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Ionic-covalent entanglement hydrogels from gellan gum, carrageenan and an epoxy-amine

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Abstract

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Keywords

ionic, gellan, gum, amine, hydrogels, epoxy, carrageenan, entanglement, covalent

Disciplines

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Ionic-covalent entanglement hydrogels from gellan gum, carrageenan and an epoxy-amine

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A 'one pot' preparation of interpenetrating polymer network hydrogels with double network characteristics is presented. A small addition of biopolymer dramatically increases the stiffness and strength of the epoxy-amine gels without affecting the large strain at failure value.

Hydrogels have been employed commercially in food[1], pharmaceuticals[2] and wearable prosthetics[3], applications that make use of the inherent non-toxic properties of many hydrogel-forming polymers. However, fully swollen hydrogels have low strength and toughness[4]. As a result, few current applications require the hydrogels to bear significant mechanical load. Overcoming this natural weakness will open the field to an array of applications including flexible electrodes, soft tissue mimics for tissue engineering and soft robotics[5,6].

In 2003, Gong *et al.* demonstrated that hydrogel materials can be toughened by interweaving two separate polymer networks to create a hybrid material[7]. The strength and strain to failure of these 'double network' (DN) hydrogels is much greater than those of the parent networks [5,7,8,9]. The formation of these gels was achieved by sequential network formation, in which a primary network was formed photo-catalytically before a second monomer solution was swollen into it and subsequently cross-linked. The process required UV irradiation in nitrogen atmospheres[7,10]. By selecting polymers whose gelling chemistries do not interact, interpenetrating polymer networks (IPN) can be formed simultaneously, rather than sequentially. However, there is currently no clear understanding of how the mechanical properties of a hybrid gel formed in this fashion will compare to those of the component gels.

Gellan gum (GG) and the carrageenans (CG) are anionic polysaccharides which are USA FDA and European Union approved as food additives, and have found wide application as gelling, stabilising and suspending agents[11,12]. Gellan is a biopolymer with carboxylic acid groups, whereas the carrageenans are sulfonated. These biopolymers form hydrogels in the presence of divalent cations such as calcium. Epoxy-amine (EA) chemistry is a simple covalently binding reaction that yields linkages similar to the natural peptide bond. Unlike most forms of acrylate polymerisation, epoxy-amine chemistry provides a simple route for forming covalent networks that are not readily disrupted by physical factors[13]. Both the epoxy-amines and the biopolymers are actively investigated as materials for future application in tissue engineering[14,15,16,17].

Interpenetrating gel networks based on cross-linked acrylates and polysaccharides have recently been reported to have high strength and toughness[18,19]. These gels are notable for a “Mullins effect”, a slow recovery of stiffness after extension to high strains[18,19].

Here, we report on IPN hydrogels based on polysaccharides and epoxy-amine chemistry that form in ‘one pot’. These ionic-covalent entanglement (ICE) gels show mechanical effects that differ from polysaccharide/acrylate and double network gels. This new combination of ionic and covalent binding (Fig. 1) allows the two polymer backbones to be cross-linked simultaneously, requiring fewer steps than sequential network formation. In this process the cross-linking of the biopolymer network is likely completed first, as they are known to gel on a timescale of minutes. The epoxy-amine curing proceeds on a slower timescale, increasing the strength and rigidity of the ICE gel over several days.

It is suggested that the stiffness, strength and curing time of the ICE systems can be tailored by tuning some or all of the following parameters: biopolymer concentration, ionic cross-linker concentration, different ionic cross-linkers (e.g. Na^+, Mg^{2+}), molecular weight of PEDGE, molecular weight of Jeffamine and weight ratio between the biopolymer and epoxy amine networks.

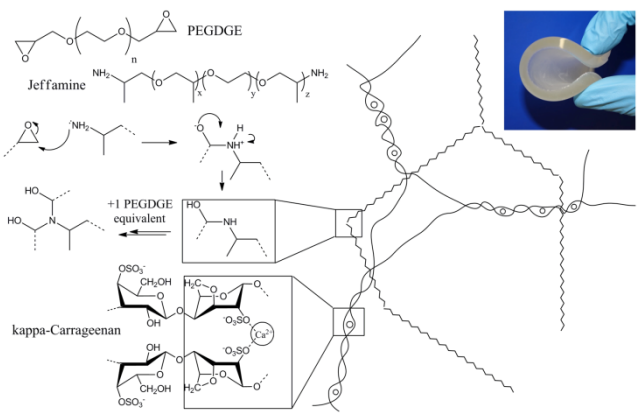

Fig. 1. Schematic representation of ionic-covalent entanglement gels: Top left, the epoxy-amine linking reaction between Jeffamine (y=39, x+z = 6) and PEGDGE (n=9). Bottom left, the ionic cross-linking sites on the kappa-carrageenan biopolymer (bottom left) and right, the resulting interpenetrating network. The photo shows a typical ICE hydrogel.

