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Bio-inspired catechol chemistry: a new way to develop a re-moldable and injectable coacervate hydrogel†

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A new way is demonstrated to develop a bio-inspired coacervate hydrogel by following catechol chemistry showing injectable and re-moldable physical properties. The formed coacervate shows potential long-term stability under water. Depending on pH, formation of the coacervate has been verified which is confirmed by XPS and zeta potential measurements.

Complex coacervation has been employed where liquid like materials are self-assembled *via* ionic interaction between two oppositely charged polyelectrolytes to generate various functional materials following spontaneous aqueous phase separation.[1] Recently, it was reported that a wide variety of drug encapsulating systems, referred to as "aqua materials", utilizes coacervate formation due to their unique physiochemical characteristics that can be easily modulated by pH, ionic strength, charge density, swelling properties and stoichiometry of interacting molecules.[2–4] Aqua materials can be molded into different shapes, while possessing moderate mechanical properties exhibiting complete self-healing property when damaged.[5,6] Unlike covalently cross-linked hydrogels that are not injectable once formed, aqua materials such as non-covalent hydrogels *via* intermolecular interactions have unique advantages, particularly as environmentally responsive drug delivery materials and injectable hydrogels. However the poor physical stability is a typical drawback of non-covalent hydrogels.[7–9]

Herein, we report the development of a novel remoldable and injectable hydrogel system based on complex coacervate formation of binary polymers: dopamine conjugated hyaluronic acid (HA-DN) and lactose modified chitosan (chitlac). Coacervation was achieved by both electrostatically driven inter-polymer chain interactions as well as Michael addition reaction of catechol molecules at alkaline pH. To the best of our knowledge, our system is the first example of a bio-inspired coacervate hydrogel system that possesses injectable/moldable property with long term underwater physical stability. HA-DN was employed as an anionic polymer in our system. The catechol derivative, such as dopamine, is a synthetic mimic of natural amino acid 3,4-dihydroxyphenylalanine (DOPA), which plays a key role in mussel foot protein adhesion in an aqueous environment.[10,11] In this study, HA was conjugated with dopamine by simple carbodiimide chemistry in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), yielding HA-catechol (HA-DN) with superior biocompatibility and degradability.[12–14] For matching polycations, biocompatible and bio-responsive chitlac was synthesized and utilized as reported elsewhere.[15]

A coacervate hydrogel was formed *via* physical gelation where catechol molecules, conjugated on the HA backbone, play a pivotal role in enhancing the stability of interpenetrating coacervate hydrogels under different conditions. In addition, the catechol molecule is highly reactive at alkaline pH and, under this condition, spontaneous catechol polymerization can be utilized.[16,17] When solution of HA-DN is prepared at neutral pH and mixed with chitlac, ionic interaction between two oppositely charged polymers dominated, resulting in the coacervate formation following electrostatic interaction (method C). Meanwhile, when pH of medium is adjusted to the alkaline pH of 8.5, catechol–quinone transition is triggered following Michael addition reaction. This process has significantly enhanced the physical stability of coacervate hydrogels providing additional crosslinking points between the polymer chains of HA-DN and chitlac. Conversely when both polymer solutions are prepared independently at pH 8.5 and mixed together (method E), coacervate formation is strongly disfavored. It is assumed that, at alkaline pH, catechol molecules in HA-DN will participate in self-crosslinking of HA-DN rather than interacting with oppositely charged chitlac. Additionally, low chain flexibility and steric hindrance generated by self-crosslinking of HA-DN will prevent the formation of an interpenetrating network between HA-DN and chitlac. Under this condition, a negligible amount of ionic interactions occur between two different polymer chains, which is not enough to form a stable intermolecular coacervate (Scheme 1a inset).

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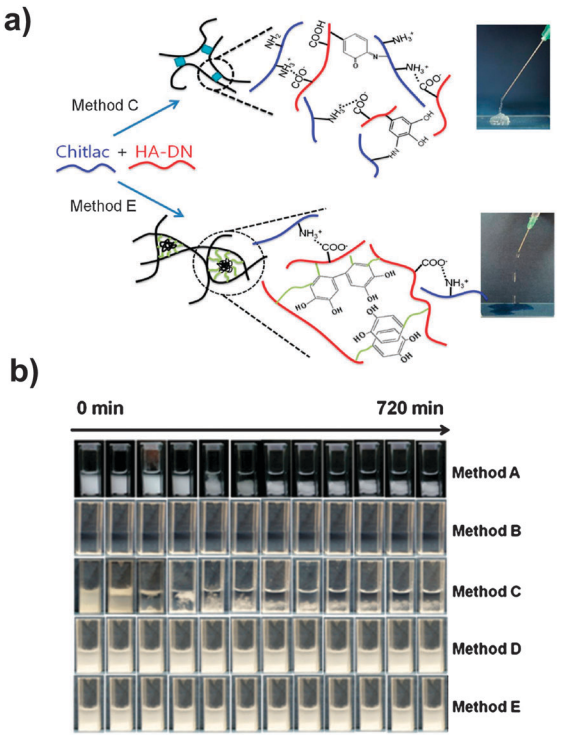

Scheme 1 (a) Proposed mechanism of coacervate hydrogel formation. (b) Optical observation of coacervate formation under different buffer conditions (0, 10, 20, 30, 40, 60, 120, 240, 360, 480, 600, and 720 min).

