Hierarchical Hydrogel Composite Interfaces with Robust Mechanical Properties for Biomedical Applications

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Cells sense and respond to a wide range of external signals, including chemical signals, topography, and interface mechanics, via interactions with the extracellular matrix (ECM), triggering the regulation of behavior and function. The ECM can be considered a hierarchical multiphase porous matrix with various components. Highly porous hydrogel-based biomaterials can mimic the critical ECM properties, to provide mechanical support for tissues and to regulate cellular behaviors, such as adhesion, proliferation, and differentiation. Herein, based on micro/nanoscale-topography-coupled mechanical action, recent advances in the fabrication and application of hydrogel composites with tunable mechanical properties and topography in biomedicine are summarized. In particular, recent findings showing that hydrogels with specifically designed structures not only influence a range of cellular processes and fit the needs of engineered tissues but also have pharmacological effects are emphasized.

1. Introduction

The extracellular matrix (ECM) is a complex hierarchical network composed of fibrous proteins; it influences a range of cellular processes, such as adhesion, proliferation, and differentiation. Biomimetic materials for the ECM hold great promise in the field of biomedicine with wide applications in drug delivery, would healing, and tissue engineering.[1] In the past few decades, extensive studies have shown that not only does the composition of the ECM affect cell behaviors, but mechanical properties also play key roles.[2,3] In particular, substrates with a suitable stiffness and topography can have regulatory effects on the physiological activities of cells and biological processes, including protein secretion, gene expression, wound healing, and tissue regeneration.[4–10] These changes are mediated by mechanotransduction at the interface of materials and cells.[11] Accordingly, building biomaterials with hierarchical networks that mimic the properties of the native ECM is important for investigating cellular physiology. However, conventional scaffolds have many limitations with respect to ECM simulation; they do not approximate the complex architecture of native tissues or provide mechanical stimuli to induce the formation of tissue-like structures.[12] Accordingly, the development of materials with a robust micro/nanoscale surface with suitable mechanical properties is still a major goal in the field of biomedicine.

Hydrogels are attractive candidates for ECM mimics because they are multiphase matrixes composed of a hydrophilic polymer with a high water content and are highly porous, similar to the native ECM.[13] The 3D networks of hydrogels not only provide good anchor sites for cells, but also deliver biologically relevant chemical and physical signals to facilitate efficient cell proliferation and differentiation. By crosslinking approaches, hydrogels can be prepared with tunable physical properties, high biocompatibility, and responsiveness to stimuli for wide uses in biomedicine.[14,15] The rational design of hydrogel networks to produce well-defined biomaterials with good mechanical properties and topography may improve the ability to mimic native tissues.

Here, based on micro/nanoscale-topography-coupled mechanical action, we review recent progress in the fabrication of hydrogel composites with tunable mechanical properties and topography for applications in biomedicine. We focus on the development of hydrogels with designed structures in tissue engineering, as we believe that they can not only influence a range of cellular processes and fit the needs of various engineered tissues, but also have pharmacological effects on cells. We further highlight various methods to prepare hierarchical hydrogel composite interfaces with robust mechanical properties and review some of the most recent and impressive applications of these hydrogels in biomedicine. Additionally, we evaluate emerging trends in the physical regulation of cell reprograming. Finally, future challenges related to the development of the next generation of hierarchical hydrogel composites are discussed.

2. Response of Cells to Robust Mechanical Hydrogel Interfaces

Since mechanotransduction and interactions at the interface between materials and cells play crucial roles in the regulation of cell behaviors and the genotype-to-phenotype relation ship, mechanical properties are important parameters in the

