Soft Matter Hot Paper

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Multi-Compartmental Hydrogel Microparticles Fabricated by Combination of Sequential Electrospinning and Photopatterning

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Abstract: *Multi-compartmental non-spherical hydrogel microparticles were fabricated by combining electrospinning and photopatterning. Sequential electrospinning produced multi-layered fiber matrices with different composition in which each layer became a compartment of the particle. Photopatterning of the hydrogel in the presence of the multi-layered fiber matrix generated multi-compartmental microparticles with different vertical functionalities. While the shapes of the hydrogel microparticles were determined by the design of the photomask, the chemical properties and size of each compartment were independently controlled by changing the molecules incorporated into each fiber matrix and the electrospinning times, respectively. The resultant multi-compartmental hydrogel microparticles could carry out not only the release of different growth factors with independent kinetics but also binding of multiple targets at different compartments.*

Well-defined anisotropic particles have recently attracted increasing attention in many areas of biomedical research, including bioimaging, drug delivery, sensing, and tissue engineering, because of the high degree of functional disparity that can be achieved on the same particle.[1] Various techniques have been used to fabricate anisotropic particles such as patchy,[2] multi-compartment,[3] and Janus particles.[4] Initially, many studies of anisotropic particles focused on Janus particles, which have biphasic properties by selective surface modifications. Although many strategies have been developed for the fabrication of these Janus particles, there are intrinsic limitations to control the physical properties of each part by just surface modification, for example, difficulty in selective control of bulk properties such as degradation and swelling. Thus, recent research related to anisotropic particles has gradually shifted towards multi-compartmentalized particles with compositional anisotropy from the surface anisotropy of Janus particles.[5] Because each individual compartment in a multi-compartmentalized particle can be composed of different materials or loaded with different additives, multi-compartmental particles can display the combined set of properties that are associated with the individual materials, and these properties are apparently different from those of particles based on the simple mixing of different materials.

Compartmentalized particles have been predominantly fabricated by fluidic processes such as microfluidic or electrohydrodynamic (EHD) co-jetting processes, both of which use a laminar flow of two or more polymer solutions. For example, Doyle and co-workers prepared multi-compartmental microparticles using stop- or continuous-flow lithography within microfluidic devices,[6] whereas Lahann and co-workers conducted pioneering work on the fabrication of anisotropic microparticles using EHD co-jetting.[7] Although fluidic processes have resulted in great successes in the fabrication of novel compartmentalized particles, these systems require the construction of combined parallel channels or needles with complex geometries and precise control over multiple flows. Furthermore, the shapes of the majority of multi-compartmental microparticles are still limited to spheres. The synthesis of non-spherical microparticles has attracted significant interest because of their myriad applications in the areas of optical light scattering, drug delivery, sensors, coatings, and catalysis because non-spherical shapes are highly desirable properties such as anisotropic responses to external fields, large surface areas, and unique structure formation.[8]

Here, we propose a simple fabrication method for generating multi-compartmental and non-spherical hydrogel microparticles in which each compartment is composed of a fiber matrix with a different composition. While different compartments are produced simultaneously in most fluidic processes, each compartment is prepared independently by the sequential electrospinning of different polymer solutions in our approach. Therefore, the properties of each compartment can be easily tuned without affecting the other compartments, not only by changing the type or the amount of polymer solution but also by incorporating different molecules into each fiber matrix. After the successful fabrication of hydrogel microparticles that were compartmentalized by an entrapped fiber matrix, a proof of concept study was performed using microparticles that were capable of releasing multiple growth factors with independent release kinetics and using microparticles with distinct binding affinity for multiple targets.

Figure 1 describes the overall procedure for the preparation of the multi-compartmental hydrogel microparticles by electrospinning and hydrogel photopatterning techniques. First, a fiber matrix incorporating a specific component is electrospun until the desired thickness is obtained. Second, a fiber matrix containing a different component is prepared on top of the first fiber matrix. This process is repeated until the desired number of fiber matrice is obtained (step 1). After

