DENTAL MATERIALS 32 (2016) 1301–1311

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/dema

Magnetic nanofiber scaffold-induced stimulation of odontogenesis and pro-angiogenesis of human dental pulp cells through Wnt/MAPK/NF-κB pathways

Hyung-Mun Yun[a,1], *Soo-Kyung Kang*[b,1], *Rajendra K. Singh*[e], *Jung-Hwan Lee*[e], *Hae-Hyoung Lee*[e,f], *Kyung-Ran Park*[a], *Jin-Kyu Yi*[c], *Deok-Won Lee*[d], *Hae-Won Kim*[e,**], *Eun-Cheol Kim*[a,*]

[a] *Department of Oral and Maxillofacial Pathology, School of Dentistry and Research Center for Tooth & Periodontal Regeneration (MRC), Kyung Hee University, Seoul 130-701, Republic of Korea*
[b] *Department of Conservative Dentistry, Kyung Hee University, Seoul, Republic of Korea*
[c] *Department of Oral Medicine, School of Dentistry, Kyung Hee University, Seoul, Republic of Korea*
[d] *Department of Oral and Maxillofacial Surgery, School of Dentistry, Kyung Hee University, Seoul, Republic of Korea*
[e] *Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Cheonan, Republic of Korea*
[f] *Department of Biomaterials Science, College of Dentistry, Dankook University, Cheonan, Republic of Korea*

ARTICLE INFO

Article history:
Received 18 November 2015
Received in revised form 7 May 2016
Accepted 22 June 2016

Keywords:
Magnetic scaffolds
Cell growth
Odontogenic differentiation
Pro-angiogenesis
Dental pulp cells

ABSTRACT

Objective. Magnetic biomaterials have recently gained great attention due to their some intriguing cell and tissue responses. However, little attention has been given to the fields of dental tissue regeneration. In this sense, we aim to investigate the effects of magnetic nanofiber scaffolds on the human dental pulp cell (HDPC) behaviors and to elucidate the underlying signaling mechanisms in the events.

Methods. Magnetic nanofiber scaffolds incorporating magnetic nanoparticles at varying contents were prepared into nanofibrous matrices to cultivate cells. Cell growth by MTS assay, odontoblastic differentiation by alkaline phosphatase (ALP) activity, mineralization, and the mRNA expression of differentiation-related genes of HDPCs, *in vitro* angiogenesis by migration and capillary tube formation in endothelial cells on the conditioned medium obtained from HDPSCs in the presence or absence of scaffolds. Western blot analysis and confocal immunofluorescene were used to asses signaling pathways.

* *Corresponding author at*: Department of Oral and Maxillofacial Pathology & Research Center for Tooth and Periodontal Regeneration (MRC), School of Dentistry, Kyung Hee University, 1 Heogi-dong, Dongdaemun-gu, Seoul, 130-701, Republic of Korea. Tel.: +82 2 961 0383; fax: +82 2 960 2324.
** *Corresponding author at*: Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Cheonan 330-714, Republic of Korea. Tel.: +82 41 550 3081; fax: +82 41 550 3085.
E-mail addresses: kimhw@dku.edu (H.-W. Kim), eckim@khu.ac.kr (E.-C. Kim).
[1] Yun HM and Kang SK contributed equally to this work as first authors.
http://dx.doi.org/10.1016/j.dental.2016.06.016

Results. The growth of HDPCs was significantly enhanced on the magnetic scaffolds with respect to the non-magnetic counterpart. The odontogenic differentiation of cells was significantly up-regulated by the culture with magnetic scaffolds. Furthermore, the magnetic scaffolds promoted the HDPC-induced angiogenesis of endothelial cells. The expression of signaling molecules, Wnt3a, phosphorylated GSK-3β and nuclear β-catenin, was substantially stimulated by the magnetic scaffolds; in parallel, the MAPK and NF-κB were highly activated when cultured on the magnetic nanofiber scaffolds.

Significance. This study is the first to demonstrate that magnetic nanofiber scaffolds stimulate HDPCs in the events of growth, odontogenic differentiation, and pro-angiogenesis, and the findings imply the novel scaffolds can be potentially useful as dentin-pulp regenerative matrices.

