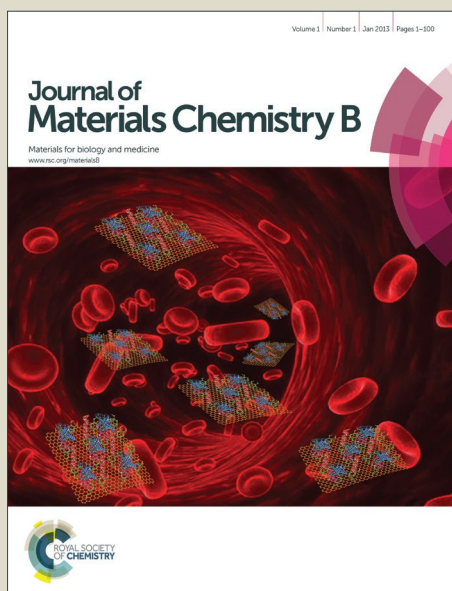


Journal of Materials Chemistry B

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

COMMUNICATION

Designed Fabrication of Super-Stiff Hybrid Hydrogel via Linear Remodeling of Polymer Networks and Subsequent Crosslinking

Cite this: DOI: 10.1039/x0xx00000x

Suji Choi and Jaeyun Kim*

Received
Accepted

DOI: 10.1039/x0xx00000x

www.rsc.org/

Super-stiff hybrid hydrogels were prepared via a linear remodeling of highly stretchable alginate/polyacrylamide hydrogel networks followed by a secondary cross-linking. The elastic modulus of the hybrid hydrogels were successfully controlled by the degree of stretch, polymer composition, and crosslinking cations.

Hydrogels are useful for diverse applications such as tissue engineering, drug delivery and immunotherapy.¹⁻⁵ The mechanical properties of hydrogels are an important aspect of their ability to fulfill the requirements of these applications. Most hydrogels are soft and weak because they possess high amounts of water within their polymer network, but several efforts have been made over the past decade to enhance the mechanical properties of hydrogels. Double-network hydrogels have been prepared by interpenetrating short and long chain polymers to efficiently dissipate the applied energy.⁶⁻¹³ Additionally, functional nano- and meso-sized inorganic nanofillers, such as carbon nanotubes¹⁴, graphene oxide¹⁵⁻¹⁷, clays^{18,19}, and others²⁰⁻²⁴, have been added to hydrogels to enhance their mechanical properties. Homogeneous network hydrogels, including tetra-arm polymer hydrogels and slide ring hydrogels, have also been proposed to achieve high stretchability via their unique structural features.²⁵⁻²⁷

Ca-alginate/polyacrylamide (PAM) hydrogels, a double-network hydrogel, have been developed recently by forming ionic crosslinking in alginate and covalent crosslinking in PAM networks. These structures produce highly-stretchable and tough hydrogels. When these hybrid hydrogels are stretched, the ionically crosslinked alginate chains are decrosslinked and broken. However, because the PAM chains stabilize deformation when alginate chains are broken, the shape of the hybrid hydrogel remains intact. Although the alginate chains which were broken would not be recovered, it could be recrosslinked by unloading. Thereby energy dissipation is dramatically increased.^{8,9} The strength of alginate/PAM hybrid hydrogels can be further enhanced

depending on the ionic crosslinking chemistry, which uses diverse multivalent cations.¹¹ For example, the elastic moduli of hybrid hydrogels crosslinked with trivalent cations, such as Al³⁺ or Fe³⁺, are 4- to 6-fold higher, respectively, than Ca-alginate/PAM hybrid hydrogels. More recently, the use of short-chain alginates, which have a lower viscosity in aqueous solution than conventional alginates, allows for a higher alginate concentration in the preparation of hybrid hydrogels. This yields a 34-fold increase in stiffness compared to previous Ca-alginate/PAM hydrogels.¹² However, finding additional methods to control the mechanical properties of alginate/PAM hybrid hydrogels still remains a challenge for many applications including bone regeneration, artificial tissue construction, or other mechanical studies of hydrogels. In this study, we propose a facile method to significantly enhance the mechanical strength of alginate/PAM hydrogels via the remodeling followed by a secondary cross-linking process (RsC process) of polymer networks, which will make it possible to widen the upper limit of hydrogel stiffness under a defined polymer composition.

