Front. Chem. China 2007, 2(1): 27–30
DOI 10.1007/s11458-007-0006-8

RESEARCH ARTICLE

XU Guofu, MOU Shenzhou, ZHOU Lingping, LIAO Susan, YIN Zhimin, CUI Fuzhai

Biomimetic strengthening polylactide scaffold materials for bone tissue engineering

Abstract In this paper, a new polylactide (PLA)-based scaffold composite by biomimetic synthesis was designed. The novel composite mainly consists of nano-hydroxyapatite (*n*-HA), which is the main inorganic content in natural bone tissue for the PLA. The crystal degree of the *n*-HA in the composite is low and the crystal size is very small, which is similar to that of natural bone. The compressive strength of the composite is higher than that of the PLA scaffold. Using the osteoblast culture technique, we detected cell behaviors on the biomaterial in vitro by SEM, and the cell affinity of the composite was found to be higher than that of the PLA scaffold. The biomimetic three-dimensional porous composite can serve as a kind of excellent scaffold material for bone tissue engineering because of its microstructure and properties.

Keywords hydroxyapatite, bone, tissue engineering, PLA-based scaffold materials

1 Introduction

Bone tissue engineering is expected to be a promising method for the repair of large bone defects [1,2]. Various kinds of scaffold materials have been used for bone tissue engineering, such as natural matrix, polymer or copolymer, bio-ceramics, etc. [3]. Among the potential scaffold materials, polylactide (PLA) polymer is a rational option for preparing the scaffold of bone tissue engineering, as it possesses excellent biodegradation, biocompatibility and forming ability, and is also approved by the Food and Drug Administration (FDA), although it has negative aspects such as low mechanical property, low hydrophilicity, tissue inflammation, etc. Design of biomaterials with surface properties similar to physiological bones would undoubtedly aid the formation of new bones at the tissue/biomaterial interfaces and improve the orthopaedic implant efficacy [4]. Based on the PLA scaffold materials [5] and nano-hydroxyapatite (*n*-HA) preparation by self-assembly [6,7], we developed a new bone tissue engineering scaffold material, n-HA/PLA (HA-PLA), in order to improve mechanical strength and biocompatibility. In this investigation, we used cell culture techniques to compare the cell affinity of the HA-PLA composite with that of the pure PLA scaffold. The detailed behaviors of the osteoblast adherence, proliferation and migration on the porous scaffold were revealed by OM and SEM. The protein synthesized by osteoblasts on the materials was also investigated to verify the normal active growth of the cells.

Translated from *Journal of Hunan University* (*Natural Sciences*), 2006, 33(2) (in Chinese)

XU Guofu, MOU Shenzhou, YIN Zhimin
School of Materials Science and Engineering, Central South University, Changsha 410083, China

ZHOU Lingping
College of Materials Science and Engineering, Hunan University, Changsha 410082, China

CUI Fuzhai, LIAO Susan (✉)
Department of Materials Science and Engineering, Tsinghua University, Beijing 100084, China

LIAO Susan (✉)
National University of Singapore, Singapore 117576, Singapore
E-mail: liaosusan@tsinghua.org.cn

2 Experimental

2.1 Materials and methods

Briefly, PLA with a molecular weight 10^5 Da was dissolved in dioxane to a final concentration of 10% (w/v), and then the solution was stirred gently at room temperature for 4−6 h. After the HA powder was added, the solution was ultrasonically homogenized and poured into a mould to be frozen at a temperature between 0°C and −20°C overnight. Finally, the solution was lyophilized to remove the dioxane crystals.

X-ray diffraction patterns of the materials were recorded on a Rigaku D/max-RB diffractometer (Rigaku, Tokyo) using Cu K_α radiation. The specimens were vacuum-dried and gold-coated for SEM examination on a Leo 1530 (Leo, Germany). The porosity was determined via a liquid displacement method taking isopropyl alcohol (isopropanol, $\rho = 0.784$ g/mL) as

the displacement liquid. As non-solvent of the composite, isopropyl alcohol can easily penetrate the pores of the scaffold and does not induce shrinkage or swelling. The compressive mechanical property was tested on an Instron 1122 mechanical tester with a 10 kN load cell according to the guidelines set in ASTM D5024–95a.

