Published in final edited form as:

J Tissue Eng Regen Med. 2017 January ; 11(1): 121–128. doi:10.1002/term.1892.

Evaluation of apparent fracture toughness of articular cartilage and hydrogels

Yinghua Xiao¹, Deena A. Rennerfeldt¹, Elizabeth A. Friis², Stevin H. Gehrke¹, and Michael S. Detamore^{1,*}

¹Chemical and Petroleum Engineering, University of Kansas, Lawrence, KS, USA

²Mechanical Engineering, University of Kansas, Lawrence, KS, USA

Abstract

Recently, biomaterials-based tissue-engineering strategies, including the use of hydrogels, have offered great promise for repairing articular cartilage. Mechanical failure testing in outcome analyses is of crucial clinical importance to the success of engineered constructs. Interpenetrating networks (IPNs) are gaining more attention, due to their superior mechanical integrity. This study provided a combination testing method of apparent fracture toughness, which was applied to both articular cartilage and hydrogels. The apparent fracture toughnesses of two groups, hydrogels and articular cartilage, were evaluated based on the modified single-edge notch test and ASTM standards on the single-edge notch test and compact tension test. The results demonstrated that the toughness for articular cartilage (348 ± 43 MPa/mm^{1/2}) was much higher than that for hydrogels. With a toughness value of 10.8 ± 1.4 MPa/mm^{1/2}, IPNs of agarose and poly(ethylene glycol) diacrylate (PEG-DA) looked promising. The IPNs were 1.4 times tougher than PEG-DA alone, although still over an order of magnitude less tough than cartilage. A new method was developed to evaluate hydrogels and cartilage in a manner that enabled a more relevant direct comparison for fracture testing of hydrogels for cartilage tissue engineering. Moreover, a target toughness value for cartilage of using this direct comparison method has been identified (348 ± 43 MPa/mm^{1/2}), and the toughness discrepancy to be overcome between hydrogels and cartilage has been quantified.

Keywords

toughness; articular cartilage; hydrogels

1. Introduction

Hydrogels used in biomaterials-based tissue engineering to regenerate articular cartilage offer great promise to address cartilage injuries (Azuma *et al.*, 2007; DeKosky *et al.*, 2010; Gong, 2010; Huang *et al.*, 2008; Sant *et al.*, 2010). Improving mechanical integrity, including both the deformation and failure of hydrogel-based constructs, is of great

*Correspondence to: Michael S. Detamore, Department of Chemical and Petroleum Engineering, University of Kansas, 1530 West 15th Street, 4132 Learned Hall, Lawrence, KS 66045, USA. detamore@ku.edu.

Conflict of interest

The authors have declared that there is no conflict of interest.

importance to cartilage regeneration. Although mechanical failure testing in outcome analyses is of crucial clinical importance regarding the success of engineered constructs, mechanical testing of hydrogel-based constructs to date has focused primarily on deformation rather than failure properties (Krupa *et al.*, 2010; Lam *et al.*, 2006; Langrana *et al.*, 2010; Suljovrujic *et al.*, 2011; Wang *et al.*, 2011; Weichold *et al.*, 2011).

Failure properties can be characterized by apparent fracture toughness, which reflects how much energy the material must absorb to fracture (Abdurrahmanoglu *et al.*, 2009) and governs the response of materials to crack propagation (Stok and Oloyede, 2007). There exist various methods to determine the apparent fracture toughness in metals and plastic films, such as the single-edge notch test, trouser tear test, indentation test, etc. Because of the lack of much-needed studies on failure properties of hydrogel-based constructs, the overall objective of this study was to develop a method to evaluate comparable apparent fracture toughness for both articular cartilage and hydrogels in cartilage tissue engineering.

There are many standard fracture toughness tests for materials of various properties. One goal in standardized fracture toughness tests is to have a specimen size sufficiently large that the geometry of the specimen does not influence the results. In a tough anisotropic composite biological material such as cartilage, the anatomy of the cartilage inherently limits specimen size and shape. To compensate for this shortcoming, one technique that can be used is to reproduce the state of stress found in a defect in cartilage through use of an identically-shaped comparison specimen. In this way, the values of fracture toughness obtained for cartilage can be directly compared to those for other materials, such as hydrogels. Such a methodology is necessary to achieve the goals of this field, which is to create an effective hydrogel for cartilage tissue engineering. Ideally, the deformation properties should *match* those of articular cartilage, and the fracture properties should *meet or exceed* those of articular cartilage. While deformation properties (e.g. aggregate modulus, dynamic moduli) are well established for hydrogels and cartilage, and have heretofore been the primary focus of cartilage tissue-engineering studies with hydrogels, fracture properties are also important and stand alongside the modulus as an important design parameter for mechanical performance.

