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Bioactive cell-derived matrices combined with polymer mesh scaffold for osteogenesis and bone healing

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ABSTRACT

Successful bone tissue engineering generally requires an osteoconductive scaffold that consists of extracellular matrix (ECM) to mimic the natural environment. In this study, we developed a PLGA/PLA-based mesh scaffold coated with cell-derived extracellular matrix (CDM) for the delivery of bone morphogenic protein (BMP-2), and assessed the capacity of this system to provide an osteogenic microenvironment. Decellularized ECM from human lung fibroblasts (hFDM) was coated onto the surface of the polymer mesh scaffolds, upon which heparin was then conjugated onto hFDM via EDC chemistry. BMP-2 was subsequently immobilized onto the mesh scaffolds via heparin, and released at a controlled rate. Human placenta-derived mesenchymal stem cells (hPMSCs) were cultured in such scaffolds and subjected to osteogenic differentiation for 28 days *in vitro*. The results showed that alkaline phosphatase (ALP) activity, mineralization, and osteogenic marker expression were significantly improved with hPMSCs cultured in the hFDM-coated mesh scaffolds compared to the control and fibronectin-coated ones. In addition, a mouse ectopic and rat calvarial bone defect model was used to examine the feasibility of current platform to induce osteogenesis as well as bone regeneration. All hFDM-coated mesh groups exhibited a significant increase of newly formed bone and in particular, hFDM-coated mesh scaffold loaded with a high dose of BMP-2 exhibited a nearly complete bone defect healing as confirmed via micro-CT and histological observation. This work proposes a great potency of using hFDM (biophysical) coupled with BMP-2 (biochemical) as a promising osteogenic microenvironment for bone tissue engineering applications.

1. Introduction

The extracellular matrix (ECM) confers a microenvironment instructive for the regulation of cellular behavior and function [1,2] Equally important, the ECM is a rich source of growth factors and other signaling molecules, providing adhesion sites as well as structural conduits for the interaction of cells with the neighboring matrix [3,4]. Specifically, the ECM in bone matrices plays a critical role in the maintenance and remodeling of bone via the storage and presentation of bioactive molecules [5–7]. Not surprisingly, current bone tissue engineering strategies center on the generation and modulation of an osteoconductive environment that mimics natural ECM. For instance, the use of decellularized ECM from animal tissues as an osteoconductive matrix has been investigated for bone regeneration [8–10]. Such tissue-derived ECM scaffolds, however, present difficulties due to the physical form of the original tissue prior to decellularization and the immunologic incompatibility with the host tissues. As an alternative, ECM obtained from *in vitro*-cultured cells is emerging as a promising biological 2D and 3D platforms for various tissue engineering fields [11,12].

For instance, various cell-derived extracellular matrices (CDM) have been used to promote osteogenic differentiation of both preosteoblasts and bone marrow mesenchymal stromal cells alike

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through their respective cellular microenvironments [13]. Thibault et al. for instance, investigated the use of an osteogenic ECM construct, composed of decellularized mesenchymal stem cells and an electrospun poly(ε-caprolactone) fiber mesh scaffold [14]. Similarly, in a study by Holtorf et al. ECM derived from rat marrow stromal cells was combined with a titanium fiber mesh scaffold and used to demonstrate the osteogenic differentiation of mesenchymal stem cells (MSCs) [15]. Indeed, while the use of CDM yields greatly advanced effects on cell proliferation and differentiation, mechanical weakness of the matrices and lack of a structural support prevent their widespread applications for bone tissue engineering. Most studies focusing on mimicking the ECM microenvironment therefore utilize supportive synthetic biomaterials such as hydroxyapatite, β-tricalcium phosphate, synthetic polymers, denatured collagen, and hydrogels [5,12,16–18]. Polymeric fibrous mesh scaffolds, in particular, provide an optimal level of porosity as well as an improved advantage for cellular migration [19,20].

However, the sub-par efficiency of such osteoconductive matrices *in vivo* has redirected the attention towards the incorporation of a stimulating factor – such as BMP-2 for osteogenesis – into the matrices, ultimately underscoring the importance of an optimal bioactive protein carrier. The field of growth factor delivery has been intensively investigated, yielding a large number of delivery systems [21–23]. Few studies, however, examine the use of cell-derived ECM in combination with an engineered 3D scaffold for a sustained release of growth factor. From this perspective, we have developed a novel platform, composed of a biodegradable PLGA/PLA mesh scaffold, functionalized with bioactive human lung fibroblast-derived matrix (hFDM). Further bioactivity is conferred to the system through the conjugation of BMP-2. We have assessed the current platform to provide an osteogenic microenvironment both *in vitro* and *in vivo* (ectopic and calvarial defect models), and in essence demonstrate the potency of cell-derived matrices on two fronts: 1) as a biomimetic biophysical template that allows for the attachment of human placenta-derived MSCs (hPMSCs), in turn providing a suitable microenvironment for osteogenic differentiation, and 2) as a biochemical reservoir from which growth factors, such as BMP-2, can be tethered and released. In our study, we demonstrate a significant improvement in the osteogenesis of hPMSCs and nearly complete calvarial bone defect healing using cell-derived matrix-based mesh scaffolds.

