the hydrogels such as collagen, gelatin, chitosan, poly(propylene fumarate) (PPF), and poly(ethylene glycol) (PEG) have been extensively investigated with respect to their gelation and cell delivery properties. The hydrogels have the merits of biomimetic structure and function and minimal incision during transplantation because they can be easily injected into the wanted sites and solidified in situ with cells and/or other bioactive components such as cell growth factors. As another type of the injectable scaffolds, the biodegradable microspheres made of poly(lactide) (PLA) and poly(lactide-co-glycolide) (PLGA) are increasingly interesting. They can simultaneously act as cell microcarriers, delivery vehicle for drugs, growth factors, and 3D scaffold by spheres piling. Fast cell proliferation can be achieved since the microspheres have larger surface area. The microcarriers having cells on their surfaces can be directly injected into the defect, piling into a three-dimensional scaffold in vivo to induce cell infiltration and tissue regeneration. The transplanted cells can proliferate and differentiate in situ in a normal histological condition as well. Nonetheless, the microcarriers only are not easy to handle and not spatially stable in the tissue defect. For this context, compounding the microspheres with a binding agent such as fibrin gel may overcome these shortcomings.

It is known that the fibrin gel is a biological adhesive widely used in various surgeries (abdominal, thoracic, vascular, oral, and endoscopic). The fibrin gel is formed by mimicking the last step of the coagulation cascade via activation of fibrinogen by thrombin. One remarkable advantage of the fibrin gel over the other types of biological hydrogels such as collagen and gelatin is that it can be obtained autologously, avoiding the potential risks of
foreign body reaction, and virus infection. Previous results demonstrate also that the cells entrapped in the fibrin gel can produce more collagen and elastin. More importantly, the clotting time can be easily mediated to match different applications. Recently, some impressing and significative progresses have been made by using the fibrin gel as an injectable scaffold. However, the commercially available fibrin glues have lower mechanical strength, tend to disintegrate in vitro and in vivo after several days, and almost completely dissolve within 3–4 weeks. Even after optimization of the gelling parameters that determine the gel stability, the obtained fibrin gel is still not stable enough for long-term cartilage repair.

Combination of the microspheres and the fibrin gel may yield an injectable composite with synergetic effects such as good mechanical strength, stabilization of the microspheres, shape-persistent ability, and biological performance. We would expect that this composite is especially advantageous in cartilage and bone regeneration. Yet to our knowledge this is an unexplored material system so far. In this work, an injectable composite of PLGA microspheres/fibrin gel is developed with the emphasis of good biocompatibility, processibility, and mechanical property. Chondrocyte culture in vitro is conducted to assess the cell responsiveness to the composite scaffold as well.

MATERIALS AND METHODS

Materials

PLGA (85/15 lactide/glycolide ratio, $M_n = 108$ kDa, $M_w = 203$ kDa) was purchased from China Textile Academy, China. Poly(vinyl alcohol) (PVA) 124 ($M_w = 85–124$ kDa, 98–99% hydrolyzed) was supplied by Shanghai Medicine and Chemical Company, China. The source of other reagents is: thrombin (400 U, Sigma-Aldrich), aprotinin (630 U/mg, Roche), and ninhydrin (Shanghai San’aisi Chemical Company, China). Fresh human plasma was kindly donated by Blood Center of Zhejiang Province. Fibrinogen was isolated from the plasma by a freeze-thaw cycle. 1,6-Hexanediamine was purified by distillation under reduced pressure. All other chemicals and reagents were used as received. Triple-distilled water was used throughout the experiment.

Preparation of PLGA Microspheres

The PLGA microspheres were prepared by an emulsion–solvent evaporation technique. Briefly, 2 g PLGA was dissolved in 40 mL methylene chloride ($CH_2Cl_2$) to obtain a transparent PLGA/$CH_2Cl_2$ solution. The solution was poured into 200 mL water containing 0.5% (w/v) PVA under mechanical agitation (400 rpm). The agitation was maintained for 6–8 h at 25°C to evaporate the $CH_2Cl_2$. The produced PLGA microspheres were collected by a membrane filtration, extensively washed with water, and frozen at -20°C for 2 h and lyophilized for 18 h to obtain the dried microspheres. The PLGA microspheres with a diameter of 70–100 μm were separated by standard sieves and used for the following studies.

