마이크로 광 조형 기술 및 표면 코팅을 이용한 향상된 인공지지체의 개발

Development of improved 3-D PPF/DEF scaffolds using microstereolithography technology and surface coating

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1. Introduction

Tissue engineering is a field that offers great opportunities for regenerative medicine. The development of three-dimensional (3-D) scaffolds that guide cells to form functional engineered tissues is among the most important areas of tissue engineering. Recently, moldless manufacturing techniques, known as solid free-form (SFF), have been used successfully to fabricate complex 3-D scaffolds [1]. One such technique, microstereolithography (MSTL) technology, has been developed to produce highly precise 3-D microstructures from functional materials, in particular, biocompatible materials. Poly(propylene fumarate) (PPF) is a UV-curable, biodegradable polymer with potential applications for bone regeneration [2, 3]. In this study, we designed 3-D scaffolds based on a PPF polymer network and then fabricated them using MSTL technology. As one of the primary components of extracellular matrix bone, apatite has good characteristics for bone reconstruction [4]. Therefore, we examined the surface modification of 3-D scaffolds applying an accelerated biomimetic apatite coating to promote cell behavior.

2. Experiments

2.1 Synthesis of materials

Poly(propylene fumarate) was synthesized via a condensation reaction, according to Gerhart *et al.* [3] with the following modifications: 2.4 mol of fumaric acid and 3 mol of propylene glycol were placed in a triple-necked 1000-mL flask with an overhead electrical stirrer, a thermometer, and a Barrett trap beneath a condenser. During synthesis, the mixture was stirred continuously at about 150 rpm. The mixture was maintained at 140°C for about 15 h, during which time about 45 mL of water were collected. Then, the temperature was increased to between 185 and 190°C to remove the excess propylene glycol and low-molecular-weight impurities. After about 4 h at this temperature, the reaction was terminated. The product was kept at room temperature overnight to prevent further polymerization. To be able to use PPF as a resin for MSTL, the cross-linking agent diethyl fumarate (DEF) was added in ratio of 70/30 to reduce the viscosity of the PPF and 1 wt% bis(2,4,6-trimethylbenzoyl)-phenylphosphine oxide (BAPO) was used as the photoinitiator.

2.2 Scaffold fabrication method

In our MSTL system, a continuous-wave Ar ion laser with a wavelength of λ = 351.1 nm (Spectra-Physics BeamLok 2065-4S; Newport Corp., Irvine, CA, USA) was used as the light source. The laser was focused on the polymer surface and the stage was moved along the X-, Y-, and Z-axes to determine the position for solidification. The photopolymer was processed in layers under laser irradiation to form 3-D structures as a physical representation of a computer-assisted design (CAD) model [5]. A hot plate was used at a working temperature of about 30°C to decrease the viscosity of the PPF/DEF mixture. A schematic diagram of the MSTL system is shown in Fig. 1.

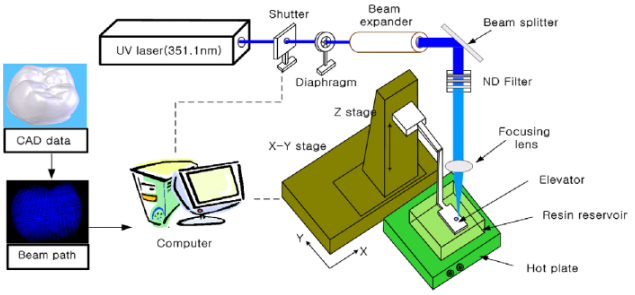
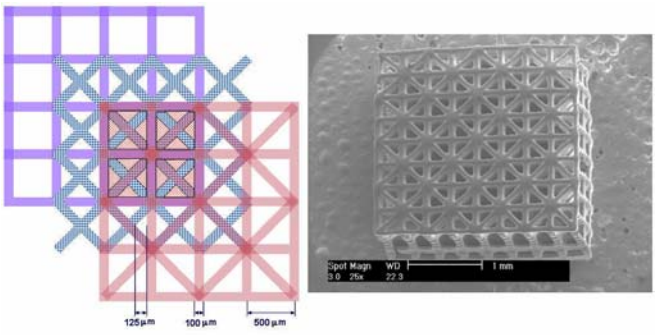


Fig. 1 Schematic diagram of the microstereolithography system

The 3-D scaffold was designed with alternating lattices and columns. Fig. 2 (a) shows the design of overlapping lattices. The lattice and column layers were each 150 μm thick. Since three columns were stacked, the final column height was 450 μm. Thirteen layers were stacked, giving a final scaffold height of 1.95 mm. A 3-D PPF/DEF scaffold was fabricated successfully using the MSTL system at a scan speed of 60 mm/min and a laser power of 350 μW. After fabrication, the structure was cleaned overnight using hot isopropanol (IPA) and ultrasound. A curing time of 10 h was required before further use. Fig. 2(b) shows a scanning electron micrograph (SEM) of the 3-D scaffold.



(a) (b)

Fig. 2 Design of the scaffold (a) and fabrication results (b)

2.3 Surface coating methods

To produce an accelerated biomimetic apatite coating, the substrate was immersed in stimulated body fluid (5SBF) containing nearly five times the inorganic ion concentrations of human blood plasma [4]. This solution was prepared by dissolving NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$, and Na_2SO_4 in distilled deionized water. The pH of solution was adjusted to 6.7 at 36.5°C with 1 M HCl and Tris.

