3D/4D printing tough hydrogel composites: a pathway to functional devices

Shannon Edward Bakarich

University of Wollongong

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Intelligent Polymer Research Institute

3D/4D Printing Tough Hydrogel Composites: A Pathway to Functional Devices

Shannon Edward Bakarich

"This thesis is presented as part of the requirements for the award of a Doctorate of Philosophy from the University of Wollongong"

January 2016
DECLARATION

I, Shannon E. Bakarich, declare that this thesis, submitted in fulfilment of the requirements for awarding the degree of Doctor of Philosophy at the Intelligent Polymer Research Institute at the University of Wollongong, is entirely my own, unless otherwise stated, referenced or acknowledged. This Document has not been submitted for qualifications at any other academic institution.

Shannon E. Bakarich

January, 2016
Hydrogels are a class of hydrated and compliant polymer materials that display a variety of desirable properties for engineering applications including actuation, conductivity, and biocompatibility. Despite their unique range of material properties, the implantation of hydrogels into real world applications has been restricted due to their poor mechanical performance. In recent years a number of toughening mechanisms have been designed to improve the mechanical characteristics of hydrogels. This thesis investigates the use 3D printing as a means to process these ‘tough hydrogels’ into complex structures and functional devices. Two different methods are designed for extrusion printing ionic-covalent entanglement hydrogels. The printing inks were formulated to match the rheological requirements of the printing machines available and the chemical composition optimised to permit rapid crosslinking of the hydrogel components. Multi-material printing techniques are then used to print these tough hydrogel inks alongside other inks of other structural polymers to create a variety of composite architectures including fibre and particulate reinforcement. The mechanical properties of a series of composite structures were measured and compared with established composites theory. The material properties of these hydrogel composites are dependent on the volume fraction of hydrogel present and can be programmed into a printed object through digital modelling software. With these printing techniques mechanical gradients have been constructed and a preliminary prototype artificial cartilage demonstrated. These printing techniques have been further developed to incorporate an ink for printing a temperature-sensitive hydrogel that actuates. Combining this ink with other structural materials, co-called “4D printing” was demonstrated and a smart valve device was designed and fabricated to illustrate the potential usefulness of this new manufacturing approach.
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<td>3D</td>
<td>Three-dimensional</td>
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<tr>
<td>4D</td>
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<tr>
<td>AAm</td>
<td>Acrylamide</td>
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<td>Alg</td>
<td>Alginate</td>
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<td>AM</td>
<td>Additive Manufacturing</td>
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<td>CAD</td>
<td>Computer aided design</td>
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<td>DN</td>
<td>Double network</td>
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<td>E</td>
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<td>Epoxy amine</td>
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<td>Emax 904 Gel-SC</td>
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<td>ICE</td>
<td>Ionic covalent entanglement</td>
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<td>IPN</td>
<td>Interpenetrating polymer network</td>
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<td>Jeffamine</td>
<td>Poly(oxyalkyleneamine)</td>
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<tr>
<td>$\kappa$</td>
<td>Consistency</td>
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<td>LSCT</td>
<td>Lower critical solution temperature</td>
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<td>$n$</td>
<td>Power law index</td>
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<td>NC</td>
<td>Nano composite</td>
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<tr>
<td>NIPAAm</td>
<td>N-isopropylacrylamide</td>
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<tr>
<td>PAAm</td>
<td>Poly(acrylamide)</td>
</tr>
<tr>
<td>PAMPS</td>
<td>Poly(2-acrylamido-2-methylpropanesulfonic acid)</td>
</tr>
<tr>
<td>PDGI</td>
<td>Poly(dodecylglyceryl itaconate)</td>
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<td>PEGDGE</td>
<td>Poly(ethylene glycol)diglycidylether</td>
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<td>PNIPAAm</td>
<td>Poly(N-isopropylacrylamide)</td>
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<td>PVA</td>
<td>Poly(vinyl alcohol)</td>
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<td>$Q$</td>
<td>Swelling ratio</td>
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<td>STL</td>
<td>Stereolithography</td>
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<td>T</td>
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<td>Tan $\delta$</td>
<td>Loss factor</td>
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<td>U</td>
<td>Work of extension</td>
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<tr>
<td>UV</td>
<td>Ultra-violet</td>
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<td>V</td>
<td>Volume fraction</td>
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<tr>
<td>$\alpha$</td>
<td>Isotropic length contraction</td>
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<td>Symbol</td>
<td>Definition</td>
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</tr>
<tr>
<td>κ-CG</td>
<td>Kappa-carrageenan</td>
</tr>
<tr>
<td>( \dot{\gamma} )</td>
<td>Shear rate</td>
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<tr>
<td>( \varepsilon )</td>
<td>Strain</td>
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<tr>
<td>( \sigma )</td>
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1 INTRODUCTION AND AIMS

1.1 Applications of hydrogels

Hydrogels are a class of materials characterised by their ‘soft and wet’ attributes.[1] Hydrogels are three dimensional networks of hydrophilic polymer swollen in large volumes of water.[2] Some examples of hydrogel are capable of retaining water contents as high as 99% by weight. The hydrophilic nature of the polymers allows the gel to absorb water but the network does not dissolve due to the presence of cross-linking. Cross-links are sites that link one polymer chain to another to build up a three dimensional network. Based on the nature of the cross-linking the hydrogels are often divided into two categories: chemical gels and physical gels.[3] Chemical gels are composed entirely of covalent bonds, while physical gels contain non-covalent cross-links. Cross-linking in physical gels can occur via hydrogen bonding, hydrophobic interactions, ionic bonding,[4-6] through the formation of crystallites[7] or by an entanglement of polymer chains.[8, 9] The extent of cross-linking within a polymer network is related to both the hydrogel’s equilibrium water content and its modulus.[10, 11] The degree of swelling within the gel is regulated by a thermodynamic balance between the driving force for dilution and an opposing force created by the stretching of elastic polymer strands between cross-link sites.[12] Gels with fewer cross-links and longer polymer chains generally have larger equilibrium water contents because they generate a smaller elastic force. Highly swollen hydrogels tend to be softer because they contain a lower concentration of the polymer to resist deformation by an applied force.

Although physically constrained as a solid by the polymer network, hydrogels maintain many of the properties of liquid water and for this reason could be described as ‘solid water’[13] and have generated much interest for use in a diverse range of applications. Hydrogels act as good thermal insulators because of the high specific heat of water.[14, 15] Water is transparent to the visible range of the electromagnetic spectrum and so optically transparent hydrogels[16] that filter UV radiation have been prepared for the application of contact lenses.[17] Hydrogels can be used as low friction surfaces due to hydrodynamic lubrication.[18] The water content inside hydrogels can be used to dissolve, store and transport small molecules. This attribute of hydrogels has been used for targeted drug delivery[19] and for the slow release of fertilisers in
soil. Ionic conduction of a hydrogel can also be achieved with the mobility of dissolved salt in the interstitial water. These conductive hydrogels can be used to form soft, transparent and stretchable electronics.

Hydrogels are sometimes thought of as ‘smart materials’ because they can sense and respond to an external stimuli. Dependent upon the polymer composition, some hydrogels are able to react to a change in environmental factors such as temperature, pH or electrical field with resulting swelling or deswelling, stress generation or release of water or other chemical species. These macroscopic events are a result of a phase change which alters the polymer network’s affinity for water. For instance when a poly(N-isopropylacrylamide) (PNIPAAm) hydrogel is exposed to a temperature above ~ 35 °C its polymer network undergoes a coil-globule transition where the polymer network exhibits a greater affinity for itself than for water. The polymer network collapses in upon itself which makes the hydrogel expel water and decrease in volume. By reversing the stimuli with cooler temperatures the polymer network reverts to its hydrophilic state so that the gel can swell with water back to its initial volume. Hydrogels have been considered for applications as actuators and sensors because of this type of dynamic behaviour.

1.1.1 Microfluidics

Microfluidics is one application where hydrogels are used as both actuators and sensors. Microfluidics deals with the control and manipulation of fluids constrained spatially within small, typically sub-millimetre channels. Lab-on-a-chip and DNA chip technologies are microfluidic devices used to replicate normal laboratory tasks on a smaller size scale. Laboratory equipment is miniaturized into a single chip which enhances efficiency, mobility and reduces the volumes of reagents and samples required. A household example of a microfluidic device is an inkjet printer head which is used to precisely dispense picoliter droplets of ink onto paper.

Traditionally fluids within microfluidic devices were moved, mixed or otherwise processed employing passive techniques like capillary forces. In more advanced systems external actuation in the form of pumps and valves are used to direct the liquid flow. Hydrogel actuators have been used in microfluidic devices to act as micropumps and microvalves. The rate of volume change in a hydrogel actuator is limited by
the diffusion of water through the polymer network. The response of the volume change approximately follows the square of the dimension as the hydrogel objects reversibly expand and contract.\textsuperscript{[33]} Hydrogels are useful for the actuators in micropumps and microvalves because at this small size scale shapes have a low volume to surface area ratio which allows for fast diffusion and quick actuation.\textsuperscript{[34]}

1.1.1.1 Microvalves

Hydrogel microvalves use volume change to block the flow of a fluid through a microchannel. In one example, a simple hydrogel microvalve device was fabricated by curing a hydrogel precursor solution into the shape of a post in the centre of a microchannel using photolithographic techniques (Figure 1:1a). To improve the efficacy of this device design the hydrogel can be cured as a jacket around a central post.\textsuperscript{[33, 34]} This design decreases the hydrogel’s volume to surface area ratio allowing faster actuation. The hydrogel valves work as sensors in these systems that open and close based upon the pH, temperature or concentration of the fluid being passed through the microchannel. \textsuperscript{[33, 35]} A number of these sensing valves can be combined to fabricate complex systems for autonomous flow control.\textsuperscript{[36]} One such example is a flow sorter which actively diverts a flow through a T-shaped channel based on the characteristics of the injected fluid. A pH flow sorter was constructed by Beebe et.al by positioning a hydrogel that expands in high pH conditions and contracts in low pH conditions in one of the channels at the end of the T and positioning a hydrogel that expands in low pH conditions and contracts in high pH conditions in the other channel (Figure 1:1b).\textsuperscript{[33]} Sensor based valves are useful in applications that cannot afford the cost or weight of an electrical power supply. Alternatively, electrical on-demand control can be achieved using heat from a resistor to stimulate a temperature sensitive hydrogel.\textsuperscript{[37]} Diamond and co-workers produced an on-demand microvalve that actuated in response to a light stimulus.\textsuperscript{[38]} Light sensitivity was achieved by incorporating a spiropyran molecule that changes pH when exposed to visible light into the polymer network of a pH sensitive hydrogel.\textsuperscript{[39, 40]}
Figure 1: Examples of hydrogel microvalves: a. Digital representation of the photolithographic process used to fabricate hydrogel post microvalves, reproduced from [34]. b. Schematic diagrams of a flow sorter with pH sensitive hydrogel post microvalves, reproduced from [34]. c. Schematic of a microvalve (i) open and (ii) closed with a hydrogel actuator in fluidic isolation, reproduced from [34]. d. Microscope images of a pH sensitive hydrogel check microvalve, reproduced from [41].

In some situations contact between a hydrogel and fluid is unacceptable for contamination purposes. In these applications a flexible and impermeable membrane can be incorporated into the device between the hydrogel actuator and the fluid. When the hydrogel expands, it pushes the membrane into the microchannel to block flow. The heat from resistors or a second set of microfluidic channels can be used to stimulate the hydrogel actuators in this type of fluidic isolation device (Figure 1:1c).[31]

Hydrogels have been formed into biomimetic valve designs that replicate structures from nature. Yu et al. used photolithography to fabricate pH sensitive check valves inside a glass microchannel with the valves replicating the structure of venous valves.[41] Check valves allow for flow to occur only in a single direction and are found in mammalian veins to stop a back flow of blood. Venous valves are a pair of angled flaps called leaflets that are pushed open by forward pressure but backward pressure
pushes the leaflets together to block flow. The artificial check valve stops back flow using two hydrogel flaps but by contracting the size of the flaps with a pH stimulus, fluid is allowed to flow back through the valve (Figure 1:1d). Similarly macroscale biomimetic valves have been made using hydrogels. Using 3D printing Lipson and co-workers replicated an aortic valve with three leaflets.\textsuperscript{[42]} The aortic valve uses the same mechanism as venous valves to stop the back flow of blood. Recently, Hockaday et al. reviewed the latest progress in the development of hydrogel based heart valves.\textsuperscript{[43]}

1.1.1.2 Micropumps

Micropumps are often used to push fluid through the channels of microfluidic devices. The majority of micropumps are made from designs that imitate displacement pumps.\textsuperscript{[44]} Displacement pumps move fluid by moving boundaries or surfaces to generate pressure and work in a periodic process. Pistons form the moving boundaries in many macroscale displacement pumps but the same kind of pressure cycle can be replicated with actuating materials. Micropumps were first developed using microelectromechanical systems in the 1980’s.\textsuperscript{[45-47]} More recently the swelling and contraction of hydrogels actuators has been harnessed to drive these pumps.\textsuperscript{[48]} Hydrogel micropumps used in lab-on-a-chip type devices can be fabricated using the same photolithographic techniques as the microvalves. Richter et.al have demonstrated micropumps of various designs that are operated by the swelling and shrinking of temperature-sensitive hydrogels.\textsuperscript{[32]}

1.1.2 Soft robotics

Hydrogel actuators are of particular interest within the emerging field of soft robotics. Traditionally, robots have been made from hard, often metallic materials and create locomotion through the movement of multiple components. In the natural world a variety of plants and animals achieve motion through soft systems devoid of rigid components.\textsuperscript{[49]} Plants cells can alter their shape when pressurized by osmosis.\textsuperscript{[50]} Muscular hydrostats which are structures like the tongue, the arms of an octopus and the trunk of an elephant are composed almost entirely of muscle and connective tissue which allows for movement with many degrees of freedom.\textsuperscript{[51, 52]} Soft robotics is a field of scientific research that is looking towards these biological systems for inspiration to create a new generation of robots from soft materials. The motivation
for soft robots is to create systems that are more compliant than the current rigid technologies. This evolving class of elastically soft, multi-purpose, and biologically inspired machines have the aptitude to change shape and apply complex locomotion strategies to overcome a diverse range of obstacles, tasks, and situations.\cite{53} It is believed that the development of soft robots will revolutionize the role of robotics in healthcare, field exploration, and cooperative human assistance.\cite{53}

1.1.2.1 Artificial muscles

To replicate the motion of biological systems soft robots use artificial muscles. Artificial muscles are actuator materials and devices that reversibly contract, expand or rotate in response to heat, light, pneumatic, chemical or electrical stimulation. To mimic muscular hydrostats soft robotic structures can be built entirely from artificial muscles. Artificial muscles can also be used to fabricate hybrid robots that contain a combination of hard and soft components. Hybrid robots that mimic the structure of vertebrates can be constructed by attaching artificial muscles to a hard and structural skeleton.

Mckibben muscles are a class of pneumatic driven artificial muscles. They were the first group of artificial muscles developed in the 1950’s for artificial limbs \cite{54} and continue to find use in prosthetics.\cite{55,56} These devices are composed of a pneumatic bladder that is partially constrained by a braided sheath of inextensible fibers (Figure 1:2a.).\cite{57} These muscles can actuate quickly, and have a length-load dependence similar to that of muscle but their only mode of actuation is to extend or contract when their pressurization is altered. To combat this limitation the artificial muscles are often used in multiples to replicate the anatomical structure of antagonistic pairs.\cite{58} A familiar example of an antagonistic pair is the combination of the biceps and the triceps in an arm. When one muscle expands, the second muscle contracts which allows for the joint to be repetitively opened and closed.
Figure 1.2 Examples of soft artificial muscles: a. Schematic diagrams of McKibben muscles when (i) stretched, (ii) relaxed and (iii) pressurized, reproduced from [59]. b. Schematic of a pneumatic network artificial muscle, reproduced from [60]. Schematic of a bending tri layer conductive polymer artificial muscle, reproduced from [61]. A (d) scanning micrograph and (e) schematic of carbon nanotube twisted yarn artificial muscles. f. A schematic of torsional actuation by constraining volumetric change, reproduced from [62].

Pneumatic networks are a second example of pressure driven artificial muscles that are commonly used to create soft robots. [60, 63] Pneumatic network muscles were first popularized by the Whitesides research group in 2011. [63] They are made up of a series of channels and voids that run through an elastomeric matrix and inflate like a balloon when pressurised for actuation. Under pressure the channels expand in the regions with the thinner walls due to their lower stiffness to accommodate asymmetric expansion...
of adjacent walls the inflating muscle actuates in a bending mode (Figure 1:2b). Pneumatic networks have been fabricated into structures that act as compliant grippers\(^6\) and tentacles\(^6\) for gently clutching fragile objects.

Pneumatic systems are often selected for use as the artificial muscles of soft robots because air pressure is a low impact strategy for supplying energy. Air has low viscosity which allows for rapid actuation, it is environmentally inert, light and compressible which makes it is easy to store. Similar pressure driven artificial muscles have also been produced that use hydraulic systems.\(^6\) The use of an incompressible fluid increases the systems rigidity and reduces its compliancy. A constraint of the pressure driven artificial muscles is the requirement for a pump to apply pressure. In most cases the pump can be tethered to the robot but this does reduce its mobility and portability. Fully mobile and untethered pneumatic networks have been made by fabricating a soft robot sufficiently large enough to carry miniature air compressors, a power source and a controller that allows autonomous operation.\(^6\)

Conductive polymers can be used to make electromechanical actuators or artificial muscles. These actuators are based on the large dimensional changes that result from the electrochemical doping of various conducting polymers, such as poly(pyrroles), poly(anilines), poly(alkylthiophenes) and poly(arylvinylene).\(^6\) Reversible bending cantilever actuators are made of a tri-layer arrangement of an anode, a cathode and a separating electrolyte.\(^6\) At least one of the anode or cathode must be a conductive polymer and the separating electrolyte contains the doping salt. Applying an electrical field causes ions form the separating electrolyte to migrate into or out of the conductive polymer layers changing their volume. The multi-layer cantilever bends in response to the asymmetric volume change (Figure 1:2c). These tri layer artificial muscles have been used to mimic the caudal or tail fin of a fish in the construction of untethered swimming robots.\(^6\)

All of the previous examples of artificial muscles have either tensile or bending modes of actuation but twisting actuators can also be used to create motion. Twisted yarns are one class of artificial muscles that can be used to produce rotation. These torsional artificial muscles were first demonstrated by Foroughi et al using an electrolyte-filled twist-spun carbon nanotube yarn.\(^6\) The twisted structure of these fibers creates torsional actuation by constraining volumetric change of the muscle (Figure 1:2d-f).
In the Foroughi et al demonstration the twisted yarn was used to spin a mixer in a microfluidic channel. Twisted yarn artificial muscles have also been produced using household materials including fishing line and sewing thread. These types of artificial muscles could possibly be used to drive a propeller for swimming robots.

1.1.2.2 Hydrogel artificial muscles

Hydrogel actuators have already been used as artificial muscles to create some basic soft robots. Hydrogels were first described as ‘chemical muscles’ by Kuhn et al. in 1950. The hydrogel’s mode of actuation is a homogeneous expansion and contraction in all three dimensions. Bending and twisting modes can be achieved in hydrogel actuators by exposing a gel with homogenous material properties to an inhomogeneous stimulus, like a gradient of temperature. Alternatively applying a homogeneous stimulus to a gel with inhomogeneous structure can also create these additional modes of actuation. The Ionov research group have created hydrogel hinges using a bilayer structure of a gel actuator and a passive gel. Taking inspiration from origami, the Japanese art of paper folding these hinges have been used to fabricate self-folding nets. The hydrogels are fabricated into 2D films that fold into 3D structures when exposed to a stimulus. Micro sized soft origami robots are very promising for application in controlled encapsulation and release of drugs, particulates and cells.

Bending hydrogel actuators have been used to fabricate mobile soft robots. In 1992, Osada et al. demonstrated an electrically driven polymer gel with wormlike motility. The soft robot was suspended from a ratchet mechanism into a bath of water. The strip of gel repetitively stretched out and bent in the same fashion as a worm in response to an oscillating electric field. This cyclic process propelled the gel strip down the ratchet bar one tooth at a time. Ma et al. made a hydrogel soft robot that could be stimulated to walk along a ratchet toothed surface with changes to humidity. Maeda et al. produced another wormlike soft robot that can walk without the constraint of an oscillating external stimulus across a similar ratchet floor (Figure 1:3a). A self-oscillating motion in the gel is produced by dissipating chemical energy from the oscillating Belousov-Zhabotinsky reaction that generates a perpetual cyclic pH change. The gel can propel itself forward because the gel strip has a heterogeneous distribution of a metal catalyst which created an asymmetric bending motion. Due to
their wet nature hydrogels have potential for applications in aqueous environments and so hydrogel actuators have been used to make swimming soft robots. Using heterogeneous pH-sensitive hydrogel actuators Kwon et al. fabricated soft robots that replicate the shape and the swimming motion of octopi and sperm.\cite{86} Like the walking robots an oscillating electric field was applied to create the swimming motion. These hydrogel robots are typically built on the microscale to allow for quick actuation. Larger hydrogel actuators with high volume to surface area ratio’s tend to be slow because it is time consuming for water to diffuse throughout the polymer network.

Figure 1:3 Examples of mobile soft robots: a. A gel actuator that can generate autonomous motility with a wormlike motion, reproduced from \cite{84}. b. A comparison between a jellyfish and a freely swimming artificial jellyfish, reproduced from \cite{87}.

In recent years hydrogels have also been used to fabricate biological actuators which are artificial muscles that are made using a combination of synthetic materials and engineered tissue. The first example of a biological actuator was reported in 2005 by Xi et al.\cite{88} To fabricate this device cultured mammalian cardiomyocytes were patterned onto a film of chromium and gold attached to a thin silicon cantilever. Cardiomyocytes are muscle cells that form heart tissue. Coordinated contractions of the cardiac muscle cells cause the heart to beat and pump blood around the body. When patterned on the metallic film the cells formed muscular bundles that contracted when electrically stimulated to move the cantilever. Parker and co-workers have adapted this approach to fabricate a number of biohybrid robots and biological machines including grippers, walkers and pumps.\cite{89} A freely swimming artificial jellyfish was produced
by culturing rat ventricular cardiomyocytes on a silicone polymer substrate. An external electrical field was applied to stimulate the muscle bundles to contract and pump the jellyfish forward (Figure 1:3b). Taking a similar approach Chan et al recently fabricated a walking biological machine. For this soft robot a single layer of cardiac cells was seeded onto a hydrogel thin film. The thin film acted as an asymmetric cantilever that would propel the walker forward when actuated.

1.1.2.3 Flexible and stretchable electronics

Robots use a computer system to control their movement. Traditional robots contain a network of metallic wiring that sends electrical signals from a motherboard to stimulate the artificial muscles. The motherboard acts as the robot’s brain and the wires mimic the function of the nervous system. In more advanced robots, the electrical circuitry can also be used to feedback information from sensors which allow for robots to autonomously interact with their surroundings. The pressure applied by a set of robotic grippers on an object can be controlled by a computer program if a feedback loop is set up between the gripper and a pressure sensor inside the gripper.

Hydrogels have a great deal of utility within the field of soft robotics because this one class of soft material can act as artificial muscles for locomotion as well as conductors and sensors that impart autonomy. Robots using stimuli responsive hydrogels can designed to actuate as a response to an environmental stimuli such as temperature or an electrical signal. Using hydrogels the Suo research group has produced an ionic cable that can send signals over long distance and an artificial skin that can measure strain when stretched and pressure when compressed. Components like these could be incorporated into computer controlled, muscular hydrostat type soft robot.

1.1.3 Biomaterials

Perhaps the most active area of research relating to hydrogels is for the development of biomaterials. A biomaterials is any kind of material that is exploited in contact with living tissues, organisms or microorganisms. Hydrogels were the first biomaterials developed specifically for human use and remain a popular choice for this application because they have been found to display excellent biocompatibility. Hydrogels were first identified for use as biomaterials because of their resemblance to natural
tissue. Both hydrogels and biological tissue have a high degree of swelling and the stiffness of a hydrogel can be tuned over a range that overlaps with the range exhibited by a variety of natural tissues. Heavily cross-linked hydrogels tend to be stiff, like cartilage, whilst highly swollen gels with a low concentration of cross-links can be very soft, like brain tissue. These likenesses allow hydrogels to form a mechanically-compatible interface with biological systems. Examples of hydrogel biomaterials include wound dressings,\cite{94,95} prosthetics implants\cite{96,97} and cell scaffolds for tissue engineering.\cite{98,99}

Hydrogels are used by tissue engineers to construct scaffolds to support the growth of cells because the hydrogel microstructure can mimic the extra cellular matrix. The voids in porous hydrogels can accommodate growing cells and the permeability of the gel can have a beneficial effect on the diffusion of nutrients and oxygen.\cite{100} In biology the extra cellular matrix is made up of a mixture of chemicals secreted by cells to provide structural and biochemical support to adjacent cells. The extra cellular matrix is made up of an interlocked network of fibrous proteins and long chain polysaccharides. The cell scaffolds fabricated by tissue engineers have been made with hydrogels formed with either synthetic polymers or biopolymers. Synthetic polymers are man-made macromolecules that are often synthesised from petrochemicals. Biopolymers are produced naturally in or by living organisms. Alginate and gelatine are two common examples of biopolymers that are harvested by breaking down tissue from plants and animals, respectively. Gellan gum is an alternate example of a biopolymer that is produced by the fermentation of a bacterium. Some protein based biopolymers contain the same chemical structures as the extra cellular matrix which acts as cues for cell growth. The cell scaffolds fabricated with synthetic polymers provide a mechanical foundation for cells. The scaffolds produced using biopolymers with physiologically relevant chemical functionality can provide both structural and biochemical stimulation.

### 1.2 Mechanics of Hydrogels

The first section of this chapter has been dedicated to listing the many favorable properties of hydrogel materials and has demonstrated how these properties have been harnessed within a diverse range of applications. However, the implementation of hydrogel materials in new technologies like soft robotics and as biomaterials is
restricted because synthetic hydrogels are typically very brittle and fragile. The benefits of wet materials have been outlined already but swelling of the polymer network has a negative effect upon mechanical integrity. In fact some hydrogels will fracture under their own weight as they swell. For this reason, hydrogels have been constrained to non-load bearing applications. As described below, however, a range of new tough hydrogels are now available that extend the application of hydrogels into load-bearing areas.

The mechanical performance of a hydrogel or any other type of material can be characterized with a tensile test. Tensile testing is a fundamental materials science test where a sample of material is stretched in a controlled, uni-axial tension until it fails. The force applied to the sample and the magnitude of extension is recorded throughout the test and reported as a stress-strain curve. Regardless of a material’s dimensions, it will produce the same strain ($\varepsilon$) when subjected to a particular stress ($\sigma$). Strain is defined as: $\varepsilon = \Delta L / L_0$ where $\Delta L$ is the change in length and $L_0$ is the initial length. Stress is defined as: $\sigma = F / A_0$ where $F$ is the applied force and $A_0$ is the initial cross-sectional area. Strictly, this definition gives the engineering stress; the true stress is given by $F / A$ where $A$ is the actual cross-sectional area. Properties calculated from a tensile test include Young’s modulus, ultimate tensile strength and failure strain. The Young’s modulus or modulus of elasticity describes the resistance of a material to deformation or what is colloquially described as stiffness. Modulus is calculated as the initial slope of the stress-strain curve and is measured in units of stress. The ultimate tensile strength is the maximum stress that a material can withstand whilst being stretched before failing. The failure strain is the maximum stress that a material can withstand whilst being stretched before failing.
Figure 1:4 Typical stress-strain curve for a tough gel illustrating Young’s modulus (E), breaking strength (\(\sigma_T\)), elongation at break (\(\varepsilon_f\)) and work of extension (U), reproduced from \[2\].

The model of a solvent swollen elastomer can be used to understand the mechanical properties of hydrogels. Elastomers or rubbers have a cross-linked polymer network molecular structure similar to hydrogels. To maximize entropy and minimize free energy, the lengths of polymer chain between cross-linking sites assemble into a random coil configuration. Elastomers oppose strain by unravelling these polymer coils under an external stress which reduces entropy. The high elongations of elastomers are a result of a change in dimensions between a relaxed random coil and a partially or fully elongated polymer chain. A polymer chain containing \(10^3\) repeat units and with a backbone of alternating C-C bonds has a length of \(\sim 10\) nm in the random coil configuration and a length of \(\sim 300\) nm when fully extended. The polymer will extended to 30 times its original length by fully unravelling the coil.\[^2\] When elastomers are relaxed they spring back through entropy-driven recoiling of polymer chains. Elastomers with a high density of cross-links display high moduli because a larger number of polymer chains per cross-sectional area need to be deformed to extend the polymer network. Dry elastomeric materials typically display moduli around 1 MPa whilst hydrogels typically display moduli in the range of 10-100 kPa. In the case of solvent-swollen elastomers like hydrogels, the modulus tends to be

reduced because there are even fewer network chains available per unit cross-sectional area to resist an applied stress and, therefore, their modulus is controlled by both the degree of cross-linking and the extent of swelling.

The extent of swelling within a hydrogel has a dramatic effect on the material’s modulus, strength, elongation and toughness. It is important to quantify the extent of swelling within the hydrogel and to quote a value of swelling ratio alongside any mechanical measurement because of this dependency. The swelling ratio is calculated in a number of ways, such as the mass ratio between the liquid water content of a hydrogel and the solid polymer content. Typically hydrogels are mechanically characterized as synthesized or when swollen to equilibrium within a particular set of environmental conditions.