Surface Aminolysis of the PLGA Microspheres

PLGA microspheres (100 mg) were immersed in 10 mL 6% hexanediamine/n-propanol solution at 40°C in a water bath for 3–30 min. They were then extensively washed with water, frozen at -20°C for 2 h and lyophilized for 18 h to obtain the dried microspheres. Amount of the introduced $NH_2$ groups on the aminolysed PLGA microspheres was quantified by a ninhydrin analysis. Briefly, 10 mg of the dried PLGA microspheres was placed in a glass tube containing 3 mL 1 mol/L ninhydrin/isopropanol solution. The tube was then maintained at 100°C for 10 min to accelerate the reaction between the ninhydrin and the amino groups. About 4 mL 1,4-dioxane was added into the tube to dissolve the microspheres after discarding the residue solution. Another 2 mL isopropanol was added to stabilize the formed blue compound. Absorbance of this mixture at 570 nm was recorded immediately by a UV-VIS spectrophotometer (UV-2550, Shimadzu, Japan). A calibration curve was obtained with 1,6-hexanediamine in 1,4-dioxane/isopropanol solution. Weight change of the microspheres was monitored as a function of aminolysis time at 40°C. The microspheres were separated from the 6% hexanediamine/n-propanol solution at a predetermined time interval (3–30 min), washed with water, lyophilized, and weighed. The weight loss ratio was defined as $(W_0 - W_t)/W_0 \times 100\%$, where $W_0$ and $W_t$ represent the initial and final weights of the microspheres, respectively.

Grafting of Fibrinogen on the Aminolysed Microspheres

The aminolysed microspheres (200 mg) were incubated in 100 mL 0.25% glutaraldehyde (GA) solution at room temperature for 3 h to transfer the $-NH_2$ groups into aldehyde ($-CHO$) groups. After extensive washing, the microspheres were incubated in 10 mL 5 mg/mL fibrinogen solution at 4°C for 12 h with occasional shaking. By the GA coupling, fibrinogen was covalently grafted on the surface of PLGA microspheres by a reaction of $-NH_2$ of the fibrinogen and $-CHO$ on the microspheres. The surface morphology of the PLGA microspheres before and after fibrinogen immobilization was observed under a confocal laser scanning microscope (CLSM, Bio-Rad Radiance 2100). For the CLSM observation, the fibrinogen immobilized microspheres were incubated in 1 mg/mL fluorescein isothiocyanate (FITC) solution at 4°C for 48 h, followed by extensive washing.

Measurement of Fibrinogen Content

The fibrinogen-immobilized microspheres (10–15 mg) were placed in a glass tube containing 3 mL 5% (w/v) sodium dodecyl sulfate (SDS) in 0.1M sodium hydroxide solution.
to decompose the microspheres. After centrifugation, absorbance of the supernatant was determined at 278 nm by UV-VIS spectroscopy. The fibrinogen content was quantified by referring to a calibration curve obtained with pure fibrinogen at the same conditions.

**Measurement of the Clotting Time**

After vortex mixing the solutions of fibrinogen and thrombin with or without fibrinogen-immobilized PLGA microspheres, the change of turbidity was recorded immediately by a UV-VIS spectrophotometer. The clotting time is defined as the time at which the maximum value appears in the differentiating curve.

**Rheological Characterization of the PLGA Microspheres/Fibrin Gel**

The PLGA microspheres were mixed with the fibrinogen and thrombin solutions. The mixture was then transferred to a 12 wells (diameter of the well is 25 mm) tissue culture plate, and allowed to gel at 37°C for 5 min. Thirty minutes later, the dynamic rheological properties were measured by an advanced rheometric expansion system (ARES, Rheometric Scientific Inc.) at 1% strain with 20-mm parallel plates. Before each rheological test, linear dependence of viscoelasticity on frequency at the test temperature was checked. For a dynamic measurement, an increasing oscillatory frequency ranging from 100 Hz to 0.1 Hz at a fixed recorded oscillatory strain of 1% at 37°C was applied, and the storage ($G'$) and loss ($G''$) modulii were recorded.

