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Bioglass®-based scaffolds incorporating polycaprolactone and chitosan coatings for controlled vancomycin delivery

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Abstract

Highly porous scaffolds have been fabricated by the replication technique using 45S5 Bioglass® (BG) powder. For the purpose of imparting a local drug release capability, the scaffolds were coated with polycaprolactone and vancomycin-loaded chitosan by a two-step procedure. Bare BG scaffolds loaded with vancomycin via a direct immersion method were used as control. The chemical composition and microstructure of bare and coated scaffolds were characterized through Fourier-transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM), respectively. The mechanical properties of the coated scaffolds were significantly improved compared with uncoated scaffolds; the compressive strength values of the coated scaffolds were about 3 times and the area under the stress–strain curve was about 7 times higher than those of the uncoated scaffolds. The scaffolds degradation behavior and the drug release profiles were studied in a phosphate buffered saline (PBS) solution. There was a sharp release of the drug in the first few hours (8 h) for both bare and coated scaffolds. For the bare scaffolds the drug was released completely in 24 h. However, the coated scaffolds showed a sustained release in a period of 11 days, suggesting the potential of the present polymer coated BG scaffolds to be used as bone tissue scaffolds with drug carrier and delivery ability.

Keywords: Bioactive glass; Scaffolds; Drug delivery; Tissue engineering

1. Introduction

The implantation of scaffolds into bone defects for inducing bone regeneration could lead to undesired infection reactions, such as osteomyelitis [1]. Local delivery of antibiotics in the implanted site can be used not only to improve the bone healing [1,2] but also to combat possible infections [3,4]. In order to act effectively in a drug release system associated to bone tissue engineering, the carrier, identified as the scaffold, needs to possess a suitable porous structure and sufficient mechanical properties [5–8]. Moreover, the drug should be released in a controlled manner and for a certain prolonged period [3,4,9–12]. Bioactive ceramics, such as hydroxyapatite, calcium phosphate and bioactive glasses, which are interesting materials for bone tissue scaffolds, have started to be considered also as drug carriers in innovative drug delivery systems [13–16].

Bioactive glass, e.g. the composition 45S5 Bioglass® (45 wt % SiO_2, 24.5 wt% Na_2O, 24.5 wt% CaO and 6 wt% P_2O_5) [17], is an attractive material for bone tissue engineering due to its ability to bond to living bone tissue without fibrous capsule

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formation, a behavior which is related to the ability of this glass to form a calcium phosphate layer with apatite-like structure when in contact with biological fluids [17–20]. However, porous 45S5 Bioglass® (BG)-based scaffolds, for example fabricated by the foam replica method, exhibit relatively low compression strength and are brittle [21]. It has been widely reported that coating the brittle scaffold structure with polymer layers is a suitable approach to overcome this shortcoming [22–24]. Moreover, loading drugs into the polymer coating can be used to impart to the scaffold a local drug delivery capability, as shown in an early study by Knowles et al. [25], in this manner the homogeneous entrapment of the drug throughout the 3D scaffold structure is possible and the drug release period can be prolonged.

Polycaprolactone (PCL) and chitosan are two of the most applied biocompatible polymers in tissue engineering and drug delivery approaches. PCL is a biodegradable aliphatic polyester [26], which is well-known for its attractive properties for biomedical applications such as nontoxicity, gradual resorption after implantation and excellent tensile properties [26–28]. Chitosan (CS) is a linear polysaccharide and is reported to be biodegradable, biocompatible, non-toxic, hydrophilic, and has a remarkable affinity for proteins [29–31].

Vancomycin is a glycopeptide antibiotic isolated from *Streptomyces orientalis* which is used to treat infections caused by Gram-positive bacteria [32,33], including methicillin-resistant staphylococcus (MRSA).

