

Photopolymerization based 3D printing of thermoreponsive hydrogel precursors

by

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Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Statement of Contributions

The research work described in Chapter 3 has been published in *Journal of Colloid and Interface Science*. **2023**, 638, 882–892.

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I am the first author of both of these journal publications.

List of Publications

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Durable Poly(N-Isopropylacrylamide) Grafted PDMS Micropillared Surfaces for Temperature-Modulated Wetting. *Colloids Surf. Physicochem. Eng. Asp.* 2021, 610, 125901. <https://doi.org/10.1016/j.colsurfa.2020.125901>.

Organohydrogels with Cellulose Nanofibers Enhanced Supramolecular Interactions toward High Performance Self-Adhesive Sensing Pads. *Carbohydr. Polym.* 2023, 320, 121211. <https://doi.org/10.1016/j.carbpol.2023.121211>.

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Antimicrobial Polymeric Composites for High-Touch Surfaces in Healthcare Applications. *Curr. Opin. Biomed. Eng.* 2022, 22, 100395. <https://doi.org/10.1016/j.cobme.2022.100395>.

Abstract

Thermoresponsive hydrogels, which alter their shape in response to temperature changes, have crucial applications in wound dressings, sensors, and other biomedical contexts due to their responsive collapse behavior, high water content, and biocompatibility. Recent advancements in 3D printing have significantly improved the complexity and precision of hydrogel fabrication beyond traditional casting methods. Bioprinting is the most prevalent method for 3D printing hydrogels but is generally expensive, low-resolution, and restricted to academic settings. One alternative photopolymerization-based 3D printing offers greater accessibility and compatibility with synthetic hydrogel systems, capable of creating micrometer-sized features. However, the mechanical limitations of the printed objects and the temperature fluctuations during polymerization pose challenges for printing thermoresponsive hydrogels.

This thesis aims to develop 3D printing methods for thermoresponsive hydrogels using a printed organo-gel precursor, which allows for enhanced mechanical properties without triggering thermoresponsive behaviors during printing. This research targets applications in wound dressings and digital health, facilitating point-of-care fabrication. Mask stereolithography was investigated for creating thermoresponsive hydrogels from poly(N-isopropyl acrylamide) (PNIPAm) and poly(oligoethylene glycol) acrylate, incorporating bio-based polysaccharides as strengthening additives and ionic crosslinkers. The first experimental system used PNIPAm with poloxamers and a double network of sodium alginate, yielding a resin capable of printing precise structures and forming patient-specific wound dressings. This system displayed superior mechanical properties at room temperature and temperature-dependent drug release and adhesion. However, the use of dimethyl sulfoxide (DMSO) and NIPAm's neurotoxicity prompted a shift to

poly(oligoethylene glycol) acrylate-based resins. In the second system, quaternized chitosan/3-sulfopropyl acrylate (QCh:SPA) salts and 2-hydroxyethyl acrylate (HEA) was investigated for producing supramolecular hydrogels along with the use of a cellulose-derived solvent Cyrene to replace DMSO making the process greener. These hydrogels exhibited enhanced elasticity and feature resolution compared to other systems, also showing conductive properties due to ionic interactions. In the third system, the studies were conducted to investigate the incorporation ethylene glycol methyl ether acrylate with HEA and the use of octylamine-grafted cellulose nanofibrils (OA-CNF) and sodium alginate to develop core-shell microparticles. This enhanced the hydrogel's mechanical properties and exhibited broad LCST behavior, offering improved stability and laying the groundwork for future enhancements aimed at refining printability and tuning LCST responses.

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Dedication

I dedicate this dissertation to my wife Vicky Bauman for her endless love and support without which it would not be possible. Given that we met at the same lab as I completed my PhD, it seems so far the most important life events have all involved the Surface Science and Bionanomaterials lab. I also thank my family for all they have done to allow me to reach this point in my life. Their patience and guidance have allowed me to pursue my passion. Additionally, I would be remiss if I did not mention our 2 cats Kuro and Chichi. Kuro for his love and affection he has provided over the past 3 years and Chichi for her innocent joy after rescuing her from the forest during the end of my degree. Even though this marks the end of a chapter in my life, looking back over the past 5 years I have seen my family grow over time and I have no doubt it will continue to grow as I start a new chapter.

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List of Abbreviations

PNIPAm: Poly(N-isopropyl acrylamide)

LCST: Lower Critical Solution Temperature

POEGA: Poly(oligo ethylene glycol acrylate)

HEA: 2-Hydroxyethyl acrylate

mEGA: Ethylene glycol methyl ether acrylate

PHEMA: Poly(2-hydroxyethyl methacrylate)

PAAm: Polyacrylamide

GelMA: Gelatin Methacrylate

PEGDA: Poly(ethylene glycol) Diacrylate

QCh: Quaternized Chitosan

SPA: 3-Sulfopropyl acrylate

IPN: Interpenetrating Polymer Network

PEG: Polyethylene glycol

PEO: Poly(ethylene oxide)

PPO: Poly(propylene oxide)

TPO: Diphenyl(2,4,6-trimethyl benzoyl)phosphine oxide

SDS: Sodium dodecyl sulphate

PVP: Polyvinylpyrrolidone

CNF: Cellulose Nanofiber

DMSO: Dimethyl sulfoxide

NMP: N-methyl pyrrolidone

DMF: Dimethylformamide

QCh:Cl: Quaternized Chitosan Chloride

SEM: Scanning Electron Microscopy

ESEM: Environmental Scanning Electron Microscopy

FTIR: Fourier-transform infrared spectroscopy

AFM: Atomic Force Microscopy

NMR: Nuclear Magnetic Resonance

CNCs: Cellulose Nanocrystals

DI: Deionized

IPA: Isopropyl Alcohol

List of Symbols

C = concentration [M]

C_{loading} = concentration of loading solution [%wt]

C_t = Concentration at a specific time [%wt]

C_d = Cure depth [μm]

D_c = Critical Exposure Dose [mJ/cm^3]

DDA = Degree of deacetylation [%]

D_p = Penetration Depth [μm]

DS = degree of substitution [%]

E = Exposure [mW/cm^2]

E_c = Critical Exposure energy [mW/cm^2]

E_m = Energy of machine light source [mW/cm^2]

F_0 = semi-infinite bulk correction factor [dimensionless]

F_1 = Geometric correction factor [dimensionless]

I = Current [A]

M_{final} = dry mass of sample [g]

M_{initial} = initial mass of sample [g]

M_{load} = mass of rhodamine b loaded in the sample [g]

S = probe spacing [mm]

t = thickness [mm]

t_{Exp} = time of exposure [s]

V = Voltage [V]

V_{AgNO_3} = volume of silver nitrate solution used [L or mL]

W = mass [g or kg]

ρ = density [g/cm³]

∂ = conductivity [S/m]

d_l = Layer Depth [μm]

f = overcure [%]

Chapter 1: Introduction and Objective

1.1. Introduction

Over the past few decades, the field of smart materials has experienced remarkable advancements in capability and application. These materials have evolved to meet the complex demands of various industries, including soft robotics and biomedical engineering. In soft robotics, there is a growing need for systems that can dynamically modify properties such as adhesion, friction, and hydrophobicity. Concurrently, integrating smart materials into biological applications has opened new avenues for medical and pharmaceutical innovations. In these applications, materials can change properties in situ, such as releasing drugs or undergoing phase changes to facilitate controlled insertion and removal. Both applications rely heavily on smart materials to achieve their respective goals, highlighting the importance of continued research and development in this area. One medical area of specific interest is mechano-active wound dressings. By providing a mechanical stimulus, the hydrogels attempt to mimic the environment observed in early human embryonic wounds, where actin cords are used to pull the wound edges, closing the wound and preventing the formation of scar tissue. Subsequently, the healing mechanism for this was found to be mechanical signalling achieved through stretching, promoting cell proliferation and differentiation within the wound, preventing scar tissue formation. These stimuli-responsive properties are typically achieved with thermoresponsive polymers that collapse at a defined temperature slightly below skin temperature.

One material that has garnered significant attention in smart materials is poly(N-isopropyl acrylamide) (PNIPAm), a thermoresponsive homopolymer. PNIPAm has been extensively researched for its potential in creating thermoresponsive hydrogels. These hydrogels can undergo significant changes in their physical properties in response to temperature variations, making them

highly suitable for applications such as drug delivery systems, tissue engineering, and responsive coatings. However, PNIPAm poses significant challenges despite its promising properties due to the neurotoxicity of N-isopropyl acrylamide (NIPAm) and its oligomers. This has led researchers to investigate alternative polymers that can offer similar or superior performance without the associated health risks.

One alternative is poly(oligoethylene glycol acrylate) (POEGA), which has shown great promise as a thermoresponsive polymer. POEGA and similar polymers exhibit tunable lower critical solution temperature (LCST) behaviour, allowing them to transition from a hydrophilic to a hydrophobic state in response to temperature changes. This tunability makes them attractive candidates for developing thermoresponsive hydrogels. These hydrogels can form networks that respond to temperature stimuli, making them suitable for various biomedical and industrial applications.

However, thermoresponsive polymers alone often form poor hydrogel networks due to their interactions with water. Below the LCST, these polymers bind significant quantities of water, which prevents the formation of strong intermolecular forces between hydrophobic moieties. Above the LCST, the system becomes energetically favourable for interactions between hydrophobic moieties to occur, leading to water expulsion from the hydrogel. This transition also results in phase separation within the polymer, where polymer-rich regions force water into surrounding areas, causing the characteristic opacity change of thermoresponsive hydrogels. Due to their high-water content and reduced intermolecular forces, neat thermoresponsive hydrogels are quite fragile when fully swollen. Therefore, developing various crosslinking and strengthening additives to improve the mechanical properties at ambient temperatures is of significant interest.

In recent years, 3D printing of hydrogels has seen a surge of interest, with significant advancements in printing techniques and hydrogel design. The ability to create complex structures with precise control over geometry and material properties has revolutionized the field. Currently, many 3D printable hydrogels utilize bioprinters that rely on ionic, physical, or chemical crosslinking. A major disadvantage of this method is the limitations of extruder geometry and the viscoelastic properties of the ink. Furthermore, although extrusion-based 3D printers have become more affordable, extrusion-based bioprinters remain expensive and are limited by proprietary software. One technology that has seen significant adoption in the consumer market is stereolithography (SLA) based printing. Unlike FDM printers that melt extruded filament and bioprinters that rely on the viscoelastic properties of the material to maintain shape before secondary crosslinking, SLA 3D printers function through photopolymerization using a controlled light source. The advantages of SLA printers include high fidelity, greater control over printing parameters, and the relative simplicity of modifying g-code. All these properties are desirable for fabricating mechanoactive wound dressings, which are commonly produced through casting techniques. Due to the complex geometries of the human body, it is desirable to develop material systems for mechanoactive wound dressing that also facilitate drug loading and provide a platform for further digital health research.

Despite these advantages, SLA-based 3D printing faces significant challenges when printing thermoresponsive hydrogels. The primary limitation is the heat generated during photopolymerization, which can interfere with the biologically relevant transition temperatures of the polymers. This challenge necessitates the use of organo-gel intermediates during fabrication. Organo-gels, which do not exhibit an LCST without water, can be printed and subsequently hydrated to form thermoresponsive hydrogels. However, the use of organo-gels introduces

difficulties, particularly when incorporating bioderived polysaccharides. These natural polymers are generally only soluble in water, necessitating processing methods that maintain their dispersion within the organo-gel to form a cohesive network in the final hydrogel.

In the 3rd chapter of this thesis, we explore the development of 3D printable PNIPAm hydrogels. PNIPAm has been widely used in various methods of hydrogel printing, including two-photon polymerization and extrusion printing. Two-photon polymerization allows polymerization to occur within the monomer solution rather than at the interface, reducing stresses on the hydrogel and eliminating the need to detach layers during printing. On the other hand, extrusion printing utilizes reinforcements such as montmorillonite or sodium alginate to gelate after extrusion, providing strength to the matrix. Despite their effectiveness, both methods are prohibitively expensive and limit the scalability of fabrication. Recent advancements in SLA-based 3D printing, which offer improvements in resolution, speed, and cost, present an opportunity to develop PNIPAm-based resins for complex hydrogel structures with commercial scalability.

In this chapter, we developed a PNIPAm-based resin reinforced with sodium alginate and cellulose nanocrystals (CNCs), capable of being printed in an SLA-type printer with mechanical properties comparable to double-network PNIPAm hydrogels. To add functionality to the hydrogel, a thermoresponsive crosslinker was incorporated to introduce multiple LCST behaviours. By exploiting these separate LCSTs, the mechanical properties of the hydrogel could be improved at ambient temperature and facilitate high drug loading at refrigerator temperatures. Furthermore, the incorporation of tannic acid enabled thermoresponsive adhesion, allowing the hydrogel to adhere to the skin at body temperature and deliver drugs transdermally before cooling to room temperature to facilitate removal. This material demonstrated desirable properties as a wound dressing, although limitations related to calcium chloride and NIPAm prevent adoption as

a point-of-care solution. While calcium chloride successfully crosslinked the alginate network, it introduced cation mobility over time, leading to changes in mechanical properties as calcium ions diffused out of the network. Additionally, NIPAm's neurotoxicity remains a concern, and although 3D printing and post-curing can lead to high conversions of NIPAm to PNIPAm, it is preferable to utilize less-toxic acrylates.

These concerns led to the development of a novel 3D printable supramolecular hydrogel, which is the focus of the 4th chapter. Based on literature exploring POEGA as a thermoresponsive linear polymer, we began investigating its use as a replacement for PNIPAm. Initial work focused on developing an ionic crosslinker for photopolymerization-based printing of 2-hydroxyethyl acrylate, with the final goal to incorporate hydrophobic monomers to enable tunable thermoresponsive behaviour. Traditionally, photopolymerization-based 3D printing uses covalent crosslinkers to provide mechanical properties during printing. Although printing structures with low crosslink densities and high elasticity are possible, these systems are generally limited to low layer counts and aspect ratios. We hypothesized that a poly(ionic salt) could be used as a crosslinker for printing organo-gels without dissociating, enabling tensile strains previously only demonstrated in bioprinting, with superior fidelity to the 3D models.

Based on initial research where chitosan was freeze-dried in the presence of a strong organic acid to facilitate solubility in polar aprotic solvents, we explored whether quaternized chitosan would exhibit similar behaviour. GTMAC chitosan emerged as a potential cationic network due to its unique position as a biologically derived quaternized polysaccharide with commercial availability, enabling the production of the poly(ionic salt) in the required quantities for 3D printing. The acrylate group selected to form this salt was 3-sulfopropyl acrylate due to the high pKa of the sulfonic acid group, with the intent that the relative strength of the cationic and anionic

components would improve the mechanical properties of the final hydrogel. In addition to demonstrating the capability of this poly(ionic salt) to form a 3D printable organo-gel, we investigated the behaviour of this salt when the ratios of the ionic components were tuned. This revealed significant differences in mechanical properties and microstructure, providing proof of concept for further exploration of poly(ionic salts) consisting of polysaccharides and ionic acrylates.

In the 5th chapter of this thesis, we integrate the findings from the previous sections by incorporating sodium alginate and thermoresponsive behaviour into POEGA hydrogels. As previously mentioned, adding ionic polysaccharides to organic systems poses significant challenges. While we had demonstrated the capability of poly(ionic salts) to solubilize polysaccharides in polar aprotic solvents, the presence of two oppositely charged soluble ionic polysaccharides leads to immediate gelation¹. This gelation is undesirable for a flowable resin used in photopolymerization-based 3D printing. To address this, sodium alginate microparticles were loaded into the precursor resin to achieve a similar network to the first study. We investigated the production of these alginate particles using lecithin and hydrophobic functionalized cellulose nanofibers (CNFs) as emulsifiers. The hydrophobic CNFs acted as Pickering emulsifiers and stabilizers for the alginate emulsion. Adding isopropanol precipitated the alginate emulsion to form alginate microparticles, which were then loaded into the organo-gel precursor resin. This form was more desirable than commercial alginate, as the smaller particle size enables better resolution in the printed structures and facilitates even distribution in the matrix.

We hypothesized that upon converting the organo-gel to hydrogel, the alginate would diffuse into the network and increase the final hydrogel's porosity and the stability of the ionic crosslinking. Furthermore, the high molecular weight of both ionic polysaccharides within the

network would prevent ion migration from affecting the mechanical properties over time. Concurrently, the resin system developed in the second section was modified by adding methyl ethylene glycol acrylate (mEGA). This system was chosen based on literature demonstrating the development of linear thermoresponsive POEGA chains. By incorporating a hydrophobic portion with an LCST below freezing, the LCST of the resulting copolymer could be decreased to a usable temperature. Additionally, mEGA was selected for its ability to achieve a broad range of LCSTs at low mass compositions. This was crucial for maintaining the solubility of quaternized chitosan in the solution.

Initially, we investigated the effect of different mEGA additions on the LCST of the resulting hydrogel, quantified by both transmittance and swelling. This was followed by characterizing the mechanical and structural properties of the thermoresponsive hydrogel loaded with alginate microparticles. Finally, the developed resin was used to 3D print organo-gels that were subsequently characterized. The thermoresponsive behavior of the final hydrogels indicates the need for further improvements, the holistic system demonstrated excellent elasticity and shows promise for wound dressing.

In the following Chapter 2, the principles of 3D printing and its applications for hydrogels will be explained. The current literature on the development of 3D printable hydrogels will also be discussed alongside a comprehensive review of different natural-synthetic networks for use in 3D printing, along with state-of-the-art thermoresponsive hydrogel systems for 3D printing. Chapters 3-5 present the investigation of 3D printing organo-gel precursors for thermoresponsive hydrogels along the evolution of the system. The dissertation ends with Chapter 6, with main concluding remarks based on the results obtained throughout the study, the main contributions of the thesis and, recommendations for future work and observations on possible improvements.

Chapter 2: Literature Review

The chapter aims to provide a review of related literature to provide contexts for the research subject of this thesis, including the introduction of different types of 3D printers and their applications, the use of 3D printing for production of hydrogel-based materials, a general overview of the main components used for UV polymerization, a comprehensive review of bioderived materials used to form double network hydrogels. The final section of the literature review focuses on the incorporation of thermoresponsive behaviour into natural, synthetic double network hydrogels and methods to achieve them.

2.1. Additive Manufacturing and 3D Printing

3D printing, a common term for the field of additive manufacturing, is a versatile tool for one-off production and the creation of geometries impossible by traditional subtractive manufacturing. It has been considered the next great revolution in production akin to the Industrial Revolution². 3D printing allows for the production of objects from computer-aided design (CAD) files that previously would have been prohibitively expensive or impossible to machine. 3D printing has steadily evolved over the past 35 years to the point where extrusion and stereolithography-based systems have become commonplace in the hobbyist marketplace. The first demonstration of a 3D printing technique was developed by Chuck Hull in 1986³ as a stereolithography-based printer, and fused deposition modelling printers have become the most affordable and common printers seen on the market today. With new developments, printers are capable of printing a vast array of materials, including metals, ceramics, hydrogels, plastics, concrete, etc., with faster lead times and lower wastage than conventional subtractive manufacturing⁴. This has led to 3D printing being used extensively in prototyping applications, allowing design flaws to be fixed earlier in the development cycle by iterating multiple times a day⁵. While 3D-printed metal parts are typically

prohibitively expensive for mass-market production runs, they are invaluable for prototyping and small production runs where the cost of tooling cannot be spread over many parts⁶.

In recent years, particularly during the COVID-19 pandemic, we have seen an explosion in the use of 3D printing for medical and healthcare-related applications. Demand quickly outpaced the supply of objects such as face shields, ventilators, masks, and isolation wards. Through crowdsourcing, many hobbyists with 3D printers started producing face shields that were distributed to healthcare workers without a reliable source. 3D-printed valves allowed the conversion of continuous positive airway pressure machines to substitute ventilators for less severe cases. Face scanning, along with 3D modelling, allows for the production of ergonomic masks that could be designed to accept replaceable filters. 3D printing was even used to fabricate temporary dwellings for patients, which could be assembled offsite and transported to where they were needed most⁷. Other developments over the past year included an open-source high-temperature extrusion printer capable of printing polyether ketone (PEEK) and polyetherimide (PEI) for under \$1000 USD, less than 1/10th the cost of comparable commercial printers. These engineered polymers are capable of withstanding extreme chemical conditions and can be sterilized in the same autoclaves used for medical tools in hospitals, allowing the printed medical devices to be reused multiple times⁸.

3D printers vary significantly in form and function. The main categories include selective laser sintering, stereolithography, 2-photon polymerization, extrusion and inkjet printing⁴.

2.1.1. Selective laser sintering

Selective laser sintering (SLS) and selective laser melting (SLM) function by depositing a thin layer of powder that is selectively fused or melted by a high-powered laser. This process is

repeated for every layer of the part until the final layer is completed and the part is removed from the bed of powder. It is capable of printing both metal and thermoplastic parts. SLS benefits from the support of the powder bed, allowing parts to be printed without the need for external support. One drawback of this technique is the relatively rough surface finish, which requires additional surface finishing⁶.

2.1.2. Stereolithography

Stereolithography (SLA) was the original 3D printing technique developed in 1986. It functions similarly to SLS, except SLA utilizes a vat of photopolymerizable resin and a laser UV light source. The UV light is controlled by a scanner to polymerize an area on the surface of the resin. The printing platform is then lowered to expose a fresh layer of resin that is then polymerized into the next layer. This process repeats until a final part is achieved⁹. There have been many improvements to this initial design, the most notable one being the inversion of the design. Modern SLA printers have a platform that raises out of the resin tank and a flexible fluorinated polyethylene (FEP) film at the bottom of the tank through which the laser can pass through¹⁰. This change allows for a greater Z-height with a lower volume of resin in the tank. Similar systems such as digital light processing (DLP) and mask SLA (mSLA) are offshoots of the SLA design. DLP and mSLA printers replace the laser light source in a SLA printer with a digital projector or an LCD mask and UV light source, respectively. These designs are significantly cheaper to produce but are limited by the resolution of the projector or LCD mask. As build volume is increased in the X and Y directions, the resolution is correspondingly reduced because there is a limit to the total number of voxels per layer. This limitation leads to newer-version SLA printers achieving more uniform surfaces and eases scale-up with the resolution only limited by the laser spot size. However, the constant resolution comes at the cost of longer build times for larger parts. DLP and mSLA printers

cure the entire layer at once, leading to consistent layer speeds¹¹. But SLA cures only a small area at a time. As the volume of the part increases, so too does the print time. One recent development in SLA-based printers comes from continuous liquid interface printers (CLIP), where the polymerization takes place above an oxygen-permeable film, creating a “dead zone” where polymerization cannot occur. This allows for the continuous printing of the part, removing the need to separate each layer from the FEP film. CLIP printers are significantly faster than typical SLA, mSLA, and DLP printers while also providing a more seamless transition between layers¹².

2.1.3. Two-photon polymerization

Differing from SLA printers, two-photon polymerization utilizes 800nm femtosecond laser pulses. When 2 of these photons are absorbed by a photoinitiator in a small volume of photopolymerizable resin, they act as a 400nm UV light source, triggering polymerization. This allows for objects to be printed within a resin vat at the focal point of the light source rather than the top or bottom¹¹. Two-photon polymerization printers are very capable of printing hydrogels. Still, they can be limited by the opacity of the resin, making it unsuitable for resins containing high solid loading or nanofillers.

2.1.4. Extrusion printing

Extrusion printing is the most common type of 3D printer available today, with fused deposition modelling (FDM) printers being the most common type of extrusion printer. FDM printers force filament through a heated tip, which is extruded as a layer on a heated bed. Stepper motors control the movement of XYZ, with the most common control scheme having the bed move on the X axis and the print head moving on the Y and Z axes. Recently, other layouts have become popular, particularly core XY printers, where the bed move in the Z dimension with the print head moving in the XY dimension⁸. FDM is limited to print plastics. 3D plotting printer is another type

of extrusion printer capable of printing other viscous materials. 3D plotting printers function by moving the printhead in all three dimensions and utilizing pneumatic pressure, a piston, or a screw to feed the material through the print head. Unlike FDM printers, 3D plotters do not generally heat the printhead but use other modifications such as ultrasonic actuators to temporarily lower the viscosity of the solution, allowing printing of viscous liquids with high solid loading¹³. 3D plotting printers are commonly used to print hydrogels with secondary networks, such as alginate, due to the high viscosity of alginate.

2.1.5. Inkjet printing

Unlike other 3D printing technologies, inkjet printing is the most capable of complex multi-material printing. Inkjet printing is classified into two categories: continuous inkjet and drop-on-demand jetting systems. Continuous inkjet systems feature a constant stream of ink exiting the nozzle under pressure, generating a jet. This jet is broken into individual droplets, and electrical signals control the direction of movement. Drop-on-demand jetting systems are modulated by a heater or piezoelectric actuator that pulses to dispense a predefined volume of ink on the substrate. This technique is commonly used to manufacture complex cell scaffolds due to the achievable resolution of 50~500µm and multi-material capability. It allows for scaffolds to be produced with very controlled heterogeneity⁴.

2.2. 3D Printing hydrogels

With the development of many 3D printing methods, almost any material has multiple methods to achieve 3D printability. One such material where 3D-printing represents significant potential are hydrogels. A hydrogel consists mainly of water and a polymeric structure that is generally water-soluble and crosslinked through physical, ionic or covalent methods¹⁴. Before the

introduction of 3D printing, hydrogels were generally produced through casting and as such the resultant materials were significantly limited in the complexity of the shape they could achieve.

Three different methods of 3D printing have been utilized for the 3D printing of hydrogels, including extrusion, 2-photon polymerization and stereolithography. By far, the most common method is with an extrusion-style printer referred to as a bioprinter. These printers typically print through a replaceable nozzle consisting of a flat-end syringe tip and are actuated pneumatically or with a syringe pump. Through the use of precise motors, the hardware is capable of 1 μ m step resolution; the viscoelastic properties of the ink prevent final structures from achieving such resolution, limiting the feature size to greater than 100 μ m^{15,16}. In addition to the fidelity limitations of such 3D printers, nozzle clogging and the pneumatic systems typically used lead to inconsistent extrusion during printing¹⁶. As such, the development of other more precise printing techniques is of interest. One such alternative is vat polymerization-based systems, including stereolithography and 2-photon polymerization. The polymer systems used for stereolithography and 2-photon polymerization are quite similar, with 2-photon polymerization capable of achieving significantly finer resolution and can utilize different initiation schemes such as cationic polymerization in addition to free radical polymerization¹⁷. 2-photon polymerization has significant advantages when compared to stereolithography-based techniques, particularly it does not need a separation step from the print surface, but its resolution is limited in build volume, feature height and the prohibitive cost of the printers¹⁸. SLA-based printers are comparatively low cost, with consumer printers costing as little as \$350¹⁹. This low cost of purchase along with the relatively high resolution achievable makes this technology desirable for scaling 3D printable systems and integration into specialized applications where customization is necessary or desirable.

2.2.1. UV Curable hydrogels

UV-curable hydrogels have received significant research effort because of the speed of the reaction and the controllability of UV polymerization. Typically, UV-curable systems use free radical chain polymerization. Other methods of UV polymerization include photo click systems such as Thiol–Norbornene hydrogels²⁰. Although such materials provide novel methods of crosslinking, they are material intensive, requiring carbodiimide chemistry to functionalize materials with norbornene groups. The other limitation of this mechanism is the resolution when stereolithography techniques are used. Since the polymerization does not require an oxygen-depleted region, UV light can easily overexpose regions, reducing the specificity of the polymerization. The following sections provide a broad overview of different components used for UV photopolymerization-based hydrogels, focusing on monomers, photoinitiators and secondary networks.

2.2.2. Monomers

Typical monomers used in UV curable systems have an acrylate group with various different functional groups. For this research, the main thermoresponsive monomers used were N-isopropyl acrylamide (NIPAM), 2-hydroxyethyl acrylate (HEA) and ethylene glycol methyl ether acrylate (mEGA). NIPAM, once polymerized, is thermoresponsive, having a lower critical solution temperature of 32°C. Across this LCST, the hydrogen bonding along the backbone switches from water to itself, leading to PNIPAM losing solubility in water above 32°C^{21–24}. In a crosslinked hydrogel, this leads to the polymer swelling when below 32°C and expelling excess water above 32°C, which are referred to as the swollen and collapsed states, respectively. HEA and mEGA, while not thermoresponsive alone, can be copolymerized to form thermoresponsive random or block copolymers. However, the lack of terminal isopropyl groups reduces the relative strength of

hydrophobic interactions when compared to PNIPAm. Alternately, due to the copolymeric nature they enable LCST tuning; by changing the ratio between HEA and mEGA or tuning the hydrophobic block length in the case of block copolymers²⁵. Other common monomers used in hydrogels are hydroxyethyl methacrylate (HEMA) and acrylamide. PHEMA hydrogels are most commonly found in disposable contact lenses but are also commonly used as a cell scaffold due to their biocompatibility^{26–28}. PAAm hydrogels are also commonly used due to their biocompatibility^{29–31}. Polyethylene glycol hydrogels are somewhat unique as they are composed almost entirely of the crosslinker polyethylene glycol diacrylate. While crosslinkers are typically added to bridge linear chains of polymers, this wide class of hydrogels has seen significant research interest as a biocompatible synthetic hydrogel³² due to PEG's high hydrophilicity and stability in aqueous systems. In addition to being highly water-soluble, PEG is also highly soluble in many organic solvents, allowing applications in many systems³³. One issue is that PEG can only be partially metabolized; due to this, the whole polymer chain must be cleared by the body. PEG chains with a MW less than 30kDa are removed by the kidneys, while the liver clears PEG chains greater than 30kDa³⁴. At molecular weights under 1000, the chemical is liquid at room temperature, with a melting point that increases with increasing MWs. This allows for tailoring the melting and glass transition points of the polymers and hydrogel properties³⁵.

Apart from these main monomers that this research focuses on, many biomolecules can be modified with acrylate groups to allow the formation of hydrogels such as GelMA^{36–39}, Hyaluronic acid methacrylate^{40–42}, Sodium alginate methacrylate^{43–45}, etc. These materials are more commonly used in bioprinting with a UV curing step, unfortunately due to the high viscosity of such materials, applications in stereolithography based printing are limited to low degrees of loading to facilitate flowability of the prepolymer resin.

2.2.3. UV initiators

Photoinitiators can be broadly classified as type 1 and type 2 initiators (Fig. 2.1). Type 1 photoinitiators include peroxides, peresters, iminosulphones, or ketones. Free radical polymerization is initiated through the fragmentation of the initiator by light. This occurs when O-O, S-S, S-N or C-C at α or β is bound to a carbonyl group. Unlike type 1 initiators, type 2 initiators do not fragment. When excited by the appropriate photon, the initiator generates a radical through hydrogen abstraction. Type 2 initiators are useful for grafting as they can generate radicals on the surface of a material. Type 2 initiators are typically slower and less efficient as they are a 2-molecule process. For this reason, most initiators used in resin-based 3D printing utilize type 1 initiators as they are more active at higher wavelengths and allow for faster polymerization⁴⁶.

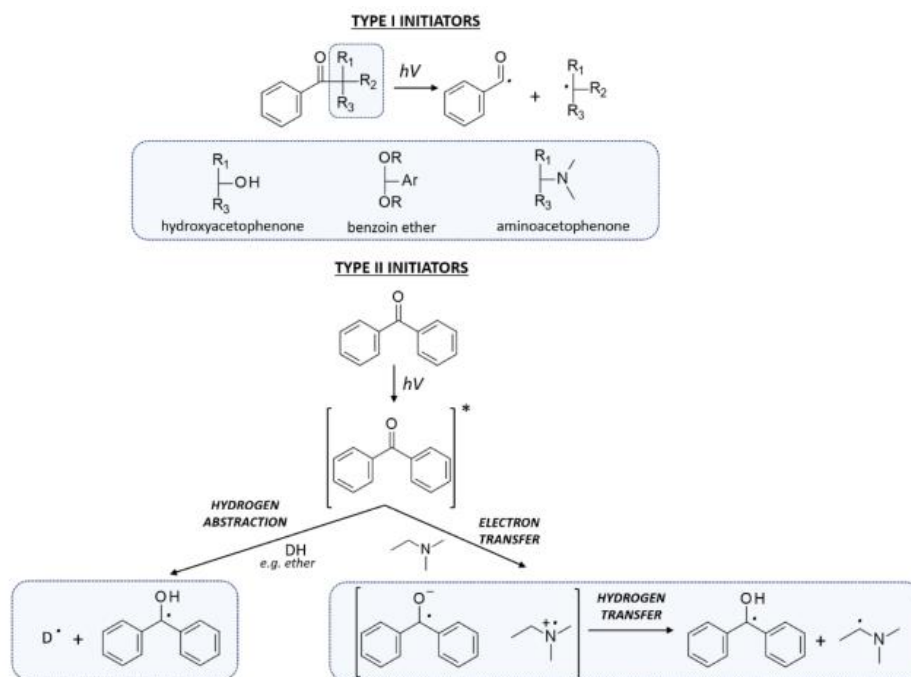


Figure 2.1 Photoinitiation process using type 1 or type 2 initiators⁴⁶ (Reproduced with permission from MDPI)

The majority of UV initiators that exist for 3D printing are insoluble in water, precluding their use in UV-curable hydrogels. This insolubility is due to the aromatic carbonyl group that

fragments from the main chemical during radical generation. This aromatic group is highly non-polar, and as such, photoinitiators require intentional design to incorporate hydrophilic groups to facilitate water solubility. While these initiators are capable of acrylate polymerization, printing hydrogels is difficult as the initiator must be homogeneously dispersed in the case of nanoparticles or soluble in the solvent in which the polymerization occurs. For this reason, water-soluble photoinitiators have seen significant development in recent years, with the most common involving the modification of Diphenyl(2,4,6-trimethyl benzoyl)phosphine oxide (TPO), a type 1 initiator. The most successful of these involve the addition of a lithium-ion to improve polar solubility⁴⁷ at the cost of efficacy or the formation of TPO nanoparticles with sodium dodecyl sulphate (SDS) and poly(vinyl pyrrolidone) (PVP) 40k MW⁴⁸. Other commercial water soluble photoinitiators include 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone, also known as Irgacure 2959. Irgacure 2959 demonstrates a low solubility of 7.6g/L in water, and a slow radical generation rate limits its use in resin-based 3D printing due to the greatly extended curing times when compared to lithium TPO or TPO nanoparticles.

2.2.4. Double interpenetrating network (IPN) hydrogels

IPN hydrogels are desirable; they have significantly improved mechanical properties compared with single network (SN) hydrogels. IPN hydrogels are composed of various networks, which can be broadly classified as Natural-Natural, Synthetic-Natural and Synthetic-Synthetic⁴. Typically, one network is stiff and brittle, allowing for the dissipation of energy by fracturing the network. The other network is capable of large deformations, holding the hydrogel together under strain⁴⁹.

Natural networks refer to a class of biologically derived polymers, including alginate^{43,44,49–52}, carrageenan^{53–55}, cellulose^{56–59}, chitosan^{60–63}, heparin⁴, hyaluronic acid^{41,64,65}, polypeptide/protein,

gelatin^{37,37,66} and silk⁶⁷. These networks can be crosslinked in various ways; some examples include alginate networks that can be ionically crosslinked in the presence of di and tri valent ions such as Fe^{3+} , Ca^{2+} and Al^{3+} ⁶⁸, hyaluronic acid methacrylate networks that can be crosslinked with typical acrylate crosslinkers, and carrageen networks that form hydrogels when a hot solution is cooled in the presence of salts^{4,22}. Synthetic networks refer to the synthesis method for the hydrogel network. These networks include homopolymer crosslinked networks that consist of a single hydrophilic monomer or a copolymer formed from the reaction of 2 or more monomers, with at least one being hydrophilic. These networks can consist of various different monomers including but not limited to PEGDA, POEGA/POEGMA, HEMA, AAm, Acrylic acid, NIPAm and 2-acrylamido-2-methylpropanesulfonic acid^{4,22}.

2.3. Natural Polysaccharides

2.3.1. Sodium Alginate Network

Alginate is a water-soluble linear polysaccharide derived from brown algae or bacteria⁵⁰. It is a specific linear polysaccharide containing carboxylate active sites, but its properties can vary significantly from source to source. This behaviour is due to the formation of 3 main blocks within the polymer with varying concentrations of mannuronic acid (M Unit) and guluronic acid (G unit), forming G unit-rich blocks, M unit-rich blocks and blocks consisting of alternating G and M units. In general, it is regarded as a biocompatible and food-safe material, forming a gel network under acidic conditions or in the presence of divalent or greater cations⁶⁹. In acidic conditions below pH 3, the carboxylic acid groups protonate to form alginic acid and intermolecular hydrogen bonds form, leading to the precipitation of a fibrous gel network⁷⁰. Conversely, in the presence of cations capable of accepting 2 or more ionic interactions, a network is formed by ionic crosslinking, where 2 or more alginate chains interact with a single cation. Alginate gels are most commonly formed

with various different divalent and trivalent cations, including Ca^{2+} , Mg^{2+} , Sr^{2+} , Ba^{2+} or Al^{3+} ^{71,72}. Ionic crosslinking is achieved by regions containing sequential G blocks, where the local region forms a ${}^1\text{C}_4$ ring conformation allowing a multivalent ion to reside within the cage and interact with 2 separate chains. It is for this reason that alginate gels demonstrate significantly different mechanical properties when crosslinked with different cations⁷³.

In alginate systems, calcium is widely used for ionic crosslinking due to the size of the cation and the relative biocompatibility of calcium ions⁶⁸. This leads to the formation of a 3-dimensional lattice of guluronic acids in different chains and calcium ions, referred to as an egg box structure⁷⁴. Furthermore, due high energy required for this conformation to rotate, the guluronic acid is the major contributor to ionic crosslinking seen in alginate hydrogels. While alginate hydrogels are desirable due to their biocompatibility and simplicity of crosslinking, they are limited by the mechanism of crosslinking. Due to the size of cations used for crosslinking, over time these ions diffuse into the surrounding medium resulting in a reduction in mechanical properties over time⁵⁰.

Alginate hydrogels are frequently used to make hydrogels with the casting method; techniques to incorporate them into 3D printing are varied. Due to the solution's viscoelastic properties of the solution one common approach is the extrusion of an alginate solution concurrently with calcium chloride. Examples of this include Faulkner-Jones et al. where sodium alginate solution and calcium chloride were dispensed through independent print heads⁷⁵, Freeman et al. where various calcium salts were mixed with alginate before bioprinting⁷⁶ and Gao et al. where the alginate is extruded into a CaCl_2 bath⁷⁷. Although extrusion is a useful method of 3D printing alginates, it is not able to achieve the same resolutions as photopolymerization-based

techniques. A second network or modification of the alginate with acrylate groups is required to achieve photopolymerizable alginate hydrogels.

In terms of double-network hydrogels, different synthetic networks have been reported, such as acrylic acid⁷⁸, 2-hydroxyethyl acrylate, polyethylene glycol diacrylate⁷⁸, n,n methylene bis acrylamide⁷⁹ and acrylamide. Alternately, if an alginate is the sole component of the network, the alginate itself can be directly acrylated^{43,80–82} and polymerized. While acrylate-based crosslinking is the most common method of photo-crosslinking alginate, other more exotic solutions exist. One such mechanism involves using a photo acid to generate cations in situ from light, as demonstrated by Valentin et al.⁸³, utilizing sodium alginate, graphene oxide and diphenyliodonium nitrate as the photoacid generator. This method is novel but only achieves millimeter-scale resolution with poor mechanical properties (40-60 kPa ultimate compressive strength).

