ENHANCING OSTEOCHONDRAL REGENERATION USING HUMAN MESENCHYMAL STEM CELLS

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Enhancing Osteochondral Regeneration Using Human Mesenchymal Stem Cells. (May 2015)

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An osteochondral defect is a joint injury characterized by the loss of cartilage along with a thin layer of bone beneath it. The cause of this condition is attributed to a number of factors, including repetitive trauma within the joint, metabolic disorders, and genetic predisposition to diseases such as osteoarthritis. Current treatments are partially successful in returning function, but more effective methods are needed for a longer lasting result and better osseointegration of grafts. We hypothesized that a collagen hydrogel synthesized with inorganic Poly(dimethyl siloxane) star-methacrylate (PDMS*-MA) and Poly(ethylene glycol)-diacrylate (PEG-DA) would promote an increased osteogenic phenotype of encapsulated human mesenchymal stem cells (hMSCs). Specifically, this osteogenic hydrogel (CPP* hydrogel) was created as an interpenetrating polymer network composed of a collagen hydrogel soaked with monomers of PEG-DA and PDMS*-MA. The CPP* hydrogel was shown to have superior mechanical properties than typical collagen hydrogels while maintaining an appropriate swelling ratio to support cell culture. Confocal imaging of the CPP* hydrogels revealed that the encapsulated hMSCs were able to survive the hydrogel formulation steps and assume a morphology characteristic of osteoblasts.

ACKNOWLEDGEMENTS

I would like acknowledge Dr. Carl Gregory for providing the human mesenchymal stem cells that were used in this study, along with PhD student Ms. Candice Haase for her contributions to this work. I would like to also acknowledge our collaborator Dr. Melissa Grunlan and the support given by TEES Strategic Initiatives Seed Grant to Dr. Kaunas.

NOMENCLATURE

PEG-DA	Poly-ethylene Glycol Diacrylate
PDMS*-MA	Poly-dimethylsiloxane Methacrylate
hMSC	human Mesenchymal Stem Cell
CPP*	Collagen/PEG-DA/PDMS*-MA
IPN	Interpenetrating Polymer Network
UV	Ultra-Violet
CCM	Complete Culture Medium
FBS	Fetal Bovine Serum
DMSO	Dimethyl Sulfoxide
BSA	Bovine Serum Albumin
PBS	Phosphate Buffer Solution

CHAPTER I

INTRODUCTION

The underlying goal of regenerative medicine is to develop technologies designed to facilitate the restoration of properly functioning tissues to an area of the body suffering insult. The specific tissue that this investigation will focus on restoring is the osteochondral interface. The interest in regenerating this tissue arose due to the prevalence and debilitating nature of cartilage damage in the injury and the relative simplicity of the tissue's function. The purpose of this project is to develop a cell scaffold designed to improve the quality, integration, and durability of regenerated tissues in joints containing osteochondral defects. To accomplish this, we proposed a two layer hydrogel system in which the bottom layer supports osseointegration between the system and the host, while the top layer supports articular cartilage production. The focus of this thesis is on the development of the bottom layer of the hydrogel system in which we developed an ideal combination of synthetic and natural materials in order to mimic the extracellular environment while maintaining necessary physical properties.

Osteoarthritis

More than 27 million adults in the U.S. suffer from osteochondral defect injury, mostly in the form of osteoarthritis. With longer life expectancies, half of all adults will experience symptomatic osteoarthritis of the knee at some point in their life[1]. Osteochondral Autologous Transfer is one of the most commonly used surgical treatments for osteochondral defects. This procedure involves harvesting a tissue graft from a non-weight-bearing section of an injured joint and transplanting it in the osteochondral defect to promote cartilage tissue regeneration.

Unfortunately, graft material for this procedure is limited due to the small area available for harvest and studies have shown that the long term outcomes of Osteochondral Autologous Transfer have resulted in incomplete osseointegration of the graft and necrosis[2]. Synthetic materials are abundantly available, but currently have their own disadvantages, including poor osteoconductivity, low host-cell adhesion properties, and immune rejection[3]. New regenerative techniques that combine both the patient's stem cells and synthetic materials show promise in offering a solution to regenerate osteochondral defects without these current limitations.

Common Scaffold Biomaterials

Poly(ethylene glycol)-diacrylate (PEG-DA) is a popular biomaterial used in hydrogel based stem cell therapies due to its biocompatibility and mechanical properties that closely match the target tissue[4]. An ideal scaffold for osteochondral tissue regeneration is composed of a zone designed to provide adequate osseointegration with the underlying subchondral bone and a zone designed to generate articular cartilage. The Grunlan Research group has shown that PEG-DA hydrogels synthesized with poly(dimethyl siloxane)-methacrylate (PDMS*-MA), shown below in Figure 1, resulted in an increase in the osteogenic activity of human mesenchymal stem cells (hMSCs)[5] due to the presence of PDMS*-MA. PDMS*-MA is an inorganic polymer containing multiple silicone groups in the polymer chain. These silicone groups act to increase the hydrophobicity of the monomer, which has been demonstrated to provide an increase in bone ECM production by osteoblasts[6]. A key feature that hMSCs encapsulated in these hydrogels did not demonstrate was normal osteoblast morphology. In-vitro hMSCs rely on a class of enzymes called matrix metalloproteinases in order to remodel the surrounding extracellular matrix proteins and assume a desired morphological shape. This does not occur in the PEG-DA/PDMS*-MA hydrogels

because these are synthetic polymers which undergo hydrolytic degradation instead of enzymatic degradation[7]. It is therefore necessary to include a network of extracellular matrix proteins in order to direct encapsulated hMSCs to behave similarly in-vitro as they would in-vivo. To accomplish this we chose to use collagen type 1 as our network of ECM due to its high content in bone tissue and the alpha-helix shape of the protein. The reason that collagen's shape is valuable in our application is because it will guide our cells to elongate out into the fibrillar morphology typical of osteoblasts.

