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Collagen-based fibrous scaffold for spatial organization of encapsulated and seeded human mesenchymal stem cells

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ABSTRACT

Living tissues consist of groups of cells organized in a controlled manner to perform a specific function. Spatial distribution of cells within a three-dimensional matrix is critical for the success of any tissue-engineering construct. Fibers endowed with cell-encapsulation capability would facilitate the achievement of this objective. Here we report the synthesis of a cell-encapsulated fibrous scaffold by interfacial polyelectrolyte complexation (IPC) of methylated collagen and a synthetic terpolymer. The collagen component was well distributed in the fiber, which had a mean ultimate tensile strength of 244.6 ± 43.0 MPa. Cultured in proliferating medium, human mesenchymal stem cells (hMSCs) encapsulated in the fibers showed higher proliferation rate than those seeded on the scaffold. Gene expression analysis revealed the maintenance of multipotency for both encapsulated and seeded samples up to 7 days as evidenced by Sox 9, CBFA-1, AFP, PPARγ2, nestin, GFAP, collagen I, osteopontin and osteonectin genes. Beyond that, seeded hMSCs started to express neuronal-specific genes such as aggrecan and MAP2. The study demonstrates the appeal of IPC for scaffold design in general and the promise of collagen-based hybrid fibers for tissue engineering in particular. It lays the foundation for building fibrous scaffold that permits 3D spatial cellular organization and multi-cellular tissue development.

1. Introduction

Spatial organization and distribution of cells within a 3D extracellular matrix are important for controlling cellular functions and neo tissue synthesis [1,2]. To achieve an organized arrangement of cells in a tissue-engineered construct, a scaffold that can facilitate cell remodelling and tissue organization would be attractive. The design and development of scaffolding materials have been constantly evolving, having progressed from an inert mechanical support to a dynamic platform for cellular adhesion, proliferation, differentiation and interaction with the physiological environment [2,3]. Fibrous biomimetic materials are popular candidates because they provide a 3D microenvironment with high surface area-to-volume ratio, offer the potential of presenting biological cues in a temporally and spatially controlled manner, and impart a controlled porous architecture for efficient waste/nutrient exchange and cell migration [4–9].

Optimal tissue development requires infiltration of cells into the scaffold, which in turn necessitates a macroporous structure with interconnected pores diameters of at least 10 μm [10–12]. Alternatively, seeded cells can migrate into the interior of the scaffold by either enzymatically degrading or displacing individual fibers, but this requires an extended culture period and appropriate chemotactic factors present within the scaffold [12,13]. As the scaffold thickness increases and the pore size decreases, the problem of hindered cell infiltration becomes significant. Various strategies have been proposed, and they are based on a common hypothesis that scaffolds embedded with cells in a controlled spatial distribution can address the current problem of limited cell infiltration and achieve a highly cellularized tissue construct. To this end, mesenchymal stem cells and smooth muscle cells have been encapsulated in photosensitive hydrogels [14,15]; fibroblasts and endothelial cells sprayed between gels [16]; smooth muscle cells sprayed between layers of electrospun fibrous mats [17]; and cells incorporated into fibers via co-axial electrospinning [18]. Cells have also been printed onto scaffolds using modified ink-jet print heads

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[19,20]. Although the reported studies have made significant progress in creating highly biofunctional scaffolds, the said processes are complex and often detrimental to cell viability. Hence a milder and simpler technique to incorporate cells into a 3D scaffold would be desirable. A recently developed technique is interfacial polyelectrolyte complexation (IPC) [4,6].

Based on electrostatic interaction of oppositely charged polyelectrolytes, IPC can produce stable fibers under aqueous and room temperature conditions for scaffold construction [4]. Unlike many current scaffold fabrication techniques [15,21,22] which involve the use of volatile organic solvents and cytotoxic photocrosslinkers that may be detrimental to the bioactivity of biologics [23] and viability of encapsulated cells, IPC is amenable to encapsulation of proteins [4], cells [6] and DNA [24] into the fibers. Encapsulation of cells with IPC technique has additional advantages over encapsulation of cells in gels; the porous architecture allows efficient nutrient/waste exchange and in essence 3D cell patterning.

