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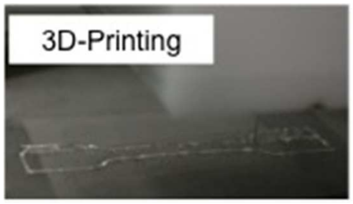

ionic-covalent entanglement hydrogels were fabricated by 3D-printing.
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Printed ionic-covalent entanglement hydrogels from carrageenan and an epoxy amine

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Carrageenan/epoxy amine ionic-covalent entanglement hydrogels were fabricated on a 3D printer. The thermal gel transition behaviour of the biopolymer kappa-carrageenan was exploited to fix the shape of the patterned ink until a covalent polymer network formed by epoxy amine addition chemistry. The printed hydrogels display a work of extension value of 1.4 ± 0.3 MJ m^{-3}.

Hydrogels are defined as materials with a solid three-dimensional (3D) network of physically or chemically cross linked hydrophilic polymer chains swollen in large volumes of water.[1] These materials have many proposed applications including use as biomedical substrates, drug-delivery systems, sensors and as artificial muscles for soft robotics.[2] However, while promising, realistic utilization of hydrogels have been hindered by difficulties in processing raw materials into complex functional devices through traditional manufacturing techniques. Recently, 3D printing has emerged as a practical tool for creating intricate hydrogel scaffolds for tissue engineering purposes[3-5] which has further demonstrated that the technology has potential to fabricate a range of hydrogel structures. 3D printing is an Additive Fabrication technique (also known as Additive Manufacturing or AM) that can build 3D objects from digital constructs by sequentially patterning layer upon layer of ink.[3]

An additional concern for fabricating with hydrogels is that they are typically brittle materials, and recent research has focused on creating mechanically robust hydrogels utilizing a variety of toughening mechanisms.[6] Examples of tough hydrogels include double-network gels,[7] nanocomposite gels,[8] slip-link gels,[9] fiber reinforced gels[10] and ionic-covalent entanglement (ICE) gels.[11-13] ICE gels in particular are interesting as they represent interpenetrating polymer network hydrogels consisting of an entanglement of one polymer network cross linked with metal cations and a second polymer network cross linked with covalent bonds.[11] ICE gels also seem amenable to novel 3D printing processes and recently a modified extrusion printer with *in situ* photopolymerisation[14] was used with tough alginate/polyacrylamide (Alg/PAAm) ICE gels.[15] Tough ICE gels have also been prepared from various other combinations of gel forming polymers such as gellan gum, carrageenan, gelatin and epoxy-amine in a 'one-pot' synthesis method[13,16] that could potentially be used in 3D printing.

This paper outlines a new method for 3D printing ICE gels that is based on optimizing the individual chemistries of the components and the rheological conditions of the ink for Additive Manufacturing. As a proof of principle of the methodology, a 3D printed composite mixture of materials (kappa-carrageenan and poly(oxyalkyleneamine) (Jeffamine)) is demonstrated. This approach exploits the thermal gelation of the kappa-carrageenan that gels first and provides structural integrity while the covalent polymer network is formed by ambient temperature epoxy-amine addition reaction.

To aid the reader a background on the materials is provided. Carrageenans are a class of water soluble biopolymers extracted from red edible seaweeds. Kappa-carrageenan (k-CG) is a dominant form of carrageenan that is uniquely identified by a single sulphonate group located on each repeat unit.[17] This group is ionized in solution, which provides cross-linking functionality between two neighboring biopolymer chains in the presence of divalent cations. Ionic cross-linking causes amorphous biopolymer chains to form an ordered double helix shaped structure, which results in the formation of a thermally reversible gel.[18]

Poly(oxyalkyleneamine) (Jeffamine) is a water soluble aliphatic diamine and poly(ethylene glycol)diglycidylether (PEGDGE) is functionalized with epoxide end groups. In solution, covalent cross-linking of Jeffamine with PEGDGE will occur between the amine and epoxide sites via an epoxy-amine addition mechanism.[19] These two polymers are to be collectively considered as a single polymer network, hereafter referred to as EA.

