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Highly Robust and Biocompatible Hydrogel for Joint Injuries

BME31112 - Research and Design Group Project Report

BSc. (Hons) Biomedical Engineering

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Abstract

Joint injuries are defined as the damage on joint tissues which includes articular cartilage, ligaments, tendons or menisci. Over the years, an array of therapeutic strategies focused on non-surgical conservative management, minimally invasive strategies and surgical strategies has been developed to treat joint injuries. However, none of these developed methods are able to restore the biomechanical properties of the tissues while being biocompatible. Therefore, a new favorable remedial strategy utilizing hydrogel mechanical and biocompatibility properties is proposed to provide an optimal medical approach for joint injuries. While there is a wide spectrum of joint injuries, this project mainly focused on a novel therapeutic device, HydroJel, for meniscus and tendon injuries through the development of highly robust and biocompatible hydrogel for joint injuries.

To optimize the formula of HydroJel, a series of characterization were done including swelling ratio test, compressive and tensile testing and simulation, cell viability assay, and adhesion test. Meanwhile, animal samples from bovines and rats were harvested and tested as the reference for the product modification. In consideration of the product commercialization, the feasibility of 3D printing and double syringe system delivery of HydroJel were explored to verify the possibility in product personalization clinical application.

Regarding the application of HydroJel in meniscus replacement, it was revealed that HydroJel was capable in attaining a compressive modulus comparable to that of bovine and human menisci. Moreover, with the employment of alginate at medium viscosity, the 3D printing of personalized HydroJel was achieved under the novel formulation. Besides, for the tendon rupture repair, histological investigation provided a strong justification in the intimate adhesion between the tendon tissue and HydroJel. The superior effectiveness of HydroJel in relinking the torn tendon and redistributing the concentrated stress which presented in a ruptured tendon, were confirmed under an array of mechanical testing and simulation study. The possible immersion of Hydrogel in the clinical setting was also supported under the double syringe system study. The biocompatibility of HydroJel is established through the cell viability assay whereas the swelling ratio was not perfectly ideal in the swelling test.

To conclude, HydroJel has exhibited a superior biocompatibility and mechanical property to provide a relatively low cost and flexibility usage to various applications worldwide. However, limitations of the product are also identified, including the incomplete characterization, high swelling ratio, and high regulatory standard before entering the

market. With this being the case, it is suggested that the completion on product characterization, continuous formula modification and clinical trials are the crucial future development directions.

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List of Abbreviations:

AT	Achilles Tendon
CAF	Central Animal Facility
CMI	Collagen meniscus implant
DMEM	Dulbecco's Modified Eagle Medium
ECM	Extracellular matrix
EU	European Union
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
GAGs	Glycosaminoglycans
HBSS	Hank's Balanced Salt Solution
hMSCs	Human mesenchymal stem cells
MSCs	Mesenchymal stem cells
MW	Molecular Weight
PAAm	Poly(acryl amide)
PBS	Phosphate-buffered saline
PEG	Polyethylene glycol
PEGDA	Poly(ethylene glycol) diacrylate
PGs	Proteoglycans
PT	Patella Tendon
SD	Sprague Dawley
TC	Tenocytes
TSCs	Tendon stem cells
UV	Ultraviolet
USA	United States of America

Chapter 1. Introduction

Joint injury, one of the musculoskeletal condition associated with the damaged joint tissues, ranging from tendons, ligaments, menisci to articular cartilages, accompanied with other musculoskeletal conditions, have been observed to be the second largest contributor towards disability in the world ("Musculoskeletal conditions," 2018). Solely considering the tendon and meniscus tissue, it is reported that an estimated healthcare expenditure exceeding €140 billion is spent for over 30 million of human-tendon related operations per year (Abbah, Spanoudes, O'Brien, Pandit, & Zeugolis, 2014). 23.8 out of each 100,000 people in the world population are diagnosed with meniscus related injury (Englund, Roemer, Hayashi, Crema, & Guermazi, 2012). With this being the case, as the global aging population booming, that the prevalence of joint injury is anticipated to boost at an astounding rate in the coming future is unavoidable, not to mention the expanding incidence rate of ligament and articular cartilage injury. Arrays of therapeutic strategies have been developed previously under three main approaches, non-surgical conservative management, minimally invasive strategies and surgical strategies, in hope of maximizing the regrowth and preservation of host tissue whenever possible. However, rarely do these treatments attain full recovery of joint tissue, generally due to the compromised functionality and mechanical property (Abbah et al., 2014; Makris, Hadidi, & Athanasiou, 2011). Therefore, the crux of prevailing treatment development has increasingly been laid on the amelioration of existing treatments or the design of a more superior measurements, through the cooperation of novel mechanical, biological or chemical factors, enabling multifunctional performance (Abbah et al., 2014; Makris et al., 2011).

One material that is frequently used to embed with these factors is hydrogel. Hydrogel is a hydrophilic, three dimensional crosslinked network which is capable of absorbing 10-20% up to thousands of times their dry weight in water (Hoffman, 2002). Despite the wide and diverse range of hydrogel composition proposed, its fabrication can fundamentally be classified into either physical mean, as an ionotropic hydrogen, or chemical mean, as a chemically crosslinked gel, through different combination of natural material such as chitosan, alginate, collagen and fibrin, or synthetic material such as PAAm, PEG and P(biscarboxy-phenoxy-phosphazene). The simple, alterable and versatile production principles and functionalization process, controllable reversibility constituted by the intrinsic responsiveness to the environment, and the biocompatibility to pattern with cells and growth factors, are three crucial characteristics entitle hydrogel as an ideal material to carry numerous factors. However, the mechanical property and bioactivity of hydrogel

have always been identified as two common concerns in the intended applications, requiring advance enhancement.

In light of this, hydrogel is possibly a potential material for superior joint injuries repair. Yet, the improvement on the mechanical property of hydrogel is the vital key in establishing their unwavering linkage. Drearily, the compensation on biocompatibility is the inveterate problem exhibited by the current techniques in boosting the mechanical strength of hydrogel. With this being the case, this study aims to examine the potential capacity of hydrogel, which has been prepared with a novel formulation, in exhibiting an adjustable mechanical property and consistent biocompatibility to repair distinct tissues under joint injury. The hydrogel formula employed in the study is dominantly comprised of PEGDA, alginate, and Type I collagen. To investigate the versatility of this hydrogel towards joint injury repair, meniscus lesion and tendon rupture have been utilized as two case studies. The meniscus lesion will be remedied using the replacement approach, and the tendon rupture is healed through the adhesive scaffold approach, the adhesive glue. An additional linker molecule, PEG-NHS is added for the case of tendon rupture. The fabricated hydrogel is first verified respectively with its mechanical properties through a series of mechanical testing, from machinery testing to animal model testing, referring to the specific cases. Subsequently, in vivo testing is exploited in studying the biocompatibility of the hydrogel.

Chapter 2. Literature Review

2.1 Structural anatomy

While the vital role of meniscus in load bearing, load transmission, shock absorption, stress reduction and joint stability maintenance is well established (Englund et al., 2012; Gray, 1999; Makris et al., 2011; Verdonk et al., 2005), challenges is continuously presented in searching for a more optimal therapeutic measurement to meniscal injuries, due to its limited self-healing potential related to the restricted vascularisation. The menisci, the medial meniscus and lateral meniscus, located in the lateral compartment and the medial compartment respectively between the femur condyle and tibia plateau within the knee joint, are two fibrocartilage disc in crescent shape (Englund et al., 2012; Gray, 1999; Makris et al., 2011). Under cross-sectional perspective, the menisci are in a wedge shape with an outer convex surface and an inner concave surface. The intrinsic structure of menisci can principally be categorized into the two zones, the superficial zone and the deep zone, according to the distinct collagen alignment demonstrated. In the superficial zone, collagen type I fiber is predominantly oriented in the radial direction in a less ordered manner. However, circumferentially arranged collagen type I fiber, which parallels to the periphery, in turn, dominates the main structure in the deep zone, with a few radial fibers perforating though (Englund et al., 2012; Fithian, Kelly, & Mow, 1990; McDevitt & Webber, 1990). Stability of the meniscus is mainly upheld by the medial collateral ligament, transverse ligament, menisiofemoral ligament and the attachment between the horns and the intercondylar region of tibia (Englund et al., 2012; Makris et al., 2011). In contrast, attachment provided by lateral collateral ligament is relatively flexible and mobile. Although the medial meniscus and lateral meniscus generally exhibit no distinct deviation in term of the specific vascularization and innervation structure, specialized ECM and cell it possessed, they illustrate a clearly different dimensions, with lateral meniscus depicting a greater range in size, shape, thickness, and mobility (Makris et al., 2011).

Distinctively, comparing with meniscus, tendon possesses a dissimilar structural architecture. Tendon has long been known as the critical element in transmitting the muscle force to bone, permitting locomotion and enhancing joint stability (Voleti, Buckley, & Soslowsky, 2012). To facilitate this function, tendon is attached firmly to the muscle and bone at its ends, forming a muscle-tendon junction and tendon-bone junction, enthesis (Wang, 2006). The internal organization of tendon is a multi-unit hierarchical structure made up of collagen molecules, which give rise to fibrils, fiber bundles, fascicles and

eventually the tendon unit that align to the long axis of tendon, interspersed with its cell content and non-collagenous molecules (Berenson, Blevins, Plaas, & Vogel, 1996). Fibrils are the basic structural unit of tendon. The fibrils then form the next level of tendon structure, fiber, when bounded by a thin layer of connective tissue, endotenons. Sequentially, the fiber bundles with endotenons form fascicles, which are joined together by a connective tissue sheath, epitenon. An outermost third layer of collective tissue, paratenon, is cooperated with epitenon to form the peripheral tissue, called peritendon (Tempfer & Traweger, 2015).

2.2 Vascular anatomy

The vascularisation and innervation structure is of utmost significance in contributing to the meniscus and tendon healing limitation. By forming the perimeniscal capillary plexus among the synovial and capsular tissues, the lateral medial genicular arteries allocate the blood towards the meniscus within an arborizing network. Despite the full vascularization of meniscus during the early developmental process, the blood and lymph vessel supply are dramatically reduced to the outer 30% of the menisci swiftly once it involved in the role in bearing the body weight around 18 months (Gray, 1999; McDevitt & Webber, 1990). Through the aging process, the vessel penetration can even possibly be reduced to 10-25%. Ultimately, an inner avascular and aneural region (red-red zone) and outer vascular and neural region (white-white zone) are defined, interconnecting by a red-white zone, with the fully vascularized and innervated anterior and posterior horn. With this being the case, as the healing capacity of menisci is depended exceedingly on the level of blood circulation (Gray, 1999; Makris et al., 2011), over 75-90% of the inner portion of the menisci is extraordinary susceptible and vulnerable to the formation of permanent lesion, without any potential form of natural repair.

