

The effects of simulated physiological conditions on the performance of novel 3D printed nanocomposite
biomaterials

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

3D printable biopolymer nanocomposites are an exciting novel class of materials with potential as biomaterials for skeletal reconstruction. In recent years, there has been an increasing demand for synthetic bone graft substitute materials. Development of these biomaterials would reduce the dependence on natural bone grafts and metal prostheses, which have significant practical and clinical issues associated with their use. Using additive manufacturing technologies, such as masked stereolithography, and biopolymer nanocomposites, complex, accurate, robust, and customizable biomaterials can be produced. Novel nanocomposite biomaterials composed of functionalized plant-based monomers and hydroxyapatite (HA) nanoparticle fillers developed previously in our laboratory have achieved mechanical performance exceeding those of commercial bone cement biomaterials. However, these materials have not been evaluated under physiologically relevant conditions. Exposure to physiological temperature of 37 °C and a hydrated environment can result in significant mechanical consequences, such as reduced mechanical strength and stiffness due to swelling, hydrolysis or osmotic cracking. To create a successful bone graft substitute, along with competent mechanical properties, it is essential that the material be degradable and resorbable to allow remodeling to natural bone tissue. One of the methods to evaluate a material's eventual *in vivo* degradation performance is to assess its susceptibility to oxidative degradation, as this is a naturally occurring mechanism in cell metabolism, inflammation responses, and osteoclast resorption. In this thesis, a set of experiments with controllable environmental factors was developed to assess the responses of our biopolymer nanocomposites to physiological temperature, water absorption, and oxidative degradation. It was determined that the biomaterials investigated in this study were susceptible to these conditions, with significant evidence of water absorption and surface degradation due to oxidation. This led to consequences in mechanical performance, in particular tensile and compressive strengths and moduli. For example, a 10 vol% HA nanocomposite exposed to elevated temperatures had 62 and 68 % reductions in compressive yield strength resulting from 14-day incubations in phosphate-buffered saline and hypochlorous acid, respectively. This thesis has demonstrated the importance of evaluating biomaterials under physiologically relevant conditions *in vitro* throughout the material development process and provides a fundamental basis and clear recommendations for further development of these materials.

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List of Abbreviations

AESO: ‘Acrylated epoxidized soybean oil’

BW: ‘Body weight’

CSBD: ‘Critically sized segmental bone defects’

DI: ‘Deionized’

DIC: ‘Digital image correlation’

DMA: ‘Dynamic mechanical analysis’

DMAP: ‘4-(dimethyl amino) pyridine’

D#: ‘#-day incubation period’

FEA: ‘Finite element analysis’

HA: ‘Hydroxyapatite’

HClO: ‘Hypochlorous acid’

H₂O₂: ‘Hydrogen peroxide’

IM: ‘Isosorbide methacrylate’

LED: ‘Light-emitting diode’

MAA: ‘Methacrylic anhydride’

mAESO: ‘Methacrylated acrylated epoxidized soybean oil’

mSLA: ‘Masked stereolithography’

MW: ‘Molecular weight’

p: ‘p-value, value of significance’

PBS: ‘Phosphate-buffered saline’

PEGDA: ‘Polyethylene glycol diacrylate’

PMMA: ‘Polymethyl methacrylate’

ROS: ‘Reactive oxygen species’

RT: ‘Room temperature’

SD: ‘Standard deviation’

SEM: ‘Scanning electron microscope’

TEGDMA: ‘Triethylene glycol dimethacrylate’

T_g: ‘Glass transition temperature’

UV: ‘Ultraviolet’

WCBSL: ‘Waterloo Composite Biomaterial Systems Laboratory’

3D: ‘Three-dimensional’

Chapter 1

Background and Literature Review

1.1 Clinical Problem

Skeletal reconstruction is an area of clinical research and practice that is experiencing increasing demand and innovation. The demand for these treatments is growing due to the rising incidences of bone disease, such as cancer, and trauma-related injuries in an aging population [1]. Ensuring proper treatment is essential, as bone fractures and defects that go untreated are highly linked with patient morbidity [2], [3]. Bone regeneration materials are predicted to globally attain a market size of 7.4 billion USD by 2023 [4]. To narrow the scope of this problem, critically-sized segmental bone defects (CSBD) will be the focus. CSBDs are injuries in diseased or damaged bone that require surgical intervention and repair with the assistance of a bone graft (Figure 1-1). These have been defined as defects that have minimum dimensions of 1 cm in length and 50% of the diameter of the cortical bone [5], however there is limited consensus in literature around this definition [6], [7]. Due to the size and locations of these defects, it is essential that they are stabilized with mechanically competent materials. Historically, these defects were repaired with natural bone grafts, mostly allografts, or metal prostheses. These methods of repair all come with their own issues and limitations which will be outlined in the subsequent sections. Therefore, there is a need for improved bone graft materials and designs.

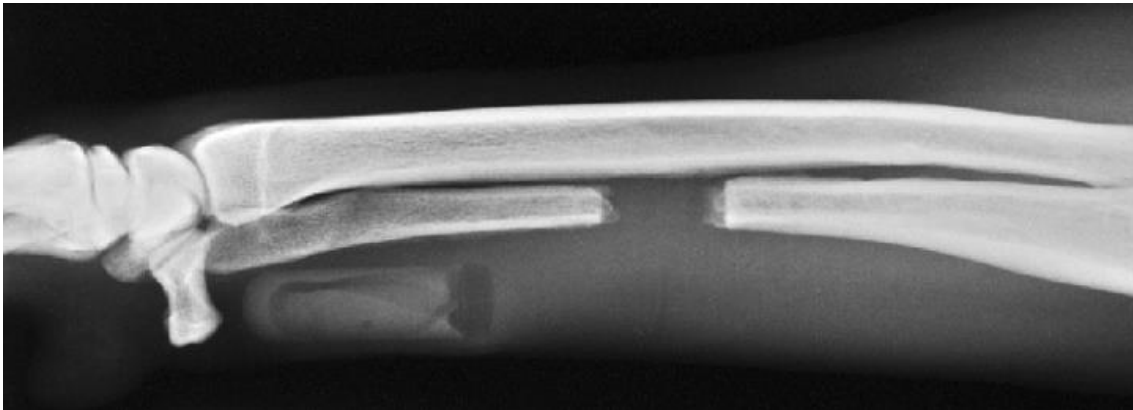


Figure 1-1 -Radiograph of a critically sized bone defect (CSBD) in an ulna. Reprinted from [8] with permission.

1.2 Bone Graft Substitutes

1.2.1 Bone

Bone is a complex and hierarchical natural composite that is primarily composed of hydroxyapatite, proteins, and collagen. This tissue is constantly undergoing remodelling which helps to maintain the skeletal system's structural integrity. Bone is constantly undergoing this process to respond to changing mechanical loading and structural microdamages [9]. This process is driven by bone cells, with resorption occurring through osteoclasts, new bone formation occurring through osteoblasts, and signalling and mechanotransduction through osteocytes [9]. Typically bone is organized in two forms: cortical and cancellous bone. Cortical or compact bone is a dense, strong, and highly organized bone that composes ~ 80 % of bone mass in adults [10]. This bone supports the majority of the mechanical function and loading of the skeletal system. Cortical bone has a longitudinal elastic modulus and compressive yield strength which range around 18 GPa and 115 MPa, respectively [11]. In contrast, cancellous or trabecular bone is a spongy and porous bone primarily found at the ends of long bones. It has an elastic modulus and strengths ranging between 10 – 3,000 MPa and 0.1 – 30 MPa, respectively [11].

1.2.2 Historic Bone Grafts and Skeletal Biomaterials

Natural bone grafts are harvested from two sources; allografts which are harvested from a deceased donor, and autografts, which are harvested from the patient. Autografts are typically considered as the bone graft standard due to their excellent fusion with the existing bone in the site [12]. These grafts have the benefits of good osteoconduction, resorption capabilities, and should have no risk of disease transmission or rejection [13]. However, these are limited by the availability of harvest sites as there are no appropriate sites for CSBD-sized grafts. Allografts offer a less invasive and available method, though typically have inferior mechanical performance to autografts, have poor comparative remodeling, and have a high risk for disease transmissions, such as human immunodeficiency virus, hepatitis C, and tuberculosis [14], [15]. Both methods are associated with post-surgical complications, such as increased blood loss, high infection risk, decreased mechanical properties, non-union of the defect, and mortality [14].

Metal is a commonly used biomaterial due to its superior mechanical properties. In skeletal applications, the materials must be able to withstand cyclic loads and fatigue caused by motion of the body. Due to this and biocompatibility considerations, stainless steel, titanium alloys, and cobalt-chromium alloys are commonly used in metallic implants for fracture fixations and joint replacement implants [16]. However, the mechanical properties of these metals far exceed that of cortical bone. In particular, their high elastic modulus causes stress shielding [17]. The higher stiffness removes stress from the surrounding bone which causes bone loss in accordance with Wolff's law, as bone remodels to accommodate the mechanical forces acting upon it [18]–[21]. This leads to implant loosening and eventual failure. They are also often prone to infection or aseptic loosening. The issues seen in metal implants highlight the importance of designing biomaterials that more closely match the properties seen in native tissue. This, along with the drawbacks of natural bone grafts, has led to the development of synthetic bone graft substitutes composed of polymers and ceramics.

1.2.3 Synthetic Substitutes

Natural bone grafts and metallic implants all possess their own issues and limitations, such as disease risk, infection, stress shielding, and mechanical failure, which has driven research into alternatives composed of polymers and ceramics. Bone graft substitutes, which replace harvested bone grafts synthetically, are predicted to increase in popularity with their global market size expanding to ~ 2100 million USD by 2030 [1]. These materials typically consist of a polymer matrix with a ceramic filler that mimics the properties of bone, such as calcium phosphate, hydroxyapatite (HA), and beta-tricalcium phosphate [1]. Benefits of these graft types include improved availability, reduced surgical site morbidity, and good sterility [22]. However, their use to date has been limited by poor clinical performance, issues with rates of resorption, and poor mechanical performance (i.e., highly brittle materials) [23].

Hydroxyapatite (HA) composes the mineral component of bone and has been used synthetically in skeletal applications for decades. HA has good osteoconductivity and is highly biocompatible. Compared to other ceramic fillers, it has good mechanical strength in compression. However, due to its slow degradation rate, which can vary due to its degree of crystallinity, it is retained in the body and can therefore impede bone remodeling [23]. Tri-calcium phosphate is mechanically weaker but has faster resorption than HA [24], [25]. This filler is typically used for repair in regions that have minimal

loading requirements, as its mechanical properties are similar to cancellous bone [26]. These materials are classified as bioactive fillers as they have been shown to support bone regrowth [27], [28]. Due to its superior mechanical properties over other applicable ceramics, HA is the preferred option for CBSDs that operate in load-bearing applications and must replace cortical bone.

To date, the majority of clinical uses of hydroxyapatite composites have occurred in dentistry and maxillofacial surgeries [29]–[34], as these applications typically require lower mechanical performances than grafts in the load-bearing skeletal system. A HA-silica gel composite, NanoBone[®], has been shown to promote bone formation from the bone-biomaterial interface in defects in the sinus cavity, with 20% new bone growth by 3 months [33], [35]. This composite has been shown to be degradable by osteoclasts in these applications [36]. Due to their mechanical properties, it is likely they would not succeed in load bearing applications.

GreenBone[™] is a commercially available synthetic biomaterial intended for structural bone grafts [37]. To date it has not been tested in humans. This material is synthesized through a biomorphic transformation of rattan wood to HA scaffolds and has been shown to promote bone regeneration in CBSDs in sheep [37], [38]. It has a compressive strength of 15 MPa [38]. Difficulties with biomaterials with only ceramic phases, such as GreenBone[™], include lack of an organic phase, limitations in processing and shaping, high brittleness, and slow degradation [39]. Therefore, a common approach to synthetic bone biomaterials includes compositing HA with different synthetic polymers. For instance, Russias et al. investigated polylactic acid composited through hot-pressing with 70 to 85 wt% HA [40]. Mechanical properties close to bone were achieved, with peak moduli and bending strengths of 10 GPa and 60 - 130 MPa achieved, respectively [40]. When exposed to degradation media (Hank's Balanced Salt Solution) for 20 days, up to ~ 17 and 70 % reductions in moduli and strength were observed, respectively [40]. However, degradation of PLA *in vivo* can lead to degradation products that trigger adverse inflammatory responses and the method of synthesis used in this study does not allow for complex structures to be fabricated [41].

1.2.4 Biomaterial Requirements: Mechanical Properties and Degradability

When designing a skeletal reconstruction biomaterial, there are a variety of design criteria that must be taken into consideration. The biomaterial should be considered as a system of properties that all interact to contribute to a successful implant. These factors are outlined in Figure 1-2 and include

biocompatibility, osteoconductivity, degradability, and mechanical, chemical, and surface properties [19]. To have the highest success with a synthetic graft, the biomechanical properties of natural bone tissue should be closely referenced. In this thesis, two of these factors will be considered: degradability and mechanical properties.

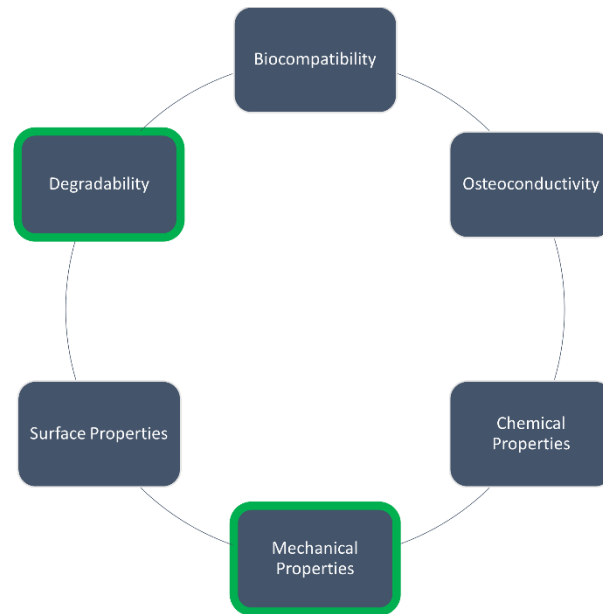


Figure 1-2 – Synthetic bone graft biomaterial system requirements. The foci for this thesis are highlighted in green.

For a biomaterial to be sufficiently mechanically robust in structural bone replacement applications, it needs to possess mechanical yield strengths greater than what is expected in physiological loading of the skeletal system. Bone tissue has a high safety factor, ranging from 1.4 to 4.1 based upon yield strain [42]. Therefore, mechanical properties of cortical bone are significantly higher than what is expected to occur in the body and what is required for bone graft materials. A better marker for biomaterial mechanical goals is to investigate the actual loading scenarios that occur *in vivo*. Mechanical stresses in the skeletal system are generated through gravitational and muscle loading and differ throughout the body [43]. These include tensile, compressive, and shear strains, which bone can respond to successfully due to its anisotropic and viscoelastic properties [44]. The femur is a significant weight bearing bone in the body, and therefore the stresses that it supports are a good upper marker of skeletal system loading. To determine the stress and strain behavior occurring internally, finite element analyses

(FEA) have been performed to model this bone under typical loading conditions [45]–[49]. Rietbergen et al. created a FEA model of a healthy proximal human femur under walking (stance phase) conditions [45]. A force of 1.4 kN was applied to the femoral head of this model, which resulted in average and maximum von Mises equivalent stresses of 4.7 and 129.1 MPa developing, respectively [45]. Forces throughout the femur in normal walking conditions are usually predicted to be ~ 236 % of body weight (BW) [45], [50]. It is recommended that hip implants are tested under these conditions and a more extreme case of climbing stairs (251 % BW) [50]. Edwards et al. have investigated the expected forces developed during running on the fatigue life of bovine cortical bone [51]. In this study, peak compressive stresses of 110, 51.6, and 82.7 MPa were used to mimic different loading rates and impacts based on the different phases of running modelled from heel-toe running at 4.4 m/s [51]. This study suggested that the stresses derived from the Rietbergen model are likely higher than what would occur during walking conditions. Due to the complexity of the environment, it is difficult to accurately determine the exact loading conditions *in vivo*, therefore the variability in literature is understandable. It is likely that stresses around 110 MPa should be expected in extreme, high impact activities. For a bone graft biomaterial, it is important for the elastic modulus to meet or be lower than that of natural bone to avoid the issues of stress shielding previously mentioned. Trabecular and cortical bone typically have elastic moduli between 10 – 3,000 MPa and 14 – 22 GPa (longitudinally), respectively [11].

Another mechanical benchmark that can be used is the performance of bone cements. Bone cements are self-curing resins that are used to fix internal prostheses, such as total hip replacements, to native bone. In accordance with ASTM F451, “Standard Specification for Acrylic Bone Cement”, cured bone cements must have an ultimate compressive strength exceeding 70 MPa [52]. ISO 5833 agrees with this requirement and also states that the minimum bending modulus and strength should be 1.8 GPa and 50 MPa, respectively [53]. Polymethyl methacrylate (PMMA) has been a standard bone cement used for over 50 years clinically. A recent study investigated the effects of monticellite and carbon nanotubes inclusion on PMMA mechanical properties [54]. They recorded maximum tensile properties of 74 MPa ultimate strength, 1.7 GPa elastic modulus, and 62% elongation at break [54]. The mechanical properties of bone cement represent a minimum benchmark for synthetic biomaterials that are developed for skeletal applications.

Previous studies in the Waterloo Biomaterial Composite Systems Laboratory (WCBSL) have achieved tensile mechanical properties of ~ 65 MPa in ultimate strength, ~30 MPa in yield strength, and a modulus of ~7 GPa [55]. These mechanical properties are close to that of the optimized PMMA,

and it is predicted that they would have compressive and flexural properties above the standards mentioned above. However, testing of these materials to date has been performed at room temperature and dry conditions. This testing does not represent the environment these biomaterials will operate in, which is 37 °C and an environment saturated with water. Polymers in particular are susceptible to temperature changes due to their viscoelastic nature. Composites can be impacted by water absorption via hydrolysis of the polymer chains or changes in the interactions between matrix and filler [56], [57]. There can also be significant mechanical consequences due to swelling. To highlight the importance of physiologically relevant testing, a recalled bone cement Kryptonite will be briefly highlighted. This product passed testing and regulatory approval with improved properties over the gold standard bone cement, however when implanted in the body it was found to behave poorly and led to numerous surgical complications. It was discovered that due to its viscoelasticity, at body temperatures, its stiffness and strength decreased by 50% compared to the room temperature conditions in which it was tested [58]. Therefore, when considering a material's suitability for clinical applications, it is important to consider the conditions in which it was tested and those it will be operated in.

Degradability is a property that must be tightly controlled in structural biomaterials that are implanted into the body. Typically, biomaterials fall under three classifications, non-biodegradable, biodegradable, and bioactive, based on their interaction with the body and their intended function. Biodegradable biomaterials are designed to degrade *in vivo* at a determined rate over a specific period [59]. These will eventually fully degrade into non-toxic products at the implantation site. To take advantage of the natural healing process of bone, synthetic bone grafts are commonly designed to be biodegradable and resorbable [60]. Resorption allows for the implant to gradually be replaced with new, native bone tissue to restore the site to its original state. This mechanism would also cause the material's stiffness to gradually decrease, allowing the bone to utilize more of the mechanical loading necessary for its growth [18]–[21].

Bone healing consists of three stages: the inflammatory response, reparative, and remodeling phases. The reparative phase, typically in weeks two to four after injury, involves the formation of a cartilaginous callus followed by ossification [19]. The remodeling phase repairs the defect to its pre-injury state, starts three weeks after injury, and can be ongoing for years [19]. These timelines are determined from fracture healing and would be extended by the presence of a graft. Therefore, the implant would need to transmit the majority of mechanical stresses through the site for at least the first month after implantation, before the new tissue is established to load share. When a bone graft is

implanted in the body, it will induce an inflammatory response in combination with the response already occurring internally due to the skeletal injury. This will recruit a variety of cells to the site, including neutrophils, macrophages, platelets, leukocytes, and mesenchymal stem cells [61]. These begin the reparative phase of bone healing with osteoblast differentiation, as well as create an environment that is capable of oxidative degradation due to cellular release of reactive oxygen species (ROS). The resorption and degradation of an implant is controlled by a variety of mechanisms including osteoclast resorption, and hydrolytic, oxidative, and enzymatic degradation. Therefore, one of the goals of these materials is to be susceptible to oxidative degradation in a controlled manner.

Biomaterials developed as bone graft substitutes must balance mechanical robustness and degradability. In the early stages of bone healing, they must have chemical and mechanical stability to support the defect site and transmit mechanical stresses. Therefore, the materials must be initially stable against degradation and have an optimized rate of degradation. To take advantage of the healing capabilities of bone tissue, during the reparative and remodeling phase the biomaterial should be capable of degradation and resorption to allow for osteoblasts to replace it with new bone. This will also take advantage of Wolff's law and should develop more bone in the site as more stress is carried by the tissue phase. Ideally the rates of degradation and new bone formation would be matched. In a study of an allograft mandible graft (Figure 1-3), after six months 44 ± 28 % of the site was composed of new bone formation along with the residual graft and connective tissue [62]. Resorption that is too slow will negatively impact bone remodeling. Resorption that is too fast will cause poor mechanical performance and could cause unwanted fibrous tissue formation [63], [64].

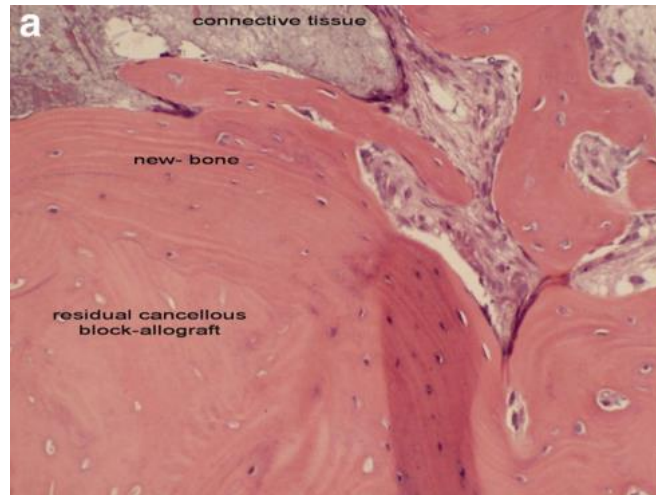


Figure 1-3 - Photomicrograph displaying residual allograft, newly formed bone, and connective tissue of a 6-month post-implant bone biopsy in an individual less than 45 years old. Reprinted from [62] with permission.

1.3 Manufacturing of Functionalized Soybean Oil/HA Nanocomposite Biomaterials.

1.3.1 3D Printing: Masked Stereolithography

Masked stereolithography (mSLA) is a specific subclass of vat photopolymerization additive manufacturing, which produces 3D objects through successive layer-by-layer curing. This class of additive manufacturing uses selective light-activated polymerization of polymer resins to create solid parts. During this process, ultraviolet (UV) light initiates crosslinking or polymerization of small monomers and oligomers to form longer polymer chains (Figure 1-4 (a)) [65], [66]. Photo initiators release free radicals upon UV light application which initiates free radical polymerization. Compared to other additive manufacturing technologies, vat photopolymerization is highly accurate and generates detailed parts with good surface finishes. Due to the nature of the materials used, i.e., thermoset resins, these reactions are irreversible post-curing. These technologies are common in dental and medical applications [67]–[70].

A variety of vat photopolymerization methods exist and are defined by their method of UV light production. mSLA printers contain light-emitting diode (LED) arrays. Their light is filtered through an

LCD screen (Figure 1-4 (b)). This system ‘flashes’ an image onto the transparent film at the bottom of the print vat for each layer. This allows for an entire layer to be processed quickly. After each layer is cured, the build platform moves in the vertical z-direction to allow for production of the next layer.

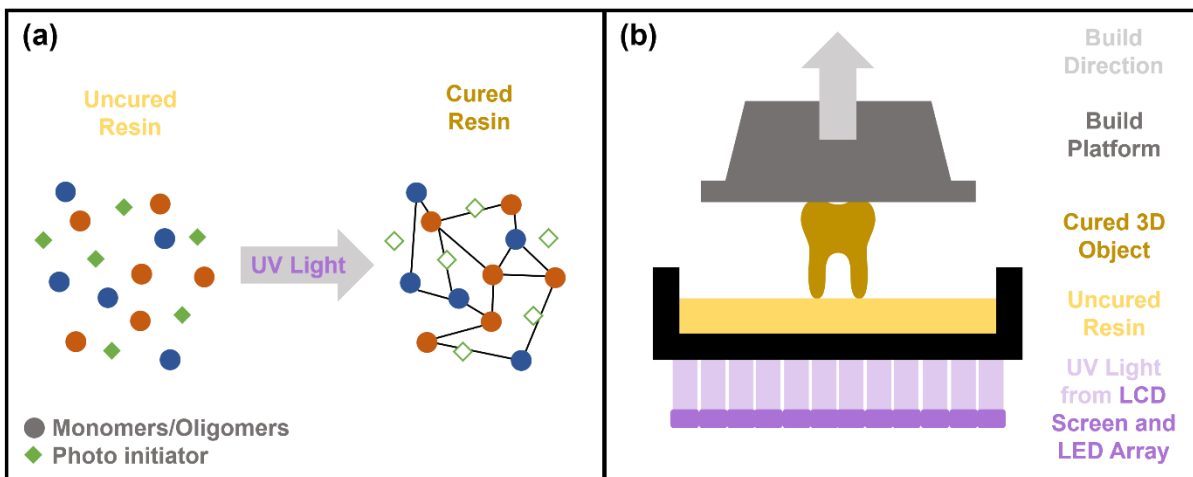


Figure 1-4 - Overview of masked stereolithography (mSLA): (a) Photopolymerization of uncured resin composed of monomers, oligomers, and photo initiator exposed to UV light, (b) diagram of mSLA printer where UV light is sourced from a LCD screen and LED array. Image created by author.

1.3.2 Material Development

Work on UV-curable biopolymer nanocomposites for 3D printing applications has been a focus of the WCBSL for a few years. Original work focused on extrudable inks for direct ink writing 3D printing [71]–[74]. While successful printing of scaffolds with adequate mechanical and cell-interaction properties was achieved, this material-printer system had difficulties with consistent and defect-free printing [75]. This was due to issues with rheology and the general issues faced with extrusion printing technologies. Therefore, focus was shifted to mSLA technology (as described in the previous section).

Originally, the resin phase of the composite inks was composed of acrylated epoxidized soybean oil (AESO) and polyethylene glycol diacrylate (PEGDA). A 10 vol% HA composite achieved tensile properties of ~ 31 MPa ultimate strength, 2 GPa modulus, and 21 MPa yield strength [75]. It was determined that HA inclusion improved mechanical properties, cytocompatibility, and bioactivity [75]. A paper by Mondal et al. expanded upon this work and investigated the effects of AESO

functionalization and alterations to the resin matrix [55]. Methacrylated AESO (mAESO) was introduced to decrease the number of residual hydroxyl groups on the polymer chain. By methacrylating these groups, viscosity of the ink was decreased, which is preferred for mSLA printing, and crosslinking of the polymer chains was increased, which increased mechanical strength. Methacrylation of AESO increased tensile fracture strength and modulus by 67 and 74 %, respectively, and greatly increased mSLA printability, fracture toughness and glass transition temperature (T_g) [55], [76], [77]. Based upon these results, mAESO was determined to be preferred over AESO when creating these printable composites.