To confirm our hypothesis, complex coacervate formation was evaluated at various pH values (Scheme 1b). Formation of the coacervate was carefully monitored by observing the phase separation of the binary polymer system. Following method 'A', using chitlac and unmodified HA, soft coacervate hydrogels were formed through weak ionic interactions between primary amine of chitlac and carboxylic acid of HA at pH 8.5. At acidic pH of 4 in method 'B' and basic pH of 10 in method 'D', there was no sign of coacervate formation, when HA-DN and chitlac solution were mixed together. In both cases, appropriate charge interactions between two polymer chains could not be maintained under such pH conditions. On the other hand, time-lapse optical images in Scheme 1b clearly exhibited complex coacervate formation as early as 2 h following method C. In addition, method 'E' did not show complex coacervate formation which translated our hypothesis into practical use, confirming the domination of intramolecular self-crosslinking of HA-DN at alkaline pH.

In order to investigate the effect of the charge ratio on the binary system, transmittance of solution and zeta-potential of the resulting coacervate were evaluated (Fig. 1). Upon increasing the ratio of HA-DN to chitlac, the transmittance and zeta-potential values decreased sharply and, at a 1 : 1 ratio, a neutral zeta-potential value was observed. It should be noted that, when hydrogels were formed by complex coacervation, a very low transmittance value was observed due to the phase separation followed by precipitation. At a 1 : 1 ratio, the zeta-potential was close to zero and the slight increase in the amount of HA-DN resulted in a very low transmittance, which we thought as the optimal condition for complex coacervate formation.

The UV-Vis spectrum indicated the formation of a stable coacervate through catechol–amine interaction. The red shift of

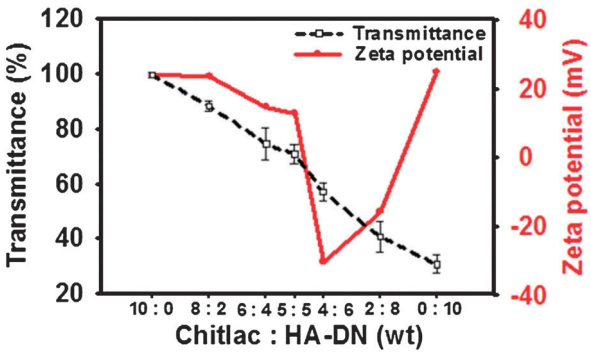

Fig. 1 Complex coacervate formation (0.1 wt%) with respect to different weight ratios between chitlac at pH 8.5 and HA-DN at pH 7.4 as indicated by transmittance and zeta-potential measurements.

the catechol peak was observed from 260 nm to 280 nm as well as increased UV absorbance over the range, indicating the presence of reacted catechol molecules (Fig. S1, ESI†). XPS data clearly indicated the presence of all possible amines such as $-NH_2$, –NH– and –N═ at 401.7, 398.8 and 398.5 eV, respectively, for method 'C' (Fig. S2a, ESI†). Primary amine can freely react with activated catechol–quinone to generate various chemical bonds to enhance the physical stability of the coacervate. However, in the case of method 'E' (Fig. S2b, ESI†), the imine peak was absent, confirming the lack of interaction between primary amine of chitlac with catechol of HA-DN. This result strongly supported that coacervate formation was only achievable *via* method 'C'. It is likely that the initial ionic interaction is necessary to form interpenetrating networks between HA-DN and chitlac prior to the covalent crosslinking of a binary polymer system by adjusting the pH of medium to initiate the reaction of activated catechol and primary amine.

The time dependent rheological property of a stable coacervate hydrogel was studied to examine the importance of initial electrostatic coacervate formation as we found the time dependency of coacervate formation from the optical observation. The G' increased rapidly for first 2 h, and then a gradual increase was observed for next 5 h and finally showed a stable condition until 12 h incubation, indicating that ionic interactions took place at an early time period for coacervate formation followed by equilibrium conditions for 12 h (Fig. 2a), which corresponds well to our previous results. We believe that, during this period, physical properties of the hydrogel become enhanced due to the formation of catechol cross-linking and covalent bond formation between binary polymers *via* Michael addition reaction.[18]

