

Spring 2019

Increasing Mechanical Properties of a Double Network Hydrogel from Polyacrylamide and Agar with Methylenebisacrylamide as a Photocrosslinker

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Recommended Citation

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Increasing Mechanical Properties of a Double Network Hydrogel from Polyacrylamide and Agar with Methylenebisacrylamide as a Photocrosslinker

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Bachelors of Science in Chemical Engineering

DEPARTMENT:

Chemical and Biomolecular Engineering

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Executive Summary

Purpose of Study

The purpose of this study is to develop a novel double network hydrogel that will be able to exhibit greater mechanical properties and hence, become more applicable in the medical field. The study will develop fundamental understanding about the double network hydrogel and materials for future development. The double network gels possess unique properties such as responsiveness to outside stimuli from thermal or acidic environments, swelling/deswelling, and anti-fouling^[1]. They have become applicable in the medical industry for usage as adhesives, drug delivery, tendon replacements, contact lenses, and repairing tissues. For example, damaged organs need tissues that are indistinguishable from the normal ones, and transplants that don't risk rejection from the immune system. Currently, there are 98,000 patients waiting for organ transplants in the US, and 20 die each day from shortage^[6]. The need for strong hydrogels to accelerate healing is imperative. This specific experiment will determine the optimal ratio of agar, water, AM, and MBAA that produce the best mechanical properties.

Results

The double network hydrogels synthesized achieved maximum tensile stress at 1.02 MPa, a tensile strain at 11.44 mm/mm, and an elasticity modulus of 234 kPa, showing promise as mechanically tough hybrid DN hydrogels that can be used in future medical applications. Gels were synthesized in 3 hours with the "one-pot" method.

Conclusions

From the completion of this project, it was affirmed that the “One-Pot” Method is an efficient and cost effective method to synthesize 3-D, hybrid, DN hydrogels. Agar was also shown to act as an excellent first, physically-crosslinked network with a brittle structure to allow for crack propagation and energy dissipation. Including photocrosslinker, MBAA, increased mechanical properties at 0.04 mol% (of PAAm) when being tested and compared to gels without the reagent. The mechanical testing of the agar/PAAm gels had a comparable mechanical toughness to that of gelatin/PAAm gels, proving that these molecules can be used to create 3-D networks and increase the fundamental understanding of hydrogels and improving their performance.

Implications

Technical skills gained include utilizing an Instron 3345 MA instrument, learning to operate Blue Hill and Origin software, and how to analyze and extract conclusions about mechanical properties from tensile stress/strain results. Also gained experience with the “one-pot” method in developing hydrogels. Skills for my career include developing a deeper understanding when reviewing literature and journals as well as how to apply conclusions from previous work to extenuate my own research. I personally plan to work with graduate students of my own with my own facility and getting to have experience in Dr. Zheng’s lab working with Ph.D candidates has taught me how to be a better lab member and as well etiquette with sharing work spaces and equipment. Working in research has taught me that frustration and failure is normal, but there are always several chances to try again and pursue new methods.

Having independent research that I could fit into my own schedule has given me a lesson in prioritizing my time and resources, as well as learning to value lab and research as a part of my day and future career. Being able to demonstrate to myself that I have the capability to function

in a lab and to produce results has given me a newfound passion and direction with my degree. Originally, I was at a loss this year towards my career and very confused. I realized I did not want to be a traditional engineer working at any company, and became enamored with academia and what it had offered. Working the lab, becoming familiar with different projects, reading journals, this has all lead me to appreciate what engineering and science can offer and the infinite avenues that are available to explore. Going outside of the curriculum to form relationships with graduate students and professors has helped me find family and support and when pursuing lab work and education I have been gifted with nothing but success. Hence, I plan to continue my education and pursue my doctorate at the University of Rochester and hopefully complete a post doctorate and find a campus I can call home and keep sharing education with students of my own.

The project presented in this report will hopefully inspire other scientists to have a better understanding of the molecular interactions that are crucial for developing tough hydrogels for future medical applications. Understanding the thermo reversible structure of gelatin's triple helical structure lead to the exploring of molecules with similar shape (like agar's double helices). The structure and allosterics of chemicals are directly linked to their function and interactions with their environment and finding further validity of these reagents will lead to optimization for better results. Strong, tough hydrogels are expected to be used for organ transplants, drug delivery, and tissues and scaffolding. The results presented are to justify double network hydrogels are strong candidates as solutions to these issues.

