

Perfusion flow culture improves growth of osteogenic cells in a gradient calcium polyphosphate scaffold

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Statement of purposes: We have developed a customized perfusion flow bioreactor to mimic physiological environment and promote cell growth. The purpose of this study was to investigate whether the perfusion flow culture improves the adhesion, growth and differentiation of murine MT3T3 pre-osteoblast cells in a gradient calcium polyphosphate (CPP) scaffold.

Materials and Methods: Gradient and homogenous CPP scaffolds' structure: The gradient scaffold has four layers analyzed by Micro-CT, 1st layer (average pore size/x: 0.38 μ m; porosity/y: 65%), 2nd layer (x: 0.13 μ m; y: 55%), 3rd layer (x: 0.10 μ m; y: 32%) and 4th layer (x: 0.08 μ m; y: 13%). The homogenous scaffold has an even structure (x: 0.21 μ m; y: 32%). 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.1). The flow rate was 1ml/s. According to the Hagen-Poiseuille relation ($T=8 \mu\text{v}/\text{ds}$) for laminar flow through a round conduit, the shear stress (T) on bone cell was 0.8Pa. Based on the average volume of pore in scaffold (0.199986cm²), the optimal amount of seeded cell was 5.8*10⁵ approximately. Cell seeding and MTT: The cell suspension with density (3*10⁶ cells/ml, seeding

efficiency: 20%) was pipetted to the top, bottom and side of each scaffold. Each scaffold was loaded the cell suspension 300 μ l. Individual cell seeded scaffolds were placed inside the bioreactor chambers separately and cultured for four days. MTT assay was used to determine the cell proliferation. Confocal microscopy: Four days after cell seeding and in culture, cells grown in the scaffolds were labeled with the Dil dye and cell distribution was measured by Leica TCS SP5 confocal microscopy. Cell differentiation test (AKP activity test): After 14 days cell cultures, AKP buffer was added in crushed scaffolds by liquid nitrogen overnight. The AKP activity in cell lysate was measured to determine the cell differentiation status.

Results: Cell distribution after manually seeding was exhibited in Fig.2-3. In gradient scaffold, the majority of cells attached to the top layer with larger pore size and porosity; fewer cells were attached to the middle-bottom layers (Fig.2). In homogenous scaffold, cell distribution on each layer was relatively even (Fig.3). Fig.4c-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.4c). After four days dynamic cell culture, no significant difference occurred between the gradient and homogenous scaffold (Fig.4d). The cell distribution results are shown in Fig.4a-b. The cells were grown deeper on the gradient scaffold when compared with homogenous scaffold. The cell differentiation level was

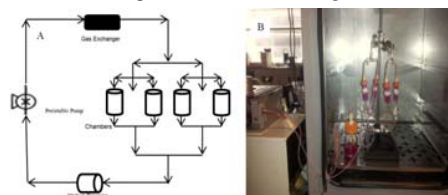


Figure 1 (A) The schematic diagram of bioreactor. (B) The perfusion flow bioreactor.

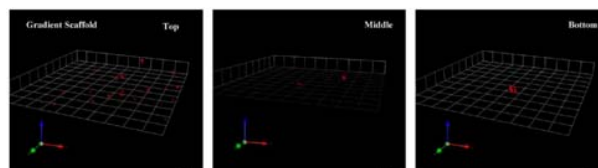


Figure 2 Cell distributions on gradient scaffold after manually seeding

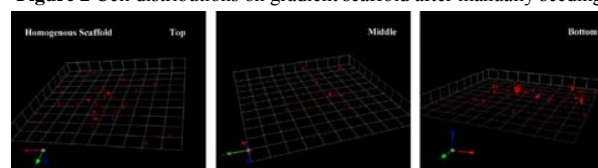


Figure 3 Cell distributions on homogenous scaffold after manually seeding

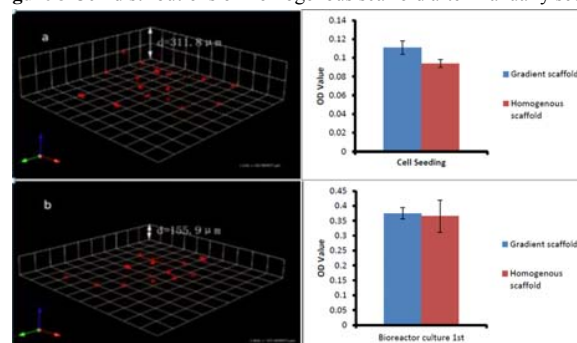


Figure 4 Cell distributions on gradient (a) and homogenous (b) scaffolds after cell cultures. The amount of cells on scaffolds after cell seeding(c). Cell proliferation after cell culture (d).

exhibited in Fig.5. The AKP level on gradient scaffold was significantly higher than the homogenous scaffold ($P<0.01$, Fig.5).

Conclusion: The perfusion flow bioreactor was successfully developed. It improved the cell cultures. The gradient structure did affect the cellular behavior.

The 1st and 2nd layers of the gradient scaffold have larger pore size and porosity which is beneficial for cellular attachment and growth. Compare to homogenous scaffold, although the gradient structure did not improved the cellular proliferation because the total space for cell growth was similar, it enhanced cellular differentiation significantly. Thus, the gradient structure impacted cellular distributions and differentiation which has potential value of clinical application.

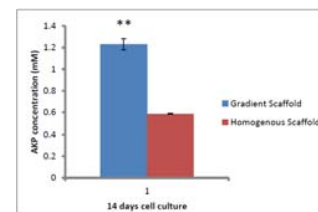


Figure 5 The results of AKP test