1.2.1 Toughness

When considering materials for use in a load bearing applications it is necessary to understand what causes mechanical failure. Generally the failure of a material occurs via a fracture mechanism where a macroscopic sized crack propagates throughout the material, causing it to break into two or more pieces. Cracks initiate at defects that concentrate stresses within a material. The resistance of a material to crack propagation is defined as toughness. Toughness is calculated as the amount of energy required to propagate a crack per unit area of new surface produced. There is a distinction between toughness and strength and this can be explained by illustrating the difference in mechanical performance of ceramics and plastics. Ceramics are typically stronger than plastics; that is ceramics are capable of sustaining greater stresses. However, ceramics are not considered to be tough. Tough materials are able to withstand relatively high levels of both mechanical load and deformation regardless of defects. Toughness can be demonstrated by considering what happens when an incision is made in material when under tension. In the case of a ceramic, the notch rapidly propagates leading to immediate fracture. In the same test a plastic may not fracture, rather the crack may be obstructed from propagating by a process such as necking. Materials with low fracture energies like ceramics are referred to colloquially by the term brittle.

When measuring the toughness of a material a crack of a known size is cut into the sample. This artificial defect is required because the size of natural defects varies from
sample to sample and so the breaking stress and strain vary likewise. Standard notch
tests are not appropriate for highly deformable materials like elastomers because
Cracks in these materials do not propagate at constant velocity and tend not to remain
in a single plane. The standard test for measuring the toughness of elastomers and
hydrogels is the trouser tear test.\textsuperscript{[2]} In this test an incision is cut part way through a
rectangle sample of material so as to divide it into two tabs or ‘trouser legs’. When the
legs are pulled apart the crack propagates through the middle of the specimen. It should
be noted that other methods do exist for measuring the fracture energy of elastomers
including the single-edge notch test\textsuperscript{[101]} and the classical pure-shear test.\textsuperscript{[102]} The values
of fracture energy measured from these different tests are generally consistent with
one another.\textsuperscript{[103]} The fracture energy measured in this type of test is reported as the
amount of energy required to propagate a crack through a material per unit cross
sectional area in units of J/m$^2$.

Some insight into the toughness of a material can also be gained from the tensile test.
The work of extension can be calculated as the area underneath the stress-strain curve.
This estimation of toughness represents the energy stored within and absorbed by a
material during a tensile test. In addition to the energy of fracture, the work of
extension includes the elastically stored energy and any energy dissipated through
plastic deformation. The work of extension is reported as the amount of energy
required to stretch a material per unit volume in units of J/m$^3$. The work of extension
is clearly distinct from fracture energy but these two parameters are related because
tougher materials will sustain both higher loads and higher extensions before failure
and, therefore, have higher work of extension.

Returning to the model of a solvent swollen elastomer, mechanical failure of hydrogels
can be described by the Lake-Thomas description of fracture in rubbers.\textsuperscript{[104]} The
fracture of elastomers occurs through a process where the polymer chains that span the
crack plane are uncoiled and then extended to the point of fracture. The fracture of
network strands allows the crack to propagate. The energy dissipated by propagating
a crack is equivalent to the energy required to fully extend the polymer chains to the
point where the bonds between backbone atoms have a stretching energy equal to their
dissociation energy. Longer polymer chains can dissipate more energy when extended
because they have a larger number of bonds to stretch. The Lake-Thomas model can
be used to calculate the minimum energy required to fracture an elastomer. The model considers both the length of the network strands between cross-links and the number of strands that cross the fracture plane. Using the Lake–Thomas theory, the fracture energy \( \Gamma_0 \) of an elastomer can be calculated as: \( \Gamma_0 = U_F m \) where \( U_F \) is the energy required to fracture a network strand in the elastomer and \( m \) is the number of polymer chains per unit of cross-sectional area that cross the crack plane in the relaxed state.\(^{[104]}\)

In the case of a solvent swollen elastomer the number of polymer chains per unit of cross-sectional area can be calculated as: \( m = m_{dry}C^{2/3} \) where \( m_{dry} \) is the number of chains across a unit area of the corresponding elastomer in the dry and undeformed state and \( C \) is the volume concentration of polymer within the hydrogel.\(^{[105]}\)

The values of fracture energy measured experimentally for some elastomers can be greater than those predicted by the Lake-Thomas model. Elastomers tend to exhibit fracture energies between \( 10^3 \) and \( 10^5 \) J/m\(^2\). According to the theory (\( i.e. U_F m_{dry} \)) dry elastomers should only exhibit values of \( \sim 50 \) J/m\(^2\).\(^{[105]}\) The discrepancy in values is due to strain hardening, a toughening mechanism which is not accounted for in the model. At high strains polymer chains begin to align with one another to form crystallites which are observed to be strain hardening.\(^{[106]}\) Strain hardening increases the energy required to propagate a crack through an elastomer by effectively increasing the strength of regions of material experiencing high strains like the areas of material surrounding a crack tips.

Dry elastomers are significantly tougher than hydrogels. The fracture energy of hydrogels is typically less than \( 10 \) J/m\(^2\). The difference in toughness between the dry and solvent swollen elastomers is a result of swelling and limited strain hardening. Swelling increases the volume of the polymer network which stretches the polymer network and creates internal stresses. The increase in volume reduces the number of network strands per unit cross-sectional area of the polymer network. Fracture energy reduces as the swelling increases because fewer polymer chains need to be stretched and fractured for a crack to propagate. The separation of polymer chains resulting from swelling also inhibits the formation of crystallites which drastically reduces strain hardening. Hydrogels are so brittle because, they have very few mechanisms available to dissipate energy.
1.2.2 Tough hydrogels

Despite the poor mechanical performance of hydrogels, there has still been a great demand for their use in load bearing applications because of their many favorable properties. Over the past few decades, this demand has stimulated concentrated research to improve the mechanical properties of hydrogels, especially their toughness. Some of the recently developed hydrogels have exhibited significant improvements in fracture energy compared to their conventional counterparts. The improvement in fracture energy of these ‘tough hydrogels’ is a result of inclusion of toughening mechanisms that serve to dissipate energy and slow crack propagation.

The many examples of tough hydrogels can be classified by toughening mechanism. Interpenetrating polymer network (IPN) hydrogels distribute applied loads throughout two or more polymer networks that are interlaced but not connected.\textsuperscript{[107]} Tetra-arm polymer hydrogels are a class of tough hydrogels that form a homogeneous polymer network.\textsuperscript{[108]} The stresses applied to homogenous polymer networks are distributed over a larger fraction of chains with a uniform strain distribution compared to the heterogeneous networks typically formed in free radical polymerization reactions. Homogenous networks also contain fewer defects for crack formation. Poly(vinyl alcohol) (PVA) hydrogels display strain hardening through the formation of crystalline domains.\textsuperscript{[109]} Slip-link hydrogels are an example of a topological hydrogel that can exhibit large elongations due to the presence of sliding cross-linkers.\textsuperscript{[8]} Fibrin hydrogels are an example of a tough hydrogel with transformable domains.\textsuperscript{[110]} These hydrogels use the unfolding of proteins to dissipate energy.\textsuperscript{[111]} Composite hydrogels receive mechanical reinforcement from other stronger or stiffer materials.\textsuperscript{[112]} Tough hydrogels can be formed using poly-functional covalent cross-linkers. Nanocomposite hydrogels are a class of hydrogels made with nano sized particles that act as cross-linkers with high functionalities.\textsuperscript{[113, 114]} Some of these toughening mechanism can be combined within the one hydrogel to produce particularly tough materials as some of the toughening mechanisms act over different length scales.\textsuperscript{[105]}

1.2.2.1 Interpenetrating polymer network hydrogels

There has been a particular focus within the development of tough hydrogels upon the formation of IPN hydrogels. Not all IPN hydrogels are tough but some hydrogels made
with IPN architecture are responsible for the highest values of fracture toughness reported for any hydrogel. The IPN architecture can be created by soaking a hydrogel having a conventional network structure in a monomer solution and subsequently initiating polymerization of the monomer solution to form a second polymer network throughout the interstitial voids of the first hydrogel (Figure 1:5).

Figure 1:5 A schematic showing the multistep synthesis of IPN hydrogels. (i) Formation of the IPN architecture starts with the synthesis of a conventional hydrogel. (ii) That hydrogel is swollen in a monomer solution and subsequently initiating a free radical polymerization of the monomer solution to form (iii) a second polymer network throughout the interstitial voids of the first hydrogel, reproduced from [2].

1.2.2.1.1 Double network hydrogels

Double network (DN) hydrogels were the first example of tough IPN hydrogels and were developed by Gong et al. in 2003.[107] DN hydrogels were first synthesized by making IPN of poly(2-acrylamido-2-methylpropanesulfonic acid) (PAMPS) and poly(acrylamide) (PAAm) but have now been made from a whole range of different polymer pairs including a jellyfish and PAAm.[115] The mechanical properties of DN hydrogels have been shown to significantly outperform the mechanical performance of the individual components (Figure 1:6a). Under an optimized structure, DN hydrogels exhibit elastic moduli between 100 and 1000 kPa, tensile strength up to 10 MPa, extensions up to 20 times their initial length, fracture energy between 100 and 4400 J/m² and work of extension values ~10 MJ/m³ with swelling ratios ~9. At an optimal composition, the PAMPS / PAAm DN hydrogels show fracture energies that are several orders of magnitudes larger than of that of single network PAMPS and PAAm hydrogels with the similar polymer concentrations. The excellent mechanical performances of these hydrogels are comparable to and even exceed some rubbers and soft loadbearing tissues.
Figure 1:6 a. Photographs comparing the mechanical performance of (i) a conventional PAMPS hydrogel and (ii) a PAMPS / PAAm DN hydrogel in compression, reproduced from [107].

b. A photograph of a DN hydrogel trouser tear test with insets illustrating Illustration of network structure of the DN gel at the crack front and damage zone, reproduced from [116].

c. Three-dimensional violet laser scanning microscope image of the damage zone around a crack tip in a DN hydrogel, reproduced from [117].

The distinction between DN hydrogels and other IPN hydrogels is that DN hydrogels are made up of two polymer networks with contrasting properties. The two polymer networks have dissimilar network stiffness, rigidity, strand length and cross-linking density. It has been determined that the toughest DN hydrogels are made using a swollen first network of a rigid polyelectrolyte and the second network made with a high molecular weight flexible neutral polymer. The first network should be cross-linked tightly while the second network should contain much fewer cross-links, which requires a very high molecular weight of the second polymer. In the optimized
systems the molar concentration of the second network is 20-30 times that of the first network.\textsuperscript{118, 119}

DN hydrogels can display a necking phenomenon when extended in tensile testing. When the hydrogel is stretched to moderate strains (~200% for the optimized PAMPS / PAAm DN hydrogels) a small portion of the tensile specimen decreases in width. The appearance of the neck is represented by a yield stress in the stress strain curve. The length of the narrowed section increases as the sample is extended further. Propagation of the neck corresponds with the formation of a plateau, or even a peak, in the stress strain curve. The necking phenomenon is a result of the accumulation of damage to the first network. The first network fails at low strains because of its intrinsically fragile properties and shorter strand lengths. The hydrogel does not fracture at this point because the second network is more elastic and can withstand greater strains. The damaged shards of the first network are held in position by the second network and act as physical cross-links similar to those in topological slip-link hydrogels. The implication of neck formation is that the damage to the first network can extend over the entire cross-sectional area as one continuous zone and that the second network is taking on the majority of the applied stress. The formation of a large necking or yielding zone is a common feature of tough materials.

The trouser tear test has provided a deeper understanding of the DN toughening mechanism resists crack propagation. A large damaged zone appears around the crack tip during the tear test (Figure 1:6b).\textsuperscript{116} During crack propagation the stress concentrated at the crack tip causes an internal fracture of the first brittle network. The first network offers sacrificial bonds that efficiently disperse the stress around the crack tip. The second network polymer chains can sustain large deformation. To completely extend the polymer chains in the second network that span the crack plane, sacrificial polymer chain from the first network that do not cross the crack plane must first be fractured. The damage zone dissipates a remarkable amount of energy by drastically increasing the number of polymer chains per unit of cross-sectional area that require fracturing for crack propagation. This damaged zone has been directly observed with a color three-dimensional violet laser scanning microscope (Figure 1:6c).\textsuperscript{117}
The formation of the yielding or damaged zone of DN hydrogels is an accumulative and irreversible process. When a bond in the first network is fractured the dissociation energy is expended and cannot be recovered. In loading-unloading tensile studies of DN hydrogels a large hysteresis has been observed during the first load-unload cycle that is absent in subsequent load-unload cycles when extended to the same maximum strain. After necking occurs the hydrogel becomes very soft. The optimized PAMPS / PAAm DN hydrogels exhibit elastic modulus approximately a tenth of that before the elongation tests. The softening is due to irreversible scission of short network strands during the first loading cycle. The damaged hydrogel maintains its high ductility and can still withstand large deformation but the toughness is severely reduced because the sacrificial network can no longer dissipate energy by fracturing bonds.

DN hydrogels show great promise for use in load bearing applications as they exhibit large fracture energies but concerns remain over the irreversible nature of their toughening mechanism. The permanent fracture of the chemical bonds might be a limitation of these hydrogels but a solution to this issue is to replace some of the brittle network with reversible physical bonds, so that damage upon loading recovers back on unloading. In this case the physical bonds serve as reversible sacrificial bonds. Following this framework the Gong group has developed a DN hydrogel based on physical association bonds. The hydrogel has a macroscopic unidomain lamellar bilayer structure of self-assembled poly(lipid), poly(dodecylglyceryl itaconate) (PDGI) stacked periodically throughout a loosely cross-linked PAAm second polymer network. Each lamellar bilayer is relatively rigid with a high modulus in the order of several MPa. The PDGI/PAAm hydrogel exhibits a large tensile strength (~600 kPa) because the dissociation of rigid bilayers during elongation requires a substantial energy cost. When the stress is removed the bilayers return to their original state and the hydrogel recovers its initial structure and properties. The mechanical properties of these hydrogels are anisotropic with the elastic modulus and tensile strength at their greatest when measured perpendicular to the bilayers.

1.2.2.1.2 Ionic covalent entanglement hydrogels

Ionic covalent entanglement (ICE) hydrogels are a second example of a tough IPN hydrogel with a combination of physical and chemical cross-linkers that show recovery from damage caused by applied strains. Inspired by the DN hydrogel
principle, ICE gels maintain a brittle and highly cross-linked polymer network that is reinforced by a second loosely cross-linked polymer network. The distinction is that covalent cross-links in the first polymer network are replaced with reversible ionic bonds (Figure 1:7a). Typically the ionic polymer network is made up of a biopolymer that forms cross-links by chelating with metallic cations. These hydrogels were first reported independently in 2012 by the in het Panhuis\textsuperscript{[120]} and Suo research groups.\textsuperscript{[103]} A wide variety of polymer pairs have been used to produce ICE gels but the alginate and PAAm IPN developed by the Suo research group has been used as a model system to study ICE hydrogels.

![Figure 1:7](image)

**Figure 1:7** a. Schematic diagrams showing the network structure of (i) alginate, (ii) PAAm and (iii) alginate / PAAm ICE hydrogels. b. Chemical structure of cross-linked alginate showing (M) $\beta$-D-mannuronate and (G) $\alpha$-L-guluronate residues. c. Schematic diagrams showing the plastic zone near the crack tip of (iv) alginate and (v) alginate / PAAm ICE hydrogels. d. Photographs of a alginate / PAAm ICE hydrogel being mechanically characterized in tension, reproduced from\textsuperscript{[103]}.

Alginate is an anionic polysaccharide that is extracted from brown algae. It is a linear copolymer of (1-4)-linked $\beta$-D-mannuronate and $\alpha$-L-guluronate residues (Figure 1:7b). The exact composition of the polymer varies dependent upon the algae source. In an aqueous solution, two pairs of the guluronate residues, one pair each from
different alginate chains form an ionic cross-link by chelating with Ca$^{2+}$ or other multivalent cation. Blocks of the guluronate residues in conjunction with divalent cations form chains of ionic cross-link coordination complexes whose structure can be described as an egg carton.$^{[121]}$ When a stress is applied to an alginate hydrogel, fracture proceeds by unzipping these chains of ionic cross-links (Figure 1:7c). After one guluronate complex unzips, the high stress shifts to the neighboring guluronate complex and causes it to unzip. For a defect to turn into a propagating crack, only the alginate chains crossing the crack plane need to unzip.

The toughness of alginate is increased dramatically by the addition of an IPN with a covalent network such as PAAm to form an ICE hydrogel. In the fracturing process of an ICE hydrogel, strands of PAAm network bridge the crack plane to stabilize deformation and enable a larger region of the alginate network to unzip. The unzipping of the alginate network, reduces the stress concentration of the PAAm network ahead of the notch and dissipates strain energy (Figure 1:7c). The alginate / PAAm hydrogels reported by Sun et al. exhibited elastic moduli between 10 and 100 kPa, tensile strength up to 200 kPa, extensions up to 23 times their initial length (Figure 1:7d), fracture energy $\sim$9 kJ/m$^2$ and work of extension values $\sim$2.5 MJ/m$^3$ with swelling ratios $\sim$9. The fracture energy of the alginate / PAAm hydrogels is much larger than the PAMPS / PAAm DN hydrogels. The increased fracture energy has been attributed to the unzipping of the alginate network. Alginate polymers used in these experiments contained a high concentration of guluronate blocks. The high concentration of guluronate blocks allows for the formation of a large number of cross-links to form between polymer chains when enough Ca$^{2+}$ ions are present. When the ICE hydrogel is stretched, the poly(acrylamide) network remains intact and the hydrogel structural integrity is maintained while the alginate network progressively unzips. Initially the ionic cross-links in close proximity to a crack tip begin unzipping, followed by an increasing number ionic cross-links located further away from the crack plane as the strain increases. The hydrogel’s fracture energy increases by increasing the number of ionic cross-links that require unzipping for crack propagation. The highest fracture energy of any hydrogel was recently reported by Li et al. $\sim$16 kJ/m$^2$.\textsuperscript{[122]} The incredible fracture energy was achieved through increasing the cross-link density of the alginate network by incorporating a combination of short- and long-chain alginates.

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ICE hydrogels have great potential for use in load bearing applications because they combine high fracture energy and a reversible toughening mechanism. In loading-unloading studies a large hysteresis is observed in the first load-unload cycle, similar to DN hydrogels. The hysteresis is due to the dissipation of energy from the unzipping of ionic cross-links in the alginate network. It is only the ionic cross-links that are broken and the alginate chains themselves remain intact so that the ionic bonds can reform when the stress is removed. The hysteresis can be observed again in subsequent load-unload cycles which is an indication of the healing of the internal damage. The modulus of the hydrogel in the subsequent load cycle is lower than in the virgin cycle which suggests that the hydrogel never fully recreates its initial structure, however after a small number of cycles the loading modulus does stabilize. The network recovery is a time dependent process as the metal cations need to diffuse to reform the ionic cross-link.

1.2.2.2 Hydrogel composites

The formation of composite materials presents an alternative means to enhance the mechanical performance of hydrogels. Composites are materials that are made by combining two or more constituent materials with significantly different properties. A motivation for stronger, lighter, and/or less expensive materials has fueled the development of composites for a wide range of applications from construction to aerospace engineering. Brittle materials can be transformed into structural composites when combined with stronger materials. These structural composites maintain many of the desired characteristics of the brittle material but inherit some of the mechanical integrity of the stronger material. Composites have been made to toughen materials for millennia. The Ancient Egyptians were the first to make structural composites by reinforcing mud with straw to make bricks. Common examples of structural composites include reinforced concrete, plywood and fiberglass. Only recently has the formation of composites been used to toughen hydrogels. Structural hydrogel composites combine the many favorable properties of hydrogels with the structural integrity of stronger materials.

Structural hydrogel composites have been made by adding fiber reinforcement to a hydrogel matrix. This process mimics biology where it is common for a hydrogel matrix to be reinforced with micro or nanofibers to produce a strong and
tough composite material. Cartilage is an example of a biological hydrogel composite that is composed of proteoglycan gel matrix reinforced by a collagen fiber network. Human cartilage is considered to be tough and exhibits fracture energy of ~ 1000 J/m². Synthetic hydrogel composites can be made using the same reinforcement techniques as fiberglass. The first hydrogel composites were reinforced with continuous and aligned fibers and displayed enhanced strength in the direction of the fibers. The addition of randomly oriented fibers can be used to produce a composite with isotropic properties. Textile processing techniques of weaving (Figure 1:8a), knitting, braiding and stitching are commonly used in fiber reinforcement technologies to produce a fiber preform with tunable mechanical performance. The Calvert research group produced a strong fiber-reinforced hydrogel through a novel method of embedding a 3D printed construct of crossed polyurethane fiber ‘log-piles’ with an epoxy amine hydrogel (Figure 1:8b).
Figure 1: Examples of fibre reinforced hydrogel composites: a. Scanning electric microscopy images of (i) a woven fibre mat and (ii) a hydrogel reinforced by a woven fibre mat, reproduced from [128]. b. (iii) A digital model and (iv) a photograph of a 3D printed fibre ‘log pile’ scaffold. (v) A photograph of a hydrogel reinforced by a 3D printed fibre ‘log pile’ scaffold, reproduced from [112]. c. Photographs of (i) alginate and (vii) alginate / PAAm ICE hydrogels reinforced with steel wool being stretched in a tensile test, reproduced from [129].

Fiber reinforcement strengthens a material by transferring an applied stress form the brittle matrix onto the fibers. The addition of strong and stiff fibers in a soft matrix means that it becomes harder to deform a hydrogel composite than a conventional hydrogel. A strong adhesion between fibers and matrix is paramount to effective reinforcement as this allows the matrix to transfer an applied stress onto the stiffer
fibers. In addition to increasing strength and modulus, fiber reinforcement also increases the toughness of a hydrogel. The fracture of and pulling out of the fibers that bridge a crack tip can dissipate significant amounts of mechanical energy in the hydrogel. The mechanical performance of hydrogel composites is heavily influenced by the volume fraction of the fiber reinforcement and the mechanical properties of the two components. It is important to note that the inclusion of solid fillers drastically reduces a hydrogel’s swelling ratio. Due to the many variables of composition and design of different hydrogel composites reported in the literature it is difficult to compare the effectiveness of reinforcement in one composite over another.

Although less common than fiber reinforcement, structural hydrogel composites have also been made with particulate reinforcement. Usta et al. have provided an example of a particulate reinforced hydrogel by adding alumina particles to gelatin hydrogel.[130] Reinforcing the gelatin with alumina in the ratio of 1:1 increased the hydrogel’s modulus from 0.1 to 0.5 MPa. Various nanoparticle composites have also been made with hydrogels, but the nanoparticles are generally considered to act as multifunctional crosslinking sites rather than particulate reinforcements.

The creation of a composite material can be used to further improve the mechanical performance of other classes of tough hydrogels.[128] The toughening mechanisms of DN and ICE hydrogels depend on molecular-scale architectures of polymer networks. Hydrogel composites on the other hand are typically made with macro-scale fibers and fillers embedded in a hydrogel matrix. These two toughening mechanisms can be combined because they operate over different length scales.[105] The Zhao research group first showed the benefits of combining molecular scale toughening mechanisms with macro-scale fibers by reinforcing alginate / PAAm ICE hydrogels and fibrin / PAAm IPN hydrogels with a woven poly(ε-caprolactone) fiber mat. The alginate / PAAm ICE hydrogels and poly(ε-caprolactone) fiber mat exhibited elastic moduli 0.4 and 0.7 MPa, respectively. An improvement to modulus was observed by the composite construct, with values reaching 1.2 MPa. The increased modulus of the composite hydrogel is an indication that the material dissipates more energy when deformed than the standard ICE hydrogel.

The benefits of using a tough hydrogel over a conventional hydrogel as a matrix material have been shown by Illeperuma et al.[129] They reinforced alginate and
alginate / PAAm ICE hydrogels with stainless steel wool composed of randomly aligned fibers (Figure 1:8c). In the brittle alginate gel the stiff fibers act like sharp knives and can readily cut through the matrix when aligned by tensioning the sample. The use of a brittle matrix results in a composite with poor mechanical properties. The ICE hydrogel deforms elastically but resists being sliced up by the moving wires, hence the tough hydrogels make for a better matrix in fiber-reinforced composites. The strength and stiffness of these ICE hydrogel composites is significantly larger than that of ICE hydrogels without fiber reinforcement.

1.2.3 Functional tough hydrogels

Tough hydrogels are exciting materials that add mechanical integrity to the unique functional characteristics of conventional hydrogels. Tough hydrogels can preserve many of the favorable properties of conventional hydrogels because they maintain a high water content. These materials have the potential to advance the development of hydrogel based technologies bringing intelligent materials to load bearing applications.

Tough hydrogels display similar biocompatibility as to conventional hydrogels. Both DN hydrogels and ICE hydrogels have been studied under cell culture and in vivo conditions. PAMPS / PAAm DN hydrogels have induced a significant inflammation when used in implantation tests on rabbits performed by the Gong research group. DN hydrogels based on PAMPS and poly(N,N′-dimetyl acrylamide) however, displayed minimal inflammatory response when implanted in the same conditions.[131] Mouse mesenchymal stem cells exposed to alginate / PAAm ICE hydrogels have been shown by the Mooney research group to maintain high viability. Implantation of these hydrogels into subcutaneous tissue of rats for 8 weeks lead to mild fibrotic encapsulation and minimal inflammatory response.[132] These results suggest that it is safe to continue exploring tough hydrogels as biomaterials. When exposed to physiological conditions the hydrogels can exhibit changes to swelling that correspond with some variation to mechanical properties. Tough hydrogels have been identified as a material that can be used to replace or treat damaged cartilage of the knee meniscus.[133] Tough hydrogels may be able to fulfil the role of an artificial meniscus because they exhibit biocompatibility, a low coefficient of friction and high strength and toughness.
Recently toughening mechanisms have been used to enhance the mechanical performance of stimuli responsive hydrogels. Most toughening mechanisms are introduced with changes to the morphology of the polymer network whereas the environmental sensitivity comes from the chemistry of the polymer itself. A variety of tough stimuli responsive hydrogels have been produced by combining one of the toughening mechanisms described above with a ‘smart’ polymer.\cite{134-136} Fei et al. produced a temperature sensitive DN hydrogel by reinforcing a PAMPS hydrogel with a responsive PNIPAAm network rather than the inert PAAm network.\cite{137} The extent of actuation in the multi-network hydrogel was impeded by the presence of the second toughening network and its response time decreased as the concentration of PNIPAAm was decreased. There are currently no reports of stimuli responsive ICE hydrogels. With mechanical robustness and a dynamic nature, tough hydrogels have the potential to create new mechanically robust hydrogel technologies and to improve use of hydrogels in their current applications.

1.3 Processing of hydrogels

Now that a variety of methods have been established for synthesizing tough and functional hydrogels, processing techniques are considered so that these materials can be fabricated into novel structures. Prior to 2012 tough hydrogels were simply set into slabs that could be cut up and analyzed in experiments. Minimal effort had gone into developing methods to form these materials into complex shapes and assemblies with other materials for real world applications. Due to their low modulus, tough hydrogels are not compatible with many traditional joining, machining and forming fabrication techniques. Adapting the processing techniques used to handle conventional hydrogels presents itself as the logical starting point for developing tough hydrogel fabrication techniques. Refining these techniques will still require a concerted effort as some tough hydrogels necessitate multi-step synthesizes that will add complexities to their processing.

Multi-material assemblies that harness the functional properties of hydrogels such as actuation, sensing and conductivity are desired for some applications including soft robotics and micro-fluidics. Creating joints between hydrogels and other materials such as metals or plastics creates an interface of mismatched elastic properties. Joints of mismatched elastic properties can form a point of weakness within a structure.
Stretching or bending of the joint can cause the two dissimilar materials to peel off one another.$^{[138]}$ It is possible to improve the connection between dissimilar materials by blending them across a gradient that forms a smoother interface by spreading the change of properties over a greater distance. Fabricating complex structures like gradients with spatially varied composition and material properties calls for the use of advanced fabrication techniques that are currently not available for hydrogels.

Developing new fabrication techniques will also allow for tough hydrogel actuators to be harnessed in a wider range of applications with increased efficacy. Tough hydrogel actuators prepared with spatially varied material properties can be designed to achieve complex modes of actuation when exposed to homogeneous stimulus.$^{[76]}$ By designing tough hydrogel actuators with pores or channels that increase their surface area will reduce the material’s response time. Voids can be introduced to tough hydrogels because a smaller volume of material is required to bear the same load as conventional hydrogel. Fabrication of these types of structures requires techniques that provide a high degree of resolution. It is through intelligent design and fabrication that the full potential of hydrogel materials can be harnessed.

1.3.1 Cast molding

The most common technique used for processing hydrogels is cast molding. Cast molding is a manufacturing process whereby a liquid material is poured into a mold that contains a cavity of the desired shape, and then allowed to solidify. This is a manufacturing technique useful for creating structures with complex geometries that can be difficult or uneconomical to make through other methods. Cast molding can make objects with any shape for which a mold can be prepared through convention processing techniques or by taking an impression of the desired shape. Cast molding can be used in a batch process to produce structures quickly and in large quantities. Plastics, glasses, metals, and raw ceramics are examples of materials that are processed with cast molding. Although cast molding is incredibly useful it is not considered to be an advanced fabrication technique.
1.3.1.1 Cast molding conventional hydrogels

Cast molding has been used so regularly with hydrogels because it is easy to pour gel precursor solutions into a mold where they can be cured solid via a number of gelation mechanisms. Thermoreversible gel precursor solutions of some biopolymers including gellan gum and carrageenan form physical gels at low temperatures. The preparation of dessert gelatin known as jelly is a household example of a thermoreversible hydrogel being formed into intricate designs by cast molding. To prepare jelly a hot solution of gelatin is poured inside a decorative mold and allowed to cool in a refrigerator. Gelation of chemical hydrogels can also be initiated by cold mixing two or more reactive components. An epoxy-amine hydrogel can be prepared by mixing solutions of epoxy and amine functionalized polymers. Gelation can also occur by initiating the polymerization of a monomer solution.

