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Effect of chitosan concentration on the properties of PLA/β-TCP scaffold produced by combination of solvent casting and salt leaching techniques

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Abstract. Polylactic acid (PLA)/beta-tricalcium phosphate (β-TCP) porous scaffold was fabricated using solvent casting and salt leaching. This scaffold is facing problems such as relatively low compressive strength and high brittleness. In order to overcome this problem, natural polymer coated porous scaffold was introduced. In this study, PLA/β-TCP scaffold was coated with four different concentration of chitosan (0.5%, 1.0%, 1.5% and 2.0% w/v). It was found chitosan coated scaffold has higher compressive strength compared to that of uncoated scaffold. The compressive strength of PLA/β-TCP coated with 1.5% w/v of chitosan indicates the highest compressive strength (0.49 MPa), which is 35% increment compared to the uncoated scaffold. Morphological observation by scanning electron microscopy (SEM) revealed that highly porous three-dimensional scaffold with well interconnected porous structure could be achieved by coated and uncoated scaffolds. The results reveal that the scaffold coated with chitosan layer achieves a highly interpenetrated polymer and scaffold network, potentially leading to a porous scaffold with higher mechanical strength and toughness.

1. Introduction

Scaffold is a porous three-dimensional structure that acts as a mechanical support for the cell growth, differentiation, proliferation, and other cellular functions. The current materials used in tissue engineering fields are metals, ceramics, composites, and natural/synthetic polymers [1]. These materials are used for replacement and repair of damaged or traumatized bone tissues. Polymer-based composite scaffolds are widely applied in tissue engineering. These composites improved the biological and mechanical performances of the scaffolds compared with other materials. Commonly, tissue engineering scaffolds are mainly made of biodegradable polymers such as poly(lactic acid) (PLA), poly(glycolic acid), and their copolymer poly- (lactic-co-glycolic acid) (PLGA) [2]. These polymers have the advantages of demonstrated host compatibility, variable physiochemical properties
and predictable resorption rates. However, these polymers also have poor cell–material interactions because of their hydrophobic nature. Calcium phosphates (i.e., hydroxyapatite (HA) and beta-tricalcium phosphate (β-TCP)) were used due to their bone-like composition. β-TCP is a bioceramic material that allows it to be resorbed and replaced by bone during the healing process. β-TCP has a good biocompatible, non-toxic, non-inflammatory and osteoconductive, but this material also facing the limitation of the biocompatibility, biodegradability and low mechanical properties [3]. In the present study, the purpose of combination of PLA and β-TCP is to achieve optimum mechanical and biological performance for bone tissue application.

There are several methods to fabricate highly porous biodegradable scaffolds including fiber-bonding, 3D-printing, replication, direct foaming, phase-separation, emulsion freeze drying, gas-foaming and solvent casting and particle leaching. The solvent casting and salt leaching are chosen as a fabrication method according to the simplicity of operation and producing scaffolds with a controlled composition, good pore interconnectivity degree, porosity and pore size [4]. Due to the limitation of the properties of PLA/β-TCP scaffolds, coating of the scaffold has been introduced. Coating method is adopted in fabricating composite scaffolds since it can improve tissue interaction, mechanical scaffold stability, and toughness [5]. Chitosan is used as a bioactive coating according to biocompatible properties, nontoxic, biodegradable and hydrophilic polysaccharide [6]. In the present study, the effects of different concentration of chitosan layer (0.5%, 1.0%, 1.5% and 2.0% w/v) with silane treatment processes on the mechanical properties of the PLA/β-TCP scaffolds were determined.

2. Materials and Methodology

2.1 Materials

The raw materials used for in the study were poly(lactic acid) (PLA), dichloromethane (DCM), chloroform, sodium chloride (NaCl) and β-tricalcium phosphate (β-TCP), chitosan, 3-(Trimethoxysilyl)propyl methacrylate (A174) and glutaraldehyde (GA). PLA was supplied by Nature Works in pellet form with a specified molecular weight average of 74,00000. DCM with > 99.5% purity, chloroform and NaCl were supplied by Merck Millipore. β-TCP, medium molecular weight of chitosan, GA and 3-trimethoxysilyl propyl methacrylate were supplied by Sigma Aldrich. β-TCP used in powder form with the weight average of 310.18 g/mol.