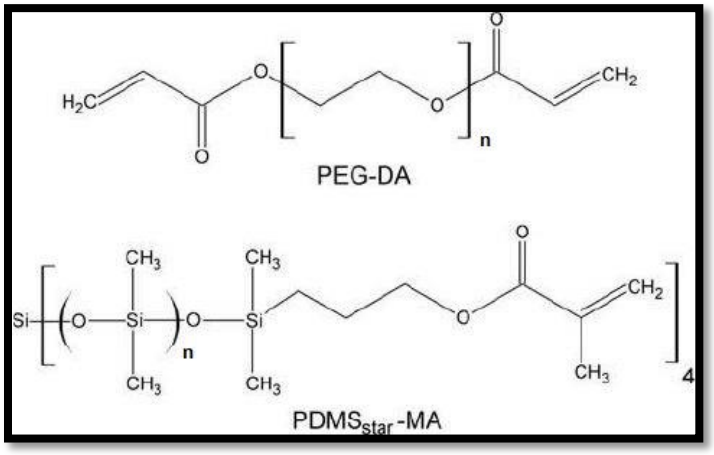


Figure 1. PEG-DA and PDMS*-MA Monomers.

Importance of Cell Morphology

The commitment of hMSCs to an adipocyte, osteoblastic, or chondrogenic fate is regulated by the transcription factors that hMSCs produce during differentiation[8]. During hMSC differentiation, the transcription factor PPAR-γ is generally considered the master regulator of adipogenesis. Likewise, the transcription factors Runx2 and SOX9 are regarded as the master regulators of osteogenesis and chondrogenesis respectively[9]. It has been demonstrated that physical stimulation of the cell's actin-tubulin cytoskeleton can lead to a cascade of intracellular signaling mediated by the RhoA/ROCKII signaling pathways which has been shown to regulate

the expression of Runx2 and PPAR- γ inside the hMSC[10,11]. Specifically, hMSCs that are allowed to flatten and spread display higher levels of Runx2 express and lower levels of PPAR- γ expression. In contrast, unspread, rounded cells have been shown to display higher levels of PPAR- γ expression and lower levels of Runx2[12].

Interpenetrating Polymer Networks

An Interpenetrating Polymer Network (IPN) is a type of polymer system that is fabricated by swelling a single hydrogel network in a solution composed of a mixture of monomers and some form of crosslinker. These monomers are then covalently crosslinked to each other to form a secondary network that is interlaced but not covalently crosslinked to the first network. This type of fabrication method allows for the resulting hydrogel to retain the distinct properties of individual networks as opposed to fabrication methods which involve mixing the components together and forming a single hydrogel network[13]. An IPN hydrogel was determined to be ideal for this experiment because we wanted our scaffold to contain a collagen component to allow encapsulated cells to flatten, spread, and proliferate while simultaneously displaying the stiffness and osteogenic properties of PEG-DA and PDMS*-MA [8]. By successfully incorporating these aspects together into one stem cell delivery system, we expect to observe a high degree of osteogenic phenotype from our encapsulated hMSCs that should lead to a greater osseointegration between the cell laden hydrogel and the subchondral bone.

CHAPTER II

METHODS

Hydrogel Preparation

In order to fabricate the Collagen/PEG-DA/PDMS*-MA (CPP*) hydrogel, a 0.3% (w/v) collagen hydrogel was synthesized to act as the initial polymer network. Rat Tail Collagen Type I (Corning Life Sciences) was first diluted down from its stock solution concentration to 3.5 mg/ml using 0.1 M acetic acid. Next, 10% (v/v) Reconstitution buffer, 20% (v/v) 5x Dulbecco's Modified Eagle Serum (Life Technologies), and 3% (v/v) NaOH were combined with the 3.5 mg/ml collagen in a Falcon™ 15mL conical centrifuge tube to produce the final collagen hydrogel solution. In order to generate cylindrical hydrogels, 300 µL of the solution was added to individual wells of a 96 well plate and placed in an incubator (37°C and 5% CO_2) to gel overnight.

Once the collagen hydrogels had fully gelled, a few were selected to act as control gels (n=3). Collagen hydrogels that were selected to be made into the CPP* hydrogels were formulated as described above but with the addition of 15% (w/v) of PEG-DA and PDMS*-MA monomers at the following ratios: 80:20 and 100:0. PDMS*-MA (MW= 7 kDa; Grunlan Research Group)[14] was incorporated in the hydrogels during the collagen hydrogel formulation step by mixing the PDMS*-MA with the final collagen hydrogel solution prior to gelation. A monomer precursor solution consisting of PEG-DA and 0.1% (w/v) 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone in ethanol (Igracure 2959; Sigma-Aldrich) was fabricated by vortexing the

reagents together and pouring them into a Fisher brand 95mm x 15mm polystyrene petri dish. The hydrogels were removed from the 96 well plate and transferred to the petri dish containing the precursor solution to soak for 2.5 hours at room temperature. After the hydrogels had been infused with the precursor solution, they were removed from the solution, dabbed dry with a Kim Wipe, and transferred back into a the 96 well plate in order to produce a stable cylindrical shape. The precursor solution inside the collagen hydrogel was then polymerized into an IPN by a 5-minute exposure to long wave UV light (~6 mW/cm^2, 365nm; Spectroline).

