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# Mechanical, material, and biological study of a PCL/bioactive glass bone scaffold: Importance of viscoelasticity

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ABSTRACT

Microsphere sintering method was used to fabricate bone tissue engineering scaffolds made of polycaprolactone (PCL)/bioactive glass 58S5Z (58S modified with 5 wt% Zinc). First, the effect of PCL/58S5Z ratio on the mechanical properties (elastic modulus and yield strength) was investigated. It was found that samples with 5 wt% 58S5Z (named 5%BG) had the highest elastic modulus and yield strength among all samples, i.e., with 0, 5, 10, and 20 wt% bioactive glass. Then, considering the importance of viscoelastic properties of bone, the viscoelastic behavior of 0%BG (scaffold with only PCL) and 5%BG samples was determined by performing compressive stress relaxation test and subsequently a Generalized Maxwell model was developed. Findings indicated a similar amount and pattern of predicted storage and loss moduli and loss factor of the composite scaffolds to those of the bone. In the next step, the analysis of biological behavior of the scaffolds using MTT assay, DAPI and Alizarin red staining demonstrated that 5%BG scaffolds had higher *in vitro* cell viability and bone formation compared to 0% BG ones. Furthermore, *in vivo* study employing H&E staining of the scaffolds implanted in rats' calvarium for 50 days, confirmed the earlier findings and showed that 5%BG-filled defects had higher and more uniform bone formation compared to both 0%BG-filled and empty defects.

## 1. Introduction

Tissue engineering is considered as the process of providing body with the means to trigger its repair and healing mechanisms [1]. Among the three main elements of tissue engineering, cell source, growth factor, and scaffold, the latter highly affects mass transport and provides the support for cell adhesion, growth, and proliferation [2]. These scaffolds should be highly porous with interconnected pores, biocompatible with degradation rate in accordance with tissue formation *in vivo*, proper surface properties for cell attachment and further functions, mechanical properties similar to that of the main tissue, and in the case of bone tissue osteoconductivity with bone bonding ability [3]. Different synthetic and natural materials including polymers, ceramics, and even metallic foams are considered as bone scaffolds, [2] while a better control over micro- and macrostructure, including porosity and material composition is achieved with synthetic materials [4]. Composite materials combine useful properties of different materials and allow to tailor physical, mechanical, and biological properties. For example, degradation of polymers could be tailored by the addition of bioactive glasses [5].

Despite ceramic and metallic scaffolds that are limited in their processability and lack in degradability, polymers have the advantage of tailorable biodegradability and flexibility in design. Among all the polymeric candidates for bone scaffolds, synthetic polymers have the advantage of controlled mechanical, degradation, and microstructural properties, with no pathogenic impurities, and excellent batch-to-batch consistency. Poly(α-hydroxy acids) are among the most popular synthetic polymers in bone tissue engineering and their hydrolytically labile ester bond has made them prone to non-enzymatic degradation [6]. Poly(ε-caprolactone) (PCL), an aliphatic polyester, shows little immunological and inflammatory stimulation and supports cell regulatory behavior [7]. PCL is one of the most studied polymers [8] with proven support of bone cells [9] that together with its composites is widely used in bone, ligament, cartilage, nerve, skin, and vascular tissue engineering applications. Its high processability [10] and biocompatibility to soft and hard tissues [11] are among the motives for its high usage as

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bone scaffolds. Despite these advantages, bone bioactivity and cellular affinity of synthetic polymers are poor and some degradation problems associated with mechanical failure and acidic cellular environment have been reported [12].

Bioactive inorganic materials including bioactive glasses are the most used synthetic materials in bone tissue engineering applications because of their high potential in bonding to bone and stimulatory effect on new bone formation [12,13] but their brittleness and low flexibility and strength make them less suitable in load bearing applications [14]. Despite this, using them with polymers would increase the structure's bioactivity and osteoconductivity, the acidic by-products of polymer degradation could be buffered, mechanical properties and degradation rate could be tailored, and microstructure can be engineered [2]. In the case of PCL, addition of bioactive glasses as a ceramic phase could compensate for its intrinsically hydrophobic nature with poor cell adhesion and also improve its mechanical properties [15].

The internal structure of the scaffold including its porous structure strongly affects structure and function of the regenerated tissue [16]. These porous scaffolds serve as a temporary template for the bone cells to perform bone formation and to provide them with mechanical support [17]. The problem with different scaffold fabrication methods including particulate leaching, solvent casting, injection molding, gas foaming, and electrospinning, is that they have little control over the porous structure with little manufacturing repeatability of the product [18]. Preparing porous scaffolds *via* sintering polymeric microspheres was first introduced by Devin et al. [19]. As a bottom-up method, this technique uses building blocks of microspheres to assemble a 3D architecture with controlled microstructure and predefined spatial pattern [20] and enhances degree of interconnectivity of the scaffold [8]. Scaffolds fabricated using this method show remarkably higher mechanical properties compared to conventional methods [21] and have 3D structure, porosity, and mechanical properties of trabecular bone [22]. Compared to 3D printing methods, the microsphere sintering method does not require complex procedures and does not have organic solvent-printhead difficulty while it has its advantages such as producibility [23].