Isosorbide methacrylate (IM) was also introduced to the resin phase of the composite. Isosorbide is a sugar monomer commonly used in high performance biopolymer materials as it contributes high stiffness due to its rigid ring structure [78]–[80]. Polymers composed of IM and mAESO have shown significantly improved properties of mechanical strength and modulus, storage modulus, and thermal stability [81]. This monomer was used to partially replace the reactive diluent phase (PEGDA) of the inks. This led to increased tensile fracture strength and elastic modulus [55].

Minimal investigation of the degradation behavior of these WCBSL composites has been previously performed on an AESO:PEGDA composite [75]. The hydrolytic, enzymatic, and oxidative degradation effects on composite mass were considered over a 28-day period. These were determined through incubation at 37 °C in deionized water (DI), lipase and esterase solutions, and hydrogen peroxide with a cobalt (II) chloride activator, respectively. After 10 days of incubation in DI, the water absorption equilibrated at 3 wt% of the original composite (Figure 1-5 (a)) [75]. Hydrolytic degradation of the materials was found to be negligible (Figure 1-5 (b)) [75]. Limited enzymatic and oxidative degradation occurred, with a maximum mass loss of 2 vol% being observed due to H_2O_2 degradation [75]. This study was performed on a different resin composition than is currently being used in the WCBSL and is very limited in its observation of the effects of swelling and degradation. As previously mentioned, it is essential to determine how these different operating conditions affect the mechanical and physical properties of these biocomposites, especially as they are intended for structural applications in vivo.

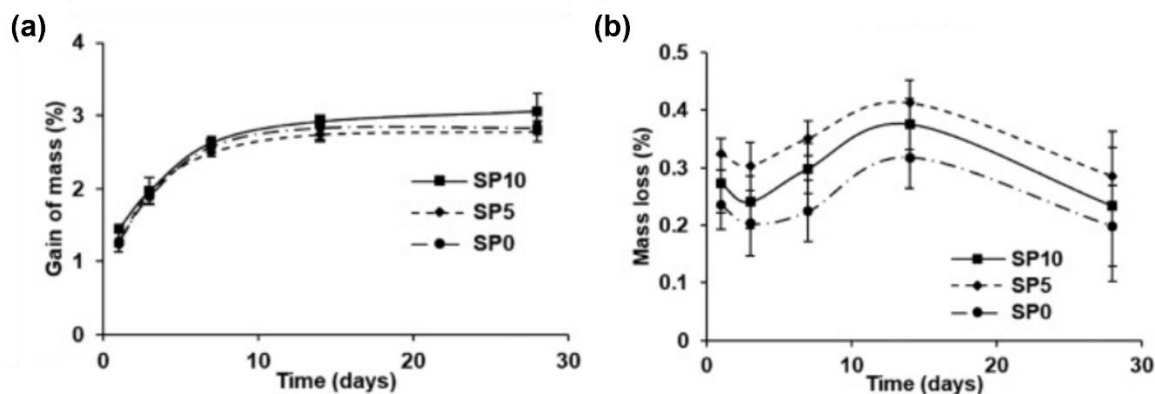


Figure 1-5 – (a) Swelling and (b) degradation behavior of AESO:PEGDA composites with varying HA content (0, 5 and 10 vol%) over time in DI water. Reprinted from [75] with permission.

1.4 Effects of Physiologically Relevant Environmental Conditions

1.4.1 Body Temperature

One of the main differences between polymers and other common structural materials such as metals is the dependence of their behaviour on temperature. Due to their viscoelasticity, their mechanical performance is dependent on their operating temperature. Viscoelastic materials display both viscous and elastic characteristics when loaded and undergoing deformation [82]. Polymers exhibit this behavior due to their ability to store and dissipate energy due to their molecular movement in their chains [83]. Viscoelastic materials are typically time and rate dependent and can be identified by hysteresis in their stress-strain curves, creep and stress relaxation behaviors, and rate dependent moduli.

When sufficient energy is present in the polymer chains, translational motion will cause polymer chains to detangle and secondary bonds to break allowing for viscous flow beyond elastic deformation. This allows polymers to typically have extensive elongation at break as the chains slide along each other and flow into new positions. The behavior of these bonds is dependent on thermal motion, and therefore the viscoelastic properties of polymers are dependent on temperature. Increased packing of polymer chains and increased functional groups along the polymer backbone typically increase the

material's strength and stiffness. The effect of temperature on the modulus of a polymer can be seen in Figure 1-6 [82]. As temperature increases through a material's glass transition temperature, the material transitions from glassy to rubbery behavior. In the glassy plateau, polymers usually exhibit brittle behavior as the molecular motion between chains is suppressed. In the rubbery plateau, materials behave much more elastically as there is sufficient thermal energy to allow chain movement.

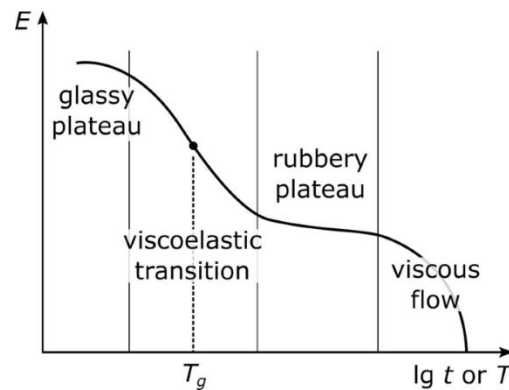


Figure 1-6 – The effect of time (t) or temperature (T) (logarithmic scale) on elastic modulus of viscoelastic polymers. Reprinted from [82] with permission.

Polymers are very susceptible to temperature changes, even when reinforced with particle fillers [84]. In particular, relatively small differences in temperature, such as from room temperature to 37 °C, can have significant impacts on mechanical properties if this change is occurring in the viscoelastic transition region (Figure 1-6). Decreased mechanical properties due to temperature change have been commonly observed in polymers [85]–[88]. The most significant impacts on mechanical properties due to temperature change are expected to be increased strain at fracture and reduced elastic modulus and strength [89]–[91].

1.4.2 Water Absorption and Hydrolysis

Polymer composites that are exposed to fluids have the potential to be impacted by water absorption, leading to material swelling, changes in matrix-filler interactions, and hydrolytic degradation. The interaction between a material and an aqueous environment are typically governed by their hydrophilicity. Hydrophilicity is the tendency of a material to be solvated by water molecules [92].

Typically, materials are classified as hydrophilic (attract and bond with water molecules), hydrophobic (repel water), and hygroscopic (attract and absorb water without bonding molecularly). AESO and mAESO are hydrophobic, while PEGDA, triethylene glycol dimethacrylate (TEGDMA), and HA are hydrophilic to different degrees [77], [93], [94]. Isosorbide is known to be hygroscopic, however dimethacrylate-functionalization has shown success in increasing the hydrophobicity of this polymer [95]. It has been proposed that water absorbed by polymers will only impact material properties if it is bonded to the polymer chains via hydrogen bonding [96].

It is a common approach in dental adhesives to bond hydrophobic methacrylate groups onto hydrophilic polymers to reduce the effects of interactions with water or other physiological environments on the material's durability [97]. However, these resins are typically still hydrophilic which has led to numerous issues with clinical uses of these dental resins due to water absorption and hydrolytic degradation [97]–[101]. The reduction in ultimate tensile strength of these adhesives has been correlated with the hydrophilicity of the resin [97]. After one year storage in water, resin-dentin materials lost 67% of their bond strength [99]. A positive correlation has also been observed between the amount of water absorption and the number of carbons in acyl groups and the number of carboxylic groups in the polymer chains [101]. The challenges faced in these dental materials with water absorption and weakening mechanical properties further highlight the importance of physiologically relevant material characterization for biomaterials.

The consequences of water absorption have also been explored in some biomaterials intended for bone graft substitution. Gorna et al. investigated porous polyurethane foams with controllable hydrophilicity with maximum compressive strengths and moduli of 340 kPa and 2 MPa, respectively [102]. It was observed that increasing hydrophilicity was correlated with increased water uptake to a maximum of 60 % uptake after immersion in phosphate-buffered saline (PBS) after six hours [102]. Calcium carbonate, glycerol phosphate calcium salt, and HA fillers reduced the amount of water uptake [102]. Mass loss due to hydrolytic degradation was also reduced by filler content and increasing hydrophobicity of the matrix [102]. Mass loss after 74 weeks ranged from 10 to 72 % [102]. By 48 weeks, the most hydrophilic foam had lost 90 % of its compressive strength, while negligible reduction in properties were seen in the materials with inorganic fillers [102]. Visually, degradation and absorption of these polyurethane foams caused cracking between pores and fragmentation and loss of shape (Figure 1-7). Extensive swelling behaviors have also been recorded in a variety of hydrogels intended for skeletal biomaterials [103], [104].

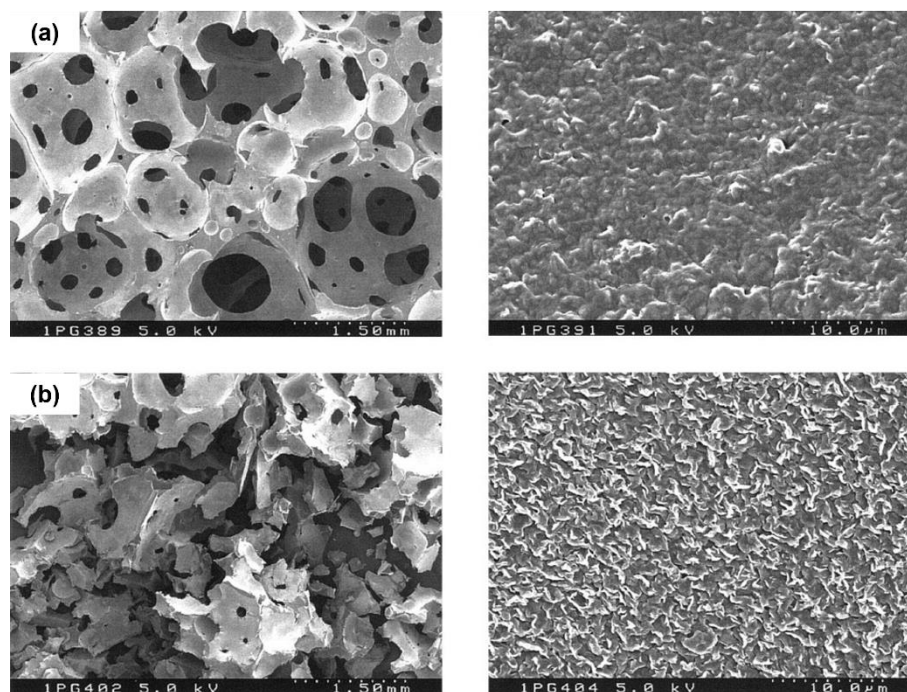


Figure 1-7 – Scanning electron microscopy (SEM) images of a porous polyurethane foam: (a) control and (b) after 74 weeks submersed in PBS. Scale bars represent 1.5 mm (left) and 10 μ m (right). Reprinted from [102] with permission.

Polyurethanes are susceptible to hydrolytic degradation of the ester, urea, and urethane functional groups within their backbone. In pharmaceutical products, carboxylate esters, carbamate and amide groups are most susceptible to hydrolysis [105]. Scissions of carboxylic ester groups leave behind shorter chains capped with carboxyl and hydroxyl groups. These carboxyl groups can lead to increased and autocatalytic hydrolysis [106], [107]. Due to these issues, polyurethanes intended for cardiovascular applications have had significant issues due to their poor hydrolytic stability *in vivo* [108]–[110]. The chemical structures for the three resin components that will be focused upon in this thesis are shown in Figure 1-8. As indicated in red, there are a number of carboxylic esters present in these compounds that could potentially be susceptible to hydrolytic degradation. In polymers, hydrolysis typically leads to a reduction in molecular weight, polymer embrittlement, and reduction in failure stresses and strains [111]–[113]. It can also affect the glass transition temperature of the polymer due to the induced changes in chemical structure [114], [115].

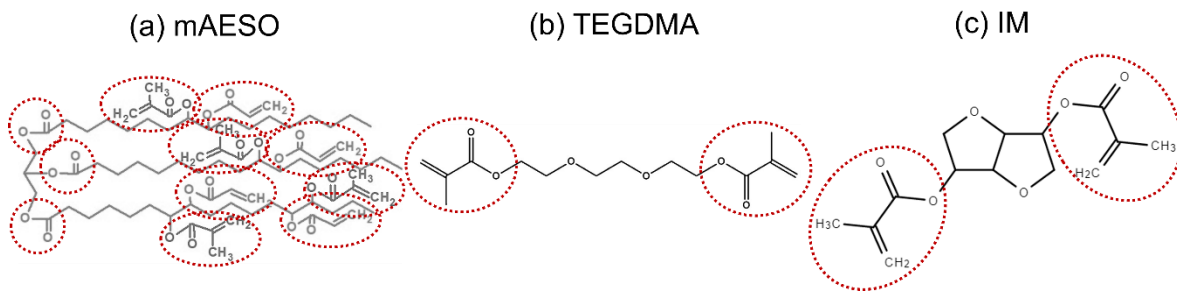


Figure 1-8 – Chemical structures for (a) methacrylated AESO (mAESO), (b) triethylene glycol dimethacrylate (TEGDMA), and (c) isosorbide methacrylate (IM). Functional groups susceptible to degradation (esters) are highlighted in red. Image created by author.

In addition to hydrolysis, swelling due to water absorption can also have significant impacts on mechanical performance of biomaterials. Swelling is determined as a change in volume caused by infiltration of solvent molecules into a material matrix [116]. This behavior can be described by the Flory-Rehner equation displayed below:

$$-[\ln(1 - v_2) + v_2 + x_1 v_2^2] = V_1 n \left(v_2^{\frac{1}{3}} - \frac{v_2}{2} \right) \quad \text{Equation 1}$$

where v_2 is the polymer volume fraction in the equilibrium swollen polymer mass (ratio of polymer mass to polymer swollen mass), V_1 is the solvent's molar volume, n is the number of polymer chains crosslinked at both ends, and x_1 is the Flory solvent-polymer interaction term [117], [118]. This theory states that increased crosslinking density will decrease the amount of swelling expected. In rubbers, the elastic modulus should decrease inversely to the inverse cube of the swelling rate [117]. This equation postulates that if no degradation occurs due to hydrolysis, a state of swelling equilibrium should be reached. The energy required to mix the polymer and solvent is dependent on the elastic free energy required to expand the polymer matrix and the mixing free energy which is dependent on the v_2 and n from Equation (1) [117]–[119]. Therefore, if the polymer resists expansion, more energy is required, which is dependent on the crosslinking density of the polymer chains.

Based on this theory, swelling of polymers is the balance of the polymer chain entropy and the enthalpy of mixing. As polymer chains rearrange and stretch due to swelling, elastic retractive forces are developed within the matrix to balance the decreased chain entropy [116]. This phenomenon leads to a weak mechanical force being applied to the material due to the effects of swelling. In synthetic soft

polymers, this has led to buckling, delamination, wrinkling and bending of thin materials [120]. Exposure to different solvents can also lead to environmental stress and osmotic cracking, in which a polymer will fail at weaker stresses than expected [121]–[123]. Recent research has also shown that tensile forces that develop in materials due to swelling can be sufficient to facilitate bond cleavage in the polymer chain [116], [124], [125]. These effects of swelling are all mechanisms that can work to decrease the mechanical properties of polymers exposed to solvents.

Compositing polymers has been shown to decrease the effects of hydration [102], [126], however they are typically still susceptible to the effects of water uptake. As solvents are absorbed into the composite, they will either bond and react with the polymer chains or they will occupy free space at the matrix-filler interface [126]–[128]. Due to this, water absorption typically reduces the interface bond strength between the matrix and fillers. In fiber-reinforced composites, this has led to reduced interlaminar strength, and flexural strength and modulus [126]. In a study on glass fiber-reinforced composites, osmotic cracking in the matrix and at the matrix-filler interface was observed (Figure 1-9) [129]. Interfacial debonding and cracking is likely caused by differential swelling and is aided by stress concentrations that may be forming in these areas [129], [130]. Increased temperature has been shown to increase the rate of moisture absorption in glass-fiber epoxy composites [131]. Introducing nanoscale fillers should reduce swelling effects as it should reduce the available void space and increase the interfacial bonding of the composites [126], [132].

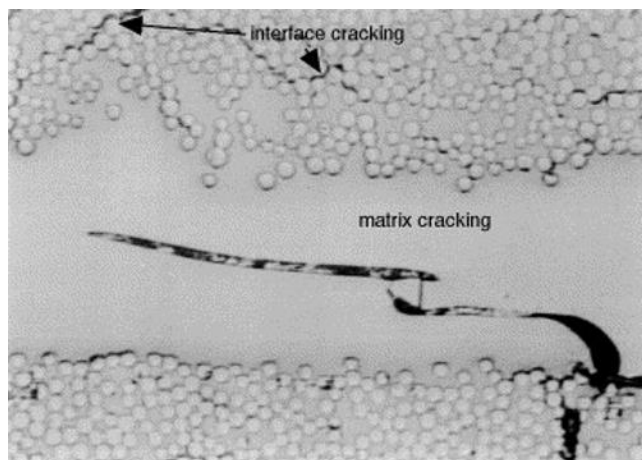


Figure 1-9 – Osmotic cracking at the matrix and filler-matrix interface in a polyester: glass fiber composite exposed to hydrothermal aging. Microscopic image with 100x magnification. Reprinted from [129] with permission.

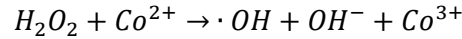
1.4.3 Oxidative Degradation

Oxidative degradation is the degradation of macromolecules by reactions with oxygen and free radicals at a material's surface [133]. Typically, these reactions function to remove hydrogen atoms from the polymer chains [133], or break carbon-carbon bonds [134]. Carboxylic acid, carboxylate ester, hydroxyl, unsaturated hydrocarbon, amide, and amine groups are the prominent functional groups susceptible to oxidation [105]. As highlighted in the above section, the polymers used in this study all contain ester functional groups which are susceptible to oxidative degradation (Figure 1-8). While isosorbide methacrylate has the susceptible groups, it has been shown to act as an antioxidant in polyurethane foams [135].

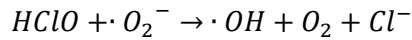
This degradation process can be initiated by the body in its warmer environment, and is caused *in vivo* by cellular metabolism from macrophages, neutrophils, osteoclasts, and giant cells in the body. These cells release superoxide anion radicals, hydroxyl radicals, hydrogen peroxide (H_2O_2), and singlet oxygen, which are known as reactive oxygen species (ROS), as metabolic byproducts [136], [137]. While ROS are formed by all cells in the body, they are specifically released by innate immune cells to target pathogens and foreign substances, such as an implanted biomaterial [138]. When immune cells are responding to a disturbance in their environment, they typically cause inflammation and elevated levels of reactive oxygen species to create an environment of oxidative stress [139], [140].

Oxidation can cause polymer mechanical properties to be significantly degraded. Scissions in a polymer's backbone and the formation of secondary crosslinking in the chain can cause embrittlement and surface softening of the material [133]. Embrittlement can occur due to preferred degradation of the amorphous regions of the polymer, correlated with the different phase densities [141]. This process can also cause fine cracking at the surface and introduce defects into the bulk material. Similar polymer responses are generated from oxidative degradation as seen from hydrolytic degradation. Typically, the effects of oxidative degradation can be quantified by reduced molecular weight, changes in chemical structure (as determined by Fourier transform infrared spectroscopy), decreased modulus, fracture strain, and impact resistance, and changes in the visual characteristics of the material (i.e., discolorations) [133], [142]. The impact and rate of oxidative degradation are dependent on the polymer's structure, morphology, and degree of crystallinity, and the number of double bonds present, respectively [133].

The hydroxyl radical is the most reactive ROS generated *in vivo* [143]. As such, it is typically used to mimic oxidative stress *in vitro*. It can be generated experimentally using less reactive ROS, such as H₂O₂, water, and a catalyst, typically iron *in vivo* [144]–[147]. In this study, a Fenton-like reaction pathway with a cobalt catalyst was used [144]:



The hydroxyl ROS will continue to react with the hydrogen peroxide to form superoxide anion radicals. These $\cdot O_2^-$ ROS will continue to react with H₂O₂ to form more hydroxyl radicals or will react with the cobalt ions to regenerate their original state. This reaction will continue until the hydroxyl ROSs are consumed. Another mechanism to mimic degradation *in vitro* is hypochlorous acid (HClO) which is produced in the *in vivo* myeloperoxidase reaction of activated neutrophils [148]–[150], and obeys the following reaction pathway:



In addition to the ROS generated by the immune response, osteoclasts will produce superoxide anion ROS to assist with bone resorption.

It is very difficult to fully mimic *in vivo* conditions *in vitro* due to the complexities of the various systems involved. Often, a high concentration, 20%, of hydrogen peroxide is used to test oxidative degradation of biomaterials [75], [151]–[156]. This concentration was originally used by Schubert et al. to mimic the oxidative environment of cell-material interfaces at an accelerated rate [153], [156]. This results in a molar concentration $\sim 10^3$ to 10^6 higher than what is expected in biological systems (Figure 1-10) [157]–[159]. Neutrophils cultured in isolation have been shown to generate μM concentrations of hydrogen peroxide [160]. Likewise, hypochlorous acid concentrations have been reported up to $\sim 50 \mu M$ *in vivo* [161]. Very few polymeric systems have shown relevant degradation results at these low concentrations [158]. As well, *in vitro* models need to account for the multitude of ROS species that are generated *in vivo* through one generated ROS. For example, the concentration of H₂O₂ has been found to have no influence on the HClO reaction pathways *in vivo* [148]. Therefore, extreme, or accelerated degradation conditions are often used to determine a biomaterial's potential for oxidative degradation.

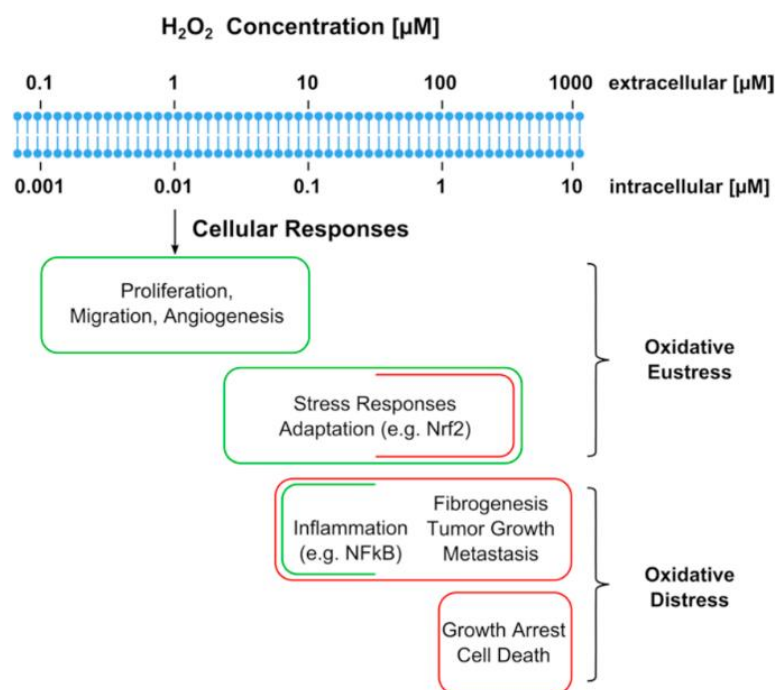


Figure 1-10 – Estimated hydrogen peroxide ROS concentration generated from cellular response to cause oxidative stress. Concentration in a normally functioning liver is highlighted by the arrow. Oxidative eustress represents normal physiological stress levels, while oxidative distress represents conditions caused by pathogens or inflammatory responses. Reprinted from [157] with permission.

Chapter 2

Objectives and Hypotheses

2.1 Objectives

The overall aims of this project were to develop testing protocols and determine the behavior of our 3D printable biopolymer nanocomposites under physiologically relevant conditions. To do this, the following objectives were defined:

- 1) Design a protocol to enable controlled, physiological temperature during mechanical testing using our lab's Psylotech uTS system.
- 2) Evaluate the mechanical properties (tensile and compressive) of WCBSL nanocomposite biomaterials under simulated physiological conditions (hydrated and 37 °C), and after exposure to oxidative degradation.
- 3) Enable controllable degradation behavior in the nanocomposite biomaterials through material modification.

Achieving these objectives would contribute to the WCBSL's overall goal of developing three-dimensional (3D) printable HA-biopolymer composites for skeletal reconstruction applications.

2.2 Hypotheses

It was hypothesized that exposure of biopolymer nanocomposite biomaterials to 37 °C would decrease their stiffness and strength. This phenomenon of decreasing mechanical properties with increasing temperature is commonly observed in polymers [85]–[88] due to their viscoelastic nature. A biomaterial with a similar composition to the ones used in this study had a T_g of 89 °C [55]. While this is higher than body temperature, the viscoelastic transition range that surrounds the T_g is typically quite wide in nanocomposites.

When investigating the effects of hydration on these materials, a variety of potential responses were considered. It was predicted that water absorption, and resulting swelling behavior, would occur in these composites as ≥ 50 vol% of the materials are composed of hydrophilic materials [93], [162]. Swelling in materials causes internal deformations and stresses to develop in the bulk material and can

result in the reduction in mechanical properties, features such as surface stress cracking, and in severe cases material failure [116], [163]. Other consequences of sample hydration can include weakened matrix-filler interactions and hydrolysis [57]. It was therefore hypothesized that incubation in PBS would decrease the mechanical performance of the biopolymer nanocomposites.

It was hypothesized that some effects due to oxidation would be observed, such as reduced mechanical strength and mass loss. This is due to the functional groups susceptible to degradation that are present in the polymers used in this study (Figure 1-8). Finally, the effects of altering the resin composition with isosorbide methacrylate (IM) on water absorption and oxidative degradation were studied. It was hypothesized that IM would reduce the effects of oxidation as it had previous been shown to act as an antioxidant in polyurethane foams [135].