1.3.1.1.1 Free radical polymerization

Free radical polymerization is a key route for synthesizing polymers and is a mechanism commonly used to create hydrogels as part of the cast molding process. It is one of the most versatile forms of polymerization available due to the relatively non-specific nature of free radical chemical interactions. According to 'Principles of Polymerization' by George Odian, about 35 % of the polymers produced by the United States in the year 2001 were synthesized by free radical polymerization. Free radical polymerization is a class of chain-growth polymerization that occurs in four stages; initiation, association, propagation and termination (Figure 1:9). Initiation of a free radical polymerization starts with the dissociation of an initiator molecule to create two radical species. Organic compounds with a liable group such as a peroxide compound are used as the initiator for free radical polymerizations. The liable bond can be broken by the addition of energy in the form of heat or irradiation such as UV (ultra violet)- or γ-irradiation. Free radical polymerization is a favorable gelation mechanism for cast molding hydrogels because the liquid precursor solution can be poured into the mold and initiated on cue by applying energy.
Figure 1: Reaction schemes showing the a. initiation, b. association, c. propagation and d. termination steps of a free radical polymerization reaction.

After radicals are formed in the initiation step, they will attack the vinyl group of a monomer in the association step. A vinyl group has one pair of electrons held securely in a σ-bond between the two carbons. The second pair of electrons is held in a looser π-bond. The free radical couples with one of the π-bond electrons to form a more stable σ bond with the carbon atom. The other electron returns to the second carbon atom, turning the whole molecule into another radical and starting the polymer chain. Propagation is the longest step of the reaction because a free radical is recycled conserved in the attack of a carbon-carbon double bond so that the chain can lengthen. Once initiated, the chain propagates until there is either no more monomer or termination occurs. During the propagation step of vinyl polymerization align heat-to-tail where the more substituted carbon atom is designated as the head. Head-to-head alignment may occur by combination at the termination step of the reaction. Free radicals from two separate chains can combine to form a covalent bond which quenches the reaction leaving a single polymer chain. The presences of other free radicals can also terminate or inhibit the reaction. Molecular oxygen is a common inhibitor and should be removed from the monomer solution prior to initiation. When present, the growing chain will react with oxygen to produce an oxygen radical, which is much less reactive and will significantly slows down the rate of propagation. The polymers produced in free radical reactions are typically linear. The chains can be cross-linked so as to form a polymer network of a chemical hydrogel by copolymerizing the vinyl monomers with molecules that have two or more carbon-carbon double bonds.
1.3.1.2 Cast molding tough hydrogels

Cast molding was the first processing technique used to shape tough hydrogels because of the versatility it demonstrated with forming conventional hydrogels. DN hydrogels are synthesized in a two-step free radical polymerization process which creates complications to the cast molding procedure. In the first step of the DN hydrogel synthesis process a brittle PAMPS hydrogel is formed. The PAMPS hydrogel acts as a skeleton that determines the approximate shape of the final DN hydrogel. Although PAMPS hydrogels can be formed into any shape through cast molding, the shape and size of the hydrogel will expand when swelling it in the PAAm monomer solution. It also becomes very difficult to eject convoluted PAMPS gels without damaging them due to their extremely poor mechanical properties. Incidentally, PAMPS gels or DN hydrogels cannot be cut or whittled into intricate shapes post synthesis due to their properties. The processing options for DN hydrogels are therefore quite limited. The Gong research group overcame this problem by applying a three-step, triple network synthesis. In the first step of the synthesis a physically cross-linked PVA is cast molded into a desired shape. The PVA hydrogel is mechanically robust enough to be ejected from a cast mold and then be used as an internal mold to determine the shape of the DN hydrogel. In the second and third steps of synthesis PAMPS and PAAm polymer networks are sequentially polymerized inside the PVA internal mold to obtain the free-shaped PVA/DN hydrogels. Following this process DN hydrogels were prepared in the shapes of a bird (Figure 1:10a), a fish (Figure 1:10b) and a Chinese knot.

![Photographs of DN hydrogels cast moulded into the complex shapes of a bird and a fish, reproduced from [141].](image)

Figure 1:10

Internal molding is not an issue when cast molding all varieties of tough hydrogels. ICE hydrogels are one of the varieties of tough hydrogels that can be cast directly into
molds because they can be synthesized in a single step, one-pot method. DN hydrogels require a two-step process because both polymer networks are typically produced in a free radical polymerization. If the gel precursor solutions of the two polymer networks were mixed and then polymerized a single network of copolymer would form rather than an IPN because the two polymer networks form via the same reaction scheme. ICE hydrogels can be synthesized with a one-pot method because the two polymer networks form using different reaction schemes, the alginate network forms by creating ionic cross-links in the presence of calcium ions and the PAAm network forms with a free radical polymerization. The IPN morphology is formed when these two gel precursor solutions are mixed and polymerized simultaneously because the two reaction schemes are independent. ICE hydrogels can be cast molded directly into intricate designs through the one-pot synthesis because the cast material is sufficiently robust enough to be ejected from the mold.

Fiber reinforced composite hydrogels have also been shaped into complex shapes with cast molding. In this process the fiber reinforcement is added to the mold in the form of loose fibers, a woven mat or as a pre-prepared scaffold. The gel precursor solution is then poured into the mold around the fiber reinforcement and cured. This process is capable of making the hydrogels in all kinds of geometry but gives little control over the spatial distribution of fibers within the hydrogel matrix. When preparing the composites loose fibers cannot be suspended in space but they tend to sink and aggregate in the bottom of the mold. This is a limitation of the cast molding process as some applications require localized reinforcement of hydrogels.

1.3.1.3 Cast molding complex structures

Cast molding is capable of shaping tough hydrogels into all kinds of designs with intricate detail provided that a mold can be made. Basic cast molding is restricted however in its ability to form more complex multi-material structures and hydrogels with spatially varied material properties. Hydrogels with spatially varied material properties are wanted for a number of purposes including asymmetric swelling of bending actuators but structures made by cast molding tend to be homogeneous.

Some control over the spatial distribution of a hydrogel’s material properties can be achieved by cast molding the structure in a multi-step process. Hydrogels made with a
different composition display different material properties; hydrogels with a higher cross-linking density have higher modulus. Variations in a hydrogel structure’s material properties can be achieved by altering the composition of the hydrogel throughout the object. A gradient structure with an incremental transition of material properties can be built up by stacking layers of hydrogels with different composition. To construct this layered structure a mold is partially filled with a gel precursor solution and cured. Once this first layer has solidified a second gel precursor solution for a slightly stiffer hydrogel can be poured into the mold and gelled on top of the first to form the second layer. The second layer of hydrogel can interpenetrate into the surface of the first during polymerization so that the two hydrogels adhere. The process can be repeated until the mold is filled. This technique has been widely used with conventional hydrogels to make bending actuators and to mimic mechanical gradients present in biological systems.\[^{142-144}\]

Naficy et al. followed the multi-step cast molding process with tough alginate / PAAm ICE hydrogels to make an artificial tendon connected to a soft alginate / poly(acrylic acid) ICE hydrogel artificial muscle. The tendon was made with three layers of ICE gel with increasing modulus and decreasing swelling ratio. \[^{145}\] The modulus of the hydrogels doubled across its length of the gradient so that the soft actuator could be attached to harder materials in the same way that muscles are attached to the skeleton in the body via tendons. However, even the stiffest hydrogels have relatively low modulus that still leaves a mismatch of properties when attempting to join them to plastics and other harder materials. Interfacing hydrogels with these stiffer materials may require the formation of a gradient in composition from hydrogel to the harder material.

Cast molding alone cannot unlock the full potential of functional tough hydrogels, despite its versatility and ability to shape materials. The multi-step cast molding procedure can be used to gain some spatial control over the mechanical properties but only in the form of laminar structures that contain variation in composition in the direction perpendicular to the stacking. The procedure lacks the versatility to fabricate more complex structures where a variation in composition is desired in more than one dimension. To achieve the full potential of tough hydrogels it is imperative that other advanced fabrication techniques are developed in parallel with cast molding.
1.3.2 3D Printing

One advanced fabrication technique that has been used by tissue engineers to process conventional hydrogels is 3D printing. 3D printing is a fabrication technique that is included under the wider umbrella of additive manufacturing technologies. It is a process that uses robotic precision to build up the structure of a digital model by successively patterning layers of material. The advantage of 3D printing over other digitally controlled manufacturing techniques is the capacity to fashion multi-component and multi-material assemblies in a single build. An adjustable spanner is just one example of the type of functional multi-component structures that has been fabricated directly through 3D printing. 3D printing has received a great deal of attention from a diverse range of fields from the fine arts to regenerative medicine and from gastronomy to aerospace engineering for its ability to fabricate complex structure in a short time scale.

1.3.2.1 Development of additive manufacturing

The earliest use of additive manufacturing was in the 1980’s for the purpose of rapid prototyping. Rapid prototyping is a group of techniques used to quickly fabricate a scale model of a physical part or assembly from a digital model. The development of rapid prototyping devices was to allow manufacturers to assess their designs sooner. Since then additive manufacturing has proved as a valuable tool for fabricating complex structure and custom one off designs.

In 1986 Charles Hull filed a patent for an apparatus used to fabricate three dimensional objects with stereolithography. The stereolithography apparatus employs a vat of photo curable resin and a UV laser to build structures one layer at a time. The laser beam traces a pattern of the objects cross-section on the surface of the liquid resin. After the pattern has been traced, the elevator platform descends by a distance equal to the thickness of a single layer and then, a resin-filled blade sweeps across the top of the vat to refill it with fresh material. The laser beam traces a pattern of a second layer and joins it to the layer below (Figure 1:11a). Stereolithography can require the simultaneous fabrication of supporting structures to attach the object to the build platform and to stop deflection due to gravity. Hull commercialized this technology
and founded the company 3D Systems Inc which remains a prominent name in additive manufacturing technology.

Figure 1:11 Examples of additive manufacturing apparatuses: a. Schematic diagrams of stereolithography apparatus in (i) top down and (ii) bottom up confirmations, reproduced from [147]. b. Schematic of the powder bed inkjet printing technique, reproduced from [147]. c. Schematic of (iii) a freeform extrusion printer with inset (iv) of the extrusion head, reproduced from [148].
The invention of stereolithography inspired the development of a range of additive manufacturing techniques starting selective laser sintering. Selective laser sintering’s development allowed a greater range of materials to be processed through additive manufacturing. Selective laser sintering uses a high powered laser to fuse together small particles of plastic, metal, ceramic, or glass powders. The same basic principles as stereolithography are used to build up the 3D structures in selective laser sintering. As in stereolithography the laser is used to trace the cross-section of the desired object which binds the particles exposed on the top layer of a bed of powder. After the pattern has been traced, the powder bed descends and a roller sweeps across the top of the powder bed leaving a fresh layer of powder. Unlike stereolithography, selective laser sintering does not require support structures because the structure being assembled is surrounded by unsintered powder at all times.

The next addition to the suite of additive manufacturing tools is called powder bed inkjet printing but is also known as binder jetting or drop-on-powder. This technique uses the head from an inkjet printer to pattern a liquid binding material onto a powder bed. Inkjet printers use either heat or piezoelectric crystals to propel 10 to 20 pL droplets of ink from the print head down onto the substrate.\textsuperscript{[149]} The liquid properties of inks must be matched to the printer before they can be jetted.\textsuperscript{[150]} Typical inkjet inks have viscosities up to 2 mPa s but printers can be designed to handle viscosities as high as 100 mPa s and a minimum surface tension of 35 mN/m.\textsuperscript{[149]} Powder bed and inkjet printing builds up three dimensional structures following the same method used in selective laser sintering. After one layer of powder is bound with the jetted ink, the powder bed lowered and a roller deposits a second layer over the top (Figure 1:11b). This additive manufacturing technique was the first to be described as 3D printing. Powder bed and inkjet printing was developed at the Massachusetts Institute of Technology in 1993\textsuperscript{[151]} and later licensed to Z Cooperation. The first of these printers used a powder bed filled with alumina powder which was bound with colloidal silica.\textsuperscript{[152,153]} These systems have since been optimized for forming objects with a wide range of materials including ceramics, acrylics, and even sugar.

All of these additive manufacturing techniques are limited in their ability to vary composition of an object fabricated in a single build. The objects tend to have homogeneous composition as they are formed out of a bin filled with a common core
material. Powder bed and inkjet 3D printing was the additive manufacturing technique with some capacity to vary the composition throughout an object. Using an inkjet printer head from a three channel color printer allowed for multiple liquid binding materials to be incorporated into the one build. A powder bed inkjet 3D printer with a multi-channel print head demonstrated color 3D printing by adding different colored dyes to binder solutions. These colors can be patterned throughout a three dimensional structure to make blends and gradients in the same way that cyan, magenta, and red colored inks are patterned by document writing printers to create complex images.

In recent times the development of 3D printing has moved away from the use of powder bed fusion techniques towards more freeform techniques. These freeform techniques build up a structure by depositing material or an ink directly onto a build stage. A number of different deposition systems have been used in the development of these freeform 3D printers. A wide range of inks and materials can be processed by these freeform 3D printing techniques because of the variety of available deposition systems.

Material jetting is a 3D printing technique that uses the same inkjet print head as used in document writing printers and powder bed inkjet 3D printers as a deposition system for freeform additive manufacturing. Material jetting uses liquid inks that can be cured solid with exposure to UV irradiation. In the printing process the inkjet head dispenses an array of ink droplets into the desired pattern. The droplets are smoothed into a flat layer with a roller and cured solid with a UV light. A second layer of droplets can then be jetted over the top of the solid layer. This process can be repeated to build up a three dimensional structure. The fabrication of supporting structures is often necessary when material jetting. The liquid ink cannot be suspended in free space but rather needs to be deposited onto a substrate. A solid platform of support structure needs to be constructed under any hollows or overhangs present in the build design. Some inks that form brittle or water soluble materials have been designed specifically for the purpose of building these sacrificial support structures. They can be patterned alongside the structural materials using the same multi-channel printed heads as used for color printing.
Fused deposition modeling is an additive manufacturing technique used for printing plastics and metals. In this process a spool of plastic filament or metal wire is unwound and passed through an extrusion nozzle. The nozzle is heated to soften or melt the tip of the filament. The extrusion nozzle deposits the material as a continuous bead rather than as droplets deposited by inkjet print heads. The bead of molten material cools and hardens when extruded out of the heated nozzle. The molten beading fuses to the previously deposited material as it cools. The fusion of the cooling material allows the beading to be stacked to build up multi layered structures. The fabrication of support structures is not always required when 3D printing with the fused deposition modeling method because molten beading can support moderate overhang and can span unsupported gaps many times the length of the beading diameter.

Extrusion printing also known as direct writing and robocasting is a third type of freeform 3D printing. Extrusion printing is similar to fused deposition modeling as it deposits ink as a continuous bead rather than as individual droplets. The difference between these two printing techniques is that extrusion printers process viscous liquids, gels and pastes. The fluid inks are stored in a syringe barrel and a pneumatic or mechanical syringe pump is used to extrude them through a syringe tip (Figure 1:11c). Once patterned the ink needs to solidify quickly so that it can support the mass of subsequent layers without deformation. Dependent on the inks composition, extrusion printing can deal with moderate overhang and the spanning of gaps like the fused deposition modeling method.[155]

Extrusion printing is the most adaptable of the 3D printing techniques and can be used to process a wide variety of materials including polymers, metals, ceramics and composites. The viscosity requirements for extrusion inks are relaxed compared to inkjet inks. An extrusion printing ink should flow when pressured by the syringe pump, hold its shape once patterned onto the substrate and harden or solidify to support the next layer of material. A number of different mechanisms have been used to solidify extrusion inks. Thermoplastic materials can be melted in a heated syringe barrel and then solidified when extruded onto a cooled substrate.[156] Ceramic pastes and caulking silicon are two examples of extrusion inks that harden by drying.[157,158] UV irradiation has been used to initiate free radical polymerizations to cure polymer precursor
solution inks solid. Extruding an ink into a reactive bath is another method that has been used to solidify liquid inks.

Material jetting, fused deposition modeling and extrusion printing freeform 3D printing techniques are all able to fabricate complex multi-material structures. Printing multi-material structures requires the use of an additive manufacturing apparatus with either a multi-channel inkjet print head or multiple extrusion nozzles. Freeform 3D printing techniques fabricate multi-material structures by patterning two materials side by side. An advantage of these freeform printing techniques over powder bed inkjet 3D printing is that the composition of the two materials can be entirely different. 3D printers can replicate the composition gradients produced with the multi-step cast moulding procedure by patterning either a series of inks or different blends of two inks side by side. The advantage that 3D printing has over the cast moulding procedure is that the additive fabrication apparatuses use robotic accuracy to precisely position the materials in three dimensional space. The resolution of most printers is only limited by the accuracy of deposition system and not the precision of the robotic platform. The droplets are \( \sim 100 \, \mu m \) in diameter and the diameter of extruded beads is determined by the internal diameter of the extrusion nozzle. Using digital modelling the resolution of material deposition can be used to assign the material composition of every point within a macroscale structure.

1.3.2.2 Operation of 3D printers

All 3D printers and additive manufacturing apparatuses are operated through the same basic three step procedure. The three steps are digital modelling, printing and post-processing.

Digital models may be prepared with a computer aided design (CAD) package or with a 3D scanner. 3D scanning is a process of digitally collecting data on the shape of a real object and creating a digital model based off that data. Digital models for 3D printing are saved in the STL (STereoLithography) file format which was created by 3D Systems Inc. The file describes the surface geometry of a digital model. A software package called a "slicer," is used to convert the shell defined in the STL file into a series of thin layers and a G-code tool path can then be determined to print each of these layers. The digital modelling step is the key to the fabrication of complex and
multi-material structures. The digital models of multi-material structures are designed as an assembly of multiple parts. Using the 3D printers software interface different materials can be added to each part with in the assembly. Digitally modelling blending of materials and gradients can be more difficult and often require the use of proprietary software.

In the second stage of the 3D printing procedure the additive manufacturing apparatus must be prepared for the build process. At this stage the printer is loaded with the resins, powders, binders, inks, polymers consumed by the printer during the build process. The G-code file can be sent to the loaded 3D printer to initiate the build process. The build process is automated and can take minutes, hours or even days to complete dependent on the size of the structure being printed and thickness of each layer that is deposited.

Post-processing is determined by the printed materials and structure printed and so one or more steps may be required to finish the printed structure. The types of post-processing steps used to finish structures include removing unbound powder or resin, removing or dissolving support structures, firing ceramics, annealing metals, chemical treatments to remove or quench unreacted monomers and painted or coated to change the outward appearance of the structure. Once the post-processing steps are completed the printed structure can be used in its desired application.

Structures with all manner of designs can be 3D printed by following these three basic steps. A wide range of materials can be incorporated into these structures because the variety of available additive manufacturing apparatuses uses different techniques to process inks. New inks can be developed for 3D printing by matching the rheological properties of materials to the processing capabilities of the different 3D printers.

1.3.2.3 Ink Rheology

Rheology is the main technique used to characterise new inks developed for freeform 3D printing. Rheology is the study of the flow of liquids and deformation of soft solids. The connection between these two processes is that deformations made by a shear force can cause viscous materials to flow.\textsuperscript{161} Rheology can offer an insight into the flow characteristics of a material being passed through the nozzle of a print head and
the solidification process of that material once deposited onto the substrate. From these insights it is possible to determine the suitability of a material for 3D printing.

Rheometry is a measuring technology used to determine rheological behaviour. Liquids and solids are both characterised using rotational and oscillatory tests. Rotational tests are performed to investigate viscous behaviour of a material and oscillatory tests viscoelastic behaviour. Viscous materials oppose shear flow and strain linearly with respect to time when a stress is applied. Elastic materials strain when stretched and quickly and fully return to their initial state when the stress is removed. Viscoelastic materials display a combination of these properties, exhibiting time-dependent strain. The difference between these three behaviours can be visualised by observing the difference in behaviour of balls of water, modelling clay and steel hitting the ground from a height. The ball of water displays ideal viscous flow behaviour and spreads out into a thin layer when it hit the ground. The ball of steel displays ideal elastic behaviour and bounces showing no deformation. The ball of modelling clay however will be permanently deformed when it hits the floor and then continue to slowly flow on the floor which is a demonstration of a viscoelastic effect. When characterising a new material as a 3D printing ink rotational tests are used to assess whether the liquid has a suitable viscosity to be propelled through a print head and oscillatory tests are used to monitor the viscoelastic properties of the material as it solidifies. The best inks will flow through the print head with little resistance yet hold their shape once patterned onto the substrate and solidify quickly.

The instrument used to collect the rheological data is called a rheometer. In one type of rheometer the test samples are loaded into the rheometer between two parallel plates. In rotational test the top plate is free to spin and the bottom plate remains stationary. For accurate calculation of the rheological parameters the fluid sample must adhere to both plates without slipping or sliding against them and display laminar flow. The parameters measured in a rotational rheology test are shear stress, shear strain and shear rate. Shear stress (τ) is defined as τ = F/A, where F is the force required to spin the upper plate and A is the cross sectional area of the material between the two plates. Shear strain (γ) is defined as γ = s/h, where s is the deflection of the plate and h is the height of the gap between the two plates. Shear modulus G of an elastic material can be calculated from these two parameters and the relationship: G = τ/γ.
Similar to the Young’s modulus, shear modulus describes the rigidity of a material. Shear rate ($\dot{\gamma}$) is the time derivative of shear strain and is defined as: $\dot{\gamma} = \nu/h$, where $\nu$ is the velocity of the spinning plate. Shear viscosity ($\eta$) of an fluid material can be calculated from the relationship: $\eta = \tau/\dot{\gamma}$. Viscosity is the parameter of most concern for the purpose of ink development.

Viscosity is the result of internal friction forces between molecules of a flowing fluid. The viscosity of low molecular weight liquids such as water, organic solvents and mineral oils is independent of shear load which is described as ideal viscous or Newtonian flow behaviour. However, the viscosity of most fluids exhibits a dependency on shear load. Controlled shear rate and controlled shear stress tests are used to determine the flow behaviour of a fluid sample. The controlled shear stress test is required for fluids that exhibit some kind of yielding behaviour. The data from both of these tests is reported in the form of a flow curve. Fluids that display an increase to viscosity corresponding to increasing shear rate are described as shear thickening and fluids that display a decrease to viscosity corresponding to increasing shear rate are described as shear thinning. Materials that behave like a solid at low shear stresses but flow like a liquid at higher shear stresses are described as Bingham plastics. Collectively these flow behaviours are described as non-Newtonian. Mathematical model functions for curve fitting are often used to describe these different flow behaviours. Ideal viscous fluids adhere to Newton’s law of viscosity: $\tau = \eta \cdot \dot{\gamma}$. Shear thickening and shear thinning fluids can be modelled by a power law model: $\tau = K \cdot \dot{\gamma}^n$, where $K$ is the flow coefficient which describes the consistency of the fluid and $n$ is the power law index. Bingham plastics are described by the function: $\tau = \tau_B + \eta_B \cdot \dot{\gamma}$, where $\tau_B$ is the Bingham yield stress and $\eta_B$ the Bingham flow coefficient. Figure 1:12 displays example flow curves of Newtonian, shear thickening and shear thinning fluids and a Bingham plastic.
Figure 1: 12 Example flow curves of (i) a Bingham plastic, (ii) a shear thickening fluid, (iii) a Newtonian fluid and (iv) a shear thinning fluid.

Shear thinning fluids and Bingham plastics are the ideal materials for use as 3D printing inks. Inks experience high shear rates when they are pushed through the print head because nozzles are narrow. The shear stress required to push fluids with a shear thinning behaviour to pass through nozzle is less than that for a Newtonian fluid with an equivalent consistency because it becomes less viscous at higher shear rates. Once patterned, the fluid is subject to smaller shear forces, such as those caused by gravity or surface tension. Shear thinning fluids can be highly viscous when exposed to little or no shear loads and Bingham plastics are resistant to flow in the absence of a shear load. This resistance to flow means that the fluid will not deform after patterning and can maintain the desired shape until it is solidified.

Temperature has a strong effect on the flow behaviour of fluids and can be used to optimise the viscosity of an ink for use in a particular 3D printer. Viscosity typically decreases as temperature is increased. Heat increases the kinetic energy of the fluid so that the molecules are able to flow with less resistance. The effect of temperature on the flow behaviour of a fluid can be observed in a rheological test by shearing a sample at a constant rate whilst applying a temperature ramp. Many additive manufacturing apparatuses are designed with temperature controlled ink wells for moderating the ink’s flow behaviour. By either heating or cooling an ink its viscosity can be tailored to pass through the nozzle of the print head.
3D printer inks need to be solidified once patterned by the print head. Fluids can be cured during a rheology test to simulate the solidification of an ink. The rheometer’s temperature control system or a radiation source can be used to initiate the solidification in situ. The solidification of a fluid is characterised by oscillatory rheometry tests. For an oscillatory test, samples are loaded into a rheometer between two parallel plates following the same method used for rotational tests. During testing the upper plate is moved back and forth along a deflection path by oscillating force whilst the bottom plate is held stationary. For accurate calculation of the rheological parameters the sample must adhere to both plates without slipping or sliding against them and the material must deform homogeneously across the shear gap. During testing the oscillations follow a pre-set sinusoidal wave function and the shear stress, shear strain and shear rate are recorded as a function of time \( t \). For tests with a controlled shear strain in the form of oscillatory sine functions: \( \gamma(t) = \gamma_A \cdot \sin \omega t \), where \( \gamma_A \) is the shear strain amplitude and \( \omega \) is the angular frequency. The resultant shear stress function is measured as phase-shifted sine function that can be modelled by the relationship: \( \tau(t) = \tau_A \cdot \sin(\omega t + \delta) \), where \( \tau_A \) is the shear stress amplitude and \( \delta \) is the phase shift angle. For ideal elastic materials this phase shift angle is \( 0^0 \), viscoelastic materials display a phase shift angle \( 0^0 \leq \delta \leq 90^0 \) and for viscous materials this phase shift angle is \( 90^0 \). For tests with a controlled shear stress in the form of oscillatory sine functions: \( \tau(t) = \tau_A \cdot \sin \omega t \), the resultant shear strain function is measured as phase-shifted sine function that can be modelled by the relationship: \( \gamma(t) = \gamma_A \cdot \sin(\omega t + \delta) \).

The parameters collected in oscillatory tests are used to calculate the complex shear modulus and the complex viscosity. The complex shear modulus \( (G^*) \) is calculated by the relationship: \( G^* = \tau(t)/\gamma(t) \), and the complex viscosity \( (\eta^*) \) is calculated by the relationship \( \eta^* = \tau(t)/\dot{\gamma}(t) \). These parameters are written in the complex form and contain both a real and imaginary component because they result from a harmonic-periodic process.

The complex shear modulus is the parameter of most concern for observing the inks as they cure. The real component of the complex shear modulus \( (G') \) is called the storage modulus and is calculated as: \( G' = (\tau_A/\gamma_A) \cdot \cos \delta \). The storage modulus is a measure of how much energy is stored within a material to reverse deformation once
the load is removed and represents the material’s elastic behaviour. The imaginary component of complex shear modulus is called the loss modulus \( (G'') \) and is calculated as: \( G'' = \left( \tau_A / \gamma_A \right) \cdot \sin \delta \). The loss modulus is a measure of how much energy is lost to a material during deformation and represents the material’s viscous behaviour. The ratio of the loss modulus to the storage modulus is called the loss or dampening factor and reveals the ratio of the viscous and elastic portion of materials viscoelastic deformation behaviour. The loss factor \((\tan \delta)\) is calculated as: \( \tan \delta = G'' / G' \). Materials with a loss factor >1 exhibit predominantly viscous deformation behaviour and are determined to be in the liquid state. Materials with a loss factor <1 exhibit predominantly elastic deformation behaviour and are determined to be in the solid state. A loss factor of 1 indicates that the material is at a transition between liquid and solid called the gel point.

The kinetics of an ink curing can be measured by a rheological test with constant shear conditions. The ink is loaded into the rheometer as a fluid and after some time the curing process is initiated. The loss factor is recorded as a function of time to determine how long the ink takes to gel. The storage modulus is also measured to determine whether the solidified ink can support the load of another layer of ink. It is desirable to minimise the solidification time of a 3D printer ink so that the deposition system can operate without pausing between layers to wait for the deposited ink to be strong and rigid enough to support the next layer of ink.

1.3.2.4 3D printing hydrogels

With these rheological considerations in mind many inks have been developed for the purpose of 3D printing conventional hydrogels. Most inks developed for printing hydrogels are gel precursor solutions containing some dissolved polymer. Polymer solutions have the rheological properties required for material jetting and extrusion printing additive manufacturing techniques because they display shear thinning flow behaviour. At rest the polymer chains adopt a random coil conformation to minimise energy. The shape of each coil is approximately spherical and contains entanglements with neighbouring polymers. The friction between entanglements is a source of flow resistance and so the polymer solutions display high shear viscosity when sheared at a low rate. During the shearing process, the molecules become orientated in the shear direction. Orientating the polymers causes the molecules to disentangle to some extent
which lowers the solution’s resistance to flow. The flow behaviour of gel precursor solutions can be optimised for use in a particular 3D printer by changing the concentration of polymer or molecular weight of polymer within an ink.