PLA/NaCl and β-TCP (Ca₃(PO₄)₂) were used as the main component in the fabrication of composite porous scaffolds. NaCl act as porogen agent while, 3-(Trimethoxysilyl) propyl methacrylate was used as silane coupling agent to produce chemical link between PLA/β-TCP scaffolds and chitosan.

2.2 Methodology

PLA/β-TCP porous scaffolds were prepared by using solvent casting and particulate (salt) leaching technique. In order to fabricate porous scaffolds, PLA pellets were dissolved in DCM/chloroform (v:v= 1:1) for two hours. About 40 wt% of β-TCP and NaCl powder with a mass ratio of polymer to NaCl of 1:5 (wt/wt) was added into the solution to form a paste-like gel. After mixing, the gel-like mixture was then cast into the Teflon mold (diameter=1.5 cm and height=2.5 cm). The scaffolds were air dried for 24 hours and de-aired inside a vacuum desiccators for 4 hours. Next, the scaffolds was dipped in ethanol about 2 hours at room temperature and immersed in distilled water for another 4 days before placed the samples in the oven at 80 °C for 24 hours to leach out of the water.

For treatment, 2% (v/v%) of silane (3-trimethoxysilyl propyl methacrylate) coupling agent was added to the 95% (v/v%) methanol and 5% (v/v%) distilled water solution. The scaffolds were soaked in this solution for 2 hours and continuously rinsed with deionized water and ethanol. After that, the scaffolds were placed in the oven at 80 °C for 2 hours. For coating process, the chitosan solution was
prepared by dissolving the powder in 99 ml of distilled water, 1 ml of acetic acid and 0.025% of glutaraldehyde in order to produce chitosan solutions (0.5%, 1.0%, 1.5% and 2.0% w/v). The composite scaffolds were then dipped into the chitosan solution; next, vacuum infiltration was carried out 24 hours to infiltrate the chitosan into the porous structure of scaffolds and finally, the scaffolds were dried in vacuum oven for 24 hours to remove any residual solvent.

The AccuPyc II 1340 Series Pycnometers were applied to identify the density and porosity of the scaffolds. The morphology of porous composite scaffolds was carried out by using field emission scanning electronmicroscopy (FESEM), model HitachiTM3000 TableTop. The compression test was conducted using Instron Universal Testing Machine; model Instron3369 according to ASTM-F-1250-13. The crosshead speed of testing was at 1 mm/min with dimensions 25 x 15 mm were used.

3. Results and discussion

Figure 1 (a-e) present the fracture surface after compressive test of coated and uncoated PLA/β-TCP scaffolds with different concentration of chitosan. All the scaffolds were treated with silane prior to chitosan coating. SEM result shows the coral-reef like structure and the distribution of interconnected macropores size in the range of 150 to 500 μm of the PLA/β-TCP scaffolds for uncoated and coated with chitosan. From Figure 1 (b-e), chitosan layer can be seen clearly on the cross-sectional surface of the scaffolds after coating with different concentrations of chitosan. The morphology of porous scaffolds coated with 1.5 w/v% (Figure 1(d)) indicates the chitosan layer on the surface of the struts compare to other coated scaffolds. However, 2.0 w/v% of the chitosan shows a thick layer of chitosan on the cross-sectional surface of the scaffolds. This indirectly proved that the chitosan is stick on the top of the surface and failed to penetrate throughout the internal micropores of the scaffolds. Zairani et al. [7] reported that the β-TCP scaffolds coated at higher concentration (6 wt%) of alginate produce rough surface of the porous structure. Penetration of alginate into the inner scaffold structure is difficult due to high alginate concentration which relates to high viscosity.