Chapter 3

Materials and Methods

3.1 Materials

Materials used for the synthesis of the functionalized monomers included acrylated epoxidized soybean oil (AESO; molecular weight (MW) = 1200 g/mol; Sigma-Aldrich, Oakville, Canada), D-Isosorbide (Alfa Aesar, Stoughton, USA), methacrylic anhydride (MAA; Sigma-Aldrich), 4-(dimethyl amino) pyridine (DMAP; Alfa Aesar), sodium hydroxide (Sigma-Aldrich), and acetone (Sigma-Aldrich). Other resin components such as triethylene glycol dimethacrylate (TEGDMA) and phenylbis (2,4,6-trimethylbenzoyl) phosphine oxide (Irgacure 819) were sourced from Sigma-Aldrich. Hydroxyapatite (HA) powder was sourced from MKNano Inc. (Mississauga, Canada). This calcium-deficient HA is in the nanoscale with average rod-shaped dimensions of 120 nm long and 30 to 40 nm wide. It has a specific gravity of 2.92, a crystallinity index of 0.52 to 0.54, and a Calcium to Phosphorus ratio of 1.52 [164]. Incubation solutions were produced from phosphate-buffered saline (PBS; 10x; Fisher Bioreagents, Pittsburgh, USA), hydrogen peroxide (H₂O₂; 30 vol%; Sigma-Aldrich), cobalt(II) chloride hexahydrate (Sigma-Aldrich), acetic acid (> 99.7%, Sigma-Aldrich), and bleach (6 % w/v sodium hypochlorite; LAVO PRO6 commercial bleach; Montreal Canada).

3.2 Ink Preparation

3.2.1 Synthesis of Methacrylate Functionalized Monomers

Methacrylation of D-Isosorbide and AESO was performed to create mAESO and IM. This was done with the methods demonstrated by Liu et al. [55], [81]. mAESO was synthesized by the reaction of 0.004 mol of DMAP, 0.25 mol of MAA, and 1.5 mol of AESO. This reaction was allowed to react under continuous stirring, 500 rpm, for 24 hours at 60 °C. Post-reaction, the solution was washed twice each with 0.5 M sodium hydroxide solution, DI water, and acetone. The resultant mAESO was dried under reduced pressure in a desiccator. IM was synthesized by the reaction of 0.2 mol D-isosorbide, 0.01 mol DMAP, and 0.6 mol MAA. This reaction occurred for six hours at 60 °C under continuous stirring of 300 rpm. The reacted solution was washed twice each with 0.5 M sodium hydroxide solution and DI water. The resultant IM was then dried under reduced pressure in a desiccator chamber.

3.2.2 Ink Processing

To create the resin component of the ink, the desired volume fractions of mAESO, TEGDMA, and IM (if required) were combined in a glass beaker. These components were mixed using a Branson Sonifier ultrasonic homogenizer (Emerson Inc., USA) for two minutes until the mAESO visually dissolved in the less viscous TEGDMA. Irgacure 819 was used as the photo initiator in these materials to enable UV curing. It was included at 1 vol% of the resin component of the ink and was dispersed into the ink using the ultrasonic homogenizer for five minutes. For the HA-containing inks, HA powder was dispersed slowly into the resin with frequent ultrasonic homogenization. Prior to inclusion, the HA powder was heated at 120 °C for an hour to remove bound water and thus reduce agglomerations in the resulting ink [77]. Directly prior to printing, the inks were mixed twice for five minutes with the ultrasonic homogenizer, and three minutes with a mechanical homogenizer (Unidrive X 1000, M. Zipperer GmbH, Germany). Inks were stored under reduced pressure in a desiccator chamber to reduce the potential of water absorption into the inks. Samples were generated from one batch of ink for each composition.

3.2.3 Ink Compositions and Nomenclature

Four material compositions were used in this thesis. Two resin compositions were investigated with varying amounts of TEGDMA and IM. A resin-only ink and a 10 vol% HA composite were trialed for each resin composition. The ratio of the resin components was kept constant between the 0 and 10 vol% HA materials within the resin phase of the composite. The compositions and nomenclature of these inks are summarized in Table 3-1.

Table 3-1 - Nomenclature and composition of the four material compositions investigated.

Nomenclature	mAESO (vol%)	TEGDMA (vol%)	IM (vol%)	HA (vol%)
MT_0HA	50	50	0	0
MT_10HA	45	45	0	10
MTI_0HA	50	20	30	0
MTI_10HA	45	18	27	10

3.3 Processing Conditions: Masked Stereolithography

To create the specimens used in the characterization experiments, the inks were 3D printed using mSLA technology. A Sonic XL 4K printer from Phrozen Technology (Taiwan) was used to print the nanocomposites (Figure 3-1 (a)). Three different geometries were printed for different tests (Figure 3-1 (b)): dog-bone tensile test specimens (width and height = 2.5 mm; gauge length = 10 mm), cylindrical compression test specimens (length = 5 mm; diameter = 2.5 mm), and beams for dynamic mechanical analysis (DMA) tests (length = 120 mm; width = 12 mm; thickness = 3 mm). 3D models of these specimens were sliced with layer heights of 50 μm using Phrozen 3D (Phrozen Technology). To reduce any defects in the gauge length, tensile specimens were printed with support materials to elevate the gauge length from the print bed. During printing, the initial six layers of each print were cured for 15 seconds with the following layers being cured for six seconds each. These print settings have been determined previously to ensure sufficient layer curing and build plate adhesion. After the specimens were 3D printed, they were removed using a razor blade and rinsed with 100 vol% ethanol. To ensure thorough curing, samples were post-print cured with a CureZone MKII curing chamber (Creative CADWORKS, Concord, Canada).

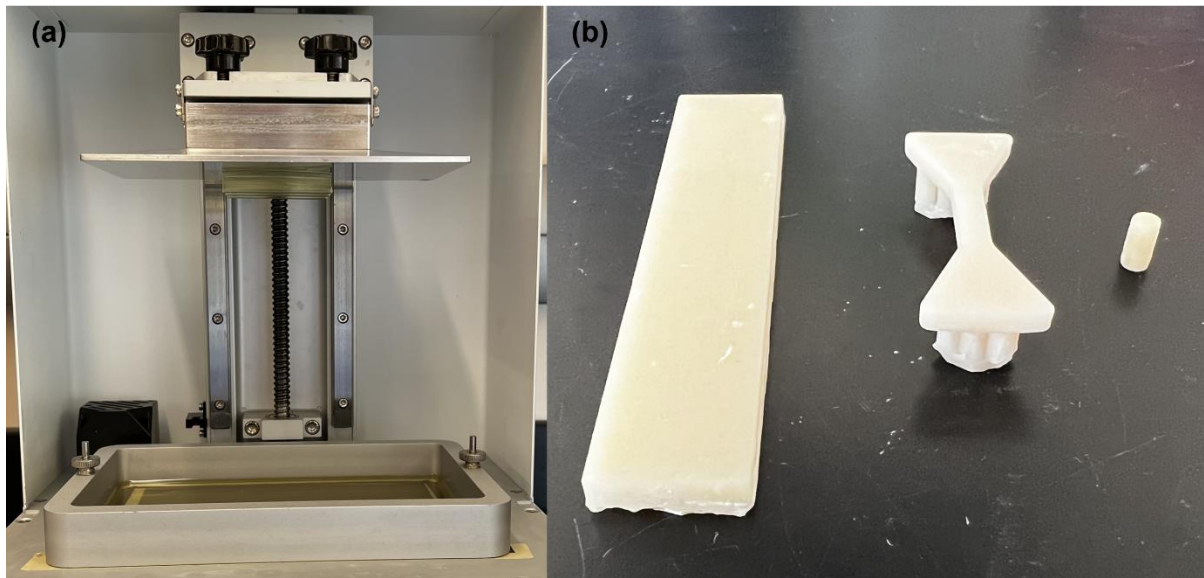


Figure 3-1 – (a) Phrozen Sonic XL 4K printer, and (b) 3D-printed beam, dog-bone, and cylindrical (left to right) MT_10HA specimens.

3.4 Material Incubation

To induce physiologically relevant conditions and oxidative degradation, tensile and compression specimens were incubated in different media at 37 °C. Various time points were investigated, with focus on 0-, 14-, and 28-day time periods. All media was changed daily to ensure consistency and continuous oxidative reactions. The ratio of media volume to specimen weight was kept constant at 10 mL : 1 g, as suggested in ISO 10993-13 [165]. Specimens incubated for mechanical testing were incubated in bulk, while the specimens used in the mass change (swelling) experiments were incubated individually.

3.4.1 Phosphate-buffered Saline

PBS was used as the media to investigate the effects of a physiologically relevant, wet environment. This is a commonly used reagent in biological experiments due to its stability, buffering capabilities, and non-toxicity. 10x PBS was diluted to 1x with DI water.

3.4.2 Hydrogen Peroxide

H₂O₂ solution was used as one of the oxidative degradation media. 30 vol% of H₂O₂ was diluted to 20 vol% with DI water. 0.1 M cobalt(II) chloride was added in an exothermic reaction. The Co²⁺ ions create a Fenton-like reaction with the H₂O₂ to create reactive hydroxyl radicals.

3.4.3 Hypochlorous Acid

Hypochlorous acid was created by combining acetic acid and sodium hypochlorite. A 5% acetic acid solution was created through dilution of 100% acetic acid with DI water. Acetic acid, sodium hypochlorite, and DI water were combined following the volumetric ratio of 1:3.19:46.87 developed at the WCBSL for other degradation work. This resulted in a 5 mM hypochlorous acid solution. The pH of the solution was measured during degradation of MT_10HA specimens using an electrochemistry meter (Orion VersaStar Pro, Thermo Scientific, Massachusetts, USA) and ranged from 7.85 to 7.47 over a 24-hour incubation period.

3.4.4 Testing Condition Nomenclature

To represent the different incubation and material testing conditions used, the following references will be used. ‘RT’ will represent room temperature mechanical testing conditions, otherwise it should be assumed that testing took place under 37 °C. Incubation media will be referred to with PBS, H₂O₂, and HClO denotations, with all other testing occurring under assumed dry conditions. The period of incubation will be referred to with ‘D14’ or ‘D28’ for 14- and 28- day incubations, respectively.

3.5 Characterization of Physical and Chemical Properties

3.5.1 Dynamic Mechanical Analysis

Dynamic mechanical analysis (DMA) was performed using a dual cantilever DMA Q800 (TA Instruments, Delaware, USA). 3D-printed beams were used (Figure 3-1 (b)). A multi-frequency strain test with a ramp rate of 3 °C/min was used from 25 to 150 °C. Analysis of data was performed using Universal Analysis (TA Instruments). Two methods of determining glass transition temperature were employed: the onset temperature in which the storage modulus decreases from its glassy state, and the peak temperature occurring in the tan delta curve [166]. Determining the onset of the storage modulus decrease provides a good marker of the lower range of temperatures in which a material’s mechanical properties will be impacted [166]. The most common method to determine T_g includes taking the peak value of the tan delta plot [166]. The peak of the tan delta curve indicates the point at which the material has the most viscous deformation response.

3.5.2 Mass Change

The dog-bone specimens were measured throughout the incubation periods to measure their mass changes. Prior to incubation, samples were measured to record their initial mass. Samples were measured on a Mettler Toledo scale (AB54-S; Columbus, USA) with 0.0001 g precision. Original dry masses of the samples ranged from 0.67 to 1.03 g, depending on their composition. The samples used in this experiment were incubated in individual 15 mL tubes to have independent measurements of n = 3. Throughout the incubation period, ‘wet’ mass measurements were taken on days 1, 3, 5, 7, 14, 21,

and 28. Then, the surface of the samples was patted dry prior to weighing. After the incubation period (D14 or D28) was completed, samples were dried under reduced pressure in a desiccator chamber for one week followed by 48 hours in a 60 °C oven. Their final, ‘dry’ mass was then measured. To determine percentage mass changes, Equation (2) was used.

$$\text{Mass Change (\%)} = \left(\frac{\text{New Mass [g]} - \text{Original Mass [g]}}{\text{Original Mass [g]}} \right) (100 [\%]) \quad (\text{Equation 2})$$

From the wet mass measurements, degradation rates were calculated by dividing the change in mass by the number of days of incubation.

3.5.3 Volume Change

Volume changes were calculated from the compressive cylindrical samples. Independent measurements were taken of each experimental group as they were measured prior to compressive testing. The length and diameter of each cylinder was measured using calipers (Mastercraft, Vonore, USA), and the volume of the samples was calculated. Percent volumetric change was calculated using the same method as in the mass change experiment (Equation 2).

3.5.4 Wettability

Prior to wettability experiments, isopropanol was used to clean and de-grease the samples using an ultrasonic homogenizer [167]. The flat top section of the dog-bone specimens was used as the testing surface. 20 µm droplets of DI water were placed using a pipette onto the surface and imaged using a Canon Rebel T7i camera (Brampton, Canada) with an EFS 55-250 mm macro lens. Images were analyzed using ImageJ (U.S. National Institute of Health, Bethesda, USA) and the ‘Contact Angle’ plugin (Marco Brugnara). This plug-in calculates the angle between the surface and the water droplet using the sphere approximation (Figure 3-2).

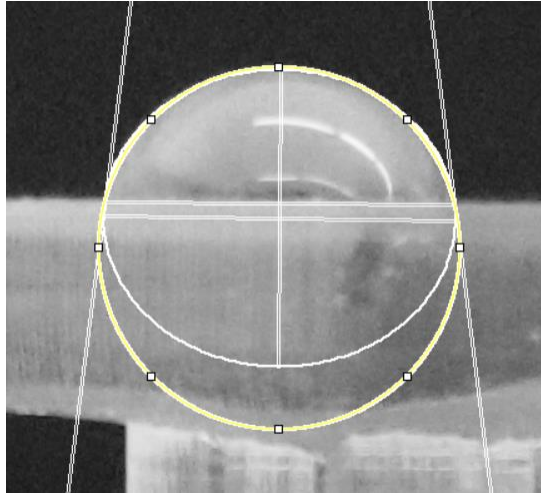


Figure 3-2 - Water droplet on MTI_0HA sample being analyzed by the Contact Angle plug-in in ImageJ.

3.6 Imaging of Surface Characteristics

3.6.1 Digital Macroscopy

For each experimental group, digital images were taken of their surfaces after the incubation period was completed. Samples were patted dry with a KimWipe and imaged using a Canon Rebel T7i camera (Brampton, Canada) and macroscope (LMscope T2 Extension 50mm, LM Macroscope, Austria). The top and bottom surfaces (oriented as in Figure 3-1 (b)) of the dog-bone gauge lengths, and the flat ends of the compression testing specimens were imaged. Visible features (i.e., pits, cracks) were analyzed using the ‘Freehand selections’ and ‘Measure’ tools in ImageJ to determine average feature areas (n = 5).

3.6.2 Scanning Electron Microscopy

Scanning electron microscopy (SEM) conducted using a LEO FE-SEM 1530 (ThermoFisher Scientific, USA) was used to image the surfaces of the dog-bone samples, prior to and following degradation in different media at 14 days. Prior to imaging, samples were cleaned in 100% ethanol for 1 minute with

an ultrasonic homogenizer to remove any remnants of dried incubation media. Secondary electron mode was used.

3.7 Characterization of Mechanical Properties

3.7.1 Mechanical Testing at Physiologically Relevant Conditions

Mechanical testing was performed using a Psylotech μ TS mechanical testing machine with a 1.6 kN load cell (Psylotech Inc., Evanston, USA). This system is typically run under room temperature conditions. To enable mechanical testing under physiologically relevant conditions, two conditions were added to the testing protocol. The first was testing at 37 °C. This was enabled through a Zoo Med Laboratories (San Jose, USA) heat lamp set at a defined distance from the testing grips (Figure 3-3). Throughout testing, the temperature at the grips was measured using an infrared forehead thermometer (SEJOY, Hangzhou, China). The temperatures in this area ranged from 37 ± 2 °C.

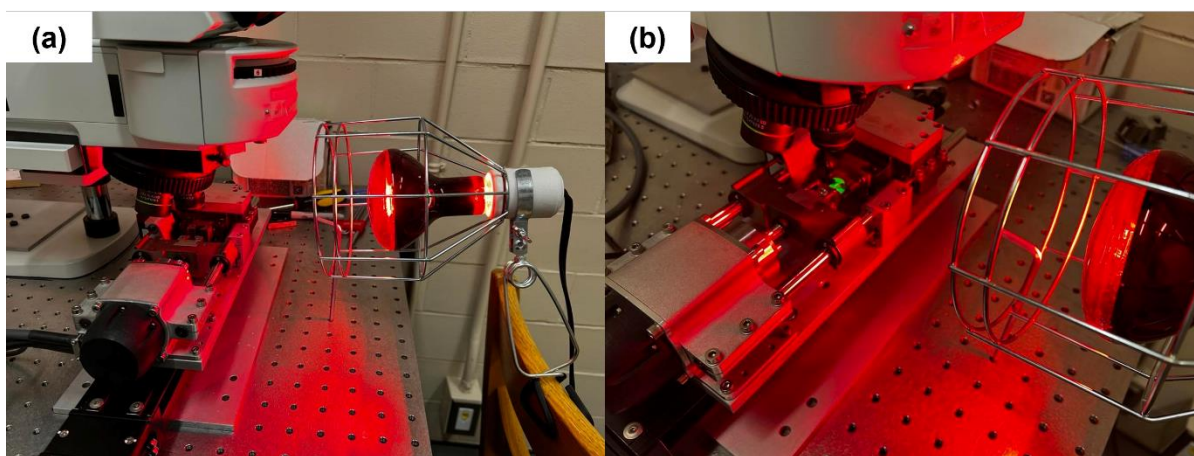


Figure 3-3 – Psylotech mechanical testing machine heated to 37 °C with an IR heat lamp from (a) side and (b) top view. The green light in (b) highlights the grip area of the machine where temperature readings were taken.

The testing machine was heated prior to testing for one hour to allow the load and displacement transducers to stabilize at the increased temperature. Under room temperature conditions, the load cell spanned a range of 35 μ N when unloaded. Under 37 °C, the load cell typically had an ~ 6 N decrease

before stabilizing within a range of 50 μN when unloaded (Figure 3-4). Samples were kept in a 37 °C oven prior to testing. To test hydrated samples, samples were kept in their incubation media until immediately prior to testing where they were patted dry with a Kim Wipe (to protect the machine from liquid and possible corrosion) and installed in the grips.

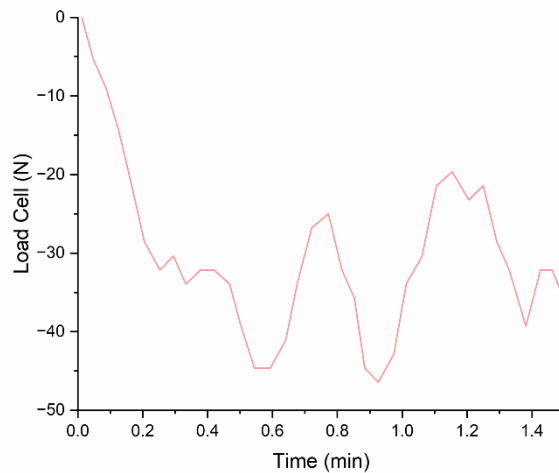


Figure 3-4 – Representative plot of load cell drift over time of the Psylotech under 37 °C conditions (after ~ 1 hr of heating with IR lamp). Immediately prior to this data, the load cell was zeroed with the grips unloaded.

3.7.2 Tensile Testing

Dog-bone tensile specimens (Figure 3-1 (b)) were tested on the Psylotech mechanical testing machine. Sample dimensions and testing strain rates followed ASTM D3039 [168]. Samples were pre-loaded to ~ 10N in the grips and exposed to preconditioning cycles at 1 Hz for 100 cycles. Prior to testing, they were speckle coated with a high-resolution toner powder (Xerox Phaser 6000). This provided a contrasting speckle pattern for digital image correlation (DIC). Images were taken with a Point Grey digital camera (Point Grey Research, Inc, Richmond, Canada; 5 MP, 2/3" detector) through a microscope (BXFM, Olympus Corp., Center Valley, USA; 2.5x magnification objective lens) at a sampling rate of 5 Hz (in sequence with the sampling rate of the load cell data). The specimens were loaded under displacement control at a rate of 16 $\mu\text{m/s}$ to induce a strain rate of 0.0016 s^{-1} until specimen failure.

To determine the displacement and strain occurring in the material throughout testing, DIC was performed using Vic 2D v6 (Correlated Solutions Inc., Columbia, USA). Transverse and axial strain measurements were determined using the ‘extensometer’ tool. Average engineering stress (σ_{ENG}) was calculated using Equation (3). Initial areas (A) were calculated from measurements of the width and height of the gauge length measured using a micrometer taken prior to testing, and force (F) was measured by the load cell.

$$\sigma_{ENG} = \frac{F}{A_{initial}} \quad (\text{Equation 3})$$

As DIC enabled transverse and axial strain measurements throughout testing, the true stress and strain could be determined. True strain (ϵ_{TRUE}) was calculated using Equation (4) with current and initial lengths (l) determined from the axial extensometer readings.

$$\epsilon_{TRUE} = \ln \left(\frac{l}{l_{initial}} \right) \quad (\text{Equation 4})$$

Transverse strain measurements were assumed to be the same as strains in the z-direction (the side the camera would not capture), due to Poisson’s ratio. Therefore, true stress (σ_{TRUE}) could be calculated using Equation (3) and substituting the initial area with the true area at each force data point. The elastic modulus (E) was calculated from the slope of the stress-strain curve in the elastic portion of the curve. This typically was 0 – 0.3% strain, however occasionally the first few data points had to be excluded due to errors from the toe region or testing setup artifacts. Ultimate tensile strength was determined as the maximum stress value reached prior to failure. Yield strength was determined using a 0.2% offset line with a slope = E . Yield strength was only measurable if the stress-strain curve proceeded from the linear elastic region and began to deflect and curve. Toughness measurements for each sample were determined from the area under the true stress-strain curve until the point of fracture. Poisson’s value (ν) was calculated from the axial and transverse strains, using Equation (5).

$$\nu = - \frac{\Delta \epsilon_{transverse}}{\Delta \epsilon_{axial}} \quad (\text{Equation 5})$$

3.7.3 Compression Testing

ASTM D695 dictates the desired dimensions for compression testing of rigid plastics [169]. It suggests testing cylindrical specimens that are 12.7 mm in diameter and 25.4 mm in length. Due to the limitations of the Psylotech's size, smaller test specimens were designed while maintaining the diameter : length ratio. Samples (2.5 mm diameter, 5 mm length; Figure 3-1 (b)) were used to ensure yielding of the material occurred within a 1 kN upper loading limit. Compressive plates were coated in translucent silicon grease (MG Chemicals, Burlington, Canada) prior to testing to ensure unconfined compression conditions. Specimens were preloaded to ~ 15 N and exposed to 100 cycles of 1 Hz preconditioning cycles with an amplitude of 25 μm . Specimens were loaded under displacement control at a rate of 8 $\mu\text{m/s}$, to maintain the same strain rate used in the tensile experiments. Samples were loaded until failure or the load reached the upper loading limit.

Engineering stress was calculated using Equation (2). Engineering strain was calculated using the crosshead's displacement divided by the sample's initial length. DIC could not be used to calculate the compressive strains as the sample faces were curved. Therefore, the compliance of the universal testing machine needed to be accounted for to gain more accurate stiffness measurements of the samples. To do this, the compression plates were loaded against each other to a preload of ~ 30 N and were compressed 50 μm at a rate of 1 $\mu\text{m/s}$ ($n = 3$). The resulting displacement (δ) and load (F) measurements were used to determine the stiffness (K_p) of the machine using Equation (6).

$$K_p = \frac{\Delta F}{\Delta \delta} \quad (\text{Equation 6})$$

The stiffness of the Psylotech compression plates (K_p) was determined to be $1.49 \pm 0.02 \times 10^4$ N/mm. The experimental stiffness data determined when compressing samples is a combination of the stiffness due to the sample and the stiffness due to the testing plates. These two stiffnesses can be modelled as a series of springs (Figure 3-5).

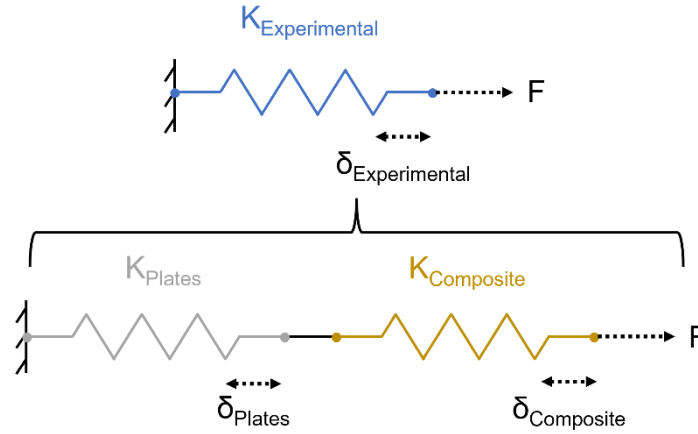


Figure 3-5 - Spring model (in series) of the combined stiffnesses (K) of the Psylotech plates and the composite samples undergoing compressive testing. F represents force and δ represents displacement.

Using this model, the following formula (Equation 7) can be derived to determine the actual stiffness of the composites (K_c) from the experimental stiffnesses (K_{exp}) determined during compression trials.

$$K_c = \frac{K_{exp} K_p}{K_p - K_{exp}} \quad (\text{Equation 7})$$

As the force remains constant throughout the model, the displacement and dependent strains must therefore be modified to account for the differences between experimental and actual stiffnesses. Therefore, the ratio of the experimental and composite stiffnesses was used to determine the corrected strain (ϵ_c) from the experimental strain (ϵ_{exp}) (Equation 8).

$$\epsilon_c = \epsilon_{exp} \left(\frac{K_{exp}}{K_c} \right) \quad (\text{Equation 8})$$

The ratio of $K_{exp} : K_c$ was determined to be 0.87 ± 0.02 ($n = 4$, calculated from MT_0HA and MT_10HA specimens tested under dry, room temperature conditions). The compressive data presented throughout this thesis include the corrected strain measurements. The effect of this compliance correction can be seen in Figure 3-6. As the majority of samples tested in this project did not fail within the loading limits of the Psylotech, only elastic modulus and yield strength were determined. These were calculated as done for the tensile testing results.

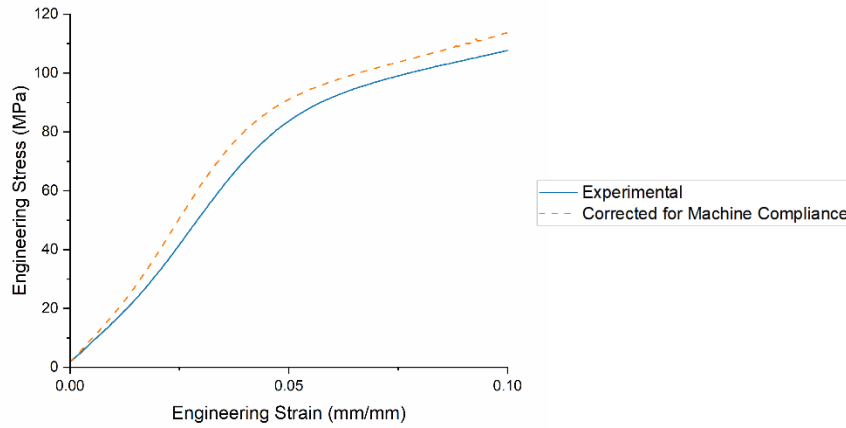


Figure 3-6 - Engineering compressive stress-strain curves of MT_10HA under dry, room temperature conditions with experimental (blue, solid) and corrected for machine compliance (orange, dashed) strains.

