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Nanofibrous Microposts and Microwells of Controlled Shapes and Their Hybridization with Hydrogels for Cell Encapsulation

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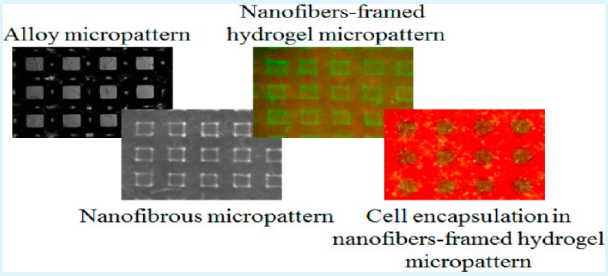
Supporting Information

ABSTRACT: A simple, robust, and cost-effective method is developed to fabricate nanofibrous micropatterns particularly microposts and microwells of controlled shapes. The key to this method is the use of an easily micropatternable and intrinsically conductive metal alloy as a template to collect electrospun fibers. The micropatterned alloy allows conformal fiber deposition with high fidelity on its topographical features and in situ formation of diverse, free-standing micropatterned nanofibrous membranes. Interestingly, these membranes can serve as structural frames to form robust hydrogel micropatterns that may otherwise be fragile on their own. These hybrid micropatterns represent a new platform for cell encapsulation where the nanofiber frames enhance the mechanical integrity of hydrogel and the micropatterns provide additional surface area for mass transfer and cell loading.



KEYWORDS: *alloy micropattern, electrospinning, nanofiber micropattern, hydrogel, cell encapsulation*

Electrospun fibers with fiber diameters from tens of nanometers to micrometers[1] have attracted intensive research interests in the past couple of decades and found broad applications including photovoltaic[2] and photonic devices,[3] catalyst supports,[4] composite reinforcements,[5] superhydrophobic surfaces,[6] immunoassay,[7] biosensing,[8] drug delivery,[9] and tissue engineering.[10−14] Generally, the electrospun fibers have random nonwoven structures, resulted from the whipping motion of the electrospinning jet.[15] However, it is highly desirable to endow these materials with more spatially organized microscale architectures in order to engineer more functional structures and devices. Examples include electronic and photonic devices[16] as well as tissue scaffolds to control cell morphology,[17] wound healing,[18] stem cells differentiation,[19] and tissue regeneration.[20]

Tremendous efforts have been made to manipulate electrospun fibers in spatially organized ways including both the alignment at the individual fiber level[18,21−23] and more complex hierarchical structures at larger length scales in 2D and 3D.[3,24−31] Of particular interest, 2D nanofibrous micropatterns have recently received much attention due to their enhanced properties such as increased surface area, roughness, and uniquely combined micro/nano structures.[25,27,30,32] The strategies to fabricate these micropatterns can be generally divided into top-down and bottom-up methods. One of the top-down methods was direct photolithographic patterning of nanofibers by mixing photoinitiator in the polymer solution and then selectively photo-cross-linking the fibers through a photomask.[24] Although various nanofibrous micropatterns were prepared, this method is only limited to photo-cross-linkable materials. In one of the bottom-up methods, nanofibrous micropatterns were fabricated through direct-write electrospinning on a 2D movable conductive collector.[26] The drawbacks were the complicated setup and manipulation as well as coarse feature resolution. Another popular bottom-up method was to use micropatterned conductive templates to collect the electrospun fibers which then formed micropatterns in situ. Despite of varying degrees of successes, limitations still exist in the robustness and versatility of templates. For example, stainless steel beads[32,33] and metal molds[28−30] were conductive but it was difficult to achieve micropatterns of controlled geometries. The feature resolution was also limited at submillimeter scale.[28,29,32,33] Although polydimethylsiloxane (PDMS) templates have the advantages of being easy to be processed into controlled micropatterns through soft lithography, intrinsically they are not conductive and hence additional treatments are required for the fiber collection.[25,34]

Here, we report a new, versatile, and robust method to fabricate nanofibrous micropatterns, particularly microposts and microwells, with controlled geometries. The key to this method is the use of an intrinsically conductive and ductile metal alloy that has a low-melting temperature and can be micropatterned, through simple imprint lithography, into controlled shapes with a relatively high resolution. When used as the substrate to collect the nanofibers, the alloy allowed

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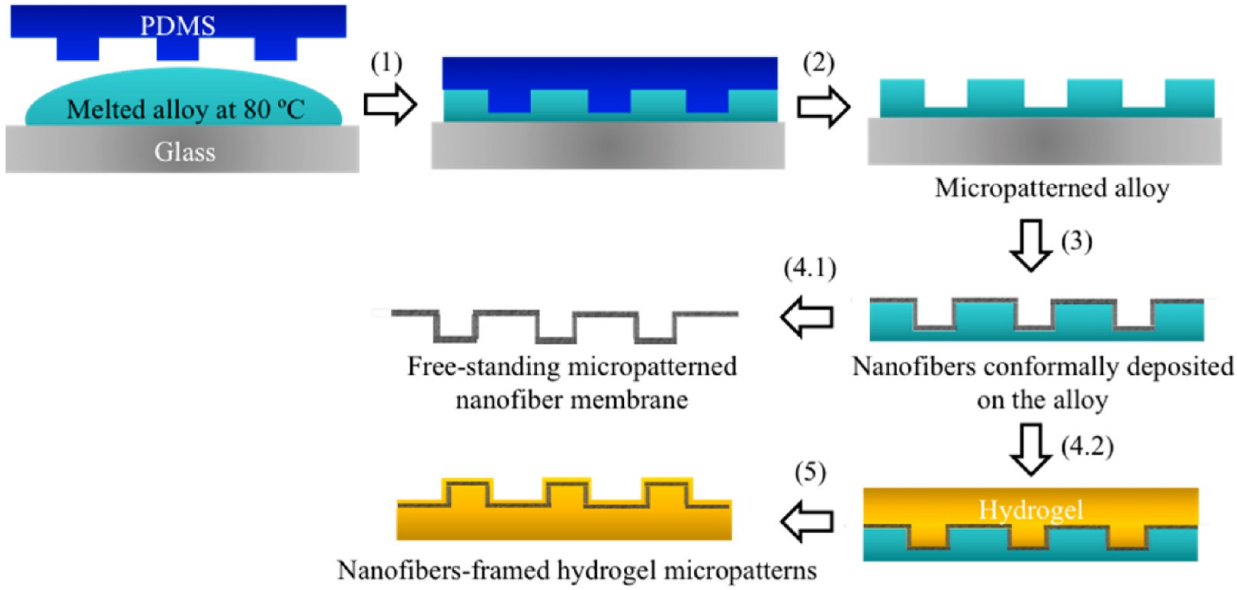
Scheme 1. Fabrication of Controllably Micropatterned Alloys, Nanofibrous Membranes, and Nanofiber-Framed Hydrogel Micropatterns[a]



