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Bilayered bioactive glass scaffolds incorporating fibrous morphology by flock technology

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ABSTRACT

We report for the first time on the combination of polymer replication method and flock technology to produce 45S5 bioactive glass (45S5 BG) scaffolds with a fibrous surface morphology suitable for tissue engineering (TE). In order to develop a fibrous morphology on the surface of the scaffolds, electroflocking was used with BG scaffolds serving as the substrate. Gelatin was used as the adhesive and short polyamide (PA) fibers were electroflocked on the top surface to produce scaffolds with anisotropic properties. In order to improve the stability of the adhesive (gelatin) and to create a better bonding between the fibers and the adhesive, a crosslinking step was carried out. The porosity on the surface could be tailored by varying the flocking time which, in turn, was shown to influence the packing density of the fibers. *In vitro* bioactivity study in simulated body fluid (SBF) was carried out and complete mineralization of the scaffold, including on the PA fibers, was detected and characterized using various techniques.

1. Introduction

Cartilage defects are commonly not repairable because a reparative matrix is not formed due to the fact that resident articular chondrocytes do not migrate into the damaged area [1]. Osteochondral tissue engineering (TE) approaches aim to repair cartilage defects at the bone interface which cannot be treated by conventional therapies and persist due to the non-existent involvement of the vasculature [2]. Driven by increasing demand, new strategies for osteochondral TE are fast developing which involve the fabrication of bilayered scaffolds [3]. In case of cartilage damage, anisotropic scaffolds with parallel fiber orientation which provide columnar pore channels represent a suitable approach [4]. These types of scaffolds can be developed by flock technology [5]. Electroflocking is the process of applying short fibers to a substrate covered with an adhesive [6]. In this process, fibers are aligned in the applied electrostatic field and accelerate towards the adhesive-coated substrate [6]. Despite its suitable application for fabricating ordered fibrous structures, electroflocking has not been yet widely considered to produce scaffolds for TE. Only few studies are available in literature [4,5,7]. Walther et al. [7], for example, showed that chondrogenesis of mesenchymal stem cells (MSC), which is determined by the synthesis of proteoglycan and deposition of collagen type II, was significantly higher on flock scaffolds than when cultivated in collagen gels.

In the present study, we have considered for the first time electroflocking technology combined with standard bioactive glass (BG) scaffolds. We developed scaffolds intended for osteochondral TE using 45S5 BG foams as substrate and gelatin as an adhesive. PA fibers were flocked perpendicularly to the three-dimensional, porous substrate to obtain composite scaffolds which will mimic both the anisotropic arrangement of collagen fibrils in the native cartilage tissue and the porous nature of the cancellous bone.

2. Materials and methods

2.1. Materials

Commercially available bioactive glass powder of 45S5 composition (in wt% – 45% SiO_2, 24.5% Na_2O, 24.5% CaO, 6% P_2O_5) with a mean particle size of 2 μm and polyvinylalcohol (PVA) (Merck Schuchardt OHG, Hohenbrunn, Germany) were used for scaffold fabrication. Fully reticulated polyurethane foams with 45 pores per inch (ppi) (Eurofoam Deutschland GmbH, Wiesbaden, Germany) acted as sacrificial templates for the final scaffolds made by foam replica method [8]. Gelatin (Sigma-Aldrich Chemie, Steinheim, Germany) was used as the adhesive and short PA fibers (Borchert+Moller GmbH u. Co.KG, Haigerloch-Stetten, Germany) were used for the flocking process. As a crosslinker, 1% N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide

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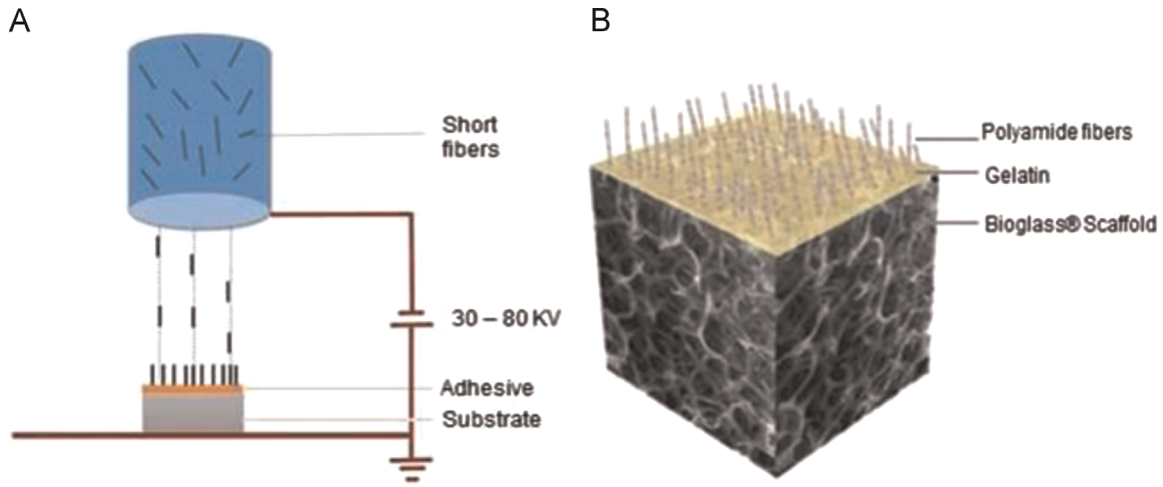

Fig. 1. Schematic diagram of (A) Electrostatic flocking and (B) fabricated bilayered scaffold.

hydrochloride (EDC) (Sigma- Aldrich Chemie GmbH, Steinheim, Germany) was used.