2.3.2. Carrageen

Like alginate, carrageenan is a linear water-soluble polysaccharide extracted from red algae⁸⁴. Where the two polysaccharides differ is in the active ionic site; alginate has a carboxylate group, and carrageenan contains a sulphate group. Carrageenan can broadly be classified into 3 major isomers: κ -, ι - and λ -carrageenan. The difference in the isomers relates to the position and number of the ester sulphate groups on the repeating units. Both κ -carrageenan and ι -carrageenan are composed of 3,6-anhydro-d-galactose and sulphated d-galactose with the gelation properties dependent on 3,6-anhydrogalactose. λ -carrageenan, unlike the other 2 is solely composed of sulphated d-galactose⁸⁵. The carrageenan hydrogels can form in the presence of various salts upon cooling a heated solution. These thermoreversible hydrogels have mechanical properties dependent on the cation concentration, cation type and carrageenan concentration. Specifically, for κ -carrageenan, it is gelled most strongly by $K^+ > Ca^{2+} \gg Na^+$. K^+ forms rigid and elastic

hydrogel, with Ca^{2+} forming a stiff and brittle hydrogel. Conversely, ι-carrageenan gels most strongly to Ca^{2+} , followed by K^{+} and finally Na^{+} . Of the different isomers, κ-carrageenan demonstrates the strongest hydrogels⁵⁴. Unfortunately, even though carrageenan demonstrates useful properties as a network for hydrogels with sulphate groups providing a superior strength of ionic interaction, thermal gelation has limited its use in UV-based 3D printing systems. Most commonly, it has seen use in extrusion-based 3D printing systems where a hot end is in use as demonstrated by Dianeze et al.⁵³, where a hydrogel solely composed of κ-Carrageenan was printed with millimeter scale accuracy. Another group recently demonstrated the use of methacrylated Carrageenan as a supplement to GelMa for extrusion-based 3D printing, reaching ultimate compressive strengths of 250 kPa with 100 μm resolution⁸⁶. While κ-Carrageenan has seen use as a possible 3D printable hydrogel, it has been limited by the low structural integrity, shape fidelity, and mechanical strength⁵⁵.

2.3.3. Sodium Hyaluronic

Hyaluronic acid (HA) is a water-soluble non-sulphated glycosaminoglycan, which can be found in almost every vertebrate tissue^{87,88}. It is commonly used in cosmetic products to improve the hydration of the skin due to its propensity to bind water molecules⁸⁹. This biopolymer is degradable through free radicals or the hyaluronidase family of enzymes, at this point, it undergoes endocytosis and lysosomal digestion. In addition to its biocompatible and biodegradable properties, HA plays an active role in various biological processes, including cell adhesion, migration, proliferation, differentiation, and angiogenesis⁹⁰. HA has commonly been utilized in the field of extrusion bioprinting^{65,91–94}, with limited research so far performed on the DLP and SLA based systems. Although initial studies have demonstrated the capability of methacrylated HA solutions to form cell-loaded hydrogels⁹⁵ utilizing SLA printing, there is not a significant body

of research. Due to the high molecular weight and viscosity of HA solutions, the methacrylated HA is more commonly used to fix structures printed through the extrusion process rather than the main method of structure formation^{91,96-99}.

2.3.4. Chitosan Network

Chitosan is a linear polysaccharide prepared by N-deacetylation of chitin⁴. There are multiple different mechanisms for the deacetylation of chitin; the most common one utilizes concentrated NaOH and high temperatures. Reaction conditions can range from 50-70% NaOH by mass and temperatures at typically 100°C. The resulting chitosan can range from 50-99% deacetylated depending on the conditions, with reduced molecular weight depending on the reaction conditions¹⁰⁰. Other methods of producing chitosan include enzymatic processes with chitin deacetylase. While chitin is virtually insoluble in almost all solvents with the exception of ionic liquids and deep eutectic solvents¹⁰¹, chitosan is soluble in acidic aqueous environments. This solubility is due to the protonation of the primary amine group on the glucosamine; as such, deacetylation is crucial to enable the solubility of chitosan¹⁰². In addition to tuning the solubility of chitosan through the degree of deacetylation. The mechanical properties and viscosity can also be varied through control of the molecular weight, with higher molecular weights improving the mechanical properties of resulting films¹⁰³ and increasing the viscosity of chitosan solutions.

Extrusion-based 3D printing of chitosan hydrogels has been achieved through various methods in the literature, including slow gelation at elevated temperatures through the addition of glycerol phosphate¹⁰⁴ or inclusion of a secondary network such as pectin to achieve suitable shear thinning properties⁶⁰. Like other polysaccharides, light-based 3D printing is achieved through 2 main pathways: 1) the inclusion of a photopolymerizable acrylate secondary network with

examples including PEGDA⁶¹ and acrylamide⁶² or 2) the modification of chitosan with acrylate or methacrylate groups to directly polymerize the polymer¹⁰⁵.

There has also been a significant body of research looking at the modification of chitosan to incorporate additional chemical groups. By far the most common modification of chitosan is the addition of quaternary ammonium groups to the polymer. Since chitosan has an intrinsic antimicrobial activity in acidic conditions due to the protonation of primary amine groups resulting from deacetylation, many groups have investigated methods of quaternization and the resulting biocompatibility and antimicrobial activity. This can be achieved through multiple different pathways, with the most common being n-alkylation with an alkyl halide or epoxidation to graft a new quaternary amine-containing molecule to the primary amine. There are multiple methods to incorporate quaternized chitosan into different hydrogels¹⁰⁶, but the current body of work utilizing quaternized chitosan in 3D printable hydrogels is limited to its use as an additive¹⁰⁷ rather than the main source of cross-linking. There is a gap in the literature where quaternized chitosan is used as the sole crosslinker in a hydrogel. Due to the potential applications of quaternized chitosan in antimicrobial and biocompatible materials, the development of hydrogels composed of quaternized chitosan is of significant interest.

2.3.5. Cellulose

Cellulose is the most abundant polysaccharide in nature extracted from plants or bacteria⁵⁶. Unlike bioderived polysaccharides such as alginate or hyaluronates, cellulose is not soluble in water at any pH and generally requires modification to achieve solubility⁴. Commonly water-soluble cellulose derivatives are produced through modification of the hydroxyl groups by esterification with methyl or ethyl groups. Examples of these polymers include ethyl cellulose, carboxyethyl cellulose, carboxymethyl cellulose, hydroxypropyl methylcellulose, hydroxyethyl

cellulose and methylcellulose⁵⁷. Methylcellulose has seen the most use as a potential 3D printing material due to its gelation properties^{58,108}; nanocellulose in the form of CNC and CNF has also seen significant use in 3D printing^{59,109,110}.

Unlike soluble cellulose derivatives, CNC and CNF are nanoparticles produced from cellulose stock. CNCs are produced through acid hydrolysis leading to the removal of amorphous regions in the cellulose. These nanoparticles are characterized by short crystals anywhere from 100-500 nm in length and 2-20 nm in diameter. CNCs demonstrate extreme tensile strengths as high as 7500-7700 MPa and elastic moduli ranging from 110-220 GPa¹¹¹. As such they are seen as a desirable strengthening additive due to their high mechanical properties and high concentration of surface OH groups enabling modification. CNF is the other commonly used cellulose nanoparticle, commonly produced through mechanical methods such as high-pressure homogenization, micro fluidization and supermasscolloiders¹¹². These methods fibrillate the cellulose and leave amorphous regions intact. CNFs are characterized by their high aspect ratios when compared to CNCs, with lengths varying from 500nm to hundreds of micrometres. Unlike CNCs, CNFs have a fibrillated appearance with diameters dependent on the method of fibrillation and pretreatments, such as TEMPO oxidation¹¹³. These high aspect ratios facilitate their use as Pickering emulsifiers in oil in water emulsions¹¹⁴⁻¹¹⁶ and as ideal bioprinting inks due to their viscoelastic properties. CNFs are preferable to CNCs when used in bioprinting due to the lower concentration required to achieve viscoelastic properties suitable for 3D printing. Furthermore, CNCs and CNFs are commonly modified through a variety of additives to incorporate additional functional groups through methods such as graft polymerization, atom transfer radical polymerization^{117,118}, reversible addition-fragmentation chain-transfer^{119,120}, ring-opening polymerization, nitroxide mediated polymerization^{121,122}, click chemistry and sialylation. Due to

this wide variety of modification methods nanocellulose has significant applications in bioprinting as a majority component of inks⁴ and stereolithography-based 3D printing as a strengthening additive²³.

2.4. Thermoresponsive hydrogels

Thermoresponsive hydrogels are desirable for biomedical applications due to their change in properties as temperature changes. Thermoresponsive polymers can be classified as positively or negatively thermoresponsive. Positively thermoresponsive polymers have an upper critical solution temperature (UCST), where below this temperature, the polymer experiences a phase transition from a soluble state to an insoluble state. An example of positively thermoresponsive polymers is gelatin, which forms a gel during cooling. Conversely, negatively thermoresponsive polymers experience a phase change when raised above their lower critical solution temperature, switching from soluble to insoluble. There are multiple examples of negatively thermoresponsive polymers, such as poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (PEO-*b*-PPO-*b*-PEO) block copolymer, also referred to as poloxamers and poly(ethylene oxide)-*b*-poly(D, L-lactic acid-co-glycolic acid)-*b*-poly(ethylene oxide) (PEO-*b*-PLGA-*b*-PEO) triblock copolymers. However, their use is limited when compared to thermoresponsive homopolymers due to the complexity of preparation¹²³. Thermoresponsive homopolymers are defined by the structure of the monomer, which is mostly hydrophilic with the inclusion of hydrophobic groups, which include methyl, ethyl and propyl. N substituted acrylamides make up the most commonly used thermoresponsive homopolymers, with examples including poly(N,N-diethylacrylamide) (PDEAM), poly(N-ethylmethacrylamide) (PNEMAM) and poly(N-isopropylacrylamide) (PNIPAm). Other thermoresponsive homopolymers include poly(methylvinylether) (PMVE),

poly(2-ethoxyethylvinylether) (PEOVE), poly(N-vinylcaprolactam) (PNVCa) and poly(N-vinylisobutyramide) (PNVIBAM). Of these thermoresponsive homopolymers, PNIPAm is the most heavily studied due to its well-characterized structure and LCST at 32°C²¹.

2.4.1. PNIPAm hydrogels

PNIPAm's thermoresponsive behaviour is a result of the amide (-CONH-) and propyl (-CH₂(CH₃)₂) groups present in the polymer's structure. At temperatures below the LCST, the amide group is solvated by water molecules leading to solubility. As temperatures increase, the hydrogen bonding is weakened, and the interactions between the propyl groups increase in strength. This reaches a critical point at the LCST when the attraction between the hydrophobic moieties of the polymer exceeds the attraction between the hydrophilic moieties and water leading to the collapse of the swelled structure and the expelling of water¹²⁴. The LCST of PNIPAm hydrogels can be modified through the inclusion of hydrophobic and hydrophilic monomers to move the LCST to a lower or higher temperature, respectively¹²⁵.

In PNIPAm hydrogels, specifically a volume phase transition is observed at a temperature slightly above the LCST. This behaviour is limited by the structure of the hydrogel. When above the LCST, the PNIPAm chains rapidly segregate and expel bound water on a molecular scale, forming a heterogenous microstructure as indicated by the opacity change of PNIPAm gels. The rate at which water is expelled from the bulk material is limited by both the poroelastic limits of the hydrogel that govern how water redistributes itself within the network¹²⁶ and the formation of a dense "skin layer" during deswelling^{127,128} that limits the diffusion of water out of the hydrogel.

The rate of swelling and expulsion of water can improve by increasing the porosity of the network or the inclusion of a second hydrophilic network such as poly(vinylalcohol) to provide expelled water paths to exit the hydrogel¹²⁹. Furthermore, the swelling of a PNIPAm network leads to an extremely low polymer concentration in the final hydrogel. These networks are weak and generally too weak to be measured²². The strength of a single network PNIPAm hydrogel can be improved by increasing crosslinking, but this comes at the expense of swelling behaviour. One common method to increase the strength is through the incorporation of a double interpenetrating network hydrogel.

2.4.2. PNIPAm IPN hydrogels

IPN hydrogels have been researched to increase PNIPAm's mechanical strength and modify its swelling properties. Zhang et al. utilized poly(vinyl alcohol) as the secondary network, composing 10-50% of the total polymer content. At higher PVA concentrations, the hydrogel was capable of deswelling to near equilibrium within 1 minute¹²⁹ expelling 95% of its water content when maintained at 49°C; this is a significant increase over pure PNIPAm hydrogels, which take over 2 hours to reduce in mass by 50% when raised above the LCST. While PVA has been utilized to improve swelling ratios, other secondary networks have been tested to improve the strength of the PNIPAm network. Many different network compositions have been tested in literature, summarized by Haq et al. in Table 1.1

Table 2.1 Mechanical properties of IPN PNIPAm hydrogel²² (reproduced with permissions from Elsevier)

Mechanical properties of IPN PNIPAm hydrogel.

Polymer	PNIPAM (%)	ESR (swollen state)	ESR (collapsed state)	Swelling time (min)	Strength (kPa) (swollen state)	Strength (kPa) (collapsed state)
Calcium alginate [60]	83	24.50	1.23	30	120 ^b	250 ^b
Cellulose [84]	25	16	5	NR	55 ^a	NR
Poly(acrylamide) [89]	12.3	6.75	6.25	NR	101 ^a	120 ^a
Poly(acrylamide) [58]	90	11	NR	NR	42.3 ^e	NR
PNIPAM [52]	100	7.8	NR	25	188 ^a	NR
P(NIPAM-co-AMPS) [52]	75	10	NR	12	341 ^a	NR
Poly(<i>N</i> -vinylpyrrolidone) [59]	90	6.7	1.95	190	20.5 ^d	NR
Silk fibroin [62]	90	9.5	0.555	250	100 ^c	NR
Silk fibroin [61]	90	9.0	1.75	0.66	40 ^a	80 ^a

2.4.3. PNIPAm nanocomposite hydrogels

Nanocomposites have also been explored as a method to increase the mechanical properties of PNIPAm hydrogels, as shown in Table 1.2. Most commonly, nanoclays are used to increase the elongation of PNIPAm networks due to their availability and low toxicity. Nanoclays also show improvements in swelling kinetics by providing channels for water to enter and leave to composite. Other additives that have seen use in recent years include cellulose nanocrystals (CNC)¹³⁰. While both these additives can significantly increase the strength of the hydrogel, their use is limited by their gelling behaviour. The gelling can be desirable in applications such as extrusion printing, where shear at the nozzle allows for flow but stabilizes the gel once extruded. In the case of stereolithography, the low shear forces present in printing leads to gelation during printing at CNC concentrations above 1-2 wt%. As such, for stereolithography-based printing, there are significant limitations to the loading of nanoparticles with 1D and 2D geometries due to their thixotropic properties. In practice, this limits the degree of loading in stereolithography to <~2%, with bioprinters using CNC concentrations above 10% to improve printability^{4,13}.

Table 2.2 Mechanical properties of PNIPAm nanocomposites.²² (reproduced with permissions from Elsevier)

Mechanical properties of nanocomposite PNIPAM hydrogel.

Nanoparticle	Concentration (%) ^a	ESR (swollen state)	ESR (collapsed state)	Swelling time (min)	Strength (kPa) (swollen state)	Elongation at break (%)
Graphene oxide [64]	2	22	2	9	TS = 250	125
Graphene oxide [155]	5	32	2	10	CS = 216	NR
Laponite XLG [156]	20	25	NR	800	TS = 42.3	1632
Laponite XLG [151]	19.8	NR	NR	80	TS = 69	1112
					$E_0 = 3.8$	
Laponite XLG [152]	11.8	NR	NR	NR	TS = 130	1500
Laponite XLG [157]	23.2	NR	NR	NR	TS = 1600	741
					$E_0 = 43,200$	
Laponite XLG [148]	6.6	18	2	10	TS = 41	857
					$E_0 = 1.5$	
Laponite XLG	Clay = 10	25	2.5	125	CS = 3000	NR
Graphene oxide [158]	GO = 1					
PNIPAM [38]	12.65	80	2	2	TS = 200	1400
Starch 300 nm [159]	100	17.2	NR	5	CS = 8440	NA
Starch 300 nm [159]	25	40.4	NR	5	CS = 2570	NA
Tetramethoxysilane [160]	50	20	2.70	10	NR	NR
Au [161]	4	NR	NR	NR	$E_0 = 6726$	NR
Fe ₂ O ₃ [162]	70	NR	NR	NR	CS = 190	NR
					$E_0 = 218$	
Fe ₂ O ₃ [163]	Fe ₂ O ₃ = 10	25	7	100	CS = 50,000	NR
Laponite XLG	Clay = 50					

^a Based on NIPAM.

2.4.4. POEGMA hydrogels

POEGMA chains have shown promise in the formation of thermal-responsive polymers through random copolymerization. Unlike PNIPAm, which is a homogeneous network, Poly(oligoethylene glycol acrylates) is a tunable thermoresponsive polymer. While there has been research into tuning PNIPAm networks through the addition of other more hydrophobic monomers, the achievable change in lower critical solution temperature is limited. The POEGMA polymer refers to a wide family of acrylates with different lengths of ethylene glycol side chains. Depending on the pendant group and the length of the glycol side chain, the hydrophobicity of each monomer can be tuned. The most hydrophilic of the monomers is hydroxy ethyl acrylate, followed by hydroxyethyl methacrylate and oligoethylene glycol acrylate. These polymers, when polymerized alone, demonstrate LCSTs above the boiling point of water. Conversely, monomers such as ethylene glycol methyl ether acrylate and diethylene glycol ethyl ether acrylate exhibit LCSTs below the freezing point of water. By polymerizing a hydrophobic and hydrophilic monomer in different ratios, the hydrophilicity of the whole polymer can be tuned, impacting the

Gibbs free energy of mixing and, therefore, the LCST. In addition to the tunable ability, these polymers have other advantages over PNIPAm. For example, regarding the toxicity of the monomers, N-isopropyl acrylamide is known to be neurotoxic and has the potential for unreacted monomers to remain in the final hydrogel. POEGMA monomers do have their own concerns, mainly the potential for chemical burns. Still, they do not demonstrate any neurotoxicity and show higher reactivity leading to a lower proportion of unreacted monomers present in the final hydrogel. For this reason, it is of interest to test the use of these thermal responsive polymers for 3D printing thermoresponsive hydrogels. So far, there is little research on the use of these polymers to form hydrogels through 3D printing, as up to this point, research has focused on the formation of linear chains of random copolymers with or grafting to form films.

2.4.5. Thermoresponsive organo-gels

In the realm of additive manufacturing, particularly in the fabrication of thermo-responsive hydrogels through 3D printing, warpage poses a significant challenge. This deformation often results from the temperature fluctuations induced by the excess heat generated during photopolymerization processes. To mitigate this issue, a novel approach has been developed involving organo-gel intermediaries¹³⁰. Unlike their hydrogel counterparts, organo-gels do not exhibit a lower critical solution temperature (LCST) when exposed to solvents other than water, thereby maintaining dimensional stability throughout the printing process. This characteristic enables the production of complex, intricate structures without the detrimental effects of swelling and shrinking that typically compromise the accuracy and fidelity of printed parts. Furthermore, the distinctive mechanical properties of organo-gel precursors, which are markedly different from those of fully swollen hydrogels, contribute to an enhanced resolution of small details. This advantage is pivotal for applications requiring high precision and structural integrity in 3D printed

materials. Thus, the utilization of organo-gel intermediaries emerges as a promising strategy for overcoming the limitations associated with traditional photopolymerization-based 3D printing of thermoresponsive materials, offering new avenues for the development of advanced functional structures.

One possible polar aprotic solvent used for organo-gel printing is dimethyl sulfoxide (DMSO). DMSO is suitable for this application due to the hydrophilic polymer precursor monomers remaining soluble in DMSO before printing. Additionally, DMSO is useful for printing thermoresponsive hydrogels as organo-gel precursors due to the lack of an LCST in DMSO¹³¹. This allows for the printing of complex geometries and long print times without the occurrence of warpage during printing. This behaviour is especially important for thermal-responsive hydrogels as photoinitiators, and the polymerization of monomers generates significant heat during curing. Printing in water leads to hydrogels adopting a collapsed state and subsequently swelling within the resin solution during the printing, causing compounding errors and inaccurate prints. As such, DMSO has significant advantages when compared to water for photopolymerization-based 3D printing. Unfortunately, the transdermal properties of DMSO¹³² This leads to serious drawbacks when the toxicity of the monomer is taken into account. Unpolymerized monomers can cross the dermal barrier and lead to adverse health outcomes. This limits the use of DMSO as an organic phase in the production of organogels for biomedical applications, requiring either time-intensive washing steps or complete conversion of monomers to the final polymer.

Cyrene is another polar aprotic solvent that has recently been commercially available. This solvent was discovered in 2016 and has seen significant increases in manufacturing, reaching commercialization in the past four years. Cyrene, also known as dihydroxylevogluconanone, is a bicyclic, chiral, seven-membered heterocyclic cycloalkanone that is derived from cellulose and is

fully biodegradable¹³³. Cyrene is most similar to dimethylformamide and N-methylpyrrolidone; it is also a suitable replacement for DMSO since it is non-toxic. While there is no significant research on the use of Cyrene as a potential replacement for polar aprotic solvents in the formation of organo-gels, its benign properties and bioderived nature make it a more desirable polar aprotic solvent when compared to alternatives such as DMSO, NMP and DMF^{134,135}.

Chapter 3: Multi-thermoreponsive PNIPAm Hydrogel¹

3.1. Introduction

Thermoresponsive hydrogels have seen significant research progress over the recent decades. In a biomedical context, thermoresponsive hydrogels are desirable due to their change in properties as temperature changes^{4,23,136–140}. Thermoresponsive hydrogels are generally classified into 2 categories, homogeneous polymers and heterogeneous polymers. Of the thermoresponsive homopolymers, poly(N-isopropylacrylamide) (PNIPAm) is the most extensively studied due to its well-characterized structure and lower critical solution temperature (LCST) at 32°C^{21,22,124,129,141}. Heterogeneous thermoresponsive polymers rely on different blocks within the co-polymer that experience a change in their interaction between blocks across the LCST. The most commonly used thermoresponsive block copolymer is poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) with molecules fitting this structure referred to as a poloxamer. When the temperature rises above the LCST in a poloxamer solution, the polypropylene oxide groups segregate leading to the formation of micelles with a hydrophobic core and hydrophilic exterior. In this work, we hypothesize that a poloxamer diacrylate (Pluronic P123) can be used to augment the thermoresponsive network composed of PNIPAm to develop a multi-thermo responsive hydrogel for 3D printing.

Early work on 3D printable thermoresponsive hydrogels utilized extrusion printing. Bakarich et. al. reported a NIPAAm/Alginate system¹⁴², while capable of printing, the resolution and finish was limited leading to parts with a surface finish inferior to cast hydrogels. Later Gladman A. et. al. reported a NIPAAm/NFC/nanoclay¹⁴³. Although the surface finish of these structures was

¹ This chapter was partially reproduced from Bauman, L.; Zhao, B. Multi-Thermo Responsive Double Network Composite Hydrogel for 3D Printing Medical Hydrogel Mask. *J. Colloid Interface Sci.* 2023, 638, 882–892. <https://doi.org/10.1016/j.jcis.2023.02.021>.

superior, the printed parts were only made of 2-3 layers (<1mm thick) and the G-code was written by hand, limiting modifications. Arslan H. et al. built on this technique to generate larger printable hydrogels out of PNIPAm¹⁴⁴ with novel shape changes, though the printed hydrogels were still limited by the extrusion nozzle with a maximum resolution of 200µm. Naficy S. et al. reported printing bilayers of PEO-PU-co-NIPAAm and PEO-PU-co-hydroxyethyl methacrylate¹⁴⁵ to form hydrogel hinges. This technique achieved thermoresponsive shape change of a solid structure, but again the printed structures were only 3 layers thick limited by the strength of the hydrogel and the printer's resolution.

While extrusion-based printing is capable of fabricating hydrogels with reasonable strength, the inability to produce fine details is a significant drawback. For this reason, stereolithography (SLA) based resin printers have gained popularity as increases in the resolution of mask stereolithography printers (mSLA) and decreases in their price have enabled the fabrication of structures with fine details. Recent developments in water-soluble photoinitiators have allowed resin-based 3D printing of hydrogels to see significant growth^{48,146,147}. In recent years, researchers started to explore 3D printing of thermoresponsive hydrogels. This can be seen in the fabrication of 4D hydrogels through programmed chemo-mechanical gradients, which can be achieved through swelling rates (through the addition of high surface area structures), crosslink density (through variation of exposure times) or chemical composition (by exchanging the resin vat) as reported by Odent et al.¹⁴⁸. Even with the significant progress in SLA-based hydrogel printing that has occurred, it has not been used to fabricate large-scale structures such as medical hydrogel masks; large 3D-printed hydrogel parts experience significant thermal cycling during photopolymerization printing, leading to water-based resins experiencing warpage during printing as each layer polymerizes above the LCST and subsequently cools and swells during the print. In

this study, we investigate the use of N-isopropyl acrylamide and Pluronic P123 diacrylate-based resin to form a double network system with sodium alginate, allowing to print a large hydrogel that can be formed into patient-specific medical hydrogel, masks for customizable reloadable drug delivery. We hypothesized that through the use of a thermoresponsive crosslinker and sodium alginate, the resulting thermoresponsive hydrogel would demonstrate improved mechanical properties at room temperature, mitigating the weak properties PNIPAm hydrogels are known for in the swollen state. Additionally, through the use of an organo-gel system, PNIPAm-based hydrogels could be prepared with mSLA techniques by preventing LCST behaviour and enabling rapid printing of large hydrogel wound dressings enabled by the swelling of the organo-gel precursor upon solvent exchange with water.

3.2. Experimental Section

3.2.1. Materials

N-isopropyl acrylamide 97% (TCI America), acrylamide 99% (Sigma Aldrich), hydroxyethyl methacrylate 97% (Sigma Aldrich), diphenyl(2,4,6 trimethylbenzoyl) phosphine oxide >98.0% (TCI America), cellulose nanocrystals (CNC) (Celluforce), sodium alginate (low viscosity)(Sigma Aldrich), Pluronic P123 (Sigma Aldrich), methacrylic anhydride $\geq 94\%$ (Sigma Aldrich), dichloromethane 99.8% (Sigma Aldrich), pentane 99% (Sigma Aldrich), sodium phosphate monobasic dihydrate $\geq 99\%$ (Sigma Aldrich), sodium phosphate dibasic heptahydrate $\geq 99\%$ (Sigma Aldrich), tannic acid ACS reagent (Sigma Aldrich), Dulbecco's phosphate-buffered saline (Sigma Aldrich), Rhodamine B 99% (Sigma Aldrich)

3.2.2. Synthesis of Crosslinker

Synthesis of Pluronic P123 diacrylate started with 0.0172 mol of Pluronic P123 that was dried at 80 °C and -25 inHg relative pressure for 24hrs. The Pluronic P123 was added to a 1 L round-bottomed flask along with 0.69 mol of methacrylic anhydride for a 20-time molar excess of methacrylic anhydride to the Pluronic P123 terminal hydroxyl groups. This solution was stirred to homogenize after which it was subjected to microwave irradiation at 1000 W in a consumer microwave (MW) at 1-minute intervals for 10 minutes. Between MW-irradiation intervals, the flask was placed on a rotary evaporator at 150 rpm under full vacuum until boiling ceased. This step served 2 purposes: firstly, it increased the conversion of the Pluronic P123 terminal hydroxyl groups to methacrylate groups since the methacrylic acid side product had the lowest boiling point of all the components; secondly, it prevented overheating during MW irradiation and thermal degradation of the intended product since the boiling at reduced pressures in the absence of heating rapidly cooled reaction mixture. Once 10 minutes of MW-irradiation had elapsed, the flask was put back to the rotary evaporator and held at 150 rpm and full vacuum in a 95°C water bath to remove the remaining methacrylic acid and any unreacted methacrylic anhydride. Subsequently, the reaction mixture was allowed to cool to room temperature before the addition of 50 ml of dichloromethane and subsequent precipitation into -40°C pentane. The precipitate was then filtered through a Buchner funnel and rinsed with fresh -40°C pentane. Finally, the precipitate was transferred to a vacuum oven and held at 40°C under vacuum for 2 hours before the heating was turned off and was kept under vacuum for 12 hours to remove any remaining pentane.

3.2.3. Synthesis of 3D printable resin

Figure 3.1 shows the key steps of synthesizing the printable resins. The resin was prepared by first dispersing 1.8 g of CNC in 250 ml of ultrapure water using a probe sonicator (Qsonica q125) with a ¼” diameter probe tip at 80% amplitude for 4 minutes. 3.6 g of tannic acid was then added to the dispersion and sonicated for another 2 minutes until fully dissolved. Concurrently 20 g of sodium alginate was added to a 2L round bottom flask along with 20 ml of glycerol, 50 ml of dimethyl sulfoxide, 2.609 g of sodium phosphate monobasic dihydrate and 8.335 g of sodium phosphate dibasic heptahydrate and mechanically stirred until homogeneously dispersed. The CNC dispersion was then added to the sodium alginate solution; and another 250 ml of ultrapure water was used to rinse out the remaining CNC dispersion and add it to the 2L flask. The resulting solution was then stirred until the sodium alginate was fully dissolved resulting in a brownish red translucent solution due to the coordination of tannic acid in a slightly basic solution as shown in Fig. 3.1. S-1). The solution was then allowed to stir for 24 hrs until the red solution turned a dark brown indicating the formation of poly(tannic acid) on the surfaces of the CNCs [seen in Fig. 3.1 S-2)]. The 2L flask was transferred to a Buchi rotary evaporator with the water bath set to 90°C at 200 rpm and full vacuum. The solution remained on the rotary evaporator until the solution increased in opacity and a film could be seen in the upper portion of the flask ~200 ml of solution remaining in the flask [seen in Fig 3.1. S-3)]. At this point, 15 g of acrylamide was added to the flask and stirred for 2 hrs. The flask was then returned to the rotary evaporator at the same conditions described above until evaporation stopped. Removal of water was confirmed by exposing a sample to molecular sieves and ensuring no change in temperature. The remaining 120 g of N-isopropyl acrylamide, 10 g hydroxyethyl methacrylate, 45 g of PluDA 6000 were added to the flask and mechanically stirred until a homogeneous solution was obtained. Finally, the flask

was returned to the rotary evaporator once more for 5 minutes to remove any pentane remaining in the synthesized PluDA. The solution was then passed through a 250 μm mesh drum sieve aided by probe sonication to break up any sodium alginate aggregates [seen in Fig 3.1. S-4)]. The filtered solution was then placed in a Thinky ARE-300 planetary mixer (THINKY U.S.A) along with 4.5 g of diphenyl (2,4,6-trimethylbenzyl phosphine oxide) and mixed for 2 minutes at 2000 rpm. Components of hydrogel precursor resin and the purpose of each component are summarized and listed in the supporting information Table 1. The prepolymer resin was printed in a Phrozen XL 4K 3D printer (Taiwan, China) with a layer thickness of 100 μm and 30 s exposure time per layer [reaction schematic shown in Fig 3.1. P-2)]. The resulting structure of the precursor can be seen in Fig. 3.1. P-3).

Once printing was completed, samples were swollen in DI water at 4°C and allowed to equilibrate for 12 hours, as seen in Fig. 3.1. PP). Once the hydrogel had swollen the temperature was increased to 40°C and equilibrated for 3 hours. Once collapsed they were transferred to a 1 M calcium chloride solution at room temperature to swell for 12 hrs and crosslink the alginate network. Finally, the hydrogel samples were transferred to room temperature DI water solution to allow for the diffusion of any free calcium and chloride ions out of the hydrogel.

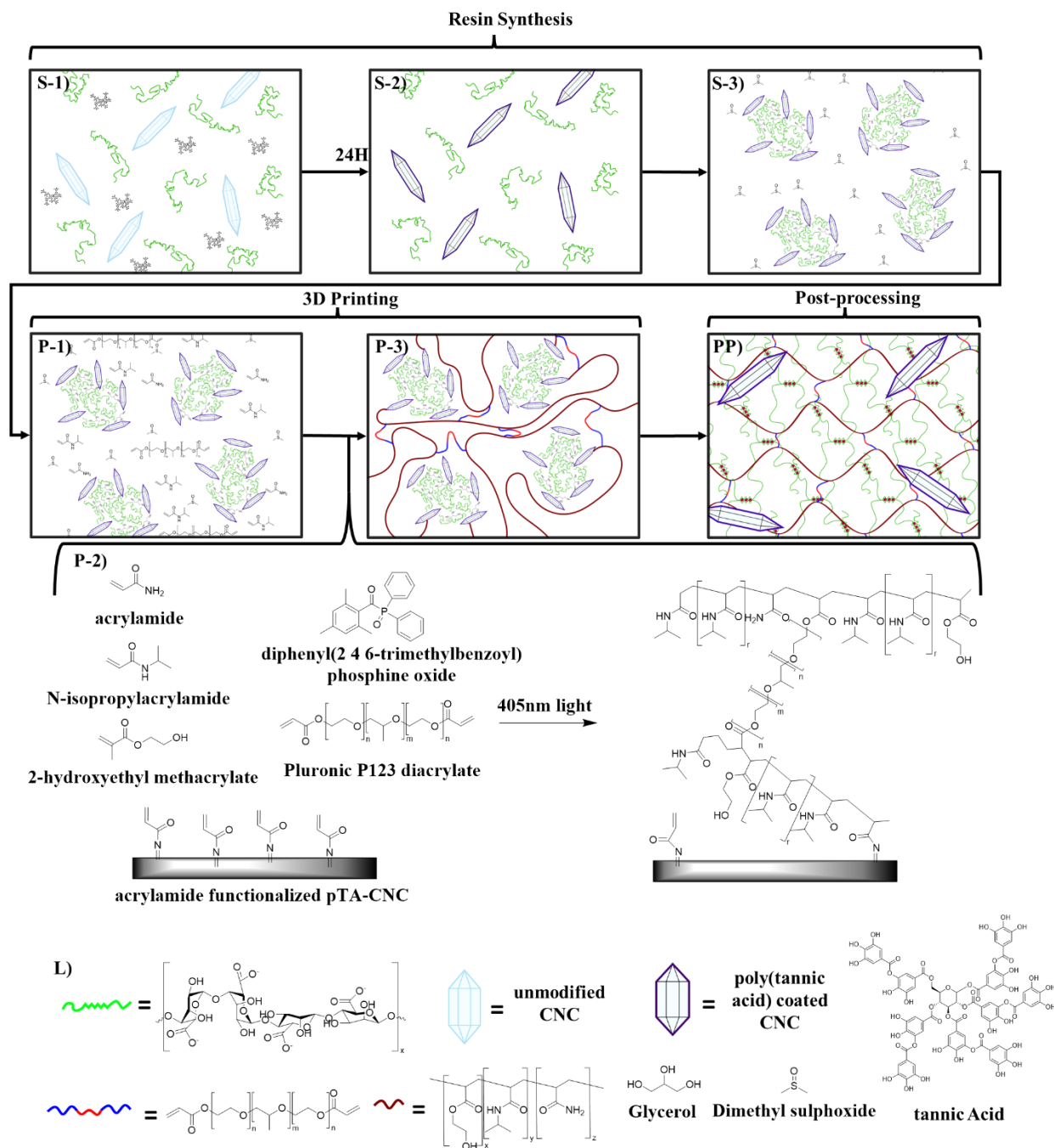


Figure 3.1 Schematic diagram of the synthesis (S) and polymerization (P) of hydrogel precursor; L) lists the key components and their symbols in the diagram. S-1) the initial state of alginate solution with additives CNC and tannic acid. The tannic acid polymerizes on the surface of the CNCs in the basic alginate solution (S-2). The water is removed forming a suspension of alginate/glycerol in DMSO (S-3). Upon removal of the water, acrylate monomers and crosslinkers are added (P-1). The polymerization occurs under UV-initiated cleavage of diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide, generating free radicals and polymerizing the precursor resin (P-2) into the final composite before solvent exchange (P-3). Once the final network is formed, water exchanges the DMSO and glycerol (PP), allowing the alginate to homogeneously distribute itself throughout the network and ionically crosslinked in the presence of Ca^{2+} ions.

3.2.4. Characterization

FT-IR spectroscopy was performed on the resin crosslinkers. Pluronic P123 and PluDA were measured by ATR with a zinc selenide crystal. DSC measurements were performed with TA instruments Q20. Hydrogel samples prepared as described in post-print processing were frozen in liquid nitrogen and ground to a fine powder. The powder was then dried and 4mg of powder was added to a hermetic Tzero pan along with 20 μ L of water and sealed. The reference sample was also prepared with a hermetic Tzero pan and 20 μ L of water. Samples were equilibrated at 1°C and heated at 5°C per minute to 60°C. The SEM measurements were performed on a FEI Quanta Feg 250 ESEM. The samples used for SEM were prepared as noted above. A slice of printed calibration cubes equilibrated at 4°C, 20°C and 40°C was cut off and placed on a Ted Pella aluminum Zeiss SEM stub with a brass clip. The samples were then transferred to liquid nitrogen bath to prevent changes in the structure during transport and measurement before being measured in the ESEM.

Tensile tests were performed with printed tensile test specimens using a tensile coupon. The samples were printed using the optimal layer times and curing methods described above. Once post-print processing was completed, the samples were equilibrated in 1 M calcium chloride solution overnight at 4°C, room temperature $\sim$ 21°C or at 40°C. During tensile testing, the samples were stretched at a rate of .5 mm/second until failure.

Swelling studies were conducted in an Espec environment chamber with samples contained in a water bath. During initial trials it was determined that the samples did not significantly change in size after 12 hrs. To ensure an equilibrium state was achieved samples were allowed to equilibrate at each new temperature for 24 hours before measurement. During measurement, the water bath was removed from the environment chamber, and samples were only removed immediately before

measurement to minimize any swelling or deswelling that would occur upon equilibration to room temperature.

