Effect of Three-dimensional Porous Composite Scaffold Applied in Bone Defect Repair

Jing-Yi Zhang, Lan-Xin Lü, Xiao-Feng Zhang, Ning-Ping Huang*
State Key Laboratory of Bio-electronics, Southeast University, Nanjing 210096, China;
*Address correspondence to Ning-Ping Huang (nphuang@seu.edu.cn)

ABSTRACT: Bone tissue engineering is one of the main biotechnology methods for bone defects treatment. PLGA (poly (lactic-co-glycolic acid)), approved by the FDA for clinical use, is currently one of the most widely used biodegradable materials for tissue engineering. This research presents a design of 3D composite scaffold which simulated the double-layer structure of long bone and its effect on rabbit radius bone defect repair. Two types of PLGA with different degradation rates was used for fabricating the internal inverse opal porous scaffold and the external nanofiber film of the composite scaffold respectively. The scaffold's effect on bone repair was assessed by X-Ray and histological tests. The results showed that the composite structure scaffold had a better effect on bone repair, compared to the single structure of the porous scaffold.
KEY WORDS: bone repair; biodegradable; PLGA; composite scaffold

Introduction

In this study, we aim to prepare a simple and effective composite scaffold, based on a deep understanding of bones' structure and composition[1,2], and previously reported researches on bone tissue engineering scaffolds[3,4]. Long bone consists of external compact bone and internal spongy bone/marrow, with basic component of hydroxyapatite (HA). Thus, PLGA with its biodegradable features and HA with its osteo-inductive proprieties, were both selected to fabricate composite scaffold.

Materials and Methods

The composite scaffold contained two parts, an internal PLGA 3D inverse opal porous scaffold prepared by solvent casting-particle leaching method and an external PLGA/n-HA nanofiber film prepared by electrospinning. Then the nanofiber film was used to wrap the PLGA porous scaffold to form the composite scaffold. The detailed procedure is described below.

Preparation of PLGA porous scaffold.

PLGA porous scaffolds were prepared by solvent casting-particle leaching method. Gelatin microspheres scaffold was used as template. PLGA dissolved in 1, 4-dioxane solution (10% wt/v) was perfused into the gelatin scaffold. After freeze-drying, gelatin was removed by hot water. The PLGA scaffold is characterized by SEM.

Preparation of PLGA/n-HA nanofiber films.

The random-aligned nanofiber films were prepared by electrospinning. HA particles (20 nm) was added into PLGA solution in the solvent of CH_2Cl_2: DMF=3:1 with concentration of 5% wt/v. The morphology of nanofibers was observed by SEM and diameter of the nanofibers was measured by ImageJ.

Preparation of composite scaffold.

Jing-Yi Zhang, State Key Laboratory of Bio-electronics, Southeast University, Nanjing 210096, China;
Tel: 15651967821; E-mail: 220174569@seu.edu.cn.

The prepared PLGA/n-HA nanofiber film was clipped into 2cm wide strips and wrapped 3-4 PLGA porous scaffold to form the composite scaffold. The length of the composite scaffold after sterilization is 1.5cm to 2cm.

Animal experiment and tests.

New Zealand white rabbits were selected to establish the 15mm radius defect model. Three groups were prepared: blank control group (maintain the defect state), porous scaffold group (implant 4 porous scaffolds), composite scaffold group (implant 1 composite scaffold). Various detection methods were used to evaluate the bone repair effect, mainly included X-Ray radiography, CT reconstruction, HE staining, and IF staining.

Results and Discussions

Scaffolds observation

The SEM characterization of PLGA scaffold and nanofiber film in Figure 1 shows that the PLGA scaffold has an inverse opal structure with good porosity and interconnection. The pore size is about 400μm as shown in Figure 1(a). The average diameter of the nanofiber was about 200nm as shown in Figure 1(b).

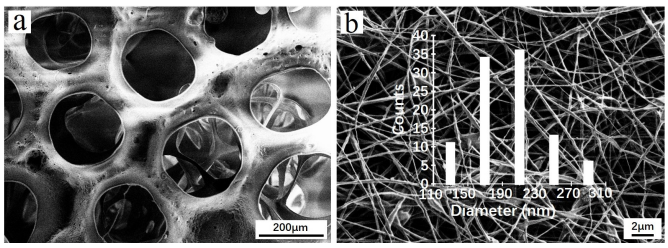

Figure 1. SEM characterization of PLGA scaffold and nanofiber film. (a) The inverse opal structure of PLGA scaffold (b) SEM characterization of nanofiber film.

