Colloids and Surfaces B: Biointerfaces 114 (2014) 316–323

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Regulation of human mesenchymal stem cells differentiation into chondrocytes in extracellular matrix-based hydrogel scaffolds

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ARTICLE INFO

Article history:
Received 29 May 2013
Received in revised form
29 September 2013
Accepted 2 October 2013
Available online 24 October 2013

Keywords:
Extracellular matrix
Human mesenchymal stem cell
Chondrocyte
Differentiation
Growth factor
Controlled release

ABSTRACT

To induce human mesenchymal stem cells (hMSCs) to differentiate into chondrocytes in three-dimensional (3D) microenvironments, we developed porous hydrogel scaffolds using the cartilage extracellular matrix (ECM) components of chondroitin sulfate (CS) and collagen (COL). The turbidity and viscosity experiments indicated hydrogel could form through pH-triggered co-precipitation when pH = 2–3. Enzyme-linked immunosorbent assay (ELISA) confirmed the hydrogel scaffolds could controllably release growth factors as envisaged. Transforming growth factor-β (TGF-β) was released to stimulate hMSCs differentiation into chondrocytes; and then collagen binding domain-basic fibroblast growth factor (CBD-bFGF) was released to improve the differentiation and preserve the chondrocyte phenotype. In *in vitro* cell culture experiments, the differentiation processes were compared in different microenvironments: 2D culture in culture plate as control, 3D culture in the fabricated scaffolds without growth factors (CC), the samples with CBD-bFGF (CC-C), the samples with TGF-β (CC-T), the samples with CBD-bFGF/TGF-β (CC-CT). Real-time polymerase chain reaction (RT-PCR) revealed the hMSC marker genes of CD44 and CD105 decreased; at the same time the chondrocyte marker genes of collagen type II and aggrecan increased, especially in the CC-CT sample. Immunostaining results further confirmed the hMSC marker protein of CD 44 disappeared and the chondrocyte marker protein of collagen type II emerged over time in the CC-CT sample. These results imply the ECM-based hydrogel scaffolds with growth factors can supply suitable 3D cell niches for hMSCs differentiation into chondrocytes and the differentiation process can be regulated by the controllably released growth factors.

1. Introduction

Mechanical trauma or degenerative disease might cause cartilage defects. Due to its avascular nature, lack of chondrocytes in extracellular matrix (ECM) and essential repair-promoting cytokine, injured cartilage cannot be easily cured through self-repair [1,2]. Surgeons have developed some methods to achieve this goal, such as autologous chondrocyte implantation/transplantation (ACI/ACT) [3], microfracture techniques (MFT) [4] and cartilage tissue engineering strategies (CTE) [5–9]. However, challenges still remain. In ACI/ACT, the weak proliferative capacity and the de-differentiation of chondrocytes; in MFT, the sacrifice of neighboring normal cartilage; in CTE, cell phenotype alteration, stress shielding, poor engraftment rate and biotoxicity, these problems limit cartilage repairing [10,11].

In CTE, a key step is restoring the damaged ECM [1]. The cartilage ECM is a three-dimensional (3D) scaffold composed of several different macromolecular constituents, such as collagen (COL) and glycosaminoglycans (GAGs), which mediate chondrocytes (cartilage-forming cells) adhesion, proliferation and subsequent functions [12,13]. For example, in cartilage ECM, chondroitin sulfate (CS) is a sulfated GAG composed of a chain of alternating sugars (*N*-acetylgalactosamine and glucuronic acid), usually found attached to proteins as part of a proteoglycan due to its negative-charge. The tightly packed and highly charged sulfate groups of CS generate electrostatic repulsion that provides much resistance of cartilage to compression. Additionally, the molecular structure of CS is similar to heparin so that it is suitable to bind and controllably release growth factors [14]. COL is important in maintaining the biological and structural integrity of the ECM. During these processes, COL

