Hypothermically Stored Human Amniotic Membrane Allograft as a Substrate for Articular Cartilage Regeneration

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Statement of Purpose: Cartilage is an avascular tissue with limited healing potential. Due to its lack of vascularization and the innate nature of adult chondrocytes, which have little migration and replication potential *in vivo*, chondral defects do not readily heal and may progress to end-stage arthritis without further intervention. Unfortunately, current treatments are limited in that they typically result in the production of fibrocartilage and may require a second surgical site as is the case with autologous chondrocyte implantation and the necessity to acquire a periosteal patch. Human amniotic membrane (HAM) may serve as a promising substrate for the promotion of chondral regeneration and is currently used in a variety of clinical applications. HAM is known to contain proteoglycans, growth factors, and progenitor cell populations that may be conducive to chondral regeneration. Thus, the purpose of this study is to investigate the utilization of a commercially available, hypothermically stored human amniotic membrane (HSAM) allograft as a potential substrate for chondral defect repair applications.

Research and Design Methods: A commercially available HSAM preparation was first acquired (Affinity, NuTech Medical, Birmingham, AL). To perform a preliminary evaluation of HSAM as a potential substrate for articular cartilage repair, normal human articular chondrocytes (NHACs) were seeded at a super-confluent density of 10^5 cells per $0.75cm^2$ of HSAM with the stromal side facing up and on tissue culture plate (TCP). NHACs were cultured on both HSAM and TCP in chondrogenic differentiation media (CDM) (Lonza, Wakersfield, MD) and supplemented with 10 ng/mL of TGF-B3. NHACs seeded on TCP without CDM (CGM) served as a control group. At days 1, 14, and 28, NHACs on HSAMs were assessed for chondrogenic differentiation. Culture media was collected and assessed for sulfated glycosaminoglycan (sGAG) content. HSAMs were then collected, cryosectioned, and stained for Collagen I (Col I), Collagen II (Col II), and CRTAC-1 using monoclonal immunofluorescent antibodies. All sections were counter-stained with DAPI. Additionally, HSAMs containing NHACs were assessed for gene expression of Col I, Col II, SOX9, and Aggrecan using qRT-PCR analysis.

Results: NHACs grown on HSAMs were shown to exhibit enhanced chondrogenic behavior relative to NHACs grown on TCP both with, and without the presence of CDM according to sGAG quantification (Fig. 1). Immunofluorescent staining confirms the presence of NHACs on the membrane along with Col II deposition (Fig. 2).

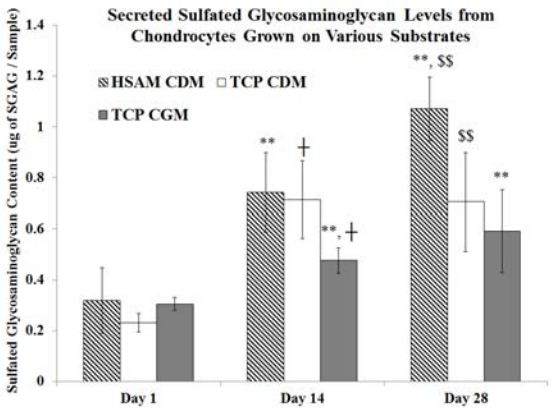

Fig. 1. sGAG secretion from NHACs over 28 days
Figure 1 demonstrates that HSAM enhances chondrogenic activity of NHACs relative to a non-substrate control (TCP). sGAG release by NHACs was enhanced when cultured on HSAM relative to both TCP control groups.

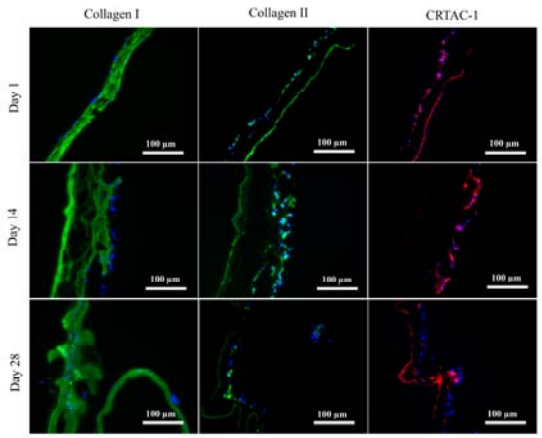

Fig. 2. Immunofluorescence for Col I, Col II, and CRTAC-1 of NHACs cultured on HSAMs in CDM over 28 days. Immunofluorescence staining confirms the presence of NHACs (CRTAC-1) and Collagen II deposition on the HSAMs. Collagen I staining demonstrates HSAM presence and DAPI demonstrates overall cell content.

Conclusions: HSAM appears to be a promising material for chondral defect repair applications. Preliminary results show that HSAM may improve chondrocyte function and encourage chondral regeneration. While initial results are promising, future studies must be performed to determine HSAM performance relative to other commonly available materials used both for chondrocyte implantation and articular cartilage repair.

References: (Jin Et. Al., Tissue Eng. 2007;13(4):693-702.)