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In-situ photopolymerization of monodisperse and discoid oxidized methacrylated alginate microgels in a microfluidic channel

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We present a simple microfluidic technique to *in-situ* photopolymerize (by 365 nm ultraviolet) monodisperse oxidized methacrylated alginate (OMA) microgels using a photoinitiator (VA-086). By this technique, we generated monodisperse spherical OMA beads and discoid non-spherical beads with better shape consistency than ionic crosslinking methods do. We found that a high monomer concentration (8 w/v %), a high photoinitiator concentration (1.5 w/v %), and absence of oxygen are critical factors to cure OMA microgels. This photopolymerizing method is an alternative to current methods to form alginate microgels and is a simpler approach to generate non-spherical alginate microgels. © *2016 AIP Publishing LLC*.
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Microgels generated by microfluidics have broad and powerful applications in cell analysis,[1] cell culture,[2] drug delivery,[3] and material engineering.[4] Although alginate has many advantages to be employed in these applications, such as mild gelation process, biocompatibility, biomimetic microscale structure, and high water content,[5] its typical gelation process is not favorable to form microgels because it needs divalent cation crosslinkers (e.g., Ca^{2+}) from an additional downstream crosslinking flow, containing either Ca^{2+} or acid, respectively, known as external and internal gelation methods.[6,7] When encountering the alginate precursor droplets to solidify them, the crosslinking flow may deform the droplets and the deformation will be preserved due to the instantaneous crosslinking process.[8] This process may reduce the microgel shape consistency and, more importantly, make it challenging to generate non-spherical alginate microgels. The robustness of ionic crosslinking methods is also relatively low because the crosslinking fluid may disturb the microfluidic steady state and generate undesired alginate microgels that can clog the microchannel.

One alternative approach can solve these issues effectively and easily: photopolymerizing or flow lithography.[9,10] We have developed a photocrosslinkable oxidized methacrylated alginate (OMA) that can be polymerized by ultraviolet (UV) light at a macroscale and degrade within weeks, as desired.[12] In this paper, we employed a flow-focusing method to generate water-in-oil OMA emulsions complemented with a photoinitiator (VA-086) in a polydimethylsiloxane (PDMS) microfluidic device and utilized a light-emitting diode (LED) UV light to

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polymerize the microdroplets into microgels inside the microchannel (i.e., *in-situ* or on-line). We also investigated the effects of concentration of OMA monomer, concentration of photoinitiator, and oxygen on curing OMA microgels. The motivation for the present work is to present improved monodispersity by using *in-situ* photopolymerization of the OMA microgels and to develop an alternative photopolymerization method to form non-spherical alginate microgels.

We synthesized the OMA monomer (with theoretical 17.5% oxidation and 45% methacrylation) based on our published protocol.[12] The OMA was dissolved in 1 ml deionized (DI) water at 4 and 8 w/v %, complemented with VA-086 (Wako, VA) at 0.5, 1, and 1.5 w/v %. Two flow-focusing PDMS microfluidic devices with different geometries were manufactured by soft-lithography to generate spherical or discoid OMA beads, respectively: a 40 μm high microchannel with 40 μm wide flow-focusing section and 100 μm wide downstream photopolymerizing section; and a 20 μm high microchannel with 20 μm wide flow-focusing section and 250 μm wide downstream photopolymerizing section. To generate OMA microdroplets, soybean oil (Sigma, MO) as a continuous oil phase fluid and VA-086 complemented OMA solution as a disperse phase fluid were driven into the microchip by two syringe pumps. A 365-nm LED UV light (Hamamatsu, Japan) was applied onto the OMA microdroplet emulsions at $\sim$600 mW/cm^2 (see supplementary material for a UV light intensity calibration),[18] while the other parts (upstream flow-focusing section and inlet and outlet tubings) were covered by aluminum foil to avoid undesired UV exposure. The outlet hole of the microchip was punched at different locations along a serpentine downstream channel (totally 30-cm-long) to adjust the UV exposure duration. The entire system was degassed (PDMS chip and fluids were degassed overnight and for 20 min, respectively) and the experiments were conducted in a nitrogen-filled glove bag. The microgels were washed by the DI water complemented with 1 v/v % Tween 20 (ThermoFisher, MA) and centrifuged (500 rpm for 3 min), followed by removing the supernatant. This washing process was repeated for three times. We fully dried and rehydrated the samples. When the shape of microgels could be preserved after shrinking and swelling during these two processes, they were considered fully cured. We observed and measured the microgels by a phase contrast microscope (Eclipse Ti-U, Nikon, IL). Since the UV light was too strong for the camera (QuantEM 512SC, Photometrics, AZ), we could not take images during UV exposure. A timer was used to record the residence time of UV exposure (from the uncovered section until the outlet) by tracking one microgel under the microscope. See Fig. 1(a) for the schematic diagram of the system.

