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# Swelling characterization of photo-cross-linked gelatin methacrylate spherical microgels for bioencapsulation

**Abstract:** The swelling behavior of biocompatible and biodegradable polymers is important for the delivery and release of cells and drugs in biomedical applications. This study reported the swelling characteristics of photo-cross-linked gelatin methacrylate (GelMa) spherical microgels. Spherical microgels were generated in a microfluidic system consisting of a co-axial flow-focusing device for microdroplet generation and an ultraviolet (UV) irradiation apparatus for polymerization. At a low flow rate ratio ( $<0.14$ ), the 9 wt.% GelMa spherical microgels were smaller than the 6 wt.% ones. In contrast, at a high flow rate ratio ( $>0.14$ ), the results were reversed. Overall, a proportional relationship was observed between the flow rate ratio and the droplet size. The increased GelMa concentration improved the mechanical properties and increased the swelling ratios. The possibility of bioencapsulation was demonstrated, with good viability of 3T3 cells encapsulated in the spherical microgels.

**Keywords:** bioencapsulation; flow focusing; photo-cross-linked gelatin methacrylate; spherical microgel; swelling.

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## 1 Introduction

Biodegradable and biocompatible polymers are very promising materials for the encapsulation of drugs and cells (1–3). In addition, scaling the polymer structure from macro- to microscale enables a variety of structural functions like swelling and shrinking. Polymer microspheres have been employed as a flexible platform for biological or medical applications such as cell delivery, drug delivery, drug release, and diagnostics (4–7). Availability, stability, and safety in microsphere bio-applications have rapidly accelerated the use of polymer microspheres.

In general, swelling is caused by expanding hydrophilic cross-linked chains. This behavior is very important in the delivery and release of cells and biomolecules for therapeutic approaches (8–10) and medical diagnostic tests because cell-controlled degradability and biomolecule release depend on swelling. For example, polymer microspheres with immobilized proteins have been used in optical biosensors to detect antibodies (11) and a point-of-care diagnostic device for the prevention of diseases has been successfully developed using microdroplet-based printing technique (12).

Polymer microspheres can be produced using several fabrication methods. Electrospraying, also called electrohydrodynamic atomization, generates droplets using electrohydrodynamic streaming and cone-jetting under an applied voltage (13, 14). However, polymer droplets need additional steps for particle formation such as consolidation and agglomeration. The solvent evaporation technique can also produce microspheres after the dissolution of a core material and a matrix material, emulsification in the continuous phase using mechanical agitation, and extraction/evaporation of solvent in the core material, but it is difficult to control the size of microspheres formed by the agitation state (15, 16). Emulsion is a technique to generate microdroplets by mechanical agitation using two immiscible liquids with a different phase (17, 18); however, it cannot guarantee the microsphere size. A flow-focusing method can also produce microdroplets hydrodynamically using a flow-focusing glass capillary device (19–21), and the method is suitable for stable size control and rapid generation of microspheres.

Therefore, in this study, a flow-focusing, glass capillary-based microfluidic device was employed to generate microdroplets made of gelatin methacrylamide (GelMa) hydrogel. Inexpensive and cell-responsive GelMa has methacrylate groups along the gelatin backbone, which allows microdroplets to be cross-linked under filtered ultraviolet (UV) irradiation. The proposed microfluidic system facilitated both the generation and the polymerization of microdroplets without an additional process. GelMa microdroplets were controlled by size with flow rates of between 50 and 300  $\mu\text{L}/\text{h}$ . The swelling

behavior of cross-linked GelMa microspheres was studied by observing microspheres in oil and phosphate-buffered saline (PBS). The possibility of bioencapsulation was investigated through 3T3 cell encapsulation in a polymer microsphere.