1. Introduction

Regenerative endodontics generally involves the treatment of infected root canal systems in combination with apical enlargement to permit revascularization, where the use of cells, growth factors and scaffolds can play significant roles [1]. Scaffolds are the essential component of tissue engineering and provide a suitable environment for stem cells in dental pulp to recapitulate the dentin-pulp generation processes [2,3]. In general, scaffolds need to satisfy physical (porous three-dimension structure), mechanical (mechanical strength and stiffness), and biological properties (biocompatibility and biodegradation) for the appropriate cellular adhesion, growth, migration and differentiation [4].

Biodegradable synthetic polymers such as polycaprolactone (PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their co-polymer have been widely used to prepare scaffolds for tissue engineering because of their biocompatibility and ability to form desired shapes [5,6]. Compared to PLA and PGA, PCL is relatively more slowly degradable, more flexible, and less expensive [7]. However, those synthetic polymers lack surface bioactivity thus have initial slow cell responses, and the hydrolytic degraded acidic products often induce inflammation. Therefore, to improve the cell interaction and tissue responses of the scaffolds, bioactive inorganic additives, including hydroxyapatite and bioactive glasses, have been incorporated [8–12]. Results have demonstrated substantial improvement in the growth and differentiation of cells including osteoblasts, bone marrow stromal cells, and dental pulp cells.

Among the additives, magnetic nanoparticles (MNPs), mainly comprised up of iron oxide magnetite, have recently gained great interest due to their excellent magnetic properties with relatively good biocompatibility [13]. The addition of MNPs to biopolymers has been shown to increase the mechanical properties such as stiffness and strength as well as the biological responses required for bone repair [14–16]. While the MNPs-incorporated biomaterials have been shown to have high potential for the stimulation of cells involved in the osteogenic process and bone formation due to the magnetic field generated which might alter the local cellular microenvironment [15,17], little has been documented for the repair and regeneration of dental tissues including dentin and pulp.

Therefore, we focus our interest in the application of the magnetic scaffolds in dental pulp-dentin regeneration. As a first step to this, we have prepared the magnetic scaffolds in a nanofiber form and examine the growth and odontoblastic differentiation of human dental pulp cells (HDPCs). Furthermore, the pro-angiogenic effects by the magnetic scaffolds are also examined. Lastly, the molecular mechanisms underlying the magnetic scaffold-induced cellular events were investigated. The aim of this study was to evaluate the hypotheses of these studies for the potential of the magnetic nanofiber scaffolds in the dentin-pulp regeneration purposes.

2. Materials and methods

2.1. *Fabrication and characterizations of magnetic nanofiber scaffolds (MNS)*

As shown in the appendix, the MNPs were first synthesized and functionalized by a citric-acid treatment to anchor carboxylic acid functional group (COOH), after which the MNS were fabricated by electrospinning, according to the previous protocol [15]. Briefly, after ferrous chloride tetrahydrate in 1 M HCl and ferric chloride hexahydratate were mixed at room temperature, the mixture was dropped into NaOH for precipitation. The MNPs were dispersed in citric acid solution for conjugation with COOH, the COOH-MNPs were precipitated by addition of acetone. 10% w/v PCL in DCM-ethanol solution were mixed with the prepared COOH-MNPs (10 or 20 wt%). This mixture was injected through the stainless steel needle connected to a high-voltage power supply (15 kV). All experiments were performed at room temperature.

The micro- and nano-structure of the samples was characterized by scanning electron microscopy (SEM, S-3000H, Hitachi, Japan) and transmission electron microscope (TEM, 7100, JEOL, USA). The crystalline phase was determined by X-ray diffraction (XRD, Danvers, MA, USA). Fourier transformed infrared (FT-IR, Perkin-Elmer) spectroscopy was used to observe the chemical bond status of the samples. The water affinity of the samples was observed by water contact angle using a Phoenix300 analyzer at 25 °C. To investigate apatite forming ability, samples were immersed in 45 ml of (1.5×) simulated body fluid (SBF) with pH 7.4 and appropriate ion concentrations (Na^+ 142.0, K^+ 5.0, Mg^{2+}1.5, Ca^{2+} 2.5, Cl^- 147.8,