HYDROGELS WITH REGIONAL MECHANICAL PROPERTIES

An Undergraduate Research Scholars Thesis

by

MCKENZIE R. JONES

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Research activities involving the use of human subjects, vertebrate animals, and/or biohazards must be reviewed and approved by the appropriate Texas A&M University regulatory research committee (i.e., IRB, IACUC, IBC) before the activity can commence. This requirement applies to activities conducted at Texas A&M and to activities conducted at non-Texas A&M facilities or institutions. In both cases, students are responsible for working with the relevant Texas A&M research compliance program to ensure and document that all Texas A&M compliance obligations are met before the study begins.

I, McKenzie Jones, certify that all research compliance requirements related to this Undergraduate Research Scholars thesis have been addressed with my Research Faculty Advisor prior to the collection of any data used in this final thesis submission.

This project did not require approval from the Texas A&M University Research Compliance & Biosafety office.

TABLE OF CONTENTS

ABSTRACT

Hydrogels with Regional Mechanical Properties

McKenzie R. Jones
Department of Biomedical Engineering
Texas A&M University

Research Faculty Advisor: Melissa A. Grunlan
Department of Biomedical Engineering
Texas A&M University

Clinical treatments for intervertebral disc (IVD) degeneration aim to relieve pain and stabilize the spine. However, recovery is limited by a lack of adequate replication of joint mechanics, donor site morbidity, and a low healing capability of the avascular tissue. Studies have shown that hydrogels are promising alternatives to typical metal or ceramic implants based on their hydration and unique mechanical properties, as well as biocompatibility. Electrostatic double network (DN) hydrogels, such as those based on negatively charged poly(2-acrylamido-2-methylpropane sulfonic acid) (PAMPS), have a distinct ability to achieve high strength through the energy dissipation in the second network. Recently, the reversible, hydrophobic interactions of poly(*N*-isopropylacrylamide) (PNIPAAm) were leveraged by the Grunlan Lab in a DN design consisting of a PAMPS first network and PNIPAAm-*co*-acrylamide (AAm) second network. A PAMPS/P(NIPAAm-*co*-AAm) (90:10 wt% ratio NIPAAm:AAm) DN yielded a unique combination of articular cartilage-like high modulus (~1 MPa), high strength (~25 MPa), and high water content (~80%). More recently, the modulus of this hydrogel was further increased to ~3 MPa – within the range of annulus fibrosus (AF) tissue - without compromising

the high strength or water content by incorporation of a cationic third network poly(acrylamidopropyl trimethylammonium chloride) (PAPTAC). The IVD has two major regions with different mechanical properties, the AF and the nucleus pulposus (NP). Thus, this present study sought to create a single hydrogel that exhibits such regional differences. This was accomplished with a triple network hydrogel for one region, and fabrication of a gelatinous interpenetrating polymer network (IPN) hydrogel for the second region. Connection of the two regions was accomplished with charged induced adhesion. Key material properties were evaluated (e.g. water content, modulus, and strength) and compared to the previously studied PAMPS/P(NIPAAm-*co*-AAm) hydrogel and mechanical properties of a healthy IVD. Results showed the creation of a hydrogel with two mechanical properties.

ACKNOWLEDGEMENTS

Contributors

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Finally, I would like to thank my friends and family for supporting me through the course of this project. They have provided me with the encouragement and guidance necessary to complete this project.

The TN-APTAC concentration series analyzed/used for Hydrogels with Regional Mechanical Properties were provided by Connor Demott. The analyses depicted in Hydrogels with Regional Mechanical Properties were conducted in part by Grunlan Research Group.

All other work conducted for the thesis was completed by the student independently.

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LIST OF FIGURES

1. INTRODUCTION

1.1 Clinical Significance

Low back pain is one of the leading disabilities in the US, affecting 52 million people annually, which can stem from the degeneration of intervertebral discs (IVDs). Degeneration of IVDs leads to a reduction in disc height causing impaired mechanics and the risk of neural damage.[1] The IVD is composed of two main sections, the annulus fibrosus (AF) and the nucleus pulposus (NP). The AF is an outer ring where lamellae fibers pull the vertebral bones together and act as a strong support between the bones. The NP, or inner region, is a fluid filled, gelatinous mass that allows for spinal articulation. Over time, tissue loss becomes more prominent due to natural degradation of the IVD. Tissue loss ultimately decreases disc height and sacrifices mechanical functions such as mobility, weight bearing capabilities, and structure of the spine.[2-5] During IVD degradation, the NP solidifies - becoming less gel-like and more brittle. With the disc becoming less elastic, the AF weakens causing tears - making the IVD prone to herniation, damage, or disease. Accordingly, surgery may be needed to relieve pressure on nerves and stabilize the spine.[3]

1.2 Current Treatments

Current treatments for IVD repair focus on removal of damaged tissue and stabilizing motion segments. Spinal fusion is one of the most common techniques, which permanently connects vertebrae, restoring disc height and stabilizing mobile segments. Bone grafts are placed between the vertebrae and metallic instrumentation are used to hold the graft in place during healing.[4] Novel approaches to spinal fusion surgery include integrating mesenchymal stem cells (MSCs) into an osteoconductive scaffold. This approach can increase biocompatibility and

integration into the native spinal tissues. However, MSCs are limited in quantity, and the quality of cells is variable depending on the patient.[6] Additionally, spinal fusion sacrifices native biomechanics and can possibly exacerbate degradation to other discs/vertebrae in the future (i.e. adjacent segment pathology).[2] Discectomies are performed when a disk is herniated and pinching a nerve. In most cases, the part of the disc pinching the nerve is removed; leaving the rest of the IVD intact. Micro endoscopic discectomy (MED) is a minimally invasive procedure using a trans muscular approach, which can be used on all forms of disc herniation. Complications relating to discectomies include possible nerve root injury, the tearing of the dura mater, and injury to organs including the ureter, small bowel, and bladder.[7] Another common alternative to spinal fusion is total disc replacement (TDR), also known as arthroplasty, which replaces the IVD with an artificial disc made of metal or a combination of metal and polymers, ultimately maintaining the segmental motion within the spine.[5] Artificial discs for TDR are mostly fabricated with Co-Cr alloys with the occasional addition of ultra-high molecular weight polyethylene (UHMWPE). Additionally, partial disc replacements (i.e. NP replacement) are emerging in the market. The prosthetic disc nucleus (PDN) is an FDA approved replacement with a hydrogel core constrained by biodegradable woven fibers.[2] NP partial disc replacements can restore disc height and mechanical support. However, NP replacement does not repair the damage in the AF, insertion is challenging and may compromise the rest of the disc. Additionally, TDR is not always reproducible, fails to replicate spinal mechanics correctly, and mechanical wear may challenge long term restoration of the disc.[3] While clinical treatments can relieve pain and provide stabilization they suffer from inadequate replication of joint kinetics and kinematics, adjacent segment pathology, donor site morbidity, and mechanical mismatch with surrounding tissue. With a 73% increase in rate of procedures in people over 65, treatments for IVD repair should be

more conducive and increase patient quality of life.[4] There is a need for the use of tissue-mimetic materials in intervertebral disc replacement procedures, such as hydrogels.

