Modulating Stiffness with Photo-Switchable Supramolecular Hydrogels[†]

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Stimuli-responsive hydrogels are attractive materials with many applications towards biomedicine, biology, construction, and manufacturing. Materials that can be cured or annealed rapidly at room temperature are of particular interest. In this work we develop a class of supramolecular coumarin-functionalised hydrogels formed *via* host-guest mediated self-assembly with cucurbit[8]uril that can photo-switch to covalent gels and reversibly toggle between the two states. A principle advantage of such materials is their ability to maintain a homogeneous chemical composition and crosslink density while selectively modulating stiffness with light. An investigation of the photo-reversibility of these functional materials elucidated that hydroxyethyl cellulose-coumarin based gels were soft and could only switch from a physical state to a covalent one, while hyaluronic acid-coumarin based gels were softer and could be photo-reversed back into a physical state after covalent curing.

Introduction

In the last few decades, many hydrogels have been proposed in the literature as materials for sensing[1–3], tissue engineering and 3D bioprinting[4–9], drug delivery[10–15], cosmetics[16], and construction or industrial use[17,18]. Coumarin (COU) functionalised polymers, whether in hydrogel form or not, have been widely developed and commercialised for fields within industrial polymer science, energy, and biomedicine.[19] COU is a light-sensitive small molecule that undergoes a [2+2] photo-dimerisation under irradiation at wavelengths >310 nm. Dimerised coumarin molecules can be decoupled with irradiation at wavelengths <310 nm, and this property has been exploited to develop light responsive materials.[19,20]

Biologically derived polymers are attractive materials on account of their availability, scalability, cytocompatibility, and biocompatibility.[3,18,21] Hydroxyethyl cellulose (HEC) is an inexpensive material that has been widely used for industrial applications such as paint formulations for the better part of a century.[22] Hyaluronic acid (HA) is a major component of the extracellular matrix, and as such is an FDA approved material. It has been heavily explored for applications in topical and parenteral drug delivery, wound dressing, tissue engineering, cancer biology, and stem cell biology.[21,23–27] HA and HEC are commonly functionalised with moieties that can be physically or covalently crosslinked because the highly entangled polysaccharides do not gel on their own.[28]

The exploitation of COU's photo-reversible dimerisation and its use as a guest in host-guest driven assembly of a linear polymer was reported by Tian *et al.*[29] In this elegant work, a bipyridine was derivatised with two COU molecules that were complexed in a 2:1 fashion with the macrocycle γ-cyclodextrin to form linear polymer chains. To date, this host-guest complexation of COU has not been used to drive dynamic self-assembly of purely supramolecular hydrogels. Furthermore, gelation of COU-based materials with cucurbiturils, which are a family of symmetric macrocycles that have many advantages over cyclodextrins including temperature and pressure stability, chemical sensitivity under acidic and basic conditions, and improved binding kinetics, has not been studied.[30]

Herein, we report the 2:1 host-guest complexation of COU with cucurbit[8]uril (CB[8]) and use this assembly to form multimodal, supramolecular and covalent biopolymer based hydrogels. We report a strategy for the pendent functionalisation of HEC and HA with COU. Upon addition of CB[8] our polymers form self-assembled, photo-tunable hydrogels. These materials are stiffened upon irradiation at wavelengths >310 nm, a useful property for ambiently photo-annealing industrial hydrogels or for tissue engineering and 3D bioprinting applications. HA-COU gels were fully photo-reversible, and could selectively toggle between a soft physical state and a stiffer covalent state.