To confirm enhanced physical properties of hydrogels, swelling kinetics of hydrogels was obtained at 37 °C by measuring percentage swelling (%) of the samples from method A and method C at various time intervals (Fig. 2b). The swelling ratio of hydrogels formed by method A was much larger than that of method C after 1 h incubation in PBS. This phenomenon can be explained as follows: the bio-inspired coacervate hydrogels formed by method C possess additional crosslinking property, resilient for chain stretching and water absorption, as compared to that of ionically generated hydrogels by method A. Within 2 h, hydrogels formed by method A were completely disappeared due to the poor

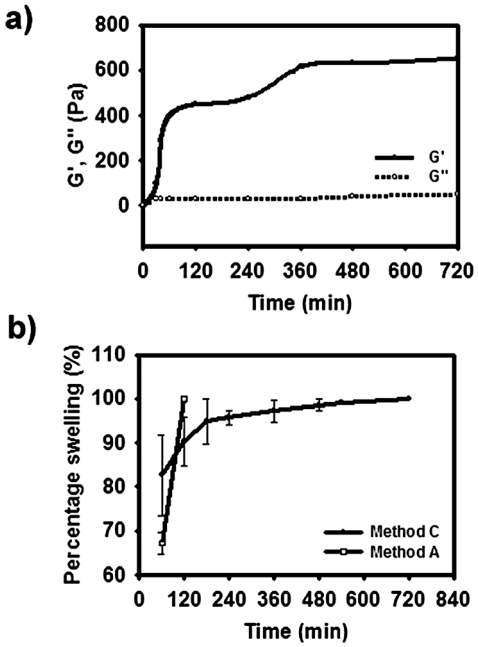

Fig. 2 Rheological behaviors of formed coacervate hydrogels. (a) G' (storage modulus) *vs.* G'' (loss modulus) at different time points, (b) percent swelling of coacervate hydrogels (method C) compared to the ionic hydrogel formed by method A.

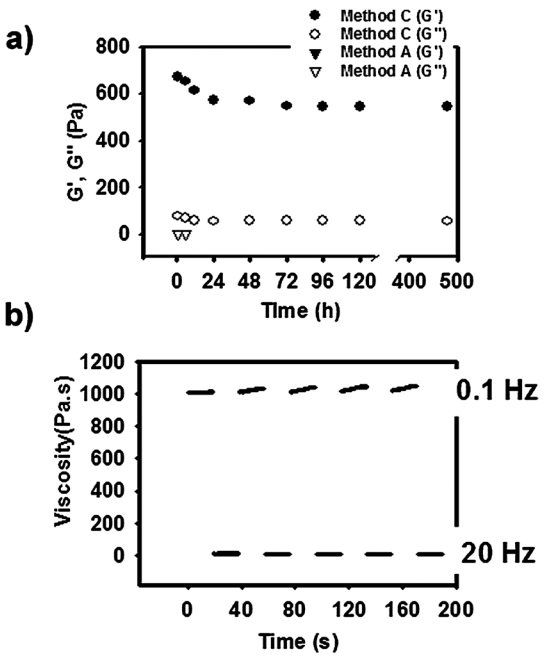

Fig. 3 Physical behaviors of bio-inspired coacervate hydrogels. (a) Dynamic modulus of coacervate hydrogels against time, (b) viscosity change of coacervate hydrogels by shear thinning and thickening at 0.1 and 20 Hz.

physical stability of an ionic coacervate formed by the chitlac–HA binary polymer system. However, bio-inspired coacervate hydrogels formed by method C maintained their physical structure until 12 h. This result suggested that covalent crosslinking by catechol chemistry provided a stronger network between HA-DN and chitlac polymers, holding enough water inside the complex coacervate while maintaining its structure.

The physical stability of the coacervate hydrogels was further examined for an extended period of time (Fig. 3a). Excellent conformity was found to support the physical stability of the hydrogels indicating the greater storage modulus values over the loss modulus for up to 20 days. Lastly, we evaluated the physical behaviors of bio-inspired coacervate hydrogels as injectable aqua materials. As shown in Fig. 3b, a shear thinning behavior of coacervate hydrogels suggests that given materials can be remolded or behave like viscous fluids with an increasing rate of shear stress. In addition, our bio-inspired coacervate hydrogels regained elastic gel-like behavior ($G'/G'' > 1$) when the rate of shear stress was reduced and their physical structure was maintained throughout the repeated experiments confirming the aqua material-like behavior exhibiting characteristics of both hydrogel and viscous fluid.

In conclusion, we have demonstrated a novel re-moldable and injectable coacervate hydrogel following catechol chemistry. Depending on pH the hydrogel was formed from HA-DN–chitlac interaction following catechol–amine reaction. Based on zeta-potential and XPS data, we concluded that this interpenetrating binary hydrogel was physically more stable and robust due to the inter-molecular polyelectrolytes complex coacervation as well as covalent crosslinking formed *via* catechol cross-linking and cyclization. Our bio-inspired coacervate hydrogels possessed the properties of aqua materials with enhanced physical properties such as an excellent long-term physical stability and hence would become an attractive candidate for various biomedical and tissue engineering applications.

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