[a](1) Press PDMS mold on melt alloy and cool to room temperature. (2) Peel off PDMS mold from solidified alloy. (3) Electrospin nanofibers on micropatterned alloy. (4.1) Peel off micropatterned nanofibrous membrane from alloy. (4.2) Cast hydrogel on nanofiber-deposited micropatterned alloy. (5) Peel off nanofiber-framed hydrogel micropatterns.

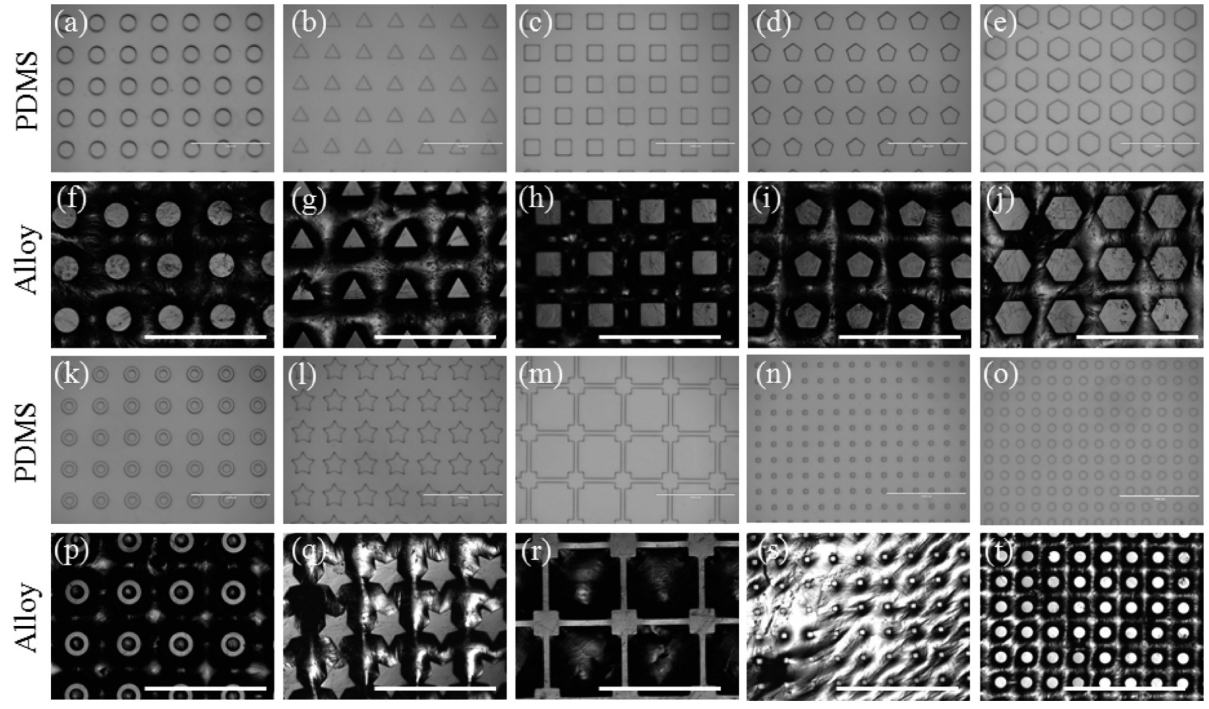


Figure 1. Microscopic images of micropatterned (a–e, k–o) PDMS molds and (f–j, p–t) alloys. Scale bars: 1000 μm.

conformal deposition of fibers on its topographical features. To our knowledge, this is the first report that metal alloy was micropatterned and used as a template to make hierarchical nanofibrous structures. We demonstrated various mechanically robust, free-standing nanofibrous microposts and microwells. In contrast to the reported microwells with smooth surface,[35] these nanofibrous microwells structurally resemble the extracellular matrix[36] and hence represent a biomimetic platform for high throughput cell culture.[37] By taking advantage of the flexibility and ductility, we also readily fabricated double-faced and rod-shaped micropatterned alloy templates and corresponding nanofibrous membranes, which may be difficult to engineer using traditional rigid metal templates.[27−30] Additionally, we demonstrated that these nanofibrous microposts and microwells could be used as structural frames to form hydrogel micropatterns for cell encapsulation applications. We showed that either non-adherent or adherent cells could be readily encapsulated in these hybrid micropatterns. Compared

