Several new platform materials have been developed to improve
the encapsulation of cells in a three-dimensional environment.
High water content, permeability, biocompatibility, and ability
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from the same two polymers, which have better mechanical strength and biological properties in
comparison to the DN hydrogels. The MR hydrogels were prepared by incorporating stiff GG microgels
into soft and ductile gelatin hydrogels. The MR hydrogels prepared at cell-compatible conditions
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2.8 times that of the gelatin hydrogels. MC3T3-E1 preosteoblasts encapsulated in MR hydrogels
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1. Introduction
Natural tissues have various mechanical properties according to
their functions. Tissues such as cartilage, tendon, and bone
have great strength because they have to sustain large loads
every day. Tissue engineering scaffolds for such load-bearing
tissues must therefore have high strength to keep their integ-
Rity after being implanted to the load-bearing positions. In this
regard, the mechanical weakness of most hydrogels is a major
limitation, although they have been considered as a promising
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Gellan gum microgel-reinforced cell-laden gelatin
hydrogels

Hyeongho Shin, Bradley D. Olsen and Ali Khademhosseini

The relatively weak mechanical properties of hydrogels remain a major drawback for their application as
load-bearing tissue scaffolds. Previously, we developed cell-laden double-network (DN) hydrogels that
were composed of photocrosslinkable gellan gum (GG) and gelatin. Further research into the materials
as tissue scaffolds determined that the strength of the DN hydrogels decreased when they were
preparation at cell-compatible conditions, and the encapsulated cells in the DN hydrogels did not function
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the mechanical strength of hydrogels, such as double-network (DN) hydrogels, polyrotaxane hydrogels, nanocomposite hydrogels, ideally homogeneous tetra-PEG hydrogels, ionically cross-linked triblock copolymer hydrogels, and shear thinning protein hydrogels reinforced by block copolymer self-

assembly. Previously we developed cell-laden DN hydrogels by using a cell-compatible two-step photocrosslinking method. Photo-
crosslinkable gellan gum (GG) was used to make the stiff first
network, and photocrosslinkable gelatin was used to make the
soft and ductile second network. The strengthening mechanism
of DN hydrogels was explained in that the first network works as
a stiff scaffold that sustains the stress throughout the construct,
and the soft and ductile second network dissipates the crack
energy preventing the failure of the construct. We adapted this
strategy for making cell-laden hydrogels from two photo-
crosslinkable biomacromolecules. The resulting DN hydrogels
exhibited significantly higher strength than single network
hydrogels, and the cells were highly viable after encapsulation
in DN hydrogels. Further research into the materials as tissue
engineering scaffolds determined that these DN hydrogels
weakened when they were prepared in cell-compatible solu-
tions, and the encapsulated cells did not function as well as in
gelatin alone.
Motivated by the need to develop better hydrogel systems in terms of both mechanical strength and biological properties, we decided to use the microgel-reinforced (MR) hydrogel strategy with the same materials that were used to make the DN hydrogels. MR hydrogels were previously fabricated by embedding stiff microgels into a soft and ductile matrix. The MR hydrogels exhibited significantly higher strength than the hydrogels with no microgels, and comparable strength to DN hydrogels. The simple difference between MR and DN hydrogels is that the stiff hydrogel is incorporated into the soft and ductile hydrogel as microparticles in MR hydrogels, not as bulk hydrogel as in DN hydrogels. By this difference, we expected two advantages of our MR hydrogels. First, although the GG component had to be prepared at relatively low polymer concentrations for DN hydrogels due to the adverse effect of GG/gelatin mass ratio on the strength of DN hydrogels, for MR hydrogels, GG hydrogels prepared at higher polymer concentration can potentially increase the strength due to their higher stiffness. Second, because cells were encapsulated not in GG but in gelatin, the cells were expected to function better in MR hydrogels than in DN hydrogels.

