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In situ formation of multilayer biocomposite with anisotropic crystal orientation

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ABSTRACT

Hydrogel composites were fabricated through a novel layer by layer spray coating process using wet chemical precursors, which were sprayed alternately onto a substrate. With this simple method the gelification of calcium alginate gel and the anisotropic crystallization of brushite within the biopolymer matrix could be obtained simultaneously. The oriented deposition of brushite crystals was confirmed by SEM and X-ray diffraction analysis, respectively. The latter showed a higher intensity of the (0 4 0) reflection compared with randomly oriented brushite powder, strongly indicating a preferred crystal orientation within the composites. These composites might be used in biomedical applications as barrier materials or possibly for mineralized interfaces like osteochondral tissue.

1. Introduction

Biopolymer composites are commonly used for biomedical applications [1]. Especially hydrogel-based materials have gained considerable interest in this field [2]. Particle size, composition, shape and orientation of the inorganic component are important factors for the composite properties and application [3,4]. In the present article hydrogel composites were fabricated by the oriented crystallization of brushite ($CaHPO_4 \cdot 2H_2O$) directed by the structure of an organic alginate matrix. Brushite, a bioresorbable mineral [5], may crystallize in a planar shape [6]. In this manifestation the basal plane of the crystal is identical with the crystallographic (0 1 0) plane [7]. Sodium alginate, which can be cross-linked by ionotropic gelation [8], is used as biocompatible organic component of the composite material. The composite synthesis was carried out using wet chemical precursors which were processed through a simple layer by layer spray coating regime whereas the crystallization process of brushite occurred simultaneously to the ionotropic gelification of the biopolymer. Since alginate membrane formation is completed rapidly the comparably slow growing brushite crystals can be guided.

2. Materials and methods

Composite fabrication: An alginate solution was produced by dissolving 3 wt% Protanal LF 10/60, a low viscosity alginate ($G/M = 70\%$, $MW = 180$ kDa; FMC Biopolymer, Drammen, Norway), in distilled water. In addition $(NH_4)_2HPO_4$ was supplemented in concentrations between 50 mmol/l and 300 mmol/l. For the layer preparation a 1 mol/l $CaCl_2$ solution was prepared. An overview of the processing steps involved is given in Fig. 1(a)–(c).

Both solutions were alternately sprayed by separate airbrush guns, each 25 times in succession ($p = 3$ bar), onto a sandblasted glass substrate to generate a multi layer structure. The required amounts of reagents to obtain a Ca/P molar ratio of 1.0 in the crystallization reaction were adjusted after each spraying step by weighting. To obtain a homogeneous mass distribution with minimum side effects, a panel with an opening in sample size of 10 cm × 15 cm, was placed between airbrush guns and substrate. On contact of the two solutions the ionotropic gelation of alginate (represented by alginate anion $C_6H_7O_6{}^-$), as well as the crystallization of brushite from $HPO_4{}^{2-}$- and Ca^{2+}-ions was induced by ion diffusion:

$$2Ca^{2+} + HPO_4^{2-} + 2C_6H_7O_6^{-} + 2H_2O \rightarrow CaHPO_4 \times 2H_2O + Ca(C_6H_7O_6)_2$$

The resulting composites were compressed unidirectional with 13 hPa for 24 h. Accordingly the specimens were transferred into a 1 mol/l $CaCl_2$ solution for further ionic cross-linking. After one day, they were washed several times in an ultrasonic bath with deionized water, followed by subsequent dehydration in acetone and covalent cross-linking in 10 vol% 1,6-hexamethylene diisocyanate and 90 vol% acetone. Unreacted isocyanate groups were eliminated by placing the samples in ethanol. These three steps were each carried out for 24 h. After another ultrasonic treatment the composites were rehydrated and stored in deionized water. A macroscopic image of the resulting composite sheets is shown in Fig. 1(d).

Scanning electron microscopy: The composites were freeze dried and gold-coated using an "Emtech K550" DC-sputtering-system.

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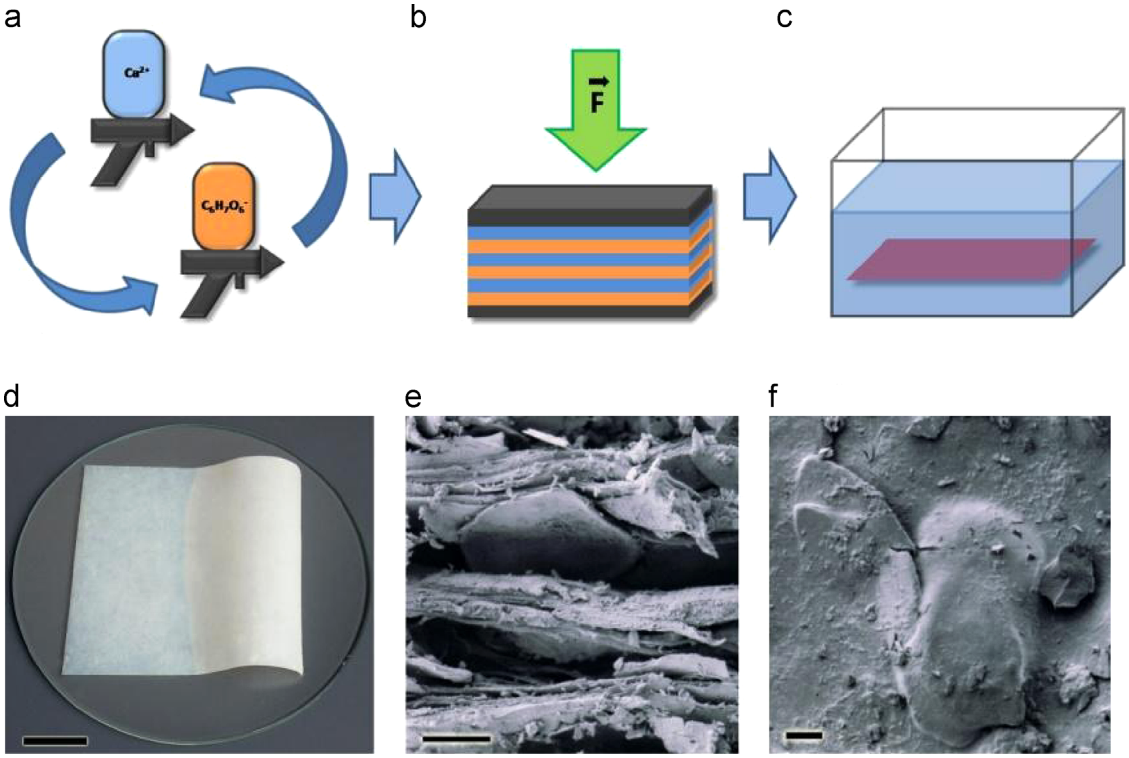