We recently reviewed fracture mechanics studies of both hydrogels and cartilage with the objective of identifying a common ground between these two disparate fields (Xiao *et al.*, 2013). We concluded that among apparent fracture toughness tests, the modified single-edge notch test was preferred for application to both hydrogels and articular cartilage because it can avoid overstressed factors induced by grips, and it is derived from the single-edge notch test, which has solid fundamental models to analyse data. Chin-Purcell and Lewis (1996) first invented the modified single-edge notch test to measure the toughness of bovine patellar cartilage, based on the work of Mai and Atkins (1980) and Srawley and Gross (1967). Adams *et al.* (2003) supplemented their work by suggesting that the thickness of the specimen did not affect apparent fracture toughness. However, the models for analysing apparent fracture toughness data were geometry-dependent. Thus, after reviewing related ASTM standards, we adopted ASTM D 5045–99 (2007), a standard test for fracture toughness of plastics, to evaluate the apparent fracture toughness for both articular cartilage and hydrogels in cartilage tissue engineering.

We recently introduced interpenetrating networks (IPNs) with drastically improved mechanical performance relative to their constituent networks of agarose and poly(ethylene glycol) diacrylate (PEG-DA), capable of successful encapsulation of viable chondrocytes (DeKosky *et al.*, 2010; Ingavle *et al.*, 2012, 2014). However, mechanical properties were evaluated with compression, and it was unknown whether IPNs would also maintain superior failure properties following a testing method more relevant to cartilage fracture mechanics studies. The objective of this study was to develop a new method to not only compare the fracture toughnesses of the IPN and PEG-DA (agarose was not tested due to its low fracture strain) but, perhaps more importantly, to enable direct comparison of the hydrogels to cartilage, establishing a quantitative benchmark or 'target' for hydrogel toughness.

It must not escape mention that cartilage failure in osteoarthritis is typically considered as a biological phenomenon following an initial impact injury, characterized by a cascade of signalling events over an extended period of time that result in the degeneration of the cartilage structure and thus the loss of mechanical integrity. However, in the fabrication of hydrogels in cartilage repair, the hydrogels must be able to withstand the mechanical environment if there is hope of patients returning to weight-bearing activity without extended delay, and in this context we therefore examined cartilage as a material. Based on the modified single-edge notch test, we established the groundwork for linking methodologies between fracture testing of cartilage and hydrogels in an effort to evaluate fracture properties for hydrogels in cartilage tissue engineering. Our hypothesis was that interpenetrating networks (IPNs) of agarose and poly (ethylene glycol) diacrylate (PEG-DA) would be significantly tougher than PEG-DA alone.

2. Materials and methods

2.1. Cartilage specimen preparation

Hog ankles from eight different individuals ($n = 8$), all males from either York or Barron strains, 5–7 months old and weighing 97–195 kg, were obtained from a local slaughterhouse. The ankles were carefully opened within 24 h of the hogs' death. The joint compartments were opened (Figure 1), wrapped in Kimwipes, soaked in 0.01 M phosphate-buffered saline (PBS) containing 0.138 M sodium chloride and 0.0027 M potassium chloride, and stored at -20°C (Allen and Athanasiou, 2005) (storage time < 1 month). Cartilage specimens (thickness 0.901–1.185 mm; Figure 2) were sectioned from the central portion of each ankle of the eight different hogs using a precision diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA).