The blood supply in the tendon tissue is achieved with blood vessels penetrated into the tendon through the enthesis and paratenon (Tempfer & Traweger, 2015). While there are a wide variety of tendons within our human body, the number of vascular branches merging into the tendon manifestly deviates from one type of tendon to another (Pang et al., 2009). Coincidentally, it is disclosed that tendon is richly supplied with capillaries network under the human embryonic stage (Peacock, 1959). However, vascular supply is drastically reduced within the intact adult tendon. The production of antiangiogenic factor and the suppression of proangiogenic factor have been described as two potential mechanisms associated with the reduction on vascularization.

2.3 Chemical composition

The specialized ECM crucially characterised the functions of both menisci and tendon, providing the key towards the development of novel treatment for injuries. Under normal condition, meniscus is a highly hydrated tissue which contains 72% of water. The remaining 28% is the specialized ECM and cells. Fundamentally, collagen occupies 75% of the ECM, together with 17% of GAGs, 2% of DNA, and less than 1% of PGs, adhesion glycoproteins and elastin (Fithian et al., 1990; Herwig, Egner, & Buddecke, 1984; Makris et al., 2011; Verdonk et al., 2005). For the collagen content within the meniscus, an array of collagen types exists and distributes throughout different zones of the menisci in varying proportion. In the red-red zone, collagen type I, II, III, IV, VI and XVIII are all present. Despite the wide variety of collagen types the in red-red zone, the majority of the collagen type is collagen type I, which dominates approximately 80% of the ECM, with the other types delicate for less than 1%. In the white-white zone, collagen type I and II constitute 70% of the ECM, with 60% of collagen type I and 40% of collagen type II. The glycosaminoglycan within the meniscus involves chondroitin-6-sulfate (60%), dermatan sulfate (25%), chondroitin-4-sulfate (15%) and keratin sulfate (15%), which are attached to the proteoglycan, aggrecan, biglycan and decorin.

In tendon, the ECM composition is primarily composed of collagen, elastin, GAGs and PGs, with slight variation in concentration among the tendons located at diverse body parts. Similarly, tendon also exhibits a wide range of collage types, mainly including collagen type I, III, V, XII, XIV and a trace quantity of type II, VI, IX, X and XI. Within all these collagen fiber types, collagen type I is noticeably the dominant type in tendon, which accounts for 60% of the total dry mass of the tissue and around 95% of the collagen component. Whilst collagen type I is found to be highly aligned along the axial direction of tendon in a form of quarter staggered fibrils, other collagen types are mostly presented at the bone insertion site, except collagen type III, which reveal in the endontenon and epitenon layer surrounding the fibrils, and type V, which is situated at the core of the fibrils. In addition to the collagen fiber, elastin has contributed to 1-2% of the total dry mass of tendon, generating the elastin fiber concurrently with microfibrillar protein. GAGs and PGs exist in a little amount within the tissue through variant assortments, depending on the mechanical environment of tendon (Berenson et al., 1996; Wang, 2006). Commonly, aggrecan is associated with a combination of dermatan sulfate and keratin sulfate, whereas decorin is bound with dermatan sulfate, and verican with chondroitin-4-sulfate.

2.4 Cellular composition

Regardless the controversy over the nomenclature of the cell types found in the menisci, three distinct cell types have been distinguished under the classification based on the shape and its surrounding ECM. In the outer zone, fibroblast like cells in an oval and fusiform shape is identified, immersed in the ECM that mainly comprised of collagen type I (Verdonk et al., 2005). In the inner zone, cells with a more round appearance are recognized as fibrochondrocytes, which are embedded in ECM that contain both significant amounts of collagen type I and II. A third cell type populated in the superficial zone is also found as another indispensable branch of meniscal cell type. This type of cell exhibits a flattened and fusiform morphology and have been postulated as a specific progenitor cell with healing capacity (Englund et al., 2012).

On the other hand, tendon is comprised of divergent cell types, including fibroblasts, TSCs, chondrocytes, vascular endothelial cells, synovial cells, smooth muscle cells and MSCs. Fibroblasts, which refer to the elongated and specialized fibroblast, TC, and their precursor cells, tenoblasts, are the leading cell population in tendon, occupying 90-95% of the total cell content. They align in rows between collagen fiber bundles to form a network of cellular protrusions (Lomas et al., 2015). TSCs, chondrocyte aligned towards the bone junction, vascular endothelial cells in the surrounding vascular network, synovial cells in the tendon sheath and smooth muscle cells aligned towards the musculotendon junction, then form the remaining 5-10% of the population. MSCs only exist scarcely in the tissue.

2.5 Etiology and Pathology

Despite the diversity in meniscus pathology, meniscal lesion, induced by meniscal tear, definitely account for the most important pathological condition of meniscus, and hence would be our targeted injury (Englund et al., 2012). Meniscal tear, which predominantly occurs in the medial meniscus (Fan & Ryu, 2000), can generally be divided into two classes, traumatic tear and degenerative tear, with respect to the underlying causes. For traumatic tear, acute sports injury, particularly ignited from football, basketball, soccer, baseball or skiing, is always regarded as the pivotal origin. Vertical, longitudinal, flat, oblique, radial, transverse, bucket handle, horizontal, flap, and complex tear are all types of tear that usually observed under this category (Englund et al., 2012; Hough & Webber, 1990; Makris et al., 2011). For degenerative tear, long-term degeneration accompanied by the aging process is another frequent cause of meniscal tear. In contrast with traumatic tear, horizontal and flap

tear presented with different degree of meniscal destruction are the more typical tear types indicated (Englund et al., 2008). As the healing potency of meniscus is correlated with the blood circulation, with patients suffer from either form of tears, they are highly susceptible to post-traumatic or degenerative injury owing to the dearth of vascular branches. Therefore, the application of external therapeutic measurements is of utmost significance to restore the healthy state of meniscus. Whilst numerous approaches have already been described in guaranteeing an appropriate recovery in the well-vascularized region, tears in the avascular zone are often the crux of ongoing study.

For tendon, although the fundamental mechanism of tendon rupture has not been completely elucidated yet, it is demonstrated that tendon rupture is closely associated with tendinopathy (Riley, 2004). Tendinopathy is used to describe a wide spectrum of tendon pathologies, stemming from overuse and excessive mechanical loading that prohibit tendon from surmounting external force (Walden et al., 2017). Likewise, tendinopathy can also be sorted into two classes, acute tendinopathy, caused by continuous overloading, and chronic tendinopathy, caused by sustained degenerative condition. Under tendinopathy, microtrauma and tear are created persistently. With this being the case, spontaneous healing of tendon is induced, following by three overlapping phases, from inflammation phase, repair phase to remodelling phase. However, instead of regaining the original property of tendon, a scar tissue is usually formed over the damaged site in the healing process, leading to a drastic reduction in mechanical strength (Lomas et al., 2015; Voleti et al., 2012; Wang, 2006). The modification of collagen fiber, cellular, molecular content and addition of adhesion site, also restrain the mechanical property of tendon at the same time. As such, without the implementation of therapy, the tendon will further be weakened through the intrinsic healing process of tendon under the surplus stress, eventually kindling tendon rupture.

2.6 Previous Research and Product Design

Out of the research concentrations laid on meniscal tear and small tendon defects, the application of tissue engineered scaffold for meniscus replacement and adhesive scaffold, the adhesive glue, for small tendon rupture repair, are two of the highlighted focuses.

With respect to the tissue-engineered scaffold, two research directions are concurrently under development: the fabrication of resorbable substitute which aims to promote tissue ingrowth with degradation, and non-resorbable substitute which serves as a

permanent prosthesis. As such, a variety of FDA approved products for meniscus replacement is available currently on the market. The CMI formed mainly by collagen fiber and polyurethanes based implant, Actifit are two traditionally example exploited for meniscus substitution. While the failure of the CMI in long-term load bearing is the undesirable issue required future modification (Rongen, van Tienen, van Bochove, Grijpma, & Buma, 2014), an incomparably biocompatibility is demonstrated by Actifit. More importantly, the over-dependence of CMI on vascular zone integration has limited its therapeutic possibility in triggering meniscus regeneration of totally impaired meniscus (Abbah et al., 2014; Makris et al., 2011). Therefore, an avalanche of research effort are still continuously paid in searching for an optimal solution. (Kobayashi, Chang, & Oka, 2005; Kobayashi, Toguchida, & Oka, 2003) have demonstrated the development of a non-resorbable synthetic meniscus with poly (vinyl alcohol) hydrogel (PVA-H). A 2-year primary study has illustrated that implantation of hydrogel replacement is able to perform comparably with meniscectomy outcome. However, inadequate intrinsic mechanical properties and fixation of meniscal implant are identified as two major problems under 1-year ovine animal experiment conducted by another research group. Despite the improvement made by Holloway, Lowman, and Palmese (2010) using varying freeze-thaw cycles, similar compressive and tensile property still have not been outright reached, with both parameters higher than that of a native meniscus.

For tendon rupture, two typical studies have been done by Scotti et al. (2009) and Simson, Crist, Strehin, Lu, and Elisseff (2013), using cellular fibrin and bone marrow as the base for bio-glue respectively. However, both of them lack sufficient evidence to show a satisfactory performance of their glue. A set of comprehensive mechanical testing is absent in Scotti et al.'s study, while the effectiveness of fibrin glue is often criticized for its weak mechanical properties (Reckers et al., 2009). Additionally, none of them have tested the performance of their glue in the knee joint. Instead, they simply implant the glue into subcutaneous tissue of the animal samples to test the biocompatibility, leaving the in vivo performance unknown. On the other hand, without directly bridging the ruptured tendon, the commercialized product, HydroJel, used as the supplement of tendon suture for further stabilization is another example of adhesive glue. It is examined that HydroJel, which expresses inferior mechanical strength, do not enhance the tensile strength of suture when applied to core and peripheral suture, and hence have no beneficial effect towards surgical operation. In conclusion, a robust bio-glue with comprehensive characterization is in need.

Chapter 3. Methodology

3.1 Materials

PEGDA with molecular weights ranging from 5,000, 10,000, 20,000 Da and PEG-NHS with molecular weight of 20,000 Da were purchased from Shanghai ToYong Bio Co. Ltd. (China). Alginate at both low and medium viscosity were purchased from Sigma-Aldrich (USA). UV initiator, Irgacure 2959, was bought from Ciba Inc. (Switzerland). Rat tail collagen type I, collagenase type II and HPSS were bought from ThermoFisher (USA). Other chemicals did not mention above were provided by the laboratory, Department of Biomedical Engineering, The Hong Kong Polytechnic University (Hong Kong, China).

