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POLYLACTIC ACID/BIOACTIVE CERAMIC BIOCOMPOSITE SCAFFOLDS FOR BONE TISSUE ENGINEERING: A BRIEF OVERVIEW

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ABSTRACT

Polylactic acid (PLA) is a highly promising material for its biodegradability, nontoxicity, high mechanical strength, ability to be absorbed into human body and its non-toxic biodegradation products. However, when it comes to bone tissue engineering, PLA has major drawbacks such as low cell adhesion caused by its hydrophobic property, and inflammatory reactions *in vivo* due to its degradation product, lactic acid. One pragmatic solution for the mentioned problems is the introduction of bioactive ceramic nanomaterials. Indeed, the incorporation of bioactive ceramic particles into PLA matrix not only can buffer the localized PH decrease due to the PLA degradation products but also improves cell adhesion, mechanical properties, and osteoconductivity. In the present study, recent proposed approaches based on developing PLA composites containing calcium phosphate ceramics such as hydroxyapatite and tricalcium phosphate are reviewed, and biodegradability, mechanical properties and bioactivity assessments of PLA-based scaffolds containing different contents of the aforementioned bioactive ceramic particles are discussed in brief. This review is written with an aim to compile the works done in this field and focus on the properties of the PLA-based composites and their fabrications methods in the field of bone tissue engineering. The scaffolds reviewed here might demonstrate the optimal solution and the suitable PLA-based composite scaffolds for bone regeneration strategies.

KEYWORDS: Bone Tissue Engineering, Bioactive Ceramic Nanomaterials, Polylactic Acid

INTRODUCTION

Tissue engineering has been defined as the implementation of scientific principle to design, construct, modify and grow the living tissues by means of biomaterials, cells and factors, alone or in combination [1]. Traditional orthopedic surgical procedures such as autograft and allograft suffer from donor site morbidity, unavailability of large tissue volumes, donor scarcity, pathogen transfer and immune rejection [2-4]. There is a growing need for bone regeneration to treat the loss of bone tissue brought about by tumors, osteonecrosis and trauma [5]. In bone tissue engineering the 3D scaffold plays an important role in the manipulation of the function of osteoblasts and guidance of new bone formation. The scaffolding materials should have sufficient mechanical properties, suitable degradation ratein order to be replaced by newly formed bone, optimized architecture and osteoconductivity[6].

Among biodegradable polymers, polylactic acid (PLA) has been widely used to develop porous scaffolds due to its excellent mechanical properties, biocompatibility, suitable biodegradability and nontoxic degradation products[7]. PLA has two optical isomers, poly-L-lactide (PLLA) and poly-D-lactide (PDLA). PLLA is well-known for its excellent mechanical properties among bioabsorbable polymers. In addition, PLLA fibers possess better mechanical properties in comparison with

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other forms of PLLA, such as cast films [8]. However, PLA has major drawbacks such as low cell adhesion due to its hydrophobic property and inflammatory reactions caused by lactic acid degradation product [9]. Therefore, the incorporation of ceramic particles into PLA is highly regarded to improve cell adhesion, mechanical properties, osteoconductivity, and buffer the localized PH decrease due to the degradation of PLA[10].

Calcium phosphate ceramics such as hydroxyapatite (HA, Ca_{10} $(PO_4)_6(OH)_2$) and tricalcium phosphate (TCP, $Ca_3(PO_4)_2$) are one of the major class of biomaterials for bone repair which are similar to the mineral component of natural bone. Although these ceramics have good osteoconductivity and bone bonding ability, they are brittle and difficult to process [6, 11]. Polymer/ceramic composite scaffolds have been developed to combine the osteoconductivity of calcium phosphate ceramics and biodegradability of polyesters through numerous fabrication techniques such as electrospinning [12], gas foaming [13], freeze drying[14], solvent casting and particle leaching [15] and thermally induced phase separation[16].

PLA/ceramics composites can be tailored to meet various bone tissue engineering application requirements. The aim of this paper is to investigate the mechanical properties and bioactivity of PLA/calcium phosphate ceramics composite scaffold such as PLA/HA and PLA/TCP produced by the aforementioned methods.

