Culture of human septal chondrocytes in a rotary bioreactor

INTRODUCTION

• Cartilaginous defects created by trauma, tumor resection, and congenital deformities must be repaired with analogous material.
• Autologous grafts are favored over synthetic and allogeneic structures.
  - Can be obtained from nasal septum, auricle, or rib.
  - Nasal septal cartilage is preferred for its superior structural properties, ease of harvest, and minimal donor site morbidity.
  - However, there is a limited supply of nasal septal cartilage.
  - Tissue engineering of autologous neocartilage offers a solution to this problem.
• Nasal septal cartilage engineering involves several key steps:
  - Cartilage harvest and chondrocyte isolation
  - Monolayer culture induces dedifferentiation
  - 3D culture in alginate beads promotes redifferentiation → formation of constructs
  - Multiple factors induce redifferentiation including media composition, growth factors, cell seeding density, 3D scaffold properties, and mechanical stimulation.
• Mechanical stimulation has been shown to favorably affect cartilage formation
  - Bioreactors (BR) control mechanical stimuli and fluid flow
  - BR culture of engineered articular cartilage improves histologic and biochemical properties compared with static conditions.

OBJECTIVES

• To show that extracellular matrix (ECM) deposition is possible in 3-dimensional culture of human septal chondrocytes cultured in a rotary bioreactor as well as in static conditions.
• To demonstrate that the biomechanical properties of human septal chondrocytes cultured in a bioreactor and static culture amplify with time.

METHODS

• Human septal specimens collected during routine surgery under IRB approval
• Cartilage digested to release chondrocytes
• Chondrocytes expanded in monolayer culture
• Release from monolayer and formation of alginate beads
• Alginate beads cultured in a bioreactor (Fig.1) and static culture

RESULTS

• Beads cultured for a total of 21 days with testing performed at day 0, 10, and 21
  - Cellularity, glycosaminoglycan (GAG) content, and types I and II collagen content determined
  - Histology (H&E and Alcian Blue) and immunohistochemistry (IHC) for types I and II collagen performed
  - Compression testing at day 0 and 21
  - ANOVA used to determine variations of DNA and GAG; paired t-test used to analyze the difference in type II collagen content
  - Post-hoc Tukey's HSD tests used to identify significant differences between time points

• Beads increased in size from day 0 to 21
• No difference in DNA content between static and BR conditions (p = 0.476)
• Initial decrease in DNA content from day 0 to 10 with a significant increase from day 10 to 21 (p < 0.01; Fig.2)
• GAG accumulation did not differ significantly between static and BR conditions (p = 0.509)
• A significant increase in GAG was observed from day 0 to 10 and 10 to 21 (p < 0.001; Fig.3)

• ELISA results for type I collagen content were below the level of detection for both conditions.
• ELISA results for type II collagen content were below the level of detection for day 0 beads (ELISA = Enzyme-Linked Immunoabsorbent Assay).
• Day 21 beads showed robust increase in type II collagen, but this did not significantly differ between culture conditions (p = 0.639; Fig.4)
• H&E staining demonstrated increasing amounts of ECM with increasing culture time
• Alcian Blue showed robust staining in day 21 beads, indicating the presence of sulfated GAGs (Fig.5)
• IHC confirmed the absence of type I collagen, but showed strong staining for type II collagen in day 10 and 21 beads. Overall, staining did not differ between the static and BR culture conditions (Fig.6)
• Biomechanical testing showed no difference between culture conditions. However, the compressive properties of the beads improved with culture time for both conditions

DISCUSSION

• Human septal chondrocytes cultured in alginate beads exhibit significant GAG and type II collagen accumulation after 21 days in both static and BR culture.
• Confirmed by multiple studies examining articular cartilage constructs.
• Non-quantitative studies performed using human septal tissue.
• The evaluation of the effect of bioreactor culture on nasal septal construct maturation is planned for the future.

REFERENCES


ACKNOWLEDGEMENTS

• AAO – HNSF Resident Research Award