3.2 Fabrication of HydroJel

HydroJel was a triple-network hydrogel made up by both natural and synthetic polymers. The photo-reactive synthetic polymer, PEGDA, served as the backbone of the product, forming the primary network. Secondary network was established by the ionic crosslinking of natural polymer, alginate. Besides, the introduction of intrinsic protein of joint tissue, collagen, added a top-up network.

To fabricate the hydrogel for meniscus substitution, 20% w/v PEGDA, 5% w/v alginate, and 0.035% w/v UV initiator were firstly added into an opaque tube containing deionized water. In a bid to uniformly disperse the mentioned chemical powders, the solution was placed on a magnetic stirrer for 15 minutes at rate of 1000 revolutions per minute. Afterwards, 0.5 mL collagen was added into the mixture. To facilitate the gelation of collagen, pH of the solution was adjusted to 7 by adding 1M NaOH solution. For the sake of uniformly distribution of collagen without disturbing its fiber, the tube was then shaken gently instead of stirring. Upon the completion of dispersion, hydrogel precursor was then exposed to UV light (365nm wavelength, 78 mW/cm²) for 10 minutes.

To equip HydroJel with the function of healing ruptured tendon, a linker molecule, PEG-NHS, was added. Similarly, PEG-NHS powder was added to the deionized water to a concentration of 15% w/v together with alginate and UV initiator at the same of concentration as mentioned above. However, the concentration of PEGDA was reduced to 15% w/v, which was meant to maintain a suitable porosity. The subsequent procedure of adding collagen and gelation followed the previously mentioned protocol for meniscus substitution.

3.3 Swelling Test

Since HydroJel is intended for joint implantation, it is necessary to exam the swelling behavior of the material in a liquid environment. For this study, the swelling ratio of the gel was defined as

$$\text{Swelling Ratio} = \frac{m_{24h} - m_{0h}}{m_{0h}} \times 100\%$$

Where m_{0h} referred to the fresh mass upon the completion of gelation while m_{24h} was defined as the mass of HydroJel after being submerged in deionized water for 24 hours.

In order to investigate the factors on swelling ratio, groups of hydrogels with different formulas underwent the test. As listed in Table 1, this study mainly focused on the influence of the concentration of PEGDA and PEG-NHS, which took up the majority part of the product aside from water.

Table 1. Setting of the hydrogels for swelling test.

Composition \ Group	1	2	3	4
20kDa PEGDA Concentration (% w/v)	20	20	15	30
20kDa PEG-NHS (% w/v)	15	0	0	0
Alginate Concentration (% w/v)	5			
Collagen Concentration (mg/mL)	1			
UV Initiator Concentration (% w/v)	0.035			
Immersion Time in Ca ²⁺ (Minute)	15			

3.4 Mechanical Testing and Simulation

Since HydroJel targeted at restoring the functions of menisci and tendons, both compressive and tensile tests were conducted with the assistance of BOSE ElectronForce® 3200 Test Instrument (BOSE, UK). The results collected by WinTest® (TA Instrument, USA) were processed and plotted into a stress versus strain curve for the ease of analysis. Young's moduli (E) were used to quantify the performance, which was calculated as the slope of the linear portion of the curve.

Confined compression test was selected in order to evaluate the performance of HydroJel for meniscus substitution. All the samples for the testing was first trimmed into a cylinder with a diameter of 8 mm and a height of 5 mm and then placed into the tailor-made model (Figure 1). During every trial, the indenter compressed in a ramp wave at a rate of 1 mm/s to 12% strain. Bovine menisci were chosen as the reference since previous researches stated that bovine menisci shared similar mechanical properties with human's (Sandmann et al., 2013). As for the hydrogel, various groups with different composition were tested in a bid to figure out the most suitable formula for meniscus hydrogel (Table 2). Among these groups, they were different in either the molecular weight or immersion time in CaCl_2 solution. For each group including animal model and hydrogel, the sample size was kept at three.

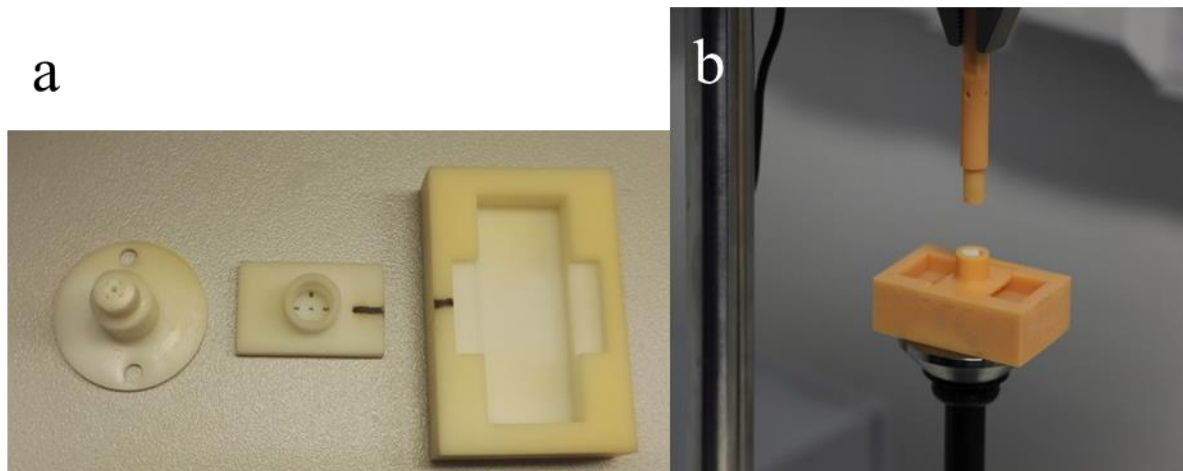


Figure 1. Illustration of confined compression test. a. 3D model of tray, indenter, bath. b. Setup of the test during experiments.

Table 2. Setting of the hydrogels for the compression testing.

Composition \ Group	1	2	3	4
PEGDA MW (Da)	5,000	10,000	20,000	20,000
PEGDA Concentration (% w/v)	20			
Immersion Time in Ca ²⁺ (Minute)	5			
Collagen Concentration (mg/mL)	1			
UV Initiator Concentration (% w/v)	0.035			
Alginate Concentration (% w/v)	10	10	10	15

In the appraisal of HydroJel for tendon repair, a cyclic tensile test was conducted. For every trail, the sample underwent 20 cycles of a 2Hz sine wave tension with the amplitude of 10% strain. To eliminate the influence of preconditioning on living tendon tissue, the Young's modulus was calculated basing on the slope of the latter stable cycles. Meanwhile, two ends of the sample were first stuck onto two metal plate and fixed onto the clamps of the machine (Figure 2). Such a setup was inspired from (Li et al., 2017). Instead of using bovine for reference, rat tendons were selected due to its availability. Both intact and injured rat tendons were test at a sample size of three. On the other hand, two different formulas of hydrogels were assessed with three samples for each group. In consideration of consistency, a hand-made glass mold was utilized to make the hydrogels for tensile test at 4.5mm wide, 2mm thick, and 15mm long.

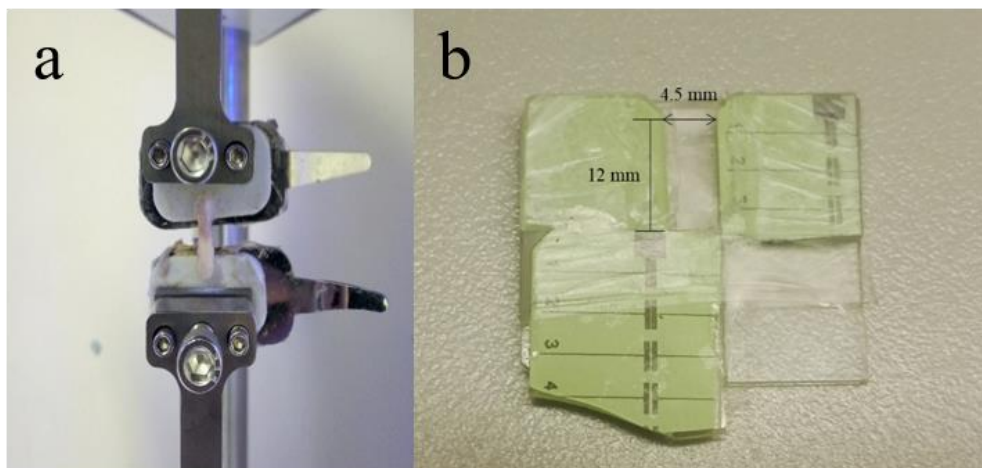


Figure 2. Illustration of tensile testing. a. Mechanical test setup. b. The hydrogel mold for tensile testing.

The fundamental mechanism of HydroJel in tendon rupture repair was further elucidated with the aid of SOLIDWORKS Simulation (SOLIDWORKS, USA). The simulated model was analyzed with its stress distribution as the indicating parameter for the HydroJel performance.

The tendon was modelled as a rectangular beam in SOLIDWORKS, based on the derived dimension of tendon as mentioned previously. To fully simulate the tendon sample, the reported elastic modulus of a tendon, accompanied with other measured mechanical properties, was applied to the material property of the constructed beam. Accordingly, through fixing one end of the created beam, a 2N tensile force is applied at the opposite end. Therefore, a tendon model mimicking the physiological tendinous loading was established. The stress distribution analysis was conducted under the SOLIDWORKS model.

3.5 Tissue Collection and Preparation

3.5.1 Meniscus

Bovine menisci (n=3) were obtained within 24 hours of the skeletally developed cattle slaughter in the local abattoir. The menisci were provided by the Department of Biomedical Engineering of The University of Strathclyde in Glasgow, United Kingdom. Prior to it used, the menisci were cleaned from connective tissues and contaminations, frozen and thawed upon usage. To prepare the sample tissues for mechanical testing, the meniscus will be carved into several cylindrical samples with a diameter of 8 mm and height of 5 mm by hammer and cylindrical chisel.

3.5.2 Tendon

Retired SD albino rats (n=6, 3 male & 3 female) were obtained from CAF of The Hong Kong Polytechnic University for this experiment. The animals were raised on a diet and water *ad libitum* and maintained in an air-conditioned room at 22 ± 2 °C with a regular 12 h light-dark cycle. Tendons were dissected from the rat and subjected to biomechanical evaluation for both healthy and injured tissue.

