Materials Science and Engineering C 79 (2017) 661–670

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Glucosamine-modified polyethylene glycol hydrogel-mediated chondrogenic differentiation of human mesenchymal stem cells

Hang Yao [a,b,c,d], Jingchen Xue [a,b], Qunfang Wang [a,b], Renjian Xie [a,b], Weichang Li [a], Sa Liu [a,b], Jinglei Cai [c], Dajiang Qin [c,*], Dong-An Wang [d,*], Li Ren [a,b,**]

[a] School of Materials Science and Engineering, South China University of Technology, Wushan RD, Tianhe District, Guangzhou 510641, China
[b] National Engineering Research Center for Tissue Restoration and Reconstruction, Guangzhou 510006, China
[c] Key Laboratory of Regenerative Biology, Guangdong Provincial Key Laboratory of Stem Cell and Regenerative Medicine, South China Institute for Stem Cell Biology and Regenerative Medicine, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510530, China
[d] Division of Bioengineering, School of Chemical & Biomedical Engineering, Nanyang Technological University, 70 Nanyang Drive, N1.3-B2-13, 637457, Singapore

ARTICLE INFO

Article history:
Received 23 January 2017
Received in revised form 29 April 2017
Accepted 9 May 2017
Available online 11 May 2017

Keywords:
Glucosamine
Polyethylene glycol hydrogel
Bone mesenchymal stem cells
Chondrogenesis

ABSTRACT

Glucosamine (GA) is an important cartilage matrix precursor for the glycosaminoglycan biochemical synthesis, and has positive effects on cartilage regeneration, particularly in osteoarthritis therapy. However, it has not been used as a bioactive group in scaffolds for cartilage repair widely. In this study, we synthesized modified polyethylene glycol (PEG) hydrogel with glucosamine and then encapsulated human bone mesenchymal stem cells (hBMSCs) in the hydrogel to induce the differentiation of hBMSCs into chondrocytes in three-dimensional culture. The GA-modified PEG hydrogels promoted the chondrogenesis of hBMSCs, particularly in the concentration of 5 mM and 10 mM. The subcutaneous transplantation of 10 mM GA-modified hydrogels with hBMSCs formed cartilage-like blocks *in vivo* for 8 weeks. Importantly, with glucosamine increase, the modified hydrogels down-regulated the fibrosis and hypertrophic cartilage markers in protein level. Therefore, glucosamine modified PEG hydrogels facilitated the chondrogenesis of hBMSCs, which might represent a new method for cartilage repair using a tissue-engineering approach.

1. Introduction

Articular cartilage lesions caused by sports injuries, degeneration and disease are major causes of joint disability. Cartilage, which has no blood vessels and low metabolic activity, consists primarily by proteoglycans and collagen matrix with a small amount of chondrocytes [1]. Poor innate access to the chondrocytes is the reason for the low regeneration capacity of damaged cartilage. Progressive cartilage loss problems dramatically increase due to the aging [2]. Cell grafts including auto- and allografts have been widely studied and have good effects. However, cell grafts only resolve small cartilage defects, allografts also generate immune rejection problems, blocking their application [2]. Many reports have demonstrated that tissue engineering cartilage represents a better method to regeneration area problems than auto- and allograft treatment [3]. However, the neo-generated cartilage is known to exhibit fibrocartilage and hypertrophic features, expressing more protein of collagen type I (COL I) and type X (COL X), but less collagen type II (COL II) and aggrecan. Thus, designing new bio-scaffolds to generate functional hyaline cartilage remains a big challenge.

Amino sugar glucosamine is the major monomer of the extracellular matrix (ECM), which consists of glycosaminoglycans (GAG), proteoglycans and glycolipids [4]. It enriches in articular cartilage, synovial fluid and intervertebral discs [5]. Glucosamine has been used as a drug or nutrition after purification or modification, and it has been approved for osteoarthritis (OA) therapy. Studies *in vitro* and *in vivo* have explored glucosamine's effects on cartilage regeneration and have demonstrated its structure-regenerating and anti-inflammatory effects [6–8]. Indeed, glucosamine not only provides the building blocks for GAG synthesis by chondrocytes but also exerts other effects, such as stimulating chondrogenesis, anti-catabolic potency and anti-inflammation [9–11]. For example, glucosamine can increase transforming growth factor β1 (*TGF-β1*) gene expression to promote the synthesis of the matrix [12]. Glucosamine was found to inhibit the secretion and activity of matrix metalloproteinase (MMP), aggrecanases and other catabolic enzymes [13,14]. However, high concentration of glucosamine results in cytotoxicity [15,16], cytotoxicity can greatly reduce by glucosamine acetylation [16,17]. Therefore, we grafted glucosamine groups into a 3D scaffold to reduce its cytotoxicity and to explore the extracellular stimulation exerted by these groups.

Polyethylene glycol (PEG) hydrogel, which has good water absorption, good biocompatibility and an excellent ability to store and transmit

* Corresponding authors.
** Corresponding author at: School of Materials Science and Engineering, South China University of Technology, Wushan RD, Tianhe District, Guangzhou 510641, China.
E-mail addresses: qin_dajiang@gibh.ac.cn (D. Qin), DAWang@ntu.edu.sg (D.-A. Wang), psliren@scut.edu.cn (L. Ren).

http://dx.doi.org/10.1016/j.msec.2017.05.043
0928-4931/

nutrients [18], has long been used as bio-scaffold [19]. PEG hydrogels have their own structure and provide fundamental 3D environments for chondrogenesis. Some disadvantages, such as lack of bioactive sites, poor mechanical properties and poor degradation properties, promote researchers to modify PEG hydrogels. PEG hydrogels can be modified by some extracellular matrices (ECMs), like hyaluronic acid (HA), or be grafted with bioactive sites to improve its function. For example, PEG hydrogels modified by collagen and HA to promote chondrogenesis [20]. Synthesized PEG/HA hydrogels with semi-interpenetrating networks improve the degradation properties during cartilage regeneration [21]. These suggest PEG-based hydrogels provide great potential application for chondrogenesis.