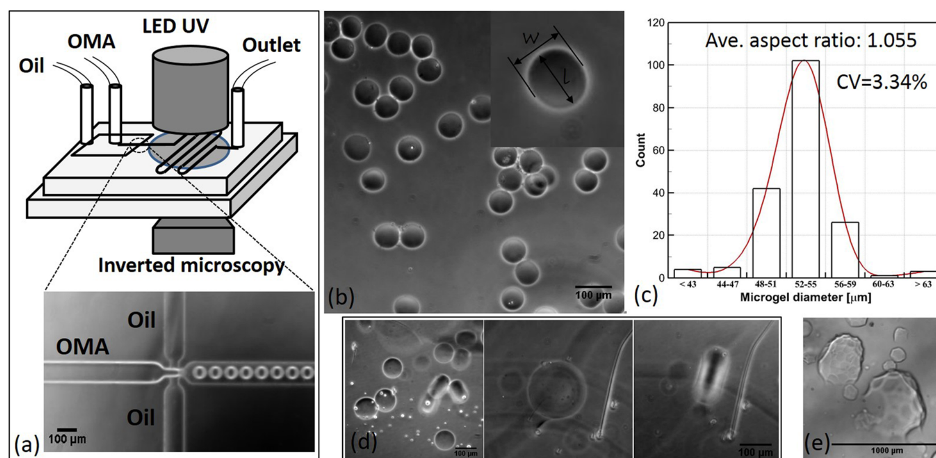

FIG. 1. Schematic diagram of experimental setup and key results. (a) Schematic diagram of experimental setup for *in-situ* photopolymerization of alginate microgels, including a PDMS microchip, one syringe pump for a continuous oil phase fluid, another syringe pump for a disperse phase fluid (OMA solution), an inverted microscope, and an LED UV light. Inset is a snapshot of the flow-focusing section taken by phase contrast microscopy (PCM). (b) Spherical OMA microgels collected from the microchannel for size measurement. (c) Size distribution of 183 randomly measured microbeads. (d) Discoid OMA microgels and two images (top and side views) of one discoid microgel. (e) Non-fully crosslinked OMA particles due to low photoinitiator concentration.

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Numerous photocrosslinkable materials have been employed to generate micro-particles by using microfluidics.[10] We have recently developed photocrosslinkable OMA, a promising material for basic biologic and tissue engineering studies due to its cytocompatibility, biodegradability, and tunable physical properties.[12] To our best knowledge, this is the first report of *in-situ* photopolymerizing OMA microbeads in a microfluidic device. Both histogram of size distribution and coefficient of variation (CV, diameter deviation divided by average diameter) of the microgels suggested their monodispersity (Fig. 1(c)). The aspect ratio (length ratio of major axis to minor axis, illustrated in Fig. 1(b) inset) was as low as 1.015, similar to the result of the internal gelation method that was considered to better control the shape consistency of microbeads than the external gelation methods.[6] The discoid microgels (Fig. 1(d)) were formed when the diameter of the OMA microdroplets exceeded the height of the microchannel because the microchannel confined them during polymerization. Note that the cured spherical microgels were 25.8% bigger than the uncured droplets on average. The thickness of the discoid microgels was even 3.85-fold greater than the height of the microchannel. This can be because the polymerized OMA microgels swelled in the DI water. The ionically crosslinked alginate microgels also exhibited similar swelling phenomenon after collection.[6] Hence, it is necessary to back-calculate the size of droplets (and the microchannel geometry) to control the final size of the microgels based on the swelling ratio.