2.2. Hydrogel specimen preparation

An effort was made to make the hydrogel geometries as similar as possible to the articular cartilage specimen, so as to enable apparent fracture toughness comparisons as closely as possible between cartilage and hydrogels. Gels were synthesized following the basic methods of our previous work (DeKosky *et al.*, 2010; Ingavle *et al.*, 2012, 2013). Briefly, 2-hydroxyethyl agarose (type VII) was obtained from Sigma-Aldrich (St. Louis, MO, USA). PEG-DA (MW 6000 Da) was obtained from SunBio (Anyang City, South Korea). The

photoinitiator Irgacure 2959 (I-2959) was purchased from Ciba (Basel, Switzerland). Agarose powder (0.2 g) was added to 10 ml PBS and autoclaved for 30 min to yield a 2% w/v agarose solution. When the agarose had cooled to 39°C, it was pipetted into rectangular silicon rubber moulds (10 mm wide $\times$ 20 mm long $\times$ 1 mm high) between glass plates. After 10 min cooling at 4°C, the gels were removed and added to a reservoir of PBS to equilibrate for at least 24 h before synthesizing PEG-DA and IPNs. A solution of 0.1% w/v I-2959 photoinitiator in deionized (DI) water was dissolved in 20% w/v PEG-DA in PBS at room temperature. One rectangular agarose gel (10 mm width, 20 mm length, 1 mm height) was cut evenly into eight pieces and placed into a 2 ml Falcon tube. 1 ml monomer solution was added into the 2 ml Falcon tube to soak those eight pieces under constant agitation using a rocker for 6 h. The eight soaked pieces were then placed four at a time into a rectangular silicon mould between optical glass microscope slides (four pieces/mould) and the surrounding space was filled with monomer solution. The gels were exposed to ultraviolet light for 5 min on each side, using 312 nm light, 3.0 mW/cm^{1/2} (XL-1000; Spectronics Corp., Lincoln, NE, USA). Four pieces of IPNs/mould were cut using a razor blade. The PEG-DA gels were made by the same exposure procedure, except for filling the moulds with only monomer solution. In total, for each outcome measure, five separate batches of hydrogels were prepared.

Hydrogel samples were then trimmed to strips using two razor blades evenly bonded together (distance between the two blades was 1 mm). Each hydrogel strip (1 $\times$ 1 $\times$ 20 mm) was then glued to a piece of marked closed-cell polyurethane foam (Thompson *et al.*, 2003) (Pacific Research Laboratories, Vashon, WA, 10 pcf) 20 mm wide, 40 mm long and 1 mm thick (Figure 3) with a cyanoacrylate adhesive. This foam is commonly used to model the elastic properties of cancellous bone (Thompson *et al.*, 2003). The crack was made through the closed-cell foam into the gels with the same series of cutting tools (Figure 4). A new razor blade was used for each sample to maintain a consistent sharp crack tip inside the hydrogel. When pressing the blade, the specimen was cut through to create the crack with a certain part of the hydrogel remaining inside the notch that was not cut. In such a way, crack lengths were reproducible.

Care was taken to ensure that hydrogel specimen dimensions matched cartilage specimen dimensions as closely as possible, which we emphasize is important for the current study and for future comparisons back to data obtained here for hydrogels and cartilage.

The thickness of the hydrogel (B'), the width of the hydrogel (w'), the width of the whole specimen (W') and the crack length (a') were measured with a micrometer under a stereomicroscope ($\sim \times 10$ magnification).

2.3. Solids content characterization

Swelling characteristics and solids content of the hydrogels were analysed to verify their suitability as a three-dimensional (3D) scaffold for tissue engineering, as solids content can help to provide a better understanding and context for mechanical properties. Cylindrical hydrogel constructs (3 mm diameter, $\sim$ 1.9 mm height) were synthesized for solids content characterization, using the methods described above. Five independent batches were made for each type of hydrogel. The swollen gel mass (m_s) was measured after soaking in excess

deionized water for 24 h, and the dry gel mass (m_d) was measured after drying in a desiccation chamber over calcium sulphate for 72 h. Measurements were made in triplicate (three separate samples) for each batch, using a high-precision balance (Shimadzu Corp., Kyoto, Japan). The swelling degree (Q) and solids content were calculated by:

$$Q = \frac{m_s}{m_d}; \quad \text{Solids content} = \frac{1}{Q} \times 100\%$$

The conversion efficiency was evaluated in terms of the conversion of the PEG-DA in the PEG-DA and IPN gels. The conversion percentage of PEG-DA in pure PEG-DA gels was calculated by:

$$\% \text{Conversion of (PEG-DA) in (PEG-DA) gels} = \frac{m_d(\text{PEG-DA})}{\phi_0 V} \times 100\%$$

