REVIEW

Amino Acids and Peptide-Based Supramolecular Hydrogels for Three-Dimensional Cell Culture

*Xiao-Qiu Dou and Chuan-Liang Feng**

Supramolecular hydrogels assembled from amino acids and peptide-derived hydrogelators have shown great potential as biomimetic three-dimensional (3D) extracellular matrices because of their merits over conventional polymeric hydrogels, such as non-covalent or physical interactions, controllable self-assembly, and biocompatibility. These merits enable hydrogels to be made not only by using external stimuli, but also under physiological conditions by rationally designing gelator structures, as well as in situ encapsulation of cells into hydrogels for 3D culture. This review will assess current progress in the preparation of amino acids and peptide-based hydrogels under various kinds of external stimuli, and in situ encapsulation of cells into the hydrogels, with a focus on understanding the associations between their structures, properties, and functions during cell culture, and the remaining challenges in this field. The amino acids and peptide-based hydrogelators with rationally designed structures have promising applications in the fields of regenerative medicine, tissue engineering, and pre-clinical evaluation.

1. Introduction

In vitro cell culture is an indispensable part of regenerative biology and pre-clinical evaluation.[1] Traditional two-dimensional (2D) cell culture has been widely studied due to its convenience and affordability. Despite this progress, 2D scaffolds cannot simulate the actual in vivo cell growth conditions and cells may alter their metabolism, gene expression patterns, production of extracellular matrix (ECM) proteins, and morphology (e.g., an increase in spreading) under 2D conditions.[2] Thus, there is an ongoing demand to engineer functional three dimensional (3D) structures for cell culture. Among 3D cell culture materials, hydrogels are one of the best candidates because of their high water content, biocompatibility, and resemblance to the gel-like character of ECM.[3,4] Ideally, a hydrogel scaffold for 3D cell culture should have the following characteristics: (a) Ease of handling under physiological conditions; (b) Mechanical properties that resemble those of natural tissue; (c) Gel uniformity at nano, micro, and macroscopic levels; (d) Compatibility with long term culture; (e) Possibility to formulate gels to match cell type; (f) Optical transparency for straightforward analysis of results.[5] Taking it a step further, cell-hydrogel constructs should be allowed to be transplanted into living systems to support in vivo cell culture. Although in vitro studies permit a simple, convenient, and detailed cell analysis, it is still challenging to extrapolate from the results of in vitro work back to the biology of the intact organism. Therefore, in vivo experiments are needed to give more information of what is really going inside living systems.

In the early stage of the development of hydrogels, the majority of hydrogel networks are formed through covalently cross-linked polymers, including poly(ethylene glycol),[6] poly(vinyl alcohol),[7] polyacrylamide,[8] and chitosan.[9] Although these chemically cross-linked hydrogels are cheaply available, the safety profile from long-term use is still unclear because of trace harmful reagents (e.g., initiator, some unidentified enzymes in the natural polymers) or catalysts, which limits their applications in the field of regenerative biology.[10]

Distinct from chemically cross-linked hydrogels, supramolecular hydrogels can be formed through non-covalent interactions between hydrogelators, such as hydrogen bonds, π–π stacking, hydrophobic, electrostatic, or van der Waals interactions, thereby no requiring additional cross-linking reagents.[11,12] These weak non-covalent interactions not only render supramolecular hydrogels susceptible to degradation, but also allow the pore size of network to respond to the mechanical force exerted by cells as they migrate through matrix.[13] Typically, the ability to respond to environmental stimuli for hydrogelation, including ions, pH, light, solvent and enzyme, facilitates in situ encapsulation of cells into truly 3D supporting hydrogel networks. The tunable bioactive behaviors by synthetic customization of gelators also ensure these hydrogels to be attractive for culturing different cell lines in 3D.

For supramolecular hydrogels, the building blocks can be either polymeric or small molecular gelators. Supramolecular polymeric hydrogels are assembled from intermolecular non-covalent interactions between polymeric gelators, such

Dr. X.-Q. Dou,[+] Prof. C.-L. Feng
State Key Laboratory of Metal Matrix Composites
School of Materials Science and Engineering
Shanghai Jiaotong University
800 Dongchuan Road., 200240 Shanghai, China
E-mail: clfeng@sjtu.edu.cn

[+]Current address: Physical Chemistry I, Department of Chemistry and Biology, University of Siegen, Adolf-Reichwein-Str. 2, 57076, Siegen, Germany

DOI: 10.1002/adma.201604062

as hydrogen bonding, metal coordination, and host-guest interactions and their potential applications in biological and electronic fields have been reported.[14] However, for polymeric gelators, the frequency and content of the functional groups along the backbone can vary from one chain to another one, and the groups may not be evenly spaced due to differences in monomer reactivity.[15] In contrast, the formation of small molecular hydrogels likely eliminates this uncertainty since the self-assembly of small molecular gelators usually results in ordered structures consisting of well-defined or regular repeats.[15] For small molecular hydrogelators, several systems have been widely reported, including cholesterol derivatives,[16] cyclodextrins,[17] imidazole derivatives,[18] urea,[19] sugar,[20] amino acids,[21] and peptide-based gelators.[22] Among these, naturally occurring amino acids and peptide-based hydrogelators[23] are the most promising candidates to support 3D cells growth due to their good biocompatibility, manipulated bioactivity, excellent gelation ability, and versatile synthetic pathway.[24] Moreover, the structural and functional properties of these hydrogelators can be modulated by varying different amino acid types and sequences, rendering them to formulate bioactive hydrogels that can mimic the structure and function of native ECM. The comparisons among conventional polymeric hydrogels, supramolecular polymeric hydrogels, and small molecular hydrogel have been summarized in **Table 1**. Herein, the current progress in constructing amino acids and peptides based supramolecular hydrogels under various kinds of external stimuli will be reviewed (**Figure 1**), with a focus on understanding the associations among the structure, properties, and function of these materials and an outlook of these hydrogels as robust 3D networks used in the field of regenerative medicine, tissue engineering, and pre-clinical evaluation.

2. Physical Interactions between Supramolecular Gelators During Self-Assembly

The self-assembly of supramolecular gelators into hydrogels is mainly driven by non-covalent interactions,[25] which are usually weaker than those of covalent bond interactions, enabling their smart responses to external stimuli. Amino acids and peptide derivatives are a class of supramolecular gelators, and intermolecular hydrogen bonds between amide bonds (—CONH—) are the main driving forces for their gelation.[26] These strong and highly directional amide hydrogen bonds are beneficial for an efficient self-assembly of hydrogelators in aqueous solution.[27] Except for amide bonds, some other functional groups (**Table 3**) are additionally introduced into the gelator structure to provide multiple hydrogen bonds for enhanced gelation ability.[28] In addition, multiple hydrogen bonding in supramolecular polymeric hydrogels can be obtained by ureidopyrimidinone (UPy) to further strengthen assemblies through quadruple hydrogen bonds.[29] Although this moiety is rarely used in amino acids and peptide-based hydrogels, it is considered as an effective group to form hydrogen bonding in the future of gelator design.

In general, amino acids and peptide-derived gelators also contain hydrophobic groups, which can provide not only

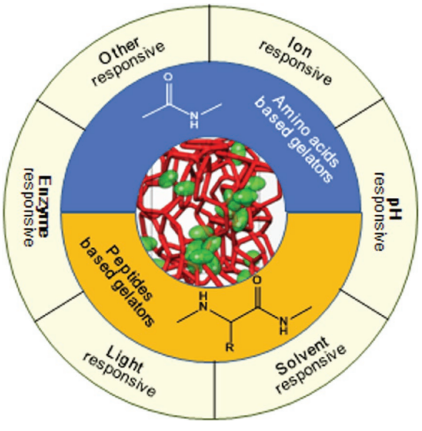

Figure 1. Explorations of 3D cell culture in amino acids and peptides based supramolecular hydrogels prepared under different external stimuli, including ions, pH, light, solvent, enzyme, and other stimuli and the advantages/disadvantages of the stimulus are shown in **Table 2**. Reproduced with permission.[63a] Copyright 2014, Wiley-VCH.

Xiao-Qiu Dou received her BSc degree in Polymeric Materials and Engineering from Ocean University of China in 2009 and her PhD degree in Materials Science and Engineering from Shanghai Jiaotong University in 2015. Since 2016, she has been a postdoctoral fellow in the Department of Chemistry and Biology at University of Siegen (Siegen, Germany), and was awarded the Humboldt Research Fellowship for postdoctoral researchers. Her research interests include development of stimuli-responsive supramolecular hydrogels for application in biomedical and biotechnology fields.

Chuanliang Feng received his doctorate from University of Twente (the Netherlands) in 2005, then he worked at the Max-Planck Institute for Polymer Research as a postdoctoral researcher (Mainz, Germany). From 1998 to 2009, he was a research scientist at Biomade Technology Foundation (Groningen, the Netherlands). In 2009, he was appointed as a full professor in the School of Material Sciences and Technology, Shanghai Jiaotong University. His research mainly focuses on functionalized polymeric nanomaterials, bioadhesion materials, and supramolecular hydrogels. Important topics are synthesis and characterization of stimuli-sensitive polymers and biomimetic materials as well as applications of biomaterials in regenerative medicine.

Table 1. Comparisons among conventional polymeric hydrogels, supramolecular polymeric hydrogels, and small molecular hydrogel.

Hydrogel category	Gelators	Crosslinking category	Advantages	Disadvantages	References
Conventional polymeric hydrogel	Polymers	Chemical crosslinking	Stable; cheaply available	Weak response to stimuli; trace harmful reagents	[6–10]
Supramolecular polymeric hydrogel	Polymers	Physical crosslinking	Response to stimuli; reversible	Inhomogeneity of polymer chain;	[14,15]
Small molecular hydrogel	Small molecules	Physical crosslinking	Response to stimuli; reversible; ordered structures; controllable assembly; easy modification	Weak stability	[15–24]

Table 2. The different stimuli used for gelation, relative gelators or functional groups, and the advantages/disadvantages of the stimulus.

Stimulus	Relative gelators or functional groups	Advantages	Disadvantages	Section
Ion or pH	EAK16-II, RAD16-I, RAD16-II, KLD12, MAX1I4, MAX1I4′, MAX1I8, P_{11}-13, P_{11}-14, C_{16}-$V_3A_3E_3$(COOH), $K(SL)_3RG(SL)_3$KGRGDS, Ac-QQKFQFQFEQQ-Am, FEFEFKFK, h9e	Responsive to ionic strength and solution pH	Challenge to gelate culture medium with many kinds of salts.	3.1
pH	COOH, NH_2	Responsive to solution pH	Undesirable low pH of pre-gel solution affecting cell viability.	3.2
Solvent	No specific group required	Gelating molecules with poor aqueous solubility	Hard to make homogeneous hydrogels	3.3
Light	—C=C—, nitrobenzy group, tet, azo	Easy or convenient operation	Toxicity of UV light	3.4
Enzyme	Depends on the kind of enzyme	Possibility for in vivo 3D cell culture	Some enzyme reactions needing a certain time.	3.5
Chemical	Disulfide bonds (cleaved by glutathione), $_D$-Ala-$_D$-Ala group (bind to vancomycin)	Hydrogel properties can be tuned by chemicals	Limited to biocompatible chemicals	3.6.1
Mechanical stimulus	No specific group required	No chemical stimulus	Weak hydrogels	3.6.3

hydrophobic interactions within gelators, but also synergistic effect on hydrogen bonds.[30] For example, Van Esch et al. coupled amino acids (with hydrophobic side chains) to a hydrophobic cyclohexane core to make a class of hydrogelators with good self-assembled ability (**Figure 2**). The hydrophobic side groups can shield the amides from water to form hydrogen bonds and hydrophobic central cores may introduce hydrophobic interactions as an additional aggregation force for self-assembly, ensuring the formation of one dimensional (1D) intermolecularly hydrogen-bonded stacks in a solvent.[31]

Table 3. Typical physical interactions in building amino acids and peptides based hydrogels. Selected references are provided.

Physical interaction category	Some specific groups	References
Hydrogen bonds	Amide bonds	[5]
	Carboxylic acid	[28a]
	Hydroxyl	[67]
	Pyridine	[68]
	Urea	[28b]
	Upy	[29]
	Nucleobase paring	[28c]
π–π Stacking	Fluorenyl	[56]
	Naphthyl	[77]
	Pyrenyl	[94]
	Phenyl	[64]
Hydrophobic interaction	Long chain alkyl	[49]
	Cyclohexane	[31]
	Benzene ring	[62]
Electrostatic (ionic) interaction	Glutamic acid and lysine	[37]
	Aspartic acid and arginine	[32]
	Aspartic acid and lysine	[42]
	lysine and PO_4^{3-}	[46]
Biorecognition: domain+ligand	Peptide + Ligand	[90]

Ionic effect and π–π stacking also play significant roles on the hydrogelation of amino acids and peptides derived gelators. RADARADARADARADA ($RADA_4$) are ionic self-complementary oligopeptides containing oppositely charged amino acids (positively charged arginine (R) and negatively charged aspartic acid (D)) and they are generally considered as effective gelators to form hydrogels through electrostatic interactions.[32] The ions have influence on the electrostatic interactions through direct participation in binding events, Debye-Huckel screening effects, or change of water activity.[33] The π–π interaction between amino acids and peptides derived gelators is another main driving force,[34] e.g., the interactions between aromatic rings in aromatic dipeptides based hydrogelators.[35] In addition, gelator-solvent interactions also play a crucial role on the self-assembly of these types of gelators. The addition of osmolytes into solution can vary equilibrium conditions due to their effect on bulk water activity and further affect the formation of hydrated self-assembling interface.[20] In sum, a number of factors those synergetically regulate gelator-gelator and gelator-water interactions are involved during the self-assembly of amino acids and peptides derived gelators, which should be taken into consideration in order to design these gelators.

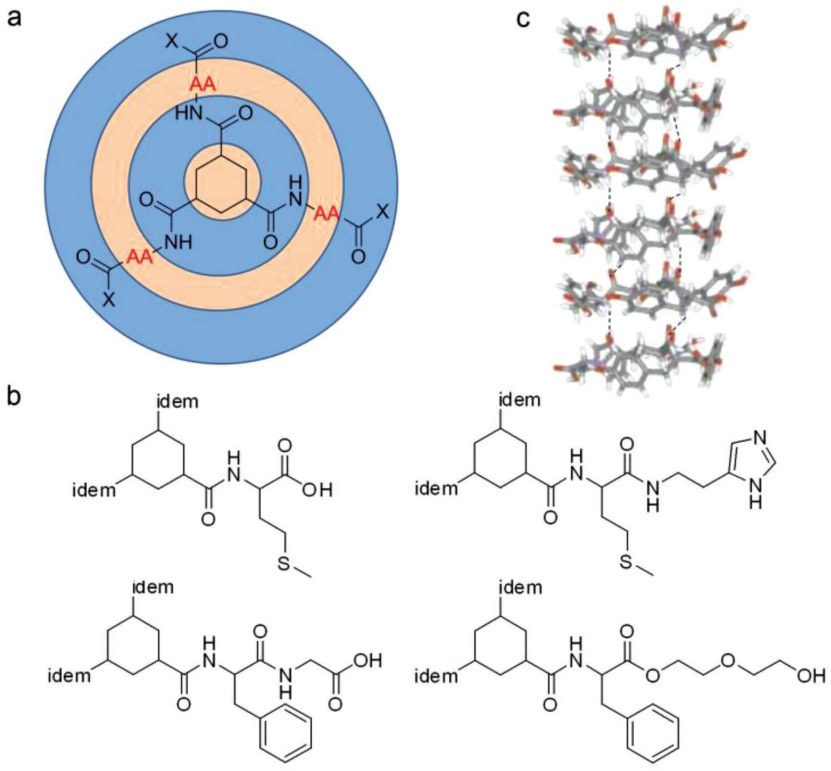

Figure 2. a) Schematic representation of cyclohexane-based hydrogelator design. Pink regions = hydrophobic; blue regions = hydrophilic; AA = amino acid (s); X = hydrophilic substituent. b) The chemical structures of the cyclohexane-based hydrogelators. idem: all the gelators have three identical side chains, for simplicity only one is shown for each getator. c) Side view of a single stack, showing the intermolecular triple hydrogen bonding chain. Reproduced with permission.[31] Copyright 2004, Wiley-VCH.

3. Supramolecular Hydrogel Construction under External Stimuli for 3D Cell Culture

Since the formation of amino acids and peptide-based supramolecular hydrogels are mainly driven by physical interactions, it allows adjustment of the 3D self-supporting matrix under external stimuli, facilitating in situ cell encapsulation into the 3D matrix in a controllable way. It may also allow the proliferated cells to be harvested from an induced solution state once hydrogels reversibly switch to sol under stimuli. Though some stimuli (such as pH and light) may have influence on cell viability, the adverse effect can be minimized through control over gelation time upon exposure to the triggers.