**Cell Culture**

Chondrocytes were isolated from cartilage tissue of rabbit ears (New Zealand Rabbit) under the institutional guideline and routinely cultured. Briefly, the chondrocytes were isolated by incubating the cartilage pieces in Dulbecco’s minimum essential medium (DMEM) containing 0.2% collagenase type II (Sigma) at 37°C for 4–6 h under agitation. The isolated chondrocytes were centrifuged, resuspended in DMEM supplemented with 10% fetal calf serum (FBS) (complete medium), 300 mg/L glucose, 50 mg/L vitamin C, 100 U/mL penicillin, and 100 μg/mL streptomycin. The chondrocytes were then seeded in 11-cm tissue culture dishes (Falcon, seeding density 2 $\times$ 10$^4$ cells/cm$^2$) and incubated in a humidified atmosphere of 95% air, 5% CO$_2$ at 37°C. After a confluent cell layer was formed (about three to four days), the chondrocytes were detached using 0.25% trypsin in PBS and were resuspended in the supplemented culture medium as described above, and used for the following experiments.

Before cell seeding, the PLGA microspheres were sterilized using 75 vol % ethanol for 2 h and washed by PBS (pH = 7.4) for several times, and then incubated in PBS for 1 day to displace the remaining ethanol. The chondrocytes were entrapped into the PLGA microspheres/fibrin gel (experimental) and fibrin gel control by mixing equal volume of thrombin (30 U/mL, 40 mM CaCl$_2$ solution, and 6 $\times$ 10$^6$ cells) and human fibrinogen (40 mg/mL, 0.9% NaCl solution; with 7.5% PLGA microspheres for the experimental) containing 50 U/mL aprotinin. The mixtures were incubated at 37°C for 10 min to form the cell containing hydrogel and composite, respectively, and then were incubated in a well of 24-well culture plate in the complete medium as described above.

To observe the cell distribution under CLSM, the cell-containing hydrogel and composite (constructs) were taken out from the culture plate and rinsed with PBS gently. Then 1 mg/mL fluorescein diacetate (FDA)/PBS solution was slowly injected into the constructs, which were then incubated for another 5 min. By this fluorescent staining, only the viable cells in the constructs can be visualized under CLSM. To observe morphology of the chondrocytes, the samples were fixed with 2.5% glutaraldehyde at 4°C for 1 h, and then sequentially dehydrated in 75% and 95% ethanol solution, each for 15 min. They were further dehydrated for two times in absolute ethanol, for one time in acetone and for one time in isoamylacetate, each for 15 min. After dried by a critical point dry method, the samples were coated with an ultrathin gold layer and observed under SEM (Cambridge stereoscan 260, UK).

The cell viability was measured by a methylthiazole-tetrazolium (MTT) method. 100 μL MTT solution (5 mg/mL) was added to each well (24-well culture plate). The samples were continually cultured for 4 h. After adding 1 mL dimethyl sulphoxide, the mixture was centrifuged at 10,000 rpm for 10 min to ensure complete separation of the formazan pigment from the constructs. Absorbance at 570 nm was measured on a microplate reader (Bio-Rad 550). Each value was averaged from three parallel experiments.

The cell number in the constructs was assessed by quantifying DNA content using a Hoechst 33258 dye (Sigma) assay. The constructs were frozen at -20°C for 1 h and lyophilized for 2 h for five circles, and each of the constructs was digested with 1 mL papain (1% w/v, with 0.09% disodium ethylenediaminetetraacetic acid (EDTA) as solvent). Shortly after 200 μL of the papain-digested solution was added into 2 mL Hoechst 33258 solution (1 μg/mL), fluorescence intensity at 480 nm was measured by a fluorescent spectrophotometer (RF-5301PC, Shimadzu, Japan). Each value was averaged from three parallel experiments.

A colorimetric method was used to measure the sulphated glycosaminoglycan (GAG) content by using 1,9-dimethylmethylene blue. Briefly, the constructs with a volume of 0.2 cm$^3$ were cut into small pieces and digested with 3 mL papain buffer solution (5 g/L papain, 0.1M KH$_2$PO$_4$, 5 mM EDTA, and 5 mM cysteine-HCl, pH 6.0) at 60°C for 6 h. A total of 200 μL of this digested solution was mixed with 3-mL dye solution prepared by dissolving 16 mg 1,9-dimethylmethylene blue in 1 L water containing 3.04 g
glycine, 2.37 g NaCl, and 95 mL 0.1 M HCl. After 5 min, absorbance at 525 nm was measured by UV-VIS spectroscopy. The GAG content was determined by referring to a calibration curve of chondroitin sulfate. Data were presented as GAG mass per volume. Each value was averaged from three parallel experiments.