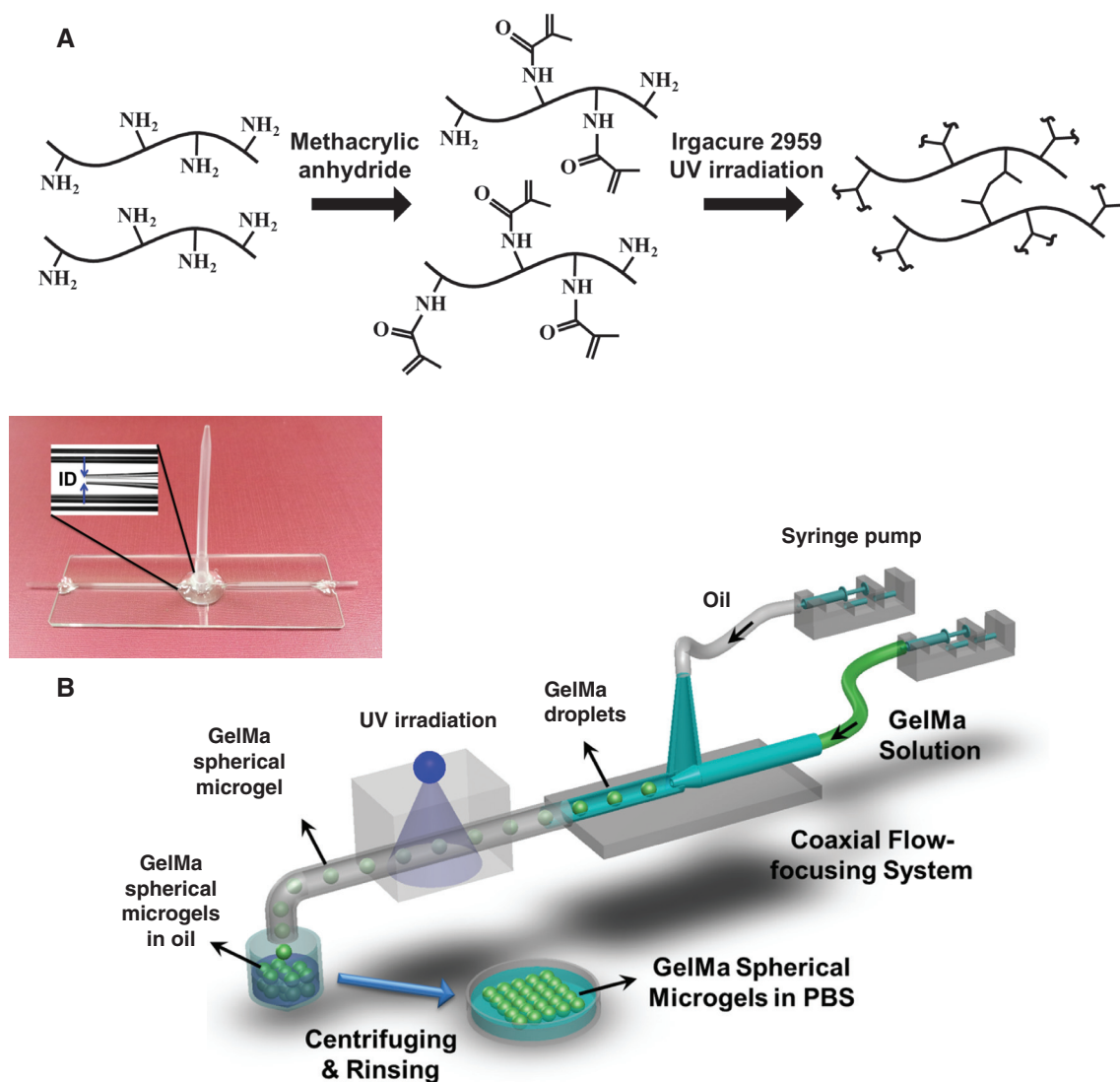
## 2 Materials and methods

### 2.1 Synthesis of methacrylamide-modified gelatin

In order to prepare photopolymerizable GelMa, a gelatin was derivatized by reaction with methacrylic functional

groups. Figure 1A shows a schematic synthesis process of photosensitive methacrylated gelatin where several amine groups on gelatin were substituted with methacrylamides. Gelatin (type A, bloom strength of 300) isolated from porcine skin by the acidic process was used.

In our experiments, 5 g of gelatin (Sigma Aldrich, St. Louis, MO, USA) was mixed into dimethyl sulfoxide (Sigma Aldrich, St. Louis, MO, USA). The solution was heated to 50°C and stirred continuously. A total of 0.5 g of 4-(dimethylamino)-pyridine (Sigma Aldrich) was then dissolved into the gelatin solution. As soon as both were fully dissolved, 2 ml of glycidyl methacrylate (Sigma Aldrich) was added to the solution at a constant flow rate of 0.5 ml/min during vigorous stirring at 50°C. After the reaction was stayed for 2 days under a dry N<sub>2</sub> gas environment,



**Figure 1** (A) Photo-cross-linking process of GelMa and (B) schematic of the microfluidic system for droplet generation and photo-polymerization (ID 100  $\mu$ m).

the mixture was dialyzed using membranes (molecular weight cut-off 12,000–14,000) against deionized water (DW) for 1 week at 40°C. The DW was changed once a day. As a result of lyophilization for 1 week, a white porous solid was obtained and stored at -80°C. The presence of methacrylate groups, which were substituted with the free amino groups on gelatin, was confirmed using  $^1\text{H}$  NMR spectroscopy at 40°C (Figure S1).

