Journal of Industrial and Engineering Chemistry 38 (2016) 113–122

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

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

Bi-compartmental 3D scaffolds for the co-culture of intervertebral disk cells and mesenchymal stem cells

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ARTICLE INFO

Article history:
Received 12 February 2016
Received in revised form 12 April 2016
Accepted 18 April 2016
Available online 25 April 2016

Keywords:
Co-culture
Intervertebral disk cells
Mesenchymal stem cells
Bi-compartmental scaffold

ABSTRACT

The combination of electrospinning and the subsequent gelation of alginate produced bi-compartmental hydrogel consisting of a nanofiber-incorporated hydrogel matrix domain and a bare alginate hydrogel domain. The co-culture system was prepared by placing intervertebral disk (IVD) cells in the bare alginate hydrogel and human bone marrow mesenchymal stem cells (hMSCs) in the nanofiber-incorporated hydrogel. Real-time polymerase chain reaction (PCR), western blotting, immunofluorescence staining, and sulfated glycosaminoglycan assays revealed that the co-cultured groups produced more collagen type II, aggrecan, glucose transporter-1 (GLUT-1), and glycosaminoglycans (GAG) than the single-cultured hMSCs, confirming the enhanced differentiation of hMSCs in the co-culture system. It is expected that our bi-compartmental 3D scaffold can be applied to heterotypic co-culture systems for the study of various cell–cell interactions.

Introduction

In native tissue, numerous homotypic and/or heterotypic cell–cell interactions typically occur through direct contacts or the exchange of soluble factors, which greatly influence cellular behavior such as survival, apoptosis, migration, proliferation, and differentiation [1,2]. A significant number of studies have been aimed at developing highly efficient cell co-culture platforms (or scaffolds) to better understand the various cell–cell interactions and mimic the cellular microenvironments where various cell types are optimally positioned relative to one another [3–6]. Most of the recent progress in co-culture platforms is based on the surface chemistry of the material in conjunction with micro-fabrication techniques that allowed the spatial patterning of multiple cell types, where the cells exist on two-dimensional (2D) flat surfaces and formed an outspread 2D monolayer [7–13]. With the emphasis on the importance and advantages of 3D culture systems over 2D cultures systems, many studies reported the use of 3D scaffoldssuch as hydrogel and nanofiber matrices, for the co-culture of different cell types [14–20]. However, most of those co-culture systems were prepared by simply mixing two or more cell types and seeding them simultaneously onto the same scaffolds, making it very difficult to localize each cell type into a specific region within the scaffold, and, therefore, precise control of the cell–cell interactions is impossible.

On the other hand, the degeneration of intervertebral disk (IVD), which is composed of a peripheral annulus fibrosus (AF) and a central nucleus pulposus (NP), is a major cause debilitating neck and/or back pain [21,22]. Although the exact pathological mechanisms are not fully understood, the decreased production of the extracellular matrix (ECM) in aging IVD cells is thought be one of the major causes of IVD degeneration [23]. In addition to the current treatments for IVD degeneration, such as palliative therapies and surgical intervention, cell-based tissue engineering approaches have received great attention for the treatment of IVD disease [24–26]. For a successful tissue engineering-based IVD therapy, sufficient amount of IVD cells as well as polymeric scaffolds are required. However, clinically, it is very difficult to quickly obtain a sufficient number of fresh cells due to the hypocellularity of the IVD [27,28]. One strategy to overcome this problem is to use mesenchymal stem cells (MSCs) that are capable of differentiating into a numberof lineages, including both

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http://dx.doi.org/10.1016/j.jiec.2016.04.013
1226-086X/

chondrocytes and NP-like cells [29–33]. Among the various methods to increase the efficacy of differentiation induction, co-culture of MSCs with IVD cells is a viable option, because the co-culture system not only enhances the MSC differentiation into IVD cells, but also significantly activates the biological properties of the NP cells such as cell proliferation, DNA synthesis and extracellular matrix (ECM) production [34–37]. Similar to other co-culture studies, most co-cultures of MSCs and IVD cells have been carried out using the 2D monolayer model [37,38], spherical pellet culture model [39,40], or a random mixture of MSCs and IVD cells within a 3D scaffold [41–45]. However, to our knowledge, there have been no reports regarding the construction of 3D scaffolds that are able to localize MSCs and IVD cells into distinct scaffold domains.

In order to develop the 3D co-culture scaffolds that allow the localization of each cell type into a specific domain, we fabricated bi-compartmental scaffolds consisting of a PCL nanofiber-incorporated hydrogel matrix and bare alginate hydrogel. In this system, the cells can be located in vertically different domains; one is within the bare hydrogel region and the other is within the nanofiber-incorporated region. A co-culture of IVD cells and hMSCs was constructed as a model system by placing the intervertebral disk (IVD) cells and hMSCs within the bare alginate hydrogel and nanofiber regions, respectively. After construction of the co-culture system, we investigated the various biological activities of the co-cultured cells, such as cell proliferation, gene expression, protein production, and GAG content.