Future Applications

A limitation to this experiment is the availability of reagents and time to test them and their abilities. Synthesis of double network hydrogels is a relatively new process and there is much unknown as to what materials would create the most optimized mechanical properties, anti-fouling abilities, etc. The hope is that this research will provide more to the understanding of how to synthesize stronger and better hydrogels, and inspiration to use more novel compositions of different reagents.

Introduction

Hydrogels are soft materials consisting of three-dimensional networks comprised mainly of water. The gels possess unique properties such as responsiveness to outside stimuli from thermal or acidic environments, swelling/deswelling, and anti-fouling. They have become applicable in the medical industry for usage as adhesives, drug delivery, tendon replacements, contact lenses, and repairing tissues. For example, damaged organs need tissues that are indistinguishable from the normal ones, and transplants risk rejection from the immune system. Each day, 20 individuals on the waiting list for organ transplants in the US die from shortage. The need for strong hydrogels to accelerate healing is imperative.

Background

Unfortunately, current hydrogels tend to be weak and brittle. Double network hydrogels have shown promise in the field of having advanced mechanical properties with a tensile stress of 1-10 MPa, a tearing fracture of 10^2 - 10^3 J m⁻², and a compression capacity of 17.2 MPa^[4]. The double network hydrogel of interest for the study is of a physically cross linked first network and a second chemically crosslinked. The first network is brittle and allows for several small fractures but has the ability to swell immensely in water. Once a second, ductile network is introduced the resulting specimen exhibits greatly increased mechanical properties. Agar was selected as the first network due to its triple helices structure that is thermoreversible^[6]. Polyacrylamide (AM) was selected to create the second network. A photocrosslinker, methylenebisacrylamide (MBAA), is necessary once the gel is subjected to UV light to chemically bind the AM to the agar^[5]. This binding is hypothesized to increase the mechanical properties of double network hydrogels for future applications.

The traditional creation of DN hydrogels is tedious, time and material consuming^[2]. The first step is to synthesize the first network with a strong polyelectrolyte (PAMPs) that will create a tightly chemically crosslinked gel. The polyelectrolyte is a long chain, charged polymer that when activated by UV light will change its architecture to adopt an expanded conformation that will ultimately affect its solvent affinity. The first network will swell in water and have the ability to absorb 20-30 times the molar concentration of the second network. The neutral polymers of the second network have long, flexible chains that slowly diffuse into the first and extend their chains away from the solution into the hydrogel. The first network's inhomogeneous structure is accredited greatly to the strength and ductility of the final product. Unfortunately, the chemical-chemical crosslinkage allows for fractures in the first network to be irreversible and have no recovery rate. The diffusion of the neutral polymer creating a second chemically crosslinked network also requires 1-2 days time and an extreme excess amount of polyelectrolyte.

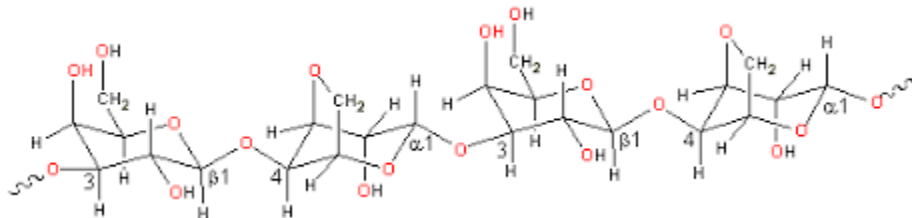


Figure 1: Shows the molecular structure of agar Credit: <http://www1.lsbu.ac.uk/water/agar.html>

Figure 1 displays the molecular dual chair conformation of agar bonded by negatively charged oxygen ions. Several of the branches have hydroxyl groups as well, and these electron affinities owe to agar's high hydrogen bonding properties that create a stable double helical structure when heated and gelled.

