Biomaterials 32 (2011) 2489–2499

Contents lists available at ScienceDirect

Biomaterials

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

Autologous extracellular matrix scaffolds for tissue engineering

Hongxu Lu [a,b], Takashi Hoshiba [a], Naoki Kawazoe [a,c], Guoping Chen [a,b,c,*]

[a] Biomaterials Center, National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan
[b] Graduate School of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8577, Japan
[c] International Center for Materials Nanoarchitectonics (MANA), National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan

ARTICLE INFO

Article history:
Received 22 November 2010
Accepted 10 December 2010
Available online 5 January 2011

Keywords:
Autologous extracellular matrix scaffold
Biomimetics
Tissue engineering
Decellularization

ABSTRACT

Development of autologous scaffolds has been highly desired for implantation without eliciting adverse inflammatory and immune responses. However, it has been difficult to obtain autologous scaffolds by tissue decellularization because of the restricted availability of autologous donor tissues from a patient. Here we report a method to prepare autologous extracellular matrix (aECM) scaffolds by combining culture of autologous cells in a three-dimensional template, decellularization, and template removal. The aECM scaffolds showed excellent biocompatibility when implanted. We anticipate that "Full Autologous Tissue Engineering" can be realized to minimize undesirable host tissue responses by culturing the patient's own cells in an aECM scaffold.

1. Introduction

Tissue engineering and regenerative medicine have been rapidly developed as an encouraging alternative practice to restore or replace lost or malfunctioning tissues and organs through the use of cells and biomaterial scaffolds [1–3]. In a common approach, the cells isolated from a patient are cultured in a biocompatible three-dimensional (3D) porous scaffold supplemented with growth factors to regenerate new tissues or organs [4,5]. The scaffold provides necessary interim support for cell adhesion, proliferation, and phenotypic differentiation; offers biochemical and biophysical cues to modulate the neo-tissue formation by mimicking the functional and structural characteristics of the native ECM [6,7], which play a crucial role in controlling and regulating cell behavior and function [8,9].

Thus far, scaffolds have been developed from synthetic biodegradable polymers such as polylactide (PLA), polyglycolide (PGA), and poly (lactide-co-glycolide) (PLGA) [10]; natural polymers such as collagen, chondroitin, and hyaluronic acid [10,11]; and acellular matrices derived from decellularized tissues and organs [12–15]. However, the synthetic polymers are limited by their biological inertness [16] and the acidic moieties, residual catalysts, and microscale particulates that accompany degradation [17]. On the other hand, although the naturally derived polymer and acellular matrices can provide abundant biological signals and degrade into physiologically tolerable compounds [18], they are exhaustively xenogeneic or allogeneic. This situation adds potential risks of pathogen transmission [19,20], and provocation of undesirable inflammatory and immunological reaction, leading to unfulfilled results from the regenerated tissues and organs [21–26].

To avoid these problems, the autologous extracellular matrix (aECM) scaffold should be a safe and reliable biomaterial candidate [27]. The development of aECM scaffolds has been strongly anticipated for use in tissue engineering and regenerative medicine [28,29]. The use of both autologous cells and autologous scaffolds would eliminate negative host responses and lead to optimal tissue regeneration. However, the availability of autologous sources of donor tissues and organs is highly limited. It has been almost impossible to use such acellular autologous matrices for tissue engineering. The ECM secreted by autologous cells would be a potential alternate to acellular autologous tissues and organs because some autologous cells can be expanded *in vitro* and maintained under a pathogen-free condition.

In this study, we developed a method of preparing ECM scaffolds by the 3D culture of cells in a selectively removable template (Fig. 1). The intracellular components and the biodegradable polymer template were selectively removed after cell culture to obtain the ECM scaffolds. To confirm the effectiveness of this method, three cell types, human bone marrow mesenchymal stem cells (MSCs), normal human articular chondrocytes (NHAC), and normal human dermal fibroblasts (NHDF) were used to prepare their respective ECM scaffolds. If autologous cells were used, the method

* Corresponding author. Biomaterials Center, National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan. Tel.: +81 29 860 4496; fax: +81 29 860 4714.
E-mail addresses: guoping.chen@nims.go.jp, guoping-chen@aist.go.jp (G. Chen).

