Materials Science and Engineering C 40 (2014) 389–397

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Surface modification of polycaprolactone scaffolds fabricated via selective laser sintering for cartilage tissue engineering

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ARTICLE INFO

Article history:
Received 3 January 2014
Received in revised form 11 March 2014
Accepted 7 April 2014
Available online 15 April 2014

Keywords:
Selective laser sintering
Gelatin
Collagen
Chondrocytes
Cartilage tissue engineering
Immersion coating

ABSTRACT

Surface modified porous polycaprolactone scaffolds fabricated via rapid prototyping techniques were evaluated for cartilage tissue engineering purposes. Polycaprolactone scaffolds manufactured by selective laser sintering (SLS) were surface modified through immersion coating with either gelatin or collagen. Three groups of scaffolds were created and compared for both mechanical and biological properties. Surface modification with collagen or gelatin improved the hydrophilicity, water uptake and mechanical strength of the pristine scaffold. From microscopic observations and biochemical analysis, collagen-modified scaffold was the best for cartilage tissue engineering in terms of cell proliferation and extracellular matrix production. Chondrocytes/collagen-modified scaffold constructs were implanted subdermally in the dorsal spaces of female nude mice. Histological and immunohistochemical staining of the retrieved implants after 8 weeks revealed enhanced cartilage tissue formation. We conclude that collagen surface modification through immersion coating on SLS-manufactured scaffolds is a feasible scaffold for cartilage tissue engineering in craniofacial reconstruction.

1. Introduction

Cartilage engineering for aesthetic or reconstructive purposes relies on the same foundation of scaffold-cell interactions found in all tissue engineering applications. However, cartilage produced for those purposes, such as the auricle and nose or cartilage for complex facial trauma, emphasizes shape, shape retention and reproduction of the mechanical characteristics found in cartilage of native origin [1].

Poly(ε-caprolactone) (PCL) is a polyester polymer that has been frequently used as tissue engineering scaffold material, especially for bone [2]. It is biocompatible, possesses excellent mechanical strength, and is used as a material for sutures. Compared with other polymers such as polylactic acid (PLA), polyglycolic acid (PGA) or poly(lactic-co-glycolic acid) (PLGA), PCL has a much longer degradation time, lasting up to three years [3]. In this aspect, PCL may be a suitable scaffold material for cartilage tissue engineering in facial reconstructive surgery. However, there are conflicting reports on its use for cartilage engineering. It has been reported to be a good choice for cartilage tissue engineering through studies of its effects on cell attachment, cell proliferation, extracellular matrix (ECM) production and mechanical performance [4–6]. However, other reports have demonstrated that there was poor biological adhesion between cellular integrin and CD44 molecules with the pristine PCL scaffold [7]. Hence, a PCL scaffold may require further optimization through advanced manufacturing or surface modification techniques.

To better enhance the interactions between cells and the scaffold, surface modification is a viable option. Cellular activity is significantly influenced by integrin-ligand interactions [8]. Since biological adhesion sites are almost completely absent on synthetic polymers, the initial interaction between cells and the scaffold is extremely important, which will depend on the presence of specific sites for cell adhesion on the scaffold surface [9]. Certain peptide sequences, such as Arg-Gly-Asp (RGD), IKVAV and YIGSR can be used to promote cell attachment to materials [10,11]. As the most abundant protein in the human body, collagen has been widely used to enhance cells-material interaction. Collagen also provides both mechanical supports to tissues in addition to serving as a provider of bioactive substrates for adhesion molecules [7]. Collagen contains binding sites such as RGD and GFOGER that help promote biological attachment of cells [12–15]. Gelatin is a mixture of polypeptides derived from the breakage of the collagen triple-helix structure [16]. Similar to collagen, gelatin is capable of promoting cell adhesion and proliferation [17]. Additional benefits of gelatin include biocompatibility and low cost [18].

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http://dx.doi.org/10.1016/j.msec.2014.04.029
0928-4931/

As one of the solid-free form fabrication methods, selective laser sintering (SLS) fuses material in a powder bed in a layer-by-layer fashion, fabricating scaffolds with a consistent design and allows for the integration of computer-aided design with more complex, customized geometries. Additionally, the requirement of interconnected channel network at the macrostructure level for mass transfer and varying microstructure for cell attachment could be met [2,19]. We hypothesize that combining PCL scaffolds manufactured by SLS with the surface-modification technique using collagen or gelatin could provide a dual scaffold design scheme that meets the degradation time requirement for shape-oriented cartilage production and addresses the issue of optimization of the PCL material for cells-scaffold interaction. Furthermore, the application of SLS-derived PCL scaffold has not been reported for cartilage tissue engineering, as it has been for bone and cardiac tissue engineering [2,20]. Therefore, this article investigates the feasibility of surface-modified PCL scaffolds fabricated through SLS for cartilage tissue engineering.

2. Materials and methods

2.1. Materials

Polycaprolactone powder (CAPA® 6501 PCL) was purchased from Solvay Interox, UK. This form of PCL has a melting point of 60 °C, a molecular weight of 50,000 Da, and 99% particle size distribution in the 10–100 μm range. Type II collagen from chicken sternal cartilage and gelatin derived from porcine skin was purchased from Sigma-Aldrich, (St. Louis, MO, USA).