Figure 2 shows the compression strength and total porosity of uncoated and coated PLA/β-TCP scaffolds with different concentration of chitosan (0, 0.5, 1.0, 1.5, and 2.0 w/v%). The concentration of chitosan at 1.5 w/v% shows the highest compressive strength (0.49 MPa) compared to uncoated and other coated scaffolds. Concentration of 1.5 w/v% of chitosan was sufficient to permit the chitosan to infiltrate the entire structure of the porous PLA/β-TCP scaffold. Infiltration of the chitosan into the scaffold improves the brittleness and biocompatibility of the porous scaffolds [8]. Besides, higher porosity of the uncoated scaffold (in Figure 2) which is 78.5% compared to other coated scaffolds which are in the range of 73 to 77.6%. Theoretically, the porosity of at least 50–90% is required for the bone ingrowth process [9]. This concludes that all the scaffolds have the minimum porosity which is more than 70% and suitable for bone tissues application.
Figure 1. SEM images of the (a) PLA/β-TCP scaffold and PLA/β-TCP prepared from different concentrations of chitosan; (b) 0.5 w/v%, (c) 1.0 w/v %, (d) 1.5 w/v % and (e) 2.0 w/v % of chitosan at different magnification (100x magnification and 500x magnification (for insert), respectively).
Figure 2. The compressive strength and percentage of porosity for PLA/β-TCP with different concentration of chitosan.

Figure 3 indicates the functional group for chitosan, silane, uncoated PLA/β-TCP scaffolds and treated-coated PLA/β-TCP scaffold. It is observed the presence of the chitosan and silane functional groups on the PLA/β-TCP scaffold structure. The silane solution used as a reference to show the presence of hydrocarbon chain (-CH) vibrations at the peak around 3000-2800 cm⁻¹. The silanol group Si-O can be seen in silanization spectrum at 1122 cm⁻¹ and the peak Si-O-Si linkage at around 500 cm⁻¹. This is because of the existence of covalent bonding between the silane coupling agent and PLA/β-TCP when coated with chitosan. The band at 1173 cm⁻¹ indicates of an amino group also indicates the possible interaction between the chitosan and PLA/β-TCP during the coating process. The IR spectrum of the scaffolds beads showed a band at 1632 cm⁻¹ which corresponds to imine bond (C=N) which might be formed between chitosan and glutaraldehyde [10].

Figure 3. FTIR spectra of (a) Pure chitosan, (b) silane solution, (c) uncoated PLA/β-TCP scaffold and treated-coated PLA/β-TCP scaffolds with different concentration of chitosan ; (d) 0.5wt% of chitosan , (e) 1.0wt% of chitosan , (d) 1.5wt% of chitosan and (d) 2.0wt% of chitosan.
Table 1. FTIR spectra that exist from the PLA/β-TCP scaffolds, treated and coated with chitosan

| Bonding type | Wavenumber (cm⁻¹) | Sample                                           |
|--------------|-------------------|--------------------------------------------------|
| OH bond      | 622, 3800-3600    | Chitosan                                         |
| C=N          | 1632              | Chitosan/ glutaraldehyde                         |
| PO₄ bond     | 607-554           | β-TCP                                            |
| Si–O, Si–O–Si| 1122, 500         | Silane                                           |
| C–H          | 1452-1353         | Silane                                           |
| NH           | 3300, 1654        | Silane, secondary amide (chitosan)               |
| C=O          | 1598,1728         | Chitosan, PLA                                    |
| C–H          | 1452-1353         | Silane                                           |

4. Conclusion

The effect of different concentrations of chitosan on the morphology, compressive strength, pore size and porosity of the PLA/β-TCP scaffolds were investigated. The PLA/β-TCP scaffold coated with 1.5 w/v% of chitosan exhibited the highest compressive strength with 75.8 % of porosity compared to uncoated and other coated scaffolds. The compressive strength shows the ascending order from 0.5 until 1.5 w/v% and the compressive strength decreased at 2.0 w/v%. That means the chitosan solution at higher concentration (2.0 w/v%) is not able to penetrate into the porous structure. FTIR analysis shows the presence of chitosan and silane functional groups on the surface of the PLA/β-TCP scaffolds.

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