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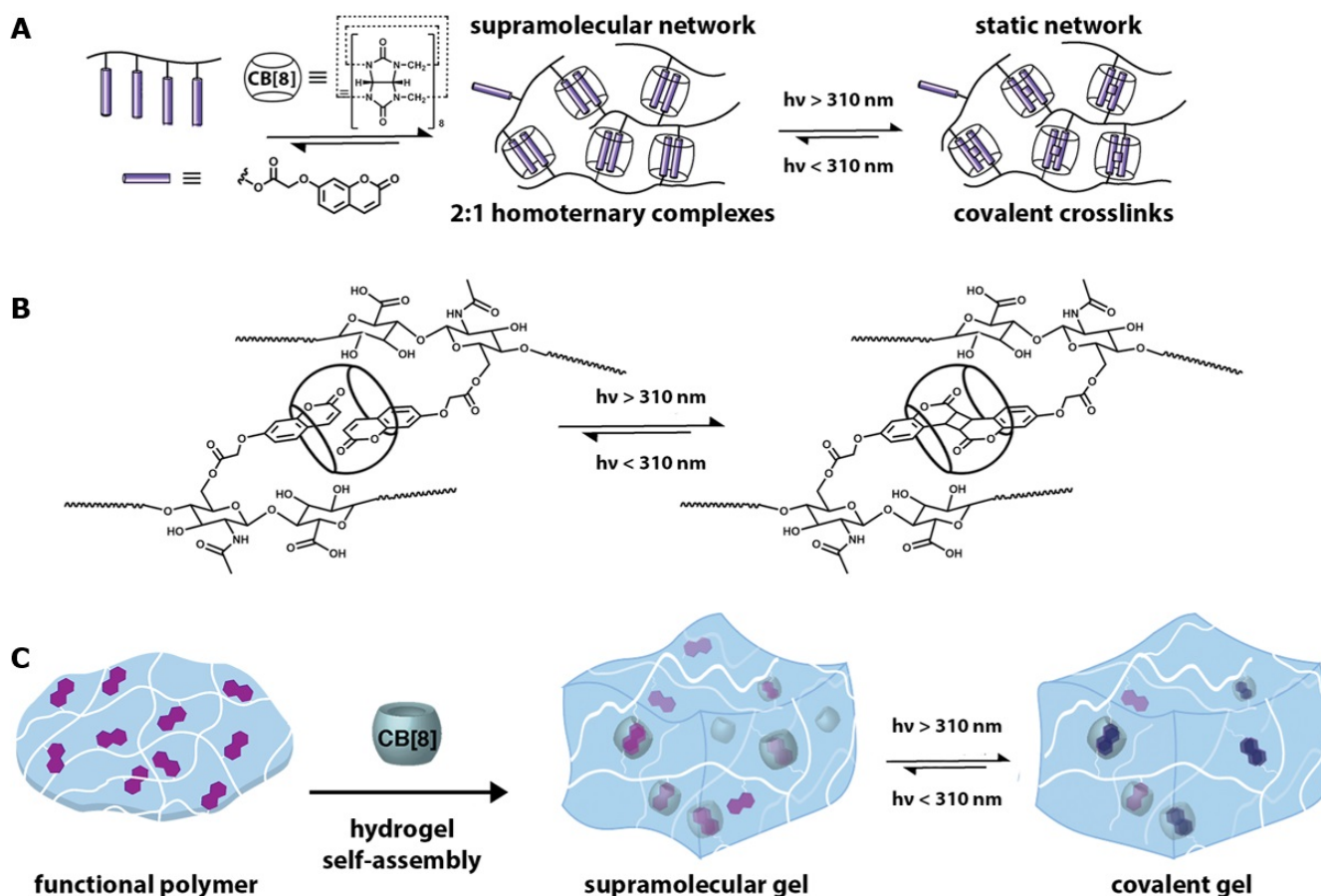
Fig. 1 Concept for coumarin-functionalised biopolymers and self-assembly using cucurbit[8]uril (CB[8]) host-guest chemistry. (A) Polysaccharides (hyaluronic acid (HA) or hydroxyethyl cellulose (HEC)) functionalised with pendant coumarin mixed in solution with cucurbit[8]uril self-assembles into a purely supramolecular gel with dynamic, physical interactions. Upon exposure to light ($\lambda > 310$ nm), coumarin undergoes a [2+2] cycloaddition to form covalent cross-links between the polymer chains, which can occur within or outside the CB[8] cavity. (B) Chemical structure of COU-functionalised HA; host-guest driven self assembly between the coumarin and cucurbit[8]uril results in supramolecular interactions; the coumarin may be reversibly photo-dimerised and decoupled. (C) Hyaluronic acid or hydroxyethyl cellulose dissolved in solution forms a viscous, entangled solution; addition of cucurbit[8]uril results in self-assembly to a supramolecular hydrogel. Dimerisation of coumarin encapsulated within cucurbit[8]uril results in a covalent network.

