Increasing the Potential of Bioactive Glass as a Scaffold for Bone Tissue Engineering with Synthetic ECM

Colin Przybylowski, Mohamed Ammar and Sabrina S. Jedlicka
Lehigh University, Bethlehem, PA

Statement of Purpose: Current bone implantation therapeutics are not able to self-repair, maintain a blood supply or modify their structure and properties (1). Bioactive glass is able to bond to bone, degrade in the body and stimulate differentiation of mesenchymal cells into osteoblasts (2-4). Bioactive glass forms a carbonated hydroxyapatite (HCA) layer on the surface and allows for controllable porosity and bioresorption. However, the differentiation process of osteoblasts on bioactive glass scaffolds needs improvement (1) for use in tissue engineering. To improve osteoblast differentiation, we have modified bioactive glass with small peptide molecules from the active regions of proteins known to stimulate osteogenesis: fibronectin and bone morphogenetic protein-2 (BMP-2). The experiment was conducted both on bioactive glass films, and on 3-D porous bioactive glass scaffolds.

Methods: Three peptide-silanes were synthesized using known techniques (5): AYAVTGRGDSPAS, ACGGKVGKACCVPTKLSPISVLYK, and ACKIPKASSVPTELSAISTLYL. Bioactive glass in the composition of 77% SiO_2–19% CaO–4% P_2O_5 was prepared by the sol-gel technique, with peptide-silane addition at 0.01% mole peptide per mole SiO_2. MC3T3 pre-osteoblasts, maintained in ATCC recommended conditions, were seeded onto thin films or porous scaffolds of this material (10,000 cells/cm^2 or 1000 cells/mm^2) (Table 1) for analysis.

Table 1. Materials and Conditions.

Material	Media
Sol-gel	Standard
Sol-gel + RGD	Standard
Sol-gel + RGD + CGG	Standard
Sol-gel + RGD + CGG + CKI	Standard
Tissue-coated glass cover-slips	Standard
Tissue-coated glass cover-slips	Differentiation

Analysis of the cells included immunocytochemistry for osteocalcin, osteopontin and osteonectin; alkaline phosphatase (AP) activity measurements; SEM analysis, and mRNA analysis for several osteogenic markers. For 3-D, diffusion of the peptides into the glass network was assessed using a fluorescent peptide label.

Results: AP activity of cells on 2-D peptide-decorated samples was enhanced or comparable to AP activity of the positive control by week 4 (Fig.1) in culture. In addition, enhanced expression of the osteogenic markers Smad4 and RunX were found in peptide-decorated samples at week 5 (Fig.2). Fluorescent peptides were added to the scaffolds and Z-stacking images confirm that they diffuse through the porous 3D network, while XPS confirms that they bind to free hydroxyl sites. SEM imaging was conducted to view the cells that had infiltrated the bioactive glass scaffolds (Fig. 3). These

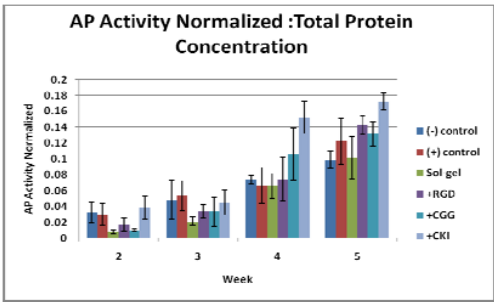

Fig. 1: AP activity.

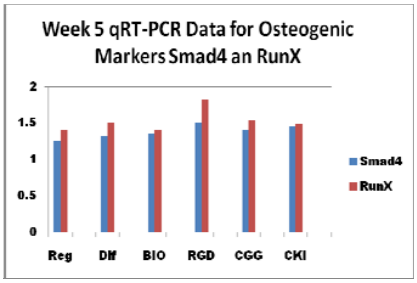

Fig. 2: Smad4 and RunX mRNA analysis using qRT-PCR.

that images indicate the cells infiltrate the scaffolds, both with and without peptides. In addition, differentiation analysis indicates that the cells are expressing mRNA and proteins consistent with osteogenesis. One unexpected result is that HCA deposition may be enhanced with the addition of the RGD peptide to the 3D samples, specifically, we believe that the RGD site is acting as a nucleation point.

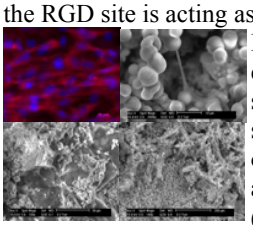

Fig. 3: (top left) Osteopontin expression in cells on RGD-decorated samples (2-D). SEM of cells on 3-D scaffolds without peptides (top right), on RGD samples (bottom left) and of an HCA layer on an RGD scaffold (bottom right).

Conclusions: In our two-dimensional analysis, the MC-3T3s appeared to differentiate on the peptide sol-gel at a rate that is equivalent to that achieved by traditional differentiation medium, indicated by comparable levels of AP activity and osteogenic marker expression among experimental and positive control samples. Additionally, cells exhibit similar adhesion and proliferation profiles on peptide-decorated 3-D samples.

References:

1. Jones, JR et al. 2006. *Biomaterials*. 27, 964-973.
2. Nishikawa, M. et al. 2004. *Cell Transplant*. 13, 367-376.
3. Roether JA, et al. 2002. *J Mater Sci—Mater Med*. 13, 1207–14.
4. Yoshikawa, H. 2009. *J.R. Soc. Interface* 6, S341-S348.
5. Jedlicka, S. et al. 2007 *J. Materials Chem.* 17, 5058-5067.