In this study, porous BG-based scaffolds fabricated by the replication method [21] were coated with a dual coating system formed by a PCL layer and a vancomycin containing CS hybrid layer. The PCL layer has as primary function the improvement of the brittleness and low strength of the BG scaffolds, while CS was used as vancomycin carrier. Incorporation of vancomycin into BG scaffolds by immersing scaffolds into a CS–vancomycin hybrid solution is expected to be a convenient alternative to attain an effective concentration of the drug at the scaffold implantation site. This is the first study considering this type of BG-based scaffolds with drug delivery capability introduced by CS coatings. The chemical composition, microstructure, mechanical properties and degradation behavior of the bare (uncoated) and coated scaffolds were investigated. In addition, the release of vancomycin from bare and coated BG scaffolds in PBS was examined.

2. Materials and methods

2.1. Preparation of BG scaffolds

45S5 Bioglass®-based scaffolds with interconnected pores were prepared by the replication method, similar to the technique introduced in an earlier study [21]. First, 0.2 g of PCL (Sigma, UK) was dissolved in 10 ml dimethyl carbonate (DMC, Sigma, Germany) to obtain a homogeneous polymer solution. Commercially available 45S5 Bioglass® powder (~2 μm in mean particle size) was added into the PCL solution in a proportion of 30 wt%. Then PU foams (Eurofoam GmbH, Germany) of 45 ppi (pores per inch) were immersed in the BG–PCL slurry for 1 min. After extracting the coated samples, these were squeezed manually to eliminate the excess slurry and dried in normal air at room temperature. Subsequently, the above steps were repeated twice. Finally, the samples ("green bodies") were sintered to produce BG-based scaffolds. The sintering schedule included an intermediate holding step at 400 °C for 1 h and a second stage at 1100 °C for 2 h [21].

2.2. Direct drug loading into BG scaffolds

Vancomycin hydrochloride (Applichem GmbH, Germany) was dissolved in distilled water with the concentration of 25 mg/ml. Scaffolds of dimensions $7 \times 7 \times 10\ mm^3$ were immersed in the drug containing solution for 10 min and dried for 24 h at 37 °C in air. These drug loaded scaffolds were fabricated for comparison purposes with the polymer coated scaffolds discussed below.

2.3. Drug loaded polymer coated BG scaffolds

As-fabricated BG scaffolds were first immersed in 4 wt%/v of PCL solution in DMC for 10 min and dried for 24 h at 37 °C. For the coating solution containing the drug, 2 wt%/v of chitosan (CS, Sigma, Japan) and two different concentrations of vancomycin (vancomycin/solvent = 25 and 50 mg/ml) were dissolved in 1% of acetic acid (Sigma, Germany). The PCL coated BG scaffolds were dipped into the solution for 10 min and dried for 24 h at 37 °C. The amount of drug loaded was determined by measuring the total amount of drug released from the scaffold using a UV spectrophotometer, as carried out in previous studies [25,34–36].

2.4. Microstructural characterization and mechanical tests

The BG scaffolds exhibited high porosity, which were determined by measurement of their mass and dimensions and applying

$$P_{scaffold} = [1 - W_1/(V_1 \rho_{solid})] \times 100\% \quad (1)$$

where ρ_{solid} (2.7 g/cm^3) is the density of solid 45S5 Bioglass® [17], W_1 is the weight of the scaffold, and V_1 is the volume of the scaffold, which was determined from the measurements of scaffold dimensions using digital calipers.

The composition of scaffolds was characterized by Fourier transform infrared spectroscopy (FTIR, Nicolet, USA). Samples were ground with KBr in an agate mortar and compressed to tablets. The spectra were collected in the 4000–400 cm^{-1} range with a resolution of 4 and 32 scans.

The morphologies of bare and coated BG scaffolds were studied by using a scanning electron microscope (Zeiss Leica, Germany) at an accelerating potential of 10 kV.

