Colloids and Surfaces B: Biointerfaces 132 (2015) 146–154

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Natural polysaccharides promote chondrocyte adhesion and proliferation on magnetic nanoparticle/PVA composite hydrogels

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ARTICLE INFO

Article history:
Received 7 February 2015
Received in revised form 15 April 2015
Accepted 7 May 2015
Available online 15 May 2015

Keywords:
Hydrogel
PVA
Hydroxyapatite
Natural polysaccharide
Chondrocytes

ABSTRACT

This paper aims to investigate the synergistic effects of natural polysaccharides and inorganic nanoparticles on cell adhesion and growth on intrinsically cell non-adhesive polyvinyl alcohol (PVA) hydrogels. Previously, we have demonstrated that Fe_2O_3 and hydroxyapatite (nHAP) nanoparticles are effective in increasing osteoblast growth on PVA hydrogels. Herein, we blended hyaluronic acid (HA) and chondroitin sulfate (CS), two important components of cartilage extracellular matrix (ECM), with Fe_2O_3/nHAP/PVA hydrogels. The presence of these natural polyelectrolytes dramatically increased the pore size and the equilibrium swelling ratio (ESR) while maintaining excellent compressive strength of hydrogels. Chondrocytes were seeded and cultured on composite PVA hydrogels containing Fe_2O_3, nHAP and Fe_2O_3/nHAP hybrids and Fe_2O_3/nHAP with HA or CS. Confocal laser scanning microscopy (CLSM) and cell counting kit-8 (CCK-8) assay consistently confirmed that the addition of HA or CS promotes chondrocyte adhesion and growth on PVA and composite hydrogels. Particularly, the combination of HA and CS exhibited further promotion to cell adhesion and proliferation compared with any single polysaccharide. The results demonstrated that the magnetic composite nanoparticles and polysaccharides provided synergistic promotion to cell adhesion and growth. Such polysaccharide-augmented composite hydrogels may have potentials in biomedical applications.

1. Introduction

Hydrogels are biomimetic materials in terms of high water content, biocompatibility and biofunctionality and thus have been widely studied as promising candidates for tissue engineering [1]. Numerous natural [2] and synthetic hydrogels [3], or a combination of both [4], have been developed. Natural hydrogels usually possess excellent biocompatibility and low toxicity, but with poor strength and toughness [5]. Synthetic hydrogels are flexible in chemical structures, functionalities and mechanical strength and toughness, but could be limited by poor biocompatibility and biofunctionality [6]. In order to fabricate scaffolds for load-bearing soft tissues, taking cartilage as an example, adequate mechanical properties have been recognized as important as the biocompatibility and biofunctionality [7,8].

Numerous strategies have been developed to create composite hydrogels with bioceramics or by blending natural and synthetic hydrogels in order to combine complementary properties to achieve ideal support to cell adhesion, proliferation and differentiation.

Polyvinyl alcohol (PVA) has been widely investigated for biomedical applications [9–11] due to its merits including excellent biocompatibility, mechanical strength and toughness, low friction and high lubricity [12,13]. However, its intrinsically cell non-adhesive nature provides poor support to cell growth and integration to peripheral tissues [14,15]. Modification of PVA with biomolecules such as arginine–glycine–aspartic acid (RGD) peptide [16], hydroxyapatite [17] or natural polysaccharide (chondroitin sulfate) has shown improvements in cell adhesion and growth [18]. For example, hydroxyapatite (HAP) is the main inorganic composition in bone and has good osteoconductivity. The incorporation of HAP into PVA hydrogel enhanced the MC3T3-E1 cell density with well spreading morphology [19]. On the contrary, iron oxide nanoparticles had little influence on chondrocyte phenotype, viability and the production of major cartilage matrix constituents [20,21]. The hybrid of magnetic iron oxide nanoparticles and hydroxyapatite had been demonstrated favorable to cell adhesion

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http://dx.doi.org/10.1016/j.colsurfb.2015.05.008
0927-7765/

