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Enhanced extracellular matrix production and differentiation of human embryonic germ cell derivatives in biodegradable poly(ε-caprolactone-co-ethyl ethylene phosphate) scaffold

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Abstract

Extracellular environment regulates cell behavior and also influences the differentiation of stem cells. Two cell lines of pluriopotent human embryonic germ cell derivatives (EBD cells) were cultured on a biodegradable poly(ε-caprolactone-co-ethyl ethylene phosphate) (PCLEEP) and non-degradable cellulose acetate scaffold. Their cell behaviors including proliferation, differentiation, cell distribution and extracellular matrix production were studied for 4 weeks and 10 months. The proliferation of the EBD cells was enhanced in both of the three-dimensional scaffolds in the first 5 weeks of culture, regardless of the material difference, compared to monolayer culture. While the gene expression profile remained multilineage for the EBD cells cultured in the cellulose acetate fibrous scaffold, much of the neuronal lineage markers were down-regulated in EBD cells cultured in the PCLEEP scaffold. On the other hand, extracellular matrix production was significantly enhanced in the PCLEEP scaffold. The study showed that the polymer substrate could influence the differentiation and growth of pluripotent stem cells in the absence of exogenous biochemical signals.

Keywords: Polymer scaffold; Biodegradability; Stem cells; Extracellular matrix; Differentiation

1. Introduction

Biomaterials can aid cell and tissue development by providing a suitable microenvironment for cell attachment, proliferation and extracellular matrix deposition [1]. Biodegradability of the biomaterials is often desired to provide room for cell proliferation and to avoid enduring biocompatibility concerns when applied in vivo.

Aliphatic polyesters, prepared by ring opening polymerization of lactones and lactides, are versatile polymers having good mechanical properties, biodegradability, and biocompatibility [2]. Adopting a similar strategy and taking advantage of the interesting phosphorus chemistry, we synthesized a biodegradable polymer with ester and phosphate linkages in the backbone, poly(ε-caprolactone-co-ethyl ethylene phosphate) (PCLEEP). [3] The phosphate units of the backbone polymer enhance degradability and hydrophilicity of the poly-ε-caprolactone (PCL). The copolymer shows an intermediate degradation rate between PCL and poly(ethyl ethylene phosphate). It is readily soluble in non-chlorinated solvents and can be processed into different forms, such as nanofibrous scaffolds [4,5], to facilitate tissue-engineering applications.

Previous studies have shown that surface chemistry and the degradation rate of polymer substrate have a significant effect on tissue formation [6]. In studying the effect on materials chemistry on a culture of human embryonic stem cells (hES), Anderson et al. examined 576 different combinations of acrylate-based polymers polymerized, using light-activated radical initiator, on poly(hydroxythyl methacrylate) [7]. Cell attachment, spreading and growth

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factor-dependent proliferation of the hES were observed to vary on these two-dimensional (2D) surfaces. Cells behave differently when cultured in a 3D configuration, and hES cells also function in a 3D environment in vivo. As of today, little is known about the influence of scaffold architecture and biodegradability on stem cell fate in a three-dimensional (3D) culture system. In this study we compared the culture of human embryoid body derived (EBD) cells on a biodegradable macroporous scaffold with a non-biodegradable fibrous scaffold.

Human embryoid body derived (EBD) cells are uncommitted precursor or progenitor cells expressing multilineage gene markers that can be maintained stably in vitro [8–10]. They represent one of the viable sources of human stem cells. These EBD cells also serve as a valuable model for studying the manipulation of human pluripotent stem cells. We have previously studied the proliferation of the EBD cells on a non-degradable 3D cellulose acetate fibrous scaffold [11]. The growth of the EBD cells is enhanced in the 3D scaffold compared to monolayer culture.

In this study, we fabricated a biodegradable PCLEEP scaffold with a salt-leaching technique. PCLEEP is a copolymer comprising ε-caprolactone and ethyl phosphate, with the latter incorporated into the backbone to accelerate the biodegradation of poly(ε-caprolactone). The proliferation, differentiation and extracellular matrix production were evaluated in this scaffold both in short-term (weeks) and long-term (months) cultures. The cell behavior of the EBD cells was compared to that on a non-degradable cellulose acetate fibrous scaffold.

