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Published in:
Chemical Science

DOI:
10.1039/c3sc52964a

Publication date:
2014

Chemical Science

EDGE ARTICLE

View Article Online
View Journal | View Issue

Cite this: *Chem. Sci.*, 2014, **5**, 1054

A carbohydrate-based hydrogel containing vesicles as responsive non-covalent cross-linkers†

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Received 25th October 2013
Accepted 16th November 2013

DOI: 10.1039/c3sc52964a

www.rsc.org/chemicalscience

In this edge article we report the preparation of a supramolecular carbohydrate hydrogel containing cyclodextrin vesicles as 3D junctions. A cellulose polymer is randomly modified with hydrophobic side groups that act as guests for the cyclodextrin hosts on the surface of the vesicles. Hence, the vesicles interconnect the polymer chains into a three-dimensional network and act as multivalent linkages. The resulting gel shows significant shear-thinning and self-healing properties, which make it highly suitable for applications that require injectability. Furthermore, SAXS and cryo-TEM measurements indicate that intact vesicles are present in the gel matrix.

Introduction

Hydrogels are biomimetic materials considering their porous structure with high water content and their viscoelastic properties similar to human tissue.[1–5] Applications of hydrogels reach from scaffolds in regenerative medicine, vehicles for storage and delivery of therapeutics to smart materials and microreactors.[6–13] The development of novel supramolecular polymer hydrogels is of particular interest as these materials possess specific non-covalent and dynamic binding motifs, which can be addressed by external stimuli and are easily tuned by modifying the architecture and density of cross-links. Furthermore, the polymer components provide excellent mechanical stability for the gel network, even at high water content.[14,15] Numerous investigations on supramolecular polymer hydrogels based on cyclodextrin (CD) inclusion complexes have been reported with various structural designs of CD and guest-functionalized polymers.[16–24] The orthogonal self-assembly of surfactants and hydrogelators as well as the preparation of vesicle polymer gels is also well-established.[25–28] In this article, we report the preparation of a novel polymer hydrogel containing cyclodextrin vesicles (CDV) as multivalent non-covalent cross-linkers. Due its supramolecular nature, the gel shows responsiveness towards a competitive host and guest. Moreover, the gel is injectable because it shows viscous flow under shear stress. To our knowledge, this is the first study on supramolecular hydrogels with vesicles as highly specific non-covalent and responsive junctions in a polymer network.

Results and discussion

Considering the biomedical applications of hydrogel materials we selected a biocompatible as well as commercially available hydroxyethyl cellulose (HEC) as the polymeric backbone of the hydrogel.[29] The second component of the gel are bilayer vesicles self-assembled from synthetic β-CD amphiphiles (β-CDA) substituted with hydrophobic *n*-dodecyl chains on the primary side and hydrophilic oligo(ethylene glycol) groups on the secondary side of the macrocycle. The hydrophobic tails are directed inwards and the hydrophilic head groups face the water so that the surface of the bilayer vesicles displays multiple CD host cavities that are available for size-specific host–guest inclusion with suitable guest molecules.[30] For this purpose, the polymer was functionalized with adamantane (AD) side groups *via* esterification (see ESI†). The inclusion of AD groups into β-CD has previously been applied to attach carbohydrates,[31,32] peptides[33,34] and guanidinium–carboxylate zwitterions[35] on the surface of β-cyclodextrin vesicles (β-CDVs) in order to mediate the adhesion and aggregation of the vesicles in aqueous solution. Also, AD-functionalized polyelectrolytes were successfully adsorbed on to the surface of β-CDVs.[36] In this study, we demonstrate that by selection of an appropriate polymer and higher concentration of vesicles, the aggregation of β-CDVs can be directed to prepare a hydrogel material with new and interesting properties. Fig. 1 shows the interaction of β-CDVs and the adamantyl-functionalized polymer HEC–AD in aqueous solution.

When a dispersion of β-CDVs is mixed with a solution of HEC–AD a supramolecular hydrogel is obtained directly. On the basis of our previous work on β-CDVs, we hypothesize that the hydrophobic adamantane side groups of the polymer form inclusion complexes with the β-CD hosts on the surface of the vesicles. Thus, several polymer chains are joined together and a non-covalent 3D polymer network is created that is able to retain the water by surface tension and capillary forces.

