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The effect of bioactive glass content on synthesis and bioactivity of composite poly (lactic-*co*-glycolic acid)/bioactive glass substrate for tissue engineering

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Abstract

Tissue engineering offers a promising new approach to bone tissue grafting. One material that has received attention in this regard is the polymer poly (lactic-*co*-glycolic acid) (PLGA). It has the advantage of controllable bioresorption and ease of processing. Another material of interest is bioactive glass (BG), which shows the ability to stimulate osteoblastic differentiation of osteoprogenitor cells. In this study, we reported on the optimal synthesis parameters and the kinetics of formation of calcium phosphate (Ca-P) phase at the surface of PLGA/BG composites. The formation of calcium phosphate layer was confirmed using scanning electron microscopy/energy dispersive X-ray analysis (SEM/EDXA). PLGA-30%BG microspheres based porous scaffolds for bone tissue engineering were examined for their ability to promote osteogenesis of marrow stromal cells (MSC). This porous scaffold supported both MSC proliferation and promoted MSC differentiation into cells expressing the osteoblast phenotype. It therefore demonstrates significant potential as a bone replacement material.

Keywords: PLGA; Bioactive glass; Calcium phosphate; Marrow stromal cells; Tissue engineering

1. Introduction

Implants are extensively used both in dentistry and in orthopaedic surgery. Early implants were metals or polymers serving primarily as replacements for bones or teeth, and designed to provide the strength appropriate to sustaining considerable loads. However, fibrous tissue interposed between the implant and bone was a frequent complication [1]. To overcome this limitation, many implants are now coated with calcium phosphates to improve bone bonding. Despite the acknowledged value of such implants, there are often problems in their clinical use. Dental and orthopedic implants with calcium phosphate coatings show a time-dependent propensity for the coating to shear-off [2]. Biphasic calcium phosphate-ceramic implants and porous hydroxyapatite have been used as synthetic grafts with variable results [3]. Studies of ceramic materials indicate that there is progressive dissolution of the implant [4–6], and rapid biodegradation is undesirable if the implant will not promote osteogenesis. However, if the implant is to serve primarily as a temporary scaffold for new bone formation, implant degradation is valuable. A useful bioresorbable implant material would therefore be one that promotes formation of new bone at a rate sufficient to balance implant resorption.

Current bone grafting techniques include using autografts and allografts typically derived from vascularized cancellous bone of the fibula or iliac crest [7]. However, these treatments have a number of limitations. Harvesting autografts is expensive and constrained both

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by limited availability and donor-site morbidity. Allografts are limited by the potential risk of introducing infection or disease, while vascularized grafts are prohibitively expensive [8]. Due to limitations associated with existing biological and synthetic grafts, bone tissue engineering has emerged as an alternative new approach in the formation of viable bone grafting systems. In this approach, a scaffold is seeded with osteogenic cells. Ideally the scaffold is a three dimensional porous structure that stimulates bone cell function and is biocompatible, biodegradable and bioactive. Poly (lactic-*co*-glycolic acid) (PLGA) polymers have been widely investigated as tissue engineering scaffolds [9–14]. Laurencin et al. [15] examined the behavior of osteoblasts on polymeric matrix and reported the attachment, proliferation and differentiation of these cells. As PLGA polymers are biodegradable, bone-grafting system based on these materials have the advantage of being replaced by host tissue. Although it has also been investigated as a bioresorbable carrier for delivering growth factors [16], PLGA does not show any osteoinductive effect. Winet and Hollinger have reported that PLGA implanted in bone chambers in rabbit tibia was absorbed within 8 weeks, but the tibia with PLGA showed delayed or decreased trabecular ingrowth and reduced neovascularization [17].

Materials such as bioactive glasses, bioactive glass-ceramics, hydroxyapatite, and polyethylene-hydroxyapatite mixtures are capable of forming an interfacial bond with bone [18]. It has been shown that bioactive glass granules of narrow size range elicited bone formation in mandibular defects as soon as 1 month after implantation [19,20]. Other reports have also demonstrated that bioactive glasses (BG) show the ability to facilitate attachment and osteoblastic differentiation of osteoprogenitor cells [21,22]. BG surface modification, which includes formation of bone-like apatite and absorption of serum proteins, was shown to be critical for the favorable effect of BG on bone cell function [22–25].

Marrow stromal cells (MSC) are multipotential progenitor cells that can commit to the osteoblast lineage. During adult bone repair, differentiating MSC are considered a major source of osteoblasts for bone deposition on implant surfaces. Positively charged surfaces have been reported to improve attachment of rat MSC, but suppress cell spreading and osteogenesis [26]. Studies of MSC osteogenesis on PLGA and poly(ethylene glycol)/PLGA copolymer foams indicate that these foams can support proliferation and differentiation of MSC [27–30]. Calcium phosphate-coated bioactive glasses have been reported to not only support but also promote osteogenesis of MSC [21,31,32]. Such studies suggest that conditioned bioactive glass not only can be osteoconductive but also osteoinductive.

Previous attempts to produce PLA/BG composite substrates showed considerable promise in forming a bioactive carbonated calcium hydroxyapatite surface that may support synthesis of bone [23]. Other reports have also demonstrated that the formation of crystalline hydroxyapatite on the surface of biopolymer/bioactive glass composites [33–35]. We reasoned that the incorporation of BG into a biodegradable PLGA might result in a composite scaffold that supports both MSC proliferation and MSC differentiation into osteoblasts. Among techniques suggested for 3D porous substrate manufacture, microsphere-based methodology provides means to control inter-connecting porosity [36,37]. In formulating a PLGA/BG microsphere-based 3D porous, osteoinductive substrate, we focused both on the effect of BG content on the morphologic properties of the microspheres, and the ability of these composite microspheres to promote the osteogenic differentiation of MSC. We hypothesized that the incorporation of BG would promote the formation of calcium phosphate-rich, serum protein-rich layer on PLGA and stimulate the attachment and osteogenesis of MSC.

2. Methods and materials

2.1. Synthesis of polymer/ceramic composites

PLGA/BG microspheres were produced by a modified emulsification method as described in previous studies [23,36,37]. PLGA of various molecular weights (5050 DL, MW: 25 kDa, PolySciences, Warrington, PA; 5050 DL, MW: 53 kDa, Tg: 44.4 °C, Medisorb, Cincinnati, OH; 6535 DL, MW: 100 kDa, Tg: 47.2 °C, Medisorb), containing (in weight %) 0%, 10%, and 30% BG were used. 45S5 bioactive glass powder with a nominal composition (in wt%) of 45% SiO_2, 24.5% CaO, 24.5% Na_2O, and 6% P_2O_5 was used. In general, PLGA was dissolved in methylene chloride ($CHCl_3$), and then BG powder ($<40\,\mu m$ particle size) was added to the solution. This mixture was added drop-wise to a stirred poly(vinyl alcohol) (PVA) solution. Microspheres were isolated by filtration, washed with deionized water, air dried and then vacuum dried. Dried particles were sieved using nylon mesh.

Porous scaffolds were produced by modifying a method used by Borden et al. [37]. PLGA with high molecular weight (MW: 100 kDa, Tg: 47.2 °C) containing 30% BG powder were used to produce well-shaped microspheres. These well-shaped PLGA-30%BG microspheres of a specific size range (350–500 μm) were poured into a 12 mm diameter Teflon mold and heated at 65 °C for 4 h to produce a porous scaffold. In addition to varying the PLGA molecular weight and the BG content, we studied the effect of stirring speed at 300,