## 2.2 Co-axial flow-focusing device

Figure 1B shows a schematic illustration of the co-axial flow-focusing device used to generate droplets. The microfluidic device using circular glass capillaries could provide several benefits for forming microdroplets, such as high chemical resistance, precise control of surface wettability, and true three-dimensional co-axial flow-focusing geometry. The device consisted of two inlets and one outlet. Each inlet was made from a glass capillary [100  $\mu\text{m}$  inner diameter (ID)] and a micropipette tip (0.1–10  $\mu\text{l}$ ) to introduce the GelMa prepolymer solution and mineral oil (M5904; Sigma Aldrich), respectively. A primary glass capillary (580  $\mu\text{m}$  ID) was installed to serve as an outlet for the fabricated droplets. The small glass capillary was inserted into the main capillary to form the co-axial flow focusing geometry. A 100- $\mu\text{m}$ -ID glass capillary was prepared by manually drawing the 580- $\mu\text{m}$ -ID glass capillary (World Precision Instruments, Inc., Sarasota, FL, USA) under a thermal treatment process. Both glass capillaries were fixed on a glass slide (25×75 mm) using epoxy. The bottom of the micropipette tip was cut to fit over the connection of the two glass capillaries and then permanently glued. The tip was connected to the GelMa prepolymer solution via tubing.

## 2.3 Fabrication of GelMa droplets

Two different concentrations of the GelMa prepolymer solution (6 and 9 wt.%) were prepared by dissolving the freeze-dried GelMa macromer in PBS (pH 7.4) at 60°C. A total of 0.2 wt.% of the photoinitiator (Irgacure 2959, CIBA Chemicals, Tarrytown, NY, USA) was then added to the solutions. Stable droplets were generated at concentration ranges from 6 to 9 wt.% of the GelMa prepolymer solution.

After they were fully dissolved, the GelMa macromer and the photoinitiator were mixed and then infused into the glass capillary inlet. In order to characterize the effect of the flow rates on the droplet formation, the GelMa prepolymer solution was injected at different flow rates of 50, 75, 100, 200, and 300  $\mu\text{l/h}$  using a syringe pump (KD

Scientific Inc., Holliston, MA, USA). The oil phase was prepared by adding an oil-soluble emulsifier, sorbitan monooleate (Span 80, Sigma Aldrich), in mineral oil. The oil was then introduced into the micropipette tip inlet at a constant flow rate of 1000  $\mu\text{l/h}$ . The GelMa prepolymer solution was defined as the dispersed phase, while the oil phase was the continuous phase. As two immiscible fluids (GelMa prepolymer solution and oil) were brought into contact at the co-axial channel, the dispersed phase (GelMa prepolymer solution) was compressed and broken up into monodisperse, spherical droplets.

## 2.4 Polymerization of methacrylated gelatin

A suitable photopolymerization process for forming a GelMa spherical microgel was conducted using UV irradiation. After exiting from the outlet of the microfluidic flow-focused device, the aqueous droplets formed with the GelMa prepolymer solution flowing out via a Tygon tubing (0.3 mm ID and 0.25 mm thick, Saint-Gobain Performance Plastics Co., Akron, OH, USA). In the presence of a water-soluble photoinitiator (Irgacure 2959), the droplets passing through the tubing were exposed to filtered UV light (320–500 nm) for 5 min (OmniCure S2000, Lumen Dynamics, Mississauga, ON, Canada). The samples were cured with a UV intensity of 0.85 W/cm<sup>2</sup>, 8 cm from the light source. The formulated GelMa spherical microgels were collected for 5 min in a 1.5-ml Eppendorf tube containing 1.0 ml of PBS. The oil in the tube was separated by centrifugation. The obtained spherical microgels were then rinsed three times with PBS to eliminate other impurities.