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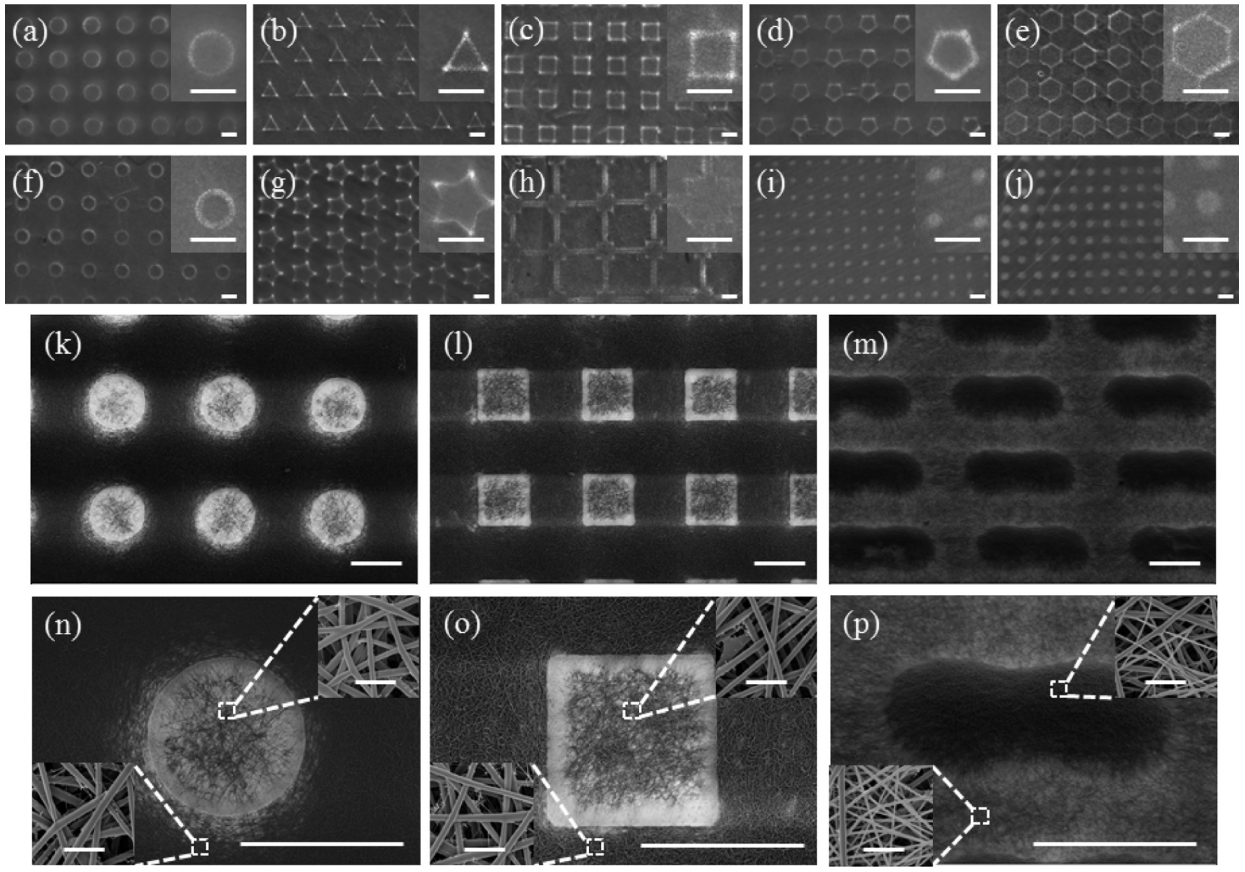

Figure 2. (a–j) Microscopic images of free-standing micropatterned nanofibrous membranes. The insert images are magnified micropatterns. Scale bars: 200 μm. (k–p) SEM images of representative micropatterned nanofibrous membranes. Images of n–p are magnified micropatterns. Scale bars: 200 μm. The inset images are individual nanofibers at high resolution. Scale bars: 2 μm.

with previous studies where fibers were simply composited within bulk hydrogels,[38,39] the nanofibrous frames not only enhance the integrity of the hydrogel as a whole but also facilitate the formation of hydrogel micropatterns, which in turn increase the surface area for mass transfer and cell loading.

Low-melting-temperature metal alloys have been utilized in a broad variety of tool and die applications including casting, tube bending, and anchoring parts, however, its potential applications in micro/nano technologies such as electrospining have never been explored. By taking advantages of its low-melting temperature and inherent conductivity, we fabricated micropatterned alloy via simple imprint lithography and used it as a template to collect electrospun nanofibers (Scheme 1). Controlled micropatterns with both round and sharp corners were replicated on the alloy with a high fidelity using PDMS molds (Figure 1). The scattered dark and bright regions on micropatterned alloy were due to the reflection of metallic luster under the microscope (Figure 1f–j, p–t). The depth of microwells on alloy was 40 $\pm$ 3 μm and controllable through the PDMS molds. The smallest width obtained on micropatterened alloy was 50 μm (Figure 1s). Unlike rigid steel beads[32,33] and metal molds[28−30] or non-conductive PDMS,[25,34] these alloy micropatterns are intrinsically conductive, flexible, and ductile, distinguishing them as attractive substrates to engineer micropatterned nanofibers with greater freedom of control. We also prepared double-faced and rod-shaped micropatterned alloy templates by pressing melt alloy with two PDMS molds in a sandwich configuration and rolling premade micropatterned alloy sheet, respectively (see Figure S1a, b in the Supporting Information). It may be difficult to engineer such intriguing and conductive templates using traditional rigid metal materials.[28−30]

We chose nylon as electrospun material because it is mechanically tough, hydrolytically stable, and has been safely used as cell encapsulation material in humans.[40] The nanofibers were conformally deposited on the alloys and free-standing micropatterned nanofibrous membranes were readily peeled off from the alloy (Figure 2). The topographical features of the membranes were corresponding to the alloy templates (Figure 2a–j). The delicate conformity of nanofibers on micropatterned alloy was characterized with SEM (Figure 2k–p). It is clearly shown that the nanofibers tightly followed the micropatterned structures on alloy, no matter the microstructures were concave microwells (Figure 2k , l) or convex microposts (Figure 2m). The inserts are high magnification SEM images of individual nanofibers on microwell/micropost and outside regions (Figure 2n–p). These controllably shaped nanofibrous microposts and microwells were in direct contrast with the previously reported nanofibrous micropatterns. The geometry of nanofibrous patterns prepared using steel beads were limited to hemispherical shape.[33] The nanofibrous micropatterns obtained by utilization of carbon black doped PDMS templates had inclined side walls.[25] In our case, the nanofibers were conformally deposited on alloy microposts or microwells with near 90° corner angles, attributing to the inherent conductivity of metal alloy. Recently, microwell technologies have received much attention for single cell analysis[41] and high throughput screening applications.[37]