In this study, we first prepared GG microgels using a water-in-oil emulsion followed by a light-initiated crosslinking. MR hydrogels were prepared by embedding the GG microgels into gelatin hydrogels and their mechanical properties were examined to compare with those of the DN hydrogels and the gelatin hydrogels with no microgels. MC3T3-E1 preosteoblasts were encapsulated in the MR hydrogels to examine their activity and osteogenic behavior by determining viability, metabolic activity, alkaline phosphatase (ALP) activity, and mineralization. Comparison with DN hydrogels from the same two polymers with the MR hydrogels indicates that the MR hydrogels have better biological performance, and may be of better biological performance, and may be of benefit as tissue scaffolds.

2. Materials and methods

2.1. Modification of GG and gelatin

GG (Gelzan™, MW: 1 000 000), gelatin (from porcine skin, Type A), and methacrylic anhydride were purchased from Sigma-Aldrich. Photocrosslinkable GG was prepared by reacting GG with methacrylic anhydride. 3 g of GG was dissolved in distilled water at 90 °C and the solution was cooled down to 50 °C. 24 ml of methacrylic anhydride was added to the solution and the reaction was allowed to proceed for 4 h at 50 °C while the pH of the solution was adjusted to 8 by adding 15 N NaOH. Then the reaction mixture was dialyzed in distilled water using dialysis membrane (MW cutoff: 12–14 kDa, Spectrum Labs, Inc.) at 4 °C for 6 days. The solution was frozen and lyophilized to obtain photocrosslinkable GG. The resulting material was kept at −40 °C until further use. Similarly, photocrosslinkable gelatin was prepared by reacting gelatin with methacrylic anhydride. 30 g of gelatin was dissolved in 300 ml of phosphate buffer saline (PBS, 1 x, Life Technologies) at 50 °C. 3 ml of methacrylic anhydride was added to the solution and the reaction was allowed to proceed for 2 h at 50 °C. The reaction mixture was dialyzed with an equal amount of distilled water and dialyzed in distilled water at 40 °C for 6 days. The resulting solution was lyophilized to obtain photocrosslinkable gelatin, and it was kept at −40 °C until further use.

2.2. Preparation and characterization of microgels

GG microgels were prepared by using a water-in-oil emulsion. Photocrosslinkable GG was dissolved in ultrapure water containing 1% (w/v) photoinitiator, 2-hydroxy-1-[4-(2-hydroxyethoxy) phenyl]-2-methyl-1-propanone (Irgacure 2959, BASF) at −40 °C to make solutions with varying GG concentrations (1.0%, 1.5%, 2.0%, 2.5%, (w/v)). 0.4 ml of the GG solution was mixed with 5 ml of mineral oil (Sigma-Aldrich) containing 0.02 ml of Span 80 (Sigma-Aldrich), and the mixture was homogenized for 3 min by using an OMNI GLH homogenizer (OMNI International). The resulting emulsion was exposed to light (325–500 nm, ~7.5 mW cm⁻², EXFO Omnicure S2000) for 120 s to obtain crosslinked GG microgels. The resulting solution was dried overnight at ~40 °C with constant stirring to evaporate the water. GG microgels were separated from mineral oil by centrifugation at 5000 rpm, and washed with isopropanol, hexane, and acetone, before they were dried under vacuum at room temperature for 3 days. The GG microgels were coded as GG1.0, GG1.5, GG2.0, and GG2.5 according to the polymer concentration in the GG microgels.

Scanning electron microscope (SEM) images of dried GG microgels were taken by using a JEOL JSM 6060 SEM with a 2.5 kV accelerating voltage at a 10 mm working distance. To prepare specimens, GG microgels were attached onto specimen stubs and sputter coated with gold/palladium (SC7640, Polaron).

Optical microscope images of microgels in PBS were taken by using an optical microscope (Zeiss AxiosObserver. D1). The size of microgels in the images was measured by using the ImageJ software.

To measure the swelling ratio, GG microgels were allowed to swell in distilled water for 1 h to reach equilibrium in a tube with known weight. After centrifugation at 5000 rpm, the excessive water was removed and the weight of the wet microgels (Wₙ) was determined. The microgels were lyophilized and the dry weight (W₀) was determined. The swelling ratio was calculated as Wₙ/W₀.

