Cell Growth on a Gradient Calcium Polyphosphate Scaffold in a Perfusion Bioreactor

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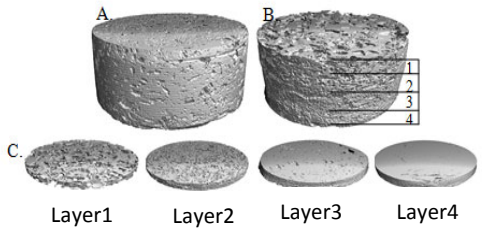
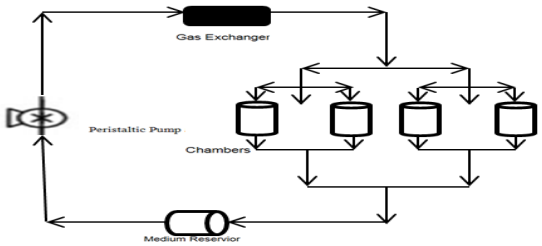
Statement of purposes: A well designed bone scaffold is crucial to bone tissue engineering. We have developed a novel gradient bone scaffold by combination of calcium polyphosphate (CPP) with different sizes of porogen (stearic acids). In this study, we used a flow perfusion bioreactor to mimic in vivo environment for murine MC3T3 osteoblast cell culture. We expected that the structure of gradient scaffold could enhance cell proliferation and distribution.

Materials and Method: Scaffold preparation: First, the scaffolds were prepared by mixing CPP power with porogen (stearic acids) of four different sizes. Second, the mixed material was loaded with 70 tons of pressure in a cylindrical model. Finally, the scaffolds were sintered in furnace to evaporate the stearic acids and form the pores. A homogenous material scaffold has an even structure with various pore sizes evenly distributed throughout the scaffold whereas the gradient scaffold contains basic four layers of material with a defined gradient of pore sizes (**Fig.1**). Flow perfusion bioreactor design and parameters for dynamic cell culture: The bioreactor system basically consisted of four parallel, vertically oriented cylindrical chambers, a cell culture medium reservoir, a peristaltic pump and a gas exchanger (**Fig.2**). The flow rate was 1ml/s. According to the Hagen-Poiseuille relation (τ=8 μv/ds)for laminar flow through a round conduit and scaffolds structure analysis by MicroCT (average pore size =0.21mm,percentage of porosity=32%), the shear stress(τ) on bone cell was 0.8Pa.Cell seeding and MTT: The cell suspension with density (1×10^5 cells/ml) was pipetted to the top, bottom and side of each scaffold. Each scaffold was loaded the cell suspension 300μl. Individual cell seeded scaffolds were placed inside the bioreactor chambers separately and cultured for four days. MTT was incubated with cell-seeded scaffolds for 2 hours, dark blue formazan crystals formed by live cells were solubulized and color measured at 560nm to determine cell proliferation. Confocal microscopy: After four days cell culture, cells grown in the scaffolds were labeled with the Dil dye and cell distribution was measured by Leica TCS SP5 confocal microscopy.

Results: A three dimensional rendering of homogenous and gradient scaffolds is shown in **Fig.1**.The average pore size of the homogenous scaffold was 0.21mm. For the gradient scaffold the pore size decreased from first layer (0.38mm) to fourth layer (0.08mm). **Fig.3c-d** show differences in cell proliferation measured by the MTT assay. There was no significant difference between the gradient and homogenous scaffold immediately after cell seeding (**Fig.3c**). After four days dynamic cell culture, no significant difference occurred between the gradient and homogenous scaffold (**Fig.3d**). The cell distribution results are shown in **Fig.3a-b**. The cells were grown deeper on the gradient scaffold when compared with homogenous scaffold.



Figture.1 3D rendering homogenous (A) and gradient (B) scaffold showing the different layers within the gradient scaffold (C).Layer 1: >300μm; Layer 2: 250-300μm; Layer 3: 75-250μm and Layer 4: <75μm.



Figture.2 Flow perfusion bioreactor design

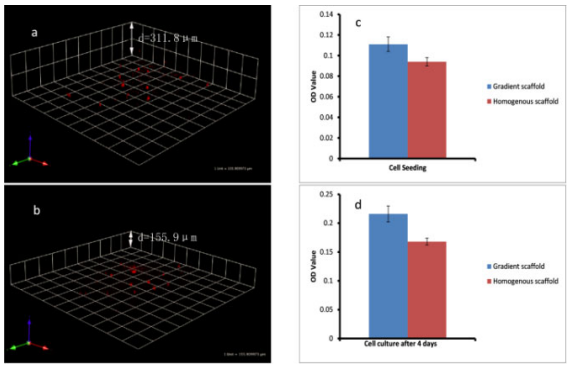


Fig.3 The MTT and Confocal Microscopy results

Conclusions: The CPP scaffold with a gradient pore size distribution is expected to better mimic nature bone structure. We hope that the layers with larger pore size can provide enough space for cell growth and the layers with small pore size can improve the mechanical strength .Our data, based on perfusion bioreactor culture conditions, showed that the majority of cells were grown deeper in the layer 1 and layer 2 (with larger pore size distribution) in the gradient scaffolds. In contrast, the cells adhered and proliferated on the surface of the homogenous scaffolds (**Fig. 3**). More efforts are required to optimize the perfusion bioreactor condition setting and develop a real time measurement of cell growth and differentiation in situ.