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Molecular co-assembly as a strategy for synergistic improvement of the mechanical properties of hydrogels†

Michal Halperin-Sternfeld, ‡ Moumita Ghosh, ‡ Rina Sevostianov, Irena Grigoriants and Lihi Adler-Abramovich *

Molecular self-assembly is a key direction for the fabrication of advanced materials. Yet, the physical properties of the formed assemblies are limited by the inherent characteristics of the specific building blocks. Here, we have applied a co-assembly approach to synergistically modulate the mechanical properties of peptide hydrogels, thereby forming extremely stable and rigid hydrogels.

Molecular self-assembly serves as a key approach for the formation of biocompatible peptide-based architectures, including nanospheres, nanotubes, nanosheets, nanofibrils and vesicles.[1–4] In addition, short peptides can form three-dimensional (3D) hydrogels. These low molecular weight hydrogelators have been widely explored in recent years.[2,3,5–7] They are particularly valuable for biotechnological and medical applications, due to their ability to act as building blocks for three-dimensional macroscopic structure formation with nanoscale order, which mimics the extracellular matrix.[2,3,5,6] Peptide-based hydrogels have been found to form a supporting scaffold for the growth of cells and are being used in the field of regenerative medicine.[8] Self-assembled ultra-short peptide building blocks have been proposed as hydrogelators due to their easy fabrication and simple chemical and biological decoration.[9,10] These advantages are unique to peptide hydrogels compared to many natural or synthetic hydrogels.[6] Recently, it has been shown that a peptide-based hydrogel could be printed in order to support human stem cells differentiation,[11] and functional motifs could be linked to self-assembled peptides to allow such differentiation and proliferation.[12] A large number of studies has focused on fluorenylmethoxycarbonyl (Fmoc)-modified oligopeptides and their ability to form hydrogels.[5,6] The common use of Fmoc as a protecting group in peptide synthesis makes this building block readily available. In addition, Fmoc-based hydrogels are very promising candidates in biomedical implementation due to their anti-inflammatory properties.[13] The supramolecular nature of these hydrogels results in typically weak materials, with storage moduli of ~1000 Pa or less. There are several examples of hydrogels with high storage modulus values, including peptide-based hydrogels reinforced with carboxylated-carbon nanotubes,[14] covalent conjugation of D-amino acid to NSAIDs,[15] peptide-based hydrogels linked by electrostatic interactions through Ca^{2+} ions, dendrimers with multiple adhesive termini for binding to clay that form a hydrogel,[16] certain di- and tripeptide sequences bearing Fmoc or indole capping groups,[9,17–19] and genetically engineered protein-based hydrogels.[20] A notable example of Fmoc-based hydrogels is the Fmoc-diphenylalanine (Fmoc-FF) peptide that efficiently assembles into a fibrous hydrogel under physiological conditions.[9,21] This building block encompasses the well-studied FF dipeptide motif that self-assembles into ordered structures and shows unique properties enabling its utilization for various applications.[1] Furthermore, Fmoc-modified aromatic amino acid analogues, namely Fmoc-Phe and Fmoc-Tyr, were shown to form ordered fibrillar assemblies,[22] consistent with the ability of single phenylalanine to form ordered structures.[23] In addition, Fmoc-modified non-coded single aromatic amino acids, including Fmoc-DOPA,[24] naphthyl modified Fmoc-alanine[25] and fluorinated Fmoc-Phe derivatives,[26] have also been investigated as hydrogelators. The fluorinated peptide derivatives of Fmoc-Phe include Fmoc-pentafluoro-phenylalanine (Fmoc-F_5-Phe), where the halogenation of the phenyl side-chain results in rapid self-assembly when compared to non-substituted Fmoc protected analogues.[26] The fluoride function has been demonstrated when short phenylalanine-containing peptides were modified to form antifouling coatings.[27] Fluoride has also been widely applied in dentistry as a non-invasive treatment of root caries lesions[28] and is incorporated into dental restoration materials[29] due to its antibacterial characteristics, including inhibition of bacterial adsorption to hydroxyapatite, interfering with bacterial metabolism and dental plaque acidogenicity.[30]

In spite of their advantages, the physical properties of short peptide- and amino acid-based hydrogels are limited due to the chemical nature of the chosen building blocks, making the modulation of these physical properties highly challenging. It has been previously shown that the co-assembly of two building blocks into

