Comparative study on the different molecule weight of poly-L-lactic acid in the biological function of composite materials☆

Zhang Ning, Li Yun-sheng

Abstract

BACKGROUND: With the development of technology, poly-L-lactic acid/β-tricalcium phosphate composite materials show good characters in the tissue engineering, which can promote osteoblast proliferation, reduce rejection reactions, and improve bone healing in a dose-dependent manner.

OBJECTIVE: To test the influence of poly-L-lactic acid with different molecule weights on the structure and function of poly-L-lactic acid/β-tricalcium phosphate composite scaffolds.

METHODS: Poly-L-lactic acid with molecule weights of 200 000 and 380 000 were combined with β-tricalcium phosphate to produce composite scaffolds by using freeze-drying method. Porosity and pore size of the samples were measured. The fetal rabbit bone mesenchymal stem cells (BMSCs) were cultured and expanded *in vitro*. They were harvested and seeded into the prepared poly-L-lactic acid/β-tricalcium phosphate scaffolds. The 3-(4, 5-Dimethylthiazol-2yl)-2, 5 diphenyltetrazolium bromide (MTT) and alkaline phosphatase were examined for comparison between normal cultured BMSCs and those cultured on the different poly-L-lactic acid/β-tricalcium phosphate scaffolds.

RESULTS AND CONCLUSION: Images of scanning electron microscope showed that the cells adhered to the scaffolds greatly. The value of MTT and alkaline phosphatase showed no significant difference ($P > 0.05$). The molecule weight of poly-L-lactic acid has no influence on the biological function of composite materials.

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INTRODUCTION

Grafting procedures can be improved using the principles of tissue engineering. Cells capable of osteogenic activity can be combined with an appropriate scaffold to stimulate bone regeneration and restore functional bone tissue[1-2]. Bone marrow stromal cells have osteogenic activity[3-7], and can be easily isolated and expanded *in vitro* [8-9].

β-tricalcium phosphate (β-TCP) is an osteoconductive, absorbable material, which, in porous ceramic form, is highly suitable for implants used in bone reconstruction or as bone substitutes. But it is brittle and easy to be fractured[10-12]. Biodegradable polymers, such as poly-L-lactic acid (PLLA), are evidenced to have a modulus of elasticity closer to that of natural cortical bone and can retain high strength over time[13]. But these materials can induce unspecific inflammatory tissue response[14-20], resulting in delayed bone healing/fusion or osteolytic reactions[21-26]. To overcome the disadvantages of these different materials, composite materials of calcium phosphates and polyesters have been developed and evaluated[27-35].

With the development of technology, the composite materials show good character in the tissue engineering[36]. PLLA/β-TCP composite materials as a new candidate of scaffold materials can increase phenotypic expression of osteoblasts, reduce foreign-body reaction, and improve bone healing in a dose-dependent manner as compared to pure materials. But most of previous studies focused on the ratio of two types of materials, different methods of manufacture, and biological characters. There is no report about the influence of molecular weight of PLLA on the character of the composite materials. The objective of our experiment was to test the influence of molecular weight of PLLA on the characters of the composite scaffold.

MATERIALS AND METHODS

Design

A cytology comparative observation.

Time and setting

The experiment was completed at the Department of Stomatology, China Meitan General Hospital in 2009.

Materials

Animals: Two 1-week-old male New Zealand white rabbits were purchased from the Animal Apartment, Capital Medical University.

Scaffold materials: The PLLA (average molecular weight=380 000 and 200 000) was purchased from Biochem-ZX, Sichuan; β-TCP (diameter=5 μm) purchased from Sichuan University.

Methods

Scaffold preparation

The PLLA and β-TCP were separately blended together with ratio of 3:7 by freeze-drying and phase separate method[37-38]. PLLA was dissolved in 3% dioxane (w/v), under stirring at –50 °C for at least 4 hours to obtain a homogeneous polymer solution. The solution was immediately frozen in liquid nitrogen and then was rapidly transferred into a freeze dry machine (Labconco Corporation, USA)[39]. Scaffolds were cut into round specimens with a diameter of 15 mm and thickness of 2 mm, washed carefully with distilled deionized water, and then dried

at 40 ℃ in a vacuum dryer machine. They were sterilized with ^{60}Co for further studies.