## 2.5 Mechanical testing

The mechanical properties of the GelMA spherical microgels were determined by an unconfined compression test. Hydrogels of 6 and 9 wt.% GelMa were prepared in five cylindrical structures (1 cm in height and 1 cm in diameter) for five compression tests. The samples were loaded on a universal material testing system (Instron Model 5542, Norwood, MA, USA). The compression test was performed at a fixed strain rate of 1 mm/min, and the applied load was measured using 10 N of the load cell. Prior to the mechanical testing, all samples were placed in PBS at room temperature for 24 h to allow for swelling. The compressive stress-strain curves of the GelMA spherical microgels were obtained based on the collected data. The Young's modulus of each GelMA concentration was calculated using the slope of the linear region in the 0–10% strain.

## 2.6 Microgel swelling analysis

The swelling behavior of the photo-cross-linked GelMa spherical microgels was analyzed under four different conditions: before cross-linking, after cross-linking, 3 h after cross-linking, and 24 h after cross-linking. The samples were collected and placed in a well plate. The samples were investigated under an inverted optical microscope (Olympus, Tokyo, Japan) to monitor the swelling characteristics of the samples. The diameters of the 30 droplets were measured for each experiment, and the data were calculated as means and standard deviations. The experiment was repeated three times. The swelling ratio, defined as the fractional increase in volume of the GelMa spherical microgels after swelling in PBS, was quantitatively calculated using the following equation:

$$Q_{t_2-t_1}(\%) = \frac{V_{t_2} - V_{t_1}}{V_{t_1}} = \frac{D_{t_2}^3 - D_{t_1}^3}{D_{t_1}^3} \quad (1)$$

where  $V$  and  $D$  are the volume and diameter of GelMa spherical microgels before and after swelling for the given times  $t_1$  and  $t_2$ , respectively. The significance of the size difference was compared based on the analysis of variance (ANOVA) at a 95% confidence level.

## 2.7 Cell culture and encapsulation in GelMa spherical microgels

NIH/3T3 fibroblasts (mouse embryonic fibroblast cell line) were cultured in Dulbecco's Modified Eagle's Medium (high glucose; Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (Invitrogen) and 1% penicillin-streptomycin (Invitrogen). The cells were incubated at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>. The cells were passaged every 4 days, and the media was replaced every 2 days in all experiments. Three different concentrations of NIH/3T3 cells (i.e., 5000, 30,000, and 70,000 cells/ml) were prepared and premixed with 6 and 9% GelMa prepolymer solutions. The mixture was then introduced into the co-axial microfluidic device, resulting in cell encapsulation in the droplets.

## 2.8 Cell viability test

The viability of NIH/3T3 cells encapsulated in spherical microgels was evaluated using the Live/Dead Viability Cytotoxicity Kit (Invitrogen). The Live/Dead assay with 0.5 µl/ml calcein AM (staining live cells with green) and

2 µl/ml ethidium homodimer-1 (staining dead cells with red) was diluted in PBS. Cell-encapsulated spherical microgels at Day 1 were collected and placed in a 12-well cell culture plate. The Live/Dead staining solution was added to the cell culture plate. Cell-encapsulated spherical microgels stained with the Live/Dead assay were incubated at 37°C for 15 min and then rinsed with PBS three times. Green- and red-fluorescent images for live and dead cells were obtained under an inverted optical microscope equipped with a fluorescent light source and filters.

## 3 Results and discussion

A three-dimensional microfluidic device was developed using a glass capillary-generated co-axial flow focusing method, and the GelMa prepolymer solution was located in the core region of the co-axial flow. The shear stress, which was caused by the continuous phase (mineral oil) in the shell region, produced uniform monodisperse microdroplets. As the flow rate of the GelMa prepolymer solution increased, the shear stress was reduced, resulting in the generation of large droplets. A surfactant was added to the oil phase to maintain a stable spherical shape. In the two-phase flow, the interfacial effect between two fluids played a crucial role in the size control of droplets due to the large surface area-to-volume ratio at the microscale. The addition of a surfactant increased the oil viscosity, which aided in the fabrication of GelMa droplets. GelMa microdroplets with 6 and 9 wt.% concentrations were fabricated. When the concentration of GelMa was <6 wt.%, cross-linked particles were unstable in oil, resulting in collapse of the spherical shape in PBS. At GelMa concentrations >9 wt.%, the viscosity was increased, generating a long jetting flow.

GelMa microdroplets flowed into the UV irradiation area where the UV irradiation activated the dissolved photoinitiator to polymerize the GelMa microdroplets, leading to the cross-linked network of the microgel. When the total energy irradiated on the microdroplets was <25.5 J/cm<sup>2</sup>, polymerization was insufficient to maintain its spherical shape in PBS. Excessive irradiation on the cell-encapsulated microdroplets exacerbated the cell viability.