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design of hydrogels for biomedical applications.[16] In recent years, substantial efforts have been made to determine the relationship between cell functions and the mechanical properties of hydrogels. For instance, the widely used polyacrylamide (PA) hydrogels with desired stiffness not only affect cell spreading, adhesion, and proliferation, but also can induce stem cell differentiation into various cell types (0.1–1 kPa for neurogenic cells, 8–17 kPa for myogenic cells, and 25–40 kPa for osteogenic cells), providing a great value in relative tissue engineering.[3,17,18] Cells adhered to stiff hydrogels experience a higher elastic modulus in their plasma membrane with a better organized actin cytoskeleton and promote faster proliferation and slower migration compared to soft hydrogels. The mechanical feedback on the effect of matrix remolding at the interface between cells and hydrogels result in determining the key molecular pathways on cell fates. Also, Huck et al. have found that the cell behaviors are also related to the nature of the hydrogels with different porosities.[8] However, Engler and co-workers revealed that the local force at the cellular mechanosensing scale via myosin contraction of cells led to the hydrogel stiffness that can regulate the cell behaviors independently (**Figure 1**a).[9] Later, Han's group proposed a loading-rate-sensing theory to explain how the cells sense ECM mechanical cues and revealed that the stiffness, pore size, and patterns which affect the effective spring constant of the hydrogel always contribute to the loading rate of integrin–ligand bonds and thus determine the cell functions (Figure 1b).[19] At the genetic level, Cooper-White and co-workers revealed that the mechanical relative mammalian target of rapamycin (mTOR) signaling plays a critical role in regulating cell fate which is related to the substrate stiffness. Modulating mechanosensitive microRNAs (miRNAs) of miR-100-5p and miR-143-3p could change mTOR pathway activity, which results in the regulation of the human mesenchymal stem cells (hMSCs) fate (Figure 1c).[20]

Elasticity and viscosity are important mechanical characteristics of substrates and their timely feedback on mechanical stimulation plays key roles in cell fate determination. More important, the ECM of various human tissues such as brain, liver, and adipose tissues is viscoelastic and exhibits significant stress relaxation to avoid damage from external forces (Figure 1d).[21–24] An adaptable hydrogel with appropriate modulus and stress relaxation properties may effectively mimic complex biological tissues.[25] Ishihara and co-workers reversed the proliferation cycle of cells to G1 phase through the modulation of the storage modulus (G′) of the poly(vinylalcohol)-based hydrogel, to improve differentiation signal sensitivity which can achieve highly efficient, lineage-restricted differentiation of stem cells for rapid tissue repairing.[26] More interesting finding by Anseth and co-workers is that hMSCs can retain the past mechanical information and enough mechanical dosing mediated by a poly(ethylene glycol)-based photodegradable hydrogel can influence future cell fate decisions regardless of the changing of mechanical environment (Figure 1e).[27] This provides a favorable basis for the transplantation of in vitro cultured tissue into body.

These findings highlight the importance of mechanical properties and inspired the development of logical hydrogel designs for biomedical applications. Simple mechanical regulation of biomimetic hydrogels not only changes the physiological state

