Composite Materials Consisting of Hydroxyapatite Impregnated Collagen Matrices Affect Osteoblast Behavior

B.L.Rogers[1], Y.Li[2], S.S.Jee[2], L.Gower[2], E.P. Douglas[2], Z. Schwartz[1], B.D.Boyan[1]
[1]Wallace H. Coulter School of Biomedical Engineering, Georgia Institute of Technology, Atlanta, Georgia
[2]Material Science and Engineering Department, University of Florida, Gainesville, Florida

Statement of Purpose: Bone is a mineralized connective tissue composed of both organic and inorganic components. The mineral phase of bone contributes to approximately two-thirds of its weight, while the remaining third is organic matrix, primarily consisting of collagen and small amounts of proteoglycan, lipid, and several noncollagenous proteins. Several composite materials have been developed and evaluated to determine their success and biocompatibility as potential bone graft substitutes, but these materials have not combined the mineral and organic components in a format comparable to mineralized extracellular matrix in vivo. A biocomposite incorporating organic and inorganic phases representating bone's native presentation of these components to osteogenic cells during bone formation and regeneration was recently described which incorporates calcium phosphate throughout type I collagen fibers, mimicking their physiological interaction during fibrillogenesis [1]. The present study assessed the effects of this material on osteoblast behavior using an in vitro assay system developed in our lab to examine cell/material interactions.

Methods: Calcium phosphate was formed using a novel polymer induced liquid-precursor (PILP) process [1,2]. Micromolar aliquots of polyaspartic acid and sodium salt were added to each solution to achieve various concentrations of the process-directing agent. Each solution was incubated at 37°C during the crystallization to simulate physiological conditions. To assess the effects of composites fabricated by coating collagen with mineral on the surface, collagen sponges (Collagen Matrix, Inc.) were placed in the crystallizing solution; after mineralization, they were rinsed with distilled water and ethanol to remove extraneous salts. The samples were then dried in air at room temperature. The PILP method was used to fabricate composites in which hydroxyapatite formed within the collagen fibrils. A collagen matrix film was created with purified type I collagen purchased as a solution of pepsin-solubilized adult bovine dermal collagen dissolved in hydrochloride acid with a concentration of 2.9mg/ml (PureCol^{TM}, Inamed BioMaterials, Fremont, CA). Collagen fibrillogenesis occurred by heating the mixture to 30°C for three days. The PILP mineralization process generated an amorphous liquid-phase mineral precursor to hydroxyapatite. This fluidic amorphous precursor was drawn into the gaps and grooves of collagen fibrils, then solidified and crystallized, leaving nanoscopic hydroxyapatite crystals embedded throughout the collagen matrix.

Human osteoblast-like MG63 cells were added to the scaffolds at a cell seeding density of 10,000/cm^2 in 500µls of media per well. An insert (CellCrown, Scaffdex Inc.) was used during cell culture to immobilize collagen scaffolds. Cells were grown on tissue culture polystyrene (plastic), collagen sponges that had not been calcified (CS), CS that were calcified by incubation in a calcium phosphate solution (CSHA), collagen sponges prepared using the PILP calcification method (CSPILP); collagen films (CF), collagen films incubated in calcium phosphate solution (CFHA); and collagen films calcified using the PILP method. When cells achieved confluence on plastic, all assays were performed.

Results: Cell number was reduced on CSHA (Fig 1a), CFHA, and CFPILP (Fig 1b) surfaces compared to plastic. Alkaline phosphatase activity was increased in cells grown on CS and to a greater extent on CSPILP; however, cells grown on CSHA had activity comparable to cells grown on plastic (Figure 1c). There was a small increase in alkaline phosphatase when cells were grown on CF or CFPILP substrates. In contrast, alkaline

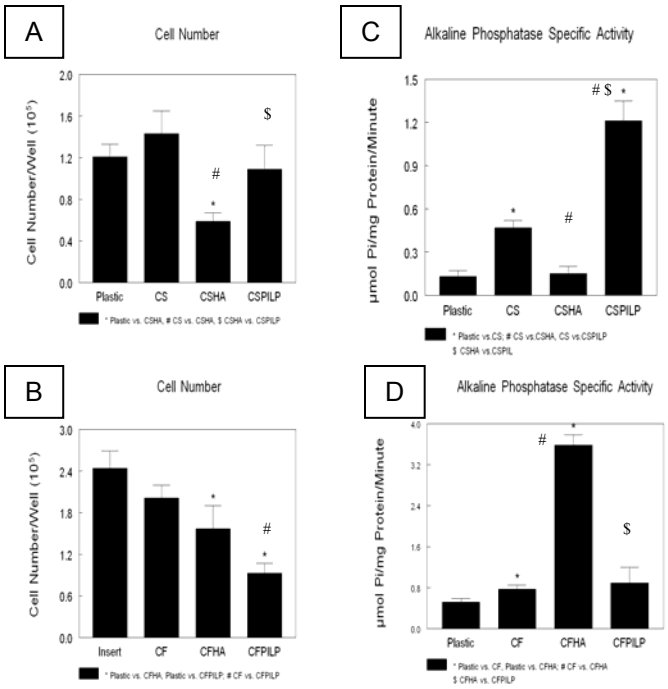

Figure 1. The effect of collagen sponge (CS) and collagen film (CF) mineralization on cell number (a,b) and alkaline phosphatase activity (c,d). Data are means ± SEM, N=6 independent cultures per variable. Results were validated in a second set of experiments.

phosphatase was increased approximately 60 fold when cells were grown on CFHA (Fig 1d).

Conclusions: Osteoblast-like cells respond differently due to the presentation of mineral and collagen. Effects on cell number differed depending on whether the mineralized collagen was formatted as a sponge or as a film. Alkaline phosphatase was increased on the collagen substrates. For the collagen sponges, the effect was greatest on CSPILP, whereas on the collagen films, the effect was greatest on CFHA. This suggests that material factors other than orientation of hydroxyapatite within the collagen fibers may play a role.

References: (1) Olszta MJ et al. J Mat Sci Eng Res 2007, 5:001 (2) Gower L.B. et al. J Crystal Growth 2000 210:719-734. (3) Rodrigues CM et al. Biomaterials 2003 24:4987-4997.

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