### Statistical Analysis

Experimental data were analyzed using two-population Student’s t-test. The significant level was set as $p < 0.05$. Results are reported as mean ± standard deviation.

### RESULTS

#### Immobilization of Fibrinogen on the PLGA Microspheres

The introduced free $—\text{NH}_2$ groups are shown in Figure 1(a). Along with prolongation of the aminolysis time the $—\text{NH}_2$ content was increased until 12 min, and then dropped slight at 15 min. Since the aminolysis in principle is a chain cleavage process, as a result of dissolution weight loss may take place when the cleaved chains are small enough. Figure 1(b) shows that the weight loss ratio was increased steadily, and reached to 10% and 23% after 15 min and 30 min, respectively.

The overall fibrinogen amount was increased along with the aminolysis time, or in other word the $—\text{NH}_2$ content [Figure 1(c)]. For example, when the $—\text{NH}_2$ content was increased from $\sim 2 \times 10^{-8}$ mol/mg to $\sim 4 \times 10^{-8}$ mol/mg, the fibrinogen was correspondingly increased from $\sim 35 \mu g$/mg to $\sim 70 \mu g$/mg.

Existence and distribution of the fibrinogen was also confirmed by CLSM observation (Figure 2), in which the fibrinogen was found to form bright skirts around the microspheres.

#### Fabrication and Physical Properties of PLGA Microspheres/Fibrin Gel Composite

After clotting at room temperature for a couple of minutes, the composite solution lost its fluidity and was transformed into an opaque hydrogel. The control results obtained from the mixture solutions of fibrinogen and thrombin is given in Figure 3(a). The absorbance at 550 nm was increased monotonously along with the time prolongation, and then leveled off to present platforms whose appearing time is...
depend on the thrombin concentration. According to [Figure 3(a)], at a higher thrombin concentration the clotting time was shorter. After the PLGA microspheres were blended, a similar alteration tendency was observed in cases that the thrombin concentration was larger than 15 U/mL [Figure 3(b)]. However, when the thrombin concentration was lower than 10 U/mL, the absorbance was unexpectedly decreased at the initial stage, and then increased again. This absorption decrease is caused by sedimentation of the PLGA microspheres. [Figure 3(c)] presents further the influence of concentration of the PLGA microspheres on the clotting process. Quantitative analysis [Figure 3(d)] found that the clotting time is almost independent on the concentration of PLGA microspheres, which is consistent with the conclusion that the clotting time is mainly controlled by the thrombin concentration.

The storage modulus $G'$ and loss modulus $G''$ as a function of oscillating frequency are shown in Figure 4. The storage modulus $G'$ and loss modulus $G''$ were increased

![CLSM image](image-url)

**Figure 2.** A CLSM image taken on cross-section of the PLGA microspheres immobilized with fibrinogen and followed by FITC staining. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

**Figure 3.** (a) Absorbance (550 nm) of fibrin gel and (b,c) PLGA microspheres/fibrin gel with variable thrombin concentration (b) and variable PLGA microsphere concentration (c) as a function of reaction time. (d) Clotting time of the fibrinogen derived from (c) at the maximum change time. In (a) the final concentrations of fibrinogen and thrombin were 20 mg/mL and 5 U/mL, respectively; in (b) the same conditions as in (a) were used with additional 2.5% PLGA microspheres in each sample; in (c) the final concentration of fibrinogen and thrombin were fixed at 20 mg/mL and 15 U/mL, respectively, supplemented with 0%, 2.5%, 5%, and 7.5% PLGA microspheres. All the hydrogel was made at 25°C.
along with the microsphere concentration [Figure 4(a,b)]. For example, when the microsphere concentration was increased from 0% to 7.5%, the storage modulus $G'$ was correspondingly increased from 287 Pa to 2850 Pa.