The Ca-alginate/PAM hybrid hydrogel is highly stretchable and reversible due to its interpenetrating polymer networks.⁸ We hypothesized that stretching the Ca-alginate/PAM hydrogel can induce a linear remodeling of the internal polymer networks and that the reorganized polymer networks can be substantially preserved by the secondary ionic crosslinking of alginate chains, even after removal of the stretching force (Fig. 1). This approach might lead to an irreversible, fixed state of stretched double polymer networks. To test this idea, interpenetrating double network hydrogels composed of 2 wt% alginate/12 wt% PAM were prepared by simultaneous ionic crosslinking of alginate with Ca²⁺ ions and photopolymerization of acrylamide. The resulting Ca-alginate/PAM hydrogel was easily stretchable and the stretched hydrogels could be returned to their initial length after being stretched by 200% (Fig. 2a). In contrast, subsequent crosslinking of the stretched hydrogels with Ba²⁺ ions resulted in an elongated morphology that did not return to its initial state. Although

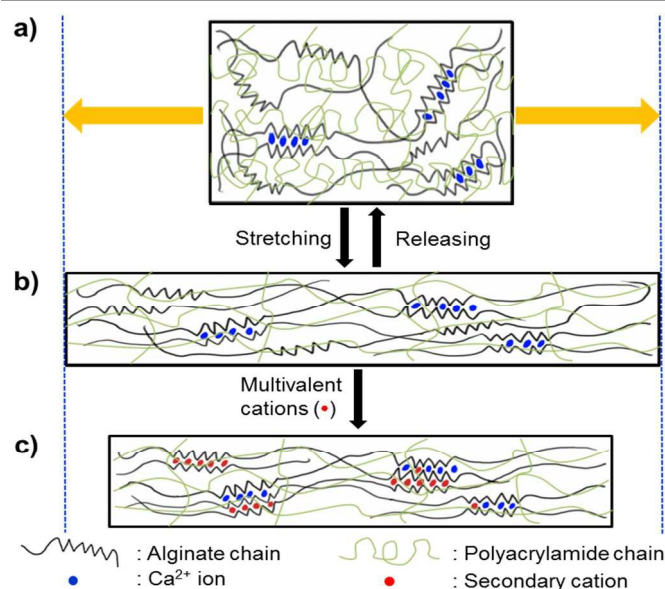


Fig. 1 Schematics of a linear remodeling of the alginate/PAM hybrid hydrogel networks and secondary cross-linking, a RsC process. (a) Alginate/PAM hybrid hydrogel. (b) Linear remodeling of the internal polymer networks by stretching. (c) Stiff hydrogel obtained by secondary crosslinking, which is a irreversible state of the linearly remodeled hydrogel networks.

the final length of the resulting Ba-alginate/PAM hydrogels (after removal of the stretching force) was shorter than the stretched length, they remained 175% and 225% of the initial length of the Ca-alginate/PAM hydrogel upon being stretched by 200% and 300%, respectively. These results indicate that the stretched polymer networks in the hybrid hydrogels can be fixed to a certain extent by the secondary ionic crosslinking of alginate chains.