The denominator represents the theoretical amount of PEG-DA in the gel for 100% conversion efficiency, where ϕ_0 is the w/v concentration of PEG-DA solution before photopolymerization (0.2) and V is the calculated volume of the preswollen sample, based on the biopsy punch diameter and mould thickness. The conversion of PEG-DA in IPNs was calculated by:

$$\% \text{ Conversion of (PEG-DA) in IPNs} = \frac{m_d(\text{IPN}) - \overline{m}_d(\text{agarose})}{\kappa \phi_0 V} \times 100\%$$

where the numerator is the estimated mass of PEG-DA in the dried IPN, κ is the partition coefficient (assumed to be 1) and the overbar denotes the average value for a given batch. Similarly, the percentage of PEG-DA in dried IPNs was also calculated:

$$\% (\text{PEG-DA}) \text{ in dry IPN} = \frac{m_d(\text{IPN}) - \overline{m}_d(\text{agarose})}{m_d(\text{IPN})} \times 100\%$$

2.4. Toughness test for articular cartilage

A crack was made through the bone to the cartilage with a series of custom-designed cutting tools (there were three cutting tools with three different notch distances, 0.25, 0.36 and 0.48 mm). The thickness of the specimen (B), the width of the articular cartilage (w), the width of the whole specimen (W) and the crack length (a) were then measured with a micrometer under a stereomicroscope ($\sim \times 10$ magnification). A new razor blade was used for each specimen to maintain a sharp crack tip. As with the hydrogel specimen preparation, part of the specimen was positioned inside the notch and remained uncut, to ensure that crack lengths were reproducible (Figure 4).

Each specimen was then marked (Figure 5), placed in a custom-built bath-grip assembly (Singh and Detamore, 2008) in an Instron Model 5848 (50 N load cell; Canton, MA), and lined up (Figure 5) in a hydrated environment with PBS at 37°C (Figure 6). For each sample,

the crack tip was consistently 30 mm away from the centre of the grips, consistent with gel specimens as noted below. Tensile loading was applied with a displacement rate of 1.5 mm/min until the specimen pulled apart. The ratio $a:W$ was varied from 0.95 to 0.98, consistent with gel specimens as noted below.

2.5. Toughness test for hydrogels

Five independent batches were made for each hydrogel group (PEG-DA and IPN) to provide a sample size of $n = 5$. Eight samples were tested from each batch. Each specimen was marked, placed in the same custom-built bath-grip assembly in the Instron Model 5848, lined up and immersed in fresh PBS. For each sample, the crack tip was consistently 30 mm away from the centre of the grips, consistent with cartilage specimens as noted above (Figure 7). The same tensile loading was applied with the displacement rate of 1.5 mm/min until the specimen pulled apart. Load and displacement were measured. The ratio $a':W'$ was varied from 0.95 to 0.98, consistent with cartilage specimens as noted above.

2.6. Calculation of apparent fracture toughness

The models from the American Society for Testing and Materials (ASTM) method D 5045–99 (2007) were adopted. Apparent fracture toughness was characterized as K_Q in units of MPa/mm^{1/2}. K_Q was calculated as follows:

$$K_Q = \left(\frac{P_Q}{BW^{\frac{1}{2}}} \right) f(x)$$

where $0.2 < x < 0.8$, and $f(x)$ is defined as follows:

$$f(x) = \frac{(2+x)(0.886+4.64x - 13.32x^2+14.72x^3 - 5.6x^4)}{(1-x)^{\frac{3}{2}}}$$

where P_Q = load determined in ASTM D 5045–99 (2007), B = specimen thickness, W = specimen width, a = crack length and $x = a/W$.

Note that, due to the inherent geometry of articular cartilage, neither the range of $a:W$ nor the specimen thickness would meet the dimension requirements of the ASTM standard. The use of the undersized specimen results in an overestimation of the actual plane strain fracture toughness. This is recognized as a limitation of the method, but is required because of the physical limitations of the biological material. For this reason, identical geometries are used for comparison tests and the term ‘apparent fracture toughness’ is used to describe the fracture toughness properties.

2.7. Statistical analyses

To compare experimental groups, a single-factor analysis of variance (ANOVA) was performed, followed by a Tukey’s Honestly Significant Difference *post hoc* test when significance was detected. Analysis was performed using the SPSS/PASW 17.0 statistical

software package. All quantitative results were expressed as mean $\pm$ standard deviation (SD).

