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Mechanical properties, biological activity and protein controlled release by poly(vinyl alcohol)–bioglass/chitosan–collagen composite scaffolds: A bone tissue engineering applications

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ABSTRACT

In the present study, composite scaffolds made with different weight ratios (0.5:1, 1:1 and 2:1) of bioactive glass (15Ca:80Si:5P) (BG)/polyvinyl alcohol (PVA) (PVABG) and chitosan (Chi)/collagen (Col) (ChiCol) were prepared by three mechanical freeze–thaw followed by freeze-drying to obtain the porous scaffolds. The mechanical properties and the *in vitro* biocompatibility of the composite scaffolds to simulated body fluid (SBF) and to rat osteoblast-like UMR-106 cells were investigated. The results from the studies indicated that the porosity and compressive strength were controlled by the weight ratio of PVABG:ChiCol. The highest compressive modulus of the composites made was 214.64 MPa which was for the 1:1 weight ratio PVABG:ChiCol. Mineralization study in SBF showed the formation of apatite crystals on the PVABG:ChiCol surface after 7 days of incubation. *In vitro* cell availability and proliferation tests confirmed the osteoblast attachment and growth on the PVABG:ChiCol surface. MTT and ALP tests on the 1:1 weight ratio PVABG:ChiCol composite indicated that the UMR-106 cells were viable. Alkaline phosphatase activity was found to increase with increasing culturing time. In addition, we showed the potential of PVABG:ChiCol drug delivery through PBS solution studies. 81.14% of BSA loading had been achieved and controlled release for over four weeks was observed. Our results indicated that the PVABG:ChiCol composites, especially the 1:1 weight ratio composite exhibited significantly improved mechanical, mineral deposition, biological properties and controlled release. This made them potential candidates for bone tissue engineering applications.

1. Introduction

Biomaterials for bone tissue engineering have been extensively studied since there is a need for these materials to repair bones which have been lost or damaged due to trauma, injuries, disease or aging. Currently the artificial biomaterials used consist of inorganic materials composited with organic materials [1–5]. For the inorganic component, synthetic calcium phosphate of hydroxyapatite (sHAp), beta tricalcium phosphate (β-TCP), dicalcium phosphate dihydrate (brushite, DCPD) and biphasic calcium phosphate (BCP) have been used. The use of these materials in both *in vitro* and *in vivo* (osteoconductivity) settings has been reviewed in ref. [6–10]. Their inherent brittleness and generally low mechanical strength when developed as three-dimensional (3-D) scaffolds have limited the use of calcium phosphate-based compounds as the bone scaffold materials for clinical application. In addition to being biodegradable, biocompatible, osteoconductive, and bioactive, scaffolding materials used for bone implantation must be mechanically matched to nature bone. This requirement has lead to the biocompositing of the calcium phosphate with polymers (synthesized polymer such as PCL, PMMA, PLA or biopolymers such as chitosan, collagen and alginate are widely used) to improve their mechanical properties [11–16].

Recently, silica based bioactive glasses (BGs) have received much attention due to their possible application in bone tissue engineering [17–22]. BG scaffolds have been shown to have excellent biocompatibility, osteoconductivity and ability to form a bone-like mineral phase at the interface when in contact with living tissue (bioactivity) [18,19]. The potential for utilizing their three-dimensional (3-D) structures to improve the mechanical properties and to provide enhanced biological response has received much attention. Biocomposite of inorganic

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bioactive glass and organic material (biopolymers and synthetic polymers) has been synthesized for bone tissue engineering applications [23–25].

In this study, the mechanical properties and *in vitro* properties of combinations of bioactive glass in soluble poly(vinyl alcohol) (PVA or PVABG) with mixtures of gel-like biopolymer of chitosan–collagen (ChiCol) have been studied. The different composites use different concentrations of the PVABG and the ChiCol components. The weight ratios of the PVABG and ChiCol in the composites employed were 0.5:1, 1:1 and 2:1 and these scaffolds will be referred to as the 0.5:1, 1:1 and 2:1 ratio scaffolds. It is well known that chitosan and collagen (a natural biopolymer) are both biocompatible and are important to the ability of the apatite to form on the scaffold [14,15]. Poly(vinyl alcohol), a water soluble polymer with good biomechanical features, good elastic properties and high degree of swelling in aqueous solution, has been used extensively as a scaffold supporting material for tissue engineering application [26–29].

To see whether PVABG:ChiCol composites having weight ratios of 0.5:1, 1:1 and 2:1 would be promising candidates for bone replacement applications, we have studied the interaction between the cells and the synthesized material's surface. PVABG/ChiCol composites were used as the substrate for the growth of rat osteoblast-like UMR-106 cell over 3 and 7 day periods. Cell morphology, adhesion and proliferation were studied using scanning electron microscopy (SEM), measurement of lactate 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetra zolium bromide (MTT) activity and alkaline phosphatase (ALP) activity. The mechanical properties and degradability of the composite scaffolds and their enhanced mineralization under *in vitro* SBF immersion were investigated. We have also looked at their potentials as drug delivery systems (DDS). In the past, other composites used for bone tissue regeneration also have been functionalized so that they exhibit controlled release of various protein growth factors attached to them. This would increase the scaffold's ability to trigger biological signals favorable for complex tissue morphogenesis. To investigate this, we have loaded bovine serum protein (BSA) (as drug model) into the PVABG/ChiCol scaffold and looked at the BSA release over a four week period.