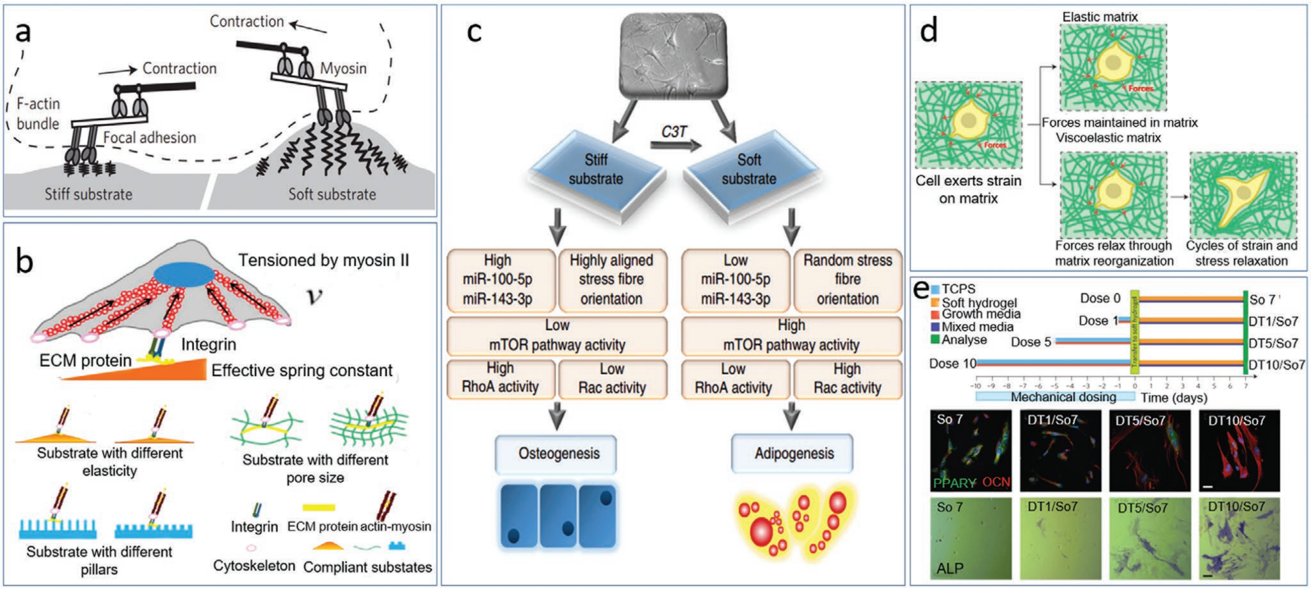

Figure 1. a) Schematic diagram of the mechanism about how cells dynamically deform substrates via myosin contractions. Reproduced with permission.[9] Copyright 2014, Nature Publishing Group. b) A schematic of the cell–integrin–ECM protein system and the contractility subject to integrin–ECM protein bonds on substrates induced by myosin with different effective spring constants, stiffnesses, pore sizes, or pillar lengths. Reproduced with permission.[19] Copyright 2015, American Chemical Society. c) Proposed model of the regulatory effects of substrate stiffness and miRNA signaling on mTOR pathway activity and hMSC differentiation. Reproduced with permission.[20] Copyright 2014, Nature Publishing Group. d) Hypothesis for how the initial elastic modulus and stress relaxation properties of matrixes regulate cellular behaviors. Reproduced with permission.[22] Copyright 2016, Nature Publishing Group. e) Immunocytochemistry of PPARγ (green) and osteocalcin (OCN) (red) in hMSCs after 7 days on soft hydrogels with various mechanical doses on past physical environments (tissue culture polystyrene (TCPS)) and staining for alkaline phosphatase (ALP) in hMSCs with mechanical dosing on TCPS before culture on soft hydrogels. Reproduced with permission.[27] Copyright 2014, Nature Publishing Group.

of cells, but also promotes the efficacy of some drugs. Han's group demonstrated that the stiffness can influence the sensitivity of breast cancer cells to targeted therapy and modulate the expression of mechanically related mRNA expression.[28,29] Moreover, they revealed that the soft PA hydrogel with larger pores can reduce the sensitive response of breast cancer cells to lapatinib because of the decreased adhesion of β4 integrin.[30] This provides a reference for antitumor drug screening in vitro and treatment of mechanically related diseases in vivo.

3. Hierarchical Hydrogel with Robust Micro/Nanoscale Interfaces

Unique structures and functions in nature have inspired biological research for centuries, and a deeper understanding of these structures and functions can contribute to progress in the field of biomedicine.[1,31,32] However, it is challenging to mimic innate tissues, which are intrinsically hierarchical micro/nanostructures with nonuniform mechanical properties.[33] Substrates with multiscale topography are designed to resolve this issue; the topography leads to variation in localized mechanical properties and associated micromechanical changes result in the manipulation of focal adhesion formation for the regulation of cell behaviors and functions (**Figure 2**a).[34–36] For instance, Han's group developed a scaffold by mixing gold nanoparticles with collagen and the formative localized stiffness could efficiently regulate the assembly of intercalated discs through the β1 integrin-mediated ILK/p-AKT/GATA4 pathway.[37] However, this method is relatively simple and has narrow application range. In recent years, many fascinating hydrogels with micro/nanotopography have been developed using various technologies, such as photolithography, micromolding, and 3D bioprinting.