The compression strength of bare and coated 45S5 Bioglass® scaffolds was measured using a Zwick/Roell Z050 universal testing machine at a crosshead speed of 1 mm/min. The samples were prismatic in shape and the dimensions were measured by Vernier calipers before loading. The nominal dimension of samples was $7 \times 7 \times 10\ mm^3$. Ten samples per group were

measured and results were averaged. The compressive stress was calculated by applying

$$P = \frac{F_{max}}{L \times B} \quad (2)$$

where P represents the compressive strength, F_{max} represents the applied maximum load, L represents the length and B represents the width of the scaffold. The work of fracture, W_{ab}, considered proportional to the area under the stress–strain curve, was calculated by using Eq. (3) [37], where $\sigma(\varepsilon)$ is the stress and ε is the strain:

$$W_{ab}(\varepsilon) = \int \sigma(\varepsilon) d\varepsilon \quad (3)$$

2.5. In vitro degradation

Prismatic-shaped specimens were placed in clean conical flasks containing 10 ml phosphate buffer saline (PBS, Sigma, USA). Specimens were incubated at 37 °C and shaken at 90 rpm. Three samples for each group were assessed. The PBS solution was replaced twice a week and after incubation, samples were washed with distilled water and dried at 37 °C. Weight loss was calculated by Eq. (4) [38], where W_0 is the initial mass and W_1 is the mass after soaking in PBS at a given time.

$$\text{Weightloss } (\%) = [(W_0 - W_1)/W_0] \times 100 \quad (4)$$

2.6. In vitro drug release study

Vancomycin loaded bare (uncoated) scaffolds and vancomycin loaded and coated (PCL/CS/BG) scaffolds were used in the *in vitro* drug release study. Three bare and coated BG scaffolds with dimensions of $7 \times 7 \times 10$ mm^3 were immersed in 10 ml PBS solution at 37 °C at pH 7.4. Each sample was incubated in an orbital shaker at a speed of 90 rpm. The drug released in the PBS medium was determined using a UV spectrophotometer at a wavelength of 280 nm. A calibration curve was obtained with vancomycin concentration in the range 0.005–0.15 mg/ml. It was observed that the calibration curve follows Beer's law: $A = 8.1505C$, where A is the absorbance and C is the concentration of vancomycin.

3. Results and discussion

3.1. FTIR analysis

In order to determine the incorporation of vancomycin in the coated BG scaffolds, FTIR spectroscopy was carried out. The absorption bands of vancomycin (Fig. 1(a)) were found at 3450 cm^{-1}, 1655 cm^{-1}, 1504 cm^{-1}, and 1231 cm^{-1} for hydroxyl stretching, C=O stretching, C=C and phenols [39]. Characteristic peaks of chitosan (Fig. 1(b)) were identified at 3450 cm^{-1}, 1660 cm^{-1}, 1596 cm^{-1}, and 1322 cm^{-1} for hydroxyl stretching, amide I, amino group and amide β, respectively. In the

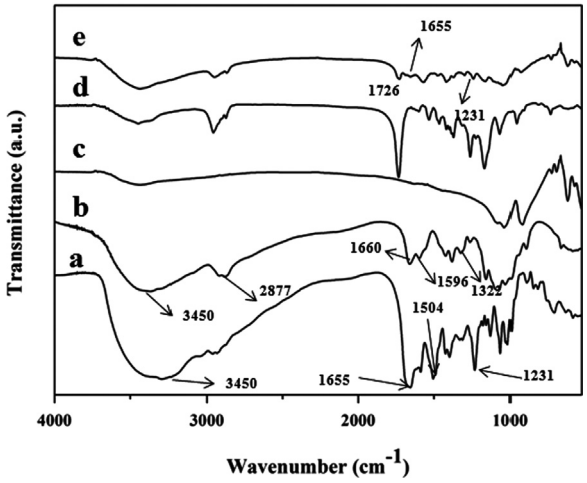

Fig. 1. FTIR spectra of (a) vancomycin, (b) CS, (c) sintered BG, (d) PCL and (e) vancomycin loaded polymer coated BG scaffolds (the explanation of the different peaks identified is presented in Section 3.1).