3. Results

3.1. Solid content analysis

The solid content analysis of the hydrogels is shown in Table 1 and the swelling degrees are reported in Figure 8. The total solids content in the IPNs was determined to be $11.41 \pm 0.23\%$ ($n = 5$). As significant differences were not observed between mould and final swelled gel dimensions (i.e. the agarose gel does not swell or deswell in the PEG-DA solution), the PEG-DA content in IPN gels could be estimated by subtracting the average solids content of the pure agarose gels (complete conversion of agarose is expected; DeKosky *et al.*, 2010) from the solids content of the IPNs. Agarose gels were composed of $\sim 2.0\%$ agarose, so the PEG-DA content of IPN gels was $\sim 9.4\%$. The swelling degree of PEG-DA was 13% higher than that of IPNs ($p < 0.05$). The conversion efficiencies in the PEG-DA and IPN gels were 82.2% and 69.1%, respectively (Table 2) ($n = 5$).

3.2. Apparent fracture toughness

Representative load-extension curves for the two hydrogel groups and cartilage are presented in Figure 9. The apparent fracture toughness for articular cartilage ($n = 8$) was $348 \pm 43 \text{ MPa/mm}^{1/2}$. The apparent fracture toughness of PEG-DA was $7.80 \pm 0.93 \text{ MPa/mm}^{1/2}$ and that of the IPN was $10.8 \pm 1.4 \text{ MPa/mm}^{1/2}$ (Figure 10). The apparent fracture toughness for articular cartilage was more than 30 times higher than that of the IPN, and the apparent fracture toughness of the IPN was 1.4 times greater than that of the PEG-DA ($p < 0.05$). Note that Figure 10 includes the apparent fracture toughness for each batch to demonstrate the range of values observed.

4. Discussion

To the best of our knowledge, this was the first effort to apply the same method to both articular cartilage and hydrogels with analogous methods for determining apparent fracture toughness. The current study introduced the approach of adhering each hydrogel strip to a piece of thin closed-cell foam in an effort to provide a reasonable model of an osteochondral specimen, and then testing these hydrogels with the modified single-edge notch test. The shape of the hydrogel specimen was trimmed in exactly the same way as articular cartilage, and the distance from the grip centreline to the crack tip was consistent, thus keeping the state of stress in the crack tip consistent in the specimens, in an effort to facilitate direct comparisons of the apparent fracture toughness values.

The IPN was tougher than the PEG-DA, as hypothesized. Using the modified single-edge notch test method in the current study, the IPN was 1.4 times tougher ($p < 0.05$), as compared to being 3.3 times tougher using the unconfined compression in our previous work (not statistically significant) (DeKosky *et al.*, 2010). The total solids content in IPNs was $\sim 11\%$ higher than that of the PEG-DA ($p < 0.05$), which may have contributed in part to the higher apparent fracture toughness of the IPNs (Gong *et al.*, 2005, 2009). However, IPNs had a lower apparent PEG-DA conversion (69% compared to 82% in PEG-DA gels). Had

the two hydrogel groups contained an equal amount of PEG-DA content, it is likely that the difference in their apparent fracture toughness would have been even greater.

The relative magnitude is less important than the advantage gained by the significant improvement in reproducibility afforded by the modified single-edge notch test. In fact, the large standard deviations with compression to failure in our previous study (80–95% of the mean!) were cited as a limitation of that testing modality (DeKosky *et al.*, 2010), as small changes in failure strain lead to large variations in toughness measurements, due to the nonlinear stress–strain behaviour at very high strains under compression. This limitation of large standard deviations was the impetus for exploring a more suitable method to evaluate the apparent fracture toughness, which ultimately led to our recent review to find the common ground between cartilage and hydrogel fracture mechanics (Xiao *et al.*, 2013), which in turn naturally evolved to the current study. With the modified single-edge notch test in the current study, we were able to achieve standard deviations in fracture toughness that were only 12–13% of the mean, a significant improvement over our previous compression-to-failure method (DeKosky *et al.*, 2010), allowing for a statistically significant demonstration of the superior toughness of IPNs compared to PEG-DA. By evaluating different batches, the reproducibility of the apparent fracture toughness from batch to batch was confirmed for both the PEG-DA and the IPN.