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3sc52964a

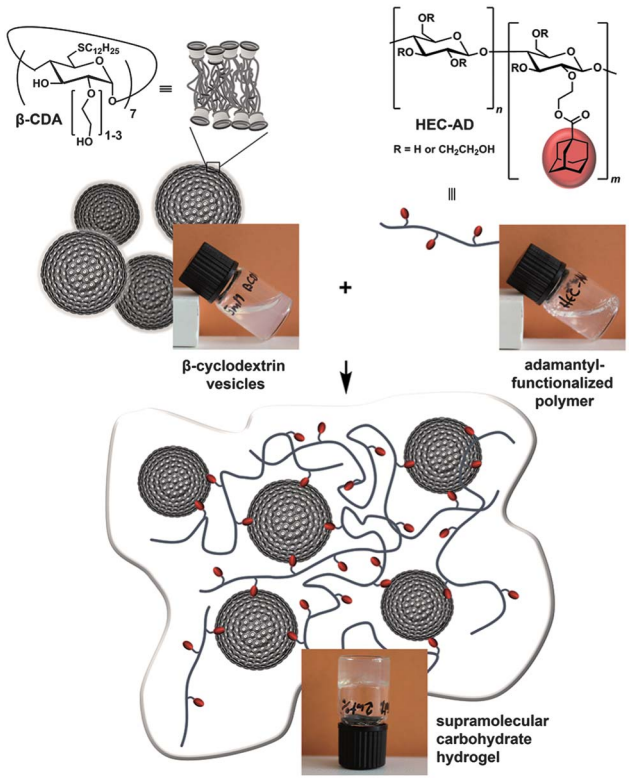

Fig. 1 Amphiphilic β-cyclodextrin host (β-CDA) and guest polymer (HEC–AD, $m:n \approx 1:8$) and formation of the supramolecular hydrogel by cross-linking of the polymer chains *via* host–guest inclusion complexes of the adamantane substituents and the macrocyclic hosts on the surface of β-cyclodextrin vesicles (β-CDV).

The preparation of the hydrogel was possible at rather low solid percentage so that the total water content was as high as 98.3 wt%. Typically, gels were prepared with 1.0–2.0 wt% of the polymer HEC–AD (corresponding to about 3.7–7.4 mM of AD substituents) and 0.75–2.25 wt% (2.5–7.5 mM) of β-CDA. By mixing and stirring the same volume of the vesicle dispersion and the polymer solution for a few seconds the aqueous solution turned into a soft material that showed self-healing properties after cutting it into pieces and bringing them back into contact. Fig. 2(a)–(d) depict hydrogel samples with different concentrations of HEC–AD and β-CDA. With increasing concentrations of β-CDA, the corresponding molar ratio of AD/CD decreases from 3 to 0.7 resulting in a higher elasticity of the material due to a higher cross-link density (*cf.* Table S1 in ESI†). It is visible to the naked eye that the turbidity of the gel also increases with the amphiphile concentration, which can be attributed to the Tyndall scattering caused by the vesicles. To ensure that the gel formation is truly based on the specific formation of host–guest inclusion complexes of β-CD with adamantyl side groups, several control samples were prepared (Fig. 2e and f). A mixture of α-CDVs and HEC–AD did not form a gel since AD is too bulky to fit into the smaller cavity of α-CD.[30] Also, an unfunctionalized HEC polymer and vesicles of β-CDA did not form a gel since in that case no guest is available for cross-linking. Furthermore, the responsiveness towards external stimuli was explored (Fig. 2g and h). Addition of an excess of competitive host (16 mM of β-CD) or guest (0.2 M of 1-adamantylamine) to the hydrogel caused disruption of the gel network as the interactions of the HEC–AD polymer chains with the vesicles were blocked. These concentrations correspond to a 6.4-fold excess of β-CD *vs.* β-CDA and a 27-fold excess of 1-adamantylamine *vs.* HEC–AD. In both cases viscous solutions were obtained. Altogether, these results clearly demonstrate the highly specific role of the vesicles as 3D junctions.