The retired SD rats were sacrificed by intravenous overdose injection of ketamine-xylazine and CO₂ asphyxiation as inspired by the procedure developed by Güngörmüş, Kolankaya, and Aydin (2015). The animal tendon tissues were obtained from two distinct harvesting sites: AT on the heel and the PT on the knee of retired SD rats. The tendon tissue of both the right and left hind limb was removed from the animal by dissection with forceps, scalpel and surgical scissors following the procedure developed by Islam et al. (2017). With

protocol inspired from Wren, Lindsey, Beaupré, and Carter (2003), the connective tissues surrounding the tendon which includes the paratenons and muscles were further removed by dissection with forceps and scalpel. The harvested tissues were then rinsed in sterile PBS and wrapped in sterile gauze. The tendons were placed inside a sealed centrifuge tube with additional saline solution and stored in $-80\text{ }^{\circ}\text{C}$ refrigerator to maintain its mechanical properties. Hasslund et al. (2008) has shown that freezing the tendon produces no detrimental effect on the biomechanical properties of the tendon. 24 hours prior to its use, the tubes will be transferred to a $4\text{ }^{\circ}\text{C}$ refrigerator to ensure that it was defrosted and flexible for the test.

3.6 Animal Injury Model

Prior to the injury model, the collagenase solution that plays an important role in the experiment was prepared. 1 mL HBSS containing calcium and magnesium were supplemented to dissolve 1g of collagenase powder in the vial. The solution was vortexed gently to dissolve the powder and transferred to a new clean tube. HBSS was further added to the collagenase solution to reduce the concentration to $100\text{ U}/\mu\text{L}$ which was the standard concentration. The collagenase solution was then stored at $4\text{ }^{\circ}\text{C}$ refrigerator for future use.

To evaluate the biomechanical properties and characterization of the tendons and glue, an injury model that represents the injury development for the tissue sample was proposed. The harvested rat tendon samples were cut in the middle perpendicular to the fibers to recreate a common macro injury and expose the collagen fiber of the tendon. The tissues were washed several times with HBSS containing calcium and magnesium to be cleaned from contamination. A sufficient amount of HBSS and $100\text{ U}/\mu\text{L}$ collagenase solutions was added to a container to dilute the concentration to $100\text{ U}/\text{mL}$. The tendon samples were then submerged in the solution to breakdown the collagen fiber through enzyme activity. Addition of 3mM of CaCl_2 were added to the solution to increase the enzyme activity and overall efficiency. The tissues were incubated for 5 hours at 37°C to imitate the micro injury produced by the enzyme in the biological body. Upon completion, the injured tendons were removed from the collagenase and washed several times with HBSS and are ready for mechanical testing.

3.7 Adhesion Verification

In order to verify whether HydroJel could attached to the tendon tissue, histology was carried out. The reliability of such an examination has been confirmed and accepted by academia (Annabi et al., 2017). In the preparation of the sample, HydroJel precursor (containing PEG-NHS) was dripped into the gap of a torn tendon. Subsequently, the ruptured tendon filled by HydroJel precursor were first exposed to UV light for 10 min (365nm wavelength, 78 mW/cm²) followed by being immersed in CaCl₂ for 15 min. Upon the completion, the sample went through the process of histology according to the published protocols (Medicine; Wen et al., 2013). In brief, the sample was dehydrated and embedded into wax. Afterwards, the wax bulk was sectioned which was further rehydrated and stained. With the sample slice stained, it was observed by optical microscope.

3.8 In Vitro Testing

In vitro test based on cell culture was conducted to evaluate the property and biocompatibility of the hydrogel sample and biocompatibility before examined the in vivo experiment. The hMSCs was used to construct the in vitro test of the bio-glue by performing the cell culture hMSCs onto the gel samples. In this experiment, culturing conditions were carefully conducted inside biological safety cabinets and controlled with sterilized equipment to assure that culturing conditions were constructed without inducing any contamination and apoptosis. Cell counting of the hMSC cells was conducted using the Haemocytometer to be able to gain an accurate estimated sample of the cell density, before the settlements of biocompatibility approval experiments included cell culturing and cell viability assay.

3.8.1 Counting Cells

The cells in a T75 flask were removed and stained with Trypan Blue to distinguish between dead cells and live cells. After the loading of samples to haemocytometer slide by micropipette which was quickly and smoothly done to prevent uneven cell distribution. The haemocytometer slide was observed under microscope and counted to derive and calculate the average number of cells simultaneously with the cell density.

3.8.2 Preparation of hMSCs and samples

Hydrogel samples were fabricated and immersed with diluted water for 24 hours inside the incubator to maximize the swelling ratio of the gel. The samples were vertically trimmed

into the thin surface before placed into 6 well plate and confocal dish. The gel samples were then immersed into PBS for 1 hour under the UV light for sterilization. The methods has been modified according to Jongpaiboonkit, King, and Murphy (2009), hMSC at the condition of passaged 7 times were detached from the original T75 flask and counted number of the cells, before seeded onto gel samples. The procedures of preparing hMSCs were constructed including added 3 mL of Trypsin for 2 minutes inside a 37 degree-Celsius, 5% CO₂ humidified incubator within a purpose to isolate the cells from T75 flask, afterwards, the observation of the cells was done under the microscope to ensure the rate of hMSCs detachment. The growth media, composed of [DMEM with glutamax (Gibco), 10% FBS (MSC-qualified, Gibco), and 1% v/v Penicillin/Streptomycin (Gibco)] was added to T75 flask in order to inhibit Trypsin reaction. As referred to Andersen, Auk-Emblem, and Dornish (2015), the cells were then centrifuged with the speed of 1,000 rpm for 5 minutes before seeded onto the gel samples.

3.8.3 Culture hMSCs onto Hydrogel samples

The monolayer cell culture of hMSCs was performed with the preparation of a thin film of fabricated hydrogel samples. The sterile fabricated gel films were seeded on a 6 well plate and 35mm confocal dish. The hMSCs at the cell density of 2×10^5 cells/cm² was delivered to each of the culture dishes. The hMSC growth media were added to each container 2 mL and 3 mL in confocal dish and 6 well plate respectively for providing nutrition and keeping cell survived. The 6 well plate and confocal dish of samples and cells were contained in the 37 degree-Celsius, 5% CO₂ humidified incubator before conducting of cell viability experiment

3.8.4 Live and Dead Cell Assay

The methodology of cell viability test was inspired by Jongpaiboonkit et al. (2009), using method of Live/Dead cell assay which provided two colours fluorescence cell viability assay including ethidium homodimer and calcein-AM. After storage of cultured hMSCs with the gel samples for 24 and 48 hours inside 37 degree-Celsius, 5% CO₂ humidified incubator, each container was washed with PBS and added add 250 mL PBS and 100 mL dye to each of the containers. The plates were covered with foil and incubate under a room temperature for 30 minutes, before the observation of cells was conducted under the fluorescent microscope.

3.9 Product Commercial Development

3.9.1 3D Printing

For the sake of personalizing of the developed meniscus substitution, the feasibility of 3D printing HydroJel was verified by 3D printing it into a cubic. Based on the above tests, the formula with the best mechanical properties was adopted. To be specific, it contains 20w/v % PEGDA (20kDa), 5 w/v % alginate and 1mg/ml collagen. As the viscosity of the pre-gel was essential to the possibility of 3D printing, alginate with both low and medium viscosity were used respectively in two trials.

Upon the completion of pre-gel preparation, the precursor was first degassed by centrifuging at 1000 rpm for 1 minute. Subsequently, it was loaded to the low temperature head of the 3D printer, 3D-Bioplotter® (Envisiontec, Germany). The supplement 365nm UV head (Envisiontec, Germany) was also installed to facilitate the crosslinking of the gel. The cubic (10×10×10mm) was printed with 90°directional change and 0.5mm diameter of strand at 1.5 mm/s under the room temperature. The UV head was set to scan over the printed layer every time after a layer was printed out.

3.9.2 Delivery system for tendon repair

In hope of evaluating the potential of HydroJel tendon glue to emerge in the clinical setting, its adaptability in delivering through the double syringe systems was studied with the utilization of a 3D printed double syringe, followed by an AB epoxy gun cartridge syringe with a putter static mixer (Taizhou Fhis Electronic, China). The performance of HydroJel within the 3D printed double syringe system, without the exploitation of mixer, was first evaluated under direct visual inspection. The UV-Vis spectrophotometer was subsequently employed to observe the performance of HydroJel under the double syringe system, in the addition of mixer.

3.9.3 3D printed double syringe system

The prototype of the 3D printed double syringe was established in the SOLIDWORKS 2016 (SOLIDWORKS, USA) (Figure 3a). The design was printed by the Makerbot Replicator 5th Gen under the instruction from IC technicians, using PLA filament with an infill density of 75% (Figure 3b). While alginate is the dominant component in determining the viscosity of the glue, the alginate solution was used to represent the glue in the trials to study the double syringe system, preventing the excessive use of material. In each trial, alginate solutions stained with violet and red colour were situated respectively into two compartment of the syringe. The compartments were filled to the same height simultaneously through

sealing the tip with paraffin. The ejected outcome from the tip was then injected over a white tissue base. The quantification was undergone based on the degree of mixing of two stained alginates visually.

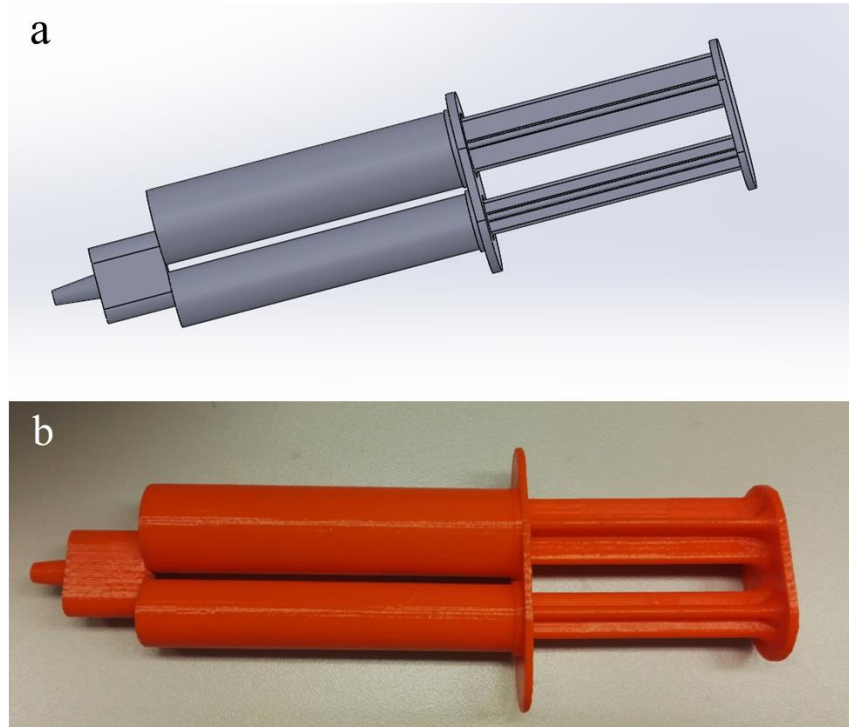


Figure 3. 3D printed double syringe delivery system. a. Solidworks 3D design b. 3D printed replica.