The major task of this study was to evaluate the apparent fracture toughness for both hydrogels and articular cartilage. In the current study, the apparent fracture toughness for porcine articular cartilage was 348 ± 43 MPa/mm^{1/2} using the modified single-edge notch test, which was more than 200 times higher than that for bovine articular cartilage measured by the single-edge notch test (Stok and Oloyede, 2007). The single-edge notch test was a different test in which only the cartilage was gripped and loaded under tension. The apparent fracture toughness of adult canine patellar cartilage in a modified single-edge notch test from ChinPurcell and Lewis (1996) was characterized by a J integral with the value of 0.14 ± 0.08 kN/m, which cannot be directly compared with the current study results, as here we developed a new model based on an ASTM standard that could be used for both cartilage and hydrogels.

The current study showed that the apparent fracture toughness of articular cartilage was 31 times higher than that of the IPN, which was in turn significantly tougher than the PEG-DA alone. With this perspective, the failure properties of synthesized hydrogels in tissue-engineering regeneration clearly need to be enhanced. Thus, factors that can significantly improve the apparent fracture toughness for the hydrogels need to be identified. It is recognized that the relative importance of scaffold fracture toughness compared to other mechanical properties, such as compressive strength and stiffness, is not yet clear and warrants further investigation. It is further recognized that compressive moduli, typically relaxed/aggregate moduli, are also a crucial design parameter for scaffolds in cartilage tissue engineering, to stand alongside, but certainly not be replaced by, fracture properties as mechanical performance design criteria.

There were some limitations with regard to testing both articular cartilage and hydrogels. For testing articular cartilage, the geometry was a limitation in terms of being unable to fit

the ASTM standard geometry. However, because of the physical size and structure of cartilage, this limitation is unavoidable, and the method was developed to ensure that the geometry of the specimens was as close to the ASTM standard geometry as possible while maintaining similar states of stress at the crack tip. In the future, longer-term studies will be valuable to evaluate whether extracellular matrix production by cell-seeded gels will significantly improve apparent fracture toughness in this testing regime. Moreover, it is clearly difficult to create apparent fracture toughness tests that will be *identical* for comparing hydrogels and articular cartilage, as cartilage has a complex gradient boundary and changing properties at the interface with the subchondral bone. Nevertheless, the approach developed here took into consideration a judiciously selected compromise among testing methods available for each material, so that boundary conditions of the cartilage in the *in vivo* state were maintained. The hydrogel testing method was carefully designed to match as closely as possible the modified single-edge notch test applied to cartilage.

Overall, a new method was developed to evaluate the failure properties of both articular cartilage and hydrogels in the context of cartilage tissue engineering. The IPNs possessed a greater toughness relative to the PEG-DA. However, their apparent fracture toughness was over an order of magnitude less than articular cartilage, so clearly there is much work to do in improving hydrogel fracture properties, unless cells are able to significantly close this gap with the production of functional extracellular matrix in clinically relevant time periods. Therefore, by developing standard testing methods that could accommodate both hydrogels and cartilage, we have a clearer target for the extent to which the failure properties of hydrogels must be improved, which will ultimately lead to better tissue replacements for damaged articular cartilage.

Acknowledgments

The authors would like to acknowledge funding from the NIH/NIBIB (Grant No. R21 EB008783).

References

- Abdurrahmanoglu S, Can V, Okay O. Design of high-toughness polyacrylamide hydrogels by hydrophobic modification. *Polymer*. 2009; 50:5449–5455.
- Adams DJ, Brosche KM, Lewis JL. Effect of specimen thickness on fracture toughness of bovine patellar cartilage. *J Biomech Eng*. 2003; 125:927–929. [PubMed: 14986422]
- Allen KD, Athanasiou KA. A surface-regional and freeze–thaw characterization of the porcine temporomandibular joint disc. *Ann Biomed Eng*. 2005; 33:951–962. [PubMed: 16060536]
- ASTM D 5045–99. Standard test methods for plane-strain fracture toughness and strain energy release rate of plastic materials. ASTM; West Conshohocken, PA: 2007.
- Azuma C, Yasuda K, Tanabe Y, et al. Biodegradation of high-toughness double network hydrogels as potential materials for artificial cartilage. *J Biomed Mater Res A*. 2007; 81A:373–380.
- Chin-Purcell MV, Lewis JL. Fracture of articular cartilage. *J Biomech Eng T ASME*. 1996; 118:545–556.
- DeKosky BJ, Dormer NH, Ingavle GC, et al. Hierarchically designed agarose and poly(ethylene glycol) interpenetrating network hydrogels for cartilage tissue engineering. *Tissue Eng C Methods*. 2010; 16:1533–1542.
- Gong JP. Why are double network hydrogels so tough? *Soft Matter*. 2010; 6:2583–2590.
- Gong JP, Tanaka Y, Osada Y. Novel hydrogels with excellent mechanical performance. *Prog Polym Sci*. 2005; 30:1–9.