2.3. Preparation and characterization of MR and DN hydrogels

GG microgels were allowed to swell in PBS containing 0.05% (w/v) photoinitiator to make solutions with varying GG concentrations [0.25%, 0.5%, 0.75%, 1.0% (w/v)]. Photocrosslinkable gelatin was dissolved in these solutions to make 10% (w/v) gelatin solutions with GG microgels. The resulting mixtures were molded into disks with ~8 mm diameter and ~1 mm thickness, and exposed to light (~7 mW cm⁻²) for 180 s. The resulting MR hydrogels were immersed in PBS and incubated at 37 °C until further experiments. The MR hydrogels were coded as MRₓᵧ, where x is the polymer concentration in the GG microgels, and y is the GG concentration in the MR hydrogels.
DN hydrogels were prepared with only minor modifications from the previously described method. In short, photocrosslinkable GG was dissolved in distilled water containing 0.05% (w/v) photoinitiator at 0.5% (w/v), and the solution was crosslinked with the same method as above. The resulting GG hydrogels were immersed in photocrosslinkable gelatin solution (10% (w/v) in PBS) containing 0.05% (w/v) photoinitiator at 37 °C for 1 day. Subsequently, the hydrogels were taken out and the excess gelatin solution was removed from the surface of the hydrogels before they were exposed to light again for 180 s. The resulting DN hydrogels were immersed in PBS at 37 °C until further experiments.

The polymer concentration of MR hydrogels was determined as \( W_d/W_s \) where \( W_d \) is the weight of the dried MR hydrogels and \( W_s \) is the weight of the swollen MR hydrogels in distilled water. Before \( W_s \) was measured, the MR hydrogels were washed with distilled water three times to remove the salts in PBS.

The mechanical properties of hydrogels were determined by unconfined, uniaxial compression tests by using an Instron 5943 mechanical tester. The compression rate was 0.5 mm min\(^{-1}\). The compressive modulus was determined as the slope of the stress–strain curve in the 0–10% strain range. The failure stress was determined as the stress at which the slope of the stress–strain curve started to decrease where the hydrogels started to break.

To observe the microstructure of the hydrogels, SEM images of the cross-section of the hydrogels were taken with a JEOL JSM 6060 SEM with a 5 kV accelerating voltage at a 10 mm working distance. To minimize changes to the hydrogel structure, the hydrogels were frozen very quickly by liquid nitrogen.

2.5. Cell behavior analysis

The cell viability was examined by using a LIVE/DEAD Viability Kit (Life Technologies) according to the manufacturer’s instruction. The cell-laden hydrogels stained with calcein AM/ethidium homodimer-1 were visualized with a Nikon Eclipse Ti fluorescence microscope (Nikon), and the cell viability was determined as the number of live cells over the number of all cells.

The metabolic activity was examined by AlamarBlue (Life Technologies) assay. The cell-laden hydrogels were incubated with 300 µM AlamarBlue reagent solution (10% (v/v) in growth media) at 37 °C for 4 h. Then the fluorescence (544 ex/590 em) of the solution was measured by using a FLUOstar plate reader (BMG). The reduction of the reagent was calculated as \( (F_s - F_b)/(F_s - F_f) \) where \( F_s \) is the fluorescence of the sample, \( F_b \) is that of the untreated reagent solution, and \( F_f \) is that of the 100% reduced solution which was prepared by autoclaving the reagent solution.

ALP activity was measured by using an ALP assay kit (Abcam). The cell-laden hydrogels were disrupted by using a TissueLyser (Qiagen) before 5 mM p-nitrophenol phosphate (pNPP) solution in the assay buffer was added to the disrupted samples. After incubation for 6 h at room temperature, the absorbance was measured at 405 nm. In order to normalize the ALP activity by the amount of DNA, the DNA was quantified by using a PicoGreen dsDNA Assay Kit (Life Technologies). The disrupted samples were incubated at room temperature for 5 min with the PicoGreen reagent solution in TE buffer before the fluorescence (485 ex/520 em) was measured.