A variety of gelation mechanisms are available to solidify the hydrogel precursor solutions once patterned. Free radical polymerisation initiated by an irradiation source is one of the common methods used to cure gel precursor inks.\[159, 162]\] This gelation mechanism can also be used in stereolithography to build up hydrogel structures.\[163-165]\] The cure time of this gelation mechanism can be optimised for 3D printing by changing the concentration of the initiator molecule within the ink or by altering the intensity of the irradiation sources. Cooling of thermally reversible hydrogels is a second method for solidifying hydrogel inks.\[166]\] The build stage of 3D printers can be cooled to decrease the curing time of these inks. These hydrogels tend to be particularly weak and so a post-processing step may be needed to cross-link the polymers. Bath printing is another method that is used to 3D print hydrogels in which case the ink must solidify upon contact with the coagulation bath.\[160]\]

The development of hydrogel inks in parallel with the development of new 3D printers has provided the capacity to fabricate a new range of complex structures. 3D printing was first performed with hydrogels for the purpose of tissue engineering which remains the primary application for the technology. Initially the extrusion printing method was used to build log pile structures for use as cell scaffolds.\[167]\] The formulations of these hydrogels are designed to mimic the extra cellular matrix to support the growth of seeded cells. Hydrogel inks have since been formulated with living cells and some printing techniques allow for individual cells to be deposited at specific positions within a cell scaffold.\[168]\] These advancements have initiated the field of computer aided tissue engineering which incorporates 3D medical imaging computer aided design and modelling and 3D printing to construct artificial tissue and organs.\[169, 170]\] In the past, the size of engineered tissues has been limited by an inability to deliver nutrients to cells inside thicker tissues but hydrogel 3D printing techniques have allowed for the fabrication of scaffolds that include vascularisation.\[171]\] Over the past decade a number of articles have been published that review the uses of 3D printing as a tool for forming complex structures that harness the biocompatibility of
hydrogels. Most of these articles have identified the poor mechanical properties of the printed hydrogels as a limitation this technology.

Prior to the beginning of this research project in 2012 a method for 3D printing with tough hydrogels had not yet been developed. The vast majority of inks and processing techniques used for 3D printing hydrogels form the conventional single network architecture. A method for extrusion printing semi-IPN hydrogels was developed by Barry III et al in 2009. Semi-IPNs are a three dimensional polymer network that is interlaced by uncrosslinked strands of a second polymer. An ink composed of straight chain PAAm and acrylamide monomer was used to print the semi-IPN. The PAAm increased the viscosity of the ink so that it could be patterned by the extrusion printer. A UV light source was used to initiate the free radical polymerisation of the acrylamide monomer and crosslinker, thereby forming a PAAm network that included the linear PAAm polymer chains originally included in the ink. The versatility of this technique for printing semi-IPNs with other polymers was shown by Hanson-Shepherd et al. who used this technique to print a semi-IPN from an ink of 2-hydroxyethylmethacrylate and poly(2-hydroxyethylmethacrylate). The versatility of this printing method establishes a basis to develop printing methods for tough IPN hydrogels.

In fact, over the past 4 years the semi-IPN printing process has been adapted to 3D print tough hydrogels by the author and by other research groups. The curing mechanism of the semi-IPN ink is analogous to the one pot synthesis of alginate/PAAm ICE hydrogels. This thesis describes the author’s work in developing the one pot synthesis of ICE hydrogels during extrusion-based 3D printing. A method for further improving the toughness of printed ICE hydrogels was demonstrated recently by Hong et al in 2015 who added nanoclay to the ink (Figure 1:13a & b). The addition of nanoclay not only added a second toughening mechanism to the cured hydrogel but also allowed the rheological properties of ink to be tailored for extrusion printing. Wei et al. have also recently reported the development of another method for printing where by a thermally reversible agar polymer network was added to an alginate/PAAm ICE hydrogel ink formulation. The development of these tough hydrogel 3D printing techniques enables the fabrication of complex structures for use in novel applications.
1.4 Aims

The main aim of this research project was to develop a way to fabricate complex structures from tough hydrogels. The following chapters of this thesis document the early development of tough hydrogel printing techniques, their integration with existing techniques used to print other materials and their use to fabricate novel structures that harness the functional properties of hydrogels.

The first aim of the research described in this thesis was to develop a methodology for processing tough hydrogels with 3D printing technology without compromising the material’s mechanical integrity compared to hydrogels formed with a casting process. Ionic covalent entanglement hydrogels have been specifically identified as the class of hydrogels to be 3D printed in this research project because they have demonstrated high toughness values and recovery of damage to their network structure. The motivation behind this initial aim was to develop a method that is capable of processing tough hydrogel materials into desirable shapes that may contain difficult geometries.

The second aim of this research project was to use a 3D printer to fabricate structural composite materials that maintain the unique properties of their hydrogel components.

Figure 1:13 a. Images of complex structures 3D printed with nanoclay alginate/PAAm ICE hydrogel. b. Photographs of a 3D printed nanoclay alginate/PAAm ICE hydrogel in compression, reproduced from[175].
The intention was to integrate the ICE gel ink formulations and the printing techniques developed in the first phase of this project with other inks of non-hydrogel materials to produce novel composites materials and multi-material structures. The objective was to utilize the spatial control of digital modelling in conjunction with 3D printing to design the internal architecture of the composite structures, to vary the volume fractions of the components within a material and to directly fabricate the inks into useful structures composed of the composite materials.

The third and final aim of this research project was to use a 3D printer to construct a multi-material assembly that not only incorporates hydrogels but harnesses the functional properties such as actuation, sensing and conductivity. The ambition for this third phase of the research project was to demonstrate that 3D printing is a viable means for fabricating hydrogel based devices to be used in real world applications. The specific objective was to combine the techniques developed in the second phase of this research project for printing multi-material structures with new functionalized hydrogel inks to produce a working device.

1.5 Chapter summaries
The next five chapters of this thesis record the author’s endeavors to achieve the above aims. The first two chapters focus on the development of inks and processing techniques for 3D printing tough hydrogels. Two different techniques for 3D printing ICE gels are described and compared in these chapters.

Chapter 2 describes the development of an extrusion based technique for 3D printing Alg/PAAm ICE gels. An ink was prepared that containing a mixture of alginate biopolymer and an acrylamide monomer solution. A UV light was used to initiate a free radical polymerization reaction of the acrylamide to solidify the printed ink. The printed hydrogels were swollen in a solution of calcium chloride to cross-link the alginate polymer chains which completed the ICE gels. This chapter explores the effects of alginate concentration on the rheological properties of the ink and compares the mechanical performance of the printed hydrogels against that of Alg/PAAm ICE gels prepared using conventional casting methods.

Chapter 3 accounts the development of a second extrusion based technique for 3D printing carrageenan/epoxy amine ICE gels. For this alternative technique an ink was
prepared that contained kappa-carrageenan biopolymer and two reactive polymers that form covalent cross-links via an epoxy-amine addition mechanism. This chapter reports on the rheological characterization of the κ-CG/EA ICE gel ink. The printing technique exploits the thermal gelation of the kappa-carrageenan to solidify the patterned ink and the epoxy-amine polymer network forms over time. The ink was stored in a heated syringe barrel and deposited onto a cooled build stage to speed up the gelation process. Tensile testing revealed that the hydrogels produced using this ink and printing technique display an improved mechanical performance compared to that of the printed Alg/PAAm ICE gels.

The next two chapters of this thesis report on the development of 3D printing techniques for fabricating hydrogel composite materials. A commercially available epoxy based UV-curable adhesive, Emax 904 Gel-SC was identified as a suitable ink for printing composite structures with the Alg/PAAm ICE gel ink because these two fluids have similar rheological properties, are both cured by UV initiated free radical polymerization reaction and display strong adhesion once solidified. The epoxy adhesive has a much higher elastic modulus than the ICE hydrogels and can be used for mechanical reinforcement or to form structural components in 3D printed objects. Despite their inferior mechanical performance, the Alg/PAAm ICE gel ink was chosen to print composite structures rather than the κ-CG/EA ICE gel ink because free radical polymerization forms the hydrogels covalent network much faster than the epoxy-amine addition mechanism.

Chapter 4 describes the development of a 3D printing technique for fabricating fiber reinforced composite materials. CAD software was used to create digital models of the composites. A multi-material 3D printer patterned the Alg/PAAm ICE gel and Emax inks within the same structure and a UV light source was used to simultaneously cure the two inks side by side which creates strong adhesion between the two solid materials. The printed composite materials were mechanically characterized and the effect of fiber volume fraction on elastic modulus was analyzed with standard composites theory. The fiber reinforced hydrogel composite 3D printing technique was used to fabricate an artificial meniscus which demonstrates how this 3D printing technique can be used to make useful structures.
Chapter 5 accounts the development of a 3D printing technique for fabricating particulate reinforced composite materials. A novel deposition system that blends inks from two separate syringe barrels into a single stream was built to print these composites. The Alg/PAAm ICE gel and Emax inks are immiscible and begun to phase separate once deposited from the extrusion nozzle. A UV light source was used to cure the ink blend immediately after deposition to limit this phase separation which leaves a particulate morphology. The printed composite materials were mechanically characterized and the effect of the particulate volume fraction on elastic modulus was analyzed with standard composites theory. Digital control over the syringe pumps allows for the extrusion rates of the two inks to be altered throughout the printing process and can be used to produce gradient structures. The hydrogel gradient printing technique was used to fabricate an artificial tendon which demonstrated how this 3D printing technique can be used to make useful structures.

Chapter 6 accounts the development of a 4D printing technique using a tough hydrogel ink. 3D printed thermally responsive tough hydrogel actuators were made by replacing the PAAm component of the Alg/PAAm ICE gel ink with PNIPAAm. Rheology was used to optimize the reaction kinetics of the UV initiated free radical polymerization of the PNIPAAm network. Tensile and actuation tests were performed to determine the effect of the NIPAAm concentration on the mechanical properties and swelling behavior of the printed hydrogels, respectively. In this chapter the 3D printing technique used to fabricate fiber reinforced composite materials was modified to incorporate the Alg/PNIPAAm ICE gel ink. The revised printing technique used CAD software to create digital models that contain structural and actuating components. A multi-material printer was used to pattern the Alg/NIPAAm ICE gel and Emax inks within the same structure and a UV light source was used to simultaneously cure the two inks side by side. A smart value that can autonomously open and close as a response to temperature to control the flow of water was fabricated to demonstrate that this 4D printing technique can produce working hydrogel based functional devices.

Chapter 7 Conclusions and Recommendations is the final chapter of this thesis. This chapter outlines the overall conclusions made by this thesis and provides recommendations for further research based off the experimental findings of this project.
2 3D PRINTING OF ALGINATE / POLY(ACRYLAMIDE) IONIC COVALENT ENTANGLEMENT HYDROGELS

This chapter has been adapted from the article “Extrusion printing of ionic-covalent entanglement hydrogels with high toughness” published in Journal of Materials Chemistry B, 2013, 1, 38, 4939-4946.

Author Statement:

The research in this chapter was performed with the assistance of Stephen Beirne, who worked on the customisation of the 3D printer used in this work and provided technical assistance with the printer’s operation. All experiments and data analysis was carried out by the author, Shannon E. Bakarich. This would not have been possible without the intellectual support and guidance of Marc in het Panhuis, Gordon G. Wallace and Geoffrey M. Spinks.

The introduction section in this chapter is a shortened version compared with the published article. The introduction has been modified to avoid duplication with the comprehensive literature review of Chapter 1.

I, Prof. Geoffrey M. Spinks (Supervisor), support and certify the above author statement.

Geoffrey M. Spinks
2.1 Introduction

As described in Chapter 1, two major limitations in the use of synthetic hydrogels are their poor mechanical robustness and the difficulty in fabricating hydrogels into complex shapes and multi-material devices. A number of toughened hydrogels have recently been reported\(^2\), as has the application of 3D printing to hydrogel formulations\(^1\). The new materials have been designed with a variety of toughening mechanisms that increase the energy dissipation during crack propagation. Examples of these tough hydrogels include slip-link gels\(^2\), nanocomposite gels\(^7\), double-network gels\(^8\) and ionic-covalent entanglement gels\(^9,10\). Toughened hydrogels have fracture energies in excess of 1000 J/m\(^2\) compared with values less 10 J/m\(^2\) for conventional hydrogels\(^2\). The synthesis of these new materials requires complex and in some cases multi-step chemical reactions. Until now toughened hydrogels have not been considered compatible for processing through additive fabrication techniques because of their complex synthesis.

The combination of additive fabrication techniques with tough hydrogels will be a significant advance in the application of these materials. The aim of the work presented in this Chapter was to determine whether ionic-covalent entanglement (ICE) gels could be prepared by extrusion printing and retain their high toughness. Ionic-covalent entanglement gels are a special case of interpenetrating polymer network gel that exhibit a remarkable mechanical performance and that can be synthesised in a relatively straightforward simultaneous dual network formation process\(^9\). Alginate/polyacrylamide (Alg/PAAm) ICE gels reported by Sun et al.\(^10\) have a reported fracture energy \(\sim 9000\) J/m\(^2\).

In this chapter ICE gels were fabricated with a UV printing technique that was designed for printing interpenetrating polymer networks\(^1\). A hydrogel precursor solution ink containing alginate biopolymer and acrylamide monomer was patterned by extrusion printing and cured \emph{in situ} by a UV light source.

2.2 Experimental

2.2.1 Materials

All materials were used as received and all solutions were prepared using Milli-Q water (resistivity = 18.2 Ω cm). Acrylamide (AAm) monomer and α-keto glutaric acid
photo-initiator were purchased from Fluka (Australia). Alginic acid sodium salt, from brown algae with Brookfield viscosity (2% in H₂O at 25 °C) of 250 mPas, N,N'-methylenebisacrylamide, calcium chloride (minimum 93.0% granular anhydrous) cross-linkers and ethylene glycol rheology modifier were purchased from Sigma Aldrich (Australia). Microscope slides (Sail Brand, Cat.No. 7101) were used as received.

2.2.2 Hydrogel ink formulation

The Alg/AAm ink was produced by adding alginic sodium salt to a stock solution of UV-curable acrylamide. The 1.8 M acrylamide solution was prepared by dissolving 6 g of acrylamide, 129 mg of N,N'-methylenebisacrylamide (1 mol% based on acrylamide monomer) and 123 mg of α-keto glutaric acid in 26.5 ml of Milli-Q water, 5 ml of the 0.1M calcium chloride stock solution and 12.5 ml of ethylene glycol. The latter was used as a non-volatile co-solvent with the ratio of ethylene glycol to water fixed at 1:2.5. Alganic sodium salt was stirred into the acrylamide solution with a spatula. Alg/AAm inks were produced with fixed amounts of acrylamide and varying alginate concentrations 1% and 5% (w/v). For each 1 ml of ink 10 mg of alginic salt was added to 1 ml of acrylamide solution for every 1% (w/v) of alginate. The weight ratio of AAm to AAm and alginate varied from 70% to 92% as the alginate concentration decreased from 5% to 1% (w/v).

2.2.3 Rheology

The flow behaviour of the Alg/AAm inks was determined using a AR2G Rheometer coupled with a cone and plate measuring system (diameter 40 mm, angle 2°06° and truncation 55 μm) and a heat controlled sample stage (TA instruments peltier plate). The viscosity was measured as a function of shear rate (1 – 500 1/s). Measurements were performed on inks at 25 °C and 35 °C.

2.2.4 Extrusion printing

A KIMM SPS1000 Bioplotter extrusion system (Korea Institute of Machinery and Material) in conjunction with a UV–curing system was used to print hydrogel structures from the Alg/AAm inks. The Dymax BlueWave 75 Rev 2.0 UV Light Curing Spot Lamp System using a 19+ W/cm² UV source with a 1 meter light guide was used to cure the printed ink. The end of the light guide was fixed to the print head
50 mm away from the nozzle by a custom-built brace (designed in SolidWorks and printed using a Dimension uPrint Plus).

Computer-aided design (SolidWorks) was used to construct a digital model of the item to be printed. Proprietary KIMM software was used to transform the digital model into a set of numerical commands that determine the printing path. This command file was then edited to accommodate for the UV–curing system. The UV light was passed over each complete layer of ink once (90 s) and over the final layer a further three times (270 s) at a height of 25 mm. UV polymerization proceeded in air and future printer designs may provide an inert atmosphere to limit oxygen inhibition.

The Alg/AAm ink was loaded into a pressurised stainless steel barrel in a temperature controlled jacket. A 23 gauge extrusion nozzle (diameter 0.337 mm) was fitted to the end of the barrel. The barrel temperature was held stable during printing at either 25 °C or 35 °C. The printing pressure (30 – 130 kPa) was optimised for each ink to deliver repeatable, unbroken and unblotted lines. The Alg/AAm ink was printed onto an untreated glass slide. The printed samples were stored in silicone oil prior to characterisation.

2.2.5 Mechanical testing

The mechanical properties of the printed hydrogels were determined using a Shimadzu EZ-L Universal Mechanical Tester. Tensile tests were performed using a 10 N load cell and spring loaded clamps. Printed dog bone samples were strained to failure at a rate of 10 mm/min. The applied stress was calculated using the total average cross sectional area of the gauge length. The elastic modulus was calculated from the initial slope of the stress-strain curve and the Work of Extension was calculated as the area under the stress-strain curve.

2.2.6 Swelling

The swelling ratio was determined after immersing printed hydrogels samples in Milli-Q water for 72 hr or in 0.1M aqueous calcium chloride for 72 hr. Swelling was also determined for samples immersed first in 0.1M calcium chloride (72 hr) followed by Milli-Q water (72 hr). Swelling ratios \(Q\) were calculated using:

\[
Q = \frac{W_S - W_D}{W_D},
\]  

(2:1)
where ‘\( W_d \)’ is the dry weight of hydrogel and ‘\( W_s \)’ is the swollen mass of the hydrogel. Samples were dried by being placed in an oven at 60 °C for 72 hr.

2.2.7 Thermal gravimetric analysis

Thermal gravimetric analysis was conducted on hydrogel samples with the TA instruments Q500 Thermal Gravimetric Analyser. The hydrogels were heated from 25 °C to 600 °C at a rate 5 °C /min. The furnace was filled with a flow of air at a rate 90 ml/min and nitrogen at a rate 10 ml/min.

2.3 Results and Discussion

This study aimed to determine whether 3D extrusion printing could be used to produce tough hydrogels formed from an ionic covalent entanglement gel system. Firstly, the pre-gel printing inks were formulated to meet the rheological requirements for extrusion printing. Using these rheological acceptable formulations, a simple dumbbell shape was printed and cured with UV radiation to both demonstrate printability and to measure mechanical properties. Control experiments were not performed on single network hydrogels because PAAm precursor solutions have insufficient viscosity to be extrusion printed and whist alginate precursor solutions can be extrusion printed they cannot be solidified by UV irradiation to support layering of the material.

2.3.1 Extrusion printing

Viscosity (\( \eta \)) measurements of the Alg/AAm printing inks were evaluated to determine their suitability for extrusion printing. The resulting flow curves (Figure 2.1a) were fitted to a power law model\[^{[161]}\]:

\[
\eta = K\dot{\gamma}^{n-1}
\]

(2:2)

where \( \dot{\gamma} \) is the shear rate, \( K \) is the consistency index, and \( n \) is the power law index. The printing solutions exhibit pseudo-plastic (non-Newtonian) shear-thinning flow behaviour typical of polymer solutions (\( n < 1 \)). As the alginate concentration increased the solutions were found to become more shear thinning (\( n \) decreases), and much thicker (\( K \) increases), see Table 2.1. The consistency index was found to be linearly proportional to concentration for concentrations between 2% w/v and 5% w/v (Figure 2.1b). The large difference in consistency between 1% w/v and 2% w/v alginate solutions could suggest that the dilute to semi-dilute transition is somewhere in this
concentration range. It was not possible to extrusion print the alginate solutions with concentrations below 2% w/v due to insufficient viscosity (low consistency) that led to unacceptable sagging and spreading of the extrudate. Alginate solutions with concentration above 2% w/v were found to possess flow characteristics compatible with the processable range of our extrusion printing system.

Table 2:1 Summary of the rheological parameters measured for the ICE gel inks.\textsuperscript{a}

<table>
<thead>
<tr>
<th>$C_{Alg}$ [% w/v]</th>
<th>$T$ [°C]</th>
<th>$K$ [Pa.s\textsuperscript{a}]</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>0.81 ± 0.20</td>
<td>0.40 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>24 ± 3.0</td>
<td>0.45 ± 0.01</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>86 ± 10</td>
<td>0.37 ± 0.01</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>152 ± 20</td>
<td>0.33 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>238 ± 40</td>
<td>0.32 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>122 ± 20</td>
<td>0.35 ± 0.03</td>
</tr>
</tbody>
</table>

\textsuperscript{a}$C_{Alg}$ is the concentration of alginate and $T$ is the print temperature. Consistency ($K$) and power law ($n$) indices were obtained through curve fitting with equation (2:2).
Figure 2: a. Flow curves of 1, 2, 3, 4 and 5% w/v alginate hydrogel precursor solutions at 25 °C and 5% w/v alginate printing solution at 35 °C. b. A plot of the hydrogel precursor solution consistency index against alginate concentration showing the linear relationship between consistency and concentration above 2% w/v. c. CAD model of 4 dog bone specimens. d-e. Patterning of hydrogel filaments to produce dog bone specimens. f. The custom UV attachment used to cure the patterned hydrogel ink. g. Completed hydrogel dog bone specimens. Photographs of Alg/PAAm ICE hydrogel dog bone specimens printed with 2, 3, 4 and 5% w/v alginate solutions (h, i, j and k respectively) printed at 25 °C. l. An Alg/PAAm ICE hydrogel dog bone specimen printed from the 5% w/v alginate solution at 35 °C.

ICE hydrogels were produced using a modified commercially-available extrusion printer. The printer was modified by mounting a fibre-optic light guide to the print head. Alg/AAm hydrogels were fabricated using the adapted extrusion printing process. Figure 2:1c-g illustrates the fabrication of dog-bone specimens for tensile testing. The resolution of the printed hydrogels improved as the consistency of the ink increased. In the computer model the reduced section of the dog-bone specimens was 2 mm in width. The reduced section of 4 separate hydrogel samples printed with the
2% w/v alginate solution (Figure 2:1h) was 2.3 ± 0.2 mm wide compared to the reduced section of dog-bones printed with the 4% w/v alginate solution (Figure 2:1j) which was 1.9 ± 0.1 mm wide. Printed ink can flow and deform from its patterned shape due to gravity of surface energy effects until the shape is fixed by the cross-linking reaction induced by UV light. The thicker (more viscous) ink deforms more slowly giving improved resolution to the final structure.

The printer produces shapes by patterning ink fibres spaced 0.3 mm apart. A solid structure is formed when these fibres sag and spread to make contact with neighbouring fibres so that the ink fuses together. At 25 °C the flow behaviour of the fibres produced by the solution containing 5% w/v alginate was too high for the fibres to combine in the time prior to UV-curing. Consequently, the hydrogel structures produced by the 5% w/v alginate solution were highly porous with voids left between the defined fibres (Figure 2:1k). The flow behaviour of the 5% w/v alginate solution was improved by heating to 35 °C, resulting in a significant reduction in consistency (Table 2:1). The flow characteristics of the 5% w/v solution at 35 °C are similar to that of a 3.5% solution at 25 °C, which is calculated using the linear relationship between consistency and alginate concentration. The resulting improved flow characteristics facilitated the formation of solid, non-porous structures from the printed fibres (Figure 2:1l).

2.3.2 Tensile testing of as-printed hydrogels

To determine the prospects of printed Alg/PAAm hydrogels in load bearing applications the printed dog bone specimens were mechanically characterised in tension. Examples of typical stress – strain curves for the printed hydrogels are included in Figure 2:2a and a summary of the Alg/PAAm hydrogel mechanical properties are shown in Table 2:2. The tensile strength, Young’s modulus and failure strain are considerably lower in hydrogels printed with 5% w/v alginate ink at 25 °C in comparison to the samples of the same formulation printed at 35 °C. The highly porous nature of the incompletely-fused samples prepared at 25 °C can account for the reduced mechanical properties. Both the Young’s modulus and failure stress are reduced since the real cross sectional area of the samples is smaller than the cross sectional area of the printed structure. Additionally, voids in the hydrogels act like flaws which dramatically lower the failure stress and failure strain of the printed
structures.

Figure 2:2 a. Typical tensile stress strain curves of printed ICE hydrogels. b. The effect of weight ratio of acrylamide to (acrylamide plus alginate), on the modulus of ICE hydrogels printed at 25 °C (diamonds) and at 35 °C (squares). All samples were printed with the same polyacrylamide content but with different concentrations of alginate: 2%, 3%, 4% and 5% corresponding to 85%, 80%, 75% and 70% AAm/(AAm + alginate) weight ratios.

Table 2:2 Summary of the mechanical properties of printed ICE gels with various weight ratios of acrylamide to (acrylamide plus alginate).\textsuperscript{b}

<table>
<thead>
<tr>
<th>$W_R$ [% w/w]</th>
<th>T [°C]</th>
<th>E [kPa]</th>
<th>$\sigma_T$ [kPa]</th>
<th>$\varepsilon_f$ [%]</th>
<th>U [kJ/m$^3$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>85</td>
<td>25</td>
<td>48 ± 3</td>
<td>91 ± 30</td>
<td>210 ± 70</td>
<td>95 ± 10</td>
</tr>
<tr>
<td>80</td>
<td>25</td>
<td>66 ± 5</td>
<td>170 ± 30</td>
<td>300 ± 20</td>
<td>260 ± 4</td>
</tr>
<tr>
<td>75</td>
<td>25</td>
<td>78 ± 10</td>
<td>110 ± 50</td>
<td>150 ± 70</td>
<td>83 ± 20</td>
</tr>
<tr>
<td>70</td>
<td>25</td>
<td>61 ± 6</td>
<td>130 ± 10</td>
<td>90 ± 5</td>
<td>59 ± 1</td>
</tr>
<tr>
<td>70</td>
<td>35</td>
<td>83 ± 10</td>
<td>140 ± 20</td>
<td>220 ± 10</td>
<td>154 ± 1</td>
</tr>
</tbody>
</table>

\textsuperscript{b}All gels were printed with the same amount of acrylamide and with varying alginate concentrations. $W_R$ is the weight ratio AAm/(AAm+alginate), T is the print temperature, E is the elastic modulus, $\sigma_T$ is the tensile strength, $\varepsilon_f$ is the failure strain and U is the work of extension.

The Young’s modulus of the non-porous hydrogels increased as the alginate concentration was increased (Figure 2:2b). The ionically cross-linked alginate forms a tighter network than the covalently cross-linked acrylamide network. Consequently, increasing the proportion of alginate to acrylamide increases the average cross-link density and, in turn, increases modulus. The effect of alginate content on failure stress and strain was less significant with comparisons made difficult by the high degree of variability in these properties. Random voids introduced by air bubbles or clogging
during extrusion likely introduced defects that influenced the failure stress and strain.

A comparison of mechanical properties shows that the printed ICE hydrogels have a smaller work of extension than the ICE hydrogels fabricated by casting and as reported by Sun et al.\textsuperscript{[103]} (Figure 2.3). For example, the hydrogels printed from the 3\% w/v alginate solution have an acrylamide to acrylamide and alginate ratio of 80\% w/w. The failure stress and failure strain of these printed hydrogels are 170 ± 30 kPa and 300 ± 20 \%, respectively. The corresponding failure stress and failure strain for an equivalent cast hydrogel were ~220 kPa\textsuperscript{[103]} and ~900 \%,\textsuperscript{[103]} respectively. The introduction of structural flaws in the hydrogel from the printing process has a significant detrimental effect on the failure strain. Although the mechanical performance of the printed ICE hydrogels is inferior to that of previously reported cast ICE hydrogels, the printed Alg/PAAm hydrogels have a superior mechanical performance compared with other printed alginate structures. For instance the hydrogels printed from the 2\% w/v alginate solution have a tensile strength 91 ± 30 kPa compared to a tensile strength of 44 ± 10 kPa for hydrogels printed from a 2.3\% alginate and 0.1\% gelatine solution used for bio-engineered cardiac pseudo tissue applications.\textsuperscript{[181]}

![Figure 2:3 A comparison of the work of extension values for the printed ICE hydrogels (open circles – this work), cast ICE hydrogels\textsuperscript{[103]} (white circles), single network hydrogels (including hydrophilic homopolymers – white diamonds and copolymers using hydrophobic co-monomer – open diamonds), double network hydrogels (dashes), nanocomposite hydrogels (triangles) and slip-link hydrogels (squares), adapted from ref.\textsuperscript{[2]}}](image-url)
2.3.3 Swelling behaviour and mechanical properties of fully-swollen hydrogels

The material properties of hydrogels and their size / shape are dependent upon their water content which is influenced by their ambient environment. Printed hydrogels are likely to be used fully immersed in aqueous solutions, so it is important to characterise the extent of swelling that occurs post-printing. In this study, the printed hydrogels were swollen in water or in 0.1 M calcium chloride solution or in water following the calcium chloride treatment. The mass swelling ratio of the equilibrated hydrogels normalised to their printed mass is shown in Figure 2.4a. Immersion of the printed hydrogels in water produces a 3 - 5 fold increase in mass, increasing with increasing alginate content. In contrast, swelling in calcium chloride solution restricts the swelling to approximately a 1.2 fold increase of the printed mass and the water uptake is insensitive to the alginate content. The free calcium ions in the calcium chloride solution likely increase the ionic cross-linking of the alginate network and restrict the amount of water absorbed. Subsequent immersion of these calcium-treated hydrogels in water has an insignificant effect on the final swelling ratio.

![Mass swelling ratio of as made hydrogels printed with 2, 3, 4 and 5% w/v alginate solutions swollen in water, 0.1M calcium chloride solution and in calcium chloride and then swollen in water.](image)

![Typical tensile stress strain curves of swollen hydrogels.](image)

The printed hydrogels immersed in water and without calcium treatment have poor mechanical properties. Figure 2.4b contains typical stress – strain curves for swollen hydrogels printed from the 3% w/v alginate solution and Table 2.3 contains a summary of their mechanical characteristics. In comparison to the as-printed hydrogels, the hydrogels swollen in water show a decrease in Young’s modulus from 66 ± 5 kPa to 23 ± 3 kPa; a decrease tensile strength from 170 ± 30 kPa to 11 ± 3 kPa and the failure
strain drops from 300 ± 20 % to 23 ± 6 % resulting in a loss to the work of extension from 260 ± 3 kJ/m³ to 1.2 ± 0.1 kJ/m³.

