Acta Biomaterialia 10 (2014) 3295–3306

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Stimulation of osteogenic and angiogenic ability of cells on polymers by pulsed laser deposition of uniform akermanite-glass nanolayer

Chengtie Wu [a,*], Dong Zhai [a], Hongshi Ma [a], Xiaomin Li [a], Yali Zhang [a], Yinghong Zhou [b], Yongxiang Luo [a], Yueyue Wang [a], Yin Xiao [b], Jiang Chang [a,*]

[a] State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai 200050, China
[b] Institute of Health & Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland 4059, Australia

ARTICLE INFO

Article history:
Received 9 December 2013
Received in revised form 27 March 2014
Accepted 31 March 2014
Available online 12 April 2014

Keywords:
Bone regeneration
Pulsed laser deposition
Polymers
Osteogenic
Angiogenic

ABSTRACT

Polymer biomaterials have been widely used for bone replacement/regeneration because of their unique mechanical properties and workability. Their inherent low bioactivity makes them lack osseointegration with host bone tissue. For this reason, bioactive inorganic particles have been always incorporated into the matrix of polymers to improve their bioactivity. However, mixing inorganic particles with polymers always results in inhomogeneity of particle distribution in polymer matrix with limited bioactivity. This study sets out to apply the pulsed laser deposition (PLD) technique to prepare uniform akermanite ($Ca_2MgSi_2O_7$, AKT) glass nanocoatings on the surface of two polymers (non-degradable polysulfone (PSU) and degradable polylactic acid (PDLLA)) in order to improve their surface osteogenic and angiogenic activity. The results show that a uniform nanolayer composed of amorphous AKT particles (~30 nm) of thickness 130 nm forms on the surface of both PSU and PDLLA films with the PLD technique. The prepared AKT-PSU and AKT-PDLLA films significantly improved the surface roughness, hydrophilicity, hardness and apatite mineralization, compared with pure PSU and PDLLA, respectively. The prepared AKT nanocoatings distinctively enhance the alkaline phosphate (ALP) activity and bone-related gene expression (ALP, OCN, OPN and Col I) of bone-forming cells on both PSU and PDLLA films. Furthermore, AKT nanocoatings on two polymers improve the attachment, proliferation, VEGF secretion and expression of proangiogenic factors and their receptors of human umbilical vein endothelial cells (HUVEC). The results suggest that PLD-prepared bioceramic nanocoatings are very useful for enhancing the physicochemical, osteogenic and angiogenic properties of both degradable and non-degradable polymers for application in bone replacement/regeneration.

1. Introduction

Synthetic polymers, such as non-degradable polysulfone (PSU) and degradable polylactic acid (PDLLA), polyglycolide (PGA) as well as their copolyesters poly(lactic-*co*-glucolic) acid (PLGA) have been widely used for bone replacement and regeneration [1–5]. These materials are generally biocompatible, with unique mechanical properties and excellent workability. However, a number of problems have been encountered regarding the use of these polymers in bone replacement and regeneration: for example, the main problems associated with these polymers are that they are quite hydrophobic and lack bioactivity, including osteogenic and angiogenic activity [6–9]. How to improve the surface bioactivity of these polymers, such as osteogenic and angiogenic properties, still remains significantly challenging.

One useful method to solve these problems is to modify the surface of polymers using functional groups, such as amino acids [10], silanol groups [11], heparin [12] and gelatin [13]. Although the functional groups on the polymers can enhance the hydrophilicity and cell attachment, the method cannot effectively solve the problem that the release of acidic degradation by-products from polymers lead to a decreased pH value in the vicinity of the implants, which may lead to inflammatory response [14,15]. Another common method of improving the bioactivity of polymer is to prepare inorganic/organic composite materials, in which most studies focus on the incorporation of bioactive inorganic particles into the matrix of polymers. However, the mixture of inorganic particles with polymers results in inhomogeneity of particle distribution in polymer matrix with limited bioactivity [16,17]. Although

* Corresponding authors. Tel.: +86 21 52412249; fax: +86 21 52413903 (C. Wu). Tel.: +86 21 52412804; fax: +86 21 52413903 (J. Chang).
E-mail addresses: chengtiewu@mail.sic.ac.cn (C. Wu), jchang@mail.sic.ac.cn (J. Chang).

http://dx.doi.org/10.1016/j.actbio.2014.03.035
1742-7061/

inorganic/organic hybrid composites can improve the homogeneity of particle distribution in the matrix of polymers by in situ compositing method [18], their bioactivity is compromised owing to the shield effect of polymers on the bioactive inorganic phase. To effectively enhance the bioactivity of polymers, the biomimetic method was mostly used to deposit apatite on the surface of polymers by soaking them in saturated SBF [19–21]. Although the method could improve the surface bioactivity of polymers, the apatite phase formed was not always uniform, with poor bonding with the polymers. Furthermore, since the prepared apatite coatings on bioploymers by the biomimetic method have non-uniform microstructures, it is difficult to modulate the cell response on the coated polymers.