3.8 Statistical Analysis

Data are presented as mean $\pm$ standard deviation (SD). Mechanical tests used sample sizes of $n = 5$ and mass studies used $n = 3$. All statistical analyses were performed in OriginPro (Version 2023b; OriginLab Corporation, Northampton, USA). Data were tested for normality using the Shapiro-Wilk test. With large sample sizes ($n > 30$), it is common to observe small, significant deviations from normality. However, these deviations will not affect the results of parametric tests, therefore with sufficient sample sizes, which are present in this thesis, this assumption can be disregarded [170].

Homogeneity of variance was tested with the Levene's and Brown-Forsythe tests. In some of the data (i.e., oxidative degradation experiments with H_2O_2), Levene's test showed that the population variances were significantly different, however the opposite was seen with the Brown-Forsythe test. The Brown-Forsythe test evaluates homogeneity of variance based on the median instead of the mean and is more robust to non-normal data while maintaining statistical power of parametric tests. Therefore, it was evaluated as an acceptable test of the assumption of homogeneity of variance when Levene's test failed.

If the variance and normality assumptions were met, parametric statistical analyses were used to further analyze the data. A variety of two- and three-way ANOVAs with Tukey post-hoc tests were employed depending on the number of relevant factors (i.e., factors = degradation media, HA content, and degradation time in the oxidative degradation study). If homogeneity of variance assumptions were not met, the data was logarithmically transformed to adjust for the skewed distribution before being statistically analysed parametrically. The p-values for each factor from the ANOVA are reported on each figure. In figures which reference control values with lines, the p-values reported are from analyses excluding these references.

Chapter 4

Results

4.1 Effects of Physiological Temperature Conditions

4.1.1 Tensile Properties

Figure 4-1 displays tensile true stress-strain curves for the MT composites tested at room and elevated temperatures. HA inclusion increased mechanical performance, while increased temperature appeared to decrease mechanical performance.

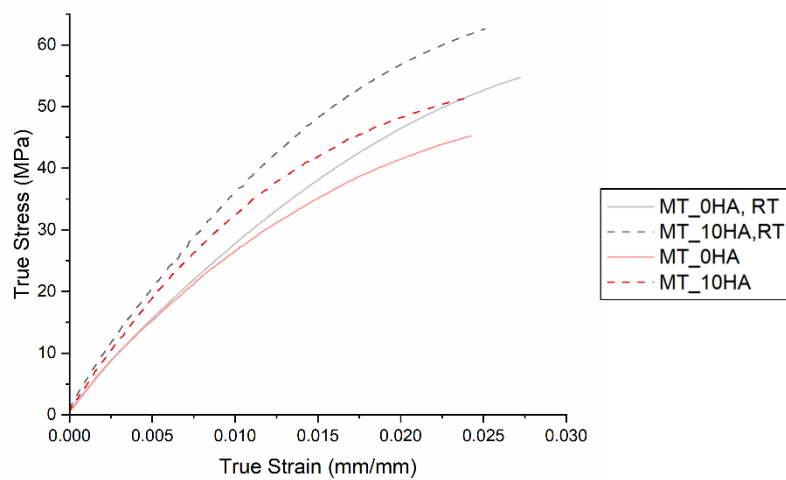


Figure 4-1 - Representative tensile true stress-strain curves for MT_0HA (light, solid) and MT_10HA (dark, dashed) samples with varying temperature: room temperature (RT; grey) and 37 °C (red).

Tensile properties were calculated for each composition/temperature and are recorded in Figure 4-2. Temperature had no significant impact on ultimate or yield strengths of the materials (Figure 4-2 (a) and (b), respectively; $p > 0.10$). The modulus of the composites at RT was increased 35.0 % by HA inclusion (Figure 4-2 (c)). No significant differences were observed in toughness or strain at fracture (Figure 4-2 (d) and (e), respectively). Interestingly, temperature increased the Poisson's ratio of both compositions (Figure 4-2 (f); $p < 0.05$).

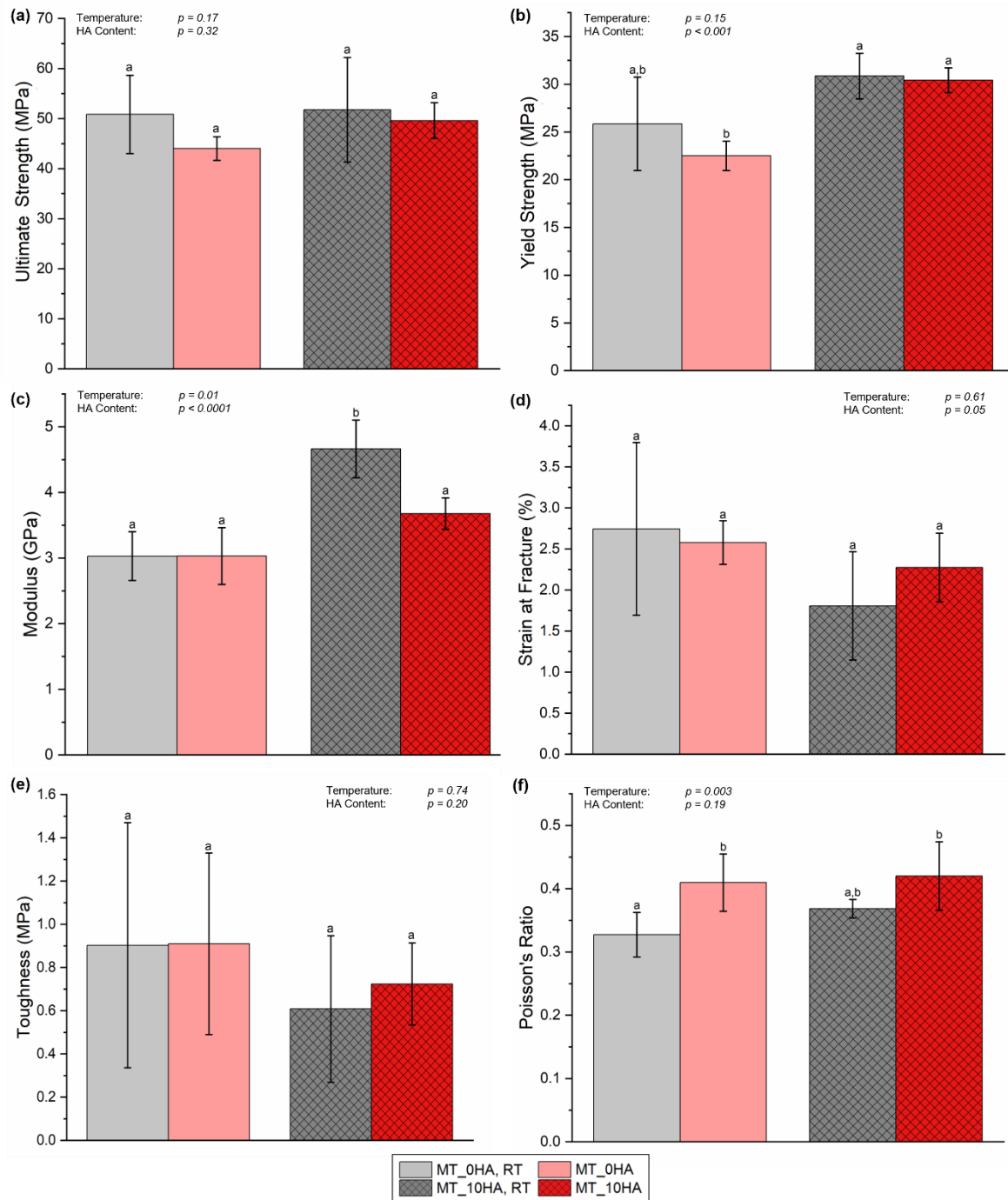


Figure 4-2 – (Previous page) – Tensile properties, (a) ultimate strengths (b) yield strengths, (c) elastic moduli, (d) strains at fracture, (e) toughness's, and (f) Poisson's ratios, of MT_0HA (light, solid) and MT_10HA (dark, cross hatched pattern) samples with varying temperature: room temperature (RT; grey) and 37 °C (red). Data are presented as mean $\pm$ SD (n = 5) with different letters representing statistical significance ($p < 0.05$).

4.1.2 Compressive Properties

MT_0HA and MT_10HA were tested under compressive loading with two different temperature conditions, room temperature and 37 °C. Representative stress-strain curves can be seen in Figure 4-3. It is observed that HA inclusion elevated the mechanical stress at each strain point. Increased temperature appeared to decrease mechanical properties in both composites.

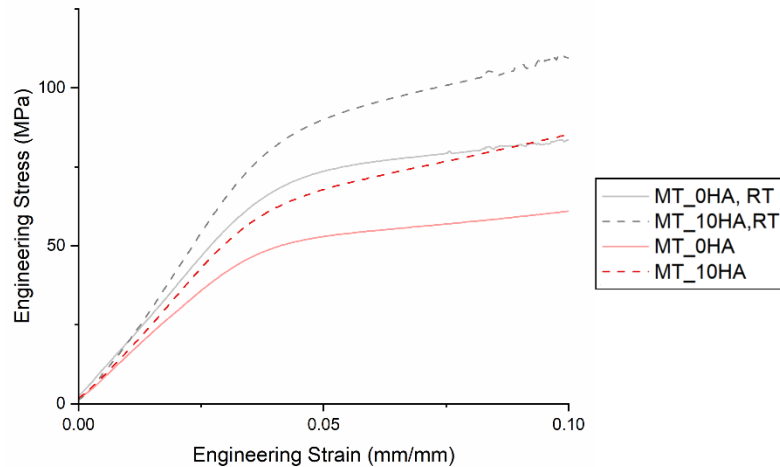


Figure 4-3 - Representative compressive engineering stress-strain curves for MT_0HA (light, solid) and MT_10HA (dark, dashed) samples with varying temperature: room temperature (RT; grey) and 37 °C (red). This figure shows a magnified view of the elastic region. Failure occurred beyond 0.1 mm/mm.

The yield strengths and moduli were calculated for these samples and are summarized in Figure 4-4 (a) and (b), respectively. The inclusion of HA had a consistent and significant increase on yield strength and elastic modulus for both temperature conditions ($p < 0.0001$). In the RT case, yield stress and

modulus increased by 22.5 and 30.5 % with HA inclusion, respectively. In contrast, increased temperature significantly decreased the mechanical properties of the compositions ($p < 0.0001$). In the resin-only composition, yield stress and modulus decreased 29.5 and 15.9 %, respectively. In the 10 vol% HA compositions, yield stress and modulus decreased 26.2 and 20.6 %, respectively. These results show more significant impacts of temperature than seen in the tensile experiments and clearly show that increased temperature has a negative impact on mechanical properties. Therefore, 37 °C was used as the baseline testing temperature for the remaining studies in this thesis.

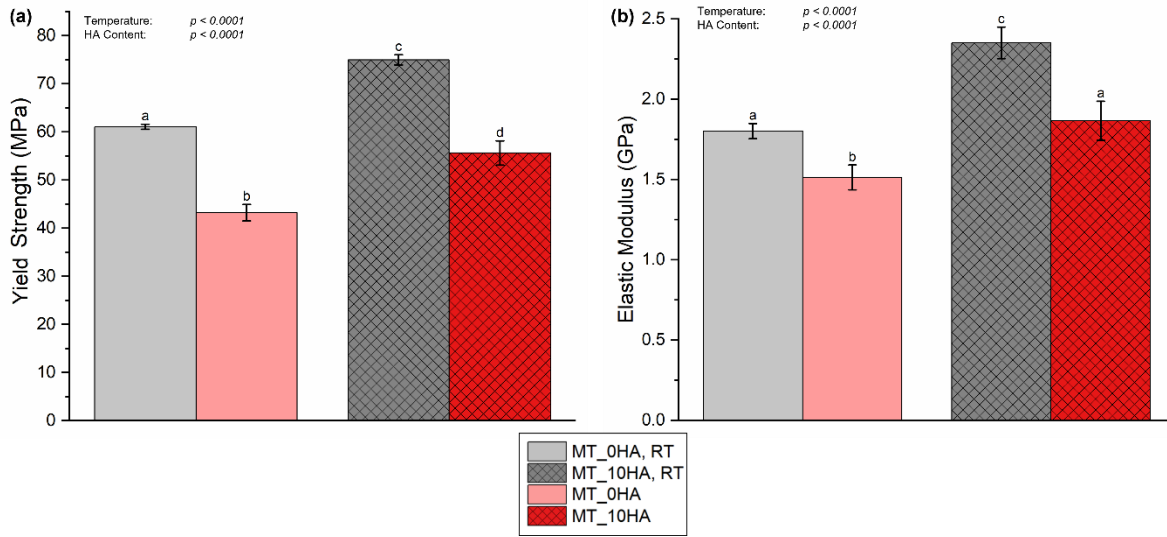


Figure 4-4 – Compressive properties, (a) yield strengths and (b) elastic moduli, of MT_0HA (light, solid) and MT_10HA (dark, cross hatched pattern) samples with varying temperature: room temperature (RT; grey) and 37 °C (red). Data are presented as mean $\pm$ SD ($n = 5$) with different letters representing statistical significance ($p < 0.05$).

4.1.3 DMA Analysis

A DMA analysis was performed on the MT_0HA and MT_10HA compositions. Plots of the storage modulus, loss modulus, and tan delta can be seen in (Figure 4-5). As the glass transition of a polymer occurs over a temperature range (Figure 1-6), a single temperature is typically reported as the T_g as reference. This was done using two methods: the peak temperature of the tan delta curve and the onset of the storage modulus decrease. From the plot of storage moduli, it is clear that the MT_0HA's onset temperature occurs beyond the testing conditions, therefore it is indicated that the transition region for

this material begins below -25 °C. MT_10HA's onset temperature occurs at -7.2 °C. The tan delta curves show a slight peak, around 82 °C, before continuing to increase to their maximum peak (Figure 4-5 (b)). This occurred at 162.4 and 156.4 °C for the 0HA and 10HA compositions, respectively. This data offered interesting observations of the thermal behavior of these materials, however a sample size of only $n = 1$ was used, therefore this data warrants repetition in the future.

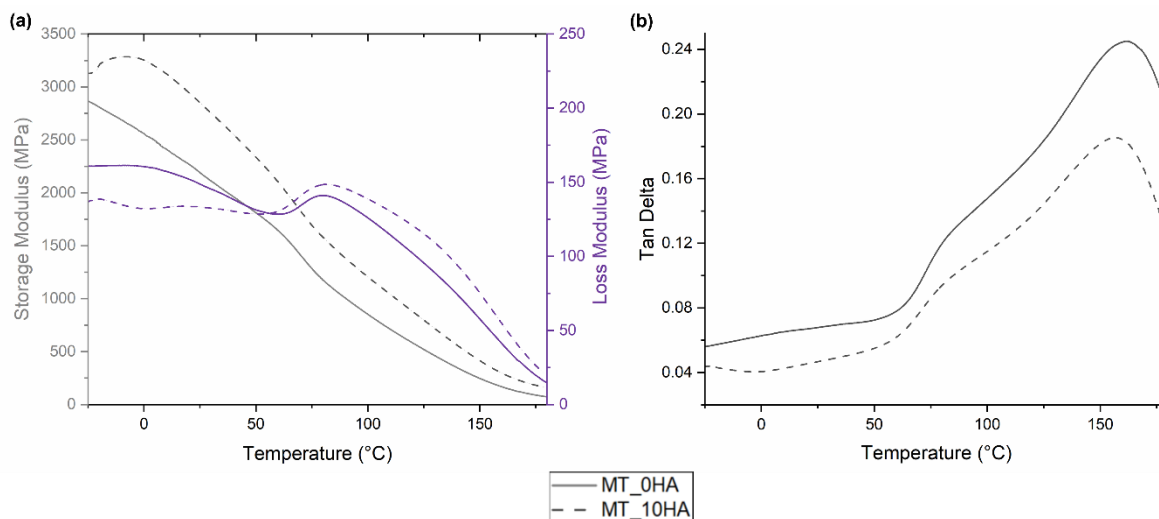


Figure 4-5 – (a) Storage (grey) and loss (purple) moduli, and (b) tan delta of MT_0HA (solid) and MT_10HA (dashed) compositions ($n = 1$). Trends are reported over a temperature sweep of -25 to 180 °C.

4.2 Effects of PBS Incubation

4.2.1 Mass and Volumetric Changes Due to Swelling

MT_0HA and MT_10HA samples were incubated in PBS for 28 days. Throughout the incubation period, their wet mass was measured and calculated as a percent mass change (Figure 4-6). After D0, the wet mass change was significantly ($p < 0.05$) different in the 0 and 10 % HA materials at each time point. After ~ 5 days, the water absorption appeared to plateau at ~ 6 and 5 vol% for MT_0HA and MT_10HA, respectively. No significant differences ($p < 0.05$) within compositions were seen after 5 and 7 days for MT_0HA and MT_10HA, respectively. Therefore, the resin-only material displayed greater swelling behavior when compared to the HA composite and appeared to equilibrate after a week.

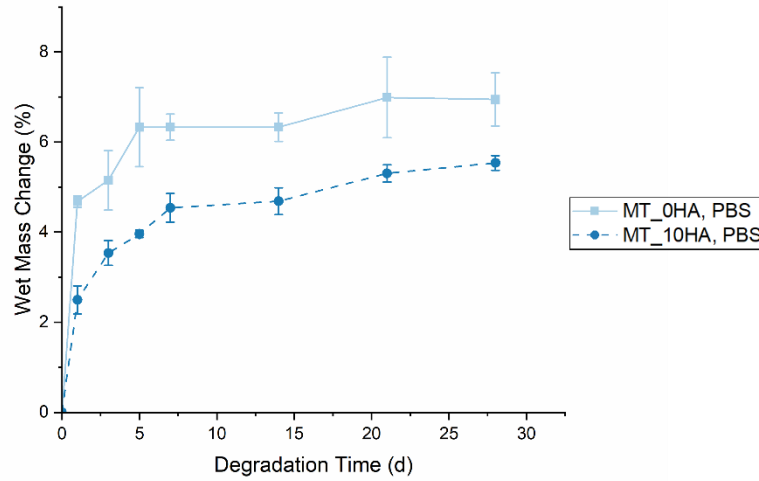


Figure 4-6 – Mass changes, recorded in ‘wet conditions’, due to swelling in PBS medium at 37 °C in MT_0HA (light blue) and MT_10HA (dark blue, dashed) compositions over a 28-day period. Data are presented as mean $\pm$ SD (n = 3).

The percent changes of mass (dry) and volume were also calculated at 14- and 28-day time points (Figure 4-7 (a) and (b), respectively) for the two compositions. The resin-only sample showed a reduction in mass of ~ 1 vol% at both time periods. This was significantly different ($p < 0.0001$) to the HA composite’s behavior which showed no significant changes from the original mass. No significant differences were seen in the volumetric data ($p > 0.05$), which could be accounted for with the dimensional variability present due to the printing process and the use of unpaired measurements. These results, along with those in Figure 4-6, suggest that the inclusion of HA introduced some resistance to water absorption.

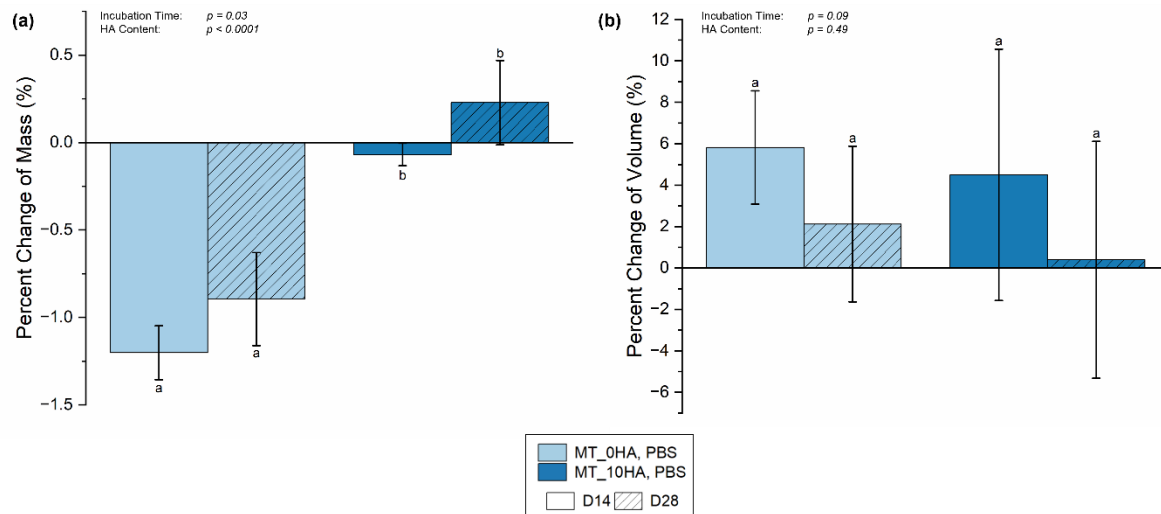


Figure 4-7 - Mass and volumetric changes due to water absorption (PBS, 37 °C) in MT_0HA (light blue) and MT_10HA (dark blue) compositions after 14 (solid) and 28 (hatched pattern) days: (a) percent change of mass in dried samples (n = 3), and (b) percent change of volume in wet samples (n = 5). Data are presented as mean $\pm$ SD with different letters representing statistical significance ($p < 0.05$).

4.2.2 Surface Properties

The bottom side of the dog-bone specimens were imaged after incubation in PBS for 14- and 28-day time periods (Figure 4-8). Images of the compositions in a dry condition, prior to any incubation, have been included as a visual comparison. In the 0 vol% HA materials, by D28 visible cracks were beginning to form along the gauge length of the specimens. No visible differences were observed in the 10 vol% HA composites. Additional macroscopic images of the top side of the dog-bone specimens and compression samples can be seen in Figures A-1 and A-2 (Appendix A), respectively.

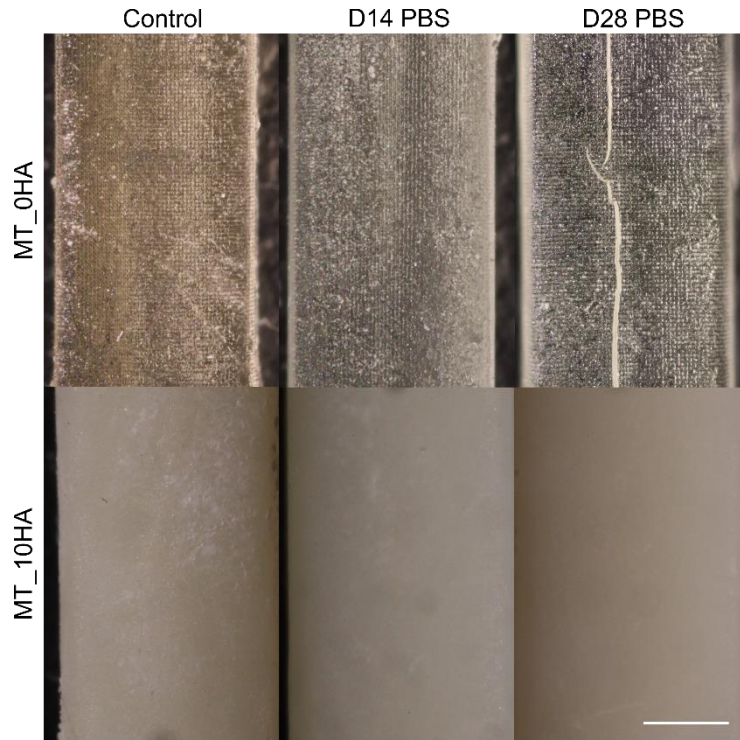


Figure 4-8 – Representative macroscopic images of the bottom surface of dog-bone MT specimens after 14- and 28-day incubations in PBS at 37 °C. Images taken prior to any incubation are shown as a control. The scale bar represents 1 mm.

To complement the macroscopic images, SEM images of the surface of these specimens were taken at 50x and 1000x magnification (Figure 4-9 and Figure 4-10, respectively). The grid pattern that develops due to the mSLA printing method is clearly seen in the 50x magnification images. In the control MT_10HA composite there is visible HA particles on the surface of the sample. After 14 days in PBS, there is clear evidence of reduced surface particles and agglomerations.

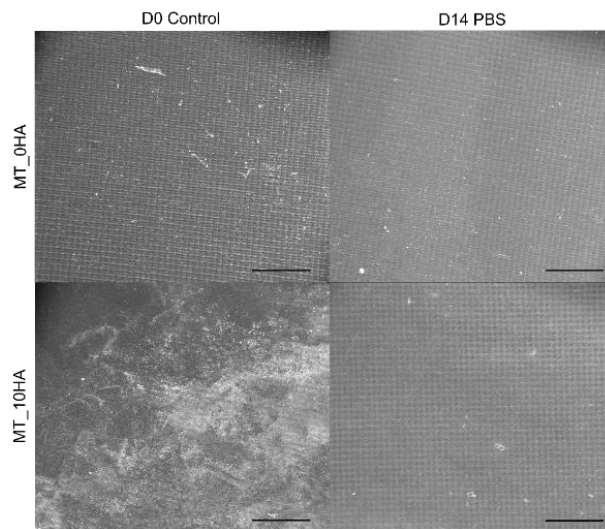


Figure 4-9 – Representative SEM images (50x magnification) of the surface of dog-bone MT specimens at D0 (control) and after 14-days in PBS at 37 °C. The scale bars represent 500 μ m.

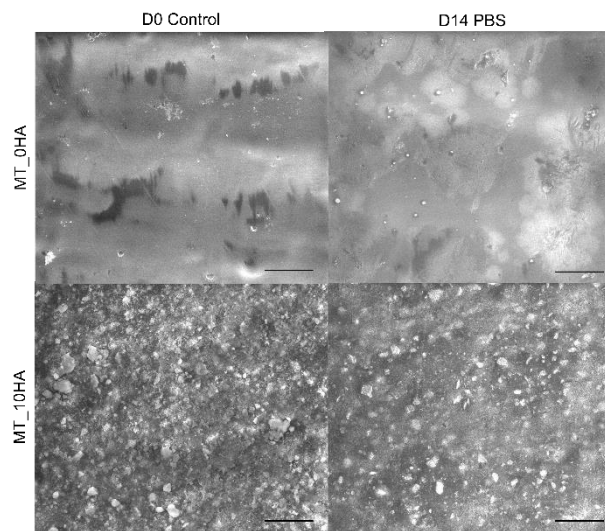


Figure 4-10 – Representative SEM images (1000x magnification) of the surface of dog-bone MT specimens at D0 (control) and after 14-days in PBS at 37 °C. The scale bars represent 20 μ m.

