Evaluation of osteoblasts on chitosan-calcium phosphate composite scaffolds in static and dynamic rotary bioreactor culture conditions

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Statement of Purpose:
Bone tissue engineering strategies are focused on developing regenerative synthetic bone grafts or scaffolds to avoid complications such as tissue morbidity and disease transmission associated with autografts and allografts. Scaffolds provide a three dimensional construct for cell attachment, proliferation, and differentiation leading to bone regeneration. Scaffolds constructed from polymers, ceramics, and their composites vary in mechanical and biological properties such as biocompatibility, biodegradability, and osteoconduction. A recent study showed that a composite scaffold composed of chitosan (a natural polymer) and calcium phosphate was biocompatible in vitro and in vivo, and had good mechanical strength and integrity[1]. The purpose of this study was to characterize the gowth and matrix mineralization of osteoblasts seeded on the chitosan calcium-phosphate composite scaffolds cultured in static and dynamic (rotary bioreactor) conditions.

Methods:
Scaffold Fabrication: Composite calcium-phosphate chitosan (Vanson, WA, 92.3% DDA) microspheres were made as previously mentioned[1] and then fused together using 0.5 wt% acetic acid to construct ~30% porous scaffolds. Thirty-six scaffolds were constructed (figure 1A).

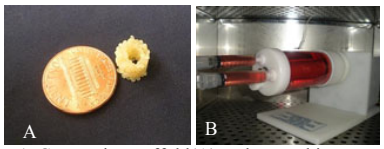

Figure 1. Composite scaffold(A) and rotary bioreactor (B).

Cell Culture: The scaffolds were seeded with human osteosarcoma cells, Saos-2, at a concentration of ~$1.0x10^4$ cells/ml. Growth media (McCoy's 5A Medium + 10% FBS + 1% AB/AM) was used during the first two weeks of the growth phase to allow for cell growth and attachment thoroughout scaffold based on an earlier pilot study. Then scaffolds were evenly divided into two groups, 16 for static culture in 12-well plates and 16 into a 250ml rotating wall bioreactor vessel (figure 1B)(Synthecon) for dynamic culture and cultured in mineralizing media (McCoy's 5A Medium + 10% FBS + 1% AB/AM + 10mM βGP + 50μg ascorbic acid). Medium was renewed every two to three days for static culture and once a week after sample collection for rotary culture.
Sampling: Cell attachment was verified using Live/Dead® Staining 24 hours after cell seeding. Then after each week of mineralizing, 4 samples were collected from each culture conditions. Three samples were ultrasonicated to lyse attached cells, and one sample was prepared for scanning electron microscopy (SEM). The lysate was collected to measure alkaline phosphatase (ALP) enzymatic activity for osteoblastic phenotype expression and to quantify DNA using Picogreen (Invitrogen) to obtain cell number. SEM images were examined for cell morphology and matrix production. Samples for the SEM were prepared according to protocol using 4% formalin and consecutive ethanol solutions (70, 80, 90, 95, and 100%) and then viewed in an environmental SEM (Philips ESEM30).

Results/Discussion:
Cell growth determined by DNA concentration per scaffold showed an increase in cell number for both culture conditions during the mineralizing phase; however, scaffolds in the rotary culture had a reduction in cell number (figure 2A). The introduction of high fluid shear stress and scaffold knocking with the vessel wall may have caused cell detachment and hence the lower numbers of cells. SEM analysis showed cells distributed throughout the scaffolds in the static culture, whereas cells on the bioreactor scaffolds were retained only on the interior of the scaffold where shear forces are likely to be minimal (data not shown). SEM images show more cellular branching and connections to the scaffold with more matrix formation for static condition (figure 3). The ALP activity (normalized to DNA concentration) peaked at week 2 of static mineralization which is characteristic of osteoblast phenotype during mineralization. Cellular ALP expression was significantly higher for static than rotary culture due in part to the larger number of cells. The bioreactor sustained a constant ALP activity for the 3 weeks of mineralization (figure 2B) indicating that the cells retained in the scaffold maintained their bone phenotype.

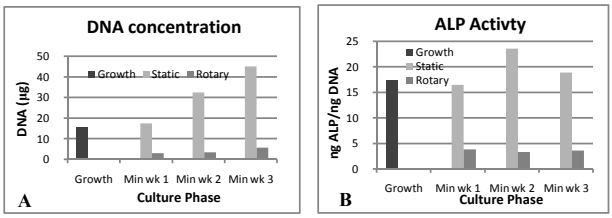

Figure 2. DNA concentration (A) and ALP activity (B).

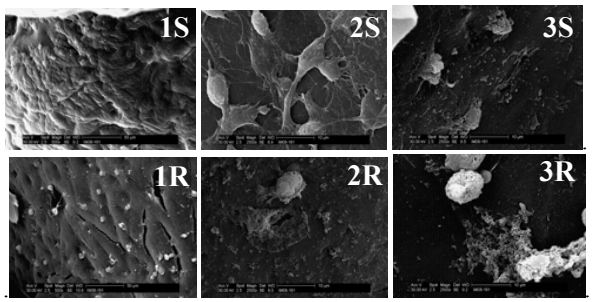

Figure 3. Cell morphology of static (1S-3S) and rotary (1R-3R) culture.

Conclusion:
The growth and matrix production observed by osteoblasts seeded on composite chitosan calcium phosphate scaffolds under static conditions confirmed the in vitro osteo-compatibility of the scaffolds. However, the high shear stresses and physical knocking of scaffolds in the dynamic culture conditions of the rotary bioreactor lead to decreased cell attachment and growth as observed by SEM and DNA analyses. Nevertheless, the cells that were retained on the interior portions of the scaffolds in the rotary bioreactor maintained their osteoblast phenotype. Studies to optimize the rotary bioreactor conditions such as speed of rotation, medium changes, etc will be pursued.

References:
1. Chesnutt, BM, et al. J of Biom Res A (2008).