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Nucleation and growth of biomimetic apatite layers on 3D plotted biodegradable polymeric scaffolds: Effect of static and dynamic coating conditions

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Abstract

Apatite layers were grown on the surface of newly developed starch/polycaprolactone (SPCL)-based scaffolds by a 3D plotting technology. To produce the biomimetic coatings, a sodium silicate gel was used as nucleating agent, followed by immersion in a simulated body fluid (SBF) solution. After growing a stable apatite layer for 7 days, the scaffolds were placed in SBF under static, agitated (80 strokes min^{-1}) and circulating flow perfusion ($Q = 4$ ml min^{-1}; $t_R = 15$ s) for up to 14 days. The materials were characterized by scanning electron microscopy/energy dispersive X-ray spectroscopy, Fourier transform infrared spectroscopy and thin-film X-ray diffraction. Cross-sections were obtained and the coating thickness was measured. The elemental composition of solution and coatings was monitored by inductively coupled plasma spectroscopy. After only 6 h of immersion in SBF it was possible to observe the formation of small nuclei of an amorphous calcium phosphate (ACP) layer. After subsequent SBF immersion from 7 to 14 days under static, agitated and circulating flow perfusion conditions, these layers grew into bone-like nanocrystalline carbonated apatites covering each scaffold fiber without compromising its initial morphology. No differences in the apatite composition/chemical structure were detectable between the coating conditions. In case of flow perfusion, the coating thickness was significantly higher. This condition, besides mimicking better the biological milieu, allowed for the coating of complex architectures at higher rates, which can greatly reduce the coating step.

Keywords: Tissue engineering scaffolding; Starch biodegradable polymer; Biomimetic; Calcium phosphate; Apatite coating

1. Introduction

The field of tissue engineering continues to advance with the discovery of new biomaterials, growth factors and scaffold fabrication techniques. In addition to demineralized bone matrix, numerous synthetic [1–3] and naturally [4–6] derived biodegradable porous scaffolds are being proposed for use as bone graft substitutes, and as delivery vehicles for bioactive factors and osteogenic cells. Among these, starch-based polymeric systems are particularly interesting due to their well-established biocompatibility [7,8] and processing versatility [9–13]. Concurrently, a variety of techniques for the processing of porous scaffolds have been reported [1–3,14–16]. However, most of the conventional fabrication techniques currently available do not meet the desired properties, as many of the processing routes still depend heavily on manual intervention. This results in inconsistent and inflexible processing procedures. Rapid Prototyping (RP) technologies are considered as potential routes for the production of complex scaffolds for tissue engineering applications, as they present superior control over design, manufacturing and reproducibility of tissue

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engineering scaffolds [16,17]. Moreover, when coupled with adequate image acquisition software, RP allows for the production of anatomically adapted three-dimensional (3D) scaffolds based on the patient's own defect. In addition, the scaffold architecture can be tailored to present different symmetries. A clear example of the potential of these technologies is the work carried out by Hutmacher et al. [1] in exploring the fused deposition modeling technique for the development of polycaprolactone (PCL) scaffolds.

From a tissue engineering point of view, the advantageous characteristics of fiber-based scaffolds include a large surface area for enhancing cell attachment and rapid diffusion of nutrients which favours cell survival and proliferation [18]. Moreover, provision of the appropriate surface chemistry for cell attachment and proliferation is another key issue that needs to be addressed. In this sense a challenging, though attractive, approach to better induce bone formation is to promote the mineralization of a bone-like calcium phosphate (generically designated as Ca–P or "apatite") layer on the outermost surface of the scaffold. Ca–P minerals found in natural hard tissues are produced spontaneously in a physiological environment at low temperatures from moderately supersaturated mineralizing solutions [19]. To learn, understand and apply these natural processes for producing Ca–P coatings biologically identical to bone apatite has been the focus of the attention of many researchers in the recent years, as reviewed elsewhere [20].

The so-called biomimetic preparation of calcium phosphate coatings on implant materials has thus emerged as a new concept. This process was initially proposed by Kokubo et al. [21] who used solutions that were able to mimic the inorganic composition of the human blood plasma, i.e. simulated body fluid (SBF) solutions. Besides its osteoconductive properties, a biomimetic apatite layer has a carrier potential due to the physiological operating conditions of temperature and pH. In fact, these coatings can serve as vehicles for the release of biologically relevant molecules such as protein growth factors, enzymes or pharmaceutical drugs used in bone-related diseases. So far, this potential has not been fully exploited.

To effectively coat an apatite layer on the surface of a biodegradable polymeric scaffold, while ensuring a homogeneous coating distribution throughout the entire surface of the pores without compromising its initial shape, represents an enormous challenge, due to complex features such as the coating composition, thickness distribution or the adhesion to the substrate. Moreover, pH changes due to possible degradation of substrates surface during the coating process have also to be taken into consideration. Nevertheless, in our research group these biomimetic coatings could be effectively produced on the surface of starch-based biodegradable polymers [22,23], namely by developing a biomimetic methodology using a sodium silicate gel as nucleating agent. This methodology has still room for improvement concerning the control of the apatite thickness and its distribution throughout the scaffold.

Most recently, the majority of the biomimetic routes proposed for coating of Ca–P layers on the surface of biomaterials have been limited to static conditions [22,24–30]. However, *in vivo*, the mineralization of bone tissue occurs in the presence of body fluids that continuously circulate in the body [31]. Therefore, an *in vitro* biomimetic approach including dynamic studies is of great significance as it comes closer to the *in vivo* scenario, where the flow of human body fluids may have an effect on the formation of bone apatite [32]. A few authors [33–37] have studied the mineralization of apatite layers under dynamic conditions, though these studies were only intended to better assess the bioactive behaviour of silica-based bioceramics. These materials are highly reactive and cause a local decrease of Ca^{2+} and PO_4^{3-} in the surrounding solution during apatite formation in static conditions, which will compromise the progress of the mineralization process. When considering 3D porous architectures that are not bioactive per se, dynamic mineralization environments can also be suitable to promote a homogeneous formation of the Ca–P layer on its interior. This work studies the nucleation of apatite layers on the surface of starch polycaprolactone (SPCL) scaffolds produced by a 3D plotting technology, and its subsequent growth under different static and dynamic mineralizing conditions.

2. Materials and methods

2.1. Preparation of the scaffolds

As polymers of natural origin, starch-based biomaterials exhibit great potential for use in bone-related applications [38–41]. In this work, the studied material is based on a blend of starch/PCL. The blend contains around 30% starch by weight. The selected biomaterial presents a good biological behaviour and has been proposed for bone tissue engineering applications by using other fiber-based processing routes such as fiber bonding [12,42–44] and by combining this technique with electrospinning to generate nano- and microfiber 3D architectures [12].

SPCL scaffolds were produced, using a 3D plotting technology (Bioplotter, EnvisionTec GmbH, Germany). In this case alternating layers with a 0°/90°/0°/90° orientation period were plotted. The resulting pattern has generated an offset that will increase the level of asymmetry. Cubic samples with a lateral side of $L = 5$ mm were produced.

2.2. Apatite coating formation

To produce an apatite coating on the surfaces of the obtained SPCL scaffolds a biomimetic methodology was used based on a previously developed sodium silicate methodology [22]. The samples were "impregnated" for 1 h with a sodium silicate gel from Sigma–Aldrich ($Na_2SiO_3{\cdot}H_2O$, containing ~14% NaOH and ~27% SiO_2, pH ≈ 13), under mild agitation. This sodium silicate gel was used herein with the concentration and SiO_2/Na_2O molar ratio as

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