3.1. Self-Assembly of Ionic Peptides

In the 1990s, Zhang et al. reported peptide-based gelators with 16 amino acids, EAK16-II (Ac-NH-AEAEAKA-KAEAEAKAK-$CONH_2$), which adopted a β-sheet configuration by self-complementary ionic interactions between positively charged lysines and negatively charged glutamic acids, together with hydrophobic interactions of alanines to form the ordered nanofibers at neutral pH.[36] Because the p*K*a values of the glutamic acid and lysine are 4.25 and 10.53, respectively, the carboxylic acid group would be negatively charged and the amino groups positively charged under pH 7. Self-assembly of the peptide occurs when the solution pH is such that the net charge of peptide molecules is near zero. Except for pH, such self-assembly is affected by ionic strength. The counter-ions and charged residues can decrease the charge distribution in aqueous solution, which further influences the self-assembly. For example, it was found that the equivalent radius of self-assembled EAK16-II fibrils changed with varying NaCl concentration. Below critical NaCl concentration, the radius of self-assembled fibrils could increase with increasing salt concentration, and above which, the opposite response was observed.[37] Since then, similar peptides were designed to make 3D nanofibrous scaffolds capable of supporting cell growth, such as RAD16-I (AcNRADARAD-ARADARADA-CNH_2) and RAD16-II (AcNRARADADARA-RADADA-CNH_2) oligopeptide with positively and negatively charged residues. Both could self-assemble into hydrogels in physiological solution (**Figure 3**a–c).[38] The stiffness of the hydrogel could be tuned by altering concentration of gelators. Human umbilical vein endothelial cells (HUVECs) suspended in ionic solution were implanted in the hydrogels. The tunable stiffness of the hydrogels can direct the differentiated cellular behavior, which is useful for vascular networks engineering in 3D.[39] RAD16-I hydrogel was also investigated as matrix scaffold that allowed ovarian cancer cells to reside in a 3D microenvironment similar to cellular niches in vivo, and was found to promote 3D cell adhesion and migration.[40] Moreover, cells in such hydrogels could maintain their cell functions after culture. Chondrocytes in KLD12 (AcKLDLKLDLK-LDL-NH_2) hydrogels not only maintained their phenotype, but also produced abundant type Π collagen and glycosaminoglycan (Figure 3d).[41] While, chondrocytes would dedifferentiate into fibroblast on 2D scaffolds and no longer produce type Π collagen and glycosaminoglycan any more. This further addressed the importance of cells culture in 3D hydrogels in tissue engineering.

In another study, Gough and Miller reported the encapsulation of chondrocytes both in vitro and in vivo into a self-assembled FEFEFKFK octapeptide hydrogel.[42] In FEFEFKFK peptide, glutamic acid and lysine were used to provide negatively and positively charged side groups, respectively. For 3D cell culture, culture medium with chondrocytes and NaOH was added in the peptide solution and further neutralize solution pH (net charge: 0) to allow gelation. On day 25, the in vitro cultured cells actively produced collagen type Π in the hydrogel matrix and a little collagen type I also deposited in some areas. It suggested that chondrocytes in 3D culture can produce both collagen type I and Π during the early stages of ECM production. This octapeptide hydrogel with the embedded chondrocytes can also be injected to faciliate cartliage repair. In addition, the

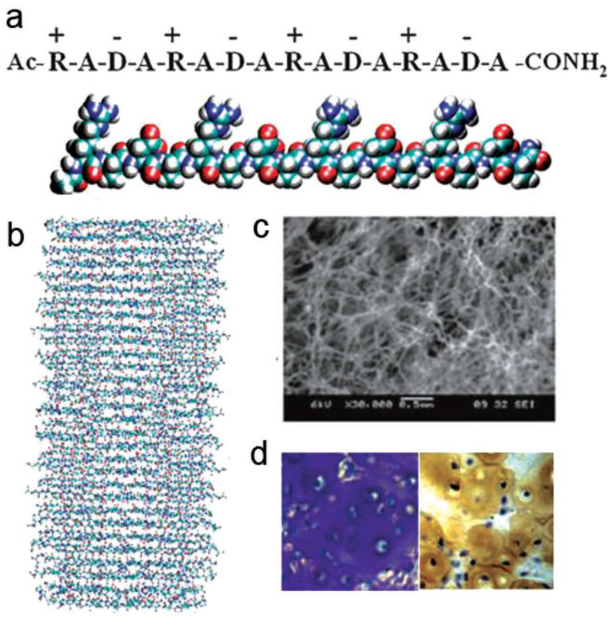

Figure 3. (a) Amino acid sequence and molecular model of RAD16-I. (b) Hundreds of individual peptides self-assembly into a nanofiber. (c) SEM image of RADA16-I nanofibrous scaffold. Reproduced with permission.[38a] Copyright 2009, Elsevier. (d) Chondrocytes in the KLD12 peptide hydrogels. The chondrocytes stained with tolustaineidine blue (TB) showing abundant glycosaminoglycan production (left panel) and type Π collagen production (right panel). Reproduced with permission.[41] Copyright 2002, National Academy of Sciences.

hydrogels from other octapeptides (such as VEVKVEVK and FEFKFEFK) also have potential to be used in 3D cell culture.[43]

Xu and his colleagues synthesized three peptides based on the well-studied self-assembling peptide MAX1 (VKVKVKVKVDPPTKVKVKVKV-NH2) by partly or completely replacing hydrophobic valine residues with isoleucines, i.e., MAX1I4 (IKIKVKVKV^{D}PPTKVKVKIKI-NH_2), MAX1I4′ (VKVKIKIKV^{D}PPTKIKIKVKV-NH_2), MAX1I8 (IKIKIKIKV^{D}-PPTKIKIKIKI-NH_2) (**Figure 4**a).[44] These peptides were unfolded in low ionic strength buffer due to electrostatic repulsion between the protonated lysine side chains. The lysine-based charge interaction can be alleviated via charge screening with salts at physiological pH (7.4), thus, allowing the peptide to undergo intramolecular folding into an amphiphilic β-hairpin with all valine residues on one face of the hairpin and lysine residues on the other. Once folded, the peptide molecules subsequently self-assembled into a fibrillar gel network, driven by lateral β-sheet hydrogen bonds, side chain-side chain hydrophobic contacts, and facial hydrophobic collapse. In order to encapsulate cells into the hydrogels, the peptides were dissolved in 2-[4-(2-hydroxyethyl)piperazin-1-yl] ethane sulfonic acid (HEPES) buffer at first. To initiate peptide structural folding, Dulbecco's modified Eagle medium (DMEM) (salt content: ≈165 mM) containing cells was added and cells could be in situ encapsulated in the hydrogels with the gelation of hairpin peptides. Figure 4b showed cell distributions in MAX1 and MAX1I8 solutions at 5 and 30 min after their hydrogelation were triggered by adding DMEM containing the prestained NIH3T3 cells. NIH3T3 cells were almost homogeneously distributed in two solutions after 5 min. Cell distribution did not change in MAX1I8 after 30 min, however, most of cells were deposited to the bottom of the field in MAX1. This can be interpreted as the difference in their hydrogelation kinetics and the lack of sufficient mechanical strength to sustain the bodies within 3D gel network for MAX1, leading to the apparent cell precipitation. Therefore, how to further improve the mechanical property and stability of hydrogel is a key factor for 3D cell culture.

Schneider's group used similar amino acids (V and K) to design peptide based gelators, such as MAX1 (VKVKVKVKV^{D}-P^{L}PTKVKVKVKV-NH_2) and MAX8 (VKVKVKVKV^{D}P^{L}PTKVEVKVKV-NH_2).[45] Because of electrostatic repulsion between positively charged lysine residues, these peptides remain unfolded in low ionic strength solution at pH 7.4. However, the folding can be triggered by screening some of lysine-based charge with adding DMEM since it contains sufficient concentrations (≈160 mM) of mono- and divalent inorganic salts to ensure effective screening. In the folded state, these peptide with hairpin conformation self-assemble both via the formation of intermolecular hydrogen bonds and van der Waals contacts and via the burial of the hydrophobic face of distinct hairpins. Therefore, the gelation of MAX1 and MAX8 can be triggered in presence of DMEM containing C3H10t1/2 mesenchymal stem cells, resulting in self-supporting and mechanically rigid gels with the impregnated cells. The resulting cell-gel constructs can be delivered via syringe to the target sites with little effect on

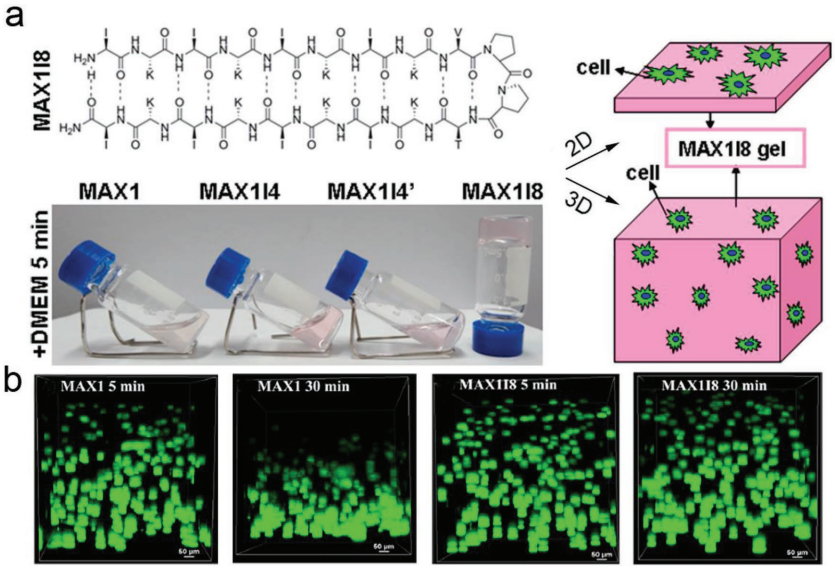

Figure 4. a) Chemical structure of MAX1I8. The peptides can self-assemble into hydrogel in the presence of ions. (b) 3D confocal microscopic images showing the cell distributions within MAX1 and MAX1I8 at room temperature with time: 5 min and 30 min. Scale bar = 50 µm. The width, height, and depth of the images fields were 1.27, 1.27, and 1.27 mm, respectively. Reproduced with permission.[44] Copyright 2014, American Chemical Society.

homogenous distribution of the cells. Cells can remain viable during the encapsulation and injection, suggesting that the designed gels are useful for delivering cells to target biological sites in tissue-regeneration efforts. Hartgerink's group utilized $K(SL)_3RG(SL)_3KGRGDS$ peptide to do the similar work on injectable hydrogels.[46] After addition of polyvalent anions (e.g., PO_4^{3-}), terminal lysine residues can have ionic interaction with PO_4^{3-} to crosslink the matrix into a hydrogel. Given the reversible nature of non-covalent bonds, the hydrogels have the merits of shear thinning and recovery, allowing the delivery of inflammatory chemokine on the injury site, such as monocyte chemoattractant protein-1 (MCP-1) and recruitment of monocytes/macrophages. This injectable cytokine loaded hydrogels are promising for use in a variety of tissue engineering applications.

Similarly, $K(SL)_3RG(SL)_3KGRGDS$ peptide can be dissolved in deionized water with sucrose and the charge of lysine-containing peptides may be screened by negatively charged heparin to form a hydrogel with the encapsulated dental pulp stem cells (DPSCs) and growth factors, e.g., vascular endothelial growth factor (VEGF).[47] **Figure 5**a displays DPSC cell in 3D after in vitro seeding into hydrogels. The cells appear elongated with stretched-out shapes and in contact with neighboring cells. The

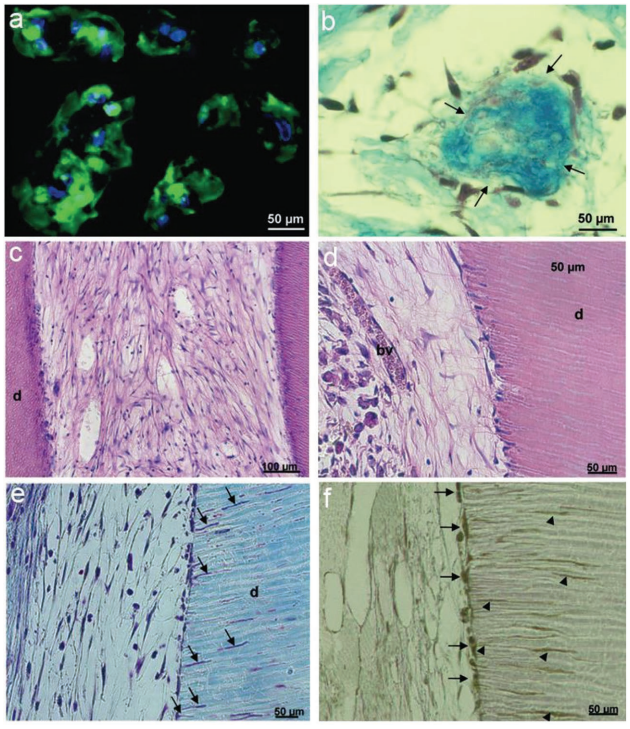

Figure 5. DPSC morphology in $K(SL)_3RG(SL)_3KGRGDS$ peptide based 3D hydrogels. (a) Confocal microscopy shows that cells are stretched out and display cell-cell contacts. (b) Histologic Masson's trichrome stain reveals collagen deposition. (c) After 5 weeks of in vivo transplantation, DPSC have formed a pulp-like soft connective tissue. (d) Higher magnification shows blood vessels (bv) and a cell layer in intimate association with the dentin wall. (e) The cells extend processes into the dentinal tubules (arrows), a characteristic of odontoblast cell morphology. (f) The cell layer adjacent to the dentin as well as the cellular processes stain positive for dentin sialoprotein. d: dentin. Reproduced with permission.[47] Copyright 2012, Mary Ann Liebert, Inc.

cell clusters in the hydrogel can produce collagen as their own ECM to replace the synthetic carrier (Figure 5b). Further histologic analysis of in vivo transplantation shows that DPSC in the peptide hydrogels with growth factors (GFs) can form a vascularized soft connective tissue as dental pulp (Figure 5c–f). The cells can further degrade the hydrogel and replace it by a collagenous ECM (Figure 5d and e). Cells at the cell-dentin interface appear flat and have an intimate association with the dentin wall, where cellular processes extend into the dentinal tubules. (Figure 5e). This custom-made, bioactive ECM at hand is able to support the formation of a vascularized soft connective tissue similar to dental pulp after transplantation in vivo. Owning to the striking resemblance between hydrogels and soft tissues, this supramolecular hydrogel is a promising material for dental treatment.

Likewise, Aggeli's group reported a binary complementary peptide, P_{11}-13 (CH_3CO-Glu-Gln-Gln-Phe-Glu-Trp-Glu-Phe-Glu-Glu-Glu-NH_2) and P_{11}-14 (CH_3CO-Gln-Gln-Orn-Phe-Orn-Trp-Orn-Phe-Orn-Gln-Gln-NH_2). Both of them have negative and positive charges under physiological conditions (NaCl, pH 7.4), respectively.[48] Upon mixing of equal quantities of P_{11}-13 and P_{11}-14, a 3D self-supporting hydrogel was instantaneously formed in culture medium due to the complementary ionic bonding between positively and negatively charged residues of the peptides. With culturing human dermal fibroblasts, they remained viable and proliferated over 28 days in P_{11}-14 hydrogels, whereas P_{11}-13/P_{11}-14 hydrogels did not support proliferation of this cell type. This highlighted the differences on cell behaviors from relatively modest changes in molecular design.

Another classical type of peptide-based hydrogel developed by Stupp's laboratory is peptide amphiphiles (PAs) containing both a long alkyl tail (e.g., C_{16} alkyl tail) and a peptide sequence (e.g., $V_3A_3E_3(COOH)$).[49] These specific PAs could self-assemble into nanofibers as a result of their β-sheet peptide domains triggered by salts. The strings of aligned PAs nanofibers could be used to direct the orientation of human mesenchymal stem cells (hMSCs) in 3D environments by dispersing the cells into PAs solutions and then dragging the solutions onto salty media (NaCl and $CaCl_2$) to form noodle-shaped strings with encapsulated cells.[50] In addition, Luo et al. prepared an enantiomer chiral self-assembling peptides d-EAK16 and l-EAK16.[51] By adding culture medium (containing Na^+, K^+...) into EAK16/cells mixture, it could induce chiral peptides to self-assemble into 3D hydrogels. It was found that D-form peptides had many advantages over L-form peptides including their resistance to protease degradation and supporting cell growth with longer time.