Results and Discussion

In this work we develop and characterise photo-sensitive supramolecular and covalent hydrogels derived from COU functionalised polysaccharides. Accardo and Kalow recently reported on dynamically tuning the stiffness of covalent hydrogels.[31] COU has been identified as a good guest for macrocycles such as cyclodextrins and cucurbiturils.[29,32] However, COU's binding affinity to macrocycles when attached to a polymer backbone and its propensity to form supramolecular, photo-reversible, and shear-reversible gels has not been thoroughly explored. Here we demonstrate that COU attached pendently to a polymer backbone continues to serve as a good guest for CB[8], forming a 2:1 homoternary complex. As depicted in Figure 1, this interaction can be exploited to make COU-based hydrogels.

7-Hydroxycoumarin (COU-OH) was used as a simplified model of backbone-bound COU to study its homoternary complexation with CB[8] in water (Fig. S1) through isothermal titration calorimetry (ITC).[33] It is challenging to obtain reliable binding information *via* a single ITC measurement for a poorly soluble compound like COU-OH (solubility in water: 0.5 mM). In order to overcome this typical limitation for hydrophobic molecules, data from three titration curves obtained using different CB[8] concentrations were simultaneously fitted according to a sequential binding model. The binding affinities for the encapsulation of the first and second COU-OH molecules are 3.9×10^3 M^{-1} ($\Delta G_1 = -20.5 \pm 0.2$ kJ/mol) and 1.5×10^4 M^{-1} ($\Delta G_2 = -23.9 \pm 0.4$ kJ/mol), respectively, exhibiting a positive co-operativity effect. Both binding processes are enthalpically driven ($\Delta H_1 = -28.2 \pm 0.9$ kJ/mol and $\Delta H_2 = -28.3 \pm 1.7$ kJ/mol) but entropically unfavorable, consistent with previous reports on CB[8] mediated binding.[34–36]

It is well known that functionalising a polymer backbone with guest moieties is a robust and facile way to form hydrogels through, for example, host-guest mediated interactions of backbone-functionalised polymers and macrocyclic
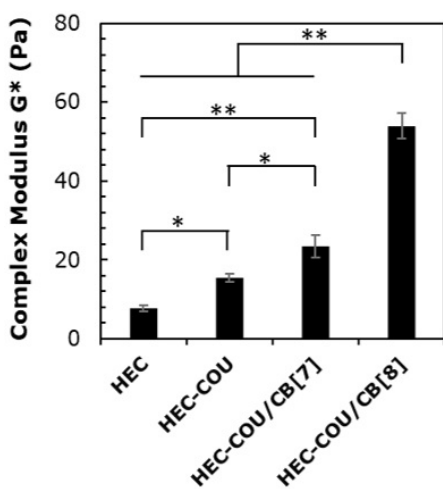
Fig. 2 Rheological data on 720 KDa HEC-based materials demonstrating the gelling effect from 2:1 homoternary biding of COU to CB[8]. Oscillatory frequency measurements in the linear viscoelastic region were taken. Complex moduli at 1 rad/s are plotted. Measurements were done with triplicate samples; *$p<0.05$; **$p<0.01$.

hosts.[8,34,37–39] Herein we functionalise HEC and HA (5-6%; Fig. S2) to create materials that self-assemble into supramolecular gels *via* COU/CB[8] 2:1 homoternary complexation and can be crosslinked into covalent gels *via* COU-COU photo-dimerisation within or outside the CB[8] cavity. We explore whether these materials may toggle between physical and covalent states (Fig. 1C).