Experimental

Materials

Polycaprolactone (PCL, MW: 80,000), calcium chloride ($CaCl_2$), 2,2,2-trifluoroethanol (TFE) and (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Sodium alginate (80–120 cP) was purchased from Wako Pure Chemical Industries (Osaka, Japan).

Preparation of electrospun PCL fibrous scaffolds

Conventional electrospinning was used to prepare the PCL nanofiber scaffolds. After PCL was dissolved in TFE to form a 20 wt% polymer solution, a 7.5 kV positive voltage was applied to the solution via an 18G metal needle and a syringe pump (NanoNC, Seoul, Korea) was used to establish a constant feeding rate in the solution (0.7 mL/h). The distance between the tip of the needle and the collecting plate was maintained at 15 cm. The electrospun fibers were collected on clean aluminum foil (connected to the ground) for different periods of time. The resulting PCL nanofibers were then treated with oxygen plasma (Femto Science Kyunggi, Korea) for 10 min prior to the hydrogel entrapping process. The radio frequency power and pressure of the plasma treatment were 40 W and 0.1 mmHg, respectively.

Fabrication of bi-compartmental scaffolds

The resulting nanofiber scaffolds were incorporated into alginate hydrogel to generate bi-compartmental scaffolds consisting of hydrogel and the nanofiber matrix. Alginate was dissolved in water to form a 3 wt% gel precursor solution. The nanofiber scaffolds were placed into 96-well plates and the alginate precursor solution was transferred to the nanofiber-containing wells. The precursor solution was crosslinked to form a hydrogel by adding a 1 wt% $CaCl_2$ solution to the alginate solution at a 2:1 volumetric ratio of alginate:$CaCl_2$. Crosslinking was performed for 15 min in a 37 °C incubator with 5% CO_2. The thickness of alginate hydrogel was controlled to be deeper than the thickness of nanofiber scaffold so that the resulting scaffolds have two distinct compartments: one is the bottom compartment consisting of the nanofiber-incorporated hydrogel matrix, and the other is upper compartment consisting of only the alginate hydrogel. The morphology of the scaffolds was observed using scanning electron microscopy (SEM) (JEOL T330A, JEOL, Ltd, Peabody, MA, USA).

Water content measurement

Water content measurements were performed on the hydrogel samples by the gravimetric method. The weighing was performed at the fully swollen and dried states. The synthesized hydrogels were first immersed in deionized water (pH 7.4) to be swollen until reaching equilibrium and were then dried overnight in a vacuum oven. The swollen weight was divided by the difference of the dried and swollen weights to obtain the water content (WC) value as

$$WC[\%] = \frac{W_h - W_d}{W_h} \cdot 100$$

where W_d is the dry weight and W_h is the swollen weight.

Mechanical test

The compression modulus of the swollen hydrogels was measured at room temperature using an Instron 5844 testing apparatus equipped with a 10 N load cell and a BioPuls bath. The sample disks were removed from PBS, and their thickness and radius were measured. The samples were placed between parallel plates and compressed at 15 mm/min until complete deformation. Load and compression measurements were collected automatically by a computer, and were used to obtain the stress–strain plot. The compression modulus was then calculated from the slope of the linear region of the resultant stress–strain plot.

Isolation of human intervertebral disk cells (IVD cells)

The intervertebral disk tissue was obtained from 15 patients (age range: 20–59 years) during surgery for lumbar spinal stenosis. The nucleus pulposus (NP) and annulus fibrosus (AF) specimens were isolated from the intervertebral disk tissue and minced with a scalpel. The tissues were then digested for 2 h at 37 °C under gentle agitation in a medium composed of equal parts of Dulbecco's Modified Eagle Medium:nutrient mixture F-12 (Ham) (1:1) (DMEM/F12, Gibco-BRL®, NY, USA) with collagenase type II (2.5 mg/ml, Sigma–Aldrich, WI, USA). The cells were filtered through a sterile nylon mesh filter (pore size: 100 μm, Falcon, MA, USA) and plated in T25 plates (Nunc™, Denmark) at a density of approximately 1.0×10^5 cells/mL. The primary cultures were sustained for two to three weeks in DMEM/F12 containing 10% fetal bovine serum (FBS, Gibco-BRL®, NY, USA), 1% v/v penicillin, streptomycin, and nystatin (Gibco-BRL®, NY, USA) in a humidified 37 °C incubator with 5% CO_2. The culture medium was changed three times per week.

Isolation of human bone marrow mesenchymal stem cells (hMSCs)

To obtain the human bone marrow mesenchymal stem cells (hMSCs), the bone marrow was collected from an iliac crest puncture during surgery for lumbar spinal stenosis, and then the hMSCs were isolated using a Ficoll-Paque (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) density gradient. All patients gave informed consent before surgery. These cells were cultured in a 100-mm flask with minimum essential medium (α-MEM) supplemented with 10% FBS, 1% (v/v) antibiotic-antimycotic,