We have previously reported the construction of alginate–chitosan PEC scaffold for cell encapsulation [6]. However, weak fiber mechanical properties, poor cellular adhesion and uneven cell distribution were observed. Hypothesizing that collagen could be an attractive cation to form PEC fibers, we study the complexation of methylated collagen with a custom-synthesized anionic terpolymer to produce a hybrid fibrous scaffold that might exploit the favorable biological properties of collagen and the tunable physical properties of a synthetic polymer.

Scaffolds made of synthetic polymers are versatile with tunable physical properties, but typically lack cell recognition signals for effective cell attachment [10]. Natural polymers such as collagen, elastin and glycoaminoglycans possess biological cues for cellular interaction but often lack the appropriate mechanical properties required as structural biomaterials for tissue regeneration [8]. A hybridization of both polymer types by IPC may produce fibers with the desired complementary properties. In this study, we examined the mechanical properties, surface morphology and collagen distribution in the collagen–terpolymer PEC fibers. We also compared the proliferation and differentiation of hMSCs seeded on (hMSC-seed) and encapsulated (hMSC-encap) within the fibrous scaffolds for three weeks in proliferating medium.

2. Materials and methods

2.1. Materials

Methyl methacrylate (MMA), hydroxyethyl methacrylate (HEMA) and methacrylic acid (MAA) monomers and 2,2′-azobisisobutyronitrile (AIBN) were purchased from Sigma–Aldrich Chemicals Ltd. (Singapore). The monomers were purified by vacuum distillation and AIBN was recrystallized in ethanol before use. Collagen Type I (Nutragen, 100 mL) was purchased from Nutacon, Netherlands.

2.2. Terpolymer synthesis

The terpolymer was synthesized in 100 mL of isopropanol via radical polymerization at 60 °C. MMA, HEMA and MAA monomers were charged into a 250 mL round bottom flask at a molar ratio of 25:25:50 [25] and 0.25 wt% of AIBN was used as an initiator. The reaction mixture was cooled for 5 min in an ice bath, purged with argon for another 5 min before it was immersed in a hot silicone oil bath. The polymerization process was carried out for 16 h, after which the mixture was cooled rapidly under running water. The terpolymer was precipitated twice in 1 L of hexane and vacuum dried. The dried terpolymer was subsequently dissolved in 1 M NaOH to convert the carboxyl acid groups to sodium carboxylate, dialyzed against deionized water for 24 h or until the external reservoir reached a pH of 5.5 and the final anionic terpolymer product was recovered by lyophilization.

2.3. Collagen methylation

12 mL of collagen (6 mg/mL in 0.012 N HCl) was precipitated in 400 mL of acetone and redissolved in 200 mL of methanol containing 0.1 M HCl. The mixture was stirred for 2 days at room temperature before it was dialyzed against deionized water (Spectrapor MWCO = 3500) until the external reservoir reached a pH of 5.5. The methylated collagen was lyophilized and stored at −80 °C before use.

2.4. Terpolymer–collagen fiber formation

Terpolymer–collagen fibers were drawn out of the interface between terpolymer and methylated collagen droplets. The terpolymer and methylated collagen concentrations used were 5.5 mg/mL and 5.0 mg/mL respectively. 5 μL of terpolymer and methylated collagen droplets were placed 1 mm apart on a Petri dish and a pair of tweezers was used to bring the 2 droplets together to draw a fiber. The fiber was attached to one of the supporting rods on a motorized roller and the fiber was drawn continuously at a rate of 10 mm/s until it was terminated by the depletion of polyelectrolyte droplets. The fibers collected on the roller were air-dried before they were removed and observed under a scanning electron microscope (SEM, FEI Quanta 200F, USA).