Recently, it has been found that long peptide h9e (FLIVIGSIIGPGGDGPGGD)-based gelators could form hydrogel on addition of cell culture medium and Ca^{2+}.[52] They can promote the hydrophobic stacked interaction by capturing the charged Asp residues and further enhance the interfiber interactions for the assembled hydrogel.[53] It provided a convenient and mild hydrogel forming process and allowed cells to be surrounded by their culture medium during cell encapsulation.

In addition, microgel from Ac-QQKFQFQFEQQ-Am (Q11) was also prepared by triggering pepetide self-assembly in

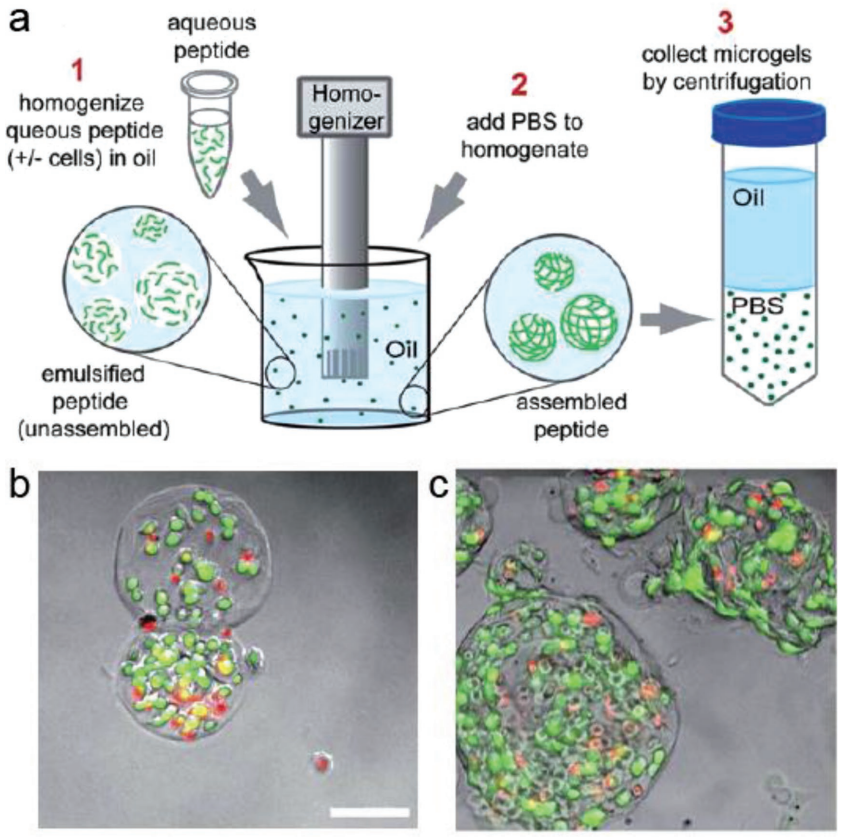

Figure 6. (a) Schematic for microgel fabrication. A solution of Q11 peptides with or without additional cells was added to mineral oil. After fomation of a water-in-oil emulsion, a small volume of PBS buffer was added, gelling the peptide in the aqueous. Finally, the microgels were extraced in excess PBS and collected by centifugation. (b) The viability of NIH3T3 cells encapsulated in Q11 microgels for 1 day (left) and 3 days (right). Both images were under the same magnification. Scale bar = 100 µm. Reproduced with permission.[54] Copyright 2011, Royal Society of Chemistry.

the aqueous phase of water-in-oil emulsions by adjusting salt strength (**Figure 6**a).[54] The formed microgel could be embedded into other self-assembled peptide matrices for generating composites of different peptide formulations. The microgel fabrication process was appropriate for cell encapsulation. NIH3T3 and C3H10T$^{1}/_{2}$ murine embryonic pluripotent stem cells have been successfully encapsulated and cultured with good viability in microgels (Figure 6b). This offers a simple route for producing spherical gels of self-assembled peptides by only using buffer addition as a trigger, making it potentially useful for constructing cell matrix with micrometer-scale dimensional control.

3.2. pH Responsive Hydrogelators

Amino acids and peptide-derived hydrogelators containing carboxyl (-COOH) or amine (-NH_2) groups are a class of pH-responsive gelators. This pH-responsive behavior is essentially attributed to the protonation/deprotonation of NH_2/COOH groups, resulting in switching between hydrogel and solution phase.[55] Various peptides and amino acid-based gelators which can form hydrogels at physiological pH (about 7.4) have been designed and prepared for 3D cell culture. It's worth noting that the ionic complementary peptides in above section are also pH responsive gelators since the charge of amino acids can be adjusted by solution pH. Amino acids will be negatively charged if pH>pK_a and positively charged if pH<pK_a. Then, self-assembly could achieved through the electrostatic interaction between charged groups (see section 3.1).

Ulijn and co-workers reported aromatic short dipeptides based on 9-fluorenyl-methoxy-carbonyl (Fmoc) (**Figure 7**a, Fmoc-FF and Fmoc-RGD).[5,56] Powders of Fmoc-peptides were dissolved in alkali (pH = 10.0 for Fmoc-FF) or acid solutions (pH = 3.0 for Fmoc-RGD) and then neutralized to pH 7.0 to make bioactive 3D hydrogels through hydrogen bonds and π–π interactions. Cells could be in situ encapuslated inside the hydrogels by mixing Fmoc-peptide solution and cell suspension (Figure 7b). The hydrogel was found to promote aggregation of the encapsulated dermal fibroblasts through RGD integrin binding (Figure 7c–e).

Tirrell et al. designed pH-responsive branched PAs composed of histidine and serine amino acids.[57] The pH-tunable PA-based scaffold could be switched between a liquid and a stable gel within physiological pH. Cells could be cultured on the top of hydrogels at physiological conditions by washing the gels with phosphate buffered saline (PBS). After 96 h, the cells grew on and into the pores of the scaffold. Similarly, Chauhan and co-workers designed the dipeptide (Phe-Δphe) containing a free amino and carboxyl group at the N- and C- termini, respectively.[58] Phe-ΔPhe could form a self-supporting hydrogel at neutral pH (pH = 7.0) but not at acidic (pH = 2.0) or alkaline (pH = 10.0) conditions. The macroscopic gel matrix was composed of a highly dense network of fibers (15–20 nm in diameter, micrometers in length). It was found that cells seeded on the top of gel could invade and migrate into three (*X*, *Y*, *Z*) planes, resulting in a 3D growth.[59]

Hartley et al. reported the gelation of a hydrophobic tripeptide (^{D}Leu-Phe-Phe) under physiological conditions (pH = 7.4).[60] Peptide ^{D}Leu-Phe-Phe was firstly dissolved in a sodium phosphate solution at pH 11.8 and gelation could be triggered by subsequent addition of an equal volume of sodium phosphate solution at pH 5.7 until final pH up to 7.4. The cultured cells on the hydrogels could infiltrate into the gel with time and showed 3D cell growth behaviors. Although the depth of cell migration is very limited compared to directly seeding cells in the hydrogels, this method is more convenient and provides a new insight to construct a 3D cell culture environment. In addition, the approach to incorporate soluble antibiotic ciprofloxacin (CIP) into these hydrogels through co-assembly

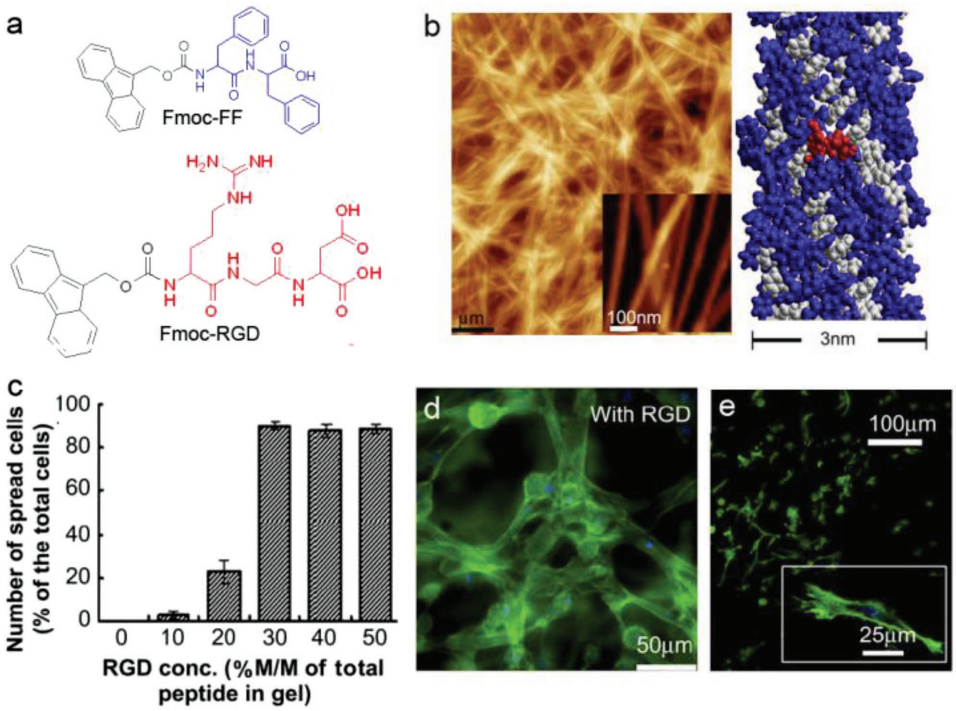

Figure 7. a) The chemical structures of the Fmoc-FF and Fmoc-RGD. b) Left panel: the AFM height image of the hydrogel showed an overlapping mesh of nanofibers, with bundles and entanglements; right panel: RGD sequences are presented on the fiber surface. c) The Fmoc-RGD concentration influenced cell spreading: in the hydrogel with 30–50% Fmoc-RGD incorporated, adequate cell spreading occurred with over 90% spread cells. d) Cell adhesion and morphology in the Fmoc-FF/RGD hydrogels: human adult dermal fibroblast (HDFa) were well spread. e) Integrin blocking experiment proved that direct interaction of the cells with RGD: cells with unblocked $\alpha 5\beta 1$ integrins were able to spread and directly attach to the RGD sites on the nanofibers. Reproduced with permission.[56a] Copyright 2009, Elsevier.

may also have important applications in the field of wound dressings and novel antimicrobial formulations.

3.3. Solvent Responsive Hydrogelators

Some hydrogelators are difficult to directly self-assemble in aqueous solution due to their poor aqueous solubility and they usually form precipitates instead of a hydrogel as a result of change in ionic strength, pH, or temperature. One approach is to dissolve them in a polar organic solvent and then mix this solution with water to form hydrogels.[61] Although a small amount of organic solvent brought into the hydrogels may change biocompatibility of resulting hydrogels, well controlled concentration and kind of organic solvent can preserve viability and function of encapsulated cells.

Varying the polarity of solvent itself to induce hydrogelation is the main method in Feng's group. They designed a novel family of effective amino acid derived hydrogelators based on a C_2-symmetric 1,4-diamide cyclohexane or benzene core.[62] These gelators could be well-distributed in aqueous solution and no trapped aggregates formed because of their typical symmetry property for molecular structures, different from the traditional amphiphilic supramolecular gelators. The gelation of C_2 gelators could be controlled by tuning the polarity of solvent. Cells may be encapsulated into the hydrogels by mixing DMEM and cells with adding the concentrated C_2 gelator dimethylsulfoxide (DMSO) solution (final DMSO concentration: 2.5–3.3%). DMSO was chosen as polar organic solvent to dissolve gelators because it is miscible with water and usually used in cell freezing culture medium.[61] Herein, DMSO concentration in the hydrogel is only 2.5–3.3% and much lower than that in cell freezing medium (10%). By this solvent-mediated method, distributions of cells in 3D environment were easily obtained within several minutes (**Figure 8**a). Further data demonstrated that the cells in such hydrogels containing small amount of DMSO had high cell viability and proliferation.

More than this, the chiral twist with right-handed and left-handed enantiomers was also obtained by incorporating chiral center into C_2 based hydrogelators.[63] By the method above, well-controlled chiral nanofibers could be obtained (Figure 8b). It is known that chirality is one of the most distinctive signatures of life and has great influence on many biological events.[64] These chiral nanofibrous hydrogels enable insight into nanofibrous chirality influences on cell behaviors in 3D ECM. Based on this, cells were in situ encapsulated inside different chiral hydrogels in one step and it was found that left-handed helical nanofibers (LPH) could increase cell adhesion and proliferation in 3D hydrogels, whereas, right-handed nanofibers (DPH) had the opposite effect (Figure 7c).

Further study demonstrated that the chirality of nanofibers may be recognized by the cells through stereospecific interaction between the chiral fibers and proteins in 3D, which may release different signals to the cells and result in different cell/substrate interaction. The stereospecific interaction

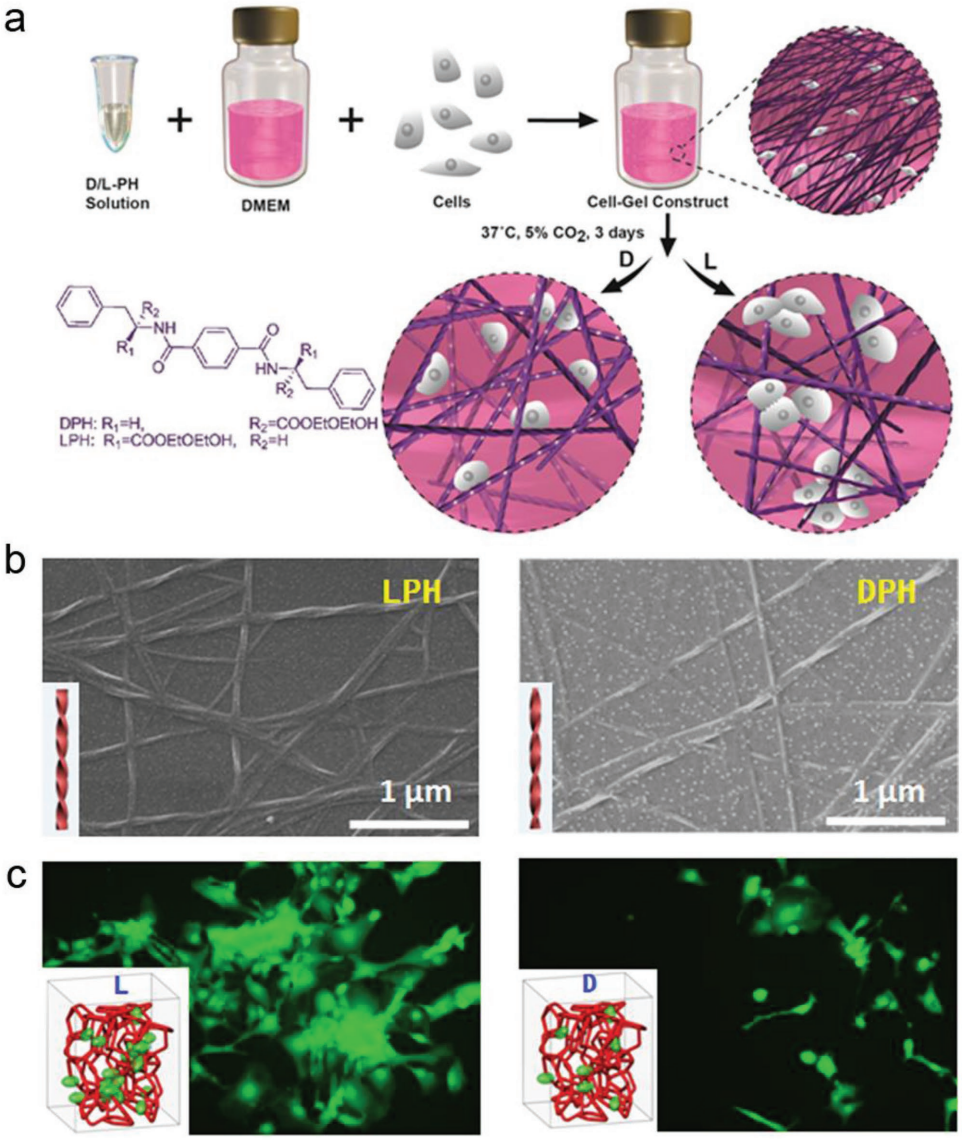

Figure 8. a) Schematic demonstration of NIH 3T3 cells cultured in supramolecular hydrogels and different cell adhesion and proliferation behaviors in enantiomeric nanofibrous hydrogels. *d* represents right-handed helical nanofibers and *l* represents left-handed helical nanofibers. Molecular structures for gelator enantiomers are inset. b) SEM images of *d*-PH xerogels with right-handed helical nanofibers and *l*-PH xerogels with left-handed helical nanofibers, respectively. c) Fluorescence microscope images of NIH 3T3 cells in *d*-PH and *l*-PH hydrogels after incubation for 3 days. Scale bar represents 50 μm. Insert: schematic demonstration of different cell adhesion behaviors in enantiomeric nanofibrous hydrogels. Reproduced with permission.[63a] Copyright 2014, Wiley-VCH.

between the cells and the chiral nanofibers is not a unique but a common effect that should be applicable to different cell types. Cells prefer to have strong interaction with left-handed nanofibers through the stereospecific recognition between cells and nanofibers. It is thus considered that the design of enantiomorphous environments may bring a novel direction for the design of biomaterials, which is complementary to the existing strategies to control cell growth density for tissue engineering. The results provided a new perspective for the influence of nanofiber chirality on cell adhesion and proliferation in 3D environments.