A PPF/DEF plate measuring ϕ9 × 0.5 mm was immersed in 20 mL of 5SBF in a plastic bottle at a temperature of 37°C. After 24 h, the specimen was removed and washed carefully with distilled deionized water and dried in air. Three 3-D PPF/DEF scaffolds were immersed for 24 h in 20 mL of 5SBF with stirring at 37°C and then washed and dried as described above.

2.4 Cell culture

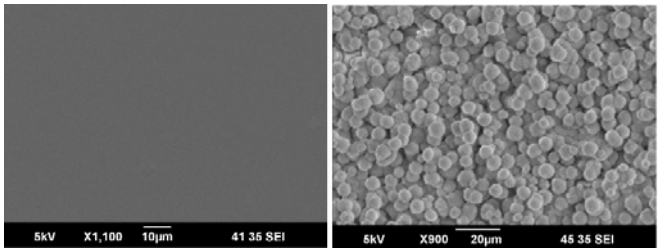
MC3T3-E1 pre-osteoblasts were used for the cell culture experiment. The cells (10^5) were suspended in 10 μl of medium, and the concentrated cell suspensions were pipetted onto scaffolds. The cells were allowed to adhere to the scaffolds for 1 h and 700 μL of medium were added to each well of 24-well plates. The culture medium was changed every 2–3 days.

2.5 MTS assays

The mitochondrial metabolic activity of the cells was determined using the MTS assay. Briefly, the scaffolds were first rinsed in PBS, and then 240 μL of MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carbonxylmethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) were added to each well. After incubating for 6 h, the MTS solution was removed. The optical density was measured at 490 nm using a plate reader.

3. Results

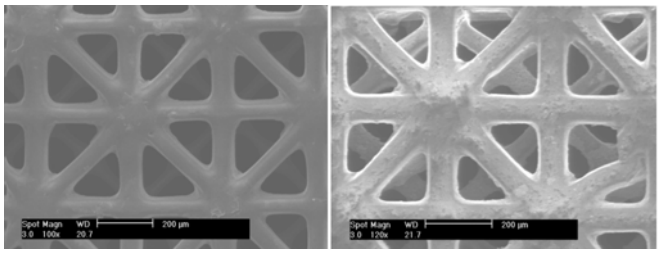
The PPF/DEF plates and scaffolds were incubated in 5SBF at 37°C for 24 h. SEM images of the PPF/DEF plates before and after incubation are shown in Fig. 3. The entire surface of the plates was covered with microparticles after a 24-h incubation.



(a) (b)

Fig. 3 SEM images of PPF/DEF plates incubated in 5SBF for 0 h (a) and 24 h (b)

Fig. 4 shows SEM images of the PPF/DEF scaffolds before and after incubation in 5SBF for 24 h. As shown in the pictures, a uniform apatite layer was generated on the surface and inside the scaffold.



(a) (b)

Fig. 4 SEM images of PPF/DEF scaffolds incubated in 5SBF for 0 h (a) and 24 h (b)

MC3T3-E1 pre-osteoblasts were cultured on the scaffolds to evaluate the cell behavior on the PPF/DEF scaffolds and the effect of the surface coating. Scaffolds were immersed in the cultures for 1 day, 1 week, or 2 weeks. The MTS assay of the effects of biomimetic apatite coating on MC3T3-E1 pre-osteoblast growth is shown in Fig. 5. The cells increased in number with culture time both with and without a coating. The biomimetic apatite coating had a good effect on the cell proliferation compared to no treatment. The cell culture result indicates that the biomimetic apatite coating is an effective way to modify the surface of PPF/DEF scaffolds to promote cell behavior.

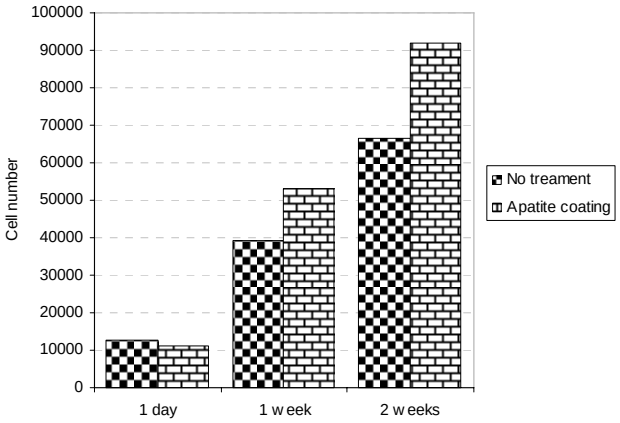


Fig. 5 Effects of a biomimetic apatite coating on MC3T3-E1 pre-osteoblast growth

4. Conclusions

We successfully fabricated 3-D PPF/DEF scaffolds using MSTL technology. Apatite was deposited on the PPF/DEF plates and 3-D scaffolds within 24 h using an accelerated biomimetic process with 5SBF. We demonstrated that the biomimetic apatite coating had a positive effect on MC3T3-E1 pre-osteoblast proliferation. In conclusion, 3-D PPF/DEF scaffolds fabricated using MSTL technology with biomimetic apatite coating can potentially be applied to bone tissue engineering.

Acknowledgments

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