HEC is an inexpensive and easily-functionalised cellulose derivative commercially available at various molecular weights; our group has previously reported on HEC-functionalised gels formed *via* host-guest interactions between backbone tethered guests and CB[8].[21,37,40] HEC-COU was first used in this work to explore gelation in COU/CB[8] systems. A 720 kDa molecular weight HEC polymer functionalised with 5% COU gelled after the addition of CB[8] (Fig. 2). There is a slight difference in stiffness between unfunctionalised HEC and HEC-COU (Fig. 2), potentially due to π-stacking within the matrix. The addition of cucurbit[7]uril (CB[7]) also resulted in a small stiffening effect. A 3-fold increase in complex modulus occurred after introduction of CB[8] with 720 kDa HEC-COU (Fig. 2). While less pronounced, a 5-6% functionalised 1.3 MDa HEC-COU polymer solution also stiffened upon addition of CB[8] (Fig. S3A). The higher molecular weight functional HEC gelled on its own when functionalised with a similar 5% COU, unlike in the lower molecular weight 720 kDa case, suggesting the presence of a critical percolation transition point in our high molecular weight polysaccharides. The HA-COU system, with a 1.5 - 1.8 MDa molecular weight, was also observed to gel on its own with 5% COU functionalizstion (Fig. S3B), with a stiffening effect occurring after addition of CB[8].

The stiffness of the higher molecular weight HEC-COU was probed with an oscillatory time sweep while irradiating the matrix with light >310 nm (320-390 nm). This transient oscillatory rheological measurement allowed for the quantification of the effect of COU dimerisation on the matrix. The HEC-COU/CB[8] system underwent major stiffening as the CB[8]-bound COU molecules were dimerised to form covalent crosslinks (Fig. 3A,B), exhibiting a storage modulus (G′) increase from 0.074 kPa to 4.1 kPa at 1 rad/s. Negligible changes in the loss modulus (G″) suggest that a similar crosslink density existed between the physical and covalent states; the change from dynamic to static crosslinks is the driving force causing a major increase in the storage modulus. Interestingly, HEC-COU/CB[8] could not be completely decoupled after COU dimerisation occurred (Fig. 3A,C). After irradiation at 254 nm, the HEC-COU/CB[8] system was reloaded onto the rheometer plate and irradiated *in situ* at 320-390 nm again to test whether the system underwent a covalent-to-physical state transition with 254 nm light, and whether the system could be redimerised to a covalent state. The inability of the material to stiffen more than once (Fig. 3C) suggests the reduction in stiffness (Fig. S4) is not caused by decoupling of COU-COU dimers but is consistent with polysaccharide matrix photo-degradation.[41] These observations were also present in the lower molecular weight 720 kDa HEC-COU/CB[8] system and in the high molecular weight HEC-COU/CB[8] system with a reduced backbone concentration of COU of 1% (data not shown).

Hyaluronic acid (HA) is a major component of the human extracellular matrix[24] and is a useful biomaterial for applications within biomedicine or biology. We leveraged the same approach as with HEC and functionalised HA with COU at 5%; we then introduced CB[8] to form a supramolecular gel (Fig. 1A,B; S3B). Similarly to HEC-COU, HA-COU gelled on its own without CB[8], but stiffened when CB[8] was introduced. The ability of HA-COU/CB[8] to photo-crosslink was then explored. Upon exposure to light >310 nm (320-390 nm), HA-COU hydrogels comparably stiffened, with an increase in G′ and negligible changes in G″ (Fig. 4A; S5). The storage modulus increased from 78 to 360 Pa upon irradiation and COU dimerisation at 1 rad/s, markedly lower than HEC-COU/CB[8] despite similar backbone functionalisation of 5%. Notably, this range of stiffness is ideal for 3D neural cell culture and tissue engineering applications.[42] While certainly the photo-reversibility of HA-COU/CB[8] occurs at wavelengths not compatible with cells and tissue, embedding cells in the physical state and curing with visible light is a potentially feasible strategy for biological applications in the future.

The ability to reversibly toggle between the physical and covalent states in HA-COU/CB[8] was explored. After irradiation at a fixed energy density to the covalent crosslinking (1.5 h), HA-COU/CB[8] could be fully reverted to its physical state, and then crosslinked again to fully recover its covalent stiffness for at least two cycles (Fig. 4A,B). When this material, however, was exposed to UV light (<310 nm) for 4 times as long (6 h), the stiffness decreased beyond the point of the original supramolecular material, and the stiffness was not able to fully recover (Fig. S6). This suggests that when there are moieties embedded within the matrix that can absorb photons (COU-COU dimers), then the integrity of the polymer backbone is not compromised. However, when the system is fully saturated and further energy is applied, dissipation occurs in the form of material breakdown.

