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Tough Hybrid Hydrogels Adapted to the Undergraduate Laboratory

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9 ABSTRACT

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11 Polymer materials are indispensable in our daily lives. This makes polymer technology of 12 critical importance in higher education. In particular, hands-on experiment-based 13 practicals/laboratories with a focus on polymer science are of tremendous value in the 14 undergraduate curriculum. Along these lines, hydrogels are highly crosslinked polymer 15 networks which show some unique properties such as water absorbance and large 16 extensibility, making them particularly well-suited in various biomedical applications. The 17 properties of hydrogels can be systematically varied via changes in composition. In this 18 practical laboratory, we use hybrid hydrogel formulations containing alginate and 19 polyacrylamide to explore the consequences of compositional changes on mechanical 20 behavior. Mechanical properties are determined using a simplified tensile test that is amenable 21 to large groups of students using standard laboratory equipment. We used marbles to induce 22 an extensional force and a ruler to measure the elongation of the gel as a function of the 23 attached weight. Hereby, stress-strain curves can be obtained and students are able to 24 compare the difference between single and double network hydrogels as well as quantify the 25 influence of network composition. This practical combines the use of chemical synthesis (i.e., 26 reactant calculations) with practical skills which makes it interesting to use in a third year 27 chemical/biomedical course. Furthermore, students can learn how to deal with chemicals and 28 gain insight in polymer chemistry and its wide applicability, particularly well-suited for students 29 coming from outside the traditional chemical science background.

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31 KEYWORDS

- 32 Undergraduate laboratory; hydrogel synthesis; biomaterials synthesis; polymer chemistry;
- 33 materials science; network formation; hybrid hydrogels; tensile testing; biomedical science

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3 INTRODUCTION

Hydrogels have properties that are ideal in a wide variety of biomedical applications, showing 5 great promise in emerging therapies and regenerative medicine.¹⁻³ They consist of a three 6 7 dimensional structural scaffold that is highly swollen with water. Hydrogels for biomaterials are 8 often composed of water compatible polymers owing to the physical/mechanical attributes 9 associated with this material class. They can be constructed from a diverse range of components, both synthetically or naturally derived.⁴⁻⁵ Biocompatibility, high water content, 10 11 permeability for metabolites, variable composition and structure similar to the biological 12 extracellular matrix make hydrogels appealing candidates in a wide range of applications from tissue engineering to drug delivery to bio(chemical)sensors.⁶⁻¹⁰ However, implementation in 13 14 some of the most mechanically demanding applications has typically been limited by 15 inadequate strength/resilience/toughness. Several innovative design strategies have been 16 developed to address this shortcoming. Of particular interest are dual network and hybrid gels 17 containing complementary networks due to (1) the astounding mechanical properties (2) high optical clarity and (3) straightforward, practical preparation.¹¹⁻¹⁵ 18

19 Owing to the wide-ranging applicability, complex molecular makeup, and unusual 20 mechanics of contemporary hydrogels, exposure to the topic in a hands-on, experimental 21 setting is valuable for students in various disciplines including chemistry, physics, materials science, and biomedical sciences.¹⁶⁻¹⁹ A basic understanding of hydrogels and visualization 22 23 of their properties would serve students across multiple disciplines and as such we introduce 24 with this work an adaptable protocol intended for the undergraduate laboratory.²⁰ This is 25 complementary to a recent laboratory experimental procedure related to self-healing hydrogels for high school students and undergraduates.²¹ Likewise, several explorations into 26 the preparation of simple hydrogels have appeared in this journal, targeting different students 27 at various educational levels.²²⁻²⁵ 28