In addition, the inherent symmetry property of C_2 gelators allows the functions of self-assembly fibers to be tuned by introducing different groups into gelator structures. Biotin molecules were chemically coupled to C-terminal carboxyl to synthesize a new gelator BG (**Figure 9**a), which could form biotin modified 3D matrix through co-assembly with C_2 based gelator G.[65] Avidin modified cells (Figure 9b) could then interact with hydrogel scaffolds through specific biotin-avidin interactions (Figure 9c), resulting in the enhanced cell adhesion and proliferation in 3D (Figure 8d). Because of the feasibility to modify different types of cells by avidin, this biotin-avidin based interaction should be not a unique but a common drive to promote cell adhesion and proliferation for most types of cells. Thus, the study not only develops a universal methodology for encouraging cell culture in a 3D matrix, but also paves

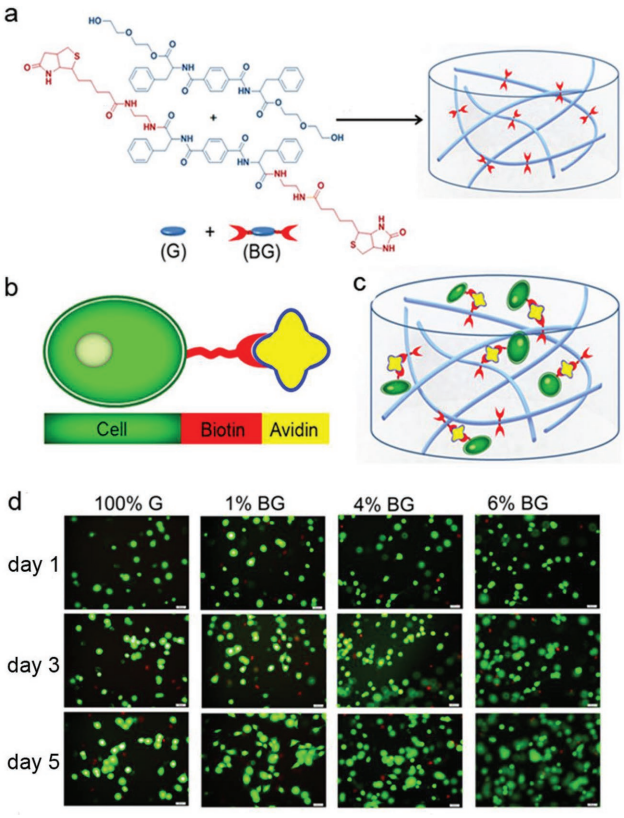

Figure 9. a) Left: Molecular structures of non-biotinylated (G) and biotinylated-gelators (BG). Right: the co-assembled biotinylated 3D networks from G and BG. b) Schematic demonstration of the avidin modified cell. c) The enhanced cell adhesion in 3D through the specific avidin-biotin interaction between the avidin modified cells and nanofibers. d) Fluorescent images of AV-MC3T3 in G and GBG hydrogels, respectively. Green staining indicates live cells and red staining indicates dead cells. Scale bars represent 20 µm. The adhesion densities of cells were increased with incorporating more biotin in the hydrogels. Reproduced with permission.[65] Copyright 2015, American Chemical Society.

a new way to functionalize 3D matrix for avoiding cell anoikis during cell culture, which may find broad applications in the fields of tissue engineering, e.g., tissue repair. Similarly, other biological signal (e.g., RGD) was also introduced in this type of nanofibers through co-assembly, which could interact with integrin on cells surfaces to improve cell adhesion.[66,67]

Further incorporation of azobenzene derivative into C_2 gelators through co-assembly made it possible to prepare light-responsive hydrogels, enabling regulation of cell behaviors in 3D environment under light irradiation (**Figure 10**a).[68] Cells could be in situ encapsulated into the hydrogels by a solvent-mediated method in one step. With exposing cell-encapsulated hydrogels to UV light, the hydrogels started to collapse after 10 min and a complete gel-to-sol phase transition was achieved after 30 min (Figure 10b), which led to the release of the cells entrapped in the hydrogel into bulk solution during the disruption process. This method allowed cells to release not only from 3D hydrogels but also from 2D gel surfaces. A rapid cell rounding was observed on 2D hydrogel surface after 30 min UV irradiation if cells were cultured on hydrogels surfaces.[68] The detachment and harvesting of cells was achieved by gently rinsing at room temperature (Figure 10c). According to the live/dead assay of the released cells from the gel surface, it suggested that weak power UV and short period irradiation did not compromise cell viability (Figure 10d). Since the experiment did not involve destructive factors to the cells, it provided a facile and biologically friendly platform for controlling cell encapsulation and release in both 3D and 2D environments under external stimuli.

Changing solvent polarity to trigger the formation of hydrogel was also suitable for other supramolecular systems, such as Fmoc-peptides reported by Gazit[69] and Liebmann et al.[70] By adding concentrated DMSO peptide solution into cell dispersion, the rapid formation of 3D fibrous network led to hydrogel formation that could adapt to the size and shape of the patterned cell container. In a word, hydrogel formation through tuning polarity of solvent can simplify the cell loading procedure into 3D microenvironments, enabling 3D cell culture to be easily achieved.

3.4. Light-Responsive Hydrogelators

Light is a particularly interesting stimulus to manipulate the transition of sol–gel, since it is a remote stimulus that can be controlled spatially and temporally with great ease and convenience. Light-responsive hydrogels have been investigated as emerging biomaterials in recent years. The dynamic nature of light-responsive hydrogels is beneficial to induce the variation of local properties, such as adhesive activity and mechanical strength, allowing for the dynamic manipulation of cells surrounded by environment.[71]

Stupp's group synthesized PA molecule containing both photocleavable 2-nitrobenzy group and bioactive epitope Arg-Gly Asp-Ser (RGDs).[72] This RGD-containing PA underwent a sol-to-gel transition in response to light and offered a pathway to seed cells into 3D. NIH3T3 mouse embryonic fibroblasts were cultured by mixing them with PA molecules and DMEM in tissue-cultured treated plate. After irradiation, light-triggered gelation of PA molecules not only encapsulated cells in a 3D environment, but also increased the bioactivity of scaffolds with the cleavage of 2-nitrobenzy group.

Gel-sol transition of hydrogel under UV irradiation can be employed to embed cells into 3D microenvironment or patterning. Hamachi et al. developed a zwitterionic amino acid tethered amphiphilic hydrogelator with a light-responsive unit (−C=C−).[73] **Figure 11** shows that photo-triggered phase transition from gel to solution can fabricate 3D channels (100–200 µm in diameter) in hydrogel 1 by UV laser irradiation. The sol containing various live cells (e.g., CHO-EGFP cells) was able to fill into the hydrogel channels through capillary force. Confocal laser scanning microscopic (CLSM) experiment demonstrated that the supramolecular hydrogel mold was gradually dissolved by immersing it in cell culture medium (DMEM) for a few days (Figure 11c). During mold dissolution, cells efficiently proliferated in the collagen microgel, while cell differentiation could be regulated into the patterned collagen gel in hydrogel 1 channel. Typically, this method allows two or more types of cells to be patterned spatially in the jungle-gym channels and to form cell patterning in 3D (Figure 11d and e).

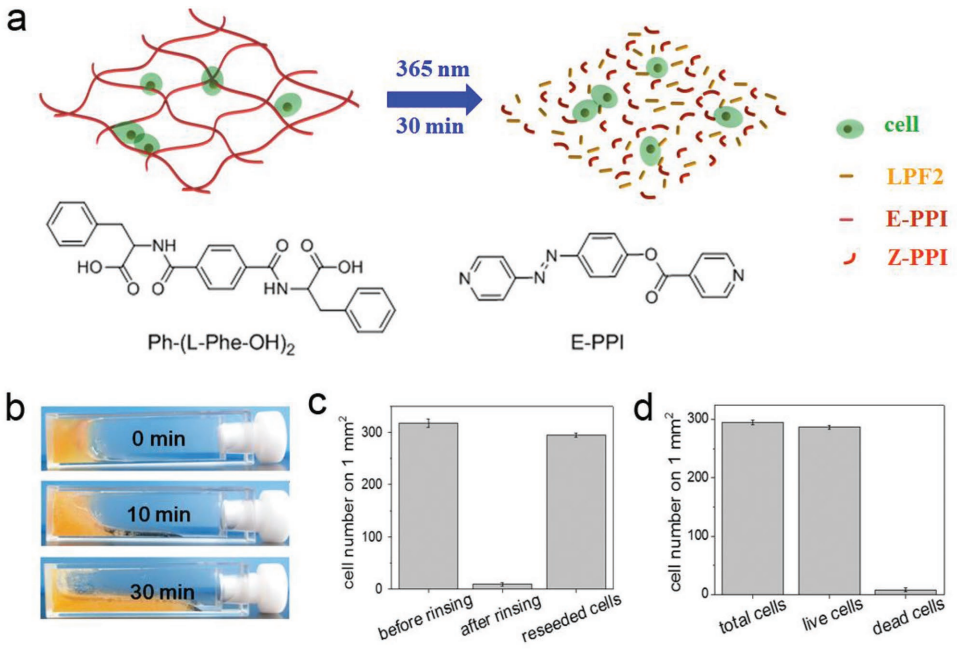

Figure 10. a) A schematic view of the encapsulated cells in the network of gel fibers before (left) and after (right) UV irradiation. b) Optical pictures of the hydrogel-cell construct after different times of UV exposure (an 8 W handed-held UV lamp emitting at 365 nm). c) Density of cells adhered on film before rinsing, after rinsing, and the harvested cell reseeded on PS plate was determined to be 318, 9, and 295 mm^{-2}, respectively. (d) The viability of the harvested cells from hydrogels after reseeding them on PS plate. Density of reseeded cells illustrated with total, live, and dead cells was 295, 286, and 8 mm^{-2}, respectively. Reproduced with permission.[68] Copyright 2015, American Chemical Society.

The advantage of hydrogel 1 mold for 3D patterning of live cells is its biocompatibility. In summary, this direct fabrication of 3D cells patterning is conducted by a solution of light-responsive hydrogelators and live cells. This method can avoid exposure of live cells to potentially harmful UV light since UV light irradiation was used before introducing cells into the gels.

Light can not only trigger the formation of hydrogel for 3D cell culture, but also tune cell behaviors (e.g., morphology). Zhang et al. explored photodegradable peptide hydrogel modified with tetrazole (Tet) moiety.[74] When the hydrogel was exposed to UV light, Tet immediately transformed it into fluorescent pyrazoline cycloadduct (Pyr). Since Pyr had slightly tilted tricyclic ring system, it could interrupt the π–π stacking interactions between aromatic Tet moieties and induce the collapse of the hydrogel (**Figure 12**a). Tet coupled with bioactive peptide sequence GFRGD (Figure 12b) could form transparent hydrogel at physiological pH, making it possible to encapsulate hMSCs cells inside gel matrix. Under UV irradiation, an obvious spreading behavior of the cultured cells was observed inside the gel because of the formation of micro-channels inside the gels, which make them attractive as smart biomaterials for spatially defined modulation on cellular microenvironments (Figure 12c and d).

3.5. Enzyme-Responsive Hydrogelators

Employing an enzyme to trigger hydrogelation of supramolecular gelators should be a useful alternative stimulus since enzymes are prevailingly present in biological environments. For enzyme-instructed hydrogels, precursors need to be converted into hydrogelators in the presence of an enzyme (such as phosphate, β-lactamase). By coupling a hydrophilic segment onto gelators, it can make a lot of precursors.[75] The removal of the hydrophilic segment by enzyme-catalyzed bond cleavage can convert the precursor to hydrogelator, which can further self-assemble into hydrogels (**Figure 13**a).

Xu et al. established a class of enzyme-triggered self-assembly based on amino acid derived precursors.[76] Tyrosine phosphate was coupled to the C-terminal of a β-amino acid derivative to give a precursor which could be hydrolyzed into hydrogelator after treatment with a phosphatase (Figure 13b).[77] The mixture of precursor and phosphatase was injected into a mouse and the in vivo gelation ability of this hydrogelator was evaluated (Figure 13c and d). The excellent biostability rendered the hydrogels as promising candidate for biomedical applications. Although the author did not directly use this hydrogel for 3D cell culture, cells encapsulated into hydrogels can be predicated by mixing precursors, phosphatase, and cells together, which provides an opportunity to proceed 3D cell culture in vivo.

Stimulated by these pioneered works, Gao et al. developed a phosphatase-catalyzed supramolecular hydrogel for 3D cell culture.[78] The precursor FEFKFEpYK was dissolved in neutral buffer solution and then converted to gelator FEFKEYK by phosphatase. The hydrogelation could happen within 5 minutes in the mixture of cell suspensions, precursor, and phosphatase to form 3D cell-gel constructs. It proved that rapid enzymatic hydrogelation ensured a homogeneous encapsulation of cells in gels. CCK-8 assay further indicated that three kinds of cells (HeLa, HepG2, and A549) kept proliferating within the gels after 5 days in culture. Moreover, the cells could be easily separated from cell-gel constructs by pipetting and

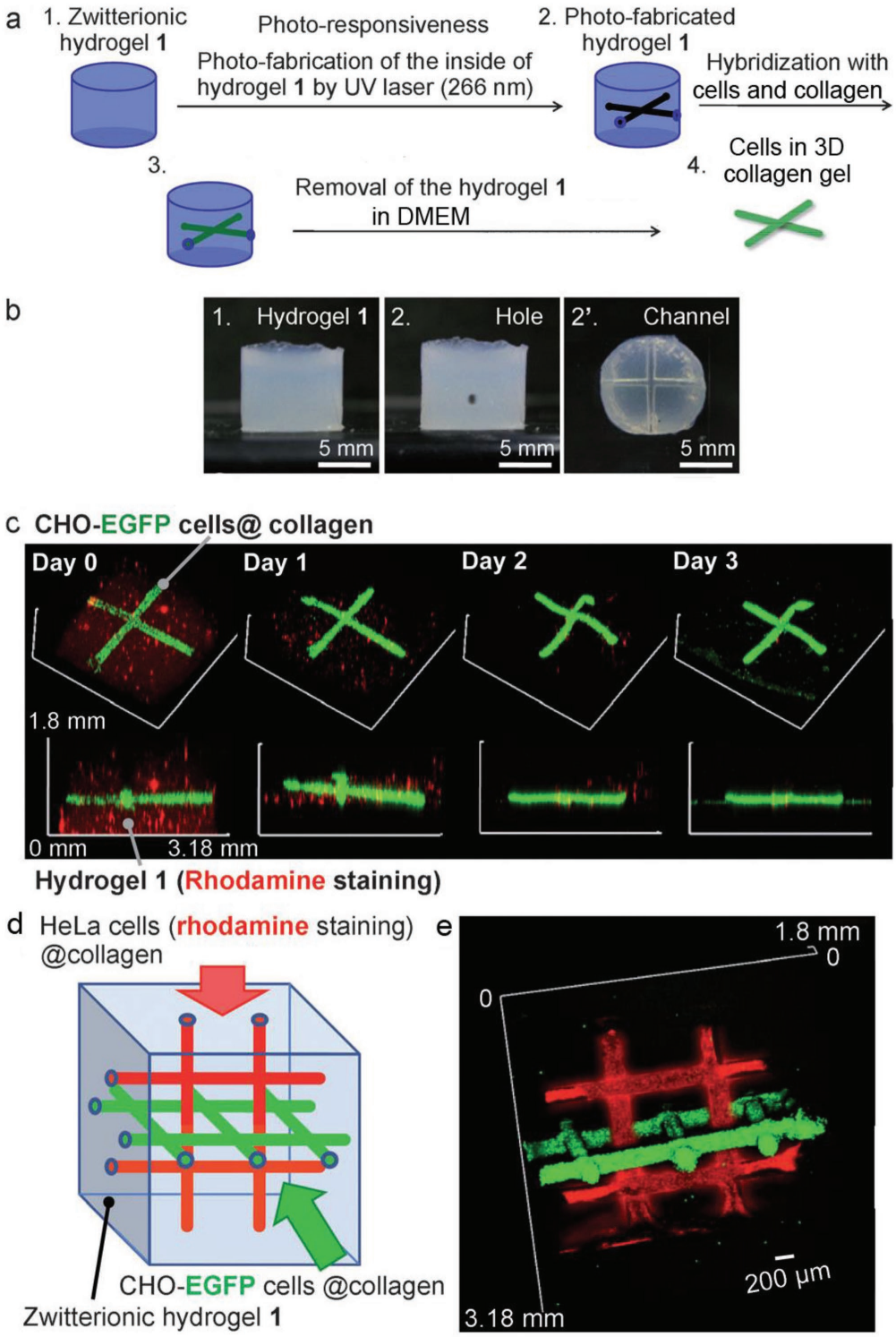

Figure 11. Molding extracellular matrices by using a fabricated zwitterionic hydrogel 1 as a molding template. a) Scheme showing photo-fabrication and the subsequent usage of hydrogel as a removable template. b) Photographs of (1) hydrogel 1, (2) hydrogel 1 after photo-fabrication. c) Time-lapse CLSM z-stack 3D images of collagen microgel containing CHO-EGFP cells molded in hydrogel 1 template. The cells were stained with green color, hydrogel 1 was stained with red color. d) and (f) showing 3D-patterning of two different cells in collagen microgel fabricated by hydrogel 1 channels. d) After a jungle-gym pattern of channels was fabricated in hydrogel 1, two types of cell lines (CHO-EGFP and HeLa cells) were poured into the separate channels from different entrances. e) CLSM z-stack image showing 3D-patterning of CHO-EGFP and HeLa cells embedded in collagen microgel molded by using hydrogel 1. Reproduced with permission.[73] Copyright 2011, Wiley-VCH.