Mineralization was examined by using Alizarin Red S (Sigma-Alrich) according to an Osteogenesis Assay Kit instruction (Millipore). The cell-laden hydrogels were fixed with 4% paraformaldehyde for 30 min, and stained for 5 min with 2% Alizarin Red S solution of which the pH was adjusted to ~4.2 by using 10% acetic acid. Then the hydrogels were washed with distilled water several times to remove all unreacted reagents. The images of the stained samples were taken by using a zoom microscope (Axio Zoom. V16, Zeiss). The samples were then left overnight with 10% acetic acid, heated to 85 °C for 10 min, and neutralized to pH of 4.1–4.5 with 10% ammonium hydroxide. Finally, the absorbance was measured at 405 nm.

2.6. Statistics

All data were expressed as mean ± standard deviation. The data were analyzed by using one-way or two-way ANOVA and
Bonferroni test to determine statistical significance (GraphPad Prism 5.02, GraphPad Software). Differences were taken to be significant for $p < 0.05$.

3. Results and discussion

3.1. Preparation and characterization of microgels

GG molecules consist of rigid repeat units that have many hydroxyl groups available for functionalization with photo-reactive methacrylate groups. Thus, hydrogels prepared by photocrosslinking highly methacrylated GG are relatively stiff with the moduli that are $>$100 kPa at the polymer concentrations of only a few percentile, which make them a potentially useful reinforcement material. GG particles were prepared at four different concentrations (1.0%, 1.5%, 2.0%, 2.5%) by using water-in-oil emulsion followed by a light-initiated photocrosslinking (Fig. 1). Since the emulsion was not transparent, a high concentration of the photoinitiator (1.0%) was used for the crosslinking. The SEM images of the GG microgels (Fig. 2(A)–(D)) showed that the resulting microgels were polydisperse and the size of the dried microgels ranged from a few micrometers to hundreds of nanometers. In PBS, the GG microgels swelled and the mean size of particles was measured to be several micrometers (Fig. 2(E)–(I)). The standard deviation of the size was large due to the high polydispersity of the microgels. No significant difference in the microgel size was observed with increasing the polymer concentration in GG microgels. This is probably because the viscosity of the aqueous GG solution was not high enough to affect the emulsion-forming process, and the GG microgels were highly crosslinked such that their

![Fig. 1 Preparation procedure of MR hydrogels.](image)

![Fig. 2 SEM (A–D) and optical microscope (E–H) images of GG microgels prepared at different polymer concentrations: (A and E) GG1.0, (B and F) GG1.5, (C and G) GG2.0 and (D and H) GG2.5. The scale bars in (A–D) represent 2 μm, and the scale bar in (E) represents 50 μm. (I) Particle size of GG microgels in PBS. (J) Swelling ratio of GG microgels in distilled water. (*) Indicates significant difference ($p < 0.05$).](image)
swelling in PBS did not depend strongly on the GG concentration. The swelling ratio of the GG microgels (Fig. 2(J)) decreased as GG concentration increased, which is reasonable because the swollen size in a solution is similar while the polymer concentration increases with GG concentration.

3.2. Preparation and characterization of MR hydrogels

Loosely crosslinked gelatin hydrogels are soft and ductile, with the modulus of a few tens of kPa at 10% polymer concentration. Various amounts of the GG microgels were added to 10% photocrosslinkable gelatin solutions in PBS before the mixture was photocrosslinked to obtain MR hydrogels (Fig. 1). The polymer concentration (w/w) of the MR hydrogels increased as the GG concentration in MR hydrogels increased, but no significant difference was observed among different GG concentrations of the microgels (Fig. 3(A)). Although the microgels can serve as additional crosslinks that constrain gelatin molecules from swelling, thus microgels with different concentrations might have led to different polymer concentrations, the gelatin hydrogels were sufficiently crosslinked that the polymer concentration of the MR hydrogels did not change significantly with the reason that microgel formulation did not affect the polymer concentration of the MR hydrogels. It is presumed that the reason that the polymer concentration increased by ∼3% with only 1% of GG concentration added in MR hydrogel is that the lower hydrophilicity of GG molecules due to the high degree of methacrylation resulted in the lower water content in the wet state.