Contact adhesion measurements were performed using a custom-built temperature control chamber for the universal mechanical tester (UMT, CETR) see Fig. S5 a) with a 5.5mm diameter flat hydrogel probe punched from a 3D printed sheet of hydrogel equilibrated in DI water after 1M CaCl_2 crosslinking described above. The disc was then superglued to a ¼” SEM stub (Ted Pella) and placed in the holder seen in Fig. S5 b) immediately before testing to prevent dehydration of the surface. Adhesion was measured against a porcine skin sample equilibrated in pH 6 or 8 phosphate buffered saline for 24 hours. The porcine skin samples were cleaned by scrubbing the skin side with a brush and ethanol to remove deposits of fat on the surface followed by DI water before equilibrating in PBS at the pH of the respective test. A 1” circular punch was then used to form disks of porcine skin followed by mounting on a microscopy slide with super glue (krazy glue). To approximate the conditions of tissue at a wound site, the top 200 μm of porcine skin (the typical thickness of the porcine epidermis is 140 μm ¹⁴⁹) was removed with a Leica vibratome (Wetzlar, Germany). Following the removal of the epidermis, the samples were rinsed with ethanol followed by DI water before equilibrating in PBS at the pH of the respective test. After equilibrating for 24 hrs, the porcine skin was removed from the PBS and rinsed with ethanol, followed by DI water to prepare the samples for testing. Before testing the surface pH of the skin was measured with an EXTECH Instruments ExStik flat-bottomed pH probe (Nashua, New Hampshire) with the samples measuring 6.2 and 7.6 pH for 6.0 (140 mM NaCl, 12 mM Na_2HPO_4 and 88 mM NaH_2PO_4) and 8.0 (140 mM NaCl, 93.2 mM Na_2HPO_4 and 6.8 mM NaH_2PO_4) pH PBS respectively. The temperature-controlled chamber consisted of an insulated enclosure containing a Peltier assembly. The temperature was controlled by means of a Horus Technologies

Inc temperature controller (Newmarket, Ontario) driving 2 Peltier elements in series. The temperature was regulated based on the temperature of the heat spreader assembly with the thermocouple seen in Fig 1. c). To ensure the measurement chamber was at the proper temperature (4°C, 20°C or 37°C), all measurements were performed within an acrylic enclosure within the insulated enclosure. Before each measurement excess water on the surface of porcine skin was removed with compressed air and then equilibrated within the enclosure with the central hole covered for 10 minutes along with a Kimwipe soaked in DI water to prevent the skin from drying. After the equilibration period the probe was removed from the DI water and dried with compressed air before attaching to the probe assembly. Indentation was performed at a constant force rate until preload of 2 g was reached over 30 s at which point the force remained constant for 30 s before retracting at a speed of 200 $\mu\text{m/s}$. After each measurement was performed with a fresh probe, a section of the porcine skin was used for the following measurement.

Characterization of the drug release behaviour of the hydrogel was performed on a Jasco J-815 CD Spectrometer (Tokyo, Japan) with a temperature-controlled holder with magnetic stirring. Absorbance measurements were performed in a 10mm quartz cuvette with a 2x7 mm stir bar and a lid modified with a copper hook to suspend the hydrogel sample above the beam path. The hydrogel samples were prepared with a 5 mm biopsy punch from a 1.8 mm thick sheet of the printed hydrogel equilibrated in DI water at 20°C. The hydrogel discs were then transferred to 1 M CaCl_2 at 20°C for 12 hours at which point the temperature was increased to 37°C for 3 hours before rinsing with DI water and transferring to a 100mg/L rhodamine B solution at 4°C. The samples were allowed to equilibrate for 24 hours before the release measurement. Before each measurement the sample chamber and the cuvette filled with 4 ml of DI water were equilibrated at the temperature 4°C, 20°C and 37°C for 20 minutes. Once the temperature stabilized, a blank

measurement was taken, and the cap and the hydrogel were placed on top and sealed with parafilm to prevent water from evaporating during the measurement. The absorbance was measured every 2 seconds with a D.I.T. of 64 ms for 8 hours. The maximum loading for each sample was calculated from the initial mass before measurement and the final mass after vacuum drying at 60°C and -12 psig by Eqn. 3.1.

Equation 3.1 Mass of rhodamine-b loaded into sample

$$M_{load} = (M_{initial} - M_{final}) * \rho_{H_2O@4^{\circ}C} * C_{loading\ solution}$$

The percent drug release was calculated by first running a calibration curve of rhodamine B in DI water at 0.01, 0.05, 0.1, 0.5, 1, 5, 10 and 50 mg/L at 4°C, 20°C and 37°C. The line of best fit was then used to calculate the concentration in the cuvette during each release test and the % drug release was calculated with Eqn. 3.2.

Equation 3.2 Cumulative % release of rhodamine-b from sample

$$\% \text{ release} = \frac{C_t * V_{water}}{M_{load}} * 100$$

3.3. Results and discussion

3.3.1. Design and fabrication of 3D printable PNIPAm-alginate double network hydrogel resin

PNIPAm hydrogels on their own are typically weak and brittle when fully swollen as the network is strained by the absorbed water and external stress can not be dissipated by means other than chain cleavage. Double network hydrogels have been used to address these mechanical limitations^{22,49,142,150–152}. In this work, we used alginate to form a double network with PNIPAm.

Alginate is a biologically derived polymer and can physically crosslinked via ionic interactions; it has often been used for tuning the mechanical properties of PNIPAm hydrogels¹⁵³. Formulating the PNIPAm and alginate precursor for resin printing is more challenging than conventional extrusion printing because resin printing builds the structure layer-by-layer via UV polymerization, which requires well-balanced resin flowability and minimal solvent evaporation. While alginate is commonly used for extrusion-based hydrogel printing^{51,77,154–160} incorporation of alginate into photolithography printing is less common as alginate aqueous solutions are very viscous.

Photolithography printing thermally cycles the printed layers across the LCST of PNIPAm. With water as a solvent, the printed PNIPAm layer forms in a collapsed state and subsequently cools, resulting in swelling and delamination, which terminates the printing process. We find dimethyl sulfoxide (DMSO) is a suitable alternative solvent since PNIPAm does not have a phase change and DMSO can be readily exchanged with water after printing to realize the final hydrogel. Note that DMSO is a good solvent for acrylate monomers but a poor solvent for sodium alginate. To disperse the alginate, water was used to dissolve the alginate in DMSO with added glycerol; the glycerol prevented the precipitation of sodium alginate during subsequent water evaporation. For high-fidelity 3D printing, the resulting resin also needs to have sufficient strength and dimensional accuracy during printing. CNCs were added along with tannic acid creating an enhanced network through the formation of poly(tannic acid) on the CNC surface with subsequent Schiff base reaction with acrylamide. Thus, CNC acts as bridging points, improving the stiffness of the hydrogel precursor. A series of FTIR analyses were performed to verify these interactions (see detailed description in the supporting information).

The dual temperature responsiveness of the hydrogel is provided by the polymers PNIPAm and Pluronic P123 (PluDA) as illustrated in Fig. 3.2 a); the incorporation of a PluDA crosslinker with a separate LCST, a 3rd equilibrium state could be realized between the 2 LCSTs, where the hydrogel mechanical properties are improved while still demonstrating a large volume change across PNIPAm's LCST. Other methods of increasing the strength of PNIPAm hydrogels in their swollen state are limited by the significant decrease in volume change. The use of a thermoresponsive crosslinker enables loading the hydrogel at 4°C and by allowing the soaking solution to exceed 9°C before application. The extra strength and elasticity provided by the PluDA above its LCST prevents damage during application with minimal shrinkage before application. The LCST of the PNIPAm has been widely studied and used in a multitude of biomedical applications as shown in Fig. 3.2. a). Pluronic P123 (P123) is a polymeric surfactant and has a critical micelle temperature (CMT) of 9°C, above which the hydrophobic interactions promote the formation of micelles with a polypropylene oxide core and polyethylene¹⁶¹ oxide shell. Of the various poloxamers, P123 was chosen as its CMT was below room temperature and it can help stabilize the precursor resin. P123's terminal hydroxyl groups can be modified to methacrylate groups forming Pluronic P123 diacrylate (PluDA). With this combination of polymers, the formed network can swell to the highest water fraction possible at temperatures below 9°C. As the temperature of the system rises above 9°C, the CMT of PluDA P123 fraction is crossed, and the hydrogel shrinks due to hydrophobic attraction between PPO blocks. These hydrophobic interactions provide additional strength to the network, reducing the low elasticity of the fully swollen hydrogel. As the temperature is raised above 32°C, the PNIPAm portion of the hydrogel expels the water surrounding it and further reduces the size of the network along with an increase in strength. The behaviour of this system is confirmed by the DSC as shown in Supplementary

Figure S2, where a large abrupt change in the heat flow occurs at 8.9°C along with a slower wider signal shown from 28-52°C. The broad peak associated with the PNIPAm is due to the addition of the hydrophilic monomers acrylamide and HEMA which modify the phase transition temperature of the PNIPAm copolymer¹⁶².

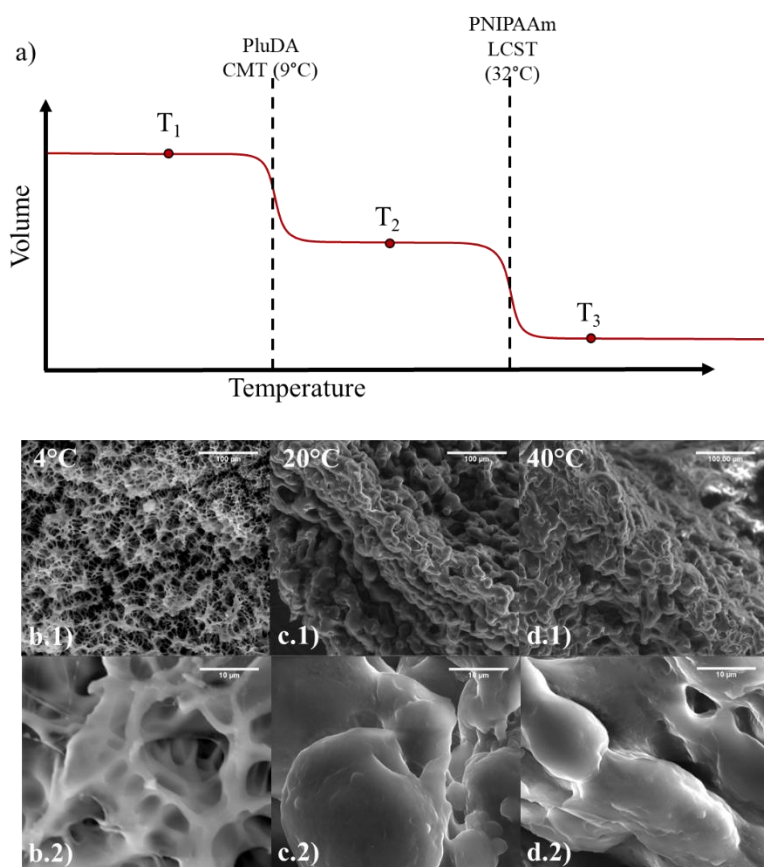


Figure 3.2 . a) Illustration of the thermoresponsive volume changes of the hydrogel with dual phase change temperature 9°C and 32 °C, b) ESEM images of the hydrogels at three temperatures- b-d.1 low magnification, b-d.2 high magnification.

To confirm the existence of separate states in the hydrogel, ESEM was performed on cryogenically frozen samples after incubation at 4°C, 20°C and 40°C as seen in Fig. 3.2. b-d). When at 4°C [Fig. 3.2. b)] the hydrogel is fully swollen and was quite porous due to the structure that remains after the alginate particles dissipate into the network. As the temperature increased to 20°C, as seen in Fig. 3.2. c), there is less porosity in the sample and shrinkage appearing from the

PluDA hydrophobic interactions has led to a partial collapse of the porous network. Finally, at 40°C in Fig. 3.2. d) we can see the network has fully collapsed with the least porosity evident of all 3 samples. The ESEM images agree with expectations of the hydrogel from the design of the resin and reinforce the reasonings behind the resin design philosophy.

The printability of the synthesized resin is shown in Fig 3.3., where a model structure with fine details and a large-size facemask are demonstrated. To test the fidelity of the model a resolution test was performed to gauge the capability of the resin to print fine structures. From comparing Fig. 3.3. a-c) it was evident that the fidelity is impressively close to the 3D model. While some fine details did not successfully print, this result is exceptional as rigid commercial photopolymers struggle to achieve such results and the failures can be prevented through proper support during printing. It was found that the resin was capable of printing for extended periods of time with minimal warpage leading to a 50% scale hydrogel precursor facemask; and the facemask fitted the model face that was used for this study exceedingly well with little gaps. Additionally, using this hydrogel precursor, it is possible to print parts in small sizes, which then can swell up to 4 times in volume to the final dimensions; this feature allows the print of large objects in a shorter period.

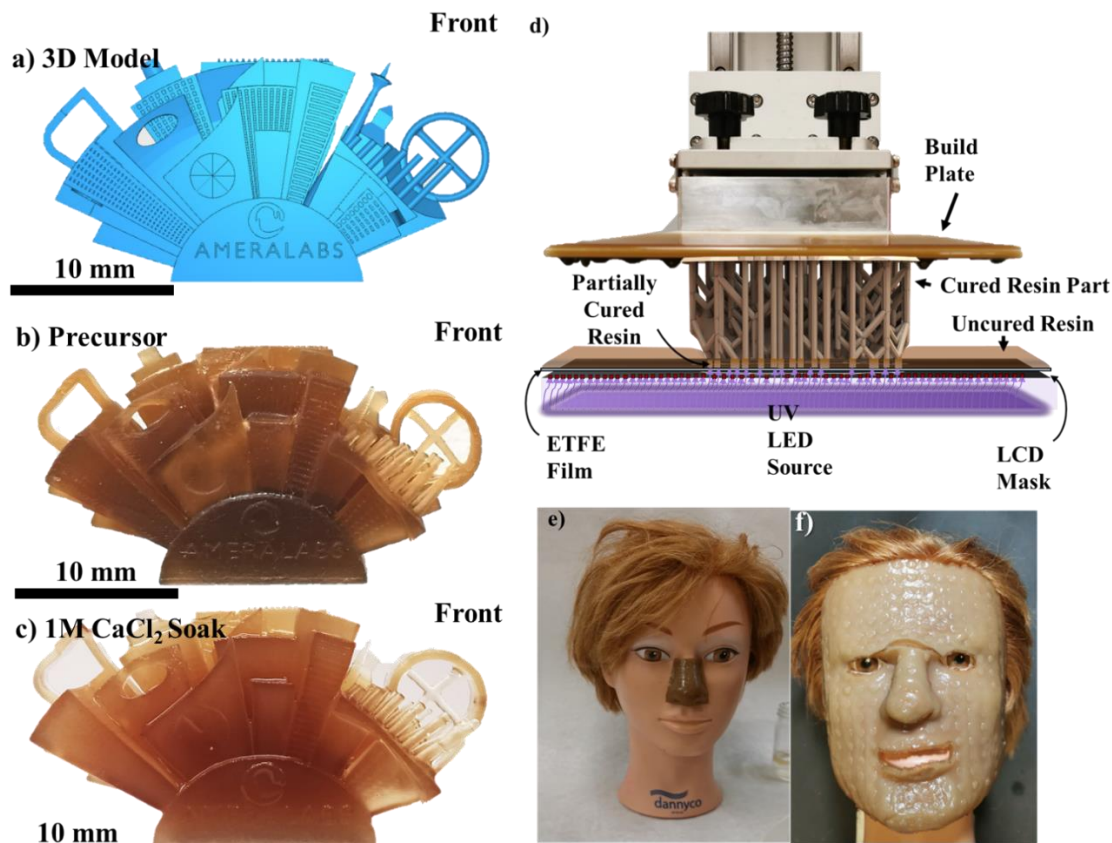


Figure 3.3 Demonstration of high printing fidelity with the hydrogel resin. a) 3D model and b) the corresponding printed hydrogel precursor and c) final hydrogel at 20°C. d) Schematic diagram of working principles of mSLA photopolymerization printing. e) a custom designed nasal mask printed from a scan of a mannequin head (3D model scaled for 1:1 after hydrogel equilibrates at 20°C) and f) a custom-designed facial mask generated from the same scan (3D model scaled for 1:1 scale at after hydrogel equilibrates at 4°C)).

3.3.2. Thermoresponsive swelling and contraction of PNIPAm-Alginate Nanocomposite Hydrogel

To investigate the temperature responsive behaviour of the hydrogels, a series of measurements of the dimensions of the samples were performed on printed hydrogel cubes at three temperatures of 4°C, 20°C and 40°C. 4°C was chosen as it was below the LCST both thermoresponsive components, 20°C was chosen as it was above the LCST of the Pluronic component but below the LCST of the PNIPAm component, 40°C was chosen as it was above the LCST of both Pluronic P123 and PNIPAm. It was found at 4°C, the samples increased in size by about 50% compared to the room temperature size as seen in Fig. 3.4. a), while at 40°C, the

samples shrunk by about 50% compared to the room temperature volume. As the samples were cycled through temperatures, the size at room temperature was reduced, likely due to the dissolution of free Ca^{2+} present in the hydrogel. As the concentration of free ions was reduced, the number of completed crosslinks in the alginate network would be increased. This would increase the cohesive forces within the hydrogel and prevent it from swelling to the same degree as when there is a greater number of ions present in the hydrogel. This hypothesis was reinforced by 2 phenomena observed when testing the hydrogel. When the cured hydrogel was swelled in DI water without Ca^{2+} ions present, the volume was significantly greater than the ionically crosslinked hydrogel at 20°C as seen in Supplementary Figure S3; this was attributed to a lower rigidity of the alginate network allowing for a greater degree of swelling. As the hydrogel was cycled through temperature gradients, the hydration % was also affected. When equilibrated in 1 M CaCl_2 the hydration reduced by 79% from 4°C to 40°C as seen in Fig. 3.4. b). It should be noted that the mechanical properties of the hydrogel were not tested after swelling cycling, though no defects in the material were observed after multiple cycles. This, however, was not true for hydrogels rapidly deswelled from 4°C to 90°C, where obvious blistering became apparent after 1 cycle, demonstrating the internal pressure associated with the hydrogel collapse could overcome the cohesive strength of the hydrogel between layers. These samples also showed a lower degree of change in the swelling volume compared to the samples cycled in DI water indicating the ions increased the rigidity of the alginate network.

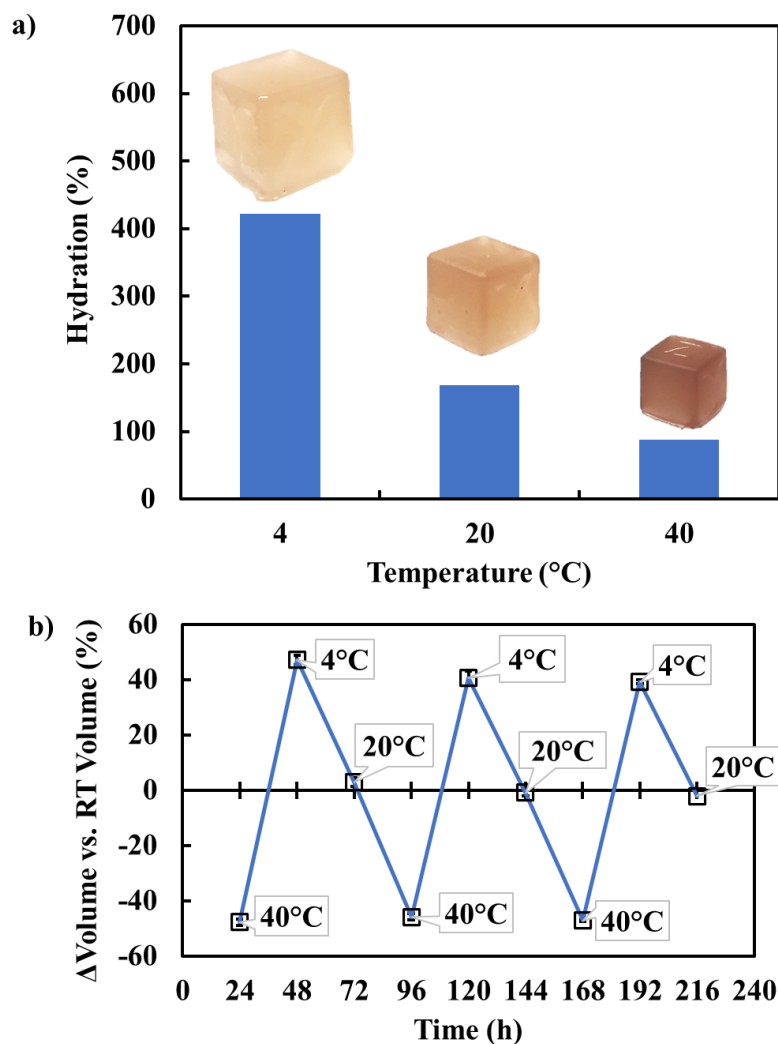


Figure 3.4 Reversible swelling/shrinking behavior of the hydrogel. a) hydration percentage of hydrogel samples equilibrated in 1M CaCl₂ at representative temperatures. b) cyclic swelling tests of 3D printed hydrogel cubes in DI water after post-curing salt treatment with error bars representing deviation over 10 replicates.

3.3.3. Thermoresponsive mechanical properties of 3D-Printed PNIPAm-Alginate

Nanocomposite Hydrogel Parts

To investigate the temperature-responsive behaviour of the 3D printed hydrogels, tensile and compression tests were performed at 4°C, 20°C and 40°C across the two LCSTs. It was found that the hydrogel had a high tensile strength in its collapsed state as shown in in Fig. 3.5. a). Initially tensile strength of the hydrogels was weak with a maximum tensile strength at 4°C of 71 kPa with a typical break point occurring at 60 kPa. At 20°C, the samples displayed an improved ultimate

tensile strength at 200 kPa (average break at 168 kPa) and an increased strain of 110%. This trend continued at 40°C with an ultimate tensile strength of 522 kPa (average break at 484 kPa) and strain of 220%. In addition to tensile properties, the hydrogel also demonstrates high compressive strength, as shown in in Fig. 3.5. b); the compressive strength of the hydrogel improved as the network collapsed. At 4°C the samples demonstrated a maximum compressive strength of 77 kPa, with cohesive failure occurring at 67% strain. At 20°C the ultimate compressive strength of the samples increased to 500 kPa as well as the ultimate strain for cohesive failure 79%. Finally, at 40°C, the samples displayed the greatest compressive strength at 6.3 MPa with no cohesive failure at 80% strain. These compressive properties are superior to other similar 3D printed systems PNIPAm systems when compared with those reported in the literature seen in Fig 3.5. b).

The observed significant increases in strength were attributed to the reduction in initial strain of the network resulting from the expelling of water at elevated temperatures along with the increased amount of hydrogen bonding present in the material. The hydrogen bonding, along with the ionic crosslinking of the Ca^{2+} ions, significantly improved the strength of the network. When the overall curve of the mechanical tests is examined, an interesting trend emerges. At both 4°C and 20°C, the samples do not display a difference in Young's modulus, yet the overall strain at break is increased. This indicates that much of the strength below the LCST of PNIPAm is due to the ionic interactions in the alginate network, while the PluDA enabled greater flexibility of the network. This translates into similar mechanical properties with a break earlier in the curve for more swollen samples. Note that this behaviour was not seen at 40°C, where there was a significant increase in stiffness and strength, indicating hydrophobic interactions of poloxamers in networks providing less strength than the PNIPAAm portion. Notably, the precursor displayed a 2-order of magnitude increase in tensile strength as shown in supporting information Figure S4 b); these

strength and stiffness are important in that they enable the printing of compliant hydrogel structures in geometries that would not bear the mechanical forces during printing, overcoming the technical hurdle that other compliant hydrogel resins struggled to achieve²³.

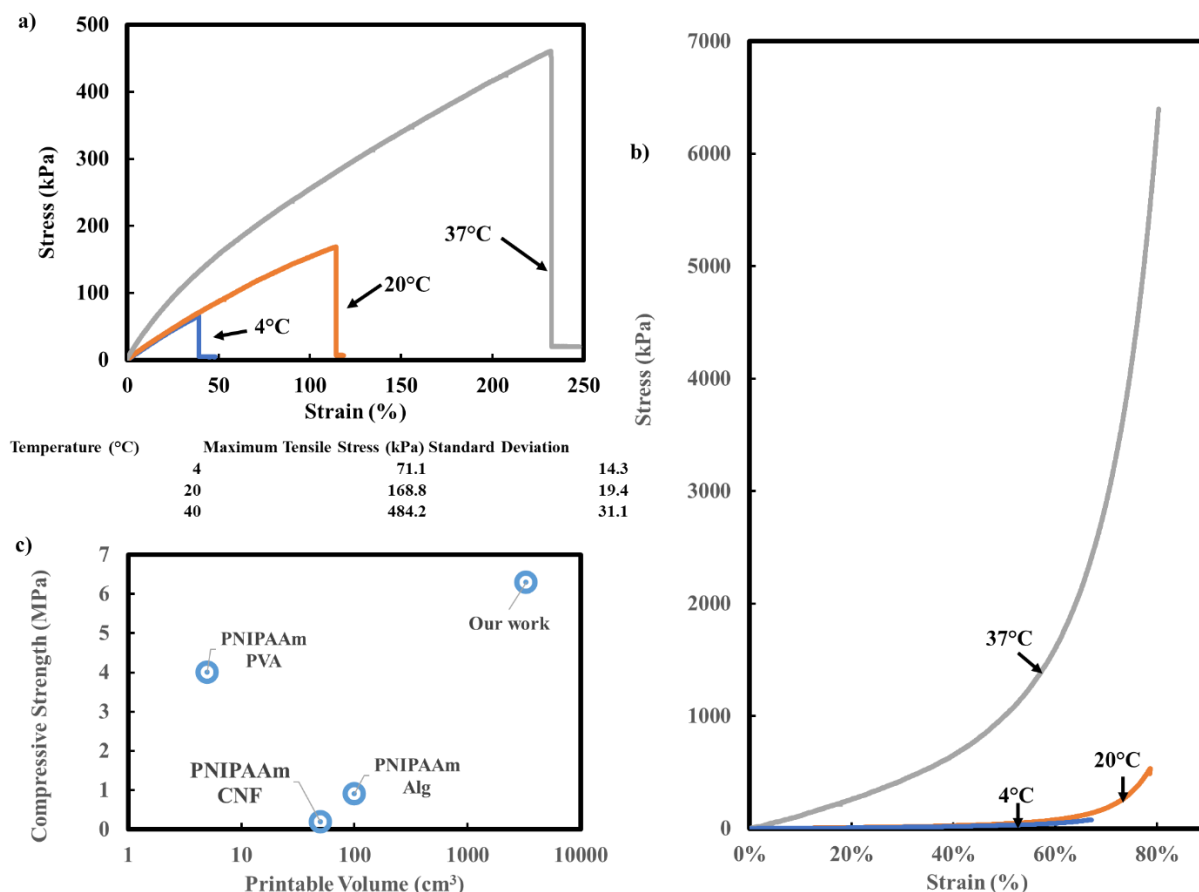


Figure 3.5 Mechanical properties of 3D-printed samples. a) tensile stress-strain curves at the representative equilibration temperatures with results of 5 replicates below. b) compressive stress-strain curves at the representative equilibration temperatures. c) A comparison of this work to other 3D printable hydrogels in literature.^{23,142,163}

3.3.4. Thermoresponsive Drug loading and release properties of PNIPAm-Alginate

Nanocomposite Hydrogel

To investigate the ability of the hydrogel to load and release drugs, a model drug, rhodamine b, was loaded by soaking a printed hydrogel cube in its water solution at 4°C. rhodamine B was chosen as a model water-soluble drug to demonstrate the loading of the hydrogel after synthesis and for its fluorescence. For the intended applications, antibiotics and painkillers

are highly desirable and generally demonstrate high water solubility; therefore, the model compound selected was water soluble. Fig. 3.6. A is a photo of the drug delivery test. At 37°C, it was found that the solution concentration significantly increased over the first hour, with about 25% of the loaded drug released, as seen in Fig. 3.6. B. Over the course of the measurement, the release rate of rhodamine consistently increased, but the rate of increase reduced in the first 5 hours as the release transitioned from pressure-driven due to the hydrogel collapsing to diffusion-driven when the hydrogel reached equilibrium. The release at 37°C displayed a significantly faster initial release with a slope of 41%/h compared to 27%/h and 14%/h for the 20°C and 4°C samples respectively. The consistent increase in the colour saturation of the solution is in the timescales similar to that associated with the use of a medical mask or wound dressing, indicating the applicability of the hydrogel medical mask. While these results indicate the applicability of this material for wound dressings in our tests the hydrogel is immersed in DI water equilibrated at the respective temperature and almost immediately reaches the test temperature. In practice, the actual release rate would be slower and more linear due to the thermal transfer only occurring on one side of the hydrogel; this would likely extend the period of hydrogel contraction-mediated release over a longer period. Additionally, the choice of model compound was not ideal due to interactions between the rhodamine b and alginate, trapping a large portion of the model compound in the hydrogel at low loading concentrations. It is likely that at physiologically relevant concentrations of loaded drugs, this interaction would be less of an issue (as the loading would be over 100mg/L). Furthermore, in realistic use cases, the wound site would be constantly absorbing released drugs, reducing the surface concentration and improving diffusion. In our drug release study, this was not accounted for as the complexity of the system made measurements difficult enough without exchanging the solution for fresh DI water and accounting for the total quantity of drug release

over multiple solution changes. Note that our work is distinguished by the strength of the hydrogel precursor Fig. S6 and the 2 distinct thermal response curves. The unique characteristics of this system make it well-suited for printing large thin-wound dressings. Furthermore, the strength of the hydrogel precursor enables the transport of printed hydrogels with little concern of damage or dehydration during shipping. This enables the hydration of the precursor to a viable mask or wound dressing with easily obtainable salts and loading the network with any desired hydrophilic drugs, a benefit that has significant uses in personalized medicine where the loaded drug can vary from one patient to the next. Additionally, they can be reloaded multiple times reducing medical waste.

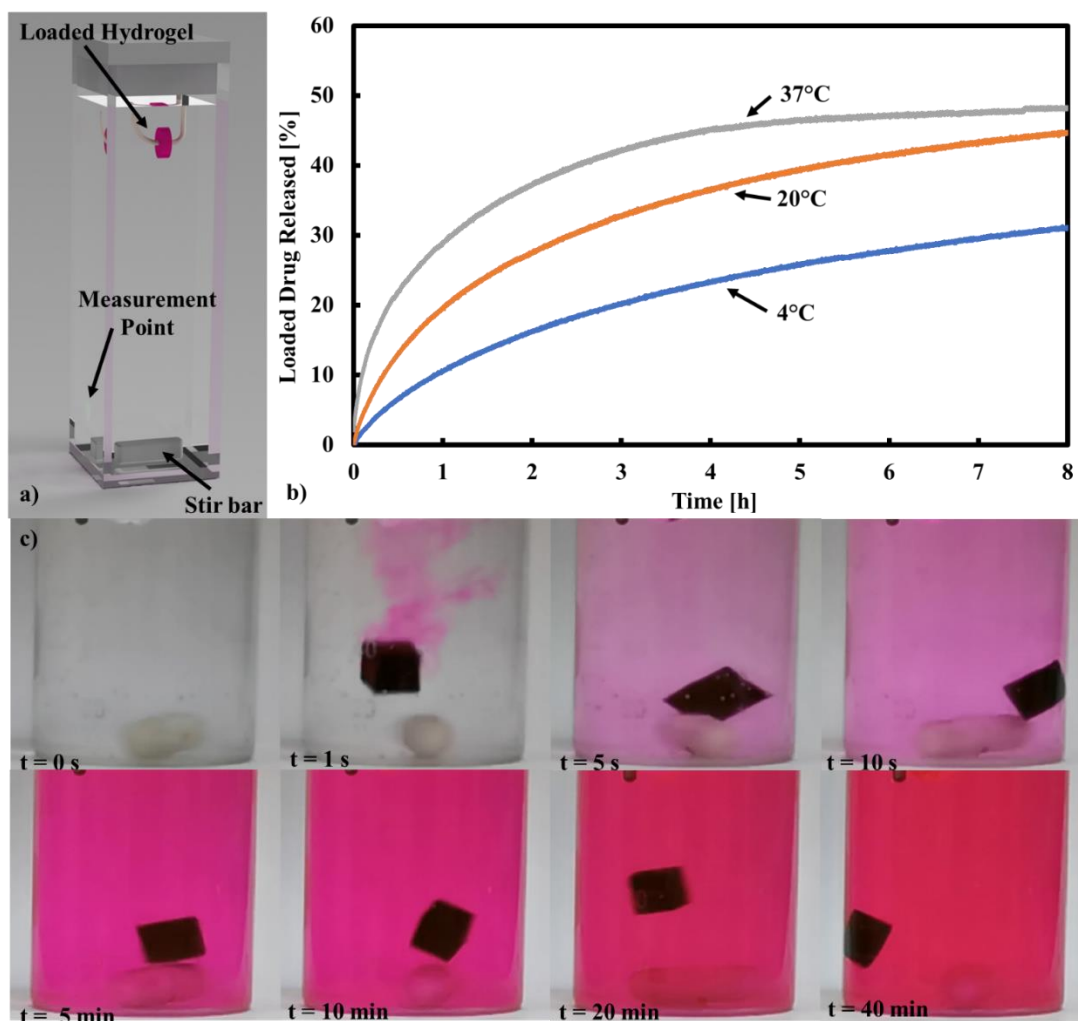


Figure 3.6 Drug release characterization of the hydrogel. a) schematic of the cuvette used for drug release measurements, b) % loaded drug release with respect to time. c) Time-series images of drug-releasing of NIP033 cube loaded with 1% rhodamine-b at 4°C placed in 100 ml of 37°C water

3.3.5. Thermoresponsive adhesion of the printed hydrogel

Finally, we investigated the adhesion properties of the printed hydrogel mask as a wound dressing. It is desirable for wound dressings to have self-adhesive properties so that no additional medical adhesives are needed. The adhesive wound dressing should also be readily peeled off as required. We found our hydrogel system can offer these desired features modulated by the natural body temperature. Supplementary Videos 1-3 and Fig. 3.7. demonstrate the thermoresponsive adhesion of the hydrogels. When in contact with dermal tissue at 4°C and 20°C (below the LCST of PNIPAm), the hydrogel showed little adhesion and detached from dermal tissue easily under acidic and basic conditions that would be seen in wound tissue (typical pH varies from 6-8 ¹⁶⁴) Fig. 3.7. c-f). This adhesion force increased upon contact with a natural dermal tissue at the body temperature of 36°C; there is no peeling during vigorous shaking and other motion gestures. Furthermore, the indentation results display around a ~300% increase in maximum pull-off force and a larger pull-off distance in Fig. 3.7. c-e) demonstrating the smart adhesion capabilities of the system. The mechanism for this improved adhesion is hypothesized to be due to both the shrinkage of the hydrogel and the conformation change of the surface composition, as shown in Fig. 3.7. b). As observed with the hydrogel cubes (Fig 3.4), the hydrogel volume shrank significantly at higher temperatures, which enables a “gripping” action resulting in a more intimate contact between hydrogel dressings and skin surface. Furthermore, there are likely some subtle conformation changes in the surface composition enhancing adhesion. At temperatures below the LCST of PNIPAm, the tannic acid-coated CNC are shielded by the hydrophilic PNIPAm portions, with few presented on the surface. When the temperature is raised above the LCST of PNIPAm, the network contracts and adopts a hydrophobic conformation. This change exposes the CNCs on the surface,

allowing the bound tannic acid groups to generate surface interactions through the numerous galloyl and catechol groups. This behaviour is particularly important for burn injuries where literature has shown the benefit of redressing burn injuries multiple times a day, but in practice, is limited by the discomfort associated with dressing changes¹⁶⁵. As such, the use of a wound dressing with switchable adhesion could facilitate frequent dressing replacements and hypothetically improve healing times with minimal waste, as 2 hydrogel dressings could be cycled rather than a fresh dressing for each use. Furthermore, at skin temperatures, we observed that delamination occurred continuously rather than spontaneously; this indicates the adhesion is not a result of capillary forces but rather adhesive interactions between the two surfaces. These adhesive characteristics and other smart properties demonstrate the hydrogel's potential as a customizable smart dressing for personalized medicine, which needs further investigation.

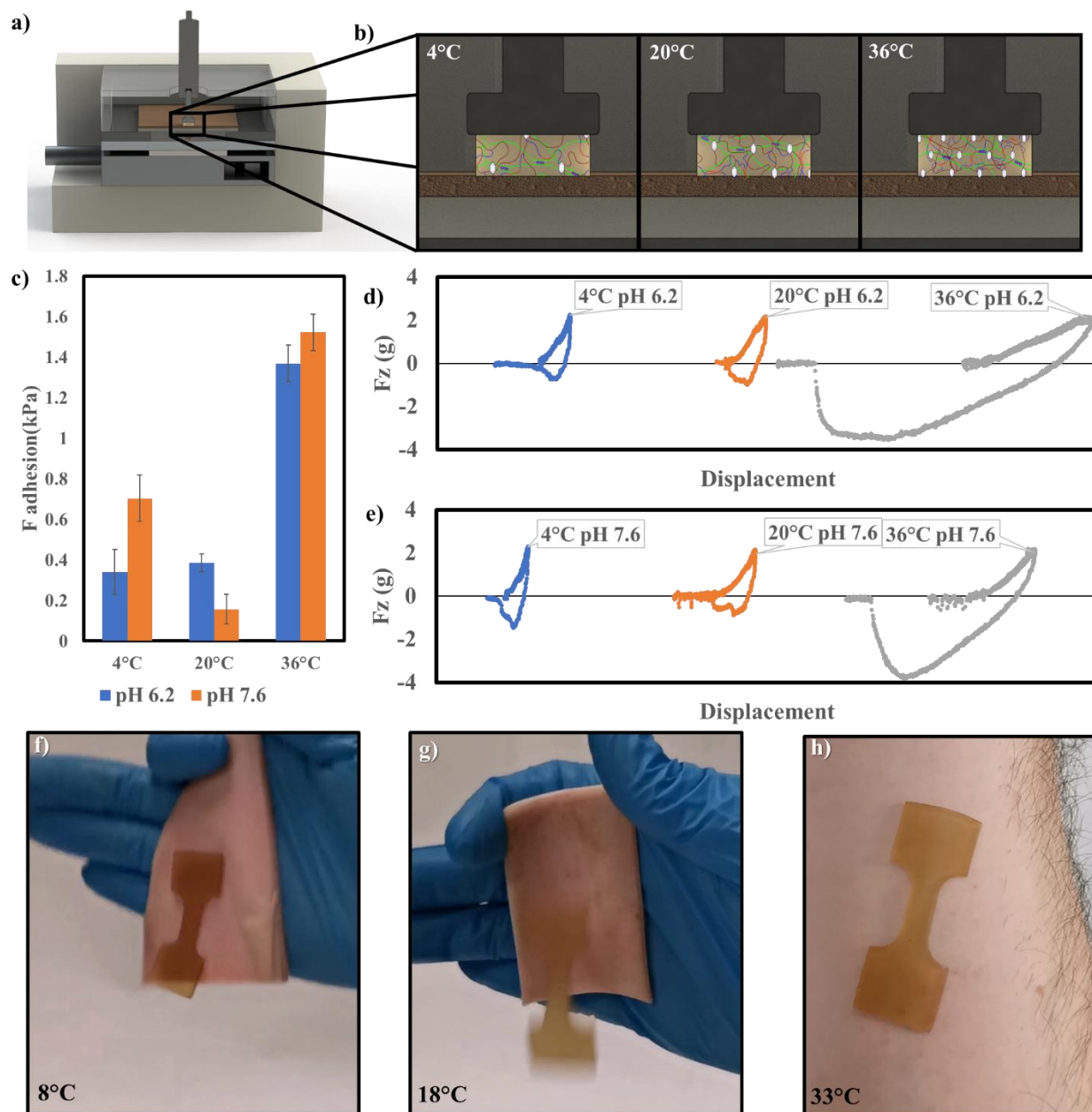


Figure 3.7 Adhesion of hydrogel on skin at various temperatures: a) 3D rendering of the temperature-controlled adhesion chamber. b) Schematic of how surface composition changes enabling temperature responsive adhesion. Above the LCST of PNIPAm, the surface conformation changes exposing the tannic acid coated CNCs. This enables the galloyl and catechol groups to interact with the skin surface. c) Summary of adhesion tests at 3 temperatures and 2 pH levels with 3 replicates each. d) Representative loading and unloading curves of the hydrogel probe on skin at pH 6.2. e) Representative loading and unloading curves of the hydrogel probe on skin at pH 7.6. f) Low adhesion to porcine skin at 8°C. g) Low adhesion to porcine skin at 18°C. h) High adhesion to human skin at 33°C. Images are frames from Supplementary Video 1.

