Spiral Structured Nanofibrous 3D Scaffolds for Bone Tissue Engineering

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Statement of Purpose: Polymeric nanofiber matrixes as the most promising analogs for native extra cellular matrix have already been widely used in tissue engineering[1]. However, the fabrication of nanofibers into complex 3D structures is restricted due to current manufacturing technique. In addition, the mechanical properties of nanofibers are not sufficient for bone tissue engineering applications. In order to overcome these limitations, we have incorporated nanofibers onto biodegradable Poly (ε-caprolactone) (PCL) 3D scaffolds. The spiral structures will be helpful for improving nutrient transport and cell penetration into the scaffolds, which are otherwise limited in the traditional scaffolds for bone tissue engineering.

Methods: PCL sheets were fabricated using solvent casting and salt leaching method [2]. PCL nanofibers were fabricated using eletrospinning and spun directly onto both sides of the PCL sheets. The nanofiber bearing sheets were then rolled into spiral structured with the aid of a piece of metal and the resultant scaffolds were referred as Fibrous scaffolds. Two other scaffolds have been made as the controls. One type of control scaffold is prepared by solvent casting and salt leaching, but without nanofiber coating. This type of scaffold is denoted as Porous PCL scaffold. The other control scaffold is prepared by solvent casting only (without salt leaching or fiber coating). This type of scaffold is denoted as Plain PCL scaffold. All scaffolds possess spiral shape as shown in Fig.1. The scaffolds were characterized by stereoscopic microscope, scanning electron microscopy (SEM) and porosity measurement. Human hFOB 1.19 osteoblast cells (ATCC) were cultured on the scaffolds. Cell proliferation was analyzed with MTS assay, the osteoblastic phenotypic development was analyzed by determining alkaline phosphatase activity, and mineralized matrix deposition was analyzed by Alzarin red staining for calcium. For statistical analysis, the student t-test was used for comparing the results between the fibrous scaffolds and other two groups. A P value of <0.05 was considered to be statistically significant.

Results/Discussion:

Spiral Scaffolds. The nanofibrous scaffold showed a spiral and open structure under stereomicroscope as shown in Fig.1. The porosities of three types of scaffolds are respectively 26.4%±1.7% for plain PCL scaffolds, 83.9%±3. 1% for porous scaffolds, and 77.8%±0. 2% for fibrous scaffolds.

Figure 1. Spiral structure of Fibrous scaffold.

Surface Morphology and Cell growth. SEM image (Fig.2 left) shows the surface of scaffold has a porous structure and consists of randomly oriented fibers. After 4 days of culture, the surface of scaffold was covered with human osteoblast cell monolayer (Fig.2 right).

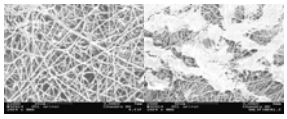

Fig.2)SEM images of fibrous scaffolds surface prior to cell seeding (left) . Human Osteoblast Cells cultured on the fibrous scaffolds at day 4 (right).

Cell Proliferation. The MTS assay indicates that the number of cells on porous scaffolds is significantly higher as compared to that on plain PCL, and the cell number on the fibrous scaffolds is further increased as compared to the other two groups for both day 4 and 8 (Fig. 3).

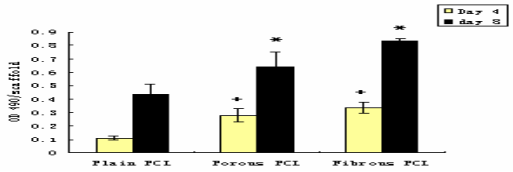

Fig.3 MTS assay for cell proliferation. (*and +: A statistically significant, higher ($P < 0.05$) MTS absorbance value than that at plain PCL scaffold at day 4 and day 8 n>3) Error bar denotes standard deviation.

Alkaline Phosphatase Activity Assay. The phenotypic expression of cells was evaluated by the enzyme assay for alkaline phosphatases by Malachite green detection system (Upstate). As shown in Fig.4, alkaline phosphatase (ALP) activity for cells on porous scaffolds is significantly higher as compared to that on plain PCL scaffold, and ALP activity for cells cultured on fibrous scaffolds further enhanced as compared with the other two groups for day 8.

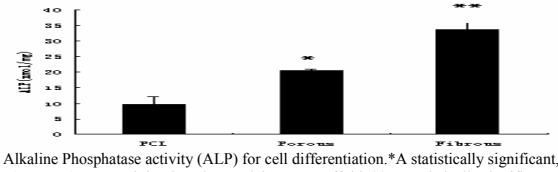

Fig.4 Alkaline Phosphatase activity (ALP) for cell differentiation.*A statistically significant, higher ($P < 0.05$) ALP activity than that at plain PCL scaffold, ** A statistically significant higher ALP activity than that of porous scaffold n=3. Error bar denotes standard deviation. Error bars denote standard deviation.

Calcium deposition by Alizarin Red Staining. After culturing for 8 days, alizarin red histochemical staining (Sigma) assay shows significant increase of calcium deposition on fibrous scaffolds as compared with those on plain PCL scaffolds and porous scaffolds without fibers (Fig.5a-c). The side image (Fig.5d) indicates uniform cell penetration into the fibrous scaffold.

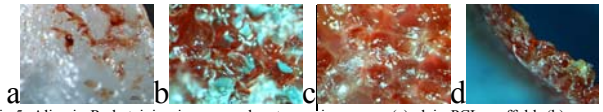

Fig.5. Alizarin Red staining images under stereomicroscope. (a) plain PCL scaffold, (b) porous scaffold (c) fibrous scaffold (d) the side image of fibrous scaffold showing calcium penetration inside the scaffold.

Conclusions: These studies suggest that our novel design of nanofibrous scaffolds with three-dimensional structure increases the osteoblastic cell proliferation, promotes osteoblastic cell phenotypic development and enhances mineralized matrix synthesis within tissue-engineered constructs. The scaffolds could be potentially used as bone grafts for repairing bone injuries.

References:

1. Ma, Z., et al., Tissue Eng, 2005. **11**(1-2): p. 101-9.
2. Laurencin, C.T., et al., J Biomed Mater Res, 1996. **30**(2): p. 133-8.