Department of Oral Biology, The Goldschleger School of Dental Medicine, Sackler Faculty of Medicine, Tel Aviv University, 69978, Israel.
E-mail: lihiA@tauex.tau.ac.il

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‡ These authors contributed equally to this work.

one ordered structure can promote the formation of a new composite material exhibiting new architectures,[31,32] higher and tunable mechanical properties,[31–35] higher stability,[34] and improved biofunctionality.[36–40] This concept is naturally occurring in biological systems, such as the extracellular matrix, the physical properties of which are modulated by combining several basic building blocks, including proteins and polysaccharides. Previous studies have shown the formation hydrogels with enhanced mechanical properties using mixed systems and the co-assembly approach.[33–35,41–44] Using two or more building blocks mixed together enabled us to achieve improved properties compared to those of each of the building blocks alone. Bing Xu's group incorporated Fmoc-F into Fmoc-Leucine to produce higher elasticity and rapid recovery after stress.[43] In another study, Tendler's group fine-tuned the mechanical properties of a peptide nanostructure composed of FF and di-D-2-napthylalanine, two aromatic peptides known to self-assemble separately, by changing their relative concentration.[45] Another method to enhance the mechanical properties of hydrogels was a pH triggered approach proposed by Adam's group.[35]

In the current study, we explored the Fmoc-F_5-Phe hydrogel (Fig. 1a). In spite of its interesting properties, its rigidity and durability are inferior to other biomolecular hydrogels. Nilsson's group demonstrated that the co-assembly of Fmoc-F_5-Phe hydrogel with a C-terminal PEG-functionalized Fmoc-F_5-Phe resulted in higher rigidities of approximately 3 kPa.[34] Here, in order to stabilize the Fmoc-F_5-Phe hydrogel, we chose to harness the mechanical properties of Fmoc-FF hydrogels and study the formation of hybrid assemblies (Fig. 1b). The Fmoc-FF hydrogels were prepared using the solvent-switch method,[9] by dissolving the peptide in DMSO and then diluting the stock solution into water. Following the dilution, a rigid hydrogel was formed (Fig. 1b and c). The Fmoc-F_5-Phe hydrogel was also prepared using the solvent-switch method, by first dissolving the building block in ethanol, followed by dilution into water. Similarly, the dilution into aqueous solution resulted in the formation of a hydrogel (Fig. 1b and c). However, this hydrogel had low stability, as after seven days it collapsed and a phase separation was observed (Fig. 1d). In order to improve the physical stability of the Fmoc-F_5-Phe hydrogel for extended periods of time, we applied the co-assembly strategy. For this purpose, hybrid hydrogels were prepared using the two building blocks, Fmoc-F_5-Phe and Fmoc-FF, at three stoichiometric ratios of 3 : 1, 1 : 1 and 1 : 3 by mixing both stock solutions and then diluting them into water. In all cases, a transparent homogenous hydrogel was formed (Fig. 1c). The stability of the hybrid hydrogels was monitored over a period of six months, and in all cases, a stable 3D structure was maintained. We further analyzed the gelation kinetics of all formed hydrogels. While Fmoc-F_5-Phe hydrogel formation occurred within seconds, the formation of a transparent rigid hydrogel using Fmoc-FF occurred only after ~4 minutes. The 3 : 1 and 1 : 3 hybrid hydrogels were found to be solid and transparent, and showed similar kinetics to that of the pure Fmoc-FF, forming after 5 minutes (Fig. 2a). In addition, in order to study the hydrogel underlying morphologies, we used transmission electron microscopy (TEM) analysis. TEM samples were prepared for the three hybrid hydrogels, as well as for the pure Fmoc-F_5-Phe and Fmoc-FF hydrogels. To this end, hydrogels were prepared at a concentration of 5 mg mL^{-1}, immediately after diluting the stock solutions in water (Fig. 2b–f). In all cases, the architectures comprised of fibrils of 15–25 nm in width. The pure Fmoc-F_5-Phe and Fmoc-FF hydrogels demonstrated tangled, several micron-long fibrils (Fig. 2b and c), while short and less tangled fibrils were observed in the 1 : 1 hybrid hydrogel samples. Notably, the fibrils of the 1 : 1 hybrid hydrogel were only a few microns long, shorter than those of the other hydrogels (Fig. 2c). The presence of fluorine within the fibrils of all hybrid hydrogels was demonstrated using energy-dispersive X-ray spectroscopy (EDS) analysis, suggesting they were indeed comprised of the two building blocks (Fig. 2g). Next, the mechanical properties of the hydrogels were examined using rheological analysis, which can also shed light on the kinetics of hydrogel formation. Strain sweep (at 5 Hz frequency) and frequency sweep (at 0.5% strain) oscillatory measurements were performed in order to optimize the appropriate measurement conditions. Fig. S1a and b (ESI†)

