

Evaluation of the zein/inorganics composite on biocompatibility and osteoblastic differentiation

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Abstract

We have previously studied the biocompatibility and mechanical properties of porous zein scaffolds. We based the study on the concept that composite scaffold materials, especially when combined with inorganic materials, are more suited to the mechanical and physiological demands of the host tissue than individual scaffold materials. We investigated the effect of zein/inorganic composite on the physical and biological properties of porous zein scaffolds, which were fabricated by salt-leaching. The composite was prepared by immersion in simulated body fluid. The hydroxyapatite (HA)-coated zein scaffold had the same porosity as the zein scaffold (over 75%). Using scanning electron microscopy, it was established that the morphology of pores located on the surface and within the porous scaffolds showed equally good pore interconnectivity with zein. However, the compressive Young's modulus decreased from 240.1 ± 96.8 to 34.4 ± 12.6 MPa, and compressive strength decreased from 7.8 ± 1.2 to 4.2 ± 0.8 MPa. From the *in vitro* test with human bone marrow stroma cells, the osteoblastic differentiation on the surface of the HA-coated zein scaffold was increased, as expressed by the alkaline phosphatase activity and reverse transcription-polymerase chain reaction analysis for marker genes. From both the mechanical and biological evaluations, the HA-coated zein scaffold was found to be the optimal biomaterial for bone tissue engineering.

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1. Introduction

Materials currently used for tissue engineering substitutes include both natural and synthetic polymers, metals and inorganic materials. The complex biological and sensitive system involved means there are strict and detailed

requirements for scaffold materials because the biocompatibility and mechanical properties are crucial when the scaffolds are used as bone tissue engineering substitutes. However, satisfying the numerous requirements for scaffold materials using only a single material is not possible; therefore, composite systems, which combine the advantages of different materials, are more promising. One such class of composites comprises natural polymers/inorganic materials which are shapable, bioactive and have adjustable biodegradation kinetics [1].

The outstanding characteristic of these inorganic materials is the fact that they have similar mineral constituents to those found in bone; these constituents provide the

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bonding interface with tissues [2]. For example, hydroxyapatite, a synthetic analog of the calcified tissues of vertebrates, has been widely used as a bone graft substitute in many surgical fields, such as orthopedic, dental and plastic surgeries. Its chemical composition, similar to that of bone, confers excellent biocompatibility and osteointegration properties [3].

Zein is a natural protein and has been utilized extensively in a wide range of products, including adhesives, biodegradable plastics, chewing gum, coating for food products, fibers, cosmetic powders, microencapsulated pesticides and drug-delivery matrices [4]. Both zein and its degraded product show good cell compatibility [5–7]. We have previously developed a three-dimensional (3D) zein scaffold in order to study its possible application in tissue engineering [7–9]. As a 3D porous scaffold for bone substitute, a zein scaffold does not interrupt the adhesion, growth, proliferation or osteogenic differentiation of rat mesenchymal stem cells [9].

As an implant material, in terms of satisfactory osteointegration and faster bone regeneration, a zein scaffold is considered to be improved by the addition of calcium phosphates such as HA or β -tricalcium phosphate (TCP) [10]. In this present study, a composite of zein and inorganic material was fabricated, the microstructure and mechanical properties of this composite were discussed, and its biocompatibility was evaluated at the cellular and molecular levels.

2. Materials and methods

2.1. Surface coating and composition analysis of zein scaffolds

Zein scaffolds were prepared by the salt-leaching method. In brief, zein was mixed with sodium chloride, and the mixture was molded into 3D scaffolds, then the samples were leached at 55 °C in a water bath and lyophilized for future use.

Similar to the method used in the work of Kong et al., porous zein scaffolds were coated with calcium phosphate layers by a solution precipitation process [11]; these are henceforth termed HA-coated zein scaffolds. In brief, simulated body fluid (SBF) containing almost five times the inorganic ion concentration of human blood plasma was prepared for the coating process. The SBF concentration levels were 710.0 mM Na^+ , 12.7 mM Ca^{2+} , 7.7 mM Mg^{2+} , 25.0 mM K^+ , 739.7 mM Cl^- , 5.0 mM HPO_4^{2-} , 21.0 mM HCO_3^- and 2.5 mM SO_4^{2-} , and the final pH of the solution was 6.5. Each disc-like HA-zein scaffold specimen, 10 mm in diameter and 2 mm thick, was immersed in 20 ml of $5 \times$ SBF for 36 h at 37 °C. The specimens were then removed from the $5 \times$ SBF solutions, carefully washed with deionized water to remove soluble inorganic ions and lyophilized. The microstructure was observed by scanning electron microscopy (SEM, JSM-6390LV, JEOL, Japan) and the elemental composition was studied using

SEM (FEI SIRION 200, FEI, USA), with an attached energy dispersive spectrometer (EDAX, Inca Oxford, Oxford, UK).

2.2. Assessment of mechanical properties

The cylindrical scaffolds 10 mm in diameter and 25 mm high were prepared, and material testing was carried out using a computer-controlled electronic universal material testing machine (Instron 5867, Instron, USA), to determine the compressive strength and the Young's modulus. The samples were compressed using a standard method with speed-controlled compression force at 1 mm min^{-1} . Data were recorded each second, while the minimum recorded displacement possible is 0.001 mm ($n = 3$).

2.3. Porosity testing of zein scaffolds

Porosity was evaluated according to the apparent densities method described previously [12,13]. The zein scaffolds were dried in an electrical oven at 120 °C for 2 h and then were cooled to room temperature in a desiccator. The weight of dry samples, the apparent weight of saturated samples and the weight of wet samples were then measured to an accuracy of 10^{-4} g ($n = 3$). The open porosity P_a was evaluated according to

$$P_a = (m_3 - m_1)/(m_3 - m_2) \times 100\%. \quad (1)$$

The total porosity P_t was calculated as follows:

$$P_t = (D_t - D_b)/D_t, \quad (2)$$

$$D_b = m_1 D_1 / (m_3 - m_2), \quad (3)$$

where m_1 , m_2 and m_3 are the mass of dry samples, the apparent mass of saturated samples and the mass of wet samples, respectively, D_b is the volume density of samples, D_t is the density of zein (1.22 g cm^{-3}), and D_1 is the density of test liquid at the testing temperature.

2.4. Seeding and culture of human bone marrow stroma cells (hBMSCs) on scaffolds

Using a modification of methods previously reported, hBMSCs were isolated and expanded [14]. All the subjects were healthy with no metabolic, inherited or other disease that might affect the current study. Bone marrow aspirates were obtained during routine orthopedic surgical procedures. Marrow aspirates (20 ml) were harvested from three individual healthy male donors (A–C) (A, 40-years-old; B, 35-years-old; C, 28-years-old) using a bone marrow biopsy needle inserted through the cortical bone. Aspirates were immediately resuspended in α -MEM (Invitrogen; Carlsbad, CA) containing 10% fetal bovine serum (FBS, PAA), 100 U ml^{-1} penicillin and 100 mg l^{-1} streptomycin (Invitrogen, Carlsbad, CA), and cultured in a humidified 37 °C/5% CO_2 incubator. The hBMSCs were selected on the basis of adhesion and proliferation on tissue culture plastic substrate. After 3 days, nonadherent cells were

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