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Selective laser sintering scaffold with hierarchical architecture and gradient composition for osteochondral repair in rabbits

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ABSTRACT

Osteochondral defects cannot be adequately self-repaired due to the presence of the sophisticated hierarchical structure and the lack of blood supply in cartilage. Thus, one of the major challenges remaining in this field is the structural design of a biomimetic scaffold that satisfies the specific requirements for osteochondral repair. To address this hurdle, a bio-inspired multilayer osteochondral scaffold that consisted of the poly(ε-caprolactone) (PCL) and the hydroxyapatite (HA)/PCL microspheres, was constructed via selective laser sintering (SLS) technique. The SLS-derived scaffolds exhibited an excellent biocompatibility to support cell adhesion and proliferation *in vitro*. The repair effect was evaluated by implanting the acellular multilayer scaffolds into osteochondral defects of a rabbit model. Our findings demonstrated that the multilayer scaffolds were able to induce articular cartilage formation by accelerating the early subchondral bone regeneration, and the newly formed tissues could well integrate with the native tissues. Consequently, the current study not only achieves osteochondral repair, but also suggests a promising strategy for the fabrication of bio-inspired multilayer scaffolds with well-designed architecture and gradient composition via SLS technique.

1. Introduction

Osteochondral defects caused by trauma, joint disease or aging severely impact the life quality of patients, due to the associated joint pain and impaired joint function [1,2]. Since cartilage is an avascular tissue with low cell density and metabolic activity, treatment of cartilaginous lesions remains a major challenge for orthopedic surgeons [3]. In addition, the complex hierarchical structure involving both articular cartilage and underlying subchondral bone is another huge challenge in reconstruction of osteochondral tissue. Several techniques are currently used to treat osteochondral defects, including autografts transplantation [4,5], autologous chondrocytes transplantation [6,7] and marrow stimulation such as subchondral drilling [8] and microfracture [9]. Although successful in some aspects, each of these techniques has its own limitations. For instance, autografts transplantation suffers from donor site morbidity, incomplete integration and degradation of the graft tissue [10], while marrow stimulation results in fibrocartilage with inferior quality [11,12].

In recent years, tissue engineering emerged as a promising alternative for osteochondral regeneration [13–16]. Osteochondral defects involve multiple tissues, including the three articular cartilage layers (superficial, transitional and deep zone), calcified cartilage and subchondral bone. Biomimetic osteochondral scaffolds were thus required to repair multiple tissues simultaneously. Among these scaffolds, the biphasic scaffold model that combines a cartilaginous phase and a bony phase was the most common strategy [17–20]. Due to the limitations of fabrication techniques and scaffold structure, the issues of layer separation and weak interface bonding frequently occur, which affect cell migration, as well as long-term and complete osteochondral repair [18,21]. To overcome these limitations, an advanced selective laser sintering (SLS) technique was applied in this study to produce a bio-inspired

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multilayer scaffold with a continuous hydroxyapatite (HA) gradient, which may be better suited for the regeneration of complex tissue interfaces. SLS is a rapid prototyping technique that has recently received a wide attention in creating diverse tissue scaffolds, mostly due to its advantages with high accuracy and customizability in manufacturing complex structures [22–24]. Compared with other 3D printing techniques, SLS employs a CO_2 laser beam to selectively sinter polymer or composite powders, producing porous scaffolds with high mechanical strength [25–27]. Consequently, SLS-derived scaffolds present a great potential for the integration of multiple properties, such as hierarchical structure from the cartilage layer to the subchondral layer, and adequate mechanical support for tissue repair.

In the current work, the poly(ε-caprolactone) (PCL) microspheres and the HA/PCL composite microspheres were used as building blocks of SLS to prepare a biomimetic multilayer scaffold for osteochondral repair (Fig. 1). PCL is one of the most commonly used polymers for bone and cartilage repair, with tailored biodegradable and mechanical properties [28,29], while HA is used to mimic the main mineral component of natural bone due to its excellent osteoconductive properties. Compared with the powder form used in conventional SLS strategies, microsphere-based SLS technique is utilized to enhance micro-scale porosity during the sintering process, which has been demonstrated by previous work [30]. The aim of this study is to verify the feasibility of using SLS to fabricate a gradient multilayer scaffold, and to assess its ability to repair osteochondral tissue in a rabbit model.