2. Materials and methods

2.1. Embryoid body derived cells (EBD)

EBD [8] cells were obtained from Dr. Gearhart's Laboratory at Johns Hopkins University. Two cell lines, SDEC and LVEC, were derived from embryoid bodies formed from embryonic germ cell cultures, which were initially isolated from 5 to 11 weeks post-fertilization human primordial germ cells [9], respectively. Cells used in the experiments were at population doubling between 35 and 36.

EGM2MV medium (Clonetics, San Diego), which included 5% fetal bovine serum (FBS), hydrocortisone, human basic fibroblast growth factor, human vascular epidermal growth factor, R(3)-insulin-like growth factor I, ascorbic acid, human epidermal growth factor, heparin, gentamycin, and amphotericin was used for the culture.

2.2. PCLEEP scaffold preparation

PCLEEP (Fig. 1) was synthesized as previously described [3]. Briefly, ε-caprolactone (Aldrich) and ethyl ethylene (EEP) were copolymerized using $Al(O^iPr)_3$ as the initiator in a dried ampoule. The mixture was allowed to react for 48 h at 100 °C. The product was dissolved in CH_2Cl_2 with 10-fold molarity of acetic acid against $Al(O^iPr)_3$. The solution was stirred at room temperature for 2 h, then washed with saturated NaCl solution three times and dried over Na_2SO_4. The product was precipitated with ether and further purified by dissolving in acetone and quenching in distilled water. The resulting polymer contained 15 mol% of EEP as confirmed by NMR, and is denoted as PCLEEP15.

PCLEEP15 was fabricated into porous scaffold by a conventional solvent-casting and particulate-leaching technique with NaCl as the porogen. Briefly, 2.5 mL acetic acid solution containing 0.3 g of PCLEEP15 was mixed with 5 g of NaCl crystals with a particle size range of 150–300 μm. The mixture was homogenized by vortexing for 1 min and frozen immediately at −80 °C. The solvent was removed by lyophilization over 3 days and the dried polymer/NaCl composites were cut into discs of around 5 mm in diameter with a razor blade. The discs were immersed in a large volume of distilled water to leach out NaCl under stirring at room temperature for 2 days. The water was changed five times per day. The discs were sterilized with 70% alcohol and lyophilized for a week to completely remove the solvent. The scaffolds were stored in a desiccator until use.

2.3. Cellulose acetate fibrous scaffold

Cellulose acetate (CA) 150/40 fiber was a gift from Celanese Acetate (North Carolina). The fiber was separated into individual filaments, less than 40 μm in diameter each, and cut into 2–4 mm segments, from which a random mesh was constructed manually.

The total porosity of PCLEEP15 scaffold and cellulose acetate scaffold was calculated using the known density of the polymers, the measured polymer mass of the scaffold, and the measured external volume of the scaffold [12]

$$\Pi = 1 - \rho_{\text{scaffold}}/\rho_{\text{polymer}}$$

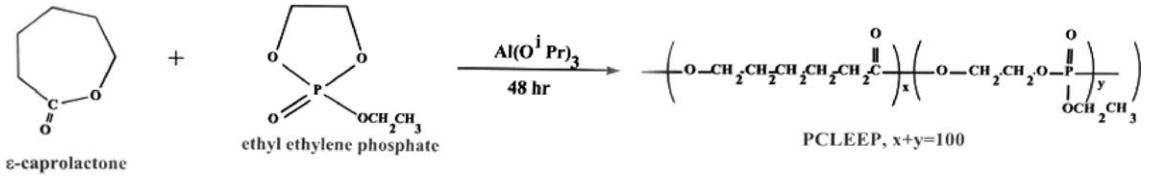

Fig. 1. Schematics of PCLEEP synthesis.

ID	Title	Pages
2658	Enhanced extracellular matrix production and differentiation of human embryonic germ cell derivatives in biodegradable poly(ε-caprolactone-co-ethyl ethylene phosphate) scaffold	12