Scaffold morphology

The internal structure of the produced sponges was examined by a scanning electron microscope (Jeol JS M-5600 LV, Japan).

Rabbit mesenchymal stem cells (MSCs) culture

Femurs and tibias from rabbits were isolated and the epiphyses were removed. Cells were incubated in a complete medium. Non-adherent cells were removed 24 hours later and the adherent cells remained. The cells were maintained in culture for 7 days, digested by Trypsin, incubated in 24-well culture dishes, and then rinsed with phosphate-buffered saline solution. After that, the cells were immersed in DMEM medium overnight. According to reference[40], isolated and cultured rabbit MSCs were identified, and then divided into three groups: control group, 20 group and 38 group. The MSCs in the latter two groups were seeded on the scaffolds with cell density of 5×10^7 cells/L.

Cell morphology

After 3 hours, the scaffolds seeded with cells were fixed with 2.5% glutaraldehyde solution in phosphate buffered saline. After phosphate buffered saline rinsing and subsequent dehydration with ethanol aqueous solutions at ethanol percent of 30%, 50%, 70%, 80%, 90%, and 100%, the dehydrated samples were dried on a critical point dryer. Then they were coated with gold/palladium and sent to be examined under the scanning electron microscope.

3-(4, 5-Dimethylthiazol-2yl)-2, 5 diphenyltetrazolium bromide (MTT) assay

The other scaffolds were incubated with MSCs for 1–7 days. The presence of viable cells into the matrices was qualitatively evaluated by the use of MTT assay. The reaction product was extracted and read on a high-efficient analyzer (HTS 7000 plus, PE) at 490 nm.

Alkaline phosphatase assay

MSCs were seeded on the scaffolds with cell density of 2×10^5 cells/mL for 10 days. At 1, 4, 7, 10 days, the medium was carefully aspirated from each well. The cell lysate was used to measure alkaline phosphatase activity, using a commercial kit (Baiding, China). The assay is based on the ability of the sample to degrade a specific alkaline phosphatase substrate. Briefly, 230 μL of phosphatase substrate was added to the 20 μL of the cell lysate samples on ice, and then it was incubated on a shaker for 1 minute in a 37 °C water bath. The absorbance density was measured at 405 nm, using the high-efficient analyzer (HTS 7000 plus, PE).

Main outcome measures

Cell morphology, cell survival and the number of alkaline phosphatase expression.

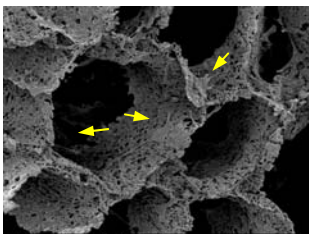
Statistical analysis

Experiments were run in triplicate per sample. All data were analyzed with SPSS 11.0 (SPSS, USA). Experimental results were expressed by mean±SD. The one-way analysis of variance and SNK inspection were done for intergroup comparison, and $P < 0.05$ was considered as a value of statistical significance.

RESULTS

Cell morphology

The images of scanning electron microscope show that the scaffolds had the interconnecting pores, which were round and homogeneous. The diameter of pore was in the range of 150–300 μm, and the porosity of materials was 70%, which were tested by liquid displacement method. We could see MSCs adhered and spread on the scaffold after 3 hours in Figure 1.



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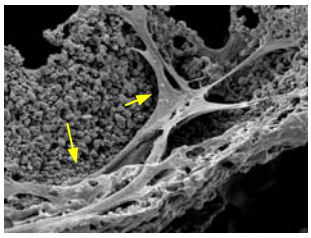

b: ×2 000

Figure 1 Mesenchymal stem cells (arrows) in 20 group adhered on the wall of pores after 3 h of culture

The number of viable cells in each group

The absorbance in MTT test for 1–7 days of the 20 group, 38 group and control group had no significant difference ($P > 0.05$; Figure 2).