**Cell Responsiveness to the Microspheres/Fibrin Gel Composite**

To assess efficacy of the composite hydrogel as a potential injectable cartilage scaffold, chondrocyte culture *in vitro* was conducted. Viable chondrocytes were observed in both the fibrin hydrogel [Figure 5(a)] and the composite scaffold [Figure 5(b)] by CLSM after 7-day culture. However, a relatively larger number of chondrocytes was found in the fibrin gel, whereas almost no cells could be found on the surface of the PLGA microspheres. In both samples, most of the chondrocytes were maintained in round morphology, which is more close to their shape in natural cartilage matrix. Similar conclusion can be made from the SEM observation, in which chondrocytes with a round shape are observable after 7-day culture [Figure 6(a,b)]. Cells with an irregular shape were also observed in the PLGA/fibrin gel composite after 14-day culture [Figure 6(c)].

MTT assay and DNA content recorded a consistent tendency of cell viability on each kind of the constructs [Figure 7(a,b)]. The optical density (proportional to cell viability, and more precisely proportional to the cell number suppose the cell activity is constant) was increased rapidly along with the culture time at the first 5 days [Figure 7(a)], and then leveled off, implying that the chondrocytes could...
normally proliferate in the fibrin hydrogel and in the composite scaffold. Significantly higher viability was recorded for the fibrin gel control than that of the composite scaffold at day 7 ($p < 0.05$), but it became eventually same after 14-day culture. DNA analysis confirmed that the cell number in the composite scaffold was significantly increased when the culture time was prolonged from 7 to 14 days ($p < 0.01$) [Figure 7(b)], whereas in the fibrin gel no difference ($p > 0.05$) was found. The fibrin gel possesses very good cell compatibility because it contains also lots of active ingredients derived from plasma. Addition of the PLGA microspheres causes more or less slower proliferation rate of the cells at the initial time. The subsequent faster proliferation rate reveals, however, the PLGA microspheres do not bring substantial side effect, which is further confirmed by the GAG secretion [Figure 7(c)]. It shows that the chondrocytes cultured in both of the fibrin gel and the composite scaffold could normally secret considerable amount of GAG, which was increased along with the culture time prolongation too. Although no difference was found at day 7, significant and nonsignificant increase of the GAG after 7-day culture was recorded from the composite scaffold and the fibrin gel control, respectively.

**DISCUSSION**

It is necessary to provide enough space and sufficient time for cells to migrate, proliferate, and differentiate for cartilage restoration by a tissue engineering way. The fibrin gels alone have been diversely used as injectable scaffolds. However, the commercially available fibrin glues have lower mechanical strength, tend to disintegrate in vitro and in vivo after several days, and almost completely dissolve within 3–4 weeks. Even after optimization of the gelling parameters that determine the gel stability, the obtained fibrin gel is still not stable enough for long-term cartilage repair. In the present study, the PLGA microspheres were compounded with the fibrin gel for the purpose of enhancement of mechanical strength and stability.

**Figure 6.** SEM images to show cell morphology in (a) fibrin gel after cultured for 7 days, (b) and (c) PLGA microspheres/fibrin gel after cultured for 7 and 14 days, respectively. The fibrinogen and thrombin concentrations were 20 mg/mL and 15 U/mL, respectively, with 25 U/mL aprotinin. (b), (c) Additional 7.5% PLGA microspheres were supplemented in (a). Arrows indicate the cells on the microspheres. Cell seeding density was $3 \times 10^6$ cells/mL.
We demonstrated that this composite scaffold indeed has a stronger mechanical strength.

Intrinsically the chemical structure of PLGA is different with that of the fibrin gel. PLGA is a synthetic polyester with a hydrophobic and bioinert surface, whereas the fibrin gel is highly hydrophilic, making them thermodynamically incompatible. Surface modification of the PLGA microspheres, for example by fibrinogen coating, is a simple but effective way to obtain a compatible composite. Here the surface aminolysis following by glutaraldehyde (GA) coupling is applied to covalently anchor the fibrinogen. In this process, part of the surface ester groups of PLGA is converted into \(-\text{NH}_2\) groups, which are further converted into aldehyde groups by large amount of GA. Following the GA coupling, the fibrinogen was covalently immobilized onto the microspheres. Physically entangled fibrinogen also existed. Aminolysis in principle is an alkaline catalyzed degradation of PLGA macromolecules. Thus weight loss is unavoidable even at rather short reaction time, as shown in [Figure 1(b)]. This weight loss is understood as a result of surface erosion, that is dissolution or dissociation of the degraded oligomers when their size is small enough.\(^{17}\) From a practical consideration, the aminolysis time should not exceed 12 min, since longer time can not further improve the \(-\text{NH}_2\) content but deteriorate the microsphere quality.

