Available online at www.sciencedirect.com
ScienceDirect
Acta Biomaterialia 5 (2009) 462–469
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Evaluations of osteogenic and osteoconductive properties of a non-woven silica gel fabric made by the electrospinning method

Young-Mi Kang [a], Kyoung-Hwa Kim [b], Yang-Jo Seol [b], Sang-Hoon Rhee [a,*]

[a] *Department of Dental Biomaterials Science and Dental Research Institute, School of Dentistry, Seoul National University, Yeongeon 28, Jongno, Seoul 110-749, Republic of Korea*

[b] *Department of Periodontology and Dental Research Institute, School of Dentistry, Seoul National University, Yeongeon 28, Jongno, Seoul 110-749, Republic of Korea*

Received 28 January 2008; received in revised form 19 May 2008; accepted 2 July 2008
Available online 17 July 2008

Abstract

Evaluations of the osteoblast-like cell responses and osteoconductivity of a non-woven silica gel fabric were carried out to determine its potential for application as a scaffold material for use in bone tissue engineering. The silica gel solution was prepared by condensation following hydrolysis of tetraethyl orthosilicate under acidic conditions. The solution was spun under a 2 kV cm^{-1} electric field. The diameters of the as-spun silica gel fibers were in the range of approximately 0.7–6 μm. The fabric was then heat-treated at 300 °C for 3 h. The proliferation of pre-osteoblastic MC3T3-E1 cells evaluated by the MTS assay was lower than on the tissue culture plate (TCP) as many cells leaked through the large voids formed by the randomly placed long, narrow silica gel fibers, which further retarded cell growth. However, the expressions of extracellular signal-regulated kinase and transcriptional factor from the cells were higher when cultured on the non-woven silica gel fabrics than on TCP. The alkaline phosphatase (ALP) activity and differentiation marker expressions assessed by amplication via the reverse transcription-polymerase chain reaction, such as type I collagen, ALP and osteocalcin, were higher for cells cultured on non-woven silica gel fabrics than on TCP. The non-woven silica gel fabric showed good osteoconductivity in the calvarial defect New Zealand white rabbit model. To this end, the non-woven silica gel fabric has good potential as a scaffold material for bone tissue engineering due to its good biological properties.

Keywords: Silica gel; Electrospinning; Non-woven fabric; Differentiation; Osteoconductivity

1. Introduction

One of the main issues when developing bone grafting materials or scaffold materials for bone tissue engineering applications has been the production of three-dimensionally (3-D) interconnected pore structures that allow for new angiogenesis to occur in vivo [1]. The replica method has been used most often for making porous ceramics; however, it is difficult to achieve good interconnectivity between pores, increase porosity, control pore size or have sufficient strength for handling. Recently, the electrospinning method has been reported to be a useful technique for producing interconnected pore structures in polymers and even ceramics. Several non-woven polymer fabrics have been examined extensively to assess their suitability for biomedical applications, and their advantages include high porosity and extracellular matrix (ECM) mimicking features [2], because the ECM milieu surrounding cells has physical and structural features in the nanometer scale and may affect several aspects of cell behavior, such as morphology, adhesion and cytoskeletal arrangements [3–8].

A number of attempts have also been made to apply electrospun non-woven polymer fabrics as scaffold materials for bone tissue engineering [3,9–13]. To be successful in bone tissue engineering applications, non-woven fabrics need to have good bone-like cell responses and

* Corresponding author. Tel.: +82 2 740 8696; fax: +82 2 740 8694.
E-mail address: rhee1213@snu.ac.kr (S.-H. Rhee).

1742-7061/$ - see front matter © 2008 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2008.07.004

osteoconductivity. In fact, electrospun non-woven polymer fabrics have exhibited better support for the attachment, proliferation and differentiation of bone-like cells [9–11,13] and mesenchymal stem cells [3,12] than tissue culture plates and solution-cast polymer films because their morphological architectures are similar to those of natural ECM, and are suitable for nutrient and metabolic waste exchange [3].

Ceramic non-woven fabrics made using the electrospinning method have been also reported [14–16], but extensive bone-like cell works and the osteoconductivity test have not yet been carried out. The reported diameters of the ceramic fibers were generally thicker than those of polymers and their physical properties were also different from polymers, so different cell responses are expected.

The purpose of this study was to evaluate the pre-osteoblastic cell responses and osteoconductivity of an electrospun, 3-D non-woven silica gel fabric to determine its potential for application as a scaffold material for bone tissue engineering. Comparisons were also made with the pre-osteoblastic cells that were cultured on tissue culture plates.