Evaluation of Hydrogel Properties

Swelling ratio was determined by placing hydrogel samples in sealed glass vials with 15 mL of de-ionized water on a shake plate (250 rpm) for 48 hours. After 48 hours, the hydrogels were removed from the vials and blotted dry with a Kim Wipe. Each hydrogel was weighed to determine the swollen weight (Ws) then placed in a vacuum oven (30 inchHg, 60°C) for 24 hours. After the water content of the hydrogels was removed, each hydrogel was again weighed to determine the dry weight (Wd). The swelling ratio was determined by using Equation (1).

Equation (1) $$SR = \frac{(Ws - Wd)}{Wd}$$

The storage modulus (G') of each hydrogel was recorded by performing Rheometric Analysis. Testing was done using a Rheometer (Physica MCR 301) equipped with a cone and plate testing system. Our various hydrogels were fabricated as previously described with the exception of using a silicone mold to form fit our hydrogels to the Rheometer's required specifications. Each silicone mold had a diameter of 10 mm and was 1.5 mm thick. The hydrogels were fabricated

and soaked while in these molds in order to generate the Collagen, CPP* 100:0, CPP* 80:20 and PEG-DA hydrogel samples. The samples were placed on the Rheometer plate and the cone was lowered down so that it came into contact with the hydrogel sample. The sample was then subjected to shear force at a frequency of 1 Hz and a strain rate ranging from 0.1-5% strain. The average value of the storage modulus was given and statistical analysis was performed on all hydrogel samples.

In order to qualitatively determine the extent of PDMS*-MA incorporation in the CPP* hydrogel we used a Nile Red Technical Grade Stain (Nile Red; Sigma-Aldrich). A precursor solution was created first by dissolving 20 mg/mL of the Nile Red powder into methanol. A 0.01% (v/v) solution consisting of Nile Red precursor solution in DI-H_2O was combined with 120 mL of PBS (Sigma-Aldrich) to create the final staining solution. Hydrogel samples were placed in the final staining solution and allowed to soak for 24 hours followed by a 3 day wash in normal 1x PBS solution where the PBS was changed daily. Hydrogel samples were imaged using a Nikon FN1 microscope and C1-plus scanning confocal unit with Argon and green Helium/Neon lasers to fluoresce the samples at 553nm.

Cell Culture

HMSCs isolated from human bone marrow aspirates are held in a hMSC repository funded by the NIH through P40 RR17447. General cell expansion was performed in accordance with our standard protocol[15]. In brief, cells were cultured in CCM consisting of alpha minimal essential medium (α-MEM; GIBCO, Invitrogen), 20% FBS (Hyclone and Altanta Biologicals), 2 mM glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin (GIBCO, Invitrogen). For each

passage, cells were seeded at 100 cm^{-2}. At 70% confluencey, cells were recovered by trypsinization followed by cryopreservation in α-MEM containing 30% FBS and 10% DMSO. Prior to encapsulation, hMSCs were thawed and grown in monolayer culture with CCM at 37°C and 5% CO_2.

Fabrication and Culture of Cell Laden Constructs

HMSCs were loaded into the CPP* hydrogel during the collagen hydrogel formulation step. Cells were first trypsinized from monoculture and counted using a hemocytometer. In a sterile cell culture hood, the cells were then added to 5x DMEM so as to yield a cell density of ~1 x 10^6 cells/mL. The collagen hydrogel was then fabricated both with and without PDMS*-MA according to the previously established protocol. The encapsulated cells were allowed to adhere and spread inside the collagen hydrogels for 24 hours before the addition of the monomer precursor solution. In order to maintain cell viability during the 2.5 hour soak, the PEG-DA precursor solution was made using CCM in place of PBS. Following the 2.5 hour soak, the cell laden constructs were polymerized into an IPN hydrogel by exposure to 5 minutes of UV light which has been demonstrated to be cytocompatible. The CPP* hydrogels were then immersed in α-MEM supplemented with 20% FBS, 2 mM glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin and maintained at 37°C and 5% CO_2. After 24 hours, samples (n=3) were selected for either live/dead staining or cell morphology characterization.

Cellular Characterization

A live/dead staining method was used to determine cell viability following cell encapsulation. The hydrogel samples were first removed from medium and washed 3 times in PBS for 15

minutes each wash. To prepare the live/dead stain, a 1 mM stock solution of Calcein AM (fluorescence= 496 nm; Sigma) in DMSO was diluted down to 1 µM using PBS with 0.1% (w/v) BSA. 5 µg/ml of Propidium Iodide (Sigma) was added to the solution and vortexed. The hydrogel samples were then soaked in the live/dead stain for 1 hour at 37°C and 5% CO_2. Following the soak, the hydrogels were removed from the stain and washed 3 times with PBS. These samples were imaged using our scanning confocal unit to fluoresce the samples at 543/632nm.