The microstructure and handling properties of the PLGA microspheres/fibrin gel were strongly influenced by the clotting time, which is mostly controlled by the concentration of thrombin.\(^{46-48}\) The PLGA microspheres/fibrin gel can be easily formed by mixing solutions of fibrinogen with PLGA microspheres and thrombin. It has been previously reported that the higher the thrombin concentration the faster the fibrin glue polymerizes.\(^{46-48}\) In the present study, we find that when the concentration of thrombin is lower than 10 U/mL, the structure of the composites was strongly affected by the PLGA microspheres [Figure 3(b)]. Lower concentration of thrombin leads to longer clotting time of the composites. Consequently, the PLGA microspheres precipitated severely (Figure 3), and thereby brought the apparent absorption decrease [Figure 3(b)]. When the concentration of thrombin was higher than 15 U/mL, the gelation time was short enough to enable a well suspension of the PLGA microspheres.

Figure 7. (a) chondrocyte viability, (b) cell number and (c) GAG content as a function of culture time. The fibrinogen and thrombin concentrations were 20 mg/mL and 15 U/mL, respectively, with 25 U/mL aprotinin for the control, and with 7.5% PLGA microspheres for the experimental. Cell seeding density was \(3 \times 10^6\) cells.
Mechanical strength of the hydrogels, especially at a dynamic state, is of critical importance for practical applications. This is most typical for cartilage restoration, since the cartilage is inevitably suffered from the dynamic force. The fibrin gels have remarkable and unique viscoelastic properties. Weisel et al. investigated the viscoelastic properties of different types of fibrin gels as a function of the oscillatory frequency. They found that the fibrin gels showed elastic dominated behavior at low frequencies, whereas the viscous response dominated at high frequencies. These results are in good agreement with the results of Eyrich and his coworkers. The present result is consistent with these reports. The $G'$ of all the fibrin gels were higher than $G''$, indicating a highly elastic dominated behavior of the hydrogel system regardless of the amount of PLGA microspheres investigated so far. However, the $G'$ and $G''$ were increased along with increase of the PLGA microsphere amount, implying that the mechanical strength of the hydrogel was definitely improved. This improvement should be resulted from the impedance of the microspheres on movement of the fibrin networks. Nevertheless, the $G'$ is still very small compared with that of the natural human articular cartilage (0.2 to 0.4 MPa). 

In vitro chondrocyte culture demonstrated that the composites can maintain the normal round shape of chondrocytes [Figures 5 and 6(a,b)] as that in their natural state, and similarly promote the cell proliferation (Figure 7). It is known that the fibrin gel contains lots of active ingredients derived from the plasma, and thus has good cell compatibility. In native cartilage the chondrocytes show normally just like hydrogel. In this case, the fibrin gel serves as an artificial ECM to embed and support chondrocytes, which is more close to the native growth environment of the chondrocytes than that of the conventional synthetic porous scaffolds. Furthermore, the composite scaffolds may provide a larger space for cell proliferation, especially for longer time when the fibrin gel is partly degraded. Indeed, no contact inhibition effect was observed at the examined time so far. Moreover, the amount of GAG was higher in the composites than that in the fibrin gel in 2 weeks [Figure 7(c)], implying that the chondrocytes in the composite scaffolds may maintain their phenotype better.

CONCLUSIONS

An injectable PLGA microspheres/fibrin gel composite is successfully fabricated by blending fibrinogen-immobilized PLGA microspheres into fibrinogen and thrombin solutions, following with incubation at 25°C within 1 min. The clotting time of the system is significantly influenced by the thrombin concentration, while less influenced by the content of the PLGA microspheres. Evenly distributed PLGA microspheres in the fibrin gel are achieved at fast enough gelation rate. Addition of the PLGA microspheres can significantly improve the elastic modulus of the scaffold, while has no side effect on the cell proliferation and GAG secretion. With these features, the PLGA microspheres/fibrin gel composite has a larger potential to be used as an injectable scaffold for cartilage regeneration.

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