The flow rate ratio of the aqueous GelMa phase to the oil phase ( $Q_{\text{GelMa}}/Q_{\text{oil}}$ ) ranged from 0.05 to 0.3 at a constant flow rate of the oil phase (1000 µl/h). At a flow rate ratio of <0.05, the dispersed phase did not penetrate the continuous phase, while at a flow rate ratio of >0.3, droplets were not generated, causing a long jetting flow. Within the flow rate ratio of 0.05 to 0.3, GelMa microdroplets with

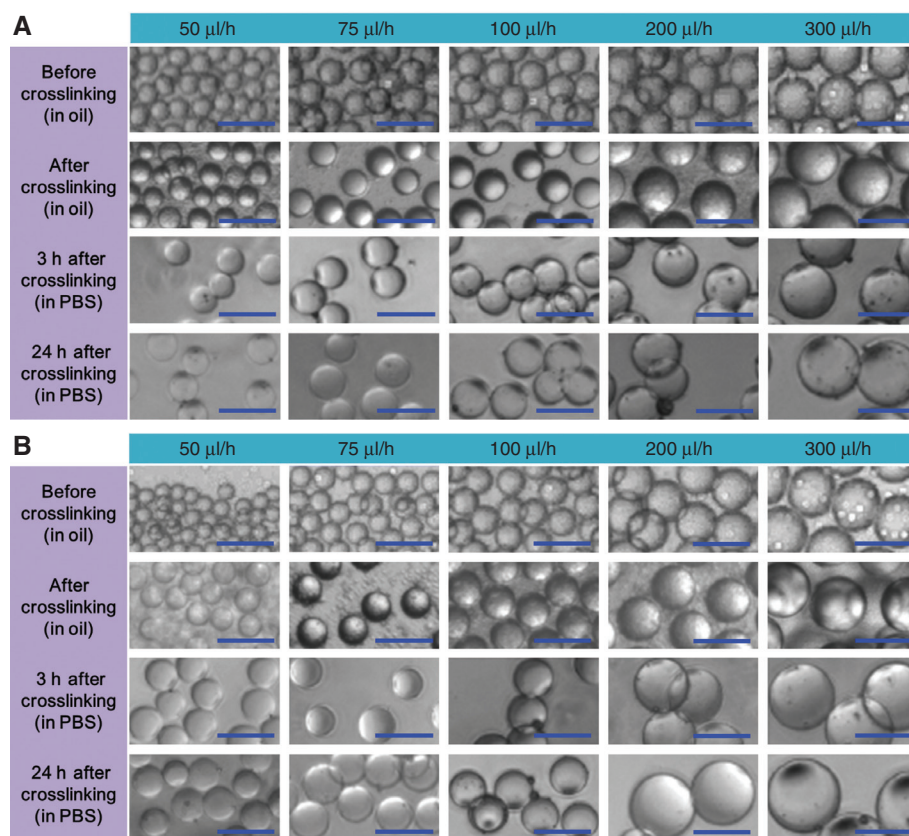


diameters from 30 to 132  $\mu\text{m}$  were generated. The droplet size was characterized according to the flow rate of the dispersed phase and swelling time. Figure 2(A) and (B) describes, respectively, the 6 and 9 wt.% GelMa spherical microgels in oil and PBS, depending on the swelling time. Overall, as the flow rate ratio increased, the droplet size and swelling rate increased. After the photo-cross-linking process of 6 and 9 wt.% GelMa microdroplets, the growth in size of GelMa microgels in oil was observed, which seemed to be caused by the polymerized hydrogel networks. When the GelMa microgels were immersed in PBS, the microgels began to swell with time and reached the equilibrium phase in swelling after 24 h. The 9 wt.% GelMa microgels in PBS showed much more swelling after 24 h than the 6 wt.% ones due to the higher cross-linking densities of hydrogels.

