Fabrication of Nanofiber Microarchitectures Localized within Hydrogel Microparticles and Their Application to Protein Delivery and Cell Encapsulation

*Hyun Jong Lee, Young Ha Park, and Won-Gun Koh**

A simple method to generate well-defined microscopic architectures composed of electrospun nanofibers is reported and their potential application to biomedical fields are described. The photopatterning of polyethylene glycol (PEG) hydrogel on electrospun polycarprolactone (PCL) nanofibers leads to the formation of two different microdomains in nanofibrous mats: a bare nanofiber region and a hydrogel-entrapped nanofiber region. The selective dissolution of bare nanofibers with an organic solvent that cannot penetrate the PEG hydrogel enables the localization of PCL nanofibers within the hydrogel microstructures, thus generating microarchitectured nanofibers. The resultant microarchitectures are easily detached from the substrate by the water-induced swelling of the PEG hydrogel. Microparticles are ultimately obtained, the size and shape of which can be easily controlled with proper photomask designs. In proof of concept experiments, bovine serum albumin(BSA)-loaded PCL nanofibers that are entrapped within the hydrogel microparticles are prepared and the sustained release of BSA from micropatterned nanofibers is successfully demonstrated, indicating the potential application of the proposed microarchitectured nanofibers to drug delivery systems. For another possible application, the capability of the nanofiber-incorporated hydrogel to encapsulate mammalian cells is investigated and the incorporation of nanofibers within the PEG hydrogel promoted cell adhesion and spreading when compared with bare PEG hydrogel is confirmed.

1. Introduction

In the past decade, electrospinning has attracted tremendous interest in the research community simply because it provides a facile and effective means by which to produce ultrathin fibers with diameters ranging from a micrometers to a few nanometers at low cost and relatively high production rates.[1,2] Various synthetic and natural polymers have been successfully electrospun into ultrathin fibers. Such structures have been proposed for a number of applications because of their inherently high aspect ratio and specific surface area. Thus far, most of the applications for the aforementioned structures have been in biomedical fields for biosensors, drug delivery, and tissue engineering.[3,4] In general, nano or submicrometer-scaled electrospun fibers are deposited on the conductive surfaces as a randomly oriented nonwoven mats size of which generally on the order of several millimeters to hundreds of millimeters, resulting in two or three-dimensional macroscopic assemblies of one-dimensional (1D) nanostructure.[5,6] There have been a number of studies on individual nanofibers and nanofibrous mats. The properties of individual electrospun nanofibers can be controlled by many factors, including the characteristics of the solution, the processing conditions, and the ambient conditions.[2] On the other hands, many studies have reported on controlling the morphology of macroscopic nanofiber mats, which has mainly focused on the fabrication of well-aligned macroscopic architecture of nanofibrous mats.[7–10] In many of the potential applications for electrospun fibers, it is desirable to achieve spatially ordered microscopic architecture of the fibers in order to obtain functional devices or structures. However, despite the extensive researches that have been conducted on nanofiber and macroscopic configurations of nanofibrous mats, only a few studies have reported on the realization of micrometer-scale architectures composed of electrospun nanofibers. For example, the selective deposition of nonwoven mats using collectors with microscopic electrode patterns could achieve dense nanofiber deposition within specific microdomain.[11,12] Furthermore, the electrospinning of photoreactive polymers and subsequent photopatterning could generate spatially well-defined fibrous micropatterns,[13–15] while multistep microcontact printing and etching techniques could be used to micropattern electrospun fibers.[16] However, such approaches have several disadvantages: 1) the availability of photoreactive polymer solution is very low and the resultant nanofibers may not be biocompatible; 2) the approaches requires complicated processes, and 3) the resultant fibrous micropatterns cannot be easily handled by being separated from the substrates, which may

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cause problems in various biomedical applications such as implantable devices. Therefore, it remains a challenge to develop simple methods of fabricating well-defined, biocompatible and independent nanofiber microarchitectures made from various polymers.

In this study, we proposed a simple method to fabricate well-defined microarchitectures composed of electrospun nanofibers. A combination of electrospinning and hydrogel lithography processes, followed by a dissolution step, allowed us to locate polycarprolactone (PCL) nanofiber only within poly(ethylene glycol) (PEG) hydrogel micropatterns, which could be easily detached from the substrates and obtained as microparticles. Since the size and shape of the microparticles can be easily controlled with a proper photomask design, it was possible to localize the electrospun nanofibers within a well-defined geometry with micrometer dimensions. For possible applications of the microarchitectured nanofibers, the sustained release of protein and cell encapsulation was successfully demonstrated. We also expect that the PEG hydrogel can also provide a protective environment for the nanofibers by preventing the non-specific adsorption of various molecules onto the nanofibers and improving the biocompatibility of microarchitectured nanofibers.

2. Results and Discussion

Since the preparation of PCL nanofibers via electrospinning and the use of nanofibers in drug delivery systems (DDSs) or cell cultures have been well-established in previous studies,[17] we focused on the fabrication and functionality of microarchitectured nanofibers.