The preparation of the materials is as follows. Initially a k-CG/EA ICE gel precursor solution was prepared following the 'one-pot' synthesis method[13]: a calcium chloride ($CaCl_2$, 0.2 mol L^{-1}) solution was generated from anhydrous calcium chloride (≥95%, Fluka Analytical, batch 0001251790) and Milli-Q water (resistivity 18.2 MΩ cm). A solution of k-CG (CP Kelco, batch SK92650) was prepared by dissolving 1 g of biopolymer in 50 ml of Milli-Q water on a laboratory hotplate with heating (70 °C) and magnetic stirring. An EA solution was then prepared by mixing 19.8 mL of molten Jeffamine (Jeffamine ED-2004, Huntsman, Batch 1F518, molecular weight 2,000 g mol^{-1}) with 7.7 mL of PEGDGE (Aldrich, Batch MKBC9721, M_n = 500 g mol^{-1}) and 17.5 mL of Milli-Q water at 70 °C. The k-CG solution was then added to the EA solution followed by the addition of 5 mL of the hot (70 °C) calcium chloride solution under magnetic stirring on a standard laboratory hot plate.

Cast k-CG/EA hydrogel samples were prepared by decanting the mixed (precursor) solution into a sealed petri dish, and stored for at least 5 days under controlled ambient conditions (21 °C, 45% relative humidity) prior to testing. For tensile mechanical analysis, samples were cut with a "dog-bone" shaped cutter with a thickness of 1.7 mm, neck width of 4 mm and gauge length of 50 mm.

Rheological testing was used to establish the optimal conditions for extrusion printing the k-CG/EA ICE gel precursor solution (i.e. the ink). Gel transition point tests were applied to determine at what temperature the ink and its constituents k-CG and EA undergo a thermal transition from solution to gel. Immediately following synthesis, ink, k-CG and EA solutions were pipetted hot (70 °C) onto the heated (70 °C) Peltier plate of an Anton Paar Physica MCR 301 rheometer to allow for its complex viscosity to be tracked during cooling. The complex viscosity of the solutions was measured at a fixed amplitude and frequency of 1 % and 10 Hz, respectively, between 70 °C and 20 °C at a cooling rate of 2 °C min^{-1}. The thermal gelation transition of the cooling k-CG solution was observed as a rapid increase in complex viscosity as the solution was cooled below 50.7 ± 0.2 °C (data not shown). No thermal gelation transition was observed for the EA solution, its complex viscosity of the remained less than 1 Pa.s when cooled to 20 °C (data not shown). The thermal gel transition of the cooling ink was observed as a significant change in the gradient of the complex viscosity between 45.0 ± 0.2 °C and 58.8 ± 0.2 °C in the gel transition point test (Figure 1a). The gel transition temperature of the ink (50.4 ± 0.3 °C) was calculated as the midpoint of this transition. This transition temperature was selected as the optimal temperature for printing the ink as its moderate complex viscosity is compatible with the extrusion process. In addition, the ink will rapidly solidify when deposited on a cool (21 °C) substrate to fix the patterned design.

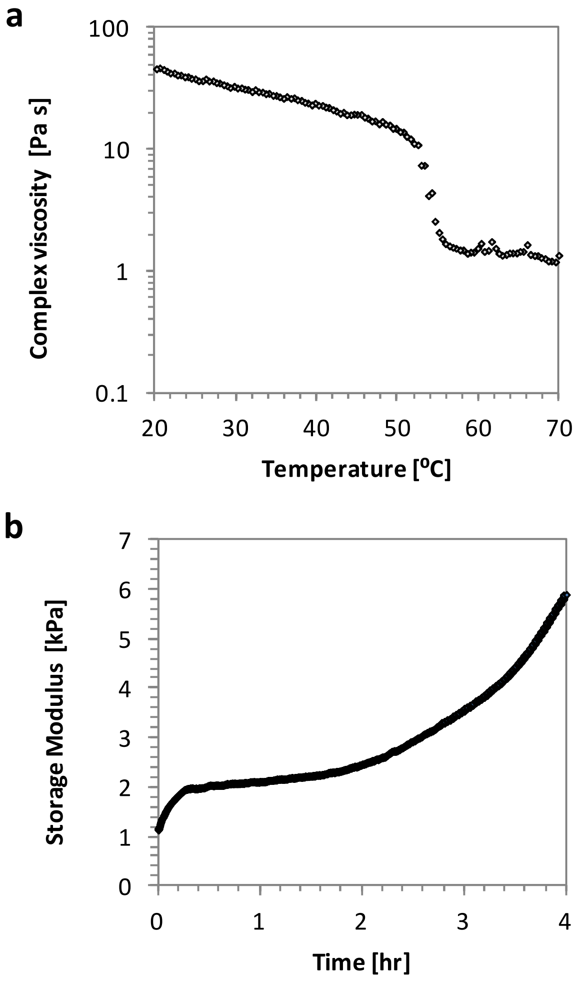

Figure 1 a. Complex viscosity of a typical ink (k-CG/EA ICE gel precursor solution) as a function of temperature under cooling from 70 to 20 °C. b. Storage modulus of a typical ink (initial temperature 70 °C) as a function of time when equilibrated at 50.4 °C for 4 hr.