To investigate linear remodeling of polymer networks in the resulting hydrogels, mesoporous silica microrods, with a high aspect ratio (L : 82 μm and D : 5 μm) and labeled with fluorescent dyes, were incorporated into the Ca-alginate/PAM hydrogel. The orientation of these fluorescent silica microrods was visualized using a fluorescent microscope before and after stretching. The mesoporous silica microrods in the intact hydrogel were randomly distributed in the hydrogels (Fig. 2b). In contrast, most silica microrods within the hydrogel were linearly aligned after stretching the hydrogel (Fig. 2c). The surface of the stretched/cross-linked Ba-alginate/PAM hydrogel also clearly showed linear patterns in the direction of stretching on SEM, while there were no recognizable patterns on control gels (Fig. S1 in the ESI†). These results indicate that the random polymer networks in the hybrid hydrogel were also likely remodeled into more-linear networks after stretching and the subsequent ionic crosslinking.

To evaluate the effects of the stretching/cross-linking process on the mechanical properties of the hybrid hydrogel, the stiffness of the hydrogels was measured using tensile testing (Fig. 3a). During tensile testing in air at room temperature, as the testing time was very short within a few minutes, the evaporation of water from the hydrogels was insignificant to induce notable change of the elastic modulus. First, to determine the effect of the Ba-crosslinker on the stiffness, Ca-alginate/PAM hydrogels were prepared and subsequently crosslinked with Ba^{2+} without a prior stretching step (Ba-alginate/PAM). As expected, Ba-alginate/PAM hydrogels showed an increase in elastic

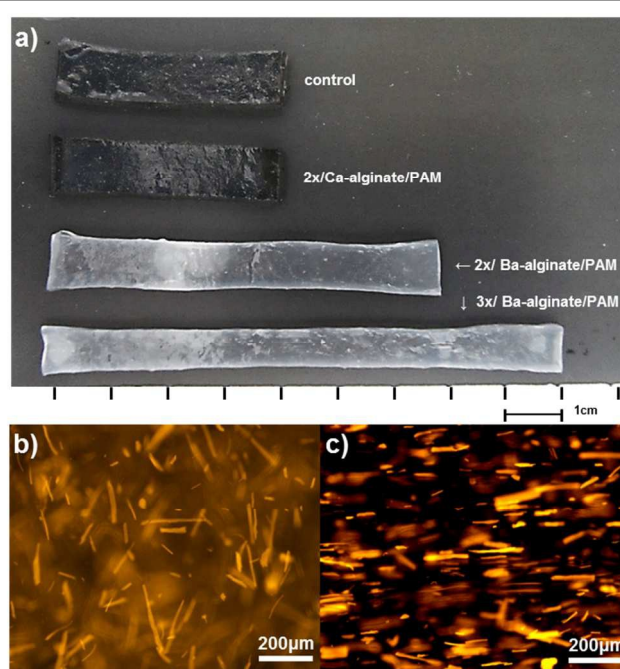


Fig. 2 (a) Photograph of Ca-alginate/PAM hydrogel (control), Ca-alginate/PAM hydrogel after 200% stretching and releasing the stretching force (2x/Ca-alginate/PAM), and two RsC hydrogels after 200% (2x/Ba-alginate/PAM) or 300% (3x/Ba-alginate/PAM) followed by barium crosslinking. (b, c) fluorescent microscope images of a Ba-alginate/PAM hybrid hydrogel prepared (b) without a stretching step and (c) with stretching to 300% of its initial length.

modulus compared to the initial gel because Ba^{2+} is known to be a stronger ionic crosslinker of alginate than Ca^{2+} .¹¹ Next, the tensile modulus of the hybrid hydrogels prepared by the RsC process was measured in the direction parallel to stretching (transverse modulus). Surprisingly, as the hydrogels were stretched longer, the elastic modulus in the resulting hydrogels was remarkably enhanced after subsequent Ba-crosslinking (Fig. 3b). For example, the elastic modulus of hybrid hydrogels stretched to 300% of their initial length and subsequently crosslinked with Ba^{2+} (3x/Ba-alginate/PAM hydrogels) was 1088 kPa, which represents a value that is almost 64 times higher than that of the original Ca-alginate/PAM hydrogels. Even compared to the 1x/Ba-alginate/PAM hydrogels, the elastic modulus was 6 times higher in 3x/Ba-alginate/PAM hydrogels (Fig. 3b). These results demonstrate that the extent of linear remodeling and fixing of the double polymer networks is critical to the remarkable enhancement of the resulting hydrogel stiffness. The water contents in the hydrogels were not significantly changed after RsC process with different extents of stretching (Fig. S2 in the ESI†), indicating that the enhancement of mechanical property of the RsC hybrid hydrogels was not attributed to the change of water content in RsC hydrogels.

