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Fabrication of single and bundled filament-like tissues using biodegradable hyaluronic acid-based hollow hydrogel fibers

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ABSTRACT

Hydrogel fibers with biodegradable and biocompatible features are useful for the fabrication of filament-like tissues. We developed cell-laden hyaluronic acid (HA)-based hollow hydrogel fibers to create single and bundled filament-like tissues. The cell-laden fibers were fabricated by crosslinking phenolic-substituted hyaluronic acid (HA-Ph) in an aqueous solution containing cells through a horseradish peroxidase (HRP)-catalyzed reaction in the presence of catalase by extruding the solution in ambient flow of an aqueous solution containing H_2O_2. The encapsulated cells proliferated and grew within the hollow core, and the cells formed filament-like constructs in both single and bundled fibers, which were obtained by collection on a rotating cylindrical tube. Single and bundled filament-like tissues covered with an additional heterogeneous cell layer were obtained by degrading the fiber membrane using hyaluronidase after covering the fiber surface with heterogeneous cells. Cellular viability was preserved during HA-Ph hydrogel fiber fabrication and filament-like tissue formation. These results demonstrate the feasibility of HA-based hollow hydrogel fibers obtained through HRP- and catalase-mediated reactions to engineer filament-like tissues.

1. Introduction

Hydrogels have emerged as leading matrices for engineered tissue scaffolds because of their hydrophilic characteristics and potential for biocompatibility [1–5]. Among hydrogels, extracellular matrix (ECM)-derived components have been recognized as ideal templates to prepare cell-laden hydrogels that can mimic the structural and biological properties of cellular environments in vivo [2,4–6]. In particular, hyaluronic acid (HA), a major ECM component in various tissues, is an attractive template for the construction of hydrogels with desired morphology, mechanical strength, and bioactivity [2,7,8]. Until now, HA-based hydrogels have been used for tissue engineering applications in various shapes and architectures such as sheets, bulk constructs, microparticles, and microcapsules [9–13]. These hydrogels provide stable three-dimensional cultivation systems for the survival, expansion, and self-renewal of cells as well as differentiation of encapsulated cells [9,11–13]. Among the various cell-laden hydrogels, fiber-shaped vehicles can be advantageous for fabrication of tissue-like biological constructs that mimic the structural and physical properties of native tissues [1,3–6]. In addition, fibers can be processed into woven, non-woven, and knitted constructs to fabricate complex tissue-like biological constructs [3–6]. To the best of our knowledge, no report has adopted an HA-based hydrogel as a fiber-shaped vehicle for cell encapsulation.

In this study, we have developed an HA-based hydrogel as a tool to fabricate a cell-laden hollow fiber-shaped vehicle. Cell-laden hydrogel fibers have commonly been fabricated from unique or composite polymers, including gelatin, collagen, alginate, amylopectin, and chitosan [1,3–6,14,15], through techniques such as electrospinning, microfluidic spinning, wetspinning, and interfacial complexation [1,3–6,13–18]. Among the various existing fabrication methods, the microfluidic spinning technique allows precise control over the size, geometry, morphology, and chemical features of fibers [1,3–6,13–18]. Previously, we produced alginate-based hollow hydrogel fibers through enzymatic crosslinking and degradation using a coaxial double-orifice spinneret [14]. In this system, horseradish peroxidase (HRP) and catalase, both of which

Abbreviations: HA, hyaluronic acid; HRP, horseradish peroxidase; HA-Ph, phenolic-substituted hyaluronic acid; gelatin-Ph, phenolic-substituted gelatin; HeLa cells, human cervical cancer cell line; 10T1/2 cells, mouse embryonic fibroblast-like cell line; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; WSCD, water-soluble carbodiimide hydrochloride; NHS, *n*-hydroxysuccinimide; MES, 2-morpholinoethane sulfonic acid.

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consume H_2O_2, were used to form a hollow in the hydrogel vehicles [14,19,20]. To this end, phenolic-substituted HA (HA-Ph) may be a useful bioactive and biodegradable material to create hollow hydrogel fibers that enable cell encapsulation while maintaining cell viability [9,12,21]. The HA-Ph fibers were made by a microfluidic spinning technique in which an aqueous solution of HA-Ph containing HRP and catalase was extruded into an ambient co-flowing aqueous solution containing H_2O_2 through a coaxial double-orifice spinneret. Subsequently, the fabricated fibers were simply bundled by rolling on a rotating cylindrical tube to prepare a higher-order fiber-shaped construct (Fig. 1B and C). The attractive point of using single and bundled HA-Ph hollow hydrogel fibers is that they provide a biomimetic environment for the growth of encapsulated cells while maintaining biodegradability.

In this study, we demonstrated the feasibility of single and bundled filament-like tissue fabrication using HA-Ph fiber-shaped hydrogel vehicles. We first investigated the diameter and membrane thickness of HA-Ph hydrogel fibers affected by changes in both the velocities of extruded solutions in the coaxial double-orifice spinneret and the concentration of reactants. Next, we examined the cytotoxicity of the fiber fabrication process and growth profiles of encapsulated cells in the HA-Ph hydrogel fibers. Finally, surface-coating with cells and the subsequent degradation of the HA-Ph hydrogel fibers were investigated to create a filament-like tissue construct covered with a heterogeneous cell layer. This study revealed the feasibility of HA-Ph cell-laden fiber-shaped vehicles for tissue engineering applications.