- Gong JP, Yu QM, Tanaka Y, et al. Direct observation of damage zone around crack tips in double-network gels. *Macromolecules*. 2009; 42:3852–3855.
- Huang AH, Yeger-McKeever M, Stein A, et al. Tensile properties of engineered cartilage formed from chondrocyte- and MSC-laden hydrogels. *Osteoarthr Cartilage*. 2008; 16:1074–1082.
- Ingavle GC, Dormer NH, Gehrke SH, et al. Using chondroitin sulfate to improve the viability and biosynthesis of chondrocytes encapsulated in interpenetrating network (IPN) hydrogels of agarose and poly (ethylene glycol) diacrylate. *J Mater Sci Mater Med*. 2012; 23:157–170. [PubMed: 22116661]
- Ingavle GC, Frei AW, Gehrke SH, et al. Incorporation of aggrecan in interpenetrating network hydrogels to improve cellular performance for cartilage tissue engineering. *Tissue Eng A*. 2013; 19:1349–1359.
- Ingavle GC, Gehrke SH, Detamore MS. The bioactivity of agarose-PEGDA interpenetrating network hydrogels with covalently immobilized RGD peptides and physically entrapped aggrecan. *Biomaterials*. 2014; 35(11):3558–3570. [PubMed: 24462353]
- Krupa I, Nedelcev T, Racko D, et al. Mechanical properties of silica hydrogels prepared and aged at physiological conditions: testing in the compression mode. *J Sol Gel Sci Techn*. 2010; 53:107–114.
- Lam KY, Li H, Ng TY, et al. Modeling and simulation of the deformation of multi-state hydrogels subjected to electrical stimuli. *Eng Anal Bound Elem*. 2006; 30:1011–1017.
- Langrana NA, Chippada U, Yurke B. Simultaneous determination of Young's modulus, shear modulus, and Poisson's ratio of soft hydrogels. *J Mater Res*. 2010; 25:545–555.
- Mai YW, Atkins AG. Crack stability in fracture-toughness testing. *J Strain Anal Eng*. 1980; 15:63–74.
- Sant S, Hancock MJ, Donnelly JP, et al. Biomimetic gradient hydrogels for tissue engineering. *Can J Chem Eng*. 2010; 88:899–911. [PubMed: 21874065]
- Singh M, Detamore MS. Tensile properties of the mandibular condylar cartilage. *J Biomech Eng*. 2008; 130:011009. [PubMed: 18298185]
- Srawley JE, Gross B. Stress intensity factors for crackline-loaded edge-crack specimens. *Mater Res Standard*. 1967; 7:155–&.
- Stok K, Oloyede A. Conceptual fracture parameters for articular cartilage. *Clin Biomech*. 2007; 22:725–735.
- Suljovrujic EH, Jovasevic JS, Dimitrijevic SI, et al. Swelling, mechanical and antimicrobial studies of Ag/P(HEMA/IA)/PVP semi-IPN hybrid hydrogels. *Acta Phys Pol A*. 2011; 120:279–283.
- Thompson MS, McCarthy ID, Lidgren L, et al. Compressive and shear properties of commercially available polyurethane foams. *J Biomech Eng*. 2003; 125:732–734. [PubMed: 14618933]
- Wang HL, Wang XZ, Brown HR. Jellyfish gel and its hybrid hydrogels with high mechanical strength. *Soft Matter*. 2011; 7:211–219.
- Weichold O, Tigges B, Popescu C. Mechanical and sorption properties of transparent nanocomposite hydrogels. *Soft Matter*. 2011; 7:5391–5396.
- Xiao Y, Friis LA, Gehrke SH, et al. Mechanical testing of hydrogels in cartilage tissue engineering: beyond the compressive modulus. *Tiss Eng B Rev*. 2013; 19:403–412.

