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PAPER

# Mechanically strong triple network hydrogels based on hyaluronan and poly(N,N-dimethylacrylamide)

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Hyaluronan (HA) is a natural polyelectrolyte with distinctive biological functions. Cross-linking of HA to generate less degradable hydrogels for use in biomedical applications has attracted interest over many years. One limitation of HA hydrogels is that they are very brittle and/or easily dissolve in physiological environments which limit their use in load-bearing applications. Herein, we describe preparation of triple-network (TN) hydrogels based on HA and poly(N,N-dimethylacrylamide) (PDMA) of high mechanical strength by sequential gelation reactions. TN hydrogels containing 81-91% water sustain compressive stresses above 20 MPa and exhibit Young's moduli up to 1 MPa. HA of various degrees of methacrylation were used as a multifunctional macromer for the synthesis of the brittle first-network component, while loosely cross-linked PDMA was the ductile, second and third network components of TN hydrogels. By tuning the methacrylation degree of HA, double-network hydrogels with a fracture stress above 10 MPa and a fracture strain of 96% were obtained. Increasing the ratio of ductile-to-brittle components via TN approach further increases the fracture stress above 20 MPa. Cyclic mechanical tests show that, although TN hydrogels internally fracture even under small strain, the ductile components hinder macroscopic crack propagation by keeping the macroscopic gel samples together.

## Introduction

Hyaluronan, or hyaluronic acid (HA), is a natural polyelectrolyte found in connectivity tissues and composed of repeating disaccharide units of  $\beta$ -1,4-D-glucuronic acid -  $\beta$ -1,3-N-acetyl-D-glucosamine (Scheme 1).<sup>1</sup> HA has distinctive biological functions, and therefore has been recognized as a potentially effective biomaterial for soft tissue regeneration.<sup>2-6</sup> Because HA is a non gelling macromolecule, it is either chemically modified or covalently cross-linked to generate a less degradable hydrogel for use in biomedical applications.<sup>7</sup> The functional groups in HA available for cross-linking are the hydroxyl and carboxyl groups. Hydroxyl groups may be cross-linked via an ether linkage and carboxyl groups via an ester linkage.<sup>8-14</sup> Methacrylation of HA with glycidyl methacrylate is another strategy for producing photocross-linkable macromers to form functional, cyto-compatible HA hydrogels (Scheme 1).<sup>15-17</sup>

One limitation of HA hydrogels is that they are very brittle and/or easily dissolve in physiological environments which limit their use in load-bearing applications. This poor mechanical performance of covalently cross-linked HA hydrogels originates from their very low resistance to crack propagation due to the lack of an efficient energy dissipation mechanism in the gel network.<sup>18, 19</sup> Weng and co-workers recently reported preparation of HA hydrogels containing 60-90% water and exhibiting a

compressive modulus of 0.5 MPa and a fracture stress of 5.2 MPa.<sup>20</sup> The hydrogels were prepared by swelling a highly cross-linked methacrylated HA first network in N,N-dimethylacrylamide (DMA) monomer solution containing a small amount of a chemical cross-linker, and then polymerizing DMA to form a loosely cross-linked poly(N,N-dimethylacrylamide) (PDMA) second network. Thus, the hydrogels consist of interpenetrating brittle (HA) and ductile (PDMA) polymer network components.<sup>20</sup> One may expect that, under large strain, the highly cross-linked, brittle first network breaks up to form many cracks while the second ductile network keeps the gel sample together,<sup>21</sup> which seems to be responsible for the improvement in the mechanical performance of brittle HA hydrogels.

The approach mentioned above is the double-network (DN) technique developed by Gong and co-workers in 2003 to prepare mechanically strong hydrogels.<sup>22-28</sup> DN hydrogels prepared from highly cross-linked poly(2-acrylamido-2-methylpropane sulfonic acid) polyelectrolyte network and linear or loosely cross-linked polyacrylamide exhibit exceptional compressive strengths of about 20 MPa and fracture energies in the hundreds of J m<sup>-2</sup>.<sup>22</sup> Our preliminary experiments showed that the reduced mechanical performance of DN hydrogels based on HA and PDMA as compared to those reported by Gong et al is due to the lesser degree of swelling of the first network hydrogels reducing the ratio of ductile-to-brittle components. For such cases, we recently

developed the triple network (TN) approach to create mechanically strong hydrogels.<sup>29</sup> TN approach bases on the loss of the translational entropy of a second monomer upon its polymerization within the first network. The entropy of second monomer, if polymerized in a first network hydrogel, decreases so that additional solvent (3<sup>rd</sup> monomer) enters into the gel phase to assume its new thermodynamic equilibrium. This means that DN will swell more than the first network so that triple networks could be prepared.<sup>29</sup>

Our aim in the present study is to improve the mechanical performance of DN hydrogels based on HA and PDMA by applying the TN approach. As such biomaterials are non-cytotoxic and highly resistant to biodegradation,<sup>20</sup> those with an excellent mechanical performance and a high degree of toughness will be a good candidate for load-bearing biomedical applications such as intervertebral disc prosthesis. As will be seen below, TN hydrogels containing 81-91% water sustain compressive stresses above 20 MPa and exhibit compressive moduli of 1 MPa.

TN hydrogels we described here consist of a highly cross-linked first HA network and loosely cross-linked PDMA as the second and third networks. This paper is organized as follows: Because the equilibrium degree of swelling and the elasticity of the first network significantly affect the mechanical strength of the resulting DN and TN hydrogels,<sup>21, 22, 29</sup> we first describe properties of the first network (or, single network) hydrogels derived from methacrylated HA macromers of various methacrylation degrees. By tuning the methacrylation degree, we were able to generate DN hydrogels exhibiting a fracture stress of around 10 MPa, which is about twice that reported by Weng et al.<sup>20</sup> Swelling these double networks in DMA solutions following polymerization of DMA in the gel phase further increases the ratio of ductile-to-brittle components, and thus produces HA/PDMA/PDMA TN hydrogels capable of sustaining above 20 MPa of compressive stress.

