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Collagenous matrix supported by a 3D-printed scaffold for osteogenic differentiation of dental pulp cells

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ABSTRACT

Objective. A systematic characterization of hybrid scaffolds, fabricated based on combinatorial additive manufacturing technique and freeze-drying method, is presented as a new platform for osteoblastic differentiation of dental pulp cells (DPCs).

Methods. The scaffolds were consisted of a collagenous matrix embedded in a 3D-printed beta-tricalcium phosphate (β-TCP) as the mineral phase. The developed construct design was intended to achieve mechanical robustness owing to 3D-printed β-TCP scaffold, and biologically active 3D cell culture matrix pertaining to the Collagen extracellular matrix. The β-TCP precursor formulations were investigated for their flow-ability at various temperatures, which optimized for fabrication of 3D printed scaffolds with interconnected porosity. The hybrid constructs were characterized by 3D laser scanning microscopy, X-ray diffraction, Fourier transform infrared spectroscopy, and compressive strength testing.

Results. The *in vitro* characterization of scaffolds revealed that the hybrid β-TCP/Collagen constructs offer superior DPCs proliferation and alkaline phosphatase (ALP) activity compared to the 3D-printed β-TCP scaffold over three weeks. Moreover, it was found that the incorporation of TCP into the Collagen matrix improves the ALP activity.

Significance. The presented results converge to suggest the developed 3D-printed β-TCP/Collagen hybrid constructs as a new platform for osteoblastic differentiation of DPCs for craniomaxillofacial bone regeneration.

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1. Introduction

Spontaneous regeneration capacity of bone tissue is only limited to small defects. Large bone defects as consequence of trauma, osteoporotic fracture, tumors, and congenital deformity require surgical intervention [1]. Allografts and autografts are the main clinical strategies to fill bone cavities. However, autografts are restricted by site morbidity and approachability of the transplantable bone. Also, allografts increase the risk of immune-rejection reactions and infectious diseases transmission [2]. To address orthopedic challenges which results in reduced quality of life and patient discomfort, synthetic graft substitutes and implants have attracted tremendous interest in the last decade [3]. An ideal synthetic bone graft should satisfy a number of optimized properties such as biodegradability, osteoconductivity, biocompatibility, interconnected porosity, and adequate mechanical strength [4–6] in order to fulfill vascularization, nutrient delivery, cellular attachment, proliferation, differentiation, integration into surrounding tissue, to be ultimately replaced with the *de novo* bone tissue [7].

An ideal scaffold for bone tissue engineering application should mimic natural bone properties such as morphology, porosity, composition, and mechanical strength [8]. Naturally, bone is composed of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) in the context of organic matrix which is mainly comprised of Collagen type I [9]. Different bone regions possess specific microstructure and function. Proximal tibial trabecular bone has typically pore size of 1 mm in diameter, 50–90% porosity, 0.30 ± 0.10 g/cm^3 apparent density, 5.3 MPa ultimate strength, and 445 MPa elastic modulus. In contrast, femoral cortical bone is compact with limited voids, 3–12% porosity, 1.85 ± 0.06 g/cm^3 apparent density, 193 MPa ultimate strength, and 17 GPa modulus of elasticity [9]. Bone is also a dynamic milieu, constantly repairing and remodelling its matrix by osteoblasts to create new mineralized bone matrix, osteoclasts disassembling and digesting the matrix, while the star-shaped osteocytes maintaining the matrix [10]. Inspired by the hierarchical structure of the bone, the designed scaffolds for bone regeneration should structurally mimic the complexity of defected site and fulfill a 3D environment for initial cell attachment, consequent cell proliferation and tissue formation to ensure fixation and integration of scaffold with the host tissue [11,12]. Generally, it has been suggested that fabrication of scaffolds with interconnected pores size over 300 μm can facilitate cell attachment and migration, vascularization, mechanical interlocking, and tissue ingrowth [13].

There have been several fabrication techniques utilized to manufacture porous scaffold, including phase separation, salt leaching, freeze-drying, gas foaming, particle sintering, and solid free-form fabrication (SFF) techniques [14]. Conventional non-SFF approaches such as freeze-drying are limited in providing control on pore size, shape, interconnectivity, and construction of complex architectures [15–18]. Furthermore, the low compressive moduli of these scaffolds hamper their application for the load bearing bone regeneration. Over the past few years, SFF techniques have gained extreme attention which the major sub-groups of this approach are selective laser sintering and 3D-printing[19,20]. Additive layer manufacturing (ALM) or 3D-printing techniques offer the advantage of creating desired levels of complexity in custom made implants according to the medical requirements [21]. In fact, 3D-printing method could be utilized in personalized medication level by obtaining individual patient computed tomography scan or magnetic resonance images [22,23]. Indeed, 3D-printing is known as a reproducible and consistent technique. The major advantages and disadvantages of different classification of ALM techniques for bone tissue engineering applications have been thoroughly summarized elsewhere [24]. 3D-printing technique enables fabrication of well-defined complex architectures both in micro and macro scales with improved mechanical properties, tuneable for different application such as soft or hard tissues [25]. In spite of several advantages, 3D-printing suffers from the lack of ability to mimic nano-fibrous structure of extracellular matrix (ECM) which mainly supports cell adhesion, proliferation, communication, and differentiation. The advantages and disadvantages of conventional non-ALM approaches and 3D-printing, have motivated researches to exploit combinatorial fabrication techniques in order to mimic the nature of bone tissue [26].

The present work aims to employ 3D-printing technique and freeze-drying method to design optimal platforms for harnessing osteogenic capacity of DPCs. To achieve this goal, hybrid constructs consisted of 3D-printed β-tricalcium phosphates (β-TCP) scaffolds filled with freeze-dried Collagen matrix were fabricated. The β-TCP was selected due to its chemical likeness to the bone mineral, which is commonly used in bone replacement applications. The in-depth physicomechanical and biological features of the developed β-TCP/Collagen hybrid constructs were investigated toward craniomaxillofacial bone regeneration.

2. Materials and methods

2.1. Ink preparation and optimization

The paste formulations were composed of water, beta-tricalcium phosphate powder (β-TCP, Sigma), sodium tripolyphospahte (TPP, Alfa Aesar), and carboxymethylcellulose (CMC, Alfa Aesar). The TCP-based formulations were composed of 15 g TCP powder, 0.5 g TPP, 75 mg CMC, and 5.25, 5.75, and 7.75 ml water, which denoted as formulation 1, 2, and 3. The ink formulations were characterized to optimize their flow-ability for the 3D-printing process. Rheological measurements were performed by a rheometer (Kinexus, Malvern) using a cone-plate geometry. Shear stress and viscosity were measured altering formulations temperature from 17 to 29 °C, and shear rate of 0–100 1/s.

2.2. Fabrication of β-TCP scaffolds using 3D-printing technique

3D-bioplotting system (Envisiontec, Germany) was utilized to fabricate scaffolds with desired geometry, size, and structure. A CAD/CAM software was used to design disk block models of scaffold. The 3D-printing was performed on paste formulations at 5 mm/s dispensing speed, optimal pressure of

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