vancomycin loaded composite scaffold (Fig. 1(e)), the peak at 920 cm^{-1} was attributed to the Si–O stretching vibration and the peak at 1035 cm^{-1} was assigned to P–O stretching vibration of the scaffold [40,41]. In addition, the composite presented the characteristic peaks of PCL (C=O, 1726 cm^{-1}), CS (amide I, 1655 cm^{-1}) and vancomycin [42] (phenolic group, 1231 cm^{-1} and amide I, 1655 cm^{-1}). The hydroxyl stretching was less pronounced in the composite; this may be due to hydrogen bonds present in both chitosan and vancomycin.

3.2. Morphology

Fig. 2 shows the SEM morphology of BG scaffolds coated with vancomycin and with a dual coating of PCL and vancomycin loaded CS solution. The uncoated BG scaffolds exhibit a well-developed open pore structure (Fig. 2(a)). When the BG scaffolds were coated with the dual layers of PCL and vancomycin–CS, the struts became smoother and thicker. In the case of 25 mg/ml vancomycin coated BG scaffolds some pores were blocked (Fig. 2(c)) while blocking of pores increased for 50 mg/ml vancomycin coated BG scaffolds (Fig. 2(d)). Comparing Fig. 2(c) and (d), it can be observed that for coatings obtained using the same concentration of PCL and CS, more pores were blocked in the case of high vancomycin concentration; this might be influenced by the increasing solution viscosity with increasing vancomycin concentration.

3.3. Mechanical tests

Typical stress–strain curves of as-sintered and polymer coated BG scaffolds are shown in Fig. 3. The sintered (uncoated) BG scaffolds exhibit the typical failure mode of brittle ceramic foams. The general shape of the stress–strain curves for the uncoated and coated scaffolds is similar. The value of W_{ab} can be calculated from the area under the stress–strain curve at a given value (e.g. 60%) of strain, with the use of Eq. (3). Compared to as-sintered BG scaffolds, the coated