The differences in energy dissipation, shear-thinning, and recovery were examined in the HA-COU/CB[8] system in the phys-
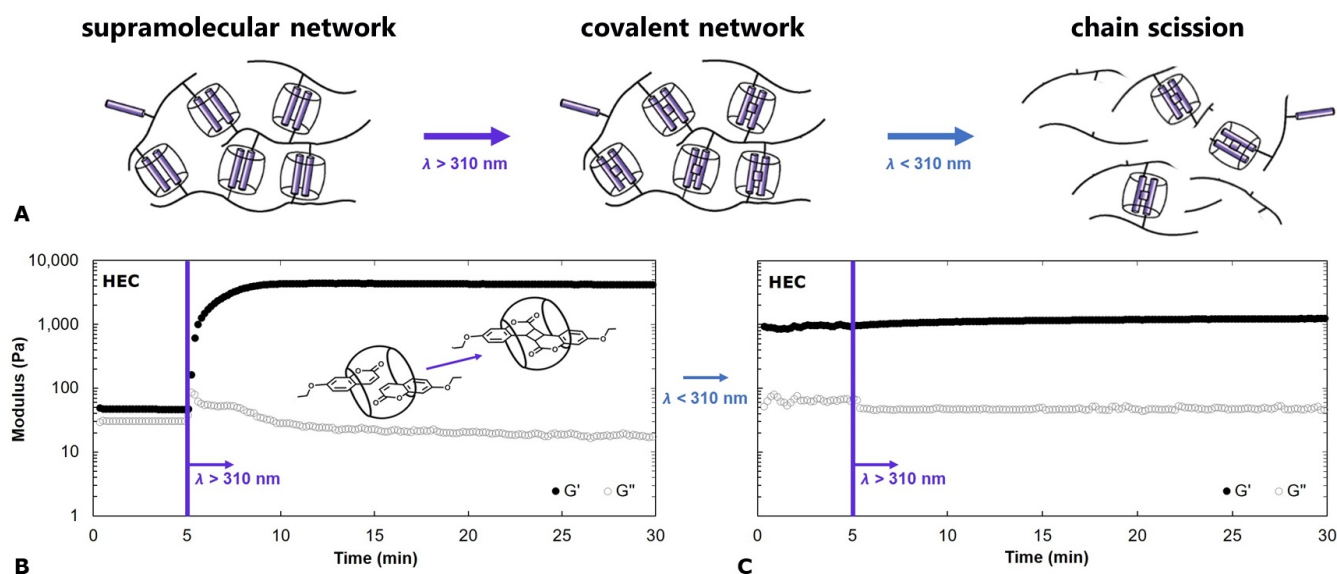
Fig. 3 Rheological analysis of HEC-COU systems. (A) Illustration depicting the dimerisation of COU upon irradiation to light between 320-390 nm, and likely chain scission upon attempted toggling back to a physical state. (B) Oscillatory UV time sweep of supramolecular HEC-COU/CB[8] dimerising into a purely covalent state. (C) Oscillatory UV time sweep of HEC-COU/CB[8] system after covalent dimerisation and after exposure to 254 nm light for 1.5 h. [ω = 5 rad/s, γ = 10%; λ = 320-390 nm; I = 140 mW/cm^2].
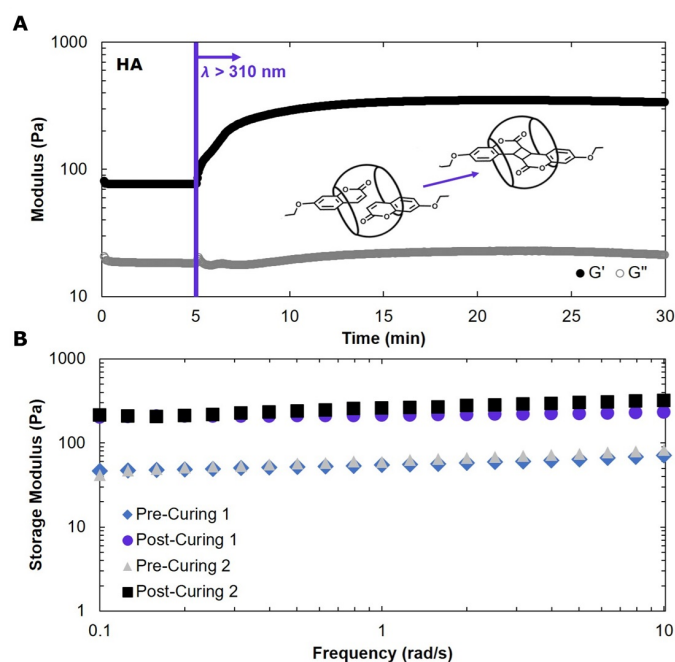

