Double-Network Strategy Improves Fracture Properties of Chondroitin Sulfate Networks
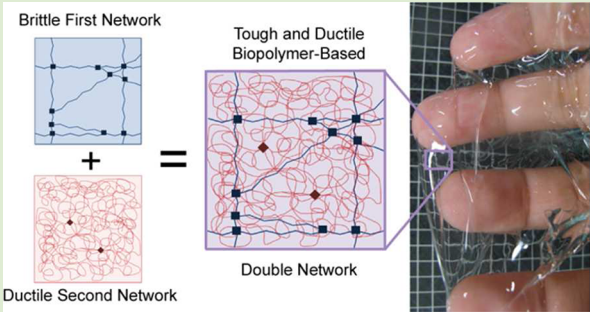
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S *Supporting Information*

ABSTRACT: A tough and ductile, ultrathin film, double-network (DN), biopolymer-based hydrogel displaying the yielding phenomenon was synthesized from methacrylated chondroitin sulfate (MCS) and polyacrylamide (PAAm). The DN of MCS/PAAm exhibited a failure stress more than 20 times greater than the single network (SN) of either MCS or PAAm and exhibited yielding stresses over 1500 kPa. In addition, the stress—strain behavior with a yielding region was also seen in a hydrogel of MCS and poly(N,N-dimethyl acrylamide) (PDMAAm). By replacing PAAm with PDMAAm, interactions known to toughen networks are removed. This demonstration supports the idea that the brittle/ductile combination is key to the DN effect over specific interactions between the networks. The MCS/PAAm and MCS/PDMAAm DN hydrogels had comparable mechanical properties to the archtypal DN hydrogels of poly(2-acrylamido-2-methylpropanesulfonic acid) (PAMPS)/PAAm. In addition, these tough and ductile, biopolymer-based, double-network hydrogels demonstrated a substantial yielding region.

In general, materials undergo either a yielding phenomenon or a brittle failure, and the strength of the material is determined by which process occurs first.[1] Many materials display yielding, however, synthetic, chemically cross-linked hydrogels always have a brittle fracture, with the exception of double-network (DN) hydrogels of poly(2-acrylamido-2-methylpropanesulfonic acid) (PAMPS)/polyacrylamide (PAAm), which also have extraordinary fracture stresses.[1,2] Anisotropic gels such as poly(2,2′-disulfonyl-4,4′-benzidine terephthalamide) (PBDT)/PAAm DN hydrogels have also shown yielding.[3] Recently, Sun et al. created hybrid gels of ionically cross-linked alginate and covalently cross-linked PAAm.[4] These alginate/PAAm hydrogels displayed high extensibility, 20 times their initial length, and a yielding region, but the fracture strength was only ~160 kPa.[4] Synthesizing tough and ductile biopolymer-based hydrogels that demonstrate the generality of yielding could lead to materials resistant to catastrophic failures, especially important in areas such as tissue engineering.[5] The goal of this work is to create a tough biopolymer-based DN hydrogel system with a yielding region using PAMPS/PAAm DN as a model and to test the hypothesis that the double-network effect is due to the combination of brittle and ductile networks. We first replaced PAMPS with MCS, thus exchanging a sulfonated synthetic polymer with a sulfonated biopolymer synthesized by cross-linking a linear polymer and then replaced PAAm with poly(N,N-dimethyl acrylamide) (PDMAAm) to eliminate the two protons of the amide group. This research shows that a biopolymer-based DN

hydrogel can be designed to have high toughness and a distinct yielding region.

DN hydrogels are formed from a highly covalently cross-linked, brittle and stiff, polyelectrolyte first network with a lightly covalently cross-linked, soft and ductile, neutral polymer second network.[2c,d,6] The second network has a molar concentration 20—30 times greater than the first network.[2c,d,6]

DN hydrogels have significantly improved toughness in comparison to either single-network (SN) alone. The improved toughness is believed to be due to the fracturing of the first network, which dissipates the strain energy, while the ductile second network holds the bulk hydrogel together and supports high strains.[2d,7] Ultrathin film DN hydrogels (~100 μm thick) are comparable to bulk, solution-cast DN gels in mechanical properties such as toughness, yielding, and necking. Thus, they have a toughening mechanism similar to the toughening mechanism found in bulk, solution-cast DN gels. However, ultrathin DN hydrogels have experimental advantages of allowing for observation of the tearing mechanism, requiring less material and equilibrating more rapidly with solutions.[2f,8]

