Strategies to engineer human cartilage using mesenchymal stem cells in fibrin hydrogels

James M Flaherty Research Scholarship Visit Report

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Field of study
Health

Introduction

Articular cartilage (AC) is the smooth tissue that lines bones within our joints. AC functions to transmit loads and allow low-friction relative motion of our bones during everyday life. AC is unable to heal back to its original state following injury, in part due to its lack of a blood supply. Instead, AC injuries may lead to the degenerative condition known as osteoarthritis (OA), which is characterized by a loss of joint function and chronic pain. OA of the knee alone afflicts over 2.5 million Canadians and Irish people combined, resulting in reduced quality of life and a burden upon our societies.

Research groups around the world are working to develop strategies to prevent or delay OA progression, for example through engineering AC replacements in the lab for transplantation. Enabled by the Flaherty Research Scholarship, I traveled to Dr. Daniel Kelly's lab in Ireland within the Trinity Centre for Bioengineering at Trinity College Dublin. My objective was to initiate a collaboration that combines their expertise in the field of AC tissue engineering with that of my home research group at the University of Alberta, headed by Dr. Adetola Adesida in Canada. Together with Ph.D. student Mr. Farhad Chariyev-Prinz, I carried out two relevant and related experiments over a 6-week period that are described below.

Human mesenchymal stem cells obtained from adult bone marrow (BMSCs) show the capacity to form AC-like tissue in the lab when supplemented with cartilage-promoting growth factors. One promising natural support material for this process is the fibrin hydrogel. Fibrin hydrogels, composed of the jelly-like material that forms during blood clotting, can be used to suspend BMSCs in 3D space to provide an environment supportive of tissue growth. Fibrin can be used to produce AC-like tissues in specific shapes such as the cylinders we used in our experiments. Tissue formation in such hydrogels is a complex process involving many controllable parameters that can be optimized to enhance the quality of the final product to be more like healthy native AC.

Accordingly, our first experiment investigated the effects of the seeding density parameter (the number of BMSCs put into the fibrin hydrogels per unit volume) on quality-related outcomes after 3-weeks' tissue growth with supplementation of TGF-β3, a potent cartilage-promoting growth factor. Based on previous work from Dr. Adesida's lab using a different supporting material (collagen sponges), we hypothesized that lower seeding densities would result in superior AC-like tissue formation.

Our second experiment aimed to overcome the unfortunate tendency of BMSC-based AC-like tissues induced by TGF-β3 to progress to a non-functional bone-like state rather than remaining as AC-like tissues. Motivated by the cyclic compressive loading experienced by AC in the knee, we investigated whether dynamic compression of BMSC-fibrin constructs may initiate AC-like tissue growth without supplementing TGF-β3. We additionally modulated the stiffness of the fibrin hydrogels used, as we suspected this would influence force transmission to BMSCs during dynamic compression with potential effects on AC-like tissue growth.

Materials and Methods

BMSCs were isolated from human bone marrow aspirate. These cells were then encouraged to expand/multiply in flasks until we had enough to carry out our experiments. All experiments took place within body temperature incubators using a standard nutritious medium to feed cells and encourage AC-like tissue growth.

For the first experiment (seeding density with growth factor supplementation), BMSCs were suspended in fibrin hydrogels at low, medium, and high seeding densities and casted into cylindrical molds (2-mm in height and 5-mm in diameter). The BMSC-fibrin hydrogels were then cultured for 3-weeks with the standard medium further supplemented with TGF-β3. At the end of the culture period, we analyzed newly-formed tissues for quality-related outcomes of shape retention, AC-related biochemical accumulation, and stiffness for comparison across seeding density groups.