2.1. Fabrication of Hydrogel-Entrapped Nanofibers

Overall procedure to prepare hydrogel microparticles entrapping PCL nanofibers was described in **Figure 1**. Nanofibers were electrospun from PCL in 2,2,2-trifluoroethanol (TFE) solutions. The TFE which is a poor solvent for PCL was used because it has been known to facilitate the solubilization of proteins such as BSA in a polymer solution.[18] In this study, all electrospinning processes were performed under the same conditions and produced an approximately 60 μm-thick but ultrathin (the diameter of the nanofiber was 285 ± 35 nm) nanofiber matrix from PCL. The ability of the PEG diacrylate (PEG-DA) to form a gel upon exposure to UV light was exploited to create hydrogel micropatterns on the PCL nanofibers via photolithography. After UV exposure, only the exposed regions underwent free-radical crosslinking and became insoluble in water. As a result, desired hydrogel micropatterns incorporating PCL nanofibers were obtained by removing the unexposed regions with water as shown in **Figure 2**a. The SEM image in Figure 2a reveals that well-defined hydrogel patterns incorporated with PCL nanofibers were obtained without any residual hydrogel precursor solution remaining in the fiber. In addition, all nanofibers were inserted at the sides of the hydrogel microstructures, leaving few fibers on the top surfaces of the hydrogel micropatterns. Therefore, two different domains were observed in the nanofiber-bound

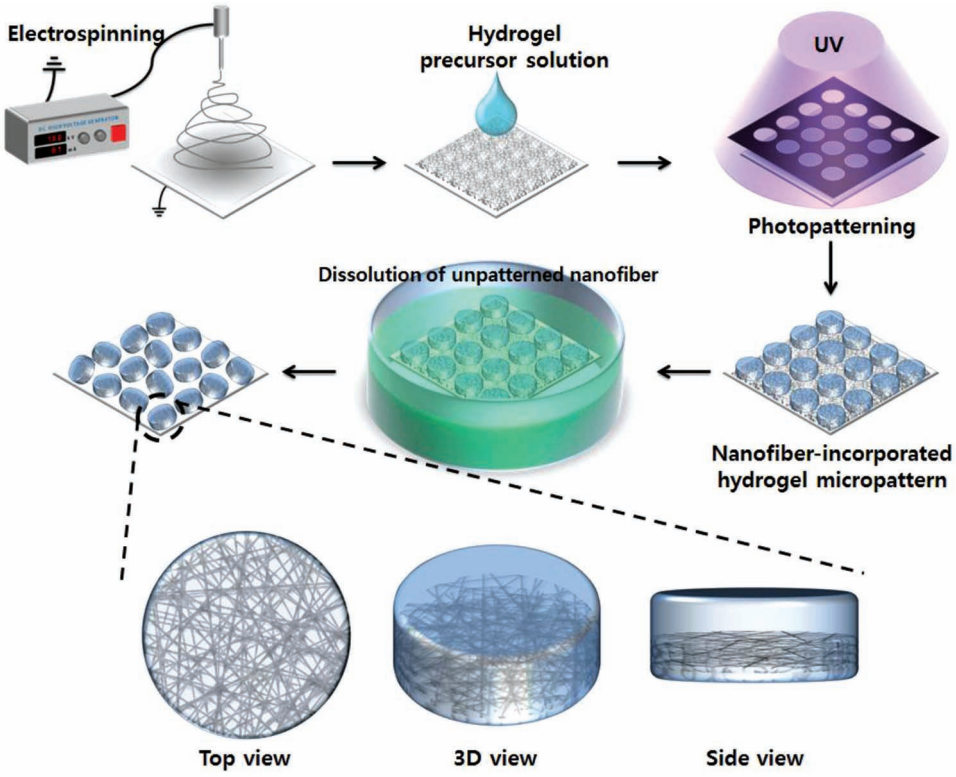

Figure 1. Schematic illustration of preparing microarchitectured PCL nanofibers that are localized within hydrogel microparticles.