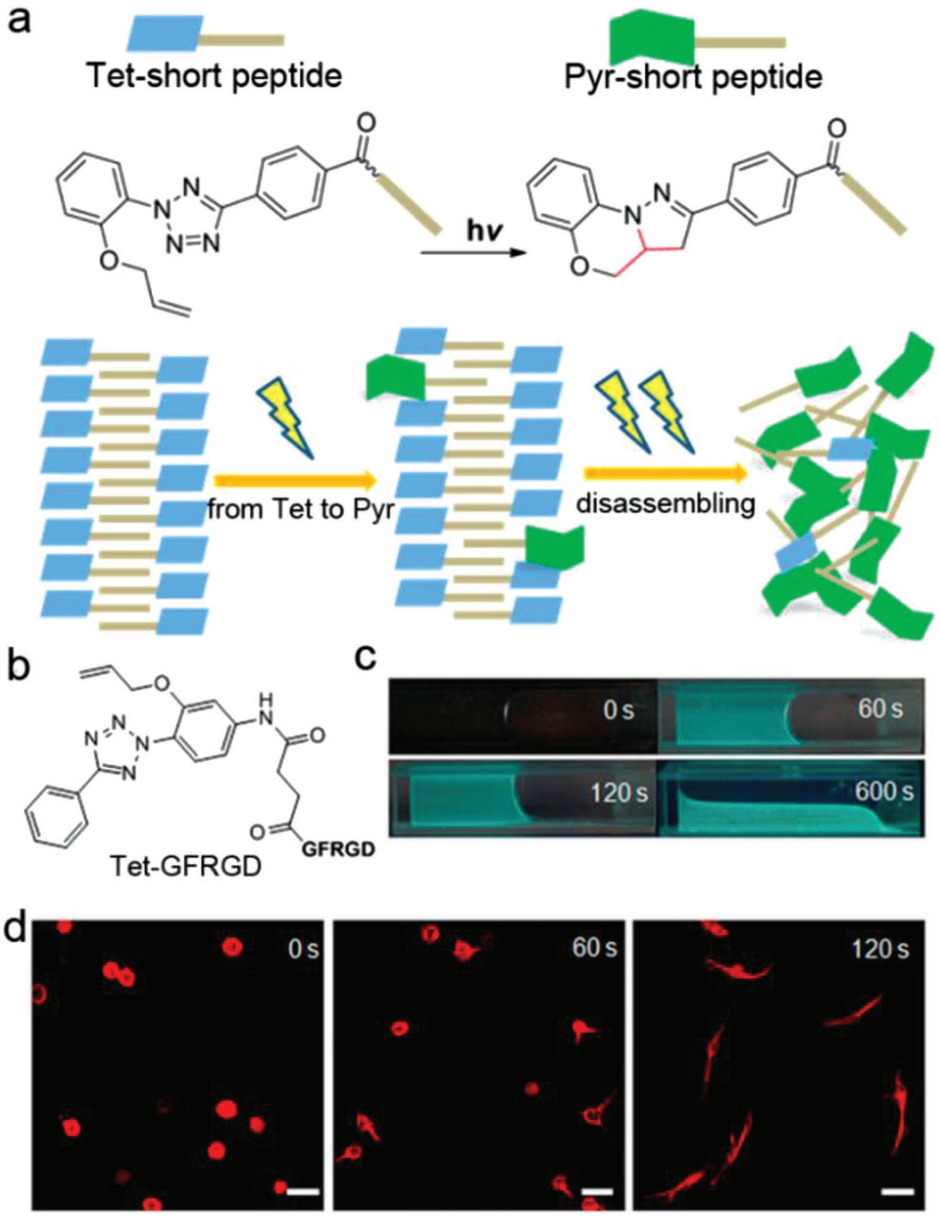

Figure 12. a) Photo-response of the self-assembled hydrogels. The gelator (Tet-peptide) transforms into nongelator (Pyr-peptide). b) Chemical structure of Tet-GFRGD. c) Fluorescent pictures of Tet-GFRGD gel with different times of UV exposure. d) Morphology of hMSCs in Tet-GFRGD gels with different times of UV exposure 3D gels (under the 8W UV light emitting at 302 nm) after 36 h of 3D culture. Scale bar = 50 µm. Reproduced with permission.[74] Copyright 2013, American Chemical Society.

then centrifuging, which was useful and convenient for subsequent cell culture.

Except for bond cleavage, bond formation is another route to convert precursors to a hydrogelator (**Figure 14**a). So far, many kinds of protease have been used to form covalent bonds between amino acids via reverse hydrolysis. The choice of protease can be guided by protease specificity of hydrolysis reactions.[79] In Ulijn's group, thermoresistant protease thermolysin or lipase was coupled with both precursors Fmoc-Phe and Phe_2 by enzyme to make a hydrogelator Fmoc-$(Phe)_3$ (Figure 14b).[80,81] It was found that Fmoc-$(Phe)_3$ hydrogel could significantly induce rat microglial cell proliferation with an increased production of neurotrophic factor NGF.[82] Interestingly, cells seeded on the hydrogels were observed to grow in a 3D space and the neighboring cells did not reside in the same focal plane. Figure 14c showed that cells localized in the regions of the gel that were several µm apart from each other along z-axix. This observation demonstrated the notion that the enzyme-responsive peptide-based hydrogels could support the adhesion and growth of microglia cells and were promising scaffolds for 3D cell culture.

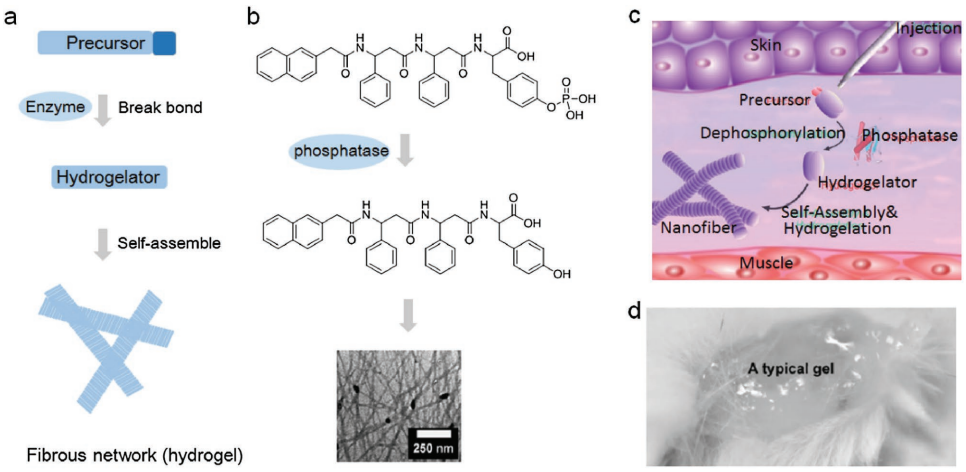

Figure 13. a) Illustration of enzyme-instructed self-assembly to form supramolecular hydrogels via bond cleavage. b) Molecular structures of the precursor and its corresponding hydrogelators, and the TEM image of the self-assembled nanofibers. The bar is 250 nm. c) Illustration of the formation of a supramolecular hydrogel in vivo. d) A typical hydrogel formed at the injection site of a mouse. Reproduced with permission.[77] Copyright 2007, Wiley-VCH.

Enzyme-induced self-assemblies are not only useful for constructing 3D cell culture scaffolds, but also used to induce cancer cell death. Xu and co-workers reported a pioneering work that enzyme-responsive peptide-based precursors could penetrate in cell membranes and self-assemble into nanofibers inside living cells,[83] which could affect cellular functions and further induce cell apoptosis due to the promiscuous interactions between nanofibers and cytoskeletal proteins resulting in the change of cytoplasm.[84] The similar phenomena were also reported by Ulijn,[85] Maruyama,[86] Yang,[87] and Wells.[88]

3.6. Other Methods of Cell Encapsulation

3.6.1. Chemicals

Except for traditional stimulus (ion, pH, solvent, light, enzyme), other stimuli (e.g., chemicals) have also been employed to make cell-gel constructs for 3D cell culture. Yang and colleagues designed several gelator precursors containing disulfide bonds Nap-GFFX-ss-EE (X = Glu (E), Lys (K), or Ser (S)), which could be converted to gelators and form 3D cell-gel constructs through disulfide bond reduction triggered by glutathione (GSH) (**Figure 15**).[89] The gelation process triggered by GSH did not affect cell viability and cell proliferation in Egel, Sgel, and Kgel. The number of metabolically active cells followed the trend of Egel>Sgel>Kgel, which was opposite to the trend of stiffness of these gels, suggesting the mechanical property of hydrogels could obviously affect cell proliferation.

Recently, Yang's group used a specific protein-peptide interaction to enhance the interaction between self-assembly nanofibers.[90] The addition of fusion protein of ubiquitin-like domain-Tax-interacting protein-1 (ULD-TIP-1) could increase cross-linking points between fibers, resulting in hydrogel formation. The hydrogels formed by this method were homogenous and suitable for encapsulating cells.[91,92]

Supramolecular hydrogelators based on N-(fluorenyl-9-methoxycarbonyl)-$_D$-Ala-$_D$-Ala can have gel-sol transition upon binding to its ligand (vancomycin) via ligand-receptor interaction, which can disturb the delicate balance between hydrophobic interactions and hydrogen bonds and in turn induce a gel-sol transition. It is believed that the ability to control the collapse of hydrogels via a biological ligand-receptor may ultimately lead to convenient collection of cultured cells from hydrogels.[93] If the Fmoc group was replaced by the pyrene group, pyrene-$_D$-Ala-$_D$-Ala hydrogel would show a completely different response to vancomycin and the storage modulus (G′) of pyrene-$_D$-Ala-$_D$-Ala hydrogel could dramatically increase after adding one equivalent of vancomycin (the value of G′ changed from 1 Pa to 10^6 Pa). In this case, vancomycin bound tightly to $_D$-Ala-$_D$-Ala group acts as a cross-linker.[94] The hydrogels may lead to convenient collection of cultured cells from 3D environments.

3.6.2. Cell Migration

The non-covalent interactions in supramolecular hydrogels allow the pore size of network to respond to the mechanical force exerted by cells when they migrate through the matrix. Feng and co-workers developed a convenient 3D cell culture by employing high swelling property of hybrid hydrogels co-assembled from C_2-phenyl based gelators and sodium hyaluronate (HA).[95] Cells seeded on the top of gels could migrate from the surface into the bulk with the swelling and the penetration depth could reach to about 65 µm (**Figure 16**). This study may enrich the existing supramolecule-based 3D environments and provide a supplementary system for achieving simple 3D cell culture.

Hydrogel scaffolds modified with cell adhesive factors (e.g., RGD, fibronectin) could also induce cells to migrate into inside 3D environment from 2D surface.[96] Hauser and co-workers introduced biologically active motifs RGD onto peptide fibers

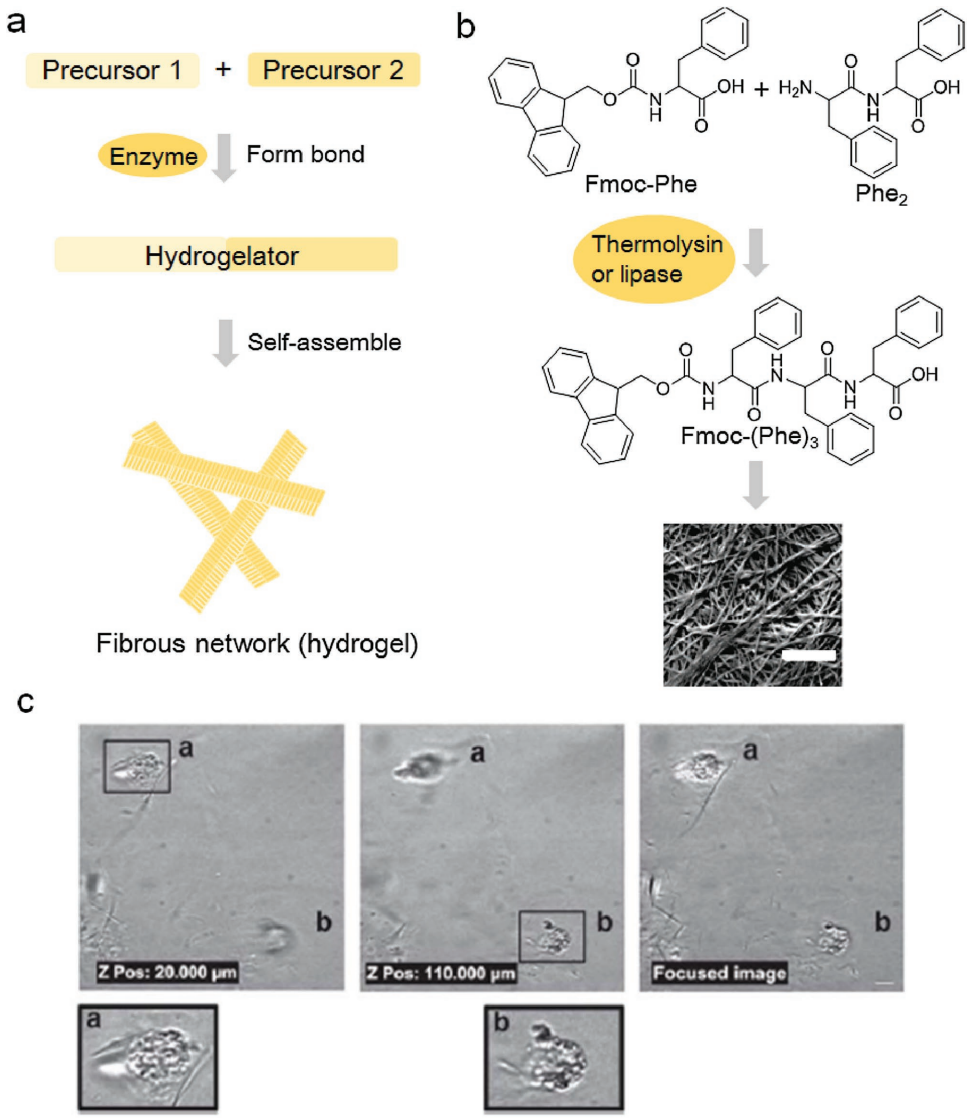

Figure 14. a) Illustration of enzyme-instructed self-assembly to form supramolecular hydrogels via bond formation. b) Molecular structures of the precursors (Fmoc-Phe and Phe_2) and its corresponding hydrogelators (Fmoc-$(Phe)_3$), and SEM image of the self-assembled nanofibers. Bar = 0.5 µm. Reproduced with permission.[80] Copyright 2006, American Chemical Society. c) Differential interference contrast (DIC) images were acquired along the z-axis at regular intervals, covering a total distance of 100 µm, and subsequently deconvolved. Cells "a" and "b" were in focus in two distinct focal planes, shown in the left and central panels, respectively. The 2-fold enlargement of the squared region was shown below each panel. The axial distance between the two focal planes was 90 µm. Scale bars: 5 µm. Reproduced with permission.[82] Copyright 2012, Royal Society of Chemistry.

via oxidation of thiols on both RGD and peptide hydrogelators (Ac-LIVAGKC). The 3D spatial cell distribution were achieved from 2D gel surface into 3D inner structures through RGD attracting cells.[97] Except for RGD, IKVAV was also appended to RADA16 for improving cell migration into nanofibrous matrix and the extensive network of migrating cells was observed with an average depth of 53 µm.[98]

3.6.3. Utilizing Thixotropic Property of Supramolecular Hydrogels

The thixotropic property is another feature of supramolecular hydrogels due to the non-covalent interactions between molecules. These hydrogels could usually be collapsed on applying mechanical stimuli, while, they would form hydrogels again and recover majority of their initial strength within a short time. Taking advantage of this unique property, Maji et al. mixed cell suspension with liquid solution containing amyloid based gelators (made by vortexing the corresponding gel) under agitation, enabling cells to be seeded with the formation of hydrogels (**Figure 17**a).[99] Confocal imaging of 3D gels implied that cells entrapped within the gels were viable (Figure 17b). The highly compliant nature of amyloid hydrogel allowed the cells to exert forces on the scaffold walls to assume spread morphology similar to cells in natural ECM matrix.