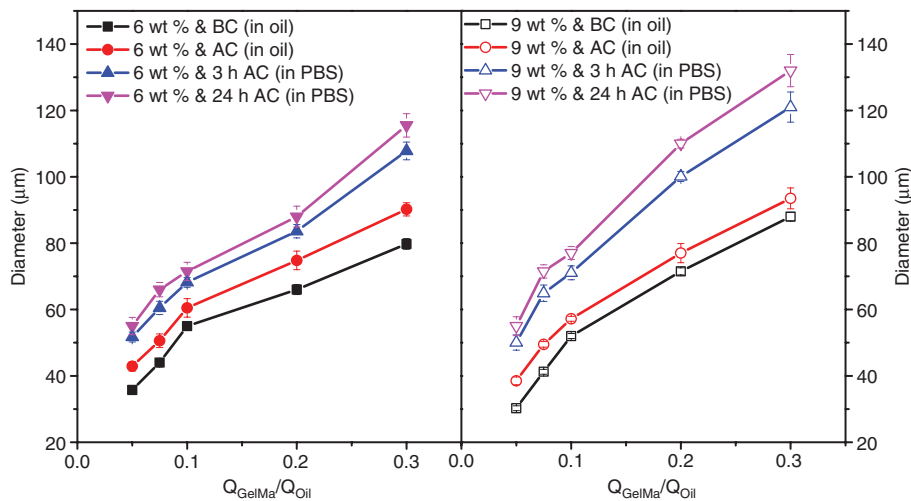
Figure 3 shows the measured diameters of 6 and 9 wt.% GelMa microdroplets and microgels that were plotted with the flow rate ratio for different swelling times, such as in oil before cross-linking, in oil after cross-linking, 3 h in PBS after cross-linking, and 24 h in PBS after cross-linking. The overall trend illustrated that the increase in flow rate ratio was proportional to the increase in diameter

of the GelMa spherical microgels with both 6 and 9 wt.% GelMa. In general, the increased viscosity of the dispersed phase generated large droplets with the increased flow rates. However, as shown in Figure S2(A), at a low flow rate ratio ( $<0.14$ ), 9 wt.% GelMa microdroplets were produced with smaller diameters than those of the 6 wt.% GelMa microdroplets. This phenomenon was observed because the dripping regime occurred at a flow rate ratio that was less than the threshold (0.14), while jetting was observed above the threshold. In the dripping regime, the higher viscosity of the dispersed phase produced uniformly smaller spherical droplets due to a larger pressure drop causing quick dripping. Alternatively, in the jetting regime with the higher viscosity, larger droplets were generated due to higher shear stress, causing long jetting.

Greater swelling ratios were observed with the greater GelMa concentration. As shown in Figure 3, as a result of the overall swelling in PBS for 24 h (from 0 to 24 h), the mean swelling ratio of 6 wt.% GelMa spherical microgels was  $94.1 \pm 27.9\%$ , while the 9 wt.% GelMa spherical microgels showed a greater mean swelling ratio of  $182.0 \pm 22.4\%$ . GelMa spherical microgels showed a high swelling ratio when they were initially



**Figure 2** Swelling characterization of spherical microgels at (A) 6 and (B) 9 wt.% GelMa. The flow rates of the GelMa prepolymer solution varied from 50 to 300  $\mu\text{l/h}$  at a fixed flow rate of oil (1000  $\mu\text{l/h}$ ) (scale bar 100  $\mu\text{m}$ ).



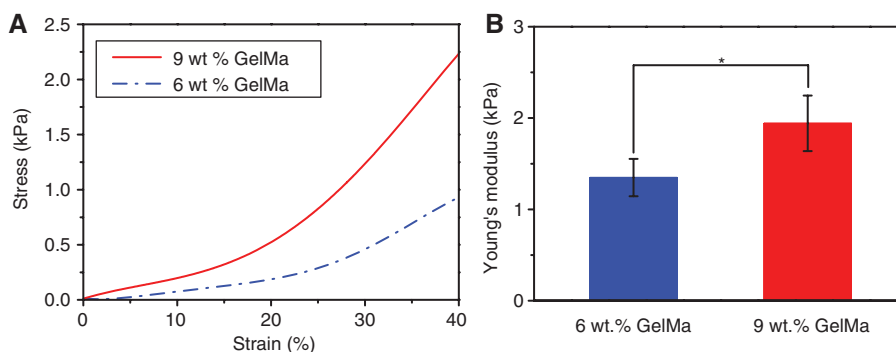
**Figure 3** Size dependence of the GelMa spherical microgels on flow rate ratio and swelling time for 6 wt.% GelMa (left) and 9 wt.% GelMa (right). BC, Before cross-linking; AC, after cross-linking.