The mechanical properties of hydrogels with varying concentrations of HEC–AD and β-CDA were studied with rheology. It should be emphasized *a priori* that the hydrogels

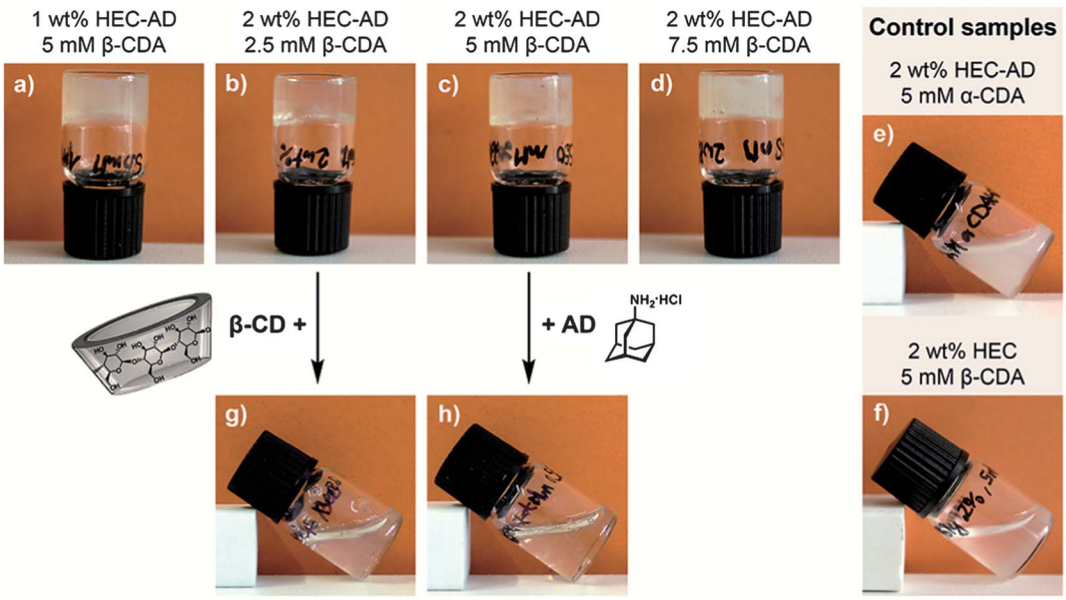

Fig. 2 (a)–(d) Hydrogels prepared with different concentrations of HEC–AD and β-CDV. (e) Control samples with α-CDV and (f) unfunctionalized polymer do not form a gel. (g) Addition of competitive host and (h) guest disrupts the gel network.