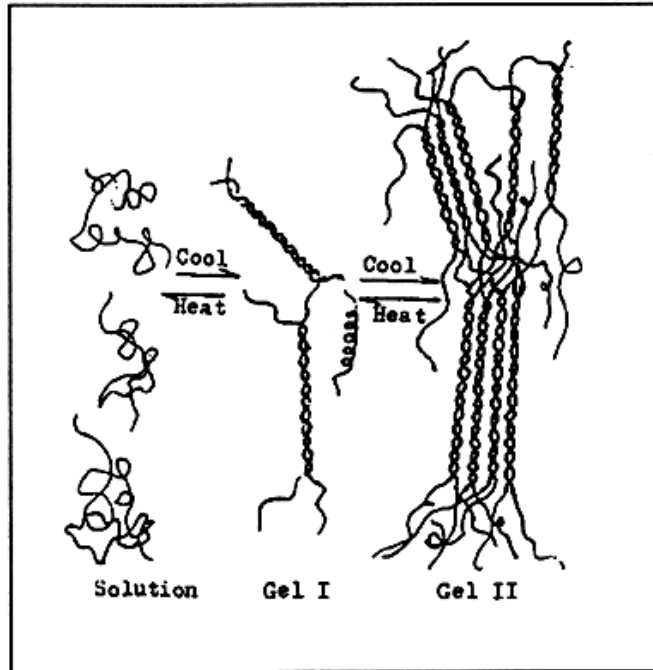


Figure 2: Shows the thermoreversible properties of agar Credit:

<http://www.fao.org/docrep/field/003/AB730E/AB730E03.htm>

Figure2 illustrates the thermo-reversible properties of the agar structure. When gelled (cooled from high heat of around 100 Celsius), the equatorial hydrogen atoms on the 3,6-galactose residues (as showed in Figure 1) are united to create a double helical conformation that can be denatured with high temperature. Denaturing, or dissolving, of the structure increases its solubility and affinity for water by freeing the charged hydroxyl groups and creating a polar molecule that favors the polarity of water. The spacing between the helices that forms during cooling is large and traps water-20 times the weight of the agar due to high solubility.

Chen and associates have developed the one-pot method to overcome these issues. The method is done by adding all reactants to “one pot” and using a heating to cooling polymerization method that accounts for the inert (non reactive) interaction of agar with the other polymers. This

is done using agar and crosslinked PAAm (Polyacrylamide), where the two networks allow for a physically and chemically crosslinked hybrid structure that does not rely on slow diffusion or exuberant amount of reactant. Agar possesses a thermo reversible solid to gel phase transition and coil to helix structural transition. Although agar is typically insoluble in cold water, heat can dissolve the structural carbohydrate and cooling (or “gelling”) causes a double helical 3D structure to form and whose framework can hold and absorb 20 times its own weight in water. The gelling property of agar is owed to the three equatorial hydrogen atoms on the 3,6-anhydro-L-galactose residues, which constrain the molecule to form a helix. It is a stable, physically crosslinked network with a slight negative charge. Once the first network is created, photopolymerization, a light activated free radical crosslinking technique, from UV light is used to non covalently crosslink PAAm with the first network. The step is a rapid liquid to solid transition of the dissolved components in the “one pot” by a rapid reaction rate that results in uniform hydrogel properties resulting in a DN with an incredible elastic modulus, strength, toughness, fracture energies, extensibility, free shapeable dual polymer. Using a hybrid physical-chemically crosslinked double network allows for fractures in the first to be reversible with thermodynamics and thus acquire a fast recovery rate with maintaining a strong and ductile hydrogels. The fabrication of the biosynthetic polymer is also weaved in 1-2 hours, exponentially less than the original 1-2 day amalgamation. This method is reliable, depends on a dilute concentration of reactants, and extremely fast in comparison to the traditional method.

These gels will have their mechanical toughness evaluated by maximum tensile stress (MPa), tensile strain maximum (mm/mm), and maximum Young’s Modulus (E, KPa). Young’s Modulus measures the resistance of a material to elastic (recoverable) deformation^[8]. In this case, a flexible material has a low Young’s Modulus and can change its shape when stress is applied. A

strong material will require high loads to permanently deform it. Tensile strain is the relative elongation of a material when subjected to stress. Tensile stress

Methodology

Double network hydrogels are to be synthesized by a one pot method: all reagents are added at once with several samples of varied compositions^[1]. It is an efficient process that minimizes labor and time. Samples will be heated to 100 Celsius and cooled for 2 hours to create the first physically cross linked network. Once at room temperature, samples will be subjected to UV light for 90 minutes to create the second, chemically cross linked network and the resulting hydrogels will undergo tensile tests to evaluate mechanical properties.

Varying compositions of reagents will be studied in independent batches to determine their effects. From Chen, it was determined that the amount of MBAA should be 0.03 mol% of AM, and thus amounts around that value were evaluated for the photocrosslinker.

