Electrospun PCL-BFP1 Nanofiber Scaffold for Bone Regeneration

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***Abstract*—Polycaprolactone nanofiber scaffolds containing bone forming peptide 1 (BFP1) prepared via electrospinning were used for *in vitro* bone regeneration from Human fetal osteoblasts (hFOBs). The aim of this study is to investigate the method for incorporating BFP1 with PCL nanofiber. BFP1 was dissolved in PCL/HFIP solution and the electrospinning was performed. hFOBs were cultured for up to 7 days under standard conditions in osteogenic differentiation media on the fibers and tissue culture plates. Results from MTS and ALP assays revealed that BFP1 immobilized on the nanofiber had same effect compared with BFP1 in the keep-changing culture media, and both two groups showed significantly higher ALP secretion than control groups. It has been shown that BFP1 is an effective and stable growth factor for bone formation, and this method has a great potential for immobilizing peptide with polymer nanofiber scaffolds.**

Keywords— Bone regeneration; polycaprolactone; nanofiber; bone-forming peptide 1; nanofiber

I. INTRODUCTION

Bone regeneration involves repairing the damaged bone, with scaffolds, cells, and growth factors used in any combination or individually to restore the injured structure and function. The combination of cells, growth factors, and scaffolds is often referred to as the tissue engineering triad [1]. The scaffold material and growth factor are commonly integrated to affect cell for tissue healing. Polycaprolactone (PCL) is a wildly used material as bone scaffold for its favorable biocompatibility and biodegradability [2]. Bone-forming peptide 1 (BFP1) is the functional group derived from bone morphogenetic protein 7 (BMP7). Compared with BMP7, BFP1 has slower clearance *in vivo* and is more stable while incorporating with nanofibers, and which make it a favorable factor for the nanofiber drug delivery system [3, 4]. It is still unclear what is the effective way for BFP1 being immobilized with PCL nanofiber mesh. The aim of this study is to investigate the efficiency of PLC nanofiber and/or BFP1 on bone regeneration and discover the optimized method for preparing composite nanofiber scaffolds.

II. MATERIALS AND METHODS

A. Preparation of electrospun nanofibers and BFP1 solution

Four situations were designed for the experiment including the group of individual PCL, PCL combined with BFP1, tissue culture plate, and tissue culture plate supplemented with BFP1. The PCL nanofiber meshes were prepared by electrospinning, and BFP1 was added to form required composite nanofiber scaffold. PCL (16%, w/v) was dissolved in 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) overnight under magnetic stirring. BFP1 (0.5mg/ml) were added into PCL solution. Early work was done to ensure the comparability of different groups by adjusting concentrations of BFP1. This study is based on the result of the early work. Electrospinning was performed with a syringe filled with electrospining solution, and with a 1.5mm inside diameter steel needle tip. A constant volume flow rate of 0.50 ml/h was maintained using a syringe pump. The voltage was kept at 11 kV and the distance between the tip and the collection screen was 10 cm. The electrospun fibers were collected on cover slips on top of aluminum foil. The content of BFP1 in tissue culture plate was equal to that in scaffold.

B. hFOB culture and bioactivity assays

Human fetal osteoblasts (hFOBs) were seeded on individual PCL nanofiber scaffold, the composite PCL nanofiber scaffolds, tissue culture plate, and tissue culture plate containing BFP1 with the osteogenic differentiation media (DMEM with low glucose, 10% fetal bovine serum, 1% penicillin-streptomycin, 50 mg/ml ascorbic acid, 0.01M glycerol-2-phosphate, and 100 nM dexamethasone) up to 7 days. SEM micrographs were taken to demonstrate the cell morphology and porous size of the nanofiber meshes. Cell viability, adhesion, and differentiation were investigated for evaluate the ability of bone regeneration. Cell viability and adhesion were determined by MTS assay on day 1, 5, and 7. Alkaline phosphatase (ALP) activity, a function of the release of p-nitrophenol from p-nitrophenol-phosphate, was measured for evaluating cell differentiation and was normalized by the MTS result on day 7.

The ANOVA and Post-hoc Test were used to assess significant differences among the groups and between every two groups based on the data of MTS and ALP tests. SPSS 2.0 and Microsoft Excel were the software for the statistical analysis. $P<0.05$ was considered significant.

III. RESULTS AND DISSCUSSION

The characterization of the nanofibers is shown using a scanning electron microscope (SEM).