Photolithography is commonly used to induce the gelation of polymer solutions through a patterned photomask, which enables the formation of hydrogels with desired shapes. Complex multiscale topographies can be created by a combination of photolithography and micromolding technology. Hydrogels prepared with micro/nanotopography not only influence cell adhesion, spreading, and migration, but can also induce cells to form different patterns or to differentiate into desired cell types.[38–40] This is because nonuniform localized stiffness caused by the topography of substrates leads to changes in the actin cytoskeleton and mechanotransduction, followed by focal adhesion rearrangement, thus resulting in changes in cellular behaviors and cell fate.[41,42] However, hydrogels with complex biomechanical properties and 3D topographies are more suitable for practical applications than hydrogels generated by conventional photolithography or micromolding. Then, stereolithography technology has been developed to enable faster hydrogel scaffold fabrication with easy changes in compositions and structural parameters.[15] In particular, Wai and co-workers developed an optical maskless stereolithography technology that allows for in situ microfabrication and large-area patterning which enable high-precision printing of poly(acrylic acid) ionic hydrogel for biosensors and microstructures (Figure 2b).[43] Hydrogels created by stereolithography have complex 3D mechanical interfaces, similar to those of natural tissues, and

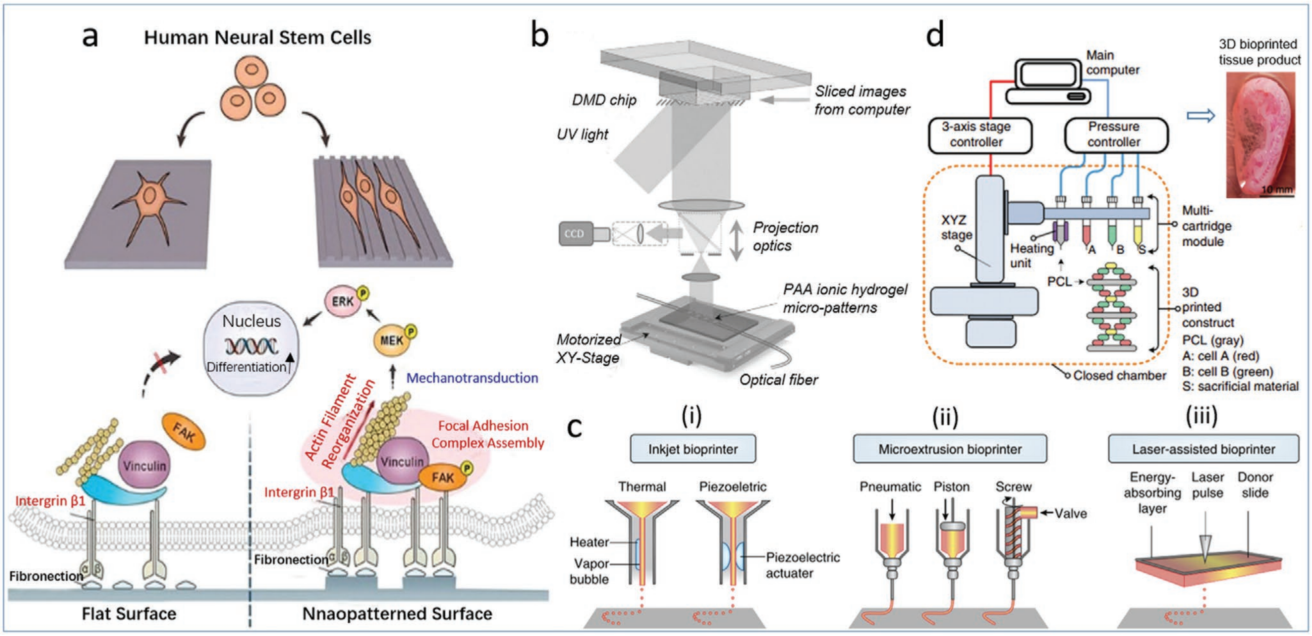

Figure 2. a) Proposed mechanism for how nanotopography influences the focal adhesion signaling pathway and differentiation of human neural stem cells. Reproduced with permission.[34] Copyright 2013, American Chemical Society. b) Schematic of the optical maskless stereolithography system: UV light illuminates the digital-mirror device (DMD) chip, and the generated optical pattern is projected on the photosensitive polymer to fabricate microstructures. Reproduced with permission.[43] Copyright 2015, Wiley-VCH. c) Different types of bioprinting tools: (i) in thermal inkjet printers, (ii) in microextrusion printers, (iii) in laser-assisted bioprinters. Reproduced with permission.[44] Copyright 2014, Nature Publishing Group. d) Integrated tissue–organ printer system consists of three major units: (i) 3-axis stage/controller, (ii) dispensing module including a multi-cartridge and pneumatic pressure controller, (iii) a closed acrylic chamber with a temperature controller and humidifier. Reproduced with permission.[47] Copyright 2014, Nature Publishing Group.

facilitate the regeneration of tissue interfaces when implanted into the body with encapsulated stem cells.