Pulsed laser deposition (PLD) is a technique specially used to prepare thin films of materials with complex stoichiometry for the application of semiconductors [22]. Using this method, it is easy to prepare uniformly nanosized ceramic films on the semiconductor substrates. PLD also allows selective coating of the substrate area with a desired shape, and ensures that the chemical composition of the starting materials and the films remains basically the same [23–25]. In addition, the degree of crystallization of the prepared films by PLD can be easily controlled by selecting different treating temperatures. Because of these important advantages of PLD, previous studies have applied the technique to prepare bioceramic coatings, such as hydroxyapatite and pseudowollastonite, on Ti alloys for improving their surface bioactivity for orthopedic application [26–28]. However, there are few studies on applying this technique to prepare nanosized bioactive glass coatings on polymers. For this reason, it is of great interest to apply the PLD technique to prepare uniform bioglass nanocoatings on the surface of polymers to enhance their bioactivity, including osteogenic and angiogenic properties.

In the past several years, silicate bioceramics, as a new family of biomaterials, have received significant attention for application in hard tissue regeneration [29–31]. Among these silicate bioceramics, akermanite, a compound containing Ca, Mg and Si, has shown the most distinct bioactivity in enhancing the proliferation and osteogenic differentiation of several stem cells, such as bone mesenchymal stem cells, periodontal ligament cells and adipose-derived stem cells by the released ionic products of the ceramic [32–36]. Previous studies suggest that the compositions containing Ca, Mg and Si are of great importance for inducing osteogenic differentiation of stem cells. Based on the advantages of the PLD technique and bioactive akermanite ceramics, it is reasonable to speculate that the combination of the PLD technique with akermanite components may lead to uniform nanocoatings on the surface of PSU and PDLLA, with significantly improved osteogenic and angiogenic differentiation. Therefore, the aim of this study is to prepare uniform akermanite glass nanocoatings on both PSU and PDLLA by the PLD method, and to systematically investigate the effect of nanocoatings on the physicochemical, osteogenic and angiogenic properties of two typical polymers with degradable and non-degradable properties.

2. Materials and methods

2.1. Preparation of PSU and PDLLA films

Two grams of PSU or PDLLA was added to 30 ml of chloroform with a concentration of 6.67% (w/v) followed by continuous stirring for 1 h. The suspensions were cast on a Teflon mold with a diameter of 10 cm, followed by standing in fume hood for 48 h to evaporate the solvent, and subsequently in a vacuum oven at room temperature for 24 h to eliminate the remaining solvent.

2.2. Preparation, characterization and surface mechanical strength of AKT-PSU and AKT-PDLLA films

An akermanite (AKT; $Ca_2MgSi_2O_7$) ceramic disk of size Ø20 × 3 mm was prepared by sintering the AKT green compacts at 1350 °C according to a previous study [32]. The AKT disk was used for the targets to coat PSU and PDLLA films.

The prepared PSU and PDLLA films were fixed on the sample stage in the chamber of the PLD instrument using double-sided adhesive. Then, the sintered AKT disk was ablated using focused laser fluence of 180 MJ with a pulsed repetition rate of 5 Hz at room temperature. The O_2 ambient was set with a pressure of 20 MPa. To control the AKT thickness on the PSU and PDLLA films, the treating time was set at 15, 25 and 40 min, respectively. The coated samples were named as AKT-PSU and AKT-PDLLA, respectively.

The surface morphology, coating thickness and surface roughness of the prepared films was observed by scanning electron microscopy (SEM; FEI Magellan 400) and atomic force microscopy (AFM; Agilent 5100). The composition of the prepared films was characterized by energy dispersive spectrum (EDS). The hydrophilicity of the prepared films was investigated by testing the water contact angle. To test the mechanical strength of the prepared films, the hardness and elastic modulus were investigated using nanoindentation analysis (G200, Agilent).

To investigate the interface morphology of AKT nanocoatings on polymer films, the prepared films were soaked in Tris–HCl buffer solution under ultrasonic treating for 10 min. Then, the surface morphology of the samples was observed by SEM.

2.3. Apatite mineralization of AKT-PSU and AKT-PDLLA films

A short-term apatite mineralization of the prepared films was carried out using acellular simulated body fluids (SBF) [37]. The samples (PSU, AKT-PSU, PDLLA and AKT-PDLLA) were immersed in SBF and kept under shaking conditions at 37 °C for 1, 3 and 7 days. The ionic concentrations of Si, Ca and Mg ions released from the films in SBF were tested by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Varian 715ES). All films soaked for 3 days were analyzed using SEM and EDS to determine their apatite mineralization.

2.4. In vitro osteogenic ability of MC3T3 on AKT-PSU and AKT-PDLLA films

2.4.1. Cell attachment and proliferation

The films were sterilized by 70% ethanol for 30 min and then UV for 30 min prior to cell experiments. MC3T3 (MC3T3-E1 Subclone 14) cells were purchased from the cell bank, Chinese Academy of Sciences. The fourth passage of MC3T3 was used to evaluate the interaction of cells with the prepared films of size 8 × 8 mm^2. To investigate the cell attachment on the films, the films were used for cell culture: 300 μl of cell suspension was added to the films at a density of 4×10^3 cells $film^{-1}$ in 48 well plates. After culturing for 3 days, the cell/films constructs were removed from the culture wells, rinsed in phosphate-buffered saline (PBS), and then fixed with 2.5% glutaraldehyde in PBS for 1 h. The fixative was removed by washing with PBS. Then the constructs were dehydrated in a graded ethanol series (30, 50, 70, 90 and 100%) and hexamethyldisilizane. The dehydrated cell/films constructs were coated with gold, and the morphological characteristics of the attached MC3T3 were observed by SEM.

MC3T3 proliferation cultured with films was analyzed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) test according to a previous publications [38]. In brief, the cells (4×10^3) were seeded on the films. The cells were cultured for 1,