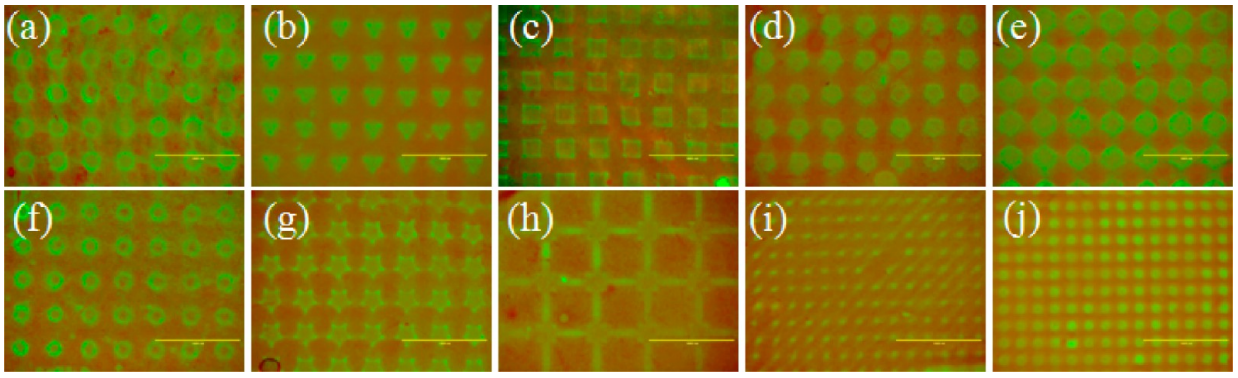

Figure 3. (a–j) Overlaid fluorescent images of nanofiber (red)-framed hydrogel (green) micropatterns of various geometries. Scale bars: 1000 μm. (See Figure S2 in the Supporting Information for individual channel images.)