## Experimental part

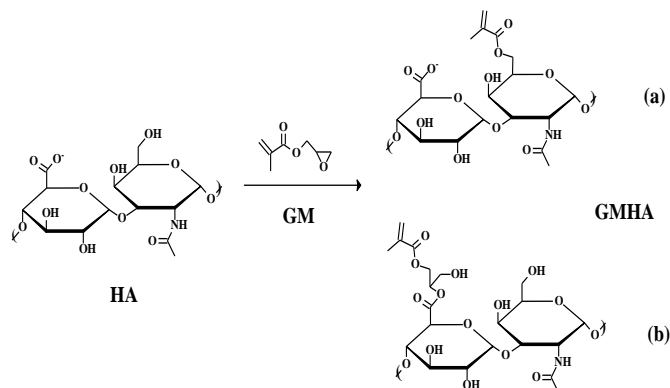
### Materials

The sodium salt of hyaluronic acid (HA) from *Streptococcus equi* was purchased from Sigma-Aldrich. Glycidyl methacrylate (GM, Sigma Aldrich), NaOH (Merck), N,N-dimethylacrylamide (DMA, Sigma-Aldrich), N,N'-methylene(bis)acrylamide (BAAm, Merck), triethylamine (TEA, Sigma-Aldrich), tetrabutylammonium bromide (TBAB, Sigma-Aldrich), 1-vinyl pyrrolidone (VP, Sigma-Aldrich) 2-oxoglutaric acid (Fluka), and Irgacure 2959 (Sigma-Aldrich) were used as received.

### Methacrylation of HA

Methacrylated HA was prepared according to the following procedure:<sup>15-17</sup> HA (0.5 g) was first dissolved in 50 mL distilled water by gently stirring overnight. Then,  $n$  mL TEA,  $n$  mL GM, and  $n$  g TBAB were added separately in that order, and allowed to fully mix for 1 h before the next addition. To vary the methacrylation degree of HA, the value  $n$  was taken as 1, 2, 4, and 8, corresponding to a molar ratio of GM to HA repeat units ( $n_{GM}/n_{HA}$ ) of 6, 12, 24, and 49, respectively. Following complete dissolution, the reaction mixture was incubated at 55 °C for 1 h.

After cooling, the solution was precipitated in acetone and the precipitate was dissolved in 30 mL water. After re-precipitation in acetone and re-dissolving in 10 mL water, it was lyophilized for 3 days to obtain methacrylated HA as a white product. Methacrylation degree of the samples was determined by nuclear magnetic resonance using a 500 MHz Agilent VNMRs spectrometer.



**Scheme 1** Disaccharide repeat unit of HA and its methacrylation using glycidyl methacrylate (GM) through ring opening (a) and transesterification modes (b) to form methacrylated HA (GMHA).

### Hydrogel preparation.

Single network hydrogels were prepared at 24 °C in aqueous solutions of the methacrylated hyaluronan (GMHA) of various methacrylation degrees using Irgacure 2959 as the initiator, and VP as a reactive comonomer and as a solvent for the initiator.<sup>15</sup> The initial concentration  $C_i$  of GMHA was set to 0.01 and 0.02 g.mL<sup>-1</sup>. Initiator concentration and the molar ratio of the initiator to VP were fixed at 2.2 wt.% (with respect to GMHA) and 0.012, respectively. Typically, GMHA (40 mg) was dissolved in 2 mL of distilled water overnight under continuous stirring. Then 34.2 μL of the initiator solution prepared by dissolving Irgacure 2959 (260 mg) in 10 mL of VP were added, and the reaction solution was transferred into plastic syringes to conduct the photopolymerization under UV light at 365 nm for 24h.

DN hydrogels were prepared by swelling the first network hydrogels in the 2<sup>nd</sup> DMA - BAAm solutions of concentration  $C_2$  between 0.10 and 0.50 g.mL<sup>-1</sup>, and photopolymerizing using 2-oxoglutaric acid initiator (0.1 mol% of DMA) at 24 °C. For this purpose, the first network hydrogel just after preparation (about 0.5 g) was immersed in 30 mL of 2<sup>nd</sup> monomer solution containing DMA, BAAm, and the initiator. After reaching the swelling equilibrium, which required about 4 days, the monomer + initiator solution containing the first network hydrogel was transferred into plastic syringes of 50 mL in volume and the photopolymerization was conducted under UV light at 365 nm for 24 h. We have to mention that due to the large volume of the 2<sup>nd</sup> DMA-BAAm solution as compared to the swollen SN hydrogel (30 mL vs 1-2 mL), the hydrogel is not in contact with the surface of the syringe and thus, the surface effects can be neglected. DN hydrogel was then separated by stripping off the external loosely cross-linked 2<sup>nd</sup> PDMA hydrogel. TN hydrogels were prepared similar to DN hydrogels by swelling DN hydrogels

in the 3<sup>rd</sup> DMA – BAAm solutions of concentration  $C_3$  between 0.10 and 0.30 g.mL<sup>-1</sup>, and photopolymerizing using 0.1 mol% 2-oxoglutaric acid initiator at 24 °C. Preparation conditions of SN, DN, and TN hydrogels are tabulated in Table S1 and S2.

### Swelling and Gel Fraction Measurements

Single network, DN, and TN hydrogel samples were immersed in a large excess of water or monomer solutions for at least 6 days by replacing solution every other day to extract any soluble species. The swelling equilibrium was tested by weighing the gel samples. All the synthesized gel samples in both as-prepared and equilibrium swollen states were transparent, indicating no macroscopic phase separation, and complete miscibility between the network components (Fig. 1A). The equilibrium relative weight swelling ratio  $m_{rel,i}$ , where the subindex  $i = 1, 2$ , and 3 stands for the first-, double-, and triple network hydrogels, respectively, was calculated as

$$m_{rel,i} = m/m_o \quad (1)$$

where  $m$  is the mass of the equilibrium swollen gel sample, and  $m_o$  is its mass after preparation.

To determine the gel fraction, the equilibrium swollen gel samples were taken out of water and dried at 80 °C under vacuum to constant mass. The gel fraction  $W_g$ , that is, the conversion of monomers to the cross-linked polymer (mass of water-insoluble polymer / initial mass of the monomer in the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> monomer solutions) was calculated from the masses of dry polymer network and from the comonomer feed.  $W_g$  was found to be close unity for all first network, DN and TN hydrogels formed at various combinations.