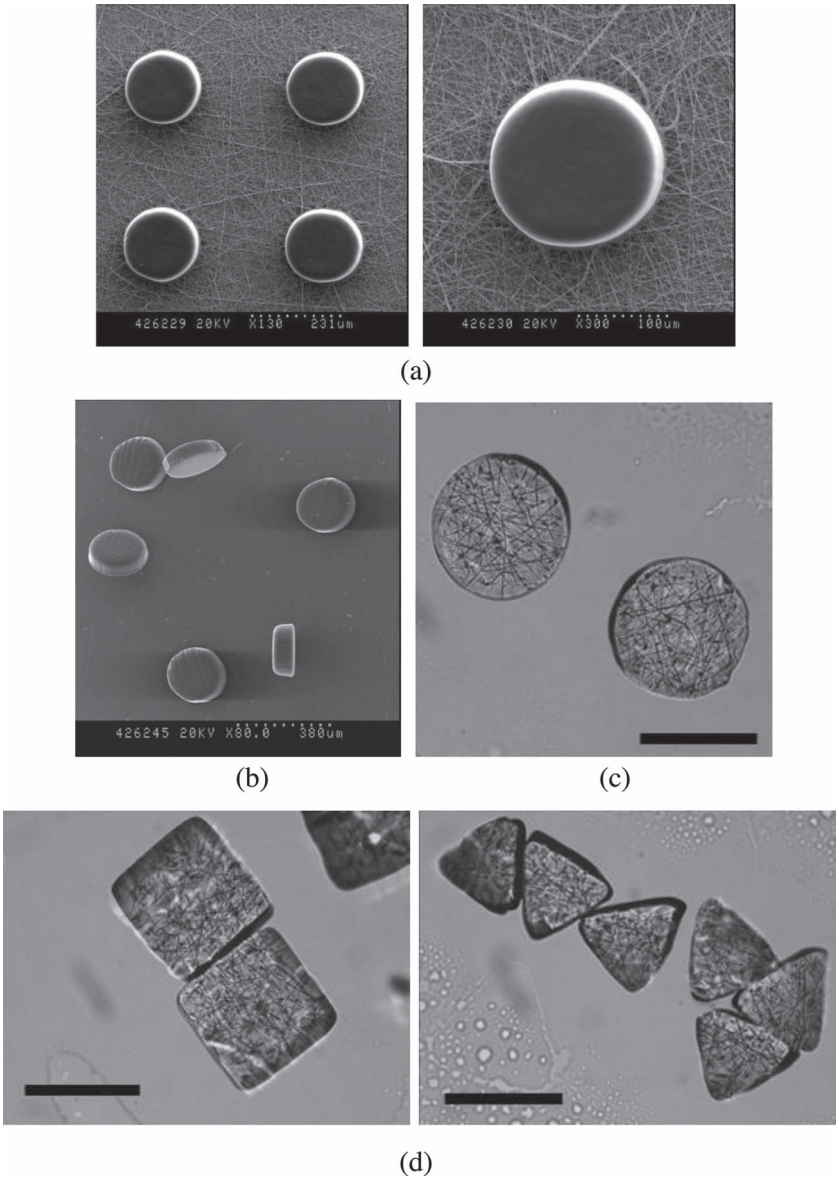

Figure 2. a) SEM images of hydrogel micropatterns incorporating PCL nanofiber prepared by photopatterning. b) SEM image of nanofiber-entrapped hydrogel microparticles obtained after selectively dissolving out PCL nanofiber region with chloroform. c) Optical image of nanofiber-entrapped hydrogel microparticles confirming presence of nanofiber within hydrogel. d) Optical images of different shapes of hydrogel microparticles entrapping PCL nanofiber. Scale bar: 200 μm.

hydrogel micropatterns: a bare PCL nanofiber region and a nanofiber-entrapping hydrogel micropatterned region. Since the PCL nanofibers are insoluble and do not swell in water, the fabrication process did not cause any change in the nanofiber structure. Reacting micropatterned nanofibers with chloroform selectively removed bare PCL nanofibers, producing microarchitectured nanofibers localized within hydrogel micropatterns. Finally, resultant microarchitectures were obtained as microparticels by scraping out hydrogel micropatterns from substrates (Figure 2b). Microarchitectures were easily detached from the substrate because the PEG hydrogels became hydrated and swollen in water during fabrication process. An optical image of the microparticles revealed the presence and localization of the PCL nanofibers within hydrogel microparticles (Figure 2c). Different shapes of the hydrogel microparticles entrapping nanofibers could also be induced, as shown in Figure 2d. The obtained results demonstrate that hydrogel micropatterning allowed for the localization of electrospun nanofibers within a controlled geometry with micrometer dimensions. While nonspherical polymeric microparticles with different shapes have been produced by several researchers,[19–22] to the best of our knowledge, the fabrication of hydrogel microparticles incorporating nanofibers has not yet been reported. The height of the hydrogel microparticles was dependent on the amount of hydrogel precursor solution, as described in our previous studies.[23] If the height of the hydrogel microparticles was similar to or lower than the thickness of the nanofiber matrix, undissolved nanofibers or marks of dissolved nanofibers were observed on the top surfaces of the hydrogel microparticles, as shown in **Figure 3**. In this study, we controlled the height of the hydrogel microparticles so as to be greater than 80 μm in order to completely entrap the 60 μm-thick nanofiber matrix. The morphology of the hydrogel microparticle entrapping nanofibers was further investigated using relatively thick (about 200 μm) hydrogel microparticles. In the SEM images shown in **Figure 4**a, very smooth surfaces without visible defects in the top and bottom sides of the cylindrical microparticles were observed. However, a trace of nanofibers was detected on the side walls of the microparticles, which confirms the thickness and location of the nanofibers within the hydrogel microparticles, as displayed in Figure 4b.

2.2. Protein Delivery from Microencapsulated Nanofibers

After the successful fabrication of microarchitectured nanofibers, we first investigated the potential application of the structures in protein delivery using BSA as a model protein. BSA was originally insoluble in TFE, but it was miscible with TFE in an aqueous solution and no BSA precipitate was apparent in the polymer solution. The electrospinning of the PCL/BSA blend solution produced nanofibers with a diameter of 435 ± 92 nm. Larger diameters and diameter variations in the PCL/BSA nanofiber resulted from the incorporation of aqueous BSA solution into the polymer solution and subsequent phase separation between the PCL and BSA during electrospinning.[24] In order to visualize the distribution of BSA in the nanofibers, FITC-BSA was incorporated into nanofibers, and fluorescence images were obtaied

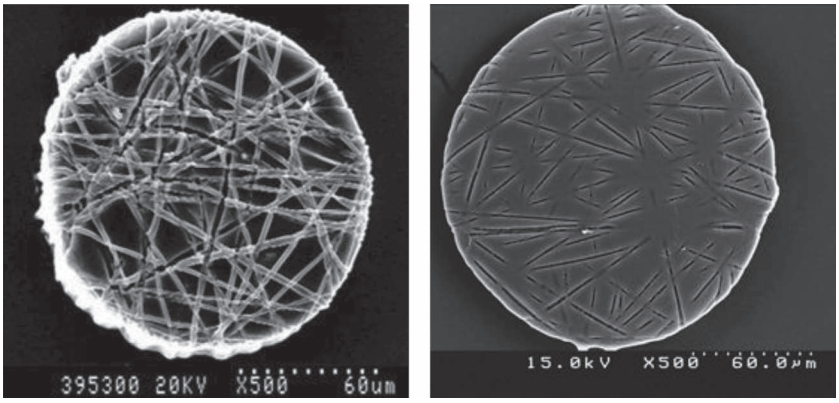

Figure 3. SEM images of nanofiber-entrapped hydrogel microparticles whose height was not tall enough to perfectly entrap the nanofiber matrix. Undissolved nanofiber (left) and marks of dissolved nanofiber (right) were observed on the hydrogel surfaces.