29 We were particularly intrigued by a seminal report describing simple protocols and formulations that led to gels with astonishing tensile and compressive properties.²⁶ The 30 concept of the work was inspired by many preceding dual network examples, and acted as a 31 springboard for several adaptations to hybrid gels with alginate.²⁷⁻³² The original work from 32 Suo and coworkers describes the combination of alginate with polyacrylamide (PAAm), 33 34 wherein the alginate (Alg) is crosslinked by ionic bonding between guluronate residues and 35 bivalent Ca⁺² ions (Figure 1). The polyacrylamide network is covalently crosslinked from the 36 UV-activated curing with methylene bis-acrylamide (MBAA). Furthermore, the two types of 37 networks are covalently joined to each other through reaction between carboxylates from 38 alginate and the amine groups of acrylamide (Figure 1A; a more detailed description of the formation of the networks can be found in the lab text included in the supplementary
 information, page S7).

3 Despite being composed of 86% water, the combination of networks gave rise to 4 remarkably tough materials, far surpassing the properties associated with the individual 5 components. This enhanced toughness is due to the energy dissipation created by the 6 unzipping of the alginate network during deformation. Extensive tensile testing was performed 7 with detailed analysis for a series of gels with variable [Alg]:[PAAm] composition.

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Figure 1. (A) graphical illustration of the chemical structures and synthetic step to make the hybrid hydrogels; (B) illustration of the procedure for preparing the gels using standard laboratory protocols.

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Hybrid alginate-polyacrylamide gels can be made via a facile two-step method using routine laboratory equipment (Figure 1B). The first network is created via free radical polymerization of acrylamide in the presence of the bifunctional crosslinker methylene bisacrylamide (MBAA) using a well-established thermal initiator ammonium persulfate (APS). Alginate is obtained commercially in polymeric form and can be used without further modification. It is ionically crosslinked in the last step of the procedure. The synthetic protocol 1 starts with making a solution of acrylamide, MBAA, APS, TEMED and alginate, which is heated 2 to initiate the radical polymerization. The poly(acrylamide) network is formed in the presence 3 of alginate, generating an entangled scaffold from the two different polymers. An intermediate hydrogel is formed during this first stage, whereby the hybrid polymer scaffold is fortified upon 4 5 introduction of a bivalent ion. Immersing the intermediate hydrogel in an aqueous solution of 6 calcium chloride serves this purpose. The calcium ions displace the sodium ions initially 7 present, thereby crosslinking the alginate by forming interchain ionic bonds between the 8 carboxyl groups on the guluronate repeating units.

9 We were inspired by the original report and identified an opportunity to adapt the 10 experimental procedure to an undergraduate laboratory experiment. Here we describe in detail the protocol that we implemented, where the actual laboratory guidelines can be found 11 12 in the supporting information. Furthermore, we provide several suggestions on additional 13 adaptations that could be made to tailor the lab for various subjects/majors/disciplines and for 14 different durations (e.g. 1-day, 2-day labs). For example, we executed the experimental protocol with a group of ca. 80 students in their 3rd (final) year of Bachelor's studies in the 15 16 Biomedical Sciences Department in Hasselt University. Students in the Biomedical Sciences 17 department at Hasselt University have a limited education in traditional chemical sciences. 18 However, tissue engineering forms an important part of their curriculum, and as such this lab 19 serves to introduce the students to one particular strategy for addressing biomaterials 20 synthesis aimed at applications in regenerative medicine/tissue engineering. This lab serves 21 as an introduction to materials synthesis and some common mechanical performance 22 benchmarking tools. We feel that this laboratory experiment offers several opportunities for 23 tailoring to a diverse range of subjects in Universities operating under a variety of structures.

The following section describes the sequence of experimental activities that we followed, including calculations used for the synthetic protocol and ultimately property evaluation using a benchtop tensile testing protocol. Within each section the detailed protocol is provided, followed by suggested adaptations that could be made based on the desired emphasis.