3.9.4 AB epoxy gun cartridge syringe with putter static mixer

Coincidentally, the alginate solutions were mixed under AB epoxy gun cartridge syringe with the putter static mixer, applying the same procedure as stated above. The ejected outcome was studied in the UV-Vis spectrophotometer compared to the alginate solution, mixed with respect to protocol in fabricating the HydroJel.

3.10 Statistics Analysis

Quantitative results from mechanical testing were analyzed by one-way ANOVA in SPSS (IBM, USA). Specifically, authors used Post-hoc Tukey test with a baseline of significance level set at $p = 0.05$. Processed mean and standard deviation values were plotted by Excel (Microsoft, USA).

4.2 Compression Test

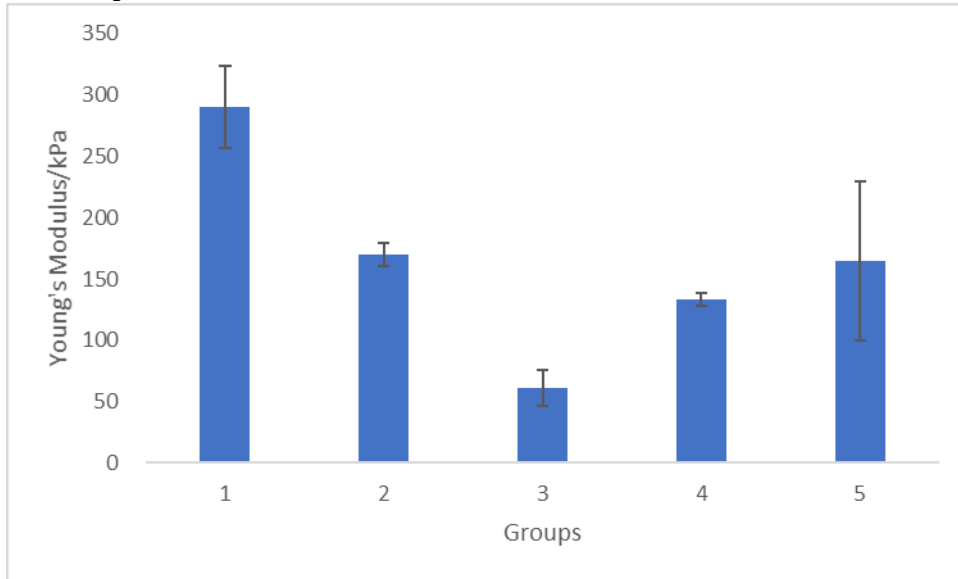


Figure 4. Results of confined compression test on hydrogels and bovine menisci. **1.** 5,000Da PEG with 10min submersion in Ca^{2+} solution; **2.** 10,000Da PEG with 10min submersion in Ca^{2+} solution; **3.** 20,000Da PEG with 10min submersion in Ca^{2+} solution; **4.** 20,000Da PEG with 15min submersion in Ca^{2+} solution; **5.** Bovine menisci.

Table 5. Statistical description of the results of confined compression test.

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	290.2267	33.53628	19.36218	206.9179	373.5354	261.10	326.89
2	3	169.6733	9.38009	5.41560	146.3719	192.9748	163.01	180.40
3	3	61.2067	14.31237	8.26325	25.6528	96.7606	46.07	74.52
4	3	133.1167	5.47169	3.15908	119.5242	146.7091	126.81	136.60
5	3	164.8333	65.01464	37.53622	3.3280	326.3387	118.20	239.10

4.3 Tensile Test

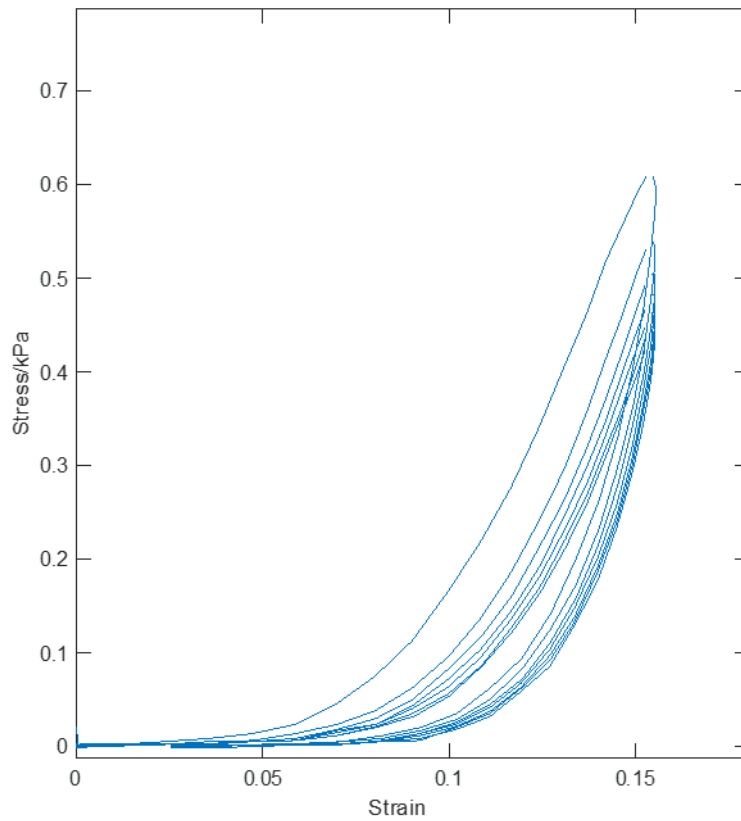


Figure 5. Stress verses strain curve of the cyclic tensile test on rat tendons.

Table 7. Statistical description of the results of tensile test.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Intact	3	8.4096	1.2774	0.7375	5.2364	11.5828	7.02	9.53
Injured	3	0.7596	0.1601	0.0925	0.3618	1.1574	0.60	0.92
HydroJel	3	0.3136	0.0892	0.0515	0.0921	0.5352	0.23	0.40

4.4 Simulation test

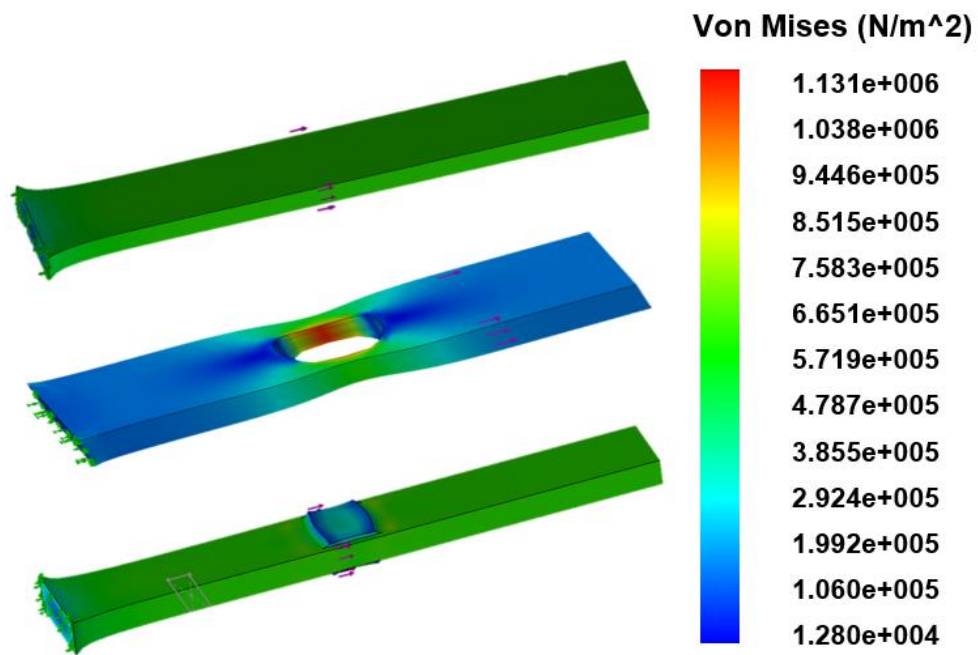


Figure 7. Results of tensile test stimulation of intact rat tendon, injured model and HydroJel treatment.

4.5 In vitro Test

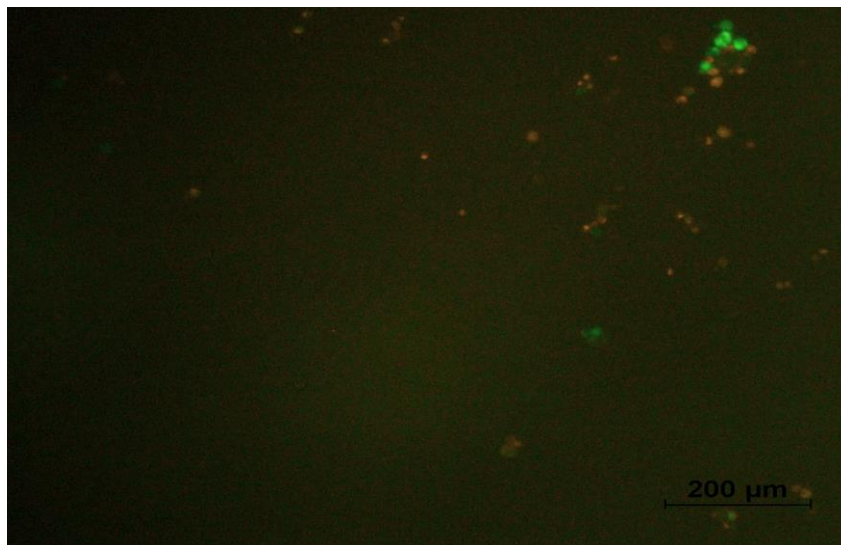


Figure 8. Human mesenchymal stem cells on hydrogel samples strain with Live and Dead cell viability assay 24 hours after cell seeding.