2.2. Scaffold processing

The substrate is the BG scaffold made by the foam replica technique [8]. In this process, a slurry of 5.7% PVA (binder) and 37% BG powder in de-iodized water was prepared. The PU foams were dipped into the slurry and the excess BG was squeezed out to avoid blocking of the pores. The green bodies were dried overnight before sintering in the furnace. The temperature profile of the sintering process involves heating up to 450 °C for 1 h to burn out the polymer foam and then sintering at 1000 °C [8].

As adhesive, gelatin was used. In the initial experiments, different gelatin solutions with different concentrations (2.5%, 5%, 10%, 15% and 20%) were prepared. The gelatin was dissolved in water under permanent stirring conditions at 50 °C. Except for the 15% gelatin solution, the other solutions were either too dilute or too viscous to be used. The optimal gelatin solution was applied on the top surface of the BG scaffold manually and a flocking box containing the fibers was placed directly over the scaffold. Fig. 1 shows the principle of flock technology as applied in the present experiments. The experimental parameters involve varied flocking durations of 10 s, 30 s and 60 s, a voltage of 60 kV and a flock distance of 12 cm.

After the flocking process, it was necessary to bind the adhesive to the substrate and to stabilize the glue. For this reason, a cross-linking step with 1% N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride followed. The EDC was dissolved in 80% ethanol and the flocked scaffolds were kept in this solution for 20 h. In order to remove toxic components from the fibers, the samples were washed in acetone once and thrice in de-iodized water.

2.3. Characterization

The surface morphology of the scaffolds before and after electroflocking was observed using a scanning electron microscope (SEM). An *in vitro* study was conducted in SBF following the procedure described by Kokubo et al. [9] in order to detect bioactivity, by assessing hydroxyapatite (HA) formation on the scaffold. The bi-layered constructs were immersed in 50 mL of SBF and kept for up to 3 weeks in a shaking incubator at 90 rpm (KS 4000 I control, IKA, Germany) and 37 °C. The pH of the solution was maintained at 7.4 and the solution was replaced twice a week. Samples were collected from the incubator after 7, 14 and 21 days, rinsed in deionized water, dried at room temperature and taken for further characterization by SEM and Fourier transform infrared spectroscopy (FTIR) analysis.

3. Results and discussion

3.1. Surface morphology

Fig. 2(A–D) shows SEM images of the as-fabricated BG scaffold, gelatin-covered scaffold and the scaffolds flocked for 10 s and 30 s.

Flocking for 10 s produced surfaces with fibers of low packing density (Fig. 2(C)). Increasing the flocking time increased the packing density of fibers (Fig. 2(D)). It was noted that higher density of fibers on the surface prevented toppling of fibers leading to a 'hairy' surface morphology.

The preparation of the adhesive is an important step in the flocking process. The 2.5%, 5% and 10% gelatin solutions were very dilute. On the other hand, the 20% gelatin solution was too viscous and started gelling even before it was applied on the scaffold. The gelatin solution with 15% concentration showed the right balance of properties and was found to provide a suitable interface for the PA fibers to adhere on the BG struts. Fig. 3(A) shows a SEM micrograph of a single pore of a BG scaffold exhibiting the gelatin layer and PA fibers attached to it, while Fig. 3(B) depicts a single PA fiber attached vertically to a BG strut, indicating a continuous defect-free interface between the fiber and the gelatin layer suggesting a strong adhesion of fibers.

3.2. In vitro bioactivity study

The flocked BG scaffolds were immersed in SBF for a period of 21 days. Cauliflower shaped structures typical for hydroxyapatite (HA) formation [10] were present on the surface of the scaffolds. Interestingly, the PA fibers were also completely covered with HA crystals. Fig. 4(A) and 4(B) show the formation of HA on the scaffold as well as on PA fibers flocked for 10 s and 30 s, respectively, and after immersion in SBF for 21 days. FTIR analysis was performed to further confirm the deposition of HA crystals. Fig. 4 (C) and 4(D) show the FTIR spectra of the scaffolds flocked for 10 s and 30 s, respectively, and after immersion in SBF for 7, 14 and 21 days. The HA formation is confirmed by the appearance of bands at ~567, 600 and 1017–1090 cm^{-1} corresponding to the bending and stretching vibration of phosphate groups (PO_4^{3-}). The band at 878 cm^{-1} corresponds to the vibration mode of carbonate groups (CO_3^{2-}) and suggests the formation of carbonated HA.