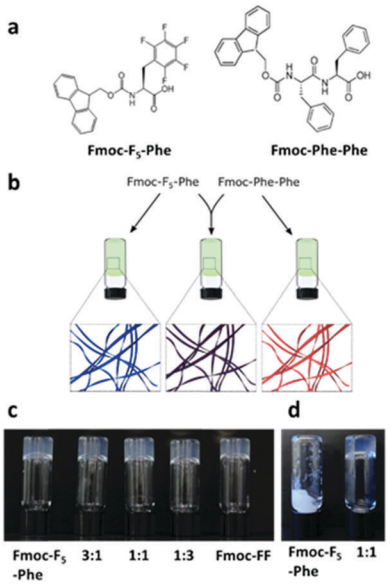

Fig. 1 (a) Molecular structures of Fmoc-FF and Fmoc-F_5-Phe. (b) Schematic presentation of the gelation process of each of the building blocks separately and of the formation of a hybrid hydrogel. (c) Inverted tubes of (left to right) Fmoc-F_5-Phe, Fmoc-F_5-Phe/Fmoc-FF 3 : 1, 1 : 1, 1 : 3, respectively and Fmoc-FF. (d) Inverted tubes of Fmoc-F_5-Phe (left) and 1 : 1 hybrid hydrogel (right) three weeks after preparation.

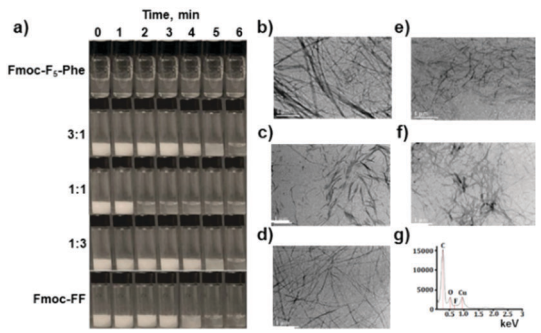

Fig. 2 (a) The formation kinetics of the different hydrogels, showing the transition from a cloudy mixture to a transparent hydrogel. (b–f) TEM micrographs of (b) Fmoc-F_5-Phe, (c) 1 : 1 hybrid hydrogel, (d) Fmoc-FF, (e) 3 : 1 hybrid hydrogel and (f) 1 : 3 hybrid hydrogel. (g) Energy-dispersive X-ray spectroscopy (EDS) analysis of the 3 : 1 hybrid hydrogel showing the fluoride peak.