Previously reported DN hydrogels made from PAMPS/PAAm have three characteristic regions, preyielding, yielding, and hardening, and display a clear yield point (transition between preyielding and yielding regions).[2c,d,f] The preyielding region is a region in which the brittle network starts absorbing

the strain energy by fracturing, allowing the gel to sustain higher stresses.[2c,d,f] The yield point in these gels is typically around 700 kPa for both bulk, solution-cast DN gels and film DN gels and is nearly independent of the elongation velocity.[2c,d] After the yielding point, the yielding zone forms, which develops as the PAMPS network breaks down entirely across a cross-section and allowing elongation of the PAAm chains.[2c,d,f] Elongation occurs at a constant nominal stress as the yielding zone grows until the PAMPS network is fully fractured over the entire length of the specimen. The yielding region typically starts at 2−3 mm/mm and ends at 8−10 mm/mm in bulk, solution-cast DN hydrogels, where in film DN hydrogels the yielding region starts at 2 mm/mm and ends around 5 mm/mm.[2c,d,f] In the hardening region, the PAAm coils become highly extended and behave according to non-Gaussian chain statistics.[2c,d,f] This region can extend to 14 mm/mm.[2c,d,f] While the DN principle is believed to be general, and many gel systems have been designed based on this concept, which have improved fracture properties,[2d] no research has demonstrated the full range of phenomena, other than with PAMPS/PAAm, much less a biopolymer-based system. Thus, this work was motivated by a desire to create a biopolymer-based system that shows a yielding region, demonstrates the generality of the brittle/ductile combination hypothesis, and reveals that the phenomena observed are not due to unique structures or interactions in the PAMPS/PAAm system.

We chose the biopolymer, chondroitin sulfate (CS) as the first component of the ultrathin film DN hydrogels because CS is a major component of cartilage that provides strength and allows for absorption of large amounts of water.[9] To create these hydrogels, methacrylated chondroitin sulfate (MCS) was formed by modifying the CS with methacrylate groups; upon photoinitiation, the cross-linked linear polymer was reacted to form a cross-linked gel.[10] The second network was formed using a copolymerization of PAAm or PDMAAm with the cross-linker N,N'-methylenebisacrylamide (BIS). AAm or DMAAm has a molar concentration 40−50 times greater than MCS disaccharide groups. A schematic of the single networks and the double network is shown in Figure 1.
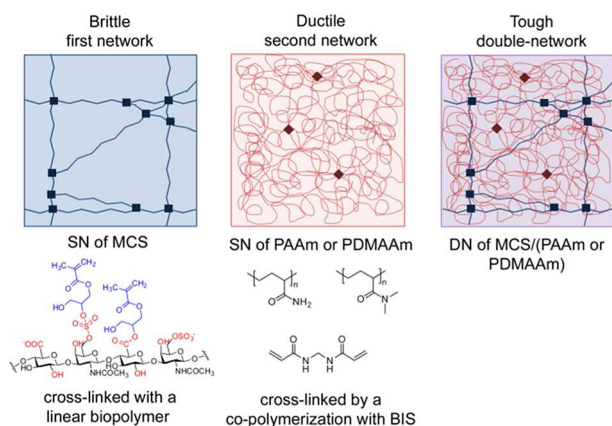

Figure 1. Scheme of single (SN) and double (DN) network gels. The first network is a highly cross-linked linear biopolymer of methacrylated chondroitin sulfate (MCS). The second network is polyacrylamide (PAAm) or poly(N,N-dimethyl acrylamide) (PDMAAm) cross-linked by a copolymerization with N,N'-methylenebisacrylamide (BIS). The double-network strategy was used to synthesize a tough and ductile DN biopolymer.

