Towards a novel bone repair product: using human placental derived adherent cells (PDACs) and mineralized collagen matrix

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Statement of Purpose: The human placenta is a rich source of both stem cells and materials. Placenta Derived Adherent Cells (PDACs) are stem cells that can be isolated from the placenta. PDACs can be induced to differentiate down adipocyte, chondrocyte and osteocyte lineages. By combining PDACs with placenta derived biomaterial scaffolds (Type I collagen) an allograft tissue engineered construct can be produced for several types of tissue including bone.

To provide PDACs with an appropriate matrix for osteogenesis, mineralized human placental collagen (HPC) was produced. In a biologically inspired process, HPC was reconstituted in fibrillar form and then mineralized. By crosslinking this composite matrix to improve mechanical properties, a promising material for PDAC osteodifferentiation was synthesized.

Methods: PDACs were isolated from the placenta by one of several methods including physical disruption of tissue from several different anatomical sites and established in a medium containing low concentrations of fetal calf serum and limited growth factors. Flow cytometry analysis showed that PDACs isolated from certain sites are CD200+ CD105+ CD73+ CD34- CD45- at $\geq$ 70%.

HPC was isolated using pepsin digestion and salt precipitation. The acid soluble HPC was reconstituted into fibers (fibrillation) at neutral pH in the presence of Na_2HPO_4 at 32ºC. The fibrillated collagen was isolated and mineralized via proprietary methods. Briefly, collagen was incubated at 40ºC, pH 9.5 in the presence of $Ca(OH)_2$ H_3PO_4, NaCl, and Na_2HPO_4 for 18 – 24 hours. The initial mineral ($Ca(OH)_2$ and H_3PO_4) to collagen ratio was varied from 60:40 to 90:10. After the product was isolated, it was crosslinked with butanediol diglycidyl ether (BDDE) at 25ºC, pH 9.5 for 24 hours. The crosslinking reaction was quenched with 0.5M glycine at pH 10 for 24 hours. The crosslinked mineralized collagen (CMC) was isolated and washed 3x with phosphate buffered saline (PBS). The CMC formulations were characterized by light and scanning electron microscopy, Thermo Gravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC) X-ray diffractometer (XRD), and Fourier Transform Infrared Spectroscopy (FTIR).

Various CMCs were sterilized with antibiotic-antimycotic. Wet samples were loaded into transwells for non-contact cytotoxicity studies using PDACs in a lactose dehydrogenase cytotoxicity assay (LDH). LDH released into the culture medium was correlated to cytotoxicity. CMC films were fabricated for PDACs adhesion and proliferation studies. Cell numbers were analyzed using a PicoGreen DNA assay at 1, 5 and 7 days.

Results/Discussion: Fibrillation of HPC reconstitutes the soluble collagen as short fibrils and long fibers (Figure 1a). During the mineralization reaction a Ca-P mineral forms along the fibers (Figure 1b). The final reaction yield is high (>80%), and the final mineral/collagen ratio of the material is close to the input mineral/collagen ratio as determined using TGA (Figure 2).

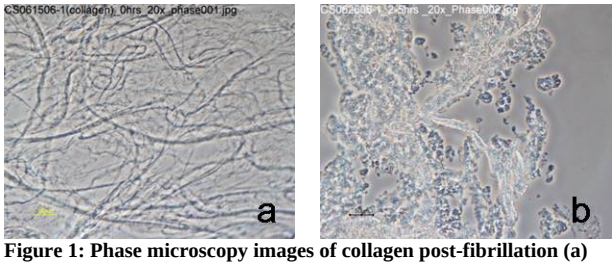

Figure 1: Phase microscopy images of collagen post-fibrillation (a) showing long fiber formation and post-mineralization (b) showing mineralized fibers. Orig mag 200x

Crosslinking was confirmed by an increase in the denaturation temperature of the collagen from ~50 to ~70ºC as determined by DSC. Crosslinking dramatically increases the durability to collagenase of non-mineralized collagen. The crosslinked material had more mechanical integrity than the non-crosslinked material and appeared more fibrous when examined by stereo microscopy and scanning electron microscopy (SEM). FTIR indicated the presence of a carbonated calcium phosphate mineral. XRD confirmed that the mineral is a poorly crystallized hydroxyapatite.

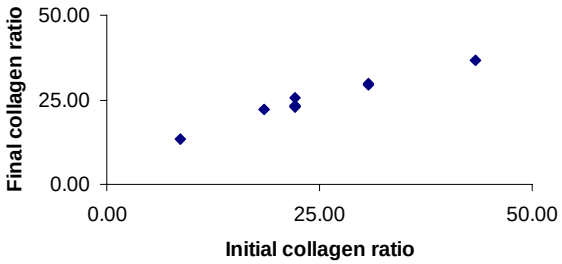

Figure 2: The final ratio of collagen to mineral is strongly correlated to the initial ratio.

PDACs showed similar LDH production when exposed to CMC as when exposed to tissue culture polystyrene (TCPS), indicating low cytotoxicity of CMCs. PDACs attached in greater numbers to CMC than to non-crosslinked mineralized collagen and all seeding densities tested. Seven days after seeding this trend continued with PDACs having the highest cell numbers on the CMC films.

Conclusions: PDACs attached and thrived on a novel biologically inspired composite synthesized from crosslinked mineralized HPC. Since this composite mimics the make-up of natural bone when combined with PDACs, it appears suitable for bone tissue engineering applications. Future studies will address PDACs driven osteogenesis on this novel composite and the development of bone repair products.