Chinese Chemical Letters Vol. 17, No. 6, pp 833-836, 2006
http://www.imm.ac.cn/journal/ccl.html

A New Route for Preparation of β-TCP/ PLLA Composite

Feng ZHANG[1,4], Fang MEI[2], Xin Zhi WANG[1], Xiao Yang HU[1],
Yong Ming LUO[3], Xu Liang DENG[1]*

[1] School and Hospital of Stomatology , Peking University, Beijing 100081.
[2]Department of Histology and Embryology, Health Science Center, Peking University, Beijing 100083
[3]Department of Environmental Science and Engineering, Kunming University of Science and Technology, Kunming 650093
[4] The 2nd Hospital of Beijing Armed Police Forces, Beijing 100037

Abstract: A new facile route for preparation of β-TCP/PLLA composites is reported in this letter. SEM images display that β-TCP particles with average diameter of 400 nm were well bonded and distributed within the pore walls of the PLLA scaffolds. The mixture of the novel complex and human dental pulp cells was transplanted subcutaneously into the dorsal surface of a nude mouse. Two months later histological examination showed that new collagen and new dentin formed. The results revealed that the new nano β-TCP/PLLA composite combined with human pulp cells could induce dentin formation, offering a new way to dental tissue engineering.

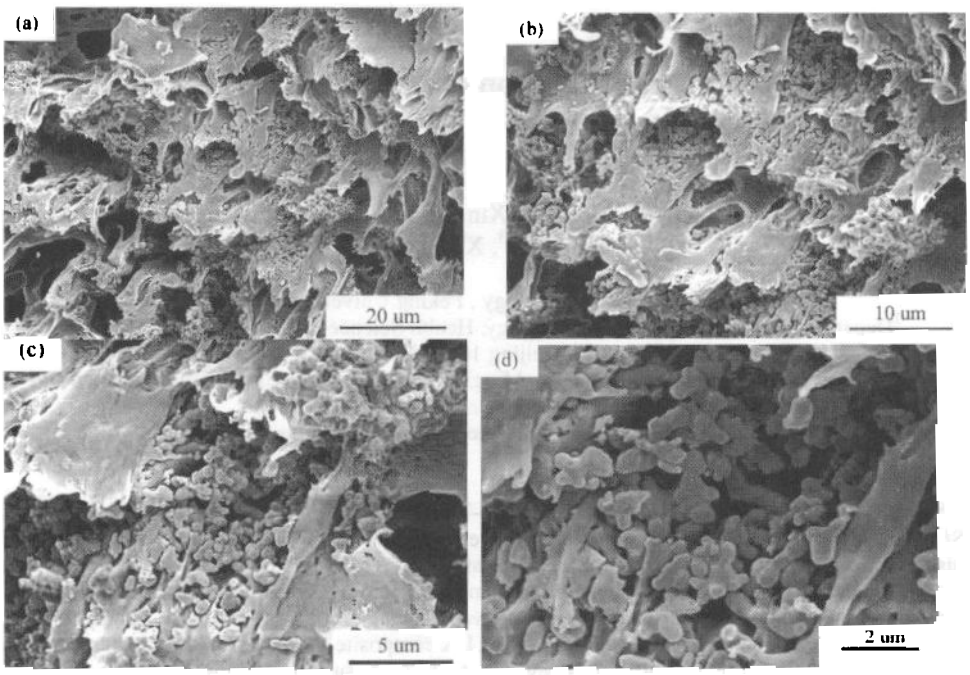
Keywords: β-TCP particles, β-TCP /PLLA composite, dental pulp cells, dental tissue engineering.

Bone tissue engineering offers a promising route to repair and replace damaged or diseased tissues in the cases including trauma, congenital and degenerative diseases, cancer, and so on. Polylactic acid(PLLA)-based materials showing bioresorbability are used for applications such as bone plates or temporary internal fixation of broken or damaged bones[1], and the bioresorption rate can be easily controlled by adjusting the degree of polymerization or copolymerization. Calcium phosphate ceramics(CPCs) such as hydroxyapatite(HA), β-tricalcium phosphate(β-TCP), and calciummetaphosphate (CMP) are well known as bioactive ceramics and are used in medical and dental fields in the form of blocks and particles. However, these ceramics are brittle and not suitable for medical use as structural materials[2]. Therefore, much effort has been devoted to synthesize calcium phosphate ceramic and PLLA composites including β-TCP/PLLA[3], HA/PLLA[2,4-6], CMP/PLLA[7], *et al.*. Among these composites, particular attention has been placed to β-TCP/PLLA due to their outstanding biological responses to physiological environments[5]. On the other hand, TCP has been proved to be resorbed *in vivo* with new bone growth. Synthesis and evaluations of β-TCP/PLLA has been reported[2,3]. However, in order to obtain high quality β-TCP, high calcination temperature is indis-

* E-mail: dengxuliang@vip.sina.com

pensable for the previous synthetic methods, which will lead to β-TCP particle agglomeration and have disadvantage impact on their osteoconductivity and bioresorbability.

