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In vitro study of 3D PLGA/n-HAp/β-TCP composite scaffolds with etched oxygen plasma surface modification in bone tissue engineering

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ABSTRACT

Three-dimensional (3D) scaffolds have many advantageous properties for bone tissue engineering application, due to its controllable properties such as pore size, structural shape and interconnectivity. In this study, effects on oxygen plasma surface modification and adding of nano-hydroxyapatite (n-HAp) and β-tricalcium phosphate (β-TCP) on the 3D PLGA/n-HAp/β-TCP scaffolds for improving preosteoblast cell (MC3T3-E1) adhesion, proliferation and differentiation were investigated. The 3D PLGA/n-HAp/β-TCP scaffolds were fabricated by 3D Bio-Extruder equipment. The 3D scaffolds were prepared with 0°/90° architecture and pore size of approximately 300 μm. In addition 3D scaffolds surface were etched by oxygen plasma to enhance the hydrophilic property and surface roughness. After oxygen plasma treatment, the surface chemistry and morphology were investigated by Fourier transform infrared spectroscopy, scanning electron microscopy, and atomic force microscopy. And also hydrophilic property was measured by contact angle. The MC3T3-E1 cell proliferation and differentiation were investigated by MTT assay and ALP activity. In present work, the 3D PLGA/HAp/beta-TCP composite scaffold with suitable structure for the growth of osteoblast cells was successfully fabricated by 3D rapid prototyping technique. The surface hydrophilicity and roughness of 3D scaffold increased by oxygen plasma treatment had a positive effect on cell adhesion, proliferation, and differentiation. Furthermore, the differentiation of MC3T3-E1 cell was significantly enhanced by adding of n-HAp and β-TCP on 3D PLGA scaffold. As a result, combination of bioceramics and oxygen plasma treatment showed a synergistic effect on biocompatibility of 3D scaffolds. This result confirms that this technique was useful tool for improving the biocompatibility in bone tissue engineering application.

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1. Introduction

With the increasingly active lifestyles, accidents, obesity and aging population, orthopedic solutions encompassing joint and bone repair, fractures, oral and maxillofacial treatment, osteoporosis and bone tumors remain to be in the greatest demand [1]. The repair of bone defects is challenging in bone tissue engineering. Among different treatment options such as autografts and allografts, bone tissue engineering that is focused on methods to synthesize and regenerate bone to restore, maintain or improve its functions *in vitro* and *in vivo* is becoming popular [2,3]. However, problems still exist, such as limited supply or donor-site morbidity with the use of autograft, and resorption or risk of disease transmission with the use of the allograft [4]. Synthetic bone grafting materials and composite materials have been developed more recently in attempts to overcome the drawbacks and limitations of autografts, allografts [5–7].

Recently, synthetic polymer/bioceramics scaffolds were used as alternative bone grafting materials in bone tissue engineering, in which bioactive ceramic particles are embedded. Among synthetic polymers, poly (lactide-co-glycolide) (PLGA), biodegradable polyester, has been studied extensively as the material of three-dimensional (3D) printed scaffold. 3D printed scaffolds produced by rapid prototyping (RP) techniques have many merits for bone tissue engineering applications, due to its controllable properties such as porosity, pore size and pore interconnectivity and structural shape [8–10]. The 3D printing system, applied to RP in structural fabrication can easily manufacture biomaterials [11].

However, PLGA has a hydrophobic nature and interaction with biological fluids result in poor cell adhesion, proliferation and differentiation. In order to enhance biocompatibility, PLGA is often used as polymer matrix in composite scaffolds including

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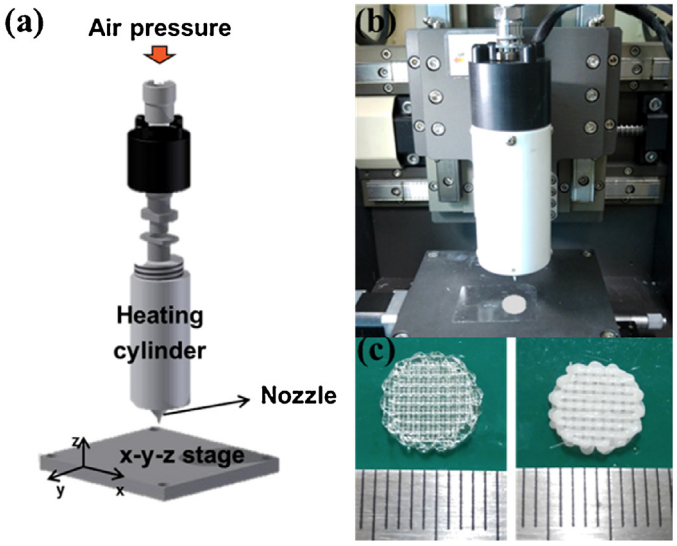

Fig. 1. (a) Schematic diagram of a 3D bio-printing system, (b) photograph of 3D printing during fabricating 3D scaffold, and (c) 3D PLGA (left) and PLGA/n-HAp/β-TCP (right) scaffolds. 3D scaffolds were fabricated with disc shape (8 mm diameter and 2 mm thickness) and pore size of approximately 300 μm.

osteogenic and osteoinductive bioceramics, such as hydroxyapatite, β-tricalcium phosphate (β-TCP), which is the main mineral component of bone tissues and confers its high bioactivity to the polymer-based composite promoting bone regeneration [12–14]. It has been reported that additions of bioceramics on 3D PLGA scaffold possess good biocompatibility and osteoconduction [15]. Also 3D printed PLGA/hydroxyapatite/β-TCP scaffolds have been proven to induce bone regeneration at *in vivo* study [16]. Alternatively, the oxygen plasma treatment is widely used to enhance the biocompatibility such as good hydrophilicity and increase of roughness on PLGA surface. Wan's study, the surface of PLGA film treated by oxygen plasma etching improved cell adhesion due to increase surface hydrophilicity and roughness [17].