PLA/CALCIUM PHOSPHATE CERAMICS COMPOSITE SCAFFOLDS

The ability to co-spin PLA with HA offers the chance to produce fibrous PLA/HA composite scaffold by electrospinning with high surface area and interconnected channels appropriate for bone tissue engineering applications. For instance, Jeong et al. developed PLA/HA scaffolds with high pore volume and interconnective pores. The composite scaffold containing 20 wt% HA had the highest tensile strength (4.71 MPa). Moreover, *in vitro* cell culture study demonstrated that the novel composites are efficacious scaffolds for the growth of pre-osteoblasts [17]. Deng et al. reported that the presence of HA particles in PLLA/HA hybrid electrospun scaffolds restrained inflammation from the acid release by autocatalytical acceleration of PLLA. However, the degradation rate was decreased significantly in comparison with neat PLLA since the dissolving of HA particles blocked off the entry of water into the scaffold[12]. Electrospun composite fibers composed of PLLA-grafted hydroxyapatite (PLLA-g-HA) nanoparticles and PLA matrix also demonstrated an improvement in mechanical properties. The increment of PLA-g-HA content in the scaffold increased the in vitro degradation rate due to the improved wettability of the composite fibers and the escape of the nanoparticles from the fiber surface during incubation [18].

McCullen et al. showed that the addition of β-TCP into electrospun PLA composite scaffolds decreased tensile strength from 847 kPa to an average of 350 kPa. However, the scaffolds containing 10 wt% β-TCP induced the highest proliferation of hASC cells, and PLA/20 wt% β-TCP showed an enhanced osteogenic differentiation and increased cell-mediated mineralization in comparison with pure PLA scaffolds [19].Dinarvand et al. coated electrospun PLA scaffolds with HA, bioactive glass (BG) and TCP and studied the bone formation induced by these scaffolds *in vivo* in a rat model. Histological and digital mammography experiments conclusively showed that PLLA/HA-BG scaffolds induced a considerably higher level of reconstruction compared with that observed in defects treated with PLLA/TCP. Moreover, PLLA/HA-BG scaffolds synergistically improved bone regeneration higher than that observed for PLLA/BG and PLLA/HA [20]. To enhance the interaction between filler and polymeric matrix, Kasuga et al. modified the surface of calcium metaphosphate fiber with NaOH, which resulted in the increment in modulus of elasticity up to 5 GPa similar to that of natural bone [21]. Habibovic et al. coated calcium metaphosphate scaffold with PLA rather than adding calcium

metaphosphate particles to the polymer base. Although the compressive strength increased from 1.5 to 2.8 MPa, the polymer coating decreased the osteoconductivity of the scaffold. [22]

Amorphous calcium phosphate (ACP) is another type of calcium phosphate candidate for PLA/calcium phosphate composites. ACP has attracted increasing attention because of its solubility and great demineralization ability. ACP is highly regarded as the metastable precursor phase for subsequent formation of calcium phosphate in biological organisms which plays an important role in the process of tissue mineralization[23]. According to Zhang et al. the incorporation of ACP nanospheres and HA nanorods in nanofibers PLA reduced tensile strength from 1.6 MPa to 1 and 0.9 MPa, respectively. The *in vitro* mineralization in simulated body fluid (SBF) showed favorable mineralization behaviors. Moreover, the *in vivo* biocompatibility assessment showed that the collagen constituent in bone defect treated with PLA/ACP and PLA HA composite nanofibers were more obvious compared with pure PLA [24]. According to Ma et al. the increment in ACP content leads to the acceleration of mineralization of PDLLA/ACP composite nanofibers. Further, the adhesion and spreading behavior of MG63 cells on the surface of the composite nanofibers occurred at the beginning of the cell/composite interaction, which is an important factor that affect the further proliferation and differentiation of the cells[25]. PLLA/ACP electrospun composites incorporated with the basic fibroblast growth factor could successfully resurface the defect with cartilage and restore the subchondral bone in rabbit model [26].

3D resorbable scaffolds with high porosities can be fabricated by thermally induced phase separation (TIPS) method to produce controlled microstructures as scaffolds for tissues. According to Ma et al. PLLA/HA composite scaffolds prepared by TIPS method possess higher osteoblast survival rate, more uniform cell distribution and growth, improved new tissue formation, enhanced bone specific gene expression, and superior mechanical properties compared with neat PLLA scaffolds [27]. Wei et al. revealed that PLLA/HA composite scaffolds produced by TIPS possess high porosity (above 90%) which can beeasily adjusted by varying phase separation parameters such as polymer concentration, volume fraction of the secondary phase, quenching temperature and solvent used. In addition, HA particles in PLA greatly enhanced protein adsorption which is of the importance in evaluating a scaffold for tissue engineering since cell adhesion and survival can be modulated by protein pre-adsorption on the substrate[6]. The scaffolds obtained by Nejati et al via TIPS method exhibited compressive strength as high as 8.67 MPa with 85% porosity which is comparable to the high end of compressive strength of cancellous bone [28].

One of the oldest methods to fabricate scaffolds with relatively high porosity and interconnectivity has been by solvent casting and particle leaching technique. Composite scaffolds of PLA and HA prepared by this method demonstrated a porosity of 86% and improved compression strength and modulus of elasticity up to 0.44 MPa and 9.8 MPa, respectively [29].