Table 1: Displays the composition of reagents as determined from Chen’s paper, and calculated from 10 mL of water to 4 mL of water for this experiment.

Composition for 10 mL of Water			Composition for 4 mL of Water		
Reagent	Amount		Reagent	Amount	
AM	7	mol/L	AM	1.99	g
Agar	17	mg/L	Agar	0.068	g
HMP	0.4	mol% of AM	HMP	0.024	g
MBAA	0.028	mol% of AM	MBAA	1.2	mg

The effects of MBAA on the mechanical toughness of the double network hydrogel were evaluate at various compositions of the component based on mol% of AM (0.00, 0.01, 0.02, 0.03, and 0.04 mol%). To accurately add the small amounts of photocrosslinker, 0.12 g of MBAA was dissolved in a beaker with 5 mL of water and covered with aluminum foil to prevent

premature degradation by UV light. A micropipette (100 micro liters) was calibrated for each of the varying mol% and is recorded in **Table 2**.

Table 2: displays the various compositions of MBAA tested.

mol%	AM (g)	Water (mL)	HMP (g)	MBAA (mL)	Agar (g)
0	1.99	4.00	0.024	0.00	0.068
0.01	1.99	4.00	0.024	0.017	0.068
0.02	1.99	4.00	0.024	0.034	0.068
0.03	1.99	4.00	0.024	0.050	0.068
0.04	1.99	4.00	0.024	0.067	0.068

The effects of agar was evaluated at different amounts (7, 17, 27, 37, 47 g/mL) and can be seen in **Table 3**.

Table 3: displays the various compositions of agar tested.

Composition	AM (g)	Agar (g)	HMP (g)	MBAA (mL)	Water (mL)
7 g/mL	1.99	0.028	0.024	0.050	4.00
17 g/mL	1.99	0.068	0.024	0.050	4.00
27 g/mL	1.99	0.108	0.024	0.050	4.00
37 g/mL	1.99	0.148	0.024	0.050	4.00
47 g/mL	1.99	0.188	0.024	0.050	4.00

The effects of polyacrylamide were also evaluated at various compositions (5, 6, 7, 8, 9 mol/L) and can be seen in **Table 4**. Note that the amounts of HMP and MBAA also fluctuated with the varied amounts of AM because in **Table 1** their compositions are based upon the molarity of the second network chemical.

Table 4: displays the compositions of hydrogels when determining effect of concentration of

AM

Composition	AM (g)	Agar (g)	HMP (g)	MBAA (mL)	Water (mL)
5 mol/L	1.42	0.068	0.018	0.036	4.00
6 mol/L	1.71	0.068	0.021	0.043	4.00
7 mol/L	1.99	0.068	0.024	0.050	4.00
8 mol/L	2.27	0.068	0.028	0.058	4.00
9 mol/L	2.56	0.068	0.031	0.065	4.00

Once each hydrogel is synthesized, they will be cut into equal sized and shaped samples with a length of 35 mm, width of 3.18, and thickness of 1 mm. These underwent tensile tests on an Instron 3345 MA. The samples were cut using a uniform tool to create a “dog bone” shape. Each end was attached to clamps evenly, and once testing began they were stretched at a rate of 100 mm/minute. Data was collected with Bluehill software and translated into visual graphs with Origin 8.5 software.

Results and Discussion

Using the one pot method, gels were created efficiently within a 2.5-3 hour process as opposed to three days and created optically transparent and testable hydrogels as seen in Figure 3.

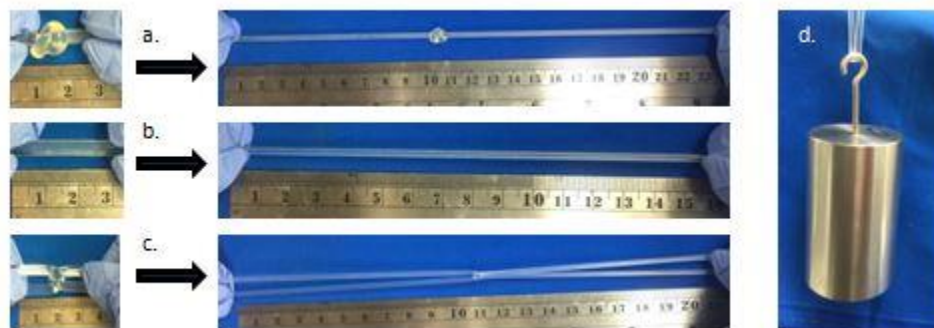


Figure 3 displays the gels being stretched to demonstrate their mechanical toughness and flexibility. 3a. shows the knotted, 3b. is the normal, 3c. is the double gels. 3d. demonstrates it withstanding 500 g of weight without breaking.

