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Preosteoblast Cell Adhesion and Proliferation on Polycaprolactone (PCL) 3D Scaffolds with Hyaluronic Acid Nano Layer

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Polycaprolactone (PCL) scaffolds have been widely used for bone tissue engineering. However, PCL is not good due to the lack of cell recognition sites and hydrophobic surface. Hyaluronic acid (HA) is a glycosaminoglycan that plays an important role in adhesion, migration and proliferation. The object of this study is to fabricate the polymeric thin film in order to improving the preosteoblast cells adhesion and proliferation on HA immobilized PCL scaffolds using amine plasma polymerization. HA immobilized PCL scaffold showed no significant effect on preosteoblast cell attachment and proliferation.

Keywords: Polycaprolactone (PCL), Scaffolds, Preosteoblast, Hyaluronic acid

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1. INTRODUCTION

In tissue engineering, the scaffold serves as a three-dimensional (3D) template for cell adhesion, proliferation and differentiation and provides an appropriate environment for the newly formed tissue [1]. The porous biodegradable polymeric scaffolds have been widely used for regenerating tissues and organs [2]. The synthetic, biodegradable polycaprolactone (PCL) has received considerable attention for tissue engineering, especially for bone and cartilage regeneration since it has appropriate mechanical properties, is less expensive and is easily fabricated into complicated shapes with appropriate porosity [3-5]. However, one of the main defects of the PCL scaffold is the highly hydrophobic surface, which can cause lack of initial cell attachment and proliferation. To improve the hydrophilicity and biological properties of PCL scaffolds various techniques have been applied [6]. One of the surface modification techniques is plasma polymerization that has proven to be a highly successful means of developing functional interfaces for the immobilization of biomolecules and drives adhesion, spreading and proliferation of cells [7,8]. Hyaluronic acid (HA) is biodegradable polysaccharide composed of repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine [9]. Furthermore, HA plays an important role in adhesion, migration and proliferation [10]. HA is negatively charged due to the carboxyl group of glucuronic acid. Therefore, a biomaterial surface with an opposite charge can boost the first contact of the cell to the surface until the cell synthesizes matrix proteins to mediate the fundamental integrin contact in focal adhesion [11].

The aim of this study is to evaluate preosteoblast cells (MC3T3-E1) attachment and proliferation on HA immobilized 3D PCL scaffolds through amine plasma polymerization.

2. EXPERIMENTAL DETAILS

2.1 Materials

3D-PCL was purchased from 3D Biotek (USA). Allylamine (AAm), EDC (1-ethyl-3-[3dimethylaminopropyl] carbodiimide hydrochloride), sodium phosphate, NHS (N-hydroxysuccinimide) were purchased from Aldrich. Methyl orange (MO) was purchased from Tokyo chemical industry. HA was obtained from MP Biomedicals (USA).

2.2 Plasma Polymerization

3D PCL surface modification was carried out by depositing a polymeric layer containing amine groups through plasma polymerization of monomer, AAm using radio frequency discharge device (MINIPLSAMA, Korea). The AAm vapor was introduced to the reactor at a flow rate of 20 SCCM. Plasma-polymerization was carried out at a discharge power of 50 W for 5-15 min. After the plasma-polymerization, AAm thin film layers formed on the 3D PCL scaffolds surface. These specimens are called "PCL/NH_2".

2.3 HA Immobilizatiom

The HA was immobilized onto the -NH2 functionalized surface in an EDC-NHS aqueous solution at a concentration of 4 mg/mL (EDC/NHS = 4/1 (w/w)) for 3 h with gentle shaking at 0℃ [12]. The samples were then immersed in a 5 g/L HA solution for 24 h with mild stirring at 0℃. The primary amine (H_2N-) of the PCL/NH_2 surface was reacted with the COOH group of the HA molecules. The prepared samples were rinsed with distilled water to remove the unbound HA and are referred to as "PCL/NH_2/HA".

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2.4 Surface Characterization

The surface morphology of the before and after plasma polymerization or HA grafting on the 3D PCL scaffolds was observed Field emission-scanning electron microscopy (FE-SEM; S-4800, Hitachi, Japan). The chemical bonding of the polymer layer was analyzed by Flourier transform infrared spectroscopy (FT-IR; Spectrum 400, PerkinElmer, UK).