X-Ray radiography

Figure 2 shows the X-Ray radiographs of all three groups at the fourth week after the operation. Lane-Sandhu standard was taken to evaluate the regeneration. It indicated that the bone formation of the composite scaffold group was more than that of the porous scaffold group.

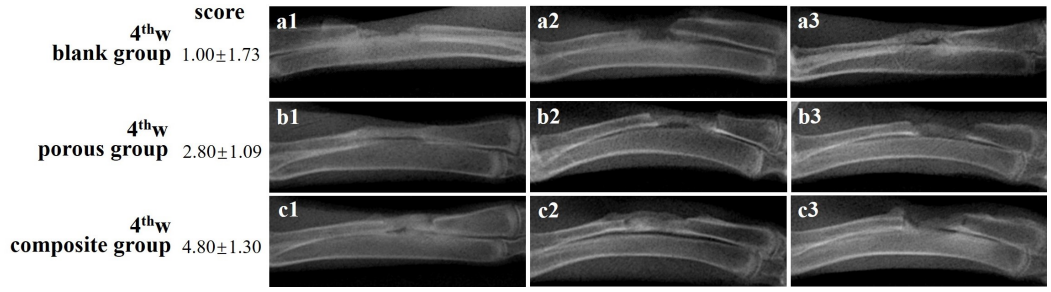

Figure 2. X-Ray images of the defect area at the fourth week postoperatively and the Lane-Sandhu Score of each group. (a) blank control group, (b) porous scaffold group, (c) composite scaffold group.

CT 3D reconstruction

CT reconstruction was required to correct the X-Ray results and to fully observe the new bone (Figure 3). From the perspective of the shape, the new bone in the porous group has a recurved state, while the new bone in the composite group has a better morphology. The reason may be that the fiber film outside the scaffold had good cell adhesion and osteoconductive, so it had a guiding effect on the morphogenesis and made the shape of new bones better.

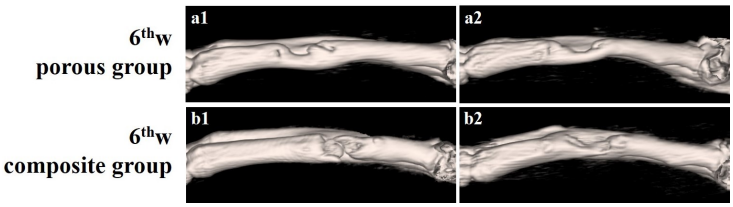

Figure 3. CT reconstruction of each two rabbits in the porous group(a) and composite group(b) at the 6th week.

From the scores of X-Ray radiography results and CT three-dimensional reconstruction, the overall osteogenesis of the composite scaffold was better than that of the single porous scaffold group. It indicates that the composite scaffolds wrapped with PLGA/n-HA nanofiber films are more conducive to bone formation and bone morphology.

HE staining & IF staining

HE staining and IF staining is aimed to test the angiogenesis and tissue regeneration. As shown in Figure 4.(a-1, b-1), and there is large amount of cell infiltration (indicated by the red arrows) between the multilayered nanofiber film of the composite scaffold. The blood vessels are indicated by the yellow arrows. IF staining targeted VWF which is the vascular markers. There are obvious VWF expression in both groups at the 4th week, means the existence of blood cells and vessels. These two staining results showed that there is no obvious difference between these two groups in angiogenesis, because interior of the composite scaffold was also a porous scaffold.

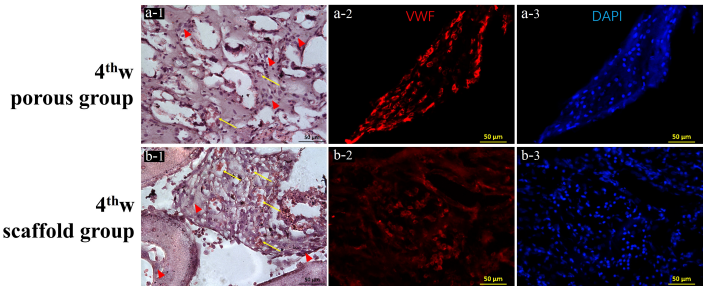

Figure 4. HE staining (a-1, b-1) and IF staining (a-2, a-3, b-2, b-3) of the scaffold at the 4th week.

Conclusions

In this study, we fabricated one novelty type of composite scaffold with double-layered structure, inverse opal structure scaffold with homogenous pores as internal layer and electrospinning nanofibers as external layer and found the double-layered scaffold had better bone repair effect and better simulated the long bone's structure than porous scaffolds, which indicating their promising application in long bone repair.

References

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