Figure 1. Harvesting of cartilage from hog ankles: (A) opening of the hog ankles; (B) sectioning the upper joint counterpart

Figure 2.
Sectioning of the articular cartilage into specimens: (A) location on the articular cartilage of the specimen to be created; (B) sectioned specimen

Figure 3.
Hydrogel specimen: the hydrogel strip was glued on the closed-cell foam with a cyanoacrylate adhesive, which was marked in the same way as the cartilage specimens

Figure 4.

Custom-machined cutting tool. There was a notch (indicated by an arrow) before the left head of the blade for inserting the hydrogel side of the specimen. There were three cutting tools with three different notch distances: 0.25, 0.36 and 0.48 mm. The specimens were inserted on the white plastic plate through the notch. When pressing the blade, the specimen was cut through with part of the specimen remaining inside the notch that was not cut. In such a way, crack lengths were reproducible

Figure 5.

Articular cartilage specimen: this was marked by pencil so as to line up straight in the grips in the Instron mechanical tester. The crack was opened along the middle of the horizontal dash line. Then grips grabbed the specimen at the bone along the vertical black line and the upper and lower horizontal black line, respectively (4 mm away from the central line and 7 mm away from the cracked cartilage)

Figure 6.

Apparent fracture toughness measurement of articular cartilage in the custom-built bath-grip assembly, immersed in PBS. Articular cartilage was placed into the custom-built bath-grip assembly in the Instron tester along the marks shown in Figure 4. PBS was filled in to equilibrate articular cartilage before testing

Figure 7.

The geometry as required in the ASTM standard: a , crack length (dashed arrow), which was measured as the horizontal distance from the crack tip to the central line of the grips; W , specimen width (solid arrow), which was measured as the horizontal distance from the end of the specimen to the central line of the grips

Figure 8.

Swelling degrees of the hydrogels. Five independent batches were made for each type of hydrogel. For each batch, three samples were taken to measure the swelling degree. One single point of data for each batch was obtained by averaging the data from those three samples. The PEG-DA gels had a significantly higher swelling degree than the IPNs ($p < 0.05$). PEG-DA, poly(ethylene glycol) diacrylate; IPN, interpenetrating network. Final values are reported as mean $\pm$ SD ($n = 5$). *Significant difference ($p < 0.05$)

Figure 9.

Representative load–extension curves for: (a) PEG-DA and IPN hydrogels; (b) cartilage. The linearity of the profiles up to maximum load demonstrates the advantage of the modified single-edge notch test over compression testing for determining the toughness of high-strength materials such as IPNs and cartilage. Note the difference in scale on the vertical axes between the two panels. PEG-DA, poly(ethylene glycol) diacrylate; IPN, interpenetrating network

Figure 10.

Apparent fracture toughnesses of the hydrogels. Five independent batches were made for each type of hydrogel. For each batch, eight samples were taken to measure the apparent fracture toughness. IPNs had a significantly higher toughness than the PEG-DA hydrogel ($p < 0.05$). For context, the apparent fracture toughness of articular cartilage was 348 ± 43 MPa/mm^{1/2}. PEG-DA, poly(ethylene glycol) diacrylate; IPN, interpenetrating network. Values are reported as mean $\pm$ SD ($n = 5$). *Significant difference ($p < 0.05$)

Table 1

Solids content analysis

Sample	Solid content (%)	Q
2% Agarose	1.99 ± 0.20	50.7 ± 4.8
6K 20% PEG-DA	10.10 ± 0.27	9.91 ± 0.26
6K 20% IPNs	11.41 ± 0.23	8.77 ± 0.18

 $n = 5$.

Table 2

PEG-DA conversion

Batch no. *	wt% PEG-DA in dry IPN	PEG-DA conversion in IPN (%)	PEG-DA conversion in PEG-DA (%)
1	89.0	72.6	85.4
2	85.7	67.3	81.4
3	86.9	69.4	82.0
4	88.5	68.1	80.7
5	88.6	68.4	81.7
Average	87.8	69.1	82.2
SD	1.4	2.1	1.8

* For each batch, data were measured in triplicate.