2.5 Cell Culture

MC3T3-E1(ATCC CRL-2953) cell, a clonal preosteoblastic cell line derived from newborn mouse calvaria, were cultured in α-MEM medium and 5 % CO_2 at 37℃ with cell seeding density of 1×10^4 cell/ml.

2.6 Cell Proliferation

The proliferation of MC3T3-E1 cells was examined by MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide to a purple formazan product, Sigma-Aldrich) assay after 5 and 15 min of culture. All samples were placed into a 12-well plate and seeded with a density of 2×10^5 cells/ml.

3. RESULTS AND DISCUSSION

3.1 Morphologies of 3D PCL Scaffolds

Fig. 1 shows the surface morphologies of pristine 3D PCL, plasma-polymerized 3D PCL, and HA immobilized 3D PCL scaffolds.

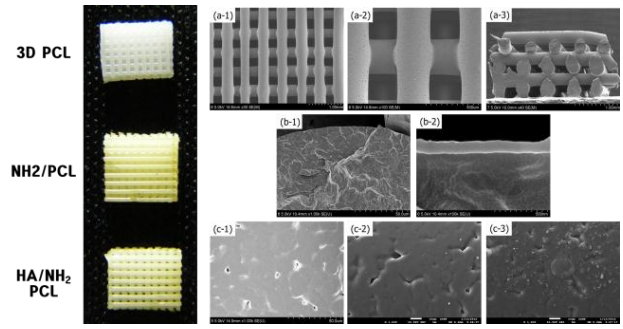

Fig. 1 – The FE-SEM micrographs of pristine 3D PCL, plasma-polymerized 3D PCL, and HA immobilized 3D PCL scaffolds. (a-1,2): 3D PCL surface, (a-3): cross-section; (b-1,2): NH_2 coated 3D PCL cross-section; (c-1): 3D PCL surface, (c-2) NH_2/PCL, and (c-3) HA/NH_2/PCL.

The left photographs show the changes of 3D PCL surface color after the AAm plasma- polymerization and HA immobilization. Fig. 1a-1, 2, 3 presents FE-SEM images of 3D PCL scaffolds with relatively flat surfaces. The AAm polymer layer is approximately 100 nm thickness (Fig. 1b-2). Fig. 1c-1, 2, 3 shows enlarged FE-SEM images of the each 3D PCL scaffold. The micro pore size of plasma-polymerized PCL scaffolds surface is smaller than that of untreated PCL scaffold. It may be effect of polymer thin films on the PCL scaffolds.

3.2 Surface Characterization

Fig. 2 presents the basic scheme for the entire reaction including plasma polymerization and HA immobilization on PCL scaffolds. Fig. 3 shows the FTIR spectra of the pristine PCL, PCL/NH_2, and PCL/HA scaffolds surfaces. To identify the chemical bonding in each step for the entire reaction process, FTIR spectrometry is a very powerful technique. The FTIR spectrum clearly shows several absorption bands characteristic of the presence of primary and/or secondary amine (3380 cm^{-1}) groups, multiple absorption bands arising from CHx bond stretches (2900–3000 cm^{-1}) in Fig. 3 [13]. The C–H bending in methylene (–CH_2–, approximately 1460 cm^{-1}), in vinyl groups (–CHCH2, approximately 1430 cm^{-1}) are also present in the AAm spectrum (the peak did not show in Fig. 3). After HA was immobilized onto PCL/NH_2 samples, the band near 1500 cm^{-1} increased.

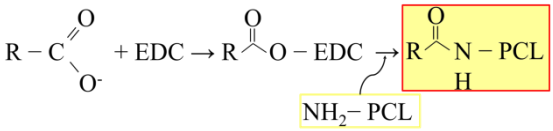

Fig. 2 – The basic process for the entire reaction including plasma polymerization and HA immobilization on PCL scaffolds