Table 2: Summary of the mechanical properties of hydrogels printed from the 3% w/v alginate solution when swollen in water, 0.1M calcium chloride solution and 0.1M calcium chloride solution followed by water.\(^c\)

<table>
<thead>
<tr>
<th></th>
<th>E [kPa]</th>
<th>(\sigma_T) [kPa]</th>
<th>(\varepsilon_f) [%]</th>
<th>U [kJ/m³]</th>
</tr>
</thead>
<tbody>
<tr>
<td>As Printed</td>
<td>67 ± 5</td>
<td>170 ± 30</td>
<td>300 ± 20</td>
<td>260 ± 3</td>
</tr>
<tr>
<td>Water</td>
<td>23 ± 3</td>
<td>11 ± 3</td>
<td>23 ± 6</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>(\text{Ca}^{2+})</td>
<td>370 ± 20</td>
<td>200 ± 20</td>
<td>89 ± 20</td>
<td>89 ± 2</td>
</tr>
<tr>
<td>(\text{Ca}^{2+}) &amp; Water</td>
<td>260 ± 20</td>
<td>130 ± 10</td>
<td>90 ± 4</td>
<td>59 ± 1</td>
</tr>
</tbody>
</table>

\(^c\)E is the elastic modulus, \(\sigma_T\) is the tensile strength, \(\varepsilon_f\) is the failure strain and U is the work of extension.

It is likely that swelling the as-printed Alg/PAAm hydrogels in calcium chloride solution increases the cross-link density of the alginate polymer network. Calcium ions diffuse from solution into the hydrogel to form cross-links with the guluronic acid units in the alginate. The mechanical characteristics of ionic – covalent entanglement hydrogels are known to be sensitive to the cross-link density of the ionic polymer network.\(^{103}\) A hydrogel printed from the 3% w/v alginate ink and swollen in 0.1 M calcium chloride solution for 72 hr shows a modulus increase from 66 ± 5 kPa (as printed) to 370 ± 17 kPa and the failure strain decreases from 300 ± 22 % to 89 ± 20 % because of the increased cross-link density. When the hydrogel is subsequently swollen in water a concentration gradient drives more water into the hydrogel which increases its volume. The dilution of elastically-active network strands creates a drop in modulus\(^{111}\) to 260 ± 20 kPa and in the work of extension to 59 ± 1 kJ/m³.

The effects of post-printing swelling on the shape of the printed objects are illustrated in Figure 2:5. Additional swelling must be compensated for in the design of models for printing to achieve the desired size and shape in the environment to which the hydrogel will be exposed during service. Figure 2:5a – d is an example of how the shape of a hydrogel printed with the 3% w/v alginate solution changes when it is swollen in water for 72 hr, 0.1 M calcium chloride for 72 hr and water after being immersed in the 0.1 M calcium chloride for 72 hr each. The dimensions of a hydrogel printed with the 3% w/v alginate solution swell to 161 ±8 % of its printed size. The printed hydrogel expands when in water because the osmotic pressure drives water...
into the hydrogel to disperse the polymer network. The hydrogel deforms until equilibrium is reached between the osmotic pressure which expands the hydrogel and the hydrogel’s elasticity which tightens the polymer network. The large deformation to the hydrogel is due to its low cross-link density.

Figure 2.5 Photographs of hydrogels printed from the 3% w/v alginate solution a. as made, b. swollen in water, c. swollen in 0.1M Calcium chloride solution and d. swollen in 0.1M Calcium chloride solution and then swollen in water.

Shape and size retention is improved for an alginate solution by first swelling in calcium chloride solution. Swelling an equivalent hydrogel printed with the 3% w/v alginate solution in a 0.1 M calcium chloride solution will only expand to 106 ± 6 % of its initial dimensions. Subsequently swelling the hydrogel in water increases its dimensions to 109 ± 9 % of its printed size. The additional cross-links in the alginate network form the calcium ions increase the modulus of the hydrogels and reduce the swelling at equilibrium with water.

2.3.4 Characterisation of swollen hydrogel composition

Finally, the composition of the hydrogels after equilibrium swelling was considered. It is known that unreacted monomer or un-cross-linked chains can be removed from the as-prepared gel during swelling. The dry masses of the equilibrium swollen gels were determined after thoroughly drying the sample. Figure 2.6a shows the dry mass of the swollen hydrogels as a ratio of the mass of polymer and monomer in the ink. The mass ratio of the hydrogels swollen in water is less than 100% which indicates a loss of solid content from the alginate network and, or loss of low molecular weight species not connected to the polyacrylamide network. The alginate polymer network breaks down during the swelling process because the osmotic pressure drives the calcium ions out of the cross-links. Break down of the polymer network structure
allows alginate polymer chains to dissolve and diffuse out of the entangled hydrogel network.\textsuperscript{[182]} When swollen in calcium chloride additional cross-links are produced so the alginate network does not break down and there is no significant change within the experimental error of the measurement between the dry mass and the solid mass in the ink.

![Figure 2:6 a. Dry mass/solid mass in Ink of as made hydrogels printed with 2, 3, 4 and 5\% w/v alginate solutions swollen in water and in 0.1 M Calcium chloride solution and then swollen in water. b. TGA derivative of an as made hydrogel printed from the 3\% w/v alginate solution before (blue) and after swelling in 0.1M calcium chloride and water (red). The dry mass of gels that have been treated by swelling in water and by swelling in 0.1M calcium chloride followed by swelling in water. Ethylene glycol was used as printing co-solvent in this study since it was found difficult to print from aqueous inks due to the rapid evaporation of water during printing. Thermal gravimetric analysis was performed on hydrogels printed with the 3\% w/v solution before and after swelling in calcium chloride and water to determine whether the ethylene glycol was retained in the hydrogel after swelling. The temperature derivative weight plot in Figure 2:6b for the as-printed hydrogel has a peak at 100 °C attributed to evaporation of water and at 200 °C which may be attributed to the loss of ethylene glycol. No peak at 200 °C was observed for the equilibrium swollen hydrogel indicating that the ethylene glycol had been removed from the hydrogel during swelling. The peaks above 200 °C are attributed to the breakdown of polymer.]

### 2.4 Summary

An additive fabrication method was designed for processing ICE hydrogels into complex structures. The inks were composed of alginate biopolymer and a photo-
curable acrylamide solution but the formulation can be extended to other chemistries. Control over pressure difference and temperature allows inks with a range of rheological properties to be extruded from the printer. By proper control of these parameters, ICE hydrogels were printed that displayed very good mechanical strength with the work of extension exceeding other types of printed hydrogels. Defects introduced during printing did restrict the mechanical strength of the printed ICE hydrogels in comparison to previously described cast ICE hydrogels. Swelling in water immediately following printing had a detrimental effect on the Alg/PAAm hydrogels because of partial dissolution of the alginate network. Post-printing immersion in a solution of calcium ions, however, can be used as a post processing technique to help preserve the properties and dimensions of the hydrogels. It is expected that this UV-extrusion printing process can be implemented to construct mechanically robust hydrogel devices.
This chapter has been adapted from the article “3D Printed ionic-covalent entanglement hydrogels from carrageenan and an epoxy amine” published in RSC Advances, 2014, 4, 72, 38088-38092.

Author Statement:

The research in this chapter was performed in a collaborative effort with Paul Balding, who performed the rheological, printing and mechanical characterisation experiments. The author Shannon E. Bakarich offered intellectual support and technical assistance during these experiments, data analysis and wrote the manuscript. Operation of the 3D printer was performed with the technical assistance of Robert Gorkin III. This would not have been possible without the intellectual input and support of Marc in het Panhuis and Geoffrey M. Spinks.

The introduction section in this chapter is a shortened version compared with the published article. The introduction has been modified to avoid duplication with the previous Chapters.

I, Prof. Marc in het Pahuis (Co-supervisor), support and certify the above author statement.

Marc in het Panhuis
3.1 Introduction

In addition to alginate and PAAm, tough ICE gels have been prepared from various other combinations of gel forming polymers such as gellan gum, carrageenan, gelatin and epoxy-amine in a ‘one-pot’ synthesis method.\textsuperscript{[179, 183]} Chapter 2 demonstrated the utility of this ‘one-pot’ synthesis method for printing ICE hydrogels. This chapter outlines a new method for 3D printing ICE gels without the use of UV-irradiation that requires an optimization of the individual chemistries of the components and the rheological conditions of the ink for Additive Manufacturing. As a proof of principle of the methodology, a 3D printed composite mixture of materials (kappa-carrageenan and poly(oxyalkyleneamine) (Jeffamine)) is demonstrated. This approach exploits the thermal gelation of the kappa-carrageenan that gels first and provides structural integrity while the covalent polymer network is formed by ambient temperature epoxy-amine addition reaction.

To aid the reader a background on the materials is provided. Carrageenans are a class of water soluble biopolymers extracted from red edible seaweeds. Kappa-carrageenan (κ-CG) is a dominant form of carrageenan that is uniquely identified by a single sulphonate group located on each repeat unit.\textsuperscript{[184]} This group is ionized in solution, which provides cross-linking functionality between two neighboring biopolymer chains in the presence of divalent cations. Ionic cross-linking causes amorphous biopolymer chains to form an ordered double helix shaped structure, which results in the formation of a thermally reversible gel.\textsuperscript{[185]}

Poly(oxyalkyleneamine) (Jeffamine) is a water soluble aliphatic diamine and poly(ethylene glycol)diglycidylether (PEGDGE) is a water soluble polymer functionalized with epoxide end groups. In solution, covalent cross-linking of Jeffamine with PEGDGE will occur between the amine and epoxide sites via an epoxy-amine addition mechanism.\textsuperscript{[186]} These two polymers are to be collectively considered as a single polymer network, hereafter referred to as the epoxy-amine (EA) network.

3.2 Experimental

3.2.1 Materials

All materials were used as received and all solutions were prepared using Milli-Q water (resistivity = 18.2 Ω cm). Anhydrous calcium chloride ≥95% (batch
001251790) was purchased from Fluka Analytical (Australia). Kappa-carrageenan (batch SK92650) was received as a gift from CP Kelco. Jeffamine ED-2004, molecular weight 2,000 g/mol (Batch 1F518) was received as a gift from Huntsman. Poly(ethylene glycol)diglycidylether, molecular weight 500 g/mol (Batch MKBC9721) was purchased from Aldrich (Australia).

3.2.2 Hydrogel ink formulation

The preparation of the materials is as follows. Initially a κ-CG/EA ICE gel precursor solution was prepared following the ‘one-pot’ synthesis method\(^\text{[179]}\): a calcium chloride (CaCl\(_2\), 0.2 mol/L) solution was generated from anhydrous calcium chloride and Milli-Q water. A solution of κ-CG was prepared by dissolving 1 g of biopolymer in 50 ml of Milli-Q water on a laboratory hotplate with heating (70 °C) and magnetic stirring. An EA solution was then prepared by mixing 19.8 mL of molten Jeffamine with 7.7 mL of PEGDGE and 17.5 mL of Milli-Q water at 70 °C. The κ-CG solution was then added to the EA solution followed by the addition of 5 mL of the hot (70 °C) calcium chloride solution.

3.2.3 Rheology

Rheological testing was used to establish the optimal conditions for extrusion printing the κ-CG/EA ICE gel precursor solution. Gel transition point tests were applied to determine at what temperature the ink and its constituents κ-CG and EA undergo a thermal transition from solution to gel. Immediately following synthesis, ink, κ-CG and EA solutions were pipetted hot (70 °C) onto the heated (70 °C) Peltier plate of an Anton Paar Physica MCR 301 rheometer to allow for its complex viscosity to be tracked during cooling. The complex viscosity of the solutions was measured at a fixed amplitude and frequency of 1 % and 10 Hz, respectively, between 70 °C and 20 °C at a cooling rate of 2 °C/min. A time sweep test was then performed upon the ink to establish the period of time during which the rheological characteristics were not significantly altered due to EA network formation. The ink was again pipetted hot (70 °C) onto the heated plate of the rheometer. The storage modulus was measured at a constant temperature (50.4 °C), amplitude (1 %) and frequency (10 Hz) for 4 hr.
3.2.4 Extrusion printing

A 4th generation 3D-Bioplotter from Envisiontec (Germany) was used to extrusion print the κ-CG/EA ICE gel precursor solutions. The Envisiontec 3D bio-plotter was selected to print the κ-CG/EA ICE gel precursor solutions rather than the KIMM SPS1000 Bioplotter used in Chapter 2 because it had a build plate with the added feature of temperature control. Before printing, the ink was loaded into a syringe barrel, which was then placed in the Bioplotter, pressurized, and allowed to equilibrate to 50.4 °C for 1 hr. The printer parameters were carefully adjusted to produce defined features. Optimal printing onto a poly(propylene) substrate held at 21 ± 1 °C required a head speed of 10-12 mm/s and pressure of 400-500 kPa when using 0.159 mm (internal diameter) sized syringe tips. Computer-aided design (SolidWorks) was used to design a model of the dumbbell shaped tensile specimens. EnvisionTEC software was then used to transform the digital model into a series of commands to dictate the print path. The print path was sliced into 3 layers with a 90⁰ shift in extrusion direction for each subsequent layer printed. Once printed the ICE gel dumbbell shapes were immediately sealed inside a Petri dish and stored for 5 days under controlled ambient conditions (21 °C, 45% relative humidity) to allow the EA polymer network to fully cure.

3.2.5 Swelling ratio

The swelling behaviour of the printed hydrogels was measured once the EA network had fully cured. The swelling ratio was calculated using equation (2:1). The samples were dried by being placed in an oven at 60 °C for 72 hr.

3.2.6 Mechanical testing

The 3D-printed gels were mechanically characterized in tension. Uniaxial tensile testing was conducted on a universal mechanical tester (Shimadzu, EZ-S) equipped with a 10 N load cell and spring loaded clamps. The tensile specimens were extended with a crosshead speed of 5 mm/min.
3.3 Results and discussion

3.3.1 Rheology

Rheological testing was used to establish the optimal conditions for extrusion printing the \( \kappa \)-CG/EA ICE gel precursor solution (i.e. the ink). A gel transition point test was applied to determine at what temperature the ink and its constituent \( \kappa \)-CG and EA undergo a thermal transition from solution to gel. The thermal gelation transition of the cooling \( \kappa \)-CG solution was observed as a rapid increase in complex viscosity as the solution was cooled below 50.7 ± 0.2 °C (data not shown). No thermal gelation transition was observed for the EA solution, its complex viscosity of the remained less than 1 Pa.s when cooled to 20 °C (data not shown). The thermal gel transition of the cooling ink was observed as a significant change in the gradient of the complex viscosity between 45.0 ± 0.2 °C and 58.8 ± 0.2 °C in the gel transition point test (Figure 1a). The gel transition temperature of the ink (50.4 ± 0.3 °C) was calculated as the midpoint of this transition. This transition temperature was selected as the optimal temperature for printing the ink as its moderate complex viscosity is compatible with the extrusion process. In addition, the ink will rapidly solidify when deposited on a cool (21 °C) substrate to fix the patterned design.

A time sweep test was then performed upon the ink to establish the period of time during which the rheological characteristics were not significantly altered due to EA network formation. An increase in storage modulus was observed in the first 0.5 hr of the time sweep test (Figure 3:1b). It is suggested that this is caused by the thermal
gelation of κ-CG solution (as a result of cooling from 70 °C to 50.4 °C). The storage modulus remained relatively constant for a period of time between 0.5 and 2.5 hr prior to a rapid increase due to formation of the EA network. Hence, the optimum window for printing the inks at 50°C is between 0.5 and 2.5 hr after preparation.

3.3.2 Extrusion printing

A 3D-Bioplotter was used to print a series of κ-CG/EA ICE gel dumbbell shapes (Figure 3:2a-b) to demonstrate that the gel precursor solutions could be processed through a 3D printer. The gel precursor solutions were extruded hot (50.4 °C) from the syringe barrel and solidified on the cooled (21 ± 1 °C) build stage. Once printed the ICE gel dumbbell shapes were immediately sealed inside a Petri dish and stored for 5 days under controlled ambient conditions to allow the EA polymer network to fully cure.

Figure 3:2 a. Photograph of extrusion printing a κ-CG/EA ICE gel dumbbell shape for tensile testing. b. Photograph of typical 3D-printed κ-CG/EA ICE gel dumbbell shapes. c. Series of photographs showing a printed κ-CG/EA ICE gel specimen during a tensile test. d. Typical stress-stretch curve of 3D printed κ-CG/EA gels. The stress-stretch curve of 3D printed Alg/PAAm ICE gel is shown for comparison (data reproduced from Section 2.3.2).
3.3.3 Swelling ratio and mechanical testing of printed hydrogels

The 3D-printed gels were mechanically characterized in tension (Figure 3:2c). Figure 3:2d is an example of a typical κ-CG/EA ICE gel stress-stretch curve. The printed hydrogels exhibited an elastic modulus of 320 ± 40 kPa, a tensile strength of 600 ± 70 kPa and failure strain of 350 ± 60 %. The work of extension (1.4 ± 0.3 MJ/m³) was calculated as the area under the curve and the swelling ratio (Q = 3.5) was calculated as the ratio of the total weight of water in the fully swollen gel to the solid dry weight of the polymer network.

The mechanical performance of the as-printed κ-CG/EA ICE gels was compared against the mechanical performances of cast Alg/PAAm ICE gels¹⁰³ and 3D printed Alg/PAAm ICE gels fabricated with in situ photopolymerisation (Chapter 2)¹⁸⁷ to assess the effectiveness of this printing method for processing mechanically robust hydrogels into useful structures (Table 3:1). The cast Alg/PAAm ICE gels exhibit a larger failure strain (~ 2300 %), work of extension (2.6 MJ/m³) and swelling ratio (6.1) compared to the printed κ-CG/EA ICE gels. However, the cast Alg/PAAm gels display a lower elastic modulus (29 kPa) and lower tensile strength (156 kPa) than the κ-CG/EA gels.¹⁰³ The κ-CG/EA gels and the printed Alg/PAAm ICE gels (300 ± 20 %)¹⁸⁷ exhibit a similar failure strains but the κ-CG/EA gels are significantly stronger than the Alg/PAAm gels (170 ± 30 kPa).¹⁸⁷ The work of extension for the κ-CG/EA ICE gels is 5.4 times greater than the work of extension of the printed Alg/PAAm gels (260 ± 10 kJ/m³) although one must note that the printed Alg/PAAm gels have a greater swelling ratio (5.7) than the κ-CG/EA gels.¹⁸⁷

Figure 3:3 demonstrates how the characteristics of the printed κ-CG/EA ICE gels compare with other tough hydrogels as well as conventional hydrogels. It is clear that ICE gels have work extension values that are superior to those exhibited by conventional gels. The modulus of the printed ICE gels is similar to the values reported for double-network (DN) gels, and higher than nano-composite (NC) gels. The ICE gels have work of extension values that are lower than most DN gels, but similar to those exhibited by NC hydrogels.
Table 3:1. Summary of the mechanical properties of printed κ-CG/EA ICE gels, printed Alg/PAAm ICE gels (data reproduced from ref. [187]) and cast Alg/PAAm ICE gels (data reproduced from ref. [103]).

<table>
<thead>
<tr>
<th></th>
<th>E [kPa]</th>
<th>$\sigma_T$ [kPa]</th>
<th>$\varepsilon_f$ [%]</th>
<th>$U$ [MJ/m$^3$]</th>
<th>$Q$</th>
</tr>
</thead>
<tbody>
<tr>
<td>κ-CG/EA ICE gels</td>
<td>320 ± 40</td>
<td>600 ± 70</td>
<td>350 ± 60</td>
<td>1.4 ± 0.3</td>
<td>3.5</td>
</tr>
<tr>
<td>Alg/PAAm ICE gels</td>
<td>29</td>
<td>156</td>
<td>2300</td>
<td>~ 2.5</td>
<td>7.1</td>
</tr>
<tr>
<td>(cast)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alg/PAAm ICE gels</td>
<td>66 ± 5</td>
<td>170 ± 30</td>
<td>300 ± 20</td>
<td>0.26 ± 0.01</td>
<td>6.7</td>
</tr>
<tr>
<td>(printed)</td>
<td></td>
<td></td>
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</table>

$^d$ E is the elastic modulus, $\sigma_T$ is the tensile strength, $\varepsilon_f$ is the failure strain, $U$ is the work of extension and $Q$ is the swelling ratio.

![Diagram showing comparison of work of extension for different types of hydrogels.](image)

Figure 3:3. Comparison of the work of extension for printed ICE hydrogels (black circle – this work, open circles – previous work), cast ICE hydrogels (white circle), single network hydrogels (including hydrophilic homopolymers – white diamonds and copolymers using hydrophobic co-monomer – shaded diamonds), double network hydrogels (dashes), nanocomposite hydrogels (triangles) and slip-link hydrogels (squares), adapted from ref. [2].

3.4 Summary

In conclusion, we have reported on a new 3D printing method that was designed to process tough ICE hydrogels into complex structures using thermal gelation. Rheology was used to observe the thermal gel transition of a gel ink synthesized with the ‘one-pot’ synthesis method. It was demonstrated that the biopolymer forms a polymer
network through a thermal gelation transition upon cooling whilst the synthetic polymer network forms over time through an EA addition chemistry reaction. This difference in behaviour was utilized to optimize the printing conditions. It was shown that the gel constituents exhibit different gelation behaviour upon cooling. The heated ink was extrusion printed onto a cooler substrate that instigated rapid solidification of the gel precursor solution fixing the shape of the patterned ink. The printed hydrogel exhibited an impressive mechanical performance with a measured work of extension in the same order of magnitude as the toughest gels produced to date. Additive Manufacturing is rapidly becoming the approach of choice in building soft materials with hydrogels, in particular in the area of biofabrication. We believe that advances in tough hydrogel materials and devices can be achieved by combining the right chemistry with the appropriate 3D printing technology. This synergistic approach would enable future applications of these materials (and devices) in diverse fields such as tissue engineering and soft robotics.
4 3D PRINTING OF FIBRE REINFORCED HYDROGEL COMPOSITES

This chapter has been adapted from the article “Three-Dimensional Printing Fiber Reinforced Hydrogel Composites” published in ACS Applied Materials & Interfaces, 2014, 6, 18, 15998-16006.

Author Statement:

The research in this chapter was performed with the assistance of Robert Gorkin III, who offered technical assistance with the operation of the 3D printer and with helped with the design of the artificial meniscus. All experiments and data analysis was carried out by the author, Shannon E. Bakarich. This would not have been possible without the intellectual support and guidance of Marc in het Panhuis, and Geoffrey M. Spinks.

The introduction section in this chapter is a shortened version compared with the published article. The introduction has been modified to avoid duplication with the previous Chapters.

I, Prof. Geoffrey M. Spinks (Supervisor), support and certify the above author statement.

______________________________
Geoffrey M. Spinks
4.1 Introduction

An advantage of 3D printing over other additive fabrication technologies is that multiple materials can be incorporated into a single build. This advantage is particularly useful for the fabrication of composite materials and complex structures that require spatially varying mechanical properties. Bioengineering is a primary example of an area where the fabrication of composites is required to mimic realistic tissues like skin, or even organs. Many soft tissues can be thought of as fibre reinforced hydrogel composites.\(^{[112]}\) For example articular cartilage is a proteoglycan gel matrix reinforced with collagen fibres. The present study investigated the preparation of fibre-reinforced hydrogels where both fibre reinforcement and matrix were 3D printed in a single-step process. Based on previous work demonstrating extrusion printing coupled with ultra-violet (UV) radiation induced gelation of a hybrid ICE gel\(^{[187]}\), the method was further developed to incorporate UV cured rigid epoxy reinforcement. A series of uniaxially oriented, continuous fibre reinforced hydrogels was prepared and the mechanical properties and swelling behaviour evaluated. The effect of fibre volume fraction on the elastic modulus was analysed using standard composites theory. In addition, a prototype articular meniscus cartilage was printed that mimicked both the complex shape and internal fibre-reinforced structure of the natural system.

4.2 Experimental

4.2.1 Materials

All materials were used as received and all solutions were prepared using Milli-Q water (resistivity = 18.2 Ω cm). α-Keto glutaric acid photo-initiator was purchased from Fluka (Australia). Acrylamide solution, 40%, for electrophoresis, sterile-filtered, alginic acid sodium salt, from brown algae with Brookfield viscosity (2% in H\(_2\)O at 25 °C) of 250 mPa s, N,N′-methylenebisacrylamide, calcium chloride (minimum 93.0% granular anhydrous) cross-linkers and ethylene glycol rheology modifier were purchased from Sigma Aldrich (Australia). A commercial epoxy based UV-curable adhesive (Emax 904 Gel-SC) was purchased from Ellsworth Adhesives (Australia).

4.2.2 Hydrogel ink formulations

The Alg/PAAm ICE hydrogel ink was prepared using a method described in Section 2.2.2. The ink was prepared by dissolving 4.50 ml of acrylamide, 38.7 mg of N,N′-
methylenebisacrylamide and 36.9 mg of α-keto glutaric acid in 5.25 ml of Milli-Q water, 1.5 ml of the 0.1 M calcium chloride stock solution and 3.75 ml of ethylene glycol. The latter was used as a non-volatile co-solvent with the ratio of ethylene glycol to water fixed at 1:2.5. A spatula was then used to dissolve 450 mg of alginic sodium salt into the printing solution.

An ink for printing a dissolvable support structures was prepared by mixing 1.5 ml of the 0.1 M calcium chloride stock solution and 3.75 ml of ethylene glycol in 9.75 ml of Milli-Q water. A spatula was then used to dissolve 450 mg of alginic sodium salt into the printing solution.

4.2.3 Rheology

The flow behaviour of each of the inks was determined following the protocols described above in chapter 2.2.3. All experimental studies were repeated in quadruplicate and the error was determined using standard deviation.

4.2.4 Contact angle

The contact angle of the extrusion inks on the printing substrate was determined using a Dataphysics Contact Angle System OCA. A square of polypropylene sheet was attached to the sample stage and a drop of the ink was placed onto the substrate. SCA20 software was used to calculate the angle between the droplet and the substrate at 1 min intervals for 5 min.

4.2.5 Extrusion printing

A 3D-Bioplotter system (EnvisionTEC, Germany) was fashioned with a commercial UV-curing system to print hydrogel composite structures from the Alg/AAm ink, the Emax 904 Gel-SC (Emax) adhesive and the alginate support gel. The Envisontec 3D bio-plotter was selected to print the κ-CG/EA ICE gel precursor solutions rather than the KIMM SPS1000 Bioplotter used in Chapter 2 because its multiple print heads allows for each of the inks to be incorporated into a single print job. A Dymax BlueWave 75 Rev 2.0 UV Light Curing Spot Lamp System using a 19+ W/cm² UV source with a 1 meter light guide was used to cure the printed ink. The end of the light guide was fixed to a custom made UV safety shield that covered the Bioplotter’s build
area. A slot in the UV safety box allowed the UV spot to be positioned directly over the patterned ink.

Computer-aided design (SolidWorks) was used to construct digital models of the tensile specimens and artificial meniscus structures that were to be printed. The composite materials were designed by creating an assembly from separate parts as illustrated in Figure 4:1 for the tensile specimens. EnvisionTEC software was used to slice the digital model into a stack of two dimensional layers (250 μm thick) to determine the print path. Material files and internal structures were then added to each part within the assembly. Tensile samples were designed with systematically varying volume fraction of reinforcing Emax fibres. The fibre diameter and length were kept constant and the number of fibres varied to achieve the desired volume fraction.

Figure 4:1. A typical example of a CAD assembly model for a 62.5% Emax hydrogel composite tensile specimen. Numbers 1 and 2 represent the Emax and ICE gel constituents, respectively, and number 3 represents a printed support structure (alginate gel) that is removed after printing.

The Alg/AAm ICE gel ink, the Emax and the alginate support gel ink were loaded into the 3D-Bioplotter in separate pressurised syringe barrels in the temperature controlled print heads. 23 gauge syringe tips (diameter 0.337 mm) were fitted to the ends of each of the barrels. The barrel temperature was held stable during printing at 25 °C. The hydrogel ink formulation was printed at 0.7 bar and with head speed of 5 mm/s. The Emax was printed at of 0.9 bar and with head speed of 5 mm/s. The alginate support gel ink was printed at of 0.6 bar and with head speed of 5 mm/s. The UV light was passed over each layer of ink for 50 s and the completed structure for an additional 200 s. A paper wipe was used to carefully remove the soft alginate support gel from the completed specimen.
The composite structures were printed onto squares cut out of polypropylene sheets attached to the build stage. The polypropylene sheets acted as release layers allowing easy separation of the printed material from the build stage. Hydrogel composites were printed with nominal Emax volume fraction of 0, 25, 37.5, 50, 62.5, 75, 87.5 and 100%. All tensile specimens were printed into dumbbell shapes with Emax end grips for ease of clamping. Printed materials were characterised immediately after the completion of the curing process.

4.2.6 Imaging

The print quality of the hydrogel composites was examined with a Leica M205 A microscope. A single drop of red food dye was added to the hydrogel ink before printing to identify the hydrogel phase. Cross-sectional images were obtained from strips of the hydrogel composites cut into 1 mm slices with a razor blade. Leica Application Suite V4 software was then used to measure the cross sectional area of the printed hydrogel composites. The true Emax volume fraction of the printed composites was calculated as the ratio between the cross sectional area of Emax and the total cross sectional area. Four repeat tests were performed on each type of composite sample with errors estimated from one standard deviation.

