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Macroporous and nanofibrous hyaluronic acid/collagen hybrid scaffold fabricated by concurrent electrospinning and deposition/leaching of salt particles

Taek Gyoung Kim, Hyun Jung Chung, Tae Gwan Park *

Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon 305-701, South Korea

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Abstract

A three-dimensional (3-D) macroporous and nanofibrous hyaluronic acid (HA) scaffold was fabricated by an electrospinning process combined with a salt leaching technique. HA and collagen were dissolved in a sodium hydroxide (NaOH)/*N,N*-dimethyl formamide (DMF) solvent mixture at a concentration of 10 wt.% and successfully electrospun into a nanofiber web with a soft, fluffy structure by the combined effects of numerous minijet evolutions and their subsequent vertical growth. To our knowledge, the formation of an extensive fluffy nanofiber morphology is the first time as a single route has been used to spontaneously generate a 3-D nanofibrous structure. By the simultaneous deposition of salt particulates as a porogen during electrospinning and subsequent chemical cross-linking and salt leaching, a water-swellable HA-based scaffold retaining a macroporous and nanofibrous geometry could be produced. Bovine chondrocytes were cultured on the HA/collagen scaffold to assessing the scaffold's cytocompatibility. The results revealed that cellular adhesion and proliferation were enhanced in proportion to the content of collagen, and the seeded chondrocytes maintained the roundness characteristic of a chondroblastic morphology.

Keywords: Hyaluronic acid; Electrospinning; Nanofibers; Salt leaching; Tissue engineering scaffold

1. Introduction

Nanofibrous structures have great potential as biomimicking architecture for promoting cell growth and maintaining cell functions, showing promise for a variety of biomedical applications, including artificial organs, biofunctional interfaces and other prosthetic devices [1–3]. It has been demonstrated that a three-dimensional (3-D) nanofibrous structure similar to that of naturally occurring extracellular matrix (ECM) provides better physical and mechanical microenvironmental surroundings for cell proliferation and differentiation [4]. Because of their high porosity and large surface area, nanofibrous network scaffolds can serve as cytocompatible template materials facilitating mass transport of oxygen and nutrients, which consequently improve cellular adhesion, proliferation and the ultimate formation of functional tissues. These nanostructured scaffolds for tissue engineering have been achieved by various methods, such as the phase-separation process [5], self-assembling peptides [6] and electrospinning [7]. Recently, an electrospinning technique has attracted much attention for the fabrication of polymeric nano-scale fibers. In this process, a polymer solution dissolved in a proper solvent is ejected through a nozzle by electrostatic repulsive forces and the solidified ultrafine fibers are deposited on a grounded metal collector in various shapes [8]. The resultant structure is a randomly oriented micro- or nanofiber network mesh with a highly open porous architecture. This structure resembles nanofibrous collagen networks embedded in proteoglycans, as observed in the natural ECM. Collagen fibers provide high tensile strength,

* Corresponding author. Tel.: +82 42 869 2621; fax: +82 42 869 2610.
E-mail address: tgpark@kaist.ac.kr (T.G. Park).

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and proteoglycan hydrogels provide resistance to compressive force. Both of these are major components of ECMs with a nano-dimensional hierarchical structure [9,10]. A wide range of biodegradable synthetic polymers (e.g. poly(L-lactic acid) (PLLA), polyglycolide (PGA), poly(D,L-lactic-co-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL) and natural biopolymers (e.g. collagen, silk fibroin, chitosan, alginate, hyaluronic acid) have therefore been electrospun for potential use as temporal biomimicking scaffolds for the regeneration of damaged or dysfunctional organs [8,11].

