CHINESE JOURNAL OF ANALYTICAL CHEMISTRY
Volume 42, Issue 2, February 2014
Online English edition of the Chinese language journal

Cite this article as: Chin J Anal Chem, 2014, 42(2), 221–226.

RESEARCH PAPER

Characterization of *In vitro* Mineralization of Porous Poly (*L*-Lactic Acid)/Bioactive Glass Composites by Attenuated Total Reflectance-Fourier Transform Infrared Mapping

WANG Qian, JIANG Xiao-Ting, XIN Yun-Zi, CUI Jun-Xian, ZHANG Pu-Dun*
School of Science, Beijing University of Chemical Technology, Beijing 100029, China

Abstract: Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) mapping was used to characterize the mineralization of porous poly (*L*-lactic acid)/ bioactive glass (PLLA/BG) composite in PBS solution. FTIR images were generated on the basis of the ratio of the absorbance of the band at 1044 cm^{-1} to the one at 1755 cm^{-1} (A_{1044}/A_{1755}) at a resolution of 8 cm^{-1} with 8-coadded scans. The imaging results showed that the amount of hydroxyapatite (HA) on the surface of the composites increased with soaking time and most of the surface area was covered by HA after being mineralized for 84 d. However, the heterogeneous mineralization increased with time lasted. The mineralization curve from the average spectra further suggested that there were four stages during mineralization. At the initial one (less than 21 d), the amounts of HA were small; at the growth one (21–70 d), BG was gradually converted to HA; at the rapid growth one (70–91 d), the mineralization was accelerated and reached up to the maximum at 91 d; and at the late stage (over 91 d), the curve became steady at first and then declined after 105 d. The results suggested that ATR-FTIR mapping was promised to be an important tool for study of the scaffold in bone tissue engineering.

Key Words: Fourier transform infrared mapping; Attenuated total reflectance; Poly (*L*-lactic acid); Bioactive glass; *In vitro* mineralization

1 Introduction

Different from other imaging techniques (e.g. electron microscopy and fluorescence microscopy), chemical imaging is used to visualize the chemical distribution of the components in a micro-domain. It contains Fourier transform infrared (FTIR) and Raman imaging. FTIR imaging appeared nearly two decades ago, and it expands FTIR technique from "point" analysis to "plane", even "stereoscopic" analysis. FTIR technique was used to study the chemical heterogeneity of a micro-region with hundreds to thousands of microns. It can be accomplished by mapping or imaging[1]. It is also suited to acquiring images for a sample with rough surface by attenuated total reflectance (ATR)-FTIR mapping[2]. FTIR imaging or mapping was applied in a number of fields, including polymers[3–6], life sciences[7,8], biomedical studies[9], archaeology[10] and forensic science[11] etc.

As a kind of bone tissue engineering material[12], bioactive glass (BG) was reported to stimulate osteogenesis *in vitro* by inducing the proliferation and osteogenic differentiation of human osteoblasts[13]. However, because of the poor processing performance, BG is usually fabricated into porous composite scaffolds by blending with natural or non-toxic synthetic polymer. Porous structure is favorable for cell growth and proliferation and for the deposition of extracellular matrix. It also facilitates the access of nutrient and oxygen and the drain of metabolites. In addition, porous composites may provide a temporary scaffold to guide the ingrowth of vascular and neural tissues[14]. When the composites are implanted into living body, BG will be gradually transformed into hydroxyapatite (HA) that is bonded strongly with bone tissue, while the polymer is gradually degraded. Due to the

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Received 26 March 2013; accepted 29 September 2013
* Corresponding author. Email: zhangpd@mail.buct.edu.cn

DOI: 10.1016/S1872-2040(13)60713-6

difficulties of in vivo study, *in vitro* mineralization under the biomimetic condition is often used[15]. The *in vivo* bioactivity of the composite material can be predicted well from the *in vitro* results.

At present, the mineralization of the composite is usually characterized by scanning electron microscopy (SEM) combined with energy dispersive X-ray analysis (EDX), X-ray diffractive (XRD) analysis and infrared spectroscopy[16]. These developed methods could not show the chemical changes of compositions in a micro region, but only provide the information on changes of morphology, elemental compositions, total crystallization and total components. In this study, the changes of chemical constituents of the porous composites of poly(L-lactic acid)/bioactive glass (PLLA/BG) were systematically investigated by ATR-FTIR mapping during the *in vitro* mineralization. The changes of the morphology were also monitored by SEM.