1.3 Hydrogel Research

Hydrogels are networks of crosslinked hydrophilic polymer chains and are widely used in biomedical applications such as scaffolds, wound dressings, and drug delivery systems. They are optimal due to their high-water content and similarity in structure to tissues; however, they tend to lack the strength and stiffness needed for load-bearing applications within the body. When looking at IVDs, a major hurdle for hydrogel replacements is to obtain the high modulus of the AF while maintaining a high-water content. Research relating to double network (DN) hydrogels showed developmental gains in the overall compressive strength, modulus, and fracture energy.[8, 9] The increase in toughness is attributed to the mixing of covalent bonds and secondary interactions (i.e. hydrophobic or electrostatic).[10, 11] The introduction of the second loosely crosslinked network increased the strength, toughness, and modulus while keeping a desirable water content.[12] Previous research in the Grunlan Lab showed promising results on using DN hydrogels to mimic tissue properties in all three categories of strength, stiffness, and hydration.[12] The electrostatic and reversible, hydrophobic interactions in the poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (PAMPS)/ poly(*N*-isopropylacrylamide-*co*-acrylamide) P(NIPAAm-co-AAm) double network allows for a high modulus (~1 MPa) and high strength (~25 MPa) while maintaining a desirable water content (~80%).

1.4 Novel Approach

Based on previous work, we now aim to develop a single hydrogel with regional mechanical properties. Two different hydrogels will be utilized to mimic the modulus, strength, and water content of the NP and AF regions of an IVD. The inner, gelatinous component will be

composed of an electrostatic interpenetrating polymer network (IPN) with PAMPS and AAm. The outer, robust region of the hydrogel will be based on the previously developed PAMPS/P(NIPAAm-*co*-AAm) DN hydrogel.[12] While improvements have been made to DN hydrogels' stiffness, it has remained a challenge to achieve ultra-high stiffness while maintaining toughness and a high water content. Recent work in the Grunlan Lab has shown that the introduction of a cationic third network of poly(3-acrylamidopropyl)trimethylammonium chloride (PAPTAC) into PAMPS/P(NIPAAm-*co*-AAm) DN hydrogels can increase the modulus to ~3 MPa, in the range of native AF tissue. The hydrogels with different mechanical properties will be connected by charged induced adhesion, while held together in a custom mold, resulting in a hydrogel with both robust and gelatinous regional mechanical properties. Evaluation of each region will be focused on the elucidation of its material properties such as water content, modulus and strength. Furthermore, the strength of the connection between the two components will be determined. With results indicated, a hydrogel with regional mechanical properties could be a good alternative to intervertebral disc replacement with the implementation of further considerations and testing.

2. MATERIALS AND METHODS

2.1 Materials

N-Isopropylacrylamide (NIPAAm, 97%), 2-acrylamido2-methylpropanesulfonic acid (AMPS, 97%), acrylamide (AAm, >99%), 3-(acrylamidopropyl)trimethylammonium chloride solution (APTAC, 75 wt % in H2O), *N,N′*-methylenebis(acrylamide) crosslinker (BIS, 99%) and 2-oxoglutaric acid photoinitiator, were obtained from Sigma-Aldrich. For hydrogel fabrication, deionized water (DI) with a resistance of 18 MΩ·cm (Cascada LS MK2, Pall) was used.

2.2 Hydrogel Fabrication

2.2.1 Interpenetrating Polymer Network Hydrogel Fabrication

IPN hydrogels were fabricated through a two-step, UV-cure process in which a single network (SN) hydrogels are soaked in a second network precursor solution and subsequently cured to form an IPN hydrogel. The SN precursor solution consisted of AMPS (1.5 M), BIS crosslinker (1 mol %), and 2-oxoglutaric acid (0.1 mol %) in DI water. The precursor solution was injected between two glass slides separated by 1 mm thick spacers and exposed to UV light (UV-transilluminator, 6 mW cm-2, 365 nm) for 5 h while being rotated at standard intervals to maintain symmetry. The SN hydrogel was removed from the mold and immediately immersed in the IPN precursor solution for 48 h at 4 °C. The IPN precursor solution consisted of AAm (1.5 M), BIS (0.1 mol %) and 2-oxoglutaric acid (0.1 mol %) in DI water. After soaking, the hydrogel was enclosed with two glass slides separated by spacers (∼1.25 mm) to form a complete seal (i.e., no air space) and then exposed to UV light for 5 h and rotated at standard intervals. The resulting IPN hydrogels were then removed from the molds and soaked in DI water for 1 week

before testing. The composition can be seen in **Figure 1**. This resulting IPN hydrogel composition is referenced as IPN-AAm.