A fluorescent phalloidin stain was used to visualize the morphology of the cells inside the hydrogel. Hydrogels were first washed 3 times in PBS for 15 minutes each and then fixed for 30 minutes in a 3.7% (v/v) formaldehyde solution in PBS. Hydrogel samples were then washed 3 times in PBS for 15 minutes each after fixation to remove the excess formaldehyde. Samples were then washed in 0.5% (v/v) Tween for 15 minutes at room temperature to premeablize the cell membranes. To prepare a working concentration of the phalloidin stain solution, a 0.5 mg/mL stock solution of phalloidin-FITC (Sigma) in DMSO was diluted to 50 µg/mL in PBS. Hydrogel samples were then soaked in the working concentration of phalloidin solution for 24 hours. Following the 24 hour soak, hydrogel samples were removed from the solution and washed 3 times in PBS for 15 minutes each before being imaged with confocal microscopy.

Statistical Analysis

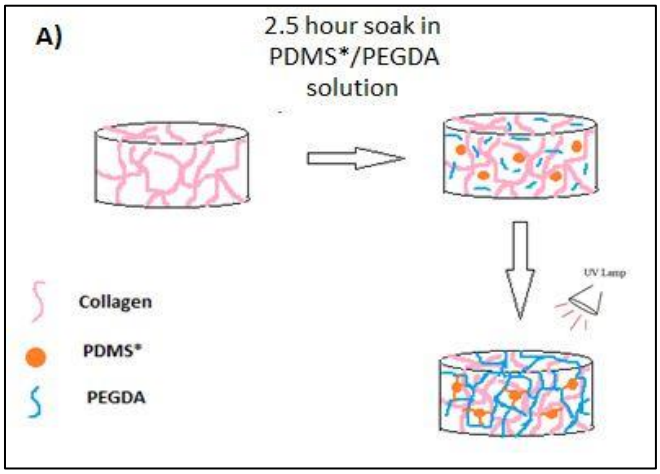
Data are reported as mean ± standard deviation. Statistical differences of swelling ratio were performed using ANOVA followed by a Student-Newmans-Keuls post hoc test, $p < 0.05$.

Rheology results were analyzed by averaging 3 sets of data and reporting as the mean ± standard deviation

CHAPTER III

RESULTS

The creation of the CPP* hydrogel had many initial challenges due to the minimally explored nature of the fabrication techniques. While other labs have developed semi-IPNs by simply mixing collagen and PEG-DA[16] together, no results have been reported on a true IPN that consists of collagen and multiple synthetic monomers networks interwoven independent of each other. The first attempts at introducing the PDMS*-MA network into the collagen hydrogel were unsuccessful and resulted in very little incorporation of the monomer. It was deduced that the high level of hydrophobicity of the PDMS*-MA made it non-conducive to the initial approach where collagen hydrogels were soaked in a monomer solution consisting of a mixture of both PEG-DA and PDMS*-MA, as shown in Figure 2A. It was determined that incorporating the PDMS*-MA into the collagen hydrogel formulation step, as shown in Figure 2B, allowed us to achieve a greater degree of PDMS*-MA integration.



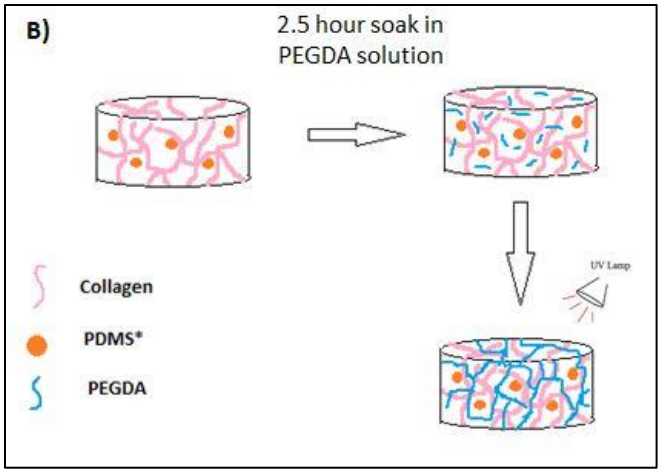


Figure 2: Two strategies for incorporating PDMS*-MA into collagen-PEG-DA IPNs. A) CPP* hydrogel formulation steps involving the addition of PDMS*-MA in the monomer soaking phase. B) CPP* hydrogel formulation steps involving the addition of PDMS*-MA in the collagen hydrogel formulation step.

Swelling Ratio

As shown below in Figure 3, the addition of an IPN network had a significant effect on the hydrogel's swelling ratio. The collagen hydrogel swelled ~3 times more than any other formulation of hydrogel due to the collagen's weak physical crosslinks, large pore size, and the presence of hydrophilic binding domains on the surface of the collagen protein[17,18]. It is because of these reasons that water can easily penetrate and be retained throughout the hydrogel. After incorporating a PEG-DA IPN network into the collagen hydrogels (CPP* 100:0), the swelling ratio decreased due to the strong covalent crosslinking of the diacrylate bonds linking the PEG-DA network and decreasing the pore size in the hydrogel. The swelling ratio of the CPP* 100:0 hydrogel was still greater than a typical PEG-DA hydrogel due to the presence of the hydrophilic collagen network. With the addition of the PDMS*-MA into the IPN network (CPP* 80:20), the swelling ratio decreased in relation to the CPP* 100:0. This was due to the hydrophobic nature of the PDMS*-MA which prevents water from penetrating into the hydrogel[19]. Through statistical analysis it was determined that the CPP* 80:20 hydrogel was statistically different than both the

typical collagen hydrogel and the CPP* 100:0, but it was found that there was no statistical difference between the CPP* 80:20 hydrogel and the normal PEG-DA hydrogel.

