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3D printed TCP-based scaffold incorporating VEGF-loaded PLGA microspheres for craniofacial tissue engineering

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ABSTRACT

Objective. Vascularization is a critical process during bone regeneration/repair and the lack of tissue vascularization is recognized as a major challenge in applying bone tissue engineering methods for cranial and maxillofacial surgeries. The aim of our study is to fabricate a vascular endothelial growth factor (VEGF)-loaded gelatin/alginate/β-TCP composite scaffold by 3D printing method using a computer-assisted design (CAD) model.

Methods. The paste, composed of (VEGF-loaded PLGA)-containing gelatin/alginate/β-TCP in water, was loaded into standard Nordson cartridges and promptly employed for printing the scaffolds. Rheological characterization of various gelatin/alginate/β-TCP formulations led to an optimized paste as a printable bioink at room temperature.

Results. The in vitro release kinetics of the loaded VEGF revealed that the designed scaffolds fulfill the bioavailability of VEGF required for vascularization in the early stages of tissue regeneration. The results were confirmed by two times increment of proliferation of human umbilical vein endothelial cells (HUVECs) seeded on the scaffolds after 10 days. The compressive modulus of the scaffolds, 98 ± 11 MPa, was found to be in the range of cancellous bone suggesting their potential application for craniofacial tissue engineering. Osteoblast culture on the scaffolds showed that the construct supports cell viability, adhesion and proliferation. It was found that the ALP activity increased over 50% using VEGF-loaded scaffolds after 2 weeks of culture.

Significance. The 3D printed gelatin/alginate/β-TCP scaffold with slow releasing of VEGF can be considered as a potential candidate for regeneration of craniofacial defects.

1. Introduction

Critically-sized bone defects created due to infection, tumor resection, or traumatic fractures cannot be healed sponta-

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neously, and external interventions are needed in most cases to regenerate new bone to restore, maintain or improve its function. Although autografts are considered as the gold standard treatment [1], it remains challenging for the clinicians to select between autografts, allografts, xenografts or engineered tissues [2–5]. The limited access to bone grafts has led to attempts to develop tissue engineering techniques using the three factors of scaffold, growth factors and/or cells for achieving favorable outcomes in bone regeneration.

Composition and physical properties of the porous scaffold can directly influence the regeneration process; biomimetic scaffolds can yield a more favorable outcome [6–8]. Additive manufacturing (AM) approaches can be used to fabricate scaffolds with tailored pore size and porosity for complex-shaped defects, the fabrication of which is problematic or impossible using other manufacturing methods reported in the literature [9–11]. 3D printing/AM allows fabrication of 3D objects of virtually any shape from a computer aided design (CAD) [12]. Two key factors for successful 3D printing fabrication are ink/binder selection and process parameter optimization. The advantage of 3D-printing is the fine control over various features of the scaffold [13]. Also, one of the widely used techniques of low temperature fused deposition modeling provides mild condition of processing, which allows plotting of drug and biomolecules such as proteins and living cells [14]. However, there is a significant challenge regarding development of suitable structural materials containing adequate amounts of bioactive components [15].

Calcium phosphate-based formulations have presented excellent osteoconductivity and biocompatibility in reconstructive surgeries for more than 30 years [11,16–25]. Tricalcium phosphate (TCP), as one of the most widely used calcium phosphates in bone tissue engineering, has demonstrated osteogenic properties, phase stability and strong bond formation with the host bone tissue in different studies [26–29]. 3D-printed TCP-based scaffolds could be considered as a proper choice for bone tissue engineering applications, since both the fine control over the structure and shape through 3D printing and osteoconductivity of the composition can be exploited. However, when encapsulation and release of sensitive biomolecules such as growth factors is required, high temperature post processes like sintering should be avoided. In this case, biopolymers such as gelatin and alginate might be used both as the binder to facilitate the printing process and as the matrix to incorporate growth factor carriers. Gelatin, a natural polymer obtained from partial hydrolysis of collagen, provides Arg-Gly-Asp (RGD) motifs that can mediate cell attachment via interaction with integrin [30–32]. The sol–gel transition of gelatin can be exploited in 3D printing procedures. On the other hand, alginate, a natural polysaccharide, has been widely used as a biomaterial for bone tissue engineering because of its biocompatibility, non-immunogenicity and biodegradability [33]. Alginate has been widely used as a thickener in the food industry [33] and can be used to adjust the viscosity and rheological properties of the ink.

The lack of functional vascularization is a major challenge in the successful clinical approach of bone tissue engineering in the practice of reconstructive orthopedic and craniofacial surgery [34]. As a result, the aim of bone tissue engineering is not only the culture of osteogenic cells on osteoconductive scaffold, but also the induction of angiogenesis to support the metabolic needs of bone. Although the main VEGF receptors expressed on endothelial cells, there are many VEGF receptors expressed on chondrocytes and osteoblasts [35]. Consequently VEGF not only promotes angiogenesis but also play an important role in bone growth and repair [36,37]. Therefore, an osteoconductive scaffold releasing VEGF could be an appropriate candidate for bone regeneration. However, encapsulation and sustained release of fragile biomolecules, such as growth factors, is very challenging. Researchers have increasingly become interested in using biodegradable polymers as host materials for drug delivery systems in the past few decades. These systems not only protect the encapsulated molecule against aggressive environments but also release the molecule in a sustainable manner. Poly (lactic-co-glycolic acid) (PLGA) has been widely used for growth factor sustained delivery in different forms including coating layers and microspheres [38–41]. Growth factor loaded PLGA microspheres can be easily incorporated into the ink to be 3D printed.

The aim of current study was to develop a 3D printed TCP-based scaffold with sustained release of VEGF for bone tissue engineering applications. Gelatin and alginate were employed as the binders to facilitate 3D printing. In fact, we hypothesized that gelatin/alginate/TCP scaffold containing vascular endothelial growth factor (VEGF)-loaded PLGA microspheres, fabricated by 3D plotting according to a predesigned CAD-model, could be a potential candidate for treatment of bone defects.

2. Experimental procedure

2.1. Materials

β-TCP, phosphate buffer saline (PBS), Alginate powder and gelatin (Type A, Bloom 300 g) were purchased from Sigma-Aldrich, USA. Dichloromethane, $CaCl_2$ and N-hydroxysuccinimide (NHS) were obtained from Alfa Aesar, USA. PLGA was purchased from Corbion Purac, PURASORB® PDLG 5010. HUVECs, Osteoblasts and VEGF were purchased from Cell Applications Inc., USA and finally, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC) was obtained from TCI America, USA. DMEM and FBS were purchased from Sigma. MTT and Alamar blue assays were obtained from Sigma and life technologies, respectively. All materials were utilized as received, without any further purification.

2.2. Formulating the ink for printing

In the formulation, β-TCP and alginate were used as the main component and thickener, respectively. Gelatin was employed as the hardening agent because of its sol–gel transition. Since sol–gel transition temperature of the ink is influenced by gelatin concentration, it plays a key role in printability of the ink. The concentration of β-TCP and alginate were set at 30% (w/v) and 5% (w/v), respectively and the gelatin concentration was altered (1, 3 and 10% (w/v)). The effect of gelatin concentration on the sol–gel transition of the ink was then investigated using rheometry. The rheological measurements