In **Figure 3**, a gel could be stretched 5x its original length. Knotted or coupled with a second hydrogel, it stretched 7x its original length and also shows the hydrogel withstanding 500 g of weight without breaking displaying its mechanical toughness and flexibility as a polymer. Retaining its shape after injection into mold implies that the gel has freeshapeable properties that can be used for future necessary conformations to tailor to a specific need. The use of a physically cross linked network provided for a brittle aggregation of agar helices that absorbed a large amount of water and AM. The second network of AM created a soft, ductile network that upon stress the sacrificial bonds created by the first network to initially dissipate energy would be absorbed and increase the overall mechanical properties.

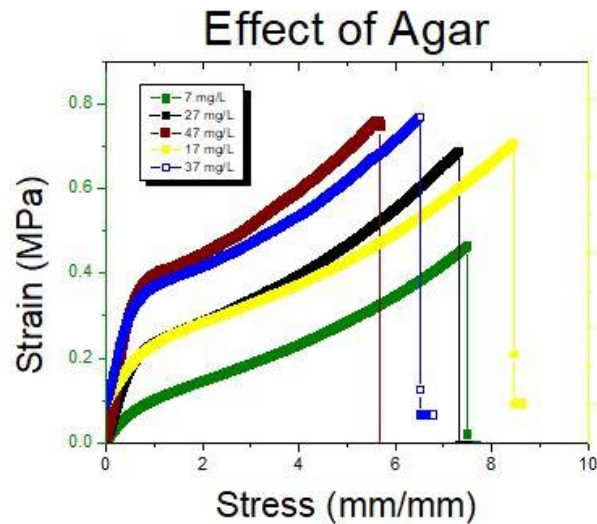


Figure 4: Displays the tensile stress/strain curve for the varied compositions of agar.

In **Figure 4**, the composition of agar was varied to determine its effects. Each sample had identical amounts of AM, HMP, water, and MBAA (0.03 mol%), but the amounts of agar tested included 7, 17, 27, 37, and 47 mg/L. It appears that the lower amounts of agar showed the highest mechanical strength with 17 mg/L showed the maximum tensile stress at 1.02 MPa, a tensile strain at 11.44 mm/mm, and an elasticity modulus of 234 kPa. Increasing the amount of agar increases the ratio of first network to second, where hydrogels with excessive amounts will be too brittle and not have enough of a second, ductile network to dissipate the energy and lowers the mechanical potential.

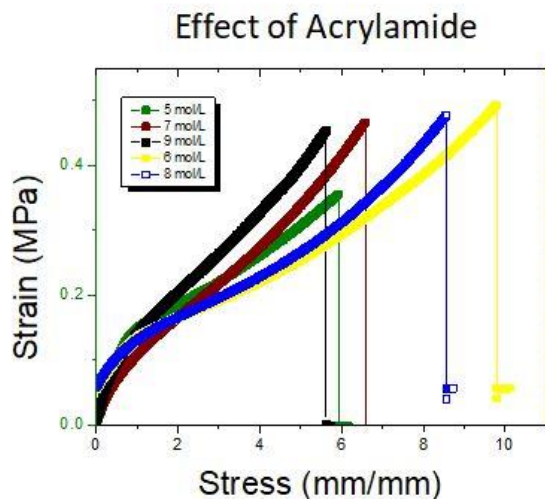


Figure 5: Displays the tensile stress/strain curve for varied compositions of Acrylamide.

In **Figure 5**, the composition of AM was varied to determine its effects. All samples had identical amounts of agar, HMP, water, and MBAA (0.03 mol%), but the amounts of AM included 5, 6, 7, 8, and 9 mol/L. Once again, more median amounts of acrylamide displayed the highest mechanical properties, with the maximum at 6 mol/L having reached maximum tensile stress at 0.796 MPa, tensile strain at 9.79 mm/mm, and $E = 198$ kPa. Increased amounts of AM will decrease the ratio of first to second network. Excessive ratio of AM will not be as tightly bound

to the first network with photocrosslinker MBAA and photoinitiator HMP, and a less organized and too soft second network will decrease the mechanical properties.