Though the photocrosslinking efficiency of OMA is very good at a macroscale, OMA microdroplets were not always cured into microgels. We found two critical factors that determined the success in photopolymerizing OMA microbeads. First, oxygen significantly negatively affects free radical reaction, hence hindering the crosslinking of the OMA molecules.[13] This effect was not evident at a macroscale because the core of the bulk OMA was cured due to the low diffusion rate of oxygen. However, the oxygen effect was critical to cure microscale precursors.[9] We found the OMA microdroplets could only be fully cured when oxygen was removed by degassing the PDMS microchip, OMA solution, and soybean oil thoroughly, and the experiments were conducted in a nitrogen-filled glove bag. Second, we found that crosslinking density played a key role. Among all material combinations tested, only the material system with a high concentration of OMA monomer (8 w/v %) and a high concentration of VA-086 (1.5 w/v %) could be cured. When using 1% VA-086, for instance, the partially cured OMA microgels coalesced into sub-millimeter-scale particles (Fig. 1(e)). Improving the crosslinking density is a general principle to ensure the microdroplets to be cured when using other photocrosslinkable materials. For example, the concentration of poly(ethylene glycol) diacrylate (PEG-DA) was used as high as 20 w/v % to form microparticles in published protocols.[14]

Our technique improved the current methods to form alginate microgels in several aspects. First, the photocrosslinking technique eliminates the ionic crosslinking flow. Hence, the microgels had better shape consistency without the deformation when encountering this additional flow. The aspect ratio of OMA microgels was as low as 1.015, as good as the internal ionic gelation method and better than external methods.[6] In addition, the photocrosslinking method is more robust. Specifically, we could start applying UV after the microfluids became steady, whereas in the ionic crosslinking methods the alginate phase of flow (in forms of streaming, plugs, and droplets) might be cured before steady state was achieved. These undesired cured alginate microgels might clog the microchannel. We can turn off the light if necessary, whereas it is impossible to remove the ionic crosslinking fluid out of the microchannel to stop further crosslinking. Second, the *in-situ* photocrosslinking feature ensured the monodispersity of microgels and highly simplifies the current method to form non-spherical alginate microgels. Methacrylated alginate has been employed to generate microgels by a visible light.[15] However, the alginate microdroplet emulsion was cured after being collected in a beaker. This "off-line" polymerizing approach could only generate spherical microgels due to the surface tension and the microgels could not be monodisperse because of coalescence during polymerization. Additionally, it took much less time (tens of seconds) by our technique to cure the microgels using the UV than using the visible light (5 min).[15] Our preliminary results suggested that such high intensity UV did not reduce the cell viability (data not shown). Liu *et al.* have successfully generated non-spherical alginate microgels (by ionic polymerization) using a delicate

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microfluidic device.[8] However, this technique needs extraordinary expertise in microchannel design and microfluidics operation to control the ionic crosslinking process. The shape consistency using this method was also poor due to the simultaneous processes of introducing crosslinker and proceeding polymerization. Additionally, degradation of ionically crosslinked alginate is uncontrollable, highly limiting its applications in tissue engineering, because a desired scaffold should degrade at a rate proportional to the new tissues formation.[11,12,16,17] Hence, our technique to photopolymerize alginate microgels can be important because it is easier to form non-spherical microgels and the degradation of OMA is controllable.

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[18]See supplementary material at http://dx.doi.org/10.1063/1.4941339 for LC-L1V3 (Hamamatsu) LED UV intensity calibration.

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