Fig. 4 Rheological characterization of hyaluronic acid conjugated with coumarin loaded with 0.5 molar equivalents of cucurbit[8]uril (HA-COU/CB). (A) Oscillatory UV time sweep of HA-COU/CB[8] demonstrating dimerisation of COU [ω = 1 rad/s, γ = 1%; λ = 320-390 nm; I = 140 mW/cm^2]. (B) Oscillatory frequency sweeps of HA-COU/CB[8] with purely non-covalent, supramolecular interactions or post-UV(>310 nm) treated until full dimerisation.

ical and covalent states (Fig. S7). The physically crosslinked material underwent shear-thinning and fully recovered its stiffness after high shear (50%, 50 rad/s) in 1 h. After the same shearing

conditions, the covalently-crosslinked polymer plateaued at 55% recovery suggesting that the supramolecular material was able to dissipate energy *via* the dissociation of the thermodynamically favourable host-guest interactions between COU and CB[8]. In the case of the 100% covalent material, this dynamic energy dissipation is not available to the system, and so energy is propagated through the system by cleavage of chemical bonds. The shear-reversibility and photo-reversibility of HA-COU/CB[8] hydrogels suggest that when there are non-covalent or covalent groups in the matrix more susceptible to dissipation or absorption of energy, the polymer backbone remains intact. [43]

Our group previously reported a photo-curable HEC-based system with an anthracene (ANC) guest [40], and it was observed that the dimerisation of HEC-ANC was not fully reversible. The reversible dimerisation of anthracene is sensitive to oxygen radicals. Therefore, it was hypothesized that this inability to reverse anthracene dimerisation was due to residual oxygen in the system. However, COU dimerisation is not oxygen sensitive, and the differences that exist between HEC-COU and HA-COU suggest that another factor, such as backbone charge, may be playing a critical role.

In summary, we explored the photo-curability, photo-reversibility, and energy dissipation of self-assembled CB[8]/COU-functionalised polysaccharides. While HEC-COU/CB[8] materials could be photo-cured, they were not reversible. On the other hand, supramolecular HA-COU/CB[8] materials were photo-reversible and shear-reversible, and chain degradation occured only after systemic energy saturation (photons or shear). Such photo-properties may limit the use of these materials in applications with extended irradiation below 310 nm, but the embedding of COU in HEC and HA systems

results in the formation of photo-active biomaterials with distinct and expanded potential applications in industry or biomedicine.

Conclusions

In this work, HA and HEC backbones were functionalised with a coumarin derivative and self-assembled into supramolecular gels with CB[8]-mediated homoternary complexes. HEC-COU/CB[8] could be dimerised from a supramolecular gel into a covalent state, but could not be reversed. HA-COU/CB[8] was fully reversible for at least two cycles, and could toggle between physical and covalent states. The HA-COU/CB[8] system is an interesting material with potential applications in additive manufacturing. The use of functional HA resins for 3D printing has been previously reported in elegant dual printed or reinforced network approaches. [44,45] The HA-COU/CB[8] system is a potentially promising resin for 4D bioprinting [46] or as a cast gel with well-defined geometry [47] owing to the *in situ* and reversible tunability of its stiffness. Most importantly, our material maintains a homogeneous chemical composition but can be selectively stiffened with spatial and temporal resolution in biologically-relevant stiffness regimes, suggesting it may be an attractive material for studying the role of stiffness in neurobiology. [48]

Conflicts of Interest

There are no conflicts of interest to declare.