Therefore, we hypothesize that the combination of bioceramics incorporation and oxygen plasma surface treatment on 3D PLGA scaffold might have a synergistic effect on biocompatibility such as preosteoblast (MC3T3-E1) cells adhesion, proliferation, and differentiation.

2. Materials and methods

2.1. Materials

PLGA (lactide/glycolide 85:15), with molecular weight of 50,000 ~ 75,000, n-HAp (nano powder, <200 nm) and β-TCP (unsintered powder) were purchased from Sigma–Aldrich. All reagents and chemicals in this study were used without any further purification.

2.2. Fabrication of 3D PLGA and 3D PLGA/n-HAp/β-TCP scaffolds

PLGA, n-HAp and β-TCP were used as 3D scaffold materials. 3D PLGA and PLGA/n-HAp/β-TCP scaffold were prepared by Bio-Extruder equipment (3D Bio Printer, M4T-100, M4T Co. Ltd., Korea), as shown in Fig. 1(a and b). Bio-Extruder equipment using computer-aided design system can produce a well-defined internal and external shape with uniform porosity for cell ingrowth. To fabricate the PLGA/n-HAp/β-TCP scaffold, the PLGA composite was prepared by blending the n-HAp and β-TCP concentration of 10 wt%, respectively. After PLGA composite was stirred by hot plate at 180 °C for 1 h and the mixture solution was poured in syringe of 3D printer. Molten polymer mixtures were extruded at 142 °C through a nozzle compressed dry air of 580 kPa pressure and feed rate was set to 220 mm/min. The nozzle size was used to 500 μm and the scaffold struts were deposited layer by layer at angles of either 0° or 90°. Finally 3D scaffolds were fabricated with disc shape (8 mm diameter, 2 mm thickness and 4 layers) and pore size of approximately 300 μm (Fig. 1c).

2.3. Oxygen plasma treatment for 3D scaffolds

The 3D PLGA and PLGA/n-HAp/β-TCP scaffolds were treated in oxygen plasma under anisotropic etching conditions for improving the preosteoblast cell affinity. The oxygen plasma treatment was carried out using radio frequency (RF, 13.56 MHz) capacitively-coupled plasma (Miniplasma Station, Korea). Reactive ion etching was applied to achieve the anisotropic profile introducing RF discharge power to a bottom electrode. The samples were placed on a stage in the vacuum chamber. For oxygen plasma etching of PLGA and PLGA/n-HAp/β-TCP scaffolds, RF discharge power was set at 100 W; oxygen gas flow rate at 20 sccm; working pressure at 13.33 Pa; treatment time for 3 min. 3D scaffolds were treated with the top and bottom surfaces so that it can be evenly inside the oxygen plasma treatment.

2.4. Surface characterization of 3D scaffolds

After oxygen plasma treatment, water contact angle measurement was used to evaluate surface hydrophilicity by measuring the degree of water spreading on the sample surface. The hydrophilicity of samples was examined by the sessile drop method using a goniometer (GS, Surface Tech Co. Ltd., Korea). A water droplet of 7 μL was dropped by a syringe mounted vertically against the sample surface. After the water was applied on the surface for 5 s, the droplet arc and the angle of contact at the interface were traced and recorded.

Before and after the plasma treatment, the surface topography and morphology was observed with a scanning electron microscopy (SEM, SNE-3200M, SEC, Korea) and Field Emission SEM (FE-SEM, S-4800, Hitachi, Japan). All samples were pre-coated with a conductive layer of sputtered gold. The micrographs were taken at accelerating voltage of 10 keV at magnifications of 50× or 5000× or 10,000×. Atomic force microscopy (AFM, XE-100, Park systems, Korea) was used to measure the surface roughness of 3D scaffolds under non-contact mode with scan rate at 0.1 Hz. In AFM, a tip attached to a flexible cantilever moves across the sample surface to measure surface morphology on an atomic scale. Scan area is 5 μm × 5 μm and 10 μm × 10 μm randomly selected from the scaffold surface. An arithmetic mean of root mean square roughness (Rq) was calculated directly from the AFM images.

Phase analysis of the fabricated 3D PLGA and PLGA/n-HAp/β-TCP scaffold was conducted using an X-ray diffractometer (XRD, X'Pert PRO MultiPurpose, Philips) to detect the phase composition and crystallinity of the n-HAp and β-TCP on the PLGA/n-HAp/β-TCP scaffold with radiation under Cu-Kα beam conditions of 40 kV and 20 mA with collection of a spectrum at $2\theta = 10°–60°$ and a step size of 0.1°.

The 3D scaffolds are examined by X-ray photoelectron spectroscopy (XPS, VG Multilab 2000, ThermoVG Scientific, UK) to obtain their elemental composition and chemical states introduced on the 3D scaffolds by oxygen plasma etching. For each specimen, a compositional survey scan was acquired using pass energy of 50 eV and core level spectra with pass energy of 20 eV. Each specimen was analyzed and averaged to obtain the reported atomic percent (at%) values.

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