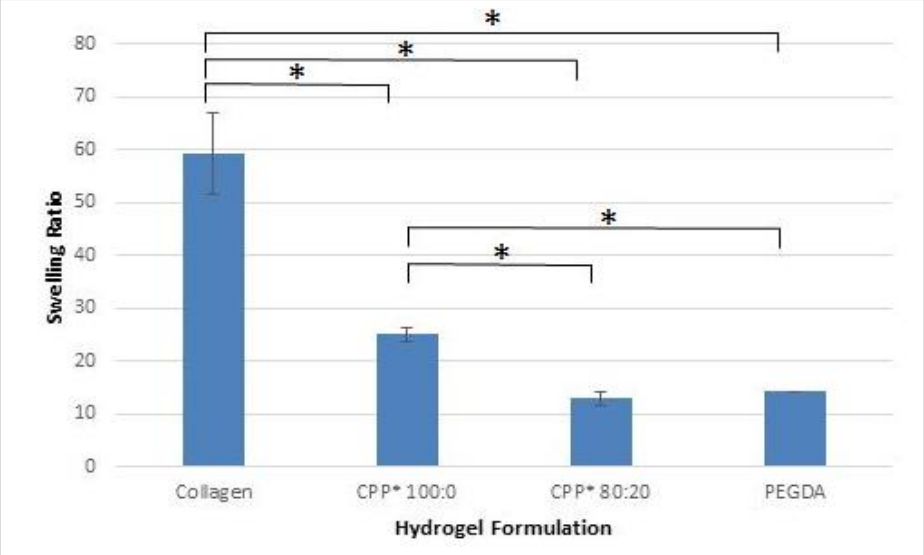


Figure 3: Swelling ratios for different formulations of hydrogels. Statistical differences were found between all of the hydrogel formulations except between the CPP* 80:20 and PEGDA hydrogels, n=3, $P<0.05$.

Hydrogel Stiffness

When we designed the CPP* hydrogel it was important for us to consider the value of the hydrogel's bulk storage modulus. The bulk storage modulus is a measure of a material's ability to resist being deformed in an elastic manner when force is applied to it. It has been demonstrated that the 3D matrix stiffness has a large impact on the differentiation of hMSCs: Softer substrates tend to result in a neural phenotype, while stronger substrates result in an osteogenic phenotype [20]. Collagen hydrogels characteristically have a lower bulk modulus than the *in vivo* environment that osteoblasts are typically exposed to. Handling these collagen hydrogels is also very difficult due to the weak physical bonds between the collagen proteins; often the hydrogel is unstable and will break apart when working with them. For these reasons the use of normal collagen hydrogels for bone regeneration application is far from optimal. PEG-

DA is a widely used biomaterial in bone regeneration applications because the material's bulk properties more closely match those of the osteoblast's natural 3D environment[21]. One of the goals in creating this CPP* hydrogel was to impart the mechanical properties of PEG-DA into a collagen hydrogel in order to generate a substrate that resists loading deformation while maintaining the opportunity for the encapsulated cells to spread. The CPP* IPN was able to successfully increase the hydrogel's mechanical integrity which is displayed below in Figure 4.

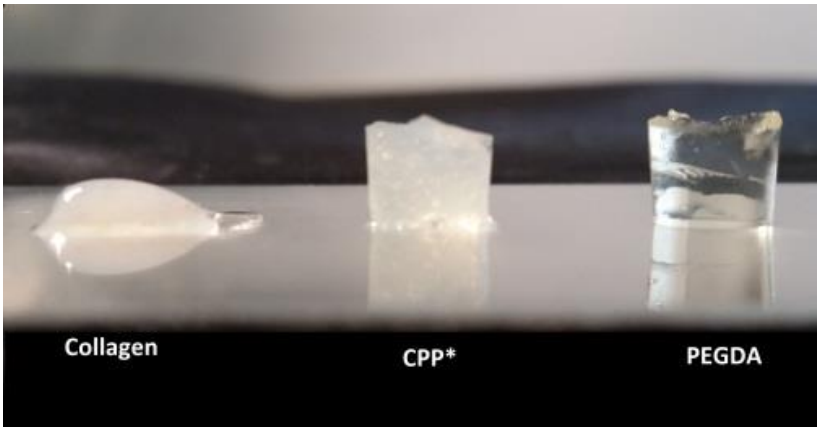


Figure 4: By introducing a PEG-DA and PDMS*-MA network into a collagen hydrogel, a notable difference can be seen in the hydrogel's mechanical integrity.

This increase in mechanical integrity of our CPP* hydrogel was verified by performing Rheology testing as shown below in Figures 5.