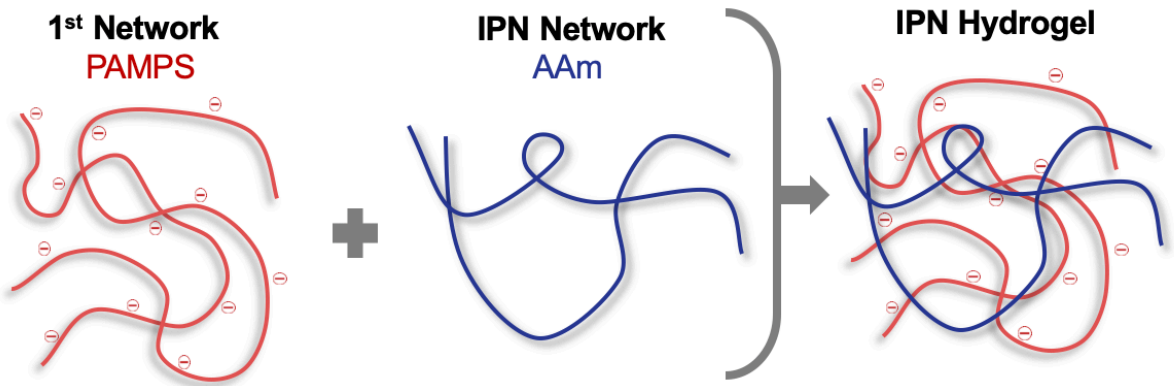

Figure 1: IPN-AAm Hydrogel Composition

2.2.2 Triple Network Hydrogel Fabrication

Triple network (TN) hydrogels were fabricated through a three-step, UV-cure process in which SN hydrogels are soaked in a double network (DN) precursor solution, subsequently cured to form a DN hydrogel which were then soaked in a TN precursor solution, which was then cured to form the final TN hydrogel. The SN precursor solution consisted of AMPS (1.5 M), BIS crosslinker (4 mol %), and 2-oxoglutaric acid (0.1 mol %) in DI water. The precursor solution was injected between two glass slides separated by 1 mm thick spacers and exposed to UV light (UV-transilluminator, 6 mW cm-2, 365 nm) for 5 h while being rotated at standard intervals to maintain symmetry. The SN hydrogel was removed from the mold and immediately immersed in the DN precursor solution for 48 h at 4 °C. The DN precursor solution consisted of NIPAAm (2.0 M), AAm (10 wt %), BIS (0.1 mol %) and 2-oxoglutaric acid (0.1 mol %) in DI water. After soaking, the hydrogel was enclosed with two glass slides separated by spacers (~1.25 mm) to form a complete seal (i.e., no air space) and then exposed to UV light for 5 h while being submerged in an ice bath (~7 °C) and rotated at standard intervals. This composition will be

referred to as DN-AAm 10%. The DN hydrogel was removed from the mold and immediately immersed in the TN precursor solution for 48 h at 4 °C. The TN precursor solution consisted of APTAC (2.0M), BIS (0.1 mol %) and 2-oxoglutaric acid (0.1 mol %) in DI water. After soaking, the hydrogel was enclosed with two glass slides separated by spacers (~1.25 mm) to form a complete seal (i.e., no air space) and then exposed to UV light for 5h while being submerged in an ice bath (~7 °C) and rotated at standard intervals. The resulting TN hydrogels were then removed from the molds and soaked in DI water for 1 week before testing. The composition can be seen in **Figure 2**. This resulting TN hydrogel composition is referenced as TN-APTAC.

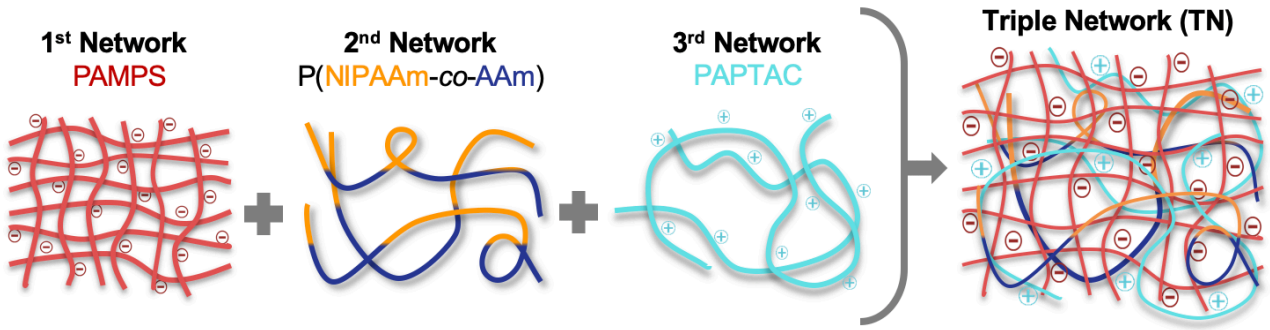

Figure 2: TN-APTAC Hydrogel Composition

2.2.3 Hydrogel Connection

Connection of the IPN-AAm and TN-APTAC hydrogels was done by using a charged induced adhesion connection process. Custom molds were made to show various connections shown in **Figure 3.** The IPN-AAm and TN-APTAC hydrogels were placed in the custom molds with ~5 mm spacers and pressed between two glass slides for 30 minutes. For interfacial lap shear testing, a custom mold shown in **Figure 4** was used between two glass slides. The silicone mold had a slit of 7 cm x 1 cm x ~3.5 mm, where 4 cm x 1 cm sized samples of IPN-AAm and TN-APTAC were connected with a 1 cm overlap. A 3 cm x 1 cm x ~1.5 mm piece of silicone was placed in the mold under the TN-APTAC gel to produce a horizontal connection. The

diagram of the custom mold is shown in **Figure 5**. The resulting connection of the two hydrogels was then soaked in DI water for 1 week before testing.

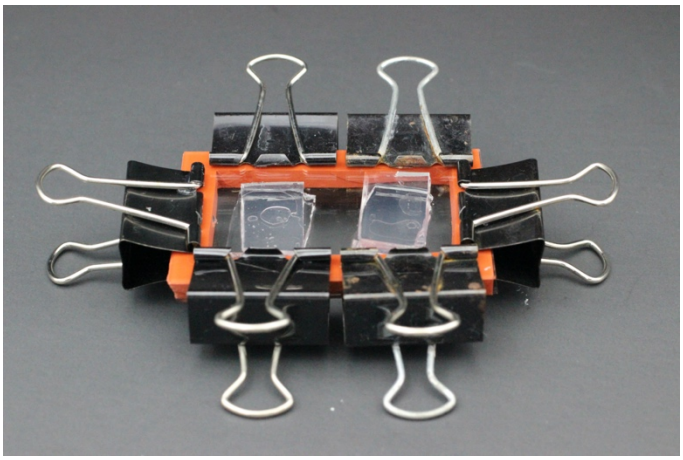

Figure 3: Square Connection of TN-APTAC and IPN-AAm hydrogel in a custom mold.