To design an ideal scaffold, a highly open porous structure with well interconnected pores is required, not only to achieve sufficient cell seeding density within the scaffold, but also to facilitate the free transport of nutrients and oxygen for subsequent cell proliferation and differentiation [12]. However, in most electrospun scaffolds, the smaller the fiber diameter becomes, the more densely the fibers are packed, resulting in interconnected pore cavities and narrow channels that are too small for the seeded cells to migrate and reside inside. In addition, thickening of the fibrous mat during electrospinning is inherently difficult and time-consuming. Since the metal collector becomes electrically insulated by deposited electrospun polymeric fibers, the resultant electrospun fibrous mat has a thin and spread structural morphology, limiting the fabrication and application of actual 3-D electrospun nanofibrous scaffolds. We previously reported electrospun PLGA nanofibers modified with cell adhesive Gly-Arg-Gly-Asp-Tyr (GRGDY) peptide for mimicking biophysical properties of the natural ECM, offering a cell-compatible fibrillar architecture for tissue engineering applications [13]. Cell attachment, spreading and proliferation were greatly enhanced with these nanofiber mats. However, as mentioned above, the non-woven structure of the mat composed of nanofiber aggregates had intrinsically much smaller pore openings than the cellular dimensions, which made it extremely difficult for the seeded cells to penetrate inside and form a 3-D cellular construct.

From this viewpoint, we developed 3-D macroporous and nanofibrous HA-based scaffolds for tissue engineering applications. First, HA/collagen blend was successfully electrospun by using a mixed solvent of sodium hydroxide (NaOH)/*N,N*-dimethyl formamide (DMF) while reducing the air gap distance between the nozzle and the collector. Subsequently, we developed a novel soft, fluffy structure of a nanofiber web inflating upward, caused by numerous minijet evolutions and their vertical growth. Because this structure was fragile and insufficient in pore size, sodium chloride salt particulates as a porogen were simultaneously deposited by a sieving method controlled by automatic vibration during electrospinning. Through chemical cross-linking and subsequent salt leaching, we could finally obtain water-swellable HA-based scaffolds that retained a macroporous and nanofibrous geometry. For in vitro assessment, bovine chondrocytes were chosen as a cell model for cellular adhesion and proliferation on HA-only and HA/collagen macroporous and nanofibrous scaffolds.

2. Materials and methods

2.1. Materials

Sodium hyaluronate (HA) (mol. wt. 680 K) was obtained from Lifecore Biomedical (Chaska, MN). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and collagen type I (from calf skin) were from Sigma (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM) and phosphate-buffered saline (PBS) were purchased from Gibco BRL (Gaithersburg, MD). Cell counting kit-8 (CCK-8) was obtained from Dojindo Laboratories (Kumamoto, Japan). Rhodamine phalloidin was purchased from Molecular Probes Inc. (Eugene, OR). All other chemicals and reagents were of analytical grade.

2.2. Methods

2.2.1. Electrospinning and deposition of salt particulates

HA and collagen were dissolved in a mixed solvent of 0.5 N NaOH and DMF with various volume ratios and different blend ratios to optimize the electrospinning conditions. A blend mixture of HA and collagen (95/5 and 80/20 weight ratio, 100 mg) was dissolved in NaOH/DMF (4/1 v/v) at a concentration of 10 wt.%. The HA/collagen solution was added into a 3 ml syringe with a metal blunt end needle (23G) and then mounted in a programmable syringe pump (model 210, KD Scientific Inc., USA) operated at 10 μl min^{-1}. The mounted syringe was tilted 30° to the horizontal plane to facilitate fiber formation by gravitation. The positive lead from a high-voltage power generator (CPS-40 K03VIT, Chungpa EMT Co., Korea) was connected to the needle tip and a DC voltage of 16 kV was applied. Electrospinning was performed at ambient temperature. Split electrified jets were deposited on a flat copper plate wrapped in aluminum foil which was also tilted at a right angle to the axis of the needle. While electrospinning, sieved NaCl salt particulates (salt particle size was 100–200 μm) were uniformly dropped into the deposited electrospun fibers at a rate of ca. 10 mg min^{-1} cm^{-2} by using a 200 μm mesh attached to a vibrating orbital shaker (IKA, model VIBRAX, Germany). The resultant nanofibers/salt composite was placed under reduced pressure to remove the residual solvent for 1 day. The morphological observation of electrospun fibers was carried out with a scanning electron microscope (SEM, Philips 535M, Netherlands) after sputter-coating with Au particles. For mechanical characterization, nanofibers were collected without the deposition of salt particulates on a mandrel-type collector rotating at 100 mm s^{-1}. The tensile test using a universal testing machine (Instron 5583) was performed on a 10 × 50 mm rectangular specimen (gauge

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