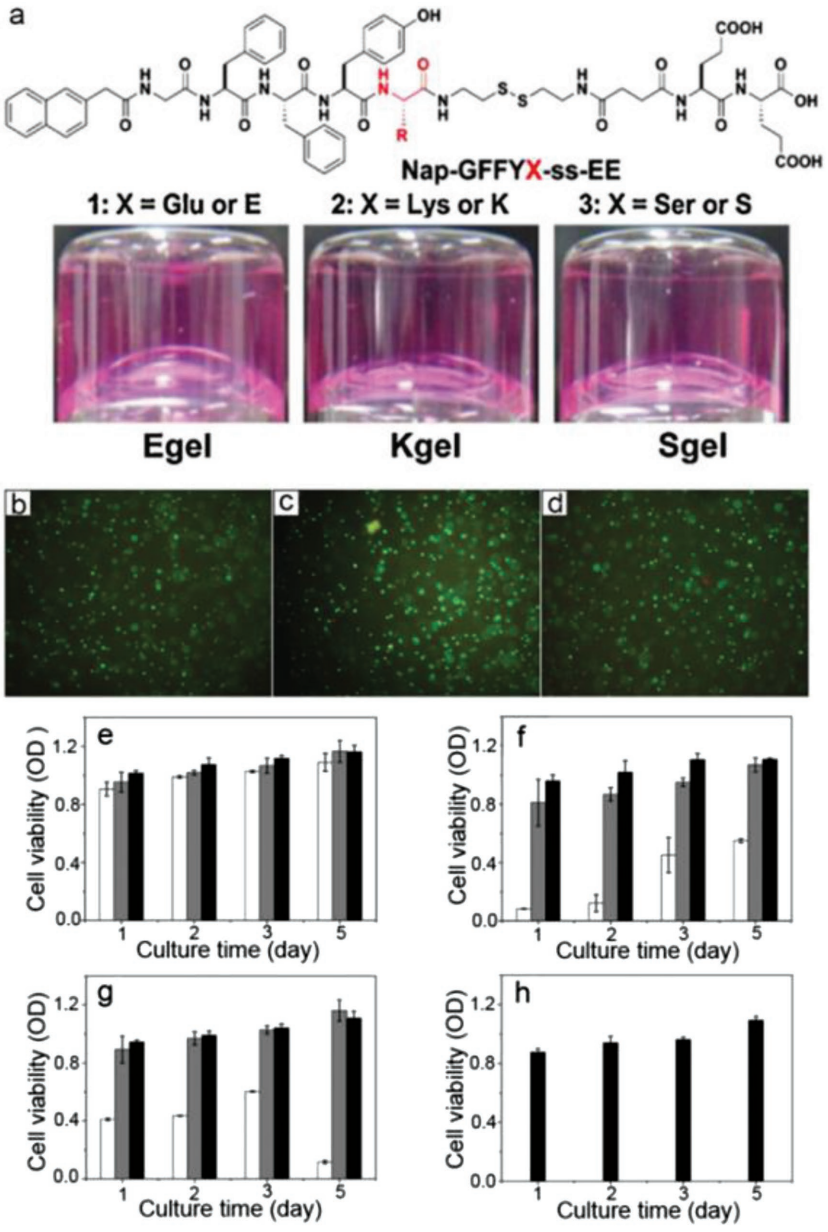

Figure 15. a) Chemical structures of the precursors of molecular hydrogelators Nap-GFFX-ss-EE and optical images of resulting hydrogels formed by treating serum-free DMEM solutions containing 0.1 wt% of different compounds with 4 equiv. of GSH (Egel from Nap-GFFYE-ss-EE, Kgel from Nap-GFFYK-ss-EE and Sgel from Nap-GFFYS-ss-EE). Live/dead assay of NIH3T3 cells cultured in hydrogels from 0.3 wt% of precursors after 4 h (live cells are shown in green and dead ones in red): (b) Egel, (c) Kgel, (d) Sgel. Proliferation rates of NIH3T3 cells cultured in different gels: (e) Egels, (f) Kgels, (g) Sgels and (h) Matrigel (white columns: gels of 0.3 wt%, grey columns: gels of 0.2 wt% and black columns: gels of 0.1 wt%). Reproduced with permission.[89] Copyright 2013, Elsevier.

Recently, Das et al. reported a class of peptide bolaamphiphiles (**Figure 18**a), which could react with p-hydroxybenzyl alcohol in the presence of lipase to form an activated diester building block (Figure 18b).[100] The activated diester building block self-assembled into nanofibrillar thixotropic hydrogel. From stress-strain experiment (Figure 18c), it was found that the kinetics of gel recovery was very rapid. Since the formation of 3D nanofibrous hydrogel was mainly driven by hydrogen bonds and other non-covalent interactions, the applied strain was capable of breaking these interactions and a large part of their meso structures without destroying the fibers, leading to breakdown of hydrogel state. The reduction of the destructive strain permitted the aligned fibers to rationally and translationally diffuse to reorganize 3D networks of nanofibers via renewed contacts.[101] This type of thixotropic property was useful for 3D cell encapsulation, and cells could be embedded in the hydrogels just by mixing and standing for several minutes. The proliferation of human umbilical cord mesenchymal stem cells was studied at three different concentrations of hydrogel (5 days culture). The detected increase in DNA suggested that the gel could promote cells proliferation, since this hydrogel could provide anchorage to cells similar to ECM. The strong cell adhesion was supposed to facilitate cell proliferation and spreading throughout the hydrogel matrix (Figure 18d).

There are a number of gelation methods for amino acids and peptide-based hydrogelators in order to provide multiple choices for 3D cell culture. Based on the increased understanding of peptide functions in cell biology, considerable efforts have focused on the incorporation of peptides or amino acids as the functional motifs on supramolecular hydrogelators for a wider range of biological applications such as cancer therapy,[102] biosensors,[103] cell imaging,[104] and drug release.[105] In addition, having deep insight into intermolecular interaction during gelation will also help to transfer research focus from molecule design to process of self-assembly, from thermodynamics to kinetics, from molecules to cells, which can integrate molecular science with bioinformatics and contribute to the use of supramolecules for better quality of life.

4. Conclusion and Outlook

Up to now, there are a wide variety of amino acids and peptide-based hydrogels with properties similar to ECM and are therefore being developed as 3D ECM mimics for cell culture and regenerative medicine. It is undoubtedly driven by their biocompatible chemical component, suitable inner-structures, tunable properties, intelligent response to stimulus, and easy preparation methods. Since synergetic hydrogen bonding interactions are essential in living processes, amide hydrogen bonds in amino acids and peptide-based self-assemblies can

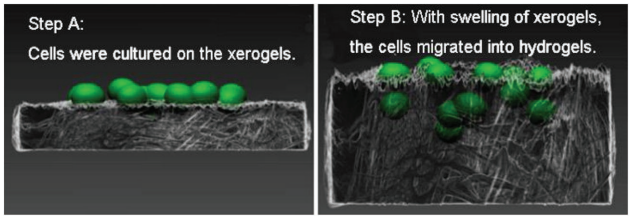

Figure 16. Schematic of the facile 3D culture strategy. Step A: cells were directly cultured on the compact thin xerogel layer. Step B: With swelling of xerogels, cells on the gels migrated from the surface into the bulk and showed 3D cell proliferation behaviors. Reproduced with permission.[95] Copyright 2014, American Chemical Society.

provide a straightforward route to mimic and manipulate basic biological environments. Thanks to the non-covalent interactions between gelators, cells can be easily cultured in 3D hydrogels through proper stimulus, such as ion, pH, solvent, light and enzyme. More than this, there are many merits for these types of hydrogels. The subtle changes in amino acid types and peptide sequences may also have dramatic effects on the physical properties (e.g., chirality, modulus, fiber diameters, hydrogel porosity) of the gels and the subsequent cell responses. The ability to introduce multiple and complex cell signaling sequences in these hydrogels can promote cell adhesion and proliferation. The tunable bioactive behaviors through synthetic customization of hydrogelators may ensure the attractiveness of hydrogels for culturing different cell lines in 3D, e.g., peptides containing epitopes or antigens which can interact with immune cells and modulate innate immunity, which is useful for immunomodulatory materials.[106,107]

Despite the significant progress for amino acids and peptide-based hydrogels, some challenges remain to be solved, including molecular design, chirality of hydrogel matrix, mechanical property, a precision "on-off", water retention, toxicity, and so on. The design methodology for these gelator structures is still in the primary stage, because the self-assembly mechanism is not fully unveiled. An exciting and promising future direction is to build direct associations among the database of supramolecular gelators, self-assembled structures (e.g., chirality), and properly cultured cell types to meet the requirement in 3D cell culture (e.g., choice of optimum hydrogel or stimuli for certain cells).

Chirality is one crucial property of ECM and has great influence on maintaining normal functions for living cells and is closely related with the relevant biological events. Thus, it should be a big challenge to make biomimetic chiral hydrogels by rationally designing molecular structures of amino acids and peptide-based gelators. Although the differential cell behaviors on enantiomorphous surfaces have been proven, how chiral properties influence cell behaviors in 3D is still questionable, including helical distance of nanofibers, the mediation role of proteins, and synergetic function with chemical composition, strength or wettability. All these need to be further explored for the hydrogels in order to deeply understand the mechanism of ECM regulated cell behaviors, which is also a necessary step to realize truly biomimetic ECM.

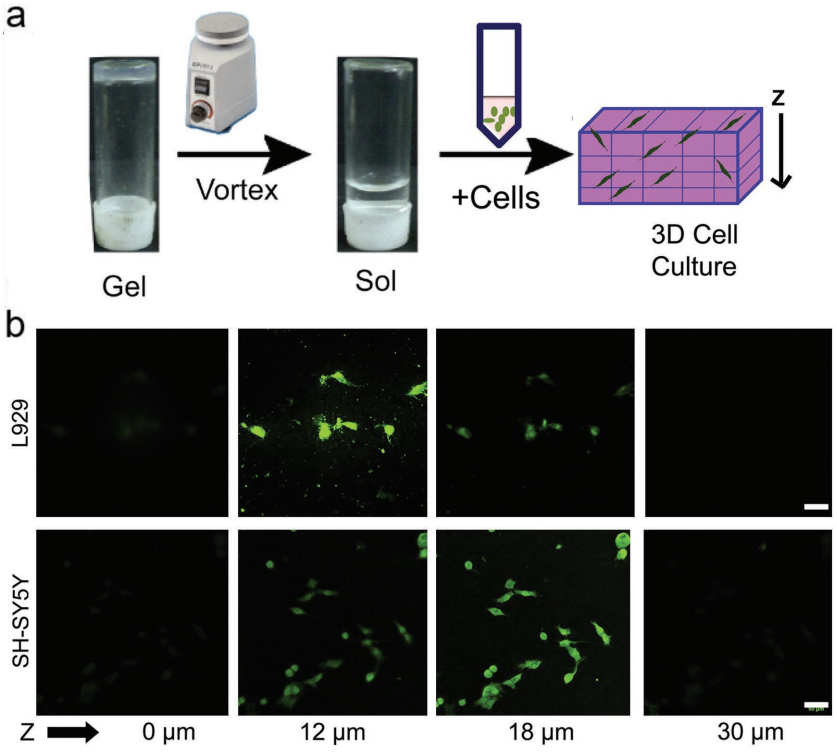

Figure 17. 3D cell culture by amyloid hydrogels. a) Schematic depicting entrapment of cells inside thixotropic peptide gels. b) 3D cell culture showing cell viability of both SH-SY5Y and L929 cells indicated by calcein AM staining (green) inside the 3D gel matrix. Scale bars are 50 μm. Reproduced with permission.[99] Copyright 2015, Elsevier.

Another main problem for self-assembled supramolecular hydrogels is their unstable and poor mechanical properties. Slight mechanical disturbances (vortex or agitation) may cause the disassembly of the structure and dissolution of supramolecular hydrogels, and in turn limit their further practical application, e.g., mimicking soft tissues, injection for regeneration of tissues. It is of great importance to improve and tune the mechanical properties of these hydrogels by properly designing gelator structures, followed by the fabrication of hydrogel networks (e.g., cross-link reaction).

For pH and solvent induced 3D hydrogels, the rate of mixing solvent was found to be slower than the rate of assembly. Local pH or solution polarity variation at the point of adding cell culture medium may lead to fast local formation of gel and inhomogeneous cell-gel constructs. To overcome this, more parameters should be considered, such as gelation rate and ion diffusion rate. For light-responsive supramolecular hydrogels, they are usually irradiated by toxic short wavelengths (UV light), in turn harmful to the cultured cells in hydrogels. Thus, there is demand for rationally designed and synthesized hydrogelators that can be irradiated by non-invasive long wavelengths. These

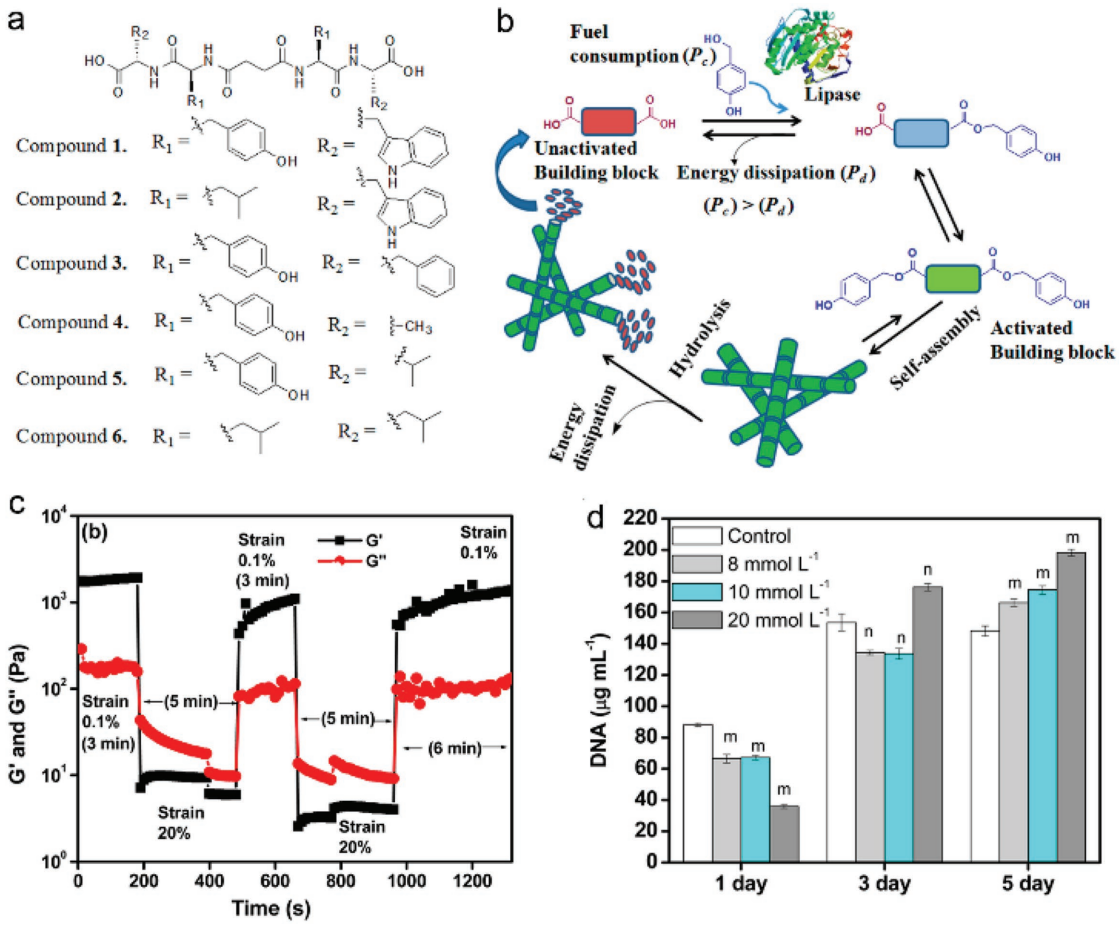

Figure 18. a) Structures of peptide bolaamphiphiles. b) Dissipative reaction of the system. Unactivated peptide bolaamphiphile incorporated energy in the form of p-hydroxybenzyl alcohol to give monoester and then diester. The activated diester self-assembled into nanofibers and formed hydrogel. The subsequent hydrolysis led to the dissipation of the energy of diester which resulted in collapse of the hydrogel. c) Step strain experiment showed the thixotropic nature of hydrogel. d) Stem-cell viability based on DNA leaching assay within the peptide hydrogels. Reproduced with permission.[100] Copyright 2015, American Chemical Society.

photo-responsive hydrogels must be potential 3D scaffolds for spatial and temporal control over cell encapsulated microenvironments through a precision "on-off" switch, which can have applications in the fields of tissue repair, cell harvesting, and cell biological study.