When the hydrogel was stretched, randomly arranged alginate chains become taut toward the stretching directions and linearly aligned, which causes the anisotropy in hybrid polymer network after secondary crosslinking (Fig. 1). The G block of alginate chains which did not participate in early calcium crosslinking could form new crosslinking sites between linearly stretched alginate chains. In addition, the secondary crosslinking ions (Ba^{2+} , Al^{3+} or Fe^{3+}) with stronger binding affinity to G blocks of alginate could replace pre-binding Ca^{2+} ions in alginate hydrogels. These procedures could lead to

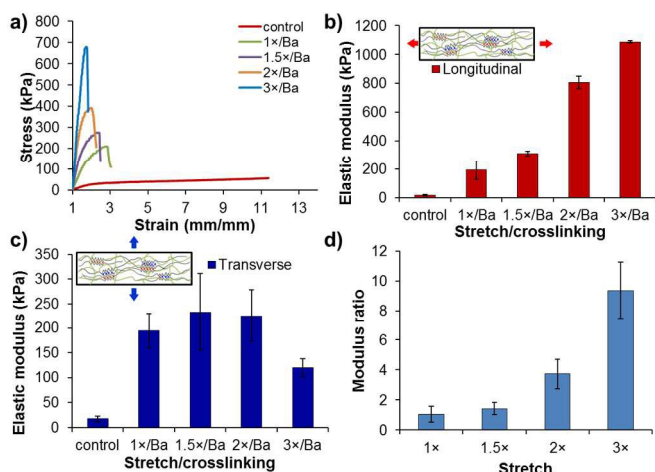


Fig. 3 Effects of the degree of stretch on the mechanical properties. (a) Stress-strain curve of Ba-alginate/PAM hybrid hydrogels prepared with different degrees of stretching. (b) Longitudinal and (c) transverse elastic moduli of the RsC hydrogels. (d) The ratio between the longitudinal and transverse elastic moduli according to the degree of stretching.

maintain the anisotropic polymer network after secondary crosslinking.

The RsC hydrogels were expected to have anisotropic properties due to the linear reorganization of the internal polymer network. To investigate their anisotropic properties, the transverse (perpendicular to the stretching direction) moduli of the stiff gels were also measured (Fig. 3c). In all stretched samples, the transverse moduli were smaller than the longitudinal moduli shown in Fig. 3b. Although all transverse moduli were higher than control Ca-alginate/PAM hydrogel, in contrast to the longitudinal moduli, the transverse moduli were nearly constant except a decrease in the 3x/Ba-alginate/PAM hydrogel. The ratios between the longitudinal and transverse elastic moduli increased steeply as the extent of stretching was increased (Fig. 3d). These differences between the longitudinal and transverse moduli of the RsC hydrogels clearly represent their anisotropic structure obtained by RsC process.

Next, we investigated the effects of polymer compositions of the RsC hydrogels on their mechanical properties. Hybrid hydrogels containing various proportions of alginate and acrylamide were prepared to find the concentration that produces the stiffest RsC hydrogel. Fig. 4a shows the stress-strain curves of the 2x/Ba-alginate/PAM hydrogels prepared using different relative ratio of alginate and PAM while the total polymer concentration was fixed at 14 wt% (the enlarged Fig. 4a was provided as Fig. S3 in the ESI†). The corresponding elastic moduli of the hydrogels were shown in Fig. 4b. As the ratio of alginate was increased, the elastic modulus of the resulting stiff gel was significantly increased. In contrast, the degree of maximum strain was inversely proportional to the alginate ratio (Fig. 4a), demonstrating that the higher stiffness compensates for the stretchability in the stiff hydrogels. We performed another set of experiments to determine the effect of the absolute alginate amount on the hydrogel stiffness under a fixed amount of PAM (12 wt%) (Fig. 4c, d). The elastic modulus of the hydrogel increased as the amount of alginate was increased, which represents that the crosslinking of the linearly aligned alginate is main reason for the enhanced stiffness of the hydrogels prepared by RsC process. However, the maximum strains of the hydrogels with different alginate amounts were all similar (Fig. 4c),

