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Chondrogenesis in scaffolds with surface modification of elastin and poly-L-lysine

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ABSTRACT

A tissue engineering cartilage is of great importance in the current diarthrodial surgery. This study presents the formation of neocartilage by cultivating chondrocytes in elastin- and poly-L-lysine-modified scaffolds. The hybrid bulk biomaterials used contained polyethylene oxide, chitin, and chitosan and were fabricated by crosslinking, pre-freezing, and lyophilization. Bovine knee chondrocytes were seeded in the scaffolds and cultured in a spinner-flask bioreactor over 4 weeks. Surface elastin showed a better efficiency in the adhesion and proliferation of bovine knee chondrocytes in the scaffolds than surface poly-L-lysine. In addition, elastin-modified constructs yielded higher quantities of secreted glycosaminoglycans and produced collagen than poly-L-lysine-modified constructs. The surface morphology demonstrated a thriving chondrogenesis in the two kinds of constructs. The staining images revealed that elastin induced larger amounts of regenerated bovine knee chondrocytes, glycosaminoglycans, and type II collagen in the constructs than poly-L-lysine. Elastin- and poly-L-lysine-grafted polyethylene oxide/chitin/chitosan scaffolds are effective in producing cartilaginous components.

1. Introduction

The surface property of tissue engineering scaffolds is the most important feature in cell culture, although the scaffolds should satisfy many requirements. This is because the majority of biochemical reactions occur at the material–medium interface. In addition, the surface properties of scaffolding substrate can be extremely influential to the cell adhesion, biocompatibility, and mitotic multiplication [1–5].

Elastin is one of the typical fibrous ingredients in extracellular matrix and provides mechanical expandability and pliable resilience to tissues, including large artery, lung, skin, cartilage, and ligament [6]. The low platelet adhesion and aggregation activity benefited elastin as a promising protein for implantable device. For example, elastin has been used in an insoluble form for autograft, allograft, xenograft, and decellularized extracellular matrix. However, the insolubility of elastin due to a high crosslinking degree restricted further developments of elastin for clinical application. The soluble elastin can be obtained by hydrolyzing with acid, alkaline, or enzyme, rendering the decomposition of its elastomeric structure. Elastin-based biomaterials were applied to surface coating for inhibiting thrombosis-related complications [7,8] and for upregulating endothelialization [9]. In addition, elastin-derived polypeptides have been employed as a key element for modifying polymeric surface to prevent infection from per-operative treatment [10]. Synthetic elastin-like sequences and tropoelastin (not crosslinked immature elastin) were also used in biomedical designs [11,12].

The cationic polyelectrolytes, including poly-L-lysine, demonstrate a strong affinity to proteins, DNA, and cell membranes and can actively interact with mammalian cells. Poly-L-lysine is water soluble due to its protonated side amine groups and has various conformations of secondary structure such as α-helix, β-sheet, and random coil [13]. In addition, isomeric poly-L-lysine could stimulate the proliferation of astrocytes isolated from aged mice [14]. Poly-L-lysine was also concluded to promote the synthesis of immunoglobulin and interferon-β and inhibit the growth of various carcinomas [15,16].

The aim of this study is to generate neocartilage by cultivating chondrocytes in elastin- and poly-L-lysine-modified scaffolds. To replace damaged and degenerated cartilage, an ideal biomaterial should imitate extracellular matrix in vivo with similar physical and biochemical properties. Thus, elastin and poly-L-lysine were grafted on biopolymer surface to establish peptide-rich surroundings for chondrocyte growth. We investigated the cell adhesion, viability, and chondrogenesis in the scaffolds. In addition, the proliferated chondrocytes, secreted glycosaminoglycans, and produced type II collagen were analyzed by histological and immunochemical staining.