A time sweep test was then performed upon the ink to establish the period of time during which the rheological characteristics were not significantly altered due to EA network formation. The ink was pipetted hot (70 °C) onto the heated plate (50.4 °C) of the rheometer. The storage modulus was measured at a constant temperature, amplitude and frequency of 50.4 °C, 1 % and 10 Hz, respectively for 4 hr. An increase in storage modulus was observed in the first 0.5 hr of the time sweep test (Figure 1b). It is suggested that this is caused by the thermal gelation of k-CG solution (as a result of cooling from 70 °C to 50.4 °C). The storage modulus remained relatively constant for a period of time between 0.5 and 2.5 hr prior to a rapid increase due to formation of the EA network. Hence, the optimum window for printing the inks at 50 °C is between 0.5 and 2.5 hr after preparation.

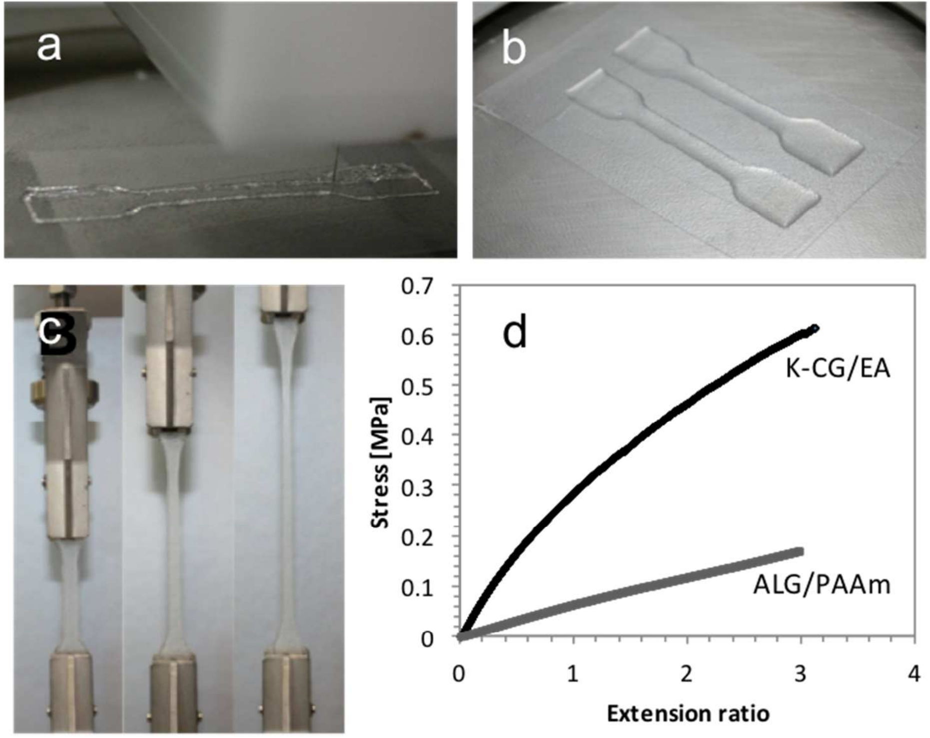

Figure 2 a. Photograph of extrusion printing a k-CG/EA ICE gel dumbbell shape for tensile testing. b. Photograph of typical 3D-printed k-CG/EA ICE gel dumbbell shapes. c. Series of photographs showing a printed k-CG/EA ICE gel specimen during a tensile test. d. Typical stress-stretch curve of 3D printed k-CG/EA gels. The stress-stretch curve of 3D printed alginate/poly(acrylamide) ICE gel (Alg/PAAm) is shown for comparison (data reproduced from ref. 15).