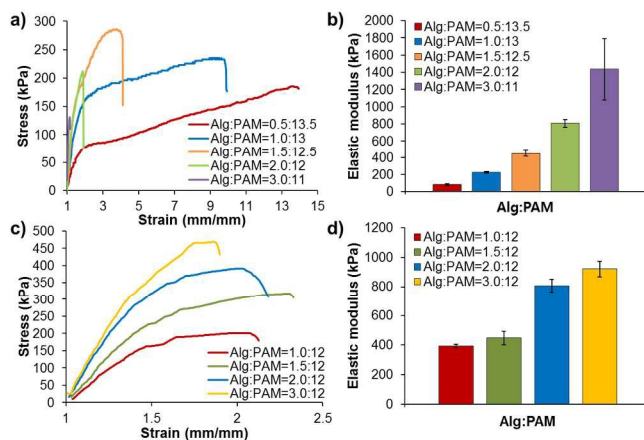


Fig. 4 Effects of polymer composition on the mechanical properties of hybrid hydrogels. (a) Stress-strain curves and (b) the corresponding elastic moduli of 2x/Ba-alginate/PAM hydrogels with various polymer ratio with a fixed total polymer concentration of 14 wt%. (c) Stress-strain curves and (d) the corresponding elastic moduli of 2x/Ba-alginate/PAM hydrogels with different alginate amounts with a fixed PAM concentration of 12 wt%.

which is probably due to the fact that there is the same amount of PAM in each hydrogel. Taken together, the alginate and PAM mainly contribute to the stiffness and stretchability of the RsC hydrogels, respectively. Based on our data, the optimized polymer composition to prepare the stiffest alginate/PAM hybrid hydrogels was 3 wt% alginate and 11 wt% PAM.

The stiffness of the ionically crosslinked alginate hydrogels is on the order of $\text{Fe}^{3+} > \text{Al}^{3+} > \text{Ba}^{2+} > \text{Ca}^{2+}$, which is caused by differences in the binding affinities between the alginate chains and the cations.^{28,29} To further enhance the stiffness of RsC hydrogels, trivalent cations were used. The same order in stiffness in pure alginate hydrogels was also observed in the RsC hydrogels prepared by 300% stretching (Fig. 5a). As observed in barium crosslinking in RsC hydrogels, more stretching led to stiffer hydrogels for subsequent crosslinking with all cations (Fig. S4 in the ESI†). By using Fe^{3+} ions and 300% stretching, we could obtain unprecedented, extremely high stiffness in the alginate/PAM hybrid hydrogels. 3x/Fe-alginate/PAM hydrogels composed of 3 wt% alginate and 11 wt% PAM showed a stiffness of 5.33 MPa (Fig. 5a); this is more than 5 times higher compared to the highest stiffness (~1 MPa) of the alginate/PAM hybrid hydrogels reported previously.¹⁸

