Materials Science and Engineering C 42 (2014) 622–628

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Biomimetic alginate/polyacrylamide porous scaffold supports human mesenchymal stem cell proliferation and chondrogenesis

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ARTICLE INFO

Article history:
Received 5 January 2014
Received in revised form 21 April 2014
Accepted 9 June 2014
Available online 19 June 2014

Keywords:
Polyacrylamide
Alginate
Hydrogel
Mesenchymal stem cell
Proliferation
Chondrogenesis

ABSTRACT

We describe the development of alginate/polyacrylamide (ALG/PAAm) porous hydrogels based on interpenetrating polymer network structure for human mesenchymal stem cell proliferation and chondrogenesis. Three ALG/PAAm hydrogels at molar ratios of 10/90, 20/80, and 30/70 were prepared and characterized with enhanced elastic and rubbery mechanical properties, which are similar to native human cartilage tissues. Their elasticity and swelling properties were also studied under different physiological pH conditions. Finally, in vitro tests demonstrated that human mesenchymal stem cells could proliferate on the as-synthesized hydrogels with improved alkaline phosphatase activities. These results suggest that ALG/PAAm hydrogels may be a promising biomaterial for cartilage tissue engineering.

1. Introduction

Regeneration of cartilage is a principal challenge in orthopedic research. A key stumbling block is the poor regenerative ability of native cartilage tissue [1]. Progressive and irreversible loss of cartilage would cause the common chronic disease-osteoarthritis in aged people [2]. Current clinical treatments for osteoarthritis include mosaicplasty, autologous chondrocytes injection and micro-fracture surgery, but the prognosis is still poor [3–5]. In vitro tissue engineering provides a promising opportunity to prepare durable cartilage replacement with potent mechanical strength and biological activities [6,7].

Interpenetrating polymer network (IPN) hydrogel is a novel type of hybrid biomaterials attracting intense attention in tissue engineering [8–14]. IPN is usually composed of at least two interpenetrating polymer networks which are either physically or chemically crosslinked. This interlocked polymer network structure results in a mechanically strong and elastic hydrogel with similar biomechanic properties to native cartilage tissues [15]. Thus, IPN hydrogels are promising for cartilage tissue engineering applications, and many synthetic and natural polymers have been investigated as IPN materials for tissue engineering, including silk fibroin [9], hyaluronic acid [11], and collagen [12]. However, there is still a strong and unmet need to understand the influence and mechanism of IPN hydrogels on cellular behaviors, such as proliferation and differentiation. Hence, in the present study, we prepared a biomimetic IPN hydrogel scaffold aiming at potential cartilage tissue engineering applications. We selected two common and low-cost biopolymers: covalent crosslinked polyacrylamide (PAAm) and ionically crosslinked alginate (ALG) to form our IPN structure (as shown in Fig. 1). PAAm and ALG were selected as the hydrogel materials in our study also due to their excellent biocompatibility and biodegradability. PAAm is a biocompatible synthetic polymer that has been widely used in plastic operations [16], drug treatment [17], and contact lenses [18]. PAAm has been clinically proved non-toxic, non-immunogenic, and stable in human body [16]. ALG is a biodegradable and naturally occurring polysaccharide extracted from the cell wall of brown algae. Due to its biodegradability and inexpensiveness, ALG is one of the most popular biomaterials that have been widely applied in pharmaceutical excipient [19], dental impression materials [20], and wound healing materials [21]. As-synthesized ALG/PAAm scaffolds exhibited potent mechanical and elastic properties, which are close to natural human cartilage tissues. We also investigated the influence of environmental pH-decrease on IPN hydrogel elasticity and swelling properties, which frequently occurs at scaffold implantation sites due to ischemic hypoxia and accumulation of metabolic waste. Moreover, we demonstrated that human mesenchymal stem cells (hMSCs) could proliferate on ALG/PAAm scaffolds with improved alkaline phosphatase (ALP) activities. hMSCs are mostly derived from bone marrow, and capable

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http://dx.doi.org/10.1016/j.msec.2014.06.013
0928-4931/

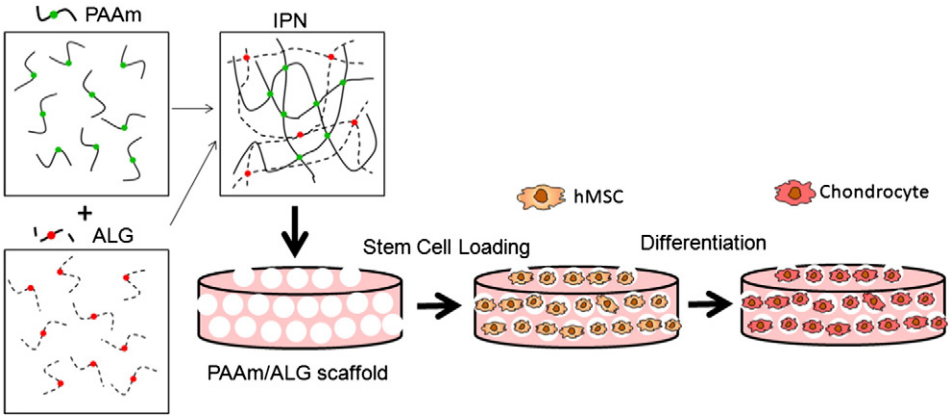

Fig. 1. Schematic illustration of the ALG/PAAm scaffold supporting hMSC proliferation and chondrogenesis.

of differentiating into multiple types of cells [22]. In cartilage tissue engineering, hMSCs are commonly utilized to generate chondrocytes in the scaffold [23–25]. We also studied the influence of the IPN hydrogel stiffness on hMSC proliferation and chondrogenesis. The results confirmed the potential of biomimetic ALG/PAAm scaffold in cartilage tissue engineering.

