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Strong tough gels for 3D tissue constructs

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Abstract

The mechanical characteristics of ionic-covalent entanglement hydrogels consisting of combinations of the biopolymers gellan gum and kappa-carrageenan, and the synthetic polymers polyacrylamide and an epoxy amine were investigated. Compression testing showed that these gels exhibited "double network" behavior, i.e. strong tough gels.

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Strong tough gels for 3D tissue constructs

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ABSTRACT

The mechanical characteristics of ionic-covalent entanglement hydrogels consisting of combinations of the biopolymers gellan gum and kappa-carrageenan, and the synthetic polymers polyacrylamide and an epoxy amine were investigated. Compression testing showed that these gels exhibited “double network” behavior, i.e. strong tough gels.

INTRODUCTION

For some tissue engineering objectives (such as cartilage tissue scaffolds), tougher and more robust hydrogels, which have the capacity to recover from mechanical deformation are required. Conventional hydrogels have poor mechanical properties because there are very few energy dissipation mechanisms to impede crack initiation and crack propagation. When under stress, forces cannot be evenly distributed over all polymer chains equally as the distance between the cross-linking points varies. When the shortest polymer chain fractures, all the load is immediately transferred to the next shortest chain, which may subsequently break.

Hydrogels can be made substantially tougher using a variety of modern hydrogel science strategies including topological hydrogels, tetra-PEG hydrogels, double network (DN) hydrogels, and microgel reinforced hydrogels [1-4].

The DN approach has been proven to be one of the most successful approaches to preparation of tough hydrogels and has been explored extensively by J. P. Gong and co-workers using poly(2-acrylamido,2-methyl,1-propanesulfonic acid) and poly(acrylamide) (PAMPS/PAAm) systems [3]. Here, gels are prepared in a two-step scheme where a PAMPS hydrogel network is swollen in acrylamide (monomer), which is subsequently photo cross-linked. They have determined that “the rigid and brittle PAMPS network serves as a sacrificial bond that fractures at a relatively low stress, while the soft, ductile PAAm network serves as hidden length that sustains stress by large extension afterwards” [3].

J.P. Gong has also hypothesised that “the introduction of any sacrificial bonds that yield and dissipate energy upon deformation will toughen the materials” [5] and that “the same DN gel concepts can, in principle, be applied to other self-healing types of materials, if the covalent bonds are replaced by reversible bonds” [3]. This hypothesis has been tested and found to be true by preparing gels with different types reversible sacrificial bonds including the hydrophobic interactions of molecules forming lamellar bilayers and ionic cross-links [6-11].

In this paper, we provide the mechanical characteristics (in compression) of strong tough hydrogel material with reversible bonds, i.e. ionic-covalent entanglement (ICE) network hydrogels.

EXPERIMENT

The hydrogels used in this study were prepared as follows. Kappa-carrageenan solutions (2% w/v) were made by dissolving kappa-carrageenan (k-CG, CP Kelco, batch SK92650) in Milli-Q water, resistivity 18.2 M Ω cm, 70 °C under stirring). 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. k-CG gel solutions (0.9 % w/v, 13.6 mM CaCl₂) were formed from 50 mL each of biopolymer solution and Milli-Q water with 10 mL of 0.15M CaCl₂.

ICE k-CG/EA gel solutions (0.9% w/v k-CG, 22.7% w/v EA, 13.6mM CaCl₂) were formed from 50 mL each of EA and k-CG solutions with 10 mL of 0.15M CaCl₂. 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.

GG hydrogels were prepared from gellan gum solutions (low-acyl gellan gum, CP Kelco, 1.0% w/v in Milli-Q water, 70 °C) cross-linked with CaCl₂ (2 mM, 70 °C). The solution was then transferred to a PVC mould to fabricate cylindrical shaped hydrogels. PAAm hydrogels were prepared from a hot (70 °C) solution of acrylamide (AAm, 3.5% w/v) and *N,N'*-methylenebisacrylamide (MBAAm, 0.9 mg/ml) to which *N,N,N',N'*-tetramethylethylenediamine (TEMED, 0.094 mg/ml), potassium persulfate (KPS, 0.68 mg/ml) were added prior to transferring to a mould in a dessicator. Following casting, all samples were evacuated to 0.100 bar (to prevent bubble formation), and held for 2 hrs.

ICE GG/PAAm hydrogels were synthesized by a simultaneous network formation technique. For example, a GG/PAAm hydrogel was prepared by combining hot (70 °C) solutions of MBAAm/AAm and GG, followed by drop-wise addition of hot (70 °C) solutions of TEMED, KPS and CaCl₂. The resulting hot solution was transferred to a mould in a dessicator, which was then evacuated (to 0.100 bar) and held for 2 hrs.