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2. Experimental

2.1. Polyethylene oxide/chitin/chitosan matrix

Three percent (w/v) polyethylene oxide (Sigma, St. Louis, MO) gel, 3% (w/v) chitin (Sigma) gel, and 3% (w/v) chitosan (Sigma) gel were prepared, respectively, by mixing 3 g polyethylene oxide, 3 g hydrochloric acid (Hanawa, Osaka, Japan)-treated chitin, and 3 g chitosan in 100 mL of 1% (v/v) aqueous acetic acid (Showa, Tokyo, Japan) solution at 40 °C [17,18]. The polyethylene oxide, chitin, and chitosan gels were mixed in a volume ratio 23:26:51 with 1% (w/w) genipin (Challenge Bioproducts, Taichung, Taiwan) for 5 min, cast in a mold of 23 mm × 23 × mm 16 mm with an exposure to ultraviolet of 365 nm (UV, Spectronics, Westbury, NY) at 40 °C for 1 h, placed at room temperature for 24 h, immersed in 95% (v/v) ethanol (Taiwan Sugar, Tainan, Taiwan) several times, and dried in biological safety cabin at room temperature. The polyethylene oxide/chitin/chitosan matrix were submerged in liquid nitrogen, and sliced by a cryostat microtome (Slee, Mainz, Germany) into scaffolds of 5 mm × 5 mm × 4 mm.

2.2. Fabrication of scaffolds modified with elastin and poly-L-lysine

One gram elastin (from bovine neck ligament, Sigma) was dissolved in 10 mL of sodium hydroxide (Mallinckrodt Baker, Phillipsburg, NJ) of 0.1 N at 100 °C and 200 rpm for 45 min, added with 10 mL of sodium hydroxide of 0.1 N, treated at room temperature and 200 rpm for 45 min, mixed with 30 mL of ultrapure water (Barnstead, Dubuque, IA) at 200 rpm for 30 min, centrifuged by a refrigerated centrifuge (AVANTij-25, Beckman Coulter, Palo Alto, CA) at 13,000 × *g* and 4 °C for 1 h. The pellet was lyophilized by a freeze dryer (Eyela, Tokyo, Japan) at 2–4 Torr for 12 h. For grafting elastin, one scaffold was injected with 90 μL of Dulbecco's phosphate buffered saline (DPBS, Sigma) containing 1% (w/w) genipin for 4 h, washed with DPBS, dried at 40 °C for 12 h, and injected with 90 μL of DPBS containing elastin at 4 °C for 12 h. For grafting poly-L-lysine (Sigma), one scaffold was injected with 90 μL of formic acid (Fisher, Fair Lawn, NJ) at 50 °C for 1 h, washed with DPBS, dried at 40 °C for 12 h, injected with 90 μL of DPBS containing 0.096% (w/v) 1-3-imethylaminopropyl)3-thylcarbodiimide hydrochloride (Sigma) and 0.023% (w/v) N-hydroxysuccinimide (Acros, Morris, NJ) at room temperature for 4 h, washed with DPBS, dried at 40 °C for 12 h, and injected with 90 μL of DPBS containing poly-L-lysine at 4 °C for 12 h. The elastin- and poly-L-lysine-grafted scaffolds were washed with DPBS and dried at 40 °C for 12 h.

2.3. Bovine knee chondrocytes

Forearm knee hyaline cartilages of calves were harvested at a local abattoir within 30 min of slaughter. The cartilaginous tissues were immersed in ice-bathed DPBS with 1% antibiotic–antimycotic solution (Sigma) and transported immediately to our laboratory. The cartilages was sliced into cubes about 1 mm^3 and digested with 0.18% type II collagenase (Sigma) in an incubator (NuAire, Plymouth, MN) with an atmosphere of 95% relative humidity and 5% CO_2 at 37 °C for 24 h. The digested suspension was centrifuged at 420 × *g* for 5 min. The bottom pellet contained bovine knee chondrocytes and was resuspended in 1 mL of Dulbecco's modified Eagle's medium (DMEM, Sigma). The concentration and viability of freshly isolated bovine knee chondrocytes were determined by a hemocytometer (Neubauer, Marienfeld, Germany) via trypan blue (Sigma) exclusion and a phase-contrast biological microscope (Motic, Richmond, BC, Canada). The excess bovine knee chondrocytes in DMEM were cryopreserved in the presence of 10% dimethyl sulfoxide (J.T. Baker, Phillipsburg, NJ) and 10% fetal bovine serum (Sigma) at −80 °C.

