Colloids and Surfaces B: Biointerfaces 147 (2016) 1–8

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Enhancing the biocompatibility of microfluidics-assisted fabrication of cell-laden microgels with channel geometry

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ARTICLE INFO

Article history:
Received 11 April 2016
Received in revised form 14 July 2016
Accepted 19 July 2016
Available online 20 July 2016

Keywords:
Microfluidics
Flow-focusing geometry
Cell encapsulation
Microgel
Biocompatibility

ABSTRACT

Microfluidic flow-focusing devices (FFD) are widely used to generate monodisperse droplets and microgels with controllable size, shape and composition for various biomedical applications. However, highly inconsistent and often low viability of cells encapsulated within the microgels prepared via microfluidic FFD has been a major concern, and yet this aspect has not been systematically explored. In this study, we demonstrate that the biocompatibility of microfluidic FFD to fabricate cell-laden microgels can be significantly enhanced by controlling the channel geometry. When a single emulsion ("single") microfluidic FFD is used to fabricate cell-laden microgels, there is a significant decrease and batch-to-batch variability in the cell viability, regardless of their size and composition. It is determined that during droplet generation, some of the cells are exposed to the oil phase which is shown to have a cytotoxic effect. Therefore, a microfluidic device with a sequential ('double') flow-focusing channels is employed instead, in which a secondary aqueous phase containing cells enters the primary aqueous phase, so the cells' exposure to the oil phase is minimized by directing them to the center of droplets. This microfluidic channel geometry significantly enhances the biocompatibility of cell-laden microgels, while maintaining the benefits of a typical microfluidic process. This study therefore provides a simple and yet highly effective strategy to improve the biocompatibility of microfluidic fabrication of cell-laden microgels.

1. Introduction

Engineering hydrogels in micrometer scales ('microgels') has attracted considerable research and industrial interest, as it takes advantage of the best of both worlds; favorable physical properties of hydrogels (e.g. hydrophilicity, biocompatibility, elasticity, and permeability), and miniaturization for enhanced processability, cost-effectiveness, high-throughput, and injectibility [1–3]. For biomedical applications, the microgels are extensively investigated as delivery vehicles for therapeutic molecules [2–4]. In addition, they are also widely used as scaffolds to encapsulate cells for tissue engineering applications [5–7].

For the fabrication of microgels, bulk emulsion polymerization is commonly employed; emulsification of a pre-gel solution within an immiscible continuous phase, followed by gelation of emulsion particles [8–10]. Generally, the emulsification is induced by applying high mechanical energy, such as sonication, to the mixture of two immiscible phases. However, this type of bulk method suffers from two critical drawbacks; particle polydispersity and damage to encapsulating species.

Microfluidic flow-focusing devices (FFD) have recently become a highly attractive tool to overcome those limitations of the traditional bulk emulsion method and generate monodisperse microgels in a reliable manner [7,11–15]. The flow-focusing channel geometry involves two intersecting channels; a fluid (dispersed phase) from one channel is broken up into monodisperse droplets by shear stress generated from a flow of immiscible fluid (continuous phase) from the other channel. The size of the droplets can be conveniently controlled by varying the flow rates of the two fluids. Furthermore, fabrication of more complex emulsions, such as Janus particles and muti-layered particles can be accomplished by adjusting the channel geometry to manipulate multiple fluid flows [16–18]. If these droplets consist of a gel precursor solution, a crosslinking method can be applied to fabricate microgels. As photocrosslinking is the most commonly used method for developing hydrogels, the droplet-generating microfluidic process coupled with photocrosslinking step could be broadly applicable for engineering microgels for various biomedical applications.

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http://dx.doi.org/10.1016/j.colsurfb.2016.07.041
0927-7765/

Several previous studies that utilized microfluidic FFD to create cell-laden microgels often displayed highly inconsistent and lower biocompatibility results, as compared with the same type of hydrogels fabricated using different methods [19–22]. However, this aspect has not been systematically investigated to date. Herein, we first demonstrated that there is indeed a significant reduction and inconsistency in the biocompatibility of cell-laden microgels, fabricated from a single emulsion ('single') microfluidic FFD, the simplest and most commonly used form of flow-focusing geometry, regardless of their physical properties. It was first postulated and then experimentally determined that during the droplet formation, some of the cells in an aqueous phase were possibly exposed to the continuous oil phase imparting a cytotoxic effect. In order to circumvent the cells from exposure to the oil phase during droplet generation, we employed a sequential ('double') microfluidic FFD in which a secondary aqueous phase containing cells is designed to enter the center of the primary aqueous phase during droplet generation. Eventually the cells would remain at the center rather than randomly within the microgels. Various physical properties as well as the viability of encapsulated cells of the microgels, developed from both single and double FFD, were measured and compared to validate the strategy.