3.4. Conclusions

We report the development of a thermoresponsive hydrogel precursor capable of large format printing with high fidelity and the investigation of its thermoresponsive properties. The hydrogel is composed of a majority of PNIPAm providing the thermoresponsive collapse of the hydrogel network seen around body temperature. A second LCST was incorporated into the polymer through the addition of a poloxamer crosslinker consisting of diacrylate functionalization of Pluronic P123, providing a sharp collapse at 8.9°C. It was found that the hydrogel displayed significant changes in strength as it collapsed as expected with PNIPAm-based hydrogels, and the 2 thermoresponsive components would display 2 LCSTs rather than a single shifted LCST. This behaviour allowed for a significant increase in the usability of these hydrogel structures at room temperature mitigating the fragility associated typically with swollen PNIPAm hydrogels. Additionally, the collapses associated with each respective LCST were shown to be repeatable with little change in swelling volume over multiple cycles. We found the resin could be used to print full-scale hydrogel medical masks with thermoresponsive release of a hydrophilic drug analog, demonstrating their capability as a loadable patient-specific wound dressing. Furthermore, we demonstrated the desired thermoresponsive switchable adhesion of the printed hydrogel on the skin. Above the LCST of PNIPAm, the hydrogel fully collapses, triggering a change in surface composition and exposing tannic acid-coated CNCs in addition to shrinkage. In this state, the hydrogel demonstrated a >300% increase in force of adhesion compared to room temperature. The use of tannic acid-modified CNCs in thermoresponsive systems for temperature-sensitive adhesion would simplify synthesis requirements compared to other block copolymer systems¹⁶⁶. Further research on this system would involve post print modification of the hydrogels to incorporate antimicrobial nanoparticles in-situ and the tuning of the resin formulation for improved strength.

Chapter 4: Quaternized Chitosan Supramolecular hydrogels ²

4.1. Introduction

Hydrogels, characterized by their hydrophilic polymer networks, are integral to various applications including tissue engineering^{4,167,168}, drug delivery^{168–171}, and soft robotics^{142,148,158}. These networks, formed through physical, ionic^{66,154,157,172}, or chemical crosslinking^{62,158,163,173} and excel in retaining substantial water quantities. Traditional hydrogel fabrication, largely limited to casting methods, has been significantly advanced with the development of 3D printing technologies. This progression has unlocked the potential for complex, precision-engineered hydrogel structures^{4,146}, surpassing the capabilities of conventional methods. Such structures are increasingly required in the fields of soft robotics and tissue engineering, where composite structures are desirable due to the unsuitability of a single material.

Hydrogel 3D printing has primarily adopted two methodologies. The first one is extrusion-based printing, which involves the deposition of hydrogel ink through a nozzle, forming structures subsequently stabilized by crosslinking. This method has the advantage of multi-material compatibility and diverse crosslinking capabilities¹⁷⁴ (physical, chemical, ionic) but is nonetheless limited by the ink's rheological properties and the resolution constraints of nozzle extrusion¹⁵⁹. The second approach is the vat polymerization, which constructs 3D hydrogel structures layer-by-layer within a bath. It encompasses three technologies: stereolithography (SLA), using a laser guided by mirrors; mask stereolithography (mSLA), employing an LCD mask; and digital light projection (DLP), utilizing a micro-mirror array. While vat polymerization achieves finer

² This chapter was partially reproduced from Conductive Supramolecular Hydrogels Enabled by Quaternized Chitosan: Acrylate Ionic Crosslinking for High Fidelity 3D Printing submitted to Carbohydrate Polymers, 2024

resolutions (2-40 μm) compared to extrusion, it demands a high degree of crosslinking to counteract the mechanical stresses of printing⁴. Advances in this field have demonstrated that multiple-material printing is possible with photopolymerization-based systems, but the mechanical limitations of current 3D printable hydrogel formulations prevent the development of all hydrogel composites. Such composites would be highly desirable for applications in tissue engineering, soft robotics and soft tissue prosthetics, where precise control over the structure would enable precise emulation of soft tissues, a common goal in bio-mimetic research.

Chemical crosslinking is common in vat polymerization to achieve high resolutions but tends to yield structures with limited elasticity due to high crosslink density or weak structures with high elasticity and high deformation¹⁰. For instance, Ge et al.¹⁷⁵ and Zhang et al. explored hydrogels composed of acrylamide and low-concentration poly(ethylene glycol) diacrylate (PEGDA)¹⁴⁶, achieving ultimate tensile strengths of around 40 kPa. These hydrogels demonstrated enhanced elasticity but were limited in strength and prone to deformation during printing. Furthermore, 3D printable conductive hydrogels provide additional limitations due to the need for conductive additives. Heo et al. demonstrated conductive gelatine methacrylate (GelMA) poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) hydrogels manufactured with SLA techniques and were able to achieve a high compressive strength of 13 MPa, with a maximum resolution of 500 μm ¹⁷⁶. Wu et al. demonstrated conductive hydrogels composed of GelMA and polyaniline prepared with DLP techniques demonstrating 100 μm resolutions¹⁷⁷ with limited mechanical characterization.

In contrast, extrusion-based methods, as demonstrated by Zhou et al., have successfully produced hydrogels with superior mechanical properties. Their work involved a hydrogen bond

crosslinked hydrogel comprising N-(pyridin-2-yl)acrylamide and methacrylic acid, which exhibited ultimate tensile strengths exceeding 4 MPa¹⁷², showcasing the potential of extrusion techniques in fabricating robust hydrogel structures.

This research addresses the need for alternative crosslinking strategies in vat polymerization of hydrogels to balance mechanical integrity with elasticity. We introduce a novel polymeric salt-based crosslinker, inspired by Sashiwa et al.'s study on chitosan solubilization in polar aprotic solvents¹⁷⁸, through the use of acidic monomers to solubilize quaternized chitosan and enable crosslinking. We employ a quaternized chitosan (QCh) and 3-sulfopropyl acrylate (SPA) system to form poly(ionic) salts that act as crosslinkers. We hypothesize that the quaternized chitosan will form a salt with 3-sulfopropyl acrylate that is soluble but does not disassociate in the polar aprotic prepolymer resin solution. This will enable the photopolymerization printing of an organo-gel at high fidelity and aspect ratios that upon solvent exchange with water forms a hydrogel with characteristics of ionically crosslinking. This strategy aims to enhance the hydrogel's elasticity without compromising their mechanical strength during printing, along with the poly(ionic) hydrogel network adding conductive properties^{179–182}. We further explore the influence of pH and ion ratios on the final mechanical properties, utilizing pTsOH with a strong anionic acrylate to modulate anion density without impacting pH. Furthermore, while our previous work utilized dimethyl sulfoxide (DMSO) for synthesis of the organo-gel precursor¹⁸³, in this work we introduce Cyrene as a sustainable alternative to DMSO¹³⁴ for synthesizing organo-gel precursors. Similar in solvating properties, Cyrene has demonstrable non-toxic properties and a lack of the transdermal transport behavior DMSO is known for¹⁸⁴. Thus, the reported material herein advances the field towards environmentally friendly, photopolymerization based 3D printable hydrogels.

4.2. Materials and Methods

4.2.1. Materials

2-hydroxyethyl acrylate (HEA) (Sigma Aldrich), 3-sulfopropyl acrylate potassium salt (SPA:K), hexane (Sigma Aldrich), diethyl ether (Sigma Aldrich), Irgacure 819 (BASF), CInd™ Chitosan Quaternary Ammonium Salt (QCh:Cl) (CD Bioparticles)(M_w = 550 kDA by viscosity, 90% degree of deacetylation and 99.7% substitution by acid base titration[confirmed by AgNO_3 titration] product information provided by supplier) Cyrene (Sigma Aldrich), Irgacure 2959 (Sigma Aldrich), SSTC60 (Purolite), Amberlyte a26 (Dupont), sodium hydroxide (Sigma Aldrich), phosphoric acid 85% (Sigma Aldrich), para-toluene sulfonic acid (pTsOH)

4.2.2. Resin formulation

All resins were formulated on a weight basis of 2.5g QChCl per 100 g HEA, with an equimolar amount of QChOH weighing 2.195 g. For QCh:SPA:pTsOH (1:1:0) 4.056g of the final salt contained an equivalent number of moles of QCh as 2.5g of QChCl a comprehensive list of amounts of QCh and SPA in each salt can be found in Supp. Table 1. In a typical synthesis of the QCh:SPA:pTsOH (1:1:0) resin, 100g of purified 2-HEA was homogenized with 4.056g of QCh:SPA:pTsOH (1:1:0) salt at 10000 rpm for 5 minutes to form a translucent slurry. At which point 12.5 g of Cyrene was added and homogenized for 5 minutes, followed by sonication for 5 minutes at 100% amplitude [Q125 ¼” probe (Qsonica, USA)], at which point the solution turned from a translucent slurry to a viscous clear yellow liquid. This was followed before the addition of 1 g Irgacure 819. Finally the prepolymer resin was mixed (2000 rpm) and degassed (2200 rpm) in an ARE-310 (Thinky, USA) planetary mixer before printing.

4.2.3. 3D Printing

All samples tested in this paper were printed with a Phrozen 4K Sonic XL (Phrozen, Taiwan) resin printer, with a 7000 ms exposure time per 50 μ m layer. The supporting information detailed print time optimization.

4.2.4. Post-print processing

Following printing, the organo-gels were rinsed with ultrapure water (UPW) followed by isopropanol. Samples were then heated to 80°C for 10 minutes to evaporate IPA and aid separation from the print stage. Samples were cured for 10 minutes under nitrogen in a purpose-built 300 W curing chamber outputting 365-405 nm. Following post-curing, the samples were equilibrated in UPW (Impedance = 18.2 M Ω) for 72 hours, exchanging every 24 hrs.

4.2.5. QCh:SPA Degradation study by NMR Spectroscopy

All samples analyzed utilized Neutral QCh:SPA:pTsOH (1:1:0) formulation as it was the most representative formulation. Water-containing samples followed weight ratios in the resin formulation section, substituting water for Cyrene and Irgacure 2959 for Irgacure 819. Samples were cast in square Sylgard 184 moulds and cured using the same curing and solvent exchange parameters as other formulations.

The Cyrene sample was prepared as described in the 3D printing section. Degradation was achieved by soaking 5g of hydrogel in 0.1 M KOH in methanol (50 ml) for 24 hours. Followed by neutralization with 1 M aqueous HCl and dialysis (14,000 MWCO) against UPW for 72 hours before freeze-drying. The dried products were then re-dispersed in d4 MeOH, D2O, or d6 DMSO at 10 mg/mL. Prior to NMR, the solutions were centrifuged at 10,000 g, then filtered with a 0.2 μ m PTFE filter to remove undissolved solids.

4.2.6. Rheometric and Mechanical measurements

Viscosity measurements utilized a CAP 2000+ viscometer (Amtech Brookfield, USA) and 1H spindle. Measurements were performed at 22°C with 200 µL and a hold of 60 seconds. Rheometric measurements were performed on a Rheospectrus C500+ (Rheolution, Canada) with 1mm thick printed sheets of hydrogel with standard post-processing punched into a 25.4 mm diameter disk. During measurement, samples were sparingly coated with 1000 cP silicon oil to prevent evaporation. Tensile tests (strain rate .5 mm/s) and compression tests (strain rate .5 mm/s) were performed on a CETR-UMT (Bruker, USA). Printed Tensile coupons and cube specimens were used for tensile and compressive tests respectively.

4.2.7. Electrical measurements

Conductivity measurements were performed on a SP-300 potentiostat (BioLogic Scientific Instruments, France) with a 4-point holder (Cascade Microtech, USA) and C4S 57/1S 3716 probe (Cascade Microtech, USA). During EIS, measurements utilized a 4 V bias at the working electrode. Conductivity was calculated from the bulk resistivity of a semi-infinite volume; the equations for the calculation are provided below. Impedance measurements of the liquid solutions were performed with a purpose-built liquid chamber seen in Fig. 4.1.

Equation 4.1 Conductivity equation

$$\partial = \frac{1}{\rho}$$

Equation 4.2 bulk resistivity equation

$$\rho = \frac{V}{I} \cdot F_0(s) \cdot F_1\left(\frac{t}{s}\right) = 2\pi s \cdot F_1\left(\frac{t}{s}\right) \cdot \frac{V}{I}$$

Equation 4.3. Thickness correction factor

$$F_1 = \frac{t/s}{\ln \left[\frac{\sinh\left(\frac{t}{s}\right)}{\sinh\left(\frac{t}{2s}\right)} \right]}$$

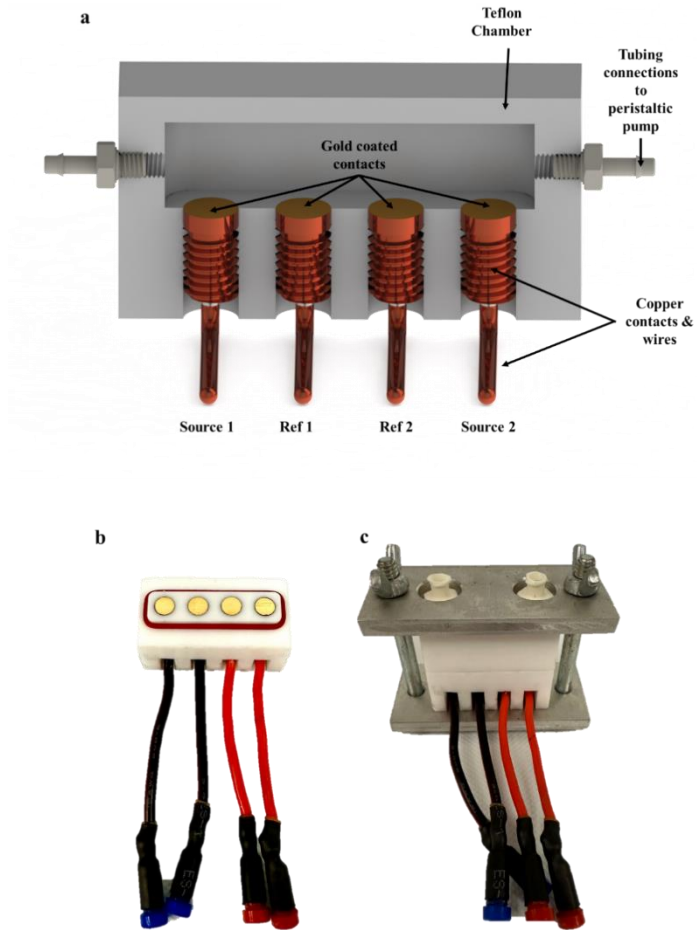


Figure 4.1 Impedance measurement cell. a) Cross-sectional 3D Model of 4-point probe liquid cell. b) 4-point probe. c) Fully assembled 4-point probe liquid cell

4.2.1. Characterization of quaternized chitosan chloride salt degree of substitution

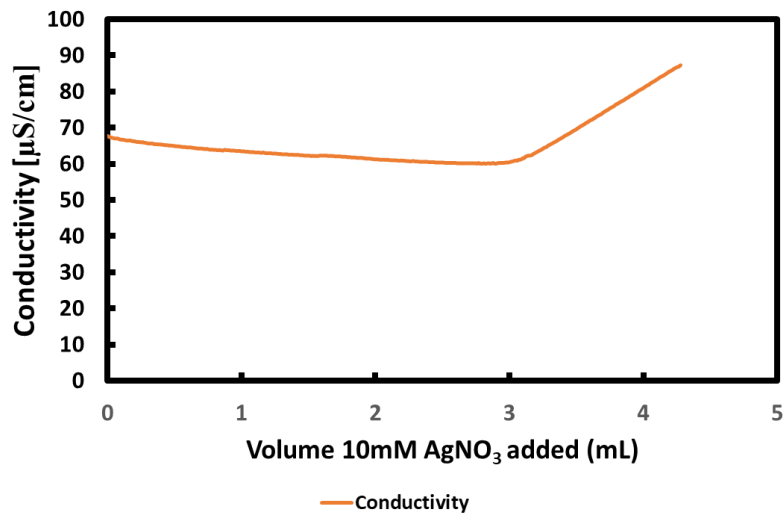


Figure 4.2 Conductometric titration of CInd™ Chitosan Quaternary Ammonium Salt with AgNO₃

The degree of substitution was calculated based on a standard silver nitrate conductometric titration. 12 mg of CInd™ Chitosan Quaternary Ammonium Salt was dissolved in 50ml of ultrapure water and titrated with 10 mM AgNO₃. The minimum was found to be 2.76 ml, which was converted to the degree of substitution using the following equation. V is the volume of solution used, c is the concentration, W is the weight of the sample being tested, and 314 is the weight of the modified glucosamide, with 162 being the weight of the unmodified glucosamide unit.

Equation 4.4.¹⁸⁵

$$DS = \frac{V_{AgNO_3} * \frac{c}{1000}}{\frac{c}{1000} + \frac{W - V * c * \frac{314}{1000}}{162}} * \frac{1}{DDA} * 100\%$$

The measured degree of substitution was calculated to be 100.7% which is similar enough to the reported degree of substitution (99.7%) to be acceptable. Overestimation of the degree of substitution is likely due to the experimental error in preparing the silver nitrate solution or human error in the determination of the onset point. Additionally, the degree of deacetylation could be slightly higher than reported by the manufacturer, leading to a degree of substitution greater than 100% since the material would have a higher number of deacetylated groups per gram than specified. For this material, there was no way to measure the degree of deacetylation as the material was received in a highly substituted form with no deacetylated groups visible in the NMR spectra reported below. As such, the only value provided for the degree of deacetylation was that provided by the manufacturer.

4.2.2. Synthesis and characterization of QCh:SPA

Q-Chitosan/acrylate salt synthesis

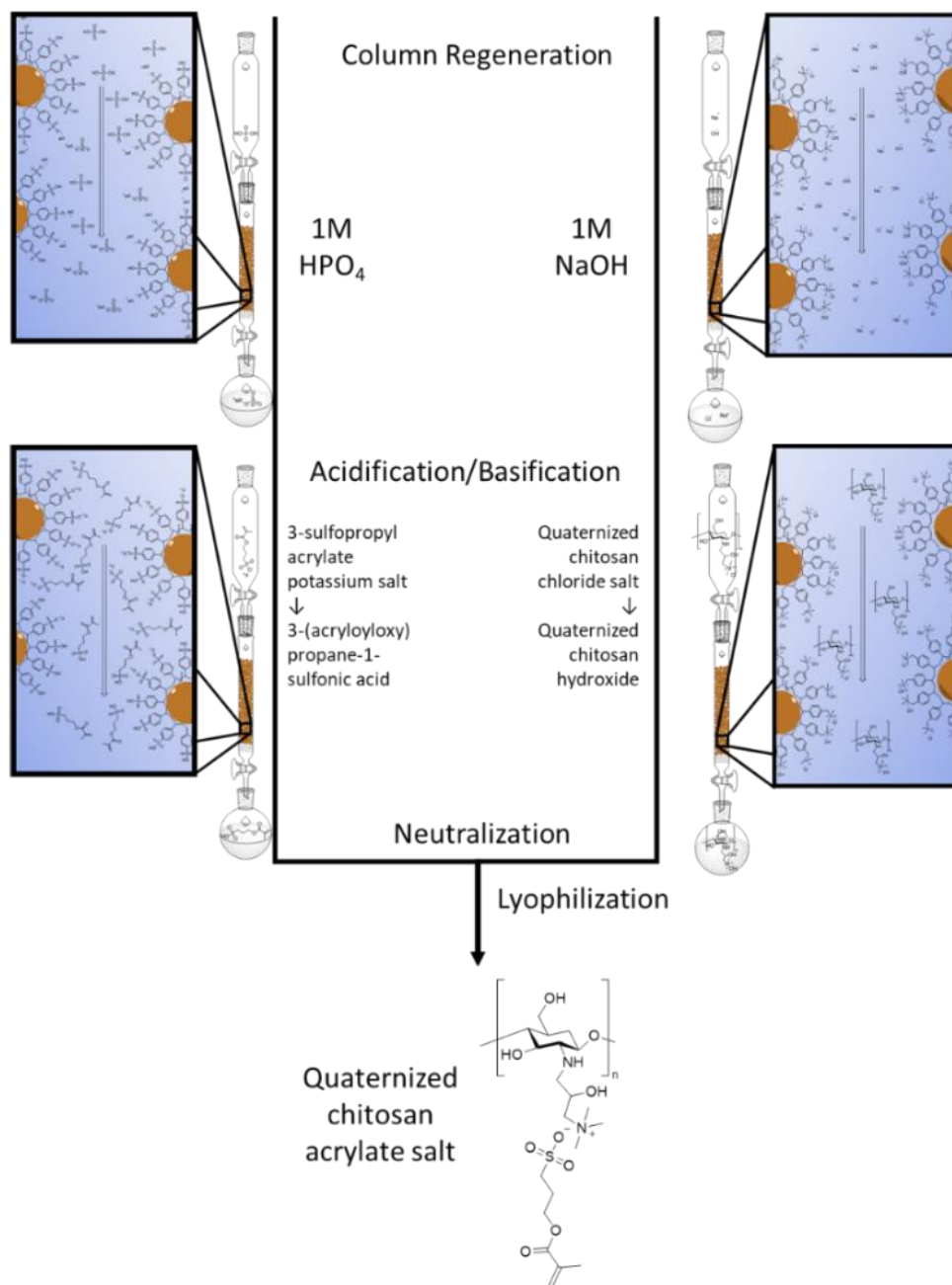


Figure 4.3 Schematic of salt synthesis.

To form a salt from Quaternized chitosan and 3-sulfopropyl acrylate potassium salt, the respective base and acid forms must first be formed by passing the materials through a cationic and anionic resin respectively [Fig. 4.3.]. Both columns were 300 mm in length with a 30mm i.d.

fed by a 500 ml reservoir at atmospheric pressure. The cationic column consisted of Amberlyte a26 converted to its OH form by passing 250 ml of 2 M NaOH through the column and rinsing until neutral before use. The anionic column consisted of purolite SSTC60 converted to its acid form by passing 250 ml of 10% wt phosphoric acid through the column and rinsing until neutral before use. This process was also used to regenerate each column between purifications to ensure the highest conversion achievable.

In a typical synthesis 15 g QChCl powder was dissolved in 700 ml of ultrapure water and passed through the cationic column followed by ultrapure water, until the product measured pH 8. Next 15 g of 3-Sulfopropyl acrylate potassium salt (SPA) was dissolved in 50 ml of ultrapure water and passed through the anionic column forming a 3-(acryloyloxy)propane-1-sulfonic acid (SPAA) solution followed by ultrapure water, until the product measured pH 6.

Table 4.1 Ideal weights and yields of QCh:SPA salts used for each resin formulation

Weight Ratio QCh:SPA 1:1	Molecular Weight	Weight (g)	% Yield	[QCh:SPA]:HEA (g:g)
QChCl (repeat unit)	330.81	2.325		
QChOH (repeat unit)	312.37	2.195		
SPA	232.29	2.500		
SPAA	194.2	2.090		
QCh/SPA		4.285	0.992	0.851:20
Weight Ratio QCh:SPA 1:0.75				
QChCl (repeat unit)	330.81	2.325		
QChOH (repeat unit)	312.37	2.195		
SPA	232.29	1.875		
SPAA	194.2	1.568		
QCh/SPA		3.763	0.997	0.750:20
Weight Ratio QCh:SPA 1:0.5				
QChCl (repeat unit)	330.81	2.325		
QChOH (repeat unit)	312.37	2.195		
SPA	232.29	1.250		
SPAA	194.2	1.045		
QCh/SPA		3.240	0.989	0.641:20
Neutral QCh:SPA:pTsOH 1:1:0				
QChCl (repeat unit)	330.81	2.325		
QChOH (repeat unit)	312.37	2.195		
SPA	232.29	2.225		
SPAA	194.2	1.860		
QCh/SPA		4.056	0.998	0.809:20
Neutral QCh:SPA:pTsOH 1:0.5:0.5				
QChCl (repeat unit)	330.81	2.325		
QChOH (repeat unit)	312.37	2.195		

SPA	232.29	1.113			
SPAA	194.2	0.930			
pTsOH	172.2	0.825			
QCh/SPA			3.950	0.990	0.782:20
Neutral QCh:SPA:pTsOH 1:0.25:0.75					
QChCl (repeat unit)	330.81	2.325			
QChOH (repeat unit)	312.37	2.195			
SPA	232.29	0.556			
SPAA	194.2	0.465			
pTsOH	172.2	1.236			
QCh/SPA			3.898	0.989	0.771:20

Neutralization

Neutralization was performed under a weight ratio or a neutral endpoint depending on the salt formed. For weight ratio salts, the weight ratio used consisted of the weight of the quaternized chitosan chloride salt to the 3-sulfopropyl acrylate potassium salt. For example, in the case of the 1:1 QCh:SPA salt, all 50ml of SPAA solution was used to neutralize the QChOH solution. For salts formed to a neutral endpoint, the SPAA solution was added to the QChOH solution slowly under stirring until a pH of 7.00 was measured by a digital pH meter (Thermo Fischer Scientific). The same method was used to form salts partially neutralized with pTsOH by splitting the QChOH solution in 1:1 or 1:3 ratios, then neutralizing each respective solution with the SPAA solution and 10 wt% pTsOH before recombining. All salt solutions formed were then flash-frozen in liquid nitrogen, followed by freeze-drying.

4.2.3. Preparation of poly(ionic) salt samples for NMR

For the NMR characterization of QCh:SPA salt, including its reactants, intermediates, and final product, samples were prepared and analyzed in deuterated water (D₂O). Based on prior observations, the intermediate acid and base are known to be stable in aqueous solutions but are prone to degradation when dried. To mitigate this, we employed a distinct set of ion exchange

columns designed for this purpose. Each column was constructed from a 25 ml glass serological pipette, with the tip removed and the neck packed with glass wool for filtration. The ion exchange resin was regenerated following a standard protocol, then rinsed with isopropanol and dried in a vacuum oven at 80°C under a pressure of -25 mmHg for four hours. Subsequently, the resin was rehydrated with D₂O overnight.

The ion exchange process involved the passage of 10 ml each of quaternized chitosan chloride (QChCl) for the cationic column and sulfopropyl acrylate (SPA) for the anionic column, both dissolved in D₂O at a concentration of 2.5 wt%. A portion of the resultant solutions was used to synthesize the neutral QCh SPA:pTsOH (1:1:0) salt, with the rest reserved for analytical purposes. All NMR spectra were acquired using a Bruker 300 MHz high-resolution NMR spectrometer.

4.2.4. Confirmation of Salt composition by NMR

NMR spectra were performed at each stage of the salt synthesis. From the spectra seen in Fig. 4.4, we can conclude that there is no significant change in the structure of SPA (Fig. 4.4. a) when converted to SPAA (Fig. 4.4. b) as with QChCl (Fig 4.4. c) when converted to QChOH (Fig. 4.4. d). It should be noted that the prominent peaks present at 1.00 ppm and 3.86 ppm indicate an isopropanol impurity, indicating the incomplete removal of the solvent used to dry the resin before rehydrating in D₂O. To confirm the impurity, the NMR of the rinse solution used to rehydrate the ion exchange resins was performed, as seen in Fig. 4.5. isopropanol was evident in both the SSTC 60 and A26 rinse solution, confirming the contaminant was not generated during the process. Unsurprisingly, there was no measurable difference between the reactant and intermediate due to the nature of the H and OH groups of SPAA and QChOH, respectively, to be exchanged with

deuterium in the D₂O, hiding the peak of interest. Finally, when observing the spectra of the QCh SPA:pTsOH (1:1:0) synthesized in D₂O and the sample dried and redissolved in D₂O, no difference is noticeable except the isopropanol contamination. These results confirm the salt formation described in the methods section and the presence of isopropanol in the D₂O columns, as the product formed from the water columns does not contain the isopropanol peak.

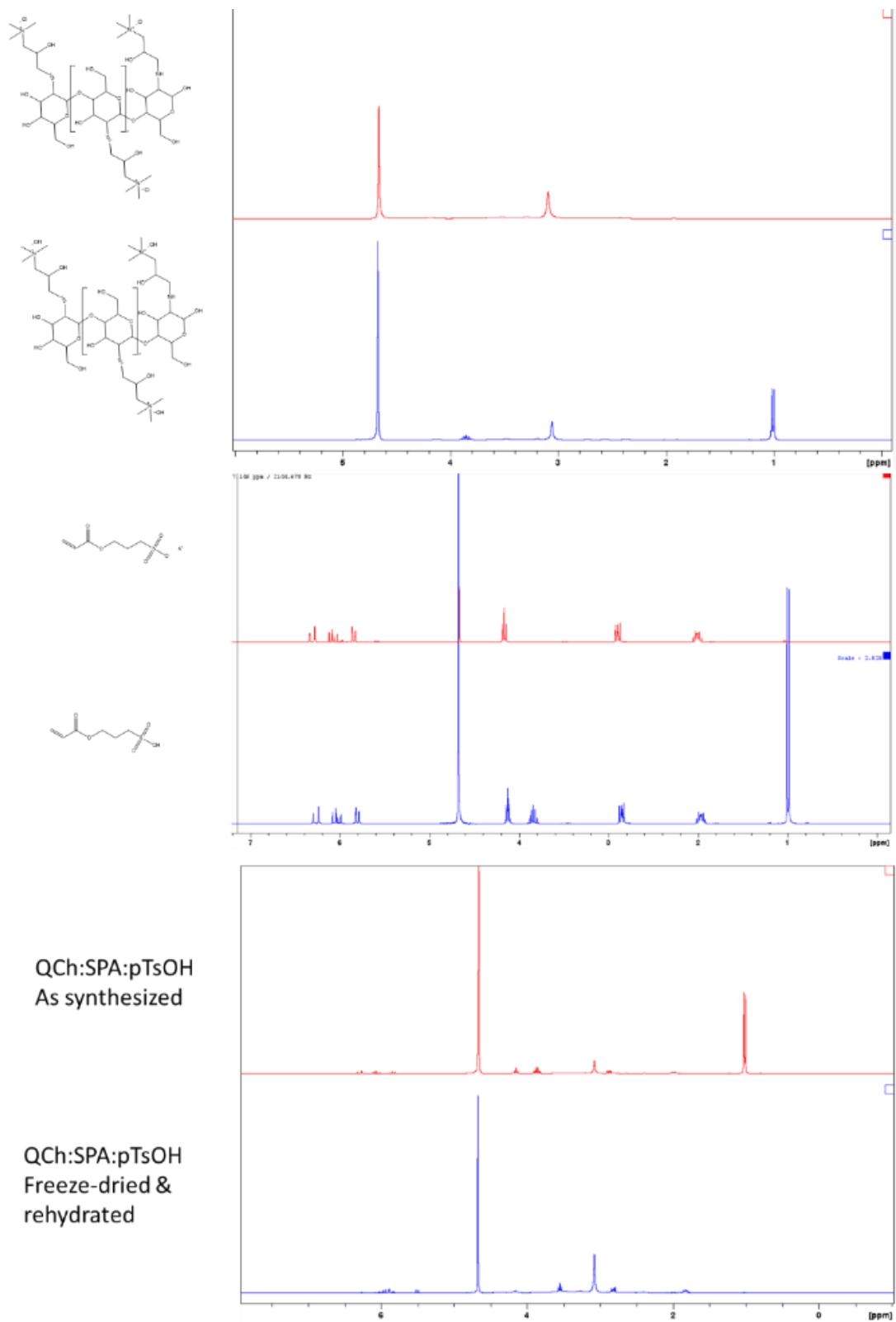


Figure 4.4 NMR spectra of the reactants intermediates and final salt products synthesized in D_2O .

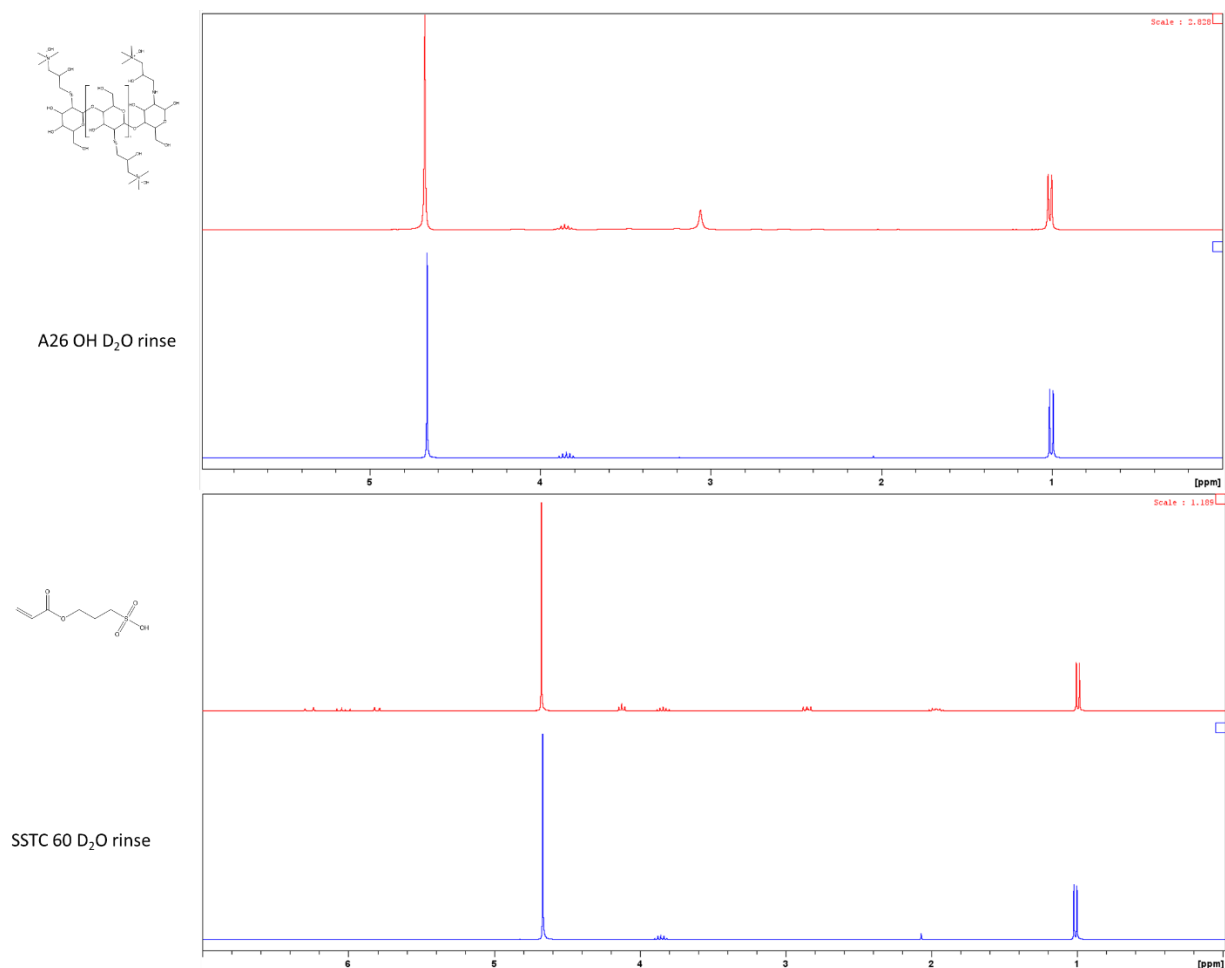


Figure 4.5 NMR spectra of D₂O used to rehydrate the a) A26 OH resin and b) SSTC H 60 compared the NMR of the respective column product, confirming isopropanol in the synthesis NMR was a result of impurities from the water removal step.

4.3. Resin composition and characteristics

Monomer Purification

Before use, 2-hydroxyethyl acrylate was purified through sequential washings to remove ethylene glycol diacrylate and acrylic acid. First, a 25 wt% solution of HEA in water was washed with hexane four times to remove ethylene glycol diacrylate impurities, followed by salting with NaCl at 200g/L and four subsequent washes with diethyl ether to remove HEA. The diethyl ether phase was then dried with magnesium sulphate and transferred to a rotary evaporator at 35°C to remove the diethyl ether. The purified HEA was stored at -20°C over molecular sieves before use.

Printing Parameter Calculation

Initial print parameters were calculated with an exposure working curve Fig. 4.6. Penetration depth (D_p) and critical exposure energy (E_c) were determined with a logarithmic line of best fit and Eqn. 4.5-7

Equation 4.5 Calculation of Penetration Depth (D_p).

$$C_d = D_p \ln\left(\frac{E}{E_c}\right) = 267.6 \ln(E) - 392.3$$

Equation 4.6 Limits to define the critical exposure energy (E_c).

$$C_d = 0 = D_p \ln\left(\frac{E}{E_c}\right) \rightarrow \ln\left(\frac{E}{E_c}\right) = 0, \quad E = E_c$$

Equation 4.7 Calculation of critical exposure energy (E_c).

$$E_c = e^{\frac{392.3 \frac{mJ}{cm^2}}{267.6 \mu m}} = 4.332 \frac{mJ}{cm^2}$$

Equation 4.8. Critical exposure energy

$$E_c = D_p * D_c * 10^{-4}$$

Equation 4.9. Critical exposure dose

$$D_c = \frac{E_c}{D_p * 10^{-4}} = \frac{4.33185 \frac{mJ}{cm^2}}{267.6 \mu m * 10^{-4}} = 161.9 \frac{mJ}{cm^3}$$

Equation 4.10. Exposure time for specified layer thickness (d_l)

$$t_{Exp} = e^{\frac{d_l}{D_p}} * \frac{E_c}{E_m} * f * 10^3$$

Eqn. 4.10. along with an exposure test Fig. 4.7 determined optimized layer exposure times. For a 50 μm layer, 1700 ms was calculated, while Fig. 4.7. suggests 7000 ms as ideal for a 50 μm layer due to layer delamination. Additional print settings can be found in Fig. 4.8.

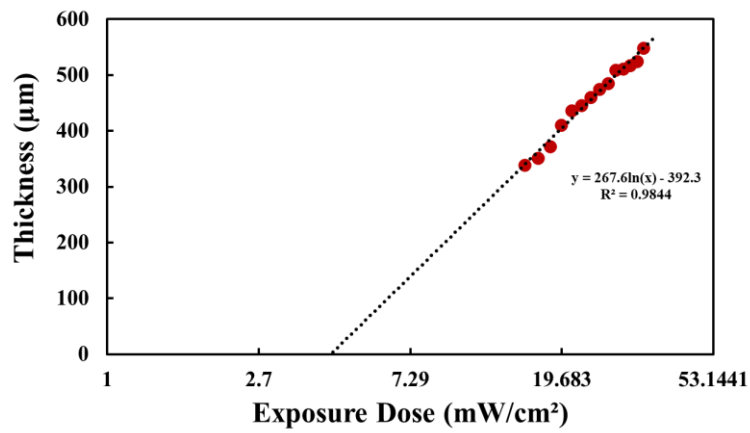


Figure 4.6 Exposure working curve results.