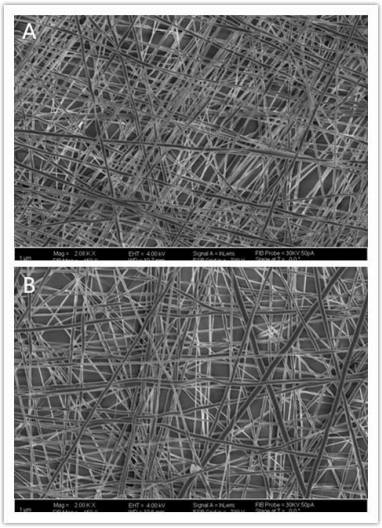

Fig1. Representative SEM photographs of nanofiber scaffold. PCL (A). PCL with BFP1 (B).

The hFOBs showed normal phenotype while treated with the differentiation medium. Compared with Tissue Culture Plate group, positive controller, Plate/BFP1 group and PCL/BFP1 group showed the significant improvement for the proliferation and differentiation of osteoblasts by both MTS and ALP assays. Among the groups, the Plate/BFP1 group showed the greatest efficiency for bone regeneration ($P<0.05$). There was no significant difference in cell viability and ALP activity between, PCL group and Tissue Culture Plate group. The decrease effect on bone regeneration in turn were: Plate/BFP1, PCL/BFP1, Plate, PCL.

It is well known that for the guidance of bone regeneration in orthopedic surgery, the biocompatible and biodegradable

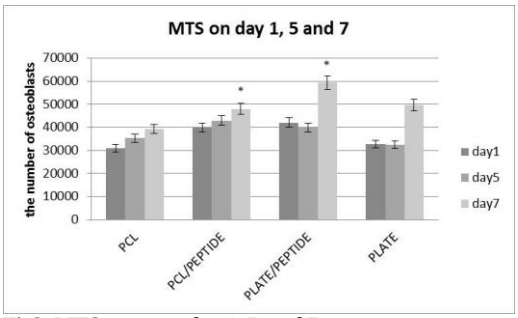

Fig2. MTS assay on day 1, 5, and 7

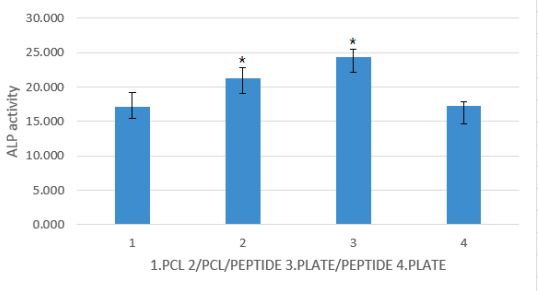

Fig3. ALP activity on day 7

artificial membranes play an essential roll. The scaffolds need to be designed with appropriate porosity for their osteoconductivity and can therefore improve proliferation and differentiation of bone cells. In this study, we fabricated scaffold based on PCL nanofiber incorporated with BFP1 for the guided bone regeneration. The results showed that BFP1 had a dominant effect on bone formation with an appropriate concentration compared with pure PCL scaffolds. The small peptide is more stable to be immobilized in polymer scaffolds and has shown its indispensable effect. It should be more effective by using PCL polymer-based composites scaffold as a situ drug delivery system. So, it is worth to involve small bone forming peptides in any future designs. On the other hand, the artificial membrane will be more beneficial by precluding the potential bone resorption in the regeneration process [5]. More factors can be introduced to this scaffold-delivery system to achieve the optimum outcome.

IV. CONLUSION

The scaffold, cells, and growth factors are core components for the development of bone regeneration, and are irreplaceable. On the other hand, they dependent on each other for effective bone regeneration. The composite scaffold with optimized component is important for clinical applications. In this study, BFP1 can be effectively incorporated into PCL nanofibrous meshes, and significantly promote the proliferation and differentiation of osteoblastic cells.

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REFERENCES

[1] Bhattacharya I, Ghayor C, Weber F E, "The use of adipose tissue-derived progenitors in bone tissue engineering-a review" [J]. Transfusion Medicine and Hemotherapy, 2016, 43(5): 336-343.

[2] Williams, J. M., Adewunmi, A., Schek, R. M., Flanagan, C. L., Krebsbach, P. H., Feinberg, S. E., ... & Das, S, "Bone tissue engineering using polycaprolactone scaffolds fabricated via selective laser sintering" [J]. Biomaterials, 2005, 26(23): 4817-4827.

[3] Kim, H. K., Kim, J. H., Park, D. S., Park, K. S., Kang, S., et al., "Osteogenesis induced by a bone forming peptide from the prodomain region of BMP-7" [J]. Biomaterials, 2012, 33(29): 7057-7063.

[4] Li, C., Vepari, C., Jin, H. J., Kim, H. J., & Kaplan, D. L, "Electrospun silk-BMP-2 scaffolds for bone tissue engineering" [J]. Biomaterials, 2006, 27(16): 3115-3124.

[5] Masquelet A C, Begue T, "The concept of induced membrane for reconstruction of long bone defects" [J]. Orthopedic Clinics of North America, 2010, 41(1): 27-37.