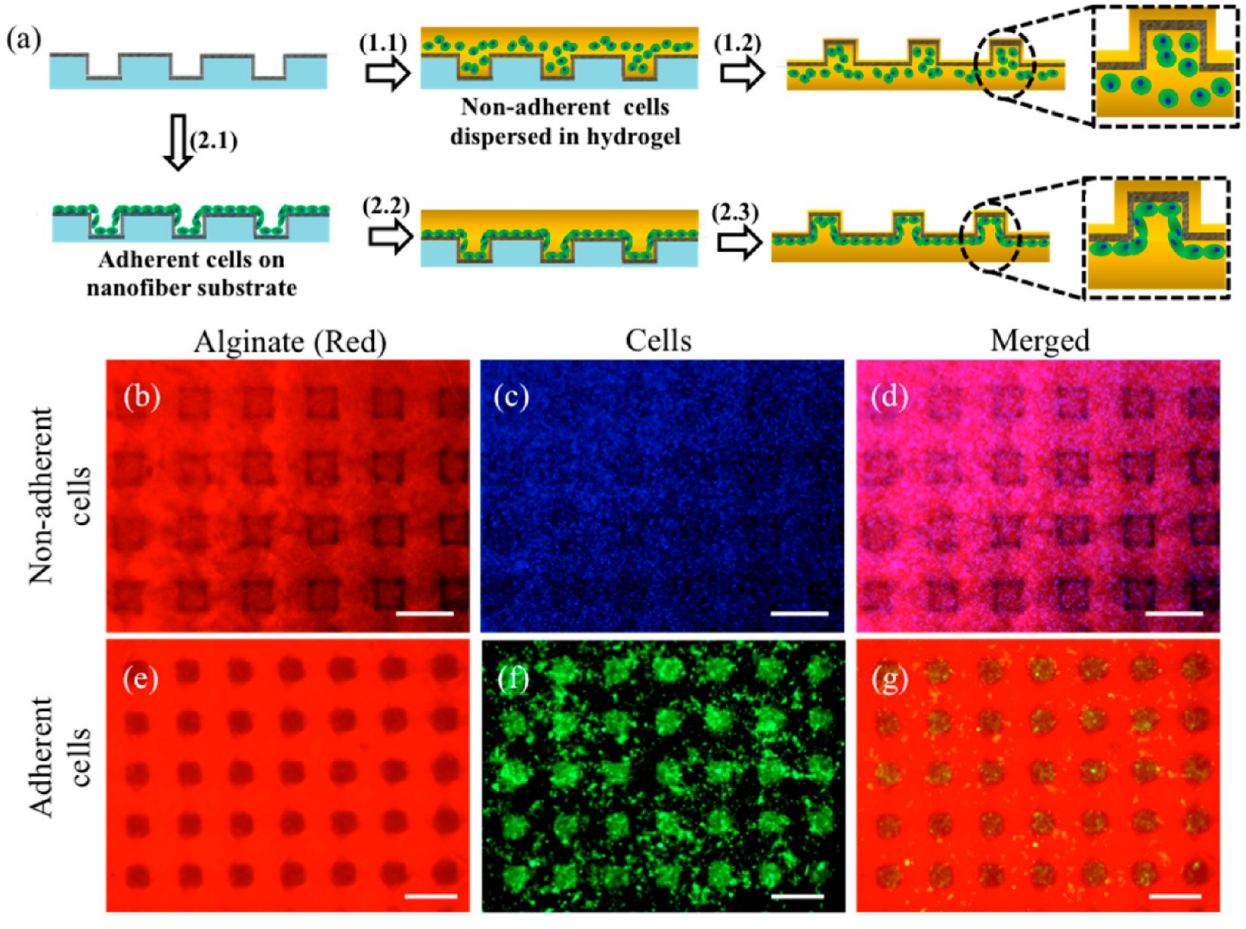

Figure 4. Cell encapsulation in nanofiber-framed hydrogel micropatterns. (a) The scheme of two cell encapsulation methods. Method 1:1.1. Disperse non-adherent cells in alginate solution; 1.2. Encapsulate cells in cross-linked nanofiber-framed hydrogel micropatterns. Method 2:2.1. Culture adherent cells on micropatterened nanofibers; 2.2. Place alginate solution on attached cells; 2.3. Encapsulate attached cells in cross-linked nanofiber-framed hydrogel micropatterns. (b–d) The fluorescent images of INS-1 cells (non-adherent model cells) encapsulated in nanofiber-framed hydrogel micropatterns. (b) Red color is alginate hydrogel and (c) the blue color indicates cell nuclei. (e–g) Fluorescent images of encapsulated MDA-MB-231 cells (adherent model cells). (e) Red color is alginate hydrogel and (f) the green color is cells expressing GFP proteins. Scale bars: 400 μm.

However, the microwells reported to date most of time only have smooth surfaces.[35] In contrast, the microwells reported here have nanofibrous surface that is structurally similar to the extracellular matrix[36] and therefore represent a new, more relevant high throughput cell culture platform. The nanofibers were also electrospun on double-faced and rod-shaped alloy by rotating the alloy template during electrospinning process (see Figure S1c, d in the Supporting Information). These unique micropatterned 3D structures may find important applications in areas such as tissue engineering.[31]

Next, we demonstrated that the nanofibrous microposts and microwells could be hybridized with hydrogels for cell encapsulation applications. Cell encapsulation holds great promises in regenerative medicine[42−44] to treat a range of

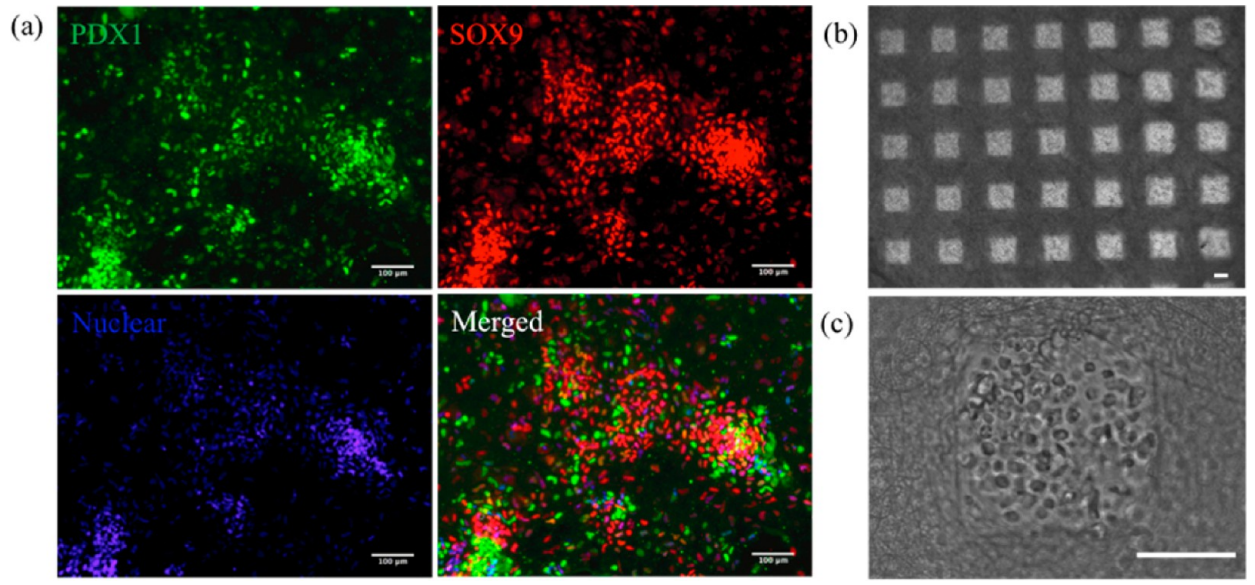

Figure 5. hESCs-PPs encapsulation in nanofiber-framed hydrogel micropatterns. (a) Immunostaining of characteristic markers of hESCs-PPs. The green color is PDX1, red color is SOX9, and blue color is cell nuclei. (b, c) Microscopic images (at different magnifications) of hESCs-PPs encapsulated in nanofiber-framed alginate hydrogel micropattern. Scale bars: 100 μm.

difficult diseases, including type 1 diabetes,[45] tumor,[46] heart failure,[47] and neurodegenerative disorders.[48] To make cell encapsulation a clinically success, one key is to develop a biocompatible encapsulation material with robust mechanical, mass transfer, and immunoisolation properties for either non-adherent or adherent cells. Instead of simply compositing fibers within bulk hydrogels as reported in previous studies,[38,39] we fabricated nanofiber-framed hydrogel micropatterns by casting and cross-linking alginate solution onto micropatterned nanofibers. The alginate solution followed the micropatterns and infiltrated the porous nanofibrous membranes. Free-standing, hydrogel micropatterns reinforced by nanofiber frames were obtained after cross-linking. Figure 3 and Figure S2 in the Supporting Information show a variety of nanofiber (red)/hydrogel (green) hybrid micropatterns. Compared with the neat hydrogel micropatterns, which are prone to shrinking and curling (see Figure S3a in the Supporting Information), the nanofibrous frames imparted the necessary mechanical robustness and durability for handling (see Figure S3b in the Supporting Information). In addition, the individual microtopographical features on the surface of hydrogel were also reinforced by the nanofibers.