0142-9612/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2010.12.016

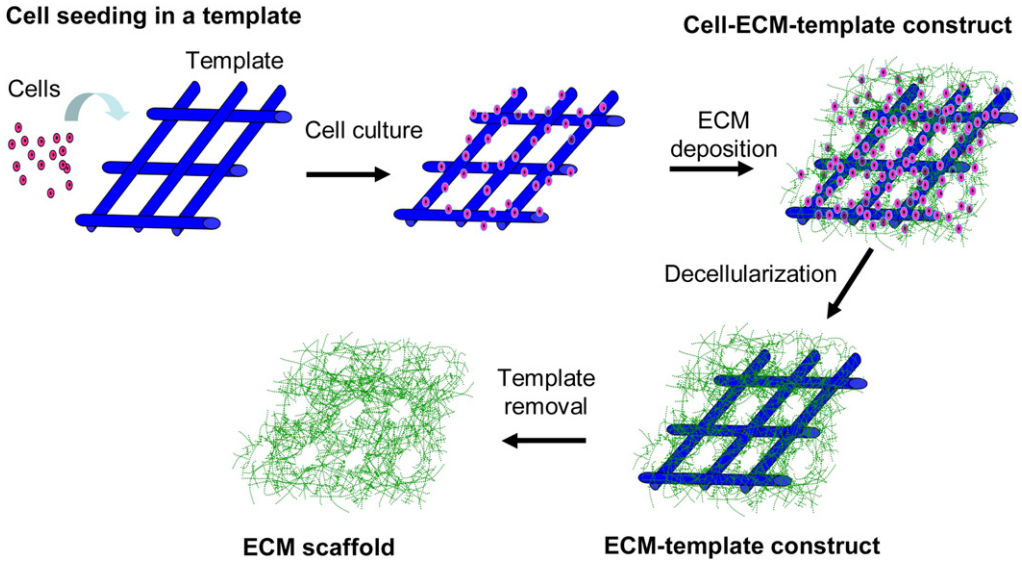

Fig. 1. Preparation scheme for ECM scaffolds.

could be used to prepare aECM scaffolds. To demonstrate the biocompatibility of the aECM scaffolds, we implanted mouse fibroblast-derived ECM scaffolds (ECM-mF) into ICR (Clrj:CD1) mice to evaluate the host tissue responses.

2. Materials and methods

2.1. ECM scaffold fabrication and characterization

MSC and NHAC, both at passage 2, were obtained from Lonza (Walkersville, MD). NHDF (derived from neonatal foreskin) were purchased from Cascade Biologics (Invitrogen, Portland, OR). The cells were seeded in 75 cm^2 tissue culture flasks and cultured using their respective proliferation media under an atmosphere of 5% CO_2 at 37 °C. MSC were cultured in MSC basal medium with MSC growth supplement; NHAC were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum; NHDF were cultured in Medium 106 with low serum growth supplement. The details were listed in Supplementary Tables 1 and 2. The cells were further subcultured thrice after confluence. Cells at passage 5 were collected by treatment with trypsin/EDTA solution and re-suspended in the medium for scaffold fabrication.

A knitted PLGA mesh (Vicryl polyglactin 910, Johnson & Johnson, Somerville, NJ) was used as a template to fabricate the ECM scaffolds. The mesh was cut into small discs with a diameter of 10.4 mm. Cells at passage 5 were seeded in discs of knitted PLGA mesh to form cell-PLGA constructs. The cell densities of MSC, NHAC, and NHDF were 3×10^5, 5×10^5, and 5×10^5 cells/ml, respectively; 200 μl cell suspension was seeded on one side of each PLGA disc. Glass rings (inner Ø = 10 mm, outer Ø = 12 mm) were covered on PLGA discs to prevent cell leakage during cell seeding. After culture for 6 h, the cell-seeded PLGA mesh discs were turned over and the other sides of the mesh discs were also seeded with the same number of cells. The cell-ECM-PLGA constructs were formed by culturing cell-PLGA constructs in DMEM supplemented with 10% fetal bovine serum (FBS), 4 mM L-glutamine, 0.4 mM L-proline, 0.1 mM MEM non-essential amino acid, 1 mM sodium pyruvate, 100 U/mL penicillin, and 100 μg/mL streptomycin. Ascorbic acid (50 μg/ml) and ascorbate-2-phosphate (150 μg/ml) (Wako, Tokyo, Japan) were added into the culture medium to stimulate ECM deposition. A mixture of ascorbic acid and ascorbic acid phosphate provides a constant concentration of ascorbate in the culture medium, and has been proved nontoxic to cells and stimulated cell proliferation and ECM deposition [30,31].