3D bioprinting is a fascinating technology that holds great promise for the creation of complex composite scaffolds in tissue engineering by the precise placement of hydrogels in a layer-by-layer manner.[44] Some tissue-like cell-laden hydrogel scaffolds fabricated by 3D bioprinting have excellent abilities to repair damaged tissues and are useful for other biomedical applications.[45,46] The most commonly used technologies for 3D bioprinting systems are inkjet (material viscosities: 3.5–12 mPa s^{-1}), extrusion (material viscosities: 30 mPa s^{-1} to $>6 \times 10^7$ mPa s^{-1}), and laser-assisted printing (material viscosities: 1–300 mPa s^{-1}) (Figure 2c).[44]

However, all existing technologies have great challenges with respect to the efficient fabrication of large-scale (>200 µm) integrated structures of cell-laden hydrogels with free-form stable shapes. To overcome these limitations, Atala and co-workers developed an integrated tissue–organ printer enabling the fabrication of stable, human-scale tissue constructs of any shape based on multiple types of cells and hydrogels (Figure 2d).[47] This substantially extends the application of hydrogel materials in tissue engineering and facilitates the fabrication of hierarchical hydrogel composite interfaces with nonuniform mechanical properties that are similar to the mechanical structure of natural tissues.

4. Conclusion

Increasing studies have demonstrated that the mechanical properties of substrates influence cell behaviors and functions independently of chemical compositions and biomolecules. The key factor determining these applications is mechanotransduction at the interface of materials and cells. We refer to this as interface mechanics, which includes not only the macroscopic mechanics of the material itself, but also the microscopic mechanics caused by the material topography, also known as localized mechanics. In recent years, the development of materials and technologies has enabled us to fabricate 2D and 3D