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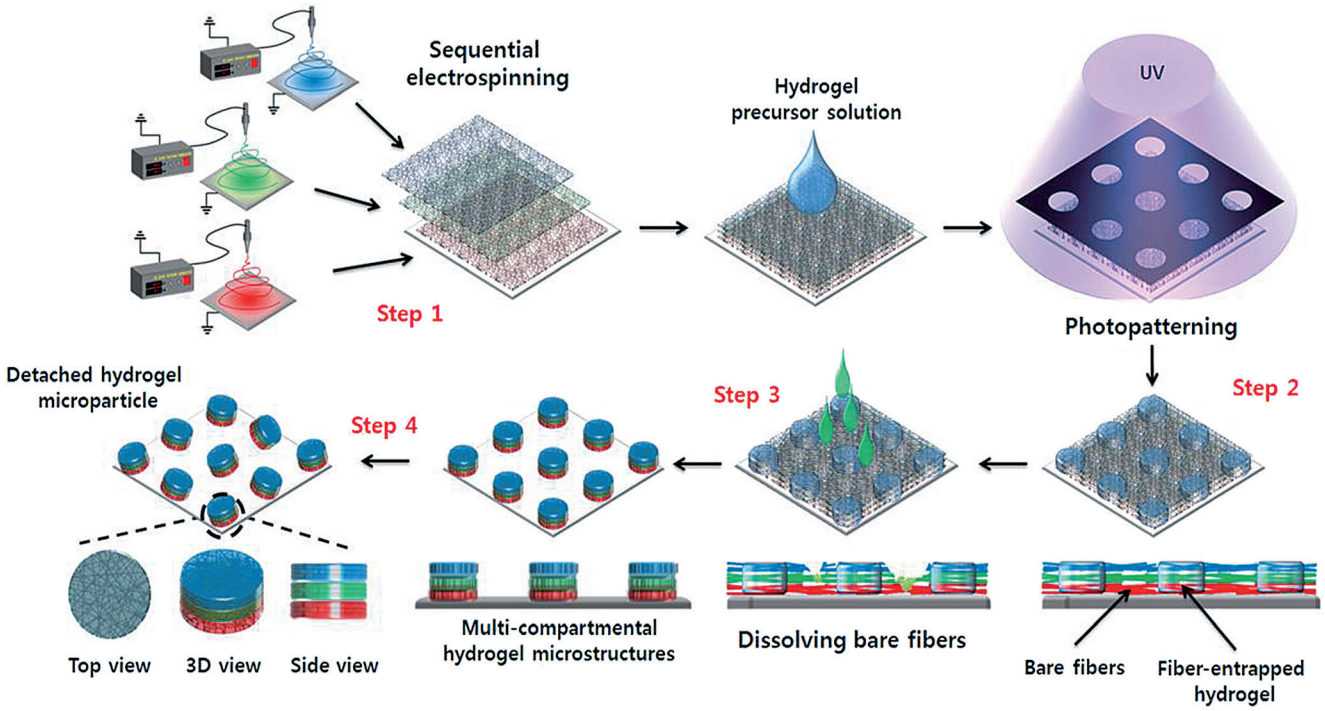

Figure 1. Schematic illustration of preparing multi-compartmental hydrogel microparticles by using sequential electrospinning and photopatterning.

the preparation of the multi-layered fiber matrix with different compositions, the hydrogel micropatterns are fabricated by using the ability of poly(ethylene glycol)diacrylate (PEGDA, molecular weight, MW, 575 g mol^{-1}) to form a gel upon exposure to UV light. The photopatterning of a PEG precursor solution through the photomask in the presence of the multi-layered fiber matrix creates two different microdomains, a bare fiber region and a fiber-entrapped hydrogel region (step 2). Reacting the fiber-incorporated hydrogel micropatterns with chloroform selectively removes the bare fibers to produce an array of hydrogel microstructures entrapping the multi-layered fibers (step 3). Because chloroform cannot penetrate into the hydrogel because of water,[9] the fibers remain intact within the hydrogel microparticles. Finally, the resultant hydrogel microstructures are obtained as microparticles by scraping the microstructures from the substrates, and each fiber matrix becomes the compartment of hydrogel microparticles (step 4).

Figure 2a shows SEM, optical and fluorescence images of PEG hydrogel microparticles that were bi-compartmentalized with different polycarprolactone (PCL; MW of 80000 g mol^{-1}) fiber matrices. For the visualization of each compartment, the PCL solutions were mixed with different fluorescence dyes (Alexa Fluor 350, 500, and 610) prior to electrospinning.