We previously modified glucosamine and synthesized *N*-acryloyl-glucosamine (AGA) by introducing a double bond into *N*-acetyl-glucosamine. The obtained monosaccharide was able to copolymerize with poly (ethylene glycol) diacrylate (PEGDA). Additionally, the glucosamine groups were grafted into PEG-based gel systems, which provide active sites [22,23]. Here, we aimed to synthesize a PEG-based hydrogel with glucosamine groups to reduce the toxicity and to maintain the good chondrogenic ability of glucosamine. We induced human bone mesenchymal stem cells (hBMSCs) differentiate into cartilage, using glucosamine modified PEG-based (PEG-g-GA) hydrogel, and examined its effect on chondrogenesis.

2. Materials and methods

2.1. hBMSCs culture and encapsulation in PEG-g-GA hydrogels

PEGDA (4000 Da) was synthesized as described previously [22]. Nuclear magnetic resonance (^{1}H NMR) and gel permeation chromatography

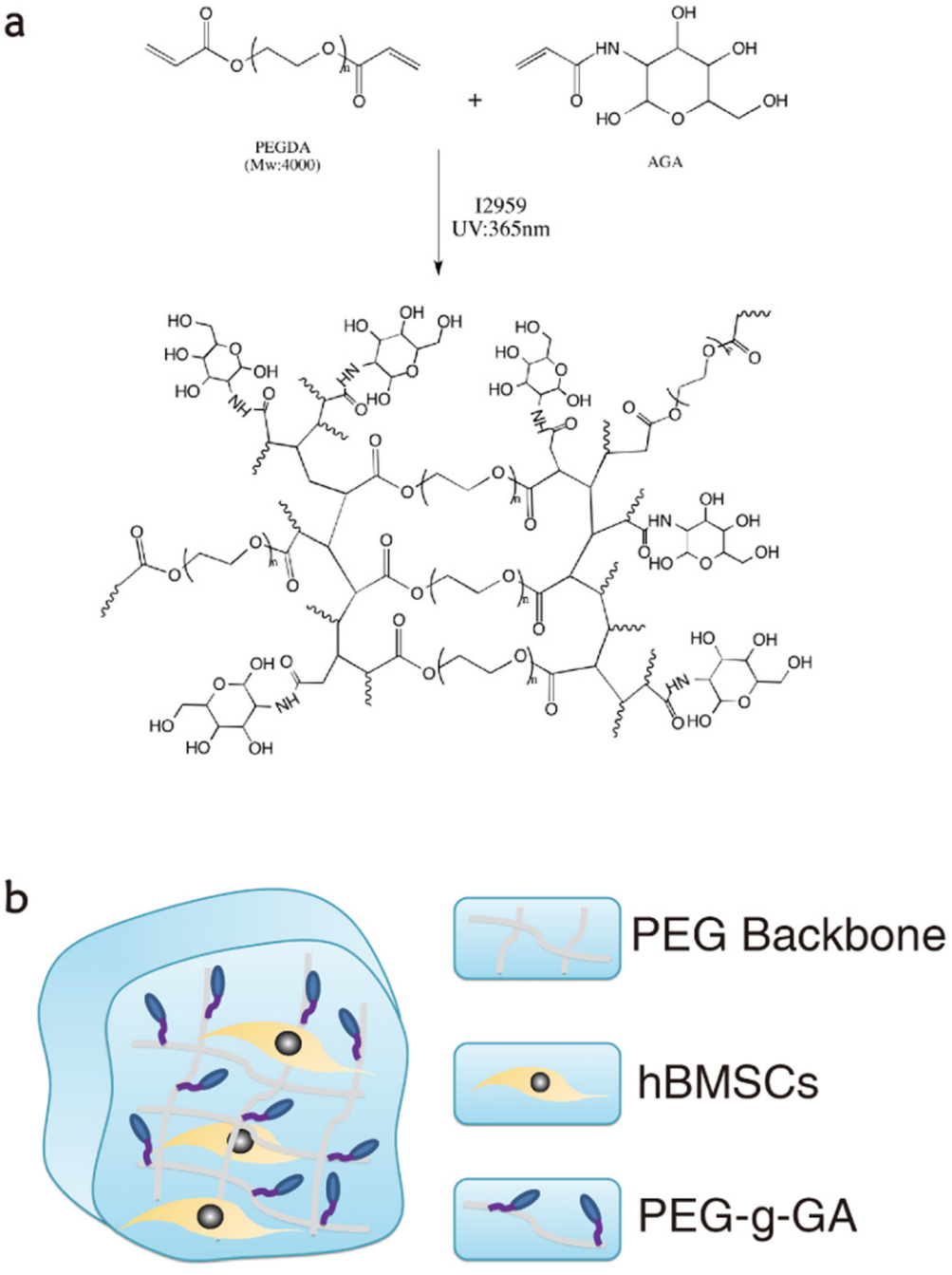

Fig. 1. (a) Photo-crosslinking reaction for fabrication of *N*-acryloyl-glucosamine modified PEGDA hydrogel. (b) Schematic structure of modified hydrogels encapsulating hBMSC.