4.2.3 Tensile Properties

Figure 4-11 displays representative stress-strain curves for MT composites exposed to PBS. It is clear that incubation in PBS decreased the mechanical strength and modulus of the materials. Similar behavior was observed at the 14- and 28-day periods, with slightly reduced fracture strains due to the longer incubation period.

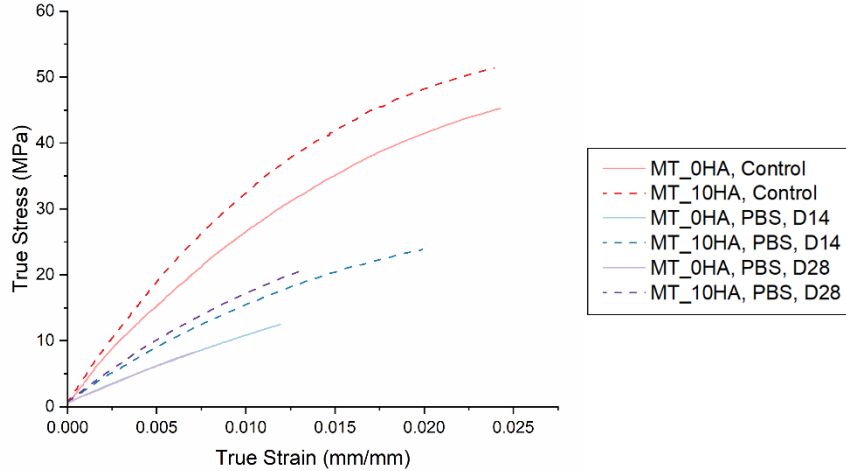


Figure 4-11 - Representative tensile true stress-strain curves for MT_0HA (light, solid) and MT_10HA (dark, dashed) compositions after 14 (blue) and 28 (purple) days in PBS at 37 °C. Reference lines (red) of the compositions' behavior tested dry at 37 °C (Figure 4-1) are included for comparison.

The ultimate strengths, yield strengths, moduli, strains at fracture, toughness's, and Poisson's ratio of the hydrated MT samples are summarized in Figure 4-12 (a) – (f), respectively. Significant decreases were observed in ultimate and yield strength after hydration ($p < 0.0001$). For instance, after 28 days in PBS MT_10HA had a 57.9 and 45.9 % decrease in ultimate and yield strength, respectively. The elastic modulus was also significantly impacted ($p < 0.0001$) and had a 62.7 and 46.2 % reduction at D28 in the 0 and 10 HA groups from their dry counterparts, respectively. No significant effects were seen on the materials' fracture strain and Poisson's ratios. Toughness was decreased due to PBS incubation ($p < 0.0001$). With the exception of yield strength, no significant variations were seen between D14 and D28 ($p > 0.05$). It is clear from these results that there is a significant impact of water absorption, swelling, and potential hydrolysis on these materials.

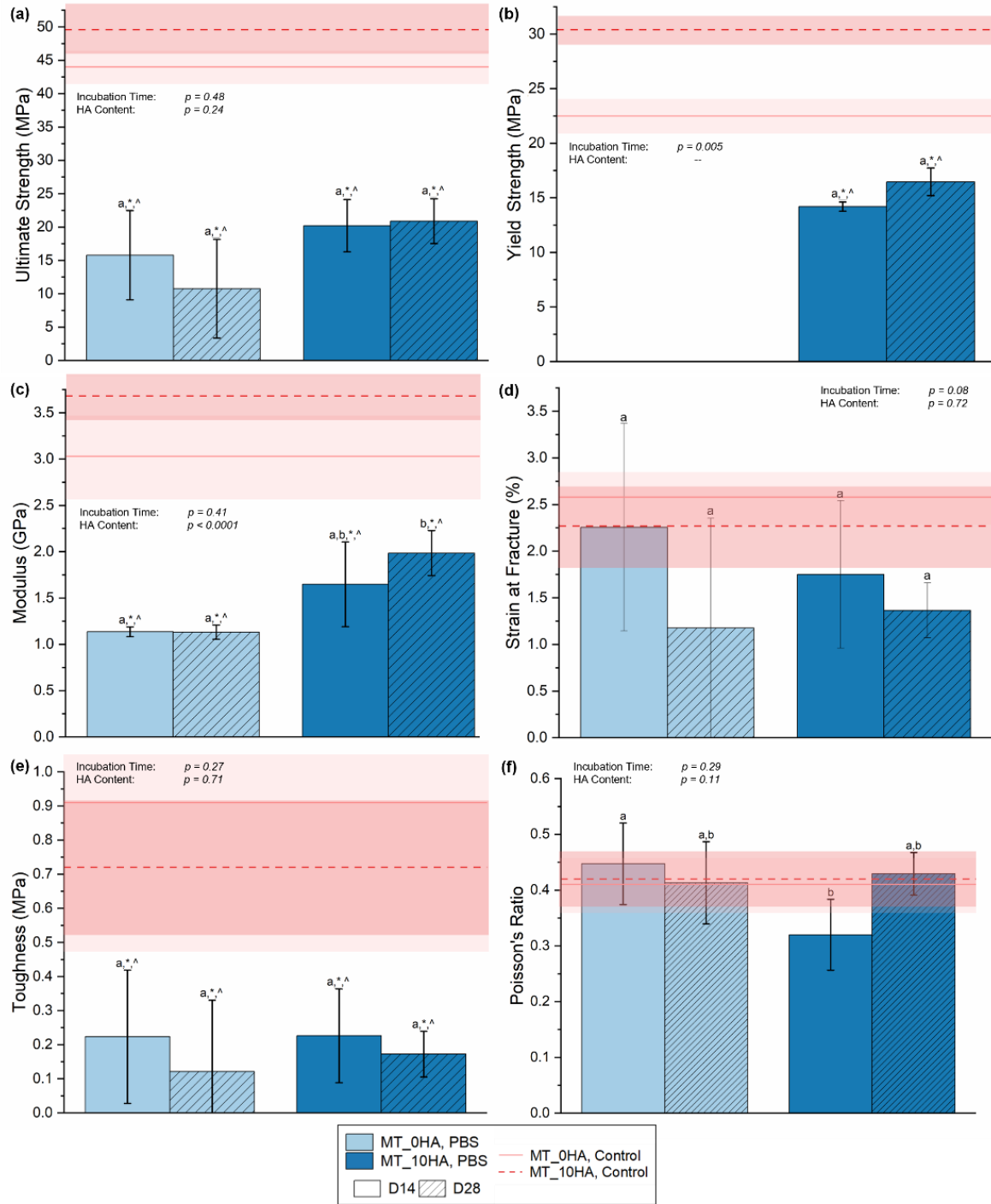


Figure 4-12 – (Previous page) – Tensile properties, (a) ultimate strengths (b) yield strengths, (c) elastic moduli, (d) strains at fracture, (e) toughness's, and (f) Poisson's ratios, of MT_0HA (light blue) and MT_10HA (dark blue) compositions after 14 (solid) and 28 (diagonal pattern) days in PBS. Data are presented as mean $\pm$ SD (n = 5) with different letters representing statistical significance ($p < 0.05$). Reference lines (red) of the compositions' behavior tested dry at 37 °C (Figure 4-2) are presented, with statistical significance from these groups represented by '*' and '^' for MT_0HA and MT_10HA, respectively. Reported p-values are from ANOVAs excluding the control data.

4.2.4 Compressive Properties

The two compositions were tested after 14- and 28- day periods in PBS and their representative compressive stress-strain curves can be seen in Figure 4-13. When compared to the compressive behavior observed under dry control conditions (Figure 4-3), significant decreases in mechanical stress and moduli were observed. The HA-containing groups consistently displayed higher mechanical properties than their resin counterparts. Minimal differences in the stress-strain curves were seen between D14 and D28 for each composition.

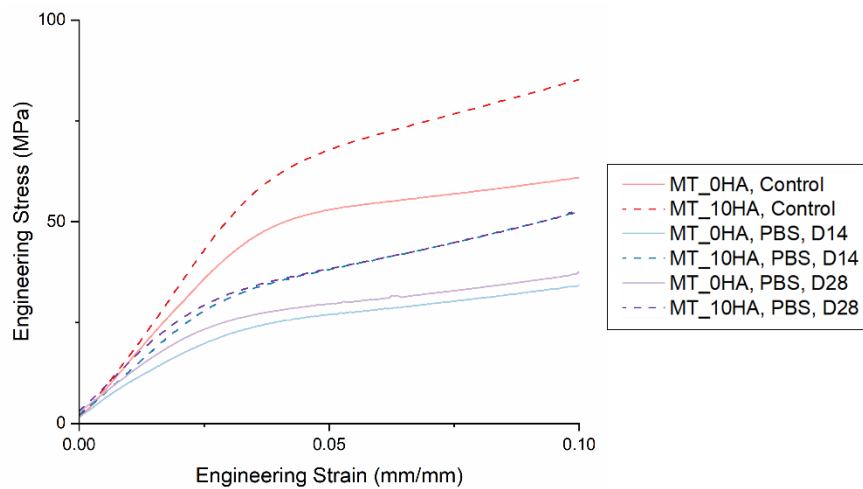


Figure 4-13 - Representative engineering compressive stress-strain curves for MT_0HA (light, solid) and MT_10HA (dark, dashed) compositions after 14 (blue) and 28 (purple) days in PBS at 37 °C. Reference lines (red) of the compositions' behavior tested dry at 37 °C (Figure 4-3) are included for comparison. This figure shows a magnified view of the elastic region. Failure occurred beyond 0.1 mm/mm.

The yield strengths and moduli were calculated to determine the compressive properties after PBS incubation and are summarized in Figure 4-14 (a) and (b), respectively. As previously seen, HA composites consistently performed better than the resin-only material ($p < 0.001$). Significant decreases were seen in both properties from the dry control comparisons (Figure 4-4; $p < 0.0001$). At D14 for the MT_0HA group, decreases of 58.2 and 49.5 % were seen in the yield strength and elastic modulus from the control group, respectively. Their HA counterpart had respective decreases of 48.7 and 42.2 %. Significant differences were seen between compositions and incubation time, with elastic moduli being particularly effected ($p < 0.005$). It is clear that the effects of PBS incubation resulted in decreased compressive strength and moduli of the materials.

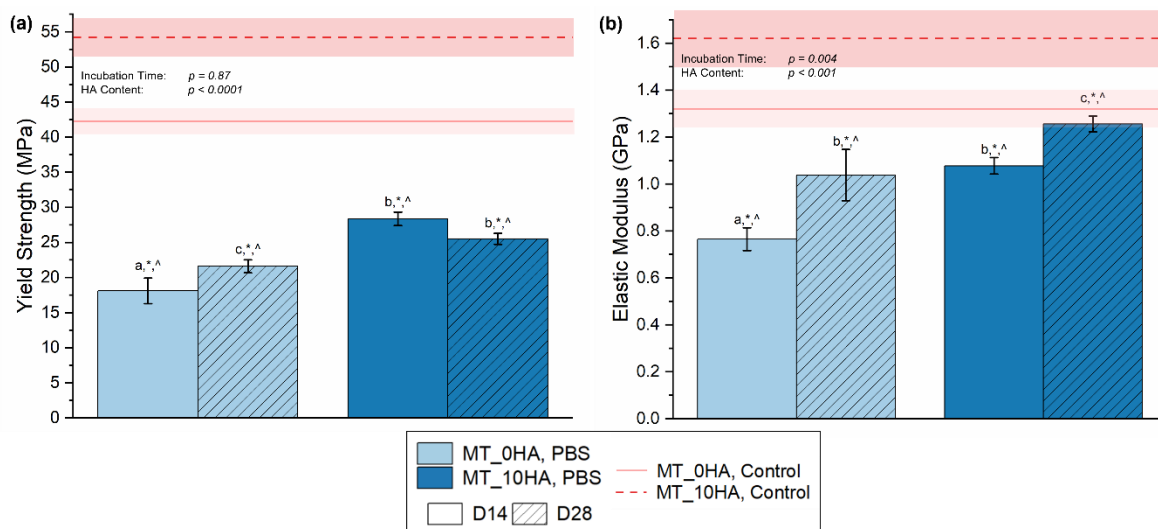


Figure 4-14 - Compressive properties, (a) yield strengths and (b) elastic moduli, of MT_0HA (light blue) and MT_10HA (dark blue) compositions after 14 (solid) and 28 (diagonal pattern) days in PBS at 37 °C. Data are presented as mean $\pm$ SD (n = 5) with different letters representing statistical significance ($p < 0.05$). Reference lines (red) of the compositions' behavior tested dry at 37 °C (Figure 4-4) are presented, with statistical significance from these groups represented by '*' and '^' for MT_0HA and MT_10HA, respectively. Reported p-values are from ANOVAs excluding the control data.

4.2.5 Wettability

The wettability of the nanocomposite surfaces was determined through contact angle measurements. These results are summarized in Table 4-1. A decrease in contact angle was seen with the addition of HA, indicating HA inclusion increased the composite's hydrophilicity. Both compositions' contact angles were less than 90 °, indicating the bulk material is hydrophilic [171].

Table 4-1 – Water contact angle of MT_0HA and MT_10HA compositions. Data are presented as mean $\pm$ SD (n = 4) with different letters representing statistical significance (p = 0.006).

Composition	Contact Angle (°)
MT_0HA	80.7 $\pm$ 0.8 ^a
MT_10HA	67.7 $\pm$ 3.4 ^b

4.3 Effects of Oxidative Degradation

4.3.1 Mass and Volumetric Properties

The changes in wet mass in the MT_0HA and MT_10HA materials due to the three incubation media were recorded over a 28-day period (Figure 4-15). When comparing the overall degradation behavior of each composition, significant differences were seen between the 0 and 10 vol% HA from D14 to D28 (p = 0.02, p = 0.007, and p < 0.0001 for D14, D21, and D28, respectively). Within the MT_0HA group, HClO drove a significant (p < 0.05) positive increase in the mass from D5 to D7 compared to the other two media (PBS, H₂O₂). At D21 and D28, significant differences were detected between the three media types (p < 0.0001, with exception of PBS and H₂O₂ with MT_0HA (p < 0.05)). Within the MT_10HA group, H₂O₂ induced a significant (p < 0.0001) negative mass change from D3 onwards compared to the other groups. From D14 to D28, the PBS and HClO behaviors were significantly (p < 0.0001) different in this composite. It is clear that while HClO causes an initial increased absorption behavior, it does degrade the material as seen in the downwards trends from D7 onwards. By D7, all mass change due to incubation media appears to have equilibrated or begun their downward degradation trends. H₂O₂ induced very different behaviors in the MT_0HA and MT_10HA compositions. It appears that a reaction occurred between the H₂O₂ and HA, causing rapid mass loss.

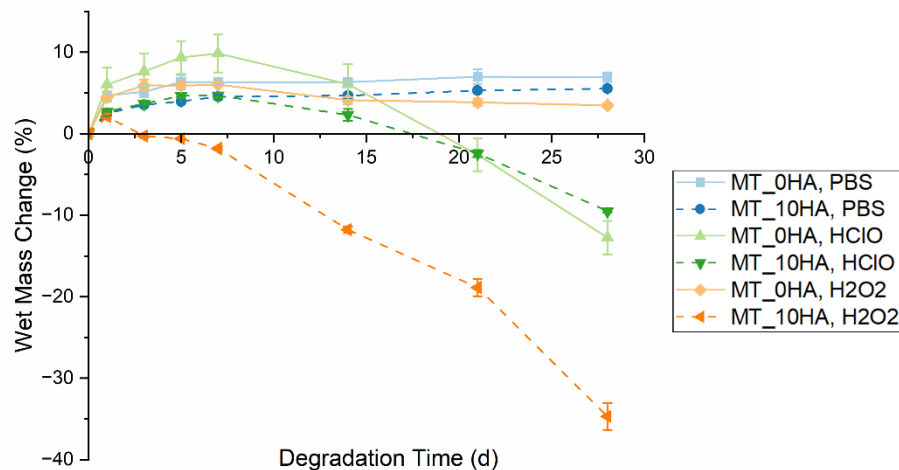


Figure 4-15 - Mass changes, recorded in ‘wet conditions’, due to oxidative degradation at 37 °C of MT_0HA (light, solid) and MT_10HA (dark, dashed) compositions over a 28-day period. Degradation media include HClO (green) and H₂O₂ (orange), with the PBS (blue) trends included from Figure 4-6 as reference. Data are presented as mean ± SD (n = 3). Reported p-values are from ANOVAs excluding the control data.

As consistent trends were seen after D7 in Figure 4-15, the rate of mass change was quantified between D7 and D28 for each composition and incubation media combination (Table 4-2). Minimal mass increases were observed for the PBS samples over this period. HClO and H₂O₂ both displayed mass losses. When incubated with HClO, the HA composite had a 25% slower degradation rate than the resin-only material ($p = 0.001$). This suggests the HA may provide some resistance to the effects of degradation by HClO. Interestingly, the MT_10HA composite had a 1514% faster degradation rate than the MT_0HA resin in the H₂O₂ media ($p < 0.0001$). As seen in Figure 4-15, this degradation rate is significantly ($p < 0.0001$) quicker than the other groups and is likely a result of an unexpected reaction occurring between the H₂O₂ and HA.

Table 4-2 - Rate of mass change from day 7 to 28 for MT_0HA and MT_10HA compositions incubated in PBS, HClO, and H₂O₂. Data are presented as mean $\pm$ SD (n = 3) with different letters representing statistical significance (p < 0.05).

Composition, Incubation Media	Rate of Mass Change (mg/day)
	(D7 – D28)
MT_0HA, PBS	0.21 $\pm$ 0.16 ^a
MT_10HA, PBS	0.41 $\pm$ 0.08 ^a
MT_0HA, HClO	-7.35 $\pm$ 0.06 ^b
MT_10HA, HClO	-5.86 $\pm$ 0.10 ^c
MT_0HA, H ₂ O ₂	-0.84 $\pm$ 0.16 ^d
MT_10HA, H ₂ O ₂	-13.56 $\pm$ 0.60 ^e

The percent changes in mass (dry) and volume are displayed in Figure 4-16 (a) and (b), respectively. All specimens exposed to degradation media displayed significant mass losses relative to the PBS-controls (p < 0.0001). By D28, the HClO degradation induced significantly different mass changes of ~ 22 and ~16 vol% in the 0HA and 10HA compositions, respectively (p < 0.0001). The H₂O₂ media followed the trends previously discussed, with extreme degradation seen in the MT_10HA samples. Within the volumetric data, significant differences were seen in the MT_10HA composite after degradation in HClO for 14 days and H₂O₂ for 14 and 28 days (p < 0.0001).

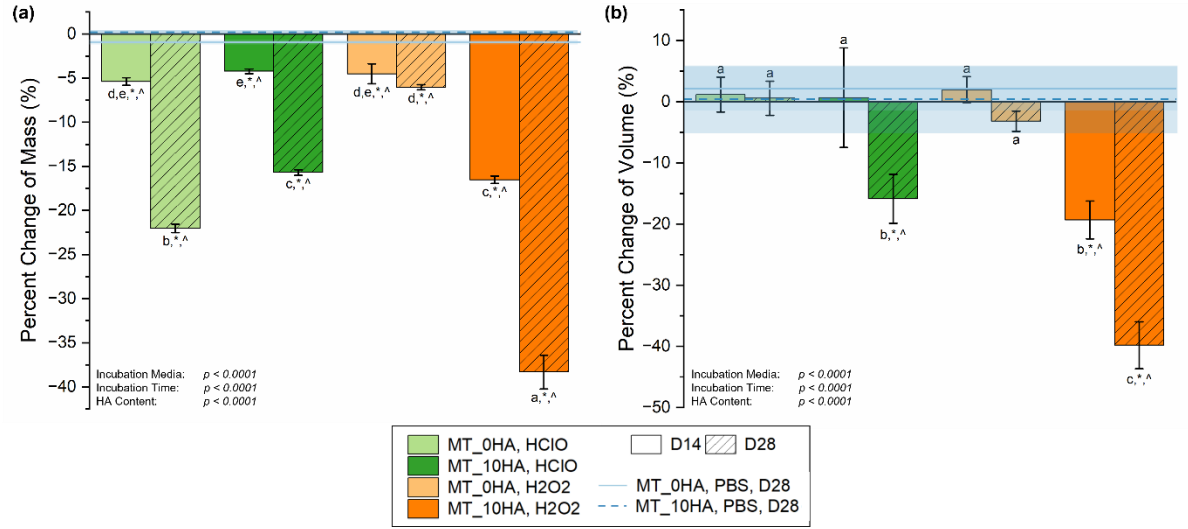


Figure 4-16 - Mass and volumetric changes due to oxidative degradation in MT_0HA (light) and MT_10HA (dark) compositions after 14 (solid) and 28 (diagonal pattern) days with HClO (green) and H₂O₂ (orange) degradation media at 37 °C: (a) percent change of mass in dried samples (n = 3), and (b) percent change of volume in wet samples (n = 5). Data are presented as mean $\pm$ SD with different letters representing statistical significance ($p < 0.05$). Reference lines from D28 PBS (Figure 4-7) are presented, with statistical significance from these groups represented by ‘*’ and ‘^’ for MT_0HA and MT_10HA, respectively.

4.3.2 Surface Properties

The degraded surfaces of the dog-bone specimens were imaged at D14 and D28 for the two oxidative media (Figure 4-17). Images of the compositions after 28 days in PBS have been added as a visual comparison. Under H₂O₂ conditions, the resin-only samples began to crack along the surface of the gauge length of the tensile specimens, with the cracks extending in no dominant direction. There were significantly more cracks at both time points than in the PBS control. The HA composite showed differing behavior, with small micron- and millimeter-scale pits forming. At D14 and D28, a sample of these defects was measured to determine void areas of 0.006 ± 0.004 and 0.012 ± 0.007 mm² (n = 5), respectively. When exposed to HClO, very similar behavior was observed between the 0 and 10 vol% HA materials. Large voids formed in the surface, with increasing density over time. The average areas of the pits in the MT_0HA inks were 0.100 ± 0.039 and 0.084 ± 0.042 mm² for D14 and D28,

respectively. For the MT_10HA composites, the areas were 0.247 ± 0.092 and 0.334 ± 0.185 mm², respectively. Therefore, the scale of the visible defects introduced is larger in the HClO condition than the H₂O₂ condition ($p < 0.001$). Additional macroscopic images of the top side of the dog-bone specimens and the compression samples can be seen in Figure A-1 and Figure A-2, respectively.

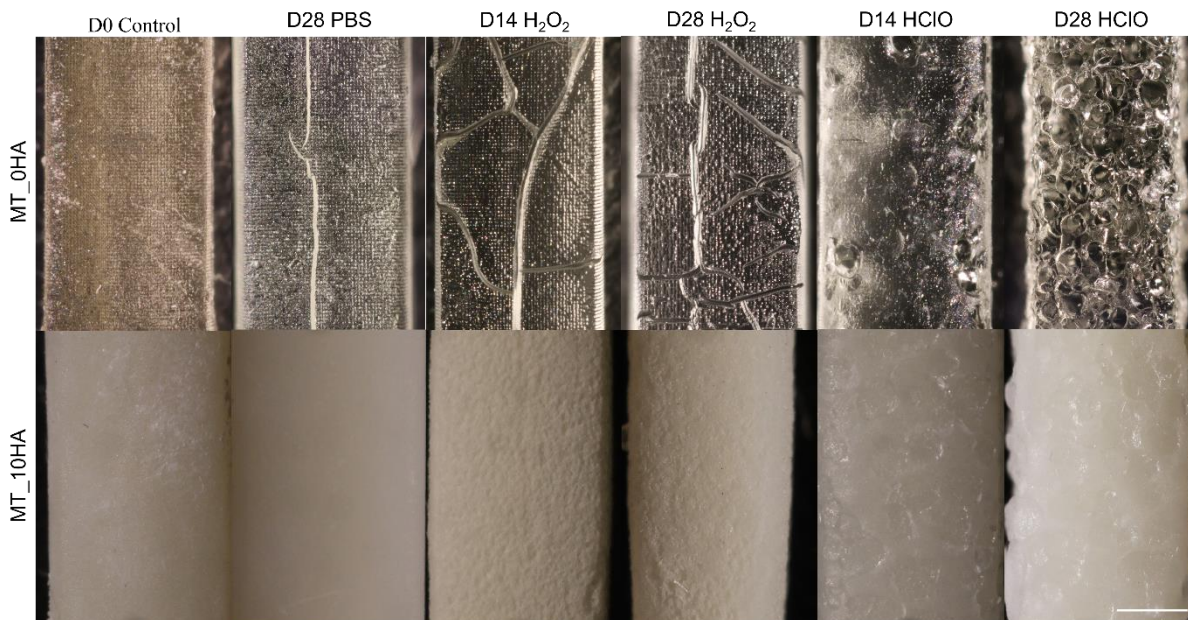


Figure 4-17 – Representative macroscope images of the bottom surface of dog-bone MT specimens degraded by H₂O₂ and HClO for 14- and 28-day periods at 37 °C. The D0 control and D28 PBS images from Figure 4-8 are included as reference. The scale bar represents 1 mm.

To further highlight the surface impacts of degradation media on these materials, SEM images of the D14 samples were taken at 50x (Figure 4-18) and 1000x (Figure 4-19) magnifications. Except for surface cracking (Figure 4-16, highlighted in orange), minimal visible effects were observed in the MT_0HA sample after H₂O₂ exposure. The grid pattern that develops on the surface due to the printing process is still observed at 50x magnification, indicating minimal bulk surface degradation has occurred. In contrast, the MT_10HA composite shows significant surface effects from H₂O₂ with small pitting occurring across the entire surface. The 1000x image shows a very rough surface, indicating aggressive degradation occurred in this sample. The HClO had similar effects on the two compositions,

with large, ~ 0.5 mm (diameter) voids appearing on the surface. It appears that the presence of HA reduces the amount of surface area covered in voids, and appears to cause less uniform, circular voids to develop. It is interesting to observe the crescent shaped features (Figure 4-18, highlighted in yellow) that are likely void initiation regions. In the resin-only specimen, the surface within the voids is very smooth. This suggests that the material is being degraded in large pieces as cracks are initiated by the HClO degradation. The HA composite has a rougher 1000x magnified surface which is likely due to the presence of filler. When incubated by HClO, it appears that HA slowed the surface degradation.