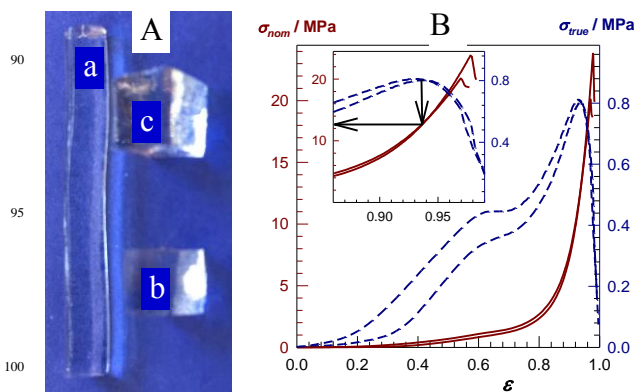
### Mechanical tests

Uniaxial compression measurements were performed on equilibrium swollen hydrogels at 24 °C on a Zwick Roell test machine using a 500 N load cell. Single network, DN, and TN hydrogels after equilibrium swelling in water were cut into cubic samples with the dimensions 3x3x3 mm. Before the test, an initial compressive contact to 0.01 N was applied to ensure a complete contact between the gel and the plates. Preliminary experiments showed that the use of Paraffin oil as lubricant to reduce friction and adhesion between the plates and the gel surface is not necessary and the deviations of the data with and without use of Paraffin oil are within the limit of experimental error. The tests were conducted at a constant crosshead speed of 0.3 and 1 mm.min<sup>-1</sup> below and above 15% compression, respectively. Load and displacement data were collected during the experiment. Compressive stress was presented by its nominal  $\sigma_{nom}$  and true values  $\sigma_{true}$  ( $= \lambda \sigma_{nom}$ ), which are the forces per cross-sectional area of the undeformed and deformed gel specimen, respectively, and  $\lambda$  is the deformation ratio (deformed length/initial length). The compressive strain  $\varepsilon$  is defined as the change in the length relative to the initial length of the gel specimen, i.e.,  $\varepsilon = 1 - \lambda$ . The strain is also given by the biaxial extension ratio  $\lambda_{biax}$  ( $= \lambda^{-0.5}$ ).<sup>27</sup> The Young's modulus  $E$  was calculated from the slope of stress-strain curves between 5 and 15% compressions. Cyclic compression tests were conducted at a constant crosshead speed

of 1 mm.min<sup>-1</sup> to a maximum strain  $\varepsilon_{max}$ , followed by retraction to zero force and a waiting time of 1 min, until the next cycle of compression. For reproducibility, at least five samples were measured for each gel and the results were averaged.

### Calculations of the fracture stress and fracture strain

Since compression tests for soft materials are easier to perform and yield more consistent results than tensile tests, we conducted uniaxial compression measurements. We report here nominal stress values to make the results comparable to those of Weng et al.<sup>20</sup> Fig. 1B shows typical stress-strain curves of a DN hydrogel as the dependences of nominal  $\sigma_{nom}$  (dark red, solid curves) and true stresses  $\sigma_{true}$  (dark blue, dashed curves) on the compressive strain  $\varepsilon$ . Results of two samples from the same gel are shown in the figure. The inset is a zoom-in to the large strain region, i.e., between 85 and 99% compressions. The fracture stresses  $\sigma_f$  of the two samples obtained from  $\sigma_{nom}$  vs  $\varepsilon$  curves are 20 and 23 MPa at strains  $\varepsilon_f$  of 96.8 and 97.7%, respectively. Thus, the gel samples apparently sustain about 20 MPa stresses at 97% compression. However, when plotted the corresponding true stresses  $\sigma_{true}$  against  $\varepsilon$  (dashed curves), maxima in the stress-strain curves appear earlier, i.e., at much lower compressions ( $\varepsilon_f = 93.5\%$ ). This indicates the onset of a microscopic failure in the samples which is not detectable in  $\sigma_{nom}$  vs  $\varepsilon$  plots. The arrows shown in the inset to Fig. 1B illustrate calculation of the real fracture stress from the maxima in  $\sigma_{true}$  vs  $\varepsilon$  curves. After such corrections conducted on 6 stress-strain curves, it was found that this DN hydrogel sustains  $12 \pm 2$  MPa stresses at  $93.4 \pm 0.9\%$  compression. Noting that, without this correction, as repeatedly reported in the literature, the fracture stress of this hydrogel is around 20 MPa. In the following, we only report the corrected fracture stresses and strains of the hydrogels.



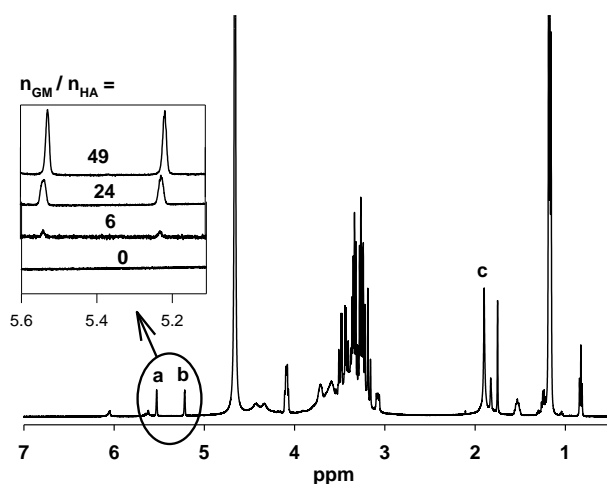
**Fig. 1 (A):** Optical images of the first network (a), DN (b), and TN hydrogels (c) in equilibrium with water. The hydrogels were derived from 4% methacrylated HA.  $C_1 = 0.02$  g.mL<sup>-1</sup>.  $C_2 = C_3 = 0.30$  g.mL<sup>-1</sup>. BAAm = 0.05 mol%. **(B):** Typical stress-strain curves of a DN hydrogel under compression as the dependences of nominal  $\sigma_{nom}$  (dark red curves) and true stresses  $\sigma_{true}$  (dark blue curves) on the compressive strain  $\varepsilon$ . Results of two samples from the same gel are shown. The inset is a zoom-in to the large strain region. The arrows illustrate the calculation of real fracture stress from the maxima in  $\sigma_{true}$  vs  $\varepsilon$  curves. Gel synthesis conditions: DM = 4%.  $C_1 = 0.02$  g.mL<sup>-1</sup>.  $C_2 = 0.30$  g.mL<sup>-1</sup>. BAAm = 0.05 mol%.  $w_{21} = 29$ .