2. Experimental section

2.1. Materials

Acrylamide, sodium alginate, Triton X-100, N,N′-methylenebisacrylamide, tetramethylethylenediamine, ammonium persulfate, and calcium chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fibronectin is purchased from Invitrogen (Carlsbad, CA, USA). MSCGM™ mesenchymal stem cell growth medium and hMSC mesenchymal stem cell chondrocyte differentiation medium were purchased from Lonza (Allendale, NJ, USA). Cell Counting Kit-8 was purchased from Dojindo Molecular Technologies (Rockville, MD, USA). Alkaline phosphatase assay kit (Fluorimetric) was purchased from Abcam (Cambridge, MA, USA).

2.2. Synthesis of porous PAAm and ALG/PAAm hydrogel

Porous PAAm and ALG/PAAm scaffolds at different molar ratios of ALG/PAAm (10/90, 20/80, and 30/70, mol/mol) were fabricated using the method published by Suo et al. with modifications [8]. 1.51 g acrylamide or sodium alginate/acrylamide mixture (10/90, 20/80, and 30/70, mol/mol) was homogeneously dissolved in 10 mL 1 wt.% Triton X-100 aqueous solution in a glass vial. To initiate the polymerization at room temperature, 6.04 mg N,N′-methylenebisacrylamide, 120.5 mg tetramethylethylenediamine and 60.5 mg ammonium persulfate were added to the acrylamide/sodium alginate solution. Sodium alginate/acrylamide foam was created by vigorous stirring. Obtained acrylamide/sodium alginate foam was cured with a portable ultraviolet light for 1 h, and dried out to a constant weight in an incubator at 37 °C for 24 h, then ionically crosslinked by calcium cation in 1 wt.% calcium chloride solution for 24 h. Finally, the scaffold was washed three times in 500 mL deionized water for 12 h. Solid PAAm and ALG/PAAm hydrogels at different molar ratios (10/90, 20/80, and 30/70, mol/mol) were prepared in the same method described above. The deionized water, instead of 1 wt.% Triton X-100 solution was used for the synthesis.

2.3. Scanning electron microscopy

Porous ALG/PAAm scaffold (10/90) was coated Pt/Pd for 2 min with 40 mA with a sputter coater (208HR, Cressington Scientific Instruments, England), and with SEM (FESEM Ultra55, Zeiss, Thornwood, NY) at a beam voltage of 5 kV. Measurements were performed in triplicate.

2.4. Mechanical testing

The elastic modulus measurement of solid PAAm and ALG/PAAm hydrogels at different molar ratios (10/90, 20/80, and 30/70, mol/mol) was performed in phosphate buffered saline at different pH (7.4, 7.0, 6.5, 6.0, and 5.5) after full hydration for three days. Samples were placed on a shaker in an incubator at 37 °C. Tensile tests of each scaffold were carried out on an Instron BioPuls 5543 (Instron, Norwood, MA, USA) using a 50 N loading cell. Scaffolds were strained to failure at a rate of 5 mm/min. Young's modulus was calculated from the initial 40% strain. Five scaffold samples of each formulation were tested. Young's modulus was calculated according to the equation:

$$E = \frac{\text{engineering tensile stress}}{\text{engineering tensile strain}} = \frac{FL}{A\Delta L}$$

E is Young's modulus (modulus of elasticity); F is the force exerted on an object under tension; L is the original length of the object; A is the original cross-sectional area of the object; and ΔL is the increased length of object under tension.

2.5. Equilibrium swelling and reswelling measurements

Mass swelling ratios of PAAm and ALG/PAAm scaffolds (10/90, 20/80, and 30/70, mol/mol) were measured in PBS of known pH, composition, and temperature. The pH of the buffer was adjusted using 0.1 N HCl to achieve pH 5.5, 6.0, 6.5, 7.0, and 7.4. Scaffold strips were placed into a glass jar containing 50 mL of buffer on a shaker with a shaking rate of 150 rpm in an incubator maintained at 37 °C. The swelling ratio of the scaffolds as a function of pH was calculated by measuring the mass of the scaffolds at 0, 1, 2, 3, 4, 24, and 48 h as follows: Swelling ratio = Ms/Mi, where Ms is the mass of the swollen scaffold in buffer and Mi is the mass of the initial scaffold before swelling. The reswelling ratios of PAAm and ALG/PAAm scaffolds at different molar ratios (10/90, 20/80, and 30/70, mol/mol) were measured and calculated using the same formula of swelling ratio as a function of time initially equilibrated at pH 5.5 and then placed in pH 7.4 buffer.

2.6. Cell culture

hMSC was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in an MSCGM™ mesenchymal stem cell growth medium at 37 °C in humidified air containing 5% CO_2. hMSCs were cultured in 100 mm culture dishes and grown to over 70% confluence within 2 weeks. The medium was changed every