2. Materials and methods

2.1. Materials

Sodium-HA (average molecular weight: 1.7×10^6 Da) was obtained from JNC Corp. (Tokyo, Japan). HRP (160 U/mg), H_2O_2 aqueous solution [30% (w/w)] and catalase (1.3×10^4 U/mg, from bovine liver) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Gelatin (type B; from porcine skin, 300 Bloom) and hyaluronidase from sheep testes (type II, ≥300 U/mg) were purchased from Sigma (St. Louis, MO, USA). HA-Ph and phenolic-substituted gelatin (gelatin-Ph) were prepared according to previously reported methods through conjugation with hydroxyl phenol groups [12,21]. HA-Ph was synthesized by amide bond formation via carbodiimide-mediated condensation of carboxyl groups of HA with amino groups of tyramine using water-soluble carbodiimide hydrochloride (WSCD) and *n*-hydroxysuccinimide (NHS) in 2-morpholinoethane sulfonic acid (MES) buffer (Fig. 1A). Gelatin-Ph was similarly synthesized by conjugation of amino groups of gelatin with carboxyl groups of 3-(4-hydroxyphenyl) propionic acid. The Ph contents in HA-Ph and gelatin-Ph were 1.9×10^{-4} mol-Ph/g-HA-Ph (7.4 Ph molecules per 100 disaccharide repeating units of HA) and 2.9×10^{-4} mol-Ph/g-gelatin-Ph, respectively.

2.2. Cell culture

Human cervical cancer cell line HeLa and mouse embryonic fibroblast-like cell line 10T1/2 obtained from the Riken Cell Bank (Ibaragi, Japan) were cultured in Dulbecco's modified Eagle's medium (DMEM; Nissui Pharmaceutical, Tokyo, Japan) containing $NaHCO_3$ and *N*-2-hydroxyethyl-piperazine-*N*′-2-ethanesulfonic acid (HEPES), 10% (v/v) heat-inactivated fetal bovine serum, 75 μg/mL penicillin, and 50 μg/mL streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C.

2.3. Hydrogel fiber fabrication

HA-Ph hydrogel fibers were prepared in a coaxial double-orifice spinneret that was designed in our laboratory. Attached were two stainless steel needles with axially flat ends in a nested configuration. The internal diameters were 260 μm and 830 μm for the inner and outer needles, respectively. The outlet tip of the outer needle was connected to a fluorine resin tube with an inner diameter of 1000 μm and 20 cm in length to hold extension of the fluid route (Fig. 1C). Phosphate-buffered saline (PBS; pH 7.4) containing HA-Ph [0.3–1.6% (w/v)], HRP (50–200 U/mL), and catalase ($2–10 \times 10^3$ U/mL) was extruded from the inner needle at 4.8 cm/s into co-flowing PBS containing H_2O_2 (0.25–2.5 mM) extruded from the outer needle at 12 cm/s. The HRP- and catalase-mediated reactions spontaneously began and crosslinking occurred among HA-Ph molecules when the two fluids merged at the intersection. The fabricated HA-Ph hydrogel fibers were discharged from the end of the fluorine resin tube and subsequently bundled by rolling around a cylindrical plastic tube (Fig. 1B and C). The bundled fibers were cut off and removed from the roller plastic tube using a surgical scalpel blade. The resultant single and bundled fibers were immersed in culture medium. The dimensions of fibers were measured from images obtained under an optical microscope. The viscosity of the prepared HA-Ph solutions for hydrogel fiber fabrication were measured at 1 s^{-1} and 25 °C using a HAAKE MARS III rheometer equipped with axial cylinder geometry hood plates (Thermo Scientific Karlsruhe, Germany).

2.4. Cytotoxicity analyses

Cell-laden HA-Ph hollow hydrogel fibers were prepared by extruding an aqueous solution containing HA-Ph [1.0% (w/v)], HRP (100 U/mL), catalase (5×10^3 U/mL), and HeLa cells (5×10^6 cells/mL) at a flow velocity of 4.8 cm/s into PBS containing H_2O_2 (0.75 mM) extruded at flow velocities of 7.5, 12, and 20 cm/s in the coaxial double-orifice spinneret. Then, bundled cell-laden fiber constructs were obtained by rolling the discharged fibers. The resultant single and bundled cell-laden hydrogel fibers were immersed in culture medium. At 2 h after preparation, the viability of encapsulated cells was determined by trypan blue exclusion of cells recovered by soaking the fibers in culture medium containing hyaluronidase (0.2 mg/mL) for 30 min. The recovered cells were seeded at 1×10^4 cells/cm^2 on a 24-well tissue culture dish filled with DMEM to evaluate the possible occurrence of adverse effects that require time to induce cell death and growth inhibition. The growth profiles of recovered cells were estimated by measuring absorbances of water-soluble formazan dye derived from tetrazolium salt released from the cells using a colorimetric assay kit (Cell Counting Kit-8; Dojindo, Kumamoto, Japan) [19]. Cells maintained through conventional subcultures were cultured at the same seeding density as a control.

2.5. Filament-like tissue fabrication

At 15 days after preparation, cell-laden HA-Ph hydrogel fibers were soaked in a solution containing gelatin-Ph [0.25% (w/v)], HRP (1 U/mL), and H_2O_2 (0.75 mM) for 3 min to provide a cell-adhesive surface on the HA-Ph hydrogel fibers. Then, they were suspended in culture medium containing 10T1/2 cells (2×10^6 cells/mL). After 2 days of incubation, the fibers were soaked for 1 h in culture medium containing hyaluronidase (0.2 mg/mL) to degrade the HA-Ph hydrogel membrane (Fig. 2). The HeLa and 10T1/2 cells were respectively stained with Cell Tracker Orange® and Cell Tracker Green® (Life Technologies, Grand