References

1 D. Buenger, F. Topuz and J. Groll, *Prog. Polym. Sci.*, 2012, **37**, 1678–1719.

2 A. N. Wilson and A. Guiseppi-Elie, *Adv. Healthc. Mater.*, 2013, **2**, 520–532.

3 N. A. Peppas, J. Z. Hilt, A. Khademhosseini and R. Langer, *Adv. Mater.*, 2006, **18**, 1345–1360.

4 K. Y. Lee and D. J. Mooney, *Chem. Rev.*, 2001, **101**, 1869–1879.

5 S. V. Murphy and A. Atala, *Nat. Biotechnol.*, 2014, **32**, 773–785.

6 T. J. Hinton, Q. Jallerat, R. N. Palchesko, J. H. Park, M. S. Grodzicki, H. J. Shue, M. H. Ramadan, A. R. Hudson and A. W. Feinberg, *Sci. Adv.*, 2015, **1**, e1500758.

7 K. Dubbin, Y. Hori, K. K. Lewis and S. C. Heilshorn, *Adv. Healthc. Mater.*, 2016, **5**, 2488–2492.

8 K. Dubbin, A. Tabet and S. C. Heilshorn, *Biofabrication*, 2017, **9**, 4.

9 V. B. Slaughter, S. S. Khurshid, A. Khademhosseini, Z. O. Fisher and N. A. Peppas, *Adv. Mater.*, 2009, **21**, 3307–3329.

10 T. R. Hoare and D. S. Kohane, *Polymer*, 2008, **49**, 1993–2007.

11 J. Li and D. J. Mooney, *Nat. Rev. Mater.*, 2016, **1**, 12.

12 G. W. Ashley, J. Henise, R. Reid and D. V. Santi, *Proc. Natl. Acad. Sci.*, 2013, **110**, 2318–2323.

13 M. W. Tibbitt, J. E. Dahlman and R. Langer, *J. Am. Chem. Soc.*, 2016, **138**, 704–717.

14 T. M. O'Shea, A. A. Aimetti, E. Kim, V. Yesilyurt and R. Langer, *Adv. Mater.*, 2015, **27**, 65–72.

15 E. A. Appel, X. J. Loh, S. T. Jones, C. A. Dreiss and O. A. Scherman, *Biomaterials*, 2012, **33**, 4646–4652.

16 M. E. Parente, A. Ochoa Andrade, G. Ares, F. Russo and A. Jiménez-Kairuz, *Int. J. Cosmet. Sci.*, 2015, **37**, 511–518.

17 A. C. Yu, H. Chen, D. Chan, G. Agmon, L. M. Stapleton, A. M. Sevit, M. W. Tibbitt, J. D. Acosta, T. Zhang, P. W. Franzia, R. Langer and E. A. Appel, *Proc. Natl. Acad. Sci.*, 2016, **113**, 14255–14260.

18 A. Sannino, C. Demitri and M. Madaghiele, *Materials*, 2009, **2**, 353–373.

19 S. R. Trenor, A. R. Shultz, B. J. Love and T. E. Long, *Chem. Rev.*, 2004, **104**, 3059–3077.

20 N. K. Mal, M. Fujiwara and Y. Tanaka, *Nature*, 2003, **421**, 350–353.

21 M. J. Rowland, M. Atgie, D. Hoogland and O. A. Scherman, *Biomacromolecules*, 2015, **16**, 2436–2443.

22 W. E. Gloor, B. H. Mahlman and R. D. Ullrich, *Ind. Eng. Chem. Res*, 1950, **42**, 2150–2153.

23 C. B. Rodell, A. L. Kaminski and J. A. Burdick, *Biomacromolecules*, 2013, **14**, 4125–4134.

24 U. Novak and A. H. Kaye, *J. Clin. Neurosci.*, 2000, **7**, 280–290.

25 J. A. Burdick and G. D. Prestwich, *Adv. Mater.*, 2011, **23**, H41–H56.

26 R. Stern, *Hyaluronan in Cancer Biology*, Elsevier, 2009.

27 S. Gerecht, J. A. Burdick, L. S. Ferreira, S. A. Townsend, R. Langer and G. Vunjak-Novakovic, *Proc. Natl. Acad. Sci.*, 2007, **104**, 11298–303.