## Results and discussion

### Single network hydrogels

Methacrylated hyaluronan (GMHA) of various degrees of methacrylation was used as a photocross-linkable macromer for the preparation of the single network hydrogels. The macromer GMHA was prepared by methacrylation of hyaluronan (HA) using glycidyl methacrylate (GM) in aqueous solutions. The reaction mechanism is quite complex and involves transesterification and ring opening modes to form GMHA (Scheme 1).<sup>15–17</sup> GM attacks both the hydroxyl groups on the N-acetyl-D-glucosamine ring via opening of the epoxide group, and the carboxylate group on the glucuronic acid ring via transesterification. Methacrylate groups are thus incorporated pendant to HA molecules of molecular weight around  $1.2 \times 10^6$  g.mol<sup>-1</sup>.<sup>8</sup> Different degrees of methacrylation were achieved by tuning the molar ratio ( $n_{GM}/n_{HA}$ ) of glycidyl methacrylate to hyaluronan in the feed. Fig. 2 shows <sup>1</sup>H NMR spectrum of GMHA prepared at  $n_{GM}/n_{HA} = 49$ . The inset shows 5.1 – 5.6 ppm region of the spectra of GMHA samples prepared at various  $n_{GM}/n_{HA}$  ratios, and native HA ( $n_{GM}/n_{HA} = 0$ ) as control. Compared to native HA, GMHA shows two new peaks at 5.2 and 5.5 ppm due to the methacrylate groups.<sup>16</sup> The degree of methacrylation (DM) was determined by integration of the methyl peak of HA at 1.9 ppm and the methacrylate peaks. The results collected in the first two columns of Table 1 reveal that increasing  $n_{GM}/n_{HA}$  from 6 to 49 also increases the degree of methacrylation (DM) of GMHA from 4 to 25%.



**Fig. 2.** <sup>1</sup>H NMR spectrum of GMHA prepared at  $n_{GM}/n_{HA} = 49$ . The inset shows 5.1 – 5.6 ppm region of the spectra of GMHA samples prepared at various  $n_{GM}/n_{HA}$  ratios together with native HA ( $n_{GM}/n_{HA} = 0$ ) as control. Peaks at 5.5 and 5.2 denoted by *a* and *b* are indicative of methacrylate groups. HA methyl peak denoted by *c* is shown at 1.9 ppm.

GMHA macromers of various methacrylation degrees were photopolymerized at a concentration  $C_I$  of 0.02 g.mL<sup>-1</sup> using Irgacure 2959 as the initiator. All the single network hydrogels were insoluble in water with a gel fraction of unity. In Fig. 3A and B, the swelling degree  $m_{rel}$  of the hydrogels and their Young's moduli  $E$  are plotted against the methacrylation degree (DM) of GMHA. Assuming a tetrafunctional phantom network, Young's modulus  $E$  is related to the effective cross-link density  $\nu_e$  of the hydrogels by<sup>30</sup>

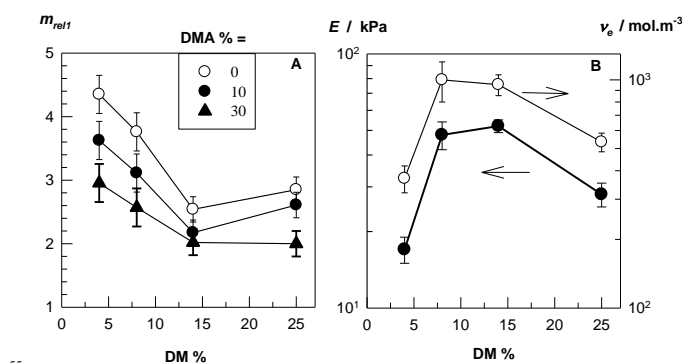
$$E = 1.5 \nu_e RT (\nu_2^0)^{1/3} (\nu_2^0)^{2/3} \quad (2)$$

where  $\nu_2^0$  and  $\nu_2$  are volume fractions of cross-linked GMHA at the gel preparation ( $\approx 0.02$ ), and in equilibrium with water ( $\approx \nu_2^0/m_{rel}$ ), respectively,  $R$  and  $T$  are in their usual meaning.

**Table 1** Characteristic data for SN and DN hydrogels

$n_{GM}/n_{HA}$	DM <sup>a</sup>	Water % <sup>b</sup>	$E^c$ / kPa		
			SN	DN <sup>d</sup>	DN <sup>e</sup>
6	4 (1)	99.5	17 (3)	211 (22)	370 (35)
12	8 (2)	99.5	48 (5)	305 (32)	549 (54)
24	14 (2)	99.2	52 (7)	611 (10)	409 (46)
49	25 (4)	99.3	28 (4)	224 (46)	442 (52)

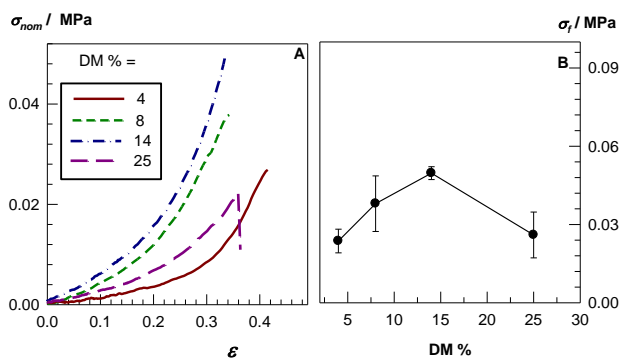
<sup>a</sup> Methacrylation degree of GMHA (in %) formed at various  $n_{GM}/n_{HA}$  ratios, <sup>b</sup> water content of SN hydrogels, <sup>c</sup> Young's moduli of SN and DN hydrogels, <sup>d</sup>  $C_2 = 0.10$  g.mL<sup>-1</sup>, and <sup>e</sup>  $C_2 = 0.30$  g.mL<sup>-1</sup>. (Standard deviations in parentheses while for water contents; they are less than 5 %.)



