Functionally Graded Beta-Tricalcium Phosphate Scaffold for Bone Regeneration

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Statement of Purpose: Critical size bone defects that result from various disease processes, genetic disorders, and trauma can be restored through placement of a graft or prosthesis. Autografts are usually considered as the gold standard in graft choice. Allografts have proven to be an alternative to autografts given their similar characteristics, but complications such as limited graft/donor availability and host immune response limit the use of such grafts. Recently, biomimetic synthetic bone grafts have become increasingly promising. Calcium phosphate ceramics (CaP), such as beta-tricalcium phosphate (β-TCP), hydroxyapatite (HA) and their composites have been frequently used in clinical settings. These materials are osteoconductive because CaP is the main inorganic component of vertebrate calcified hard tissues. As bone substitutes, CaP grafts are also desired to have a macroporous structure to allow for bone in-growth. It is very important to understand how varied pore size and arrangement of macroporous scaffolds will influence cell response and migration. It is our hypothesis that varied pore size and arrangements across scaffolds will influence cell migration and response within the scaffolds. This study investigated the effect of various types of biomimetic β-TCP scaffolds on cell migration and cell behavior *in vitro*.

Methods: Completely interconnected, macroporous biodegradable β-TCP scaffolds were prepared. The architecture and chemistry were fully manipulated by varying templates, casting materials and methods. Four types of β-TCP scaffolds were fabricated from two different pore sizes (big pore, approximately 600-800 μm, and small pore, approximately 350 - 500 μm). The four arrangements are uniform big pore (Big); uniform small pore (Small); horizontally graded peripheral big pore and central small pore (BSB); and horizontally graded peripheral small pore and central big pore (SBS). All samples were autoclaved and pre-vacuumed. W-20 mouse osteoblast precursor cells were used to evaluate the cell response and cell migration. To evaluate cell response to the scaffolds, the cells were pre-seeded onto the scaffolds, and the cell proliferation and differentiation were measured at designated time points over 3 weeks by using dsDNA assay and alkaline phosphatase (ALP) assay. To evaluate cell migration into scaffolds, well plates were seeded with the cells and, 4 days after incubation, the scaffolds were placed on top of the cells in the well plates. Cell migration was estimated by the amount of dsDNA produced by the cells migrating into the scaffolds and residing on the well plates for 7 and 14 days.

Results/Discussion: In pre-seeded cell response experiment, the dsDNA amounts produced by the pre-seeded cells continued to increase on the 4 groups of scaffolds for the first 2 weeks, and then reached a peak at day 19. There were no significant differences in the dsDNA amount among the four groups. The ALP specific activities continued to increase during the whole period of experiments. At day 19, the cells on the SBS exhibited significantly greater ALP specific activity compared to those on other scaffolds. In this experiment, we seeded the same amount of initial cells on the 2-dimensional (2D) well-plates as controls and the 3-dimensional (3D) porous scaffolds as experimental groups. The data show that the cells on the scaffolds (test groups) exhibited significantly greater amounts of dsDNA compared to those on the well plates (controls) within 2 weeks, suggesting there was significantly more cell growth on the 3D porous scaffolds compared to those on the 2D well-plates. This is because the scaffolds provide significantly more surface area for cells to grow. On the other hand, the differentiation of the osteoblast precursor cells was delayed on the 3D porous scaffolds compared to those on the 2D well-plates because the cells on the scaffolds did not reach confluence during the experimental period. This suggests that higher amount of cells need to be seeded on the 3D porous scaffolds in order to retain the higher proliferation and un-delayed differentiation. It is very interesting that the SBS scaffold enhanced osteoblast differentiation compared to the other scaffolds.

In The cell migration experiment, cells were allowed to become 80-90% confluence on the well plates before the scaffolds were placed on top of the cells. The dsDNA amounts continued to increase on the 4 groups of scaffolds during the two weeks. All scaffold groups exhibited significantly greater cell growth compared to those on the well plates (2D controls) because of more room for cell growth. There were no significant differences in the dsDNA amount among the 4 groups on day 7. But, at day 14, the cells on the Big, Small, and BSB groups exhibited significantly greater dsDNA amounts compared to those on group SBS. At day 14, in the scaffold containing well plates, the cells on the well plates decreased compared to those on the well plates in the absence of scaffolds (controls), suggesting that the cells did migrate into the scaffolds. All scaffold groups show very low ALP specific activities and there were no significant differences among the 4 groups for the 2 weeks. This is likely because the scaffolds have not reached confluence yet. A longer incubation period will be needed to observe any differences between specific ALP activities.

Conclusions: This study confirms that the pore size and the arrangement of the macroporous scaffolds does influence cell behavior and cell migration. A longer in vitro cell culture and in vivo study will be needed to further study the architectural effect of the scaffolds in the future.

References:

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