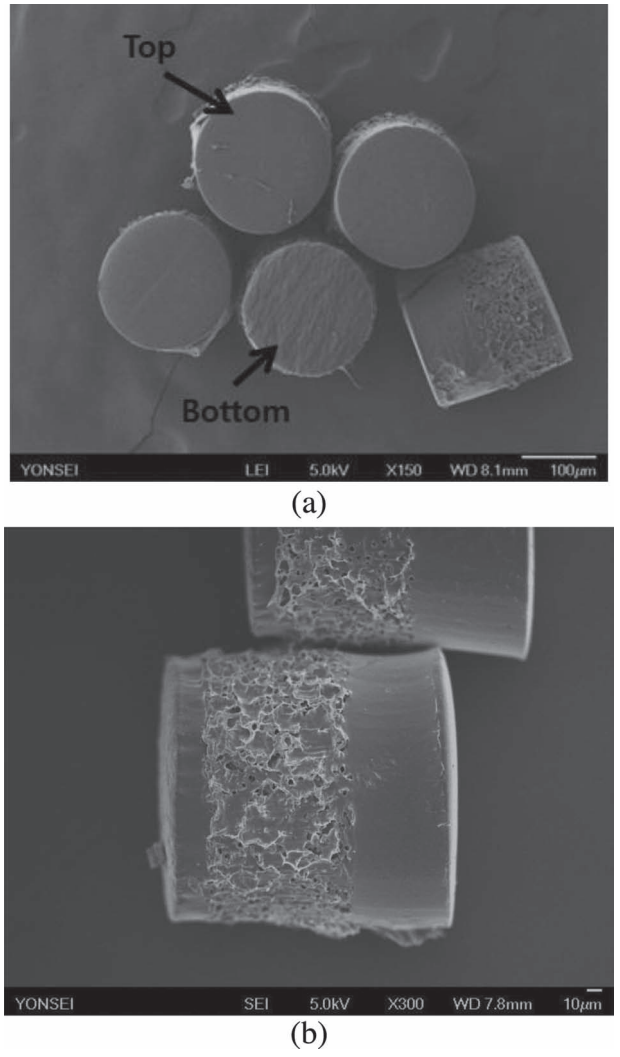

Figure 4. a) SEM images showing top and bottom surface as well as side wall of hydrogel microparticles entrapping nanofiber. b) High magnification image of a side wall.

with fluorescence microscope. As shown in **Figure 5**a, BSA molecules were homogeneously loaded into the nanofibers with the presence of a few BSA aggregates at certain locations in the nanofibers. It should be noted that BSA aggregates produced by phase separation caused the formation of beads, which may be one of the reasons why the PCL/BSA nanofiber had larger diameters and diameter variations when compared to the PCL nanofibers. A subsequent photopatterning process produced BSA-loaded nanofibers within spatially well-defined hydrogel microparticles as shown in Figure 5b. The release behavior of the BSA from the microarchitectured nanofibers was studied and compared to that from bare PCL nanofibers. It is well known that a severe initial burst release occurs when drug-incorporating nanofibers are prepared by electrospinning blends of drugs and polymer solution.[25,26] As shown in Figure 5c, the bare nanofiber system showed a high degree of initial burst (about 63%) and BSA release continued for only 200 hours. On the other hands, Pishko and coworkers reported that the sustained release of BSA for 280 days was possible from highly-crosslinked PEG hydrogel because the mesh size of the hydrogel is large enough to allow for the diffusion of BSA and small enough to achieve long-term release of BSA.[27] The role of highly crosslinked PEG hydrogel as an additional diffusion barrier enabled the hydrogel-entrapped nanofiber system to have a smaller burst release (about 22%) and achieve sustained release of BSA over 1000 h, as shown in Figure 5c.

2.3. Cell Encapsulation within Nanofiber-Containing Hydrogel

In addition to protein delivery, another proposed application of microarchitecture composed of nanofibers and hydrogel is cell encapsulation because this material is very similar to an extracellular matrix (ECM) composed of fibers and hydrogel-like interfibril molecules such as proteoglycan and glycoproteins. While PEG hydrogels have been popularly used as biomimetic matrices for cell encapsulation, complicated chemistry was usually introduced to incorporate cell-adhesive molecules into the hydrogels with the loss of the innate biocompatibility of PEG hydrogel because of the non-adhesive property of the PEG hydrogel toward cells and proteins.[28–32] We expect that this problem would be easily resolved by incorporating cell-adhesive nanofibers within the hydrogel because PCL nanofibers have been widely used for cell adhesion and growth. To investigate the effect of nanofiber incorporation on cell encapsulation, the morphology of encapsulated cells was examined. Cells were judged to be spread if they were enlongated or possessed a polygonal morphology. When fibroblasts were encapsulated within bare PEG hydrogel, they appeared rounded (**Figure 6**a), while most of cells were evenly dispersed along nanofibers and remained spread inside nanofiber-incorporated hydrogel, as shown in Figure 6b after 24 hours. This result demonstrates that the incorporation of the nanofibers within PEG hydrogel can enable cell adhesion and spreading inside the hydrogel