Calcium phosphate glasses are also well suited for bone repair because they have a chemical composition close to that of the mineral bone and their degradation can be adjusted by modifying their chemical formulation; therefore, PLA/calcium phosphate glass composites are also highly considered for bone tissue engineering. PLA/calcium phosphate glass composites fabricated by solvent casting and particle leaching technique exhibited an interconnected structure with a porosity as high as 97% and an enhanced surface roughness in comparison with neat PLA matrix. The incorporation of calcium phosphate glass in PLA improved the compressive modulus from 74.5 to 120 kPa and enhanced cell viability.In addition, glass particles induced the formation of a calcium phosphate precipitate which facilitates the interaction between

the material and bone tissue. Cell culture evaluation indicated that the MG63 cells seeded in pure PLA showed a very flat and extended morphology, while the cells seeded in rough surfaces demonstrated a more rounded or cuboidal shape with long cytoplasmic extensions [30, 31].In order to investigate the influence of solvent in the preparation method on the properties of scaffolds, solvent casting and salt leaching and phase-separated scaffolds of PLA/calcium phosphate glass were prepared and characterized by Charles-Harris et al. The porosity and stiffness of solvent cast composite scaffold were 95% and 0.19 MPa, respectively, while those of phase-separated scaffolds were 90% and 7.1 MPa, respectively. The MG63 cell cultures performed during 21 days indicated that the phase-separated scaffolds induced less proliferation during first week of culture; however, from then on the proliferation levels were similar for both scaffolds. In addition, MG63 cells in the solvent cast scaffolds tended to spread towards their interior while the cells in phase-separated scaffolds tended to remain on their surface and form a thick layer there. Hence, the solvent cast scaffolds were easily colonized by the cells which were distributed in the pores of scaffold and produced extracellular matrix inside the scaffolds. The level of Alkaline phosphatase activity of solvent cast scaffolds reached its maximum at day 14 while that of phase-separated scaffolds attained its maximum at day 7, and then decreased. Therefore, the phase-separated scaffolds sustained more and earlier cell differentiation than the solvent cast ones. [32].

Emulsion freeze-drying technique is another method to fabricate PLA scaffolds with porosity higher than 90% and a pore size ranging from 20 to 200 µm [33]. According to Haimi et al. the culture of human adipose stem cells (ASCs) on freeze-dried PLA/β-TCP scaffolds produced higher DNA content and alkaline phosphatase activity relative to pure PLA scaffold [34]. A study on PLA/β-TCP containing different filler ratios and prepared by freeze-drying displayed that the amount of filler did not affect the pore distribution or pore size in the scaffolds. Moreover, after 26 weeks of hydrolysis*in vitro*, all samples lost only 5% of their weight. During preparation of the composite scaffolds,β-TCP was allowed to be dispersed only at the bottom of the scaffolds, which may encourage the growth of bone cells on the porous bottom surfacewhile may inhibit the ingrowth of osteoblast and encourage the ingrowth of chondrocytes on the dense surface skin of the scaffold[35].Cao et al. reported that that freeze-dried composite scaffolds containing 30 wt% and 50 wt% β-TCP demonstrated enhanced ingrowth of new bone, and had similar biological performance in terms of osteogenesis. However, the scaffold containing 50 wt% filler content was shown to be very brittle that cannot be used in bone repair [36].

Supercritical gas foaming is a solvent-free fabrication method to produce scaffolds with controlled architecture and properties suitable for bone tissue regeneration applications. Mathieu et al. prepared PLA/HA and PLA/β-TCP scaffolds using supercritical gas foaming method to mimic natural bone structurewith a porosity of about 80%. The scaffolds showed anisotropy in the morphology, which making longitudinal modulus up to 1.5 times greater than transverse modulus. Biocompatibility studies demonstrated that the scaffolds supported cell growth, proliferation and differentiation of human primary osteoblast cells [37]. Montjovent et al. seeded human fetal and adult bone cells on the composite scaffolds prepared by gas foaming method. After 4 weeks of culture, it was found that fetal cells penetrated inside the scaffolds better than did adult cells. In addition, the composites induced a higher enzymatic activity level in comparison with pure PLA [38]. Using supercritical gas foaming method, porous scaffold based on PLA reinforced with 5 wt% β-TCP was obtained. The porosity and elastic modulus of PLA/β-TCP were 83% and 121 MPa, respectively, while those of pure PLA scaffold obtained by gas foaming method were 83% and 50 MPa, respectively. Human fetal and adult bone cells were seeded on the scaffolds and both foams supported adhesion, intenseproliferation and differentiation of seeded cells *in vitro*. The addition of β-TCP resulted in

higher ALP activity for fetal bone cells and a stronger production of Gla-osteocalcin for adult bone cells. The *in vivo* behavior of PLA/β-TCP foams in combination with human fetal cells in rat model indicated that the degradation rate was coupled to the rate of tissue regeneration which led to a structural integrity of the constructs [39].