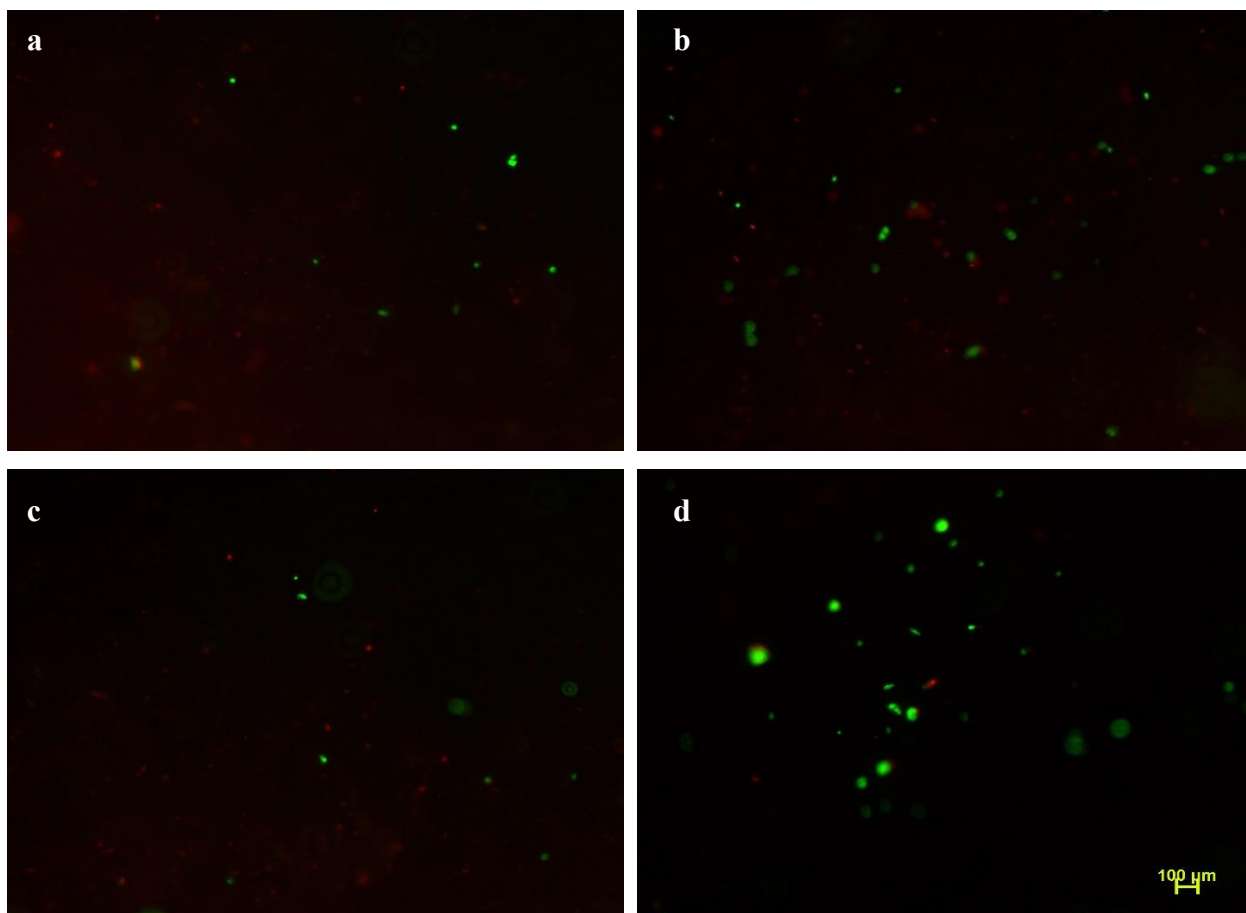


Figure 9. Human mesenchymal stem cells on hydrogel samples strain with Live and Dead cell viability assay 48 hours after cell seeding.

4.6 Adhesion Verification

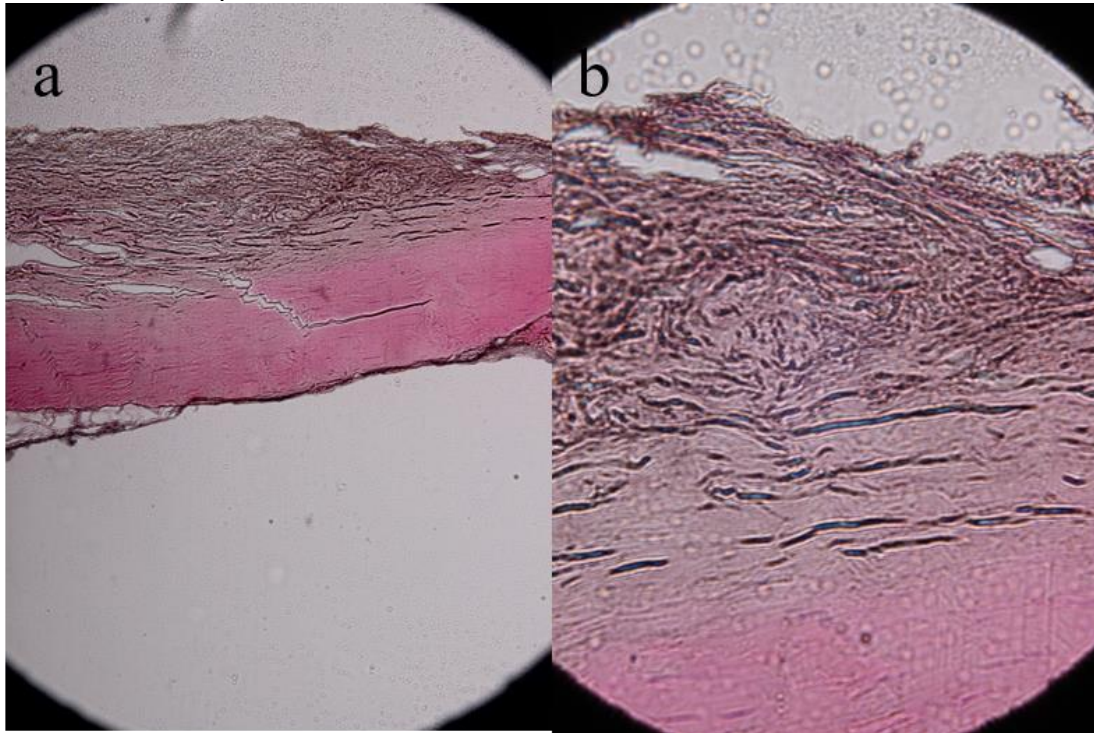


Figure 10. Histology section of tendon tissues healed with HydroJel. **a.** 5 times magnification. **b.** 10 times magnification.

4.7 Product Commercial Development

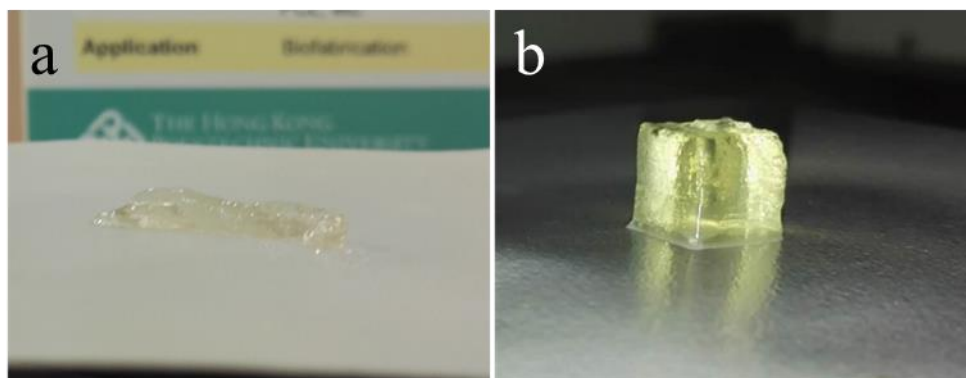


Figure 11. The 3D printed cubic by 3D-Bioplotter with HydroJel. **a.** HydroJel made up from low viscosity alginate. **b.** HydroJel made up from medium viscosity alginate.



Figure 12. Double syringe system mixed solution.

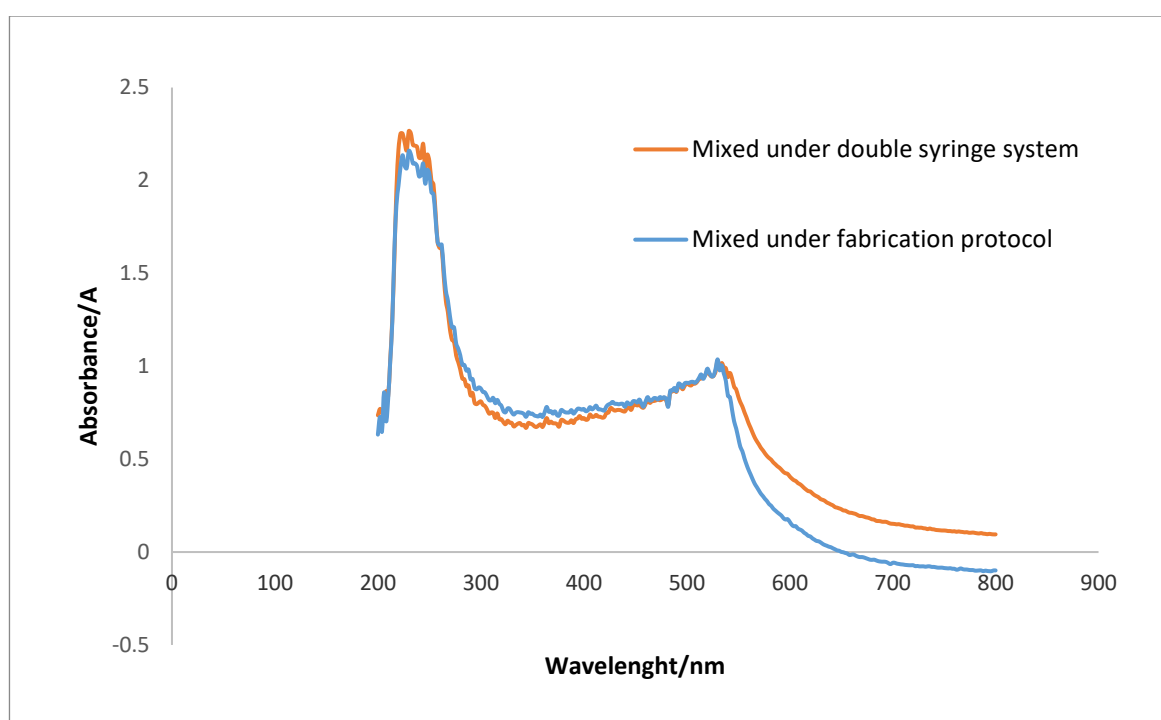


Figure 13. UV-Vis Spectrophotometer results of the mixed solutions.