We devised two methods to demonstrate the feasibility of cell encapsulation using nanofiber-framed hydrogel micropatterns (Figure 4a). To encapsulate non-adherent cells such as islet cells, the cells were first suspended in alginate solution and the cell mixture was then poured on the nanofibrous microwells followed by cross-linking. As shown in Figure 4b−d, INS-1 832/13 cells, a model cell mimicking islet cells, were encapsulated in this way. For encapsulation of adherent cell, we first cultured the cells and attached them on the nanofiber substrates and then an alginate solution was infused into the nanofibrous membranes and cross-linked. The green MDA-MB-231-GFP cells were used as model cells, cultured on micropatterned nanofibers, and encapsulated in alginate hydrogel (Figure 4e−g). Moreover, we encapsulated fibroblasts and human umbilical vein endothelial cells (HUVECs) which may be useful for skin disease treatment[49] and regeneration of ischemic tissues,[50] respectively (see Figure S4a, b in the Supporting Information). Interestingly, fibroblasts attached on nanofibers and HUVECs suspended in the solution can be co-encapsulated in nanofiber-framed hydrogel micropatterns (see Figure S4c in the Supporting Information), representing a potential vascularization model.[51] It is worth mentioning that the nanofibers, because of their structural similarity to extracellular matrix, have been shown as an excellent growth substrate for many types of cells[36] and therefore this approach provides a new platform to encapsulate and deliver these cells. Furthermore, the cells attached to the nanofiber membranes are close to the surface and have easy access to oxygen and nutrients, greatly reducing the risk of necrosis.[43] In a microarray composed of thousands of microposts, the lateral area of all microposts is appreciable and substantial. Therefore, compared with traditional encapsulation of cells in planar structures, the microtopographical structures remarkably increase the surface area for mass transfer and cell loading. By taking these advantages, human embryonic stem cell (hESC)-derived pancreatic progenitors (PPs), an unlimited and practical cell source for type 1 diabetes treatment, were cultured on Matrigel-coated nanofibrous micropatterns and then encapsulated in alginate hydrogel (Figure 5). The characteristic markers of hESC-PPs were confirmed with immunostaining of PDX1 and SOX9 (Figure 5a). As shown in b and c in Figure 5, hESCs-PPs adhered on nanofibers and were encapsulated in alginate hydrogel micropattern. The encapsulated hESCs-PPs were expected to mature into glucose responsive, insulin-producing β-like cells after transplantation in vivo.[52,53]

In conclusion, we have demonstrated a simple, robust, and cost-effective approach to controllably micropatterning the nanofibers by taking advantages of the intrinsic conductivity, ductility, and flexibility of low-melting temperature alloys. These nanofibrous microposts and microwells represent an interesting and biomimetic platform for cell culture and high-throughput applications. We also demonstrated that the nanofiber-framed hydrogel micropatterns can be engineered for cell encapsulation applications for both non-adherent and adherent cells. The nanofibers not only enhance the integrity of the hydrogel as a whole for durability and easy handling but

also improve the mechanical robustness of individual microtopographical hydrogel structures. The increased surface area due to the micropatterns is beneficial for mass transfer and cell loading.

■ ASSOCIATED CONTENT

Supporting Information

Experimental section and supplemental figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Li, D.; Xia, Y. Electrospinning of Nanofibers: Reinventing the Wheel? *Adv. Mater.* **2004**, *16*, 1151−1170.

(2) Onozuka, K.; Ding, B.; Tsuge, Y.; Naka, T.; Yamazaki, M.; Sugi, S.; Ohno, S.; Yoshikawa, M.; Shiratori, S. Electrospinning Processed Nanofibrous TiO_2 Membranes for Photovoltaic Applications. *Nanotechnology* **2006**, *17*, 1026−1031.

(3) Di Benedetto, F.; Camposeo, A.; Pagliara, S.; Mele, E.; Persano, L.; Stabile, R.; Cingolani, R.; Pisignano, D. Patterning of Light-Emitting Conjugated Polymer Nanofibres. *Nat. Nanotechnol.* **2008**, *3*, 614−619.

(4) Formo, E.; Lee, E.; Campbell, D.; Xia, Y. Functionalization of Electrospun TiO_2 Nanofibers with Pt Nanoparticles and Nanowires for Catalytic Applications. *Nano Lett.* **2008**, *8*, 668−672.

(5) Liu, J.; Yue, Z.; Fong, H. Continuous Nanoscale Carbon Fibers with Superior Mechanical Strength. *Small* **2009**, *5*, 536−542.

(6) Ma, M.; Hill, R. M.; Lowery, J. L.; Fridrikh, S. V.; Rutledge, G. C. Electrospun Poly(Styrene-Block-Dimethylsiloxane) Block Copolymer Fibers Exhibiting Superhydrophobicity. *Langmuir* **2005**, *21*, 5549−5554.

(7) Yang, D.; Niu, X.; Liu, Y.; Wang, Y.; Gu, X.; Song, L.; Zhao, R.; Ma, L.; Shao, Y.; Jiang, X. Electrospun Nanofibrous Membranes: A Novel Solid Substrate for Microfluidic Immunoassays for HIV. *Adv. Mater.* **2008**, *20*, 4770−4775.

(8) Mondal, K.; Ali, M. A.; Agrawal, V. V.; Malhotra, B. D.; Sharma, A. Highly Sensitive Biofunctionalized Mesoporous Electrospun TiO_2 Nanofiber Based Interface for Biosensing. *ACS Appl. Mater. Interfaces* **2014**, *6*, 2516−2527.

(9) Son, Y. J.; Kim, W. J.; Yoo, H. S. Therapeutic Applications of Electrospun Nanofibers for Drug Delivery Systems. *Arch. Pharm. Res.* **2014**, *37*, 69−78.

(10) Yang, W. J.; Fu, J.; Wang, D. X.; Wang, T.; Wang, H.; Jin, S. G.; He, N. Y. Study on Chitosan/Polycaprolactone Blending Vascular Scaffolds by Electrospinning. *J. Biomed. Nanotechnol.* **2010**, *6*, 254−259.

(11) Ahvaz, H. H.; Mobasheri, H.; Bakhshandeh, B.; Shakhssalim, N.; Naji, M.; Dodel, M.; Soleimani, M. Mechanical Characteristics of Electrospun Aligned PCL/PLLA Nanofibrous Scaffolds Conduct Cell Differentiation in Human Bladder Tissue Engineering. *J. Nanosci. Nanotechnol.* **2013**, *13*, 4736−4743.

