Human Mesenchymal Stem Cell Proliferation, Differentiation, and Mineralization on 3-dimensional Nano Hydroxyapatite-Polymeric Composite Scaffolds for Tissue Regeneration

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Statement of Purpose: Recent advances in human stem cell research have demonstrated that human mesenchymal stem cells (HMSCs) have great potential in bone tissue engineering because of their high proliferation rate, self-renewal capacity, and specialized differentiation under given conditions [1]. In addition, mesenchymal stem cell growth and differentiation are influenced by substrate signals [2]. Previously, we have developed and characterized poly (lactide-*co*-glycolide) (PLAGA) / nano hydroxyapatite (n-HA) composite microsphere scaffolds suitable for bioreactor-based bone tissue engineering [3]. The objective of the present study was to evaluate the effects of the incorporation of nanohydroxyapatite (n-HA) into PLAGA scaffolds on HMSC proliferation, differentiation, and mineralization.

Methods: PLAGA and PLAGA/n-HA scaffolds were fabricated using a sintered microsphere technique [3]. HMSCs (Cambrex, East Rutherford, NJ) were expanded and maintained in the Mesenchymal Stem Cell Basal Medium (Cambrex). Scaffolds were soaked in 70% ethanol for 15 minutes and washed with sterile water twice for 15 minutes, and then treated with UV light for 30 minutes on each side of the scaffolds. HMSCs (Passage 4) were seeded at a density of 1×10^5 cells per scaffold in well plates. After 24 hours, the scaffolds were transferred to 50-ml high-aspect-ratio vessels (Synthecon, Houston) and cultured under static condition in osteogenic media (Cambrex) at 37°C and 5% CO_2. The media were changed every 3 days, and the cultures were maintained for 21 days. At day 7, 14, and 21, scaffolds were taken out for characterization. Cell morphology and distribution on scaffolds were visualized by scanning electron microscopy (SEM). Cell proliferation on scaffolds was quantified by MTS assay. Alkaline phosphatase (ALP) activity was measured as an indication of osteogenic differentiation. Mineralization was visualized and quantified by Alizarin red (ALZ) staining. Statistical analysis was performed using a one-way ANOVA with Tukey test for multiple comparison ($p<0.05$).

Results/Discussion: SEM micrographs (not shown here) showed that HMSCs attached and proliferated on both PLAGA and PLAGA/n-HA scaffolds. The MTS assay results are shown in Figure 1a. On day 14, cell number on PLAGA/n-HA scaffolds was significantly higher than PLAGA scaffolds, and the cell numbers were comparable on day 21. This observation suggests that PLAGA/n-HA scaffolds can induce higher rates of proliferation of HMSCs at early time points as compared to PLAGA scaffolds. In addition, the presence of nano-HA led to a significant increase in differentiation potential of scaffolds (Figure 1b). On day 21, PLAGA/n-HA scaffolds showed significant increases in alkaline phosphatase activity as compared to PLAGA scaffolds. This is further corroborated by an increase in mineralization on PLAGA/n-HA as compared to PLAGA scaffolds. Figures 2a and b show alkaline phosphatase staining of cells on PLAGA and PLAGA/n-HA scaffolds after 21 days in static culture. PLAGA/n-HA scaffolds showed more intense staining as compared to PLAGA scaffolds demonstrating higher calcium content. Figure 2c shows quantitatively the extent of calcium deposition on the scaffolds (after subtracting readings of blank scaffolds). PLAGA/n-HA scaffolds showed an approximately 5-fold increase in calcium deposition as compared to PLAGA at day 21.

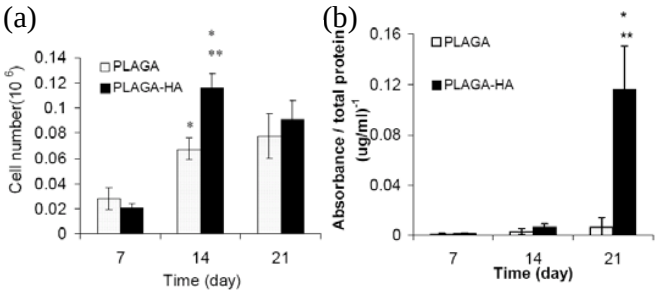

Figure 1. (a) Cell proliferation by MTS assay. (b) Relative alkaline phosphatase activity. (*) indicates significantly higher than previous time point. (**) indicates significantly higher than PLAGA scaffolds. $p<0.05$

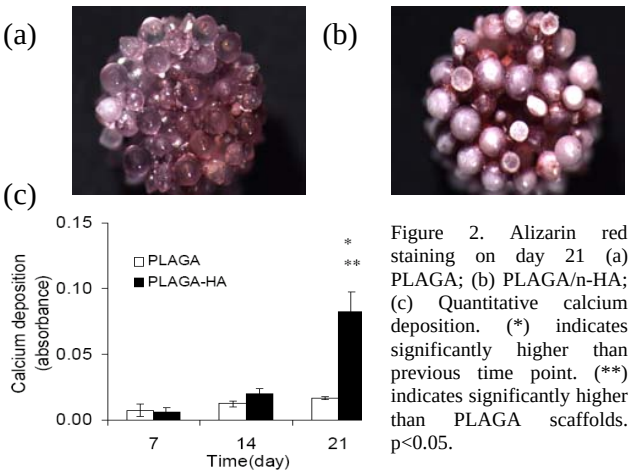

Figure 2. Alizarin red staining on day 21 (a) PLAGA; (b) PLAGA/n-HA; (c) Quantitative calcium deposition. (*) indicates significantly higher than previous time point. (**) indicates significantly higher than PLAGA scaffolds. $p<0.05$.

Conclusions: The incorporation of nano-sized hydroxyapatite in PLAGA sintered microsphere matrices promoted the proliferation and osteogenic differentiation of HMSCs on the scaffolds. This, along with their improved mechanical properties [3], demonstrates that PLAGA/n-HA sintered microsphere matrices are excellent scaffolds for bone tissue engineering applications.

References: [1] Zhao F, et al, Biotechnol. Bioeng, 2005, 91(4), 482-93. [2] Datta N, et al, Biomaterials 2005,26, 971-977. [3] Lv Q, et al, Transactions of the 31st annual meeting of SFB, Volume XXIX, 50, 2006.

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