2. Materials and methods

2.1. Preparation of specimens

Tetraethyl orthosilicate (Aldrich), the silica precursor, was hydrolyzed and condensed through reaction with water, ethanol and HCl (at a molar ratio of 1:4:1:0.02, respectively) at 60 °C for 3 h. Following the reaction, the solution was transferred to a syringe (spinneret) that was connected to a high-voltage supply, generating a high electric field between the spinneret and a ground-collecting drum covered with aluminum foil. The silica gel fibers were spun under an electric field of 2 kV cm^{-1}. After spinning, the non-woven silica gel fabrics were dried for at least 24 h under ambient conditions. The fabric was then heat-treated at 300 °C for 3 h at a heating rate of 2 °C min^{-1}. The as-heat-treated, non-woven silica gel fabric is referred to as NSF. The microstructure of the specimen NSF was observed by field emission scanning electron microscopy (FE-SEM; S-4700, Hitachi).

2.2. Cell testing

2.2.1. Cell proliferation

For the proliferation assay, mouse calvaria-derived pre-osteoblastic MC3T3-E1 cells (CRL-2593™, ATCC®) [17] were seeded at a density of 10^5 cells $well^{-1}$ on NSF, which was 11.5 mm in diameter, in 48-well plates and cultured at 37 °C with 5% CO_2 for various periods of time (1, 3, and 7 days). The tissue culture plate (TCP) was used as a control. α-Minimum essential medium (α-MEM; Fresh Media™, Welgene) containing 10% fetal bovine serum (FBS; Gold Serum™, Welgene), 100 μg ml^{-1} penicillin (Gibco) and 100 μg ml^{-1} streptomycin (Gibco) was used as the culture medium.

The number of viable cells was measured using the MTS assay (CellTiter 96® AQ_{ueous} One Solution Reagent, Promega). Briefly, the cells were washed twice with phosphate-buffered saline (PBS), then 30 μl of MTS solution was added to each well containing 200 μl of PBS. The cells were incubated at 37 °C with 5% CO_2 for 1 h. Absorbance was measured at 490 nm with an ELISA microplate reader (Model 550, Bio-Rad).

2.2.2. Cell morphology

For FE-SEM observations, cells grown on TCP and NSF were fixed in a 2.5% glutaraldehyde solution and rinsed three times with 0.2 M sodium cacodylate buffer (pH 7.4). The specimens were post-fixed with 1% osmium tetraoxide and dehydrated in a graduated series of alcohol solutions and amyl acetate before critical-point drying. After coating with gold, the microstructure was observed by FE-SEM.

For confocal laser scanning microscopy (FluoView-300, Olympus) observations, cells were fixed in 10% formalin, the nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI, Vector) and the actin filament was stained with rhodamine phalloidin (Molecular Probes) after culturing 10^5 cells $well^{-1}$ for 1.5 h. Confocal laser scanning microscopy was applied to observe cell morphology and the cytoskeletal arrangement.

2.2.3. Western blotting

Cells cultured on TCP and NSF for 24 h, 24 h 10 min, and 24 h 20 min at a density of 10^6 cells $well^{-1}$ were lysed in lysis buffer (0.1% Triton X-100 in PBS), fractionated by 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride (Pierce) membrane by electroblotting. Antibodies against mouse ERK-1/2 and ERK-1/2 phosphorylation (p44/42 and phosphor-p44/42 MAP kinase antibody, Cell Signaling Technology) were added at concentrations suggested by the manufacturer. Proteins were visualized with horseradish peroxidase-conjugated anti-mouse immunoglobulin (R&D systems) and an enhanced chemiluminescence detection system (West-Zol® plus, Intron Biotechnology).

2.2.4. Alkaline phosphatase activity assay

For the alkaline phosphatase (ALP) activity assay, the cells were seeded on TCP and NSF at a density of 10^6 cells $well^{-1}$ within 48-well plates. α-MEM containing 10% FBS, 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin, 100 nM dexamethasone (Sigma), 50 μg ml^{-1} ascorbic acid (Sigma), and 10 mM β-glycerol phosphate (Sigma) was used as an osteogenic culture medium.

The differentiation of pre-osteoblastic MC3T3-E1 cells was evaluated by the expression of ALP activity after 7 days of culture. The cultured cell layer was rinsed twice with Hanks' balanced salt solution (Cellgro®, Mediatech) and ALP buffer was added to lyse the cells. Estimation of the protein content was performed using the Bradford assay method (Bio-Rad protein assay kit, Bio-Rad) [18].

ID	Title	Pages
2386	Evaluations of osteogenic and osteoconductive properties of a non-woven silica gel fabric made by the electrospinning method	8