The mechanical properties of MR hydrogels were determined by unconfined, uniaxial compression tests. Fig. 3(B) compares the stress–strain curves of two representative MR hydrogels and the gelatin hydrogel with no microgels. When a relatively small amount of microgels was added (MR2.5–0.25 (MRx-y: GG concentration is x% in microgels and y% in MR hydrogels)), the modulus did not increase significantly, but the failure stress increased significantly (statistical analysis is shown in Fig. 3(C) and (D)). When a relatively large amount of microgels was added (MR2.5–1.0), both the modulus and the failure stress increased significantly, but the failure occurred at a lower strain resulting in not as high failure stress as in the previous formulation (statistical analysis is shown in Fig. 3(C) and (D)). Fig. 3(C) shows the compressive modulus of all the MR hydrogel formulations prepared. GG1.0 microgels were not stiff enough so that adding them with up to 1% GG concentration in MR hydrogel did not make significant increase in the compressive modulus of the MR hydrogels. GG1.5, GG2.0, and GG2.5 microgels did not increase the compressive modulus of the MR hydrogels significantly at only 0.25% GG concentration in MR hydrogel; however, at more than 0.25%, as more amount of the microgels were added, the modulus of MR hydrogels increased. At higher GG concentrations (0.75% and 1%) in MR hydrogel, GG2.5, which is the stiffest microgels, resulted in highest compressive modulus among the all microgel formulations. Fig. 3(D) shows that all formulations of MR hydrogels prepared exhibited higher strength than the gelatin hydrogels. When the microgels of the highest GG concentration (GG2.5) were added, the failure stress abruptly increased at the lowest GG concentration in MR hydrogel (0.25%), and it clearly decreased as the GG concentration in MR hydrogel increased. The maximal strength was 3.2 MPa, which was 2.8 times that of the gelatin hydrogels. However, as the GG concentration of the microgels decreased, the failure stress at the lowest GG concentration in MR hydrogel decreased, and it did not decrease as much with the increase of the GG concentration in MR hydrogel.
At 1% GG concentration in MR hydrogel, MR hydrogels with GG1.0 showed even higher strength than those with GG2.5. The strengthening mechanism of MR hydrogels has been previously elucidated: the GG microgels provide sacrificial bonds that can resist the crack propagation in the gelatin network. Although the GG microgels are brittle, so cracks can grow in the microgels, the microgels are dispersed in the gelatin network so the cracks of microgels do not propagate into macroscopic ones until the crack of gelatin network starts to grow.

Comparison with DN hydrogels from the same two polymers with MR hydrogels showed that MR hydrogels have significantly higher strength. The compressive modulus and the failure stress of a DN hydrogel were showed in each dotted box in Fig. 3(C) and (D). The formulation of the DN hydrogel was 0.5% GG/10% gelatin, which is optimal for high strength at 10% gelatin concentration. If the GG concentration increases, the modulus becomes much higher, but the failure stress decreases. But, even with the optimal formulation, the failure stress of the DN hydrogels is only 1.4 times that of the gelatin hydrogels. In previous work, the DN hydrogels were prepared from GG and gelatin solutions in distilled water. However, it turned out that the DN hydrogels prepared from the GG solution in PBS and the gelatin solution in media, which are cell-compatible solutions, exhibited significantly lower strength than those prepared from water solution. We determined that this is because the GG molecules collapse when they are in a solution with high ionic strength such as PBS or media. GG molecules have negative charges on their backbone, so they exist as extended form in distilled water due to the repulsive force between the charges. However, when they are in a solution with many ions, the electric interactions are screened by the ions and the molecules collapse. The MR hydrogels were also prepared from gelatin solutions with GG microgels in PBS, so the strength might be lower than that of the MR hydrogels prepared form water solutions, but the higher GG concentration of the microgels (thus higher stiffness of microgels) enhanced the strength of the MR hydrogels, which was not the case for the DN hydrogels. This is the mechanical advantage of the MR hydrogels over DN hydrogels.