To further demonstrate the stiffness of the RsC hydrogels, the

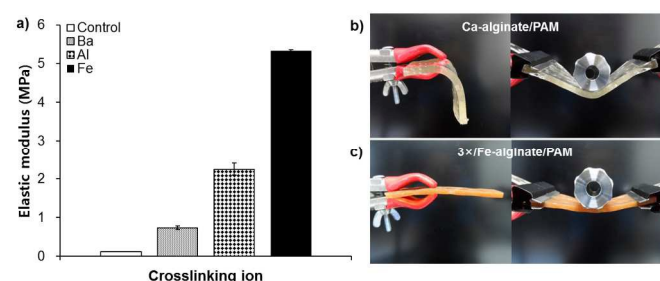


Fig. 5 (a) Elastic modulus of RsC hydrogels prepared via crosslinking with different cations under same 300% stretching. The control corresponds to the initial Ca-alginate/PAM hydrogel. Photographs of (b) Ca-alginate/PAM hydrogel and (c) 3x/Fe-alginate/PAM hydrogel to show an enhanced mechanical property in RsC hydrogel.

3×/Fe-alginate/PAM hydrogel was prepared and compared with the control Ca-alginate/PAM hydrogel. When one end of the Ca-alginate/PAM hydrogel (length: 80 mm, width: 50 mm, thickness 3 mm) was held by a clamp, the other end of the hydrogel could not retain its shape and bent due to gravity because it was very soft (Fig. 5b). In contrast, the 3×/Fe-alginate/PAM hydrogel retained its shape, exhibiting higher stiffness (Fig. 5c). Taken together, the simple RsC approach could make it possible to substantially enhance the stiffness of alginate/PAM hybrid hydrogels in a controlled manner. The super-stiff hydrogels prepared by the RsC process have great potential for utilization in various applications where substantial mechanical properties are demanded.

Conclusions

In conclusion, we report a simple method to control the mechanical properties of alginate/PAM hybrid hydrogels through linear remodelling and secondary crosslinking of the internal polymer networks in the hydrogels. The polymer networks were linearly remodelled by stretching and fixed by the subsequent ionic crosslinking of alginate chains. This simple process led to significant enhancement of the elastic modulus of the hydrogels compared to control hybrid hydrogels. The elastic modulus was strongly dependent on the extent of stretching of the polymer networks. In addition, the type of secondary crosslinking cations and the polymer composition ratio could further control the elastic modulus. This simple approach will increase the range of hydrogel stiffness under a defined polymer composition, which may be beneficial in many applications including bone regeneration, artificial tissue construction, or other mechanical studies of hydrogels.

Acknowledgements

This work was supported by the NRF grants (2010-0027955, 2012R1A1A1042735) funded by the National Research Foundation under the Ministry of Science, ICT & Future Planning, Korea.

Notes

Affiliation: School of Chemical Engineering, Sungkyunkwan University, Suwon 440-746, Korea. E-mail: kimjaeyun@skku.edu

† Electronic supplementary information (ESI) available: Experimental, SEM images of the surface of hydrogels before and after the RsC process (Fig. S1), the water contents of RsC hydrogels with different degrees of stretch (Fig. S2), the enlarged version of Fig. 4a (Fig. S3), the effects of crosslinking ions on the elastic modulus of the RsC hydrogels (Fig. S4).