**Fig. 3.** The swelling degrees  $m_{rel}$  of SN hydrogels in water and in DMA solutions (A), and their Young's moduli  $E$  in equilibrium swollen state in water (filled symbols, B) both plotted against the methacrylation degree (DM) of GMHA. Open symbols in B show the cross-link density  $\nu_e$  of the hydrogels calculated using eqn (2) plotted against DM.  $C_I = 0.02$  g.mL<sup>-1</sup>.

Open symbols in Fig. 3B show the cross-link density  $\nu_e$  of the hydrogels plotted against DM. The lowest cross-link density  $\nu_e$  and thus, the highest swelling ratio was obtained at 4% DM, i.e., at the lowest degree of methacrylation.  $\nu_e$  first increases with increasing DM but then decreases, which is attributed to the favourable intramolecular cross-linking reactions at high local concentration of methacrylate groups decreasing the number of effective cross-links.<sup>31</sup> Moreover, increasing monomer (DMA) concentration in the external solution decreases the swelling ratio of the hydrogels due to the osmotic pressure of DMA molecules in the external solution. Single network (SN) hydrogels were also subjected to uniaxial compression tests. Typical stress-strain curves of the hydrogels are shown in Fig. 4A where the nominal stress  $\sigma_{nom}$  is plotted against the compressive strain  $\varepsilon$ . In Fig. 4B, the fracture stress  $\sigma_f$  of the hydrogels is plotted against DM. The fracture stress  $\sigma_f$  of SN hydrogels is between 0.02 and 0.05 MPa while their fracture strains  $\varepsilon_f$  are around 0.4, i.e., the hydrogels rupture at around 40% compressions. This poor mechanical performance of SN hydrogels is typical for classical, chemically cross-linked hydrogels due to the lack of an efficient energy

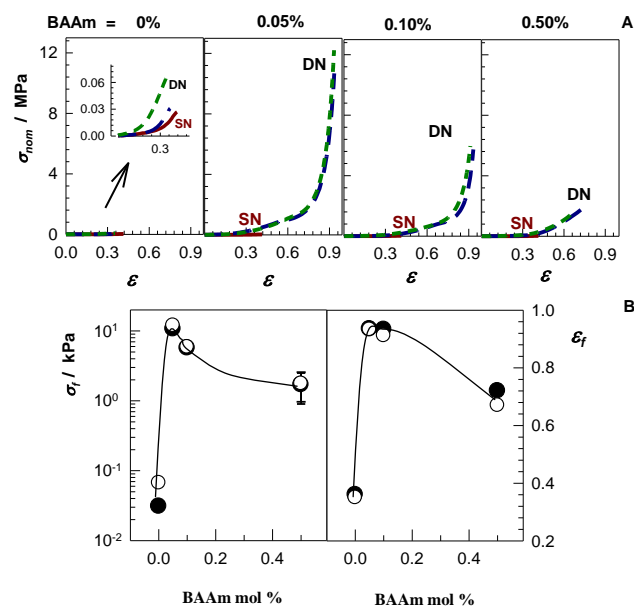
dissipation mechanism.



**Fig. 4.** (A): Typical stress-strain curves of SN hydrogels formed from GMHA macromers of various methacrylation degrees (DM) as indicated.  $C_1 = 0.02 \text{ g.mL}^{-1}$ . (B): The fracture stress  $\sigma_f$  of SN hydrogels plotted against DM.

### Double- and triple-network hydrogels

DN hydrogels were prepared by swelling SN hydrogels in DMA solutions containing the cross-linker BAAm and the initiator 2-oxoglutaric acid, following photopolymerization. DN hydrogels in equilibrium with water contained 87-95% water. We first fixed the methacrylation degree of GMHA at 4% while the cross-linker (BAAm) concentration in the second monomer solution was varied. Fig. 5A shows typical compressive stress-strain curves of SN (solid curves) and DN hydrogels (dashed curves) formed in DMA solutions at a concentration  $C_2$  of 0.10 and 0.30  $\text{g.mL}^{-1}$ , containing various amounts of BAAm. In Fig. 5B, the fracture stress  $\sigma_f$  and strain at break  $\epsilon_f$  are plotted against BAAm concentration. In the absence of a cross-linker, the fracture stress of SN hydrogels slightly increases from 0.03 to 0.07 MPa after double networking (see the inset to Fig. 5A), while after addition 0.05 mol% BAAm cross-linker in the DMA solution,  $\sigma_f$  increases dramatically and becomes  $12 \pm 2 \text{ MPa}$  at a DMA concentration  $C_2$  of 0.30  $\text{g.mL}^{-1}$ . The fracture strain also increases from 40 to 94% compression. Further increase of the cross-linker content again decreases  $\sigma_f$  of DNs. This result highlights importance of the presence of a small amount of a chemical cross-linker in the second network solution. Previous work indeed shows that formation of mechanically strong DN hydrogels requires strong chain entanglements or covalent links between the 1<sup>st</sup> and 2<sup>nd</sup> polymer networks.<sup>20-22</sup> In case of SN hydrogels formed by vinyl-divinyl monomer copolymerization, no additional cross-linker was needed in the second network solution.<sup>29</sup> This is due to the initiator molecules remaining in the first network as well as the pendant vinyl groups of divinyl monomer units acting as potential cross-link points between 1<sup>st</sup> and 2<sup>nd</sup> networks.<sup>29</sup> For the present DN system, methacrylate groups of GMHA macromer seem to be sterically unable to effectively link the first to the second network in the absence of a chemical cross-linker. This could be related to the high molecular weight of HA ( $1.2 \times 10^6 \text{ g.mol}^{-1}$ )<sup>8</sup> and thus, high viscosity of the gelation solutions.