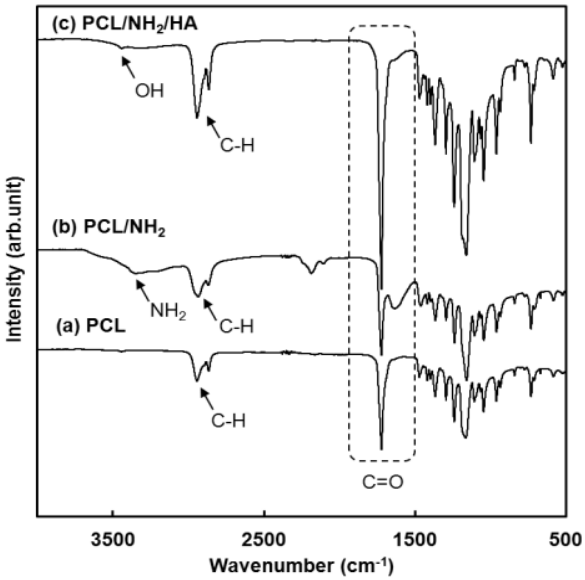

Fig. 3 – FTIR spectra of (a) PCL, (b) PCL/NH_2, (c) PCL/NH_2/HA scaffolds

3.3 Biological Activity

To investigate the cell proliferation, we used to MTT assay. Fig. 4 shows the proliferation of preosteoblast MC3T3-E1 cells cultured on pristine 3D PCL scaffold (control), PCL/NH_2, and PCL/HA scaffold for 5 and 15 min. For PCL/NH_2 scaffold, as the increase culturing time, cell proliferation is increased. However, it could be seen that the initial proliferation of MC3T3-E1 cell on the HA immobilized PCL scaffold was almost equal or decrease. The MTT results in the present study indicate

that the immobilization of HA have no significant effect on preosteoblast cell initial attachment and proliferation. It may be that presence of HA as the outermost layer appeared to have a more negative effect on osteoblast attachment than control groups. The anti-adhesive nature of HA has been previously reported and unalloyed Ti grafted with HA was shown to inhibit both osteoblast and fibroblast attachment [14]. To understand this phenomenon of poor osteoblast adhesion on PCL/HA scaffold surfaces, MC3T3-E1 cells adhesion assays were carried out on control, PCL/NH_2, and PCL/HA samples. PCL/HA surface exhibited significantly low cell coverage as compared to pristine 3D PCL scaffolds. Cassinelli et al. have reported that although HA does not support cell adhesion, it does not adversely affect the viability of cells [15].

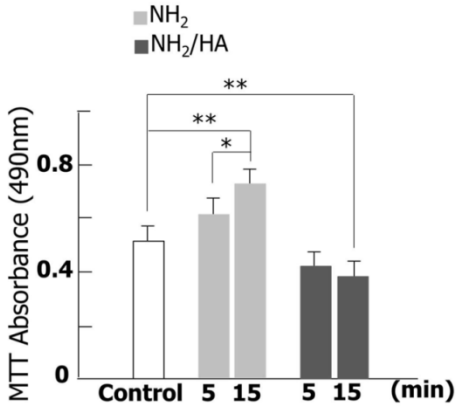

Fig. 4 – Effects of surface modification of PCL/NH_2, PCL/HA using plasma on MC3T3-E1 osteoblast-like cell proliferation. *P<0.05; **P<0.01.

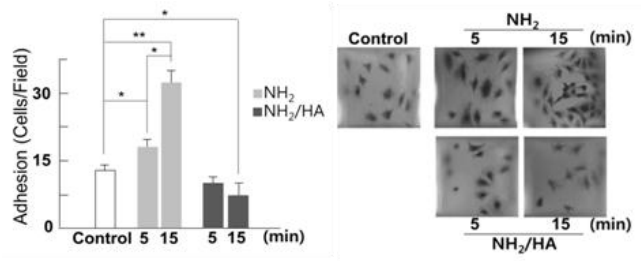

Fig. 5 – Effects of surface modification of PCL/NH_2, PCL/HA using plasma on MC3T3-E1 osteoblast-like cell adhesion. *P<0.05; **P<0.01.

4. CONCLUSION

Allylamine plasma-polymerization provides suitable functional group such as -NH_2 and charge on the surface of 3D PCL scaffolds. In addition, HA was successfully immobilized on polymer films containing the amine groups. However, HA immobilized PCL scaffolds showed no significant effect on MC3T3-E1 cells of initial stage attachment and proliferation. HA immobilization could be useful method where cell adhesion is undesirable, such as orbital fractures, where muscles should avoid adhesion to the implant.

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