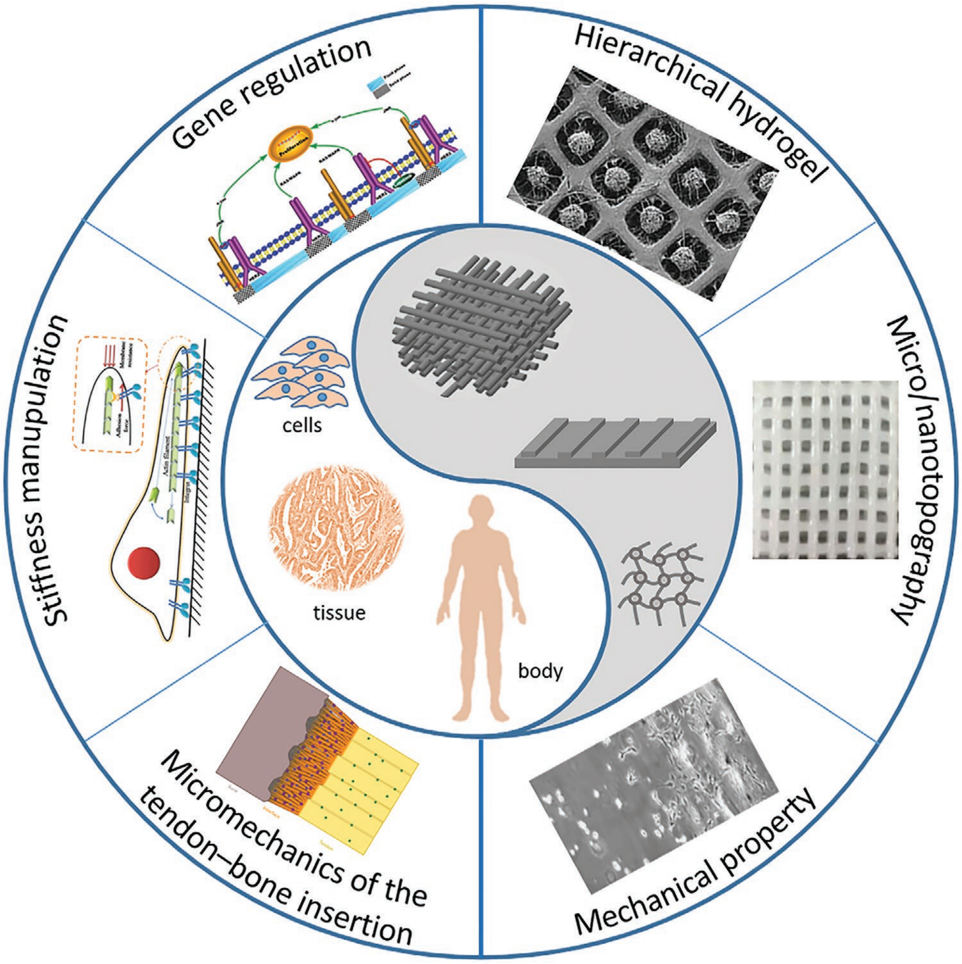

Figure 3. Recent exploration about the mechanical interfaces of nature tissues and relevant development of scaffolds for cell reprograming. Hierarchical hydrogel composite interfaces with micro/nanoscale surface and robust mechanical properties stand as promising materials for biomedical applications. Image for stiffness manupulation: reproduced with permission.[16] Copyright 2014, Nature Publishing Group. Image for gene regulation: reproduced with permission.[30] Copyright 2016, Wiley-VCH. Image for micromechanics of the tendon–bone insertion: reproduced with permission.[33] Copyright 2014, Nature Publishing Group. Image for micro/nanotopography: reproduced with permission.[45] Copyright 2015, Wiley-VCH. Image for hierarchical hydrogel: reproduced with permission.[48] Copyright 2016, Elsevier B.V. Image for mechanical property: reproduced with permission.[49] Copyright 2015, Wiley-VCH.

hydrogel scaffolds with a variety of mechanical properties. However, the ultimate goal is to build heterogeneous biomechanical interfaces that resemble natural tissues (**Figure 3**).[33,45,48,49] The fabrication of hierarchical hydrogel composite interfaces with robust mechanical interfaces has important implications for biomedical applications.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

biomedical engineering, drug screening, hierarchical interfaces, hydrogel composites, mechanics