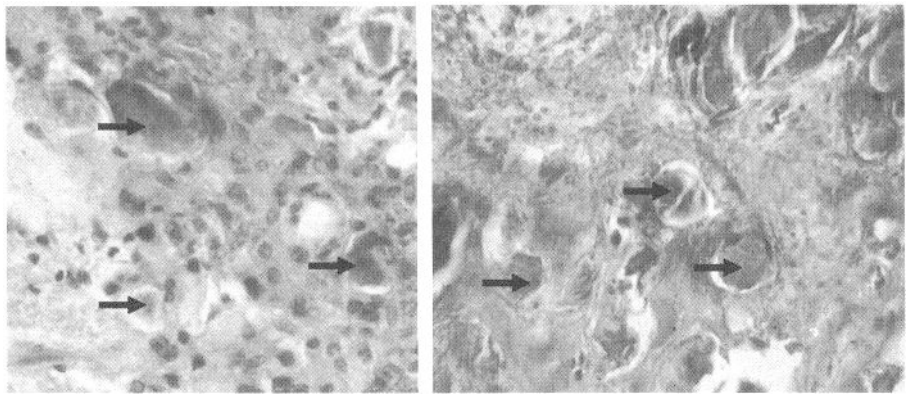
Figure 1 SEM images of β-TCP / PLA composites at different magnifications



In this letter, the preparation of β-TCP/PLLA composite involves two steps: the first step consists of the preparation of β-TCP particles at room temperature (RT,~25℃); the second step is preparation of β-TCP/PLLA composite. In the first step, 0.01mol H_3PO_4 was first dissolved in anhydrous methanol. Then, 0.015 mol $(CH_3COO)_2Ca{\cdot}xH_2O$ powder was slowly added into the mixture solution to keep Ca/P molar ratio of 1.5 with constant vigorous stirring for 12h, and the reaction product was aged for 24h. All the above procedures were processed at RT. Subsequently, the product was filtered and dried at 80℃ overnight. In the second step, β-TCP particles(300-500 nm in diameter) were dispersed in chloroform solution by sonication. After that, PLLA [MW=3.74×10^5] was dissolved in the β-TCP suspended solvent at about 60℃ to make homogeneous solutions, and the ratio of PLLA/β-TCP was 75/25 by weight. The mixture was rapidly put into a teflon vial and transferred into liquid nitrogen to induce solid-liquid or liquid phase separation for 10-30 minutes. Finally, the solidified mixture was freeze-dried to remove solvent.

The XRD patterns for as-synthesized β-TCP particles (not shown) exhibited that all the peak positions were in good agreement with those for β-TCP powder obtained from the Joint Committee on Powder Diffraction Standards Card (No: 9-169). No peaks assigned to the other materials and TCP crystalline forms were detected in the patterns. These results implied that the product contains only β-TCP one crystalline phase (a =10.42 Å, c = 37.38), which was formed at RT in anhydrous methanol media.

Figure 2 Histological examination images of transplantation site



The morphology and microstructure of the composites were measured with SEM, as shown in **Figure 1(a,b,c,d)**. It can be seen that the β-TCP particles with average diameter of 400nm were distributed within the pore walls of the PLLA scaffolds and no large aggregates appeared in the pores. As can be clearly seen from **Figure 1d**, no β-TCP particles observed in the scaffold surface indicated that the particles were well bonded with PLLA scaffolds.

Dental pulp cells were obtained from one tooth of an 11-year-old patient extracted for orthodontic treatment. Approximately 4×10^6 DPSCs population were mixed with 40mg β-TCP/PLLA composite and then transplanted subcutaneously into the dorsal surface of 10-week-old immunocompromised beige mice[9,10]. Two months later, histological examination was carried out. The new formed dentin was shown in **Figure 2a**. Sample was stained with hematoxylin and eosin: azury homogeneous area is the new dentin formation (arrows). In **Figure 2b**, sample was stained with mallary: homogeneous black areas are the new dentin formation (arrows). All the results showed the new β-TCP / PLLA composite was a choice for dental tissue engineering scaffold.

In conclusion, a new and facile route for preparation of β-TCP nano-particles has been performed at room temperature, and the particles were well distributed within the pore walls of the PLLA scaffolds by sonication to form β-TCP/PLLA composite. The animal test resulted that the composites combined with human pulp cells induced new dentin formation, indicating the composites offered a new way to dental tissue engineering.

Acknowledgments

We gratefully acknowledge the support of this research work by National High Technology Research and Development Program of China (No. 2003AA302220, 2004AA304G50) and the National Natural Science Foundation of China (No. 30471907).

