Cartilage Matrix Gels Promote Chondrogenesis of Human Mesenchymal Stem Cells

Olivia A. Burnsed, René Olivares-Navarrete, Sharon L. Hyzy, Katherine O. Marchand, Zvi Schwartz, Barbara D. Boyan
Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA

Statement of Purpose: A limited supply of healthy autologous or allogeneic cartilage sources has inspired a growing interest in xenogeneic decellularized cartilage as a biological scaffold. Though decellularized matrices may provide functional mechanical support, they also act as reservoirs for growth factors (Roughly PJ. Eur Cell Mater. 2006;12:92-101). Formation of functional cartilage while preventing calcification and osteochondral bone formation is one of the major obstacles in tissue engineering, perhaps because the growth factors that are typically used stimulate both chondrogenesis and osteogenesis (Hogan BL. Genes Dev. 1996;10:1580). No studies have investigated the ability of decellularized cartilage extracellular matrix (ECM) to stimulate chondrogenesis of human mesenchymal stem cells (MSCs) without the addition of or pre-treatment with exogenous factors. Sharks are an interesting model for cartilage formation since their entire skeleton is composed of cartilage and they contain multiple anti-angiogenic compounds. The objectives of this study were (1) to determine if shark and pig cartilage extracellular matrix (ECM) scaffolds can stimulate chondrogenic differentiation of MSCs without the addition of exogenous factors, and (2) to determine if the soluble factors retained by these ECM scaffolds are responsible for this induction.

Methods: Shark skull, pig articular, and pig auricular cartilage were processed with a series of enzymatic and chemical washes (modified from Reing 2010), lyophilized, digested in pepsin, and made into a 6 mg/mL gel. Decellularization was verified through decreased visualization of nuclei in DAPI and H&E staining, and DNA quantification through PicoGreen analysis and DNA gel electrophoresis (data not shown). ECM digest gels were then used to coat the bottom of 24 well plates (n=6/gel). Culture on ECM gels: After complete gelation within the wells, human bone marrow-derived MSCs were cultured on the ECM gels for seven days. Growth media with 2% or 10% FBS were changed every three days. MSCs cultured on tissue culture polystyrene served as a control. RNA was collected 12 hours after the last media change for qRT-PCR analysis of chondrogenic and angiogenic gene expression. Media were collected 24 hours after the last media change to quantify the amounts of angiogenic proteins secreted by ELISA. Conditioned Media Experiment: To determine the effect of soluble factors released by the ECM gels, growth media with 2% or 10% FBS was left over ECM gels for 12h and directly used (100%) or diluted 1:2 (50%) to treat plated MSCs. After 12 h, RNA was collected and qRT-PCR performed. All experiments are n=6/variable, analyzed by ANOVA and post hoc Bonferroni's modification of Student's t-test.

Results: Culture on ECM gels: Human MSCs cultured on shark and pig ECM gels had increased expression of chondrogenic mRNA and decreased collagen I, had higher total DNA and lower secretion of angiogenic factors VEGF-A and FGF-2. Conditioned Media Experiment: Soluble factors released by the matrices caused a decrease in VEGFA and FGF2 mRNA levels (Fig 1), and an increase in most chondrogenic mRNA levels (Fig 2). Interestingly, treatment with diluted conditioned media (50%) increased ACAN and COMP mRNA levels over undiluted (100%) conditioned media treatments.

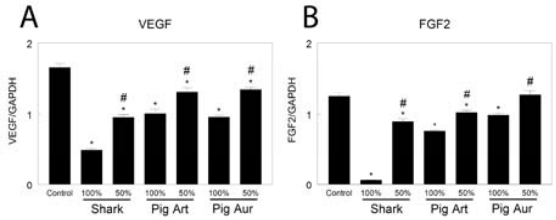

Figure 1. Levels of angiogenic mRNA determined by qRT-PCR analysis of MSCs treated with undiluted (100%) or diluted (50%) conditioned media from shark, pig articular, or pig auricular cartilage ECM gels.

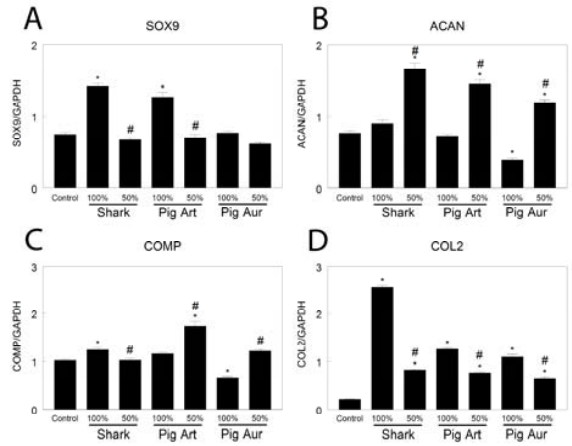

Figure 2. Levels of chondrogenic mRNA determined by qRT-PCR analysis of MSCs treated with undiluted (100%) or diluted (50%) conditioned media from shark, pig articular, or pig auricular cartilage ECM gels.

Conclusions: MSCs cultured on cartilage ECM gels or treated with conditioned media from cartilage ECM gels had lower secretion of angiogenic factors in comparison to the control, indicating that soluble factors remaining in cartilage ECM have anti-angiogenic properties. MSCs cultured on cartilage ECM gels or treated with conditioned media from cartilage ECM gels had higher chondrogenic gene expression without the addition of exogenous factors, demonstrating that the effects of culturing MSCs on ECM gels are not due to the scaffold structure alone. These results indicate that decellularized cartilage from xenogeneic sources may serve as an effective matrix to support chondrogenic differentiation of MSCs while inhibiting production of angiogenic factors.

Acknowledgements: Department of Defense; Presidential Undergraduate Research Award, Georgia Tech.