2. Materials and methods

2.1. Materials

Poly(L-lactide-co-glycolide) (PLGA; lactic to glycolic acid molar ratio, 50:50) and poly(L-lactide) (PLA) was purchased from Boehringer Ingelheim (Ingelheim, Germany). Heparin sodium and bone morphogenetic protein 2 (BMP-2) were purchased from Acros Organics Inc. and R&D Systems (USA), respectively. 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), N-hydroxysuccinimide (NHS), and 4-morpholineethanesulfonic acid (MES) were supplied from Aldrich–Sigma (St. Louis, MO, USA). All other chemicals were analytically graded and were used as received. For cell experiments, Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), trypsin/EDTA, penicillin/streptomycin (P/S), and phosphate buffered saline (PBS, pH 7.4) were purchased from Gibco BRL (Carlsbad, CA, USA). Cell Counting kit-8 (CCK-8) was purchased from Dojindo Co. Ltd (Kumamoto, Japan). Alkaline phosphatase (ALP) LabAssay kit was purchased from Wako Chemicals Inc. (USA). PKH26 red-fluorescent cell linker mini kit was obtained from Aldrich–Sigma. Balb/c nude mice and Sprague dawley (SD) rats were supplied from Nara Biotech (Seoul, Korea).

2.2. Preparation of PLGA/PLA mesh scaffolds

PLGA and PLA fibers, 2–2.5 mm in length, were prepared by using a rotary cutter and their nonwovens were produced via modified wet-laid process [24]. PLGA and PLA fibers were mixed in an aqueous solution with a dispersing agent (1 wt.% pluronic F127; Sigma–Aldrich) and randomly laid on a wire mesh to filter the liquid. The formed web was subsequently processed through a thermal bonding, in which the web was transferred to a heater and cured at 170 °C for 5 min (Fig. 1A). The resulting mesh was cut into sheets (5 × 5 × 3 mm, L × W × H) and they were sterilized by soaking in 70% ethanol under ultraviolet (UV) light.

2.3. Fabrication of hFDM-coated mesh scaffolds

hFDM was obtained from *in vitro*-cultured WI-38 human lung fibroblasts as previously described [13,25]. After the decellularization process, hFDM was harvested by gently scraping with a cell scraper, transferred to 50 mL tubes, and vigorously agitated using a homogenizer (20,000 rpm; HG-3000, SMT, Japan) until a

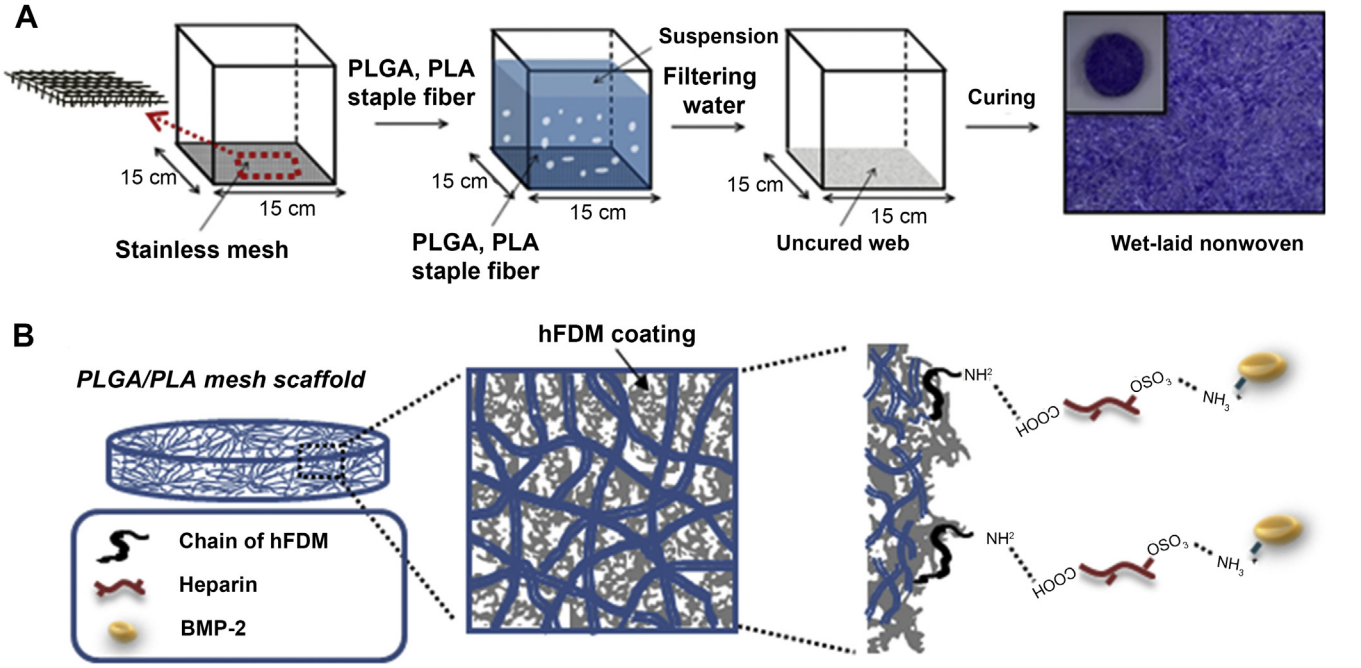

Fig. 1. Schematic Illustration of PLGA/PLA Mesh Scaffold Fabrication and Subsequent Immobilization of BMP-2. (A) An aqueous suspension of PLGA and PLA fibers is filtered onto a stainless mesh and thermally cured at 170 °C to yield PLGA/PLA mesh scaffolds. (B) hFDM-coated PLGA/PLA mesh scaffolds are functionalized with heparin via EDC chemistry, onto which BMP-2 is immobilized.