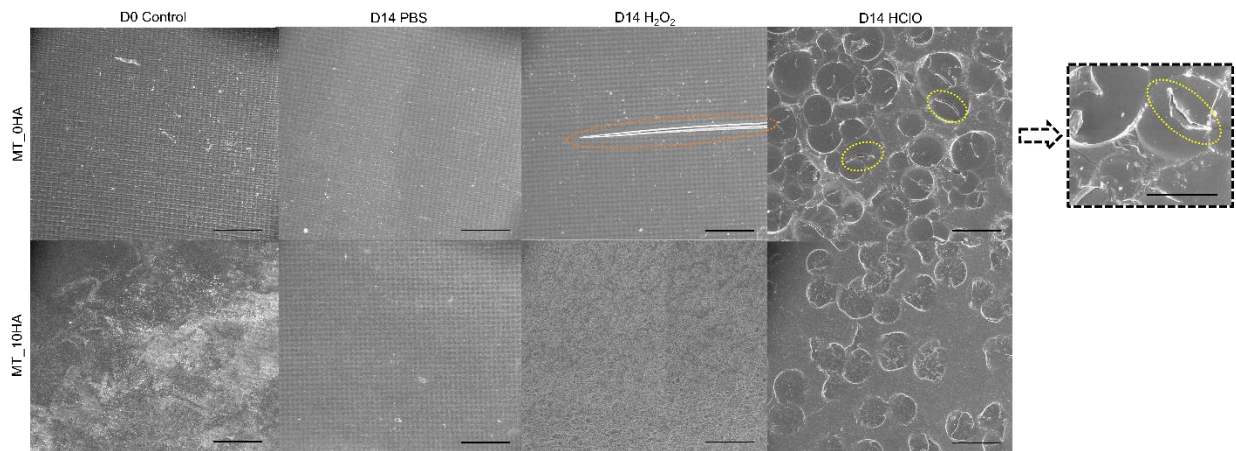


Figure 4-18 – Representative SEM images (50x magnification) of the surface of dog-bone MT specimens after degradation by H₂O₂ and HClO for 14 days at 37 °C. A magnified (200x) view of the MT_0HA D14 HClO sample is provided (right). D0 control and D14 PBS images from Figure 4-9 are included as reference. Features indicative of void initiation cracks from HClO incubation are highlighted in yellow. Surface cracks developed from H₂O₂ are highlighted in orange. The scale bars represent 500 μm.

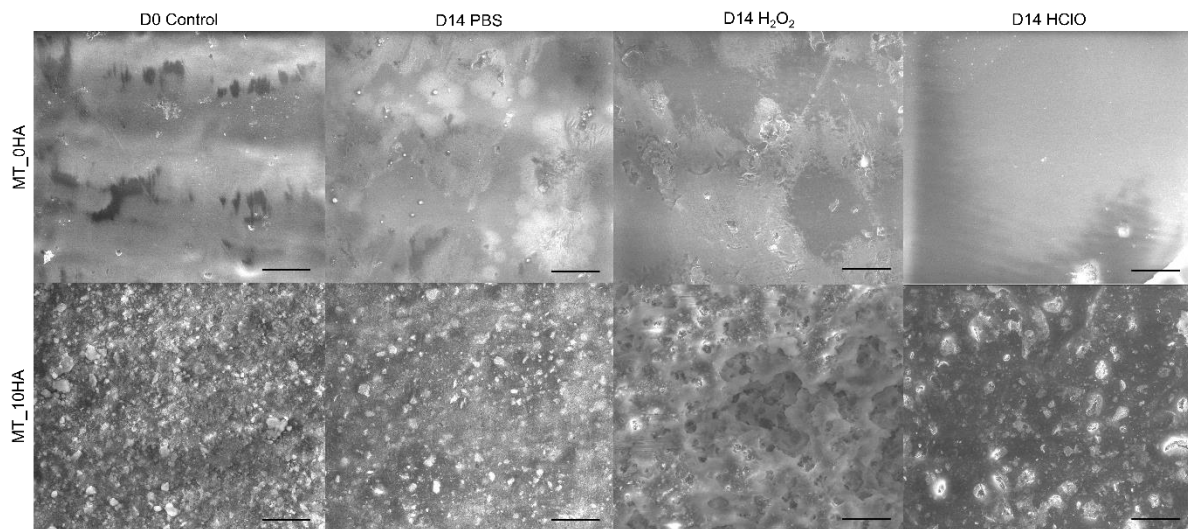


Figure 4-19 – Representative SEM images (1000x magnification) of the surface of dog-bone MT specimens after degradation by H_2O_2 and HClO for 14 days at 37 °C. When applicable, images were taken inside of the pits observed in Figure 4-18. D0 control and D14 PBS images from Figure 4-10 are included as reference. The scale bars represent 20 μm .

4.3.3 Tensile Properties

Representative true stress-strain curves are presented in Figure 4-20 to demonstrate the effects of oxidative degradation on the tensile behavior of MT_0HA and MT_10HA. When compared to samples incubated in PBS, a minor, negative impact on mechanical properties is observed due to oxidation effects. There does appear to be a small increase in ductility in the materials when exposed to oxidation. It is important to note that the mechanical data for this study was generated using the post-incubation dimensions of the specimens.

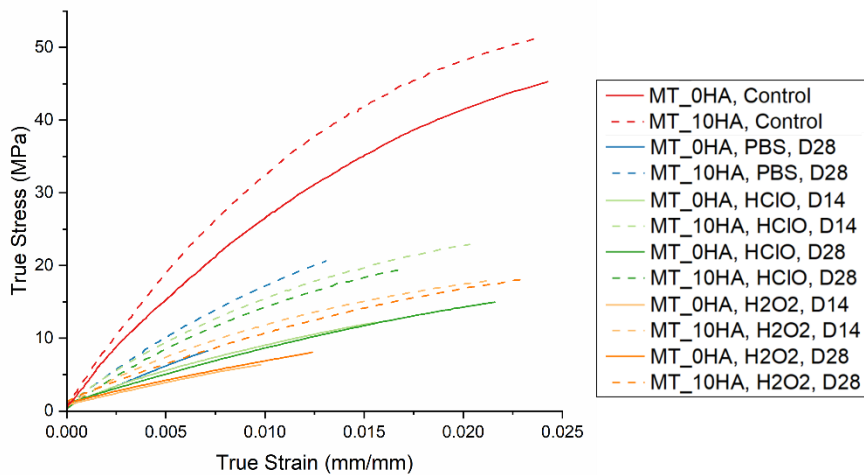


Figure 4-20 – Representative true tensile stress-strain curves for MT_0HA (solid) and MT_10HA (dashed) compositions after 14 (light) and 28 (dark) days in HClO (green) and H₂O₂ (orange) at 37 °C. Reference lines of the compositions’ behavior tested in dry, control conditions (red; Figure 4-1) and after 28 days in PBS (blue; Figure 4-12) are included for comparison.

Tensile properties of the samples exposed to oxidative media are displayed in Figure 4-21. Incubation in HClO and H₂O₂ had minimal effects on ultimate tensile strength, when compared to incubation in PBS (Figure 4-21 (a)). However, yield stress, when it could be determined, was reduced in the 10 vol% HA composites with D28 decreases of 21.4 and 30.7 % from the PBS control in the HClO and H₂O₂ media, respectively ($p < 0.001$). As seen in Figure 4-20, exposure to any incubation media generated very brittle materials with slight or no yield point before failure. The moduli of the composites were reduced slightly with oxidative exposure (Figure 4-21(c)). No significant changes were observed in fracture strain, toughness, or Poisson’s ratio. A minor, insignificant increase in fracture strain was observed, indicating a potential small plasticization effect of oxidation. The scale of mechanical performance reduction due to addition of oxidative degradation is minimal when compared to the impact of hydration effects only on the materials. While it is difficult to separate the impacts of oxidative degradation from absorption effects, it is clear that the majority of the changes in mechanical performance of these materials is due to the effects of hydration.

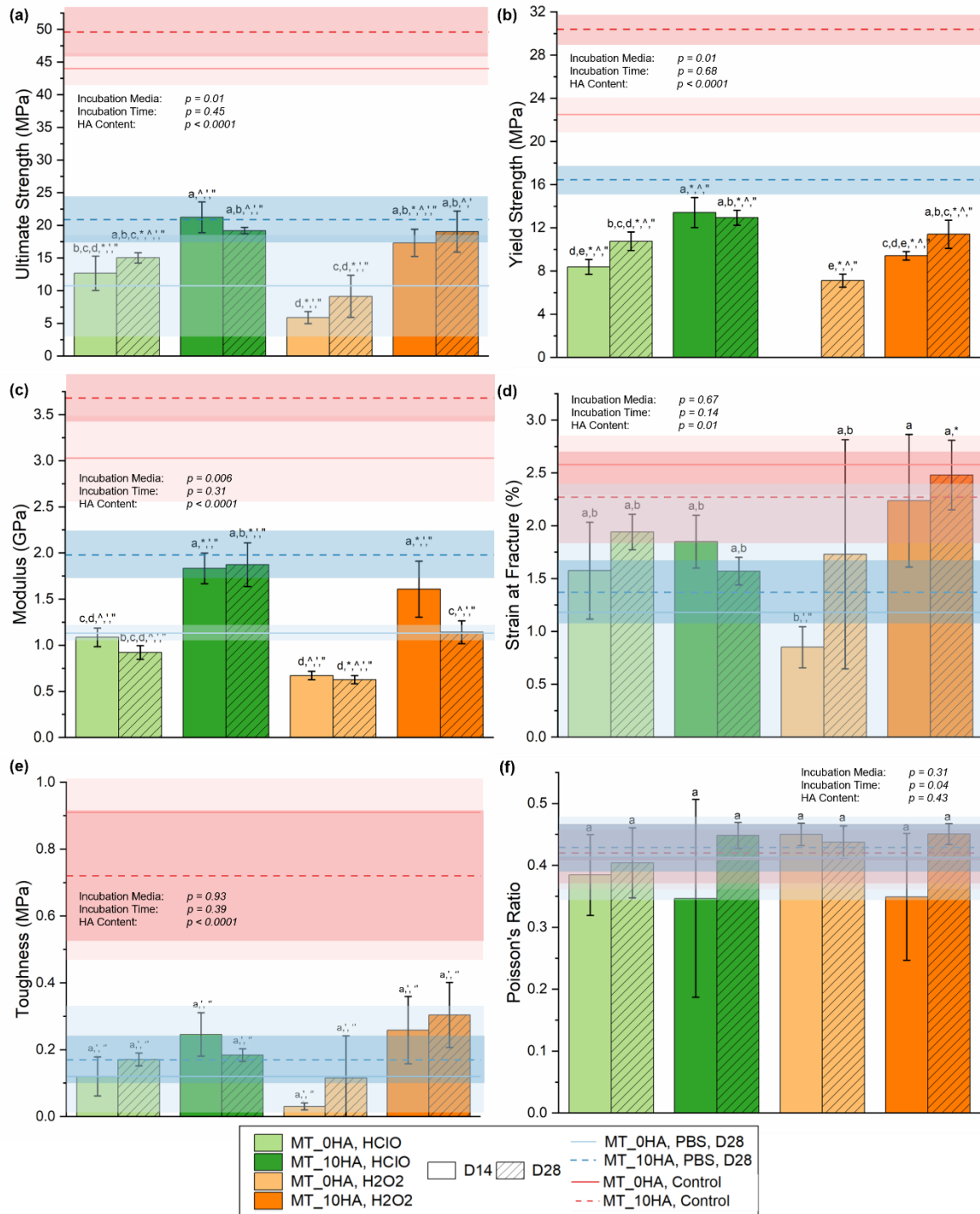


Figure 4-21 – (Previous page) – Tensile properties, (a) ultimate strengths (b) yield strengths, (c) elastic moduli, (d) strains at fracture, (e) toughness's, and (f) Poisson's ratios, of MT_0HA (light) and MT_10HA (dark) compositions after 14 (solid) and 28 (diagonal pattern) days in HClO (green) and H₂O₂ (orange) at 37 °C. Data are presented as mean ± SD (n = 5) with different letters representing statistical significance (p < 0.05). Reference lines (red) of the compositions' behavior from their dry control condition (Figure 4-1) are presented, with statistical significance from these groups represented by ‘’ and ‘’’ for MT_0HA and MT_10HA, respectively. Reference lines (blue) of the compositions' behavior from D28 PBS (Figure 4-12) are presented, with statistical significance from these groups represented by ‘*’ and ‘^’ for MT_0HA and MT_10HA, respectively. Reported p-values are from ANOVAs excluding the control data.

4.3.4 Compressive Properties

Hypochlorous acid and H₂O₂ were used to degrade MT compressive samples for 14- and 28-day periods, and their representative compressive stress-strain curves can be seen in Figure 4-22. When compared to the compressive behavior observed after 28 days in PBS (Figure 4-13), a slight decrease in mechanical properties is seen after HClO exposure. A larger reduction in mechanical properties is seen in the H₂O₂ groups. As seen previously, MT_10HA had superior mechanical performance to its resin-only counterparts. Minimal differences in the stress-strain curves were seen between the two time points for each composition.

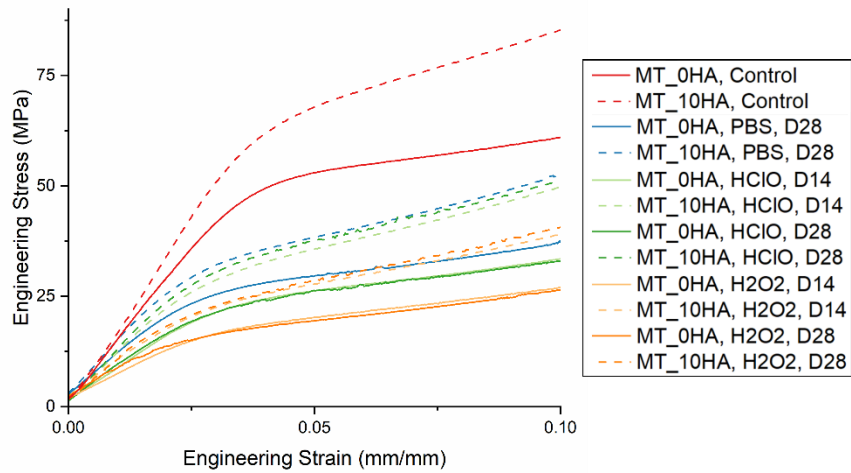


Figure 4-22 - Representative engineering compressive stress-strain curves for MT_0HA (solid) and MT_10HA (dashed) compositions after 14 (light) and 28 (dark) days in HClO (green) and H₂O₂ (orange). Reference lines of the compositions' behavior tested in dry, control conditions (red; Figure 4-3), and after 28 days in PBS (blue; Figure 4-13) are included for comparison. This figure shows a magnified view of the elastic region and failure points occurred beyond 0.1 mm/mm.

Figure 4-23 displays the calculated yield strengths and moduli in (a) and (b), respectively. Significant reductions ($p < 0.0001$) in yield strength from the PBS control were observed in the H₂O₂ incubated samples, but not the HClO incubated samples. In the majority of groups, there was a significant reduction in elastic modulus due to oxidative media. H₂O₂ appeared to have a stronger negative impact on mechanical properties ($p < 0.0001$). It is interesting to observe in the HA-containing composites that an increase in modulus occurred from D14 to D28 in oxidative media. While this could be due to changes in the polymer structures, it could also be a result of the significant changes in volume observed at these time points (Figure 4-16). While reductions in strength and moduli were observed due to oxidative degradation from the PBS controls, they are minimal when compared to the overall decrease in properties observed due to hydration from the dry control samples (Figure 4-14).

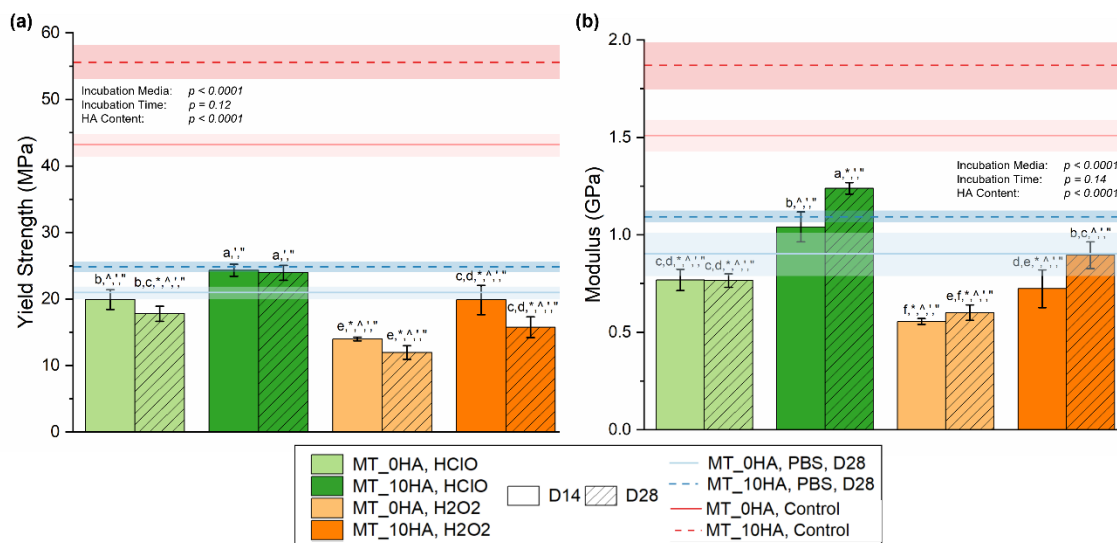


Figure 4-23 - Compressive properties, (a) yield strengths and (b) elastic moduli, of MT_0HA (light) and MT_10HA (dark) compositions after 14 (solid) and 28 (diagonal pattern) days in HClO (green) and H₂O₂ (orange) at 37 °C. Data are presented as mean ± SD (n = 5) with different letters representing statistical significance (p < 0.05). Reference lines (red) of the compositions' behavior from their dry control condition (Figure 4-4) are presented, with statistical significance from these groups represented by '' and '''' for MT_0HA and MT_10HA, respectively. Reference lines (blue) of the compositions' behavior from D28 PBS (Figure 4-14) are presented, with statistical significance from these groups represented by '' and '^' for MT_0HA and MT_10HA, respectively. (a) and (b) were analyzed with non-parametric statistics. Reported p-values are from ANOVAs excluding the control data.**

4.4 Effects of Isosorbide Methacrylate

4.4.1 Mass and Volumetric Properties

Figure 4-24 displays the mass change of the MTI compositions over time when incubated in PBS and HClO. Throughout the incubation period, the 0HA and 10HA groups had significantly (p < 0.0001) different behaviors. In PBS, the resin-only sample reached a maximum mass change 43% higher than the HA composite. Within the composition groupings, at D14, the HClO induced significant (p < 0.05) reductions in mass from their PBS counterparts. This mimics the behavior seen in the MT resins, with

higher absorption in the resin-only samples, and clear degradation due to HClO occurring after a week. Higher mass changes due to water absorption were seen than in the MT samples, with MT_0HA plateauing at ~ 6 % (Figure 4-6) for comparison.

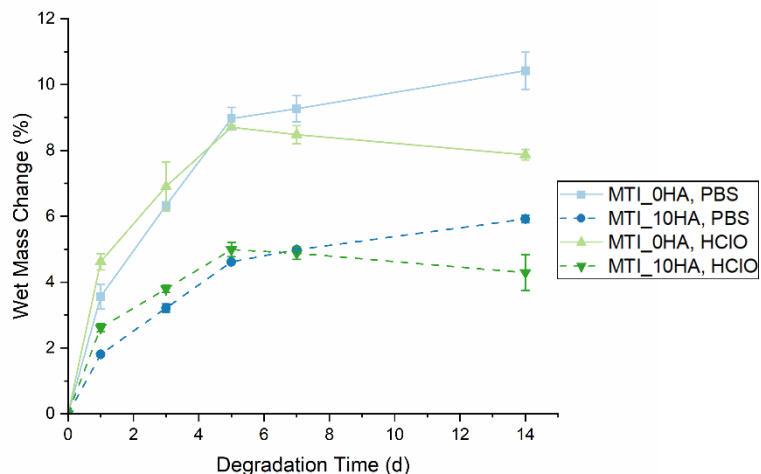


Figure 4-24 - Mass changes, recorded in ‘wet conditions’, due to water absorption and oxidative degradation in MTI_0HA (light, solid) and MTI_10HA (dark, dashed) compositions over a 14-day period at 37 °C. Incubation media include HClO (green) and PBS (blue). Data are presented as mean ± SD (n = 3).

To capture the degradation behavior, the rate of mass change between D7 and D14 was calculated (Table 4-3). A greater increase in mass change was seen due to PBS in the MTI compositions than in their MT counterparts (Table 4-2; $p = 0.03$ and $p = 0.05$ for 0 and 10 HA compositions, respectively). This larger positive rate of change suggests that the water absorption in these samples had not equilibrated after a week, as seen previously. However, HClO appeared to induce a slower degradation rate in these samples, decreased by 91 and 86 % for the 0HA and 10HA compositions, respectively. These rates were significantly different from the MT comparisons ($p < 0.0001$).

Table 4-3 - Rate of mass change from day 7 to 14 for MTI_0HA and MTI_10HA compositions incubated in PBS and HClO. Data are presented as mean $\pm$ SD (n = 3) with different letters representing statistical significance ($p < 0.05$). Statistically significant differences from the sample's MT counterpart (Table 4-2) are represented with a ''.**

Composition, Incubation Media	Rate of Mass Change (mg/day)
	(D7 – D14)
MTI_0HA, PBS	1.24 $\pm$ 0.60 ^{a, *}
MTI_10HA, PBS	1.33 $\pm$ 0.07 ^a
MTI_0HA, HClO	-0.67 $\pm$ 0.47 ^{b, *}
MTI_10HA, HClO	-0.81 $\pm$ 0.47 ^{b, *}

The percent changes of mass (dry) and volume are displayed in Figure 4-25 (a) and (b), respectively. The mass and volumetric changes seen in the samples exposed to PBS were consistent to the behavior seen in the MT compositions (Figure 4-7). The HClO samples displayed significant ($p < 0.0001$) mass losses when compared to the PBS groups. However, significantly less mass was lost in the MTI samples than in the MT compositions due to HClO incubation (Figure 4-16; $p < 0.05$). No significant differences were seen in the volumetric data across all groups. Based on all the mass and volumetric properties investigated, it can be inferred that the inclusion of IM increases the sensitivity of the material to water absorption but decreases its sensitivity to oxidation.

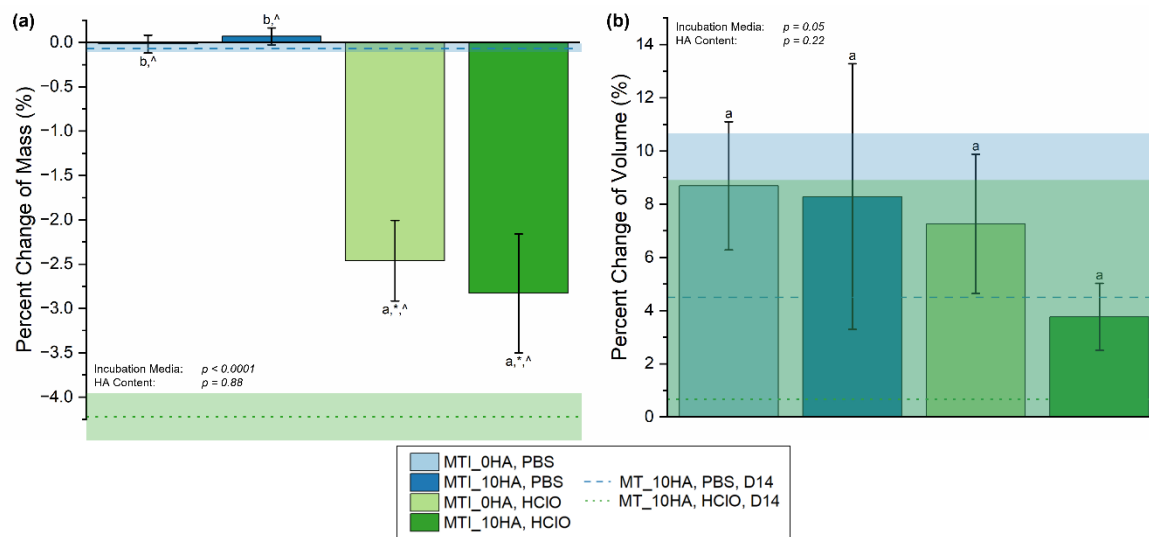


Figure 4-25 - Mass and volumetric changes due to water absorption and oxidative degradation in MTI_0HA (light) and MTI_10HA (dark) compositions at 14 days with PBS (blue) and HClO (green) incubation media at 37 °C: (a) percent change of mass in dried samples (n = 3), and (b) percent change of volume in wet samples (n = 5). Data are presented as mean $\pm$ SD with different letters representing statistical significance ($p < 0.05$). Reference lines of the MT_10HA composition's behavior from D14 (Figure 4-7 and Figure 4-16) are presented, with statistical significance from these groups represented by '*' and '^' for the PBS and HClO media, respectively. Reported p-values are from ANOVAs excluding the control data.

4.4.2 Surface Properties

The degraded surfaces of the bottom side of the MTI dog-bone specimens were imaged at D14 after incubation in PBS and HClO (Figure 4-26). Images of the compositions in a dry condition, prior to any incubation, have been included as a visual comparison. Under PBS conditions, no significant visible changes were observed. This is consistent with the behavior seen on day 14 in the MT inks (Figure 4-8). The 0 vol% material had visible degradation pits after 14 days in HClO. The voids were measured and had an average area of $0.099 \pm 0.045 \text{ mm}^2$, respectively. The degraded voids had very similar dimensions to those seen in the MT inks, however, appear sparser throughout the imaged surface. Interestingly, no visible defects were observed in the 10 vol% HA composites, which contrasts with the results seen in the previous MT study (Figure 4-17). Additional macroscopic images of the top side of

the dog-bone specimens and the compression samples can be seen in Figures A-3 and A-4 (Appendix A), respectively.

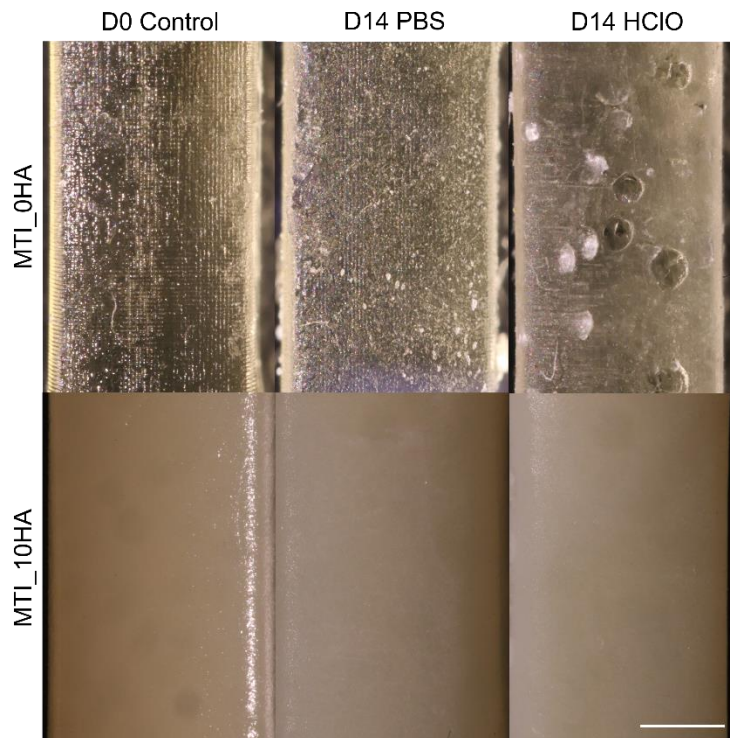


Figure 4-26 – Representative macroscopic images of the bottom surface of dog-bone MTI specimens incubated in PBS and HClO for a 14-day period at 37 °C. Images taken prior to any incubation are shown as a control. The scale bar represents 1 mm.