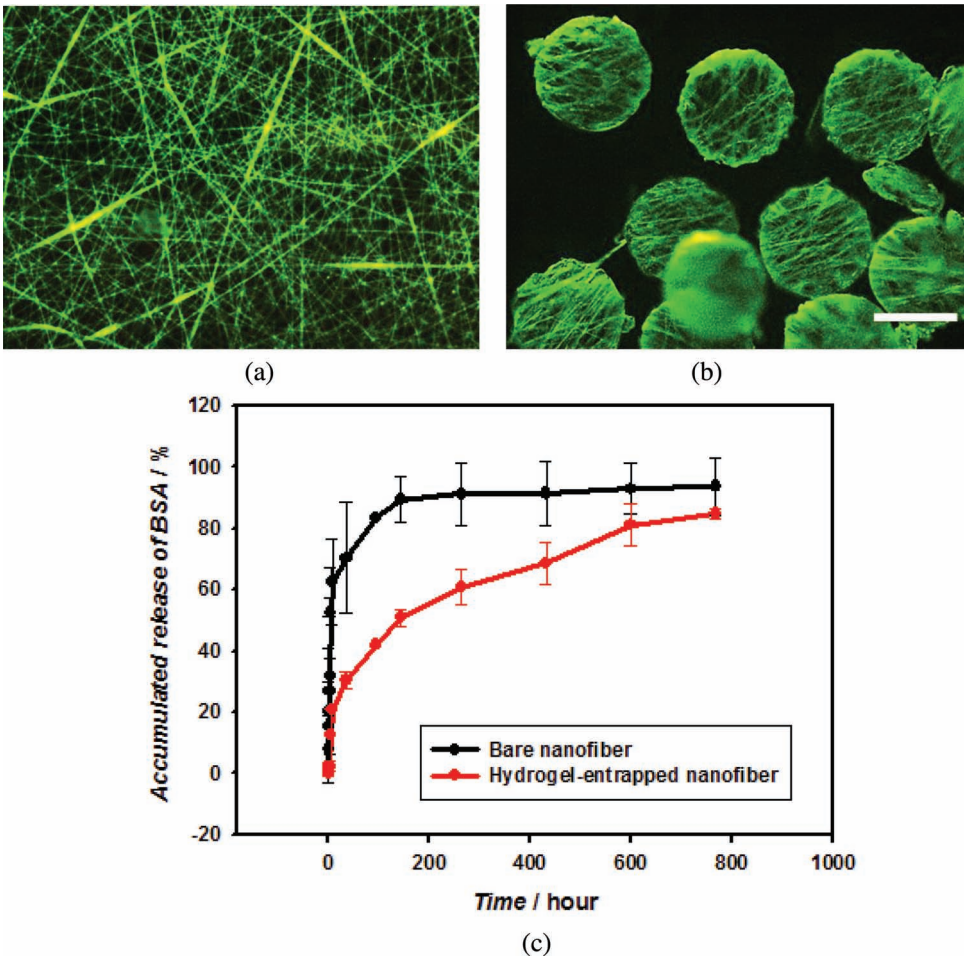

Figure 5. Application of hydrogel-entrapped nanofiber to controlled release of protein. a) Fluorescence image of nanofiber loading FITC-BSA. b) Hydrogel microparticles entrapping FITC-BSA-loaded nanofibers. Scale bar: 100 μm. c) Release behavior of BSA from bare nanofiber and hydrogel-entrapped nanofiber.

without need for a chemical modification step. The viability of encapsulated cells was investigated using Live/Dead Viability/Cytotoxicity fluorescence assay that stains living cells green and dead cells red, respectively. As shown in fluorescence images of Figure 6c, there are many more green spots (living cells) than red spots (dead cells), demonstrating that most of cells remained viable within nanofiber-containing hydrogels. We also observed cell spreading inside the hydrogel microparticles incorporating nanofibers, as shown in Figure 6d. The existence of cells in a three-dimensional environment was further confirmed by the image in Figure 6e, which shows the side view of the hydrogel microparticles. This figure clearly reveals that cells existed not only on the top region but also in the middle and bottom region of the nanofiber matrix entrapped within the hydrogels, thus demonstrating that the cells were dispersed throughout the entire thickness of the nanofiber matrix. Here, encapsulated cells were stained with calcein AM for fluorescence imaging. The calcein-AM diffuses through the membrane of living cells and reacts with intracellular esterase to produce a green fluorescence. Therefore, fluorescence images not only showed the morphology of the cells, but also indicated that the cells remained viable after the photopatterning process. Viability assay revealed that percentage of viable cells was about 80.2 ± 3.2% after photopatterning process. However, using a toxic organic solvent such as chloroform is not desirable for cell-related research although it was reported that hydrogel micropattern prevented the penetration of chloroform.[33] Therefore, for cell encapsulation purposes, it would be better to use nanofibers that can be dissolved or degraded via cell-friendly method in future study.