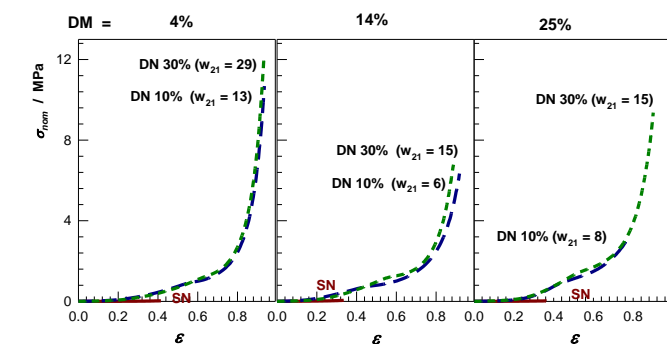
**Fig. 5.** (A): Compressive stress-strain curves of SN (solid curves) and DN hydrogels (dashed curves) formed from 4% methacrylated HA.  $C_1 = 0.02 \text{ g.mL}^{-1}$ .  $C_2 = 0.10$  (long dashed blue curves) and  $0.30 \text{ g.mL}^{-1}$  (short dashed green curves). (B): The fracture stress  $\sigma_f$  and strain at break  $\epsilon_f$  of DN hydrogels plotted against BAAm concentration.  $C_1 = 0.02 \text{ g.mL}^{-1}$ .  $C_2 = 0.10$  (●) and  $0.30 \text{ g.mL}^{-1}$  (○). Note that most of the error bars are smaller than the symbols.

As a next step, we fixed the cross-linker content at 0.05 mol% while the degree of methacrylation (DM) of GMHA was varied. Fig. 6 shows the stress – strain curves of SN (solid curves) and DN hydrogels formed at DM = 4, 14, and 25% (dashed curves). DN hydrogels were prepared by swelling SN hydrogels in DMA solutions of concentration  $C_2 = 0.10$  and  $0.30 \text{ g.mL}^{-1}$  containing 0.05 mol% BAAm cross-linker. SN hydrogels formed from GMHA with the lowest methacrylation degree (4%) produces DNs with the highest fracture stresses, e.g.,  $11 \pm 1$  and  $12 \pm 2 \text{ MPa}$  at  $C_2 = 0.10$  and  $0.30 \text{ g.mL}^{-1}$ , respectively, while increasing methacrylation degree of GMHA deteriorates the mechanical performances of DN hydrogels. In the pioneering work by Weng and co-workers on hyaluronan based DNs,<sup>20</sup> the maximum fracture stress achieved was 5.2 MPa which was obtained using methacrylated hyaluronan with DM of 10%. Thus, the results in Fig. 6 indicate that decreasing methacrylation degree down to 4% results in a 2.3-fold increase in the mechanical strength of DN hydrogels.

What is the reason for this improvement? Previous work shows that the molar or mass ratio of the second to the first network units is an important parameter determining the mechanical strength of DN hydrogels.<sup>22</sup> An extraordinary mechanical performance requires a high concentration of the second network as compared to the first one, i.e., a high ratio of these networks. For the present DN system, the mass ratio  $w_{21}$  of the second to the first network units can be estimated by<sup>29</sup>

$$w_{21} = \frac{(m_{rel} - 1)C_2}{C_1} \quad (3)$$

In Fig. 6,  $w_{2/1}$  ratios of DN hydrogels are indicated next to the curves within parenthesis. Because of the relatively high swelling ratio  $m_{rel1}$  of SN hydrogel formed using 4% methacrylated hyaluronan (Fig. 3A), it produces DN hydrogel with the highest  $w_{2/1}$  ratio so that the maximum improvement in the mechanical performance was achieved. Young's modulus  $E$  of the hydrogels also increased significantly after double-networking at  $C_2 = 0.10$  and  $0.30 \text{ g.mL}^{-1}$  (Table 1). For instance, SN hydrogels formed using 4% methacrylated hyaluronan exhibit a Young's modulus of 17 kPa, while after double-networking at  $C_2 = 0.30 \text{ g.mL}^{-1}$ , it increases to 370 kPa. The drastic increase of the modulus  $E$  upon formation of double network structures indicates a high degree of physical and chemical connectivity between the network components of DN hydrogels.

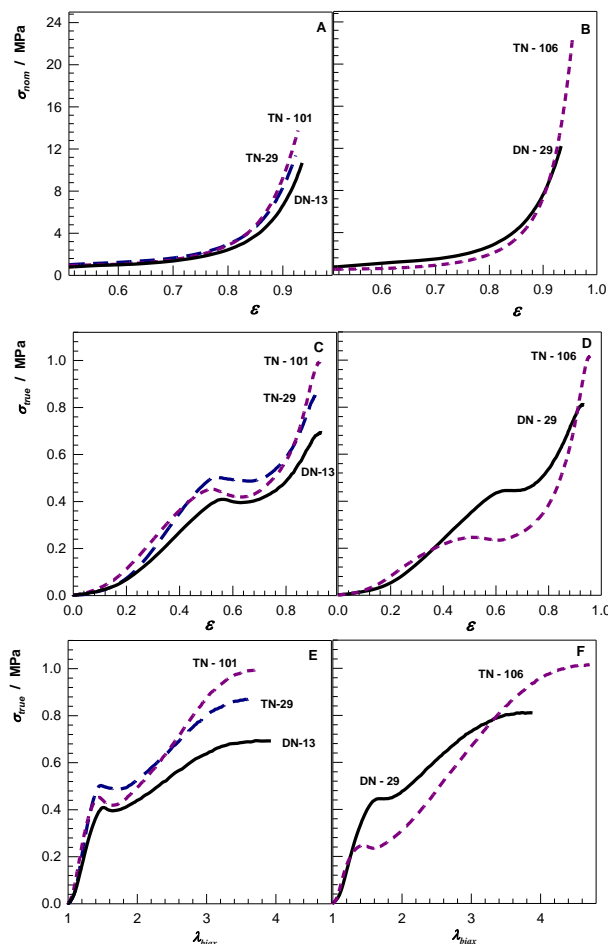


**Fig. 6.** Compressive stress-strain curves of SN (solid curves) and DN hydrogels (dashed curves) formed from HA with various degrees of methacrylation (DM), as indicated.  $C_1 = 0.02 \text{ g.mL}^{-1}$ .  $C_2 = 0.10$  (long dashed blue curves) and  $0.30 \text{ g.mL}^{-1}$  (short dashed green curves). BAAM = 0.05 mol%.  $w_{2/1}$  ratios calculated using eqn (3) are also indicated in the figures.