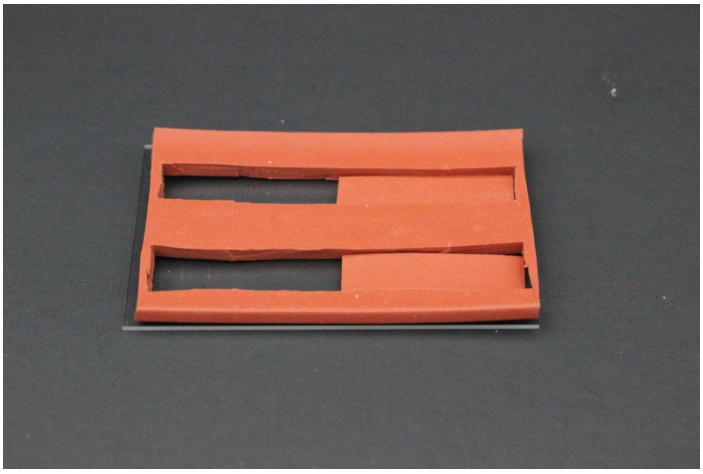

Figure 4: Interfacial Lap Shear Testing Custom Mold

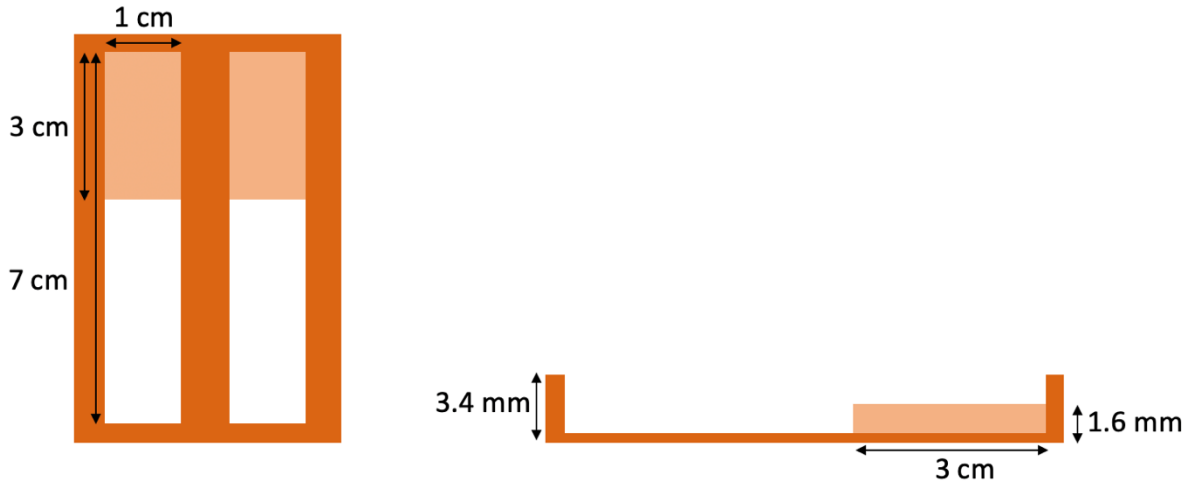

Figure 5: Interfacial Lap Shear Testing Custom Mold Diagram

The resulting connection from the interfacial lap shear custom mold can be seen in **Figure 6**, a 10 mm biopsy punch from the square connection can be seen in **Figure 7**, and a circular connection mimicking the intervertebral disc connection can be seen in **Figure 13**.

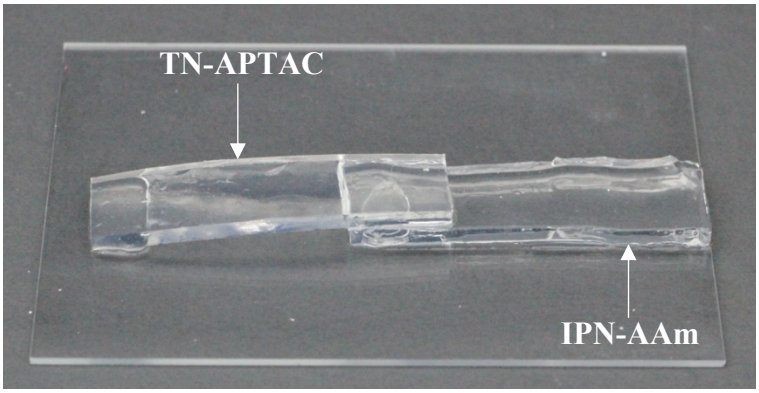

Figure 6: Connection from Interfacial Lap Shear Custom Mold

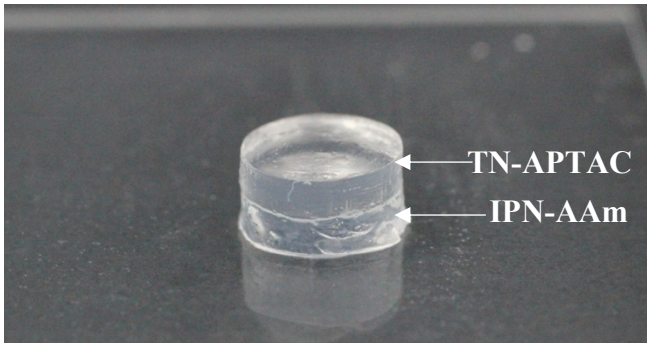

Figure 7: 10 mm biopsy punch from Square Connection Mold

2.3 Hydrogel Characterization

2.3.1 Volume Phase Transition Temperature

Differential scanning calorimetry (DSC, TA Instruments Q100) was used to determine the volume phase transition temperature (VPTT) of swollen IPN and TN hydrogels. A small square hydrogel specimen (~8-10 mg, cut with a razor blade) was blotted dry with a Kim Wipe and sealed in a hermetic pan for both samples. The samples were first cooled to 0 °C, then the temperature was ramped up to 65 °C and back down to 0 °C at a rate of 3 °C/min for two continuous cycles. The VPTT was characterized by the peak temperature of the endotherm (T_{max}) and the initial temperature at which the endothermic phase transition peak starts (T_o). Reported data are from the second heating cycle to ensure any thermal history has been erased and to simulate an arbitrary n^{th} heating cycle.

2.3.2 Equilibrium Water Content

The values for equilibrium water content were calculated as $[(W_s - W_d)/ W_s] \times 100$ where W_s was the swollen weight of the hydrogel or cartilage disc and W_d was the dry weight of the hydrogel disc after exposure to high vacuum at 60 °C overnight.

2.3.3 Unconfined Compression

Compressive mechanical properties, such as elastic modulus and strength were evaluated with an Instron 5944 at room temperature (RT). IPN-AAm hydrogels were punched into 3 discs with a 6 mm biopsy punch. TN-APTAC hydrogels were punched into 3 discs with a 6 mm biopsy punch. Each disc was blotted to remove surface water and then placed between the parallel plates with an initial preload force of 0.5 N. The samples were compressed at a strain rate of 1 mm/min until point of fracture. The elastic compressive modulus (E) was obtained from the slope of the linear portion of the stress−strain curve (0−5% strain). The ultimate compressive strength (σ_f) and the % strain at break (ε_f) were defined, at the point of fracture. Finally, the toughness (U_t) was obtained from the integration of the stress−strain curve.