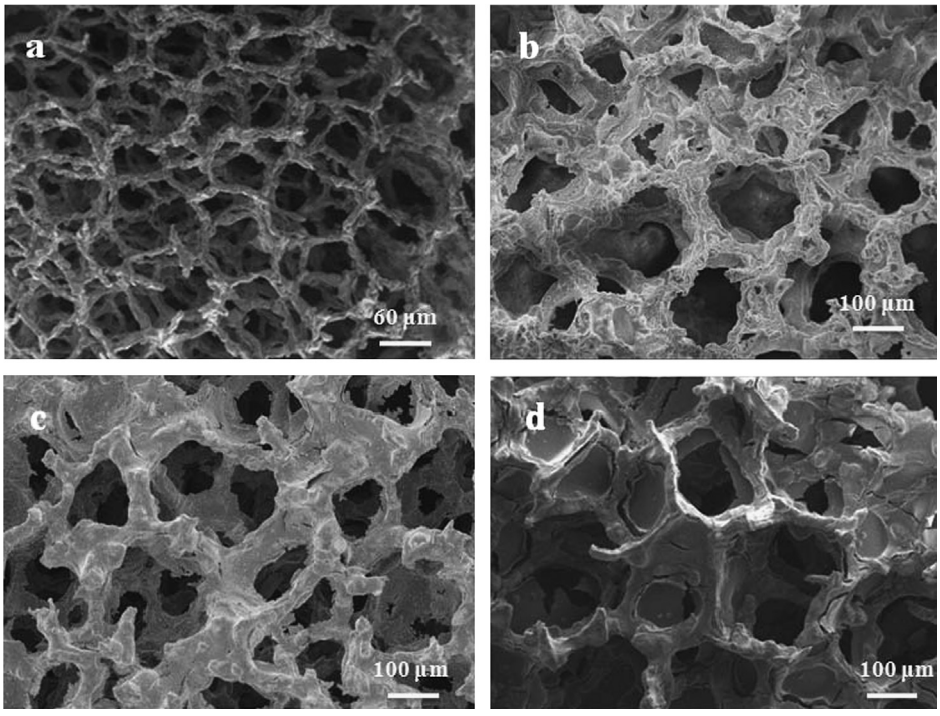

Fig. 2. SEM morphologies of vancomycin-containing BG scaffolds: (a) bare (uncoated) scaffold, (b) PCL–CS coated scaffold and (c) and (d) PCL–CS coated scaffolds (25 mg/ml and 50 mg/ml of vancomycin, respectively).

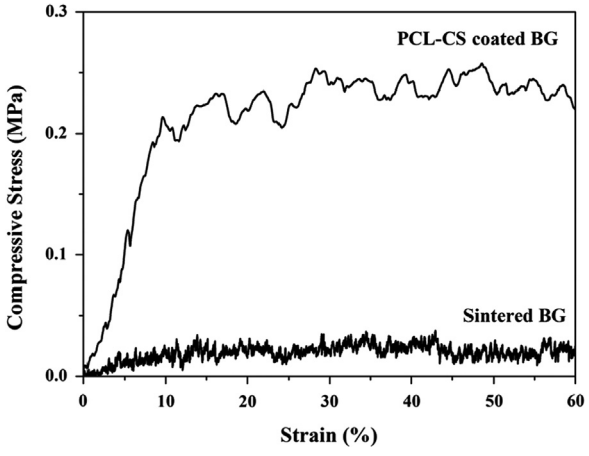

Fig. 3. Typical stress–strain curves of sintered (uncoated) BG and PCL–CS coated BG scaffolds.

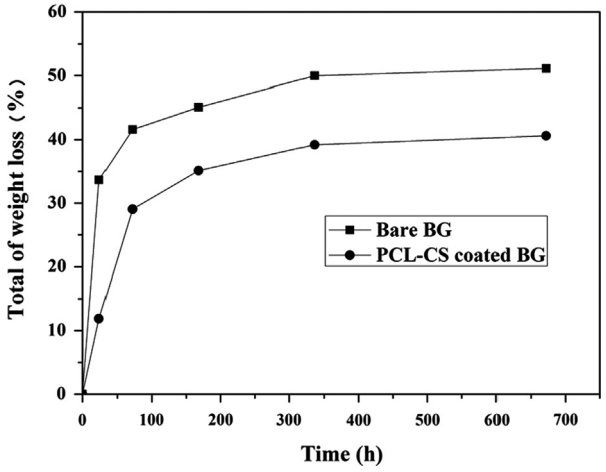

Fig. 4. Weight loss of bare (uncoated) and PCL–CS coated BG scaffolds after immersion in PBS solution for 28 days.

BG scaffolds had higher compressive strength, elastic modulus (slope of the stress–strain curve) and work of fracture. The compressive strength value of coated scaffolds was 0.20±0.04 MPa. The value of W_{ab} of the uncoated scaffolds was ~1.8 N cm whist the coated BG scaffold had a value of ~12 N cm, which indicates a substantial increase of the toughness of the scaffold induced by the presence of the polymer coating, in agreement with literature reports [22–24].

3.4. In vitro degradation

Fig. 4 shows the degradation rates of uncoated and PCL–CS coated BG scaffolds in PBS at 1, 3, 7, 14 and 28 days. The data show that mass loss gradually increased over time for both bare and coated BG scaffolds. At day 1, the total weight loss of uncoated and coated BG scaffolds was 33.7% and 11.9%, respectively. However, the weight loss of the coated scaffolds was almost 2 times higher than that of bare scaffolds from day 1 to 3. This result is likely due to the dissolution of CS in the PBS solution. From the 3rd day to the 28th day, the degradation rates of the two groups were similar. After 28 days of degradation, the total weight loss values for bare and coated BG scaffolds were 51.1% and 40.6%, respectively. Overall, the coated scaffolds showed lower total weight loss than bare scaffolds.