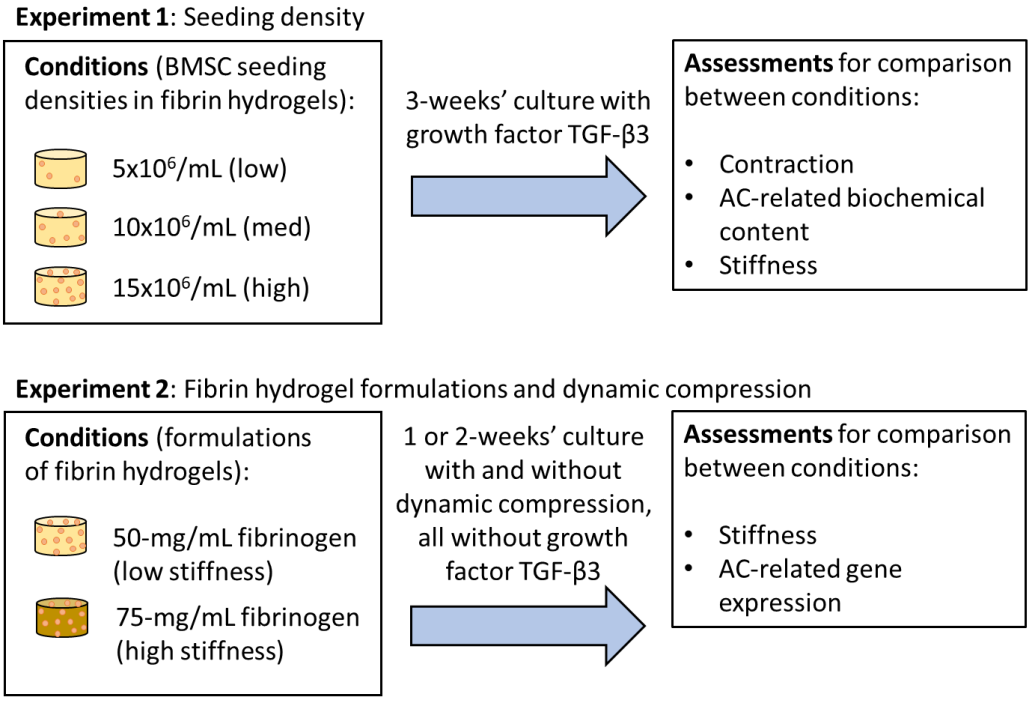

Figure 1: Experiments carried out with bone marrow mesenchymal stem cells (BMSCs) in fibrin hydrogels to form articular cartilage (AC)-like tissues.

For the second experiment (fibrin hydrogel formulation and dynamic compression), BMSCs were suspended in fibrin hydrogels of low and high stiffness at the high seeding density from study 1 and again cast into cylinders. The BMSC-fibrin hydrogels were then exposed to dynamic compression vs. no compression, for either one or two weeks. We then compared any AC-like tissue growth in dynamic compression groups relative to the controls for each hydrogel formulation/stiffness and time point.

Results

For our first experiment, we visually observed hydrogels from all conditions become opaque over time, indicating that BMSCs were actively forming new tissue. After 3-weeks' culture, in the low and medium seeding density groups, the newly-formed tissues had contracted by up to a third whereas in the high seeding density group they had increased in size by about 10% (Fig. 2A & B).