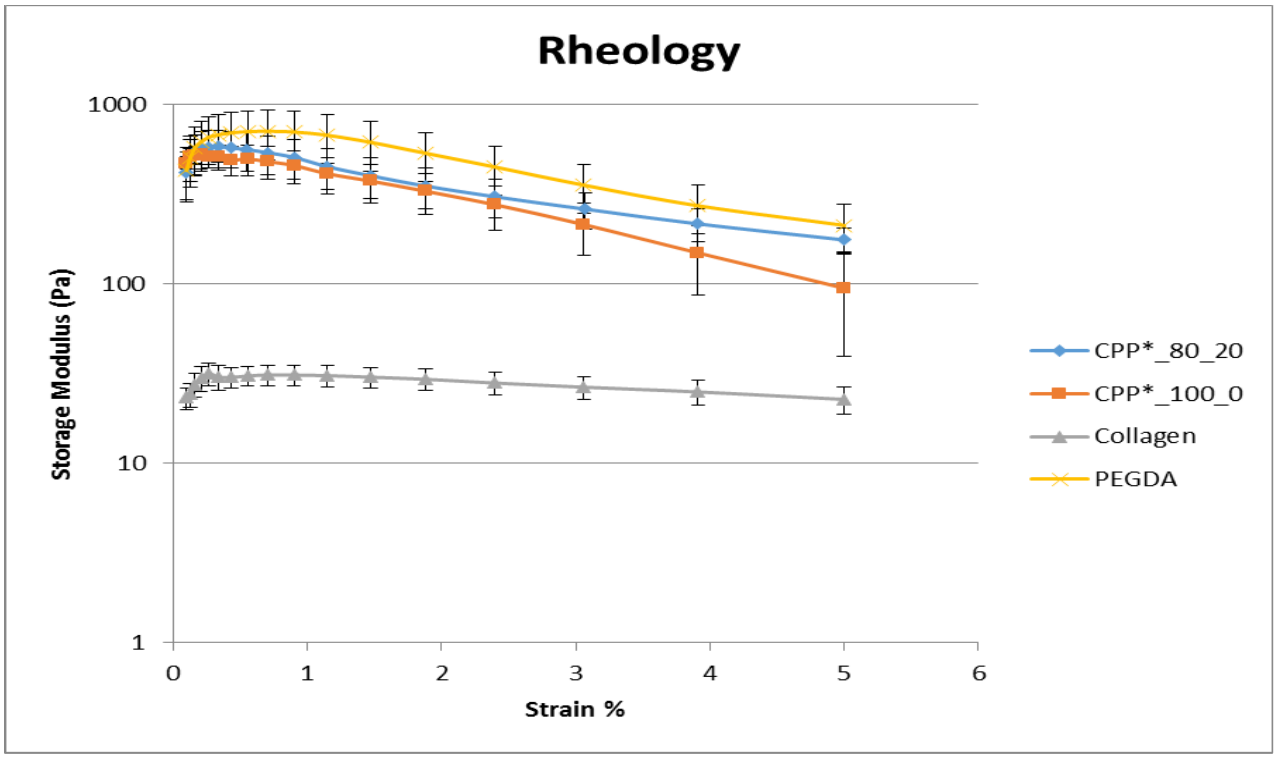


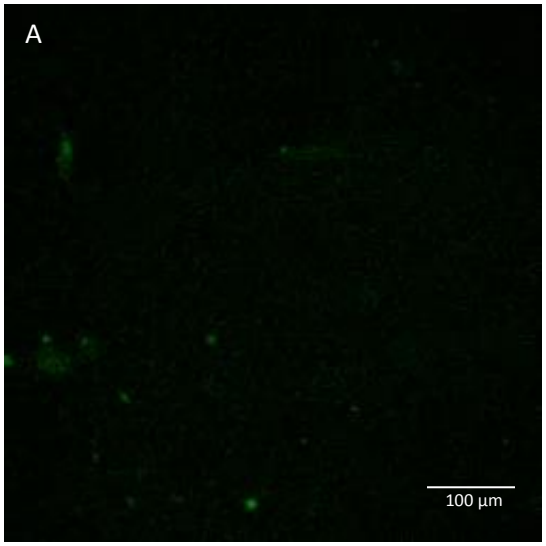
Figure 5: Storage modulus for various hydrogels over a percent increasing in applied strain.

As shown in Figure 5, the hydrogel's stiffness varied according to the materials used in the fabrication method. Based on the results obtained from the swelling ratios, we expected the stiffness of the hydrogels to follow the same corresponding pattern. This was indeed the case since the collagen hydrogels had the lowest storage modulus while the PEG-DA hydrogels had the highest storage modulus. Both the CPP*100:0 and CPP*80:20 had a storage modulus in the same range as PEG-DA, but the CPP* 80:20 was slightly higher than the CPP* 100:0.

Incorporation of PDMS*-MA

We used a Nile Red stain in order to fluorescently stain the incorporated hydrophobic PDMS*-MA. Our first attempts at incorporating PDMS*-MA, as previously depicted in Figure 2A, resulted in minimal incorporation of the polymer, as shown below in Figure 6A. Upon

incorporating the PDMS*-MA in the collagen hydrogel step we observed that the polymer was introduced more completely and formed spheroids ranging up to 75 µm in diameter inside the hydrogel, as shown below in Figure 6B. These spheroids were expected based on earlier studies by our collaborator Dr. M. Grunlan, who's lab demonstrated that when PDMS*-MA and PEG-DA are dissolved in DI-H_2O and polymerized into a hydrogel, the PDMS*-MA forms "discrete micro-droplets" throughout the entirety of the hydrogel[22]. The fact that the PDMS*-MA is not homogenously distributed throughout the hydrogel is of minimal concern for the project. It was demonstrated by the Grunlan lab that PDMS*-MA introduced at an 80:20 ratio with PEG-DA dissolved in DI-H_2O was sufficient to induce an osteogenic response without needing the polymer to be spread homogenously throughout the hydrogel[23].