Magnified SEM images of the surface of the MTI composites after 14 days incubated in HClO can be seen in Figure 4-27 and Figure 4-26 at 50x and 1000x magnification, respectively. As seen in the MT_0HA material (Figure 4-18), large voids formed on the resin-only sample. However, significantly less surface area is occupied by voids on the MTI specimens. The HA-containing IM composite had no visible voids present at 50x magnification. For both compositions, their 1000x magnified surfaces were similar to the MT composites (Figure 4-19). The beneficial impact of HA on reducing surface degradation is continued in the MTI composites. The inclusion of IM also appears to decrease the surface degradation from HClO.

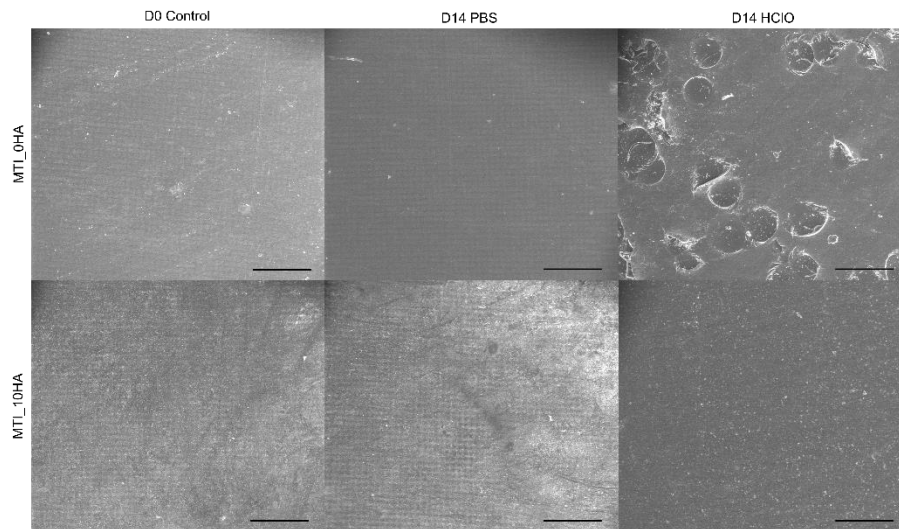


Figure 4-27 – Representative SEM images (50x magnification) of the surface of dog-bone MTI specimens at D0 control, and after incubation in PBS and HClO for 14 days at 37 °C. The scale bars represent 500 μm .

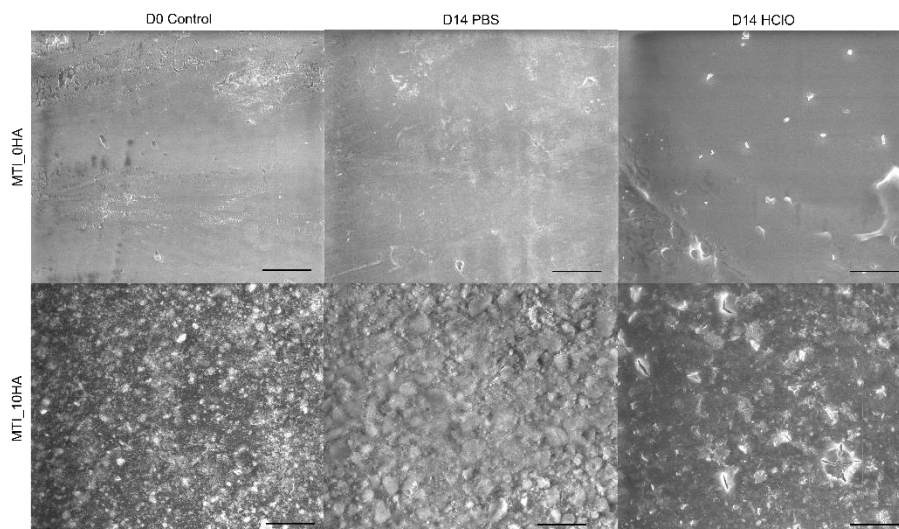


Figure 4-28 – Representative SEM images (50x magnification) of the surface of dog-bone MTI specimens at D0 control, and after incubation in PBS and HClO for 14 days at 37 °C. When applicable, magnified images were taken inside of the pits. The scale bars represent 20 μm .

4.4.3 Tensile Properties

The true stress-strain curves for the tensile behavior of MTI_0HA and MTI_10HA exposed to different conditions can be seen in Figure 4-29. Exposure to PBS and HClO decreased the strength of the composites. Hydration of the samples with PBS increased the ductility of the samples, observed in their increased strain at fracture. This is in contrast to the behavior observed in the MT composites (Figure 4-11). In the resin-only samples, PBS incubation after 14 days decreased the strain at fracture of MT_0HA by 17.8 %. In contrast, the MTI samples' fracture strain increased by 17.1 %. The oxidatively degraded samples were weak and brittle.

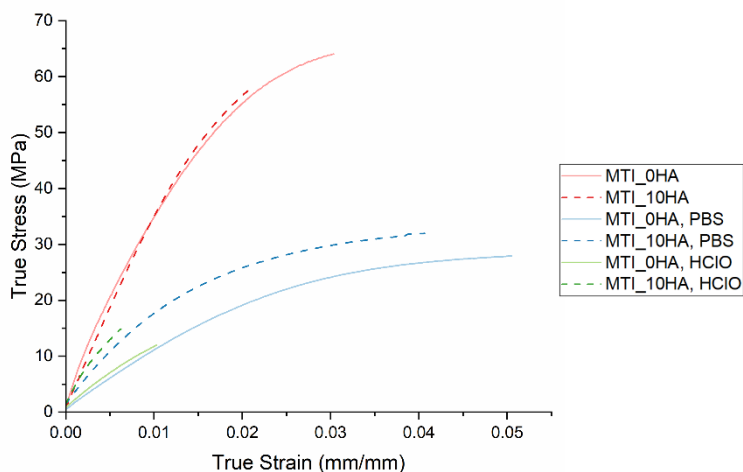


Figure 4-29 - Representative true tensile stress-strain curves for MTI_0HA (light, solid) and MT_10HA (dark, dashed) compositions after 14 days in PBS (blue) and HClO (green) at 37 °C. Reference lines (red) of the compositions' behavior tested dry are included for comparison.

The ultimate strengths, yield strengths, moduli, strain at fracture, toughness, and Poisson's ratio were calculated from the stress-strain curves (Figure 4-30 (a)-(f), respectively). Significant reductions ($p < 0.0001$) in ultimate strengths and moduli were observed in the materials after exposure to media. Exposure to HClO had a more significant impact on the ultimate strength than exposure to PBS ($p < 0.0001$). Due to induced weakness from HClO, no yield strength could be determined. Hydration with PBS increased the strain at fracture of the samples, with large significant effects ($p < 0.0001$) in the HA composite. HClO had significant negative impacts on material toughness consistent with loss of

strength ($p < 0.0001$). With the exception of the resin-only group tested under dry and PBS conditions ($p = 0.02$), no significant variations were seen in Poisson's ratio.

When contrasted with the behavior of MT composites exposed to PBS and HClO, there are clear similarities. The reduction in ultimate tensile strength due to PBS hydration after 14-days was 64.2 and 56.2 %, in the MT and MTI resin-only samples. Likewise, their moduli were reduced 62.4 and 63.5 %, respectively. However, as mentioned above, the fracture strain of the materials is drastically increased by hydration. For instance, MT_0HA and MTI_0HA soaked in PBS and tested at D14 had strains at fracture of 2.26 and 4.17 %, respectively. A greater decrease in ultimate strength due to HClO incubation was observed in MTI_0HA (58.3 %) than in MT_0HA (19.7 %). However, the modulus of MTI_0HA had no (0.3 %) change due to oxidative effects. It is clear that there are some additional interactions occurring between IM and the incubation media that are causing altered behavior, such as seen in the surface images.

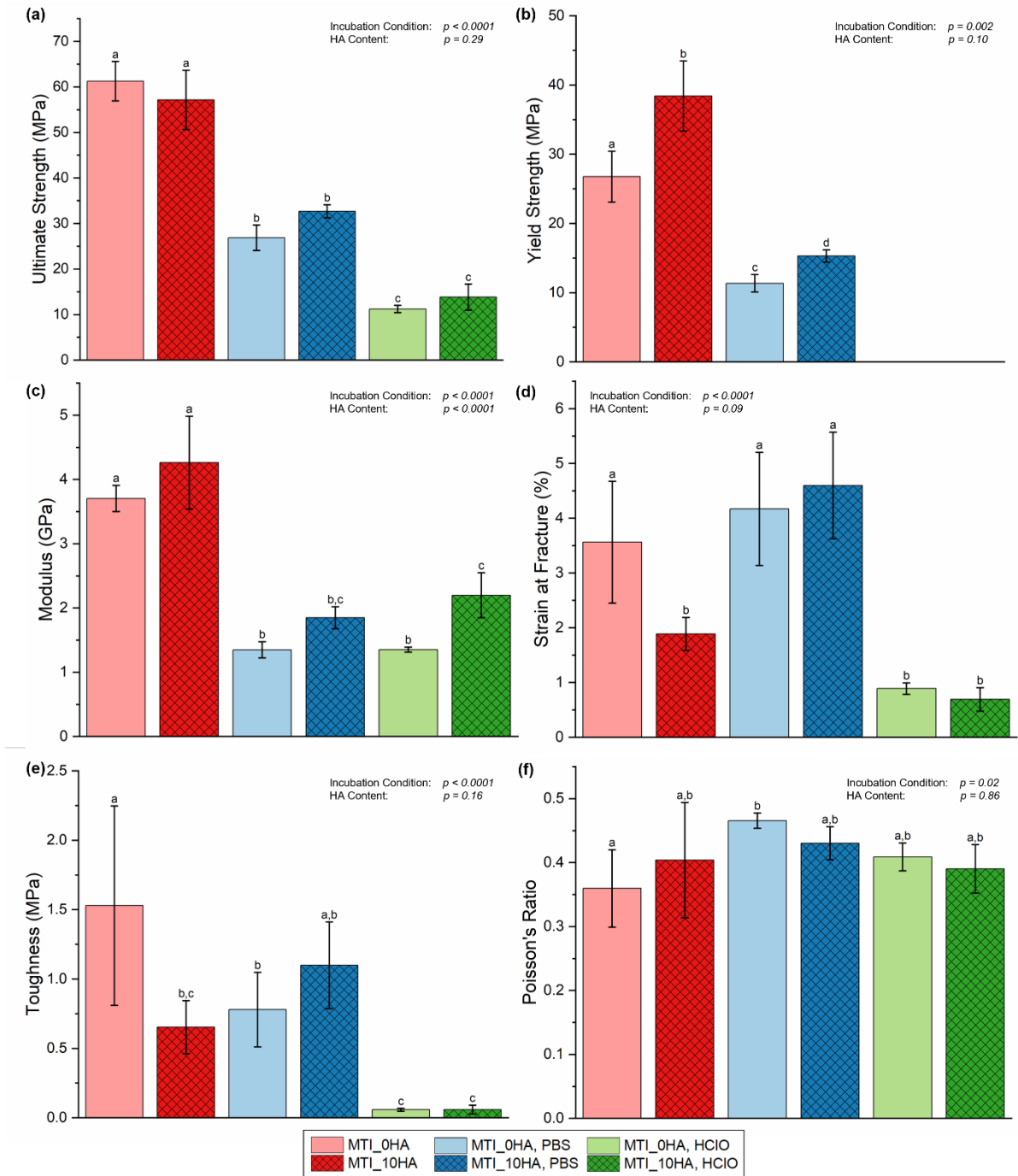


Figure 4-30 – (Previous page) – Tensile properties, (a) ultimate strengths, (b) yield strengths, (c) elastic moduli, (d) strains at fracture, ϵ toughness's, and (f) Poisson's ratios, of MTI_0HA (light) and MTI_10HA (dark, cross-hatched) compositions after 14 days in PBS (blue) and HClO (green) at 37 °C. The compositions' dry (red) mechanical properties are included. Data are presented as mean $\pm$ SD (n = 5) with different letters representing statistical significance ($p < 0.05$).

4.4.4 Compressive Properties

The dry, D14 PBS, and D14 HClO compressive stress-strain curves for the MTI composites can be seen in Figure 4-31. It appears that the addition of IM increased the yield strength of these materials. The trends due to hydration and oxidation observed are similar to those seen in the MT composites. Therefore, a significant reduction in mechanical performance is seen after incubation in either medium.

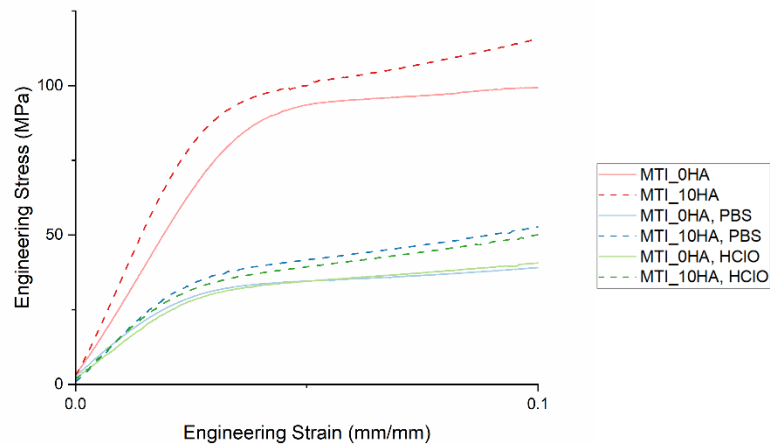


Figure 4-31 - Representative engineering compressive stress-strain curves for MTI_0HA (light, solid) and MT_10HA (dark, dashed) compositions after 14 days in PBS (blue) and HClO (green) at 37 °C. Reference lines (red) of the compositions' behavior tested dry are included for comparison. This figure shows a magnified view of the elastic region. Failure points occurred beyond 0.1 mm/mm.

The yield strengths and moduli of the MTI composites are seen in Figure 4-32 (a) and (b), respectively. In the dry control condition, the HA-containing group had a significantly higher yield strength and elastic modulus of 80.0 MPa ($p = 0.003$) and 2.8 GPa ($p < 0.0001$), respectively. These properties increased from the MT_10HA composite by 47.6 and 72.8 %, respectively. Therefore, there is a clear increase in mechanical performance due to IM inclusion. Within each composition, no significant differences were seen between the reductions in properties from PBS and HClO incubation. This indicates that the oxidative medium had minimal effects on mechanical performance when compared to the impacts of hydration. In the MTI_10HA composite, 66.1 and 53.9 % reductions in yield strength and modulus were seen after 14 days in PBS. This appears to be a larger reduction than seen in the MT_10HA composite after 14 days incubation in PBS. Reductions of 48.7 and 42.0 % were observed in the MT case (Figure 4-14). While it appears that IM inclusion has increased the impacts of water absorption on the compressive properties of the composites, it appears to have decreased the impacts of oxidative degradation on mechanical properties.

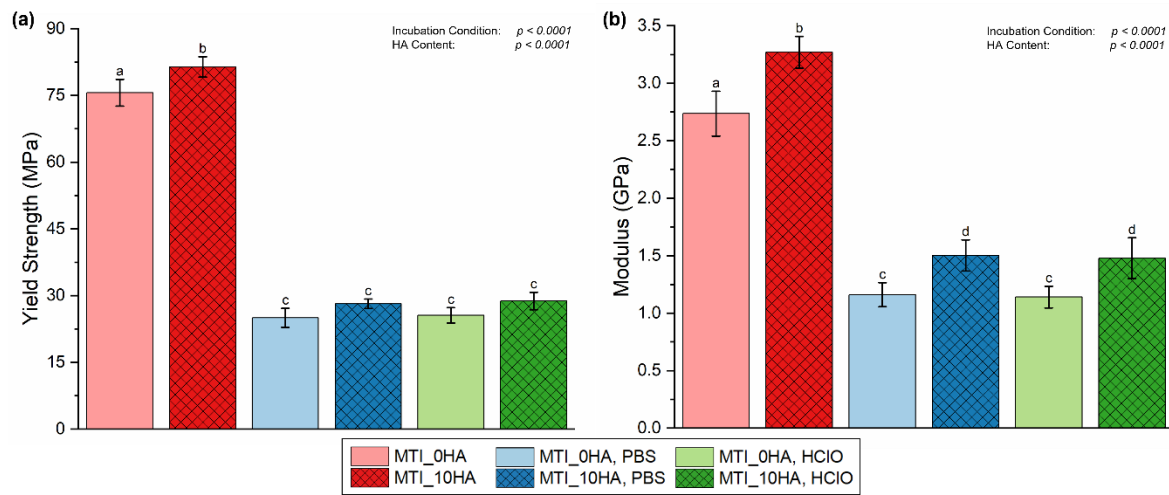


Figure 4-32 - Compressive properties, (a) yield strengths and (b) elastic moduli, of MTI_0HA (light) and MTI_10HA (dark, cross-hatched) compositions after 14 days in PBS (blue) and HClO (green) at 37 °C. The compositions' dry (red) mechanical properties are included. Data are presented as mean $\pm$ SD ($n = 5$) with different letters representing statistical significance ($p < 0.05$).

4.4.5 Wettability

It can be seen in Figure 4-33 that MTI compositions follow very similar wettability trends to those observed in MT compositions. Negligible reductions in contact angle were observed with IM inclusion. HA inclusion increased the hydrophilicity of the materials, with a 17.5 % decrease in contact angle between the two MTI compositions. All compositions in this study are hydrophilic.

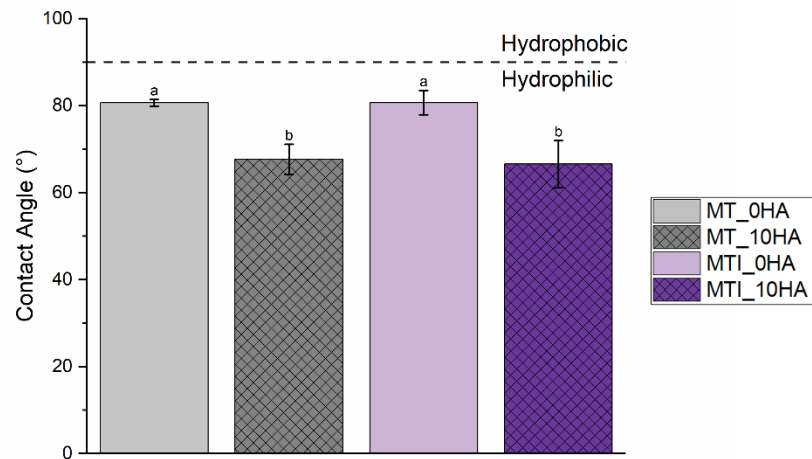


Figure 4-33 - Contact angle of MT (grey) and MTI (purple) compositions with DI water. The angle at which behavior switched from hydrophilic to hydrophobic is indicated. Data are presented as mean $\pm$ SD (n = 4) with different letters representing significance ($p < 0.05$).

Chapter 5

Discussion

5.1 General Discussion

The biopolymer nanocomposites developed in this study have demonstrated useful mechanical properties when tested under ambient conditions. The MT_10HA composite achieved a compressive yield strength of 75.0 ± 1.1 MPa. This exceeds the recommended ultimate compressive strength for bone cements of 70 MPa [52]. This composite also had an ultimate tensile strength of 51.8 ± 10.4 MPa. If you consider that bending strengths are typically larger than tensile strengths in materials [172], this composite should also exceed the ISO recommended bending strength for bone cements of 50 MPa [53]. Therefore, without the effects of physiological conditions, these biomaterials meet the minimum requirements for skeletal reconstruction materials. The elastic modulus of the MT_10HA composite was approximately a third of that of cortical bone, while it significantly surpassed the moduli of cancellous bone [11].

When compared to other synthetic bone graft substitutes, the mechanical properties of the MT composites far exceed the properties of materials developed for dental and maxillofacial applications [25]–[30]. However, the mechanical properties of other biomaterials developed for structural applications meet or exceed what was achieved in this study. PLA:HA composites developed by Russias et al. achieved bending strengths up to 130 MPa [40]. Polyurethane:HA composites have shown compressive yield strengths of 90 MPa [173]. The high mechanical properties achieved in these two studies are likely due, in part, to their high HA loadings (> 60 wt%). However, due to these high HA loadings, the processing techniques of these materials are limited due to their high viscosities. This significantly reduces the practical functionality of these materials and highlights the benefits of the mSLA printing technology employed with the MT composites in this thesis. The mAESO:TEGDMA:HA composite system is a competitive material when compared to other synthetic bone reconstruction materials, especially when considering mechanical properties and processing abilities.

It is interesting to note the variations between tensile and compressive properties. For instance, the RT yield strength of the HA composite was 30.9 ± 2.4 MPa under tension and 75.0 ± 1.1 MPa under compression. This indicates that these materials are strength asymmetric. Strength asymmetry refers to

the differences in deformation seen when a material is placed under tension or compression. This is a common phenomenon observed in polymers and composites, and therefore modified yield criterion have been developed to model the behaviour of these biomaterials [174]–[176].

Likewise, the elastic moduli measured in tension and compression were different (RT MT_10HA; 4.7 ± 0.4 and 2.4 ± 0.1 MPa, respectively). There are a variety of potential explanations for these differences. When tested in compression, there is a potential for friction to develop between the specimens and plates resulting in barreling of the sample. The effects of this were minimized by greasing the plates prior to each test to allow for unconfined compression conditions. There is also compliance introduced into tests that use the crosshead displacement of a mechanical testing machine to determine strains, as done in the compressive tests [177]. This causes the recorded stiffness of a material to be influenced by the machine's own stiffness. This was accounted for by correcting the experimental strains as discussed in Chapter 3 (Figure 3-5Figure 3-6). Based on the sources of variability, the moduli values derived under tension are more accurate representations of the material's true properties.

5.2 Effects of Physiological Temperature Conditions

It is clear from the mechanical results that there is an impact of increased temperature on these materials as expected. Increased temperature tended to reduce mechanical strength and elastic modulus. This agrees with the literature, with the most significant impacts on mechanical properties being observed in stiffness and strength [89]–[91]. This effect is seen significantly and most clearly in the compression trials (Figure 4-4). However, compression results were only reported in the elastic region, therefore the effect of temperature on plasticity was not fully evaluated. This is because approximately half of the test runs completed for this thesis did not fail within the allowable load limits of the testing apparatus. Beyond the yield point of the materials, the tests became more inconsistent with each other as the samples began to buckle.

In contrast, the entire range of the tensile tests could be observed. In addition to the changes in moduli and strengths due to physiological temperature, an interesting effect occurred of increased Poisson's ratio with temperature (Figure 4-2 (f)). A more significant effect is seen in the resin-only specimens, which is likely due to the stabilizing effect of fillers on thermal stability [178], [179]. Increased Poisson's ratios indicates that the material is more susceptible to deformation in the directions

perpendicular to loading, due to increased chain motion as a result of higher temperatures. This along with decreased moduli indicates the materials have reduced stiffness at 37 °C.

This reduction in stiffness agrees with the expected temperature behavior of viscoelastic materials (Figure 1-6). With increased temperature there is more energy present in the polymer system to allow for chain movement, therefore increasing the flexibility of the materials. As previously discussed, the viscoelastic or glass transition temperature ranges can be quite wide. This is clear from the DMA analysis performed in this study, which showed transition behavior from -7 to 156 °C for the MT-10HA composition. A broader transition region was seen in the 0HA composition. Therefore, it is clear that 37 °C would fall within the glass transition region of the materials. This is supported by the changes in mechanical properties observed from RT to 37 °C testing conditions.

An interesting phenomenon was observed in the tan delta curves of the materials (Figure 4-5 (b)), where an intermediate peak was seen before the main peak indicating T_g . While these peaks are typically quite sharp and clear, it is possible that they can be broadened by additives or processing conditions [166]. It is also possible that this is due to a second glass transition occurring due to separated matrix phases. These results differ from a previous composition's (mAESO:PEGDA:HA) behavior, which showed a clear and definitive tan delta peak at 89 °C [55]. However, this previous study did not test the DMA at as high of a temperature as done in this work. It is likely that replacing PEGDA with the more organized and tightly packed TEGDMA monomer has reduced the chain mobility therefore increasing the T_g of the blend. The beginning of the glass transition range, indicated by the storage modulus behavior, were similar in this study and the previous [55].

As previously mentioned, the mechanical properties achieved in these composites when tested at RT and dry conditions are competent and similar to those found in literature and standards. However, due to the influences of temperature, testing under RT conditions could lead to erroneous confidence in the material's performance, which in drastic cases could lead to potential implant failure when exposed to physiological conditions. For example, the compressive yield strength of the MT_0HA and MT_10HA compositions decreased by 29.5 and 26.5 % from RT to 37 °C, respectively. Therefore, due to the significant effects observed in this study, it is recommended that these composite materials be tested under physiologically relevant temperature conditions. Throughout the remainder of this thesis, materials were tested at 37 °C.

5.3 Effects of PBS Incubation

The effects of PBS incubation were evaluated with a variety of methods, including mass and volumetric change, surface effects, and mechanical properties. It is apparent from the wet mass results (Figure 4-6), that water absorption occurred in both compositions. Mass increases of ~ 6 and 5 % were observed in the MT_0HA and MT_10HA materials, respectively. These swelling plateaus are representative of the equilibrium reached between the polymer and solvent, as described by Flory-Rehner [117], [118]. It took approximately a week for the swelling behavior to equilibrate. The absorption in the MT compositions is greater than the ~ 3% increase seen previously with AESO/PEGDA inks [75]. This could be due to the altered matrix composition, different processing and printing processes, or the use of PBS instead of DI water as the incubation medium [180]. The slight decrease in absorption in the presence of filler is consistent with previous literature [102], [126]. Very minimal dry mass reductions were observed (< 1 and 0 % for MT_0HA and MT_10HA, respectively), indicating that minimal if any loss of due to hydrolytic degradation occurred. A study by Correlo et al., showed similar results with HA inclusion resulting in significantly reduced water uptake and degradation [181].

The effects of water absorption and swelling were also visually observed in the materials. As seen in Figure 4-8, large, macroscopic cracking occurred in the 0 vol% HA material. Osmotic surface cracking has been seen in polymer matrices exposed to aqueous environments [123], [129], [130]. This can be a result of the development of internal stresses due to swelling. There is also the potential that different phases of a material can have differential swelling, resulting in uneven internal stress distributions in the composite. At this time, it is unclear whether the mixture of mAESO/TEGDMA results in a homogenous mixture or whether there is phase separation within the matrix, though high miscibility is expected. Interestingly, the HA composite had no visual macroscopic cracks. HA particles could play a role in preventing these large cracks from developing through mechanisms such as crack pinning or crack deflection [182], [183]. Fillers can increase the energy required for a crack to propagate or cause cracks to deflect around them.