4.2.7 Swelling ratio

The swelling ratio was determined after immersing printed hydrogel composite samples in 0.1M calcium chloride (72 hr) to fully cross-link the alginate followed by immersion in Milli-Q water (72 hr). A single drop of red food dye was added to the Milli-Q water to aid in imaging the swollen hydrogels. The swelling ratio was calculated using equation (2:1). The samples were dried by being placed in an oven at 60 °C for 72 hr.

4.2.8 Mechanical testing

The mechanical properties of the printed composites were determined using a Shimadzu EZ-L Universal Mechanical Tester. Tensile tests were performed using a 50 N load cell for low modulus/strength samples, and a 500 N load cell for high modulus/strength samples. Printed tensile specimens were strained to failure at a rate of 10 mm/min. The applied stress was calculated using the average cross sectional area of the unstrained specimen at the gauge length. The failure stress and failure strain
were calculated from the onset of failure. The Work of Extension was calculated as the area under the stress-strain curve to failure. Four repeat tests were performed on each type of composite sample with errors estimated from one standard deviation. An adhesion test was performed to determine the strength of the bond between the ICE gel matrix and Emax fibres in the printed composites. A tensile specimen was printed with Emax ends and a band of ICE gel. The tensile specimen was extended in the direction perpendicular to ICE gel band until failure. The position of failure was noted in each of these tests.

4.3 Results and discussion

The aim of the present study was to evaluate the effectiveness of fibre-reinforcement of 3D printed hydrogel structures where both the fibre reinforcement and the matrix were printed in a single process. Initially Emax 904 Gel-SC adhesive was identified as a material with suitable UV cure characteristics, appropriate rheological properties for extrusion printing, adhesion to the Alg/AAm ICE gel and acceptable mechanical properties for fibre reinforcement. Utilising a multi-barrelled extrusion printer, dumbbell shaped tensile specimens were printed and cured with UV radiation to demonstrate composite printability and to measure mechanical properties.

4.3.1 Extrusion printing fibre reinforced hydrogels

The viscosity ‘\( \eta \)’ of the Emax and alginate support gel inks was measured to determine the optimal conditions for extrusion printing. The observed viscosity data (Figure 4:2a) were fitted to the power law model described by equation (2:2). Both the Emax and alginate support gel inks display shear-thinning flow behaviour (\( n < 1 \)) similar to the Alg/AAm ICE gel ink characterised in Section 2.3.1. In that previous rheological study it was determined that our extrusion printing system in conjunction with a 23 gauge syringe tip could process inks with consistency index values between 20 and 150 Pa s\(^n\) and power law index values between 0.35 and 0.45.\(^{14}\) The alginate ICE gel ink has a consistency index 80 ± 10 Pa s\(^n\) and power law index 0.37 ± 0.01 (Table 1). The new inks were also found to have flow characteristics compatible for extrusion printing with consistency index and power law index values of 41 ± 1 Pa s\(^n\) and 0.44 ± 0.01, respectively, for Emax and 50 ± 10 Pa s\(^n\) and 0.38 ± 0.02, respectively, for the alginate support ink.

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The contact angle between the polypropylene sheet and each of the extrusion inks was measured over a 5 minute period (Figure 4:2b) to determine their wetting behaviour. The contact angle indicates whether the ink will spread on the substrate or bead up in the time period between printing and curing of the ink. Wetting is an important printing parameter because the accuracy of a printer is limited by the extent of which an ink will spread once deposited. After 5 minutes the Emax had a lower contact angle (63 ± 3°) and hence larger degree of wetting than the Alg/AAm ICE gel (88 ± 6°) and the alginate support gel (90 ± 3°).

![Figure 4:2](image_url)

Figure 4:2. a. Experimental viscosity data (symbols) and flow curves fitted to equation (2:2) for Emax (open squares), alginate support gel (closed diamonds) and Alg/AAm ICE gel (open diamonds). b. Contact angle measurements of Emax (open squares), alginate support gel (closed diamonds) and Alg/AAm ICE gel (open diamonds) on the polypropylene sheet measured as a function of time.

<table>
<thead>
<tr>
<th>Material</th>
<th>K [Pa s^n]</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emax</td>
<td>41 ± 1</td>
<td>0.44 ± 0.01</td>
</tr>
<tr>
<td>Alginate support gel</td>
<td>50 ± 10</td>
<td>0.38 ± 0.02</td>
</tr>
<tr>
<td>Alginate ICE gel</td>
<td>80 ± 10</td>
<td>0.37 ± 0.01</td>
</tr>
</tbody>
</table>

Consistency (K) and power law (n) indices were obtained through curve fitting with equation (2:2).

Fibre reinforced hydrogel composites were fabricated with a commercially available 3D printer and cured with a UV light source. Initially the composite structures were printed with the Emax and Alg/AAm ICE gel inks. The patterned ink was cured after the entire structure had been printed. In Figure 4:3 cross sections of the printed...
composites were compared with cross sections of their CAD model (Figure 4:3a & d) to assess the accuracy of this printing method. Two types of defects were noted. Firstly, examination of the exposed Emax edges revealed that the Emax ink was spreading once patterned, this can be attributed to its low contact angle. This surface wetting produced a tapered edge rather than the desired square shape (Figure 4:3b). A more defined vertical edge (Figure 4:3c) was achieved by using a removable alginate support ink at the edges to prevent spreading of Emax. The support ink was not UV-curable and was easily removed after printing. Secondly, inspection of the fibre structure of the composite hydrogels (Figure 4:3e) revealed that the fibres were collapsing prior to curing when UV exposure was performed after the printing of several layers. A better defined fibre structure (Figure 4:3f) that was closer to the CAD model was achieved by partially curing each layer of ink for 50 s during the fabrication process. After analysing these results all further fibre reinforced hydrogel composite structures were printed with an alginate support ink mould against all exposed Emax edges and each layer of ink was partially cured prior to printing the following layer.

Figure 4:3. a. The cross section of a CAD model of a 100% Emax sample compared to microscope images of the edge of 100% Emax samples printed b. with no support gel mould and c. with a support gel mould. d. The cross section of a CAD model of a 50% Emax sample compared to microscope images of 50% Emax samples e. cured after printing 4 layers of ink and f. cured after printing each layer of ink. In the CAD models component 1 defines Emax and component 2 defines ICE gel. For the microscope images red food dye was used to highlight the ICE gel.

Cross sections of the fiber reinforced hydrogels were also used to calculate the true Emax volume fraction of the printed composites. The true volume fraction was calculated as the ratio between the cross sectional area of Emax and the total cross
sectional area of the printed composite. Figure 4.4 is a plot of the true Emax volume fraction against the Emax volume fractions of the CAD models. The plot shows that the true Emax volume fraction is greater than the intended Emax for all of the printed hydrogel composites. This result indicates the Emax was deposited through the printer at a higher rate than the ICE gel. In the following the true Emax volume fraction is used to identify each of the fiber reinforced hydrogel composites.

![Figure 4.4](image)

Figure 4.4 The true Emax volume fraction calculated from the cross sections microscope images as a function of the volume fraction from the CAD models.

### 4.3.2 Swelling ratio and mechanical testing of fully swollen hydrogel composites

Hydrogel mechanical properties are strongly dependent upon swelling ratio which is in turn influenced by the environment. The swelling ratio and mechanical properties of the printed hydrogel composites were characterised when fully swollen because hydrogel materials are most commonly implemented in aqueous environments. Fully swollen hydrogel composites were prepared by immersing the as-printed composites in 0.1M calcium chloride for 72 hrs to crosslink the alginate polymer network and to enhance their mechanical properties. The hydrogels were then allowed to equilibrate in water for a further 72 hrs. Based on the mass loss during heating at 60°C for 72 h to fully dry the hydrogel the swelling ratios were determined using equation (2:1). The ICE gel exhibited a swelling ratio of 3.5 ± 0.3 % and the Emax exhibited a swelling ratio of 0.19 ± 0.03 %. As expected, the swelling ratios of hydrogel composites decreased as the Emax volume fraction was increased. The plot in Figure
4:5 demonstrates that the swelling ratios of the composites ‘\( Q_c \)’ closely approximates the simple rule of mixtures for swelling ratio which was calculated using:

\[
Q_c = Q_f V_f + Q_m V_m \tag{4:1}
\]

where ‘\( Q_f \)’ and ‘\( Q_m \)’ are the swelling ratio of the fibre (Emax) and the matrix (ICE gel) respectively and ‘\( V_f \)’ and ‘\( V_m \)’ are the fibre and matrix volume fractions, respectively. The rule of mixtures is a weighted mean which assumes that each component’s contribution to the total swelling ratio is additive.

Figure 4:5. The swelling ratio of swollen hydrogel composites measured as a function of Emax volume fraction. The broken line indicates the rule of mixtures (equation (4:1)).

Tensile tests on the swollen hydrogel composites in the fibre direction were performed to determine the effect of the Emax volume fraction on the composites’ mechanical properties. Figure 4:6a - d includes photographs of the hydrogel composite tensile specimens when clamped in the tensile tester. Examples of typical stress-strain curves of the swollen fibre reinforced hydrogels are shown in Figure 4:6g and a summary of the mechanical data is included in

Table 4:2. The ICE gel exhibited an elastic modulus of 260 ± 20 kPa and the Emax had an elastic modulus of 2700 ± 200 kPa. The ICE gel is soft and wet whilst the Emax is hard and dry. The tensile strength of the 100% ICE gel was 80 ± 10 kPa with a failure strain of 48 ± 4% compared with a tensile strength of 2400 ± 100 kPa and a failure strain of 230 ± 10% for the 100% Emax. The printed composite structures display a combination of properties in between these two extremes with an increase in
elastic modulus, failure strength, failure strain and work of extension corresponding with an increase in Emax volume fraction and decrease in swelling ratio. By varying the Emax volume fraction of the CAD models the mechanical properties of the printed composites can be varied over several orders of magnitude.

To determine the efficiency of printed Emax fibres for reinforcing printed ICE gel, the experimental mechanical data was compared with standard composite theory. Maximum fibre reinforcement requires a perfect bond between matrix and fibre reinforcement so that the matrix and the fibre reinforcement achieve equal strains. An adhesion test was performed upon an ICE gel / Emax tensile specimen (Figure 4:6e) to determine the strength of the bond between the ICE gel matrix and Emax fibres in the printed composites. Figure 4:6f shows that mechanical failure occurred within the hydrogel band of the tensile specimen and not at the ICE gel / Emax interface indicating that the bond between the ICE gel and Emax is stronger than ICE gel. Due to the strength of this bond it can be assumed that there is little slipping between the matrix and fibre reinforcement so that equal strain is observed by the matrix and the fibre reinforcement when under tension.

When the strain between the matrix and fibre reinforcement is equal the fibre reinforcement experiences a greater stress than the matrix because the fibre reinforcement has a larger elastic modulus than the matrix. Based on this condition, the upper bound elastic modulus ‘\(E_{\parallel}\)’ of a perfectly reinforced composite was calculated using the rule of mixtures:\[118\]

\[
E_{\parallel} = E_f V_f + E_m V_m ,
\]

(4:2)

Where ‘\(E_f\)’ and ‘\(E_m\)’ are the elastic modulus of the fibre and the matrix, respectively. The rule of mixtures assumes that both the matrix and fibres behave linear elastically.

Ineffective fibre reinforcement occurs when stress from the matrix is not transferred to the fibre reinforcement; which could be caused by slipping between the matrix and fibre reinforcement, fibre end effects or off-axis alignment of the reinforcing fibres. Minimum fibre reinforcement occurs when a tensile load is applied perpendicular to the fibre direction. In this case the load is shared uniformly across the composite so that the stress in the fibres is equal to the stress in the matrix. Based on this condition,
the lower bound elastic modulus ‘\( E_\perp \)’ of an ineffectively reinforced composite was calculated using the transverse rule of mixtures:[188]

\[
E_\perp = \frac{E_f E_m}{E_f V_m + E_m V_f}
\]  

(4:3)

The measured elastic modulus of the swollen hydrogel composites was compared with the rule of mixtures in Figure 4:6h. This plot demonstrates that printed Emax fibres provide effective reinforcement to the printed ICE gel and adhere to the upper bound rule of mixtures as the experimental values for elastic modulus very closely match the theoretical values calculated from equation (4:2).

Figure 4:6. Photographs of 3D printed hydrogel composite tensile specimens with (a) 0, (b) 28, (c) 64 and (d) 100% Emax volume fractions clamped for tensile testing. Photographs of ICE gel / Emax adhesion test sample (e) before and (f) after mechanical failure. For the photographs red food dye was used to highlight the ICE gel. g. Typical stress strain curves for swollen hydrogel composites with 0, 28, 64, 87 and 100% Emax volume fractions. h. Mechanical properties of fully swollen hydrogel composites as a function of Emax volume fraction elastic modulus including the theoretical upper (equation (4:2)) and lower (equation (4:3)) bounds for composite elastic modulus.
Table 4.2 Summary of the mechanical properties of swollen hydrogel composites.

<table>
<thead>
<tr>
<th>$E_{\text{Emax}}$</th>
<th>E</th>
<th>$E_{\parallel}$</th>
<th>$E_{\perp}$</th>
<th>$\sigma_T$</th>
<th>$\varepsilon_f$</th>
<th>U</th>
</tr>
</thead>
<tbody>
<tr>
<td>[%]</td>
<td>[MPa]</td>
<td>[MPa]</td>
<td>[MPa]</td>
<td>[MPa]</td>
<td>[%]</td>
<td>[kJ/m$^3$]</td>
</tr>
<tr>
<td>0</td>
<td>0.26±0.02</td>
<td>0.26</td>
<td>0.26</td>
<td>0.08±0.01</td>
<td>48±4</td>
<td>22±6</td>
</tr>
<tr>
<td>28±2</td>
<td>0.77±0.07</td>
<td>0.86</td>
<td>0.34</td>
<td>0.31±0.04</td>
<td>74±6</td>
<td>140±20</td>
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<tr>
<td>43±5</td>
<td>1.4±0.2</td>
<td>1.2</td>
<td>0.40</td>
<td>0.51±0.05</td>
<td>81±8</td>
<td>243±50</td>
</tr>
<tr>
<td>64±3</td>
<td>1.7±0.2</td>
<td>1.5</td>
<td>0.48</td>
<td>0.65±0.01</td>
<td>89±8</td>
<td>320±30</td>
</tr>
<tr>
<td>68±2</td>
<td>1.9±0.2</td>
<td>1.8</td>
<td>0.61</td>
<td>1.0±0.10</td>
<td>110±6</td>
<td>580±70</td>
</tr>
<tr>
<td>87±3</td>
<td>2.2±0.1</td>
<td>2.1</td>
<td>0.81</td>
<td>1.30±0.03</td>
<td>140±3</td>
<td>1000±50</td>
</tr>
<tr>
<td>91±2</td>
<td>2.3±0.1</td>
<td>2.4</td>
<td>1.3</td>
<td>1.66±0.04</td>
<td>170±10</td>
<td>1500±30</td>
</tr>
<tr>
<td>100</td>
<td>2.7±0.2</td>
<td>2.7</td>
<td>2.7</td>
<td>2.40±0.10</td>
<td>230±10</td>
<td>2800±200</td>
</tr>
</tbody>
</table>

$E_{\text{Emax}}$ is the Emax Volume Fraction, E is the elastic modulus, $\sigma_T$ is the tensile strength, $\varepsilon_f$ is the failure strain and U is the work of extension. Upper limit ($E_{\parallel}$) and lower limit ($E_{\perp}$) indicate elastic modulus calculations using equations (4.2) and (4.3), respectively.

4.3.3 Swelling ratio and mechanical testing of as-printed hydrogel composites

As 3D-Bioprinting has been touted for its ability to produce parts “as needed,” it is important to understand the mechanical properties of the as-printed hydrogel composites to assess potential applications for immediate use. The as-printed ICE gel exhibited a swelling ratio of 2.6±0.3 and the Emax had a swelling ratio of 0.05±0.01. These two swelling ratios are less than the swelling ratios of the swollen materials which indicates that both the Emax and the ICE gel absorb water when exposed to an aqueous environment. Figure 4.7 demonstrates that the as-printed composite materials adhere to the law of mixtures for swelling (equation (4.1)) as expected.
Figure 4:7. The swelling ratio of as-printed hydrogel composites measured as a function of Emax volume fraction. The dotted line indicates the rule of mixtures (Equation (4:1)).

The mechanical characteristics of the as-printed materials differ drastically to the mechanical characteristics of the swollen composites. Examples of typical stress-strain curves for the as-printed reinforced hydrogels are shown in Figure 4:8a and a summary of the mechanical data is included in Table 4:3. The elastic modulus of the as-printed ICE gel ($70 \pm 4$ kPa) is significantly lower than the elastic modulus of the swollen hydrogel because the as-printed alginate network is not cross-linked until it is treated with calcium chloride. The Emax elastic modulus ($76 \pm 9$ MPa) is significantly larger than the elastic modulus of the swollen Emax. The vast difference between the elastic modulus of Emax when printed and when swollen indicates that the absorption of small volumes of water by Emax has a considerable plasticizing effect the mechanical properties of Emax.
The as-printed composite materials display similar behavior to the swollen composites with increasing elastic modulus, failure strength, failure strain and work of extension corresponding to an increased Emax volume fraction. The experimental values of elastic modulus for the as-printed hydrogel composites were also compared to the rule of mixtures in Figure 4:8b. In this case the measured elastic moduli fell below the upper bound rule of mixtures values. This discrepancy between experimental and theoretical elastic modulus is likely a result of the Emax phase absorbing water from the adjacent ICE gel phase during the printing process. The theoretical values given in Figure 8b use the modulus of fully-dry Emax and may not represent the true modulus for as-printed Emax in the composite samples, since it is shown above that small amounts of water absorption significantly decreases the elastic modulus of Emax. To demonstrate the effect of moisture absorption by Emax from adjacent ICE gel, a 91% Emax hydrogel composite samples was printed and tested in tension after all of the ICE gel had been carefully removed by a scalpel. The Emax phase of this sample had an elastic modulus of 29 ± 2 MPa, which is appreciably smaller than the elastic modulus of the fully dry Emax material. These results indicate that water can be absorbed by the Emax from adjacent ICE gel, thereby lowering the reinforcing efficiency and overall modulus of the as-printed composites.

Figure 4:8. a. Typical stress strain curves for as-printed hydrogel composites with 0, 28, 64, 87 and 100% Emax volume fractions. b. The elastic modulus for as-printed hydrogel composites as a function of Emax volume fraction including the theoretical upper (equation (4:2)) and lower (equation (4:3)) bounds for composite elastic modulus.
Table 4.3. Summary of the mechanical properties of as-printed hydrogel composites.$^g$

| $V_{Emax}$ | E   | $E_{||}$ | $E_{\perp}$ | $\sigma_T$ | $\epsilon_f$ | U     |
|-----------|-----|---------|-----------|----------|------------|------|
| [%]       | [MPa] | [MPa]   | [MPa]     | [MPa]    | [%]        | [kJ/m$^3$] |
| 0         | 0.070 ± 0.004 | 0.070 | 0.070 | 0.046 ± 0.003 | 62 ± 7 | 13 ± 2 |
| 28 ± 2    | 0.64 ± 0.09  | 19    | 0.093 | 0.26 ± 0.01 | 64 ± 7 | 90 ± 20 |
| 43 ± 5    | 2.4 ± 0.3    | 29    | 0.11  | 0.71 ± 0.08 | 60 ± 7 | 240 ± 50 |
| 64 ± 3    | 4.4 ± 0.9    | 38    | 0.14  | 0.81 ± 0.04 | 90 ± 10 | 480 ± 90 |
| 68 ± 2    | 5.6 ± 0.7    | 48    | 0.19  | 1.2 ± 0.1  | 96 ± 6 | 720 ± 90 |
| 87 ± 3    | 14 ± 3       | 57    | 0.28  | 1.5 ± 0.1  | 110 ± 10 | 1200 ± 80 |
| 91 ± 2    | 53 ± 2       | 67    | 0.56  | 3.5 ± 0.2  | 170 ± 4 | 4200 ± 400 |
| 100       | 76 ± 9       | 76    | 76    | 5.5 ± 0.4  | 190 ± 20 | 6800 ± 400 |

$^g$ $V_{Emax}$ is the Emax Volume Fraction, E is the elastic modulus, $\sigma_T$ is the tensile strength, $\epsilon_f$ is the failure strain and U is the work of extension. Upper limit ($E_{||}$) and lower limit ($E_{\perp}$) indicate elastic modulus calculations using equations (4.2) and (4.3), respectively.

4.3.4 Fabrication of artificial meniscus

An artificial meniscus was 3D printed to demonstrate how this fibre reinforced hydrogel printing technique can be adapted to fabricate complex hydrogel structures for real world applications. The meniscus is a crescent shaped cartilage structure in the knee (Figure 4.9a) that acts to disperse the weight of the body and reduce friction within the knee joint. Meniscal injuries that do not heal and that cannot be repaired are treated by removing the damaged meniscus and replacing it with either an allograft or
a synthetic replacement. 3D-printing is a valuable tool for fabricating such a synthetic implantable structure because patient specific implants can be easily made by tailoring the digital model of the implant to match the size and shape of an individual patient’s knee. Fibre reinforced hydrogels are a logical choice of material for use in an artificial meniscus because the natural meniscus is also a fibre reinforced hydrogel. The meniscus contains a collagen fibre network that is made up of circumferential fibres and radial or ‘tie’ fibres (Figure 4:9b) which give the cartilage strength and stiffness in the desired directions.

To print a fibre reinforced hydrogel artificial meniscus CAD software was used to create a digital model of the meniscus. The simplified digital model of an artificial meniscus contained both circumferential fibres and radial fibres so as to mimic the structure of natural meniscus (Figure 4:9c). The concentration of Emax was spatially varied throughout the digital model so as to control the mechanical properties in different regions of the artificial meniscus. A low Emax concentration was used around the arc of the meniscus in order to replicate the low modulus of soft tissue. The ends of the meniscus were designed with a high Emax concentration to create a hard site that could be used to attach the artificial meniscus to hard bone. The artificial meniscus was fabricated by printing alternating layers of the circumferential fibres and the radial fibres (Figure 4:9d-e). A photograph of the completed 3D printed fibre reinforced hydrogel artificial meniscus is shown in Figure 4:9f. The printed structure was loaded in compression to 500 N (50 kg) without any observable damage.
4.4 Summary

3D printing in conjunction with digital modelling was used to fabricate fibre reinforced hydrogels in a single-step process. By altering the ICE gel and Emax volume fractions of the digital models a full spectrum of soft / wet through to hard / dry composite materials were prepared. When the mechanical characteristics of these printed composites were compared with standard composite theory the swollen fibre reinforced hydrogels were found to adhere to the rule of mixtures. An apparent discrepancy was observed, however, between the as-printed materials and the rule of mixtures estimation of elastic modulus. During the printing process the Emax fibres absorb water from the adjacent ICE gel matrix and absorption of water was shown to drastically reduce the elastic modulus of Emax. The discrepancy between the as-printed composites and the rule of mixtures can be accounted for by the softening of the Emax phase by the absorption of water from the adjacent hydrogel.
This chapter has demonstrated the capability of 3D printing of simple fibre composites based on hydrogel materials. An example application was demonstrated by printing an artificial meniscus cartilage which mimicked the complex 3D shape and incorporated spatially varying fibre reinforcements. Improvements in print resolution when matched to appropriate ink rheology and solidification methods offer the potential to print more complex composites structures, such as particulate reinforced, 3D reinforcement and cellular reinforcements, including honeycomb structures. The further development of these 3D modelling techniques is expected to be useful for the fabrication of multi-component hydrogel structures or devices with multiple applications in micro-fluidics (pumps and valves), robotics (artificial muscles) and bionics (tissue scaffolds and artificial organs). Ultimately, we hope to be able to produce 3D printed versions of soft tissues like tendons, cartilage, skin and muscle where spatial variation in composition and properties is a major contributor to function.
This chapter has been adapted from the article “3D printing of bio-inspired tough hydrogel composites with spatially varying materials properties: towards biofabrication of tendons” submitted to Additive Manufacturing.

Author Statement:

The research in this chapter was performed with the assistance of Robert Gorkin III and Sina Naficy designed and constructed the 3D printer and offered intellectual support in the writing of this manuscript or Reece Gately who wrote the software to operate the printer. All experiments and data analysis was carried out by the author, Shannon E. Bakarich. This would not have been possible without the intellectual input and support of Marc in het Panhuis and Geoffrey M. Spinks.

The introduction section in this chapter is a shortened version compared with the submitted article. The introduction has been modified to avoid duplication with the previous Chapters.

I, Prof. Geoffrey M. Spinks (Supervisor), support and certify the above author statement.
5.1 Introduction

A major focus of biofabrication is dedicated to creating material systems that replicate biological functions.\[^{192}\] However, many bio-inspired structures are made from single materials in limited configurations and do not adequately mimic the complex heterogeneous structure and properties of biological tissue.\[^{142}\] Overcoming this limitation is a challenge to identify both suitable material candidates and appropriate fabrication mechanisms amenable to create advanced structures. Tough hydrogels have typically been formed using a casting process to produce a homogeneous block of material\[^{107, 112, 120}\], yet natural tissue tends to show more complex and heterogeneous compositions.\[^{193}\] Tendons are a good example of a heterogeneous tissue with a gradual transition or gradient of modulus throughout the length. The graduated stiffness allows the tendon to form a seamless interface with soft muscle at one end and hard bone at the other.\[^{194}\] One approach to create a gradient of mechanical properties within a hydrogel structure is achieved by casting a stack of hydrogels in a step-by-step process so that each hydrogel layer has a different modulus.\[^{142, 145, 195}\] This method is cumbersome and produces a staggered transition rather than an optimal smooth and continuous gradient. The development of an additive manufacturing method for fabricating tough hydrogels with a continuous gradient of material properties will allow for the production of a new generation of bio-mimetic materials to be used in bio-inspired models.

Material blends have been printed using the extrusion printing technique and a process where inks from two separate syringe barrels are extruded through a novel tip that mixes the two inks together before patterning them onto a substrate.\[^{196}\] Coarse gradients in material properties have been produced using these printing methods by patterning different inks in incremental steps.\[^{196-198}\] However, no technique has yet been reported that can produce a smooth and continuous gradient in a hydrogel based material. In this Chapter, a tough hydrogel composite with spatially varying mechanical properties that was produced using a custom built extrusion-based 3D printing system. The resulting tough hydrogel materials are characterized using mechanical testing and modelled with composite theory. A prototype artificial tendon was fabricated and characterized to demonstrate the ability of our printing approach and hydrogel materials to mimic naturally occurring gradient structures.
5.2 Experimental

5.2.1 Materials

All materials were used as-received and all solutions were prepared using Milli-Q water (resistivity = 18.2 Ω cm). α-Keto glutaric acid photo-initiator was purchased from Fluka (Australia). Acrylamide solution (40%, for electrophoresis, sterile-filtered), alginic acid sodium salt (from brown algae with Brookfield viscosity 2% in H₂O at 25 °C of 250 mPa s), calcium chloride (minimum 93.0% granular anhydrous), ethylene glycol (rheology modifier) and N,N'-methylenebisacrylamide crosslinker were purchased from Sigma Aldrich (Australia). A commercial epoxy based UV-curable adhesive (Emax 904 Gel-SC) was purchased from Ellsworth Adhesives (Australia).

5.2.2 Hydrogel ink formulation

An Alg/AAm ICE gel ink was prepared using the method described in chapter 4.2.2. A single drop of red food dye was added to the ink to aid in imaging the printed hydrogel.

5.2.3 Extrusion printing

Ribbons of hydrogel composite materials were extrusion printed using a custom built 3D printer. A novel custom printer was constructed to print the hydrogel composites because the neither of the printers used earlier in this thesis are able to blend materials. The 3D printer was built around a Sherline 8020 CNC milling stage and a novel syringe controlled deposition system. The deposition system was made up of two Zaber T-LA60A-KT03 Miniature Linear Actuators mounted to the CNC gantry by a custom designed 3D printed rig. Designs were made in Solidworks and the system was 3D printed on an uPrint system (Stratasys, USA). The rig allowed for insertion of 30mL or 5mL standard syringe barrels preloaded with material. Plastic tubing and a Y-adaptor was used to direct the flow from the two syringes into a single stream. A disposable static chaotic mixer with 12 mixing elements (Nordson EFD) was added below the Y-adaptor to insure blending of the inks from the two syringes. A custom 3D-printed nozzle (fabricated with a Form1 3D printer, Formlabs) was attached to the bottom of the deposition system. This nozzle was designed to extrude a flat ribbon of material having a rectangular shaped outlet with dimensions 1.5 mm by 15 mm. An
Opsytec Dr. Gröble 365 nm UV-LED smart light source was also integrated near the deposition system so that its illuminated area followed the print head to cure dispensed ink. Figure 5:1 shows the custom built printer with Figure 5:1 b providing a close-up view of the deposition system.

Figure 5:1 a. Photograph of the custom built 3D printer showing the Zaber pumps (i), syringe barrels (ii.), UV light source (iii) and the printer head (iv). b. Photograph of the print head showing the position of the ‘Y’ adaptor (i.), the static chaotic mixer (ii.) and the custom nozzle (iii.).