3. Conclusions

We demonstrated one possible method to generate well-controlled microscopic architectures composed of electrospun nanofibers. A combination of electrospinning and hydrogel lithography produced a micropatterned PCL nanofiber matrix divided into two different nanofiber regions; a bare PCL nanofiber region and a hydrogel-entrapped nanofiber region. Bare nanofibers were selectively dissolved out by chloroform due to the fact that the hydrogel pattern prevents the penetration of organic solvents, thus producing nanofibers that were localized

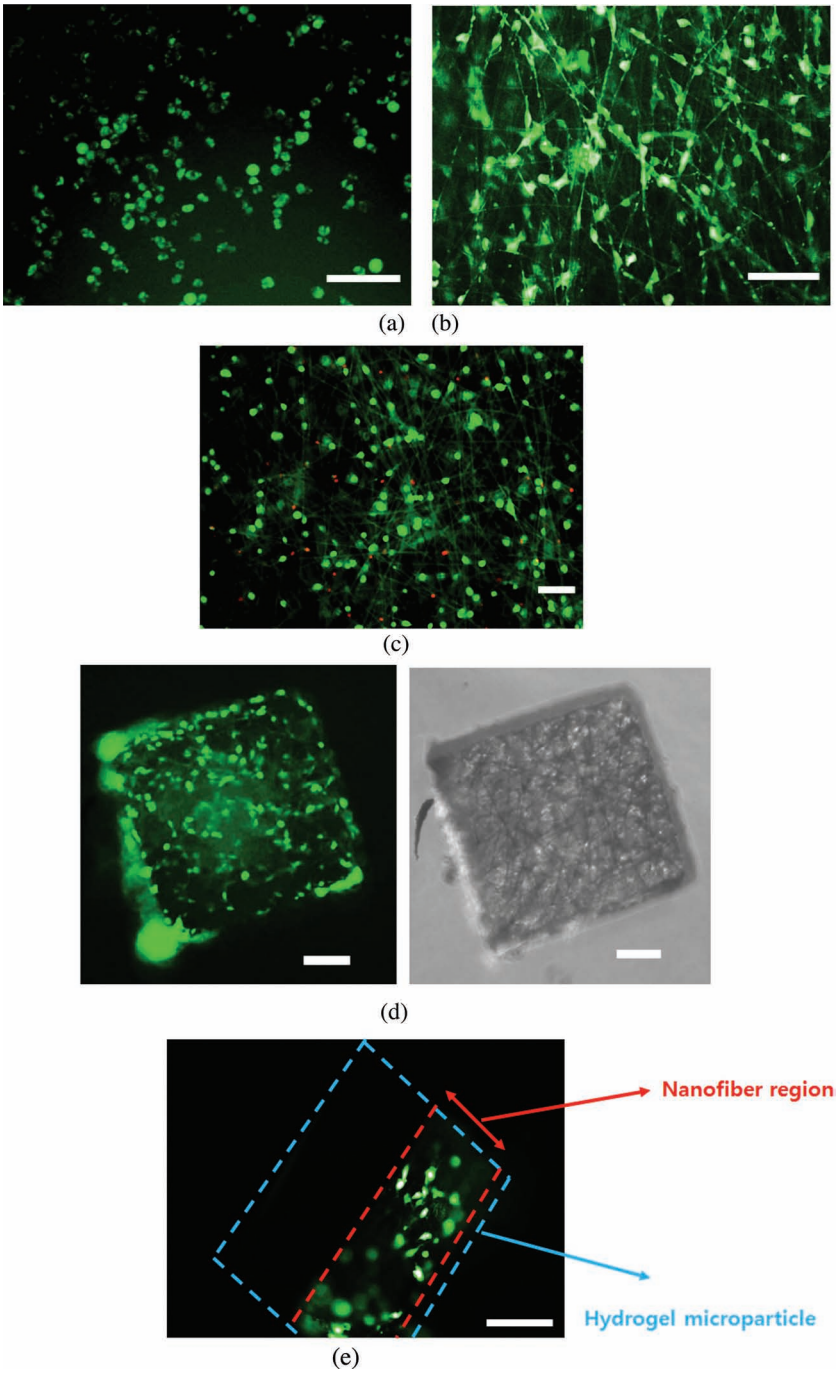

Figure 6. Application of hydrogel-entrapped nanofiber to cell encapsulation. a) Fluorescence image of fibroblasts encapsulated within bare PEG hydrogel. b) Fluorescence image of hydrogel encapsulating nanofiber-attached fibroblasts. c) Live/Dead fluorescent viability assay which stained living cells green and dead cells red. d) Top view and e) side view of hydrogel microparticles encapsulating nanofiber-attached fibroblasts. Scale bar: 100 µm.

within the hydrogel microstructures with a controlled geometry. As one example of these microarchitectured nanofibers, we prepared BSA-loaded PCL nanofibers that were entrapped within hydrogel microparticles and investigated the potential application of controlled protein release. Hydrogel-entrapped nanofiber microarchitectures showed a more sustained release of BSA than bare macroscopic nanofibers with a smaller extent of burst release. We also investigated the possibility of encapsulating fibroblasts within nanofiber-containing PEG hydrogel microparticles and demonstrated that the incorporation of PCL nanofibers within the hydrogels enabled cell adhesion and spreading inside the PEG hydrogels.