DN hydrogels were also prepared starting from SN hydrogels formed at a lower GMHA concentration ( $C_1 = 0.01$  instead of  $0.02 \text{ g.mL}^{-1}$ ). However, no further improvement in the mechanical properties was observed due to the limiting value of  $w_{2/1}$  ratio (Fig. S1 – S3). Because the key to obtain mechanically strong hydrogels is to increase the ratio of ductile-to-brittle network components, we used triple-network (TN) approach developed recently in our group.<sup>29</sup> Thus, DN hydrogels were first swollen in DMA solutions containing the cross-linker BAAM (0.05 mol%) and the initiator until equilibrium is reached, following photopolymerization to obtain TN hydrogels. The relative swelling ratios  $m_{rel2}$  of DN hydrogels in water and in DMA solutions were between 1.4 and 2.9. For a given methacrylation degree of GMHA,  $m_{rel2}$  increased with increasing  $w_{2/1}$  ratio of the DNs (Table S3). Increasing  $w_{2/1}$  ratio means that larger amount of the second monomer (DMA) is polymerized during the formation of DN hydrogels. Consequently, a larger decrease in the entropy occurs due to the monomer-to-polymer conversion within the gel phase so that more DMA solution can enter into the DN hydrogel leading to larger  $m_{rel2}$  values. The mass ratio  $w_{32/1}$  of the second and third to the first network units was estimated as<sup>29</sup>

$$w_{32/1} = \frac{m_{rel}(m_{rel2} - 1)C_3 + (m_{rel} - 1)C_2}{C_1} \quad (4)$$

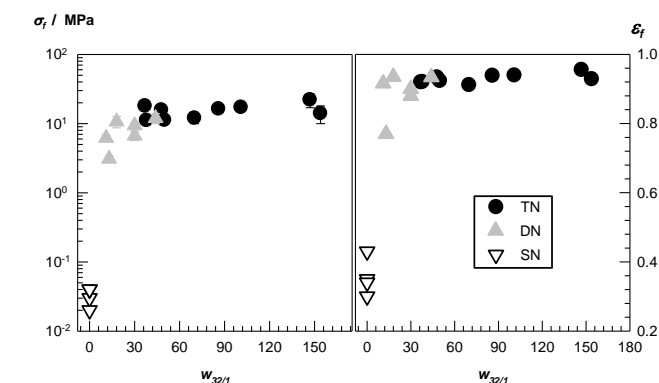
The mass ratio  $w_{32/1}$  of the second + third to the first polymer units was varied by changing DMA concentration  $C_3$  in the 3<sup>rd</sup> monomer solution at a fixed  $w_{2/1}$  ratio of DN's. Similar to DN hydrogels, the gel fraction  $W_g$  was close to unity for all TN hydrogels formed at various  $w_{32/1}$  ratios. In the swollen state, TN hydrogels contained 81 to 91% water; similar to the DN's, the swelling ratio  $m_{rel3}$  of TN hydrogels in water increased with increasing  $w_{32/1}$  ratio (Table S4). This also means that quadruple-network hydrogels could also be prepared by swelling TNs in a 4<sup>th</sup> monomer solution.



**Fig. 7.** (A, B):  $\sigma_{nom}$  vs.  $\epsilon$  plots for DN (solid curves) and TN hydrogels (dashed curves) formed from 4% methacrylated HA. DNs were prepared in DMA solutions at a concentration  $C_2 = 0.10$  (A) and  $0.30 \text{ g.mL}^{-1}$  (B) both containing 0.05 mol% BAAM. TNs were prepared in the presence of 0.05 mol% BAAM at  $C_3 = 0.10$  and  $0.30 \text{ g.mL}^{-1}$ .  $C_1 = 0.02 \text{ g.mL}^{-1}$ . Hydrogel samples are denoted in the figures as DN-x or TN-y, where x and y are  $w_{2/1}$  and  $w_{32/1}$  ratios, respectively. (C-F):  $\sigma_{true}$  vs.  $\epsilon$  (C,D) and  $\sigma_{true}$  vs.  $\lambda_{max}$  plots derived from the curves given in A and B, respectively.

Fig. 7A and B show stress-strain curves of DN (solid curves) and TN hydrogels (dashed curves) formed from 4% methacrylated hyaluronan. Hydrogel samples are denoted in the figures as DN-x or TN-y, where x and y are  $w_{2/1}$  and  $w_{32/1}$  ratios, respectively. The fracture stresses  $\sigma_f$  of DN hydrogels formed at  $w_{2/1} = 13$  and 29 are  $11 \pm 1$  and  $12 \pm 2$  MPa, respectively.  $\sigma_f$  further increases to  $15 \pm 3$  and  $22 \pm 5$  MPa after triple-networking at  $w_{32/1}$  ratios of 101 and 106, respectively. These values are the highest fracture stresses for such hydrogels reported so far in the literature. Thus,

TN synthesis starting from the DN's leads to 4- to 8-fold increase in the mass ratio of ductile-to-brittle network components ( $w_{32/1}/w_{21}$ ), and produces hydrogels exhibiting very high fracture stresses. The results also show that the mechanical performance of DN hydrogels could be further strengthened by incorporation of additional ductile component (loosely cross-linked PDMA) via TN approach. Noting that, as in the case of DNs, no improvement in the mechanical performances of DN hydrogels was observed if the cross-linker BAAM is not included into the third monomer solution (Table S2, Fig. S4).