2.2 Cell culture and co-culture with material

Osteoblasts were isolated via sequential digestions of neonatal rat calvaria according to established procedures [8,9], and cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) under standard cell culture conditions (i.e., 37°C, humidified, 5% CO_2). Osteoblasts were used at population numbers 2−4. The composite was cut into a disc 1−2 mm in thickness, and then sterilized by γ-ray irradiation (2.5 Mrad). The osteoblasts were seeded into the dish composite 3.5 cm in diameter at a concentration of 5×10^4 cells/cm^2. The medium was changed twice a week, and routinely examined using phase contrast microscopy.

Samples for SEM were fixed with 2.5% glutaraldehyde in 0.1 mol/L phosphate buffered saline (PBS) (pH = 7.4), followed by 1% osmium tetroxide in acetone. The specimens were dehydrated through a graded series of ethanol and acetonitrile, vacuum-dried and gold-coated for examination on a Leo 1530 (Leo, Germany).

2.3 Total protein assay

Osteoblasts (4×10^3 cells/cm^2) were seeded in the 24-well plate and cultured in the DMEM + 10% fetal calf serum (FCS) medium under the standard cell cultured conditions for 2, 4, 6 days. At the end of the prescribed time, osteoblasts were trypsinized using 0.25% trypsinase from each substrate. Total protein content in the cell lysates was determined on a spectrophotometer [4]. For this purpose, aliquots of each protein-containing, distilled-water supernatant were incubated with a solution of 1 mol/L NaOH at 100°C for 30 min, and stained bicinchoninic acid (BCA) protein assay reagent (BCA Kit No. 23225, PIERCE). Light absorbance of these samples was measured at 562 nm on an Ultrospec 3100 pro UV/visible Spectrophotometer (Biochrom Ltd., England). Total intracellular protein (expressed as μg) synthesized by osteoblasts cultured in the medium was determined from a standard curve of absorbance versus known concentrations of albumin by BCA kit. Total protein synthesized by osteoblasts cultured in DMEM (supplemented with 10% FBS) served as blank control.

3 Results and discussion

The phase development in the composite is shown in Fig. 1. The PLA peaks appear only at 16.6° and 18.9°. Compared with the HA crystal, the broadening of the diffraction peaks of HA-PLA implies a small grain size and low crystallinity. The pattern of the HA-PLA presents the broadening of the peaks, which is also similar to the pattern of the natural bone [10,11]. The biomimetic synthesis of HA helps us to obtain the *n*-HA that keeps the initial state in the composite. The scaffold materials prepared by the freeze-drying technique consist of n-HA and PLA.

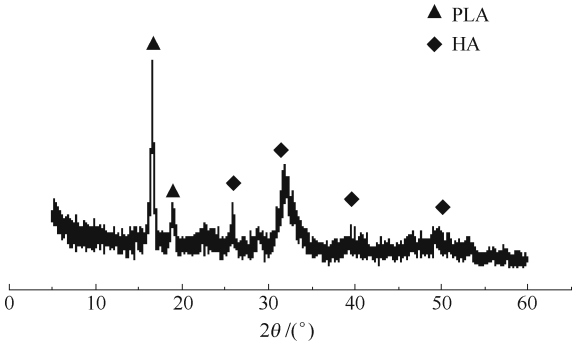

Fig. 1 X-ray diffraction results of the HA-PLA composite

Table 1 provides the comparable results of the pure PLA and the HA-PLA composite. The compressive strength and elastic modulus values of the composite is distinctly about two times higher than that of the PLA, while the porosities of both materials have almost the same level. High porosity of materials is one of the essential factors for bone tissue engineering. The interconnected pores also provide the tunnels to carry the nutrient and cell in vivo [12]. By adding the n-HA particles, the composite can be strengthened and shows a performance more similar to that of natural bone tissues. The composite also has good toughness useful in clinical application. The excellent forming ability of this composite is another advantage for clinical application, while bio-ceramics with well-crystallized and coarse grains are too hard for remedy materials. Moreover, the n-HA is also biodegradable, while the degradation of normal tissue culture polystyrene (TCP) and HA ceramics is too slow to be measured. The high porosity makes it possible to remove the outcome current of the composite degradation in vivo.

