Dynamic Culturing of Chitosan-Glycosaminoglycan Scaffolds with Bone Marrow Mesenchymal Stem Cells for Heart Valve Tissue Engineering

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Introduction: The questions: "what cell source?" and "what cell type?", continue to be major issues in tissue engineering. Bone marrow-derived mesenchymal stem cells (bmMSCs) have been shown recently to be a promising cell source for tissue-engineered heart valves. However, directing their differentiation into valvular interstitial cells is still a challenge. Several studies have reported successful differentiation of MSCs into endothelial cells (ECs), myofibroblasts or smooth muscle cells (SMCs) in static 2D cultures by using specific growth factors, substrate stiffness or co-culturing approaches. However, differentiation within dynamic 3D cultures has not been fully investigated. In this study, we investigated the feasibility of differentiating bmMSCs into SMCs within glycosaminoglycan-chitosan scaffolds under dynamic perfusion culture conditions.

Materials and Methods: Ovine bone marrow collected from the femurs of freshly slaughtered adult sheep was used to isolate MSCs based on preferential adhesion to tissue culture plastic. After 15 days of culturing, cells were characterized based on the ability to differentiate into chondrogenic, adipogenic and osteogenic lineages. After characterization, MSCs were grown and subcultured up to passage 6. Differentiation of MSCs into SMCs was initiated immediately upon seeding by culturing for 7 days in SMC differentiation medium consisting of IMDM supplemented with 20% FBS, 1 ng/ml TGFβ-1, 30 μM ascorbate-2-phosphate, and antibiotics. Differentiation into SMCs was confirmed by immunostaining for α-smooth muscle actin (α-SMA). Porous chitosan tubular scaffolds were fabricated by freezing 3 ml of chitosan solution (2 wt% medium MW chitosan in 3.5% acetic acid) in a dry ice-methanol bath followed by lyophilization. The tubular scaffolds were then covalently derivatized with EDC-activated heparin at a 1:1 heparin:chitosan mass ratio. MSCs were seeded into the scaffolds and cultured in a custom perfusion bioreactor for 1 week. Experimental scaffolds were cultured in SMC differentiation medium, while control scaffolds were cultured in MSC proliferation medium consists of IMDM supplemented with 10% FBS and antibiotics. At the end of both cultures, scaffold samples were processed for hematoxylin and eosin (H&E) staining.

Results: MSCs isolated from ovine marrow demonstrated the capacity to differentiate into osteocytes, adipocytes and chondrocytes as confirmed by Von Kossa, Oil Red O, and Safranin O staining and morphological characteristics (data not shown). Differentiation into SMCs was indicated by positive α-SMA staining as shown in Fig. 1. H&E staining of both dynamic scaffold cultures showed that the MSCs maintained viability throughout the 1 week culture. Cells cultured in the MSC proliferation medium grew as dense aggregates (Fig. 2) while those cultured in SMC differentiation medium exhibited an elongated morphology typical of SMCs in three-dimensional culture (Fig. 2). Longer term cultures are under way for evaluation of growth kinetics and extracellular matrix deposition.

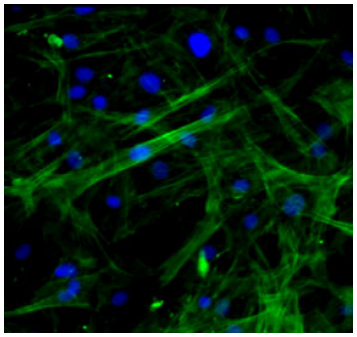

Fig. 1: α-SMA immunofluorescent staining of ovine MSCs in 2D static culture after 7 days of culture.

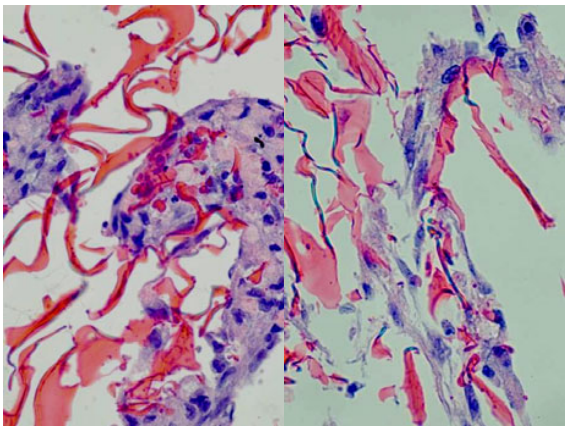

Fig. 2: H&E staining of MSC-seeded heparin-chitosan scaffolds. (Left): Cells cultured in MSC proliferation medium. (Right): Cells cultured in SMC differentiation medium.

Conclusions: Our early results demonstrated the ability to differentiate ovine MSCs into SMC-like cells in static dish cultures. In addition, the study illustrates the feasibility of culturing bmMSCs in heparin-chitosan scaffold under perfusion conditions for at least 1 week. These results provide a promising technology platform for engineering of MSC-derived large vessels and heart valves and their subsequent testing in a large animal (ovine) model.

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