immersed in PBS for 3 h after cross-linking. However, as the GelMa spherical microgels reached a swelling equilibrium during 24 h, the swelling ratio showed a drastic decrease. In the comparison of Figure S2(A) and (B), only a slight increase in diameter was observed after cross-linking, which was caused by a stabilizing process of the polymerized hydrogel. In Figure S2(B) and (C), the swelling ratios ( $Q_{0-3\text{ h}}$ ) of the spherical microgels from 0 to 3 h in PBS increased significantly because of water absorption under hydrophilic condition. After swelling for 3 h in PBS, the swelling ratios ( $Q_{0-3\text{ h}}$ ) of 6 and 9 wt.% GelMa spherical microgels were 39.6–75.0% and 91.7–125.4%, respectively, depending on the flow rate ratios (0.05 to 0.03). As shown in Figure S2(C) and (D), the swelling ratios ( $Q_{3-24\text{ h}}$ ) from 3 to 24 h in PBS were rapidly reduced due to the limited capacity for water absorption. After swelling for 21 h (from 3 to 24 h) in PBS, the swelling

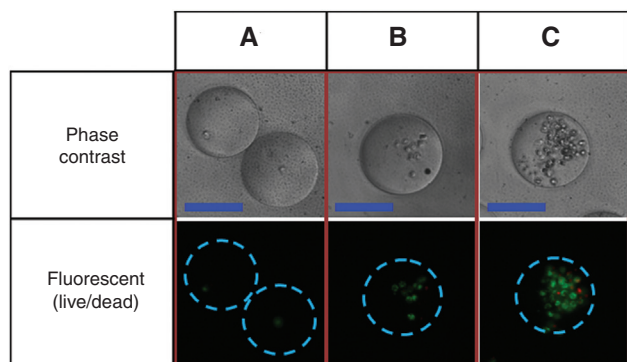
ratios ( $Q_{3-24\text{ h}}$ ) decreased to 15.2–29.8% and 27.2–33.7% for the 6 and 9 wt.% GelMa spherical microgels, respectively.

Although the 9 wt.% GelMa spherical microgels were smaller in PBS below the threshold flow rate ratio (0.14), greater swelling of the 9 wt.% GelMa spherical microgels led to a diameter identical to that of the 6 wt.% GelMa spherical microgels. After swelling for 24 h in PBS, the diameters of the 9 wt.% GelMa spherical microgels increased more at all flow rate ratios than those of 6 wt.% GelMa spherical microgels, as shown in Figure S2(D).

The stiffness of the GelMa spherical microgels was investigated using compressive strength tests. As shown in Figure 4, compressive stress-strain curves were obtained for two different GelMa concentrations (6 and 9 wt.%). From the stress-strain curves, the elastic modulus was calculated as  $1.35 \pm 0.20$  and  $1.94 \pm 0.31$  kPa for the 6 and 9 wt.% of GelMa hydrogels, respectively ( $p < 0.01$ ). As the



**Figure 4** Compressive strength test. (A) Stress-strain curves of GelMa hydrogels and (B) Young's modulus of 6 and 9 wt.% GelMa hydrogels. (\* $p < 0.01$ ).



**Figure 5** Phase contrast and fluorescent images of NIH/3T3 cells encapsulated in GelMa spherical microgels with encapsulation of (A) a single cell, (B) a small number of cells, and (C) a large number of cells (scale bar 100  $\mu\text{m}$ ).

concentration of the GelMa increased, the rigidity of the spherical microgels showed a significant increase due to the increase in cross-linking chains.

In order to demonstrate the possibility of bioencapsulation, NIH/3T3 cells were encapsulated inside the spherical microgels and their viability was evaluated using the Live/Dead Viability Cytotoxicity Kit. GelMa spherical microgels were produced at a flow rate ratio of 0.3 using the 6 wt.% GelMa prepolymer solution for this study. Figure 5 shows both the phase contrast and the fluorescent microscopic images of the live and dead cells inside the spherical microgels on Day 1. The encapsulated cell number per spherical microgel could be controlled by cell density premixed with the GelMa prepolymer solution. Figure 5(A–C) indicates the encapsulation of a single cell, a small number of cells, and a large number of cells,

respectively, which represented the homogeneous encapsulation of the cells driven by co-axial flow focusing. Encapsulated NIH/3T3 cells stained with green showed high cell viability inside the GelMa spherical microgels. These results demonstrated that the microdroplet generation and polymerization processes using GelMa were non-cytotoxic and biocompatible in the microfluidic system.

