Ex Vivo Generation of Three Dimensional Human Mesenchymal Stem Cells/Nano-hydroxyapatite Composite Scaffold Constructs in HARV Bioreactors as Potential Bone Graft

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Introduction:

A common bone tissue engineering approach involves culture of autologous cells from small biopsy with three-dimensional (3D) scaffolds in *ex vivo* environment and re-implantation back to the body. In this study, we employed high aspect ratio vessel (HARV) bioreactor as the *ex vivo* culture method because it has been shown to improve cell in-growth, osteoblastic cell differentiation, and mineralization [1]. To mimic the structure of natural bone consisting organic/inorganic phases, we designed and characterized poly (lactide-*co*-glycolide) (PLAGA) / nano hydroxyapatite (n-HA) composite scaffolds for HARV bioreactor based bone tissue engineering applications [2]. Human mesenchymal stem cells (HMSCs) were seeded on composite scaffolds and cultured in HARV bioreactor for 21 days with pure polymeric scaffolds as control. Cell distribution, proliferation, differentiation and mineralization were investigated.

Materials and Methods: PLAGA/n-HA scaffolds were fabricated using a sintered microsphere technique and sterilized before seeding as previously described [2]. Pure PLAGA scaffolds were fabricated in a similar fashion. HMSCs (Cambrex, East Rutherford, NJ) were expanded and maintained in the MSC Basal Medium (Cambrex). HMSCs were seeded at a density of 5×10^4 cells per scaffold in well plates. After 24 hours, the scaffolds were transferred to 50 mL HARVs (Synthecon, Houston) and cultured dynamically with a rotating speed of 36 rpm in Cambrex osteogenic media at 37°C and 5% CO_2. Cell proliferation on scaffolds was quantified by Picogreen DNA assay. Alkaline phosphatase (ALP) activity was measured as an indication of osteogenic differentiation. Mineralization was visualized by Alizarin red (ALZ) staining. Actin fibers and cell nuclei were stained and visualized using confocal microscopy. Statistical analysis was performed using a one-way ANOVA with Tukey test for multiple comparison ($p<0.05$).

Results and Discussion: Figure 1 shows DNA and ALP activity as a function of culture time. HMSCs showed significantly higher amount of DNA on composite scaffolds than PLAGA scaffolds on day 21. Normalized ALP activities of HMSCs on composite scaffolds were significantly higher than on PLAGA scaffolds at all time points indicating greater osteogenic differentiation. Moreover, significantly more calcium deposition of HMSCs were measured on composite scaffolds, as shown in Fig 2a. Fig 2b shows the picture of a composite scaffold stained with ALZ staining on day 21. These results demonstrated that HMSCs on composite scaffolds exhibited enhanced cell proliferation, differentiation, and mineralization as compared with PLAGA scaffolds in HARV bioreactors. Fig 3a shows actin and nucleus staining of HMSCs at the center of a composite scaffold after culture in HARV bioreactors for 21 days, suggesting that dynamic culture in HARV bioreactor can improve nutrient transport and waste removal, therefore, promote cell in-growth into scaffolds. The superior cellular responses on PLAGA/n-HA scaffolds may be attributed to enhanced surface roughness [3] and mechanical properties [2]. PLAGA scaffolds were found not to be intact after long-term culture in bioreactor. The inferior mechanical properties may help explain the monotonic cellular responses over culture time. Both types of scaffolds were incubated in simulated body fluid (SBF) for 28days. The results showed that composite scaffolds have superior ability to absorb the calcium ions from the solution than PLAGA scaffolds (as shown in Fig 3b). Therefore, composite scaffolds can accelerate the initialization of mineralization process of HMSCs.

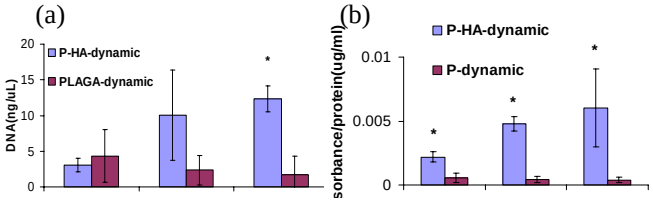

Figure 1. (a) Cell proliferation by DNA assay. (b) Relative alkaline phosphatase activity. (*)indicates significantly higher than PLAGA scaffolds. $p<0.05$

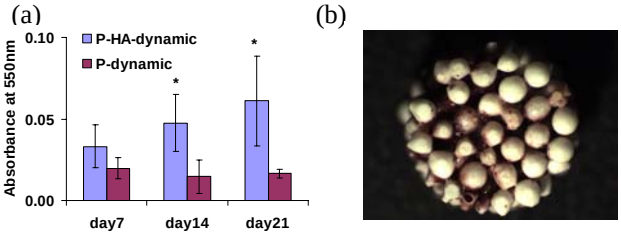

Figure 2. (a) Calcium deposition by ALZ staining with culture time. (b) ALZ staining of HMSCs on composite scaffold on day 21. $p<0.05$

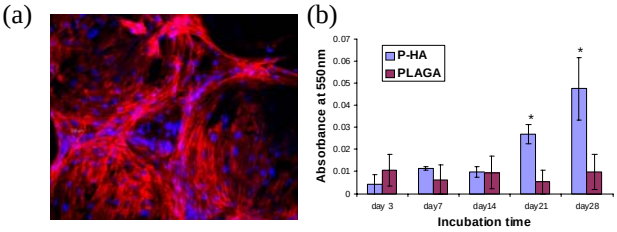

Figure 3. (a) Actin (red) and nucleus (blue) staining of HMSCs at the center of a PLAGA/n-HA scaffold on day 21 indicating deep cell penetration into scaffold. (b) Calcium absorbtion from SBF with time. (*) indicaties significantl difference compared with PLAGA scaffolds. $P<0.05$

Conclusions: The study demonstrated enhanced proliferation, differentiation, and mineralization of HMSCs on PLAGA/n-HA as compared to PLAGA scaffolds under dynamic culture conditions in HARV bioreactor. We postulate that the surface properties, mechanical properties, and the ability to absorb calcium ions (proved by biomimetic study) may contribute to the difference.

References: 1. Yu, X., et al., PNAS, 101, 11203-8, 2004. 2. Lv, Q., et al., Transactions of the 31st annual meeting of SFB, Volume XXIX, 50, 2006. 3. Zhao, F., et al, Biotechnol. Bioeng, 91(4), 482-93, 2005.

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