Materials Science and Engineering C 59 (2016) 514–523

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Continuous cellularization of calcium phosphate hybrid scaffolds induced by plasma polymer activation

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ARTICLE INFO

Article history:
Received 5 August 2015
Received in revised form 13 October 2015
Accepted 15 October 2015
Available online 19 October 2015

Keywords:
3D printing
Calcium phosphate
Plasma technology
Perfusion cell reactor
Tissue engineering
Human osteoblasts
Cell migration

ABSTRACT

The generation of hybrid materials based on β-tricalcium phosphate (TCP) and various biodegradable polymers like poly(L-lactide-co-D,L-lactide) (PLA) represents a common approach to overcoming the disadvantages of pure TCP devices. These disadvantages lie in TCP's mechanical properties, such as brittleness. The positive characteristic of PLA — improvement of compressive strength of calcium phosphate scaffolds – is diametrically opposed to its cell attractiveness. Therefore, the objective of this work was to optimize osteoblast migration and cellularization inside a three-dimensionally (3D) printed, PLA polymer stabilized TCP hybrid scaffold by a plasma polymer process depositing amino groups via allylamine. MG-63 osteoblastic cells inside the 10 mm hybrid scaffold were dynamically cultivated for 14 days in a 3D model system integrated in a perfusion reactor. The whole TCP/PLA hybrid scaffold was continuously colonized due to plasma polymerized allylamine activation inducing the migration potential of osteoblasts.

1. Introduction

Synthetic bone substitutes provide an alternative to the limited resources of autografts and the problems which arise when using allogenic and xenogenic grafts [54,61]. The successful implantation of a biomaterial into bone is determined by the intimate interaction between the implant and the host tissue at the implant/tissue interface. The process of bone cell ingrowth is highly influenced by surface properties and the architecture of the scaffold. A bone graft material should therefore possess sufficient porosity and permeability to allow integration within the native tissue and vascular invasion, and it must satisfy the transport demands for oxygen and nutrients [25,69].

To provide bone grafts of synthetic calcium phosphates (CaP) which are similar to the mineral bone phase, additive manufacturing techniques like three-dimensional (3D) printing have been used successfully to realize 3D bone-like structures [58]. These technologies allow the design and fabrication of complex scaffold geometries with a fully interconnected pore network [31]. 3D printing is a powder-based process that builds physical 3D models directly from computer data. Layers of a fine powder are selectively bonded by printing a liquid binder from a micro-dispensing valve in the shape of each cross-section of a 3D dataset [66]. This is a promising method for the direct fabrication of custom-made implants, where the outer shape of the implant can be adapted to a given bone defect and the inner channels of the scaffold are optimized for adequate ingrowth of bone cells and for vascularization [24,34]. Porous tricalcium phosphate (TCP) ceramics are known to have excellent biocompatibility and are gradually eliminated by degradation with a velocity similar to osseous ingrowth [21]. Thus TCP scaffolds can be used as 'intermediate phase implants' [35], stimulating bone growth and remodelling. Despite these benefits, the brittleness of these materials is a serious drawback and 3D printed TCP scaffolds cannot as yet be used in load-bearing applications.

The creation of hybrid materials based on CaP and various biodegradable polymers like polycaprolactone [47,48,72], polylactide [37,39], and poly(lactide-co-glycolide) [41] represents a common approach to overcoming the disadvantages of the mechanical properties of pure CaP devices [39,53]. In particular polylactide, a clinically approved biodegradable polymer, has been used in orthopaedic and trauma surgery applications for years [27]. The coating of porous CaP ceramics with polylactide, maintaining pore interconnectivity for bone cell ingrowth, seems to be an interesting approach to reduce the brittleness of CaP ceramic scaffolds [26]. However, polylactide surfaces do not present

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http://dx.doi.org/10.1016/j.msec.2015.10.048
0928-4931/

favourable properties for cell interaction [3,70] and low osteoconductivity hampers bone cell ingrowth into polylactide-stabilized CaP scaffolds [37].

The enhancement of bone cell adhesion is a prerequisite for the osteoconductivity of artificial implant materials. The functionalization of polymers with N-containing groups is known to influence surface properties like hydrophilicity, free surface energy and surface charge positively, thereby favouring cell adhesion and spreading [17,33,60]. Amino group-functionalized surfaces are of special interest for the control of cell attachment and physiology due to their basic character and positive charging in an aqueous environment at physiological pH [55]. Direct functionalization by low temperature plasmas sustained in N_2 or N-containing gases was used to generate amino groups on polylactide [63,65,70]. The positive effect on 3D cell migration of the introduction of amino groups to polymer surfaces by ammonia plasma was shown on artificial scaffolds made from polycarbonate [5].

Coating of biomaterials by plasma polymerized allylamine (PPAAm) was proven to generate positively charged amino groups on the surface resulting in a positive zeta potential [17,42]. It was demonstrated that the cell-supportive effects of PPAAm thin films on titanium (Ti) were maintained even after storage for up to one year and after γ-sterilization [18]. PPAAm is beneficial for the adhesion and spreading capacity of cells also with impaired cytoskeleton – artificially induced by cytochalasin D – providing a potential application for bio-vitalizing specific implants for morbid bone tissues [30]. Long-term experiments of up to six weeks in rats recently revealed that this PPAAm nanolayer on Ti alloys induced bone growth and improved the bone-to-implant contact area decisively [20].