Fig. 1. Processing steps of composite fabrication: (a) Alternating spray coating of calcium and biopolymer solution with airbrush guns, (b) unidirectional compression, (c) wet chemical treatments (ionic cross-linking, washing, subsequent dehydration and covalent cross-linking). Resulting composites: (d) Photography of hydrated (left) and partial dried (right) composite; (e) SEM image of cross section through intentionally delaminated composite structure, (f) SEM image of top view on the composite. Planar brushite crystals are predominantly aligned parallel to the enclosing matrix layers (Scale bars: (d) 2 cm, (e) and (f) 20 μm)

A “Digital Scanning Microscope 940” (Zeiss, Germany) was used to record images with an acceleration voltage of 5 kV.

X-ray diffraction: Two theta scans of the samples were recorded with an X-Ray Diffractometer, “D5005” (Siemens, Karlsruhe, Germany) in Bragg–Brentano geometry. As radiation source served an X-ray tube type “FK60-04 × 12” with a copper anode. At an acceleration voltage of 40 kV, an electron current of 30 mA was realized. The XRD diffraction patterns were recorded at a resolution of 0.02° in a 2Θ angle range from 20° to 40°.

Mechanical testing: Static tensile testing was performed using a Zwick 1440 (Zwick, Ulm, Germany) with a preliminary test force of 0.3 N and a maximum load of 12 N. The cross head speed was set to 10 mm/min. Rectangular specimens with dimensions of 13 mm × 60 mm and an average thickness of 0.2 mm were used to determine tensile strength and elastic modulus, respectively.

DSC/TG: DSC/TG measurements were carried out on a STA 409 PC (Netsch, Selb, Germany) with a heating rate of 5 K/min and an argon flow of 150 ml/min.

3. Results

SEM investigations of the mineralized region showed plate like brushite crystals incorporated between alginate matrix layers, being aligned parallel to the composite surface. An intentionally delaminated cross section reveals the anisotropic mineralization between the ca. 1 μm thick alginate layers (Fig. 1(e)), whereas the top of the composite is covered with flat aligned crystals and calcium alginate (Fig. 1(f)).

An existing anisotropy within the spatial orientation of the crystallites can be analyzed by comparison of the composite X-ray diffraction (XRD) pattern with the XRD pattern of polycrystalline brushite powder prepared by aqueous precipitation (Fig. 2). The powder shows a random orientation of the crystallites, whereas the respective reflection intensities primarily match those of the database [9] (Fig. 2(b)). In contrast to this and in relation to the other occurring reflections of the composite diffraction pattern, a significant higher intensity of the (0 4 0) reflection is indicating a preferred crystal orientation within the composite (Fig. 2(a)). The diffraction pattern of the composite is superimposed with a stronger background due to the biopolymer content.

Considering the mineral content, the composites were incinerated (see exemplary DSC/TG curve in Fig. 3(a)). The residual mass after incineration and the mineral content of the composite were directly proportional to the concentration of $HPO_4{}^{2-}$ ions used for synthesis (see Fig. 3(b)). Structures produced with molar hydrogen phosphate concentrations from 0.05 mol/l to 0.3 mol/l were analyzed regarding their tensile strength and elastic modulus. The tensile strength decreased linearly from 4.9 MPa, at 0.05 mol/l $HPO_4{}^{2-}$, to 3.5 MPa, when 0.3 mol/l $HPO_4{}^{2-}$ was used. Corresponding to these results, the elastic modulus increased linearly from 63.5 MPa, in the case of highest mineral content, to 325 MPa, at the lowest mineral content. (Fig. 3(c) and (d)).

4. Discussion

Anisotropic mineralization reveals to be a crucial attribute of many biocomposites [10,11]. As demonstrated in the current study, the biopolymer matrix may guide the spatial orientation of growing brushite crystals (see Fig. 4). At contact of alginate solution and calcium solution a thin gel membrane is formed at the interface. Through this membrane, with pores smaller than 5 nm [8], ion diffusion takes place, whereas the larger alginate polyanion cannot penetrate the gel membrane. Concentration gradients accelerate calcium ions towards the membrane and into the biopolymer phase, while macromolecular alginate ions (hexagonal structures/$C_6H_7O_6{}^-$) and hydrogen phosphate ions move in opposite direction. Inside the