The hydrogels used in this study were prepared as follows. A calcium chloride ($CaCl_2$, 0.15 mol/L) solution was generated from anhydrous calcium chloride (≥95%, Fluka Analytical, batch 0001251790) and Milli-Q water (resistivity 18.2 MΩ cm). Biopolymer solutions (2% w/v) were made by dissolving low-acyl gellan gum (CP Kelco, batch 9K6968A), iota carrageenan (i-CG, CP Kelco, Batch SK93842) or kappa-carrageenan (k-CG, CP Kelco, batch SK92650) in Milli-Q H_2O on a laboratory hotplate (Stuart, CB162) with heating (70 °C) and magnetic stirring before capping and storing overnight at 70 °C in a laboratory oven (Binder Inc., 1.2 kW). A solution of epoxy-amine (50% w/v) was then prepared by mixing 50 mL Milli-Q, 34 g of a molten (40-50˚C) polyetheramine (Jeffamine ED-2003, Huntsman, Batch 1F518, molecular weight 2000 g/mol), and 14 g of poly(ethylene glycol) diglycidyl ether (PEGDGE, Aldrich, Batch MKBC9721, M_n = 500 g/mol). These precursor solutions were then combined hot (70 °C), at ratios dictated by the gel type: Epoxy-amine gel solutions (25% w/v EA) were formed from 50 mL each of EA solution and Milli-Q. Biopolymer gel solutions (0.9 % w/v biopolymer, 13.6 mM $CaCl_2$) were formed from 50 mL each of biopolymer solution and Milli-Q water with 10 mL of 0.15M $CaCl_2$. ICE gel solutions (0.9% w/v biopolymer, 22.7% w/v EA, 13.6mM $CaCl_2$) were formed from 50 mL each of EA and biopolymer solutions with 10 mL of 0.15M $CaCl_2$. Once mixed, gel solutions were decanted into PVC molding wells, sealed and stored under controlled ambient conditions (45% relative humidity, 21˚C) for 1 week prior to testing. All gels were tested as-made.

Compression testing was conducted on a universal mechanical tester (Shimadzu, EZ-S). Quadruplicate samples were placed into individual 60 mm Petri dishes lined with moist, absorbent paper and linearly compressed with a 15 mm diameter head at a rate of 25 mm/min to a maximum compression of 8 mm (80%). Ionic-covalent entanglement gels containing i-CG and k-CG (Fig. 2a, Fig. S1, Supporting Information and Table 1) showed substantially higher moduli and strengths than the epoxy-amine networks, with the reinforcement effect being most prominent at high strain. For example, the stress at 80% strain value of the k-CG/EA gels (930 ± 130 kPa) is 5.3 fold higher than the corresponding EA hydrogels. This observation combined with reinforcement compared to k-CG gels is characteristic of double network behaviour[7]. Although forming the strongest network as a single network (Fig. 2b, Table 1), gellan gum does not significantly change the modulus and failure properties of the epoxy-amine gels.

Hydrogels produced in this study were further examined by rheological testing using a controlled strain rheometer (Anton Paar Physica MCR 301, 15 mm parallel plates). Quadruplicate samples were tested under a dynamic amplitude sweep mode between 0.01% and 100% strain at a constant angular frequency of 5 Hz (Fig. 3 and Fig. S2, Supporting Information). Using this technique, complex shear modulus may be separated into its elastic 'storage modulus' (G') and viscous 'loss modulus' (G"), which reflect the solidity and liquidity of the material, respectively. Soft, non-Newtonian materials are known to exhibit a finite 'linear viscoelastic' (LVE) region in which the storage and loss moduli are invariant with strain[20]. The end of the LVE region (determined as the strain where the G' decreases by more than 10% from the plateau value) corresponds with the maximum shear strain that can be applied to the material before it undergoes irreversible deformation. As shown in Table 2, the addition of a small amount of the carrageenans (to the EA) greatly increases the modulus compared to that of the epoxy-amine gel without reducing the yield strain. As with the compression tests, k-CG was more effective than i-CG in stiffening the EA gel. However, this change was relatively modest when compared to the large difference in the moduli of i-CG and k-CG gels. The modulus of single network gels of i-CG is 1/50 the modulus of k-CG gels, but when combined with the epoxy-amine system the relative improvement is much greater (Table 1).

Table 1. Summary of values for water content (WC), stress at failure (σ_f), strain at failure (ε_f) and tangent modulus (E_{tan}, 20-30% strain) of the hydrogels formed from gellan gum (GG), kappa-carrageenan (k-CG), iota-carrageenan (i-CG), and epoxy-amine (EA). Where failure was not observed, stress at 80% (σ_{80}) is reported in lieu of failure stress. The data was Q-tested (confidence interval ≥ 95 %) and the reported values and numerical errors are averages of the values obtained and ± 1 standard deviation, respectively.