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30 EXPERIMENTAL PROCEDURE

Materials: Sodium alginate (NaAlg), acrylamide (AAm), ammonium persulfate (APS), N,N'methylenebisacrylamide (MBAA), tetramethylethylenediamine (TEMED), CaCl₂,
demineralized water, acetone, mold + glass plates, clamps, grip seal bags, marbles, a long
ruler, ring stand, pipets, pipet tips, 500 mL beaker, stirring bar, weighing boats, spatula, gloves,
5 mL volumetric flasks, syringe, large gauge needles (diameter 1.2 mm), stirring plate, lab

coat, safety glasses, gloves (see Supporting Information for further details on specific items
 used).

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Synthetic Procedure: The experimental procedure conducted in our laboratories was carried 4 5 out by students working in groups of two. Each group was assigned two gel compositions; one 6 single network gel (see Figure 2D; gel #1 or #8) and one dual network gel (#2–7). For each 7 gel composition, three individual samples were prepared to provide statistical comparison, 8 giving a total of six gels to be tested for each group, provided they had enough time. An 9 overview of the different steps of the practical and the time distribution are provided for a 1-10 day (i.e. 8 h) experiment (Figure 2A). The students start the practical by calculating how much of each of the components they need to make stock solutions and the precursor solutions of 11 the hydrogels they were assigned. For these calculations the students should consider the 12 total mass of monomer/polymer and the water content (e.g., 86 wt %). (see Supporting 13 14 Information for an example calculation sheet). Before starting with the preparation of the 15 hydrogels the students prepared stock solutions in 5 mL volumetric flasks for the following chemicals: MBAA (0.01 g/mL), APS (0.03 g/mL), TEMED (0.02 g/mL). A stock solution of 16 alginate (0.075 g/mL) was made by the lab supervisors in advance, owing to the long 17 18 dissolution time required (~24 h) for the particular source of starting material used (Sigma-19 Aldrich product number W201502). Ultimately, the final material properties will depend acutely 20 on the source and purification protocols of the alginate. However, the trends in tensile property 21 variation with composition will likely be consistent, regardless of the source.



Calculations Stock solutions & hydrogel composition 45 min Prepare of stock solutions 30 min Prepare hydrogels Prepare hydrogel precursor solution 1 hour Heat hydrogel solution in mold 1 hour (inactive) Prepare CaCl₂ solution 10 min (during 1 hour heating time) --- Break ---

Soak gels in CaCl₂ solution 30 min (inactive) Cleaning of glassware 15 min (during soaking time)

Tensile Testing

Prepare set-up 15 min (during soaking time) Measure tensile strength 1.5 hours

Data processing

Making stress-strain curves 2 hours (at home)



Figure 1. A) Timeline of the activities performed during a one-day lab. B) Chemical structures
 of the components used to synthesize the poly(acrylamide) network. C) Chemical structure of
 sodium alginate, the major constituent of the second network. D) Compositions of the gels.