Since cell-based tissue engineering strategies for bone repair have been until now time-consuming and economically and logistically infeasible for clinical applications [40], it seems to be more lucrative to develop resorbable, mechanically stable, osteoconductive scaffolds with interconnecting pores which are easily occupied by cells from the bordering bone tissue after implantation. In earlier in vitro investigations concerning cell ingrowth into scaffold materials we developed a 3D cell culture model system with two scaffolds fixed by a clamping ring. This 3D model can be used for the observation of cell migration and proliferation inside a porous scaffold and is well suited for non-destructive investigations of cells in a 3D environment [6,44]. The cell colonization towards the center of a porous scaffold is of particular importance. With increasing scaffold size, cell proliferation and differentiation in the core region of the scaffold is impaired [38,62]. We were recently able to show that cell occupation of the inner scaffold surface is limited under static culture conditions [28]; this accentuates the need for dynamic culture conditions [2,14,71].

Here we describe a new TCP-based hybrid scaffold comprising a mechanically stabilizing internal polylactide coating and a cell adhesion stimulating PPAAm nanolayer realized by following three fabrication steps: 3D printing, solvent coating and plasma polymerization. The scaffold properties were characterized by scanning electron microscopy (SEM), porosity analysis, mechanical compression tests and XPS analysis. The occupation of the bioactive hybrid scaffold by human bone cells was determined in a dynamic in vitro 3D model system with perfusion cultivation.

2. Materials and methods

2.1. Hybrid scaffold

2.1.1. 3D printing

Porous scaffolds (Fig. 1) were produced from ß-tricalcium phosphate (TCP) using three-dimensional (3D) printing technology [66] and the CAD-software SolidWorks (DS SolidWorks, Massachusetts). Details of the 3D printing test setup have been published [56]. Spray-dried granulate TCP4 (BioCer, Bayreuth, Germany) was utilized as the raw material. An aqueous solution of dextrin (20 wt-%) and saccharose (2.5 wt-%) was used as printing binder. The resolution of the printing raster and the layer thickness were 0.25 mm. The scaffolds had a complex internal structure. The channels were aligned straight in the x–y-direction and diagonally (58° angle) in the z-direction. The 3D printed ceramic green bodies were sintered for 2 h at 1250 °C in a high-temperature furnace (Nabertherm, Germany) in ambient air. Analyses of biocompatibility were conducted successfully in a previous study [66]. After sintering, the porosity and the pore size of the 3D printed scaffolds reached nearly 50% and 500 μm, respectively [57]. The scaffolds were designed to fit into the 3D model system (Fig. 2) and were 28 mm in diameter and 5 mm in height. For the mechanical characterization, scaffolds of the dimensions 20 × 10 mm were produced. These cylinders also have a complex internal structure of horizontal and vertical cavities.

2.1.2. Polylactide infiltration, mechanical test and disc preparation

2.1.2.1. Infiltration. TCP scaffolds were mechanically stabilized by infiltration with a medical grade poly(L-lactide-co-D,L-lactide) (PLA, 70/30, Boehringer Ingelheim, Germany). For this purpose the scaffolds were dipped in a 2% (w/w) solution of PLA in dichloromethane for 15 min. Afterwards the pores were re-opened with slight air pressure and the samples were dried at 40 °C for 2 h and subsequently at room temperature overnight in vacuum to remove the solvent completely. A thin polymeric layer is formed on the inner scaffold surface by this process. The polymer content in the formed composite was 0.96% ± 0.17%.

2.1.2.2. Scanning electron microscopy (SEM). Materials were gold sputtered with a coater (SCD 004, BAL-TEC, Lichtenstein). The samples were characterized by scanning electron microscope SEM DSM 960A (Carl Zeiss, Germany).

2.1.2.3. Mechanical test. The average compressive strength of the scaffolds (n = 5) was determined with a uniaxial testing system (zwicki-Line, Zwick, Ulm, Germany).

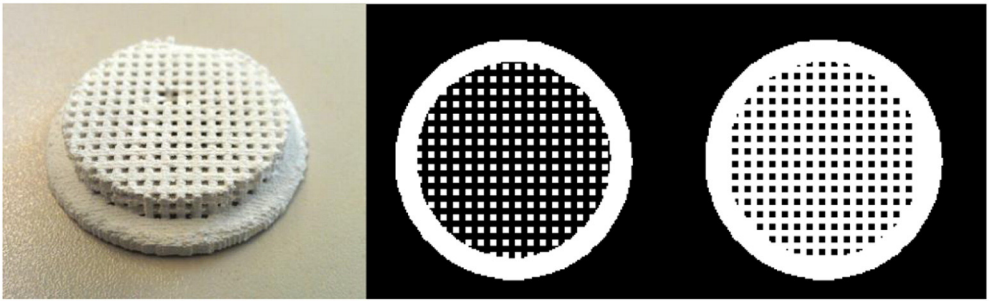

Fig. 1. Scaffold sample from the 3D printing process with quadrangular pores of 500 μm size constructed at a 58° angle in the z-direction (left) and two typical slices from the stack of bitmaps of the specimen with interconnecting cavities (right).