2.3.4 Confined Shear

Shear strength was tested with the Instron 5944 at RT of the IPN-AAm control. A biopsy punch was used to create a 10 mm diameter cylinder and cut to a height of 8 mm for shear evaluation, seen in **Figure 8**. Specimens were evaluated in a custom confined shear set-up, where the lower arm remained stationary as an upper arm was displaced. The upper and lower arms were fabricated from ¼ inch thick aluminum bars (McMaster Carr) cut to 5 in x 1 in. Each mount had a 10 mm diameter hole, with the upper arm's hole machined to a depth of 4 mm and the lowers arm's cut through the bar. The arms were affixed in tension clamps on an Instron 5944 with a 2580-2kN load cell. To ensure alignment of the hole in each arm, a 10 mm diameter rod was placed between the holes and the arms were clamped together. Furthermore, to ensure vertical alignment, the arms were affixed to the tension clamps using a square and level. Insertion of the hydrogel into the fixture was done while the holes in each arm were aligned. Hydrogels were pushed through the hole in the lower arm until it was up against the back hole in

the upper arm. A silicone spacer (10 mm diameter x ~2.5 mm in length) was then pressed to fit into the lower arm to prevent movement of the hydrogel. Once inserted, the upper arm was displaced at a rate of 2 mm/min – applying a shear strain to the interface of the hydrogel – until failure.

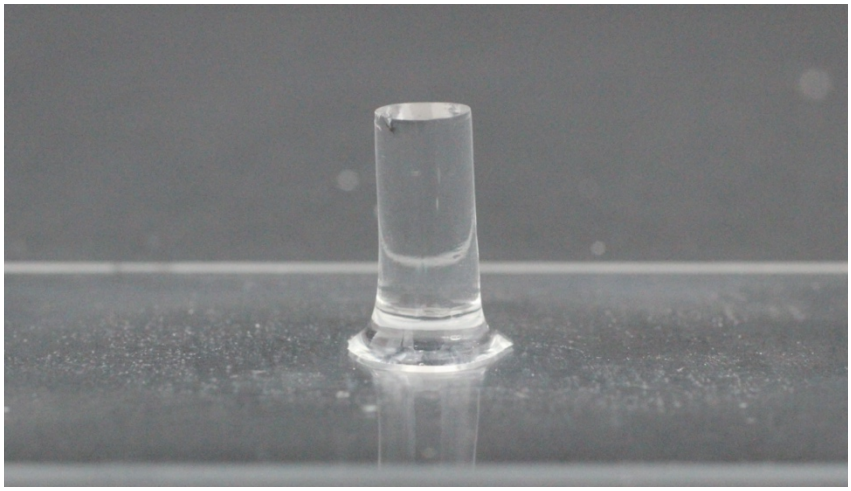

Figure 8: IPN-AAm hydrogel prepared for Interfacial Shear Testing

2.3.5 Interfacial Lap Shear

Interfacial shear strength at the site of connection was tested with the Instron 5944 at RT. The IPN-AAm and TN-APTAC hydrogel samples were connected in an interfacial lap shear custom mold, as seen in **Figure 6**. Specimens were evaluated in a modified custom lap shear set-up,[13] where the lower arm remained stationary as an upper arm was displaced. The upper and lower arms were fabricated from 1/8 inch thick aluminum bars (McMaster Carr) cut to 0.5 in x 2.75 in. Each bar had sandpaper attached to one side, to prevent displacement of the hydrogel. The arms were affixed in tension clamps on an Instron 5944 with a 2580-2kN load cell. Each arm and hydrogel were clamped with about 1 cm in the tension clamp. The set up of the test can be seen in **Figure 9**. Furthermore, to ensure vertical alignment, the arms were affixed to the

tension clamps using a square and level. Once inserted, the upper arm had a preload force of 0.05 N and was displaced at a rate of 10 mm/min – applying a shear strain to the interface of the hydrogel – until failure.

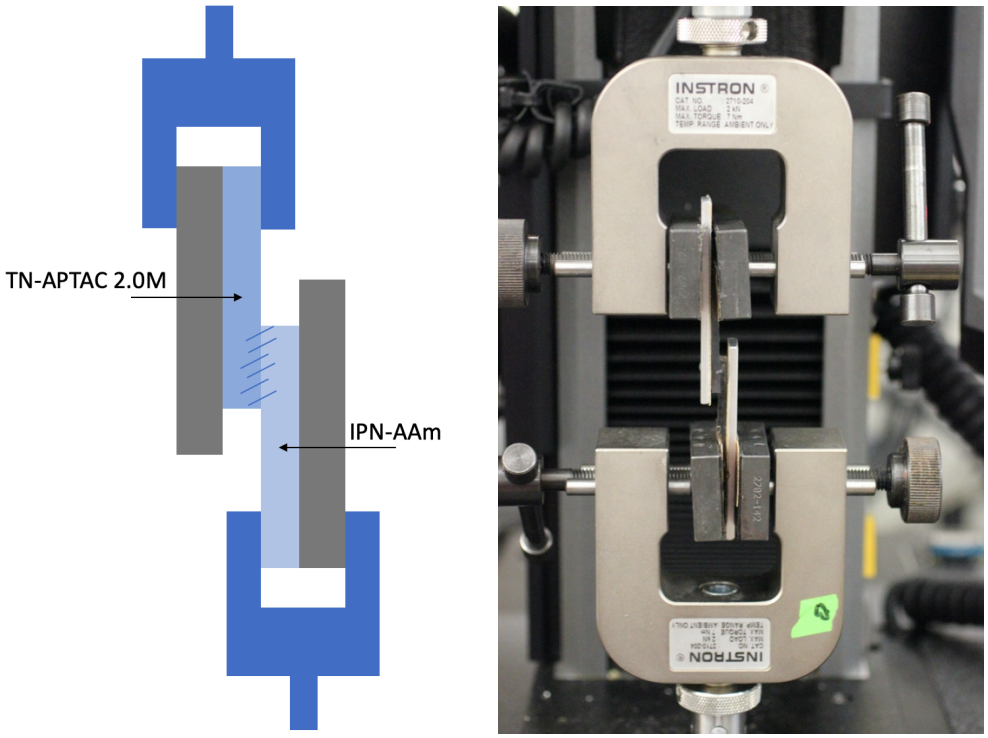

Figure 9: Interfacial Lap Shear Testing Set Up

2.3.6 Statistical Analysis

For shear and compression testing, statistical analysis values were compared using one-way ANOVA with Dunnett's correction to determine p-values by using GraphPad.