A 4th generation 3D-Bioplotter from Envisiontec (Germany) was used to print a series of k-CG/EA ICE gel dumbbell shapes (Figure 2a-b) to demonstrate that the gel precursor solutions could be processed through a 3D printer. Before printing, the ink was loaded into a syringe barrel, which was then placed in the Bioplotter, pressurized, and allowed to equilibrate to 50.4 °C for 1 hr. The printer parameters were carefully adjusted to produce defined features. Optimal printing onto a poly(propylene) substrate held at 21 ± 1 °C required a head speed of 10-12 mm s^{-1} and pressure of 400-500 kPa when using 0.159 mm (internal diameter) sized syringe tips. Computer-aided design (SolidWorks) was used to design a model of the dumbbell shaped tensile specimens with thickness of 2 mm, neck width of 10 mm and gauge length of 60 mm. EnvisionTEC software was then used to transform the digital model into a series of commands to dictate the print path. The print path was sliced into 3 layers with a 90° shift in extrusion direction for each subsequent layer printed. The printing of the entire dumbbell shaped tensile specimen is completed in approximately 5 min. The total fabrication time (including ink equilibration in the syringe barrel) is then 65 min. Once printed the ICE gel dumbbell shapes were immediately sealed inside a Petri dish and stored for 5 days under controlled ambient conditions (21 °C, 45% relative humidity) to allow the EA polymer network to fully cure.

The 3D-printed and cast gels were mechanically characterized in tension (Figure 2c). Uniaxial tensile testing was conducted on a universal mechanical tester (Shimadzu, EZ-S) equipped with a 10 N load cell and spring loaded clamps. The tensile specimens were extended with a crosshead speed of 5 mm min^{-1}. Figure 2d is an example of a typical printed k-CG/EA ICE gel stress-stretch curve. The printed hydrogels exhibited an elastic modulus of 320 ± 40 kPa, a tensile strength of 600 ± 70 kPa and extension ratio of 3.5 ± 0.6. The work of extension (1.4 ± 0.3 MJ m^{-3}) was calculated as the area under the curve, and the swelling ratio (Q = 3.3) was calculated as the ratio of the total weight of the hydrogel to the solid dry weight of the hydrogel.

The mechanical performance of the as-printed k-CG/EA ICE gels was compared against the mechanical performances of cast k-CG/EA, cast alginate/polyacrylamide (Alg/PAAm) ICE gels[12] and 3D printed Alg/PAAm ICE gels fabricated with *in situ* photopolymerisation[15] to assess the effectiveness of this printing method for processing mechanically robust hydrogels into useful structures (see Table 1). The printed k-CG/EA ICE gels exhibit a lower swelling ratio, similar extension ratio (~3.3), but higher modulus, tensile strength and modulus compared to the cast k-CG/EA ICE gels. It is suggested that the difference in swelling ratio between cast and printed materials may play a role in this. The printed k-CG/EA gels and the printed Alg/PAAm ICE gels (3.0 ± 0.2)[15] exhibit similar extension ratios but the k-CG/EA gels are significantly stronger than the Alg/PAAm gels (170 ± 30 kPa).[15] The work of extension for the k-CG/EA ICE gels is 5.4 times greater than the work of extension of the printed Alg/PAAm gels (260 ± 10 kJ m^{-3}) although one must note that the printed Alg/PAAm gels have a greater swelling ratio (5.7) than the k-CG/EA gels.[15] In our previous work (reference 15) we demonstrated that the introduction of structural flaws during the printing process of the Alg/PAAm is responsible for the observed reduction in mechanical properties compared to the cast Alg/PAAm hydrogels (see Table 1). Therefore it could be possible that part of the difference in the characteristics between the printed ICE gels could be attributed to the difference in printing equipment, i.e. Envisiontec 3D-Bioplotter (this work) versus KIMM SPS1000 Bioplotter.[15]

Figure 3 demonstrates how the characteristics of the printed k-CG/EA ICE gels compare with other tough hydrogels as well as conventional hydrogels. It is clear that ICE gels have work extension values that are superior to those exhibited by conventional gels. The modulus of the printed ICE gels is similar to the values reported for double-network (DN) gels, and better then nano-composite (NC) gels. The ICE gels have work of extension values that are lower than most DN gels, but similar to those exhibited by NC hydrogels.

Table 1. Summary of the mechanical properties of printed k-CG/EA ICE gels, cast k-CG/EA ICE gels, printed alginate/polyacrylamide ICE gels (data reproduced from ref. 15) and cast alginate/polyacrylamide ICE gels (data reproduced from ref. 12) where E is the elastic modulus, σ_{max} is the tensile strength, λ is the extension ratio, U is the work of extension and Q is the swelling ratio.