(12) Nirmala, R.; Kalpana, D.; Navamathavan, R.; Lee, Y. S.; Kim, H. Y. Preparation and Characterizations of Silver Incorporated Polyurethane Composite Nanofibers Via Electrospinning for Biomedical Applications. *J. Nanosci. Nanotechnol.* **2013**, *13*, 4686−4693.

(13) Ghasemi-Mobarakeh, L.; Prabhakaran, M. P.; Balasubramanian, P.; Jin, G.; Valipouri, A.; Ramakrishna, S. Advances in Electrospun Nanofibers for Bone and Cartilage Regeneration. *J. Nanosci. Nanotechnol.* **2013**, *13*, 4656−4671.

(14) Pham, Q. P.; Sharma, U.; Mikos, A. G. Electrospinning of Polymeric Nanofibers for Tissue Engineering Applications: A Review. *Tissue Eng.* **2006**, *12*, 1197−1211.

(15) Shin, Y. M.; Hohman, M. M.; Brenner, M. P.; Rutledge, G. C. Experimental Characterization of Electrospinning: The Electrically Forced Jet and Instabilities. *Polymer* **2001**, *42*, 09955−09967.

(16) Huang, Y.; Duan, X. F.; Wei, Q. Q.; Lieber, C. M. Directed Assembly of One-Dimensional Nanostructures into Functional Networks. *Science* **2001**, *291*, 630−633.

(17) Zhong, S.; Teo, W. E.; Zhu, X.; Beuerman, R. W.; Ramakrishna, S.; Yung, L. Y. An Aligned Nanofibrous Collagen Scaffold by Electrospinning and Its Effects on In Vitro Fibroblast Culture. *J. Biomed. Mater. Res., Part A* **2006**, *79*, 456−463.

(18) Xie, J.; Macewan, M. R.; Ray, W. Z.; Liu, W.; Siewe, D. Y.; Xia, Y. Radially Aligned, Electrospun Nanofibers as Dural Substitutes for Wound Closure and Tissue Regeneration Applications. *ACS Nano* **2010**, *4*, 5027−5036.

(19) Ren, Y. J.; Zhang, S.; Mi, R.; Liu, Q.; Zeng, X.; Rao, M.; Hoke, A.; Mao, H. Q. Enhanced Differentiation of Human Neural Crest Stem Cells Towards the Schwann Cell Lineage by Aligned Electrospun Fiber Matrix. *Acta Biomater.* **2013**, *9*, 7727−7736.

(20) Liu, W.; Thomopoulos, S.; Xia, Y. Electrospun Nanofibers for Regenerative Medicine. *Adv. Healthcare Mater.* **2012**, *1*, 10−25.

(21) Katta, P.; Alessandro, M.; Ramsier, R. D.; Chase, G. G. Continuous Electrospinning of Aligned Polymer Nanofibers onto a Wire Drum Collector. *Nano Lett.* **2004**, *4*, 2215−2218.

(22) Li, D.; Wang, Y.; Xia, Y. Electrospinning Nanofibers as Uniaxially Aligned Arrays and Layer-by-Layer Stacked Films. *Adv. Mater.* **2004**, *16*, 361−366.

(23) Yang, D.; Lu, B.; Zhao, Y.; Jiang, X. Fabrication of Aligned Fibrous Arrays by Magnetic Electrospinning. *Adv. Mater.* **2007**, *19*, 3702−3706.

(24) Carlberg, B.; Wang, T.; Liu, J. Direct Photolithographic Patterning of Electrospun Films for Defined Nanofibrillar Microarchitectures. *Langmuir* **2010**, *26*, 2235−2239.

(25) Cheng, Q.; Lee, B. L.; Komvopoulos, K.; Li, S. Engineering the Microstructure of Electrospun Fibrous Scaffolds by Microtopography. *Biomacromolecules* **2013**, *14*, 1349−1360.

(26) Lee, J.; Lee, S. Y.; Jang, J.; Jeong, Y. H.; Cho, D. W. Fabrication of Patterned Nanofibrous Mats Using Direct-Write Electrospinning. *Langmuir* **2012**, *28*, 7267−7275.

(27) Liu, Y.; Zhang, L.; Li, H.; Yan, S.; Yu, J.; Weng, J.; Li, X. Electrospun Fibrous Mats on Lithographically Micropatterned Collectors to Control Cellular Behaviors. *Langmuir* **2012**, *28*, 17134−17142.

(28) Vaquette, C.; Cooper-White, J. J. Increasing Electrospun Scaffold Pore Size with Tailored Collectors for Improved Cell Penetration. *Acta Biomater.* **2011**, *7*, 2544−2557.

(29) Wu, Y.; Dong, Z.; Wilson, S.; Clark, R. L. Template-Assisted Assembly of Electrospun Fibers. *Polymer* **2010**, *51*, 3244−3248.

(30) Zhang, D.; Chang, J. Patterning of Electrospun Fibers Using Electroconductive Templates. *Adv. Mater.* **2007**, *19*, 3664−3667.