4 To start the preparation of the hydrogel precursor solution for gels #2-7, acrylamide is 5 dissolved in water, as calculated according to the concentrations provided. For safety reasons 6 the acrylamide is first weighed in a 50 mL bottle under a separate lab hood and is transported 7 to the students own hood while sealed with a septum or secure cap. Acrylamide is a highly 8 toxic monomer, and extreme caution should be taken in handling this chemical. This should 9 be highlighted and emphasized, particularly for students who may be less familiar with 10 precautions associated with handling toxic materials (i.e. Biomedical students, Materials 11 Science students, etc.). Next the students add the water directly to the acrylamide in the 50 12 mL bottle. A stirring bar is added and the solution is stirred until the monomer dissolved. The 13 alginate is added to the AAm solution using a syringe with a wide-gauge needle (diameter 1.2 14 mm) because of the high viscosity of the alginate stock solution. The required volume of stock 15 solutions of MBAA and TEMED are added using micropipets. Thereafter the bottle is sealed 16 with a septum and flushed with nitrogen gas for 5 minutes using a balloon. The required 17 volume of APS is added with a micropipet while minimizing exposure to air as much as 18 possible. It is important to add APS after deoxygenation, as it can cause premature gelation 19 in the beaker during the deoxygenation step. To make the gels more visible, food coloring can 20 also be added. To obtain dog bone shaped samples the hydrogel precursor solution is poured 21 into a PMMA mold (Figure 3). This procedure should be carried out inside a fume hood with 22 the bench covered with paper to absorb possible spillage of the hydrogel precursor solution. 23 A glass plate is put on top of the paper and some drops of water are placed on top. Next, the 24 PMMA mold is placed on top of the wet glass. The water ensures good contact between the 25 mold and the glass plate and thus reduces spillage of the hydrogel precursor solution. The 26 monomer/polymer precursor solution can then be poured into the mold. The mold is covered 27 by a second glass plate while ensuring as little air as possible remains inside the mold. The 28 plates are clamped together using foldback clips and placed in an oven at 50 °C for 1.5 hours. 29 Meanwhile, a 0.3 M CaCl₂ solution can already be prepared by the students. After the break, 30 the molds are taken out of the oven and allowed to cool down for 10 min. The glass covers 31 are removed and the mold is placed inside the 0.3M CaCl₂ solution for 15 min. The gels are 32 removed from the mold and equilibrated for another 15 minutes inside an aqueous solution of 33 CaCl₂. Thereafter the excess water is removed from the gels by gently dabbing with a paper 34 towel.

The preparation of the hydrogel precursor solution for gel #8 (100% acrylamide) is the same as for the other gels, except that alginate excluded. Therefore, gel #8 is not submerged in the CaCl₂ solution. The gel can be measured directly after thermal curing. The preparation

- 1 of the hydrogel precursor solution for gel #1 (100% alginate) is comparatively straightforward.
- 2 This sample requires that the 0.075 g/mL stock solutions are poured directly into a mold and
- 3 placed inside a 0.3M CaCl₂ solution without cover. After one hour the gels are removed from
- 4 the mold and allowed to equilibrate for another 20 minutes to ensure homogeneous
- 5 crosslinking. Excess water is removed from the gels by dabbing with a paper towel.



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Figure 2. Protocol for preparing hydrogels in preformed dog-bone shaped molds for consistent
 sample quality. The gels precursors in the photo array have been artificially colored for visual
 clarity. Alternatives to these home-made molds can be found in supporting information.

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Tensile testing: The procedure for deoxygenation procedure and tensile testing is 11 12 summarized in Figure 4. The tensile properties of the gels are measured with a simple setup, 13 which is amenable to large groups (Figure 4E). Other innovative alternatives can be found in 14 literature.³³⁻³⁵ Students measure the thickness (t) and width (w) of the sample to obtain cross-15 sectional area (Figure 4D). These values are subsequently used to calculate the stress (σ = 16 F/A, where A is the cross-sectional area). The grip sections of the dog bone sample are 17 clamped with foldback clips, in which one end is hung from a ring stand. The students place 18 marbles into a plastic bag, measuring the mass of each sequential marble addition (the lower 19 foldback clip and paperclip included), and attach it to the lower end of the hydrogel using a 20 paperclip. The weight will be increased gradually and the elongation of the hydrogel sample 21 is recorded for each iterative marble addition. Alternatively, if the practical can be conducted in small groups of up to 10 students the tensile tests can be demonstrated to the students using a standard tensile tester. Depending on the limits of the tensile testing equipment the gels can be stretched until they break. However, this is not necessary for the students to complete their assessment. The student can use the data they obtain from this experiment to calculate the stress and strain at each point measured and make a corresponding stress-strain curve. Optionally, the students can compare the mechanical properties of different compositions using the collective data obtained by other groups.

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Figure 4. A) Deoxygenation step using a nitrogen-filled balloon. B) Dogbone shaped mold cut
out of a PMMA sheet (3mm thick) using a laser cutter. C) Mold filled with precursor solution
(artificially colored) and clamped in between two glass plates. D) Left: example of end product
for gel #2. Right: dimensions of the dog-bone shape. E) Custom mechanical tensile testing
setup.