2. Materials and methods

2.1. Microfluidic fabrication of microgels

The detailed fabrication process and channel geometries for the polydimethylsiloxane (PDMS)-based microfluidic devices are described in the Supplementary material. The inlet and outlet of the microfluidic device were connected by plastic tubing (0.3 mm inner diameter and 0.76 mm outer diameter, Tygon®). The solutions were injected and their flow rates were controlled by electronic syringe pumps (KDS100, KD Scientific). For single flow-focusing geometry, the aqueous phase consisted of photocrosslinkable methacrylic gelatin ('MA-Gel', 5 or 8% (w/v)) and Irgacure® 2959 (0.1% (w/v) in phosphate buffered saline (PBS, pH 7.4), and the oil phase consisted of Span®80 (20% (v/v) in mineral oil (Sigma Aldrich). For a double flow-focusing geometry, two separate aqueous phases were used; the first aqueous phase consisted of MA-Gel (5 or 8% (w/v) and Irgacure® 2959 (0.1% (w/v), and the second aqueous phase consisted of MA-Gel (4 or 6% (w/v) and Irgacure® 2959 (0.1% (w/v). The solutions were filtered (0.2 μm syringe filter) prior to use. Detailed synthesis of MA-Gel is described in the Supplementary material [23–25]. The droplet formation was monitored with an inverted optical microscope (XDS-3FL, Optika). The experiment was performed at 37 °C to maximize the cell viability during experiments. Due to the presence of methacrylic groups on gelatin chains, it prevented MA-Gel chains from physical association, and thus MA-Gel solution did not undergo physical gelation and remained in solution throughout the fabrication process.

The droplets were then exposed to UV for five minutes (850 mW/cm^2, S1500, OmniCure®) to crosslink the droplets to form microgels. The microgels were collected from the oil phase by centrifugation, and washed with PBS three times to remove residual oil.

2.2. Mechanical characterization of microgels

Mechanical properties of MA-Gel microgels were evaluated by measuring their elastic moduli using atomic force microscopy (AFM) based nanoindentation (Agilent 5100 ILM) [24,26]. The spring constant of a standard silicon cantilever used for indentation was 0.20 N m^{-1} [27]. After placing the microgel onto a sample stage, the cantilever was positioned at the center of each microgel, and the piezo-based scanner is moved at the rate of 3 μm s^{-1} to indent the microgel. The applied force vs. indentation depth was obtained. The elastic modulus (E) of each microgel was calculated using Hertz contact theory for the spherical elastic solid,

$$F = \frac{4}{3}\left(\frac{E}{1-\nu^2}\right) R^{1/2} h^{3/2}, \quad (1)$$

where R is the radius of a microgel, h is the indentation depth, and ν is the Poisson's ratio of the microgel and equals to 0.5 assuming the microgel follows the behavior of an ideal rubber [28].

2.3. Biocompatibility evaluation of cell-laden microgels

NIH3T3 fibroblasts (ATCC) were encapsulated within MA-Gel microgels by dispersing the cells in the pre-gel solution prior to microgel fabrication (2×10^6 cells mL^{-1}). For the double flow-focusing geometry, the cells were dispersed in the secondary aqueous phase to focus the cells to the center of the microgels. The cell-laden microgels were then immersed in cell culture media (Dulbecco's Modified Essential Medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin, Gibco®) and cultured at 37 °C with 5% atmospheric CO_2.

The viability of cells within the microgels was evaluated using LIVE/DEAD® Viability/Cytotoxicity Kit (Molecular Probes). Briefly, cell-laden microgels were collected at a designated time point, and treated with calcein-AM and ethidium homodimer-1 to visualize and count the live (green fluorescence) and dead (red fluorescence) cells, respectively, using a fluorescence microscope (XDS-3FL, Optika). The viability was reported as the percentage of live cells from the total number of cells.

3. Results and discussion

3.1. Microgel fabrication using a single microfluidic flow-focusing device (FFD)

Photocrosslinkable gelatin, which is conjugated with methacrylic functional groups ('MA-Gel'), was used to fabricate microgels in this study. Previous studies have demonstrated that photocrosslinkable gelatin hydrogels prepared via microfabrication techniques such as photolithography and micromolding could be successfully utilized as cell-laden scaffolds for tissue engineering applications [29–32]. Gelatin, derived from natural collagen via hydrolysis, retains cell adhesion sequences (e.g. RGD peptides) and matrix degradation sequences (e.g. matrix metalloproteinase recognition domains), therefore is a highly attractive choice as a scaffold material for tissue engineering [33,34].

Monodisperse cell-laden MA-Gel microgels were engineered using a microfluidic flow-focusing device (FFD) by photocrosslinking the pre-gel solution droplets generated from a single microfluidic FFD (Fig. 1, Fig. S2a, Video S1). The pre-gel solution consisting of 3T3 fibroblasts dispersed in MA-Gel and a photoinitiator was used as the dispersed aqueous phase, and flowed from inner channel, which was intersected by a flow of continuous oil phase (*i.e.* mineral oil with Span®80 as a surfactant). The use of a relatively high concentration of Span®80 was necessary for the following reasons. First, due to the high viscosity of MA-Gel pre-gel solution, the aqueous flow formed a jet instead of droplets when the surfactant was absent from the oil phase (Fig. S3a). With the increasing amount of the surfactant, the jetting gradually transitioned into the dripping regime as the increased shear stress imparted by the increased viscosity of the oil phase overcame the interfacial tension of the aqueous phase. Even with the dripping regime, the resulting droplets were not stable, often resulting in coalescence (Fig. S3b). Only when the surfactant concentration was significantly raised, above 15% (v/v), stable and monodisperse droplets were formed.