Chapter 5. Discussion

5.1 Design of HydroJel

As illustrated in Figure 14a, the primary structure of HydroJel was formed by the covalent binding of PEGs under the exposure to UV light. The reason for choosing such a kind of polymer to construct the backbone is its superior mechanical properties. It has been found that PEG can undergo an excessive level of compression or stretch and recover to original morphology immediately (Chen, Poon, Setiasabds, Panyang, & Wen, 2016; Hong et al., 2015). Besides, Hong et al. realized that the elasticity of the PEG-based hydrogel increased with the molecular weight of PEG. Therefore, the 20kDa PEG is believed to be of sufficient elasticity for both meniscal and tendinous applications. Apart from that, crosslinking of PEG in HydroJel was based on the formation of covalent bonds, which enabled the materials for long-term usage (Zhao et al., 2016).

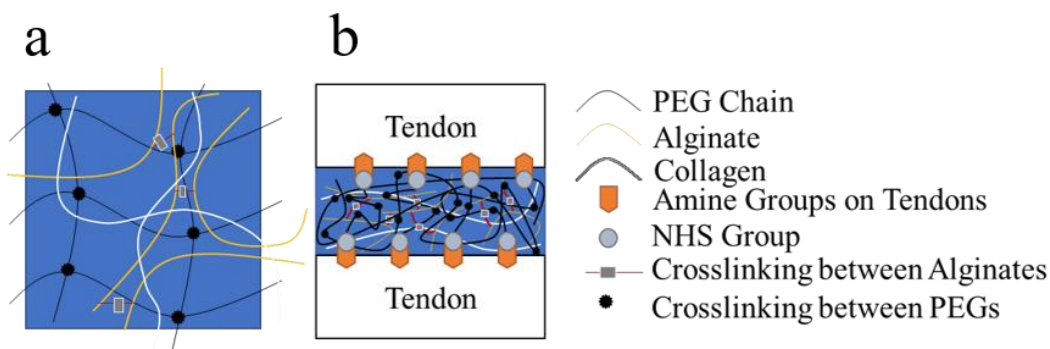


Figure 14. Illustration of HydroJel design. **a.** Structure of the HydroJel. **b.** Working mechanism of HydroJel for tendon repair.

Although the hydrogel made up by 20kDa PEGDA owned a satisfactory elasticity, it seemed to be weak in stiffness based on the result of compression test (Figure 4). Previous study has suggested that an effective and efficient method to deal with this drawback is to add extra networks. Therefore, alginate was introduced into the hydrogel in an expectation to enhance the stiffness by building up a secondary network. Comparing Group 3 and Group 4 in Table 5, the Young's modulus improved significantly when being submerged in calcium solution for a longer time. Such a phenomenon is likely to be resulted from the higher crosslinking of alginate because of the longer immersion time. A immersion time of 15 minutes could not only eliminate the influence of swelling while providing sufficient calcium ion to alginate for crosslinking (Wei et al., 2015). Furthermore, the irreversible ionic crosslinking of alginate breaks under the deformation induced by external forces

(Hong et al., 2015). As a result, HydroJel displayed hysteresis in the tensile test (Figure 5), mimicking the pattern of living tissue.

Aside from mechanical properties, biocompatibility is also another important criterion for the design of the product. In terms of chemical structure, neither PEGDA nor alginate has the domain for cell attachment (Shi, He, & Liu, 2015). With this being concerned, collagen was added since such a protein consisted of the site for cell adhesion (Jia & Kiick, 2009). Also, this top-up network is believed to, to some extent, contribute to the mechanical properties (Chan, Wippich, Wu, Sivasankar, & Schmidt, 2012).

Compared to meniscus substitution, tendon repair requires the materials to be capable of adhering to the tendon tissue. Consequently, there was an additional molecule, PEG-NHS, in the tendinous application. This is because that the reaction between NHS group and the amine group in living tissue has been popularly used for bio-adhesive (Ghobril & Grinstaff, 2015). As shown in Figure 14b, NHS group of the molecule is expected to serve as the linker molecule, binding to the amine groups in tendon tissue while connecting to the backbone covalently.

5.2 Performance of HydroJel

5.2.1 Meniscus Substitution

Due to the physiological functions of meniscus, robust mechanical properties are the most basic and essential requirement. Judging from Table 6, the p value between Group 4 and Group 5 was 0.776, indicating absence of significant difference in the mean value of Young's modulus. In other words, HydroJel was as stiff as the bovine sample. Moreover, the Young's modulus of human menisci at the strain of 12% was measured at around 137.6kPa (Chia & Hull, 2008). Given such a datum, HydroJel was further verified to be strong enough for meniscus substitution.

5.2.2 Tendon Repair

To assess the performance of HydroJel in healing ruptured tendons, a developed equation was used (Yuk, Zhang, Lin, Parada, & Zhao, 2016):

$$\Gamma = \Gamma_0 + \Gamma_D$$

In the presented equation, Γ refers to toughness of breaking an adhesive on a surface while Γ_0 and Γ_D denote the energy of binding and the toughness of the adhesive respectively. As discussed previously, HydroJel was expected to form a covalent bond with the tissue (Figure 14b). Such a binding was visually verified in the result of histology. In Figure 10, the upper

porous part was likely to be HydroJel. Its original structure might be destroyed during the dehydration process, leaving an unorganized mesh in the histology section. From observation, there was a gradual transition from HydroJel to the lower uniform tendon tissue. Without obvious gap between the gel and the tissue, it implied that covalent bonds were formed between them.

With F_0 being verified visually, F_D was analyzed quantitatively with the assistance of cyclic tensile test and simulation. From Figure 5, it could be told that HydroJel shared an analogous pattern in stress-strain curve in response to the tension. Meanwhile, based on Table 7, the formulated injured model efficiently reduced the Young's modulus. Furthermore, the analysis of the Young's modulus was not confident to confirm the significant difference between HydroJel and injured tendon tissues (Figure 6 and Table 8). In a bid to visualize the effect of applying such a hydrogel onto the injured tendon, simulation was conducted. As shown in Figure 7b, the loading was highly concentrated in the injured sites, being likely to induce further injuries. By applying HydroJel onto the injured site, the loading was redistributed uniformly (Figure 7c). Combining all the results of mechanical testing, HydroJel exhibited the capability to heal tendon rupture like glue.

5.2.3 *In vitro* test

Cell viability considered to be the important component of *in vitro* experiments which not only depending on biocompatibility of the materials but also culture conditions and hMSCs treatments by directly or indirectly induced apoptosis or cytotoxicity (Nuttelman, Tripodi, & Anseth, 2005). In this experiment, culturing conditions were carefully conducted inside biological safety cabinets and controlled with sterilized equipment to assure that culturing conditions were constructed without inducing any contamination and apoptosis. The result of Live and Dead cell viability assay which was conducted 24 and 48 hours after cell seeding onto the gel samples, displayed as two colours of fluorescent probes which appearance fluorescent colour characterized viable and dead cells (King, Jongpaiboonkit, & Murphy, 2010). The dyes used in this experiment included red fluorescent ethidium homodimer (EthD-1) and green fluorescent calcein-AM which measuring recognized parameters of cell viability, plasma membrane integrity and intracellular esterase activity respectively (Sanfilippo et al., 2011).

The settlement of experiment was constructed with different settlings which in the Figure 8 displayed the first trial result of cell seeded on uncut sample and conducted cell viability after 24 hours. According to the results from Figure 8, which Live/Dead cell

viability staining was conducted 24 hours after cell seeding onto 6-well plate, which only few number of the cells were discovered under fluorescent microscope due to various conditions including the small amount of cell seeding, and lack of collagen during gel fabrication led the gel to absorb growth medium during the incubation time. Solution of swollen issue was constructed by immersing the gel samples in incubator for 24 hours before the settlement of live and dead cell assay staining to be able to reduce swelling rate of gel samples and increase effectiveness level of *in vitro* experiment. During the second experimental period, the gel samples were cut into thin films to cover surface of 6-well plate container and conducted Live/Dead cell viability assay after 48 hours of cell seeding. The results in Figure 9, exhibited that majority of the cells seeded onto the gel samples indicated to be viable cells which specify a better biocompatible property of the biomaterial.

Further testing of cell viability, proliferation, and differentiation requires to be constructed before conducting the *in vivo* experiment as the properties of the material is still under development for a better clarification of effectiveness and property of hydrogel (Barminko et al., 2011). Cell encapsulation in three-dimensional culture can be examined in the future which would lead to further characterization of material. Therefore, further *in vitro* testing can be the indicators to appropriate adjustment of material for a better development of the sample's biocompatibility property.

5.3.4 Limitations

The materials were observed to easily take in abundant water based on the test of swelling. It is not desirable to have a high swelling ratio as the mechanical properties of the materials may be weakened after absorbing extra water. The results from Group 2, 3, and 4 implied that PEGDA seemed to be dominant in affecting the capability of absorbing water (Table 3). Comparing Group 1 and 2, it could be concluded that the linker molecule, PEG-NHS had no influence on the swelling ratio (Table 4). The less PEGDA was contained, the lower swelling ratio was. It is highly likely that the hydrophilic polymer PEGDA chain containing quantities of oxygen attracts the water. Although the swelling effect was eliminated by reducing the amount of PEGDA, there was another concern in the reduction of elasticity. Therefore, a balance between the swelling ratio and elasticity shall be investigated.

5.3 Product Commercial Development

5.3.1 3D Printing

According to Figure 11, only the pre-gel using alginate of medium viscosity could be 3D printed successfully. The major reason for that could be the insufficient viscosity of low viscosity alginate which was not strong enough to hold the structure before being crosslinked by UV after being ejected from the head. Therefore, the gel collapsed after being printed out. Conversely, the medium viscosity trail successfully printed a well-defined 1cm³ cubic. Although previous mechanical test was based on HydroJel with low viscosity alginate, it is believed that there would be no significant difference in the mechanical properties between HydroJel made up by alginate with medium and low viscosity. This is because that the viscosity and stiffness are independently controlled based on a systemic review (Lee & Mooney, 2012). Thus, with medium viscosity alginate, HydroJel is able to be customized by 3D printing while preserves its mechanical properties.

5.3.2 Product Delivery System

Without the use of mixer in the 3D printed double syringe system, an inferiorly mixed alginate solution is exhibited under visual inspection (Figure. 12). The poor intrinsic mixing property of the mixing region in the system is inspected to be the principle reason accounted for this phenomenon. Additionally, the uncontrolled diffusion of internal solution from the wall of the syringe system have been recognized as the minor problem of the device. It is believed that the insufficient level of infill density of the 3D printed double syringe is the underlying cause. As a result, some of the solution is forced to squeeze through the pores on the wall of syringe under the applied force. On the other hand, fundamental to the peak superposition under UV-Vis spectrum is the enhanced mixing performance stemming from the use of mixer (Figure 13). As the overlapping of peak under UV-Vis spectrum indicates the two solution is of the equivalent color. This implies that the syringe system equipped with the mixer are able to merge the solution comparably as that mixed under the fabrication protocol. With this being the case, the result provides a preliminary insight in verifying that HydroJel is able to perform equally, at the laboratory level, in the clinical setting in term of the mixing of solution with the use of double syringe system.