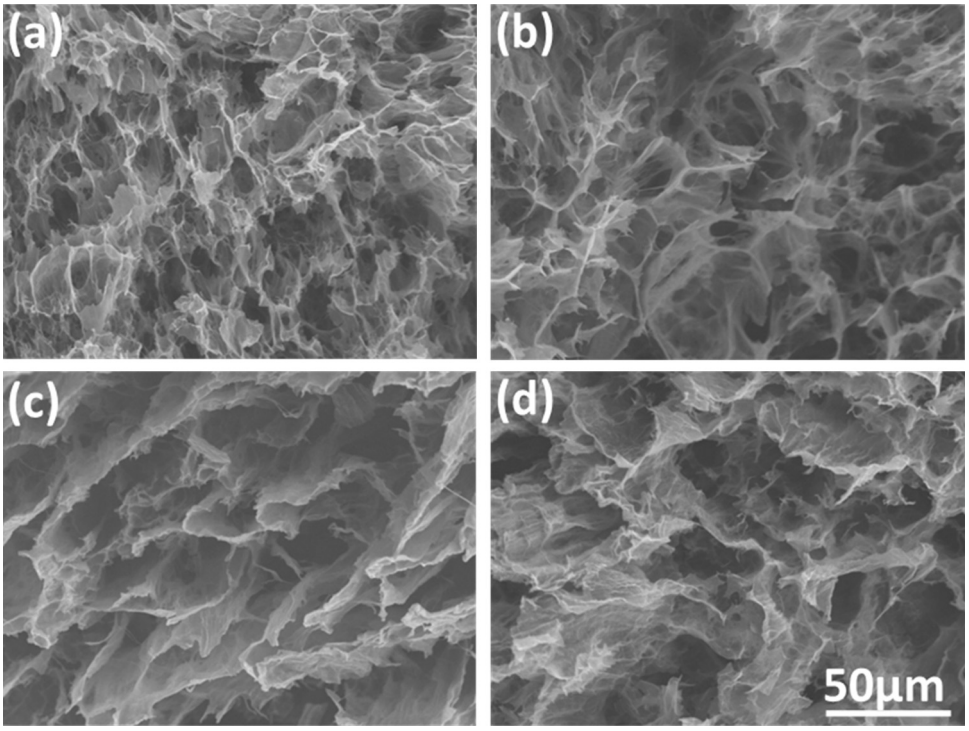

Fig. 1. SEM images of (a) PVA, (b) HA/PVA, (c) CS/PVA and (d) HA/CS/PVA.

and proliferation on composite hydrogels. For instance, magnetic scaffolds (FeHA/collagen) were fabricated by doping Fe^{2+}/Fe^{3+} ions into HAP nanocrystals nucleated on self-assembling collagen fibers. Such a composite hydrogel provided better support to cell adhesion, distribution and proliferation than the control HA/collagen scaffolds [22]. Our previous studies also demonstrated promoted osteoblast adhesion and proliferation on Fe_2O_3/HAP/PVA hydrogels than on HAP/PVA and PVA hydrogels [23].

In contrast, polysaccharides such as hyaluronic acid (HA) and chondroitin sulfate (CS) are two important biomolecules in most tissues including cartilage extracellular matrix (ECM). They are critical for cell adhesion, differentiation, migration and tissue integration [24,25]. Hydrogels based on HA and CS or their derivatives have been widely investigated as scaffolds to engineer or repair tissues including cartilage or osteochondral defects [26–28]. Interestingly, aldehyde-modified CS conjugated with amines could act as strong bio-adhesives to glue tissue interface in cartilage defect of rats [25]. Moreover, a combination of HA and CS has been demonstrated to achieve better results of cell growth than single HA or CS. For instance, compared with hyaluronic acid/silk fibroin (HA/SF, 20/80) (w/w) scaffolds, chondroitin sulfate/hyaluronic acid/silk fibroin (CS/HA/SF, 5/15/80) (w/w/w) scaffolds improved the angiogenesis and collagen production and promoted dermis regeneration [29]. Despite of these advantages of polysaccharides in cell growth, extracellular matrix secretion and tissue repair, inadequate mechanical properties remain as major limits to applications in tissue engineering [30]. It is desired to develop strong and tough hydrogels with excellent bioactivities and biofunctionalities for biomedical applications.

In this article, we combined polysaccharides (HA and CS) with previously reported strong and tough Fe_2O_3/nHAP/PVA nanocomposite hydrogels in order to further enhance the biofunctionality of these hydrogels. HA, CS and a combination of both were incorporated into PVA hydrogels and nanocomposites prior to freeze-thawing. The morphology, swelling and mechanical properties of these composite hydrogels were investigated. Neat PVA hydrogels and those composited with Fe_2O_3, nHAP and Fe_2O_3/nHAP hybrids were used for chondrocyte adhesion and proliferation study. Confocal laser scanning microscopy (CLSM) and cell counting kit-8 (CCK-8) assay results demonstrated that an appropriate content of polysaccharides (HA) could significantly improve the adhesion and proliferation of cells. When single or combined polysaccharides were incorporated into PVA hydrogels, the influence of chondrocyte adhesion and proliferation on PVA hydrogels was studied. Finally, the synergistic effect of polysaccharides and nanoparticles in promoting the adhesion and proliferation of chondrocytes is discussed.

2. Materials and methods

2.1. Materials

Poly(vinyl alcohol) (PVA, degree of polymerization: 1750 ± 50, analytical reagent, ≥99.0%) was purchased from Sinopharm Chemical Reagent Co., Ltd. Hyaluronic acid sodium (HA, *M*w = 380 kDa) was obtained from Shan Dong Freda Biopharm Co., Ltd. Chondroitin sulfate (CS) was obtained from Shaanxi Sciphar Natural Products Co., Ltd. $FeCl_2{\cdot}4H_2O$ (≥99.0%), $FeCl_3{\cdot}6H_2O$ (≥99.0%), $Ca(NO_3)_2{\cdot}4H_2O$ (≥99.0%), $(NH_4)_2HPO_4$ (≥99.0%) and NH_4OH (25.0%) solutions were of analytical reagent grade (Aladdin Chemistry Co., Ltd). All other chemicals and solvents were of analytical reagent grade.

2.2. Preparation of nanocomposite hydrogels and magnetic nanocomposite hydrogels

Magnetic Fe_2O_3/nHAP nanoparticles were prepared as described previously [23]. PVA was dissolved in deionized water with mechanical stirring at 90 °C for 6 h to yield a 3.3 wt% solution, which was then slowly cooled down to 25 °C. HA and CS were separately dissolved in deionized water with magnetic stirring at 25 °C for 2 h to yield a 2.0 wt% solution. Subsequently, single (HA or CS) or combined (HA and CS) polysaccharide solutions were, respectively, added into PVA solution to fabricate