Assessment: With the collected data the student should be able to make the stress-strain curves of the different gels and plot the results in a graph. The students are also asked to calculate the Young's modulus for the various samples. A comparison between samples with different compositions can be made, depending on the desired complexity of the analysis and the targeted learning emphasis. Additional calculations for advanced materials science students could include the work of extension (i.e., toughness).

- 22
- 23 HAZARDS
- 24

1 The use of chemicals always involves risks. Therefore, it is important that the students 2 are aware of the safety precautions of each chemical compound that will be used. The most 3 hazardous chemical of the practical is acrylamide because it is a carcinogenic powder which 4 can cause gene mutations and organ damage. To prevent exposure, this compound is 5 weighed on an enclosed balance placed under a fume hood. Alternatively, an aqueous stock 6 solution of acrylamide monomer could be provided to the students instead of the powdered 7 product, prepared by the lab instructor prior to the lab. This would mitigate some of the risk 8 associated with handling this highly toxic substance. In any case, the potential hazards should 9 be emphasized repeatedly to students. Also, TEMED should be handled with great care due 10 to its flammability and corrosive properties; it is also kept inside the fume hood at all times. 11 While MBAA is also toxic if swallowed or inhaled, alginate and CaCl₂ don't have special 12 precautions. Since the required chemicals can have some harmful effects in contact with skin 13 or eyes, students should wear personal protective equipment (gloves, eye protection and a 14 lab coat) at all times. In addition, all synthetic procedures were performed under a fume hood 15 to prevent inhalation of potential hazardous fumes. After the gels have solidified, they were 16 allowed outside the fume hood but students should still wear gloves while performing the 17 tensile tests. Gloves should be switched regularly if needed. The acrylamide waste, including 18 the hydrogels which can contain acrylamide monomer, are disposed in a separate waste 19 container for hazardous chemicals. All other waste is collected in the correct container: 20 aqueous, non-halogenated, halogenated or solid waste. The primary risks during this practical 21 are related to the chemicals, which are minimized by the protective clothing, fume hood, 22 recommended glassware and awareness. The students are also informed once more of the 23 safety precautions before they are allowed to start. Examples of safety sheets are provided in 24 the supporting information.

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Results: With the collected data the student should be able to make the stress-strain curves of the different gels and compare the results in a graph. Figure 5 illustrates an example

RESULTS AND DISCUSSION

28 curves of the different gels and compare the results in a graph. Figure 5 illustrates an example 29 of the stress-strain curves and mechanical properties associated with the different 30 compositions explored in this experiment. The data displayed in Figure 5 were obtained from 31 a standard tensile tester. Using this procedure gels with a tensile strength of 10-500 kPa and 32 stretch of up to 1000% and higher can be prepared. A clear correlation between the modulus 33 and extension of the gels and the amount of alginate present in the gels is shown. The work of extension, calculated as the area under the stress-strain curve, shows the composition of 34 35 85 wt % acrylamide / 15 wt % alginate as being the toughest hydrogel.

These results demonstrate the strong contrast in mechanical properties of single network compared with double network hydrogels. PAAm gels are known to be very soft and 1 elastic, typically exhibiting very low Young's modulus (particularly at low crosslink densities). 2 The 100% alginate network is stronger yet very brittle, breaking at low strain but higher stress 3 compared to PAAm (Figure 5). These properties contrast sharply and can also be seen in the double network hydrogels (#2-7). Increasing alginate content leads to increasing modulus and 4 5 decreasing strain. The hybrid gel thus becomes stronger, but also more brittle. By adjusting 6 the ratio of the two networks, an optimal combination can be found in terms of toughness (i.e., 7 a gel that is both strong and soft). This is nicely displayed in the work of extension (i.e., 8 toughness) graph in Figure 5. As mentioned earlier, this shows that gel #5 is the toughest 9 hydrogel amongst the compositions explored in this exercise.