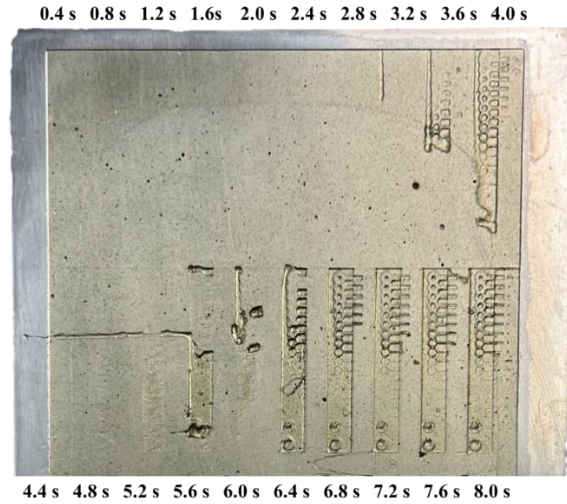


Figure 4.7 Multiple exposure test for single layer with 6.8s demonstrating suitable adhesion to preceding layer. Bubbles evident in the test were due to a lack of degassing before printing.

50 Micron

Name
Name
0001 HEA QcSPA Cyrene

Resin properties
Price per liter 50.00 EUR
Density in gram per milliliter 1.00 [gr/ml]
Color

Layer thickness Movement & exposure Part pre processing Slice post processing

Setup
☒ Split movement in 2 stages

Bottom layers 0
Exposure transition layers 0

Normal layers
Exposure time Calculate 7000 [ms]
Rest time after retract / off time 2500 [ms]
Rest time before lift 0 [ms]
Rest time after lift 0 [ms]
Z Lift Distance 3.00 10.00 [mm]
3.00 10.00 [mm]
Z Lift speed (peel) 50 150 [mm/min]
Z Retract speed (back in resin) 50 150 [mm/min]

Bottom layers
Exposure bottom layers 7000 [ms]
Rest time after retract bottom layers / off time 1000 [ms]
Rest time before lift bottom layers 0 [ms]
Rest time after lift bottom layers 0 [ms]
Z Lift Distance Bottom 5.00 0.00 [mm]
5.00 0.00 [mm]
Z Bottom Speed (peel) 50 1 [mm/min]
Z Retract speed bottom (back in resin) 50 1 [mm/min]

Cancel Save

Figure 4.8 Printing parameters

4.4. Results

4.4.1. The design of QCh:SPA Salt for Crosslinking HEA Polymer in Cyrene

For 3D printing of conductive supramolecular hydrogels, crosslinking of the polymer network is a critical element for high-fidelity printing of the desired structure due to the stresses associated with the photopolymerization printing process. During the separation of the printed structure from the resin tank, the force of delamination and viscous forces from movement can deform the printed structures and prevent the proper alignment of subsequent layers. These forces are the major limiting factor in fabrication of high-fidelity structures with the vat polymerization process. To mitigate these factors, large amounts of covalent crosslinkers such as N,N-methylene

bisacrylamide, ethylene glycol diacrylate or poly(ethylene glycol) diacrylate are used to enhance the network rigidity, enabling the printing of overhanging structures and higher resolutions at the expense of network elasticity. Conversely, when a smaller amount of crosslinkers is used to improve the elasticity, it is at the expense of rigidity, limiting printable structures to low aspect ratio objects with minimal overhangs. Furthermore, for 3D printable organo-gel precursors, the solvent is essential for the sustainability of the 3D printing process because many polar aprotic solvents are derived from the fossil fuel industry. Our previous work aimed to mitigate the impact of these factors through the printing of an intermediary organo-gel using DMSO¹⁸³. While DMSO improves the mechanical properties during printing, it is both unsustainable and introduces toxicity concerns due to DMSO's propensity to facilitate dermal transport of toxic monomers through the skin. Through the use of Cyrene in place of DMSO, these concerns can be mitigated due to Cyrene's non-toxic properties and sustainable production, furthering the field of sustainable photopolymerization-based hydrogel 3D-printing.

To facilitate high-fidelity printing with high toughness, a new type of crosslinker was designed that based on the solubility of a poly(ionic) salt containing an anionic monomer and a cationic biopolymer. During polymerization, the anionic monomer component of the salt copolymerizes into the primary network, providing ionic interactions between the biopolymer and the primary network. Increased rigidity is achieved by polymerization in a polar aprotic solvent, where the strength of the ionic interactions is increased dramatically due to the absence of water, preventing disassociation and enabling high-fidelity printing through increased rigidity. For this purpose, 3-sulfopropyl acrylate (SPA) and quaternized chitosan were chosen as the anionic monomer and cationic biopolymer, respectively. During polymerization in Cyrene, the SPA monomers are incorporated into the growing pHEA chain. As each SPA monomer is associated

with a quaternized amine group on chitosan, this crosslinking provides significantly greater rigidity than the ionic interactions or physical crosslinking alone. After solvent exchange with water, these associated groups can disassociate, considerably improving the elasticity of the network. Through this mechanism, the hydrogels can behave like a covalently crosslinked network during printing as an organo-gel and convert to a flexible ionically crosslinked network after solvent exchange with water. While multiple formulations of the crosslinking salt are possible and in preliminary tests, all could act as a crosslinker for photopolymerization-based 3D printing. This work focused on improving the toughness of the resulting hydrogel through a poly-ionic crosslinker. We hypothesized that by tuning the ratio of the anionic to the cationic component, increased network elasticity could be achieved through variation in the number of free ionic sites. Initially, the weight ratio of QCh:SPA was considered a suitable variable to tune, enabling an excess of cationic or anionic sites. Since chitosan is well known for its pH responsiveness, the variable pH could impact its properties. For this reason, we also explored using pTsoH as a non-polymerizable anionic component to enable the formation of salts with variable ionic ratios with a constant pH.

To validate the crosslinking potential of the QCh:SPA salt for hydroxyethyl acrylate (HEA), two distinct samples, one with and one without the QCh:SPA salt, were synthesized. Polymerization was induced using a 200 mW/cm² UV LED point source for 5 min, as depicted in Figure 4.9. a & b. These comparative tests elucidated the crosslinking role of QCh:SPA; there are notable differences in the mechanical properties of the two samples. The HEA polymer sans QCh:SPA exhibited characteristics akin to physically crosslinked polymers, marked by high elongation and limited structural memory. In contrast, including QCh:SPA in the organo-gel resulted in a material that retained its shape post-polymerization, enabling detachment from the glass slide without notable structural alterations. We speculate that the QCh:SPA salt disperses

within the HEA resin, maintaining the integrity of the quaternary amine-sulfo ionic bond, leading to high localized SPA concentrations near quaternized chitosan, forming a quasi-block copolymer, as illustrated in Figure 4.9. e. This configuration contrasts with the linear HEA network (Figure 4.9. c). Evidence of crosslinking was further observed in Figure 4.9. a & b, where the salt-free organo-gel dissolved in methanol over 12 hours, whereas the QCh:SPA containing sample exhibited only swelling, indicating the efficacy of QCh:SPA as an ionic crosslinker.

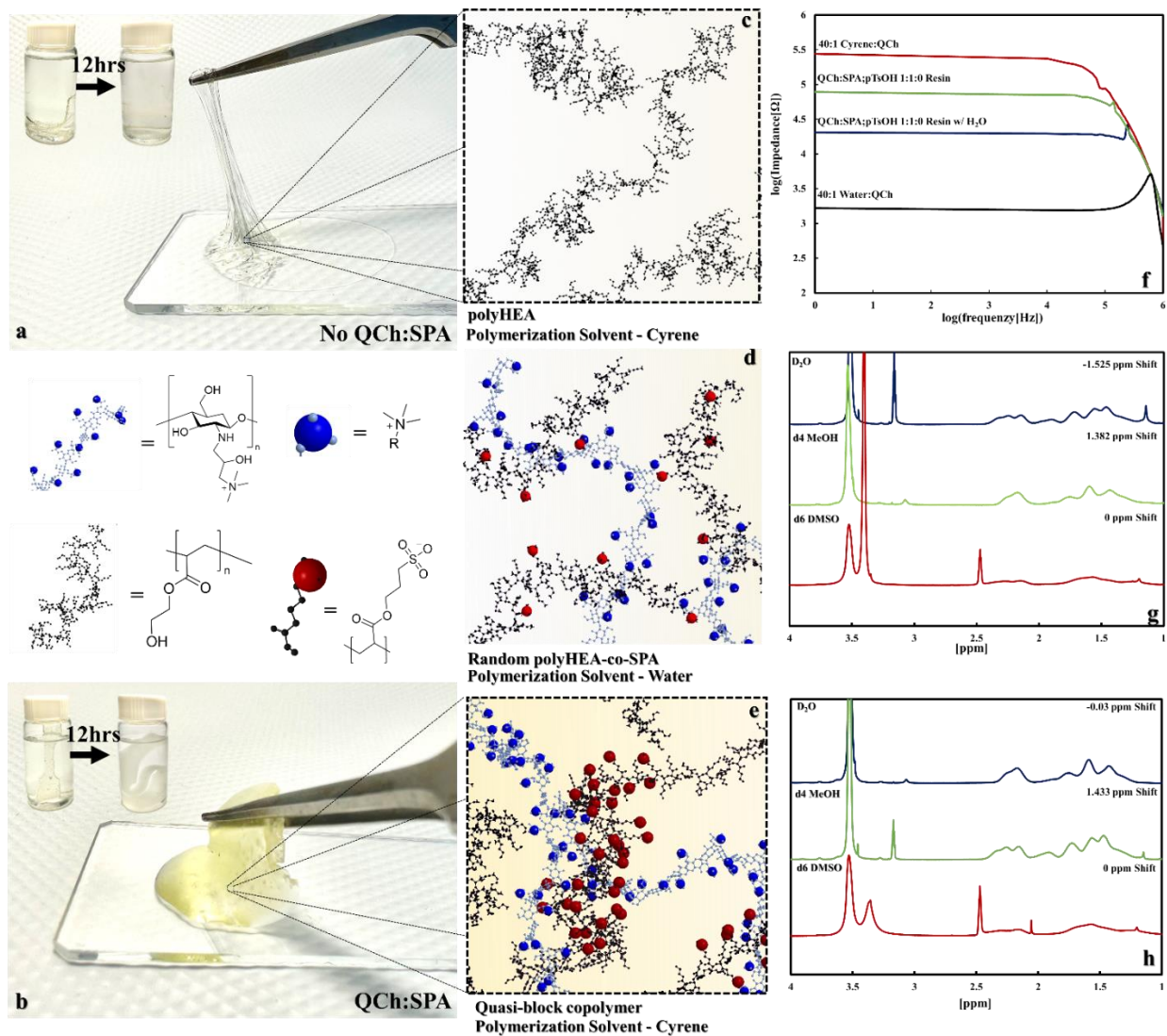


Figure 4.9 Method of QCh:SPA crosslinking HEA & analysis of polymer structure . a) HEA organo-gel prepared with 10g HEA, 1.25g Cyrene and 100 mg Irgacure 819 cured with 365 nm at 200 mW/cm⁻² for 5 minutes. Inset image on top left demonstrates complete dissolution after 12 hours in methanol b) QCh:SPA:pTsOH (1:1:0) resin cured with 365 nm at 200 mW/cm⁻² for 5 minutes. QCh:SPA containing salt exhibits higher elasticity and exhibits elastic rather than plastic deformation. The inset image on the top left demonstrates swelling after 12 hours in methanol. c-e) 3D Schematic of the different hydrogel networks representing no QCh:SPA [c], disassociated QCh:SPA (H₂O) leading to a random copolymer [d] and dissolved but associated QCh:SPA:pTsOH (Cyrene) causing molecular templating and a quasi-block-co-polymer structure [e]. f) Impedance measurements of the neutral QCh:SPA:pTsOH 1:1:0 salt in different formulations, red= 20:0.809 Cyrene:[QCh:SPA:pTsOH 1:1:0] by weight, green= QCh:SPA:pTsOH 1:1:0, blue= [QCh:SPA:pTsOH 1:1:0] 12.5% water substituted for Cyrene, black= 20:0.809 Water:[QCh:SPA:pTsOH 1:1:0]. All solutions have a ratio of Major Component: eq. wt. of QChCl of 40:1. g) Spectra of the water-polymerized hydrogel degradation products. h) Spectra of the Cyrene polymerized hydrogel degradation products.

To elucidate its macromolecular structure, seen in Figure 4.9. c-e, a series of degradation studies Figure 4.9. f & g were performed where ionic interactions in the hydrogel were reduced through the introduction of KOH in methanol, allowing the networks to dissolve; after purification with dialysis, the resulting polymer was dissolved in various solvents to determine what sections of the network were soluble. We hypothesized that a quasi-block copolymer structure would affect the solubility of the resulting degradation products, as demonstrated with block copolymers in the literature¹⁸⁶. When polymerized in a polar aprotic solvent, the sulfo group was anticipated to remain associated with the quaternary amine, leading to a quasi-block-copolymer structure. Conversely, polymerization in water was expected to result in salt dissociation and a more random structure. Spectral analysis in various solvents revealed two primary trends: degradation affected the ionic bonds and the synthetic network, and the DMSO spectra (Figure 4.9. g & h) showed a notable difference in peak ratios at 3.5 and 3.4 ppm. These spectra served as controls, with subsequent analyses in D₂O and d₄ methanol (Fig. 4.9. g & h) indicating a reversal in spectral patterns depending on the polymerization solvent. This suggests variations in the molecular order and solvation properties of the polymer. Moreover, a significant peak shift was observed in deuterium oxide (D₂O), where polymers formed in Cyrene and water exhibited shifts of -0.03 ppm and -1.525 ppm, respectively, attributed to differing degrees of hydrogen bonding. Impedance measurements (Fig. 4.9. f) on various prepolymer resins and QCh:SPA:pTsOH (1:1:0) formulations further supported these findings. QCh:SPA:pTsOH in water demonstrated the lowest impedance, followed by the water-based prepolymer resin. The Cyrene-based systems showed higher impedance, with QCh:SPA:pTsOH in Cyrene exhibiting the highest. These results indicate that salt dissociation is significantly hindered in Cyrene, suggesting the formation of a networked structure where SPA groups cluster near the QCh.

4.4.2. Impact of Cation: Anion Ratio on Hydrogel Structure

To understand how the different components of bioderived poly(ionic) salt affect the crosslinking performance, we varied the ratio of QCh:Cl to SPA:K without pTsOH. Before curing, the prepolymer resins demonstrated varying viscosities based on the ratio of SPA in the salt, as seen in Fig. 4.10. a) with the 1:0.75 & 1:0.5 ratios displaying the lower viscosities, which was attributed to the QCh not fully dispersing into the network. After solvent exchange with water, the mechanical properties of the various ratio hydrogels can be seen in Fig. 4.10. b, c & d. Note that pH changed with the varied ratios, significantly affecting mechanical and electrical properties. The mechanical and electrical results indicated interesting behaviour, with 1:0.75 QCh:SPA demonstrating the highest stiffness and inductive behaviour. Environmental scanning microscopy (ESEM) (Fig. 4.10. g-l) was performed to further investigate these trends to reveal the structural differences. Acidic QCh:SPA 1:1 hydrogels, observed at 4000x magnification (Fig. 4.10. g & j), exhibited a porous network with fibrous structures. These fibres, resulting from QCh:SPA salt solubilization in the resin, led to a secondary network within the hydroxyethyl acrylate (HEA) matrix. In contrast, QCh:SPA 1:0.75 & 1:0.5 hydrogels (Fig 4.10. h, i, k & l) showed nodular formations instead of fibres, indicating less QCh dispersion in the resin, with the nodules acting as crosslinkers.

These microstructural variances shed light on the distinct mechanical properties of the hydrogels. The QCh:SPA 1:0.75 & 1:0.5 samples exhibited higher tensile and compressive strengths and a steeper loss modulus slope with frequency, as detailed in Fig. 4.10. b-d. These mechanical traits correlated with the pH changes in the salt during freeze-drying, as observed in pH measurements post-salt synthesis and post-freeze-drying. After redissolving in water (Fig.

4.10.). Basic salts showed decreased pH and incomplete redispersion, suggesting instability of QCh in basic form during freeze-drying, leading to reduced hydroxyl ions and irreversible aggregation in the network. Consequently, distinct salt nodules formed in the polymer, markedly altering its mechanical and electrical properties. These findings highlight the pivotal role of salt composition and pH in determining QCh:SPA hydrogel microstructures, which directly influence their mechanical and electrical characteristics Fig 4.10. e-f.

2 Component Variable Anion Concentration

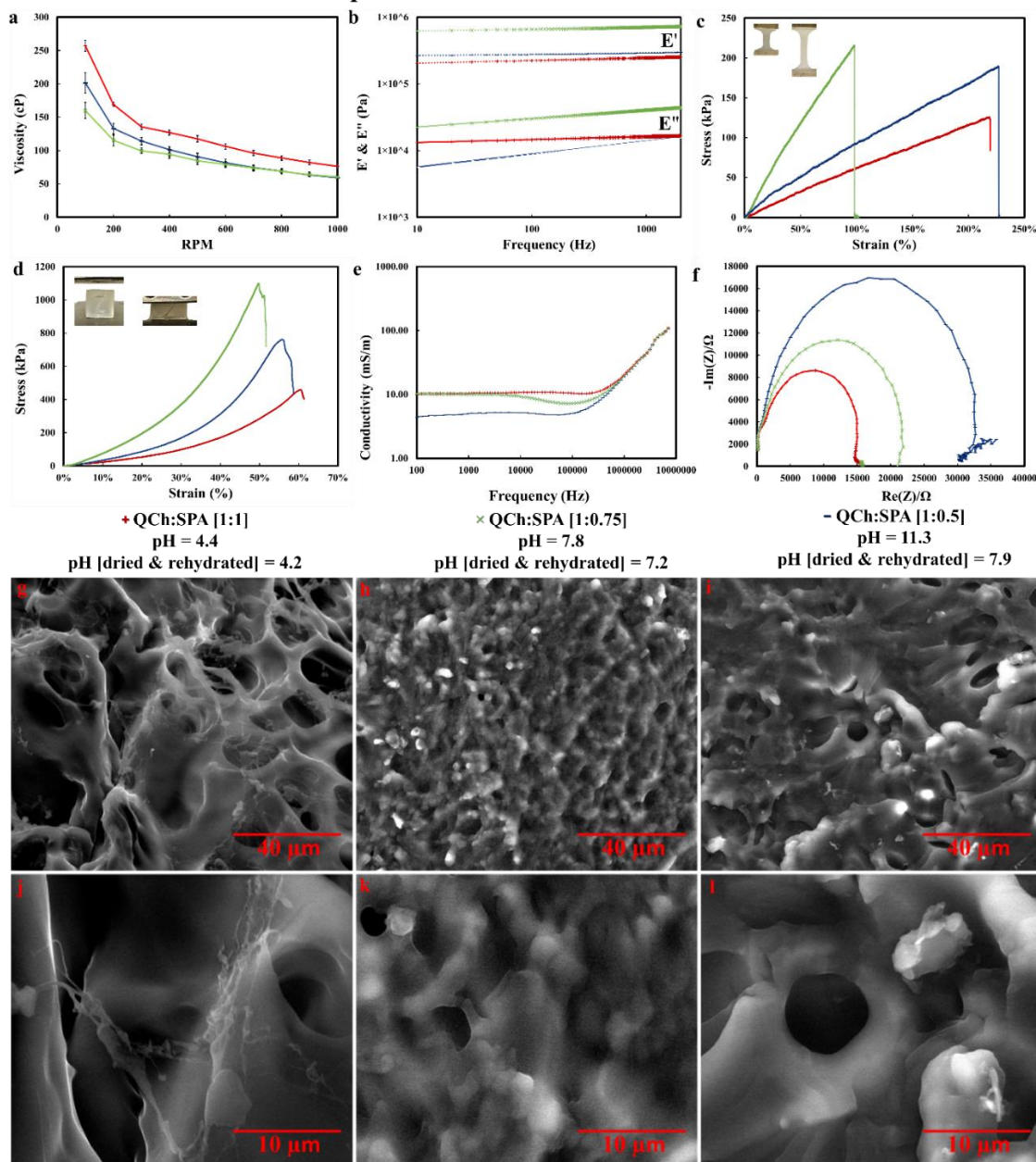


Figure 4.10 Mechanical & structural characterization of quaternized chitosan 3-sulfopropyl acrylate-co-2-hydroxyethyl acrylate hydrogels with varying QCh:SPA salt formulations. . a-c) mechanical properties after varying weight ratio of quaternized chitosan hydroxide to 3-sulfopropyl acrylate before acidification (red=1:1, green=1:0.75, blue=1:0.5). a) Viscosity curve as a function of RPM on a cone plate viscometer. b) Storage and loss modulus of the weight ratio salt hydrogels. c) tensile stress-strain curves, d) compression stress-strain curves to 80% strain (replicate measurements available in Supplementary Figure 9), e) 4-point conductivity measurements of the hydrogels at varying frequencies. f) Nyquist impedance plots of the hydrogels indicative of behaviour similar to model circuits.. g-l) Environmental SEM of the frozen HEA hydrogel cross-section crosslinked with 1:1, 1:0.75, 1:0.5 QCh:SPA (left to right). The images in the upper row (g-i) were taken at 1000x magnification, and those in the lower row (j-l) were taken at 4000x magnification. The pH of the salt used in each hydrogel is reported at the bottom of each column. pH refers to the pH of the aqueous solution that, after freeze drying, forms the salt at ~1 wt%, while the pH [dried & rehydrated] refers to the pH of the freeze-dried salt redissolved in water at 2 wt%.

4.4.3. Impact of Acrylate Anion Concentration on Hydrogel Structure and Mechanical Properties at Neutral pH

The above investigations revealed that the pH of the salt solution before freeze-drying significantly influences the structural composition of the resulting hydrogel. The addition of varied amounts of SPA being the sole acidic component led to variations in the molar ratio of acidic to basic groups. To investigate the role of acrylate anion concentration on the hydrogel structure, we formulated the salts with equal anionic and cationic groups but with the addition of pTsOH to replace a portion of SPA forming neutral SPA salts with varying anionic acrylate density. Viscosity measurements, as illustrated in Figure 3.11. a) showcased a notable dependency on salt composition. The neutral SPA salt exhibited a peak viscosity of 300 cP, surpassing the 250 cP observed in the previous 1:1 QCh:SPA resin. Interestingly, other neutral salts containing pTsOH (Neutral 1:1 SPA:pTsOH & Neutral 1:3 SPA:pTsOH) mirrored the viscosities of the ratio salts. All neutral salts displayed enhanced shear-thinning properties compared to their ratio counterparts (Fig. 4.12), indicating a significant dissolution of quaternized chitosan within the resin, suggesting altered salt structures in the prepolymer resin.

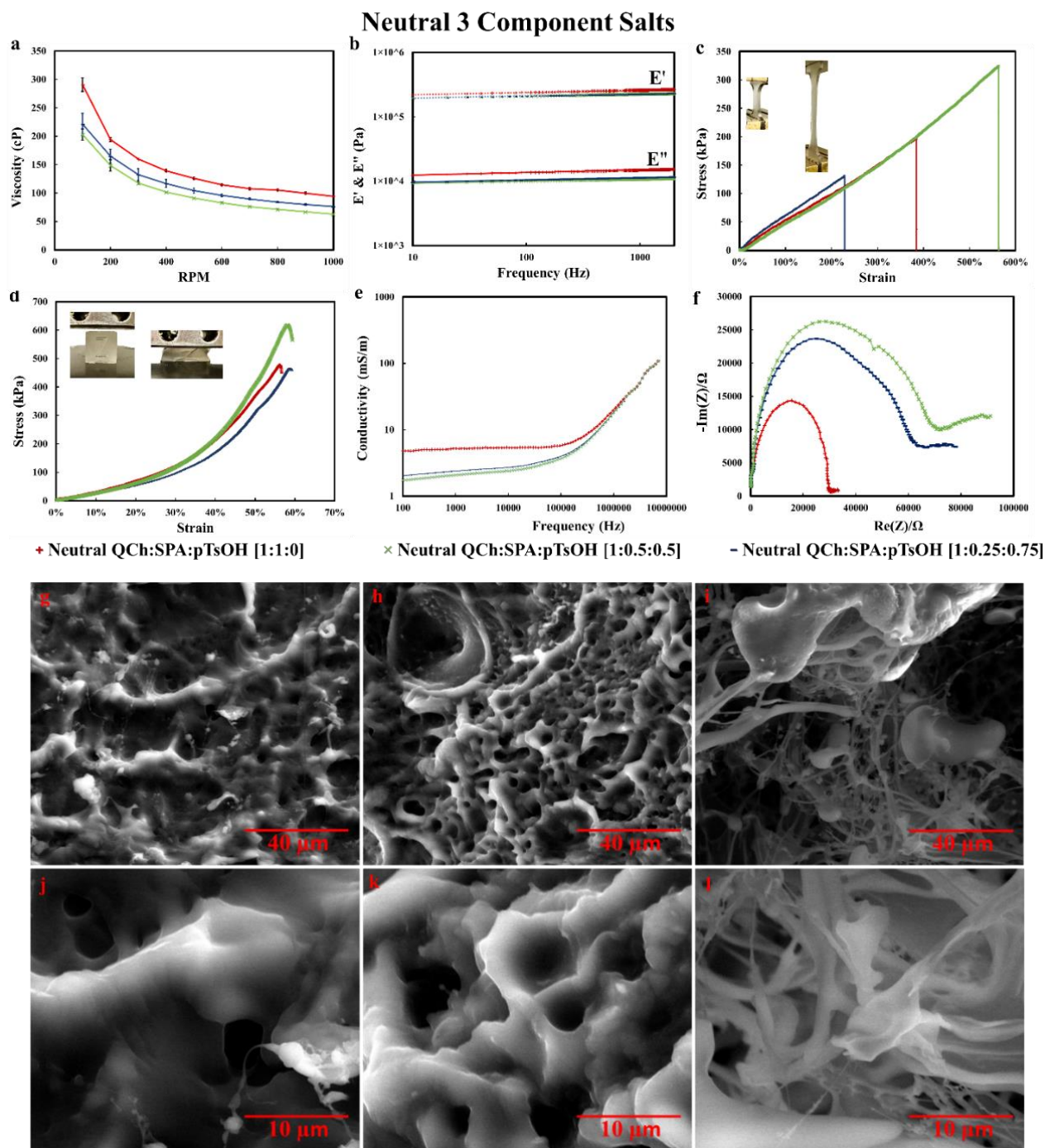


Figure 4.11 Mechanical & structural characterization of quaternized chitosan 3-sulfopropyl acrylate-co-2-hydroxyethyl acrylate hydrogels with varying the molar ratio of 3-sulfopropyl acrylate to pTsOH used during neutralization (red=1:1:0, green=1:0.5:0.5, blue=1:0.25:0.75). a) Viscosity curve as a function of RPM on a cone plate viscometer. b) Storage ($\cdots$ line) and loss modulus ($-$ line) of the neutral salt hydrogels. c) Tensile stress-strain curves, d) compression stress-strain curves to 80% strain (replicate measurements available in Supplementary Figure 9), e) 4-point conductivity measurements of the hydrogels at varying frequencies. f) Nyquist impedance plots of the hydrogels. g-l) Environmental SEM of the frozen HEA hydrogel cross-section crosslinked with 1:1:0, 1:0.5:0.5 and 1:0.25:0.75 QCh:SPA:pTsOH (left to right) the ratios are based on the number of ionic sites in the salt. The images in the upper row (a-c) were taken at 1000x magnification, and the pictures in the lower row (d-f) were taken at 4000x magnification.

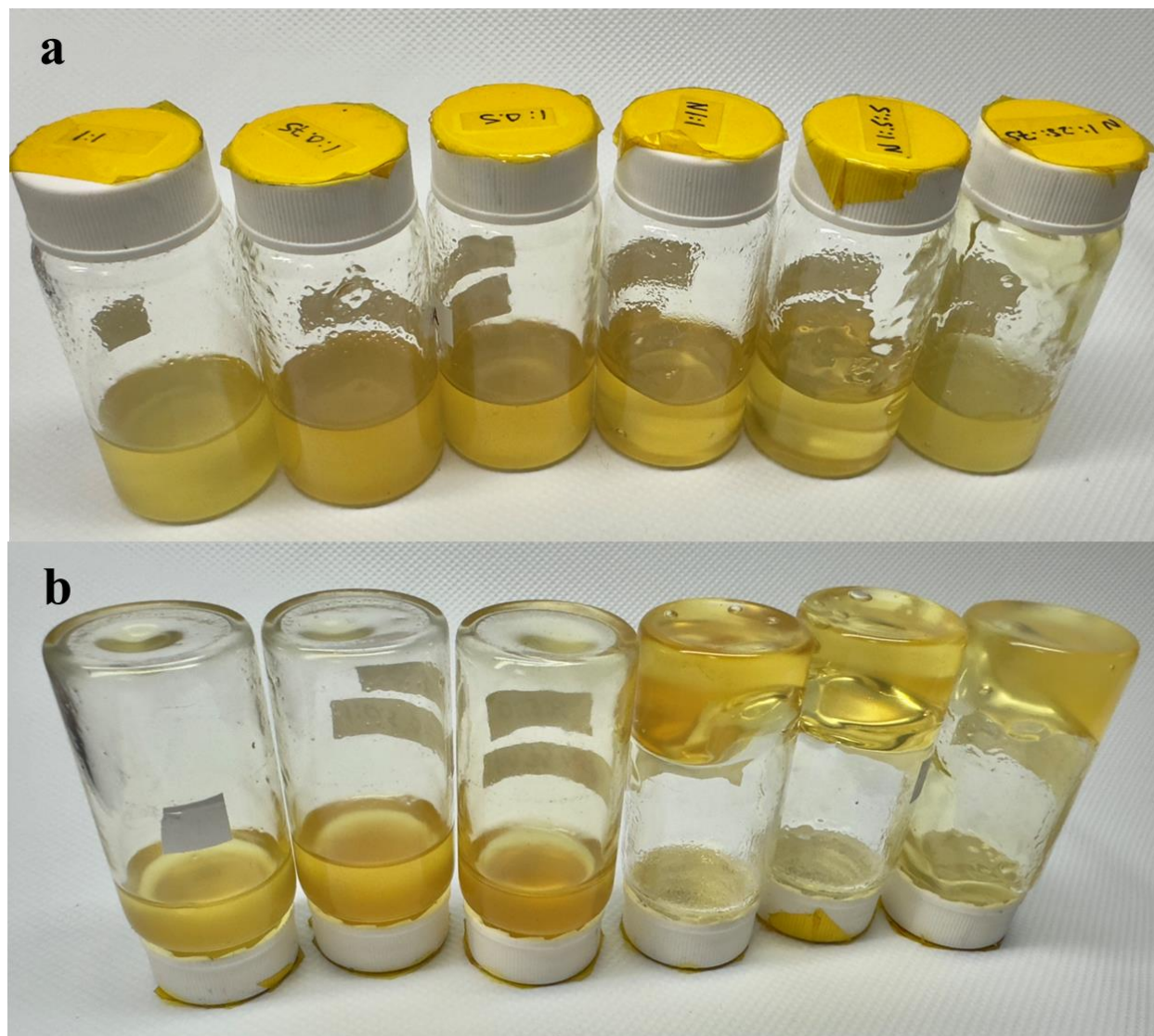


Figure 4.12 Demonstration of the viscoelastic properties of the neutral salts. From left to right, the salt used are as follows: WR 1:1, WR 1:0.75, WR 1:0.5, N 1:0, N 1:1, N 1:3.

Rheometric analysis (Fig. 4.11 b) revealed consistent storage and loss moduli across different neutral salt hydrogels, with the formulation lacking pTsOH exhibiting marginally higher moduli. This trend is attributable to a denser ionic crosslink network with fewer free cationic sites. Surprisingly, tensile and compressive tests indicated that while the initial elasticity of the network remained relatively unchanged across different anionic acrylate concentrations, the ultimate tensile strain and stress varied substantially with SPA concentration. The optimal SPA concentration was

found at a 1:1 ratio with pTsOH, yielding an ultimate tensile strength of 300 kPa, compared to 200 kPa for the SPA salt and 100 kPa for the 1:3 SPA:pTsOH salt. Similarly, in compressive tests, the 1:1 SPA:pTsOH formulation exhibited the highest compressive strength of 625 kPa. However, the increase was not as pronounced as in the tensile tests, with all samples failing at similar compressive strains.

The structural changes are evidenced in conductivity measurements (Fig. 4.11. e). Unlike the ratio salts, none of the formulations exhibited the previously observed frequency/resistance dependence from 10 kHz to 500 kHz. Instead, conductivity gradually decreased with reduced frequency. Nyquist plots (Fig. 4.11. f) suggested a Randles equivalent circuit characteristic of an electrochemical system, deviating from the Warburg region observed in ratio salts. Environmental Scanning Electron Microscopy (ESEM) on the frozen hydrogels (Fig. 4.11. g-l) provided insights into how microstructural variations impact mechanical and electrical properties. The porous structures differed markedly from basic ratio salts (QCh:SPA 1:0.75 & 1:0.5), with a closer resemblance to the QCh:SPA 1:1 sample evident in the neutral salt compositions. As the ratio of SPA:pTsOH decreased, increased porosity was observed in the hydrogel, with the network shifting to a fibrillar composition at the lowest concentration of SPA.

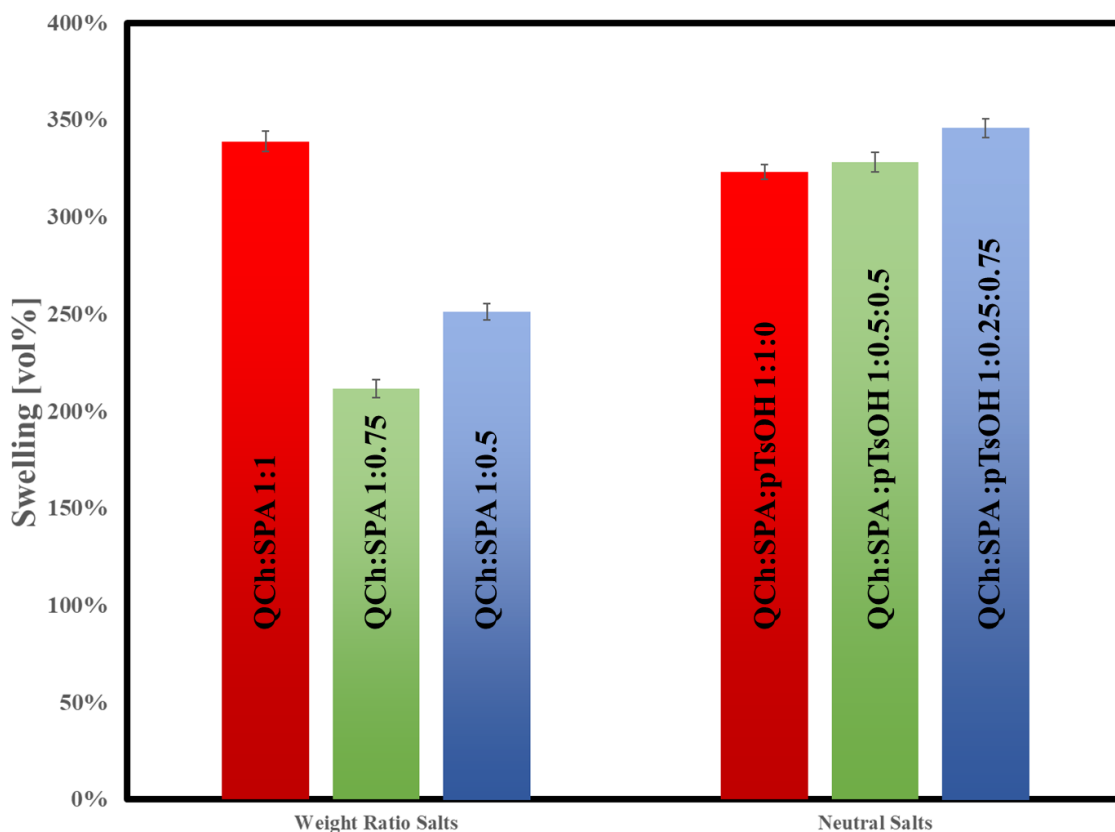


Figure 4.13 Swelling ratio of different formulations.

Equilibrium swelling behaviour (Fig. 4.13) also varied, with basic salts swelling to a lesser extent. Fibrillar regions, indicative of quaternized chitosan, were observed in QCh:SPA:pTsOH 1:1:0 but absent in other neutral salts. Differences in porosity and swelling degree likely contribute to the increased ultimate tensile strength and elongation at break observed in specific formulations, with the change to a fibrillar network significantly impacting mechanical properties. Overall, these results demonstrate that the interaction between anionic groups and quaternized chitosan significantly affects the hydrogel's mechanical and electrical properties, with the pH of the salt solution and the concentration of the anionic acrylate at neutral pH playing a pivotal role in determining the hydrogel's structural and functional characteristics.