present the results for the 1 : 1 hybrid hydrogel at 25 °C. Similar results were obtained for the other hydrogels, and the conditions for time sweep measurements were thus set to be 0.5% strain and 5 Hz frequency. The rheological analysis showed that a significant portion of gel rigidification is achieved within less than 6 minutes (Fig. 3a). However, the completion of the gelation and final rigidification, *i.e.* the time in which the storage modulus G' reaches its plateau, is a longer process. The individual Fmoc-FF hydrogel exhibited high rigidity, with a G' value of more than 9 kPa and a short gelation time of approximately 10 min (Fig. 3a). The Fmoc-F_5-Phe hydrogel reached a G' value of approximately 3.5 kPa after 2 hours, yet it continued to rise slowly, reaching a value of 4.2 kPa after 3 hours. A possible explanation for this phenomenon is the slow diffusion of the building blocks during the process of structural organization into fibers within a viscous solution, as the Fmoc-F_5-Phe solution became very viscous immediately following the dilution in water, while the Fmoc-FF solution remained in a liquid state for several minutes. The storage modulus of the 3 : 1 hybrid hydrogel reached a plateau within 6 min, with a G' value of approximately 22.5 kPa. The storage modulus of the 1 : 3 hybrid hydrogel reached a plateau after ~50 min, with a G' value of approximately 68.8 kPa. Strikingly, the 1 : 1 hybrid hydrogel reached a plateau after 40 min, with a considerably higher storage modulus of approximately 190 kPa (Fig. 3a). Notably, the final storage moduli of all hybrid hydrogels were higher than those of the pure single building block gels, reaching their plateau after 60 min. The 1 : 1 hybrid hydrogel was extremely rigid, with a very high storage modulus value at the endpoint (Fig. 3b), placing this hydrogel as one of the most rigid supramolecular hydrogels reported to date. This high mechanical rigidity at a low gelator concentration of 5 mg mL^{-1}, along with the easy tunability using various hybrid hydrogel ratios, would be of particular interest in tissue engineering and cell culture applications, where the nanoscale morphological and mechanical environment is vital for controlling various processes, such as stem cell differentiation.[46] Recently, it has been shown that stem cells can undergo stiffness-directed fate differentiation into neuronal, chondrogenic, and osteogenic lineages on soft (1 kPa), stiff (13 kPa) and rigid (32 kPa) hydrogels, respectively.[47,48] To further probe the internal organization of the hybrid hydrogels, we monitored their fluorescence spectra in the near-UV region, taking advantage of the characteristic fluorescence emission of the Fmoc aromatic moiety.[24] Generally, all hydrogels showed an emission peak at 320 nm (Fig. 3c and d). However, the 1 : 1 hybrid hydrogel spectra presented a second, more intense peak at 360 nm. The spectra of both the 1 : 3 and 3 : 1 hybrid hydrogels also presented increased emission at 360 nm, yet it manifested in combination with the 320 nm peak. These results suggest that new aromatic interactions arise in the hybrid hydrogels as compared to the individual hydrogels, further indicating that the hybrid hydrogels are formed through the co-assembly of the two building blocks. This co-assembly is most likely mediated by π–π interactions between the aromatic Fmoc groups in the two building blocks, as well as between the aromatic phenylalanine in Fmoc-FF and the halogenated phenylalanine side chains in the Fmoc-F_5-Phe, similar to previously reported co-assembly systems.[49,50] Time dependent fluorescence emission of the 1 : 1 hybrid hydrogel (Fig. 3d) showed that 6 min after the start of gelation, the fluorescence pattern was similar to that of either of the individual hydrogels, with a single peak at 320 nm, while the 360 nm peak emerged after 10 min, increasing gradually and concomitantly with the gelation kinetics, until reaching a maximum peak at 20 min. This further supports the notion that new molecular interactions arise as a result of a co-assembly process. The rigidity of all hybrid hydrogels prepared in this study is extraordinary, pointing out the Fmoc-F_5-Phe/Fmoc-FF hybrid hydrogel as one of the most rigid supramolecular hydrogels reported to date. Fig. 4 compares the storage moduli of different classes of hydrogels, namely synthetic (green region), including chitosan crosslinked with glutaraldehyde,[51] thiol modified hyaluronic acid,[52] Fmoc-FF and polyacrylamide gels,[53] natural (orange region), which includes collagen,[54] and hybrid gels (yellow region), such as chitosan/PEG,[55] Fmoc-FF/alginate,[56] hyaluronic acid /fibrinogen[52] and the Fmoc-F_5-Phe/Fmoc-FF hybrid gels reported in this study (blue region).

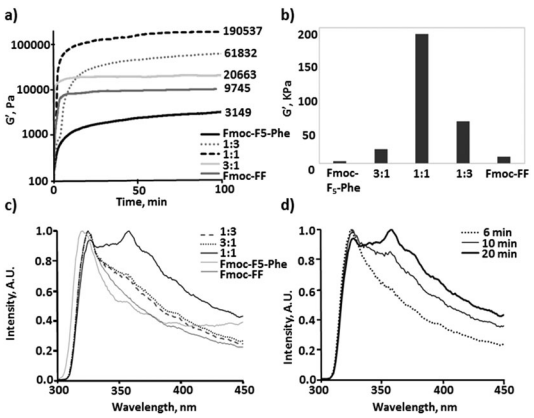

Fig. 3 Rheological and spectroscopic analysis of the hydrogels. (a) *In situ* time sweep oscillation measurements of hydrogel formation. (b) The end-point storage moduli G' of the various hydrogels, as determined by rheology. (c) Fluorescence spectra of different hydrogels taken 20 minutes after gel preparation. (d) Fluorescence spectra of the 1 : 1 hybrid hydrogel during gel formation taken 6, 10 and 20 min after gel preparation.