Table 1 The property results of pure PLA and HA-PLA

Sample	Strength σ /MPa	E /MPa	Porosity /%
PLA	0.89	15.2	91.8
HA-PLA	2.07	30.1	86.1

The microstructures of the two scaffold materials are shown in Figs. 2 and 3. The pore sizes of the two materials also stay at the range of 100−300 μm. It has been suggested that the pore size range of 100−400 μm is preferred by osteoblasts because it provides the optimum compression and tension performance on the osteoblast mechanoreceptors [13]. The pore walls of the PLA scaffold appear more continuous than that of the HA-PLA composite. In other words, there are more little pores on the large pore walls in the composite,

which will help the inter-connection of different pores. The roughness of the composite is higher than that of the pure PLA scaffold material.

Fig. 2 SEM micrograph of the pure PLA scaffold

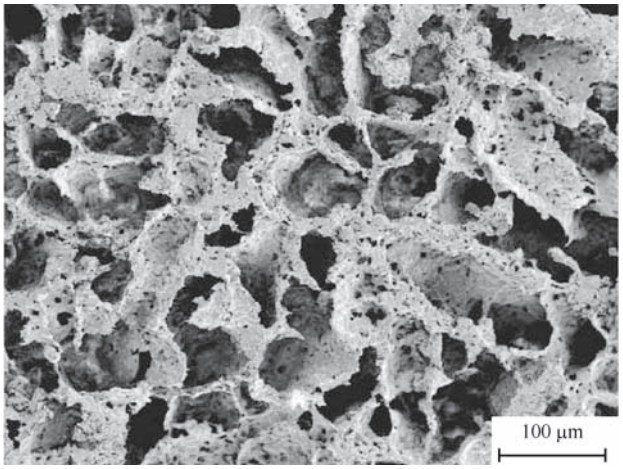

Fig. 3 SEM micrograph of the HA-PLA composite scaffold

In order to investigate the cell affinity of such two scaffolds, we seeded osteoblasts on them. Figures 4 and 5 show the cells on the scaffolds by one-week culture. The arrows in the two figures are directed towards the osteoblasts on the materials. Although all filopodia are developed by the cells, on the PLA scaffold, cells adhered on the surface only by filopodia; but on the HA-PLA composite, there were whole cells including the filopodia, cytoplast and nucleus. Thus, the reaction with high cell affinity for the composite has been clearly demonstrated. In the culture media, the composite surface gradually becomes smoother because of biodegradation. In the process of co-culture, the outcome of the biodegradation did not apparently change the pH value, which is one of the essential factors for keeping the normal growth of the cells. From preliminary studies, the biodegradation behaviors of HA-PLA maintain a steady rule that matches the bone-assembly processes. The possible reason is that the acidic outcome of the PLA degradation could be neutralized by partial alkaline substances of the n-HA degradation in this composite. This character of the composite may decrease the inflammatory response of the polymer in vivo.