2.5. Fiber characterization

2.5.1. Mechanical property measurement of fibers

The tensile properties of the fibers were performed according to ASTM D 3822 using a nanotensile testing system (Nano Bionix System, MTS, USA). The sample preparation for the tensile test was described previously [26]. Briefly, 10 single strands of terpolymer–collagen fiber were individually mounted onto a cardboard frame and secured with adhesive tapes at the ends before the frame was mounted on the nanotensile tester. Each fiber has a gauge length of 10 mm. The sides of the frame were snipped off before the fiber was subjected to uniaxial tensile loading at a strain rate of $3.7 \times 10^{-2}\ s^{-1}$ until they break.

2.5.2. AFM imaging

Atomic force microscope (AFM) (Dimension 3100, Digital Instruments, USA) was used to study the fiber surface morphology. Fibers were collected on a mica surface, dried in a desiccator for a day before imaging in tapping mode. Silicon nitride tips with resonance frequencies of 279–300 kHz were used for this study.

2.5.3. Quantum dot labelling of collagen

Biotinylated anti-Collagen Type I (Rabbit) antibody (Rockland Immunochemicals, Inc. USA) was added to the methylated collagen solution in a dilution ratio of 1:3000. The mixture was incubated at room temperature for 3 h and dialyzed against deionized water. Streptavidin-conjugated quantum dot 605 nm (Invitrogen, Singapore) was added at a dilution of 1:50 and incubated for 10 min in the dark. 5 μL of the quantum dot labelled methylated collagen and 5 μL of terpolymer were used to draw the fibers. The fibers were collected on glass coverslips, washed with PBS and mounted onto glass slides for confocal imaging.

2.6. hMSC culture studies

hMSCs were purchased from Cambrex (Poietics; Lonza, Switzerland), cultured and expanded in mesenchymal stem cell growth medium (MSCGM™). The cells used in the experiments were between passages 4 and 7.

2.6.1. Seeding hMSCs on fibers

Dried PEC fibers were immersed in 70% ethanol for 30 min before 3 h of UV irradiation. They were washed 3 times with sterile PBS and placed in 24 well culture plates. 100 μL of cell suspension containing of 10^4 cells hMSCs was added to the top of the fibers and after 30 min of cell seeding, fresh MSCGM was added to the culture well. The cultures were incubated at 37 °C with 5% CO_2.

2.6.2. Encapsulating hMSCs in fibers

hMSCs cultured in 25 T flasks were trypsinized and washed with PBS before adding to 100 μL of methylated collagen to make a cell suspension of 3×10^5 cells/mL. 5 μL of methylated collagen containing cells were placed on a sterile Petri dish next to 5 μL of terpolymer solution. A pair of tweezers was used to bring the 2 droplets together and draw a cell-encapsulated fiber. The fiber was collected on a glass coverslip and washed with PBS before immersing in cell culture medium immediately. This was repeated until 20 cell-encapsulated fibers were collected and the samples were transferred from the glass coverslip into a 6-well Transwell plate and incubated at 37 °C with 5% CO_2.

2.7. Cell viability and proliferation studies

The viability and proliferation of hMSCs-seed and hMSCs-encap were verified by Alamar Blue and live/dead cell assay (Molecular Probes and Sigma–Aldrich, Singapore). 40 μL of Alamar Blue solution was added to 400 μL cell culture medium and the samples were incubated for 3.5 h. The medium was removed and the fluorescence intensity readings were taken at excitation and emission wavelengths of 544 nm and 590 nm respectively. For live/dead assay, CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate) and propidium iodide (PI) were used. In brief, the cells were incubated in 300 μL of cell culture medium containing 0.02 mM CMFDA for 45 min at 37 °C, washed with PBS before incubating in 300 μL of 0.05 mg/mL PI for 5 min. The samples were washed twice in PBS and viewed under fluorescence microscope immediately.