The SEM image shows the fabrication of hydrogel microparticles without any residual fibers, and the optical image confirms the presence and localization of the fibers within the hydrogel microparticles. The fluorescence image shows that each compartment was spatially segregated, and the interfaces between the different compartments were very well-

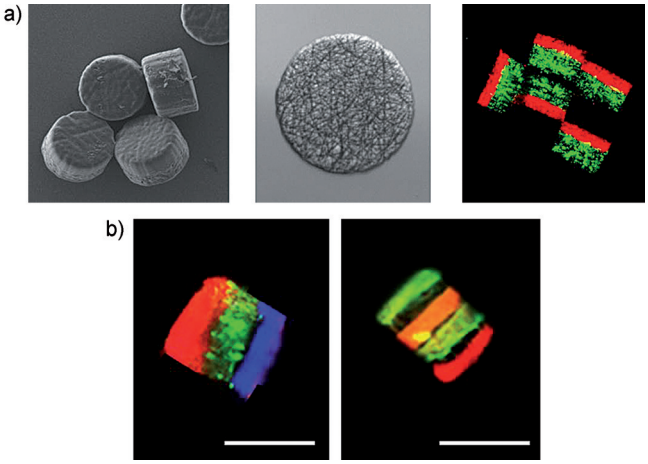

Figure 2. Fabrication of non-spherical hydrogel microparticles compartmentalized by entrapped fiber matrix. a) SEM, optical, and fluorescence images of bi-compartmental hydrogel microparticles. b) Fluorescence image of tri- and tetra-compartmental hydrogel microparticle. (Scale bar: 200 μm).

defined without any significant overlap. The numbers and compositions of the compartments were controlled by the number of electrospinning processes and the incorporated molecules within each electrospun fiber matrix, respectively, as demonstrated by the tri- and tetra-compartmental hydrogel microparticles shown in Figure 2b. The thickness of the fiber matrix and the height of the hydrogel microparticles could be tuned by changing the collection time of electrospinning and the volume of the hydrogel precursor solution, respectively, as shown in Figure 3a.

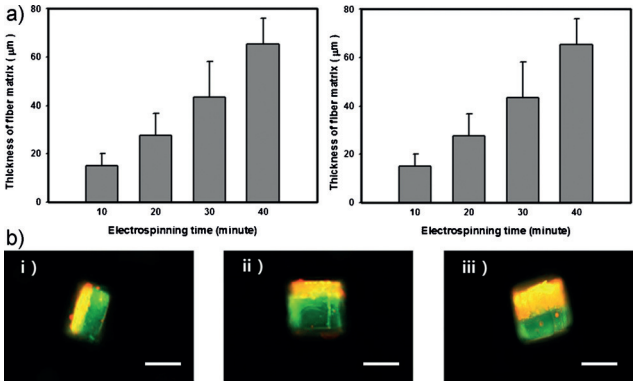

Figure 3. Control of the thickness of the fiber matrix and the height of the hydrogel microparticles. a) Effect of the electrospinning time on the thickness of fiber matrix (left) and effect of the amount of precursor solution on the height of the hydrogel microparticle (right). b) Fluorescence images of fiber-entrapped hydrogel microparticles with different height of fiber matrix and hydrogel microparticle. Orange dye and green dye were incorporated into the fibers and the hydrogel, respectively. (Scale bar: 100 μm).

Therefore, fiber-entrapped hydrogel microparticles with different height of fiber matrix and hydrogel microparticle could be easily fabricated as shown in the fluorscence images (Figure 3b). For example, in case of fluorescence images (i) and (ii), the electrospinning time was 20 minutes producing an approximately 30 μm-thick fiber matrix, but more volume of the hydrogel precursor solution [50 μL for image (i) and 100 μL for image (ii)] was used for hydrogel microparticles in image (ii), producing higher hydrogel microparticles (ca. 200 μm) than those in image (i) (ca. 100 μm). On the other hands, in fluorescence images (ii) and (iii), the volume of hydrogel precursor solution was the same but a longer electrospining time (60 min) was used for image (iii), producing hydrogel microparticles with the same height (ca. 200 μm) but a fiber matrix with different thickness (ca. 100 μm). Furthermore the shapes and lateral dimensions of the hydrogel microparticles could be easily controlled by changing the design of the photomask (see Figure S1 in the Supporting Information). The size of each compartment was independently tuned by controlling the duration of each electrospinning step, as shown in Figure S2. If we compare the yield and reliability between our approach and other fluidic processes in producing multi-compartmental particles, our process would have lower yield because of the relatively long electrospinning process. However, we are confident that our process is a more reliable method in controlling the size and shape of microparticles as well as the number and the property of the compartments.