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[1] J. J. Green, J. H. Elisseeff, *Nature* **2016**, *540*, 386.
[2] F. Chowdhury, S. Na, D. Li, Y. C. Poh, T. S. Tanaka, F. Wang, N. Wang, *Nat. Mater.* **2010**, *9*, 82.
[3] A. J. Engler, S. Sen, H. L. Sweeney, D. E. Discher, *Cell* **2006**, *126*, 677.
[4] F. Wang, L. Shi, W.-X. He, D. Han, Y. Yan, Z.-Y. Niu, S.-G. Shi, *Appl. Surf. Sci.* **2013**, *265*, 480.
[5] H. Gao, J. Zhang, F. Liu, Z. Ao, S. Liu, S. Zhu, D. Han, B. Yang, *J. Mater. Chem. B* **2014**, *2*, 7192.
[6] Q. Zhang, H. Dong, Y. Li, Y. Zhu, L. Zeng, H. Gao, B. Yuan, X. Chen, C. Mao, *ACS Appl. Mater. Interfaces* **2015**, *7*, 23336.
[7] J. Feng, L. Lin, P. Chen, W. Hua, Q. Sun, Z. Ao, D. Liu, L. Jiang, S. Wang, D. Han, *ACS Appl. Mater. Interfaces* **2015**, *7*, 8961.
[8] B. Trappmann, J. E. Gautrot, J. T. Connelly, D. G. Strange, Y. Li, M. L. Oyen, M. A. Cohen Stuart, H. Boehm, B. Li, V. Vogel, J. P. Spatz, F. M. Watt, W. T. Huck, *Nat. Mater.* **2012**, *11*, 642.
[9] J. H. Wen, L. G. Vincent, A. Fuhrmann, Y. S. Choi, K. C. Hribar, H. Taylor-Weiner, S. Chen, A. J. Engler, *Nat. Mater.* **2014**, *13*, 979.
[10] G. Chen, L. Zhao, J. Feng, G. You, Q. Sun, P. Li, D. Han, H. Zhou, *PLoS One* **2013**, *8*, e67360.
[11] N. Huebsch, P. R. Arany, A. S. Mao, D. Shvartsman, O. A. Ali, S. A. Bencherif, J. Rivera-Feliciano, D. J. Mooney, *Nat. Mater.* **2010**, *9*, 518.
[12] M. Mehrali, A. Thakur, C. P. Pennisi, S. Talebian, A. Arpanaei, M. Nikkhah, A. Dolatshahi-Pirouz, *Adv. Mater.* **2017**, *29*, 1603612.
[13] N. Annabi, A. Tamayol, J. A. Uquillas, M. Akbari, L. E. Bertassoni, C. Cha, G. Camci-Unal, M. R. Dokmeci, N. A. Peppas, A. Khademhosseini, *Adv. Mater.* **2014**, *26*, 85.
[14] J. Thiele, Y. Ma, S. M. Bruekers, S. Ma, W. T. Huck, *Adv. Mater.* **2014**, *26*, 125.
[15] A. Vedadghavami, F. Minooei, M. H. Mohammadi, S. Khetani, A. Rezaei Kolahchi, S. Mashayekhan, A. Sanati-Nezhad, *Acta Biomater.* **2017**, *62*, 42.
[16] J. Li, D. Han, Y. P. Zhao, *Sci. Rep.* **2015**, *4*, 3910.
[17] L. Han, X. Lu, M. Wang, D. Gan, W. Deng, K. Wang, L. Fang, K. Liu, C. W. Chan, Y. Tang, L. T. Weng, H. Yuan, *Small* **2017**, *13*, 1601916.
[18] B. Xu, P. Zheng, F. Gao, W. Wang, H. Zhang, X. Zhang, X. Feng, W. Liu, *Adv. Funct. Mater.* **2017**, *27*, 1604327.
[19] L. Jiang, Z. Sun, X. Chen, J. Li, Y. Xu, Y. Zu, J. Hu, D. Han, C. Yang, *ACS Nano* **2016**, *10*, 207.
[20] J. E. Frith, G. D. Kusuma, J. Carthew, F. Li, N. Cloonan, G. A. Gomez, J. J. Cooper-White, *Nat. Commun.* **2018**, *9*, 257.
[21] O. Chaudhuri, L. Gu, M. Darnell, D. Klumpers, S. A. Bencherif, J. C. Weaver, N. Huebsch, D. J. Mooney, *Nat. Commun.* **2015**, *6*, 6364.
[22] O. Chaudhuri, L. Gu, D. Klumpers, M. Darnell, S. A. Bencherif, J. C. Weaver, N. Huebsch, H. P. Lee, E. Lippens, G. N. Duda, D. J. Mooney, *Nat. Mater.* **2016**, *15*, 326.
[23] H. P. Lee, L. Gu, D. J. Mooney, M. E. Levenston, O. Chaudhuri, *Nat. Mater.* **2017**, *16*, 1243.
