Osteogenic Differentiation of Placental-Derived Adherent Cells (PDACs) on 3D Calcium Phosphate based Scaffolds

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Statement of Purpose: Tissue engineering using stem cells is emerging as a promising alternative to tissue or organ transplantation. We have described the isolation and culture of novel stem cells from postpartum placenta (Placenta-Derived Adherent Cells, PDACs) with characteristics and phenotype of pluripotent stem cells. PDACs have been shown to differentiate along the adipogenic, chondrogenic, and osteogenic lineage. Therefore, PDACs constitute an important and non-controversial source of pluripotent stem cells that could be used as a therapeutic for the repair of damaged or diseased tissue. In the present study, we investigated the cellular behavior and osteogenic potential of PDACs on two-dimensional tissue culture polystyrene and 3 dimensional calcium phosphate-based scaffolds.

Methods: PDACs were isolated from the placenta by one of several methods including physical disruption of tissue from several different anatomical sites within the placenta. PDACs were established in proprietary Anthro1B medium that contains low concentrations of fetal calf serum and growth factors. Flow cytometry analysis showed that PDACs isolated from certain sites express the following phenotypic markers: CD200+ CD105+ CD73+ CD34- CD45-. For osteogenesis studies, PDACs were seeded in either basal medium (Cambrex, East Rutherford, NJ) or proprietary Anthro1B medium, then maintained in either Anthro1B medium or induced with Osteogenic differentiation (OS) medium (Cambrex,) for 4 weeks. Alkaline phosphatase (AP) activity on cell lysates was assessed with a colorimetric assay (Cell Biolabs, San Diego, CA) and normalized to DNA using the PicoGreen dsDNA fluorescent assay (Invitrogen, Eugene, OR). Mineralization of the extracellular matrix in the form of bone nodule formation was determined by scanning electron microscopy (SEM) used in conjunction with x-ray spectroscopy (JEOL JSM-6400F field emission SEM). For studies on 3 dimensional scaffolds, PDACs were seeded on calcium phosphate (CaP, BD Biosciences, San Jose CA) or β-tri-calcium phosphate (TCP, Therics, Akron, OH) scaffolds and cultured as described above. The number of cells attached to scaffolds, AP activity, and bone nodule formation for PDACs cultured on 3D scaffolds was assessed as described above.

Results/Discussion: PDACs seeded and maintained in Anthro1B throughout the entire 4-week time course showed the highest AP activity, suggesting that Anthro1B medium stimulates the greatest PDAC osteogenic differentiation. SEM imaging (Figure 1) demonstrated PDAC matrix mineralization (in the form of bone nodule formation), rich in calcium and phosphate as denoted by the colocalization of calcium and phosphate elemental mapping by x-ray analysis. Next, we examined PDAC behavior on 3 dimensional calcium phosphate based scaffolds. To determine whether there were differences in PDAC attachment and proliferation on the CaP or TCP scaffolds, PDACs were cultured on each of the scaffolds for up to 28 days. The results showed that the TCP scaffolds supported greater cell numbers compared to the CaP scaffolds during all stages of in vitro culture.

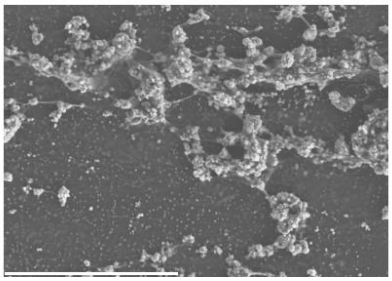

Figure 1. SEM imaging of induced PDACs form mineralized matrix. Bar = 70 μm

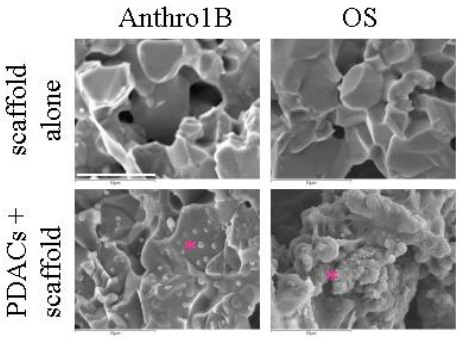

Figure 2. PDAC bone matrix formation on TCP scaffolds. Bar = 10 μm. *nodules of bone matrix formation

AP activity was similar in PDACs seeded on TCP scaffolds whether cultured in Anthro1B or OS medium, supporting data obtained on tissue culture polystyrene; no AP activity was detected on CaP scaffolds. SEM imaging of PDACs cultured on TCP scaffolds revealed the presence of bone nodules (*, Figure 2), indicative of bone matrix formation. Scaffolds cultured in Anthro1B or OS medium (without PDACs) did not show evidence of bone nodules and were instead characterized by sharp edges of the TCP crystals.

Conclusions: PDACs differentiate functionally along an osteogenic pathway given the appropriate stimuli, and show higher viability and better osteogenic behavior on TCP compared to CaP scaffolds. Therefore, from these studies we conclude that pluripotent PDACs can be used in bone tissue engineering together with proper scaffolds.