To investigate the microstructure of the hydrogels, SEM images of the cross-section of the GG, gelatin, DN and MR1.5 hydrogels were taken (Fig. 4). All the hydrogels presented interconnected porous structure, which accelerates the transport of nutrients and waste products. However, except for GG hydrogels, all observed pores were smaller than approximately 10 μm, so the cell growth and migration in the hydrogels might be impeded. Thus, some degradation of the hydrogels might be needed to facilitate cell behaviors and the formation of extracellular matrix.

3.3. Cell behavior in MR hydrogels

The MC3T3-E1 cell line is a preosteoblast derived from Mus musculus (mouse) calvaria. It has been widely used as a model to evaluate the capacity of substrates for osteogenic differentiation. It exhibits high levels of differentiation after culture in media with ascorbic acid and phosphate producing osteogenic markers such as ALP and depositing minerals. We encapsulated these cells in five hydrogel formulations which are GG, gelatin, DN, MR1.5–0.5, MR1.5–1.0 (MRx-y: GG concentration is x% in microgels and y% in MR hydrogels) hydrogels. The images of the calcine AM/ethidium homodimer-1 live/dead stained hydrogels are shown in Fig. 5(A). At all formulations of hydrogels, the cell viability was over 70% after 14 days of culture (Fig. 5(B)), which is on par with typical photocrosslinked hydrogels. This shows that the photocrosslinking conditions such as the intensity of the light, the exposure time and the photoinitiator concentration were well adjusted for the MC3T3-E1 cells. The microgels contained negligible amount of toxic chemicals that could harm cells, and the microgels themselves were not harmful to cells. In addition, requirements for cell survival such as the transport of nutrients and wastes were met. It is presumed that the higher viability in the GG hydrogels than that in other hydrogels at day 7 and 14 is because the initial damage by the photocrosslinking was less in GG hydrogels due to more amount of methacrylate groups which can react with free radicals that is harmful to cells. It was observed that higher concentration of the photoinitiator led to even lower viability in the gelatin hydrogels, while the viability remained at the same level in the GG hydrogels (data not shown). However, the metabolic activity of the cells showed significant difference between varying formulations as seen in Fig. 5(C). In the gelatin and the two MR hydrogels, the metabolic activity started to increase at day 14, and ended up with about four-fold increase at day 21 compared to day 1. However, the metabolic activity in the GG and the DN hydrogels significantly decreased at day 7, and remained low until day 21. The difference between these two groups was that the cells were encapsulated in gelatin for the former group, while the cells were encapsulated in GG for the latter group. The MC3T3-E1 cells are anchorage-dependent cells of which the activity, function, and differentiation are highly affected by the adhesion to a substrate. It is well known that gelatin is a great material
for this kind of cells because it has many adhesion sites such as RGD peptide. Thus the higher metabolic activity in the former group is presumed that because MC3T3-E1 cells were better attached to the gelatin environment than to the GG environment. Although gelatin molecules penetrated into the GG hydrogel so the cells might face partly the gelatin environment in DN hydrogels, it is speculated that the gelatin could not strongly interact with the cells when the cells were already stuck in the GG network. Seeing that the level of the metabolic activity in the gelatin, MR1.5–0.5, and MR1.5–1.0 hydrogels were all similar, it is concluded that the GG microgels did not interfere significantly with the cell–gelatin interaction at up to 1.0% concentration.