A critical issue for amino acids and peptide hydrogels is that they prefer to spontaneously release water (syneresis), which seriously limits the related applications (e.g. tissue repair, drug release, and biosensor). How to circumvent the syneresis problem is a challenge and urgent target in order to broaden their application. In addition, visualizing the performance of ECM in 3D environments is also necessary in many fundamental physiological studies, e.g., reciprocal interactions between cells and matrix. To make fluorescent hydrogels assembled from amino acids and peptides hydrogelators for imaging 3D cell-matrix interaction should be a new research field.

Finally, many of amino acid and peptide based hydrogels can be injectable for tissue regeneration studies, such as for cardiac tissue, nerve and cartilage. Although these challenges still exist for practical application, including viscosity and stability of hydrogels, cell survival and functions in the 3D gels. In addition, toxicity/biocompatibility of supramolecular hydrogels should be also considered for in vivo and in vitro cell culture. Researchers found that amyloid hydrogels could support cell proliferation and differentiation in in vivo 3D hydrogels, however, it has some risks in terms of biocompatibility for in vivo cell culture, since amyloid peptide may be deposited in body with the degradation of the hydrogels into amyloid peptide.[99]

Overcoming the above mentioned issues is a great challenge, however, it may come true with the comprehensive development of supramolecular chemistry, polymer chemistry, and materials science. Interdisciplinary combination will facilitate the design and development of next-generation functional supramolecular hydrogels for the purpose of being used in regenerative medicine, tissue engineering, pre-clinical evaluation.

Acknowledgements

We thank the NSFC (51573092, 51273111), the National Basic Research Program of China (973 Program 2012CB933803), the Shanghai Jiao Tong

University (SJTU)–University of Michigan (UM) Collaborative Research Program, and the Program for Professor of Special Appointment (Eastern Scholar) at the Shanghai Institutions of Higher Learning and the Alexander von Humboldt Foundation (postdoc stipend to X.Q. Dou).

Received: July 30, 2016
Revised: October 16, 2016
Published online: January 23, 2017

[1] a) 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; b) F. Z. Volpato, T. Fuhrmann, C. Migliaresi, D. W. Hutmacher, P. D. Dalton, *Biomaterials* **2013**, *34*, 4945; c) E. Arslan, I. C. Gariop, G. Gulseren, A. B. Tekinay, M. O. Guler, *Adv. Healthc. Mater.* **2014**, *3*, 1357.
[2] M. W. Tibbitt, K. S. Anseth, *Biotechnol. Bioeng.* **2009**, *103*, 655.
[3] a) K. Yue, G. Trujillo-de, M. M. Alvarez, A. Tamayol, N. Annabi, A. Khademhosseini, *Biomaterials* **2015**, *73*, 254; b) H. Storrie, M. O. Guler, S. N. Abu-Amara, T. Volberg, M. Rao, B. Geiger, S. I. Stupp, *Bioamterials* **2007**, *28*, 4608; c) R. J. Williams, A. M. Smith, R. Collins, N. Hodson, A. K. Das, R. V. Ulijn, *Nat. Nanotechnol.* **2009**, *4*, 19.
[4] a) D. Seliktar, *Science* **2012**, *336*, 1124; b) O. Guillame-Gentil, O. Semenov, A. S. Roca, T. Groth, R. Zahn, J. Vörös, M. Zenobi-Wong, *Adv. Mater.* **2010**, *22*, 5443.
[5] V. Jayawarna, S. M. Richardson, A. R. Hirst, N. W. Hodson, A. Saiani, J. E. Gough, R. V. Ulijn, *Acta Biomater.* **2009**, *5*, 934.
[6] C. Cha, J. H. Jeong, J. Shim, H. Kong, *Acta Biomater.* **2011**, *7*, 3719.
[7] a) W. C. Hsieh, J. J. Liau, *Carbohyd. Polym.* **2013**, *15*, 574; b) T. H. Kim, D. B. An, S. H. Oh, M. K. Kang, H. H. Song, J. H. Lee, *Biomaterials* **2015**, *40*, 51.
[8] J. R. Tse, A. J. Engler, *PloS One* **2011**, *6*, e15978.
[9] a) L. A. Reis, L. L. Y. Chiu, Y. Liang, K. Hyunh, A. Momen, M. Radisic, *Acta Biomater.* **2012**, *8*, 1022; b) Z. F. Sun, F. C. Lv, L. J. Cao, L. Liu, Y. Zhang, Z. G. Lu, *Angew. Chem. Int. Ed.* **2015**, *54*, 7944.
[10] H. Urakami, Z. Guan, *Biomacromolecules* **2008**, *9*, 592.
[11] a) X. W. Du, J. Zhou, J. F. Shi, B. Xu, *Chem. Rev.* **2015**, *115*, 13165; b) M. Ma, Y. Kuang, Y. Gao, Y. Zhang, P. Gao, B. Xu, *J. Am. Chem. Soc.* **2010**, *132*, 2719; c) J. D. Hartgerink, E. Beniash, S. I. Stupp, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5133.
[12] K. J. Skilling, F. Citossi, T. D. Bradshaw, M. Ashford, B. Kellam, M. Marlow, *Soft Matter* **2014**, *10*, 237.
[13] A. Horii, X. M. Wang, F. Gelain, S. G. Zhang, *PloS One* **2007**, *2*, e190.
[14] a) T. Aida, E. W. Meijer, S. I. Stupp, *Science* **2012**, *335*, 813; b) E. A. Appei, J. del Barrio, X. J. Loh, O. A. Scherman, *Chem. Soc. Rev.* **2012**, *41*, 6195; c) J. Li, *NPG Asia Mater.* **2010**, *2*, 112; d) X. F. Ji, B. B. Shi, H. Wang, D. Y. Xia, K. C. Jie, Z. L. Wu, F. H. Huang, *Adv. Mater.* **2015**, *27*, 8062; e) C. Li, M. J. Rowland, Y. Shao, T. Y. Cao, C. Chen, H. Y. Jia, X. Zhou, Z. Q. Yang, O. A. Scherman, D. S. Liu, *Adv. Mater.* **2015**, *27*, 3298; f) R. E. Kieltyka, A. C. H. Pape, L. Albertazzi, Y. Nakano, M. M. C. Bastings, I. K. Voets, P. Y. W. Dankers, E. W. Meijer, *J. Am. Chem. Soc.* **2013**, *135*, 11159; g) S. Tamesue, Y. Takashima, H. Yamaguchi, S. Shinkai, A. Harada, *Angew. Chem. Int. Ed.* **2010**, *49*, 7461.
[15] X. W. Du, J. Zhou, B. Xu, *Chem. Asian J.* **2014**, *9*, 1446.
[16] a) F. M. Xu, H. B. Wang, J. Zhao, X. S. Liu, D. D. Li, C. J. Chen, J. Ji, *Macromolecules* **2013**, *46*, 4235; b) J. L. Yan, J. Liu, P. Jing, C. K. Xu, J. M. Wu, D. Gao, Y. Fang, *Soft Matter* **2012**, *8*, 11697.
[17] a) Z. X. Zhang, K. L. Liu, J. Li, *Angew. Chem. Int. Ed.* **2013**, *52*, 6180; b) K. L. Liu, Z. X. Zhang, J. Li, *Soft Matter* **2011**, *7*, 11290; c) C. B. Rodell, A. L. Kaminski, J. A. Burdick, *Biomacromolecules* **2013**, *14*, 4125; d) T. Kakuta, Y. Takashima, A. Harada, *Macromolecules* **2013**, *46*, 4575.
[18] Y. Q. Liu, T. Y. Wang, M. H. Liu, *Chem. Eur. J.* **2012**, *18*, 14650.
[19] a) D. Higashi, M. Yoshida, M. Yamanaka, *Chem. Asian J.* **2013**, *8*, 2584; b) M. Yamanaka, N. Haraya, S. Yamamichi, *Chem. Asian J.* **2011**, *6*, 1022; c) F. Rodriguez-Llansola, B. Escuder, J. F. Miravet, D. Hermida-Merino, I. W. Hamley, C. J. Cardin, W. Hayes, *Chem. Commun.* **2010**, *46*, 7960.
[20] a) N. Goyal, S. Cheuk, G. J. Wang, *Tetrahedron* **2010**, *66*, 6962; b) Y. Ogawa, C. Yoshiyama, T. Kitaoka, *Langmuir* **2012**, *28*, 4404; c) G. J. Wang, S. Cheuk, H. Yang, N. Goyal, P. V. N. Reddy, B. Hopkinson, *Langmuir* **2009**, *25*, 8696; d) M. Ikeda, R. Ochi, Y. Kurita, D. J. Pochan, I. Hamachi, *Chem. Eur. J.* **2012**, *18*, 13091.
[21] a) P. Li, X. Q. Dou, C. L. Feng, D. Zhang, *Soft Matter* **2013**, *9*, 3750; b) S. Mukherjee, T. Kar, P. K. Das, *Chem. Asian J.* **2014**, *9*, 2798; c) I. Ziemecka, G. J. M. Koper, A. G. L. Olive, J. H. Van Esch, *Soft Matter* **2013**, *9*, 1556; d) A. Friggeri, B. L. Feringa, J. van Esch, *J. Controlled Release* **2004**, *97*, 241; e) V. J. Nebot, J. Armengol, J. Smets, S. F. Prieto, B. Escuder, J. F. Miravet, *Chem. Eur. J.* **2012**, *18*, 4063; f) P. S. Kubiak, S. Awhida, C. Hotchen, W. T. Deng, B. Alston, T. O. McDonald, D. J. Adams, P. J. Cameron, *Chem. Commun.* **2015**, *51*, 10427.
[22] a) S. Koutsopoulos, S. G. Zhang, *Acta Biomater.* **2013**, *9*, 5162; b) T. Muraoka, C. Y. Kohr, H. Cui, S. I. Stupp, *Angew. Chem. Int. Ed.* **2009**, *48*, 5946; c) J. Raeburn, T. O. McDonald, D. J. Adams, *Chem. Commun.* **2012**, *48*, 9355; d) Y. Nagai, H. Yokoi, K. Kaihara, K. Naruse, *Biomaterials* **2012**, *33*, 1044; e) S. Grigoriou, E. K. Johnson, L. Chen, D. J. Adams, T. D. James, P. J. Cameron, *Soft Matter* **2012**, *8*, 6788; f) E. K Johnson, D. J. Adams, P. J. Cameron, *J. Mater. Chem.* **2011**, *21*, 2024; g) E. K. Johnson, D. J. Adams, P. J. Cameron, *J. Am. Chem. Soc.* **2010**, *132*, 5130.
[23] a) C. A. E. Hauser, S. G. Zhang, *Chem. Soc. Rev.* **2010**, *39*, 2780; b) S. G. Zhang, *Nat. Biotechnol.* **2003**, *21*, 1171; c) J. Boekhoven, S. I. Stupp, *Adv. Mater.* **2014**, *26*, 1642; d) J. Gao, W. T. Zheng, J. M. Zhang, D. Guan, Z. M. Yang, D. L. Kong, Q. Zhao, *Chem. Comm.* **2013**, *49*, 9173; e) A. M. Smith, R. J. Williams, C. Tang, P. Coppo, R. F. Collins, M. L. Turner, A. Saiani, R. V. Ulijn, *Adv. Mater.* **2008**, *20*, 37.
[24] E. C. Wu, S. G. Zhang, C. A. E. Hauser, *Adv. Funct. Mater.* **2012**, *22*, 456.
[25] J. M. Lehn, *Science* **2002**, *295*, 2400.
[26] E. L. Bakota, O. Sensoy, B. Ozgur, M. Sayar, J. D. Hartgerink, *Biomacromolecules* **2013**, *14*, 1370.
[27] a) J. T. van Herpt, M. C. A. Stuart, W. R. Browne, B. L. Feringa, *Chem. Eur. J.* **2014**, *20*, 3077; b) A. Mahler, M. Reches, M. Rechter, S. Cohen, E. Gazit, *Adv. Mater.* **2006**, *18*, 1365; c) D. J. Adams, M. F. Butler, W. J. Frith, M. Kirkland, L. Mullen, P. Sanderson, *Soft Matter* **2009**, *5*, 1856.
[28] a) X. Q. Dou, P. Li, S. Q. Lu, X. B. Tian, Y. T. Tang, J. D. Mercer-Chalmers, C. L. Feng, *J. Mol. Liq.* **2013**, *180*, 129; b) M. Yamanaka, *Chem. Rec.* **2016**, *16*, 768; c) D. Yuan, X. W. Du, J. F. Shi, N. Zhou, J. Zhou, B. Xu, *Angew. Chem. Int. Ed.* **2015**, *54*, 5705.
[29] H. Y. Wang, S. C. Heilshorn, *Adv. Mater.* **2015**, *27*, 3717.
[30] a) V. J. Nebot, J. Armengol, J. Smets, S. F. Prieto, B. Escuder, J. F. Miravet, *Chem. Eur. J.* **2012**, *18*, 4063; b) D. J. Adams, L. M. Mullen, M. Berta, L. Chen, W. J. Frith, *Soft Matter* **2010**, *6*, 1971.
[31] K. J. C. Van Bommel, C. Van der Pol, I. Kuizebelt, A. Friggeri, A. Heeres, A. Meetsma, B. L. Feringa, J. Van Esch, *Angew. Chem. Int. Ed.* **2004**, *43*, 1663.
[32] M. Kabiri, I. Bushnak, M. T. McDermot, L. D. Unsworth, *Biomacromolecules* **2013**, *14*, 3943.