References

- K. Y. Lee, D. J. Mooney, *Chem. Rev.* 2001, **101**, 1869-1880.
- Y. Qiu, K. Park, *Adv. Drug Deliv. Rev.* 2001, **53**, 321-339.
- J. S. Lee, J. Shin, H. Park, Y. Kim, B. Kim, J. Oh, S. Cho, *Biomacromolecules* 2013, **15**, 206-218.
- O. Jeon, K. H. Bouhadir, J. M. Mansour, E. Alsberg, *Biomaterials* 2009, **30**, 2724-2734.
- H. Miyajima, T. Matsumoto, T. Sakai, S. Yamaguchi, S. H. An, M. Abe, S. Wakisaka, K. Y. Lee, H. Egusa, S. Imazato, *Biomaterials* 2011, **32**, 6754-6763.
- J. P. Gong, Y. Katsuyama, T. Kurokawa, Y. Osada, *Adv. Mater.* 2003, **15**, 1155-1158.
- M. A. Haque, T. Kurokawa, G. Kamita, J. P. Gong, *Macromolecules* 2011, **44**, 8916-8924.
- J. Sun, X. Zhao, W. R. Illeperuma, O. Chaudhuri, K. H. Oh, D. J. Mooney, J. J. Vlassak, Z. Suo, *Nature* 2012, **489**, 133-136.
- S. E. Bakarich, G. C. Pidgeon, P. Balding, L. Stevens, P. Calvert, M. Panhuis, *Soft Matter* 2012, **8**, 9985-9988.
- N. Russ, B. I. Zielbauer, K. Koynov, T. A. Vilgis, *Biomacromolecules* 2013, **14**, 4116-4124.
- C. H. Yang, M. X. Wang, H. Haider, J. H. Yang, J. Sun, Y. M. Chen, J. Zhou, Z. Suo, *ACS Appl. Mater. Interfaces* 2013, **5**, 10418-10422.
- J. Li, W. R. Illeperuma, Z. Suo, J. J. Vlassak, *ACS Macro Lett.* 2014, **3**, 520-523.
- Y. Zhao, T. Nakajima, J. J. Yang, T. Kurokawa, J. Liu, J. Lu, S. Mizumoto, K. Sugahara, N. Kitamura, K. Yasuda, A. U. D. Daniels, J. P. Gong, *Adv. Mater.* 2014, **26**, 436-442.
- R. Du, J. Wu, L. Chen, H. Huang, X. Zhang, J. Zhang, *Small* 2014, **10**, 1387-1393.
- S. R. Shin, B. Aghaei-Ghareh-Bolagh, T. T. Dang, S. N. Topkaya, X. Gao, S. Y. Yang, S. M. Jung, J. H. Oh, M. R. Dokmeci, X. S. Tang, A. Khademhosseini, *Adv. Mater.* 2013, **25**, 6385-6391.
- C. Cha, S. R. Shin, X. Gao, N. Annabi, M. R. Dokmeci, X. S. Tang, A. Khademhosseini, *Small* 2014, **10**, 514-523.
- H. Cong, P. Wang, S. Yu, *Small* 2014, **10**, 448-453.
- K. Haraguchi, R. Farnworth, A. Ohbayashi, T. Takehisa, *Macromolecules* 2003, **36**, 5732-5741.
- T. Wang, S. Zheng, W. Sun, X. Liu, S. Fu, Z. Tong, *Soft Matter* 2014, **10**, 3506-3512.
- T. Huang, H. Xu, K. Jiao, L. Zhu, H. R. Brown, H. Wang, *Adv. Mater.* 2007, **19**, 1622-1626.
- Z. Hu, G. Chen, *Adv. Mater.* 2014, **26**, 5950-5956.
- Y. Sun, G. Gao, G. Du, Y. Cheng, J. Fu, *ACS Macro Lett.* 2014, **3**, 496-500.
- S. Lin, C. Cao, Q. Wang, M. Gonzalez, J. E. Dolbow, X. Zhao, *Soft Matter* 2014, **10**, 7519-7527.
- T. Sawada, S. Kang, J. Watanabe, H. Mihara, T. Serizawa, *ACS Macro Lett.* 2014, **3**, 341-345.
- Y. Okumura, K. Ito, *Adv. Mater.* 2001, **13**, 485-487.
- T. Sakai, T. Matsunaga, Y. Yamamoto, C. Ito, R. Yoshida, S. Suzuki, N. Sasaki, M. Shibayama, U. Chung, *Macromolecules* 2008, **41**, 5379-5384.
- T. Sakai, Y. Akagi, T. Matsunaga, M. Kurakazu, U. Chung, M. Shibayama, *Macromol. Rapid Commun.* 2010, **31**, 1954-1959.
- B. Lee, G. Min, T. Kim, *Arch. Pharm. Res.* 1996, **19**, 280-285.
- Y. A. Mørch, I. Donati, B. L. Strand, G. Skjåk-Bræk, *Biomacromolecules* 2006, **7**, 1471-1480.