The level of the osteogenic behavior of the MC3T3-E1 cells in each hydrogel formulation was assessed by examining ALP activity and mineralization. As seen in Fig. 6, ALP activity in the gelatin and the two MR hydrogels again increased significantly over 21 days of culture, while that in the GG and DN hydrogels remained significantly lower. It is most likely because of the same reason as the difference in metabolic activity, the cell–gelatin interaction. The facilitation of the differentiation of MC3T3-E1 cells or osteogenic differentiation of mesenchymal stem cells (MSCs) by using a cell-adhesive substrate has been shown in previous studies. The enhanced osteogenic behavior in the gelatin and MR hydrogels was also confirmed by the Alizarin Red S staining for minerals (Fig. 7 and 8). At day 28 of culture, all the surface of the gelatin and the MR hydrogels turned red, while the GG and DN hydrogels still have unstained areas (Fig. 7). Quantitative analysis in Fig. 8 shows that adding up to 1% of GG microgels into gelatin hydrogels did not affect the amount of the mineralization significantly. Although the amounts of Alizarin Red S stained in gelatin hydrogels did not affect the amount of the mineralization significantly. Although the amounts of Alizarin Red S stained in gelatin hydrogels did not affect the amount of the mineralization significantly. Although the amounts of Alizarin Red S stained in gelatin hydrogels did not affect the amount of the mineralization significantly. Although the amounts of Alizarin Red S stained in gelatin hydrogels did not affect the amount of the mineralization significantly. Although the amounts of Alizarin Red S stained in gelatin hydrogels did not affect the amount of the mineralization significantly.
depending on the properties of the cells encapsulated, the effect of the hydrogel formulation on the function or the differentiation of the cells can be different. For example, RGD peptides that exist on gelatin molecules promote early stages of chondrogenesis of MSCs, but their persistence in the scaffold can limit complete differentiation of MSCs.38,39 Thus, the properties of the cells always need to be considered when a hydrogel formulation is selected for a certain purpose. Finding other materials that have different biological properties and can be used as the stiff reinforcing microgels or the soft and ductile matrix will thus broaden the range of applications of MR hydrogels.

Finally, another advantage of MR hydrogels as tissue scaffolds is that they are potentially injectable as previously studied photocrosslinkable polymers,40,41 which is not the case for DN hydrogels. By adding the GG component in the gelatin hydrogels as microgels not as bulk hydrogels, injection of the gelatin solutions with GG microgels and cells into a body followed by a photocrosslinking in situ became possible. This enables the minimally invasive implantation, which is one of the advantages of hydrogel-based tissue scaffolds over those from different types of material.1,40,42 However, it should be noted that, although the feasibility of photocrosslinking of injected polymers in vivo by transdermal light exposure was confirmed,43,44 further studies to overcome inefficient light penetration through skin and develop biocompatible photo-initiators are needed for clinical use as injectable tissue scaffolds.

4. Conclusion

We developed mechanically strong MR hydrogels by embedding stiff GG microgels into soft and ductile gelatin hydrogels. The MR hydrogels exhibited higher strength than the DN hydrogels and the gelatin hydrogels with no microgels. The strength of MR hydrogels varied with the polymer concentration in the GG microgels and the GG concentration in the MR hydrogels. MC3T3-E1 preosteoblasts were encapsulated in the MR hydrogels with a high cell viability. They exhibited as high metabolic activity in the MR hydrogels as in the gelatin hydrogels after culture, while their metabolic activity remained low over time in the DN hydrogels. The osteogenic behavior determined by measuring ALP activity and mineralization was facilitated as greatly in the MR hydrogels as in the gelatin hydrogels, while the osteogenic behavior was not as strong in DN hydrogels. These results suggest that the MR hydrogels may have high potential as load-bearing tissue scaffolds.

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Notes and references

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Fig. 7 Alizarin Red S staining on MC3T3-E1 cells/hydrogel constructs after 1 and 28 days in culture. The scale bar represents 500 μm.

Fig. 8 Quantification of Alizarin Red S stained in MC3T3-E1 cells/hydrogel constructs. (*) Indicates significant difference (p < 0.05).