The stress−strain behavior of a DN of MCS/PAAm to a SN of MCS and a SN of PAAm is compared in Figure 2a,b. Figure
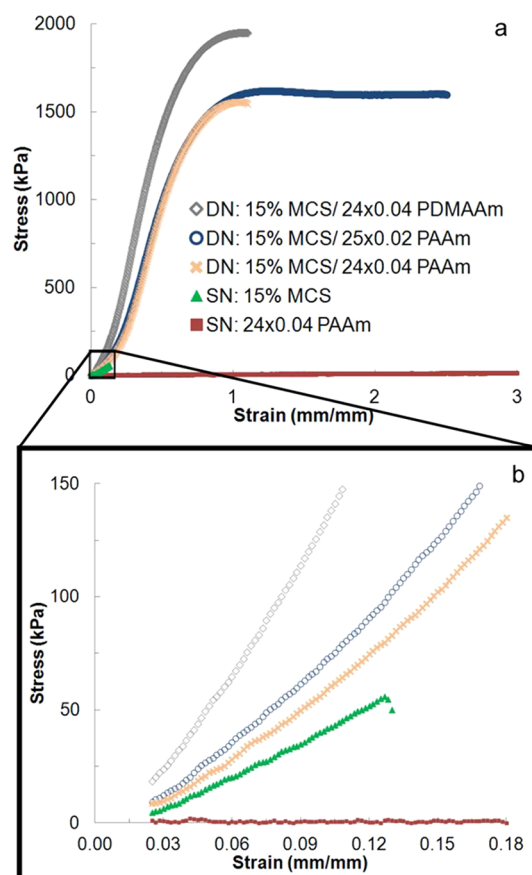

Figure 2. Stress−strain response under tension single (SN) and double (DN) network gels. All of the curves have been truncated after failure. (a) Combining brittle MCS with ductile PAAm into a double-network increases fracture stress over 30 times and introduces a yielding region. Failure of the SN of PAAm was 10.6 mm/mm and failure stress of 83 kPa. Replacing PAAm for PDMAAm shows the generality of the DN and yielding effect. (b) Stiffness of the DNs were slightly better than SN of MCS and significantly greater than the SN of PAAm. The data before a strain of ∼0.03 mm/mm is not recorded due to equipment inaccuracies at the low strain.

2a, shows dramatic differences between the DN and the SN. The specific values for the swelling and mechanical properties for SN MCS, SN PAAm, and select formulations of DN hydrogels are listed in Table 1. The failure stress of the DN MCS/PAAm was 16 times that of a SN of PAAm and 40 times that of a SN of MCS. The failure strain of the DN of MCS/PAAm, ∼100−250%, was somewhat a mixture of the SN of MCS, ∼10%, and the SN of PAAm, ∼1000%. Figure 2b shows the initial stress−strain of the networks. The Young's modulus (E), from the initial data of a strain of 5−15%, is 100 times higher for the MCS/PAAm DN than it is for the SN of PAAm. E is also slightly higher in the DN of MCS/PAAm than the SN of MCS but can be attributed to the fact that the swelling of the DN is much less than the SN, which normally increases the modulus. Besides that, the tearing energy was ∼100 times greater in the DN of MCS/PAAm than the SN of PAAm. Due to the extremely brittle nature of the SN of MCS, the tearing experiment could not be performed. In general, the DN of

Table 1. Swelling and Mechanical Properties of Different Hydrogel Networks

MCS formulation wt%	PAAm formulation TxC	swelling Q (g/g)	Young's modulus E (kPa)	toughness (kJ/m³)	yielding point stress (kPa)	failure stress (kPa)	failure strain (mm/mm)	tearing energy T (J/m²)
15		34.1 ± 4.3	487 ± 50	1.89 ± 1.59	N/A	40.1 ± 22	0.10 ± 0.04	a
	24 × 0.04	26.5 ± 0.1	7.83 ± 0.3	349 ± 92	N/A	96.7 ± 19	10.8 ± 1.2	67.7
15	24 × 0.04	7.0 ± 0.3	748 ± 103	709 ± 467	1680 ± 165	1670 ± 150	1.09 ± 0.08	695
15	25 × 0.02	6.9 ± 0.2	1040 ± 62	2940 ± 528	1610 ± 22	1600 ± 12	2.27 ± 0.34	585
15	25 × 0.04 (PDMAAm)	7.1 ± 0.2	1700 ± 125	1160 ± 176	1930 ± 47	1930 ± 47	1.00 ± 0.08	185

^{a}Too brittle to be measured (mean ± standard deviation).