To set up the print of composite ribbons, the Alg/AAm ICE gel ink was loaded into one of the two syringes and Emax was loaded into the other; each syringe was capped with a piston. The ribbon volume fraction and geometry was controlled using two programs running in parallel: custom software that allowed the two inks to be dispensed in different ratios, and an off-the-shelf CNC control program (LinuxCNC EMC2) controlling the movement of the print gantry. The custom program (developed in C#, using Visual Studio 12.0) worked by adjusting the speed of the two linear actuators; which in turn delivered mechanical pressure to the pistons in the syringes and extruded material out of the Y connector, through the mixer, and ultimately out of the ribbon nozzle. The net flow rate of combined material out of the nozzle had to remain constant to maintain consistency; thus the individual flow rates of the two
materials from each barrel were adjusted relative to each other throughout the print. For gradient printing the flow rate of the two materials had to be linearly ramped; the speed of depression in one barrel was decreased from 100% to 0% while simultaneously the other barrel experienced a speed increase from 0% to 100%. The moving stage was controlled independently from the pumping actuation, and as such the flow rate and print speed were then optimized to extrude material at the appropriate speed to maintain a consistently thick layer. If the speed was too fast or too slow the print would clump or pull and show a varying thickness through the print. The optimal combined total rate of extension of the two linear actuators was set at a constant 180 µm/s when the milling stage was moved with a speed of 80 mm/min. The UV light using 100 % intensity followed the extrusion nozzle to set the blended composite structure by partially curing the two inks.

5.2.4 Post printing processing

A processing scheme was applied to the composite ribbons after printing to improve their mechanical properties. A Dymax BlueWave 75 Rev 2.0 UV Light Curing Spot Lamp System using a 19+ W/cm² UV source with a 1 meter light guide was used to complete the curing process. Each 30 mm length of ribbon was exposed to the spot of UV irradiation for 30 s. The composites were then immersed in 0.1 M aqueous calcium chloride solution for 72 hr to fully crosslink the alginate polymer network and were then immersed in Milli-Q water for a further 72 hr to reach their equilibrium swelling ratio.

5.2.5 Swelling ratio

The swelling ratio of the printed composite samples was determined after the immersion in 0.1M calcium chloride and fully equilibrating in Milli-Q water. The swelling ratio was calculated using equation (2:1). The samples were dried by being placed in an oven at 60 °C for 72 hr.

5.2.6 Imaging

Image analysis software was used to calculate the volume fraction of the printed composite materials. Ribbons of the printed composites were imaged with a Leica 216
Macroscope. The images were loaded into MATLAB R2011a to measure the color intensity of the blue channel in images of composite printed with different ratios of the red Alg/AAm ICE gel ink to the blue Emax. The Emax volume fraction of the printed composites was determined by comparing the intensity of the blue channel in images of the composite materials to the intensity of the blue channel in images of 0 % Emax samples (blue intensity normalized to 0) and 100 % Emax samples (blue intensity normalized to 100).

5.2.7 Mechanical testing

The mechanical properties of the fully swollen printed composites were determined using a Shimadzu EZ-L Universal Mechanical Tester. Tensile specimens were prepared by cutting swollen printed composite ribbons into 30 mm lengths. Strips of poly(propylene) sheet were glued onto both ends of the composite tensile specimens to act as grips. The tensile specimens were attached to the mechanical tester with screw loaded clamps. Tensile tests were performed using a 500 N load cell and the tensile specimens were strained to failure at a rate of 10 mm/min. The applied stress was calculated using the average cross sectional area of the unstrained specimen. The failure stress and failure strain were calculated from the onset of failure. The work of extension was calculated as the area under the stress-strain curve to failure. Four repeat tests were performed on each type of composite sample with errors estimated from one standard deviation.

5.3 Results and discussion

The aim of the present study was to develop and evaluate a custom built 3D printer equipped with a novel extrusion based deposition system to print blends of hydrogels and other materials to form gradient structures and spatially varying materials properties. Epoxy Emax 904 Gel-SC and Alg/AAm ICE hydrogel inks were identified as suitable materials to perform these tests, based on recent work where these materials were used to print fiber reinforced composites.\textsuperscript{[200]} The new custom-built printer has been designed to form blends of the two extruded materials where the volume fraction of each phase could be controlled through newly developed software. The print quality has been examined using imaging techniques and the mechanical properties of the printed materials have been characterized and analyzed using standard composites theory.
5.3.1 Extrusion printing

The extrusion printer produced uniform rectangular shaped ribbons composed of a blend of the two inks. The width of the extruded rectangles was $14 \pm 1 \text{ mm}$ as set by the width of the extrusion head. Figure 5:2a shows examples of images of lengths of the extruded ribbons printed with varying ICE hydrogel: Emax ratios. The ratio of the materials was varied by keeping the total rate of extrusion constant, while varying the extrusion rate of the Emax ink with respect to ICE hydrogel between 0% and 100%. The appearance of the extruded samples showed a transition from red (alginate) to blue (Emax). Figure 5:2b shows that the calculated value for the Emax volume fraction obtained from image analysis closely agrees with the expected Emax fraction obtained by controlling the extrusion ratio of the two inks. This result shows that the digitally controlled print heads can accurately dispense the two inks in a programmed ratio. The use of stepper motors for syringe pump control provides a higher level of accuracy of the dispensed volume compared to that of the syringe pumps driven by pneumatic pressure used in previous projects.\[200\]

Figure 5:2 a. Macroscope images of sample extruded ribbons printed with Emax content of 0, 25, 37.5, 50, 62.5, 75 and 100 % in blends with Alg/PAAm ICE hydrogel. For clarity the Alg/PAAm ICE gel is colored red and the Emax is colored blue. b. Emax volume fraction obtained from image analysis as a function of the software controlled Emax extrusion ratio. c. The swelling ratio of the extruded composite materials (calculated after completion of the post printing processing) as a function of Emax volume fraction obtained from image analysis.
The Alg/AAm ICE gel ink and the Emax ink prove to be immiscible and phase separate following deposition. When the ink blend is exposed to UV irradiation the two inks begin to solidify to set the composite structure. This structure can be easily visualized as the Alg/AAm ICE gel ink is dyed red in color and the Emax has a blue color. Figure 5.2 a. includes microstructural images of the extruded ribbons printed with Emax extrusion rates of 0, 25, 37.5, 50, 62.5, 75 and 100 % of the total extrusion rate. The Emax phase produces small round particles in the composite materials printed with Emax extrusion rates of 25 and 37.5 % of the total extrusion rate. The maximum diameter of the particles are up to 220 ± 20 μm and 510 ± 30 μm, respectively, for the composite materials printed with Emax extrusion rates of 25 and 37.5 % of the total extrusion rate. A continuous Emax phase is produced in all the composite materials printed with Emax extrusion rates of 50 % or greater of the total extrusion rate. The Alg/AAm ICE gel phase does not appear to be dispersed evenly in these composites. Future modifications to the static mixer length and design could be used to modify the microstructure formed in these composites.

The extruded composite ribbons were exposed to a post printing process to complete the crosslinking of each phase[187] before undergoing mechanical characterization. A UV spot lamp was used to complete the curing process of the two inks, and then the composite ribbons were immersed in 0.1 M calcium chloride solution to crosslink the alginate polymer network with the metal cations. All samples were then immersed in water and allowed to reach equilibrium swelling. The swelling ratio of the swollen composite materials was calculated using equation (2:1). Ribbons printed entirely of the Alg/AAm ICE gel ink displayed a swelling ratio of 10 ± 0.7 and ribbons printed entirely of the Emax ink displayed a considerably lower swelling ratio of just 0.20 ± 0.04. Figure 5:2c shows that the swelling ratio of the composite materials decreases as the volume fraction of Emax in the composite material was increased. This result demonstrates that by varying the ratio in which the two inks are dispensed that the swelling ratio of a printed composite can be controlled over several orders of magnitude. The swelling ratio follows a linear fashion as a function of ICE hydrogel content up to the point where the Emax ink forms the continuous phase. After this point (50 % or greater Emax), the ICE phase is isolated within the continuous Emax phase and swelling ratio deviates from the rule of mixtures.
5.3.2 Mechanical testing

Tensile testing was performed on segments of the printed ribbons to determine the effect of the Emax volume fraction on the mechanical properties of the composite materials. The tensile tests were performed after the completion of the post printing process and once the composites had been equilibrated in water. The segments of the composite materials were loaded in the long direction of the ribbon. Examples of typical stress-strain curves for the printed composite ribbons are shown in Figure 5:3a and a summary of all data is provided in Table 5:1. Hydrogel ribbons printed entirely from the Alg/AAm ICE gel ink displayed an elastic modulus of 350 ± 30 kPa, a tensile strength of 90 ± 20 kPa, a failure strain of 38 ± 3% and a work of extension of 19 ± 2 kJ/m³. At the other extreme, samples printed entirely from Emax displayed higher elastic modulus of 2300 ± 100 kPa, tensile strength of 1100 ± 40 kPa, failure strain of 75 ± 7% and work of extension of 660 ± 30 kJ/m³. The reduced strength and stiffness of the alginate hydrogel can be related to its considerably higher swelling ratio compared with the Emax.\[2\] As expected, the mechanical properties of the ribbons printed from a blend of the two inks varied between the two extremes. The composite materials displayed an increase in elastic modulus, failure strength, failure strain and work of extension corresponding to an increase in Emax volume fraction and decrease in swelling ratio. These trends demonstrate that by varying the ratio in which these two inks are dispensed that the mechanical properties of a printed composite can be controlled over a wide range of values.

![Typical stress-strain curves for printed composites with Emax volume fraction of 0, 25, 52, 79, and 100 %.](image1)

![Elastic modulus of the printed composites as a function of the Emax volume fraction.](image2)

Figure 5:3 Typical stress-strain curves for printed composites with Emax volume fraction of 0, 25, 52, 79, and 100 %. b. Elastic modulus of the printed composites as a function of the Emax volume fraction. Solid and dotted lines represent the theoretical upper (evaluated using equation (4:2)) and lower (evaluated using equation (5:1)) bounds for elastic modulus, respectively.

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The experimental data taken from the tensile testing was compared with composite theory to determine how well the Emax component reinforces the hydrogel matrix. The extent of agreement between the measured modulus values and the theoretical predictions depends upon the type of microstructure formed in these printed composites. At higher Emax compositions, the rigid epoxy phase is continuous and the composite conforms to the rule of mixtures\(^{[188]}\) model of an ideal fiber-reinforced composite which is given by equation (4:2). This model assumes that both phases experience identical strains under load and requires excellent adhesion between the two phases so that no slippage can occur at the interface. It has previously been shown that there is strong adhesion between the Alg/PAAm ICE gel and Emax when the two inks are simultaneously cured.\(^{[200]}\) The rule of mixtures can be used to describe the elastic modulus of these composites when these composite ribbons are strained in the print direction because the Emax phase acts as continuous fibers.

The composite materials printed with Emax volume fractions of 40% or less can be described as particulate reinforced composites of rigid Emax phase formed within a softer, hydrogel matrix. The reinforcing efficiency of particulate reinforcement is less than that of fibre reinforcement of the same volume fraction because the strain is not equally distributed between the two phases. Einstein was the first to develop a model to predict the elastic modulus of particulate composites\(^{[201]}\) and in the years since a number of empirical and semi-empirical models have been proposed.\(^{[202, 203]}\) One such model for calculating the minimum elastic modulus \(E_{MIN}\) for a particulate reinforced composite is the Ishai and Cohen equation\(^{[204]}\) given by:

\[
\frac{E_{PRC}}{E_m} = 1 + \frac{V_f}{\rho/(\rho - 1) - V_f^{1/3}},
\]

\(5:1\)

where \(\rho\) is calculated as \(E_f/E_m\), and \(E_{PRC}\) is the lower limit for the composite modulus. This model has previously been shown to satisfactorily fit moduli data for epoxy/glass bead composites and epoxy/rubber composites\(^{[202]}\) and also gives good agreement for the Emax particle reinforced alginate hydrogels formed with Emax compositions of 40% or less.

The theoretical predictions from these two composite models are shown as dashed lines in Figure 3b. It can be seen that the experimentally measured moduli values follow the lower bound predictions closely for Emax volume fractions of 40% and
lower. At Emax volume fractions of 52% and higher, the measured values correspond closely to the upper bound predictions.

Table 5:1. A summary of the mechanical properties of printed composite materials.$^b$

<table>
<thead>
<tr>
<th>$V_{E\text{max}}$ [%]</th>
<th>$Q$</th>
<th>E [MPa]</th>
<th>$E_\parallel$ [MPa]</th>
<th>$E_{PRC}$ [MPa]</th>
<th>$\sigma_T$ [kPa]</th>
<th>$\epsilon_f$ [%]</th>
<th>U [kJ/m$^3$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10 ± 0.7</td>
<td>0.35 ± 0.03</td>
<td>0.35</td>
<td>0.35</td>
<td>90 ± 20</td>
<td>38 ± 3</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>25 ± 2</td>
<td>5.7 ± 0.2</td>
<td>0.54 ± 0.01</td>
<td>0.98</td>
<td>0.52</td>
<td>160 ± 20</td>
<td>42 ± 7</td>
<td>35 ± 3</td>
</tr>
<tr>
<td>40 ± 3</td>
<td>2.9 ± 0.3</td>
<td>0.84 ± 0.07</td>
<td>1.4</td>
<td>0.70</td>
<td>270 ± 20</td>
<td>47 ± 2</td>
<td>73 ± 9</td>
</tr>
<tr>
<td>52 ± 2</td>
<td>1.5 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>1.7</td>
<td>0.90</td>
<td>340 ± 30</td>
<td>48 ± 7</td>
<td>110 ± 10</td>
</tr>
<tr>
<td>58 ± 2</td>
<td>0.9 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>1.8</td>
<td>1.0</td>
<td>610 ± 30</td>
<td>66 ± 6</td>
<td>140 ± 20</td>
</tr>
<tr>
<td>79 ± 4</td>
<td>0.6 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>2.3</td>
<td>1.6</td>
<td>680 ± 20</td>
<td>67 ± 8</td>
<td>250 ± 30</td>
</tr>
<tr>
<td>100</td>
<td>0.20 ± 0.04</td>
<td>2.9 ± 0.1</td>
<td>2.9</td>
<td>2.9</td>
<td>1100 ± 40</td>
<td>75 ± 7</td>
<td>660 ± 30</td>
</tr>
</tbody>
</table>

$^b$ $V_{E\text{max}}$ is the Emax volume fraction, $Q$ is the swelling ratio, E is the elastic modulus, $E_\parallel$ is the theoretical maximum elastic modulus (evaluated using equation (4:2)), $E_{PRC}$ is the theoretical minimum elastic modulus (evaluated using equation (5:1)), $\sigma_T$ is the tensile strength, $\epsilon_f$ is the failure strain and U is the work of extension.

5.3.3 Gradient printing

One advantage of 3D printing is the ability to produce products with spatially varying material properties. In some cases a gradual transition, or gradient, in properties is desirable with many such examples seen in nature. For example, tendons are a tough band of connective tissue that connects soft muscle to hard bone. Tendons contain a gradient of mechanical properties between the two extremes of hard and soft which make a smooth interface between the muscle and bone. Here, an anatomical model of a tendon was printed to demonstrate how the custom built printer can be used to fabricate gradient structures. Using software control, a sample was prepared where the relative extrusion rates of the Emax and Alg/AAm ICE gel inks were varied along the length of the build stage. Figure 5:4 a. shows the Emax extrusion rate as a percentage of the total extrusion rate along the full 18cm length of the artificial tendon. The extrusion profile was designed to produce the spatially varying structure. Figure 5:4 b includes a digital representation of the artificial tendon and Figure 5:4 c includes a photograph of the printed structure. Both ends of the artificial tendon were designed
to be 100% Emax to act as rigid attachment points. Two transition zones of different length were formed adjacent to each rigid end to provide a gradient from 100% Emax to 100% Alg/PAAm. Transitions in color from blue to purple to red along the length of the artificial tendon are a visual indication of the gradient from hard Emax to the soft Alg/AAm ICE gel. A wedge shaped pattern is visible in the structure of the gradients in the artificial tendon which results from the laminar flow through the rectangular dye nozzle. The pattern could be removed by optimizing the flow dynamics through the nozzle.

Figure 5:4 a. Emax extrusion rate as a percentage of the total extrusion rate as a function of the length for an artificial tendon. b. A digital representation of an artificial tendon that shows variation in the material composition through color. The model represents gradients by the transition of blue to red. c. A photograph of an artificial tendon printed with Alg/PAAm ICE gel and Emax gradient structures. d. An illustration of the arm showing the pronator teres muscle attached to the skeleton by two tendons. e. Photograph of a 3D printed artificial tendon attached to a skeleton as the pronator teres.

The artificial tendon was attached to the arm of a model skeleton in the position of the pronator teres muscle. The pronator teres muscle functions to rotate the forearm. Figure 5:4 d. illustrates the anatomical position of the pronator teres muscle and it is attachment to the skeleton by two tendons. Figure 5:4 e. is a photograph of the artificial tendon when attached to the arm as the pronator teres muscle. Connection of the
artificial tendon attached to the bone can be made through the regions of Emax as it is mechanically more robust than the Alg/PAAm ICE gel. It is possible to add additional function to the artificial tendon by using a stimuli responsive tough hydrogel in place of the Alg/PAAm ICE gel which could cause the artificial tendon to expand and contract in a muscle-like fashion.\textsuperscript{[145, 205]}

5.4 Summary
We have developed a new extrusion based gradient printing system, and demonstrated its function by 3D printing a range of tough hydrogel composites. A spectrum of soft and wet through to hard and dry particulate reinforced composites were prepared by altering the ratios of a soft Alg/AAm based hydrogel to a hard UV-curable ink (Emax) in the materials. The printed materials were mechanically characterized in tension and were modeled by composite theory. The 3D printer platform allowed the composites to be fabricated directly into functional structures and this was demonstrated by printing an anatomical model of a tendon. The artificial tendon contained a gradient of composition and mechanical properties between the extremes of the hydrogel and the Emax. The printer itself fills a void within the current suite of 3D printers as the first extrusion based deposition system capable of producing these smooth and continuous gradient structures. An important aspect of the printer function is the use of two mechanically driven syringe pumps in conjunction with new software that allows for independent control over the rate of extrusion from each of the pumps into a static mixer. Each syringe pump can be loaded with a different ink, which is then mixed together in desired ratios by the static mixer. This technique not only opens a pathway towards new functionally graded materials for use in the next generation of anatomical models, but also could ultimately lead the way towards the fabrication of multifunctional single material systems that would replace the need for objects requiring a complex assembly of multiple parts with homogeneous properties and discrete functionality.\textsuperscript{[196, 206]}
This chapter has been adapted from the article “4D Printing with Mechanically Robust, Thermally Actuating Hydrogels” published in Macromolecular Rapid Communications, 2015, 36, 12, 1211-1217.

Author Statement:

The research in this chapter was performed with the assistance of Robert Gorkin III, who helped with the design and testing of the smart valve. All experiments and data analysis was carried out by the author, Shannon E. Bakarich. This would not have been possible without the intellectual support and guidance of Marc in het Panhuis, and Geoffrey M. Spinks.

The introduction section in this chapter is a shortened version compared with the published article. The introduction has been modified to avoid duplication with the previous Chapters.

I, Prof. Geoffrey M. Spinks (Supervisor), support and certify the above author statement.
6.1 Introduction

Four dimensional (4D) printing is an exciting emerging technology for creating dynamic devices that can change their shape and, or function on-demand and over time.\cite{207-209} 4D printing combines smart actuating and sensing materials with Additive Manufacturing techniques to offer an innovative, versatile and convenient method for crafting custom-designed sensors,\cite{210} robotics,\cite{211} and self-assembling structures.\cite{207} Stimuli-responsive volume-change materials incorporated into multi-material structures can be harnessed to create movement in the same way that biological muscles achieve motion in animals and nastic movements are generated in plants.\cite{76, 81} Current 4D printed examples utilize water absorption\cite{207} or thermal shape memory\cite{208} to demonstrate impressive shape change, but are slow to respond, show limited reversibility, and restricted to bending type motions of flexible structures that generate little force. We here describe relatively fast and reversible, skeletal muscle-like linear actuation in 3D printed tough hydrogel materials and their incorporation into a smart valve that can control the flow of water.

In this Chapter, 4D printing using an actuating hydrogel based ink is demonstrated for the first time. A new alginate and poly(N-isopropylacrylamide) (PNIPAAm) ICE gel ink is formulated for this demonstration. The thermally-responsive PNIPAAm functions as both the toughening agent and to provide actuation through reversible volume transitions. PNIPAAm is a widely studied temperature-sensitive hydrogel that exhibits a large reversible volume transition at the lower critical solution temperature (LSCT), (\(\sim 32-35 \, ^{\circ}C\)).\cite{24, 212, 213} The volume change is caused by a coil–globule transition of the polymer network strands\cite{213} and results in a large decrease in the water content when the temperature is increased above the LCST.\cite{24}

6.2 Experimental

6.2.1 Materials

All materials were used as-received and all solutions were prepared using Milli-Q water (resistivity = 18.2 \(\Omega\) cm). \(\alpha\)-Keto glutaric acid photo-initiator was purchased from Fluka (Australia). Acrylamide solution (40%, for electrophoresis, sterile-filtered), alginic acid sodium salt (from brown algae with Brookfield viscosity 2% in \(\text{H}_2\text{O}\) at 25 \(^\circ\text{C}\) of 250 mPa s), calcium chloride (minimum 93.0% granular anhydrous),
ethylene glycol (rheology modifier), N-isopropylacrylamide and N,N’-methylenebisacrylamide crosslinker were purchased from Sigma Aldrich (Australia). A commercial epoxy based UV-curable adhesive (Emax 904 Gel-SC) was purchased from Ellsworth Adhesives (Australia).

6.2.2 Hydrogel Ink formulations

A range of PNIPAAm hydrogel precursor solutions with NIPAAm concentrations between 10 and 20 % (w/v) were prepared by dissolving between 1.5 and 3.0 g of NIPAAm, 15 mg of N,N’-methylenebisacrylamide and 90 mg of α-Keto glutaric acid in 15 ml of 0.01 M calcium chloride. Alg/PNIPAAm ICE gel precursor solutions were prepared by adding 450 mg of alginic acid sodium salt was mixed into the PNIPAAm hydrogel precursor solutions with a spatula. The ICE gel precursor solutions were then left to rest for 24 hr before use as extrusion printer ink. An Alg/AAm ICE gel ink and an alginate based ink for printing sacrificial support structures were prepared using the methods described above in chapter 4.2.2.

6.2.3 Rheology

The flow behaviour of each of the inks was determined following the protocols described above in chapter 2.2.3. All experimental studies were repeated in quadruplicate and the error was determined using standard deviation.

The gelation kinetics of the inks were determined using an Anton Paar Physica MCR 301 Rheometer coupled with cone (diameter 50 mm, angle 1° and truncation 97 μm) and quartz plate measuring system, a temperature control hood (Anton Paar H-PTD200) and a Dymax BlueWave 75 Rev 2.0 UV Light Curing Spot Lamp. Fluid samples were loaded onto the quartz plate and were sheared with constant conditions (oscillatory shear strain of 5% and a frequency of 20 Hz). The storage modulus and loss factor were measured as a function of time and 60 s after the UV source was switched on to cure the sample. Rheology tests were performed at different constant temperatures between 5 to 25 °C.

6.2.4 Extrusion printing

Alg/PNIPAAm ICE hydrogels and a multi-component valve were fabricated with a 3D-Bioplotter system (EnvisionTEC, Germany) that was coupled with a commercial
UV-curing system.[200] A Dymax BlueWave 75 Rev 2.0 UV Light Curing Spot Lamp System using a 19+ W/cm² UV source with a 1 meter light guide was used to cure the printed ink. Digital models of tensile specimens and the multi-component valve were prepared with computer-aided design software (Solidworks). EnvisionTEC software was used to slice the digital model into a stack of two dimensional layers (250 μm thick) to determine the print path. Material files and internal structures were then added to each part within the assembly.

The extrusion inks were loaded into the 3D-Bioplotter in separate pressurized syringe barrels which were positioned into the temperature controlled print heads. Each barrel was fitted with a 23 gauge syringe tip (diameter 0.337 mm) and maintained at stable temperature of 10 °C during printing. A pressure between 0.5 and 1 bar was used to extrude the inks and a head speed between 5 and 20 mm/s was used to pattern the inks with a 0.5 mm resolution onto a glass sheet that was cooled to 10 °C. The UV light was passed over each layer of patterned ink for 50 s and 200 s for the final layer. A spatula was used to remove any of the alginate based ink for printing sacrificial support structures once the print was finished. Finally the printed ICE hydrogels were immersed in 0.1M calcium chloride (72 hr) to fully crosslink the alginate.

6.2.5 Swelling ratio

The swelling ratio of the printed Alg/PNIPAAm ICE hydrogels in water was determined at 20 °C and 60 °C. The hydrogels swelling ratios were calculated after they were immersed in water for 30 min using equation (2:1). Samples were dried by being placed in an oven at 60 °C for 72 hr. The isotropic length contraction ‘α’ of the hydrogels was calculated using:

\[
\alpha = \frac{L_{20} - L_{60}}{L_{20}} \times 100\%\text{, }
\]

where ‘L_{20}’ is the length of hydrogel when swollen at 20 °C and ‘L_{60}’ is the length of hydrogel when swollen at 60 °C.

6.2.6 Mechanical testing

The mechanical properties of the printed alginate/PNIPAAm ICE hydrogels were determined using a Shimadzu EZ-L Universal Mechanical Tester. Tensile tests were
performed using a 50 N load cell. Printed tensile specimens were strained to failure at a rate of 10 mm/min. The applied stress was calculated using the average cross sectional area of the unstrained specimen at the gauge length. The failure stress and failure strain were calculated from the onset of failure. Work of extension was calculated as the area under the stress-strain curve to failure. Four repeat tests were performed on each material with errors estimated from one standard deviation.

6.2.7 Actuation testing

The actuation properties of the printed Alg/PNIPAAm ICE hydrogels were determined using a Shimadzu EZ-L Universal Mechanical Tester with a 50 N load cell and water sleeve. Printed tensile specimens were clamped to the universal mechanical tester inside a sleeve that was filled with 20 °C water. The force was measured over time as each specimen was extended to a stroke of 1 mm at t=0 to ensure that the hydrogels were kept under tension. The water was drained from the sleeve around the hydrogel and replaced with water heated to 60 °C in ~2 min while the stroke was maintained at 1 mm for 30 min. The force – stroke behavior of the hydrogels was then determined by cycling the stroke between 1 mm and a stroke with an equivalent stress of zero at a stroke rate of 10 mm/min for a total of three unload and load cycles. Finally, the stroke was maintained at 1 mm for a further 120 min and the water sleeve was drained and refilled with 20 °C water.

6.2.8 Valve characterization

The valve’s performance was characterized by measuring the flow rate of water through the valve. The flow rate was calculated by measuring as the time required for a 5 ml column of water to pass through the valve. The valve was closed by passing 60 °C water over the hydrogel actuators for up to 5 min and opened by passing 20 °C water over hydrogel actuators.

6.3 Results and discussion

6.3.1 Ink rheology

Rheology was used to characterize the Alg/PNIPAAm ICE hydrogel precursor solutions to optimize the conditions for extrusion printing. The experimental viscosity data (Figure 6:1) was fitted to the power law model described by equation (2:2). The
hydrogel precursor solutions exhibit non-Newtonian, shear thinning behaviour \((n < 1)\) typical of polymer solutions. The hydrogel precursor solutions became thinner \((K\) decreases) and increasingly shear thinning \((n\) decreases) as the N-isopropylacrylamide concentration was increased (Table 6:1). The hydrogel precursor solutions displayed 

\[ K \text{ values between 45 and 150 } \text{Pa s}^n \text{ which is consistent with the } K \text{ values of Alg/AAm ICE gel precursor solutions (between 20 and 150 } \text{Pa s}^n \text{) that have previously been printed through our extrusion printing system with a 23 gauge syringe tip.}^{[2]}

![Graph showing viscosity vs. shear rate for hydrogel precursor solutions](image)

Table 6:1: Summary of the rheological parameters measured for different Alg/NIPAAm ICE hydrogel precursor solutions.\(^1\)

<table>
<thead>
<tr>
<th>(C_{NIPAam}) [% (w/v)]</th>
<th>(K) [Pa s(^n)]</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>150 ± 10</td>
<td>0.31 ± 0.01</td>
</tr>
<tr>
<td>15</td>
<td>62 ± 2</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td>20</td>
<td>45 ± 1</td>
<td>0.29 ± 0.03</td>
</tr>
</tbody>
</table>

\(^1C_{NIPAam}\) is the concentration of NIPAAm. Consistency \((K)\) and power law \((n)\) indices were obtained through curve fitting with equation (2:2).

The gelation of the Alg/NIPAAm ICE hydrogel precursor solutions was observed with an Anton Paar Physica MCR 301 Rheometer coupled with cone (diameter 50 mm, angle 1\(^0\) and truncation 97 \(\mu\)m) and quartz plate measuring system, a temperature
control hood (Anton Paar H-PTD200) and a Dymax BlueWave 75 Rev 2.0 UV Light Curing Spot Lamp. The ICE gel precursor solutions were loaded onto the quartz plate and after 60 s continuously irradiated with UV light as the storage modulus was tracked over time. The increase in the storage modulus (Figure 6:2a) and decrease in tanδ (Figure 6:2b) indicates formation of the PNIPAAm polymer network. The maximum storage modulus achieved after 180 s of UV irradiation increased as the reaction temperature was lowered from 25 to 5 °C. Figure 6:2c shows that each of the three Alg/NIPAAm ICE hydrogel precursor solutions displayed this same behaviour and that the storage modulus was higher for the hydrogels polymerised with a higher NIPAAm concentration. At the completion of the test the hydrogels were observed by photographing them through the quartz plate. When irradiated with UV light at 25 °C the ICE hydrogel precursor solutions formed an opaque white paste (Figure 6:2d) which is indicative of phase separation occurring during the polymerisation reaction. At temperatures 20 °C and below the Alg/NIPAAm ICE hydrogel precursor solutions gelled to form a transparent solid (Figure 6:2e).
Figure 6.2. Storage modulus (a.) and Tan δ (b) as a function of time for 10 % (w/v) N-isopropylacrylamide, Alg/NIPAAm ICE hydrogel precursor solution at 5, 10, 15, 20 and 25 °C. The Alg/NIPAAm ICE hydrogel precursor solution was irradiated with UV light after 60 sec. The storage modulus of Alg/NIPAAm ICE hydrogel precursor solutions as a function of solution temperature for concentrations of 10 (diamonds), 15 (squares) and 20 (circles) % (w/v) N-isopropylacrylamide when irradiated with UV light for 180 s. c. and d. Photographs taken of hydrogel samples formed between the rheometer’s quartz plate and stainless steel cone during the rheological testing. An opaque gel was formed at 25 °C that is indicative of phase separation. (d.) An optically transparent gel formed at 10 °C (e.).