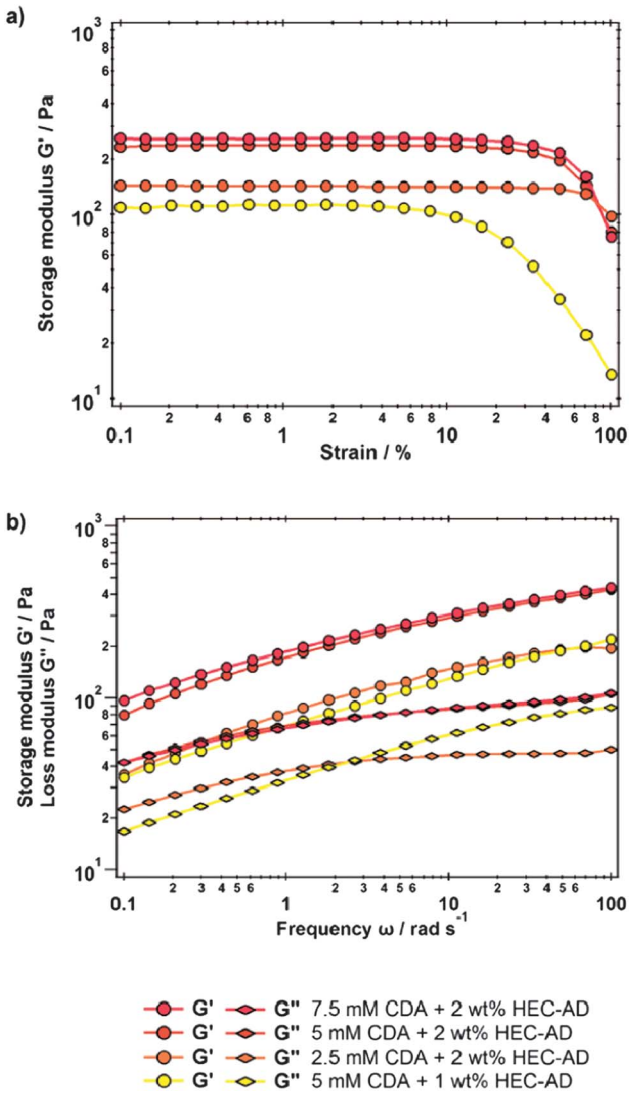

Fig. 3 Oscillatory rheological measurements of the hydrogels with varying concentrations of HEC–AD and β-CDV. (a) Storage modulus G' obtained from a strain-amplitude sweep performed at 10 rad. s^{-1}. (b) Storage G' and loss G'' moduli obtained from a frequency-sweep performed at 5% strain.

contain two components that interact through relatively weak and dynamic non-covalent host–guest complexation. For each individual cyclodextrin inclusion complex, the equilibrium binding constant $K_a \sim 10^4$ M^{-1}, the on-rate k_{on} is diffusion controlled, and the off-rate k_{off} is on a μs time scale. This implies that two time scales operate in the hydrogel: a rather long relaxation time for the HEC–AD polymer and a rather short relaxation time for the non-covalent cross-links. Therefore, it would be expected that at time scales longer than the relaxation time of the cross-links, the hydrogel behaves like a polymer fluid while at time scales shorter than the relaxation time of the cross-links, the hydrogel behaves like a cross-linked viscoelastic material. Indeed, rheology confirmed this hypothesis.

For a detailed picture of the viscous and elastic portions of the gels, oscillatory rheological measurements were performed. A strain-amplitude sweep revealed that the extent of the linear viscoelastic region strongly depends on the polymer content. For gels with 1 wt% HEC–AD a breakdown of the gel structure was observed at 10% strain, while for 2 wt% polymer content the hydrogel structure was maintained up to 40–50% strain (Fig. 3a). The values of the plateau modulus G' increased with the polymer or vesicle concentration reflecting a rise in rigidity and a growing number of non-covalent cross-links. It should be noted that the increment of the elastic modulus G' was much higher from 2.5 mM to 5.0 mM β-CDA content ($\Delta G' = 90$ Pa) than from 5.0 mM to 7.5 mM β-CDA content ($\Delta G' = 20$ Pa). This can be explained by an increasing saturation of the available AD side chains as the increment of the AD/β-CD ratio also decreases from 1.5 to 0.5, respectively. In Fig. 3b frequency sweep measurements of the storage and loss moduli (G' and G'') are shown. The elastic component G' dominates the viscous component G'' and the curves are parallel and almost linear, confirming the gel character of the materials. Considering the modest difference of G' and G'', the viscoelastic character of the hydrogel is limited in comparison to *e.g.* a permanently cross-linked polymer hydrogel. Furthermore, temperature-dependent rheological measurements revealed a decrease in mechanical strength with increasing temperature (10–50 °C), which can be explained with an increase of the bilayer fluidity of the vesicles in combination with a decrease in association constant of the host–guest inclusion complexation (see Fig. S5 in ESI†). This is yet another observation indicating that specific non-covalent interactions are responsible for the hydrogel architecture and its mechanical properties.