	E [kPa]	σ_{max} [kPa]	λ	U [MJ m^{-3}]	Q
k-CG/EA ICE gels (printed)	320 ± 40	600 ± 70	3.5 ± 0.6	1.4 ± 0.3	3.3
k-CG/EA ICE gels (cast)	29 ± 7	106 ± 17	3.3 ± 0.6	0.21 ± 0.06	5.0
Alg/PAAm ICE gels (cast)	29	156	23	~ 2.5	6.1
Alg/PAAm ICE gels (printed)	66 ± 5	170 ± 30	3.0 ± 0.2	0.26 ± 0.01	5.7

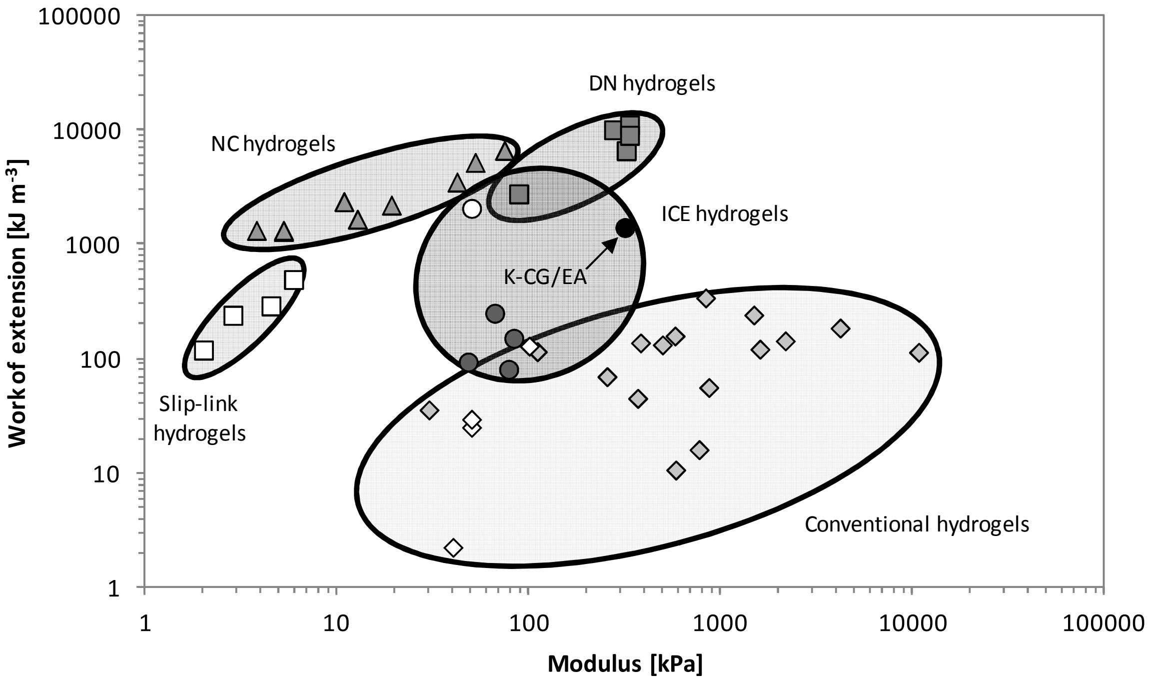

Figure 3. Comparison of the work of extension for printed ICE hydrogels (black circle – this work, shaded circles – previous work[15]), cast ICE hydrogels[12] (white circle), single network hydrogels (including hydrophilic homopolymers – white diamonds and copolymers using hydrophobic co-monomer – shaded diamonds), double network hydrogels (shaded squares), nanocomposite hydrogels (triangles) and slip-link hydrogels (white squares), adapted from ref. 20.

Conclusions

In conclusion, we have reported on a new 3D printing method that was designed to process tough ICE hydrogels into complex structures using thermal gelation. Rheology was used to observe the thermal gel transition of a gel ink synthesized with a 'one-pot' synthesis method. It was demonstrated that the biopolymer forms a polymer network through a thermal gelation transition upon cooling whilst the synthetic polymer network forms over time through an EA addition chemistry reaction. This difference in behaviour was utilized to optimize the printing conditions. It was shown that the gel constituents exhibit different gelation behaviour upon cooling. The heated ink was extrusion printed onto a cooler substrate that instigated rapid solidification of the gel precursor solution fixing the shape of the patterned ink. The printed hydrogel exhibited an impressive mechanical performance with a measured work of extension in the same order of magnitude as the toughest gels produced to date.[12]

Additive Manufacturing is rapidly becoming the approach of choice in building soft materials with hydrogels, in particular in the area of biofabrication. We believe that advances in tough hydrogel materials and devices can be achieved by combining the right chemistry with the appropriate 3D printing technology. This synergistic approach would enable future applications of these materials (and devices) in diverse fields such as tissue engineering and soft robotics.

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Notes and references

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