References

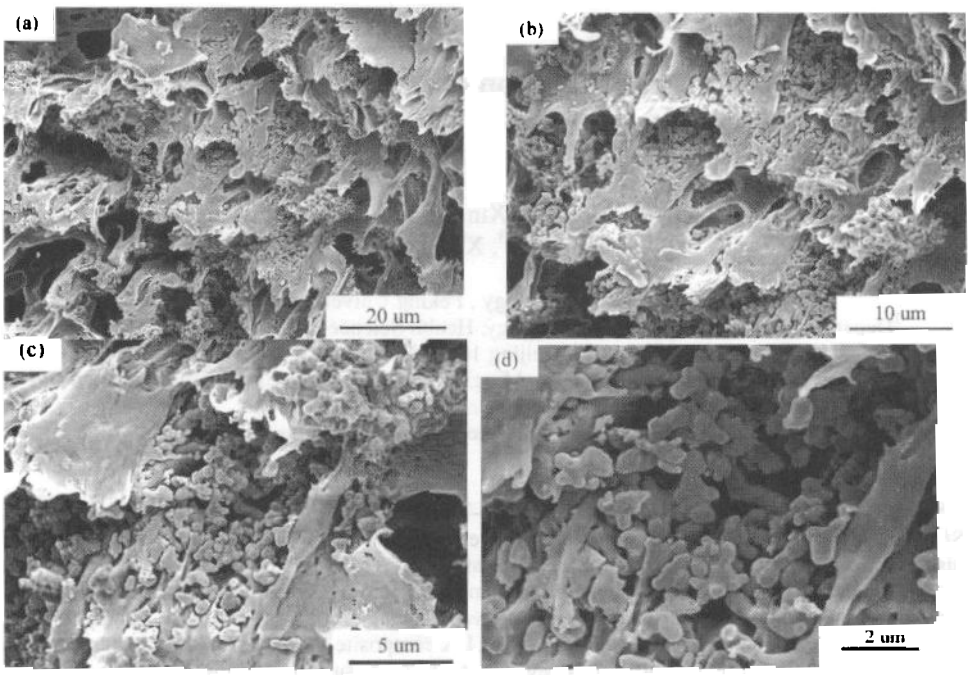
1. S. Vainionpää, P. Rokkanen, P. Törmälä, *Prog. Polym. Sci.*, **1989**, *14*, 679.
2. M. Kikuchi, Y. Suetsugu, J. Tanaka, M. Akao, *J. Mater. Sci. Mater. Med.*, **1997**, *8*, 361.
3. S. J. Lee, Y. J. Park, S. N. Park,*et al.*, *J. Bio. Med. Mater. Res.*, **2001**, *55*, 295.
4. S. Higashi, T. Yamamuro, T. Nakamura, *et al.*, *Biomaterials*, **1986**, *7*, 183.
5. G. B. Wei, P. X. Ma, *Biomaterials*, **2004**, *25*, 4749.
6. T. Kasuga, Y. Ota, M. Nogami, Y. Abe, *Biomaterials*, **2001**, *22*, 19.
7. Y. Jung, S. S. Kim, Y. H. Kim, *et al.*, *Biomaterials*, **2005**, *26*, 6314.
8. N. Kivrak, A. C. Tas, *J. Am. Ceram. Soc.*, **1998**, *81*, 2245.
9. S. Gronthos, J. Brahim, W. Li, L. W. Fisher, *et al.*, *Journal of Dental Research*, **2002**, *81*, 8, ProQuest Medical Librarypg., 531.
10. P. H. Kresbach, S. A. Kuznetsov, K. Satomura, *et al.*, *Transplatation*, **1997**, *63*, 1059.

Received 7 November, 2005

1.
2.
3.
4.
5.
6.

pensable for the previous synthetic methods, which will lead to β-TCP particle agglomeration and have disadvantage impact on their osteoconductivity and bioresorbability.

Figure 1 SEM images of β-TCP / PLA composites at different magnifications



In this letter, the preparation of β-TCP/PLLA composite involves two steps: the first step consists of the preparation of β-TCP particles at room temperature (RT,~25℃); the second step is preparation of β-TCP/PLLA composite. In the first step, 0.01mol H_3PO_4 was first dissolved in anhydrous methanol. Then, 0.015 mol $(CH_3COO)_2Ca{\cdot}xH_2O$ powder was slowly added into the mixture solution to keep Ca/P molar ratio of 1.5 with constant vigorous stirring for 12h, and the reaction product was aged for 24h. All the above procedures were processed at RT. Subsequently, the product was filtered and dried at 80℃ overnight. In the second step, β-TCP particles(300-500 nm in diameter) were dispersed in chloroform solution by sonication. After that, PLLA [MW=3.74×10^5] was dissolved in the β-TCP suspended solvent at about 60℃ to make homogeneous solutions, and the ratio of PLLA/β-TCP was 75/25 by weight. The mixture was rapidly put into a teflon vial and transferred into liquid nitrogen to induce solid-liquid or liquid phase separation for 10-30 minutes. Finally, the solidified mixture was freeze-dried to remove solvent.

The XRD patterns for as-synthesized β-TCP particles (not shown) exhibited that all the peak positions were in good agreement with those for β-TCP powder obtained from the Joint Committee on Powder Diffraction Standards Card (No: 9-169). No peaks assigned to the other materials and TCP crystalline forms were detected in the patterns. These results implied that the product contains only β-TCP one crystalline phase (a =10.42 Å, c = 37.38), which was formed at RT in anhydrous methanol media.