Characterization of a Full Thickness Bovine Cartilage Defect Model

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Disclosures:

Introduction: Cartilage regeneration remains a significant clinical challenge due to the intrinsically poor repair potential of native cartilage and the lack of graft integration with subchondral bone. To evaluate the efficacy of cartilage grafts, physiologically relevant in vitro models are needed which can be used to investigate how cells in the native tissue respond and interact with the graft, and how the graft integrates with the surrounding native tissue. The objective of this study is to characterize an in vitro full thickness defect model, focusing on the effect of in vitro culture on tissue properties, as well as the effect of acellular cartilage graft presence on the surrounding native tissue. Commonly used scaffolds for cartilage repair include both hydrogel[1-6] and polymer nanofiber-based grafts[7,8]; in this study, an agarose-nanofiber scaffold system was investigated.

Methods: Osteochondral explant harvest, defect creation, and culture: Immature bovine metacarpophalangeal joints were obtained from a local abattoir and a diamond-tipped drill bit (Starlite) was used to core osteochondral plugs (d=7mm) from the articular surface. The bone portion of each plug was trimmed to obtain a final height of 5 mm for each osteochondral plug. A high velocity water jet (Waterpik) was used to remove the marrow and a full thickness defect was created (d=5mm) in the cartilage using a biopsy punch. Full thickness defect models were cultured for 14 days at 37°C with 5% CO2 in DMEM with 10% fetal bovine serum which was changed three times weekly with timepoints at 1, 7, and 14 days. Scaffold/explant interactions: Sterile PLGA nanofiber disks (d=5mm, thickness=150μm) were placed into defects immediately after defect creation (n=5) and acellular 2% agarose hydrogel constructs (n=5, d=5mm) were press fit into the defects on top of the nanofiber inserts. Explants with scaffolds in the defect were cultured for 14 days in the same conditions as scaffold-free explant controls. Cell Viability and Number: Total DNA (n=5) was measured using the Picogreen dsDNA assay (Molecular Probes) and cell viability (n=2) was evaluated using the Live/Dead cytotoxicity kit (Invitrogen) and the solution LDH assay kit (Fisher). Matrix Production: Glycosaminoglycan (GAG) production (n=5) was measured with the dimethylmethylene blue dye-binding assay. Collagen production (n=5) was quantified using a modified hydroxyproline assay. Alcian blue and picrosirius red were used to stain for GAG and collagen distribution (n=2). Mineralization: Alkaline phosphatase (ALP) activity (n=5) was detected using a colorimetric assay for mineralization potential. Statistical Analysis: ANOVA and the Tukey-HSD post-hoc test were used for all pair-wise comparisons (p<0.05).

Results: On average, per joint, four osteochondral plugs were harvested and full thickness defects were reproducibly generated in each. Cells within both tissue regions (bone, cartilage) remained viable throughout the culture period, albeit with a zone of death observed at the cut edges of the cartilage for all samples by day 1. The zone of death produced by the drill appeared to penetrate further into the tissue when compared to the zone created by the biopsy punch. Consistent with the live/dead cell imaging, the cell number measured in both the cartilage and bone regions remained unchanged over 14 days of culture (p<0.05). As expected, ALP activity was higher in the bone region than in the cartilage region, but no change was observed over time for either tissue type. Similarly, no changes were detected for the matrix content over time for either region (p<0.05) which was consistent with histological staining with picrosirius red as well as alcian blue (Figure 1). The nanofiber and hydrogel components which were fitted in the defect remained seated in the defect site during culture; however, the acellular agarose hydrogel did not integrate well with the surrounding tissue. The scaffold components (hydrogel and nanofiber) in the defect site did not affect the viability of the explant and no significant differences were measured in terms of cell number, GAG content, or collagen content between the explants with and without scaffold components present in the defect site (Figure 2).
Discussion: The results of this study suggest that it is feasible to reproducibly generate osteochondral explants with full thickness defects and to use this as a model system to study scaffold/host interaction over time. A similar full thickness model was recently reported by de Vries-van Melle et al. [9]; with a focus on characterization through media assays and histology. Building on this work, the study presented here examines the biochemical content of both cartilage and bone regions independently which was not previously reported. The observation of a zone of death in cartilage agrees with previous accounts [10, 11] and similar explant matrix content has been reported for undamaged cartilage [12] and bone [13] explants, indicating that the harvest and defect creation process does not compromise future viability of either the cartilage or bone tissue. Moreover, stability of this model was maintained over a two-week culture period, demonstrating that it can be used to study scaffold interactions with host tissue with high fidelity. While the acellular scaffold included in this validation experiment was not integrated with host tissue, future studies will evaluate mature tissue engineered constructs that stimulate host cell migration and integration with bone.

Significance: There is a need for in vitro defect models that enable the optimization of cartilage grafts prior to in vivo animal studies and clinical trials. In vitro defect models which better approximate in vivo conditions can facilitate more informative in vitro testing and optimization. The full thickness defect model presented here provides a viable and stable model system which can be used to study the interactions between scaffold and host tissue.

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