3. RESULTS AND DISCUSSION

3.1 Hydrogel Characterization

3.1.1 Volume Phase Transition Temperature

For testing, the IPN-AAm hydrogel was evaluated independently from the TN-APTAC hydrogel. The addition of the AAm second network to AMPS did not show a VPTT around 37 °C which is in the body's physiological range. A VPTT was not expected in the IPN-AAm hydrogel as there was no thermoresponsive polymer within the composition. The TN-APTAC hydrogel contains a thermoresponsive polymer, NIPAAm, within it is composition. Therefore, the VPTT needed to be evaluated to ensure there was no swelling or deswelling within the body. Testing showed that the VPTT was tuned out of the physiological temperature range. The DN-AAm 0%, showed a control of a non-tuned VPTT, which puts the implant at risk for failure within the body. The analysis of the VPTT is shown in **Figure 10**.

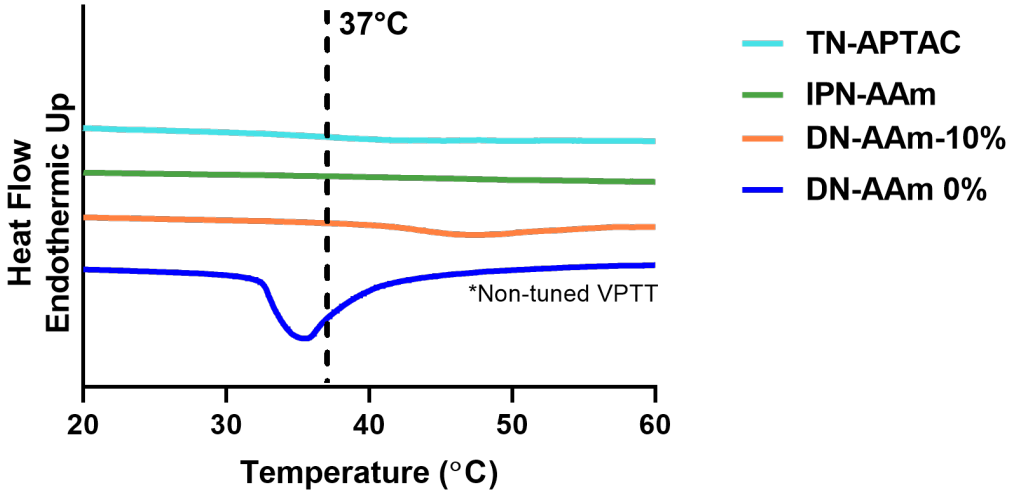

Figure 10: VPTT analysis at physiological body temperature.

3.1.2 Equilibrium Water Content

Water content was evaluated at ~97% for the IPN-AAm hydrogel which can be attributed to the hydrophilicity of the polymers and their loosely crosslinked nature. Mimicking the hydration of the inner region of the IVD is crucial to providing a disc that can improve joint kinematics within the spine. The water content of the NP region of the IVD ranges from 70-90%.[2] The TN-APTAC hydrogel in this experiment resulted in a water content of ~80% which is comparable to the water content of the AF region of the IVD which ranges from 60-80%.[2] The high-water content of both hydrogels is important for the implementation of the gel within the body.

3.1.3 Unconfined Compression

Studies have reported that hydrogels with high water contents are not able to produce high strength and stiffness. However, previous research in the Grunlan Lab has combated this issue by introducing a second, interpenetrating network to increase the strength and stiffness.[12] Notably, the IPN-AAm hydrogel displayed a modulus of ~0.14 MPa and a strength of ~1.8 MPa at a strain of ~75%. (**Figure 11**) A corresponding toughness of ~0.3 MJ/m^3 was shown. This was compared to the modulus of the NP region of the IVD which was evaluated of ~0.03 MPa.[2] Although, the IPN-AAm hydrogel is more robust than the NP region of the IVD, it is gelatinous in nature.

The incorporation of an APTAC third network into the previously studied DN-AAm 10% hydrogel[12] gives way for more robust mechanical properties. Compressive testing on the TN-APTAC hydrogels showed that altering the concentration of APTAC resulted in tunable mechanical properties. A modulus plateau of ~3 MPa was seen at a 2.0M concentration of APTAC, thus the 2.0M concentration was used within this study. The analysis of compressive

testing of the TN-APTAC series is shown in **Figure A1**. The modulus of ~3 MPa was evaluated with a one-way ANOVA compared to the DN-AAm 10% hydrogel and is shown in **Figure 11** to be statistically different with a p-value less than 0.05. The modulus of the AF region of the IVD was evaluated of ~2.3 MPa,[2] concluding that the TN-APTAC hydrogel is tissue mimetic. TN-APTAC strength of ~30 MPa was achieved at a strain of ~90%. This resulted in a corresponding toughness of ~4 MJ/m^3. Electrostatic interactions between the positive and negative networks allows for the ability to tune hydrogels for various load bearing applications within the body. These tests and statistics are shown to compare mechanical properties of the IPN-AAm and TN-APTAC hydrogels with the NP and AF regions of the IVD to conclude that they are tissue mimetic and can be used in further studies.