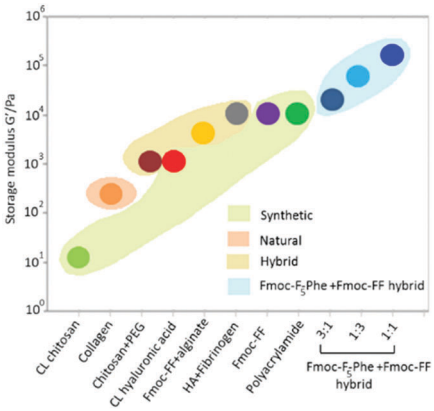

Fig. 4 The storage modulus (G') of different classes of hydrogels, namely synthetic (green region), which includes chitosan crosslinked with glutaraldehyde,[51] thiol modified hyaluronic acid,[52] Fmoc-FF and polyacrylamide gels,[53] natural (orange region), which includes collagen,[54] hybrid gels (yellow region), which include chitosan and PEG,[55] Fmoc-FF and alginate,[56] hyaluronic acid and fibrinogen,[52] and the hybrid Fmoc-F5-Phe/Fmoc-FF gels (blue region) reported in this study.

To summarize, in the present study we demonstrated, for the first time, the synergistic effect of the co-assembly of Fmoc-F_5-Phe and Fmoc-FF on the mechanical properties of the resulting hybrid hydrogels. The 1:1 hybrid formulation exhibited remarkable mechanical properties with a storage modulus as high as 190 kPa, an order of magnitude higher than hydrogels formed by each of the individual building blocks. Taken together, the different characteristics of the hybrid hydrogels demonstrate the supramolecular co-assembly approach to result in ultra-rigid hydrogels with controllable mechanical properties that can be utilized for tissue engineering and other biotechnological applications. We provide a conceptual framework for the significant expansion of the repertoire of nanomaterials to fully exploit the prospects of multi-component supramolecular chemistry.