After being cultured for 5 days (MSC) or 6 days (NHAC and NHDF), the cell-ECM-PLGA constructs were washed with phosphate buffered saline (PBS) and MilliQ water (Millipore, Bedford, MA). Freeze-thaw cycling plus NH_4OH aqueous solution treatment was used for the decellularization. The cell-PLGA constructs were frozen at −80 °C in MilliQ water for 3 h, thawed at room temperature, and washed with MilliQ water. The freeze-thaw cycles were repeated 6 times. The constructs were then immersed in an aqueous solution of ammonia (25 mM) for 20 min on a slowly moving four-way shaker. After the ammonia solution treatment, the constructs were washed with MilliQ water 6 times. The constructs were immersed in a 0.5 M aqueous solution of trisodium phosphate and incubated at 37 °C for 48 h to remove the PLGA template. The ECM scaffolds were obtained after being washed with MilliQ water. Finally, the ECM scaffolds were freeze-dried for 24 h on an EYELA FDU-2200 freeze-drier (Tokyo Rikakikai, Tokyo, Japan) and sterilized with ethylene oxide gas (EOG) using an Eogelk SA-1000 sterilizer (Elk Corp., Osaka, Japan) for cell culture use.

2.2. Characterization of ECM scaffolds

The ECM scaffolds were coated with Pt and their porous structures were observed by a scanning electron microscope (SEM) (JSM-5610; JEOL, Tokyo, Japan). The removal of PLGA was examined by ATR-FTIR on an IR Prestige-1 FTIR spectrophotometer (Shimadzu, Kyoto, Japan). To evaluate the removal of cellular components after decellularization, the ECM scaffolds were stained with Hoechst 33258 (for cell nuclei), Alexa Fluro 488-labeled phalloidin (for F-actin), and DiI (for cell membrane). The cell-ECM constructs without decellularization and PLGA were also stained as controls. The fluorescent staining was observed under an Olympus BX51 fluorescence microscope with a DP-70 CCD camera (Olympus, Tokyo, Japan). The images were manipulated by DP controller software.

Immunofluorescence staining was performed to confirm the existence of ECM components in the scaffolds, including type I collagen, type II collagen, type III collagen, fibronectin, vitronectin, laminin, aggrecan, versican, decorin, and biglycan. The antibodies are listed in Supplementary Table 3. Briefly, the ECM scaffolds were incubated with 2% BSA/PBS for 30 min for blocking. The ECM scaffolds were sequentially incubated with the first antibodies for 1 h and second antibodies for 40 min at 37 °C in dark. Then the ECM scaffolds were mounted with Vectashield® mounting medium (Vector, Burlingame, CA) and observed.

For DNA and GAG analysis, the ECM scaffolds were digested with papain solution at 60 °C for 6 h. The cell-ECM-template constructs before decellularization and template removal were digested in papain solution as controls. Papain (Sigma, St. Louis, MO) was dissolved at 400 μg/ml in 0.1 M phosphate buffer (pH 6.0), with 5 mM cysteine hydrochloride, and 5 mM EDTA. The lysates were used for detection of the DNA and GAG amount. A Hoechst 33258-based DNA quantification kit (Sigma) was used to measure the DNA. Five μL lysate was added to 2 ml Hoechst 33258 solution and the fluorescence intensity was recorded by a FP-6500 spectrofluorometer (JASCO, Tokyo, Japan). The excitation wavelength was set at 360 nm and the emission wavelength was set at 460 nm. The DNA amount was calculated based on a standard curve obtained with the standard DNA supplied with the kit ($n = 3$). The sulfated glycosaminoglycan (GAG) amounts were measured by a Blyscan™ sGAG assay kit (Biocolor, Newtonabbey, UK) according to the manufacturer's manual. The specimen lysate was mixed with Blyscan™ dye to bind the GAG. The GAG-dye complex was then collected by centrifugation. After the supernatant was removed and the tube drained, the dissociation reagent was added. Then 200 μl solution was transferred into a 96-well plate. Absorbance against the background control was obtained at a wavelength of 656 nm on a Benchmark Plus™ microplate spectrophotometer (Bio-Rad, Tokyo, Japan) and the GAG amount ($n = 3$) was calculated based on a standard curve obtained with the standard GAG supplied with the kit.

Collagen contents in the ECM scaffolds were determined using the Sircol collagen assay (Biocolor) according to the manufacturer's instructions. In brief, ECM scaffolds were incubated for 48 h at 48 °C in 0.5 M acetic acid containing 0.1 mg/ml pepsin. The samples were added to Sircol dye reagent, and collagen-dye complexes formed and precipitated out from the soluble unbound dye. After centrifugation, the pellet was washed once with Acid-Salt Wash Reagent and suspended in alkali