[24] E. E. Charrier, K. Pogoda, R. G. Wells, P. A. Janmey, *Nat. Commun.* **2018**, *9*, 449.
[25] D. D. McKinnon, D. W. Domaille, J. N. Cha, K. S. Anseth, *Adv. Mater.* **2014**, *26*, 865.
[26] H. Oda, T. Konno, K. Ishihara, *Biomaterials* **2015**, *56*, 86.
[27] C. Yang, M. W. Tibbitt, L. Basta, K. S. Anseth, *Nat. Mater.* **2014**, *13*, 645.
[28] J. Feng, Y. Tang, Y. Xu, Q. Sun, F. Liao, D. Han, *Clin. Hemorheol. Microcirc.* **2013**, *55*, 121.
[29] C. Liu, X. Li, J. Feng, F. Liao, D. Li, D. Han, *Clin. Hemorheol. Microcirc.* **2016**, *63*, 399.
[30] C. Liu, X. Li, W. Hua, J. Li, X. Han, Q. Ha, J. Feng, F. Liao, D. Li, D. Han, *Small* **2016**, *12*, 4675.
[31] L. Chen, D. Han, L. Jiang, *Colloids Surf., B* **2011**, *85*, 2.
[32] L. Lin, M. Liu, L. Chen, P. Chen, J. Ma, D. Han, L. Jiang, *Adv. Mater.* **2010**, *22*, 4826.
[33] L. Rossetti, L. A. Kuntz, E. Kunold, J. Schock, K. W. Muller, H. Grabmayr, J. Stolberg-Stolberg, F. Pfeiffer, S. A. Sieber, R. Burgkart, A. R. Bausch, *Nat. Mater.* **2017**, *16*, 664.
[34] K. Yang, K. Jung, E. Ko, J. Kim, K. I. Park, J. Kim, S. W. Cho, *ACS Appl. Mater. Interfaces* **2013**, *5*, 10529.
[35] M. J. P. Biggs, M. Fernandez, D. Thomas, R. Cooper, M. Palma, J. Liao, T. Fazio, C. Dahlberg, H. Wheadon, A. Pallipurath, A. Pandit, J. Kysar, S. J. Wind, *Adv. Mater.* **2017**, *29*, 1702119.
[36] J. Deng, C. Zhao, J. P. Spatz, Q. Wei, *ACS Nano* **2017**, *11*, 8282.
[37] Y. Li, X. Shi, L. Tian, H. Sun, Y. Wu, X. Li, J. Li, Y. Wei, X. Han, J. Zhang, X. Jia, R. Bai, L. Jing, P. Ding, H. Liu, D. Han, *Adv. Mater.* **2016**, *28*, 10230.
[38] M. K. Lee, M. H. Rich, J. Lee, H. Kong, *Biomaterials* **2015**, *58*, 26.
[39] C. Zhao, J. Hou, R. Chen, Z. Xin, H. Shi, S.-C. Wong, J. Yin, Q. Shi, *J. Mater. Chem. B* **2017**, *5*, 2315.
[40] Z. Ming, J. Fan, C. Bao, Y. Xue, Q. Lin, L. Zhu, *Adv. Funct. Mater.* **2018**, *28*, 1706918.
[41] R. A. Gittens, T. McLachlan, R. Olivares-Navarrete, Y. Cai, S. Berner, R. Tannenbaum, Z. Schwartz, K. H. Sandhage, B. D. Boyan, *Biomaterials* **2011**, *32*, 3395.
[42] B. K. K. Teo, S. ThaiWong, C. K. Lim, T. Y. S. Kung, C. H. Yap, Y. Ramagopal, L. H. Romer, E. K. F. Yim, *ACS Nano* **2013**, *7*, 4785.
[43] M. J. Yin, M. Yao, S. Gao, A. P. Zhang, H. Y. Tam, P. K. Wai, *Adv. Mater.* **2016**, *28*, 1394.
[44] S. V. Murphy, A. Atala, *Nat. Biotechnol.* **2014**, *32*, 773.
[45] F. Gao, Z. Xu, Q. Liang, B. Liu, H. Li, Y. Wu, Y. Zhang, Z. Lin, M. Wu, C. Ruan, W. Liu, *Adv. Funct. Mater.* **2018**, *28*, 1706644.
[46] X. Liu, H. Yuk, S. Lin, G. A. Parada, T. C. Tang, E. Tham, C. de la Fuente-Nunez, T. K. Lu, X. Zhao, *Adv. Mater.* **2018**, *30*, 1870021.
[47] H. W. Kang, S. J. Lee, I. K. Ko, C. Kengla, J. J. Yoo, A. Atala, *Nat. Biotechnol.* **2016**, *34*, 312.
[48] H. S. Shin, Y. M. Kook, H. J. Hong, Y. M. Kim, W. G. Koh, J. Y. Lim, *Acta Biomater.* **2016**, *45*, 121.
[49] N. Zaari, P. Rajagopalan, S. K. Kim, A. J. Engler, J. Y. Wong, *Adv. Mater.* **2004**, *16*, 2133.