One of the most important functions of the bone tissue is its mechanical behavior, thus, evaluating the mechanical properties of bone scaffolds have been a crucial step of such studies. Bone also exhibits time-dependent characteristics (viscoelastic behavior) [24], and scaffolds as its replacement should have the same behavior. Evaluating the viscoelasticity of the scaffolds as well as modeling these experimental data could be beneficial in predicting their behavior and function in the body which it has not received much attention. Scaffolds' viscoelasticity also affects bone cells' normal functions which are highly sensitive to their environment [25].

In an earlier study [26], we fully investigated bone scaffold fabrication using PCL and microsphere sintering method. According to these results, sintering PCL microspheres with diameters in the range of 350–600 μm in 64.5 °C for 100 min would result in scaffolds with highest possible Young's modulus and yield strength. The optimal pore size for bone ingrowth in scaffolds has been reported to be at least 100 μm, a requirement of bone tissue engineering [27,28]. The study also showed that the mechanical behavior of this scaffold is linear viscoelastic and a Maxwell model with 3 branches can properly describe its behavior. Although the proposed scaffold showed higher mechanical properties compared to many other PCL scaffolds prepared by other fabrication methods, its Young's modulus and yield strength were still less than those of the bone and its viscoelasticity mismatched the bone either. To overcome these dissimilarities and to boost its biological behavior, creating composite scaffolds were considered.

In the present study PCL/58S5Z (bioactive glass 58S modified with 5 wt% Zn) bone composite scaffolds were prepared *via* microsphere sintering method and their mechanical, structural, and biological properties where evaluated. The aim of the study was to investigate the effect of incorporating bioactive glass into PCL on improving its mechanical properties (elastic and viscoelastic) as well as biological properties (*in vitro* and *in vivo*). Finally, because of the importance of the viscoelasticity in bone tissue, a linear viscoelastic model was developed to predict scaffolds' mechanical behavior and compared to bone in different loading conditions.

## 2. Materials and methods

### 2.1. Materials

Materials required for preparation of bioactive glasses including tetraethylorthosilicate (TEOS), triethyl phosphate (TEP), Ca$(NO_3)_2{\cdot}4H_2O$, $NaNO_3$, $Zn(NO_3)_2{\cdot}6H_2O$, and reagents including $HNO_3$ and ethanol were purchased from Merck Inc. and used without further modification. Other materials including poly(ε-caprolactone) (average $M_n$ 80,000), Polyvinyl Alcohol (PVA) (Mw 9000–10,000, 80% hydrolyzed), and dichloromethane were purchased from Sigma-Aldrich.

### 2.2. Bioactive glass synthesis

The 5 wt% zinc-modified 58S Bioactive glass (58S5Z: 58 wt% $SiO_2$, 28 wt% CaO, 9 wt% $P_2O_5$, 5 wt% ZnO) was prepared using sol-gel method proposed by Li et al. [29]. Briefly, $HNO_3$ (2 M) and ethanol (99.5%) were mixed and stirred for half an hour. The resultant mixture was then, added to the mixed solution of water and TEOS. Subsequently, the reactants, TEP, Ca$(NO_3)_2{\cdot}4H_2O$, and $Zn(NO_3)_2{\cdot}6H_2O$ were, added to the solution while being stirred. The final sol was maintained at room temperature for 14 days, then aged and dried at 90 °C for 50 h. Glass powder was thermally treated at 700 °C for three hours (heating rate of 5 °C/min).

### 2.3. Microsphere preparation and scaffold fabrication

PCL/58S5Z microspheres were prepared with single emulsion process in which 1.2 g of PCL/58S5Z with different weight ratios (100:0, 95:5, 90:10, and 80:20 named 0%BG, 5%BG, 10%BG, and 20%BG, respectively) was dissolved in 10 mL dichloromethane (12% w/v) and stirred for 1 h to obtain complete homogenized solution. The polymeric solution was added dropwise to the aqueous solution of PVA (200 mL of 0.02% w/v), while stirring at 400 rpm. Solvent was completely evaporated after stirring for 4 h and solid microspheres were formed. They were then washed with deionized water and dried in room temperature. Next, the microspheres in the range of 350–600 μm were selected using proper sieves for scaffold fabrication.

The selected microspheres were poured into the steel mold (5 mm diameter by 10 mm height cylinder) and heated for 100 min at 64.5 °C. Next, the mold was allowed to reach the room temperature and the scaffold was removed from the mold.

### 2.4. Structural characterizations

The ethanol displacement method (Eq. (1)) was used to measure the porosity of the scaffolds:

$$\text{porosity} = (V_1 - V_3)/(V_2 - V_3) \times 100 \quad (1)$$

$V_1$ is the initial volume of ethanol before submerging the scaffold in it, $V_2$ is the volume of the impregnated scaffold and the surrounding ethanol after 2 h, and $V_3$ is the final volume of the remaining liquid after removing the scaffold.

Crystallinity of the microspheres in the 5–55° 2θ range was investigated. FTIR spectrum was used to identify the presence of functional groups of both bioactive glass and polymer in the composite structure. Using Bomem MB 100 spectrometer the FTIR absorbance mode of PCL and composite microspheres as well as 58S5Z powders were obtained in KBr pellets in the range of 400 to 4000 $cm^{-1}$.

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