ENHANCED CELL SHEET TECHNOLOGY FOR CARTILAGE REPAIR

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INTRODUCTION

Cartilage is one of the most important bodily components in allowing one to walk painlessly. It is the connective tissue in your joints, providing smooth articulations and minimalizing friction between bones. Injuries to this tissue are very common and can be detrimental if not treated, due to its lack of intrinsic healing ability. There exist various known causes of cartilage injury, including joint dislocation, ligament tear, fall/impact, infection, and inflammation [1]. Osteoarthritis is the most common cartilage defect, afflicting approximately 27 million Americans today [2]. It is a degenerative disease seen most prevalently in elderly, obese, and former athletes due to excessive wear and tear on the joints.

Currently, there are several techniques being used to reduce the impacts of defective cartilage; however, they all have shortcomings. Surgical intervention, for instance, whether that be grafting, microfracture, or injection, all expose patients to the risk of rejection, infection, and deterioration [2]. Ideally, an implant should consist of the patient’s own cells/tissue to reduce these risks. Autologous Chondrocyte Implantation (ACI) is a procedure that uses the patient’s own cells/tissue to boost the repair process in the body. Though this is an improvement from most surgical techniques, contamination and lack of cell number still limit this technique’s success [3].

Further advancements to the field of cartilage regeneration can be seen in the research currently being conducted by several groups in Japan. They hypothesized that layering sheets of mesenchymal stem cells (MSCs) will increase the rate and ability of differentiation into chondrocyte cells [4], [5]. However, none of these groups were able to obtain constructs that expressed mechanical properties suitable for implantation after chondroinduction.

OBJECTIVE

Based on previous literature and past studies, further analysis of the effects of the cell sheet technology on cartilage differentiation needed to be conducted. To expand upon prior studies’ findings, our lab decided to test two different cell types, chondrocytes and MSCs, and see which would result in the best, mechanically-sound 3D construct. Our goal was to obtain a structure that could be implanted into the patient and be comparable to naturally occurring cartilage.

HYPOTHESIS/SUCCESS CRITERIA

It was hypothesized that previous research had been missing a condensation-like process in their trials. This process, in essence, is the induction of cell-rounding, which promotes aggregation, cell-to-cell contact, and therefore also ultimately improving differentiation and chondrogenesis. The aim of this project was to compare and contrast chondrogenic expression between cell types, trypsin treatment, and exposure to growth factor TGF-β and see which variables yielded the best results. The success criteria consisted of high cell viability, chondrogenic gene expression, glycosaminoglycan (GAG) deposition, low hypertrophy, and suitable mechanical properties, as determined by qualitative measures.

METHODS

The study was designed to test the effects of several different variables on cartilage formation. There was no conclusive evidence based on previous research verifying one cell type to result in superior cartilage deposition over the other type. Consequently, both chondrocytes and MSCs were tested. The assessment of the primary hypothesis on the addition of a condensation-like process was achieved by trypsinization. Trypsin is a digestive enzyme that breaks down proteins, but short cell exposure time causes the desired cell rounding effect. Furthermore, the experiment was to observe the effect of the presence of TGF-β.

In addition to the varying cell cultures that were examined, the second part of the project was to determine a method that resulted in the best 3D construct. Based on previous research, our group tested two construct techniques: large sheet and beads-based. In both cases, the cells were cultured in growth medium for ten days, trypsinized, exposed to the growth factor, and then placed in the bioreactor to further develop. The large sheets were created by scraping the cell cultures from their respective flasks once they reached a confluent state. This was to ensure the cellular networks remained intact, hypothesized to aid in the forming of a solid structure. The beads-based trials were founded on the concept of fusing tiny ‘beads’ together into one construct, utilizing 60-well plates and either coalescing the beads with a UV-activated methacrylated gelatin (mGL) or not.

Both construct methods, the large sheet and the beads-based, were both ultimately formed into the 3D cylindrical structure by means of a porous cassette filled with agarose gel and punched with two small cylindrical holes. The cells were placed in these holes and cultured in the cassettes in growth medium. See Figure 1.

Figure 1. Experimental setup
RESULTS
The trypsinization resulted in the cell rounding that was desired for this experiment. Visual comparison of the chondrocyte and MSC morphologies with and without trypsin treatment and with and without the growth factor can be seen in Figure 2. In the cases with TGF-β, there is visible growth compared to without. In addition, the trials with trypsin treatment are slightly smaller than those without; this is due to the condensation effect on the cells.

Figure 2 Cell morphology

The live/dead staining showed that the TGF-β treatment is able to increase the cell viability. Additionally, only the cultures with the growth factor present led to expression of chondrogenic gene markers, which is exemplified in Figure 3, showing two of the four tested gene marker results. Aggrecan (AGC) expression was seen regardless of trypsinization; however, the hypertrophic marker Collagen X is significantly decreased when trypsinized. These were positive results, supporting out hypothesis that trypsin treatment would be beneficial for chondrogenesis.

Figure 3 Chondrogenic and hypertrophic gene markers

The histology of the safranin-o staining for GAG deposition supported the conclusion that without the growth factor there was no chondrogenesis. Furthermore, the longer the cells cultured the higher the deposition of cartilage.

The results for part two of the experiment, forming the construct were not nearly as successful. The large sheets showed nearly no GAG deposition, and the beads-based trials only showed good chondrogenesis externally. The ‘beads’ on the inside of the constructs were not fully fused and further testing to quantify the mechanical properties of the structures could not be performed.

DISCUSSION
The results of this study supported the initial hypothesis. Trypsinization resulted in condensation-like changes, leading to a more tightly compact cell structure. As a result, the mechanical property of the structures were seen to improve as compared with those not treated with trypsin. Also, there was no effect on the TGF-β driven chondrogenesis as a result of trypsin treatment. This chondrogenesis was supported by the excellent chondrogenic gene (Collagen II and AGC) expression and low hypertrophic marker (Collagen X and MMP13) expression.

This research concluded that the cassette used to contain the construct was not good for a long term culture. There was a perfusion issue with the culture medium not being able to reach the center of the construct. Moving forward, further trials will involve finding a better method to form the cylindrical construct. To solve the perfusion issue, one possible solution was to culture the constructs in the cassettes for only a week, take them out, then cut them in half. Another possible solution was to use an alternative to the cassettes that would be more permeable to the medium.

CONCLUSION
The cell sheet technology, paired with the trypsinization treatment has the potential to drastically improve the treatment for cartilage defects, such as osteoarthritis. Upon the improvement of the construct method, the design will be implantable, utilizing the patient’s own cells and tissues for a maximal probability of success. This technique will ideally eliminate the need for multiple invasive surgeries, painful grafting, and high risk implantations.

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REFERENCES