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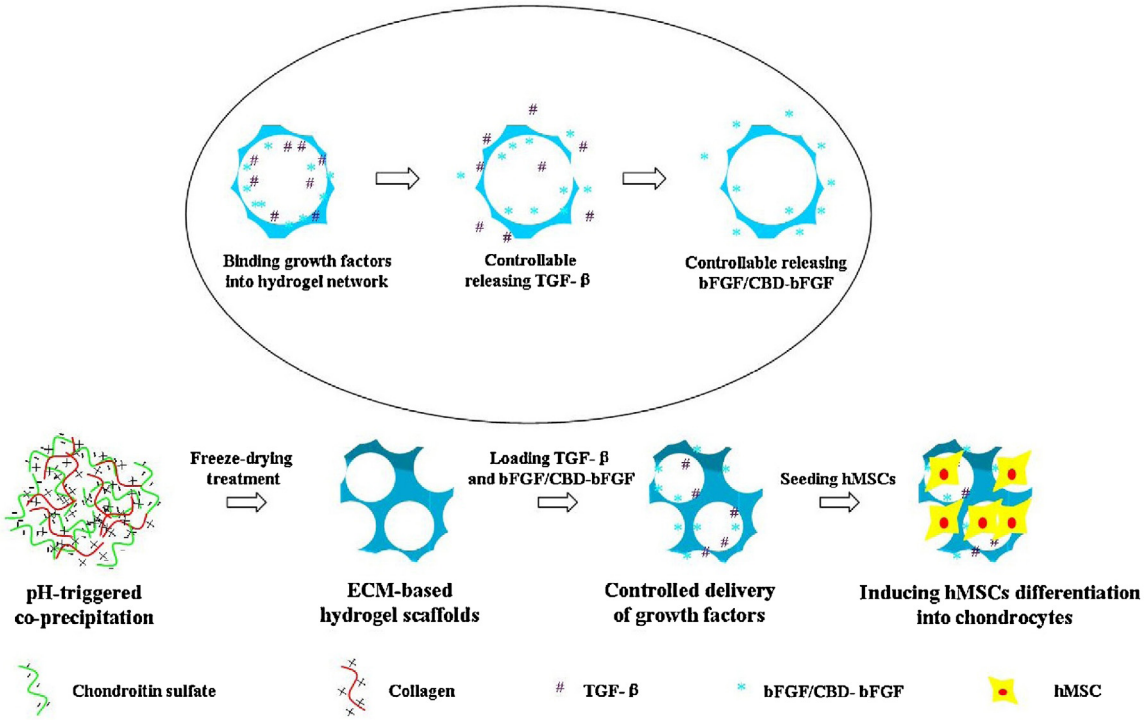
http://dx.doi.org/10.1016/j.colsurfb.2013.10.001



Scheme 1. Schematic representation of fabricating ECM-based hydrogel scaffolds for hMSCs differentiation into chondrocytes.

functions as a highly organized, dynamic remodeled, 3D architecture which provides anchorage sites and structural guidance for cell adhesion, migration and differentiation [15].

Though the numerous side groups in the polymer chains of tissue engineering scaffolds can be chemically modified with the bioactive ECM molecules to construct the cell niches [14], some shortcomings, such as organic reagents or high temperature, might destroy bioactivity of the derivatives. To improve these limitations, we developed ECM-based hydrogel scaffolds through pH-triggered co-precipitation of the cartilage ECM components of CS and COL illuminated as Scheme 1. In this way, the bioactivity, the thermal and mechanical stability of the assembled molecules can be better preserved at normal temperature in the aqueous system. Moreover, hydrogels are increasingly employed as biologically inspired 3D cell scaffolds those mimic natural ECM [16–24]. Engineered tissues have been created as *in vitro* 3D physiological models to provide more biologically relevant complexity than traditional 2D culture [25,26].

On the other hand, human mesenchymal stem cells (hMSCs) have shown their multiple differentiation potential, rapid proliferation and easy availability, which make them a promising candidate for CTE [27–30]. To induce chondrogenic differentiation, hMSCs require the appropriate signals. Several previous studies have demonstrated that a variety of growth factors such as basic fibroblast growth factor (bFGF), bone morphogenic proteins (BMP), insulin-like growth factor-I (IGF-I) and transforming growth factor-β (TGF-β) can induce the differentiation of mesenchymal cells into chondrocytes under certain culture conditions [31–36]. Herein, we envisage to controllably releasing TGF-β and bFGF to regulate hMSCs differentiation into chondrocytes in the ECM-based hydrogel scaffolds. TGF-β was released to stimulate hMSCs differentiation into chondrocytes; and then bFGF was released to improve the differentiation and preserve the chondrocyte phenotype.

For this aim, a short collagen binding domain (CBD) TKKTLRT derived from mammalian collagenase was designed to make bFGF specially bind collagen [37]. With this domain, collagen binding domain-basic fibroblast growth factor (CBD-bFGF) shows a notable collagen-binding ability and also remains excellent biological activity. In our previous studies, some growth factors, such as vascular endothelial growth factor (VEGF), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), BMP and bFGF were fused with CBD. The recombinant proteins are delivered in a site-specific manner with collagen, retaining the effective concentration at the target site and showing more effective tissue regeneration [38–43]. In *in vitro* 3D cell culture experiments, hMSCs were seeded into the fabricated scaffolds to investigate hMSCs differentiation into chondrocytes.

2. Materials and methods

2.1. Materials

Collagen type I (COL) was purified from the mouse tail as reported [44]. Chondroitin sulfate (CS) was purchased from Sigma. Human transforming growth factor-β (TGF-β) was obtained from Wuhan Boster Biological Engineering Co., Ltd. Human mesenchymal stem cells (hMSCs) in placenta were obtained as a gift from Prof. Dr. Qin Shi (Institute of Orthopaedics, Soochow University). The following experiments were performed using cells from passages 3–5. Other reagents not mentioned were purchased from Sinopharm Chemical Reagent Co., Ltd. The water used in all experiments was treated in a three-stage Millipore Milli-Q plus 185-purification system and had a resistivity higher than 18.2 MΩ cm.

2.2. Preparation of bFGF and CBD-bFGF

Preparation of bFGF and CBD-bFGF was performed as described previously [37,43]. Briefly, genes of human native bFGF and CBD-bFGF which contained a His_6 Tag were amplified by polymerase chain reaction and inserted into vector pET-28a (Novagen, Madison), respectively. After transformation of expression vectors