4.4.4. Printability of Neutral QCh:SPA:pTsOH 1:1:0 Resin Formulation

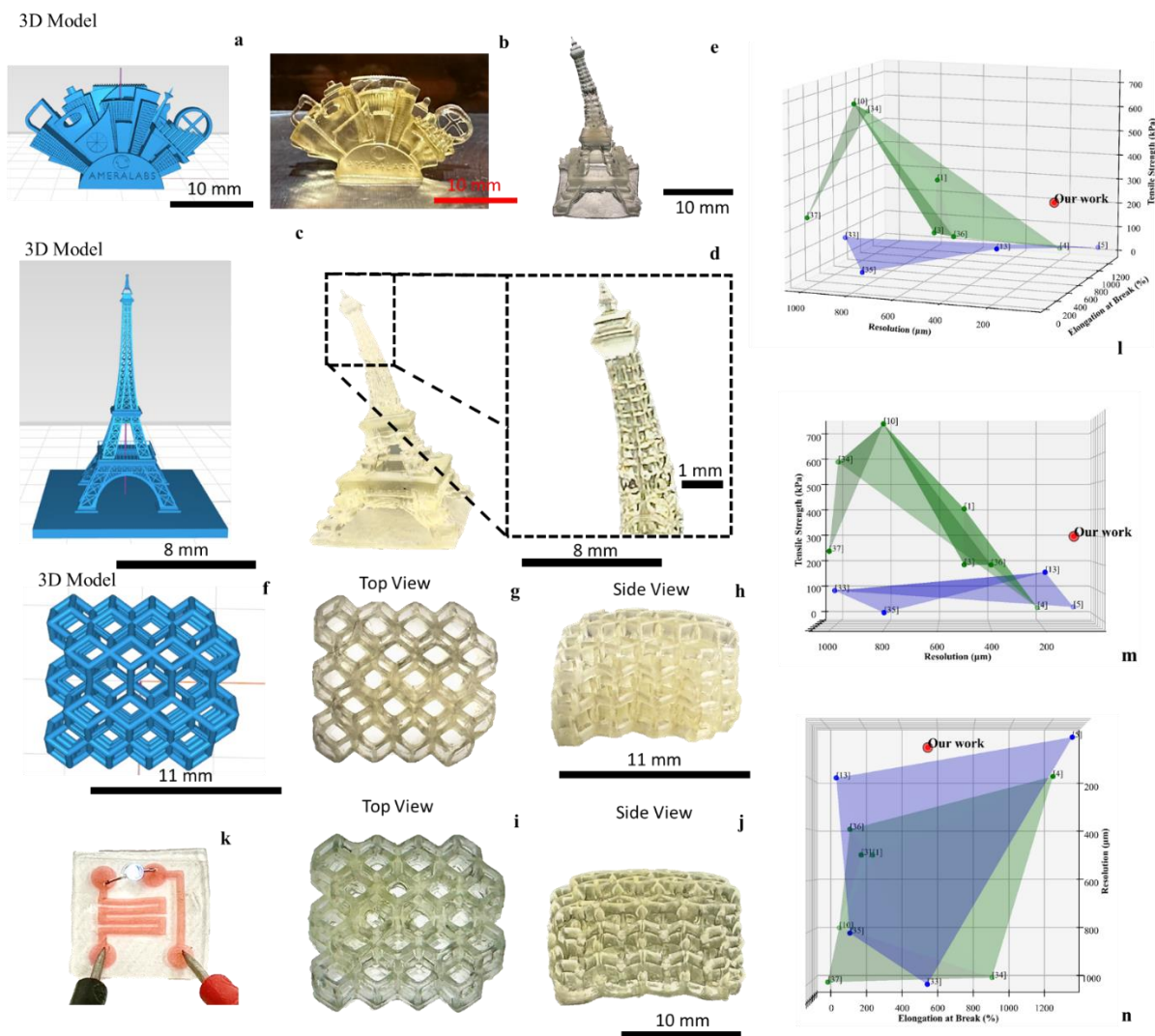


Figure 4.14 Demonstration of QCh:SPA:pTsoH 1:1:0 printing resolution. a) commonly used resolution test. b) 3D printed organo-gel generated from the previous model. c) Eiffel tower model. d) 3D printed organo-gel generated from the Eiffel tower model. e) 3D printed Eiffel tower hydrogel after solvent exchange f) geometric mesh model g-h) Top and side views of 3D printed organo-gel generated from the geometric mesh. i-j) Top and side views of 3D printed hydrogel generated from the geometric mesh after solvent exchange. k) Demonstration QCh:SPA:pTsoH [1:1:0] to act as a circuit material, Circuit 3D printed with 0.125 wt% Sudan III for visibility, encapsulated with acrylamide hydrogel and soaked in UPW. l-n) Comparison of different 3D printable hydrogels in literature along with the region occupied by the reported system. l) isometric view, m) side view, n) top view. Blue region includes 3D printable hydrogels utilizing photopolymerization based methods, green region includes 3D printable hydrogels utilizing extrusion based methods.

To demonstrate the fidelity capabilities of the QCh:SPA:pTsOH resins, several small structures were printed as seen in Figure 4.14. , showcasing its potential for intricate 3D printing applications. The resolution test (Fig. 4.14. a) demonstrated the hydrogel's proficiency in printing

detailed geometries. Most features of the model were accurately replicated apart from the thin tower at the back, indicating a limitation in printing extremely slender structures. This outcome suggests a high degree of resolution in the x and y directions, highlighting the hydrogel's potential for detailed fabrication. For the Eiffel Tower model (Fig. 4.14. c), the printed organo-gel (Fig. 4.14. d) exhibited a remarkable resemblance to the original design, especially in the upper portion where intricate details were successfully captured. However, inaccuracies were observed in the lower observation decks, attributable to over-polymerization in the z-direction. This phenomenon, while impacting the precision of finer details, is a common challenge in photolithography-based 3D printing and reflects the depth of penetration and curing of light in the hydrogel material. The geometric mesh model (Fig. 4.14. e) and its corresponding printed organo-gel (Fig. 4.14. f-g) further reinforced these observations. Both the top and side views of the printed sample displayed significant resolution in the x and y planes. However, similar to the Eiffel Tower model, over-polymerization in the z-direction was evident, affecting the print's vertical precision. It is important to note that the tendency for over-polymerization in the z-direction is primarily due to a low mass absorption coefficient, resulting in light penetration through to previously cured layers. This issue could potentially be mitigated by incorporating a colored or transparent photo absorber. However, such modifications were beyond the scope of this study and present an opportunity for future research to enhance the z-directional resolution of this material while maintaining its transparent properties.

A comparison of different 3D printable hydrogel systems is provided in Fig. 4.14. h-j, comparing the mechanical properties and XY resolution of the printed hydrogels with other work in the literature. By comparing our work to the regions occupied by photopolymerization (blue) and extrusion-based printing (green), it is clear that the QCh:SPA system is capable of bridging

the gap between the two methods, facilitating mechanical properties similar to extrusion-based methods at resolutions achievable with photolithography based printing. The introduction of 3D printable poly(electrolyte) hydrogels opens up many possibilities for photopolymerization-based 3D printing, facilitating the production of high-resolution complex hydrogel structures with tunable mechanical and electrical properties. Thus, the QCh:SPA:pTsOH 1:1:0 hydrogel demonstrates promising printability with high resolution in the x and y directions, though improvements in z-directional accuracy are needed. These findings showcase the hydrogel's potential in detailed 3D printing applications and highlight areas for further material and process optimization.

4.4.5. Discussion

When observing the effect of hydrogels crosslinked through double network polyelectrolytes, we can see that ionic interactions between the quaternized chitosan and the sulfo groups in 3-sulfopropyl acrylate provide ionic crosslinking of the network. These ionic interactions enable the 3D printing of hydrogels with high elasticity at high fidelity using consumer-grade equipment, an achievement not previously reported. To our knowledge, this system is the first reported use of polyelectrolyte salts as the sole crosslinker in a hydrogel network formed by photopolymerization. We can derive four main scientific insights from the systems reported in this study. First, the use of a non-toxic polar aprotic solvent (Cyrene) prevents the SPA from disassociating from the QCh, leading to the concentration of SPA within regions containing high concentrations of QCh molecules, as seen from the NMR degradation studies. This enables higher elasticity of the final network as regions with low SPA are majorly crosslinked through physical interactions. In contrast, regions high in SPA contain larger quantities of ionic crosslinking, forming the supramolecular hydrogel. The use of Cyrene is particularly important, as previous

work has utilized other toxic or non-renewable polar aprotic solvents for organo-gel 3D printing. Through the use of a bio-renewable, non-toxic polar aprotic solvent, this work makes steps towards the development of safer majority biobased systems for photopolymerization-based 3D printing. Second, the pH of the salt solution before freeze-drying directly impacts the hydrogel structure. While unstable at basic pH values of the QCh salt, the resulting hydrogel demonstrates stiff properties with higher conductivity, which could act as a strain sensor. Third, with the neutral salts we observed that the tensile strength, electrical properties and network morphology could be tuned by partially neutralizing the QCh with a non-polymerizable acid. Depending on the ratio, a porous or fibrous network could be achieved, along with a significant modulation of the ultimate strain at break, with little change to the elastic characteristics of the network. This change in morphology was attributed to interactions in the hydrogel between the two networks during the solvent exchange with water. Although the neutral sample without pTsoH had visible quaternized chitosan regions, when the SPA was partially replaced with pTsoH, these regions were no longer visible. Therefore, it was concluded that the HEA network was forming around the positively charged chitosan as pTsoH no longer screened the charge after leaching out of the network. As the quantity of unscreened charge increased, the HEA network became more porous and finally assumed a similar fibrillar form to the dispersion of quaternized chitosan within the hydrogel. This behavior could also be utilized to improve the percolation characteristics of networks. Normally, a large quantity of additive is required to reach percolation for a finite volume as opposed to this system, where the morphology change improves percolation, resulting in increased conductivity at low SPA concentrations. One promising continuation of this work is the inclusion of electrically conductive additives that template with the network, allowing lower concentration percolation. Fourth, depending on the pH and ratio of non-polymerizing acid, different characteristic circuits

could be achieved with a single system as seen in the Nyquist impedance results, demonstrating that different formulations of this hydrogel could be used to emulate different circuits when embedded in soft materials. Overall, this work demonstrated the capability of natural polymer: synthetic acrylate salts to be utilized as a crosslinker for ionic hydrogel formation and 3D printing. This design has many possible applications and could be modified to different natural polymers such as sodium alginate or sodium hyaluronate. As such, there are many potential bioderived polysaccharide:acrylate salt combinations that would allow further development of this system. Furthermore, other iterations potentially include the use of peptide chains with cationic or anionic groups to replace the polysaccharide component. As such, this novel method of ionic crosslinking has the ability to open a new class of photopolymerization-based 3D printable materials, enabling material properties previously restricted to extrusion-based systems.

As for the technical implications for practical applications, the adaptability of these hydrogels as an iontophoresis medium offers a promising avenue for localized drug delivery. The customization potential through 3D printing enhances the applicability of this technique, making it more patient-specific and efficient. The reduced risk of transdermal compound transfer further underscores the clinical viability of this approach since no small molecules contribute to the conductivity. Future applications include the prospect of using these hydrogels in smart contact lenses as circuit materials, which is particularly intriguing. While further research is needed to optimize transparency and conductivity, the potential of creating less obtrusive and more biocompatible circuits in soft materials is an exciting development. This could lead to breakthroughs in wearable technology and biomedical devices.

It is worthwhile to note that the poly(ionic) salt crosslinker design could be applied to many different natural polymers and opens a new class of crosslinkers for 3D printing utilizing ionic polysaccharides. This differs significantly from previous work as the polysaccharide acts as a core component of the main hydrogel network rather than a secondary strengthening network seen in typical double-network hydrogels. Thus, our findings extend the fundamental understanding of hydrogel behaviour in not only the presence of associated poly(electrolytes) but also open, diverse possibilities for their application in medical and technological fields. The tunability of these materials, in terms of both physical and chemical properties, makes them a promising candidate for future innovations in soft material science.

4.5. Conclusion

In this study, we reported the development of a novel crosslinking scheme utilizing poly(ionic) salts derived from natural sources. These salts were capable of ionically crosslinking 2-hydroxyethyl acrylate hydrogels to a degree that facilitated their production with vat-polymerization-based 3D printing techniques, enabling the fabrication of high-fidelity 3D hydrogels with high elasticities. Printability studies confirmed the capability of the QCh:SPA salt to facilitate printing of high fidelity organo-gels as hypothesized, with NMR and conductivity measurements indicated the structural differences between the solvent exchanged organo-gel and the hydrogel formed in water indicating a change in copolymer structure of 2-hydroxyethyl acrylate and 3-sulfopropyl acrylate along with the lack of disassociation in the prepolymer resin. This was attributed to the association of the sulfo groups in 3-sulfopropyl acrylate to the quaternized amine groups in quaternized chitosan during polymerization. A comprehensive study of the effect of salt composition on material properties was performed. This revealed the significant impact of the salt solution's pH on morphology and material properties, along with the effect partial

substitution of the acrylate anion with an inert anion had on final material properties. Due to the quaternized chitosan:3-sulfopropyl acrylate interactions, the hydrogel demonstrated tunable conductive properties, enabling its possible use as a circuit material in hydrogel composites. This application was facilitated by the high elasticity and transparency of the final hydrogel compared to other conductive hydrogel systems.

To our knowledge, this is the first time that polysaccharide salt is used as a crosslinker for light-based 3D printable hydrogels. This development differs significantly from previous work as the polysaccharide acts as a core component of the main hydrogel network rather than a secondary strengthening network seen in typical double-network hydrogels. This type of poly(ionic) salt crosslinker design could be applied to many different natural polymers and opens a new class of crosslinkers for 3D printing utilizing ionic polysaccharides.

In summary, this work proves the capability of quaternized chitosan:acrylate salts to function as a crosslinker for organo-gel 3D printing. This enables the fabrication of solely ionically crosslinked hydrogels through photopolymerization-based 3D printing. This work demonstrates the first step in poly(ionic salt) crosslinkers for 3D printing opening the possibility for other polysaccharide:acrylate salt systems use as photopolymerization crosslinkers.

Chapter 5: Thermoresponsive poly(oligo ethylene glycol acrylates) hydrogels loaded with alginate microparticles

5.1. Introduction

In this chapter, we expand on our previously developed system utilizing quaternized chitosan and 3-sulfopropyl acrylate as poly(ionic salts) to form supramolecular hydrogels. While PNIPAm is commonly used for thermoresponsive hydrogels, its monomers are known for their

neurotoxicity¹⁹². Alternatively, poly(oligo ethylene glycol acrylates) (POEGA) have emerged as promising replacements due to their tunability and lack of neurotoxic properties¹⁹³.

Unlike PNIPAm, which has a limited tunable LCST range, POEGA polymers demonstrate a broader LCST spectrum. Steinhauer et al. reported LCSTs ranging from 5.7°C to 58°C with copolymers of HEA and ethylene glycol methyl ether acrylate (mEGA)¹⁹⁴, with Boyer et al. achieving LCSTs from 22°C to 70°C using diethylene glycol ethyl ether acrylate and oligo ethylene glycol acrylate¹⁹⁵. This tunability stems from the varied derivatives of ethylene glycol in the side chains. The LCST behaviour results from the transition of the polymer from a coil to a globule state, expelling water and forming polymer-rich regions. The balance between the enthalpy of hydrogen bonding and the entropy of mixing governs this transition.

By modifying the hydrophilicity of the polymer, it is possible to adjust the temperature at which the LCST occurs, which is determined by the Gibbs free energy of mixing. When the enthalpy contribution of the polymer hydrogen bonding with the water dominates the equation, a negative Gibbs free energy enables spontaneous solubilization of the polymer in water. As the temperature increases, the entropy term becomes dominant, resulting in a positive Gibbs free energy occurring and manifesting as phase separation¹⁹⁶. Tuning of this crossover point enables control over the LCST and is achieved by altering the polymer's backbone, side chain, and end groups. Within the POEGA family, acrylate and methacrylate backbones are common^{197–200}, though other backbones like styrenics, vinyl ethers, and carbonates have seen use²⁵. Methacrylate groups are generally more hydrophobic and stable compared to acrylates²⁰¹. The major contributor to the hydrophilicity of the polymer is determined by the side chain length, e.g. the number of ethylene glycol units and the end groups (methyl, ethyl, or hydroxyl). Specifically, the hydrophilicity is proportional to the number of repeat ethylene glycol units with terminal hydroxyl groups, such as in HEA,

significantly increasing the hydrophilicity and longer terminal hydrocarbons, such as ethylene, increasing the hydrophobicity¹⁹⁶.

The application of POEGA in 3D printing is significant due to its biocompatibility and well-defined thermoresponsive behaviour. However, POEGA polymers are traditionally produced using fossil fuel-based technologies. As green production methods gain traction, there is a growing need to develop stimuli-responsive materials incorporating biobased components, ultimately aiming for fully biobased polymers.

One natural polysaccharide showing promise as an additive to acrylate-based hydrogels is sodium alginate. Derived from algae, alginate can crosslink with divalent and trivalent cations due to its carboxylate groups⁷⁴. However, this crosslinking method poses challenges, such as cation mobility and salt presence, which can degrade mechanical properties over time and cause pain when exposed to open wounds⁴⁴. These issues limit the application of alginate-containing hydrogels in fields like soft robotics and wound dressings, where mechanical stability and minimal pain response are crucial.

To address these challenges, we propose a system in the loading of alginate microparticles in the POEGA organo-gel crosslinked with quaternized chitosan. These particles were insoluble in the polar aprotic prepolymer resin, enabling high loadings of alginate to be achieved, which could then interact with the hydrogel network once solvent exchange with water was performed. The intended goal of this microparticle was to improve the mechanical properties of the final hydrogel while introducing controlled porosity. This would be achieved through the formation of alginate microparticles formed through an emulsion process assisted by a hydrophobic CNF Pickering emulsifier and lecithin.

Cellulose nanofibers (CNFs), derived from the most abundant biopolymer on earth, have emerged as a promising, environmentally friendly stabilizer for emulsions. Their high aspect ratio, mechanical strength, and modifiable surface chemistry make them ideal candidates for this purpose. These bioderived surfactants have seen increased research activity in O/W^{114,202–207} and water in water emulsions, yet their use in water in oil [W/O]^{208–212} emulsions typically require intensive synthesis steps. Specifically, to function as a W/O emulsifier, the CNFs need hydrophobic surface modification. This can be achieved through various surface modification treatments, such as grafting²¹³, silanization^{214,215}, EDC coupling²¹⁶ and esterification²¹⁷ enhances their compatibility with oil phases, thus facilitating the stabilization of water-in-oil (W/O) emulsions.

Hydrophobic CNF particles were chosen as a potential emulsifier due to the previously reported capabilities of unmodified CNF particles for stabilizing oil in water [O/W] emulsions. These bioderived surfactants have seen increased research activity in O/W and water in water emulsions, yet their use in water in oil [W/O] emulsions is lacking. Most commonly, polyglycerol polyricinoleate (PGPR), Tween 80 or Span 20 are used for W/O emulsions²¹⁸. While surfactant-based methods are suitable for emulsions, the structure provided by an interfacial assembly of CNF particles would assist in maintaining the shape of the particles during solidification and preventing coalescence.

Of different modification schemes the one-pot synthesis demonstrated by Cranston²¹⁹ has significant desirability compared to other methods mentioned due to the use of plant polyphenols and ease of purification. This method utilizes tannic acid to form a conjugate with the surface of the CNF and facilitates grafting of amine-containing molecules under basic conditions through Schiff base formation.

In this chapter, we aim to demonstrate the capability of HEA/mEGA copolymer to form thermoresponsive supramolecular hydrogels with quaternized chitosan poly(ionic salts). Furthermore, through the formation of alginate/CNF microparticles, we aim to improve the porosity of the resulting hydrogels, along with their mechanical properties and the speed of thermal response.

5.2. Materials and methods

5.2.1. Materials

2-hydroxyethyl acrylate (HEA) (Sigma Aldrich), ethylene glycol methyl ether acrylate (mEGA), 3-sulfopropyl acrylate potassium salt (SPA:K), hexane (Sigma Aldrich), diethyl ether (Sigma Aldrich), Irgacure 819 (BASF), CInd™ Chitosan Quaternary Ammonium Salt (QCh:Cl) (CD Bioparticles, Cyrene (Sigma Aldrich), Irgacure 2959 (Sigma Aldrich), SSTC60 (Purolite), Amberlyte a26 (Dupont), sodium hydroxide (Sigma Aldrich), phosphoric acid 85% (Sigma Aldrich), para-toluene sulfonic acid (pTsOH), Sodium alginate(low viscosity) (Sigma Aldrich), lecithin technical grade (Fischer Scientific), white paraffin oil , cellulose nanofibers (Cellulose lab) 3% weight slurry, octyl amine $\geq 99.0\%$ (Sigma Aldrich), tannic acid ACS reagent (Sigma Aldrich)

5.2.2. Thermoresponsive hydrogel precursor resin

Neutral QCh:SPA:pTsOH [1:0.25:0.75] was prepared as described in Chapter 2. Once prepared the 200g resin was stirred vigorously in a round bottom flask kept at -70°C by a dry ice acetone bath. To this 244.4 g of mEGA was added to the flask dropwise over the course of 40 minutes. This blend formed the 45:55 HEA:mEGA, referring to the weight ratio of HEA resin to mEGA monomer. Other blends were formed by mixing HEA resin with the 45:55 HEA:mEGA

resin. In the case of the 42:58 blend, additional mEGA was added to 45:55 HEA:mEGA resin to reach the required ratio.

5.2.3. LCST Transmittance measurements

To test the LCST of the HEA/mEGA blends, thin films of 150 μ m were cast between 2 glass slides using glass coverslips as spacers. The films were cured in a custom-built UV curing chamber and swollen in ultrapure water to separate the films from the glass slides. After swelling, the films were placed in a glass cuvette and held in place with an aluminum clip. Transmittance measurements were performed on a Jasco J-815 CD Spectrometer (Tokyo, Japan) with a temperature-controlled holder with magnetic stirring. Each sample was measured with a temperature wavelength sweep from 3°C-95°C at a heating rate of 2°C/min with a wave scan every 3°C from 600-800nm.

5.2.4. Equilibrium swelling measurements

To test the equilibrium swelling of the HEA/mEGA blends, specimens were cast in PDMS moulds and cured in a custom-built UV curing chamber, followed by demolding and subsequently swollen in ultrapure water. After swelling each sample was placed in a glass vial filled with fresh ultrapure water and equilibrated at 5°C for 72 hours in an environment chamber. The mass and volume of each sample were measured, followed by increasing the temperature to 10°C and allowing it to equilibrate for at least 8 hours. From this point onwards, the mass and volume were recorded every 10°C after at least 8 hours of stabilization.

5.2.5. OA-CNF Alginate Microparticle Synthesis

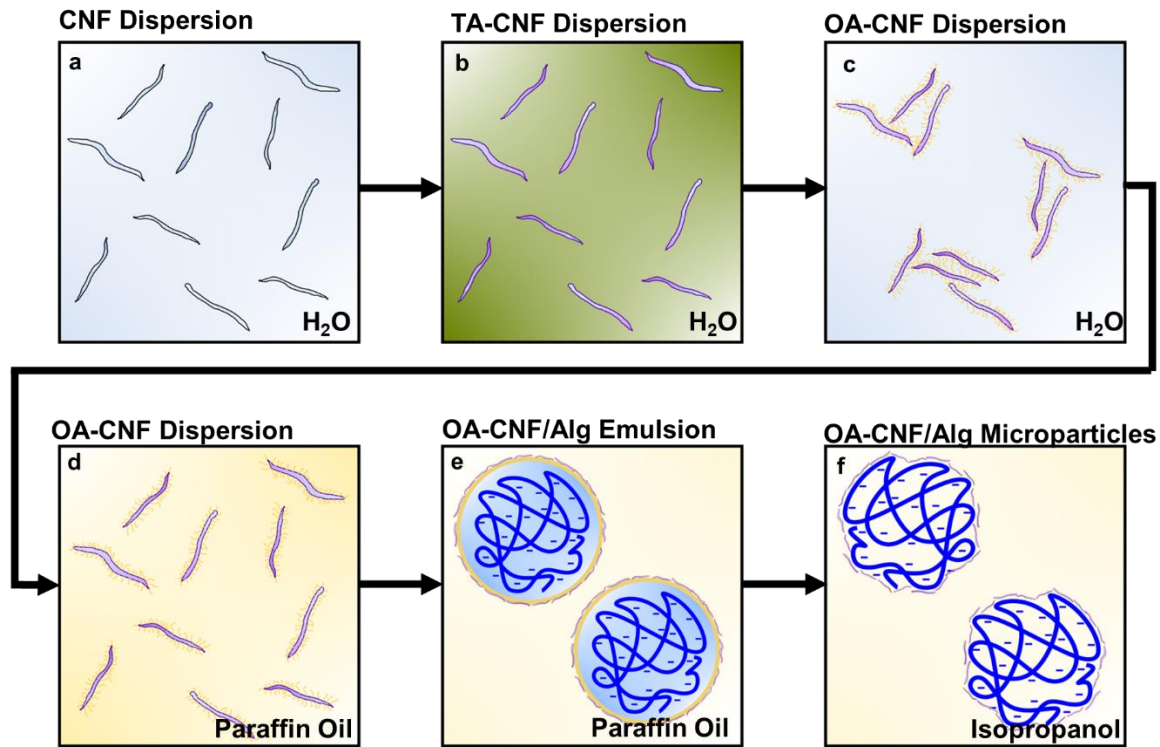


Figure 5.1 Synthesis schematic of OA-CNF/Alg microparticles. Initially CNFs are dispersed in water at 1 wt% [a]. After the addition of tannic acid at a pH of 8 the tannic acid coordinates with the surface of the CNF forming what is referred to as TA-CNF [b] with change in solution color to greenish brown. Octylamine reacts with TA-CNF surface to form hydrophobic OA-CNF which aggregates in the aqueous solution [c]. Following purification, the OA-CNF nanoparticles are redispersed in paraffin oil [d], and lecithin is added. The alginate water phase is then added and emulsified forming OA-CNF/Alg emulsion [e]. Finally the emulsion was solidified with the addition of isopropanol and subsequently purified [f].

Cellulose nanofibers (CNF) were prepared by diluting 333.3 g of CNF slurry (containing 10 g of CNF) with 666.6 g of ultrapure water to form a 1% solution. This solution was sonicated for 5 minutes at 70% amplitude using a Q Sonica Q125 probe sonicator with a ¼” probe to ensure sufficient dispersion. [Fig 5.1. a]

Modification Process:

Following sonication, the solution was adjusted to pH 8.0 by adding 1M NaOH. Subsequently, 1 g of tannic acid was dissolved in 5 ml of deionized water and added to the CNF solution (10 wt% of CNF). The mixture was vigorously stirred for 6 hours until the colour changed from light yellow to brownish green, indicating the onset of tannic acid oxidation [Fig 5.1. b]. After this period, 100 mM octylamine was added to the solution and stirred overnight, resulting in a colour change to deep yellow [Fig 5.1. c].

Purification and Concentration:

The hydrophobically modified CNF, now agglomerated at the top of the reaction vessel, was purified by performing three centrifugations with water followed by three with ethanol. The samples were then centrifuged at 22,000 G for 15 minutes to concentrate the CNFs. The concentrated CNFs were dispersed in paraffin oil at a concentration of 15 mg/mL using a high-speed homogenizer. Residual ethanol was removed by drying the samples in a vacuum oven at 70°C and -29 inHg overnight [Fig 5.1. d].

Aqueous Phase Preparation:

The aqueous phase was prepared by mixing 60 g of sodium alginate with 40 g of glycerol until fully wetted. This mixture was then combined with 300 ml of ultrapure water under vigorous stirring and mechanically stirred overnight to ensure complete dissolution of the sodium alginate. The oil phase was prepared by diluting 15 mg/mL of octylamine-modified CNF in paraffin oil to 10 mg/mL and adding 10 mg/mL of lecithin to the mixture.

Emulsion Production:

Emulsions were produced by mixing the two phases at a volume ratio of 60:40 using a high-speed homogenizer at 10,000 rpm for 5 minutes. In a typical synthesis, 200 ml of the alginate solution was added to 300 ml of paraffin oil containing 10 mg/ml OA-CNF and 10 mg/ml lecithin, followed by initial emulsion formation by high-speed homogenization. The mixture was then passed through a UP400St (Hielscher ultrasonics, Germany) with an FC22K flow cell and S24d22D probe at 90% amplitude 3 times with a peristaltic pump at 10 ml/min through a ¼” silicon tube [Fig 5.1. e].

Particle Solidification and Purification:

Alginate particles were solidified by adding an equivalent volume of the aqueous phase of isopropanol dropwise while stirring at 600 rpm. The particles were then purified by centrifugation at 5,000 rpm with three washes of isopropanol followed by three washes of ethyl acetate to remove any remaining lecithin and paraffin oil. The purified particles were dried at 50°C and -29 inHg for 4 hours before characterization [Fig 5.1. f].

5.2.6. OA-CNF/Alginate microparticle Characterization

Characterization of the CNF and OA-CNF was performed with FTIR, AFM and contact angle. For FTIR, 1% CNF dispersion in water or isopropyl alcohol for unmodified and modified CNFs, respectively, were added to potassium bromide in a mortar and pestle and ground into a slurry. The resulting slurry was dried in a vacuum oven at 80°C and -700 mmHg overnight to remove any solvent. The resulting powder was ground with fresh KBr and pressed into a pellet

before measuring on a thermoscientific Nicolet FTIR. AFM was performed on a mica slice after depositing a 0.0005 wt% OACNF or CNF dispersion by spin coating at 1000 rpm. Particle size distributions were measured using a Shimadzu SALD-201V particle size analyzer with isopropyl alcohol as the working fluid. SEM was performed on a Tescan VEGA TS-5130 SEM at 20 keV after depositing the particle dispersions in isopropyl alcohol on a 5 mm x 5 mm piece of silica wafer, followed by air drying and subsequent sputter coating with gold for 2 minutes at 20 keV. The creaming study was initially homogenized by the same method as the flow cell emulsions and final emulsions were prepared with probe sonication at 80% amplitude for 2 minutes on a q125 probe sonicator (Qsonica, USA) with a ¼ in the probe.

5.2.7. OA-CNF/alginate Microparticle Loaded HEA/mEGA resin

Dried OA-CNF/alginate microparticles were loaded into the 45:55 HEA:mEGA resin on a weight basis. In a typical synthesis, 12.5g of OA-CNF/alginate microparticles were added to 87.5 g of 45:55 HEA:mEGA resin before mixing for 10 minutes in a Thinky ARE-310 (Thinky, USA) planetary mixer for 10 minutes at 2000 rpm.

5.2.8. Viscosity and Mechanical Measurements

Viscosity measurements utilized a CAP 2000+ viscometer (Amtech Brookfield, USA) and 1H spindle. Measurements were performed at 22°C with 200 µL and a hold of 60 seconds. Tensile tests (strain rate 2 mm/s) were performed on a CETR-UMT (Bruker, USA). Unless otherwise specified, tensile coupons were cast in PDMS moulds, followed by 10 minutes in a custom-built 300 W curing chamber before demolding and solvent exchange with water. 3D-printed tensile coupons were printed with a layer time of 8.5s per layer on a Phrozen 8K mini before cleaning with isopropyl alcohol and curing for 10 minutes in a custom-built 300 W curing chamber.

5.3. Results and discussion

5.3.1. Characterization of CNF

FTIR and AFM analyses were conducted on raw and modified CNFs to confirm the successful grafting of octylamine. The FTIR spectra revealed characteristic changes at 2800 cm^{-1} , indicative of aldehydes, and 1640 cm^{-1} , indicative of C=N bonds (Fig. 5.2. a). While these results do not conclusively confirm the modification, they strongly suggest successful grafting. Additionally, contact angle measurements exhibited a significant increase from $45^{\circ}\pm 9^{\circ}$ in unmodified CNFs to $135^{\circ}\pm 7^{\circ}$ in modified CNFs (OA-CNF), indicating a substantial rise in hydrophobicity (Fig. 5.2b & c), providing further evidence of successful grafting of octylamine on the surface of CNFs.

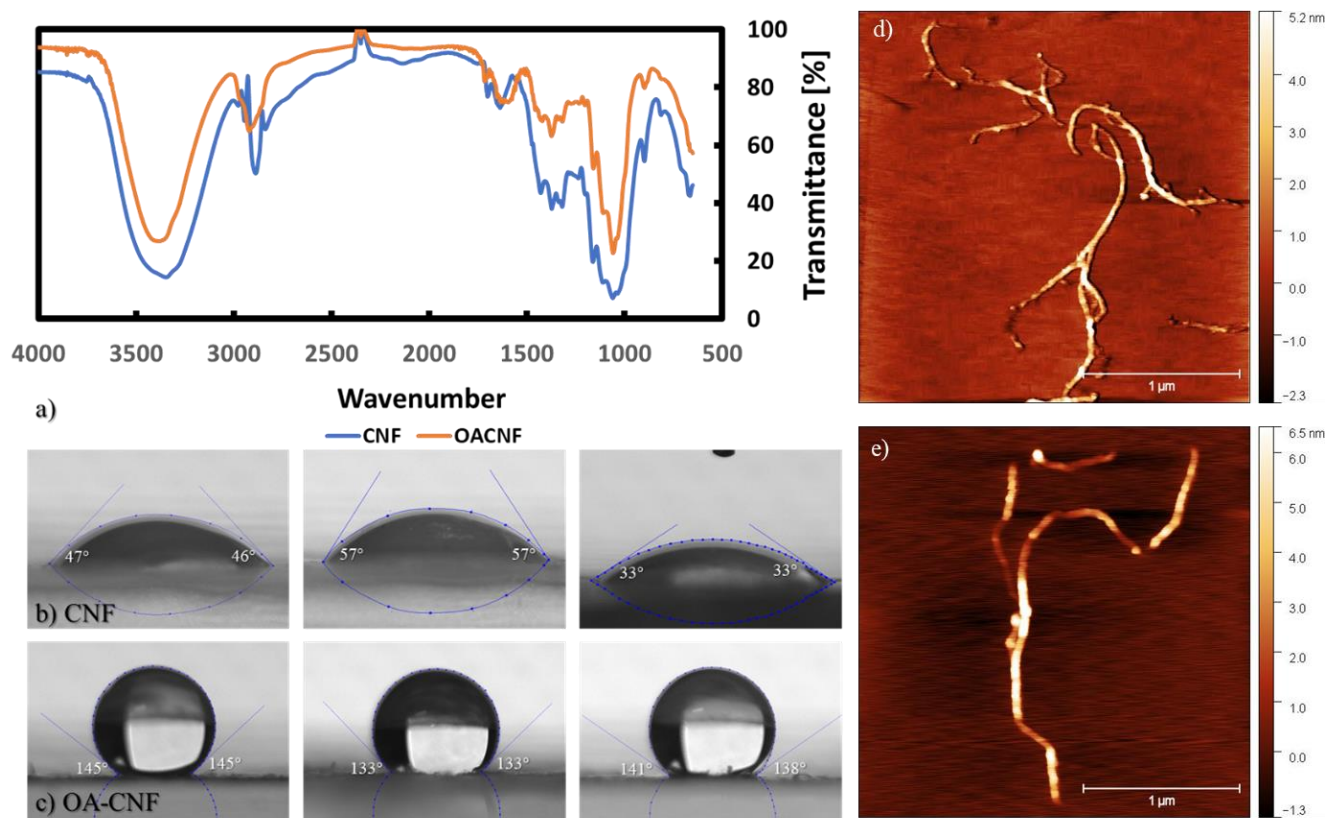


Figure 5.2 Characterization of the unmodified CNF and OA-CNF particles. A) FTIR measurements of the CNF (blue) and OA-CNF (orange). Contact angle measurements of the CNF films (b) and OA-CNF films (c). AFM images of the CNF (d) and OA-CNF(e) deposited on mica slides.

After confirming hydrophobic modification, AFM was employed to analyze the structural characteristics of dilute solutions of spin-coated CNFs on mica. The modified CNFs retained similar lengths (1-2 μm) and thicknesses (10-20 nm) compared to the unmodified CNFs, indicating that the tannic acid and octylamine modification did not significantly alter the CNF structure (Fig. 5.2. d & e). Furthermore, the formation of nodule-like structures on the modified CNFs suggests a non-uniform modification pattern, which is beneficial for interfacial assembly applications, enhancing the stability of macroemulsions.

Although lecithin alone could form emulsions suitable for solidification, the resulting powder could not be purified. During centrifugation with isopropyl alcohol, lecithin-only

emulsions aggregated and could not be redispersed, even with sonication. Conversely, emulsions with OA-CNF exhibited superior dispersibility after centrifugation, requiring only gentle stirring during purification.

SEM and particle size analyses revealed the effect of mixing methods on particle size distribution and shape. Mechanical stirring produced more homogenous particles in terms of sphericity but with a broader size distribution, with many particles exceeding 100 μm (Fig. 5.3a & c). Flow sonication, however, resulted in smaller, more uniformly sized particles, with none exceeding 100 μm (Fig. 5.4a). High shear mixing methods produced better dispersions and smaller particles, attributed to the high viscosity of the sodium alginate solution. These findings confirm that mechanical mixing alone was insufficient for creating small sodium alginate particles required for printing.

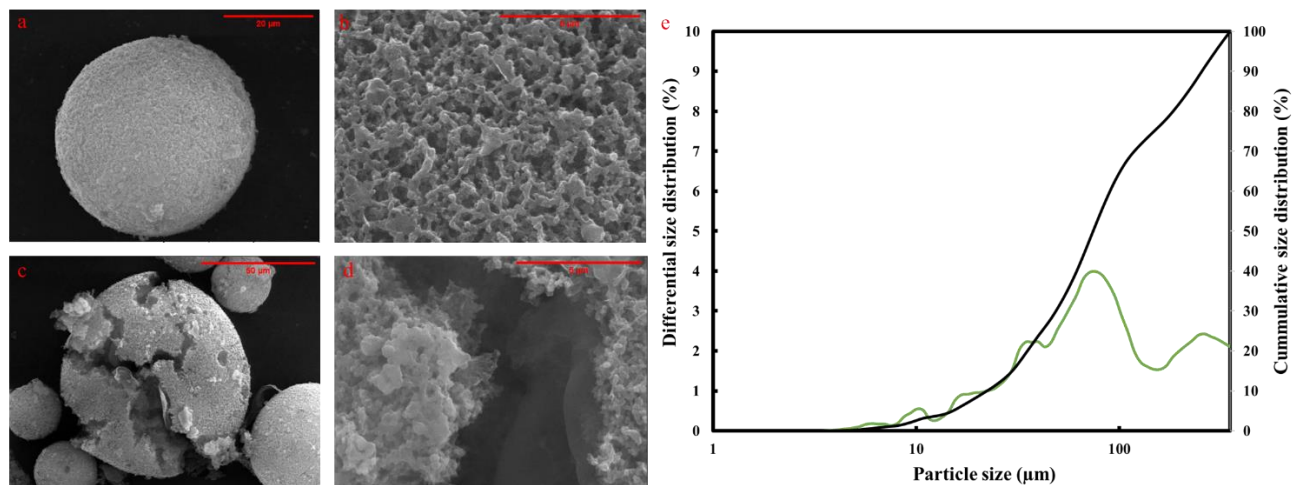


Figure 5.3 Characterization of OA-CNF/Alg microparticles prepared through mechanical stirring at 600 RPM. (a) SEM image of OA-CNF/Alg particle at 4x magnification. (b) Surface at 25x magnification. (c) Image of a fractured OA-CNF/Alg microparticle at 2x magnification. (d) Magnified area of the fracture showing different morphologies. (e) Particle size distribution of microparticles, with the majority between 50-100 μm .

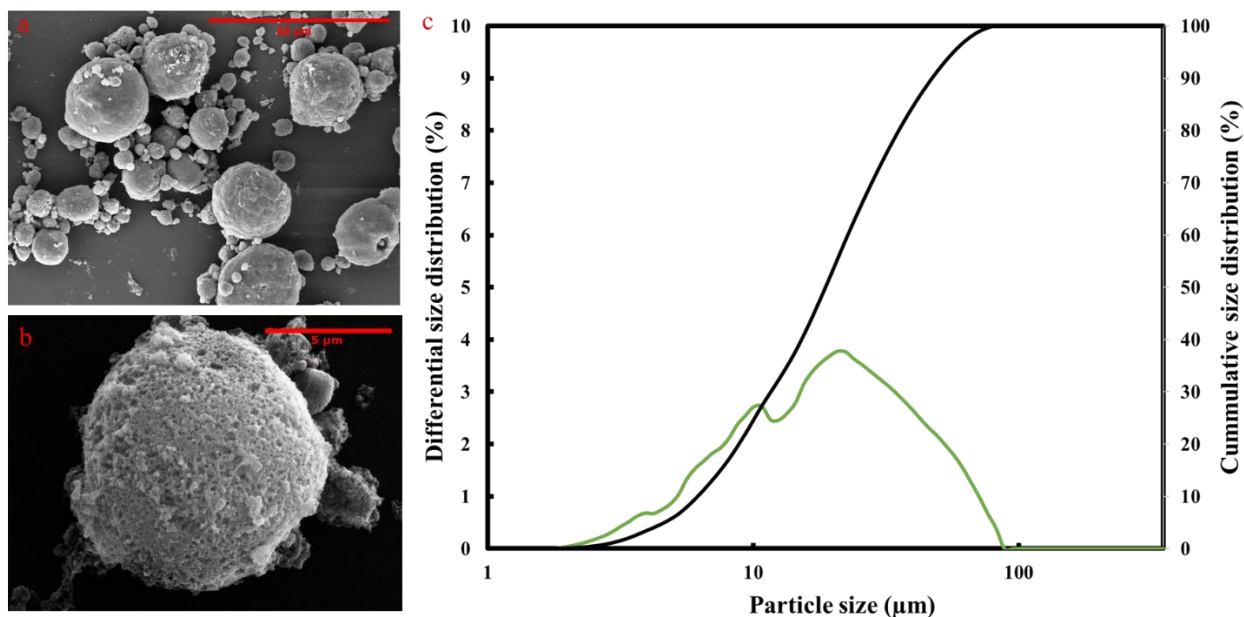


Figure 5.4 Characterization of OA-CNF/Alg microparticles prepared by flow sonication. (a) SEM image of OA-CNF/Alg microparticles at 2.5kx magnification. (b) Surface at 17.5kx magnification. (c) Particle size distribution of microparticles, with the majority between 10-50 μm .

