Biodegradable Polymer-Magnesium Composite scaffolds for Bone Tissue Engineering: Effect of Magnesium on Osteoblast Proliferation, Maturation and Mineralization

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Statement of Purpose: Metals that are present as trace elements in human body have great promise as biodegradable materials. In particular, magnesium with density and mechanical properties similar to human cortical bone can serve as an ideal scaffold material for bone repair/regeneration[1]. In spite of its excellent physical properties, magnesium still suffers from the clinically observable hydrogen gas release. One of the ways to minimize the gas release is by forming Mg composites with biodegradable polymers. The aim of the present study was to explore the possibility of forming PLGA-Mg composite scaffolds and further evaluate the effect of Mg on the scaffold osteocompatibility *in vitro*.

Methods: Briefly, PLGA microspheres with different loadings of Mg (0, 1 and 3 wt%) were prepared using emulsion/solvent evaporation method[2]. The composite microspheres (size range: 425-600 μm) were heat-treated at 80 °C/1 h to form three-dimensional (3D) and porous scaffolds. Composite nature of the scaffolds was analyzed using Scanning Electron Microscopy (SEM). For cell adhesion, growth and osteocompatibility evaluation, scaffolds were sterilized with Ethanol & UV light exposure, and subsequently cultured using Ham's F-12 media supplemented with 12% FBS, 1% penicillin/streptomycin, 3mM of β-glycerophosphate, and 10μg/ml of ascorbic acid. Primary rat osteoblasts were isolated from calvaria of 2-3 day-old rat pups and cultured for 3, 7, 14, and 21 days, after which scaffolds were removed from culture and analyzed for cell proliferation (PicoGreen dsDNA assay), maturation (alkaline phosphatase expression) and calcium deposition (Alizarin red staining).

Results/Discussion: SEM investigations on PLGA-magnesium composites revealed the presence of Mg particle loading into the polymer microspheres, which was further confirmed by elemental analysis. These studies established the possibility of forming PLGA-Mg composite microspheres. The microspheres were subjected to thermal sintering and developed into three dimensional and porous matrices with porosity ≈ 30% and mean pore diameter ≈ 130 μm. Scaffolds were seeded with primary rat osteoblasts and cell performance was studied with respect to Mg loading. The study was carried out on three different scaffold types - polymeric PLGA and composites PLGA-1wt% Mg and PLGA-3wt% Mg. As shown in Figure 1(a) PLGA and PLGA-Mg scaffolds supported progressive osteoblast growth, however the composite scaffolds showed lower proliferation at early time points and attained higher or PLGA comparable growth by day 21. This was further corroborated by the SEM micrographs (as shown in Figure 1(b)) recorded on the cellularized composite scaffolds. The ALP activity normalized to the cell number (Figure 2(a)) peaked on day 14 with significantly higher expression levels for 3% Mg composites. The calcium deposition estimated (by Alizarin red staining and quantification method) for all the scaffolds is shown in Figure 2(b). Although PLGA polymer scaffolds showed higher calcium levels at day 14, the composites ended up exhibiting significantly higher amounts of calcium deposition on day 21. The observed osteoblast proliferation and significantly higher levels of osteoblast maturation and mineralization on the composite scaffolds clearly confirm the positive influence of Mg on the scaffold osteocompatibility. The specific cell-matrix interactions that are responsible for this phenomenon are under investigation. However, this is an important finding that could be effectively used to design various Mg-biodegradable polymer composite scaffolds for accelerated bone defect repair/regeneration.

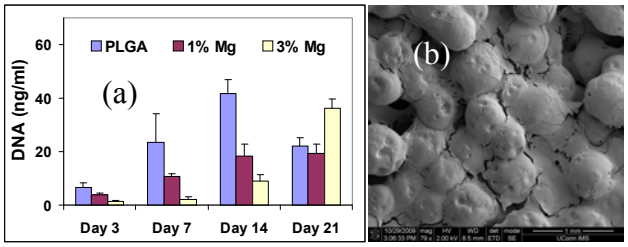

Figure 1: (a) Primary rat osteoblast proliferation on PLGA and PLGA composite scaffolds containing 1% and 3% of Mg. (b) A representative SEM (recorded on a PLGA-1%Mg composite scaffold at day 14) showing a fully cellularized construct.

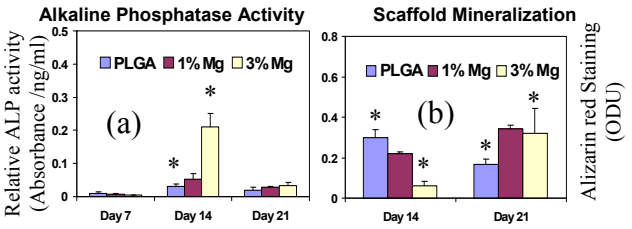

Figure 2: (a) Normalized Alkaline Phosphatase (ALP) activity and (b) calcium deposition of the polymeric PLGA and PLGA-Mg composite scaffolds. The higher levels of ALP activity and calcium deposition confirm the Mg enhanced osteoblast maturation and mineralization. (*) denotes significant difference with $p<0.05$.

Conclusion: We have demonstrated for the first time the feasibility of forming biodegradable polymer-magnesium composite scaffolds with suitable porosity and pore diameter for their use in bone tissue engineering. Primary osteoblast performance confirmed the magnesium enhanced scaffold osteocompatibility, which is a desired feature to design grafts that accelerate bone defect repair and regeneration. In addition, integration of Mg with PLGA could further achieve mechanically stronger scaffolds with neutral degradation by-products.

References: ***1.*** *Williams D., Med Device Technol. 17 (2006) 9.*
2. *Nukavarapu SP, et al., Biomacromolecules 9(2008)1818.*