2 Experimental

2.1 Instruments, materials and reagents

A Nexus 8700 FTIR spectrometer coupled with a Continuμm XL FTIR imaging microscope (Thermo Electron, USA) was used to acquire the FTIR maps. An S-4700 field emission scanning electron microscope (SEM, Hitachi, Japan) was used to observe the morphology changes of the materials. The mineralization experiment was conducted in a DK-420S three-way water bath (Shanghai Jinghong, China). Poly (*L*-lactide) with an inherent viscosity (η) of 1.22 dl g^{-1} and a weight-average molecular weight (M_w) of about 121000 g mol^{-1} was purchased from Jinan Daigang Biomaterials Co. Ltd. (Jinan, China). BG powder was obtained from NovaMin Technology, Inc. (FL, USA). 1,4-Dioxane, ethanol, $AgNO_3$ and all the reagents for prepared phosphate buffered saline (PBS) solution were of analytical grade.

2.2 Preparation and *in vitro* mineralization of porous PLLA/BG composites

Similar to the reference[17], solvent casting-salt leaching method was applied to prepare the porous PLLA/BG composites in this experiment. Briefly, 3 g of PLLA were dissolved with 30 mL 1,4-dioxane at room temperature, and 3 g of BG were also homogeneously suspended in another 30 mL 1,4-dioxane using an ultrasonicator. The two solutions were then mixed under vigorous magnetic stirring for 30 min. Then 24 g of ground NaCl powder with particle size of 150–250 μm (80–100 mesh) were added into mixed solution and stirred for 30 min. The as-prepared mixture was cast onto several petri dishes to a certain thickness and dried under natural conditions for 24 h. The dried slices were immersed in deionized water to remove the salt. The water was replaced every 12 h till the residual chloride ion was no longer detected (using 0.1 M $AgNO_3$ solution). Finally, the obtained porous composites were dried in a vacuum oven at 45 ºC for 48 h.

The prepared composite materials were soaked in a beaker with 250 mL PBS (pH 7.4), and then placed in a water bath at 37 ºC for mineralization. A few samples were removed every 7 d from the buffer and then were rinsed with deionized water to remove the residual PBS salts. The adsorbed water on the surface was then absorbed using filter paper and the samples were dried in a vacuum oven at 45 ºC for 24 h. The PBS solution was also replaced by the fresh one every 7 d.

2.3 ATR-FTIR mapping

After soaking in PBS for serial days, PLLA/BG samples were cut into sheets with size of 1.5 cm × 1.5 cm and fixed onto a clean glass slide which was placed on the sample stage of the imaging system. MCT detector was cooled by liquid nitrogen. Before mapping, a slide-on silicon ATR accessory fitted to a 15 × IR objective (N.A. = 0.58) was pulled out and let IR beam focus on the sample. The mapping area (100 μm × 100 μm) was thus selected and the ATR crystal was then pulled in. FTIR images were acquired at a resolution of 8 cm^{-1} in the wavenumber range of 4000–650 cm^{-1} with 8 co-added scans for samples and 64 scans for background. The acquiring, the processing and the analysis of the FTIR images were implemented using the Atlμs software of Omnic 7.2 (Thermo Electron).

2.4 Scanning electron microscopy

The dried sample was fractured after being immersed into liquid nitrogen and was fixed onto the sample stage using conductive adhesive and was then sputter-coated with Pt under high vacuum. After different mineralization days, the morphology of PLLA/BG composites was observed at 20 kV and 10 μA.

3 Results and discussion

3.1 FTIR analysis of PLLA/BG composites

In PBS solution, PLLA could be degraded into lactic acid, CO_2 and H_2O, which causing the difficulty in direct detecting PLLA, and BG could be gradually mineralized to HA[12,13], a major insoluble inorganic element ($\lg K_{sp(HA)} = -57.5$[18] at 25 ^{o}C) of human bones and teeth with high crystallinity. The micro ATR-FTIR spectra of PLLA, BG and HA are shown in Fig.1. The characteristic absorption bands of PLLA include the ones at 1755 cm^{-1} ($\nu_{C=O}$), 1184 cm^{-1} ($\nu_{as(C\text{-}O\text{-}C)}$), 1090 cm^{-1} ($\nu_{s(C\text{-}O\text{-}C)}$) and the shoulder band at 1044 cm^{-1} ($\nu_{C\text{-}CH3}$)[19]. BG is a sintered amorphous powder of several inorganic chemicals and the weak band at 1595 cm^{-1} was typical for characterisation[20]. However, the wide bands at 1460 cm^{-1}