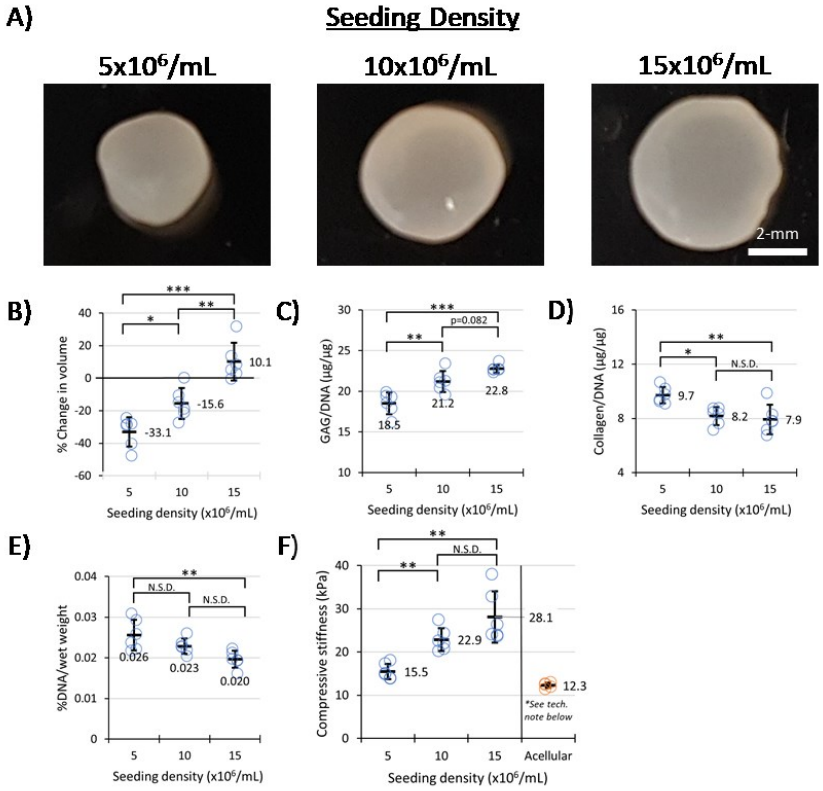

*Figure 2: Seeding density affected AC-like tissue formation by BMSCs. Increased seeding density tended to lead to: (A,B) less contraction, (C) increased normalized accumulation of glycosaminoglycans (GAGs), (D) decreased normalized accumulation of collagen, (E) decreased cellularity by weight, and (F) increased stiffness. Technical note: the acellular group was prepared using a different batch of reagents. Statistical arguments were made using analysis of variance on means or ranks (depending on distributions within groups) with application of the Bonferroni correction, with n=6-8 biological replicates per group. *: p<0.05, **: p<0.01, ***: p<0.001. N.S.D.: no significant difference.*

We observed the greatest accumulation of glycosaminoglycans (GAGs), a biochemical component of AC, in the high seeding density group; in fact, we found the same trend even after normalizing to account for the increased number of cells initially seeded into the hydrogels (Fig. 2C). At the same time, we observed the greatest normalized accumulation of collagen, another biochemical component of AC, in the low seeding density group (Fig. 2D).

Remarkably, tissues within the high seeding density group had the lowest cell content, measured as DNA content, when normalized to weight (Fig. 2E). This was in part because they increased in size rather than contracting as the tissues did in the lower seeding density groups. Finally, the hydrogels in the high seeding density condition demonstrated the greatest stiffness (Fig. 2F).

For our second experiment, we prepared two formulations of fibrin hydrogels and verified that they were of different stiffnesses (Fig. 3). After one and two weeks of dynamic compression, however, we could not visually observe tissue formation in any condition; rather, the BMSC-fibrin constructs remained almost entirely translucent, which indicated that little-to-no tissue growth had occurred. Thus, rather than relying on protein-level metrics as in the first experiment, we measured how the BMSCs in the fibrin hydrogels expressed the precursors of AC-related biochemicals at the gene expression level.

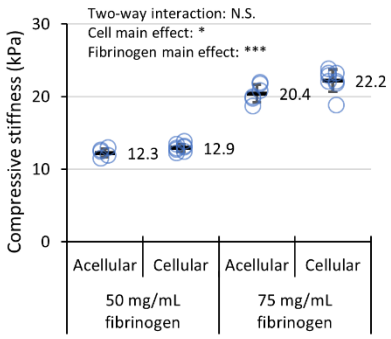

Figure 3: Fibrinogen concentration had a large positive effect on stiffness, whereas the presence of cells had a small positive effect. N.S.: not significant. n=5-8 replicates per group.

We did not observe a compelling trend of AC-related gene regulation by dynamic compression regardless of the hydrogel stiffness (data not presented). One possible explanation of this finding is that the hydrogel stiffnesses we used were too low to appropriately transmit forces for initiation of AC-like tissue growth without TGF-β3 supplementation.

Conclusion

In our first experiment, we demonstrated that seeding density is an important factor in AC-like tissue formation by BMSCs in fibrin hydrogels with significant effects on contraction, AC-related biochemical content, and stiffness. Our immediate next step for this experiment is to compare the synthesis of AC-specific collagen across our seeding density groups using histology and immunofluorescence techniques.

In our second experiment, we showed that dynamic compression as applied in our culture model does not promote AC-like tissue formation by BMSCs, even at the early gene expression level. We will use the knowledge gained to improve the loading regime for a future experiment.

Plans for continuing collaboration

We intend to test the reproducibility results from both studies across institutions and with additional BMSC donors. We hope to then disseminate our collective technical results in a peer-reviewed publication. Finally, Mr. Chariyev-Prinz and I hope to maintain in close contact as we continue with our main Ph.D. research projects to mutually benefit from each other's respective skillsets.

Contacts made

This ICUF research award allowed me (far right side below) to connect and work alongside Mr. Farhad Chariyev-Prinz (fourth from the right side), Dr. Kelly (centre in a plaid shirt) and over two dozen members of his research group. I also had the pleasure of meeting many researchers working in other groups within the Trinity Centre for Bioengineering. I hope that the new collaboration with Dr. Kelly's laboratory will lead to further student travel between our research groups. This will allow good collaborative use of the equipment, resources, and expertise available at our respective institutions.

Acknowledgement

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