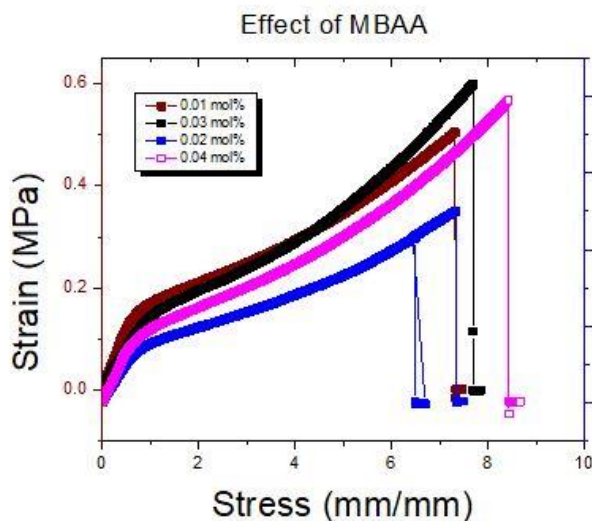


Figure 6: Displays the tensile stress and strain curve for the varied compositions of MBAA.

In **Figure 6**, it is seen that an MBAA composition of 0.04 mol% of AM with a maximum tensile stress of 0.789 MPa, tensile stress of 8.47 mm/mm, and elasticity modulus of 260 kPa. Samples with 0.00 mol% of MBAA were too brittle to test, implying that for these proposed compositions the second, ductile network of AM is too soft and requires the photocrosslinker. Traditionally, MBAA concentrations beyond 0.03 mol% caused excessive crosslinking leading to overly tightly wound second networks that resulted in two brittle structures, both the first and second networks, completely contradicting the point of having a hybrid double network with gelatin/PAAm. Gelatin, however, has a triple helix structure as opposed to agar. The triple helix would have more molecular surface area that could potentially lead to the “over cross-linking” phenomenon observed in gelatin, whereas the double helix in agar does not allow the photocrosslinker to overly bind the long, linear polymer chain of PAAm, and a higher concentration of MBAA

would result in heightened mechanical properties of agar/PAAm gels. Another important note is that the physically crosslinked network of agar is formed before the second network, whereas gelatin does not form its network until after the chemical crosslinking in PAAm under UV light.

Conclusion

Double Network hydrogels are three dimensional networks of a soft, mechanically tough material that have been used for drug delivery, agriculture, adhesives, and other widely applicable uses. Using the one-pot method, a single hydrogel can be produced in 3 hours as opposed to the once demonstrated three days. It has been found that with a first, physically cross linked network and a second, chemically cross linked, hybrid double network hydrogels exhibit high mechanical properties and are freeshapeable. Agar is a thermoreversible organic molecule with a triple helix structure that provides an excellent first network that organizes into aggregate bundles once cooled to room temperature (after heated to 100 Celsius) to reform its hydrogen bonds and absorbs 20-30x its weight in water. AM is a long polymer that readily dissolves in water to form a second, chemically crosslinked network (with photoinitiator HMP) that wraps and forms more hydrogen bonds. The increase in chemical activity with long polymer chains forms an organized structure around the lattice matrices created by the first network as reinforcement so that once stress is added to the hydrogel to help dissipate energy from crack propagation in the first, brittle network to create a ductile and soft polymer. Photocrosslinker, methylenebisacrylamide, activated with UV light organizes and increases chemical bonding of AM polymer chains with agar helices to increase the tensile stress/strain of a hydrogel without needing to alter the agar/AM ratio. These gels can achieve maximum tensile stress at 1.02 MPa, a tensile strain at 11.44 mm/mm, and an elasticity modulus of 234 kPa, whereas gelatin/PAAm gels achieve a tearing tensile stress of 0.268 MPa, a tensile strain of 40.69 mm/mm, and $E = 84$

kPa. Agar/PAAm show promise of being a tough double network hydrogel, and have their mechanical strength increased by using photocrosslinker MBAA. Differences in mechanical properties in relation to MBAA concentration may be attributed to structural differences in the previously tested gelatin/PAAm gels versus agar/PAAm gels and sequencing of network formations.

Special Acknowledgements

A thank you to Dr. Jie Zheng who has supported this project through his facility, resources, and as a Principle Investigator.

Also a thank you to Fengyu Yang, a Ph. D candidate at The University of Akron who has helped guide through the project and provided insight and assistance in lab.

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