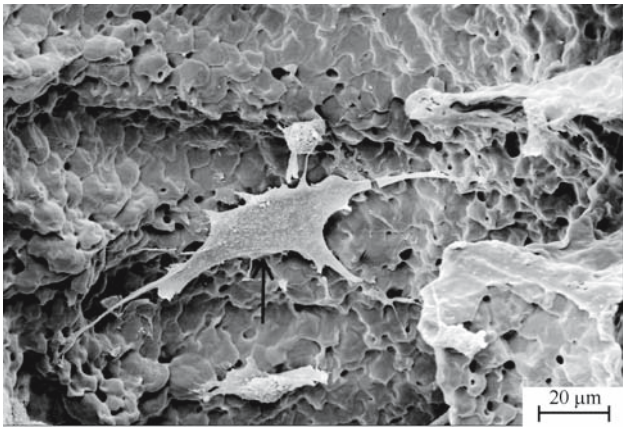

Fig. 4 The osteoblast (directed by an arrow) on the PLA scaffold

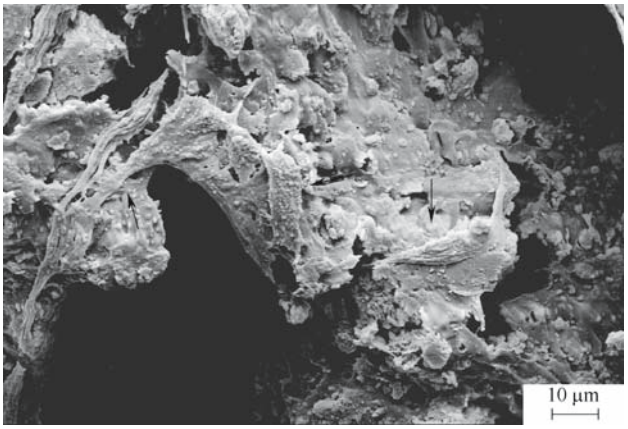

Fig. 5 The osteoblasts (directed by arrows) on the HA-PLA scaffold

In order to confirm the normal activity of osteoblasts on the scaffold materials, we compared the protein content of the cells on pure PLA and HA-PLA scaffolds with that of the blank control. Table 2 shows the changes of protein content for the above three groups. The lowest value of HA-PLA at 2 days increases to the highest value at 4 days, but decreases in the following days. The increase may be due to the fast growth of the cells at this time, while the cells on the PLA maintain a smooth growth. In short, both PLA and HA-PLA have high compatibility for their protein content values comparable to the blank control for the whole experimental period.

Table 2 Total protein contents on the scaffold /μg

Sample	2 /days	4 /days	6 /days
Blank control	61.5	71.4	68.0
PLA	73.2	75.6	65.2
HA-PLA	54.4	78.6	62.4

4 Conclusions

Based on the PLA scaffold material, we have fabricated a new type of nano-composite scaffold HA-PLA by biomimetic

strategy for bone tissue engineering. The composite shows some features of natural bones in both composition and microstructure. Moreover, both the compressive mechanical property and the cell affinity of the composite are higher than those of the PLA scaffold. By cell culture, it has been demonstrated that the composite is biocompatible and biodegradable. The HA-PLA composite scaffold is proved to be a promising material for bone tissue engineering.

Acknowledgements This work was supported by the National High-technology Foundation of China (863, No. 2001AA320605), and the National Natural Science Foundation of China (Grant No. 20031010).

References

1. Stock U A, Vacanti J P. Tissue engneering: current state and prospects. Annu Rev Med, 2001, 52:443
2. Langer R, Vacanti J P. Tissue engineering. Science, 1993, 260: 920
3. Robert F. Tissue engineers build new bone. Science, 2000, 289: 1498
4. Webster T J, Siegel R W, Bizios R. Osteoblast adhesion on nanophase ceramics. Biomaterials, 1999, 20: 1221
5. Chen G P, Ushida T, Tateishi T. Development of biodegradable porous scaffolds or tissue engineering. Mater Sci Eng C, 2001, 17: 63
6. Du C, Cui F Z, Zhang W, Feng Q L, Zhu X D, de Groot K. Formation of calcium phosphate/collagen composites through mineralization of collagen matrix. J Biomed Mater Res, 2000, 50: 518
7. Wang R Z, Cui F Z, Lu H B, Wen H B, Ma C L, Li H D. Synthesis of nanophase hydroxyapatite collagen composite. J Mater Sci Lett, 1995, 14: 490
8. Ishuaug S L, Yaszemski M J, Bizios R, Mikos A G. Osteoblast function on synthetic biodegradable polymers. J Biomed Mater Res, 1994, 28: 1445
9. Puleo D A, Holleran L A, Doremus R H, Bizis R. Osteoblast responses to orthopaedic implant materials in vitro. J Biomed Mater Res, 1991, 25: 711
10. Mitchell R. An evaluation of bone healing in cavities in the jaws implanted with a collagen matrix. Br J Oral Maxillofac Surg, 1992, 30: 180
11. Landis W J, Song M J, Leith A, et al. Mineral and organic matrix interaction in normally calcifying tendon visualized in three dimensions by high-voltage electron microscopic tomography and graphic image reconstruction. J Struct Biol, 1993, 110: 39
12. Shea L D, Wang D, Fraceschi R T, Mooney D J. Engineering bone development from a pre-osteoblast cell line on three-dimensional scaffolds. Tissue Engineering, 2000, 6: 605
13. Dennis J E, Haynesworth S E, Young R G, Caplan A I. Osteogenesis in marrow-derived mesenchymal cell porous ceramic composites transplanted subcutaneously: effect of fibronectin and laminin on cell retention and rate of osteogenic expression. Cell Transplant, 1992, 1: 23