

ASSESSING STEM CELL POTENTIAL FOR A TMJ DISC CONSTRUCT

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INTRODUCTION

Jaw joint disorders affect more than 10 million Americans and can have a significant effect on quality of life. The Temporomandibular Joint, or TMJ, is behind the pain, clicking, and grinding of the jaw that so many individuals find aggravating and in some cases, debilitating. Up to 70% of patients with jaw joint disorders suffer from some displacement of the TMJ disc, which can lead to deterioration of the native joint tissue. (Farrar et. al., 1979). Like other cartilagenous tissues, the poor regenerative capabilities of the TMJ disc make it a good candidate for tissue engineering. For patients who must undergo discectomy, or removal of the TMJ disc, there is currently no available tissue engineered construct with which to replace the degenerated disc.

Current research in the TMJ realm has turned to seeking cell sources that could be potentially developed into a TMJ disc construct. A healthy TMJ must withstand a biomechanical environment unique from other joints in the body, and as a result develops a distinctive extracellular matrix (ECM) composition. Mature chondrocytes extracted from the autologous site are in some ways promising because they display the imperative joint properties, but these show limited ability to expand in culture (Mao, 2005).

A second cell source under consideration is mesenchymal-derived stem cells. These show great capability of differentiating into multiple cell lineages, including cartilage, bone, and tendon (Pittenger et. al., 1999). Through combinations of scaffold choice, type of media, mechanical stimuli, and growth factors, these cells can be manipulated to display a directed phenotype upon culturing. One type of mesenchymal stem cells, adipose-derived stem cells (ASCs), are particularly promising due to their abundance, ease of attainability, and capability of undergoing differentiation into fibrocartilage.

One study has already employed ASCs to examine their potential for tissue engineering the TMJ disc. This study by Mäenpää et. al. in 2010 showed limited success in getting the ASCs to show similar ECM composition as cells extracted from the TMJ disc. This indicated inadequate differentiation of the cells into fibrocartilage. This study employed the micromass and pellet seeding techniques on a poly(L/D)lactide (PLA) disc, which may have affected the outcome.

Objective

The present in vitro study sought to assess the attachment potential of ASCs to a novel biomaterial, the Polyglycerol Sebacate (PGS) scaffold. Seeding efficiency was examined specifically for two seeding techniques, a rocker and spinner flask. The objective of this study was to determine if the experimental design could serve as a viable alternative to that of the Mäenpää study. More specifically, this study sought to qualify which of the two seeding techniques, rocker or spinner flask, should be employed for a more meaningful pilot.

Success Criteria

There were three distinct success criteria for this study. First, the optimal seeding efficiency was set at 40%. Second, the threshold for maximum variability was set at 25% of the mean. Third, cells needed to be visualized on the scaffold.

METHODS

The adipose-derived stem cells used in this study were isolated from a non-diabetic female patient. Fat tissue was taken from the patient during liposuction and from this, ASCs were isolated after many rounds of centrifugation and aspiration. Cells were expanded in a culture solution of Dulbecco's modified Eagle's medium (DMEM)/low glucose (Thermo Scientific), 10% fetal bovine serum (Atlantic Biologicals), 1% penicillin-streptomycin (Lonza), and 25 µg/mL L-ascorbic acid (Sigma-Aldrich). Cells were expanded up to passage number three in order to obtain 3 million cells for the seeding study.

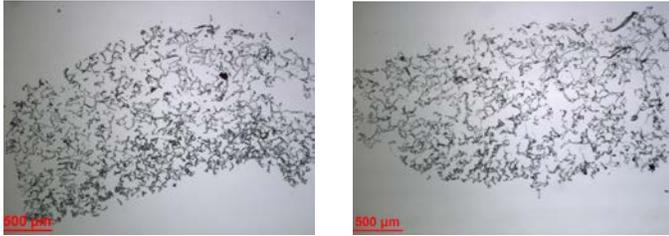
Scaffolds were cut from sheets of PGS with a 4 mm biopsy punch, at 1.3 mm thickness. Cells were seeded at 375,000 cells/scaffold, or about 23 million cells/mL scaffold. Chondrogenic media was used for cell seeding culture and consisted of DMEM/high glucose (Thermo Scientific), 1% penicillin-streptomycin (Lonza), 1% nonessential amino acid solution (Thermo Scientific), 1% insulin-transferrin-selenium+premix (BD Biosciences), .1 µM dexamethasone (MP Biomedicals), 40 µg/mL L-proline (Acros Organics), and 50 µg/mL ascorbate 2-phosphate (Sigma-Aldrich). For one of the two seeding groups, 1.5 million cells in 100 mL of chondrogenic media were cultured with n=4 scaffolds in a spinner flask. For the second group, 375,000 cells in 1.5 mL of media were cultured with 1 scaffold each in n=4 wells of a 24 well plate. The former group was cultured on a magnetic stir plate at 60 revolutions per minute and the latter group was cultured on a rocker at 70 revolutions per minute. Culture occurred for three days at 37°C and 5% CO₂ for both groups.

Histological testing was performed on n=1 scaffold from each group. The scaffolds were set in optimal cutting temperature compound (Tissue-Tech) and frozen at -80°C. They were then cryosectioned at 6 µm, fixed in cold acetone, and stained with hematoxylin and eosin. From these stained sections, cell distribution could be visualized.

