INTRODUCTION:

Cell-based strategies for bone tissue engineering have utilized various delivery constructs including both synthetic and naturally-derived materials. While synthetic materials are advantageous for their ease of manufacturing and tunable properties, natural extracellular matrices (ECMs) may better facilitate cell viability, tissue regrowth and remodeling. This study compared the ability of a natural and synthetic membrane to support short-term survival of in vitro human bone marrow-derived mesenchymal stem cells (hMSCs) as well as their subsequent osteogenic differentiation. 2D constructs composed of (a) randomly-oriented polycaprolactone (PCL) nanofibers and (b) porcine small intestinal submucosa (SIS) ECM were compared. Previous studies have shown nanofiber meshes to facilitate both attachment and osteogenic differentiation of hMSCs. Porcine SIS material has demonstrated a consistent ability to support cardiovascular tissue repair in a clinical setting. However, few studies have compared the performance of natural and synthetic membranes. In anticipation of in vivo implantation studies, hMSCs labeled with GFP and luciferase reporter genes were evaluated on the nanofiber constructs as well.

METHODS:

Matrix Preparation: PCL solution (12% w/v) was electrospun at a rate of 0.75 mL/hr for 3 hours. ECM sheet material was supplied by CorMatrix, Inc. hMSC Seeding: hMSCs were obtained from Texas A&M (Prockop Lab) and fully characterized to confirm multipotency. Cells were seeded on 2D membranes at a density of 40,000 per cm².

Quantitative Assays: Seeded constructs were imaged in control or osteogenic media and changed biweekly over a period of 4 weeks. Calcium deposition, alkaline phosphatase (ALP) activity and cell viability were assessed upon conclusion of culture period. Calcium and ALP assays were performed using a standard and p-nitrophenol, respectively. Quantities were normalized to construct surface area and DNA content (determined using a PicoGreen assay with values for calcium taken from parallel culture wells). hMSC Transduction: hMSCs were co-transduced with GFP and luciferase reporter genes by the Gazit Laboratory using a lentiviral vector. Viability Staining: Live/dead staining followed 4 weeks of culture was performed using calcein/ethidium homodimer (Invitrogen). Visualization of labeled cells was conducted following 7 days of culture in control media. Statistics: Data were analyzed using a two-way ANOVA with post-test comparisons. Bars represent SEM. Asterisk denotes p<0.05.

RESULTS:

hMSCs, seeded on nanofiber mesh and CorMatrix, were differentiated over a period of 4 weeks. Subsequently, ALP activity was quantified as an early indicator of osteogenic differentiation. Although peak ALP expression for differentiation on tissue culture plates occurs at day 7 or 14, previous nanofiber mesh studies have found levels to remain high through 4 weeks of culture. ALP activity was significantly higher for cells grown in osteogenic media in comparison to control for both membrane types (Fig. 1). These results are consistent with those found qualitatively using a von Kossa stain (data not shown). Additionally, ALP expression for the osteogenic CorMatrix group was significantly higher than that of the nanofiber mesh group. Results from the calcium assay further supported this effect (Fig. 1).

Live/dead stains show that cell viability is maintained on both synthetic and natural membranes (Fig. 2). Interestingly, cell number appeared higher for both osteogenic groups, a phenomenon confirmed by DNA quantification (Fig. 1). Staining of labeled hMSCs (Fig. 3) displays similar cell survival in addition to confirming high transduction efficiency as seen by fluorescence microscopy, FACS analysis and bioluminescence imaging (data not shown).

DISCUSSION:

The ability of hMSCs to undergo osteogenic differentiation on both membranes is demonstrated by the ALP activity data. The significantly higher values for ALP activity and calcium deposition on CorMatrix material could indicate either an improved extent of or longitudinal variation in supported differentiation behavior. Further work at additional timepoints is required to discern this mechanism. Enhanced osteogenic differentiation of hMSCs on the naturally-derived ECM may be due to a fundamental difference in membrane properties such as topography, surface-binding chemistry or pH.

Live/dead images indicate the ability of either material type to support cell viability in vitro. Visualization of labeled hMSCs confirms a high transduction efficiency as well as unhindered cell viability resulting from the labeling procedure. Next steps will utilize bioluminescence imaging to assess membrane effect on the short-term survival of these cells delivered subcutaneously. Consequence of construct type on the functional repair of bone tissue will subsequently be examined using a critically-sized segmental defect model.

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