[33] M. T. Record, C. F. Anderson, T. M. Q. LOhman, *Rev. Biophys.* **1978**, *11*, 103.
[34] a) A. P. H. J. Schenning, E. W. Meijer, *Chem. Commun.* **2005**, *26*, 3245; b) R. L. E. Furlan, S. Otto, J. K. M. Sanders, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4801.
[35] F. J. M. Hoeben, P. Jonkheijm, E. W. Meijer, A. P. H. J. Schenning, *Chem. Rev.* **2005**, *105*, 1491.
[36] a) S. G. Zhang, T. Holmes, C. Lockshin, A. Rich, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 3334; b) S. G. Zhang, C. Lockshin, R. Cook, A. Rich, *Biopolymers* **1994**, *34*, 663.
[37] a) M. López de la Paz, K. Goldie, J. Zurdo, E. Lacroix, C. M. Dobson, *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 16502; b) Y. Hong, M. D. Pritzker, R. L. Legge, P. Chen, *Colloids Surf. B Biointerfaces* **2005**, *46*, 152.
[38] a) Y. L. Yang, U. Khoe, X. M. Wang, A. Horii, H. Yokoi, S. G. Zhang, *Nano Today* **2009**, *4*, 193; b) H. Yokoi, T. Kinoshita, S. G. Zhang, *Pro. Natl. Acad. Sci. USA* **2005**, *102*, 8414; c) T. C. Holmes, S. de Lacalle, X. Su, G. S. Liu, A. Rich, S. G. Zhang, *Pro. Natl. Acad. Sci. USA* **2000**, *97*, 6728.
[39] A. L. Sieminski, A. S. Was, G. Kim, H. Gong, R. D. Kamm, *Cell Biochem. Biophys.* **2007**, *49*, 73.
[40] Z. Yang, X. Zhao, *Int. J. Nanomedicine* **2011**, *6*, 303.
[41] J. Kisiday, M. Jin, B. Kurz, H. Hung, C. Semino, S. G. Zhang, A. J. Grodzinsky, *Pro. Natl. Acad. U. S. A.* **2002**, *99*, 9996.
[42] A. Mujeeb, A. F. Miller, A. Saiani, J. E. Gough, *Acta Biomater.* **2013**, *9*, 4609.
[43] a) D. Robert, C. Rochas, A. Saiani, A. F. Miller, *Langmuir* **2012**, *28*, 16196; b) A. Mohammed, A. F. Miller, A. Saiani, *Macromol. Symp.* **2007**, *251*, 88; c) A. Saiani, A. Mohammed, H. Frielinghaus, R. Collins, N. Hodson, C. M. Kielty, M. J. Sherratt, A. F. Miller, *Soft Matter* **2009**, *5*, 193.
[44] C. X. Chen, Y. F. Gu, L. Deng, S. Y. Han, X. Sun, Y. C. Chen, J. R. Lu, H. Xu, *ACS Appl. Mater. Interfaces* **2014**, *6*, 14360.
[45] a) L. Haines-Butterick, K. Rajagopal, M. Branco, D. Salick, R. Rughani, M. Pilarz, M. S. Lamm, D. J. Pochan, J. P. Schneider, *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 7791; b) D. J. Smith, G. A. Brat, S. H. Medina, D. Tong, Y. Huang, J. Grahammer, G. J. Furtmüller, B. C. Oh, K. J. Nagy-Smith, P. Walczak, G. Brandacher, J. P. Schneider, *Nat. Nanotechnol.* **2016**, *11*, 95; c) S. H. Medina, S. E. Miller, A. I. Keim, A. P. Gorka, M. J. Schnermann, J. P. Schneider, *Angew. Chem. Int. Ed.* **2016**, *55*, 3369; d) S. H. Medina, S. Li, O. M. Z. Howard, M. Dunlap, A. Trivett, J. P. Schneider, J. J. Oppenheim, *Biomaterials* **2015**, *53*, 545.
[46] a) V. A. Kumar, N. L. Taylor, S. Y. Shi, N. C. Wickremasinghe, R. N. D'Souza, J. D. Hartgerink, *Biomaterials* **2015**, *52*, 71; b) V. A. Kumar, Q. Liu, N. C. Wickremasinghe, S. Y. Shi, T. T. Cornwright, Y. X. Deng, A. Azares, A. N. Moore, A. M. Acevedo-Jake, N. R. Agudo, S. Pan, D. G. Woodside, P. Vanderslice, J. T. Willerson, R. A. Dixon, J. D. Hartgerink, *Biomaterials* **2016**, *98*, 113; c) M. K. Kang, J. S. Colombo, R. N. D'Souza, J. D. Hartgerink, *Biomacromolecules* **2014**, *15*, 2004; d) E. L. Bakota, Y. Wang, F. R. Danesh, J. D. Hartgerink, *Biomacromolecules* **2011**, *12*, 1651.
[47] K. M. Galler, J. D. Hartgerink, A. C. Cavender, G. Schmalz, R. N. D'Souza, *Tissue Eng. Part A* **2012**, *18*, 176.
[48] S. Kyle, S. H. Felton, M. J. McPherson, A. Aggeli, E. Ingham, *Adv. Healthcare Mater.* **2012**, *1*, 640.
[49] J. D. Hartgerink, E. Beniash, S. I. Stupp, *Science* **2001**, *294*, 1684.
[50] S. M. Zhang, M. A. Greenfied, A. Mata, L. C. Palmer, R. Bitton, J. R. Mantei, C. Aparicio, M. O. De la Cruz, S. I. Stupp, *Nat. Mater.* **2010**, *9*, 594.
[51] Z. L. Luo, Y. Y. Yue, Y. F. Zhang, X. Yuan, J. P. Gong, L. L. Wang, B. He, Z. Liu, Y. L. Sun, J. Liu, M. F. Hu, J. Zheng, *Biomaterials* **2013**, *34*, 4902.
[52] H. Z. Huang, Y. Ding, X. Z. Sun, T. A. Nguyen, *PloS One* **2013**, *8*, e59482.
[53] H. Z. Huang, A. I. Herrera, Z. P. Luo, O. Prakash, X. Z. S. Sun, *Biophys J.* **2012**, *103*, 979.
[54] Y. F. Tian, J. M. Devgun, J. H. Collier, *Soft Matter* **2011**, *7*, 6005.
[55] Y. Zhao, H. Yokoi, M. Tanaka, T. K inoshita, T. Tan, *Biomacromolecules* **2008**, *9*, 1511.
[56] a) M. Zhou, A. M. Smith, A. K. Das, N. W. Hodson, R. F. Collins, R. V. Ulijn, J. E. Gough, *Biomaterials* **2009**, *30*, 2523; b) V. Jayawarna, M. Ali, T. A. Jowitt, A. F. Miller, A. Saiani, J. E. Gough, R. V. Ulijn, *Adv. Mater.* **2006**, *18*, 611.
[57] B. F. Lin, K. A. Megley, N. Viswanathan, D. V. Krogstad, L. B. Drews, M. J. Kade, Y. C. Qian, M. V. Tirrell, *J. Mater. Chem.* **2012**, *22*, 19447.
[58] J. J. Panda, A. Mishra, A. Basu, V. S. Chauhan, *Biomacromolecules* **2008**, *9*, 2244.
[59] J. J. Panda, R. Dua, A. Mishra, B. Mittra, V. S. Chauhan, *ACS Appl. Mater. Interf.* **2010**, *2*, 2839.
[60] S. Marchesan, Y. Qu, L. J. Waddington, C. D. Easton, V. Glattauer, T. J. Lithgow, K. M. McLean, J. S. Forsythe, P. G. Hartley, *Biomaterials* **2013**, *34*, 3678.
[61] F. Zhao, Y. Gao, J. F. Shi, H. M. Browdy, B. Xu, *Langmuir* **2011**, *27*, 1510.
[62] a) C. L. Feng, X. Q. Dou, D. Zhang, H. Schonherr, *Macromol. Rapid Commun.* **2012**, *33*, 1535; b) X. Q. Dou, P. Li, D. Zhang, C. L. Feng, *Soft Matter* **2012**, *8*, 3231; c) X. Q. Dou, X. M. Yang, P. Li, Z. G. Zhang, H. Schonherr, D. Zhang, C. L. Feng, *Soft Matter* **2012**, *8*, 9539.
[63] a) G. F. Liu, D. Zhang, C. L. Feng, *Angew. Chem. Int. Ed.* **2014**, *53*, 7789; b) G. F. Liu, L. Y. Zhu, W. Ji, C. L. Feng, Z. X. Wei, *Angew. Chem. Int. Ed.* **2016**, *55*, 2411.
[64] a) D. Hanein, B. Geiger, L. Addadi, *Science* **1994**, *263*, 1413; b) R. M. Hazen, D. S. Sholl, *Nat. Mater.* **2003**, *2*, 367; c) T. L. Sun, D. Han, K. Rhemann, L. F. Chi, H. Fuchs, *J. Am. Chem. Soc.* **2007**, *129*, 1496.
[65] X. Q. Dou, J. Zhang, C. L. Feng, *ACS Appl. Mater. Interfaces* **2015**, *7*, 20786.
[66] X. Q. Dou, P. Li, D. Zhang, C. L. Feng, *J. Mater. Chem. B* **2013**, *1*, 3562.
[67] X. Q. Dou, D. Zhang, C. L. Feng, *Langmuir* **2013**, *29*, 1539.
[68] G. F. Liu, W. Ji, W. L. Wang, C. L. Feng, *ACS Appl. Mater. Interfaces* **2015**, *7*, 301.
[69] R. Orbach, L. Adler-Abramovich, S. Zigerson, I. Mironi-Harpaz, D. Seliktar, E. Gazit, *Biomacromolecules* **2009**, *10*, 2646.
[70] T. Liebmann, S. Rydholm, V. Akpe, H. Brismar, *BMC Biotechnology* **2007**, *7*, 88.
[71] I. Tomatsu, K. Peng, A. Kros, *Adv. Drug Delivery Rev.* **2011**, *63*, 1257.
[72] T. Muraoka, C. Y. Koh, H. G. Cui, S. I. Stupp, *Angew. Chem. Int. Ed.* **2009**, *48*, 5949.
[73] H. Komatsu, S. Tsukiji, M. Ikeda, I. Hamachi, *Chem. Asian J.* **2011**, *6*, 2368.
[74] M. T. He, J. B. Li, S. Tan, R. Z. Wang, Y. Zhang, *J. Am. Chem. Soc.* **2013**, *135*, 18718.
[75] a) C. Vigier-Carriere, T. Garnier, D. Wagner, P. Lavalle, M. Rabineau, J. Hemmerle, B. Senger, P. Schaaf, F. Boulmedais, L. Jierry, *Angew. Chem. Int. Ed.* **2015**, *54*, 10198; b) Y. Gao, Y. Kuang, Z. F. Guo, Z. H. Guo, I. J. Krauss, B. Xu, *J. Am. Chem. Soc.* **2009**, *131*, 13576; c) J. F. Shi, D. Yuan, R. Haburcak, Q. Zhang, C. Zhao, X. X. Zhang, B. Xu, *Chem. Eur. J.* **2015**, *21*, 18047; d) J. Zhou, X. W. Du, Y. Gao, J. F. Shi, B. Xu, *J. Am. Chem. Soc.* **2014**, *136*, 2970.
[76] a) Z. M. Yang, H. W. Gu, D. G. Fu, P. Gao, K. J. K. Lam, B. Xu, *Adv. Mater.* **2004**, *17*, 1440; b) Z. M. Yang, G. L. Liang, L. Wang, B. Xu, *J. Am. Chem. Soc.* **2006**, *128*, 3038; c) F. Zhao, C. S. Weitzel,

Y. Gao, H. M. Browdy, J. F. Shi, H. C. Lin, S. T. Lovett, B. Xu, *Nanoscale* **2011**, *3*, 2859.
[77] Z. M. Yang, G. L. Liang, M. L. Ma, Y. Gao, B. Xu, *Small* **2007**, *3*, 558.
[78] G. Q. Chen, J. X. Chen, Q. C. Liu, C. W. Ou, J. Gao, *RSC Adv.* **2015**, *5*, 30675.
[79] X. Qin, W. C. Xie, S. Tian, J. L. Cai, H. Yuan, Z. Yu, G. L. Butterfoss, A. C. Khuong, R. A. Gross, *Chem. Commun.* **2013**, *49*, 4839.
[80] S. Toledano, R. J. Williams, V. Jayawarna, R. V. Ulijn, *J. Am. Chem. Soc.* **2006**, *128*, 1070.
[81] L. Chronopoulou, S. Lorenzoni, G. Masci, M. Dentini, A. R. Tongana, G. Togna, F. Bordic, C. Palocci, *Soft Matter* **2010**, *6*, 2525.
[82] L. Chronopoulou, A. R. Togna, G. Guarguaglini, G. Masci, F. Giammaruco, G. I. Togna, C. Palocci, *Soft Matter* **2012**, *8*, 5784.
[83] a) Y. Gao, J. F. Shi, D. Yuan, B. Xu, *Nat. Commun.* **2012**, *3*, 1033; b) Z. M. Yang, G. L. Liang, B. Xu, *Acc. Chem. Res.* **2008**, *41*, 315; c) J. F. Shi, X. W. Du, D. Yuan, J. Zhou, N. Zhou, Y. B. Huang, B. Xu, *Biomacromolecules* **2014**, *15*, 3559; d) J. Zhou, X. W. Du, N. Yamagata, B. Xu, *J. Am. Chem. Soc.* **2016**, *138*, 3813.
[84] a) Y. Kuang, M. J. C. Long, J. Zhou, J. F. Shi, Y. Gao, C. Xu, L. Hedstrom, B. Xu, *J. Biol. Chem.* **2014**, *289*, 29208; b) J. F. Shi, B. Xu, *Nano Today* **2015**, *10*, 615.
[85] a) R. A. Pires, Y. M. Abul-Haija, D. S. Costa, R. Novoa-Carballal, R. L. Reis, R. V. Ulijn, I. Pashkuleva, *J. Am. Chem. Soc.* **2015**, *137*, 576; b) S. Fleming, R. V. Ulijn, *Chem. Soc. Rev.* **2014**, *43*, 8150.
[86] A. Tanaka, Y. Fukuoka, Y. Morimoto, T. Honjo, D. Koda, M. Goto, T. Maruyama, *J. Am. Chem. Soc.* **2015**, *137*, 770.
[87] a) C. Yang, M. Bian, Z. Yang, *Biomater. Sci.* **2014**, *2*, 651; b) C. Ren, J. Zhang, M. Chen, Z. Yang, *Chem. Soc. Rev.* **2014**, *43*, 7257.
[88] J. A. Zorn, H. Wille, D. W. Wolan, J. A. Wells, *J. Am. Chem. Soc.* **2011**, *133*, 19630.
[89] L. N. Lv, H. X. Liu, X. M. Chen, Z. M. Yang, *Colloids Surf., B* **2013**, *108*, 352.
[90] X. L. Zhang, X. L. Chu, L. Wang, H. M. Wang, G. L. Liang, J. X. Zhang, J. F. Long, Z. M. Yang, *Angew. Chem. Int. Ed.* **2012**, *51*, 4388.
[91] H. M. Wang, Z. M. Yang, *Nanoscale* **2012**, *4*, 5259.
[92] J. Y. Wang, J. X. Zhang, X. L. Zhang, H. Zhou, *PloS One* **2013**, *8*, e75727.
[93] Y. Zhang, H. W. Gu, Z. M. Yang, B. Xu, *J. Am. Chem. Soc.* **2003**, *125*, 13680.
[94] Y. Zhang, Z. M. Yang, F. Yuan, H. W. Gu, P. Gao, B. Xu, *J. Am. Chem. Soc.* **2004**, *126*, 15028.
[95] P. Li, Z. Q. Yin, X. Q. Dou, G. D. Zhou, C. L. Feng, *ACS Appl. Mater. Interfaces* **2014**, *6*, 7948.
[96] S. Marchesan, C. D. Easton, K. E. Styan, L. J. Waddington, F. Kushkaki, L. Goodall, K. M. Mclean, J. S. Forsythe, P. G. Hartley, *Nanoscale* **2014**, *6*, 5172.
[97] W. Y. Seow, C. A. E. Hauser, *Adv. Healthcare Mater.* **2013**, *2*, 1219.
[98] Z. X. Zhang, Q. X. Zheng, Y. C. Wu, D. J. Hao, *Biotechnol. Bioprocess Eng.* **2010**, *15*, 545.
[99] R. S. Jacob, D. Ghosh, P. K. Singh, S. K. Basu, N. N. Jha, S. Das, P. K. Sukul, S. Patil, S. Sathaye, A. Kumar, A. Chowdhury, S. Malik, S. Sen, S. K. Maji, *Biomaterials* **2015**, *54*, 97.
[100] A. K. Das, I. Maity, H. S. Parmar, T. O. McDonald, M. Konda, *Biomacromolecules* **2015**, *16*, 1157.
[101] V. A. Mallia, M. George, D. L. Blair, R. G. Weiss, *Langmuir* **2009**, *25*, 8615.
[102] Y. Kuang, J. F. Shi, J. Li, D. Yuan, K. A. Alberti, Q. B. Xu, B. Xu, *Angew. Chem. Int. Ed.* **2014**, *53*, 8104.
[103] J. H. Kim, S. Y. Lim, D. H. Nam, J. Ryu, S. K. Ku, C. B. Park, *Biosens. Bioelectron.* **2011**, *26*, 1860.
[104] Y. Gao, J. F. Shi, D. Yuan, B. Xu, *Nat. Commun.* **2012**, *3*, 1033.
[105] K. J. C. van Bommel, A. Friggeri, M. C. A. Stuart, B. L. Feringa, J. van Esch, *J. Controlled Release* **2005**, *101*, 287.
[106] a) J. S. Rudra, Y. F. Tian, J. P. Jung, J. H. Collier, *Pro. Natl. Acad. Sci. USA* **2010**, *107*, 622; b) F. Zhao, B. A. Heesters, I. Chiu, Y. Gao, J. F. Shi, N. Zhou, M. C. Carroll, B. Xu, *Org. Biomol. Chem.* **2014**, *12*, 6818.
[107] F. Zhao, J. Y. Li, N. Zhou, J. Sakai, Y. Gao, J. F. Shi, B. Goldman, H. M. Browdy, H. R. Luo, B. Xu, *Bioconjugate Chem.* **2014**, *25*, 2116.