Biochemical analysis was performed on n=3 scaffolds from each group. The scaffolds were dried in a speed vacuum for ten hours and then digested at 60°C in papain buffer with 125 µg/mL papain (Sigma-Aldrich), 5 mM N-acetyl-L-cysteine (Sigma-Aldrich), and 5 mM EDTA (Sigma-Aldrich) for 48 hours. The scaffolds were tested for DNA content using a PicoGreen dsDNA quantification kit (Molecular Probes, Inc.). The DNA content was used to determine attachment efficiency, and the efficiency of the two groups was compared with a significance level of p<0.05.

RESULTS

Staining of the scaffolds from the spinner flask and rocker groups revealed very low seeding density, with no cells or matrix visualized at the 3 day time point. The spinner flask and rocker group scaffolds are shown below in Figures 1 and 2, respectively.



Figures 1 (left) and 2 (right). Hematoxylin and Eosin stains of PGS scaffold after 3 days of culture in the spinner flask and rocker environments, respectively. Scale bar (red) is 500 μm .

The DNA assay revealed an attachment of $67,000 \pm 4000$ cells/scaffold and $71,000 \pm 8000$ cells/scaffold for the spinner flask and rocker groups, amounting to a seeding efficiency of $21\% \pm 1\%$ and $22\% \pm 2\%$, respectively; these are shown below in Figure 3. The standard deviation was found to be 10% and 19% of the mean for the spinner flask and rocker groups, both below the threshold of 25%. The cell attachment mean percent and variability did not show a statistically significant difference between the two groups.

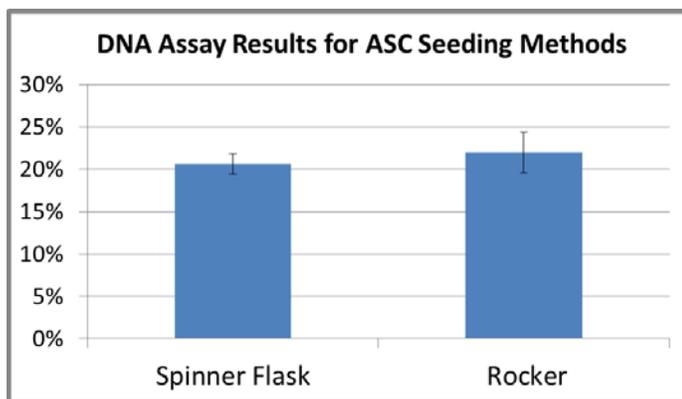


Figure 3. Cell seeding efficiency for spinner flask and rocker seeding methods from $n=3$ scaffolds/group.

DISCUSSION

The objective of the study was met; the results revealed a potential for both seeding techniques to be used with PGS as the experimental design of choice in future ASC studies for TMJ applications. Although there was no significant difference observed between the two groups in terms of attachment efficiency, the variability for each group was less than one quarter of the mean, indicating dependability in the results, and satisfying one success criterion. Two other success criteria, visualization of cells within the scaffold and attachment efficiency of $>40\%$, were not achieved during this preliminary study. The ASC attachment efficiency was just over 20% for the two groups, indicating a lower level of success in initial attachment than studies using mature fibrocartilage cells (Hagandora et al., 2012).

Low attachment efficiency did not necessarily signify reduced potential of ASCs in TMJ research. This was the first study of its kind assessing ASC attachment on PGS, so the true threshold of success remains unknown. Past studies with other seeding techniques have shown saturation potentials above 23 million cells/mL scaffold, such as one study conducted with native TMJ disc cells (Almarza et al., 2005), but as ASCs are a unique and under investigated cell type, it is possible that PGS could have sustained higher numbers of cells than anticipated, and thus a greater cell count per mL scaffold could have resulted in better attachment efficiency.

This study was limited in that results beyond the three days of attachment remain unknown. The major limitation of the 2010 Mäenpää et al. study were that the ASCs did not show full differentiation compared to native TMJ disc cells in culture and the TMJ disc itself. But these results could not be established until the 3 week/ 6 week time points. As these points were not reached during our study, an assessment of collagen content could not be conducted. Thus, our study cannot serve as a valid contrast to the Mäenpää study. Differentiation potential of ASCs with our experimental design remains unknown.

CONCLUSION

In conclusion, this study highlighted not only a potential for ASCs to be used for TMJ applications, but a necessity for more thorough experimentation with the two seeding techniques employed. An ideal experimental design would maximize both ASC attachment and differentiation into TMJ disc cells. If this could be accomplished, leading to design of a TMJ disc construct, it would likely have a tremendous impact on the community of patients who continue to suffer the deleterious effects of jaw joint disorders.

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REFERENCES

- Almarza, A., & Athanasiou, K. Effects of initial cell seeding density for the tissue engineering of the temporomandibular joint disc. *Ann Biomed Eng* 33(7): 943-50, 2005.
- Farrar, W., & McCarty, W. The TMJ dilemma. *J Ala Dent Assoc* 63(1): 19-26, 1979.
- Hagandora, C., Gao, J., Wang, Y., Almarza, A. Poly (glycerol sebacate): a novel scaffold material for temporomandibular joint disc engineering. *Tissue Eng* 19(5-6): 729-37, 2012.
- Mäenpää, K., Ellä, V., Mauno, J., Kellomäki, M., Suuronen, R., Ylikomi, T., & Miettinen, S. Use of adipose stem cells and polylactide discs for tissue engineering of the temporomandibular joint disc. *J R Soc Interface* 7(42): 177-88, 2010.
- Mao, J. Stem-cell-driven regeneration of synovial joints. *Biol Cell* 97(5): 289-301, 2005.
- Pittenger, M., Mackay, A., Beck, S., Jaiswal, R., Douglas, R., Mosca, J., Moorman, M., Simonetti, D., Craig, S., Marshak, D. *Science* 284(5411): 143-7, 1999.