11 12

Figure 5. Tensile testing/stress-strain curves for various gel compositions. Measurements were made on a standard tensile tester (500 N load cell, 100 mm/min load rate). Evaluation of several key parameters are provided from the stress-strain measurements, including modulus, stress at break, strain at break and work of extension. Percentages of alginate are expressed in terms of total solids concentration. 100% alginate means 100% of the total solids are comprised of alginate. The solids concentration in water were held constant at 86 wt %.

20 Depending on the level of the course, the toughening mechanism can be explored/discussed in depth. There are several key reports that extensively describe the 21 toughening mechanism in these systems, as well as the trade-off between strength and 22 toughness.^{16-17, 36-38} In general double network hydrogels are composed of a brittle/stiff 23 polyelectrolyte network and a soft/stretchable neutral network. The toughness results from the 24 25 internal fracture of the polyelectrolyte network. Disrupting the ionic crosslinks in this instance dissipates energy during deformation. In the meantime, the elasticity of the neutral 2nd 26 27 network maintains the general shape and endows the material with some degree of 28 recoverability. The mechanical properties are therefore dependent on the ratio of the two 29 networks as well as the crosslink density of the respective networks. Exploring these parameters in detail is a natural extension of this laboratory experiment for more advanced
 students. On the other hand, simply investigating the composition–mechanics relationships
 with the provided protocol is ideal for introductory laboratory courses.

4 During deformation of the hybrid hydrogels the alginate chains remain intact. However, 5 the dynamic reversible nature of the ionic crosslinking enables this network to partially recover 6 its strength upon release. The ionic bonds can reform after deformation, while the breaking of 7 the ionic bonds provides an energy dissipation mechanism. The materials exhibit mechanical hysteresis properties as reported by Sun et al²⁶ and this offers an appealing avenue for further 8 9 exploration in a more advanced version of the lab, tailored toward students with an emphasis 10 on mechanics and/or gels. This would require performing repeated elongations, and would be 11 best performed with a standard tensile testing instrument to capture the real nuanced 12 hysteresis and demonstrate its dependence on gel composition.

13 Adaptations: The emphasis and protocol of this lab are highly adaptable, depending on 14 the target student discipline. For example, chemistry students could benefit from 15 systematically altering the chemical makeup. This could be done by introducing alternate comonomers (e.g. acrylic acid³⁹) and monitoring the influence on the mechanical properties. 16 Likewise, crosslinking density could by systematically varied via the MBAA concentration²⁶ 17 18 and CaCl₂ concentration. Network density is clearly correlated with extensibility and modulus, 19 and could be investigated in this manner. The effect of different ions on the double network properties could be explored.²⁷ A more in depth explanation of the toughening mechanism of 20 21 these double network hydrogels could be provided in an adapted version of the lab text, 22 individually tailored to students with a different background. Additional hysteresis measurements²⁶ could also be performed to test the influence of the ionic crosslinks on the 23 mechanical properties. As part of the learning goals, students could be asked to speculate as 24 25 to the origin of hysteresis and provide a summary of potential strategies to curb this 26 phenomenon.

The procedure can readily be adapted to a multi-day lab. Examples of layouts can be found in supporting information (page S25). However, some attention must be paid to storage of the gels prepared in a multi-day format. The hydrogels should always be stored in a humid environment in a well-sealed container to prevent extensive water from evaporating. This can be done with parafilm-sealed petri-dishes stored in a refrigerator or a sealed desiccator.

Learning goals: We present here a list of several learning goals that were set for students in their 3rd (final) year of Bachelor's studies in the Biomedical Sciences Department. Students were graded through observation during the lab practical (correct calculations, safety, independence) and a post-lab assessment. Bear in mind that these students have very little experience performing chemical lab protocols and mechanical testing. More experienced students with a strong background in chemical sciences may benefit from additional learning goals and their knowledge can be tested in the post lab assessment or in a full lab report. This
is open to adaptation, and should be tailored to fit the format of the curriculum in which it is
used. However, we provide some suggestions here.