Table I: PLA/HA Composites for Bone Tissue Engineering

Method	HA Content	Modulus (MPa)	Strength (MPa)	Cell Type	Ref
Electrospinning	10 wt%			MG63	[12]
Electrospinning	5, 20 wt%	1.8-4.71	0.157-0.262	MC3T3-E1	[17]
Electrospinning		118	2.86	MG63	[40]
Electrospinning	0.25,0.5 wt%		0.52- 0.65	L929, MC3T3-E1	[41]
Thermally Induced Phase Separation	10-30wt%	0.3-0.63			[42]
Thermally Induced Phase Separation	10-70 wt%	6.1-11	0.22-0.4		[43]
Thermally Induced Phase Separation	50 wt%	10.87	0.39	MC3T3-E1	[27]
Thermally Induced Phase Separation	10-70 wt%	4.3-8.3			[6]
Thermally Induced Phase Separation	50 wt%	14.9	8.67	MSCs	[28]
Solvent Casting and Particle Leaching	10-50 wt%	4.72-9.87	0.29-0.44		[29]
Supercritical Gas Foaming	5 wt%	50-200	2.5-6		[37]
Supercritical Gas Foaming	5 wt%	133.2		hFOB	[38]
Supercritical Gas Foaming	10-50 wt%	0.081-0.122			[44]
Supercritical Gas Foaming	2,4 wt%		0.852-1.014		[45]

Table II: PLA/ TCP Composites for Bone Tissue Engineering

Method	TCP Content	Modulus (MPa)	Strength (MPa)	Cell Type	Ref
Electrospinning	5,10,20 wt%	4.526-8.509	0.269-0.447	hASC	[19]
Electrospinning	50 wt% (ACP)		1	MG63	[24]
Electrospinning	50 wt% (ACP)			*in vivo*	[26]
Freeze Drying	10,20 wt%			ASCs	[34]
Freeze Drying	10,30,50 wt%		1.2-2.1	*in vivo*	[36]
Solvent Casting and Particle Leaching	40 wt% (Gass)	0.075-0.12	0.175-0.201	SAOS-2 MG63	[30, 31]
Solvent Casting and Particle Leaching	50 wt% (Glass)		0.19	MG63	[32]
Solvent Casting and Particle Leaching	10-40 wt%	0.3-0.8		MG63	[46]
Solvent Casting and Particle Leaching	50 wt%			MG63	[47]
Solvent Casting and Particle Leaching	20-50 wt%		0.057-0.207		[48]
Supercritical Gas Foaming	5 wt%	121		hFOB	[38, 39]

Table I and II demonstrate the influence of different types of composite manufacturing methods and the amount of HA andTCP content on mechanical properties of PLA composite scaffolds discussed above. The strengthening influence of HA and TCP in PLA polymeric matrix can be explained by the fact that the PLA matrix is a load transfer medium; therefore, it transfers the load to the intrinsically rigid HA and TCP ceramics. However, it has to be mentioned that the addition of HA and TCP can lead to the decreases in tensile strength of PLA matrix due to the brittleness of the ceramic particles [36, 49]. Another issue is the amount of incorporated filler in the polymeric matrix. Indeed, when the filler content is low, the PLA matrix is continuous, which ensures suitable interfacial bonding with HA or TCP and thus leads to the enhancement in mechanical strength. In composites with higher filler content, however, the amount of PLA matrix surrounding the filler particle is lower. This results in poor integrity of the composite and subsequently, leads to the lower mechanical properties in comparison with cancellous bone[50]. According to the tables, although many of the synthesized composites exhibit poor mechanical properties, most of them show appropriate in vitro bioactivity. It has to be mentioned that the fabrication methods and its parameters, grade of polymer and filler immensely affect the mechanical properties of obtained composite [51].

CONCLUSIONS

PLA/calcium phosphate ceramic materials have attracted much interest for bone tissue engineering applications.

The bioactivity, mechanical properties, degradability and architecture of the composite scaffoldsimmensely depend on the fabrication techniques, the interfacial interaction between fillers and matrix and properties and contents of raw materials. Generally, the mechanical properties of these scaffolds are worse than those of cancellous human bone; therefore, they are unable to provide minimal mechanical support. Many of these composite scaffolds, however, have been shown to be bioactive using different tests from soaking in SBF through to in vivo implantation. It has been shown that PLA/calcium phosphate ceramic composites can be tailored to meet various bone tissue engineering requirements. However, a number of issues such as mechanical strength, long-term degradation and inflammatory responses have to be improved for wider application of PLA/calcium phosphate ceramic composites scaffolds in bone tissue engineering.

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