2.4. Adhesion efficiency of bovine knee chondrocytes

The elastin- and poly-L-lysine-grafted polyethylene oxide/chitin/chitosan scaffolds were sliced into samples of 5 mm × 5 mm × 2 mm, disinfected with 70% (v/v) ethanol, and dried under ultraviolet. The suspension of bovine knee chondrocytes was injected in various positions of the scaffolds at a density of 4.8×10^6 cells/construct. The constructs were incubated for 1 h and 12 h. After incubation, the constructs were immersed in the culture medium with a volume of 0.2 mL/construct. The number of bovine knee chondrocytes released into the culture medium was determined by the hemocytometer associated with the phase-contrast biological microscope. The adhesion efficiency of bovine knee chondrocytes was calculated by $[(4.8 \times 10^6 - \text{number of released bovine knee chondrocytes})/(4.8 \times 10^6)] \times 100\%$.

2.5. Viability of bovine knee chondrocytes

After adhesion of bovine knee chondrocytes for 4 h, a construct was immersed in 0.4 mL of culture medium and incubated in the CO_2 incubator for 8 h and centrifuged at 420 × *g*. One construct was reacted with 100 μL of MTT solution (Sigma) at 5 mg/mL in darkness for 4 h. After removal of MTT solution, 900 μL of MTT solubilization solution (Sigma) were added into the construct in darkness for 10 min. The light absorbance at 570 nm of 200 μL of the resultant solution was evaluated by an ultraviolet–visible detector (Bio-Tek, Winooski, VT). The cell viability was determined by $A_{12}/A_4 \times 100\%$, where A_4 and A_{12} are, respectively, the light absorbance after adhesion for 4 h and that after subsequent incubation for 8 h.

2.6. Culture of bovine knee chondrocytes

The elastin- and poly-L-lysine-grafted polyethylene oxide/chitin/chitosan scaffolds were disinfected with 70% (v/v) ethanol and dried. The suspension of bovine knee chondrocytes was injected into the scaffolds at a density of 8.3×10^6 cells/construct. After seeding for 12 h, the constructs were cultured in flasks with DMEM containing 50 μg/mL penicillin G (Sigma), 50 μg/mL streptomycin sulfate (Sigma), 10 mM 4-2-hydroxyethyl-1-piperazineethanesulfonic acid (J.T. Baker), 50 μg/mL L-(+)-ascorbic acid (J.T. Baker), 10% fetal bovine serum (Sigma), 0.1 mM minimum essential medium non-essential amino acid (Sigma), and 0.4 mM L-proline (Sigma). The volume of the culture medium per flask was 250 mL. The culture medium in one flask was stirred by two paddles in a spinner system (Integra Biosciences, Wallisellen, Switzerland) at 60 rpm in the CO_2 incubator. A half of the culture medium was replaced every 3 days. The constructs were fixed in the center of the flask with stainless wires.

2.7. Morphology of cultured constructs

The images of the cultured constructs were obtained by a field emission scanning electron microscope (FE-SEM, JSM-6330 TF, Jeol, Tokyo, Japan). The constructs were sliced, washed with DPBS, treated with 2.5% glutaraldehyde (Fluka, Buchs, Switzerland) for 4 h, desiccated stepwise with ethanol (Tedia, Fairfield, OH) from 70% to 99.8% (v/v), vacuum-dried for 10 min, and sputter-coated with gold at 5 mA and at an accelerating voltage of 1 kV for 7 min.