Learning Goals: 1. Looking up safety sheet of the chemicals used and adjust the handling of the chemicals accordingly; 2. Perform calculations to obtain the values needed during the experiment; 3. Accurately making stock solutions; 4. Follow the lab instruction to prepare double network hydrogels; 5. Performing simple tensile tests; 6. Calculating stress and strain, making a stress-strain graph; 7. Interpretation the obtained data (calculation of the elastic modulus, ...); 8. Demonstrate understanding of the formation of the two networks.

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11 **Observations:** We executed the experimental protocol with a group of ca. 80 students in their 12 3rd (final) year of Bachelor's studies in the Biomedical Sciences Department at Hasselt 13 University in Belgium. Students successfully prepared double network hydrogels using this 14 procedure. Careful reading of the instructions turned out to be essential (as usual!), and the 15 laboratory manual was modified for the second year to highlight some important points. For 16 example, the procedure had to be repeated by 4 of the 80 students because gelation occurred 17 inside the beaker during the deoxygenation step. This indicated how important it is to add APS 18 (initiator) at the right time perform subsequent steps in a timely manner. A few students (2 out 19 of 80) had no gelation after heating, possibly due to a missing component such as initiator or 20 crosslinker. These students joined another group to perform the tensile tests, and as such still 21 managed to participate in a key activity. This also highlights the crucial role of each reactant, 22 and the acute dependence of performance on chemical reactions. Which components are 23 responsible for which chemical processes? This provides key learning points, particularly for 24 students without a traditional chemical education/background.

25 An example of data sets obtained by students is available in the supporting 26 information. During tensile testing it is important that the students gradually increase the mass 27 of the marbles. Increasing the mass (force) by large increments may lead to rupture with very 28 few data points along the curve. Inaccurate evaluation of modulus is thus exacerbated. The 29 most common error made by the students in the post-lab assessment was failures in unit 30 conversions or wrong calculations. Mistakes during the synthesis of the hydrogels can also 31 lead to divergent data. Despite these minor deviations, most students were able to deduce the 32 general trend and used the correct terminology (soft, tough, brittle) for each of the measured 33 gels. The students were typically capable of describing relationships between composition and 34 mechanics, and were importantly able to calculate the Young's modulus and work of extension 35 (toughness), despite this being their first exposure to mechanics/materials science generally. 36 Two additional data sets were also added to the supporting information. These

37 datasets were obtained using hydrogels that were synthesized using a slightly different

protocol. For these gels a petri dish was used as a mold. The circular intermediate hydrogel was soaked for only 5 minutes in a CaCl₂ solution. Rectangular shapes were cut out of the circular gel for tensile testing. This gives rise to slightly different mechanical properties, but the general trend is still observed.

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CONCLUSION

8 The aim of this laboratory is to prepare a double network hydrogel by free radical 9 polymerization and explore the influence of the composition on the mechanical properties. 10 This lab procedure gives the students an opportunity to gain experience in both polymer 11 chemistry and material science. The hydrogels are prepared using an easy procedure and the 12 students are made aware of the safety precautions associated with handling hazardous 13 chemicals. A simple setup allows the students to explore tensile testing in a fun and hands-on 14 manner. The adjustable procedure makes the experiment interesting for an undergraduate 15 laboratory in a diverse range of subjects from chemistry, to polymer and materials sciences, 16 to biomedical sciences.

17

18 ASSOCIATED CONTENT

19 Supporting information

- 20 Detailed list of materials and equipment used, student handouts, safety rapport, 21 example calculation sheet, student results, description of adaptations to the procedure.
- 22
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Tough Hybrid Hydrogels Adapted to the Undergraduate Laboratory 4

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