Hydrogel	WC (%)	E_{tan} (kPa)	σ_f (kPa)	σ_{80} (kPa)	ε_f (%)
EA	75	79 ± 39	-	175 ± 43	≥ 80
i-CG	99	1.3 ± 0.4	1.8 ± 0.2	-	79 ± 2
k-CG	99	77 ± 9	57 ± 2	-	54 ± 1
GG	99	86 ± 35	180 ± 130	-	40.0 ± 0.3
i-CG/EA	76	250 ± 100	-	592 ± 7	≥ 80
k-CG/EA	76	360 ± 150	-	930 ± 130	≥ 80
GG/EA	76	59 ± 32	-	300 ± 170	≥ 80

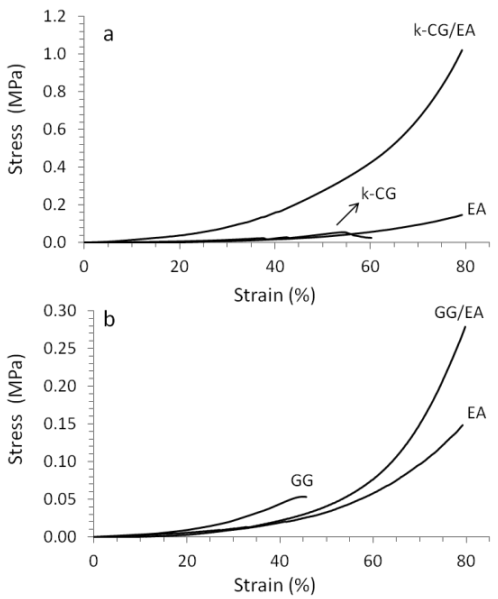

Fig. 2. Typical compressive stress-strain curves of (a) kappa-carrageenan (k-CG), epoxy-amine (EA) and k-CG/EA ICE hydrogels and (b) gellan gum (GG), EA and GG/EA ICE hydrogels.

Although gellan gum is the stiffest polysaccharide gel, both compression and rheological analysis revealed it has almost no effect when incorporated into the epoxy-amine gels. At present we do not have a definitive explanation for this behaviour, however, it is suggested that this may be caused by GG losing its ability to become cross-linked by the Ca^{2+} ions. The absence of this occurrence for the carrageenans may point towards a deactivation or complexation of the GG carboxyl functionality

that prevents the cross-linking of the GG network.

Table 2. Summary of rheological properties. Linear viscoelastic storage modulus (G'), linear viscoelastic loss modulus (G") and linear viscoelastic region endpoint (γ_{max}) of hydrogels formed from gellan gum (GG), kappa-carrageenan (k-CG), iota-carrageenan (i-CG), and epoxy-amine (EA).

Hydrogel	G' (kPa)	G" (kPa)	γ_{max} (%)
EA	21.2 ± 5.0	0.074 ± 0.021	41 ± 37
i-CG	0.131 ± 0.013	0.0059 ± 0.0043	≥100
k-CG	72.4 ± 6.3	6.73 ± 0.74	0.61 ± 0.22
GG	176 ± 25	14.7 ± 1.6	0.288 ± 0.053
EA/i-CG	92 ± 26	0.188 ± 0.054	44 ± 13
EA/k-CG	120 ± 57	2.3 ± 1.0	46 ± 23
EA/GG	25 ± 17	0.219 ± 0.021	58 ± 10

Previous research on the polysaccharide/acrylate hybrid gels showed a slow recovery effect from large strains[18,19], whereas double network gels display irreversible softening under cyclic loading[21,22]. It has been suggested that hybrid gels have the potential ability to withstand strain without any significant reduction in mechanical characteristics, which is important for the future application of these materials as load tolerant hydrogels[18,19]. This was investigated by subjecting the hydrogels to a cyclic testing regime between 20% and 40% compressive strain (rate 50 mm/min). The gels were held for 2 s between loading and unloading cycles. The evolution of typical EA, k-CG and k-CG/EA gels is shown in Fig. 4. The tangent moduli of the loading and unloading cycles are approximately constant for the EA and k-CG/EA gels, but decrease significantly for k-CG gels. The relative mechanical characteristics are maintained, i.e. ICE gels of k-CG/EA have higher tangent modulus compared to EA gels. These results indicate that the mechanical characteristics of the EA and ICE gels are not adversely affected by the imposed testing regime over 10 cycles. In contrast, the k-CG gels are unable to withstand repeated mechanical compression.