Even though approximately half of these materials is composed of hydrophobic materials [77], the materials still have contact angles less than 90° (Table 4-1). While these materials are classified as hydrophilic, their contact angles are near the transition to indicate relative hydrophobicity. This characterization is commonly seen in dental materials, even after hydrophobic methacrylation has occurred [97], as in the case of mAESO and TEGDMA. This result is expected based upon the

significant water absorption seen in these materials. It is common when hydrophilic and hydrophobic monomers are combined to have a resulting hydrophilic matrix. It has been observed that the hydrophilic phases encourage water movement throughout the entire material [184]. This was observed in the materials used in this study, with high levels of absorption observed (Figure 4-6). HA inclusion increased the hydrophilicity of the composite, which is a common observation [185].

The consequences of water absorption are clearly seen in the mechanical results. A significant reduction in mechanical performance was seen under tension (Figure 4-11) and compression (Figure 4-13) with PBS incubation. For example, the tensile ultimate and compressive yield strengths of MT_0HA decreased 64.2 and 58.2 % from their dry counterparts after 14 days in PBS. Absorption also decreased the elastic moduli and toughness. HA inclusion appeared to slightly increase the material's resistance to hydrolytic degradation. This phenomenon agrees with what has previously been seen in literature and demonstrates the beneficial effect of compositing on absorption behavior [102], [126]. For instance, in contrast to the above case, MT_10HA had 59.2 and 48.7 % decreases in ultimate tensile strength and compressive yield strength, respectively. It is clear that the swelling behavior of these materials and the resultant mechanical performance is a significant issue.

It is difficult to separate the effects of hydrolysis, swelling, and absorption on filler-matrix interfaces into their specific influences on mechanical properties. There is potential that hydrolysis is occurring, though likely very minimally due to the dry mass loss results (Figure 4-7 (a)). Hydrolysis can result in embrittlement and reduced failure stresses [111]–[113], both of which were observed in the MT compositions. Swelling induces mechanical stresses, osmotic cracking, and potentially polymer bond cleavage [116], [122]–[125]. These effects could result in reduced ultimate and yield strengths, as well as altered moduli and embrittlement. Finally, compositing of polymers can both reduce the effects of hydration but increase stress concentrations, interfacial debonding, and cracking, resulting in reduced mechanical properties [129], [130]. Therefore, there is likely a combination of mechanisms occurring in the material-solvent interaction that are leading to the significant differences in mechanical results observed.

The combined effects of temperature and hydration on these specimens was very significant. While both conditions contributed to reduced mechanical properties, there are also compounding effects that can occur due to their interactions. Water absorption and hydrolysis can alter the glass transition range of a polymer, and increased temperatures have been shown to increase the rate of moisture absorption

[114], [115], [131]. When considering implantable materials, both of these conditions apply. From the ambient conditions, significant decreases in mechanical behavior were seen. 78.9 and 62.7 % reductions in MT_0HA ultimate tensile strength and modulus were observed after 28 days in PBS when tested at 37 °C . The resultant mechanical properties when tested under physiologically relevant conditions are below the recommended values from ASTM and ISO standards [52], [53]. Therefore, there is a significant requirement to test materials intended for biological applications in conditions representative of their application's environment. It is rare in literature for these factors to be considered in the early phases of biomaterial development, in particular their impacts on mechanical performance, which is a significant methodological deficiency.

5.4 Effects of Oxidative Degradation

It is clear that degradation of the MT compositions occurred in the two oxidation media. In Figure 4-15, after 7 days significant mass losses were observed due to HClO and H₂O₂. HClO appears to induce increased initial solvent absorption in MT_0HA. In both compositions, this oxidative media induced a -5.9 to - 7.4 mg/day degradation rate. H₂O₂ induced less degradation of MT_0HA. However, a very large degradation rate was observed in the 10 HA composite (-13.6 mg/day). This degradation rate was unexpected when compared to the other material/media trends. Throughout the degradation study, it was common for the HA samples to increase the rate, intensity, and temperature of the H₂O₂ reaction when compared to the resin-only materials. It is therefore likely that an unanticipated reaction occurred between H₂O₂ and HA. Hydroxyapatite's chemical structure is composed of phosphate ions bonded via calcium (Ca²⁺) ions. A potential mechanism is proposed of an additional reaction occurring due to the calcium ions acting as a catalyst in a Fenton-like reaction pathway, leading to the development of additional hydroxyl ROS. HA has been previously used as a catalyst for oxidation reactions [186].

This extreme effect due to H₂O₂ and HA interactions was also observed in structural changes in the material, seen in the dry percent changes of mass and volume (Figure 4-16). A decrease in mass of - 38.3 % was observed after a 28-day period in this degradation media. In a previous study, the HA agglomerations present in similar composites were determined to be 2 – 10 µm [55]. The voids observed in the surface of the degraded HA composite appear to be a similar scale to these agglomerations (Figure 4-19). Therefore, there is the potential that the HA particles and agglomerations were targeted by degradation action and released from the composite into the degradation media. Interestingly, a lower

reduction in mechanical properties was observed when compared to the effects of HClO. The significant reduction in volume of the H₂O₂ degraded samples allowed for higher strengths to be obtained at the reduced loading the HA composites were able to support.

In contrast to the 10 HA composite's interactions, H₂O₂ had significantly more controlled and uniform effects on the resin-only material. At day 14, no significant differences were observed in the mass loss of MT_0HA exposed to H₂O₂ and HClO (Figure 4-16 (a)). In the macroscopic view, cracking was observed across the surface of the samples (Figure 4-17). The cracking was greater than what was previously seen in the PBS-incubated samples. This could potentially be due to more aggressive or unbalanced stresses arising within the material's surface due to swelling and degradation mechanisms of the H₂O₂. Interestingly, the microscopic images displayed a smooth, mostly undegraded surface. This is indicated by the grid pattern that arises due to the mSLA printing technology still being visible on the degraded surface in Figure 4-18. It is possible that mass loss and degradation are occurring within the cracks and surface defects that arise, and not upon the bulk surface. Within mechanical properties, decreases in strength and modulus were observed. This could be a result of increased osmotic and stress cracking, or due to oxidative degradation altering the bonds within the polymer backbone. Decreased elastic modulus is a key indicator of oxidative degradation [133]. While expected and comparable results to HClO degradation were observed with H₂O₂ and MT_0HA, the extreme and unexpected reaction that occurred with HA is a significant issue. It is difficult to determine the validity of these results. Therefore, the HClO results were determined to provide a clearer and more trustworthy view of the degradation potential of these biomaterials.

One of the most significant differences between the samples exposed to PBS and HClO is apparent in the final dry mass loss (Figure 4-16). There was clear mass loss occurring due to oxidation with D28 losses of 22.0 and 15.7 % for MT_0HA and MT_10HA, respectively. HA appears to reduce the amount of oxidative degradation. This degradation is clearly seen macroscopically and microscopically (Figure 4-17 and Figure 4-18, respectively). The HClO appears to degrade the surface of the specimens resulting in large micron to millimeter sized voids. Due to this degradation pattern, large particles are released from the surface of the specimens. This suggests that these samples may have low durability *in vivo* and could potentially create issues in the surrounding tissue.

Under SEM, crescent shaped crack initiation regions were observed, suggesting the mechanism of degradation of the material's surface. Interestingly, the inclusion of HA appears to reduce the number

of voids and decrease the uniformity of their shape. This could be due to HA inhibiting or redirecting the growth of the defects. At 1000x magnification, the surface of the voids is smooth (Figure 4-19). It is possible that this degradation pattern occurred due to phase separation of the mAESO and TEGDMA with one having a preferred degradation over the other. Preferred degradation has been correlated with different phase densities previously [141]. The presence of these cracks suggests a mechanical mechanism influencing the properties of these materials, in addition to the chemical effects of degradation. The defects that are formed due to HClO, in particular the cracks (Figure 4-18; highlighted in yellow), are likely to cause of reduced mechanical performance (strength) as these cracks propagate under tensile loading. The presence of cracks leads to areas of stress concentration leading to increased likelihood of brittle failure [187]. Under tension, the cracks will open perpendicular to the applied force. Under compression, cracks will open parallel to the direction of loading due to Poisson's effect. Therefore, it is expected that the impact of these defects should be more pronounced under tension than compression.

Small, mostly insignificant reductions in tensile and compressive strengths were seen with HClO incubation when compared to the effects of PBS incubation (Figure 4-21Figure 4-23). Likewise, minimal changes were observed in moduli, toughness, or Poisson's ratio due to oxidation. Interestingly, a slight increase in fracture strain was observed, indicating that oxidative degradation of these materials might have a minor plasticization effect (Figure 4-21 (d)). When compared to the reduction in properties from swelling effects only, the additional oxidative degradation is minor. It is impossible to isolate the effects of oxidative degradation from the mechanical consequences of swelling, therefore the exact effect of oxidative reactions is unclear. When considering the overall effects of the oxidative media, in particular the mass and surface properties, it is clear that these materials are susceptible to oxidative degradation.

5.5 Effects of Isosorbide Methacrylate Inclusion

Isosorbide methacrylate was trialed as a means to control degradation in these composite biomaterials due to its reported antioxidant properties [135]. It also increased mechanical strength and stiffness in a previous study [55]. This increase on mechanical performance was seen in this study with increased tensile strengths and moduli. Under 37 °C test conditions, the MT_10HA composite had a tensile elastic modulus of 4.3 GPa and an ultimate strength of 57.1 MPa. Unexpectedly, strain at fracture and

toughness also increased with IM addition. These increases in mechanical performance are due to the short, cyclic structure of IM, which acts as a stiff segment within the matrix and increased covalent bonding within the polymers due to the increased number of oxygen molecules [55], [78], [188]. A similar composite (with PEGDA in place of TEGDMA) demonstrated a modulus and ultimate strength of ~ 7.5 GPa and 66 MPa, respectively, under tension at room temperature [55]. Fracture strain decreased upon IM inclusion and was ~ 2.1 % at 30 vol% IM [55]. Interestingly, in this study, IM inclusion increased the fracture strain. These altered effects are likely a result of the differences in polymer chain arrangement due to TEGDMA and the increased temperature testing conditions employed in this thesis.

When exposed to PBS, it appears that the inclusion of IM increases the absorption and swelling of the materials (Figure 4-24). The resin-only MTI composition reached ~ 11 % mass increase after 14 days in PBS which is significantly higher than what was observed in MT_0HA. Additionally, the absorption does not appear to plateau within a week, as observed with MT compositions. This could be a result of the effect that different levels of crosslinking can have on the swelling ability of a material [117], [118]. Additionally, the presence of additional oxygen atoms in the IM rings (Figure 1-8 (c)) may result in increased hydrogen bonding between the polymer and water molecules [189]. Interestingly, the wettability of the material was not significantly different from MT_0HA (Figure 4-33).

In contrast to its effect on water absorption, IM inclusion did appear to slow degradation due to HClO incubation. The rate of degradation between D7 and D14 was an order of magnitude slower than what was observed with MT degradation (Table 4-3). Overall degradation mass loss at D14 was also decreased by IM inclusion. This is supported by the surface properties of these materials. It was observed in Figure 4-26, that MTI_0HA has significantly less surface voids when compared to MT_0HA (Figure 4-17). No observable voids were present in the MTI_10HA composite. These trends were supported by SEM images (Figure 4-27). Therefore, it is apparent that IM acts as an antioxidant.

As seen with the MT compositions, significant reductions in mechanical strength and modulus occurred due to PBS and HClO incubation. Interestingly, the effects of PBS hydration on the strain at fracture are drastically different. When MT compositions were exposed to PBS, this caused severe embrittlement and reduced fracture strains which resulted in no discernible yield points. In contrast, PBS incubation increased the fracture strain immensely (i.e., 143.4 % increase in MTI_10HA fracture

strain). This has led to the development of a clear yield point in these samples, and an increased plasticity region. The significant increase within the composite is likely due to weakened HA-matrix interfacial bonding resulting in easier deformation of the material [190]. In contrast, the HClO incubated samples demonstrated brittle failure. This was likely due to the presence of cracks and stress concentrations in the material, which increase the potential for brittle failure of the material. The compressive properties (Figure 4-32) present a clear image that the majority of the mechanical property reductions in this material system are due to the effects of water absorption and swelling, and not oxidation. There was only a 3.3 and 0.8 % difference between the yield strength and modulus of MTI_10HA exposed to PBS and HClO, respectively.

From these results, it appears that IM has contrasting influences on the performance of these materials when exposed to hydration and oxidative media. While it has been shown that IM inclusion can be used to decrease and control oxidative degradation due to its antioxidant properties, it also increases the water absorption and swelling of these materials. This leads to increased mechanical consequences of swelling and other water absorption effects. Therefore, the benefits of reduced oxidation on mechanical properties are minimal when compounded with the negative effects of swelling.

5.6 Limitations

One key limitation of this study is the method that was used to impose environmental conditions upon the samples. The method of heating the testing machine, a reptile heat lamp (Figure 3-3), could be refined in further studies. Due to the setup of the system, the heat lamp was only applied to one side of the testing specimens, therefore non-uniform heating throughout the sample was likely to occur. Using infrared heating and pre-heating the samples in a low temperature oven likely negated this effect. There is also a possibility that heating of the load and displacement cells lead to data creep, however, this was reduced by preheating the machine and allowing the transducers to equilibrate at the increased temperature (Figure 3-4).

Additionally, testing of hydrated samples might be improved by a system that would allow for the samples to be continuously hydrated throughout the mechanical tests. Samples were kept in their incubation media until the last possible moment to reduce the effects of evaporation. To reduce these potential inconsistencies and to represent an immersed, heated environment more accurately, an environmental testing chamber could be employed. These allow for controllable temperature and fully

immersed testing specimens. Another limitation of this testing system is the stronger materials used in this study exceeded the loading limit of the machine when tested in compression. This prevented the investigation of behaviour under compression beyond the yield point, such as failure stress and strain.

Due to the complexities that exist inside the body, it is doubtful that these *in vitro* experiments fully capture the behavior they would display *in vivo*. *In vivo*, a variety of concurrent degradation mechanisms occur simultaneously. There is the potential for hydrolytic, oxidative, and enzymatic degradation. Also, the effects of osteoclast and osteoblast resorption and remodeling, and mineralization of the materials could all have additional impacts on the behavior of the implanted biomaterials. Even when focusing solely on oxidative degradation, many more ROS are generated during natural cell metabolism and inflammation responses than are mimicked by HClO or H₂O₂. This highlights the limitation of using *in vitro* conditions to model actual *in vivo* physiological conditions.

Another limitation of the oxidative degradation experiments is that they tested the material's response to an extreme concentration of ROS. As previously discussed, both the H₂O₂ and HClO concentrations used in this study are higher than would be found *in vivo*. The concentrations of H₂O₂ and HClO were three to six, and four orders of magnitude higher than anticipated *in vivo* [157], [161]. Elevated concentrations of ROS are commonly used *in vitro* to gain meaningful and significant comparisons between different experimental groups [75], [151]–[156]. While these *in vitro* degradation systems are limited in their ability to fully mimic *in vivo* conditions, they are a good indicator of a material's susceptibility to these degradation pathways.

Chapter 6

Conclusions

6.1 Conclusions

This thesis studied the impacts physiologically relevant testing conditions can have on the behavior of biopolymer nanocomposite biomaterials. A testing system was developed to mechanically characterize samples incubated in different media at human body temperature, 37 °C. When tested at room temperature and dry conditions, biopolymer HA composites exceeded the suggested mechanical properties for standard bone cements. Note that these strength and moduli requirements were tested under ambient conditions according to the standards [191]. It has been demonstrated that an acrylic bone cement demonstrated 10% and 3% reductions in compressive ultimate strength when exposed to physiologically relevant temperatures and moisture, respectively [192]. As seen in bone cements, it was demonstrated that temperature, water absorption and oxidative degradation all impacted the composite materials, in particular their tensile and compressive strengths and moduli. For example, after 14 days of incubation in PBS at 37 °C, MT_10HA demonstrated a 49 and 42 % decrease in tensile yield strength and modulus, respectively. It is clear from the results observed in this thesis that exposure of these materials to physiological conditions might lead to failure under relevant loading conditions.

It was hypothesized that increased temperature would decrease mechanical strength and stiffness. This was supported in both compression and tension, with significantly lower ultimate strengths, yield strengths, and moduli measured. Interestingly, Poisson's ratio for both MT compositions was increased at 37 °C. It was theorized that the mechanical properties of these materials would be temperature dependent due to the viscoelastic nature of polymer composites, and the impact that temperature can have on their mechanical properties. It was confirmed with DMA that these materials at 37 °C are in their glass transition range, therefore it is understandable that these differences in mechanical properties would be observed.

As hypothesized, water absorption and swelling occurred in these composite materials and caused inferior mechanical properties. As predicted, based on the nature of the phases in the material, the MT compositions were minimally hydrophilic. This led to high levels of water absorption, which ultimately led to decreased mechanical performance. It is likely that the reduction in mechanical properties is due to the implications of swelling, osmotic cracking, and weakened matrix-filler interphase. While the

monomers in these material systems have functional groups susceptible to hydrolysis, it is the hydrolytic degradation was negligible. Very minimal overall mass changes were observed for the specimens incubated in PBS.

Greater impacts of oxidative degradation were observed than anticipated. Greater mass loss was seen when compared to previous studies [75]. Large mass changes occurred, with MT_10HA losing -15.7 ± 0.3 % after 28 days when incubated in HClO solution. The significant degradation was confirmed with surface imaging, where large voids were seen from HClO degradation action. While there were some changes in mechanical performance between the oxidatively degraded and PBS hydrated samples, the most significant reduction in properties appears to occur due to the effects of water absorption. Unexpectedly, H₂O₂ produced a relatively extreme reaction with HA and therefore excluding it from future trials with these materials.

Finally, as anticipated, IM acted as an antioxidant. In addition, IM increased the mechanical performance of the biopolymer nanocomposites. The most significant differences in degradation were noted for the mass change and surface properties. For example, in the 10 HA composites 33 % less mass was lost due to degradation upon IM inclusion. In contrast, IM inclusion did appear to increase absorption of water, therefore minimal overall improvements in mechanical properties were observed.

6.2 Recommendations

A significant problem has been identified in the development of these biopolymer nanocomposites. The mechanical performance of these biomaterials is significantly impacted by testing under physiologically relevant conditions. The main issue appears to be the water absorption and resultant swelling. Therefore, the main recommendation for future work would be to negate the impacts of water absorption. This could be done in a variety of ways, such as increasing the hydrophobicity of the resin or increasing the crosslinking to reduce the swelling. As has been done with methacrylation of dental resin materials, it is possible to functionalize materials to reduce their hydrophilicity. This has been done in this and previous works with the methacrylation of AESO [55], however it is unlikely this had a drastic effect on hydrophilicity of the overall resin as AESO is hydrophobic [77]. Additionally, hydrophilic components of the resins, such as TEGDMA, could be replaced with more hydrophobic alternatives. Increasing the interface strength and stability of the matrix-filler interface and reducing the size of particle agglomerations should also be explored through HA functionalization. Decreasing

the filler size to the nanoscale has been shown to reduce the space in the composite that water can penetrate [123], [129]. However, in future work, when varying the hydrophilicity of the materials it is important to consider the challenges this may cause, such as potentially negatively impacting the biocompatibility of the biomaterial.

Using simple ROS generating media in *in vitro* experiments is a good initial evaluation of a material's degradation potential. However, as previously mentioned, this is not representative of the conditions occurring *in vivo*. Therefore, further *in vitro* evaluations of these materials is required to determine their susceptibility to enzymatic degradation and osteoclast resorption mechanisms. Collectively, these three markers can provide a more complete *in vitro* perspective on the degradation potential of these materials and their suitability as a degradable and resorbable biomaterial. To further this investigation, *in vivo* trials would also be recommended to obtain a more accurate view of these materials' response to physiological environmental conditions. Additional concerns that should be addressed include furthering the investigation into the mechanical suitability of these materials. This could include investigating the effect of mineralization, a natural process on bone graft materials, on mechanical stability. Other considerations include investigating the impact of temperature, hydration, and oxidation on additional mechanical properties such as viscoelasticity, fatigue, and fracture toughness.

6.3 Contributions

The work presented in this thesis demonstrates a comprehensive investigation into the effects of physiological operating conditions on biomaterial functionality. The effects of temperature, hydration, and oxidation are often neglected in the early development of biomaterials. Neglecting these effects has led to significant and high-risk implant defects and failures in the past. In particular, the impact on mechanical properties is often unexplored. As shown in this thesis, mechanical properties can be more than halved through testing in environmentally relevant conditions. Implementing these considerations into the early stages of material development is essential to ensure the properties you are observing are relevant to their designed applications.

To characterize the impacts of these factors, a testing system was developed to allow for mechanical testing under 37 °C conditions. It was shown that this system, coupled with samples incubated in media prior to testing, provides relevant data displaying clear and significant differences from samples tested at room temperature and dry conditions. An *in vitro* system to provide *in vivo* context to material

development, in particular mechanical degradation, has been developed. This thesis clearly demonstrates previously unforeseen issues present in the WCBSL's functionalized monomer and HA biocomposites. This will provide a fundamental basis of the effects of physiological conditions for the WSBCL to improve these materials in future works.

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Appendix A

Supplementary Data

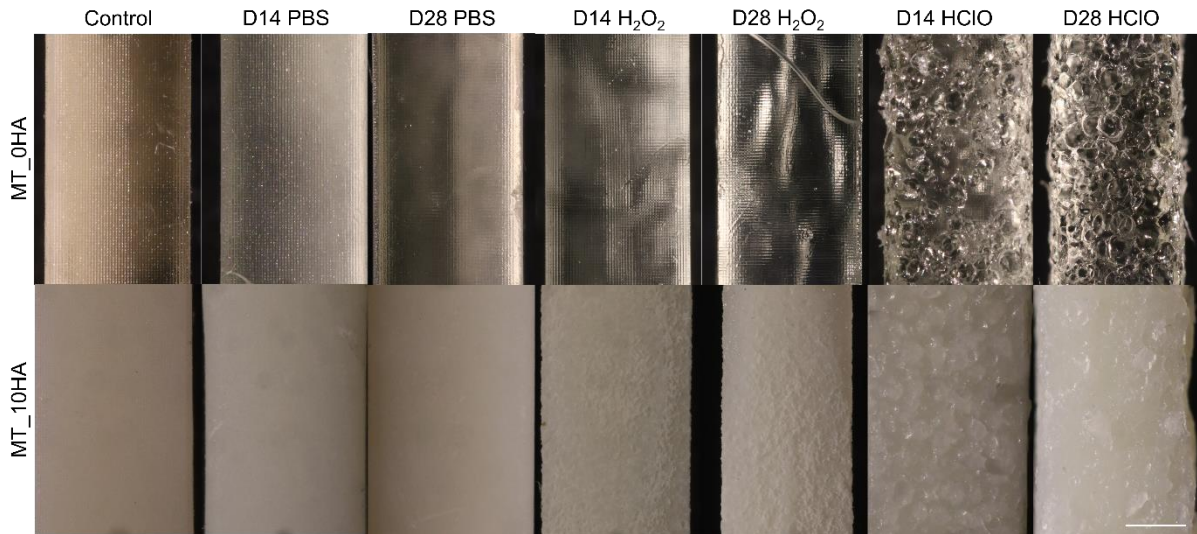


Figure A-1 - Macroscopic images of the top surface of MT dog-bone specimens after 14- and 28-day periods in PBS, H₂O₂, and HClO. Images taken prior to any incubation are used as a control. The scale bar represents 1 mm.

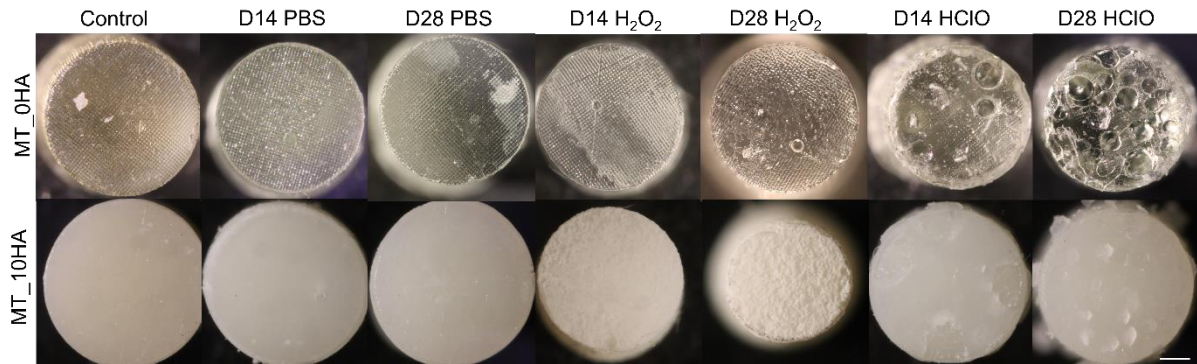


Figure A-2 - Macroscopic images of the flat surface of MT cylindrical compression specimens after 14- and 28-day periods in PBS, H₂O₂, and HClO. Images taken prior to any incubation are used as a control. The scale bar represents 0.5 mm.

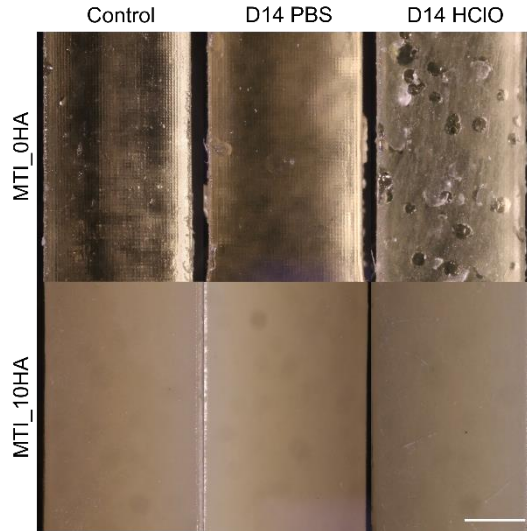


Figure A-3 - Macroscopic images of the top surface of MTI dog-bone specimens after 14 days in PBS and HClO. Images taken prior to any incubation are used as a control. The scale bar represents 1 mm.

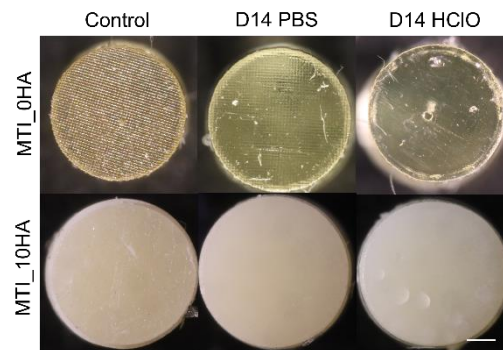


Figure A-4 - Macroscopic images of the flat surface of MTI cylindrical compression specimens after 14 days in PBS and HClO. Images taken prior to any incubation are used as a control. The scale bar represents 0.5 mm.