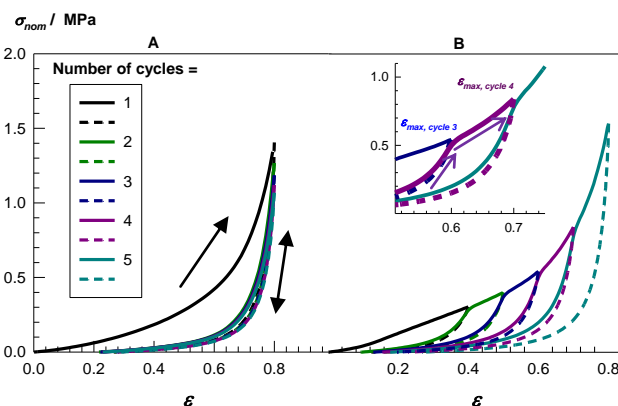


**Fig. 8.** Fracture stress  $\sigma_f$  (left) and fracture strain  $\epsilon_f$  (right) of TN hydrogels formed using 0.05% BAAM plotted against  $w_{32/1}$  ratio (filled circles). For comparison, fracture data obtained from SN ( $w_{32/1} = 0$ , open down-triangles) and DN hydrogels ( $w_{32/1} = w_{21}$ , gray up-triangles) are also shown in the figure.

In Fig. 8, the filled circles show the fracture stress  $\sigma_f$  and fracture strain  $\epsilon_f$  of all TN hydrogels formed using 0.05% BAAM cross-linker plotted against  $w_{32/1}$  ratio. For comparison,  $\sigma_f$  of SN ( $w_{32/1} = 0$ , open down-triangles) and DN hydrogels ( $w_{32/1} = w_{21}$ , gray up-triangles) are also shown in the figure. All TN hydrogels sustain 10 - 20 MPa compressive stresses at 95% compressions and exhibit a Young's modulus  $E$  up to 1 MPa (Fig. S5).

Another characteristic of high strength DN and TN hydrogels is the appearance of a yielding region in stress-strain curves between 50 and 65% compression (Fig. S6). This feature becomes more apparent when the nominal stress  $\sigma_{nom}$  is converted to its true value  $\sigma_{true}$  and then plotted against  $\epsilon$  or biaxial extension ratio  $\lambda_{biax}$ . This is illustrated in Fig. 7C – F derived from stress – strain curves in Fig. 7A and B. The yielding behavior of mechanically strong DN and TN hydrogels is attributed to a significant internal fracture under strain. To demonstrate this fracture, DN and TN hydrogels were subjected to cyclic compression tests by successive loading/unloading cycles up to a maximum strain  $\epsilon_{max}$ . In Fig. 9A, five successive loading – unloading cycles of a TN hydrogel sample prepared at  $w_{21} = 29$  and  $w_{32/1} = 106$  are shown up to a maximum strain of 80% ( $\epsilon_{max} = 0.8$ ). The loading curve of the first compressive cycle is different from the unloading curve indicating damage in the gel sample and dissipation of energy during the first cycle. The energy dissipated in this cycle, calculated from the area between the loading and unloading curves, is  $160 \text{ kJ.m}^{-3}$ . However, the following cycles are almost elastic with a small amount of hysteresis ( $22 - 24 \text{ kJ.m}^{-3}$ ), and they closely follow the path of the first unloading. The results show that an irreversible internal

damage occurs in the gel sample. In Fig. 9B, the same but virgin hydrogel sample was subjected to five successive loading – unloading cycles with increasing maximum strain from 40 to 80%. After the first compressive cycle, each successive loading curve consists of elastic and damage regions due to the irreversible damage done during the previous cycle. Elastic region follows the path of the unloading curve of the previous cycle while the damage region continues the loading curve of the previous cycle (see the inset to Fig. 9B). The transition from elastic to damage region occurs at the maximum strain of the previous cycle. Thus, due to the irreversible damage done during the previous cycle, additional damage only occurs at a higher maximum strain. All these indicate the occurrence of a significant extent of internal fracture in the hydrogels even at 40% compressions where the single network hydrogels rupture (Fig. 4B). Thus, the ductile, loosely cross-linked PDMA second and third network components hinder macroscopic crack propagation by keeping the macroscopic gel sample together, while the sample internally fractures. This internal fracture is responsible for the extraordinary mechanical properties of the present DN and TN hydrogels based on HA and PDMA.



**Fig. 9.** Five successive loading / unloading cycles of a TN hydrogel up to a maximum compression of 80 % (A) and with increasing compression from 40 to 80% (B). The up and down arrows in A indicate loading and unloading curves, respectively. The inset to B is a zoom-in to highlight the damage and elastic regions of the 4<sup>th</sup> cycle. Synthesis parameters of TN hydrogels:  $w_{21} = 29$ ,  $w_{32/1} = 106$ . For clarity, loading and unloading curves are shown by the solid and dashed curves, respectively.

## Conclusions

DN and TN hydrogels based on HA and PDMA with extraordinary mechanical properties were prepared by DN and TN approaches. The single network (SN) hydrogels were prepared by polymerization of HA of various degrees of methacrylation in aqueous solutions. SN hydrogels can sustain up to 40% compression and break at a stress of 0.02 – 0.05 MPa. By tuning the methacrylation degree of HA, DN hydrogels with a fracture stress above 10 MPa and a fracture strain of 96% were obtained. Triple-networking of DN hydrogels further increases the ratio of ductile/brittle components, and thus produces mechanically strong HA/PDMA/PDMA TN hydrogels. TN hydrogels contain 81-91% water and sustain compressive stresses above 20 MPa. Cyclic mechanical tests conducted on DN and TN hydrogels show a significant mechanical hysteresis and



irreversible loading/unloading cycles, even under small strain conditions where the single network hydrogels rupture. The results indicate that the loosely cross-linked PDMA second and third network components hinder macroscopic crack propagation by keeping the macroscopic gel sample together while it internally fractures. This internal fracture is responsible for the extraordinary mechanical properties of the present DN and TN hydrogels based on HA and PDMA.

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Triple network hydrogels based on hyaluronan and poly(N,N-dimethylacrylamide) containing 81-91% water sustain compressive stresses above 20 MPa and exhibit Young’s moduli up to 1 MPa.

