

EFFECT OF 3D HYDROGEL SCAFFOLDS ON THE CHONDRODIFFERENTIATION OF SWINE MESENCHYMAL STEM CELLS *IN VIVO*

+*Mesa, J M.; ** Nuttelman, CR; **Anseth, KS; *Yaremchuk, M J; *Randolph, M A
 +*Massachusetts General Hospital, Harvard Medical School. Boston, Massachusetts, USA

INTRODUCTION: Bone marrow-derived adult mesenchymal stem cells (MSC) are a potential source of chondrogenic cells for reconstruction and repair of cartilage structures in the face, neck and extremities. MSC have been shown to differentiate into a chondrogenic lineage following a micropellet mass culture (3D) with specific chondrogenic media¹, but not in monolayer culture (2D). Previous studies in our laboratory demonstrated that swine Mesenchymal Stem Cells express type II collagen mRNA in micropellet mass culture without the addition of chondrodifferentiation factors (TGF β). This suggests that chondrodifferentiation of MSC requires a 3 dimensional environment. Fibrin gel (FG)² and Polyethylene Glycol (PEG)³ have been shown to be favorable 3D hydrogel scaffolds for tissue engineering cartilage from chondrocytes *in vivo*. The aim of this study is to evaluate the effect of FG and PEG 3D scaffolds in the chondrodifferentiation of swine MSC (sMSC) *in vivo*.

MATERIALS AND METHODS: All procedures involving animals were approved by the Institutional Animal Care and Use Committee (IACUC) of the Massachusetts General Hospital following the NIH Guide for the Care and Use of Laboratory Animals. Bone marrow was aspirated from the iliac crest of swine. sMSC were isolated by percoll gradient and *in vitro* culture. 2nd passage non-chondrodifferentiated sMSC were harvested from plates, encapsulated in both FG and non-degradable photopolymerizable PEG at 40×10^6 cells/ml of hydrogel, and implanted into the subcutaneous tissue of nude mice for 6 weeks. After harvest, samples were analyzed for cartilage matrix formation by histology (H&E, Safranin O, Masson's Trichrome staining) and biochemistry (GAG, and hydroxyproline content).

RESULTS: Samples made with FG were soft and transparent in appearance, while samples made with PEG resembled cartilage in color and texture (Figure 1, gross appearance). sMSC encapsulated in PEG accumulated basophilic matrix, glycosaminoglycans, and collagen in the pericellular area (Figure 1, H&E, Safranin O, Trichrome respectively). sMSC encapsulated in FG did not produce cartilage matrix. Biochemical analysis demonstrated production of glycosaminoglycans (GAG) and collagen (hydroxyproline) in samples made with sMSC encapsulated in PEG (Figure 2).

CONCLUSION: This study demonstrated that sMSC can be induced to a chondrogenic lineage *in vivo* when encapsulated in PEG, but not in FG, even without the use of chondrodifferentiation factors. This study demonstrates that the type of 3D scaffold has a strong influence in the chondrodifferentiation of swine MSC *in vivo*.

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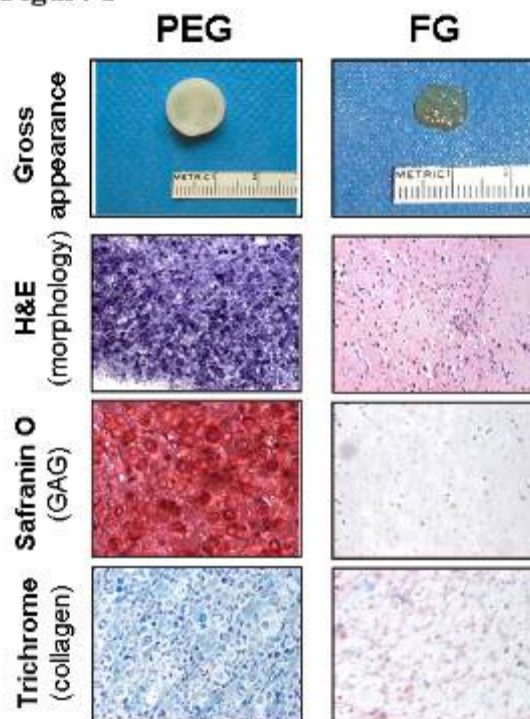
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AFFILIATED INSTITUTIONS:

** University of Colorado. Boulder, Colorado.3

REFERENCES:

- 1- Pittenger MF, et al. Science. 284(5411):143-7, 1999 Apr 2.
- 2- Silverman RP, et al. Plas. Reconstr. Surg. 103(7): 1809-18, 1999 Jun
- 3-Bryant SJ and Anseth KS. J Biomed Mater Res. 2002 Jan;59(1):63-72.

Figure 1**Figure 2**