5.4. 3D Printable POEGA Hydrogels Results

After initial success with the formation of supramolecular hydrogels composed of HEA and QCh:SPA salt, as described in Chapter 4, attention was focused on the addition of mEGA to form a thermoresponsive 3D printable system. While the 1:1 SPA:pTsOH neutral salts demonstrated significantly higher strengths when compared to the 1:3 SPA:pTsOH, the latter shown higher compatibility with the mEGA monomer. When adding the mEGA monomer, the neutral QCh SPA:pTsOH 1:1 salt tended to precipitate out of solution indicated by a change in opacity and a significant decrease in viscosity. During initial trials, it was found that the 1:3 SPA:pTsOH salt demonstrated the best compatibility with the mEGA and tended to form a more homogeneous resin than the 1:1 SPA:pTsOH.

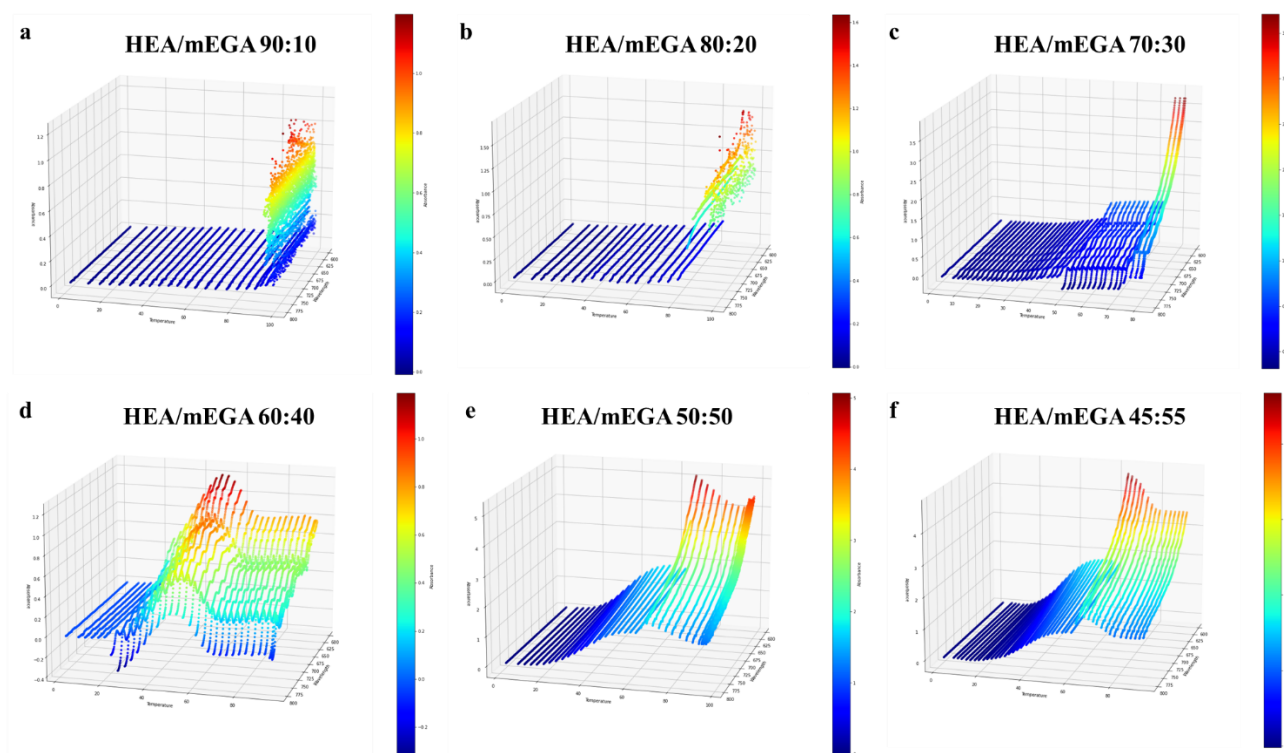


Figure 5.5 Absorbance temperature scans of HEA/mEGA blends crosslinked with neutral QCh SPA:pTsOH [1:3]. [a] 90 wt% HEA resin : 10 wt% mEGA monomer, [b] 80 wt% HEA resin : 20 wt% mEGA monomer, [c] 70 wt% HEA resin : 30 wt% mEGA monomer, [d] 60 wt% HEA resin : 40 wt% mEGA monomer, [e] 50 wt% HEA resin : 50 wt% mEGA monomer, [f] 45 wt% HEA resin : 55 wt% mEGA monomer.

As POEGMA hydrogels were typically produced as random copolymers using reversible addition–fragmentation chain-transfer polymerization or atom transfer radical polymerization²⁵, samples prepared by free radical polymerization and the LCST behaviour of the formed hydrogels were investigated. Various blends modifying the ratio of HEA resin to mEGA monomer were prepared to see the effect of increasing hydrophobic content in the network on the thermal response. The thermal responsive properties were measured with two different techniques to ensure the LCST opacity change was associated with the shrinkage seen in thermoresponsive polymers. At a low mEGA content of 10% [Fig. 5.5. a] no significant change in opacity was observed. The spike in opacity was attributed to the boiling of the water within the cuvette. At increasing mEGA contents [Fig. 5.6. b&c], an observable LCST occurs, with 20 wt% mEGA demonstrating an LCST at 80°C and 30 wt% mEGA showing LCST behavior in the 50-70°C

range. As mEGA content increased, the temperature onset of the LCST decreased, and the degree of opacity increased with the 45:55 HEA resin to mEGA by weight, demonstrating the lowest thermal response of the blends tested with onset at 20°C. While each polymer blend demonstrated a different LCST temperature, the blends do not behave as expected of thermoresponsive homopolymers such as PNIPAm. Due to the random nature of the blends, the polymers reorient their networks as the temperatures increase, leading to variations in the opacity. This behaviour was most pronounced in the 60:40 and 45:55 blends, where above the measured LCST temperature, the 60:40 film began to decrease in opacity at around 60°C as seen in other POEGMA systems²²⁰. This behaviour contrasted with the 50:50 and 45:55 blends, where there was a significant increase in opacity at lower wavelengths around 60°C. The variation of the LCST behaviour indicates that the use of POEGAs in this system was likely not ideal for the generation of 4D printable hydrogels as the opacity change was significantly more gradual than thermoresponsive homopolymers. This characteristic is unsuitable for 4D printable hydrogels, as a sharp LCST is required to trigger a usable shape change. Since the LCST occurs gradually, the LCST of different sections can overlap, preventing the selective collapse of specific regions. Alternatively, this hydrogel demonstrated thermoresponsive properties over a large range, which is suitable for applications where a proportional response is desirable. For example, a valve could be composed of this material with an orifice that allows a greater flow at low temperatures, allowing the device to self-modulate.

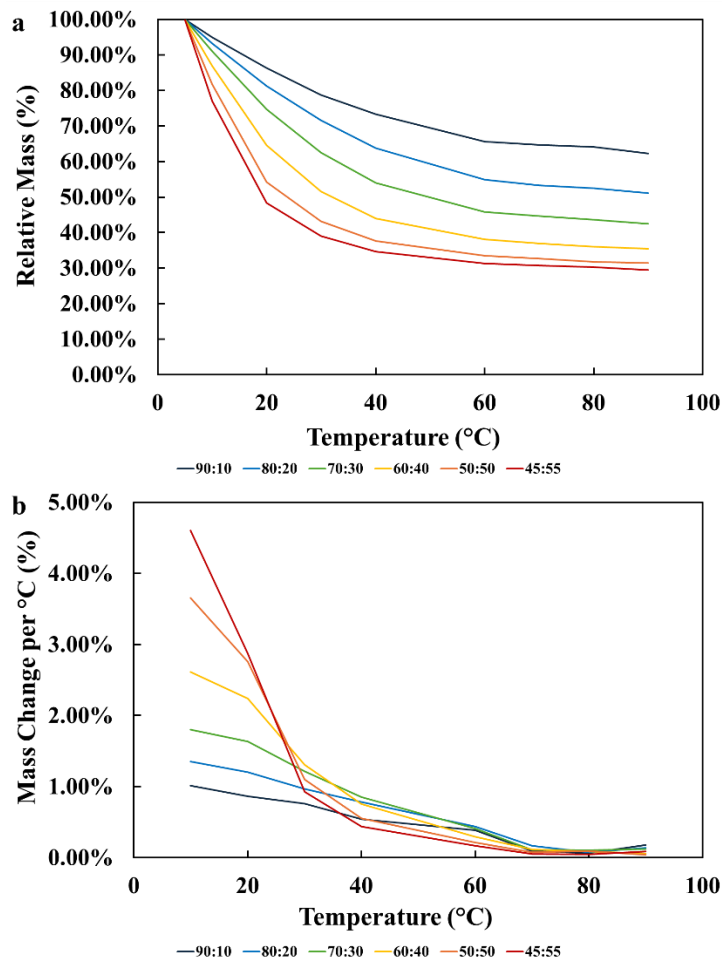


Figure 5.6 Equilibrium swelling graphs of different HEA:mEGA blends at increasing temperature. [a] Relative mass compared to 5°C of each hydrogel sample at increasing temperatures. [b] Mass change of the sample per °C [dark blue] HEA:mEGA 90:10, [blue] HEA:mEGA 80:20, [green] HEA:mEGA 70:30, [yellow] HEA:mEGA 60:40, [orange] HEA:mEGA 50:50, [red] HEA:mEGA 45:55.

Due to the irregular behaviour of the POEGMA hydrogels, a swelling study was then conducted to investigate the change in equilibrium swelling volume at different temperatures [Fig 5.6. a]. We see responses From the swelling tests that do not align with the previously performed opacity tests. The maximum degree of shrinkage occurred throughout heating, rather than at a defined LCST. Surprisingly, the largest change in mass was observed in the 5-20°C range rather than the >20°C range, as demonstrated by the opacity results [Fig 5.6. b]. These behaviours are significantly different than other thermoresponsive polymers and give insight into the function of POEGA polymers. In the literature, POEGA polymers have demonstrated self-assembly behaviour

when dispersed in solution as linear polymers, particularly the tendency to precipitate at a defined temperature before rearranging at higher temperatures and increasing in size²²⁰. Surprisingly, the LCST is not correlated with the equilibrium swelling mass in the prepared blends. This indicates that phase segregation at the LCST was not a driving factor of water expulsion; rather, the increased intermolecular interactions between hydrophobic groups drove water expulsion as it occurred at significantly lower temperatures than the LCST. While there is significant initial shrinkage in the hydrogel, samples containing lower concentrations of mEGA displayed a wider shrinkage range, with significant shrinkage still occurring at 60°C before stabilizing near zero at higher temperatures. This behavior indicates a segregated structure of the final hydrogel due to the compatibility of the mEGA with the QCh salt, which led to phase separation within the polymer resulting in a wide range of LCST temperatures within the polymer and the irregular thermal response.

Although this was not expected behavior when compared to thermoresponsive homopolymers such as PNIPAm, this result is interesting as it demonstrates these thermal responsive hydrogels can be tuned in both the LCST and the degree of shrinkage based on the structure of the polymer produced. Surprisingly for the tested samples, the thermal responsive volume change was significantly different than the measured opacity change indicating that in the case of this network the LCST and the equilibrium swelling percent are distinct temperatures. Interestingly, although all the equilibrium swelling measurements demonstrated similar behavior, the absorbance at higher temperatures significantly varies. This behavior is most obvious when comparing the 60:40 HEA/mEGA to the 45:55 HEA/mEGA. The former demonstrated a decrease in opacity at elevated temperatures, while the latter saw a significant increase in opacity above 60°C. This behavior points to a significant variation in microstructure, where the blends containing

higher portions of hydrophilic monomers initially displace water forming a phase separated structure, before expelling sufficient water where the size of the phase separations decreases. Conversely networks containing greater proportions of hydrophobic monomers phase separate at lower LCSTs before a significant increase in opacity was seen at 60°C. This is quite different from PNIPAm networks where these two behaviors occur at the same temperature. Unlike linear POEGA copolymers, swelling results of this material indicate that the control of LCST for this POEGA system was not as simple as varying the ratio of HEA and mEGA due to the phase separation of the monomers prior to printing, the second goal of this section was to incorporate sodium alginate into the hydrogel with the goal of increasing the strength of the network in addition to the speed of the networks thermal response [Fig. 5.8].

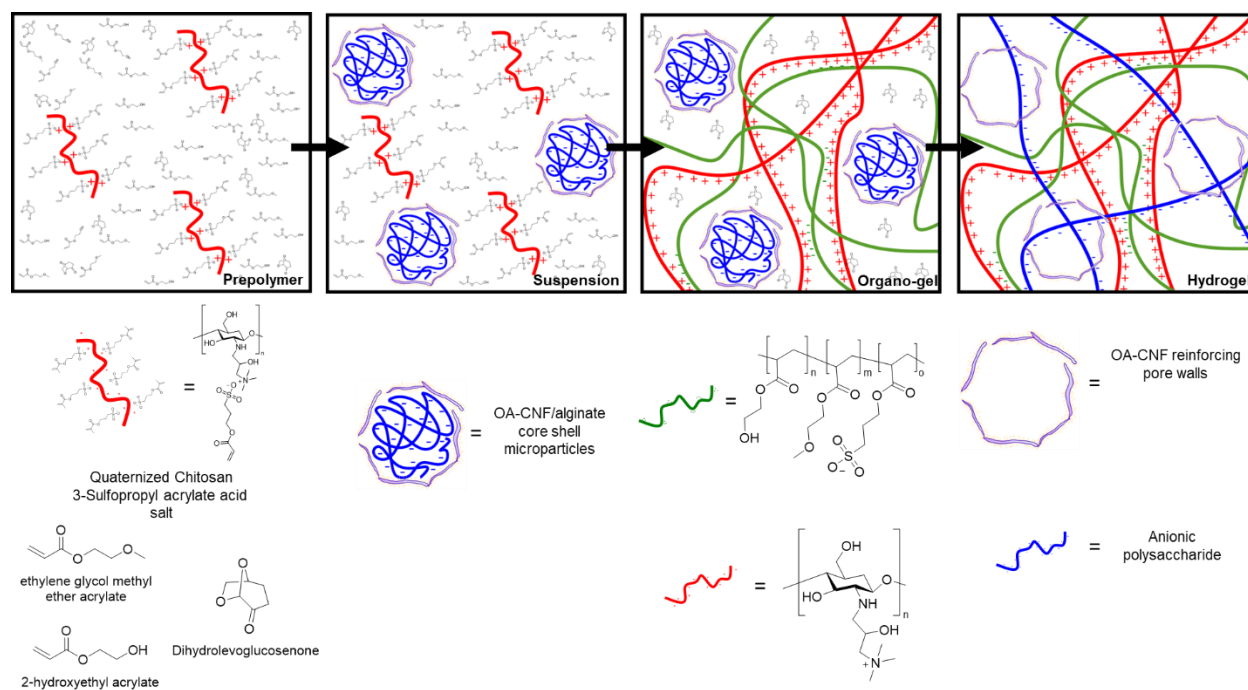


Figure 5.7 Synthesis steps of the ionically crosslinked hydrogel. [Prepolymer] HEA/mEGA 45:55 prepolymer resin. [Suspension] Dried OA-CNF/Alg microparticles are added to the prepolymer resin and dispersed with planetary mixing. [Organo-gel] The monomers are then photopolymerized by 405nm light forming an organo-gel with bonds to the Q-Chit acrylate salt. Due to the nature of the salt the resulting thermoresponsive network has templated anionic charges at the regions that interact with Q-Chit. [Hydrogel] After a solvent exchange with water, the anionic polysaccharide is able to integrate into the network and form ionic bonds to the Q-Chit network. The spaces occupied by the anionic polysaccharide then form a controlled pore network based on the size of the initial microparticles. These void spaces are then reinforced by the remaining nanoparticles.

To determine a suitable concentration of sodium alginate particles to load into the network, the viscosity of the pre-polymer resin was tested (Fig. 5.8). The 45:55 HEA resin exhibited a dynamic viscosity range from 8.5 ± 0.5 to 40 ± 4.1 cP, inversely proportional to the shear rate. Lower shear rates were not tested as the viscometer displayed inaccurate results outside the linear power curve range of the motor. The viscosity increased proportionally to the loading concentration of alginate microparticles, with 12.5 wt% alginate demonstrating the highest viscosity, ranging from 70.7 ± 2.4 to 154.0 ± 4.5 cP. Although the loaded resins' viscosity was not significantly different from previous work using quaternized chitosan in HEA, the resulting resin's shear-thinning properties limited its printability. At concentrations above 15 wt%, the resin would not flow under its weight when inverted, preventing its use in SLA-style machines, though suitable for devices like bioprinters where shear-thinning resin is desirable.

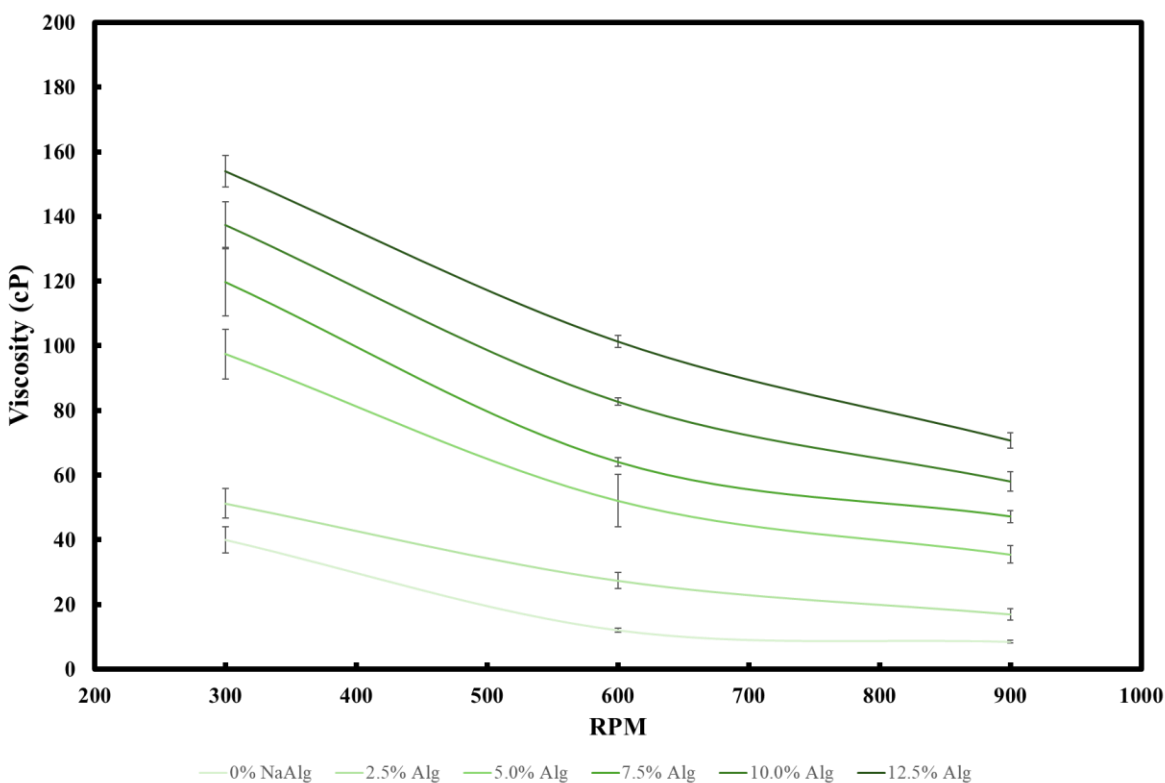


Figure 5.8 Viscosity measurements of HEA/mEGA 45:55 containing different loadings of OA-CNF/Alg microparticles.

Following viscosity characterization, the tensile properties of loaded hydrogels were measured (Fig. 5.9). Surprisingly, the unloaded hydrogel performed poorly compared to previous hydrogels containing only HEA, with an ultimate tensile strength of 40 kPa and an ultimate strain of 156%. Higher sodium alginate microparticle loading increased tensile strength and elasticity, with a trend of increasing tensile strength and Young's modulus as loading increased.

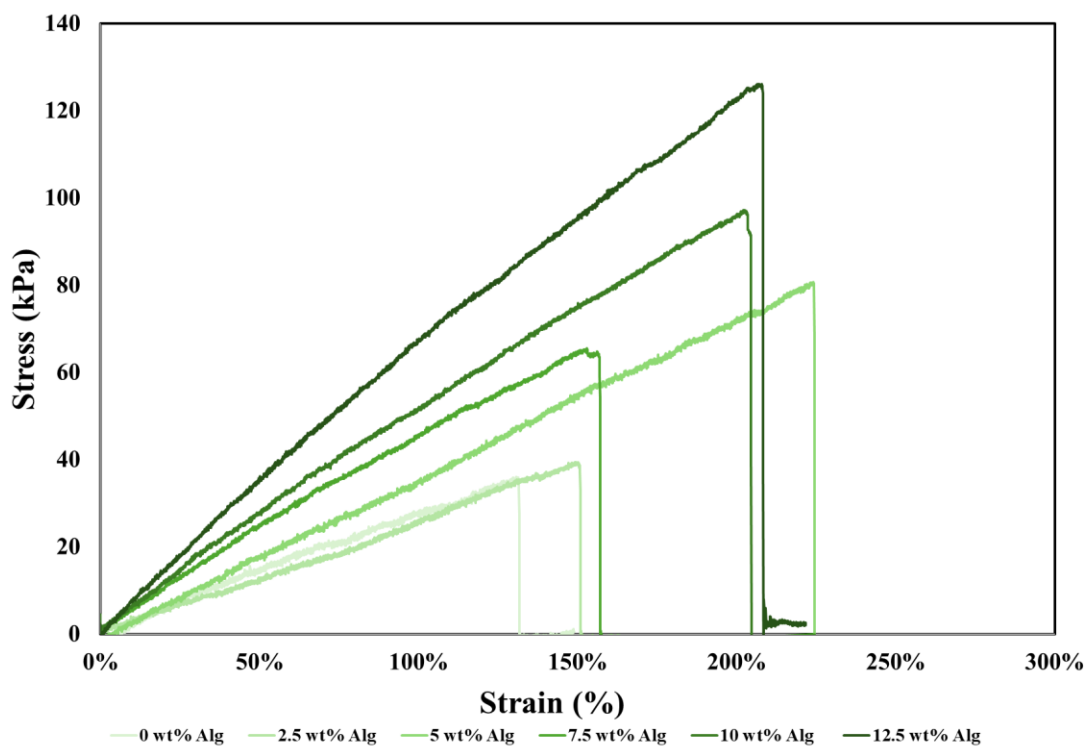


Figure 5.9 Tensile measurements of cast HEA/mEGA 45:55 hydrogels containing different loadings of OA-CNF/Alg microparticles.

Table 5.1 Average ultimate tensile strength and strain of cast samples with various alginate loading percentages.

Alginate (wt%)	Loading	Tensile strength (kPa)	Standard Deviation	Ultimate Strain (%)	Standard Deviation
0.0%		40.8	±5.4	156 %	±39.7%
2.5%		40.3	±1.4	164%	±10.9%
5.0%		84.2	±12.5	194 %	±16.0%
7.5%		72.6	±10.0	140%	±14.1%
10.0%		95.8	±13.4	202%	±8.7%
12.5%		113.1	±10.5	171%	±27.2%

While the tensile properties of the cast hydrogels were unexpectedly low, the microstructure of the cast organo-gel and final hydrogel were investigated by SEM and ESEM, respectively. The cross-sections of the cast organo-gel (Fig. 5.11a-d) showed visible alginate particles as white particles at 2.5 wt% loading. As loading increased, more particles were visible in the cross-section, and the smooth matrix surface displayed rougher characteristics, indicating smaller alginate particles interacting with the matrix and stiffening the surface. These samples were cryogenically cooled in LN2 and subsequently fractured. Due to the brittle nature of the polymer at these temperatures, the samples did not display the expected geometry of alginate particles seen earlier. Despite this, the increasing alginate loading varied the fracture plane of the matrix, and an even distribution of OA-CNF/Alg microparticles was observed.

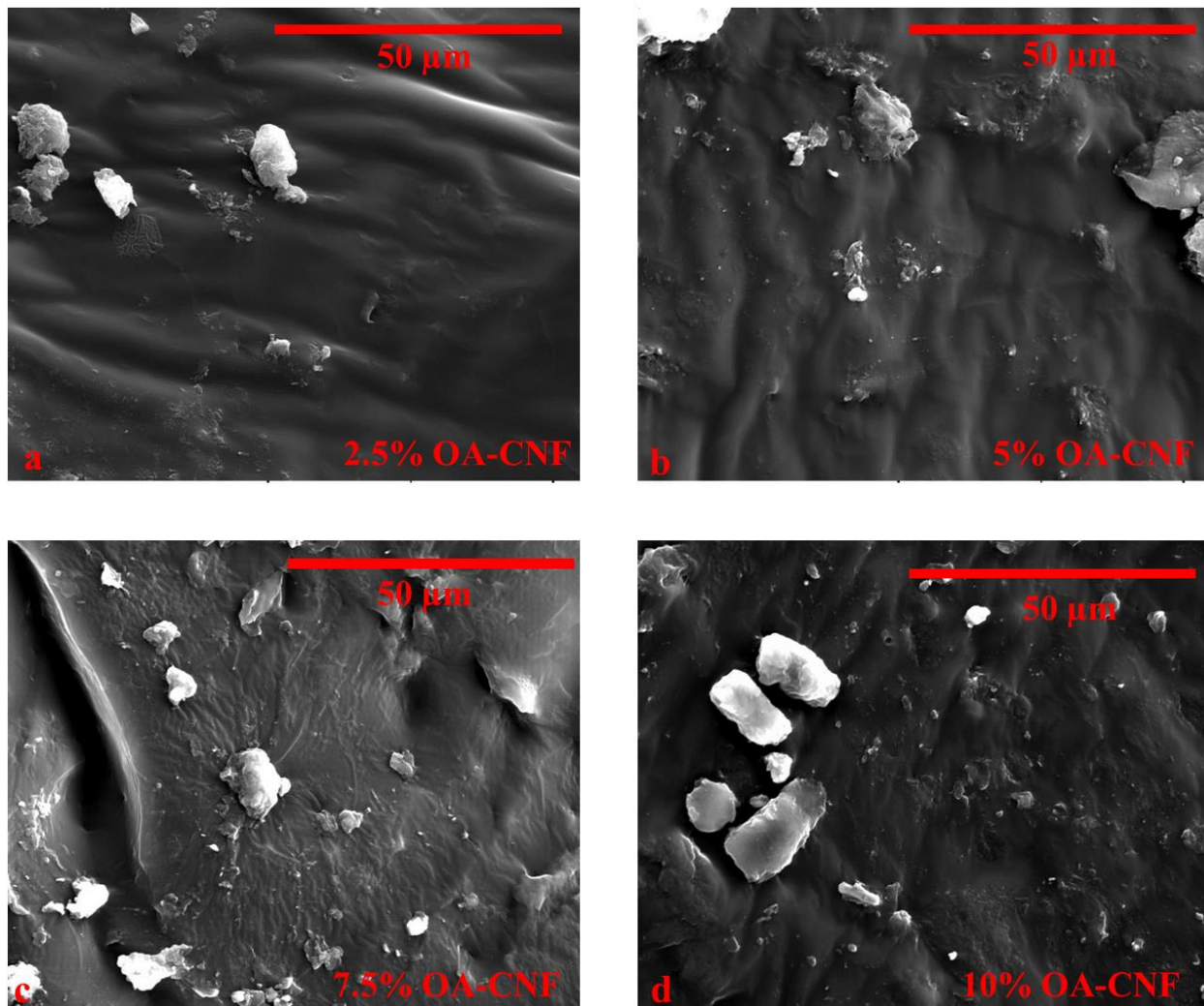


Figure 5.10 SEM images of cast HEA/mEGA 45:55 organo-gels containing 2.5 wt% [a], 5 wt% [b], 7.5 wt% [c] and 10 wt% [d] loadings of OA-CNF/Alg microparticles.

To further investigate the composite material's microstructure, samples that had undergone solvent exchange with water were cooled in liquid nitrogen, and the fracture plane was imaged with ESEM (Fig. 5.11). Initial images of samples without alginate particles showed a rough surface with small pores within 5-10 μm . The 5 wt% alginate sample indicated larger pores, correlating with the mechanical tests where 2.5 wt% did not affect mechanical properties measurably. As alginate loading increased, porosity increased, with 7.5%-12.5% loading demonstrating more ~ 20 μm pores.

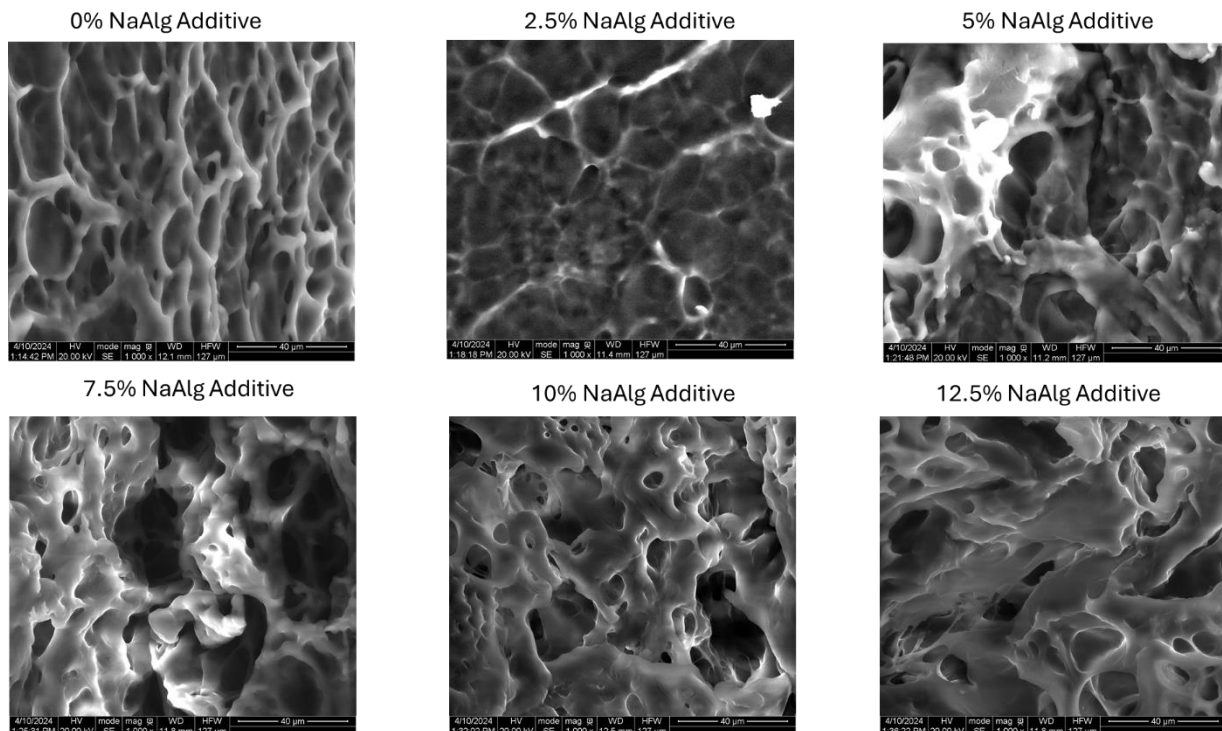


Figure 5.11 ESEM images of cast HEA/mEGA 45:55 hydrogels containing different loadings of OA-CNF/Alg microparticles at 1000x magnification.

These increasing porosity results from the ESEM, along with the water content of each hydrogel reported in Fig. 5.12, lead to the conclusion that the addition of alginate densifies the network due to the development of holes within the network while maintaining the same water content. This behaviour and the hydrophobic interactions between the octylamine-modified CNF and the mEGA component of the material demonstrate a definitive strengthening behaviour of the network.

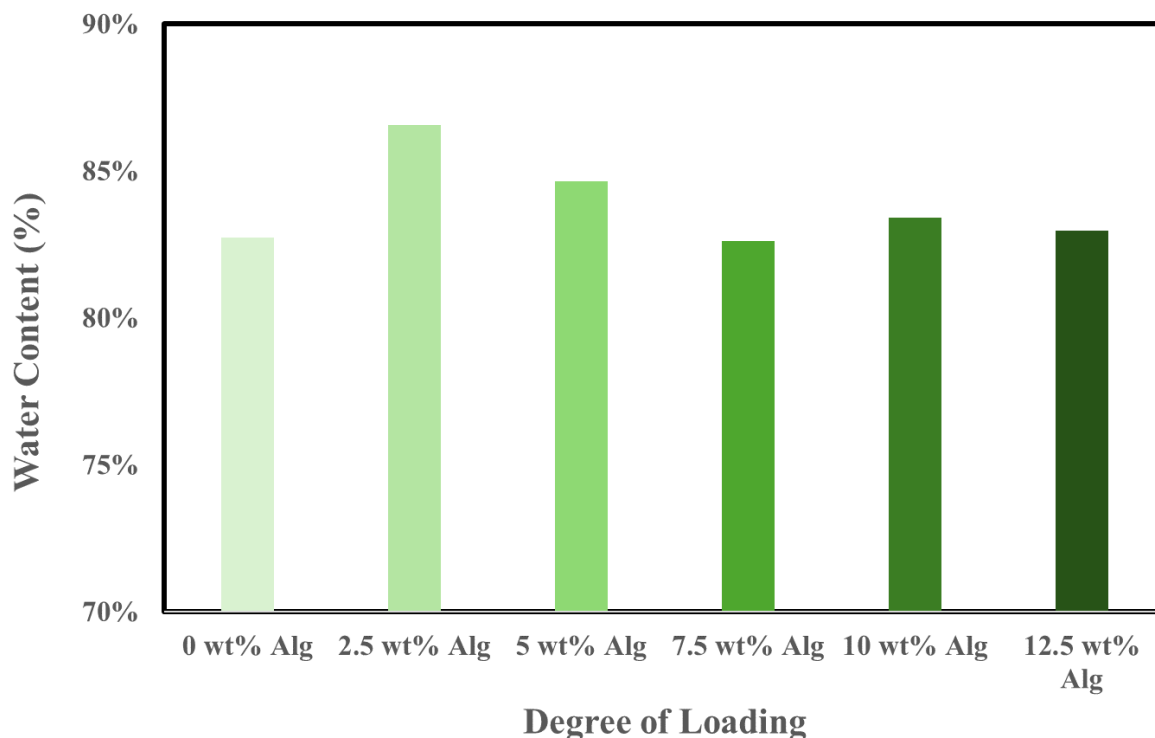


Figure 5.12 Water content of cast HEA/mEGA 45:55 hydrogels containing different loadings of OA-CNF/Alg microparticles.

Following resin characterization through casting, a larger quantity of the 12.5% alginate resin was synthesized to test 3D printability. While the material was 3D printable, high resin loading complicated accurate prints. The 5x5x5 mm calibration cube exhibited accurate X and Y dimensions (4.95 mm), but the Z dimension was significantly larger (6.2 mm), attributed to high solids loading and large microparticle size. Particles above 50 μm increased layer thickness, compounding inaccuracies in the Z dimension. Despite this, the mechanical properties of printed organo-gels were superior to cast samples (Fig. 5.13a). After a 3-day solvent exchange with water, 3D printed alginate-loaded hydrogels showed an ultimate tensile strength of 205 ± 3 kPa and an ultimate strain of $441 \pm 30.35\%$, an 81% increase in tensile strength and more than double the strain compared to cast samples. These improvements were attributed to the uniform alginate dispersion in printed samples and consistent polymerization, reducing matrix defects.

Comparisons of 3D printed samples stored in DI water for 72 hours and 720 hours (Fig. 5.14. b) showed a slight decrease in tensile strength over time but an increase in ultimate strain, suggesting the cationic biopolymer mitigates mechanical property reduction in alginate hydrogels. Thermo-mechanical properties showed a significant increase in ultimate strain ($861 \pm 41\%$) and modest tensile strength increase (272 ± 9 kPa) upon network collapse and water expulsion. Surprisingly, the POEGA network demonstrated significantly different stiffness behaviors from the PNIPAm hydrogels investigated earlier. When collapsed, the POEGA networks demonstrated a more pronounced stress dissipation region above 100% strain, while the stiffness was significantly lower than the swollen network above 100% strain. This behaviour is opposite to that seen in the PNIPAm hydrogels earlier in the thesis, where a significant increase in stiffness was observed above the LCST.