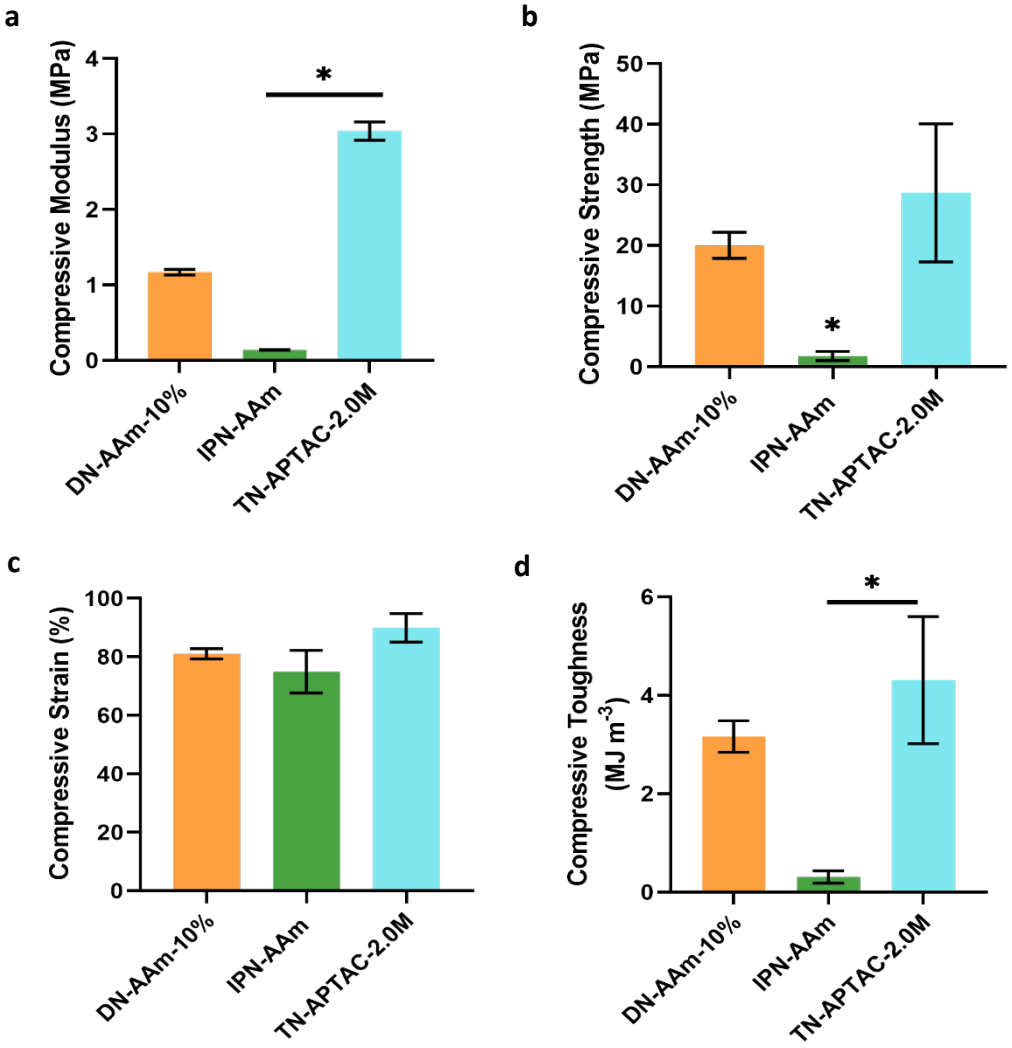

*Figure 11: Compressive mechanical properties of the IPN-AAm and TN-APTAC hydrogel alongside DN-AAm 10% comparing (a) modulus, (b) strength, (c) strain, and (d) toughness. All *'s represent a p-value less than 0.05, showing a statistical significance between the sample and DN-AAm 10%.*

3.1.4 Hydrogel Connection

The strength of connection between the IPN-AAm and TN-APTAC hydrogels was elucidated with mechanical testing, using shear tests of the IPN-AAm hydrogel as a control. Custom molds were fabricated and used to hold the hydrogel connections during attachment. Layered connections were used for interfacial shear testing and circular connections were used for proof of concept. In lap shear testing, failure was seen in the IPN-AAm hydrogel by a tension

break. The connection was still intact, shown in **Figure 12**, meaning that when failure occurred there was a cohesive break. This failure occurred during all shear tests at the site of connection. Max shear stress of the layered connection was reached at a stress of ~13 kPa before failure. The connection was compared to the IPN-AAm control which reached a shear stress of ~28 kPa before failure in the confined shear test. The connection shear stress is lower than the IPN-AAm control, however, the connection exceeded tensile properties of the IPN-AAm hydrogel. The tensile stress of ~48 kPa for the IPN-AAm hydrogel was achieved. This showed that the connection was robust and could be used for further applications.

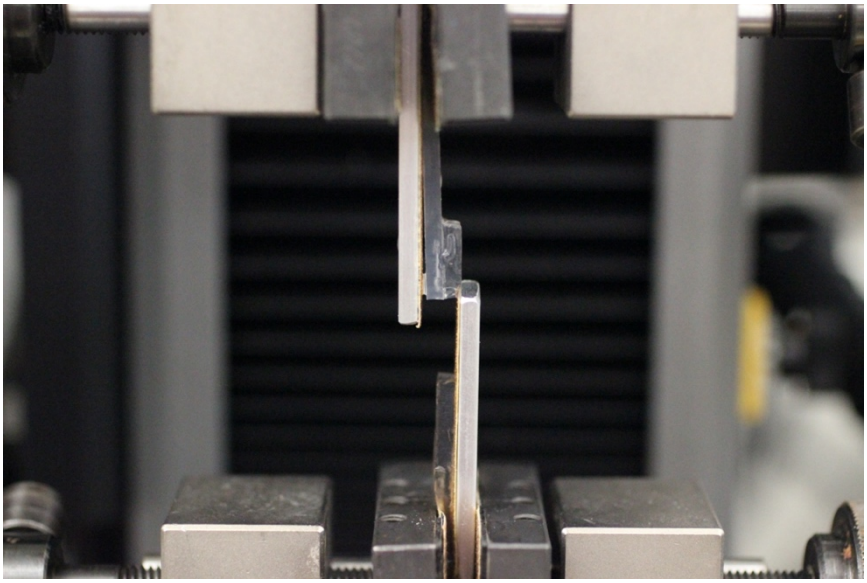

Figure 12: Cohesive failure of the IPN-AAm and TN-APTAC hydrogel connection during Interfacial Shear Testing. The IPN-AAm hydrogel broke in tension before the connection failed.

4. CONCLUSION

In summary, this study sought to fabricate tissue mimetic hydrogels as an alternative implant for IVD replacement by recapitulating the mechanical properties of the AF and NP as well as achieving adherence of the two. Through TN-APTAC hydrogel fabrication, a robust modulus (~3 MPa) was achieved and is comparable to the strong and stiff AF region of the IVD. Similar to the NP of the IVD, through IPN-AAm hydrogel fabrication, a gelatinous modulus (~0.14 MPa) and water content (97%) were achieved. These values were comparable to the gelatinous NP region of the IVD. Both hydrogels maintained desirable water contents and a VPTT not within the range of physiological body temperature, the latter ensuring dimensional stability due to lack of deswelling or swelling upon implantation.

The concept of self-healing hydrogels was researched and was shown to be successful for electrostatic hydrogel interactions.[14-16] This resulted in a charged induced adhesion which was used to create a single hydrogel with regional mechanical properties. This approach was utilized to merge the TN-APTAC and IPN-AAm hydrogels. Connection of the hydrogels to obtain regional mechanical properties was obtained by vertically stacking the hydrogels in between two glass slides and a custom mold. The interfacial shear testing of the connection was compared to the control of the IPN-AAm hydrogel, and was shown that there was a robust connection between the IPN-AAm and TN-APTAC hydrogels. The electrostatic interactions between the IPN-AAm and TN-APTAC hydrogels led to a charged induced adhesion. A circular mold, with an outer region of TN-APTAC and an inner region of IPN-AAm was fabricated to show proof of concept for IVD replication. (**Figure 13**) The charged induced adhesion that is implemented, shows to be a promising candidate for hydrogels with regional mechanical properties. This

technique can be used in further directions for various load bearing areas within the body and to reduce the risk of mechanical mismatch which is an issue with current IVD implants.