4. Experimental Section

Materials: Poly(ethylene glycol) diacrylate (PEG-DA, MW 575), PEG-methacrylate (PEG-MA) (MW 1000), photoinitiator 2-hydroxy-2-methylpropiophenone (HOMPP), polycarprolactone (PCL) (MW 80 000), 2,2,2-trifluoroethanol(TFE), dimethylsulfoxide (DMSO), bovine serum album (BSA, MW 66 000), and Bradford reagent were purchased from Sigma-Aldrich (Milwaukee, WI, USA). For cell encapsulation, 2-hydroxy-1[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (Irgacure 2959) (Ciba, Tarrytown, NY, USA) was used as a photoinitiator. A Live/Dead Viability/Cytotoxicity Kit (L-7013), phosphate buffered saline (PBS, 0.1 M, pH 7.4), calcein-AM was purchased from Invitrogen (Carlsbad, CA, USA). Murine 3T3 fibroblasts were obtained from the American Type Culture Collection (Manassas, VA, USA). The photomasks for photolithography were prepared using AutoCAD and were printed on transparencies using a standard laser jet printer (LaserWriter 16/600 PS, Apple Inc., Cupertino, CA, USA).

Preparation of PCL Nanofibers: Nanofibers were electrospun from a PCL solution. The electrospinning apparatus used in this study consisted of a plastic syringe (10 mL) capped with a flat-ended 23-G metal needle, a syringe pump (KD Scientific, Holliston, MA, USA) for controlling the feeding rate, a stainless steel substrate as a collecting plate, and a high voltage power supply (NanoNC, Seoul, Korea). The polymer solution was prepared by dissolving PCL (800 mg) in TFE (6.0 mL), which was then placed in an 80 °C convection oven overnight. The solution was transferred to a syringe for electrospinning. To electrospin PCL fibers, a 10 kV positive voltage was applied to the solution via the needle, and a constant solution feeding rate (0.3 mL h^{-1}) was maintained by the syringe pump. The distance between the tip of the needle and the collecting plate was 20 cm. The electrospun fibers were collected for 30 min on clean aluminium foil placed on the collecting plate. The temperature and humidity were set to 25 °C and 50%, respectively. For the BSA-releasing experiments, BSA (25 mg) was dissolved in water (1.0 mL) and mixed with the polymer solution so as to homogeneously incorporate the BSA. The electrospun nanofibers were characterized using scanning electron microscopy (SEM) (JEOL, Ltd., Peabody, MA, USA) and laser scanning confocal microscopy (LSCM, LSM 510 META, Carl Zeiss Inc., Thornwood, NY, USA).

Hydrogel Microparticles Entrapping Nanofibers: PEG Hydrogels were prepared by UV-initiated free radical polymerization. Purified PEG-DA (MW 575) was dissolved in PBS to form a 50% w/v solution, and HOMPP (20 µL) was added to the PEG-DA solution (1.0 mL) to initiate

photopolymerization. This precursor solution was dropped onto a 1.8 cm × 1.8 cm PCL nanofiber matrix and then exposed to 365 nm, 300 mW cm^{-2} UV light (EFOS Ultracure 100ss Plus, UV spot lamp, Mississauga, Ontario, Canada) through photomasks. Complete cross-linking of the PEG-DA required a time of less than 2 s, producing the desired hydrogel microstructures incorporating PCL nanofibers after the unreacted precursor solution was washed away with water. Hydrogel microparticles containing the PCL nanofibers were ultimately obtained by dissolving out nanofibers between the hydrogel microstructures with chloroform. For this procedure, a drop of chloroform was placed onto the nanofiber-incorporated hydrogel for 5 s and immediately washed away with a PBS solution or cell culture media.

In Vitro BSA Release Study: A bare nanofiber mesh with a fixed size or hydrogel-entrapped nanofibers loaded with BSA were placed, in triplicate, in a 12-well tissue culture plate and immersed in 1 mL of the PBS solution at 37 °C in a humidified 5% CO_2 environmental incubator. At pre-determined time intervals, 200 μL of the release mediums were collected and replaced with an equal volume of fresh PBS solution. The amount of BSA in the collected solution was measured with Bradford reagent.

Cell Encapsulation: The gel precursor solution for cell encapsulation was prepared as previously described.[34] The solution is composed of PEG-MA (MW 1000) (20% w/v) and Irgacure 2959 (1% w/w) in cell culture medium. To prepare cell-containing hydrogel microparticles incorporating the nanofiber, cell suspension (1.0×10^5 cells mL^{-1}) was dropped onto the nanofiber matrix and incubated with cell culture medium for 5 h. After confirming that cells adhered onto nanofiber matrix, the cell-containing nanofibers were removed from the cell-culture dish and subjected to a photopatterning process with a UV exposure time of 30 seconds. As a control, cell-containing hydrogel microparticles without nanofibers were prepared following a published protocol.[34] For fluorescence imaging of the encapsulated fibroblasts, calcein-AM was used. Briefly, calcein-AM solutions (10 μM) were prepared by diluting the calcein-AM stock solution dissolved in DMSO with the cell culture medium. Encapsulated cells were incubated in this solution for 30 min and then examined with a fluorescence microscope. A Live/Dead Viability/Cytotoxicity fluorescence assay was carried out as previously described.[35]

Cell Culture: Fibroblasts were cultured in DMEM with glucose (4.5 g L^{-1}) and FBS (10% w/w) and were incubated at 37 °C in 5% CO_2 and 95% air. Cells were grown to confluence in 75 cm^2 polystyrene tissue culture flasks and confluent cells were subcultured every two to three days by trypsinization with trypsin/EDTA.