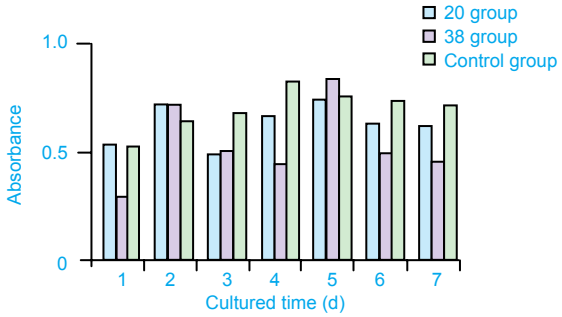

Figure 2 3-(4,5-Dimethylthiazol-2yl)-2,5 diphenyltetrazolium bromide assay of the number of viable cells in each group

The changes in activities of alkaline phosphatase

The alkaline phosphatase activities of the 20 group, 38 group and control group had no significant difference ($P > 0.05$; Figure 3).

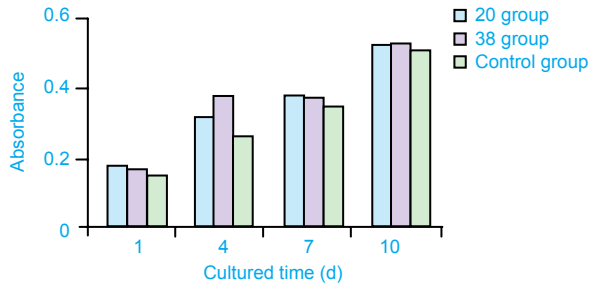

Figure 3 The activity of alkaline phosphatase in each group

DISCUSSION

Once the materials and their ratio are confirmed, the molecule weight of PLLA cannot change the biological character of the scaffolds. Molecule weight of PLLA may influence the mechanical property and degradation[41], but the biological function of the materials is not related to the molecule weight of PLLA[42].

The rate of degradation, however, is determined by many factors such as configurational structure, copolymer ratio, crystallinity, molecular weight, morphology, stresses, amount of residual monomers, porosity and site of implantation. Molecular weight become a more important factor determining the rate of degradation same as the other factors[43]. Slight decrease of the molecular weight of the PLLA probably leads to easier dissolution of PLLA molecules into the culture medium; but it does not increase the lactic acid concentration of the cell cultures in a short time[44]. This may be the reason of the similar results of these two groups. PLLA/β-TCP composite materials have good biological property which can act as a carrier for bone tissue engineering scaffold. The molecular weight of PLLA does not influence the biological character of the composite materials in a short time. But further experiments need to be done to investigate the influence of the molecule weight of PLLA on the other properties and biological characters *in vivo* for a long time.

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不同聚乳酸相对分子质量对其构建复合支架材料生物学功能的影响☆

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摘要

背景：聚乳酸/β-磷酸三钙复合材料作为支架，可以增加成骨细胞的增殖，减少排异反应，提高骨愈合，并具有剂量依赖性。

目的：检测不同聚左乳酸相对分子质量对于聚左乳酸-β-磷酸三钙复合支架材料功能及其结构的影响。

方法：选用相对分子质量为 200 000 和 380 000 的聚左乳酸通过冻干法与 β-磷酸三钙制备成聚左乳酸-β-磷酸三钙复合支架材料，检测样本的孔隙率和孔隙直径，将乳兔的骨髓间充质干细胞与相对分子质量为 200 000 和 380 000 的聚左乳酸构建的支架材料复合培养，并与正常培养的乳兔的骨髓间充质干细胞进行形态学、细胞增殖以及细胞中碱性磷酸酶表达水平的对比观察。

结果与结论：电镜结果显示，兔的骨髓间充质干细胞均能在相对分子质量为 200 000 和 380 000 聚左乳酸构建的支架材料表面形成很好的黏附。MTT 检测显示各组细胞培养 1~7 d 时细胞增殖差异没有显著性意义($P > 0.05$)，且各组细胞碱性磷酸酶表达水平接近($P > 0.05$)。说明含有不同相对分子质量的聚左乳酸-β-磷酸三钙复合支架材料对于兔骨髓间充质干细胞的黏附与增殖无影响。

关键词：聚左乳酸；复合材料；高分子聚合材料；相对分子质量；骨髓间充质干细胞
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伦理要求：实验符合动物伦理学要求。

本文创新性：本文研究的材料是新型聚乳酸-β-磷酸三钙复合支架材料。国内外大部分的研究都集中在不同相对分子质量对于材料机械性能及孔隙率等的影响，并未见其对于支架材料生物学性能的影响。因此本文的创新点就在于研究了不同聚乳酸相对分子质量对于聚乳酸-β-磷酸三钙复合支架材料生物学功能的影响。