28 A. Tabet, K. Sokolowski, D. Hoogland, J. Shilts and O. A. Scherman, *F1000Research*, 2018.

29 Q. Zhang, D. H. Qu, J. Wu, X. Ma, Q. Wang and H. Tian, *Langmuir*, 2013, **29**, 5345–5350.

30 S. J. Barrow, S. Kasera, M. J. Rowland, J. Del Barrio and O. A. Scherman, *Chem. Rev.*, 2015, **115**, 12320–12406.

31 J. Accardo and J. Kalow, *Chemical Science*, 2018, **9**, 5987–5993.

32 R. Wang, D. Bardelang, M. Waite, K. A. Udachin, D. M. Leek, K. Yu, C. I. Ratcliffe and J. A. Ripmeester, *Org. Biomol. Chem.*, 2009, **7**, 2435.

33 C. A. Brautigam, H. Zhao, C. Vargas, S. Keller and P. Schuck, *Nat. Protoc.*, 2016, **11**, 882–894.

34 F. Biedermann, V. D. Uzunova, O. A. Scherman, W. M. Nau and A. De Simone, *J. Am. Chem. Soc.*, 2012, **134**, 15318–15323.

35 G. Wu, M. Olesińska, Y. Wu, D. Matak-Vinkovic and O. A. Scherman, *J. Am. Chem. Soc.*, 2017, **139**, 3202–3208.

36 Z. Walsh-Korb, Y. Yu, E. R. Janeček, Y. Lan, J. Del Barrio, P. E. Williams, X. Zhang and O. A. Scherman, *Langmuir*, 2017, **33**, 1343–1350.

37 E. A. Appel, F. Biedermann, U. Rauwald, S. T. Jones, J. M. Zayed and O. A. Scherman, *J. Am. Chem. Soc.*, 2010, **132**, 14251–14260.

38 J. L. Mann, A. C. Yu, G. Agmon and E. Appel, *Biomater. Sci.*, 2017, **6**, 10–37.

39 E. A. Appel, J. del Barrio, X. J. Loh and O. A. Scherman, *Chem.*

Soc. Rev., 2012, **41**, 6195.

40 F. Biedermann, I. Ross and O. A. Scherman, *Polym. Chem.*, 2014, **5**, 5375.

41 A. Sionkowska, A. Płanecka, K. Lewandowska, B. Kaczmarek and P. Szarszewska, *Progress on Chemistry and Application of Chitin and its Derivatives*, 2013, **18**, 21–28.

42 M. J. Rowland, C. C. Parkins, J. H. McAbee, A. K. Kolb, R. Hein, X. Jun Loh, C. Watts and O. A. Scherman, *Biomaterials*, 2018, in press.

43 J. Liu, C. Soo Yun, Z. Yu, Y. Lan, C. Abell and O. A. Scherman, *Adv. Mater.*, 2017, 1604951.

44 S. Stichler, T. Böck, N. Paxton, S. Bertlein, R. Levato, V. Schill, W. Smolan, J. Malda, J. Teßmar, T. Blunk and J. Groll, *Biofabrication*, 2017, **9**, 4.

45 L. Ouyang, C. B. Highley, C. B. Rodell, W. Sun and J. A. Burdick, *ACS Biomater. Sci. Eng.*, 2016, **2**, 1743–1751.

46 B. Gao, Q. Yang, X. Zhao, G. Jin, Y. Ma and F. Xu, *Trends Biotechnol.*, 2016, **34**, 746–756.

47 A. Tabet, M. Gardner, S. Swanson, S. Crump, A. McMeekin, D. Gong, R. Tabet, B. Hacker and I. Nestrasil, *F1000Research*, 2017, **5**, 2811.

48 D. E. Koser, A. J. Thompson, S. K. Foster, A. Dwivedy, E. K. Pillai, G. K. Sheridan, H. Svoboda, M. Viana, L. D. F. Costa, J. Guck, C. E. Holt and K. Franze, *Nat. Neurosci.*, 2016, **19**, 1592–1598.