Steady-shear measurements (Fig. 4a) revealed the shear-thinning behavior of the materials. For all concentrations the viscosity of the gels decreased with increasing shear rate. Up to a shear rate of $\dot{\gamma} = 0.1$ s^{-1} the viscosity remained constant. Here, the differences in absolute values of the zero-shear viscosities ($\eta_0 = 100$–1000 Pa s) again indicate a dependency on the cross-link density. For increasing shear rates the gels showed viscous flow under shear stress, as the non-covalent interactions are easily broken. This is a common phenomenon for supramolecular polymeric hydrogels due to their dynamically cross-linked polymer chains.[15] The recovery of the hydrogel materials after applying a high shear rate was furthermore examined (Fig. 4b). Initially, the viscosity at a shear rate in the linear regime ($\dot{\gamma} = 0.5$ s^{-1}) was measured. Next, the bulk structure of the hydrogel was disrupted by applying a high-magnitude shear rate ($\dot{\gamma} = 500$ s^{-1}) causing a decline in viscosity. In the last step, again a shear rate of $\dot{\gamma} = 0.5$ s^{-1} was used to study the reorganization of the material. The viscosity was found to recover within only a few seconds to 90% of the initial values for gels containing 2 wt% HEC–AD and 70% for the gel with 1 wt% of the polymer after the first cycle.

Hence, the supramolecular hydrogel material possesses shear-thinning and self-healing properties. These results provoked us to test whether the hydrogel can be pressed through a syringe with a narrow needle (26 G), which would be suitable for injection of the material (Fig. 4c). We found that the gels could easily be injected by applying a gentle force. In accordance with the rheological recovery experiments the

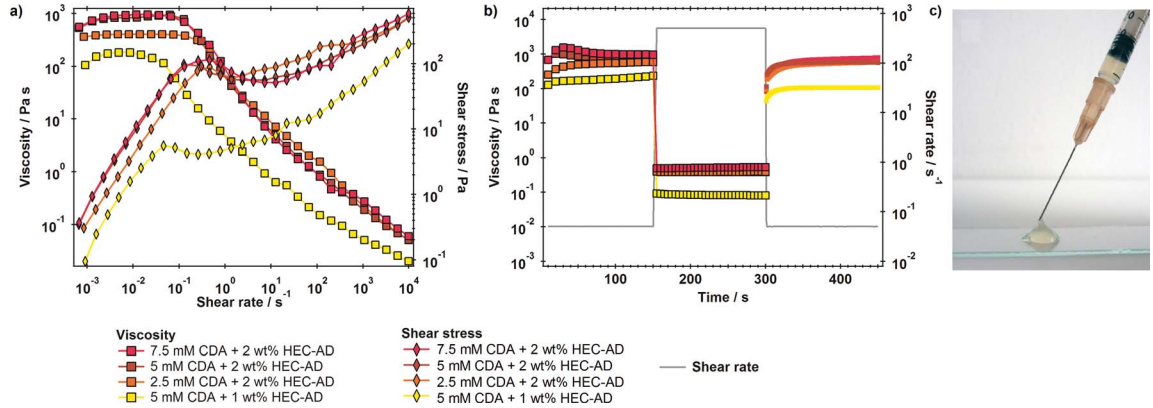

Fig. 4 (a) Steady shear rheological measurements reveal the response to increasing shear stress. (b) Step-rate measurements display the recovery of the hydrogel structure immediately after disruption due to a high-magnitude shear-rate. (c) Due to their shear-thinning and self-healing properties the hydrogels are injectable through a narrow 26 G needle.