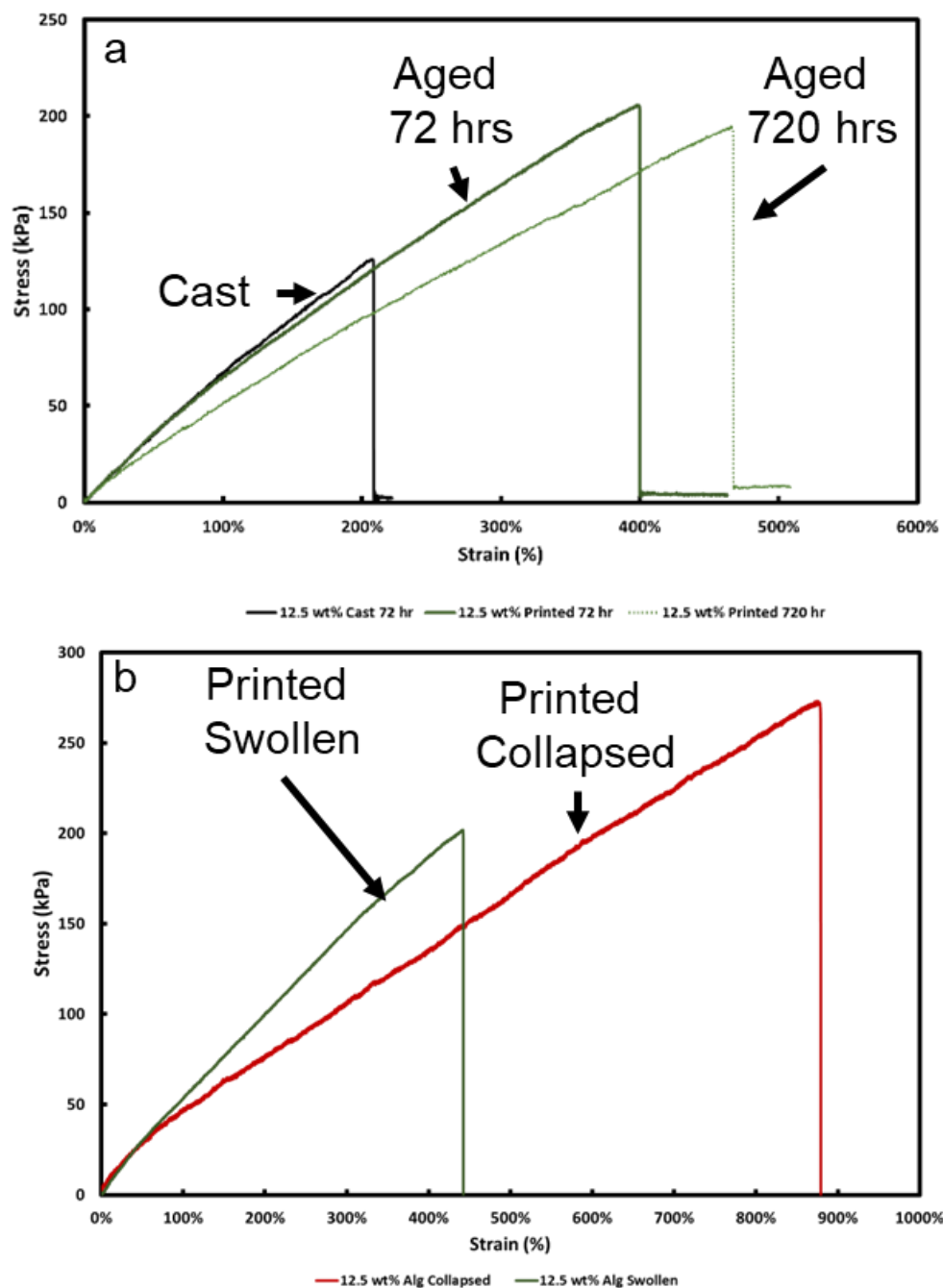


Figure 5.13 Tensile measurements of 3D printed HEA/mEGA 45:55 hydrogels with 12.5 wt% OA-CNF/Alg microparticles. (a) Comparison of stress-strain curves of cast samples stored in DI water for 72 hours (black), 3D printed samples stored in DI water for 72 hours (dark green), and 3D printed samples stored in DI water for 720 hours (green). (b) Comparison of stress-strain curves of swollen hydrogels stored in DI water for 72 hours (green) at 5°C to collapsed hydrogels stored in DI water for 72 hours (red) at 60°C.

SEM was performed on 3D-printed organogels to determine microstructure. SEM images (Fig. 5.14) revealed a significantly different structure compared to previous cross-sections. Samples sectioned at room temperature with a scalpel showed clear alginate microparticles, with circular regions composed of a rough surface well dispersed throughout the organogel. These images prove that the OA-CNF/Alg microparticles are well distributed within the network and maintain the shape seen after initial synthesis.

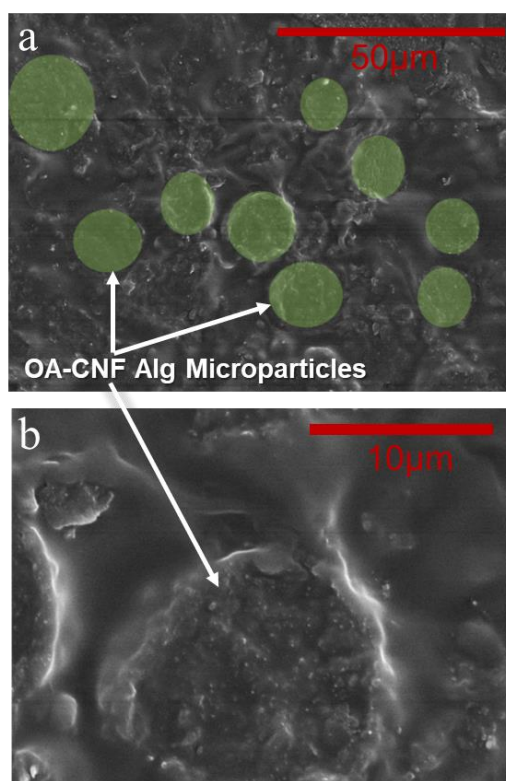


Figure 5.14 SEM images of a cut cross-section of the 3D printed organo-gel with OA-CNF microparticles emphasized in green at 2.5kx (a) and 10kx (b).

Discussion

Overall, this section investigated using mEGA as a co-monomer for 3D printing organo-gels and following the confirmation of the HEA:mEGA copolymer forming a thermoresponsive

hydrogel. The prepolymer was loaded with core-shell OA-CNF/alginate microparticles to improve the mechanical properties of the final hydrogel and improve the stability of sodium alginate-bearing thermoresponsive hydrogels. The thermoresponsive properties of the resulting hydrogel demonstrated a more extensive range than expected. The secondary goal of improving the mechanical properties of the HEA:mEGA copolymer and ionic stability alginate containing IPN hydrogels were successful, with significant improvements in the ultimate tensile stress and strain. Furthermore, the use of quaternized chitosan enabled the ionic crosslinking of alginate networks that were not susceptible to cationic diffusion over extended periods. When applied to digital health, these properties provide desirable improvements for alginate-based wound dressings. This furthers the initial goals of this research, which are to develop robust thermoresponsive wound dressings for drug delivery. At the same time, one potential application of thermoresponsive hydrogels is their use in shape-change materials. In the literature, the formation of random copolymers of mEGA and HEA have demonstrated sharp LCST behavior¹⁹⁴, unfortunately the characteristics of these linear polymers does not translate to the hydrogels synthesised. One likely reason these polymers do not display suitable characteristics is the use of quaternized chitosan as an ionic crosslinker to form the supramolecular hydrogels. Due to the phase separation of mEGA from the QCh and HEA-rich phase, a broad response was achieved. However, This limitation can be seen as a positive for applications in self-regulating systems where the size of the hydrogel impacts the temperature and digital health, where the skin temperature can be significantly lower than body temperature. The wide range of thermal responses these systems provide provides a greater range with which drug delivery could be effective. Additionally, using QCh has numerous benefits for the final polymer, namely conductive characteristics and potential degradability, as

demonstrated by the HEA hydrogels. Finally, using quaternized chitosan provides the potential for antimicrobial properties and stabilizing the alginate network.

5.5. Conclusions

In this chapter, we successfully demonstrated the formation of thermoresponsive supramolecular hydrogels by incorporating mEGA into the previously reported system. The resulting hydrogels demonstrated broad thermal responses with tunable LCST behaviour. Furthermore, through the development and subsequent loading of core/shell alginate microparticles, the mechanical properties of the resulting hydrogels could be significantly improved, reaching 400% when swollen and 860% when collapsed. The HEA/mEGA hydrogels provide a promising system for thermoresponsive wound dressings with potentially antimicrobial properties. In contrast, the core-shell alginate particles offer a method for incorporating an alginate network into applications where solubilized alginate is undesirable due to its viscous properties.

Chapter 6: Thesis Conclusions, Remarks and Future work

This research program aims to investigate the use of organo-gel precursors to develop 3D printable thermoresponsive hydrogels with desired properties for digital health and the rapidly growing field of personalized medicine. The concluding remarks and some insights into the development of 3D printable thermoresponsive hydrogel precursors acquired from this research are listed below.

6.1. Multi-thermoresponsive PNIPAm Hydrogel

Experimental studies were performed to develop 3D printable PNIPAm-based materials. This work first reported 4 main findings. First, dimethyl sulfoxide is a superior solvent for organo-gel 3D printing compared to isopropanol used in the literature. The low volatility and compatibility with different acrylates enable the printing of large-scale hydrogels over long periods at high fidelity. Second, the use acrylated poloxamers as crosslinkers for the DMSO organo-gels maintained the original thermoresponsive properties of the poloxamer independent of the main network. Third, by adding glycerol, alginate dispersions could successfully form in DMSO, allowing high loading levels of alginate in organo-gels. Additionally, the presence of tannic acid-modified CNCs modulates the size of the alginate dispersion, preventing agglomeration and improving the system's fidelity. Finally, the tannic acid-modified CNCs provide both thermoresponsive adhesion in the PNIPAm network and act as a photo absorber during printing, further improving fidelity. This work benefits the field of photopolymerizable hydrogels for 3D printing, specifically where ionic polysaccharides are used as a secondary network. We published this work in the *Journal of Colloid and Interface Science*. **2023**, 638, 882–892.

6.2. Quaternized Chitosan Supramolecular hydrogels

Systematic studies on the use of quaternized chitosan:acrylate salts as an ionic crosslinker for 3D printable organo-gels provided insight into the mechanism with which it ionically crosslinked HEA hydrogels. By modulating the composition of the quaternized chitosan : acrylate salt, the final hydrogel's mechanical, structural and conductive properties could be tuned for desired applications. This work first reported using a poly(ionic) salt composed of an ionic polysaccharide and an ionic acrylate and the first work to use Cyrene in the 3D printing of hydrogels. Through the formation of salt from N-(2-hydroxy-3-trimethyl ammonium) propyl chitosan and 3-sulfopropyl acrylate, the resultant poly(ionic) salt was soluble in the polar aprotic resin while remaining associated. This enabled the high-fidelity printing of a highly elastic hydrogel through an intermediate organo-gel. This work significantly benefits the field of 3D printing hydrogels as the crosslinking scheme opens a new category of crosslinkers for photopolymerization-based 3D printing. Furthermore, this work demonstrates the use of Cyrene for organo-gel 3D printing as it provides numerous health and environmental benefits compared to DMSO for digital health and personalized healthcare applications. This work is under revision with *Carbohydrate Polymers*. **2024.**

6.3. Thermoresponsive poly(oligo ethylene glycol acrylates) hydrogels loaded with alginate microparticles

After understanding how quaternized chitosan:acrylate salt functioned with HEA to form a supramolecular hydrogel, mEGA was incorporated within the resin to produce thermoresponsive supramolecular hydrogels. This work first reported using poly(oligo ethylene glycol acrylates) as a thermoresponsive hydrogel network for photopolymerization-based 3D printing. While previous work has focused on POEGA to form linear polymers or as

a graft, they have not previously been used in photopolymerization-based 3D printing. Concurrently, a biobased system for large-scale production of sodium alginate/OA-CNF microparticles was reported. This system utilized hydrophobically modified CNFs to act as a Pickering emulsifier along with lecithin to produce alginate microparticles. This was the first reported use of hydrophobic CNFs to act as a water-in-oil pickering emulsifier. This work has significant implications in the cosmetics, food, and emulsions industries as it provides a simple method of stabilizing water-in-oil emulsions containing high ion concentrations with biobased surfactants. Finally, the resultant core/shell microparticles significantly increased the mechanical properties of the thermoresponsive poly(oligo ethylene glycol acrylates) hydrogels and prevented degradation of the alginate network over time due to cation diffusion. This work significantly benefits biobased hydrogel research as it addresses core issues with the stability of alginate hydrogels while also providing methods to form hydrogels from 2 oppositely charged polysaccharides accurately.

Overall, the research presented in this thesis contributes to advancing the field of 3D printable thermoresponsive hydrogels, providing insights into new crosslinking methods for photopolymerization-based 3D printing and methods of incorporating of different LCST behaviour in the resulting hydrogels. Specifically, this work pioneers organo-gel precursors for 3D printing natural-synthetic double network hydrogels. Due to the solubility limitations of bioderived polysaccharides and dispersion limitations of bioderived nanoparticles, an informed design must be utilized to achieve compatible systems. Two approaches used in this thesis were the design of polysaccharide microparticle systems in the 3rd and 5th chapters that were insoluble in the prepolymer resin and diffused into the network upon solvent exchange and the formation of polysaccharide acrylate salts, which facilitate solubilization of the polysaccharides

in a polar aprotic resin. This provides two alternate methods of incorporating high biomaterial loading within 3D printed hydrogels and provides a clear path forward for further development of environmentally friendly thermoresponsive hydrogels. Furthermore, organo-gel 3D printable systems enable the fabrication of large hydrogel objects with reduced printing time while preventing LCST behaviours from affecting the fidelity of the printed structures. As such, this work furthers the field of mechanoactive wound dressings, demonstrating the benefits of 3D printing and methods to reduce the degree to which harmful compounds are used in manufacturing.

6.4. Future work

Throughout this thesis, two comprehensively different 3D printed thermoresponsive hydrogels were developed. Initially, chapter 3 focused on a thermoresponsive hydrogel with bioderived components, but the use of PNIPAm, CaCl₂, and DMSO limited its applicability in biomedical and wound dressing applications. While the work demonstrated valuable properties, further characterization of the drug release mechanism should be conducted. This mainly focuses on removing geometric constraints and defining the hydrogel's intrinsic properties that govern the drug release mechanism. Chapters 4 & 5 acted as a natural evolution of the thermoresponsive systems developed, replacing PNIPAm and DMSO with a HEA mEGA copolymer and Cyrene, respectively, and the use of a supramolecular crosslinking system, removing the need for CaCl₂ to act, as a crosslinker for the alginate network. Future work for chapter 4 includes using 2D NMR to characterize the supramolecular hydrogel better. While solid-state carbon NMR was performed on the dried hydrogel, the spinning field lines obscured the areas of interest and prevented conclusions from being reached. Future work would focus on the further characterization of the supramolecular hydrogel.

The HEA/mEGA system presented has multiple potential approaches that could be used to improve the hydrogel described in this section and improve the properties of the resulting hydrogel. While the photopolymerization-based 3D printing techniques traditionally utilize free radical polymerization, the addition of QCh led to regions of varying composition of mEGA and HEA, as evident by the broad thermal response seen in the LCST behaviour of the polymer. Recently, Asadi-Eydivand et al. demonstrated the applicability of RAFT polymerization for light-based 3D printing²²¹. Using RAFT techniques, it would be possible to form poly(HEA-co-mEGA) oligomers separate from the QCh and add these to the HEA resin. This approach could potentially solve the phase separation of the thermoresponsive monomers within the resin. Furthermore, by polymerizing the mEGA beforehand with HEA, the thermoresponsive behaviour of the final hydrogel could be determined beforehand, leading to more controllability and reducing the volatility of mEGA, preventing changes in the resin composition during long prints. The second improvement that could be implemented for the HEA-co-mEGA system would be the testing and implementing of different quaternized chitosan acrylate salts. Quaternized chitosan neutralized with 3-sulfopropyl acrylate was used for all the work reported and composed most salt variations tested; initial tests with acrylic acid indicated improved solubility in HEA. Other potential salts could be utilized with 3-sulfopropyl methacrylate or methacrylic acid. Adding the methyl group would increase the hydrophobicity of the resulting QCh salt and improve solubility within HEA/mEGA systems. While initial results testing acrylic acid salts were promising with the QCh:AA salts solubilizing within HEA without the use of high-power sonication, there was not sufficient time to redevelop the 3D printing system as significant characterization and optimization work had been performed on the SPA-based salt. Additionally, non-polymerizable acids other than pTsOH could neutralize the quaternized chitosan to improve compatibility with mEGA. In the

original work investigating solubilizing chitosan in polar aprotic solvents, camphor sulfonic acid also enabled solubility in DMSO, possibly replacing pTsOH. Additionally, although quaternized chitosan has seen significant applications as an antimicrobial agent with limited cytotoxicity, we could not perform such studies on the system developed. As such, the characterization of the antimicrobial and cytotoxic characteristics of the reported systems would significantly improve the relevance of the hydrogels in digital health. Furthermore, further characterization of the OA-CNF particles should be performed, specifically transmission electron microscopy with energy-dispersive X-ray spectroscopy. This would allow the visualization of the modification of CNFs by mapping the nitrogen groups on the fibrils.

Overall, this work takes steps to develop novel thermoresponsive hydrogels capable of synthesis through the printing of intermediate organo-gels. The developed system was capable of producing thermoresponsive hydrogels with high elasticity and stability, and the thermoresponsive characteristics broadly provided further potential within the wound dressing and digital health space. One potential application of these materials is as a replacement for traditional stitching and wound dressings. Due to their high elasticity and stability, patient-specific tubular bandages could be possible applications where the tension of the hydrogel could replace the need for wound closures such as stitches.

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Appendix (Supporting information)

Pluronic Diacrylate Synthesis Schematic

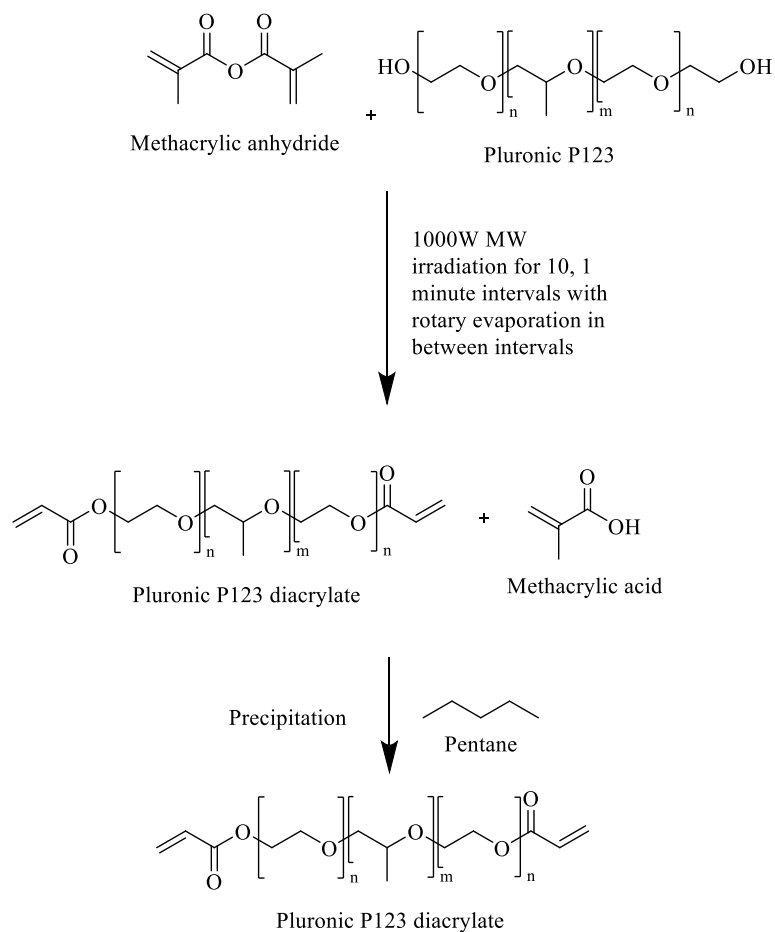


Figure S1 Synthesis scheme for Pluronic P123 diacrylate.

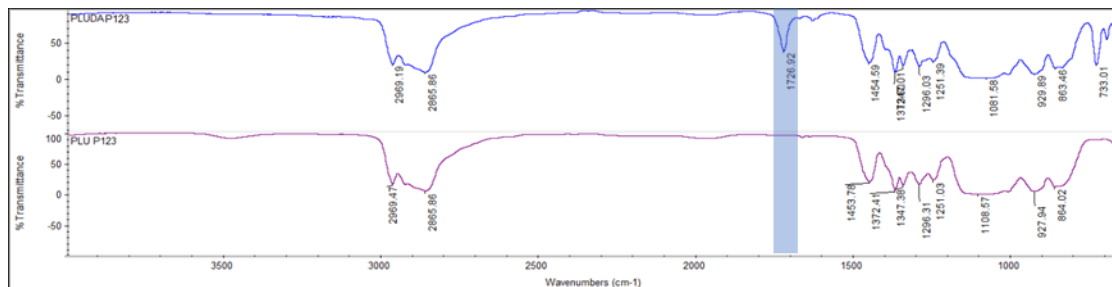


Figure S2. FTIR spectra of the Pluronic P123 Diacrylate, Pluronic P123 [listed from top to bottom]. The blue highlighted region highlights the region C=O stretching is displayed.

Comprehensive overview of the reasoning behind the design of the resin

Natural components

As illustrated in Figure 1, we used N-isopropyl acrylamide and pluronic p123 diacrylate-based resin to form a double network system with sodium alginate. Sodium alginate was selected as a secondary network to increase the networks flexibility once ionically crosslinked by Ca^{+} ions. While extensively used as a double network for cast hydrogels and extruded hydrogels, alginate's rheological properties preclude its incorporation into waterborne photolithography resins in significant concentrations and its limited solubility complicates its use with non-water systems. To address this challenge, our approach utilized both CNCs and glycerol to form micro-phase separated regions containing higher concentrations of alginate and glycerol with CNCs acting as nucleation points. Previous research within our group had found it possible to dissolve sodium alginate in a glycerol by first creating a glycerol/water mixture and slowly removing the water through vacuum drying. The resulting gel though was not dispersible in dimethyl sulfoxide, the chosen solvent for this system. Building on this approach, sodium alginate was first dissolved in a water/glycerol/dmso solution (40:1:2 volumetric ratio) and allowed to fully disperse at which point the water was slowly removed by rotary evaporation. This technique generated a viscous paste with relatively large aggregates that could be reduced through sonication assisted filtration, though the resulting printed hydrogels still displayed poor surface finish and print resolution due to the size of the filler.

To mitigate this issue CNC nanoparticles were incorporated into the water solution before the addition of alginate. The hypothesised mechanism would function by providing either nucleation points for the formation of the alginate/glycerol phase or a pseudo-Pickering emulsion preventing aggregation of alginate/glycerol particles by stabilization at the phase transition. As hypothesized the addition of CNC nanoparticles reduced the particle size in the resulting slurry improving resolution, though the printable resolution was still limited by the material rather than hardware limitations. Concurrently tests were performed on incorporation of various free-radical scavengers to prevent over polymerization. During testing it was found tannic acid showed promise as an additive due to the high concentration of hydroxyl groups. These groups provided significant strength improvements to cast hydrogel precursors made of the resin. This would be beneficial during printing as improved mechanical properties would allow for thinner features to be printed, further improving resolution. While promising, the acidity of the tannic acid led to significant settling of the alginate particles in the resin during printing. This was hypothesised to be due to the formation of alginic acid from sodium alginate in an acidic environment. Rather than select a different free-radical scavenger, it we decided to phosphate buffer to maintain a pH of 7.6 in the alginate solution. Interestingly, this solution turned an off red color, likely the result of a tannic acid coordination complex forming. While after evaporation, the slurry regained its light brown color indicating the complex was stable in water and not DMSO. The resulting resin at this point displayed superior strength and dimensional accuracy during printing leading to fewer failed prints from layer adhesion failures. Based on these results, the decision to scale up the synthesis for comprehensive testing was made.

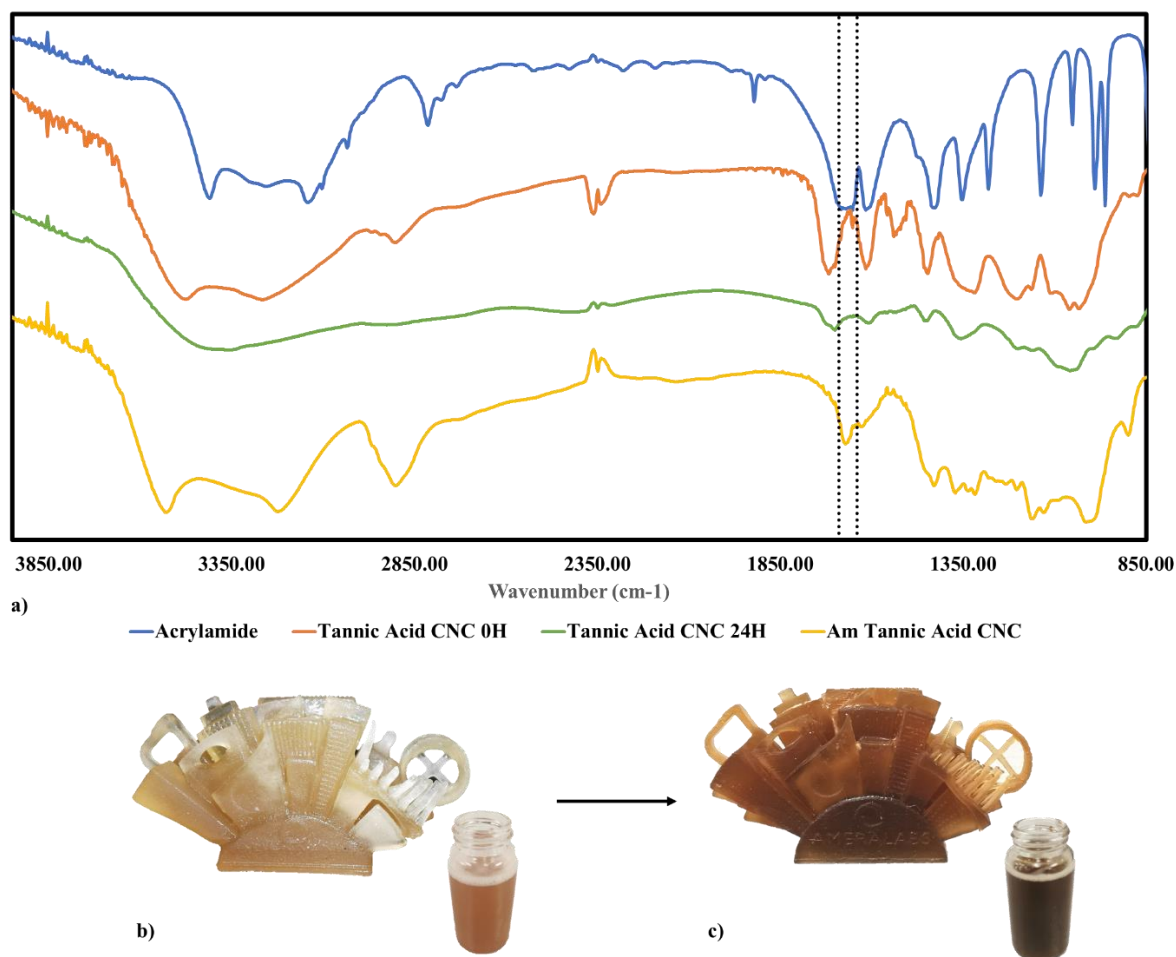


Figure S3. FTIR spectra of CNCs modified with tannic acid and subsequently acrylamide [a)] and demonstration of the fidelity change in the resin that occurs with the addition of the 24 h stirring step [b) & c)] and the visual change in the water solution before evaporation [lower right hand corner of each section].

During this synthesis, evaporation step was taking longer than expected and the solution was kept under stirring overnight, before continuing the next day. Unexpectedly, the solution transitioned to a dark brown as the basic solution had allowed to tannic acid to undergo oxidative polymerization. The resulting resin displayed a significantly lower viscosity and better surface finish due to the formation of smaller alginate/glycerol particles. Furthermore, the mechanical strength of the preswollen parts increased, reducing

the amount of additional crosslinkers required for printing. It was suspected that acrylamide in the synthetic components were reacting in a Schiff base scheme to form poly(tannic acid) coated CNCs with surface bound acrylate groups. To investigate this behaviour, FTIR spectra were collected of the tannic acid/CNC solution in phosphate buffer immediately after mixing, after 24 h of mixing and after 6 h of subsequent mixing with acrylamide in the ratios equivalent to that in the alginate solution. After mixing with acrylamide, the samples were centrifuged and washed with water over 3 cycles and a further 3 cycles with ethanol to remove any unbound acrylamide. As seen in Fig S3. from observing the hydroxyl peaks at $3550\text{--}3200\text{ cm}^{-1}$, $3200\text{--}2700\text{ cm}^{-1}$, and the C=O peak at 1800 cm^{-1} we can see the reduction in intensity after 24 h of mixing. Furthermore, the purified acrylamide poly(tannic acid) CNC mixture displayed a N=C peak at 1670 cm^{-1} indicative of a Schiff base reaction as well as peaks indicative of acrylamide. To our knowledge this is the first reported synthesis of acrylamide functionalized poly(tannic acid) CNC's and the first reported use of functionalized CNC's generated in-situ with no further purification. While previous works have shown one-pot synthesis of functionalized CNCs, they are typically purified after synthesis and not incorporated into a sub-synthesis within an ongoing one-pot synthesis.

Key insights gained from this investigation into the natural phase of the double networked hydrogel can be summarized as follows. While alginate is not traditionally soluble in glycerol, it can form an alginate glycerol dispersion in DMSO, facilitating high loading in polar aprotic solvents. Alone the alginate/glycerol microparticles are too large to form a homogeneous material on a macroscale, but the presence of CNC nanoparticles during their formation reduces their size by an unknown mechanism. This mechanism is suspected to

be through either the presence of nucleation sites, or the formation of a pseudo-Pickering emulsion. Finally, the incorporation of tannic acid in photopolymerizable pre-hydrogel resins provides a multifunctional role acting as both a free-radical scavenger and a reinforcing agent through hydrogen bonding. Additionally, under the reported conditions. Tannic acid can be polymerized on the surface of CNCs in-situ and form acrylamide functionalized CNCs, that can provide additional crosslinking and stiffness, improving printability.

Synthetic components

While prior discussion on the resin design focused on the development of the alginate base. This section focuses on how the acrylates were chosen for the resin. The initial design of the resin focused on the use of solely NIPAm and 1 wt% N,N'-Methylenebis(acrylamide) and 2.5 wt% Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide. During initial tests, it was found that the NIPAm tended to crystallize at the concentrations present in the resin. While this could be mitigated by the addition of more DMSO, this led to significantly reduced print quality and mechanical properties. Alternatively, acrylamide was chosen as an additive to prevent the crystallization of the NIPAm in the resin. It was found that at concentrations of 12.5 wt% of NIPAm, the crystallization could be prevented. As discussed above, the aggregation and settling of the alginate/glycerol particles caused multiple issues during printing. To improve the stability of the suspension, the addition of other monomers and crosslinkers were tested. From these exploratory studies, it was found that 2-hydroxyethyl methacrylate (10:120 mass ratio to PNIPAm) and high mw poly(ethylene glycol) diacrylate (molar eqv. to MBAm) improved the suspension characteristics during printing. This allowed for long prints to

be performed without settling. While the resulting hydrogels displayed satisfactory strength at 37°C. In the swollen state their poor elasticity significantly reduced their strength limiting real world applications. To address this problem, the poly(ethylene glycol) diacrylate was replaced with the dimethacrylated poloxamer Pluronic P123 dimethacrylate. Pluronic P123 was chosen as the ideal poloxamer as its critical micelle temperature is 8.9°C, below room temperature and the molecular weight promoted flexible rather than rigid crosslinking. Additionally, the lower molecular weight enabled a sufficient crosslink density while allowing for NIPAm to remain the major constituent of the resin. Through its incorporation the resulting hydrogel displayed 3 distinct regions swollen, partially collapsed and fully collapsed. This enabled the hydrogel to display greater mechanical properties at room temperature. The key insight from this section focuses on the use of acrylated surfactants to improve dispersions in resins. Traditionally surfactants are only added in minimal quantities as excess surfactant can reduce the interfacial bond between the filler and matrix. Through the use of thermo-responsive acrylated surfactants, significant portions can be added to utilize their dispersive properties while also imparting distinct thermal responses to the network without sacrificing mechanical properties.

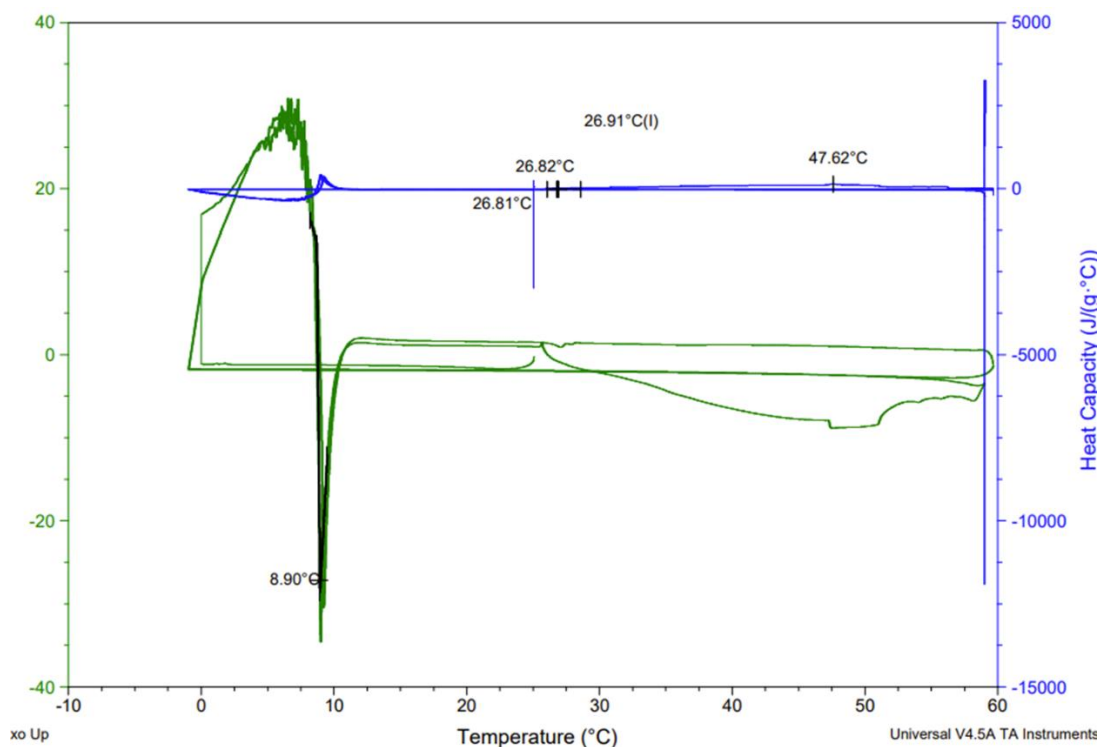


Figure S4 DSC measurement of dried hydrogel and known volume of DI water in the reference and measurement sample.

Table S1. Components of hydrogel precursor resin and the purpose of each component

Final Formulation	Resin	Weight (g)	Purpose
Sodium alginate		20	ionically crosslinked secondary network
Dimethyl sulfoxide		55	solvent/viscosity reduction
Glycerol		26	stabilizing sodium alginate in DMSO
Sodium phosphate monobasic dihydrate		2.609	sodium phosphate buffer .1M 7.1 pH prevents phase separation and reduces viscosity
Sodium phosphate dibasic heptahydrate		8.335	sodium phosphate buffer .1M 7.1 pH prevents phase separation and reduces viscosity
Tannic acid		3.6	crosslinker and radical scavenger reducing over polymerization
Cellulose nanocrystals		1.8	increasing hydrogel strength

N-isopropylacrylamide	110	Thermo-responsive behavior
Acrylamide	15	stabilizing N-isopropyl acrylamide in DMSO (prevents phase separation and crystallization)
Hydroxyethyl methacrylate	10	increases hydrogel strength, reduces viscosity
Pluronic P123 Diacrylate mw 6000	45	surfactant crosslinker with thermo-responsive behavior
Diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide	4.5	photoinitiator

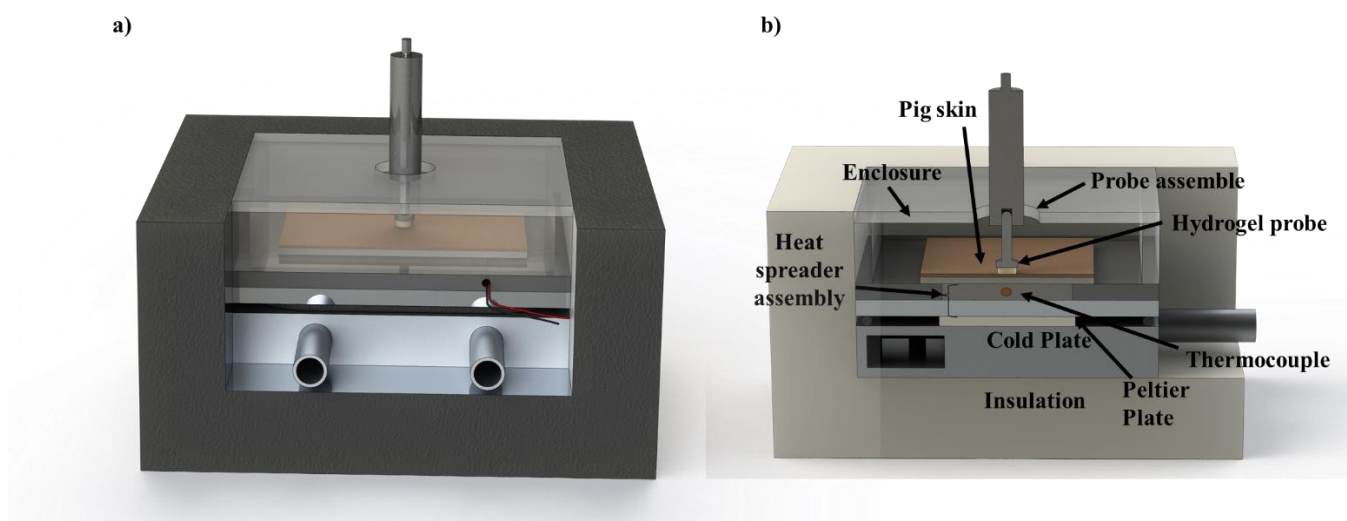


Figure S5. a) 3D renderings of the temperature-controlled adhesion measurement setup,
 b) cross-section of the setup with important components labelled.

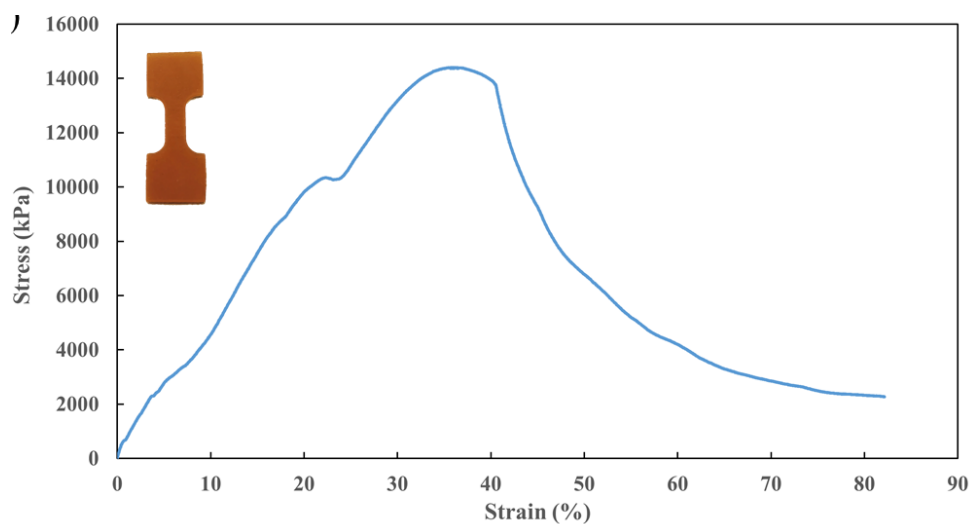


Figure S6 tensile test of the fully cured hydrogel precursor before swelling in water [measured using tensile test specimen pictured in top left-hand corner of graph].

Supplementary Video 1 (SV 1.mp4) Demonstration of thermoresponsive adhesion of 3D printed hydrogel to porcine & human skin at 8°C, 18°C and 33°C.

Supplementary Video 2 (SV 2.mp4) Peeling demonstration of 3D printed hydrogel on human skin.

Supplementary Video 3 (SV 3.mp4) Adhesion demonstration of 3D printed hydrogel on finger during extension and contraction.