2.2. Porous scaffolds design and fabrication

The PCL scaffolds used in this study were fabricated by an in house-built SLS, rapid prototyping machine. Solidworks 2007 software was used to design the dimensions and pores distribution of PCL scaffolds. A scaffold design parameter utilizing a stack angle of 0°/45°/90°/135°, as previously investigated in our laboratory, was used. Cylindrical scaffolds possessing 3-dimensional orthogonal periodic porous architectures were manufactured, each with 12 layers of 0.2 mm layer distance, 0.8 mm line distance and a combined height of 2.4 mm and a base diameter of 12 mm. Manufacturing parameters were set at a laser power of 2 W, particle bed temperature of 40 °C and a scanning speed of 500 mm/s. After the SLS process was completed, the scaffolds were allowed to cool inside the processing chamber for approximately 1 h and were then removed from the particle bed. Excess powder surrounding the scaffold was brushed off and the scaffold was cleaned by compressed air.

2.3. Surface modification of scaffolds

For gelatin modification, samples were immersed in 2.5%(v/v) gelatin dissolved in double distilled water at 37 °C for 2 days. Scaffolds were removed and oven-dried at 37 °C for 1 day, followed by rinsing with phosphate buffered saline (PBS) solution. To modify the scaffolds with type II collagen, collagen solution was prepared by dissolving 0.05 g of collagen in 10 ml 0.3%(v/v) acetic acid titrated to pH 7.4 with 1 N NaOH. Afterwards, the solution was stored at 4 °C overnight before use. Scaffolds were immersed in collagen gel at 4 °C and placed in a shaker for 24 h, then removed and allowed to dry at room temperature in a hood. Three types of scaffold were used for comparison, pristine PCL scaffold (PCL), gelatin-modified PCL scaffold (GEL + P), and collagen-modified PCL scaffold (COL + P). Sterilization of the scaffold was performed by immersion in 50% ethanol for 30 min and 75% ethanol for 60 min, followed by air-drying overnight.

2.4. Characterization of scaffolds

The volume of each scaffold was measured by calculating the difference between the volume of an alcohol solution with or without the scaffold using a microbalance. Scaffold density was calculated by the mass of the scaffold over its volume. Porosity was determined as the difference between 100% and the percent ratio of the PCL scaffold density versus the PCL powder density ($n = 6$). Instron 5544 (Instron Inc., Carton, MA, USA) was used to determine the compressive strength of six scaffolds of each type in the dry state from the stress-strain curve. Instrument conditions were set for speed = 1 mm/min, sampling frequency = 0.1 s, pre-set force = 0.1 N, load = 450 N. The wettability of the scaffold was determined using a First Tech Angstroms FTA-125 goniometer (First Ten Angstroms, Portsmouth, Virginia). The contact angles were measured at room temperature 10 s after dropping 10 μl deionized distilled (DDI) water onto each scaffold and calculated using an automated fitting program. All reported water contact angles were averaged over 2 measurements for 3 replicate of each scaffold.

To determine the water uptake of the scaffold, scaffolds were measured for net dry weights and then put in a 24-well plate. They were then soaked in 10 ml DDI water. Changes in weight were measured at 5-min intervals for the first 50 min followed by measurements at 10 min-intervals for the next 20 min. The scaffolds were measured again at 100 min and then measured after 1, 3, 6, 12 and 24 h. The swelling ratio (%) was determined for six scaffolds of each type as the increase in weight after saturated in DDI water compared to the weight of the dry scaffold using the equation, swelling ratio = $(\text{weight}_{\text{saturated}} - \text{weight}_{\text{dry}})/\text{weight}_{\text{dry}} \times 100$. The chemical structure of the pristine PCL scaffold and surface-modified variants were analyzed using FT-730 Fourier-transform infrared (FTIR) spectroscopy (Horiba, Ltd., Kyoto, Japan). One milligram of sample was packed into a pellet form with KBr and the transmittance was recorded with an accumulation of 10 scans, resolution at 2 cm^{-1} and spectral range from 4000 to 400 cm^{-1}. To observe the layer interfaces and surface morphologies of scaffolds with or without modification, the microstructure of each type of scaffold was observed by scanning electron microscope (SEM) (Philips XL-30, Amsterdam, Netherlands) after sputter coating for 60 s.

2.5. Chondrocyte isolation and culture

Animal experiments were performed with approval from the Institutional Animal Care and Use Committee of Chang Gung Memorial Hospital and conformed to the standards of the Association for Assessment and Accreditation of Laboratory Animal Care. Chondrocytes were isolated by enzymatic digestion of the knee joint articular cartilage from hind limbs of 3-week-old swine. The cartilage was minced and washed twice in a PBS supplemented with penicillin-streptomycin (100 units/ml penicillin and 100 mg/ml streptomycin; Gibco BRL, Grand Island, NY, USA) for 5 min at room temperature. Chondrocytes were released by overnight digestion in 0.3% collagenase solution (Sigma-Aldrich) in an orbital shaker for 12 h. The remaining chondrocytes were centrifuged (1500 rpm for 7 min for 3 times) and re-suspended in Dulbecco's Modified Eagle Medium/Nutrient Mixture Ham-12 (DMEM/F12; Sigma) containing 10% fetal bovine serum (FBS, Hyclone, Thermo Scientific) and 1% penicillin-streptomycin solution at 37 °C in a humidified atmosphere containing 5% CO_2.

2.6. In vitro and in vivo studies

Chondrocytes were plated at a density of 1×10^6 cells/ml and cultured until passage 2 for further experiments. Harvested chondrocytes were adjusted to a final concentration of 1×10^7 cells/ml and a 200 ml cell suspension (2×10^6 cells) was gently pipetted onto each scaffold. The cell-scaffold constructs were then incubated for 4 h at 37 °C with 95% humidity and 5% CO_2 to allow for complete adhesion of the cells to the scaffolds. Then the constructs were moved to a different well,