2. Materials and methods

2.1. Preparation and characterization of PCL microspheres and HA/PCL composite microspheres

The PCL microspheres and the HA/PCL composite microspheres with 5 wt% to 30 wt% HA were separately prepared using a modified protocol of S/O/W emulsion solvent evaporation as previously described [30,31]. Briefly, PCL (Mw = 50,000) (Daigang Biomaterial Co., Ltd, China) was dissolved in dichloromethane. HA nanoparticles with the size distribution of 80–100 nm were synthesized using previously published protocol [32–34]), and dispersed sufficiently in ethyl alcohol by ultrasonication. The two solutions were mixed sufficiently and added dropwise into a solution of 1.5% (w/v) poly(vinyl alcohol). After continuous stirring for 4–6 h, the microspheres were collected by centrifugation, washed and freeze-dried at −80 °C. The surface morphology, chemical structure and thermodynamic properties of the resultant microspheres were characterized by scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FT-IR) and thermogravimetric analysis (TGA), respectively.

2.2. Design and fabrication of SLS-derived scaffolds

A cylindrical scaffold (4 mm in diameter, 2.8 mm in thickness) with pore size of 500 μm was designed using a three-dimensional (3D) modeling software Pro/E and then exported into the STL format. The PCL microspheres and the HA/PCL composite microspheres were used as basic building blocks to fabricate the multilayer scaffolds using the SLS system (HRPS-IV, BinHu Mechanical & Electrical Co., Ltd, China). The HA content throughout the depth of the scaffold was designed to increase from 0 to 30 wt% with 5% increments from the top (articular cartilage) to the bottom (subchondral bone) layer, for a total of seven layers (Fig. S1). The sintering parameters were optimized for different microspheres in each layer, and were listed in Table S1. Also, the top PCL layer was set to be 400 μm thick, which is close to the thickness of rabbit's articular cartilage. The continuous HA gradient was achieved by controlling the powder supply for each layer manually. Briefly, the PCL microspheres were first put on the work platform, spread out

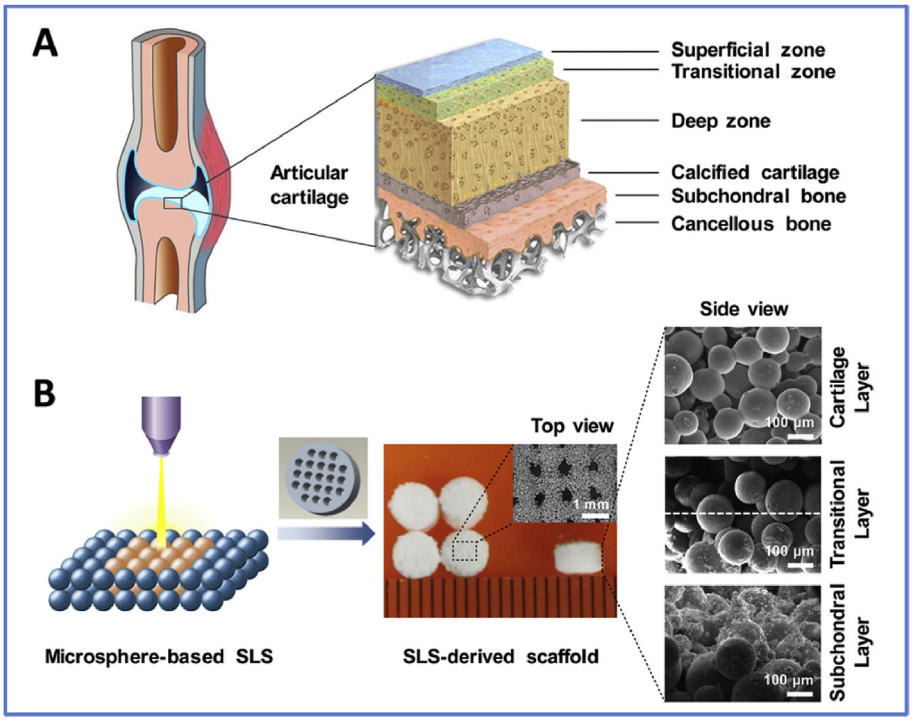

Fig. 1. Hierarchical architecture of natural osteochondral unit and its biomimetic replication in this work. **(A)** Natural osteochondral unit consists of several diverse tissue layers including superficial cartilage, middle calcified cartilage and deep subchondral bone, as well as transitional zones between different layers. **(B)** The PCL microspheres and HA/PCL composite microspheres were used as building blocks to fabricate bio-inspired multilayer scaffolds via SLS technique. The precisely-designed multilayer scaffold featured a macroporous cylinder with a continuous HA gradient from the articular cartilage layer to the subchondral bone layer.