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Notes and references

1 L. Adler-Abramovich and E. Gazit, *Chem. Soc. Rev.*, 2014, **43**, 6881–6893.
2 J. Lee, I. R. Choe, N.-K. Kim, W.-J. Kim, H.-S. Jang, Y.-S. Lee and K. T. Nam, *ACS Nano*, 2016, **10**, 8263–8270.
3 H.-S. Jang, J.-H. Lee, Y.-S. Park, Y.-O. Kim, J. Park, T.-Y. Yang, K. Jin, J. Lee, S. Park, J. M. You, K.-W. Jeong, A. Shin, I.-S. Oh, M.-K. Kwon, Y.-I. Kim, H.-H. Cho, H. N. Han, Y. Kim, Y. H. Chang, S. R. Paik, K. T. Nam and Y.-S. Lee, *Nat. Commun.*, 2014, **5**, 3665.
4 C. A. Hauser and S. Zhang, *Chem. Soc. Rev.*, 2010, **39**, 2780–2790.
5 S. Fleming and R. V. Ulijn, *Chem. Soc. Rev.*, 2014, **43**, 8150–8177.
6 G. Fichman and E. Gazit, *Acta Biomater.*, 2014, **10**, 1671–1682.
7 D. Li, H. Wang, D. Kong and Z. Yang, *Nanoscale*, 2012, **4**, 3047–3049.
8 R. G. Ellis-Behnke, Y. X. Liang, S. W. You, D. K. C. Tay, S. Zhang, K. F. So and G. E. Schneider, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 5054–5059.
9 A. Mahler, M. Reches, M. Rechter, S. Cohen and E. Gazit, *Adv. Mater.*, 2006, **18**, 1365–1370.
10 V. Jayawarna, M. Ali, T. A. Jowitt, A. F. Miller, A. Saiani, J. E. Gough and R. V. Ulijn, *Adv. Mater.*, 2006, **18**, 611–614.
11 Y. Loo, A. Lakshmanan, M. Ni, L. L. Toh, S. Wang and C. A. E. Hauser, *Nano Lett.*, 2015, **15**, 6919–6925.
12 F. Gelain, D. Silva, A. Caprini, F. Taraballi, A. Natalello, O. Villa, K. T. Nam, R. N. Zuckermann, S. M. Doglia and A. Vescovi, *ACS Nano*, 2011, **5**, 1845–1859.
13 R. M. Burch, M. Weitzberg, N. Blok, R. Muhlhauser, D. Martin, S. G. Farmer, J. M. Bator, J. R. Connor, M. Green and C. Ko, *Proc. Natl. Acad. Sci. U. S. A.*, 1991, **88**, 355–359.
14 S. K. Mandal, T. Kar, D. Das and P. K. Das, *Chem. Commun.*, 2012, **48**, 1814–1816.
15 J. Li, Y. Kuang, Y. Gao, X. Du, J. Shi and B. Xu, *J. Am. Chem. Soc.*, 2013, **135**, 542–545.
16 Q. Wang, J. L. Mynar, M. Yoshida, E. Lee, M. Lee, K. Okuro, K. Kinbara and T. Aida, *Nature*, 2010, **463**, 339–343.
17 V. Jayawarna, S. M. Richardson, A. R. Hirst, N. W. Hodson, A. Saiani, J. E. Gough and R. V. Ulijn, *Acta Biomater.*, 2009, **5**, 934–943.
18 G. Cheng, V. Castelletto, C. M. Moulton, G. E. Newby and I. W. Hamley, *Langmuir*, 2010, **26**, 4990–4998.
19 A. D. Martin, A. B. Robinson, A. F. Mason, J. P. Wojciechowski and P. Thordarson, *Chem. Commun.*, 2014, **50**, 15541–15544.
20 M. A. Gonzalez, J. R. Simon, A. Ghoorchian, Z. Scholl, S. Lin, M. Rubinstein, P. Marszalek, A. Chilkoti, G. P. López and X. Zhao, *Adv. Mater.*, 2017, **29**, 1604743.
21 V. Jayawarna, M. Ali, T. A. Jowitt, A. E. Miller, A. Saiani, J. E. Gough and R. V. Ulijn, *Adv. Mater.*, 2006, **18**, 611–614.
22 E. R. Draper, K. L. Morris, M. A. Little, J. Raeburn, C. Colquhoun, E. R. Cross, T. O. McDonald, L. C. Serpell and D. J. Adams, *CrystEngComm*, 2015, **17**, 8047–8057.
23 L. Adler-Abramovich, L. Vaks, O. Carny, D. Trudler, A. Magno, A. Caflisch, D. Frenkel and E. Gazit, *Nat. Chem. Biol.*, 2012, **8**, 701–706.
24 G. Fichman, T. Guterman, L. Adler-Abramovich and E. Gazit, *CrystEngComm*, 2015, **17**, 8105–8112.
25 R. Orbach, L. Adler-Abramovich, S. Zigerson, I. Mironi-Harpaz, D. Seliktar and E. Gazit, *Biomacromolecules*, 2009, **10**, 2646–2651.
26 D. M. Ryan, S. B. Anderson and B. L. Nilsson, *Soft Matter*, 2010, **6**, 3220–3231.
27 S. Maity, S. Nir, T. Zada and M. Reches, *Chem. Commun.*, 2014, **50**, 11154–11157.