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 10-25 mm/min to a maximum compression of 8 mm (80%).

DISCUSSION

Figure 1 shows typical stress-strain curve of GG, PAAm and ICE GG/PAAm ICE hydrogels. This data shows that these ICE gels exhibit “double network” behavior, i.e. the values of the mechanical properties of the ICE gels is significantly better than those of the constituent gels. For example, at failure, the GG/PAAm hydrogels can withstand significantly more stress (216 ± 31 kPa) than the corresponding GG and PAAm hydrogels (see Table 1). The fracture

strain of the GG/PAAm hydrogel is more than double that of the GG hydrogel and comparable to the PAAm hydrogel.

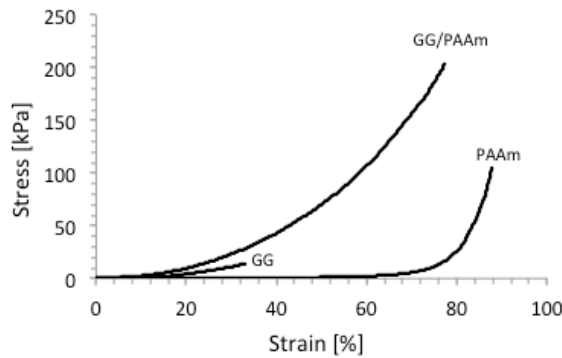


Figure 1. Typical compressive stress-strain curves of single network gellan gum (GG), poly(acrylamide) (PAAm) and ICE GG/PAAm hydrogels [8].

Typical stress-strain curves k-CG, EA and ICE k-CG/EA hydrogels are shown in Figure 2. The ICE gels showed substantially higher moduli and strengths than the k-CG and 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 significantly higher than the corresponding EA hydrogels (see Table 1). Similar to the GG/PAAm gels, the k-CG/EA gels also exhibit “double network” behaviour. The ICE gels formed from k-CG/EA are tougher (as evident from the area under the curve) compared to the GG/PAAm gels.

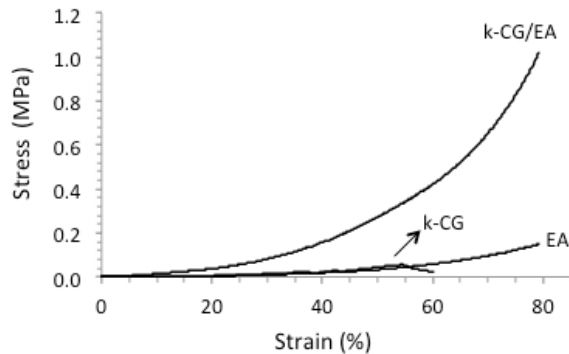


Figure 2. Typical compressive stress-strain curves of (a) kappa-carrageenan (k-CG), epoxy-amine (EA) and ICE k-CG/EA hydrogels [9].

Table 1. Summary of mechanical characteristics. Summary of values for 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), polyacrylamide (PAAm), and epoxy-amine (EA). Where failure was not observed (indicated by ≥ 80), stress at 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.

Hydrogel	E_{tan} (kPa)	σ_f (kPa)	ϵ_f (%)
GG	90 ± 3	17 ± 1	37 ± 3
PAAm	1.6 ± 0.2	110 ± 6	≥ 80
GG/PAAm	121 ± 10	216 ± 31	77 ± 3
k-CG	77 ± 9	57 ± 2	54 ± 1
EA	79 ± 39	175 ± 43	≥ 80
k-CG/EA	360 ± 150	930 ± 130	≥ 80

CONCLUSIONS

We have introduced ionic covalent entanglement (ICE) hydrogels based on interpenetrating polymer networks of a covalently bound synthetic polymer and an ionically cross-linked polysaccharide. Networks of biopolymers were entangled with covalently linked epoxy-amine and polyacrylamide in a ‘one pot’ synthesis with minimal processing. It was found that these ICE gels provided substantial mechanical reinforcement (“double network” behavior) compared to their constituent gels. Our results demonstrated that ICE hydrogels exhibit two distinct types of reinforcement: (i) combining a low modulus/high strain PAAm network with high modulus/low strain GG network results in a high modulus/high strain hybrid GG/PAAm gel; and (ii) combining a low modulus/high strain EA network with low modulus/low strain k-CG network results in a high modulus/high strain hybrid k-CG/EA gel. These strong tough gels are excellent candidates for the development of 3D tissue constructs.

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