In Vitro Tissue Generation by Adult Equine Multipotent Stromal Cells on Collagen Scaffolds

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The versatility and suitability of collagen I scaffold for supporting diverse equine tissue regeneration was confirmed in this study by generation of distinct mesenchymal tissues by both adipose-derived and bone marrow–derived multipotent stromal cells. Authors’ addresses: Laboratory for Equine and Comparative Orthopedic Research, Equine Health Studies Program, Department of Veterinary Clinical Sciences, Louisiana State University, Baton Rouge, LA 70803 (Xie, Zhang, Lopez); and Department of Biomedical Engineering, Columbia University, New York, NY 10032 (Marsano, Vunjak-Novakovic); e-mail: mlopez@vetmed.lsu.edu. *Corresponding and presenting author. © 2012 AAEP.

1. Introduction
To derive the greatest benefits from equine multipotent stromal cells (MSC) and propel the technology into clinical trials, it is necessary to optimize equine MSC-scaffold constructs in vitro. The hypothesis tested was that in vitro adipogenesis, osteogenesis, and chondrogenesis of both cell constructs have identical microstructures and ultrastructures.

2. Materials and Methods
After characterization of cell surface markers with flow cytometry, bone marrow–derived multipotent stromal cells (BMSCs) and adipose-derived multipotent stromal cells (ASCs) were loaded by perfusion bioreactor. Replicate constructs were assessed immediately after loading and following 7, 14, and 21 days of culture in stromal and induction medium. Cell number, viability, distribution, and differentiation were evaluated with confocal laser, light, scanning electron microscopy, and reverse transcriptase polymerase chain reaction (RT-PCR). Data were analyzed by 3-way ANOVA.

3. Results
Uniform cell distribution was achieved for both cell types. The mRNA levels of collagen I and sex determining Region Y-box 9 were significantly higher in BMSC constructs, whereas lipoprotein lipase levels were significantly higher in ASC constructs at all time points. Tissue specific microstructural and ultrastructural changes were evident and similar in both constructs cultured in induction medium.

4. Discussion
The ability of adult equine MSC-scaffold constructs to form different mesenchymal tissue types was critically assessed in vitro, revealing that customized equine tissue engineering can be achieved to meet current needs of efficient therapeutic treatment in horses.