2. Experimental

2.1. Materials

Chemicals used in this experiment are chitosan from the crab shells having a degree of deacetylation > 85% obtained from Sigma, USA, collagen made from calf skin (Sigma, USA), $Ca(NO_3)_2 \cdot 4H_2O$ (calcium nitrate) (Fluka Chemika, Switzerland), $(NH_4)_2HPO_4$ (diammonium hydrogen phosphate) (Fisher Scientific, UK), sodium hydroxide (NaOH) (Merck, Germany), hydrochloric acid (HCl) (Mallinckrodt chemicals, USA), poly(vinyl alcohol) (PVA) (98% hydrolyzed, average molecular weight of 72,000 g mol^{-1}), sodium dodecyl sulfate (SDS, $CH_3(CH_2)_{11}OSO_3Na$) (QRëC™, New Zealand). These were used without any further purification. Tetraethoxysilane (TEOS, 99%, Sigma, USA) was chosen as the silica precursor.

2.2. Synthesis of bioactive glass (BG) and preparation of PVABG composite particles

BG of molar composition 85%SiO_2–10%CaO–5%P_2O_5 was synthesized by sol–gel method using the procedure described elsewhere [23,30]. The precursors for the BG components were 3.7 g of tetraethyl orthosilicate (TEOS), 0.34 g of $(NH_4)_2HPO_4$ (diammonium hydrogen phosphate), 0.49 g of $Ca(NO_3)_2 \cdot 4H_2O$ (calcium nitrate) and 0.5 M HCl (1.5 g). Sodium dodecyl sulfate (SDS, $CH_3(CH_2)_{11}OSO_3Na$) (2 g) was used as structure-directing agent. These chemicals were dissolved in 60 g of ethanol solution and stirred at room temperature for 2 days to obtain CaO–SiO_2–P_2O_5 sol. The raw powders were obtained by evaporating the ethanol from the sol at 110 °C until it was completely removed. The porous structures were then heated at 1000 °C for 3 h. This stage was done to decompose and burn out the SDS which had supported the structure of the porous bodies in the BG scaffolds. To obtain the PVA/BG composite powder, 2:1 weight ratio of the PVA solution (7 wt.%) and the BG particles were mixed together and stirred for 4 h at room temperature into a homogeneous composite. The supernatant was removed and the composite PVABG powder was obtained by freeze-drying.

2.3. Preparation of gel-like chitosan–collagen (ChiCol) composite

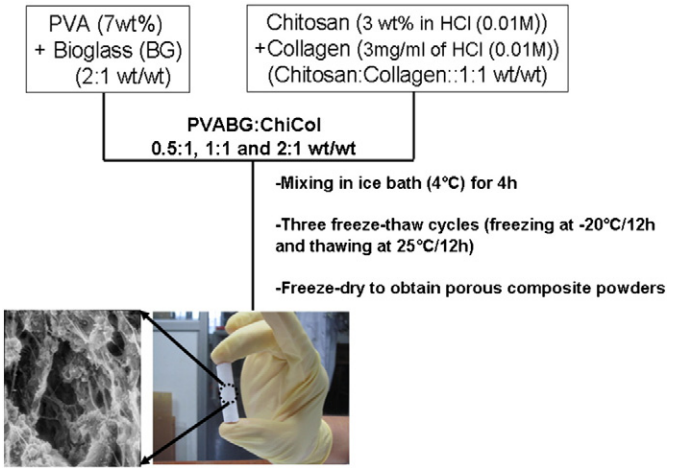
The chitosan–collagen (ChiCol) composite was obtained by mixing the two solutions. No cross-linking occurred. Mixture of collagen (3 mg/mL of 1×10^{-3} M HCl) and chitosan (1 g/100 mL of 0.01 M HCl) having the weight ratio (Chi:Col = 1:1) was prepared as a precursor for the fabrication. Neutralizing the mixture was done by adding NaOH (1 M) to the precursor blend at 4 °C. The final pH was kept at 7 (at this pH, gel like ChiCol formed). The neutralized ColChi hybrid gel was kept at 4 °C for further experimentation.

2.4. Preparation of PVABG/ChiCol composite scaffolds

Composite scaffolds of different amounts of PVABG and ChiCol (at weight ratios of 0.5:1, 1:1 and 2:1) were prepared by three mechanical freeze–thawing steps (12 h at −20 °C and 12 h at room temperature (25 °C) each step). The porous scaffolds were obtained by freeze drying in the steps shown in Scheme 1. The necessary amounts of the PVABG powders were poured into the gel-like solutions of the chitosan–collagen. The resultant mixture was stirred for 4 h in an ice bath. The resulting milky white solution then underwent three freeze/thawing cycles which were carried out (12 h at −20 °C and 12 h at room temperature (25 °C) each cycle). The samples were then frozen at −80 °C for 48 h and lyophilized with a freeze dryer to form porous scaffolds. In this way, PVABG/ChiCol scaffolds with a well developed porous structure were obtained.

2.5. In vitro biomineralization and biodegradable of PVABG/ChiCol composite scaffolds

To study the bioactive behavior of PVABG/ChiCol composite scaffolds, each of the prepared powders was pressed in pellet (with diameter of ~1.3 cm and ~2.5 mm in thickness) and soaked in a simulated body fluid (SBF) for 7 days. The SBF solution we used is one of the more extensively used ones (See ref. [31]). It contains the following chemicals: NaCl (136.8 mM), $NaHCO_3$ (4.2 mM), KCl (3.0 mM),



Scheme 1. Flow chart for the synthesis of bioactive glass (15Ca:80Si:5P) (BG)/polyvinyl alcohol (PVA) (PVABG) and chitosan (Chi)/collagen (Col) (ChiCol) (PVABG:ChiCol) composite scaffolds.