(31) Zhang, D.; Chang, J. Electrospinning of Three-Dimensional Nanofibrous Tubes with Controllable Architectures. *Nano Lett.* **2008**, *8*, 3283−3287.
(32) Ma, B.; Xie, J.; Jiang, J.; Wu, J. Sandwich-Type Fiber Scaffolds with Square Arrayed Microwells and Nanostructured Cues as Microskin Grafts for Skin Regeneration. *Biomaterials* **2014**, *35*, 630−641.
(33) Xie, J.; Liu, W.; MacEwan, M. R.; Yeh, Y. C.; Thomopoulos, S.; Xia, Y. Nanofiber Membranes with Controllable Microwells and Structural Cues and Their Use in Forming Cell Microarrays and Neuronal Networks. *Small* **2011**, *7*, 293−297.
(34) Ding, Z.; Salim, A.; Ziaie, B. Selective Nanofiber Deposition through Field-Enhanced Electrospinning. *Langmuir* **2009**, *25*, 9648−9652.
(35) Charnley, M.; Textor, M.; Khademhosseini, A.; Lutolf, M. P. Integration Column: Microwell Arrays for Mammalian Cell Culture. *Integr. Biol.* **2009**, *1*, 625−634.
(36) Stevens, M. M.; George, J. H. Exploring and Engineering the Cell Surface Interface. *Science* **2005**, *310*, 1135−1138.
(37) Woodruff, K.; Fidalgo, L. M.; Gobaa, S.; Lutolf, M. P.; Maerkl, S. J. Live Mammalian Cell Arrays. *Nat. Methods* **2013**, *10*, 550−552.
(38) Agrawal, A.; Rahbar, N.; Calvert, P. D. Strong Fiber-Reinforced Hydrogel. *Acta Biomater.* **2013**, *9*, 5313−5318.
(39) Lee, H. J.; Park, Y. H.; Koh, W. G. Fabrication of Nanofiber Microarchitectures Localized within Hydrogel Microparticles and Their Application to Protein Delivery and Cell Encapsulation. *Adv. Funct. Mater.* **2013**, *23*, 591−597.
(40) Prochorov, A. V.; Tretjak, S. I.; Goranov, V. A.; Glinnik, A. A.; Goltsev, M. V. Treatment of Insulin Dependent Diabetes Mellitus with Intravascular Transplantation of Pancreatic Islet Cells without Immunosuppressive Therapy. *Adv. Med. Sci.* **2008**, *53*, 240−244.
(41) Lindstrom, S.; Andersson-Svahn, H. Single-Cell Culture in Microwells. *Methods Mol. Biol.* **2012**, *853*, 41−52.
(42) Chang, T. M. Therapeutic Applications of Polymeric Artificial Cells. *Nat. Rev. Drug Discovery* **2005**, *4*, 221−235.
(43) Orive, G.; Hernandez, R. M.; Gascon, A. R.; Calafiore, R.; Chang, T. M.; De Vos, P.; Hortelano, G.; Hunkeler, D.; Lacik, I.; Shapiro, A. M.; Pedraz, J. L. Cell Encapsulation: Promise and Progress. *Nat. Med.* **2003**, *9*, 104−107.
(44) Read, T. A.; Sorensen, D. R.; Mahesparan, R.; Enger, P. O.; Timpl, R.; Olsen, B. R.; Hjelstuen, M. H.; Haraldseth, O.; Bjerkvig, R. Local Endostatin Treatment of Gliomas Administered by Microencapsulated Producer Cells. *Nat. Biotechnol.* **2001**, *19*, 29−34.
(45) Calafiore, R. Alginate Microcapsules for Pancreatic Islet Cell Graft Immunoprotection: Struggle and Progress Towards the Final Cure for Type 1 Diabetes Mellitus. *Expert Opin. Biol. Ther.* **2003**, *3*, 201−205.
(46) Joki, T.; Machluf, M.; Atala, A.; Zhu, J.; Seyfried, N. T.; Dunn, I. F.; Abe, T.; Carroll, R. S.; Black, P. M. Continuous Release of Endostatin from Microencapsulated Engineered Cells for Tumor Therapy. *Nat. Biotechnol.* **2001**, *19*, 35−39.
(47) Wollert, K. C.; Drexler, H. Cell-Based Therapy for Heart Failure. *Curr. Opin. Cardiol.* **2006**, *21*, 234−239.
(48) Kishima, H.; Poyot, T.; Bloch, J.; Dauguet, J.; Conde, F.; Dolle, F.; Hinnen, F.; Pralong, W.; Palfi, S.; Deglon, N.; Aebischer, P.; Hantraye, P. Encapsulated GDNF-Producing C2C12 Cells for Parkinson's Disease: A Pre-Clinical Study in Chronic MPTP-Treated Baboons. *Neurobiol. Dis.* **2004**, *16*, 428−439.
(49) Yun, E. J.; Yon, B.; Joo, M. K.; Jeong, B. Cell Therapy for Skin Wound Using Fibroblast Encapsulated Poly(Ethylene Glycol)-Poly(L-Alanine) Thermogel. *Biomacromolecules* **2012**, *13*, 1106−1111.
(50) Kraehenbuehl, T. P.; Ferreira, L. S.; Zammaretti, P.; Hubbell, J. A.; Langer, R. Cell-Responsive Hydrogel for Encapsulation of Vascular Cells. *Biomaterials* **2009**, *30*, 4318−4324.
(51) Sukmana, I.; Vermette, P. The Effects of Co-Culture with Fibroblasts and Angiogenic Growth Factors on Microvascular Maturation and Multi-Cellular Lumen Formation in HUVEC-Oriented Polymer Fibre Constructs. *Biomaterials* **2010**, *31*, 5091−5099.
(52) Kroon, E.; Martinson, L. A.; Kadoya, K.; Bang, A. G.; Kelly, O. G.; Eliazer, S.; Young, H.; Richardson, M.; Smart, N. G.; Cunningham, J.; Agulnick, A. D.; D'Amour, K. A.; Carpenter, M. K.; Baetge, E. E. Pancreatic Endoderm Derived from Human Embryonic Stem Cells Generates Glucose-Responsive Insulin-Secreting Cells In Vivo. *Nat. Biotechnol.* **2008**, *26*, 443−452.
(53) Chen, S.; Borowiak, M.; Fox, J. L.; Maehr, R.; Osafune, K.; Davidow, L.; Lam, K.; Peng, L. F.; Schreiber, S. L.; Rubin, L. L.; Melton, D. A Small Molecule That Directs Differentiation of Human ESCs into the Pancreatic Lineage. *Nat. Chem. Biol.* **2009**, *5*, 258−265.