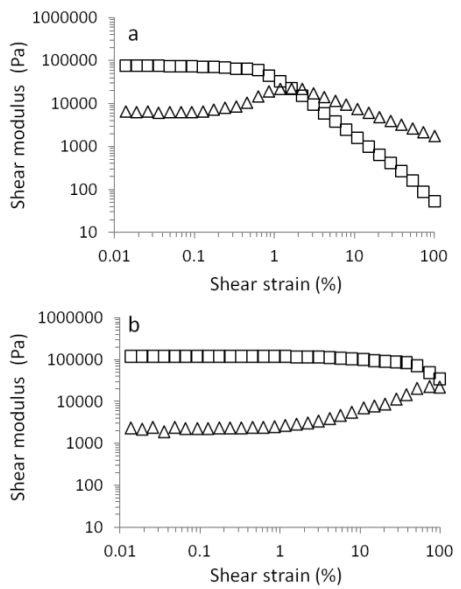

Fig. 3. Typical amplitude sweeps of (a) kappa-carrageenan, and (b) kappa-carrageenan/epoxy-amine ICE hydrogels. Storage and loss moduli are indicated by squares and triangles, respectively.

Similar results were obtained for the other types of gels, i.e. biopolymer gels were unable to withstand the cyclic compression, but i-CG/EA and GG/EA ICE gels were not affected (Fig. S3, Supporting Information). The average ratios of the tangent moduli of the unloading to the loading part of EA, k-CG/EA, i-CG/EA and GG/EA ICE gels over 10 cycles are 0.93 ± 0.09, 0.71 ± 0.06, 0.84 ± 0.06, and 0.60 ± 0.07, respectively. This could suggest that the ICE gels dissipate more energy compared to the EA gels when cycled between 20 and 40% strain.

Previous research on the behaviour of interpenetrating gels has revealed two distinct modes of reinforcement: (i) double network hydrogels, in which the initial moduli of double network gels are similar to the component gels, but they exhibit a dramatic increase in stiffness in the high strain region [7]; and (ii) gellan gum/poly(acrylamide) hydrogels, combining gellan gum with poly(acrylamide) into a hybrid gel resulted in a modest increase in initial modulus, but a large increase in the strain to failure value compared to gellan gum gels[18]. Hence, combining a low modulus/high strain poly(acrylamide) network with high modulus/low strain gellan gum network results in a high modulus/high strain hybrid gel. The results presented in this paper suggest a third variant, the carrageenan/epoxy-amine hybrid gels are much stiffer than either gel alone, and retain the high strain to failure of the epoxy-amine gels.

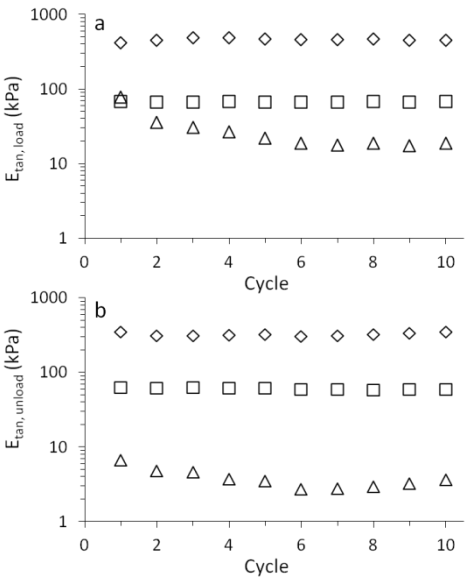

Fig. 4. Typical tangent moduli (20-30% strain) calculated using the (a) loading and (b) unloading parts of the cyclic testing regime for kappa-carrageenan (k-CG, triangles), epoxy-amine (EA, squares) and k-CG/EA ICE (diamonds) hydrogels.

Conclusions

In summary, we have introduced ionic covalent entanglement (ICE) hydrogels based on interpenetrating polymer networks of covalently bound epoxy-amines and ionically cross-linked polysaccharides. Networks of biopolymers of the carrageenan and gellan gum families were entangled with covalently linked epoxy-amine in a 'one pot' synthesis with minimal processing. It was found that carrageenan biopolymers provided substantial mechanical reinforcement when entangled with epoxy-amine systems, whereas gellan gum did not. Specifically, the addition of kappa-carrageenan to the epoxy-amine yielded a 5-6 fold increase in tangent modulus and compressive stress at failure value when

compared to epoxy-amine gels of comparable water contents. The ICE gels were formed under mild conditions and are seemingly tough, flexible and resilient. Consequently, ICE gels provide a promising avenue for research towards load tolerant hydrogels.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [typical compressive strain curves for i-CG/EA hydrogels, typical strain sweeps for i-CG/EA and GG/EA hydrogels and cyclic testing of GG/EA hydrogels]. See DOI: 10.1039/b000000x/

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