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[1] A. Greiner, J. H. Wendorff, *Angew. Chem. Int. Ed.* **2007**, *46*, 5670.
[2] D. H. Reneker, A. L. Yarin, *Polymer* **2008**, *49*, 2387.
[3] S. Agarwal, J. H. Wendorff, A. Greiner, *Polymer* **2008**, *49*, 5603.
[4] J. W. Xie, X. R. Li, Y. N. Xia, *Macromol. Rapid Commun.* **2008**, *29*, 1775.
[5] A. Frenot, I. S. Chronakis, *Curr. Opin. Colloid Interface Sci.* **2003**, *8*, 64.
[6] D. Li, Y. N. Xia, *Adv. Mater.* **2004**, *16*, 1151.
[7] W. E. Teo, S. Ramakrishna, *Nanotechnology* **2006**, *17*, R89.
[8] S. P. Zhong, W. E. Teo, X. Zhu, R. W. Beuerman, S. Ramakrishna, L. Y. L. Yung, *J. Biomed. Mater. Res., Part A* **2006**, *79A*, 456.
[9] D. Li, Y. L. Wang, Y. N. Xia, *Adv. Mater.* **2004**, *16*, 361.
[10] D. Y. Yang, B. Lu, Y. Zhao, X. Y. Jiang, *Adv. Mater.* **2007**, *19*, 3702.
[11] Z. W. Ding, A. Salim, B. Ziaie, *Langmuir* **2009**, *25*, 9648.
[12] D. M. Zhang, J. Chang, *Adv. Mater.* **2007**, *19*, 3664.
[13] B. Carlberg, T. Wang, J. Liu, *Langmuir* **2010**, *26*, 2235.
[14] C. S. Sharma, A. Sharma, M. Madou, *Langmuir* **2010**, *26*, 2218.
[15] L. Jiang, M. Zhang, J. X. Li, W. J. Wen, J. H. Qin, *Adv. Mater.* **2012**, *24*, 2191.
[16] J. Shi, L. Wang, Y. Chen, *Langmuir* **2009**, *25*, 6015.
[17] A. Cipitria, A. Skelton, T. R. Dargaville, P. D. Dalton, D. W. Hutmacher, *J. Mater. Chem.* **2011**, *21*, 9419.
[18] Y. Z. Zhang, X. Wang, Y. Feng, J. Li, C. T. Lim, S. Ramakrishna, *Biomacromolecules* **2006**, *7*, 1049.
[19] D. Dendukuri, S. S. Gu, D. C. Pregibon, T. A. Hatton, P. S. Doyle, *Lab Chip* **2007**, *7*, 818.
[20] D. Dendukuri, D. C. Pregibon, J. Collins, T. A. Hatton, P. S. Doyle, *Nat. Mater.* **2006**, *5*, 365.
[21] H. Lee, J. Kim, H. Kim, J. Kim, S. Kwon, *Nat. Mater.* **2010**, *9*, 745.
[22] C. L. Lewis, C. H. Choi, Y. Lin, C. S. Lee, H. Yi, *Anal. Chem.* **2010**, *82*, 5851.
[23] H. J. Lee, H. S. Kim, H. O. Kim, W. G. Koh, *Lab Chip* **2011**, *11*, 2849.
[24] C. M. Valmikinathan, S. Defroda, X. J. Yu, *Biomacromolecules* **2009**, *10*, 1084.
[25] E. R. Kenawy, G. L. Bowlin, K. Mansfield, J. Layman, D. G. Simpson, E. H. Sanders, G. E. Wnek, *J. Controlled Release* **2002**, *81*, 57.
[26] H. L. Jiang, D. F. Fang, B. J. Hsiao, B. J. Chu, W. L. Chen, *J. Biomater. Sci., Polym. Ed.* **2004**, *15*, 279.
[27] M. B. Mellott, K. Searcy, M. V. Pishko, *Biomaterials* **2001**, *22*, 929.
[28] I. Mironi-Harpaz, D. Y. Wang, S. Venkatraman, D. Seliktar, *Acta Biomater.* **2012**, *8*, 1838.
[29] L. M. Weber, K. N. Hayda, K. Haskins, K. S. Anseth, *Biomaterials* **2007**, *28*, 3004.
[30] W. G. Koh, L. J. Itle, M. V. Pishko, *Anal. Chem.* **2003**, *75*, 5783.
[31] Y. Fu, K. D. Xu, X. X. Zheng, A. J. Giacomin, A. W. Mix, W. Y. J. Kao, *Biomaterials* **2012**, *33*, 48.
[32] P. Panda, S. Ali, E. Lo, B. G. Chung, T. A. Hatton, A. Khademhosseini, P. S. Doyle, *Lab Chip* **2008**, *8*, 1056.
[33] S. W. Lee, H. J. Lee, J. H. Choi, W. G. Koh, J. M. Myoung, J. H. Hur, J. J. Park, J. H. Cho, U. Jeong, *Nano Lett.* **2010**, *10*, 347.
[34] Y. A. Du, E. Lo, S. Ali, A. Khademhosseini, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 9522.
[35] W.G. Koh, M. Pishko, *Anal. Bioanal. Chem.* **2006**, *385*, 1389.