5.4 Globalization

Hong Kong, a metropolitan city in the center of Asia, is recognized to have one of the best healthcare system in the world. The excellent health education and developed healthcare system and services are reflected by the outstanding performance in various health indicators. In 2016, the life expectancies of Hong Kong resident were 87.3 years for female and 81.3 for male, one of the highest in the world ("Major Health Indicators in 2015 and 2016," 2017). In addition, low infant mortality rate of 1.7 and zero maternal death exhibited an exquisite and developed healthcare system ("Major Health Indicators in 2015 and 2016," 2017).

Comparing the healthcare system of Hong Kong to that of England, there are many similar values found at the core of the system. The National Health Service (NHS) provides all UK citizens free access to quality healthcare and is globally considered to be one of the best health systems in the world. Over the decades, the healthcare system provided by the NHS have improved the life expectancy while dramatically reducing the mortality rates of respiratory, cancers and circulatory diseases of British (Boyle, 2011).

The extended period of British colonial rule of the city has resulted in close resemblance of the Hong Kong health care system to the NHS of the United Kingdom (Kong et al., 2015). The fundamental value of Hong Kong's healthcare system is to provide lifelong holistic healthcare and medical treatment for all citizens (Kong et al., 2015). To achieve its goals, the government adopted the uniform healthcare system with a central governed structure to manage all the medical institutions (Zhang & Wang, 2007). The Hong Kong Food and Health Bureau is the governing body that is responsible in maintaining the comprehensive healthcare system of Hong Kong. The Bureau forms and execute various health policies that promotes public health and provide preventive, curative and rehabilitative services for all in need ("Vision, Mission and Core Values," 2013). In addition, the bureau is responsible to manage and allocate the government resources to efficaciously operate the health services of all levels (Kong et al., 2015). The Hong Kong public healthcare spending budget was 5.8 billion US dollars or 17% of government budget in 2013 ("HK healthcare is a dual-track system," 2013). When the budget is proportioned to the population of around 7 million people, Hong Kong spends 250% more per capita than other developed countries such as the United States of America (Hoft, 2014). The Hong Kong healthcare system runs on a dual-track of both public and private sectors. The public hospital and health institutes are regulated by the Hospital Authority under the bureau to provide a uniform health services for all. The sustainable development of the private sector contributes

to a part of the healthcare need, providing quality medical services while alleviating pressure of public finances.

The UK government aims to provide universal and free at point of delivery and establish the NHS to achieve these goals (Grosios, Gahan, & Burbidge, 2010). In the same way as the Hong Kong Food and Health Bureau, the Department of Health of the United Kingdom is the government body responsible for the establishment and operation of new policies of the NHS, public well-being and other health related areas (Boyle, 2011). The system is funded by the government through general taxation and the Department of health is responsible in allocation and disbursement of the money (Boyle, 2011). In 2008 alone, the government allocate 8.7% of the total gross domestic product (GDP) of the nation for the NHS. However, the major difference between the system is the structure where the NHS medical clinic care are divided into three different sections based on the patients' needs (Grosios et al., 2010). The primary level involves general practitioner (GP), dentist and pharmacist in local clinics while the secondary level provides hospital interventions. The tertiary section provides specialist healthcare with the recommendations from their respective GP (Grosios et al., 2010).

The high level of government spending on healthcare and the corresponding system of both cities greatly affect the opportunity for our product to make a breakthrough. While the UK government provides full medical coverage in which includes prosthetics and joint replacement coverage, the Hong Kong government provides free operation services but requires the patient to pay for the prosthetics devices ("Fees and Charges," 2017). Our product, HydroJel, provides a low-cost alternative to joint injuries that includes tendon, meniscus and articular cartilage problem. The product also allows the prevention of further joint deterioration such as osteoarthritis that will require a more complicated treatment that includes a total joint replacement. With a limited medical coverage, HydroJel will be more accepted in the Hong Kong market as a low-cost alternative to the normal treatment of joint injuries compared to the UK market. However, as the NHS wants to provide the best treatment and universal coverage for all their patients, they will also accept the product as a treatment for joint injuries.

Despite the exemplary healthcare system that provides excellent services, Hong Kong and United Kingdom also faces several major challenges to sustain the high standard of medical care. In the same manner as other developed countries, Hong Kong and United Kingdom faces a serious challenge in healthcare due to the rapidly aging population. The percentage of elders in the Hong Kong population is currently 12.5% and it is projected to

double to 25% by 2030, putting more strain on the healthcare cost of the city ("HK healthcare is a dual-track system," 2013). The aging population also increases chronic diseases such as cancer and Alzheimer's that increases the health care cost of the population (Grosios et al., 2010). As the country is developing further, lifestyle diseases such as obesity and cardiovascular diseases increases. In the United Kingdom alone, these diseases cost 16% of the total health expenditure and resulted in 40-70% mortality (Wanless, 2004). Furthermore, the swift advancement of health technology and medical procedures resulted in an elevated medical cost to buy the machine and adopt the best procedure, putting more strain on the government resources ("HK healthcare is a dual-track system," 2013). These advancements in medical technology also causes a gap between the healthcare as it is improving much faster for the rich (Grosios et al., 2010). The public hospitals in Hong Kong are managed and heavily subsidized by the government in the aim to provide quality services at a minimal cost for the residents. This low user charge attracts all patients, even those who are financially capable to afford private medical care, causing a high demand and waiting time for the healthcare services (Wong et al., 2012). The NHS also faces similar problem as they provide universal free access to healthcare which causes many patients to misuse the services for personal benefit. These challenges produce a strain on the government healthcare expenditure which have already experienced a 40% increase from 2007 to 2012 ("HK healthcare is a dual-track system," 2013).

As a developed region in terms of health and economics, both Hong Kong and the UK have a population demography that is shifting towards the aging population. With an increasing ratio of elderly and their diminishing health, the aging population puts a strain on the healthcare spending of both the UK and Hong Kong. Diseases such as joint injuries will develop to be a more severe condition such as osteoarthritis that requires a more expensive medical procedure. To prevent further development of joint injuries, HydroJel can be adopted in both the Hong Kong and UK market as an early treatment for joint injuries. HydroJel can be adopted in the hopes to prevent further deterioration of joints that requires expensive medical procedure, reducing the economical strain on the government health spending. Furthermore, HydroJel provides a new technology and procedure to help heal various joint injuries at a relatively low cost. With the swift advancement of technology that resulted in a higher cost of spending to provide the best medical procedure, HydroJel has a competitive advantage over the other products. It is a better and relatively low-cost solution to the current joint injuries procedures. Therefore, both government and respective health

department will be more likely to accept and embrace HydroJel to provide a better and cheaper alternative.

Aside from the healthcare system and government spending, another consideration for the adoption of HydroJel is the rules and regulations governing medical devices. In the interest to ensure the safety of the people, the UK and European government developed strict guidelines and rules for medical devices (*Regulation of medical implants in the EU and UK*, 2012). Medical devices, especially implants with higher medical classification, were required to have the EU certification and provide transparent clinical evidence of performance and safety (*Regulation of medical implants in the EU and UK*, 2012). On the other hand, the Hong Kong government does not have an independent government body that regulates the safety of the medical devices (*Regulation of Medical Devices*, 2010). However, the Hong Kong government have several guidelines and safety protocols that has to be followed (*Regulation of Medical Devices*, 2010). Based on the global classification of medical devices, HydroJel is considered as a Class III medical device as it is an implantable device that are placed within the body, requires surgery and designed for continuous use (*Regulation of medical implants in the EU and UK*, 2012). With a higher classification of medical device, a longer period of clinical trials and a greater amount of evidence is required to exhibit the performance and safety of HydroJel. This also resulted in a longer time to receive the EU certification that is enforced by UK and the EU region. Therefore, this will inhibit the adoption of the HydroJel product in both Hong Kong and UK regions. However, once the highly perceived EU certification was received for the product, it will be able to make a breakthrough in the other regions of the world.

Chapter 6. Conclusion

In this study, the developed HydroJel can be used for not only meniscus substitution but also tendon repair. Throughout this research and design process, a series of testing and experiments were conducted in order to achieve the ideal product properties, including mechanical testing on both HydroJel and animal tissue samples, swelling ratio test, cell viability test, and product development investigation. With regards to meniscus substitution, HydroJel has a compressive modulus at 133.11 kPa, which exhibited no significant difference with the bovine menisci and human menisci. Besides, the feasibility of the 3D printing HydroJel has been verified for the one made up from alginate of medium viscosity. With the collected positive results, HydroJel is believed to be a patient-specific device for meniscus substitution. In terms of tendon repair, the modified HydroJel was visually inspected to adhere on the tendon tissue based on the histology. Meanwhile, the results of mechanical testing and simulation supported the efficacy in linking the ruptured tendons and eliminating the originally unevenly distributed loading. Furthermore, the practicability of HydroJel in clinical usage was proved in the double syringe system test. As for the overall properties of HydroJel, cell variability assay confirmed the biocompatibility whereas the swelling ratio was not perfectly ideal. Aside from the experimental aspect, it has been realized that HydroJel, as a Class III medical device, is a highly adaptive product for distinct regions over the world. It has provided a low cost and prevailing strategy in allowing both preventive and therapeutic treatment for joint injuries. Dimly, as HydroJel is deemed as a Class III medical device, unwavering challenge is still revealed in being certified by various regulations, which hinder the expansion of Hydrogel across the world market.

Future directions for HydroJel development can be laid on three main aspects: the complete product characterization, further formulation and material modification, and clinical trials. Due to the time limit of this project, in vivo test of the performance of HydroJel has not been completed yet. Future investigation may first focus on small animals such as rats and then move forwards to large animals like sheep. In response to the results from animal tests, the formula should be further modified. Besides, the high swelling ratio shall be solved in prior to the in vivo test by adding coating. Upon the completion of all the mentioned preclinical tests, the clinical trial is expected to carry out. Since human subjects will be involved in the clinical tests, cooperation with local hospital in Hong Kong will be needed. Furthermore, abundant funding shall be secured as such a clinical test is extremely costly. With all the procedure achieved, the final step in product development falls to pursuing the FDA approval.

In conclusion, plenty of promising laboratory results of HydroJel have been collected while systematic future investigation is still in need.

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