## 4 Conclusions

The swelling characteristics of photo-cross-linked GelMa spherical microgels are reported in this study. A microfluidic system consisting of a flow-focusing device and a UV irradiation apparatus generated homogeneous GelMa spherical microgels of a controlled size. The increased flow rate ratio produced larger microgels. The increased GelMa concentration led to stronger mechanical properties of microgels with higher swelling ratios. The initial rapid swelling ratio of GelMa spherical microgels decreased gradually, approaching a swelling equilibrium. Application for bioencapsulation was demonstrated with good viability of NIH/3T3 cells encapsulated in the spherical microgels. Thus, the characterized swelling behavior of GelMa spherical microgels could be valuable for use as a flexible platform in biological or medical applications.

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## References

- Mano JF, Sousa RA, Boesel LF, Neves NM, Reis RL. Bioinert, biodegradable and injectable polymeric matrix composites for hard tissue replacement: state of the art and recent developments. *Compos Sci Technol.* 2004;64:789–817.
- Ramakrishna S, Mayer J, Wintermantel E, Leong KW. Biomedical applications of polymer-composite materials: a review. *Compos Sci Technol.* 2001;61:1189–224.
- Jagur-Grodzinski J. Biomedical application of functional polymers. *React Funct Polym.* 1999;39:99–138.
- Mathiowitz E, Jacob JS, Jong YS, et al. Biologically erodable microspheres as potential oral drug delivery systems. *Nature.* 1997;386:410–4.
- Radovic M, Vranjes-Duric S, Nikolic N, et al. Development and evaluation of 90Y-labeled albumin microspheres loaded with magnetite nanoparticles for possible applications in cancer therapy. *J Mater Chem.* 2012;22:24017–25.
- Freiberg S, Zhu XX. Polymer microspheres for controlled drug release. *Int J Pharmaceut.* 2004;282:1–18.
- Kawaguchi H. Functional polymer microspheres. *Prog Polym Sci.* 2000;25:1171–210.
- Malafaya PB, Silva GA, Reis RL. Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. *Adv Drug Deliver Rev.* 2007;59:207–33.
- Gyawali D, Nair P, Zhang Y, et al. Citric acid-derived in situ crosslinkable biodegradable polymers for cell delivery. *Biomaterials.* 2010;31:9092–105.
- Yao R, Zhang R, Lin F, Luan J. Biomimetic injectable HUVEC-adipocytes/collagen/alginate microsphere co-cultures for adipose tissue engineering. *Biotechnol Bioeng.* 2012;110:1430–43.
- Basinska T, Slomkowski S. Design of polyglycidol-containing microspheres for biomedical applications. *Chem Pap.* 2012;66:352–68.

12. Martinez AW, Phillips ST, Whitesides GM, Carrilho E. Diagnostics for the developing world: microfluidic paper-based analytical devices. *Anal Chem.* 2009;82:3–10.
13. Park CH, Lee J. Electro sprayed polymer particles: effect of the solvent properties. *J Appl Polym Sci.* 2009;114:430–7.
14. Bock N, Woodruff MA, Hutmacher DW, Dargaville TR. Electro spraying, a reproducible method for production of polymeric microspheres for biomedical applications. *Polymers.* 2011;3:131–49.
15. Feng S-S, Ruan G, Li Q-T. Fabrication and characterizations of a novel drug delivery device liposomes-in-microsphere (LIM). *Biomaterials.* 2004;25:5181–9.
16. Liao Y, Fukuda T, Kamata N. Effect of solvent evaporation on the self-assembly of poly(3-hexylthiophene-2,5-diyl) and on the film morphology during electrospray deposition. *Phys Status Solidi RRL* 2013;8:154–7.
17. Lopac SK, Torres MP, Wilson-Welder JH, Wannemuehler MJ, Narasimhan B. Effect of polymer chemistry and fabrication method on protein release and stability from polyanhydride microspheres. *J Biomed Mater Res B.* 2009;91B:938–47.
18. Zeng Y, Novak R, Shuga J, Smith MT, Mathies RA. High-performance single cell genetic analysis using microfluidic emulsion generator arrays. *Anal Chem.* 2010;82:3183–90.
19. Duncanson WJ, Zieringer M, Wagner O, et al. Microfluidic synthesis of monodisperse porous microspheres with size-tunable pores. *Soft Matter.* 2012;8:10636–40.
20. Seo M, Paquet C, Nie Z, Xu S, Kumacheva E. Microfluidic consecutive flow-focusing droplet generators. *Soft Matter.* 2007;3:986–92.
21. Park JI, Saffari A, Kumar S, Günther A, Kumacheva E. Microfluidic synthesis of polymer and inorganic particulate materials. *Annu Rev Mater Res.* 2010;40:415–43.