Conditions suitable for printing the Alg/PNIPAAm ICE gel precursor solutions were determined from these rheological studies. The gel precursor solutions were compatible with extrusion printing as their flow characteristics were similar to other ICE gel extrusion inks that had been successfully printed. To form a strong hydrogel and to avoid phase separation of the patterned ink the gel precursor solutions needed to be cooled to 10 °C in the temperature controlled print heads and patterned onto a glass plate that was also cooled to 10 °C.
6.3.2 Extrusion printing

Alg/PNIPAAm ICE gel inks with various concentrations of NIPAAm were prepared for extrusion printing. The inks had a constant alginate concentration of 4% (w/v) and NIPAAm concentrations of 10%, 15% or 20% (w/v) with fixed amounts of covalent crosslinker and UV initiator. Samples of each ink formulation were printed and UV cured at sub-ambient temperatures (10°C) with an Envisiontec 3D-Bioplotter coupled with a UV-light source. The printed hydrogels were immersed in 0.1 M calcium chloride solution to crosslink the alginate polymer network and to enhance their mechanical properties. The fully crosslinked gels were then rinsed and further immersed in water at 20 °C and swollen to equilibrium.

6.3.3 Swelling ratio and mechanical testing of Alg/PNIPAAm ICE hydrogels

Swelling ratios were determined for each of the ICE gels to determine their suitability for thermally-induced actuation. Increasing the PNIPAAm concentration in the ICE gel ink from 10% to 20% (w/v) made no significant difference to the swelling ratio of the printed hydrogels when swollen in water at 20 °C (Figure 6:3), since the swelling at this temperature was controlled by the more tightly crosslinked alginate network. Swelling ratios were in the range of 8.7-9.3 and were of a similar magnitude to previously described printed Alg/PAAm ICE gels. In contrast, when swollen in water at 60 °C the swelling ratio of the Alg/PNIPAAm gels was reduced significantly to 0.52 – 1.5 with a greater contraction occurring in the gel with the higher PNIPAAm content. The chosen temperature was well above the PNIPAAm thermal transition so as to rapidly heat the gel and generate fast actuation. Volumetric contraction ratios on heating from 20 °C to 60 °C increased from 6.1 to 9.3 to 16.7 for inks with NIPAAm contents of 10, 15 and 20% (w/v). The alginate portion of the ICE gel resists the contraction of the thermally-responsive PNIPAAm phase, so the contraction ratio decreased as the alginate fraction increased. The gels dramatically changed in size when heated as shown in Figure 6:3a and b. Increasing PNIPAAm content in the ICE gels resulted in larger isotropic length contraction at 60 °C based on their equilibrium size at 20 °C of 41 ± 1 %, 44 ± 1 % and 49 ± 1 %, for the gels prepared from 10%, 15% and 20% NIPAAm inks, respectively. The reversible nature of the thermally-induced actuation was observed when the Alg/PNIPAAm ICE gels swelled to their initial equilibrium conditions when cooled from 60 °C to 20 °C.
Figure 6:3. Photographs of a 3D printed 10 % (w/v) N-isopropylacrylamide, Alg/PNIPAAm ICE hydrogel tensile specimen swollen in water at 20 °C (a.) and 60 °C (b.). c. Swelling ratios of Alg/PNIPAAm ICE hydrogels with 10, 15 and 20 % (w/v) N-isopropylacrylamide concentrations at 20 °C and 60 °C. Typical tensile stress strain curves of Alg/PNIPAAm ICE hydrogels with 10, 15 and 20 % w/v N-isopropylacrylamide concentrations when swollen in water at 20 °C (d.) and 60 °C (e.).

The mechanical performance of printed Alg/PNIPAAm ICE gels was characterized in tension to evaluate their potential for use in load bearing applications. Mechanical tests were performed at temperatures both above and below the PNIPAAm swelling transition. As expected, the tensile strength and modulus of all ICE hydrogels increased considerably in the less-swollen state at 60 °C in comparison with the highly swollen state at 20 °C (Figure 6:3d and e and Table 2). The fully swollen gels had similar mechanical properties to printed Alg/PAAm hydrogels described previously.\textsuperscript{187} As the NIPAAm concentration in the printing solutions was increased from 10% to 20 % (w/v) the tensile strength and modulus decreased considerably when tested at 20 °C. Since the swelling ratio of the fully swollen gels at 20 °C were similar, the differences in mechanical properties can be attributed to the different volume fractions of alginate which strongly influence ICE gel modulus and tensile strength as recently reported.\textsuperscript{122} The ionically crosslinked alginate forms the more tightly
crosslinked network in comparison with the PNIPAAm network in the expanded state at 20°C. As a consequence, the hybrid gel modulus and strength decrease as the relative alginate fraction of the swollen gel is decreased. In contrast, it is seen in Figure 6:3d that at 60 °C the tensile strength increased with increasing PNIPAAm content. This strength increase can be attributed to the significantly reduced swelling ratio of these hybrid gels. It is well-known that the magnitude of strength and modulus are inversely proportional to the swelling ratio.\[2\]

Table 6:2 A summary of the mechanical properties of printed Alg/PNIPAAm ICE gels.\[3\]

<table>
<thead>
<tr>
<th>C_{NIPAAm} [% (w/v)]</th>
<th>T [°C]</th>
<th>Q</th>
<th>E [MPa]</th>
<th>(\sigma_T) [kPa]</th>
<th>(\varepsilon_f) [%]</th>
<th>U [kJ/m³]</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>20</td>
<td>9.2 ± 0.2</td>
<td>0.29 ± 0.04</td>
<td>70 ± 4</td>
<td>42 ± 7</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>1.5 ± 0.1</td>
<td>1.4 ± 0.3</td>
<td>340 ± 50</td>
<td>90 ± 20</td>
<td>200 ± 50</td>
</tr>
<tr>
<td>15</td>
<td>20</td>
<td>9.3 ± 0.1</td>
<td>0.26 ± 0.02</td>
<td>60 ± 6</td>
<td>47 ± 8</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>15</td>
<td>60</td>
<td>1.0 ± 0.1</td>
<td>1.8 ± 0.2</td>
<td>650 ± 70</td>
<td>120 ± 20</td>
<td>410 ± 60</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>8.7 ± 0.2</td>
<td>0.17 ± 0.03</td>
<td>46 ± 4</td>
<td>54 ± 9</td>
<td>16 ± 4</td>
</tr>
<tr>
<td>20</td>
<td>60</td>
<td>0.52 ± 0.02</td>
<td>1.9 ± 0.4</td>
<td>890 ± 50</td>
<td>180 ± 10</td>
<td>960 ± 60</td>
</tr>
</tbody>
</table>

\[3\] \(C_{NIPAAm}\) is the concentration of NIPAAm, T is the swelling temperature, Q is the swelling ratio, E is the elastic modulus, \(\sigma_T\) is the tensile strength, \(\varepsilon_f\) is the failure strain and U is the work of extension.

6.3.4 Actuation testing of Alg/PNIPAAm ICE hydrogels

The performance of the Alg/PNIPAAm ICE gels as thermally-stimulated actuators was next investigated to assess their suitability for use in 4D printing applications. Although the free swelling of hydrogels has been extensively studied, few investigations have considered their swelling when subjected to an external load.\[24, 216\] We have developed a method to observe the constrained swelling of the Alg/PNIPAAm ICE gels with a Universal Tensile Tester equipped with a water sleeve. Hydrogel samples were clamped to the mechanical tensile tester inside the sleeve which was filled with water at 20 °C. To ensure that the hydrogels were under tension each specimen was extended to a stroke of 1 mm (4 % strain). To begin the actuation testing the water was drained from the sleeve and immediately replaced with water heated to 60 °C. The increase in temperature caused the hydrogels to de-swell, however the hydrogels could not contract because the length was fixed. Consequently,
a force was produced as a result of the constrained de-swelling. Stress was determined from the measured force and the initial cross-sectional area of the sample (at 20 °C) and is shown as a function of time for one sample in Figure 6.4a. The generated stress reached a constant value after approximately 15 minutes and provided a measure of the blocked stress. Increasing PNIPAAm content in the ICE gels resulted in higher blocked stresses of 10.2 ± 0.6 kPa, 17.3 ± 0.4 kPa and 20.9 ± 0.6 kPa, n=3 for the gels prepared from 10%, 15% and 20% NIPAAm inks, respectively. The magnitude of the blocked stress is determined from the extent of volume contraction on heating and the elastic modulus of the gel in the heated state. The much larger de-swelling of the gels with higher PNIPAAm contents accounts for their higher observed blocked stresses.
Figure 6: a. Stress as a function of time for a 10 % (w/v) N-isopropylacrylamide, Alg/PNIPAAm ICE hydrogel specimen in an actuation test. The sample was first immersed in 20 °C water and tensioned by stretching 1mm. The water was then replaced with hot water (60 °C) and the stress increase measured while maintaining a constant sample length. The inset highlights the second stage of the actuation test where the sample unloaded and reloaded three times between an extension of 1 mm and a sample length at which the stress reduced to zero. The actuation test was then completed by re-immersing the sample in 20 °C water and measuring the stress decay at constant sample length. b. Typical stress-stroke curves recorded at 60 °C for Alg/PNIPAAm ICE hydrogel specimens with 10, 15 and 20 % (w/v) N-isopropylacrylamide concentrations. The force is normalized to a stress and the stroke is converted to a contraction strain based on the geometry of the swollen gel at 20 °C. Free strain ($\epsilon_f$) and blocked stress ($\sigma_b$) values were determined from intercepts with the abscissa and ordinate, as indicated for the 20% PNIPAAm hybrid gel. c. Typical stress-stroke curves recorded over 3 cycles at 60°C for an Alg/PNIPAAm ICE hydrogel specimen printed with 20 % (w/v) N-isopropylacrylamide concentration. The force is normalized to a stress and the stroke is converted to a contraction strain based on the geometry of the swollen gel at 20 °C.

Stress–stroke curves were prepared in the second stage of the actuation test (Figure 6:4b). Here the stress generated from the constrained de-swelling of the hydrogels was relaxed to zero by steadily reducing the separation between clamps while recording force and crosshead displacement. The samples were then immediately re-stretched to the initial strain and this unloading, reloading process was repeated 3 times. Typical
stress-strain curves for each of the three hydrogels are shown in Figure 6:4b. The free strain was calculated as the strain when the stress was equal to zero and the elastic modulus (at 60 °C) was calculated as the average slope of the unloading curve. As expected, free strains increased with increasing PNIPAAm content in the gels: 12 ± 2 %, 19 ± 2 % and 35 ± 3 %, n=3 for the gels prepared from 10%, 15% and 20% NIPAAm inks, respectively.

Several unusual features were observed from the actuation tests and these are likely related to the labile nature of the ionic crosslinks in the ICE gels under load. Firstly the free strain values determined from the stress-stroke curves were smaller than measured from unconstrained free swelling experiments and the 60 °C elastic moduli obtained from these curves were lower than measured in tensile tests. In addition, it was noted that the slope of the reloading stress-stroke curve was steeper than the unloading curve which resulted in larger free strain and blocked stress values in the second and third stress-strain cycles (Figure 6:4c). These observations may be explained by the known dissociation of ionic crosslinks in the alginate network under load and their reformation when unloaded.\[103,120] When the ICE gels were constrained during de-swelling the internal forces caused some of the ionic crosslinks to fail in a stress relaxation process.\[217,218] The resulting reduced crosslink density accounts for the lower elastic modulus of the hydrogels measured in the stress-stroke curves than that determined from tensile tests. Partial reformation of the ionic crosslinks when the ICE gel was unloaded\[103,120] accounts for the steeper slope in the reloading stress-stroke curve than in the previous unloading. Finally, the free strain determined from the loaded actuation tests is smaller than the isotropic length contraction from the unconstrained swelling is because of the stress relaxation during the initial constrained, isometric swelling. The free strain (\(\varepsilon_F\)) is determined as \(\varepsilon_F = \sigma_b/E_T\). The free strain is reduced because the blocked stress (\(\sigma_b\)) and modulus (\(E_T\)) of the hydrogel are lowered because of stress relaxation. From the isotropic length contraction measured in the unconstrained swelling tests and elastic modulus at 60 °C from the tensile tests, the predicted blocked stress are 590 kPa, 770 kPa and 930 kPa for the ICE gels prepared from inks containing 10%, 15% and 20% NIPAAm, respectively. These values are more than 50 times larger than the measured blocked stresses and demonstrate the very significant stress relaxation that occurs during constrained
swelling. Similarly, large stress relaxation has been observed in alginate networks with ionic crosslinks.\cite{217-219}

The final step of the actuation testing was the re-swelling of the hydrogels in 20 °C water. The stroke was maintained at 1 mm for 120 minutes and the water sleeve was drained and quickly refilled with 20 °C water. The hydrogels absorbed water which resulted in a relaxation of the stress. Hydrogels printed with a 10 % (w/v) NIPAAm concentration showed a stress relaxation of $13.3 \pm 0.7$ kPa over $25 \pm 1$ min. The hydrogels printed with a 20 % (w/v) NIPAAm concentration relaxed $27.0 \pm 0.3$ kPa of stress over a larger response time of $78 \pm 2$ min. These stress relaxations through cooling / swelling were larger than the corresponding stress generated during heating / contraction of the same gel. The loss of ionic crosslinks under load at 60 °C allow for a greater swelling at 20 °C than occurs in the pristine gel. Typical of PNIPAAm hydrogels the swelling rate of cooling hydrogels in 20 °C water was slower than the de-swelling rate of the heating hydrogels in 60 °C water.\cite{213} A summary of all of the actuation testing data is included in the Table 3.

Table 6:3 A summary of the constrained swelling properties of printed Alg/PNIPAAm ICE gels.\textsuperscript{k}

<table>
<thead>
<tr>
<th>$C_{NIPAAm}$ [% (w/v)]</th>
<th>$\Delta \sigma_H$ [kPa]</th>
<th>$\Delta T_H$ [s]</th>
<th>$\Delta \sigma_C$ [kPa]</th>
<th>$\Delta T_C$ [s]</th>
<th>$E_{60}$ [kPa]</th>
<th>$\epsilon_F$ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10.2 ± 0.6</td>
<td>8 ± 1</td>
<td>13.3 ± 0.7</td>
<td>25 ± 1</td>
<td>97 ± 6</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>15</td>
<td>17.3 ± 0.4</td>
<td>11 ± 1</td>
<td>24.6 ± 0.7</td>
<td>53 ± 5</td>
<td>88 ± 4</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>20</td>
<td>20.9 ± 0.6</td>
<td>18 ± 2</td>
<td>27.0 ± 0.3</td>
<td>78 ± 2</td>
<td>60 ± 4</td>
<td>35 ± 3</td>
</tr>
</tbody>
</table>

\textsuperscript{k} $C_{NIPAAm}$ is the concentration of NIPAAm, $\Delta \sigma_H$ is the stress of heating, $\Delta T_H$ is the heating response time, $\Delta \sigma_C$ the stress of cooling, is $\Delta T_C$ the cooling response time, $E_{60}$ is the elastic modulus at 60 °C and $\epsilon_F$ is the free stroke.

6.3.5 Fabrication and characterisation of a smart valve

The Alg/PNIPAAm ICE gels show promise for application as actuators because they can repetitively achieve a large free strain and reasonable blocked stress. We demonstrated how these materials can be fabricated into a smart valve for control of water flow\cite{32-34, 220, 221} with 4D printing. A multi-component computer aided design
(CAD) model of the smart valve was prepared with Solidworks software (Figure 6:5a) and printed with an Envisiontec 3D-Bioplotter (Figure 6:5b). Each of the four components was assigned a different material ink as follows: rigid Emax epoxy compound for the valve design contained tubing and a cap;{[214]} Alg/PNIPAAm ICE gel ink (10 % (w/v) NIPAAm) for actuators that open and close the valve; Alg/PAAm ICE gel ink for the gasket and a channel for the flow of water; and an alginate based ink for the sacrificial support structures. The valve was printed in the open configuration (Figure 6:5c), and when hot water made contact with the actuator components the valve experienced a 4D printing transformation. During heating the actuators contracted, to close the outlet and blocking the flow through the water channel (Figure 6:5d).

![Figure 6:5](image_url)

Figure 6:5 a. Computer aided design model of hydrogel valve. Labels indicate components to be printed with Alg/PNIPAAm ICE hydrogel ink (i), Emax (ii), Alg/PAAm ICE hydrogel ink (iii) and alginate based ink for printing sacrificial support structures (iv). Photographs of (b.) the Bioplotter printing the valve; (c.) the 4D printed valve swollen in water at 20 °C; and (d.) the 4D printed valve swollen in water at 60 °C. e. Flow rate of water through the 4D printed valve as a function of time swollen in water at 60 °C.

The valve design was evaluated by measuring the flow rate of water through the printed valve. The flow rate was calculated by measuring the time required for a 5 ml
column of water to pass through the valve. Water passed through the open valve at a rate of 1.40 ± 0.04 ml/s (Figure 6:5e). The flow rate was then assessed after exposing the valve to 60 °C water for 1 minute. The flow rate through the valve dropped by more than 50% to 0.63 ± 0.07 ml/s. The reversible nature of the valve was demonstrated by passing 20 °C water over the hydrogel actuators which caused the valve to re-open. The average flow rate through the closed valve was 0.60 ± 0.10 ml/s after 1 minute heating time and when repeated over 5 cycles of opening and closing. The valves show a reproducible degree of control. The valve could be almost completely closed by increasing the amount of time that 60 °C water was passed over the hydrogel actuators. After 2 min in the 60 °C water the flow rate through the valve reduced to 13 ± 2 µl/s (or over 99% reduction in flow rate) and after 3.5 minutes the flow rate stabilised at 8 ± 1 µl/s (or 99.4 ± 1.2 % closed). This behaviour can be understood from the first step of the Alg/PNIPAAm ICE gel actuation test. When 60 °C water passes over the open valve the hydrogel is free to contract until it caps the end of the flow cannel. Once capped the hydrogel continues to undergo constrained de-swell until a maximum stress is produced. The flow of water through the valve decreases until this maximum stress is achieved. Modifications to the valve design to improve the sealing surface are likely to allow complete 100% closure.

6.4 Summary

In summary, we have designed a new ink for 3D printing of hydrogels that are both mechanically robust and thermally actuating. By modifying the amount of thermally responsive PNIPAAm network in the hydrogels, the gels showed reversible length changes of 41% - 49 % when heated and cooled between 20 °C and 60 °C. Blocked stresses generated in tension were in the range of 10 kPa to 21 kPa. We were able to fabricate a smart valve that autonomously controls the flow of water by printing a dynamic Alg/PNIPAAm ICE gel ink alongside other static materials. The valve automatically closed upon exposure to hot water, reducing the flow rate by 99%, and opened in cold water. With CAD modeling this 4D printing technique can be easily extended to make other types of moving structures. The ability to 4D print robust, actuating hydrogel materials opens a new avenue for fabricating hydrogel based sensors, soft robots, medical devices and self-assembling structures.
7 CONCLUSIONS AND RECOMMENDATIONS

The ultimate goal of this Ph.D. project was to construct objects and devices that not only incorporate hydrogel materials within their structure but that harness their unique properties and functionality. This thesis focused on the use of 3D printing technology as the fabrication tool for creating complex geometries and useful objects. The aims of the thesis were achieved in a progression of three steps. Initially a number of inks were formulated for extrusion printing tough ICE gels. Next, new composite materials were produced using techniques that printed the ICE gel inks in the same structure as other materials. Finally these printing techniques were used to fabricate functional structures and devices. Developing new techniques for 3D printing tough hydrogels required the customization of 3D printers, manipulation of software and the formulation of inks.

This research project has shown that ICE gels formed with a one-pot synthesis method can be 3D printed. ICE gel precursor solutions were optimised for use as extrusion inks by rheological characterisation of their flow behaviour and gelation mechanisms. Extrusion printing of two ICE gel precursor solution inks with different gelation mechanisms was demonstrated in chapters 2 and 3. An Alg/PAAm ICE gel precursor solution was extrusion printed with a customised 3D printer with an UV light that was used to initiate a free radical polymerisation of AAm monomers to solidify the patterned ink. A κ-CG/EA ICE gel precursor solution was printed using the thermal gel transition of κ-CG to solidify the patterned ink. The ability of these 3D printing techniques to fabricate the ICE gels into complex shapes was demonstrated by directly printing the inks into dogbone shaped tensile testing shapes.

The mechanical performance of the printed Alg/PAAm ICE gels exceeded those of previous examples of printed alginate hydrogels. For instance ICE gels printed with a 2% w/v alginate concentration have a tensile strength 91 ± 30 kPa whereas hydrogels printed by Xu et al. from a 2.3% alginate and 0.1% gelatine solution used for bio-engineered cardiac pseudo tissue applications exhibit a tensile strength of 44 ± 10 kPa. However, the mechanical performance of the printed Alg/PAAm ICE gels
did not match that Alg/PAAm ICE gels cast under optimised laboratory conditions. Alg/PAAm ICE gels printed with an ink formulation optimised for maximum mechanical performance displayed an ultimate tensile strength and a work of extension values of 170 ± 30 kPa and 260 ± 10 kJ/m³, respectively. The work of extension of these printed hydrogels is significantly lower than that of cast Alg/PAAm ICE gels which display values of ~ 2.5 MJ/m³. The introduction of some small bubbles and voids formed by the printing process that act as defects and oxygen inhibition are believed to be at least partly responsible for the discrepancy between mechanical properties of the hydrogels formed with these two fabrication techniques. Considerable effort is spent to isolate the free radical polymerisation reaction from the presence of oxygen when casting hydrogels under optimised laboratory conditions but this was not practical with the 3D printer apparatus used in this research project.

The κ-CG/EA ICE gels that were synthesised without a free radical polymerisation reaction and exhibited a superior mechanical performance compared to the printed Alg/PAAm ICE gels. The printed κ-CG/EA ICE gels displayed an ultimate tensile strength and a work of extension values of 600 ± 70 kPa and 1.4 ± 0.3 MJ/m³, respectively. The epoxy-amine addition reaction from mixing Jeffamine with PEGDGE assured the inclusion of long polymer chains between cross-links chains in the covalent polymer network. In the future some effort should be spent to exclude oxygen in the UV curing step of the Alg/PAAm ICE gel ink. Removing oxygen form the curing ink would avoid the occurrence of oxygen inhibition and encourage the formation of long polymer chains between cross-links chains in the hydrogels covalent polymer network, producing a tougher hydrogel.

Since the publication of this research, Wei et al. have provided a solution to avoid oxygen inhibition with a triple network hydrogel printing technique. In this new technique the components of a thermally reversible agar polymer network are added to an Alg/PAAm ICE gel precursor solution which enabled the solution to retain its patterned shape upon cooling. UV-irradiation was later used to fully cure the thermally reversible hydrogel inside a protective nitrogen atmosphere. The hydrogels fabricated using this amended ICE gel printing technique exhibit the greatest record mechanical performance of all printed hydrogels with values of ultimate tensile strength and a work of extension values of ~ 1.1 MPa and ~ 3.9 MJ/m³, respectively.
This thesis has shown that 3D printing can be used for processing hydrogels alongside other materials to make structural composites and to fabricate multi-material structures. The development of two 3D printing techniques that were used to make composite materials of different morphologies from the Alg/PAAm ICE gel ink and Emax, a commercially available epoxy based UV-curable adhesive was reported in chapters 4 and 5. Despite the inferior mechanical performance of the hydrogels printed with the Alg/PAAm ICE gel ink, it was chosen to print composite structures rather than the κ-CG/EA ICE gel ink because UV initiated free radical polymerization forms the covalent network much faster than the epoxy-amine addition mechanism. Emax 904 Gel-SC was selected for printing composite structures with the Alg/PAAm ICE gel ink because these two fluids have similar rheological properties, are both cured by an UV irradiation source and display strong adhesion once solidified.

The development of the hydrogel composite printing techniques required the manipulation of software and customisation of 3D printing hardware. UV irradiation sources were added to all the printers used to fabricate composites so that the patterned inks could be cured in situ. CAD software was used to create digital models of fibre Reinforced hydrogel composites and a multi-material printer was used to pattern the Alg/PAAm ICE gel and Emax inks with in the one structure. The particulate reinforced materials were made by blending the two immiscible inks in different volume ratios. Blending the two inks required the construction of a novel deposition system that funnelled the flow of ink from two syringe barrels into a single stream and through a static mixer. Custom software was written by R. Gately to alter and ramp the extrusion rates of the two inks during printing to form gradient structures. The further development of new 3D printing software and hardware is a space that can be advanced in the future to improve the capacity for 3D printers to produce hydrogel composite materials.

The mechanical properties of the printed Alg/PAAm ICE gel and Emax composites were characterised in tension to assess their potential for use as structural materials. The Young’s modulus and ultimate tensile strength of both types of composites was larger for composites with larger Emax volume fractions. Analysis with standard composite theory showed that the fibre reinforced composites modulus followed the ‘Rule of Mixtures’ and the modulus of the particulate reinforced composites adhered
to the Ishai and Cohen equation. These composites were shown to maintain the properties of the hydrogel matrix with high swelling ratios that adhered to the simple rule of mixtures for swelling ratio.

These composites materials were directly fabricated into functional structures through 3D printing. A prototype artificial meniscus with a biomimetic fibre structure was fabricated using the fibre reinforced hydrogel printing technique and an artificial tendon was prepared with the particulate reinforced printing technique. The artificial meniscus contained two composition gradients that formed a transition of modulus between the soft hydrogel and the hard Emax. The printed structures displayed the utility of these printing techniques for fabricating complex structures that incorporate the unique properties of hydrogels.

This thesis has alluded to the use of hydrogels and hydrogel composites as biomaterials however there has been no biocompatibility testing performed on any of the inks used in these experiments. Toxicology studies would be required before any of the structures fabricated in this thesis could be considered for application as a biomaterial. Darnell et al. have performed preliminary studies where Alg/PAAm ICE gels were implanted into subcutaneous tissue of rats for 8 weeks and showed minimal inflammatory response. These promising results support the need for the further exploration of extremely tough alginate/PAAM ICE gels as biomaterials. The Emax photo adhesive was initially selected as a model ink to demonstrate the fabrication of composite materials by simultaneous curing of two inks and so its biocompatibility was not considered for these experiments. Emax was not designed as a biomaterial but rather for use on circuit boards. It is likely that Emax contains toxins and be incompatible as a biomaterial. In the future it will be necessary to formulate inks for the reinforcing fraction of hydrogel composite with a particular application in mind whether that be for use as a biomaterial or otherwise.

This thesis also demonstrated how 3D printers can construct multi-material devices that harness the properties of hydrogels as functionality. An ink for printing tough and thermally responsive hydrogels was used to demonstrate a new 4D printing technique in chapter 6. The AAm monomer component of the Alg/PAAm ICE gel ink was replaced with NIPAAm. 3D printed Alg/PNIPAAm ICE gels displayed a free strain of 35 ± 3 % and a blocking stress of 20.9 ± 0.6 kPa in constrained swelling testing when
heated above the LSCT of PNIPAAm. The experimental value of blocking stress was significantly lower than the theoretical value of 930 kPa calculated using the hydrogel’s free swelling isotropic length contraction of 49 ± 1 %. The difference in values was a result of a stress relaxation mechanism caused by the unzipping of ionic bonds in the alginate polymer network which is not considered in standard actuator theory. The hydrogel was incorporated into a smart valve that can autonomously open and close as a response to the temperature of the flowing water using the same printing technique that was used to print fiber reinforced hydrogels. The printed devise channels the actuation of the Alg/PNIPAAm ICE gel to open and close the valve. This demonstration highlights the potential of hydrogels for use in real world applications.

In further research the 4D printing technique could be used to fabricate microfluidic devices and soft robots. In the case of microfluidics this printing technique offers a new means to fabricate pumps and valves in a series of 3D printed microchannels. Soft robots with hydrogel based artificial muscles attached to a hard skeleton could also be constructed with this printing technique. With further development 4D printing of hydrogel actuators may also be a useful tool for fabricating soft robots that mimic muscular hydrostats. The composition and as result, the swelling properties of the Alg/PNIPAAm ICE gel actuators printed in this thesis were homogeneous which creates isotropic expansion and contraction of the hydrogels when exposed to change in temperature. More complex forms of actuation such as bending or twisting are possible by spatially altering the swelling properties of an actuator throughout a structure. With 3D printing it will be possible to fabricate heterogeneous hydrogels programed with specific modes of actuation by patterning inks for hydrogels with different swelling properties within the one structure.

In this thesis actuation was stimulated by heat generated by an external source in the hydrogels environment. Many applications however will require on demand control of the actuation through a digital interface. It may be possible to stimulate the Alg/PNIPAAm ICE gels with an electronic stimulus through the process of Joule heating. Joule heating is process where the passage of electric current through a conductor releases heat. A new area of research would be to develop a conductive ink that could be incorporated into a 3D printed devise to provide heat to the hydrogel actuators from an electronic signal.
In conclusion 3D/4D printing of tough hydrogels provides a viable means for fabricating devices that harness the unique properties of hydrogels as functionality. With further development 3D/4D printing of tough hydrogels could be used to manufacture of microfluidics, soft robots and bionic devices. The enhancement of this technology will be possible through the design of new 3D printers and software in parallel with the formulation of new tough hydrogel inks.
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