28 R. J. Wierichs and H. Meyer-Lueckel, *J. Dent. Res.*, 2015, **94**, 261–271.
29 F. A. Shah, *Mater. Sci. Eng., C*, 2016, **58**, 1279–1289.
30 C. Van Loveren, *Caries Res.*, 2001, **35**, 65–70.
31 S. Yuran, Y. Razvag and M. Reches, *ACS Nano*, 2012, **6**, 9559–9566.
32 L. Adler-Abramovich, P. Marco, Z. A. Arnon, R. C. Creasey, T. C. Michaels, A. Levin, D. J. Scurr, C. J. Roberts, T. P. Knowles, S. J. Tendler and E. Gazit, *ACS Nano*, 2016, **10**, 7436–7442.
33 J. Z. Gasiorowski and J. H. Collier, *Biomacromolecules*, 2011, **12**, 3549–3558.
34 D. M. Ryan, T. M. Doran and B. L. Nilsson, *Chem. Commun.*, 2011, **47**, 475–477.
35 C. Colquhoun, E. R. Draper, E. G. Eden, B. N. Cattoz, K. L. Morris, L. Chen, T. O. McDonald, A. E. Terry, P. C. Griffiths, L. C. Serpell and D. J. Adams, *Nanoscale*, 2014, **6**, 13719–13725.
36 V. Jayawarna, S. M. Richardson, A. R. Hirst, N. W. Hodson, A. Saiani, J. E. Gough and R. V. Ulijn, *Acta Biomater.*, 2009, **5**, 934–943.
37 M. J. Webber, J. Tongers, M. A. Renault, J. G. Roncalli, D. W. Losordo and S. I. Stupp, *Acta Biomater.*, 2010, **6**, 3–11.
38 D. J. Toft, T. J. Moyer, S. M. Standley, Y. Ruff, A. Ugolkov, S. I. Stupp and V. L. Cryns, *ACS Nano*, 2012, **6**, 7956–7965.
39 T. Shekhter Zahavi, M. Oron-Herman, G. Kostenich, E. Rub, Y. Salitra, L. Buzhansky, A. Orenstein, E. Gazit and L. Adler-Abramovich, *ChemNanoMat*, 2017, **3**, 27.
40 H. Wang, Z. Luo, Y. Wang, T. He, C. Yang, C. Ren, L. Ma, C. Gong, X. Li and Z. Yang, *Adv. Funct. Mater.*, 2016, **26**, 1822–1829.
41 D. Li, J. Liu, L. Chu, J. Liu and Z. Yang, *Chem. Commun.*, 2012, **48**, 6175–6177.
42 V. Sedman, X. Chen, S. Allen, C. Roberts, V. Korolkov and S. Tendler, *J. Microsc.*, 2013, **249**, 165–172.
43 Z. Yang, L. Wang, J. Wang, P. Gao and B. Xu, *J. Mater. Chem.*, 2010, **20**, 2128–2132.
44 Y. Zhang, Z. Yang, F. Yuan, H. Gu, P. Gao and B. Xu, *J. Am. Chem. Soc.*, 2004, **126**, 15028–15029.
45 V. L. Sedman, X. Chen, S. Allen, C. J. Roberts, V. V. Korolkov and S. J. B. Tendler, *J. Microsc.*, 2013, **249**, 165–172.
46 P.-Y. Wang, L. R. Clements, H. Thissen, A. Jane, W.-B. Tsai and N. H. Voelcker, *Adv. Funct. Mater.*, 2012, **22**, 3414–3423.
47 A. J. Engler, S. Sen, H. L. Sweeney and D. E. Discher, *Cell*, 2006, **126**, 677–689.
48 E. V. Alakpa, V. Jayawarna, A. Lampel, K. V. Burgess, C. C. West, S. C. J. Bakker, S. Roy, N. Javid, S. Fleming, D. A. Lamprou, J. Yang, A. Miller, A. J. Urquhart, P. W. J. M. Frederix, N. T. Hunt, B. Péault, R. V. Ulijn and M. J. Dalby, *Chem*, 2016, **1**, 298–319.
49 D. M. Ryan, T. M. Doran and B. L. Nilsson, *Langmuir*, 2011, **27**, 11145–11156.
50 S. Fleming, S. Debnath, P. W. J. M. Frederix, N. T. Hunt and R. V. Ulijn, *Biomacromolecules*, 2014, **15**, 1171–1184.
51 W. Argüelles-Monal, F. Goycoolea, C. Peniche and I. Higuera-Ciapara, *Polym. Gels Networks*, 1998, **6**, 429–440.
52 Y. Ma, M. P. Neubauer, J. Thiele, A. Fery and W. Huck, *Biomater. Sci.*, 2014, **2**, 1661–1671.
53 Y.-H. Lee, J.-R. Huang, Y.-K. Wang and K.-H. Lin, *Integr. Biol.*, 2013, **5**, 1447–1455.
54 M. Sawkins, W. Bowen, P. Dhadda, H. Markides, L. Sidney, A. Taylor, F. Rose, S. Badylak, K. Shakesheff and L. White, *Acta Biomater.*, 2013, **9**, 7865–7873.
55 B. Yang, Y. Zhang, X. Zhang, L. Tao, S. Li and Y. Wei, *Polym. Chem.*, 2012, **3**, 3235–3238.
56 X. Gong, C. Branford-White, L. Tao, S. Li, J. Quan, H. Nie and L. Zhu, *Mater. Sci. Eng., C*, 2016, **58**, 478–486.