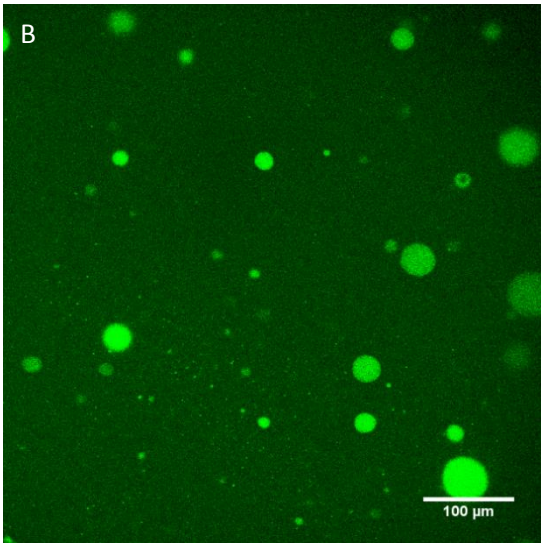


Figure 6: Confocal image of PDMS*-MA incorporation using two methods. A) Introduction of PDMS*-MA using a diffusion mediate soak. B) Introduction of PDMS*-MA in the collagen hydrogel formulation step.

Cell Viability Analysis

Another important aspect of the CPP* hydrogel is that the encapsulated hMSCs have to remain viable long enough to impart a therapeutic effect on the regeneration of the osteochondral defect. This means that we had to ensure that the hMSCs could survive throughout the entire hydrogel fabrication process. The risk for hMSC death was highest during the PEG-DA precursor soaking step where the hydrogels were exposed to PEG-DA monomer and photoinitiator for an extended period of time. We initially tried an overnight soak to ensure that the PEG-DA monomers had ample time to diffuse completely into the hydrogel, but we found that this was an unnecessarily long time frame that resulted in a very high level of cell death (data not shown). Based on searches through the existing literature regarding IPN fabrication with encapsulated cells, it was determined that a soak time ranging between 2-3 hours was an appropriate time frame to ensure monomer diffusion and cell viability[24,25].

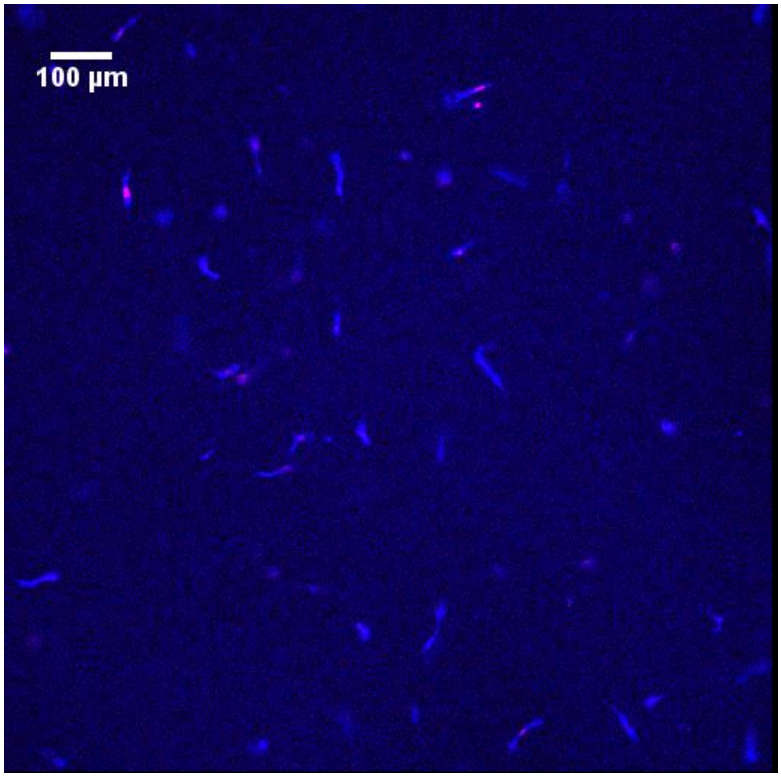


Figure 7: Confocal cross section of a CPP* hydrogel showing live-dead staining of hMSCs.

The live dead assay utilizes Calcein AM and Propidium Iodide to discriminate between live and dead cells. Calcein AM is transported across the membrane of the hMSC into the cell's cytosol causing enzymatic hydrolysis that result in the cell fluorescing at 496 nm. The nucleuses of dead cells are stained with Propidium Iodide which fluoresces under red light. The results of this assay, as shown above in Figure 7, demonstrated that the encapsulation process and fabrication of the CPP* hydrogel was minimal harmful to the hMSCs.

Morphology

After acquiring images of encapsulated hMSCs using confocal microscopy and phalloidin-FITC staining, we are able to visualize the cell morphology in both a CPP* 80:20 hydrogel and a normal PEG-DA hydrogel. As shown in Figure 8A, the encapsulated hMSCs inside the CPP*

hydrogel were able to spread and displayed the characteristic spindle-shape that is typical of an osteoblast[26]. Comparatively, Figure 8B shows encapsulated hMSCs inside a normal PEG-DA hydrogel.