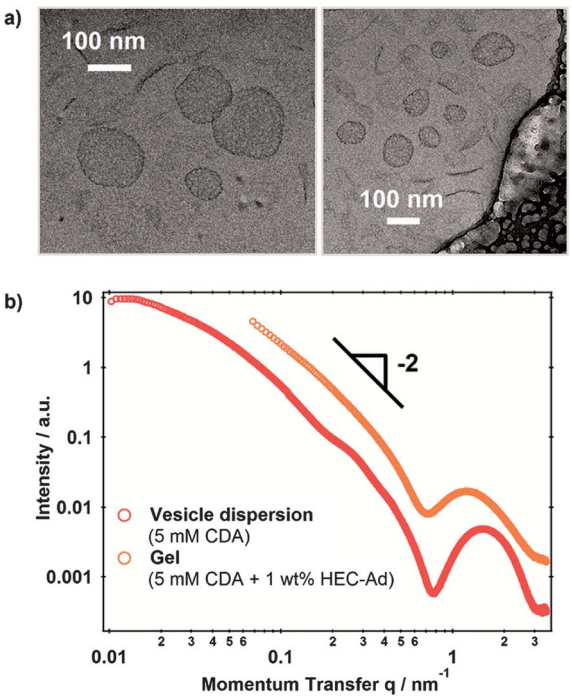

Fig. 5 (a) Cryo-TEM images of diluted hydrogel with 2 wt% HEC–AD and 5 mM β-CDA show intact vesicles. (b) Small-angle X-ray scattering profiles of a β-cyclodextrin vesicle dispersion containing 5 mM β-CDA and a hydrogel made of 5 mM β-CDA and 1 wt% HEC–AD.

material immediately regained its gel structure after escaping the needle (see video in ESI†). Considering that the hydrogels are self-assembled from simple carbohydrate precursors, a cellulose polymer and amphiphilic cyclodextrins, this makes them highly suitable for biomedical applications: the hydrogels can be readily injected to a specific target site and are potentially biocompatible. Moreover, the mechanical properties are readily tuned by varying the concentration of the polymer or the number of β-CDVs as responsive cross-linkers. Considering that there are only few examples of CD-based shear-thinning hydrogels showing fast recovery kinetics in the range of seconds, our system makes an important contribution to the field of injectable CD hydrogels.[17,37,38]

To prove that intact vesicles are present within the gel network, cryogenic transmission electron microscopy (cryo-TEM) and small-angle X-ray scattering (SAXS) measurements were performed. Fig. 5a shows cryo-TEM pictures of a hydrogel with 5 mM β-CDA and 2 wt% HEC–AD. Intact vesicles of 50–150 nm in diameter as well as some disrupted vesicles were found. We emphasize that for preparation of the cryo-TEM samples the gel had to be diluted about 10-fold with stirring. We assume that some of the vesicles were torn into pieces during this step resulting in the observed vesicle fragments.

The scattering profiles of a 5 mM β-CD vesicle dispersion and a hydrogel sample containing 5 mM β-CDA and 1 wt% HEC–AD are shown in Fig. 5b. For both samples, the scattered intensity $I(q)$ showed a q^{-2} decay at moderate q values, which is characteristic for bilayer scattering. The correlation peak at small q values furthermore corresponds to a bilayer thickness $d = 2\pi/q_{peak}$ of the vesicles and is determined to 5.0 nm for the vesicles in the gel matrix and 4.2 nm for the vesicles in aqueous solution. These values are in good agreement with previously made small-angle X-ray diffraction measurements of air-dried multilayers of β-CDVs[30] and overall the results confirm the presence of intact vesicles interconnected by polymer chains within the gel matrix.

Conclusion

In summary, a novel cyclodextrin-based polymer hydrogel was prepared containing vesicles as specific non-covalent and responsive junctions. A cellulose polymer was functionalized with adamantane side groups and cyclodextrin vesicles served as a multiple 3D cross-linker. Moreover, the gel network showed responsiveness as it could readily be destroyed by the addition of a competitive host and guest. The material properties were studied with rheology and found to be easily tuned by varying the concentration of the components. Most striking, the hydrogel showed shear-thinning and self-healing properties and was injectable with a fast recovery of the gel bulk structure.

The presence of vesicles within the gel network as well as the biocompatibility of the components may have further advantages for drug delivery purposes. We propose that therapeutic agents can be loaded inside the vesicles and controlled release can be induced due a distinct transport resistance of both the vesicle bilayer and the gel network.

Acknowledgements

We are grateful for financial support from the Graduate School of Chemistry in Münster (fellowship to S. Himmelein). We thank PD Dr Cornelia Cramer-Kellers for her kind introduction into rheological measurements. The authors acknowledge the European Synchrotron Radiation Facility in Grenoble (France) for the beam time at the high-brilliance beamline ID02. We are grateful for local assistance from Dr. G. Lotze and Dr. T. Narayanan as well as help with the measurements by Dr. I. Voets and Dr. P. Besenius.

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