Since the composition of each compartment could be independently controlled, we were able to fabricate multi-compartmental hydrogel microparticles in which each compartment contained different nanomaterials. For example, we fabricated bi-compartmental hydrogel microparticles where one compartment contained qunatum dots (QDs) with emission wavelength of 600 nm and the other compartment contained magnetic nanoparticles, as described in Figure 4a.

The incorporation of nanoparticles into the different fiber matrices was confirmed by fluorescence and TEM images, as

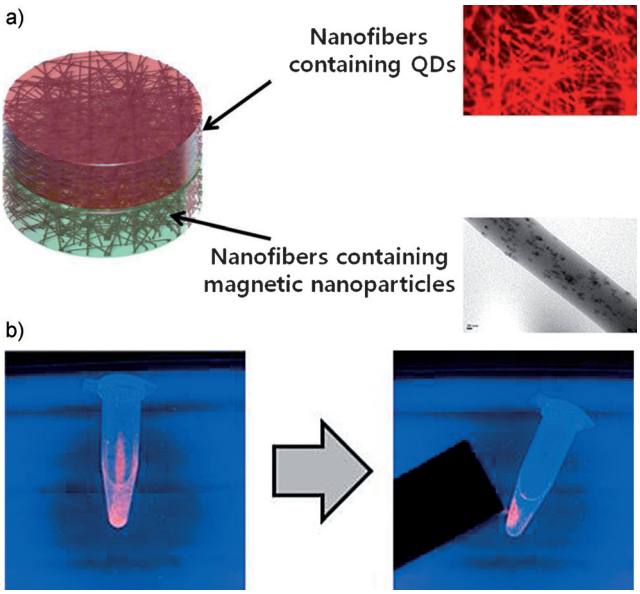

Figure 4. Bi-compartmental hydrogel microparticles containing different nanomaterials in each compartment. a) Scheme of bi-compartmental microparticles with fluorescence and TEM images. b) Bi-compartmental hydrogel microparticles being attracted by a magnet

shown in Figure 4a. Because of the incorporated magnetic nanoparticles, the resultant bi-compartmental hydrogel microparticles were easily separated by a magnet as shown in Figure 4b.

The multi-compartmental architecture can be applied in various biomedical fields, such as drug delivery and biosensing. As first proof-of-concept experiment, we fabricated dual growth factor-loaded bi-compartmental hydrogel microparticles that were capable of releasing two different growth factors with independent release kinetics. In this system, the size of each compartment was the same, but the chemistry of each compartment was different. One compartment was based on fibers of poly(DL-lactide-co-glycolide) with lactide/glycolide ratio 65:35 (PLGA 65:35) and the other compartment was based on fibers of PLGA 85:15. Figure 5 displays the controlled release of bFGF and BMP-2 which were loaded in each compartment. In Figure 5a, bFGF and BMP-2 were incorporated in PLGA 65:35 and PLGA 85:15, respectively. The release for the opposite loading case (i.e., BMP-2 in PLGA 65:35 and bFGF in PLGA 85:15) is seen in Figure 5b. In both cases, bFGF and BMP-2 in PLGA 65:35 were released faster than in PLGA 85:15 because the PLGA with the lower lactide/glycolide ratio degraded faster than the PLGA with the higher lactide/glycolide ratio.[10] These results showed that multiple growth factor-releasing hydrogel microparticles can be generated by different immobilizing growth factors within each compartment, and the release of each growth factor can be controlled independently by adjusting the properties of the fibers in each compartment.

As second proof-of-concept experiment, we fabricated hydrogel microparticles consisting of two compartments with different binding affinity, which can be applied for a high-throughput bioassay in the future. Here, each compartment was made from IgG-immobilized and IgM-immobilized