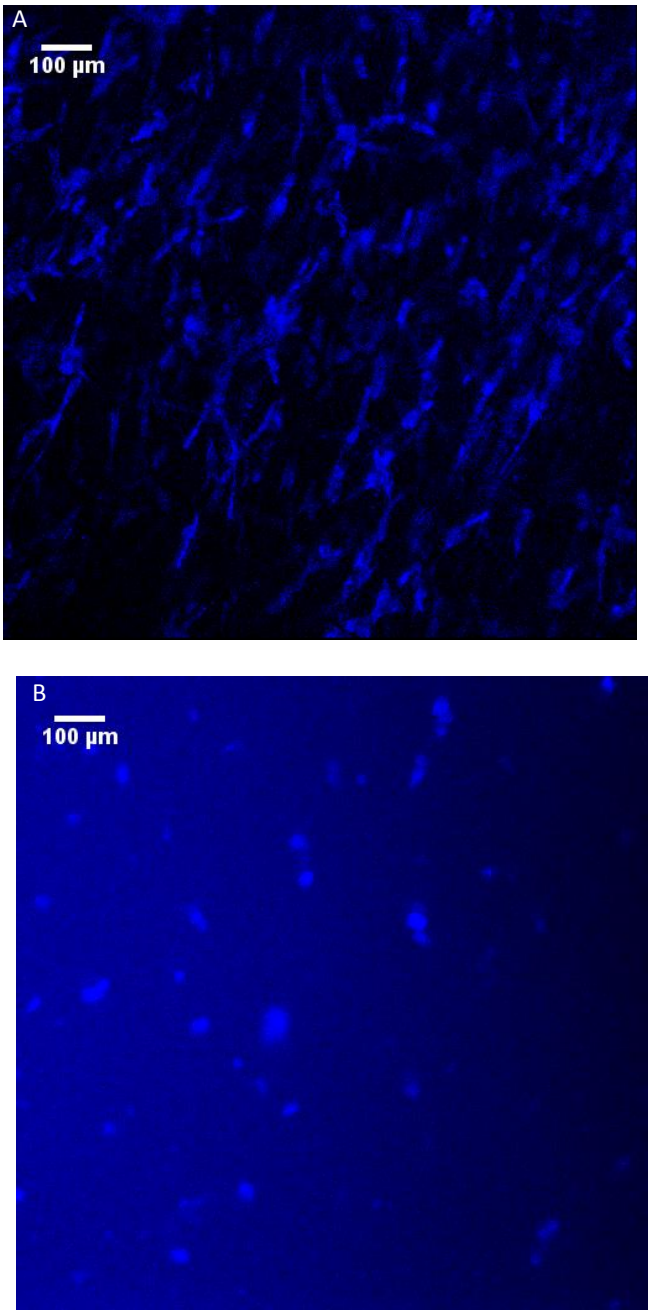


Figure 8: Confocal image of hMSC morphology using a fluorescent Phallodine stain. A) CPP* hydrogels displayed hMSCs spreading and forming cell-cell interactions. B) PEG-DA hydrogels displayed rounded hMSCs morphology.

CHAPTER IV

CONCLUSIONS

Through our investigation, we were able to successfully develop a method for introducing PEG-DA and PDMS*-MA polymer networks into a collagen hydrogel to form a bioactive IPN hydrogel. We found that this stem cell delivery system allowed us to improve the morphology of encapsulated hMSCs while maintaining scaffold stiffness similar to that of osteochondral tissue[27].

Interpretation of hMSC Spreading

As we had hypothesized, the incorporation of collagen into our stem cell delivery system facilitated encapsulated cell spreading. Along with playing an active role in regulating osteogenic gene expression, cell spreading also allows hMSCs to form physical cell-cell cadherin interactions through the extension of lamellipodia. We believe that this is a desired result due to the critical role that cadherin interactions play in the osteogenic differentiation of hMSCs[28, 29]. In our control PEG-DA hydrogel, we verified that encapsulated hMSCs were unable to spread throughout the hydrogel and maintained a spherical morphology. This spherical morphology prevented the hMSCs from coming into contact with other cells, thus inhibiting encapsulated cells from receiving cadherin interactions. We concluded that by promoting both cell spreading within our CPP* hydrogel, we were able to ultimately create an environment that promotes encapsulated cells to undergo osteogenesis.

Interpretation of Increased Scaffold Stiffness

In order for our stem cell delivery system to be considered a feasible solution for regenerating osteochondral defects we had to ensure that the scaffold had adequate mechanical integrity. We chose to use PEG-DA to impart this mechanical integrity to our system because it is a widely used material in this field of research. We hypothesized that by incorporating this material into our collagen scaffold, we could generate a system that is able to be handled without damage and be stiff enough to be comparable to standard treatments. We were successfully able to increase the modulus of our collagen hydrogel to the same order of magnitude as a PEG-DA hydrogel. Upon incorporating the second polymer, PDMS*-MA, into the system, we found that the hydrophobic nature of the polymer offset the hydrophilicity of the collagen network and gave us a swelling ratio and stiffness similar to that of a PEG-DA hydrogel.

Future Work

After demonstrating that the encapsulated hMSCs displayed an osteogenic morphology and that our scaffold showed desired mechanical properties, we have moved on to running biochemical analysis in order to verify that the encapsulated hMSCs are differentiating into osteoblasts. In order to determine this quantitatively, we are performing an enzyme-linked immunosorbent assay to detect the levels of secreted osteoprotegerin. This protein is a mid-stage indicator that hMSCs are undergoing osteogenesis. This data will allow us to further demonstrate that the incorporation of a collagen network has an effect on the differentiation of hMSCs.

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