3.5. In vitro drug release

The percentage of vancomycin released from bare and coated BG scaffolds was normalized to the total amount of vancomycin incorporated into the BG scaffolds. Results of cumulative release in PBS are shown in Fig. 5. Scaffolds coated with CS incorporating high (vancomycin/solvent = 50 mg/ml) (*H*) and low (vancomycin/solvent = 25 mg/ml) (*L*) vancomycin concentrations were investigated. Three stages of the release profile were observed. In the first stage, all samples showed rapid drug release during the first 12 h, which is ascribed to the dissolution of the surface incorporated vancomycin; however the coated scaffolds showed lower initial burst release (coated *L*: 51% and coated *H*: 35%). In the second stage, the release rate decreased and the curve changed its slope. In this stage, the coated scaffolds exhibited a longer release period than the bare scaffolds. In the third stage, the release from bare scaffolds was complete, and the release rate from the coated scaffolds was much lower than the one measured in the earlier stages.

It is observed that during the release period investigated, the drug in the bare BG scaffolds was released completely in just 24 h while the drug entrapped in the polymer coated BG scaffolds was released in a controlled manner over the whole 11-day period. It was also confirmed that the release rate from scaffolds coated at the high vancomycin concentration (*H*) was lower than that of low concentration (*L*) coatings. This result may be due to partial blocking of pores by the polymer coating, which occurs to a lesser extent in scaffolds with lower vancomycin content (*L*) compared with the high concentration coated scaffolds (*H*), as indicated also in Fig. 2(c) and (d). Thus the L-coated scaffolds present a higher area of contact with PBS. However, the overall trend of drug release of the two coated scaffolds was similar.

4. Conclusions

Porous BG scaffolds coated by PCL and CS were developed for use in bone tissue engineering applications. The antibiotic vancomycin was entrapped into the BG scaffolds via CS coating to confer the scaffold controlled drug release capability. The compressive strength, elastic modulus and work of fracture of the coated scaffolds were higher than those of bare scaffolds. The *in vitro* degradation of coated scaffolds in PBS was lower than that of bare scaffolds in a period of 28 days. Vancomycin in the polymer coated scaffolds was released in a controlled and sustained manner and the release rate was dependent on the vancomycin concentration in the CS coating. The present scaffolds exhibiting the intrinsic biactivity of bioactive glass and controlled release ability of an antibiotic are proposed for bone tissue engineering and represent a new scaffold type in the emerging field of tissue engineering therapeutics based on bioactive drug eluting scaffolds.

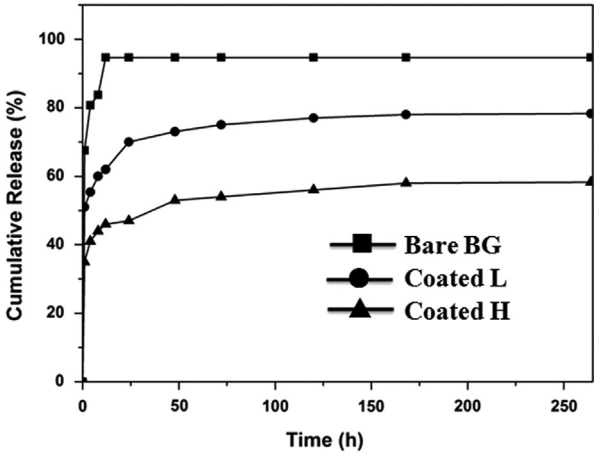

Fig. 5. *In vitro* vancomycin released from bare and coated BG scaffolds. Coated BG scaffolds were loaded with the drug at two different concentrations (vancomycin/solvent = 25 mg/ml (*L*) and 50 mg/ml (*H*)).

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