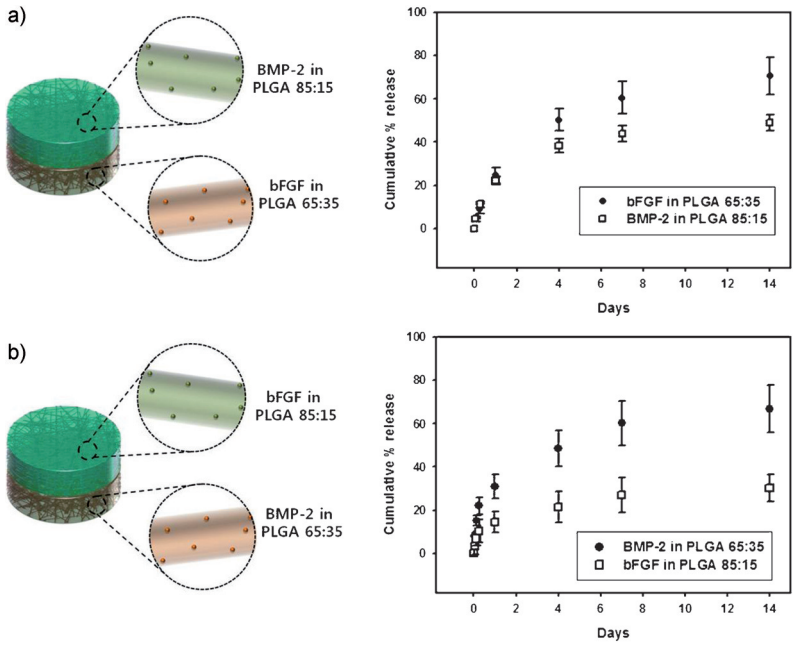

Figure 5. Bi-compartmental hydrogel microparticles capable of controlled release of different growth factors independently. a) bFGF in the PLGA 65:35 and BMP-2 in PLGA 85:15. b) BMP-2 in the PLGA 65:35 and bFGF in PLGA 85:15.

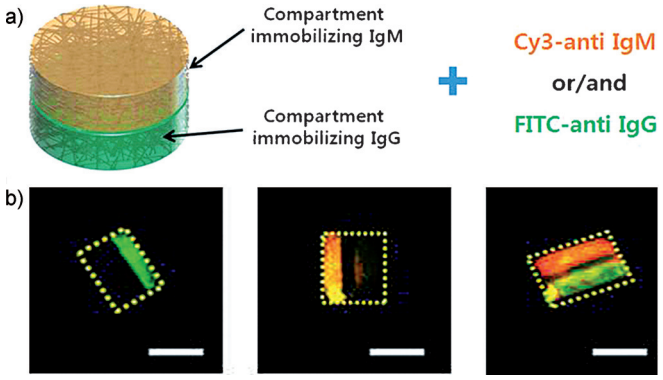

Figure 6. Binding assay with bi-compartmental hydrogel microparticles immobilizing IgG and IgM. a) Schematic illustration of the binding assay. b) Fluorescent images obtained after the microparticles were reacted with a solution of FITC-anti IgG only (left), Cy3-anti IgM only (middle), and a solution containing both FITC-anti IgG and Cy3-anti IgM (right).

fibers, respectively, as illustrated in Figure 6a. Antibodies were physically immobilized onto PCL fibers, while PEG (MW 200 g mol^{-1}) was added to the precursor solution as porogen to facilitate the diffusion of targets through hydrogel.[11] As shown in Figure 6b, when bi-compartmental hydrogel microparticles were incubated with a solution containing only FITC-anti IgG, green-fluorescent signals were observed only within the IgG-immobilized compartment without detectable signals in the IgM-immobilized compartment because of specific binding between the antibody and the antigen. On the other hand, strong fluorescent signals were observed only within the IgM-immobilized compartment when bi-compartmental hydrogel microparticles were incubated with a solution containing only Cy3-anti IgM. The incubation of hydrogel microparticles with solutions containing both FITC-anti IgG and Cy3-anti IgM resulted in fluorescent signals in both compartments. Since multiple assays are possible even with single microparticles, the use of developed multi-compartmental hydrogel microparticles for bioassay is expected to significantly enhance the assay throughput.

In summary, we developed a novel process for fabricating multi-compartmental hydrogel microparticles that use electrospinning and photopatterning processes. Sequential electrospinning processes produced a multi-layered fiber matrix that was able to incorporate different molecules. Subsequent photopatterning of the hydrogel microstructures in the presence of the multi-layered fiber matrix generated multi-compartmental microparticles with vertically differentiated functionalities. Chemical properties and sizes of each compartment were independently controlled by altering the molecules incorporated in each fiber matrix and by altering the electrospinning time, respectively. As potential applications of these multi-compartmental hydrogel microparticles, sequential release of multiple growth factors and binding of different targets to each compartment were successfully demonstrated. We expect that the resulting multi-compartmental hydrogel microparticles would play an important role in the development of novel biomaterials with precisely designable physical and chemical properties.

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