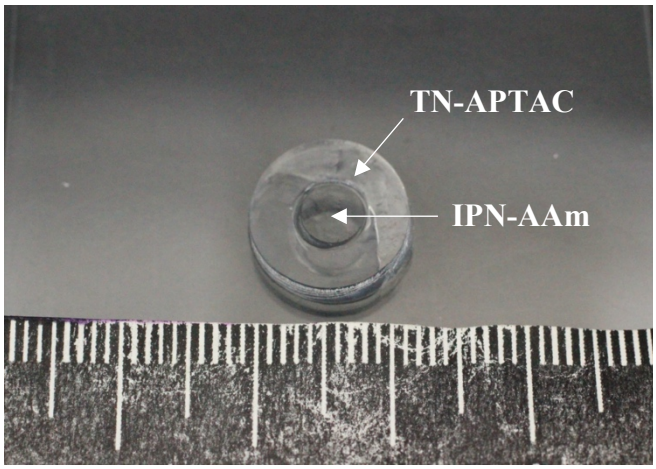

Figure 13: Circular IVD Connection

Further studies will be done with hydrogels with regional mechanical properties for use in IVD repair and replacement. An interest is to perform a double lap shear test to further evaluate the cohesion failure at the interfacial surface. Performing a double lap shear test will eliminate the tension failure of the IPN-AAm hydrogel, resulting in a quantitative interfacial shear stress. Providing an interfacial shear stress value, without tension failure, will confirm the charge induced adhesion connection approach and validate previous testing. To maintain the original mechanics of the spine, integration with the adjacent vertebrae and the TN-APTAC hydrogel will need to be successful. Thus, fabrication and design will be done on the TN-APTAC hydrogel to implement a surface porosity component that is needed for integration between the hydrogel and the surrounding tissues. Further testing can also be done to evaluate the replication of joint kinematics and the risk of mechanical mismatch once the hydrogel is integrated with the surrounding tissue. With a double lap shear test and the addition of porosity,

the concept of hydrogels with regional mechanical properties can be implemented into IVD replacement studies.

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APPENDIX

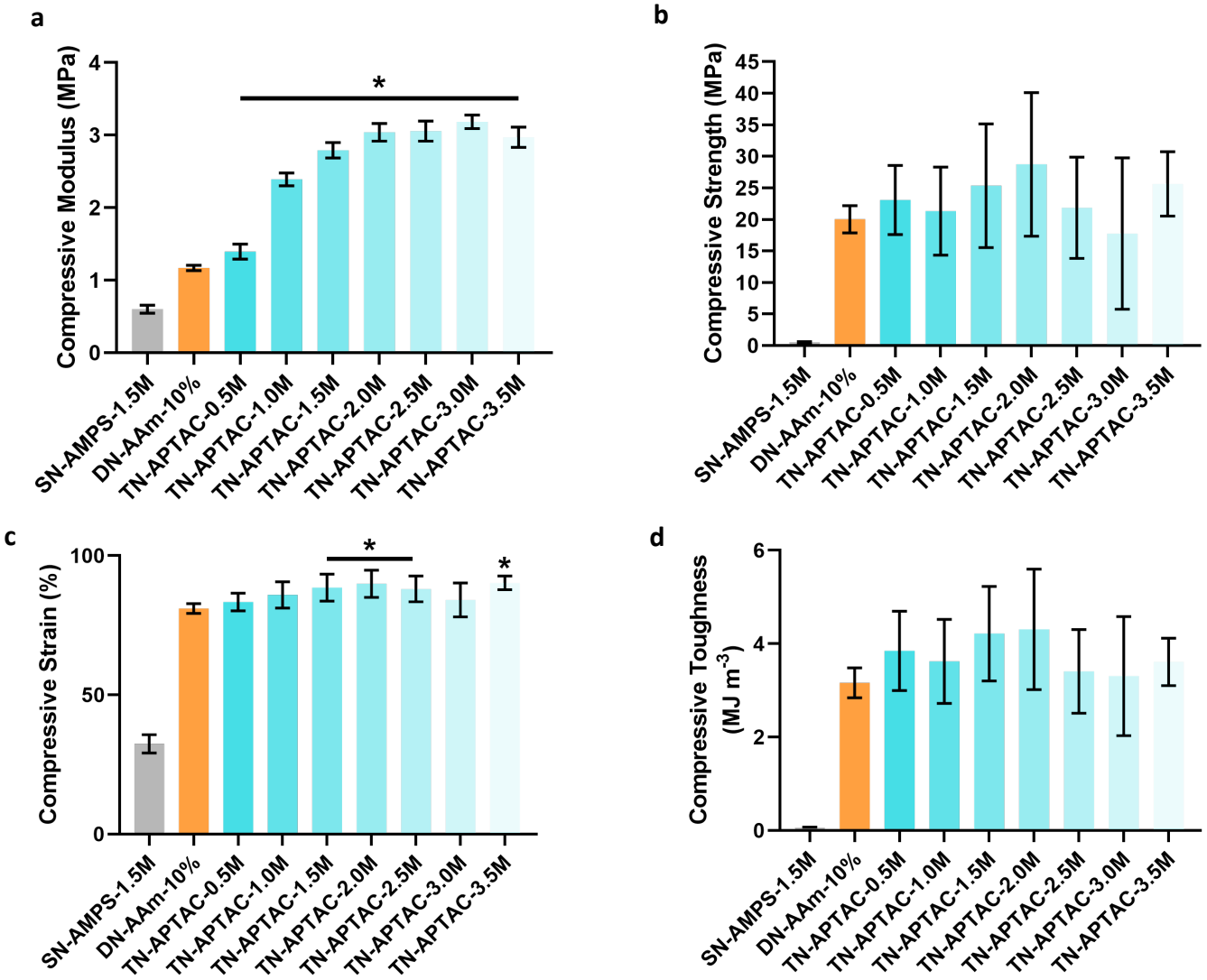

*Figure A 1: Compressive mechanical properties of the TN-APTAC hydrogel series alongside DN-AAm 10% and SN-AMPS 1.5M comparing (a) modulus, (b) strength, (c) strain, and (d) toughness. All *'s represent a p-value less than 0.05, showing a statistical significance between the sample and DN-AAm 10%.*