MCS/PAAm showed superior properties to SN of MCS or SN of PAAm.

Further, the observed trend for the MCS/PAAm DN shows that two of the three characteristic regions can be achieved, open circles in Figure 2a. Similar to previous literature, we attribute the modulus of the preyielding region as dominated by the densely cross-linked polyelectrolyte MCS network.[2f,11] The initial part of the preyielding region or toe region of the curve is approximately elastic. The DN is shown to have a "J-shaped" curve that is not commonly seen in hydrogels but is often observed in biomaterials, such as cartilage, which occurs upon realignment of the chains to the same orientation as the direction of extension.[1,12] In synthetic DN hydrogels, this may indicate a different mechanism of toughening from the fracturing mechanism that is observed at higher strains. At the yielding point, the MCS/PAAm DN hydrogels were observed to have a stress of 1500−2400 kPa, nearly double that of the reported PAMPS/PAAm DN hydrogels; however, the strain (~100%) is only half that of the PAMPS/PAAm DN hydrogels.[2f] The MCS/PAAm DN hydrogels also exhibited a yielding region where we believe the MCS network is fully fractured across a cross-section and the hydrogels undergo elongation of the PAAm coils.[2d,f] The similar behavior of MCS/PAAm and PAMPS/PAAm suggests that a similar mechanism is occurring, even though the first network of MCS/PAAm is a cross-linked, linear biopolymer with a quite different microstructure than PAMPS. Even though the hardening region was not observed in the MCS/PAAm system, we believe that with modifications of the formulations a longer yielding region, potentially stretching to a strain of 4−7 mm/mm, and a hardening region that increases the fracture stress dramatically, can be achieved. The early failure of the MCS/PAAm could be attributed to the large yield stress created from a highly cross-linked MCS network, which may not allow for high strain at such a high stress without prematurely breaking. Therefore, to reach the hardening region, the fracture stress of the PAAm network needs to exceed the yield stress of the MCS/PAAm DN, which can be obtained by increasing the concentration of PAAm or decreasing the cross-linking density of MCS. Nevertheless, this is the first demonstration of a biopolymer-based network that shows a substantial yielding region, which leads to a ductile rather than brittle failure.

We then investigated changing the second network to *N,N*-dimethyl acrylamide (DMAAm) to test the hypothesis that hydrogen bonding between networks is responsible for the gel toughness. The $CONH_2$ group on acrylamide has a high hydrogen bonding capability and its interactions with the first network could significantly increase toughness, as has been seen in other systems.[13] Furthermore, DMAAm is potentially useful for biomedical applications since it is less toxic than AAm.[13a,14] A typical MCS/PDMAAm DN stress−strain curve is shown as open diamonds in Figure 1. MCS/PDMAAm has a similar stress−strain curve to MCS/PAAm thus has comparable mechanical properties, Figure 2a. The DN of MCS/PDMAAm also has similar swelling (~7 g/g) to the DN of MCS/PAAm. Further, DN of MCS/PDMAAm exhibits a fairly high toughness of 1160 kJ/m³. This supports our hypothesis that the specific interaction between PAMPS/PAAm or MCS/PAAm is not essential to the toughening mechanism, but rather the brittle/ductile network combination.

This is the first demonstration of a tough and ductile biopolymer-based hydrogel with a distinct yielding phenomenon. The MCS/PAAm DN hydrogels show two of the three characteristic regions − preyielding and yielding regions, with significantly improved mechanical properties. Besides that, this work supports the hypothesis that the brittle/ductile combination of networks is key to obtaining the DN effect, over specific interactions or particular microstructures of the networks. Replacement of PAAm with PDMAAm removes specific interactions (known to toughen networks) without significantly altering the stress−strain curves of MCS/PDMAAm, supporting the idea that the brittle-ductile combination is crucial relative to noncovalent interactions between the networks. MCS/PAAm and MCS/PDMAAm DN hydrogels are comparable to our previous work on tough double-network gels of PAMPS/PAAm and have proved to provide excellent toughness and strength with the use of biopolymers.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental methods and materials supplied. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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