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The effect of oxidation on the degradation of photocrosslinkable alginate hydrogels

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ABSTRACT

Recently, we reported on a new photocrosslinkable alginate-based hydrogel, which has controllable physical and cell adhesive properties. The macromer solution containing cells can be injected in a minimally invasive manner into a defect site and crosslinked while maintaining high cell viability. The number of hydrolyzable ester bonds in the formed crosslinks may be controlled by altering the degree of methacrylation on the alginate polymer backbone. However, the degradation rate of the hydrogels has been found to be slower in vivo than in vitro. The purpose of this study was to develop photocrosslinked alginate hydrogels with an increased range of biodegradation rates for more rapid in vivo biodegradation in regenerative medicine and bioactive factor delivery applications. Therefore, we oxidized alginate prior to methacrylation to change the uronate residue conformations to an open-chain adduct, which makes it more vulnerable to hydrolysis. Here, we demonstrate that the swelling behavior, degradation profiles, and storage moduli of photocrosslinked hydrogels formed from oxidized, methacrylated alginates (OMAs) are tunable by varying the degree of alginate oxidation. The OMA macromers and photocrosslinked OMA hydrogels exhibited cytocompatibility when cultured with human bone marrow-derived mesenchymal stem cells (hBMMSCs). In addition, hMSCs derived from bone marrow or adipose tissue photoencapsulated within these hydrogels remained viable, and their proliferation rate was a function of alginate oxidation level and initial hydrogel weight fraction. Oxidation permits a wider range of photocrosslinked OMA hydrogels physical properties, which may enhance these therapeutic materials' utility in tissue engineering and other biomedical applications.

1. Introduction

Recently, we developed alginate macromers that can be photopolymerized to form hydrogels with controllable mechanical properties, swelling ratios, and degradation rates in vitro [1]. Cells could be easily encapsulated via suspension in aqueous macromer solution followed by in situ photocrosslinking, and the resultant hydrogels showed excellent cytocompatibility. We have also demonstrated the capacity to control the cell adhesivity of these hydrogels by covalently coupling cell adhesion ligands, such as those containing the RGD sequence, to the methacrylated alginate backbone [2]. RGD modification of photocrosslinked alginate hydrogels promoted cell adhesion and spreading on the surface of the photocrosslinked alginate hydrogels, and enhanced the proliferation of encapsulated cells. Additionally, we presented an affinity-based growth factor delivery system using photocrosslinked alginate hydrogels by incorporation of methacrylated heparin, which can be coupled to alginate hydrogels through photopolymerization, to allow for the controlled and prolonged release of heparin binding growth factors, such as fibroblast growth factor-2, vascular endothelial cell growth factor, transforming growth factor-β_1, and bone morphogenetic protein-2. The release profiles of growth factors from the heparin-modified hydrogels were sustained over three weeks with no initial burst release, and the released growth factors retained their biological activity [3]. The implantation of growth factor-laden hydrogels in an animal model resulted in enhanced new tissue formation, however, the degradation rate of these hydrogels was found to be much slower in media than in water and in vivo than in vitro, limiting new tissue formation in the hydrogels [3].

Since alginate is not naturally enzymatically degraded in mammals, ionically crosslinked alginate hydrogels exhibit a remarkably slow degradation rate, which is typically months to years for their complete removal from injection sites [4,5]. Thus,

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the degradation kinetics of alginate hydrogels has been previously controlled, for example, by varying alginate molecular weight [6] and incorporation of biodegradable crosslinks [1]. In another approach, oxidized alginates (OAs) were synthesized to accelerate the degradation rate of alginate [7–11]. When alginate is oxidized by reacting with sodium periodate, the carbon–carbon bonds of the *cis*-diol groups in the uronate residues are cleaved [7,10] and changed to dialdehyde groups. This approach offers control over the degradation rate by varying the oxidation degree, as increasing the oxidation degree can increase the vulnerability of alginate hydrogels to hydrolysis [8,10].

The purpose of this study was to develop photocrosslinked alginate hydrogels with an increased range of biodegradation rates for more rapid in vivo biodegradation in tissue engineering and bioactive factor delivery applications. Therefore, we oxidized the alginate prior to methacrylation to change the uronate residue conformations to an open-chain adduct, which would be more prone to hydrolytic degradation [10]. In this manner, hydrolytic degradation could take place both at the alginate polymer backbone and at the ester bonds in the photopolymerized crosslinks [12]. In this study, we investigated whether the swelling behavior, degradation profiles, and storage moduli of photocrosslinked hydrogels formed from oxidized and methacrylated alginates (OMAs) are tunable by varying the degree of alginate oxidation. The potential applicability of these photocrosslinked OMA hydrogels as a carrier of human mesenchymal stem cells (hMSCs) derived from bone marrow and adipose tissue was also examined in vitro.

2. Materials and methods

2.1. Preparation of OA

The OA was prepared by reacting sodium alginate (Protanal LF 20/40, 196,000 g/mol, FMC Biopolymer, Philadelphia, PA, USA) with sodium periodate (Sigma) using a modification of a previously described method [10,13]. Briefly, sodium alginate (20 g) was dissolved in ultrapure deionized water (diH_2O, 1800 ml) overnight. Sodium periodate (2.0, 3.5, 5.0, and 10.1 g) was dissolved in 200 ml diH_2O and added into separate alginate solutions to achieve different degrees of theoretical alginate oxidation (10, 17.5, 25, and 50%, respectively) under stirring in the dark at room temperature (RT), and the reaction was stopped after 24 h by the addition of ethylene glycol (molar ratio of ethylene glycol:sodium periodate = 1:1). The OA was purified by dialysis (MWCO 3500; Spectrum Laboratories Inc., Rancho Dominguez, CA, USA) against deionized ultra pure water (diH_2O) for 3 days, filtered (0.22 μm filter), and lyophilized.

2.2. Synthesis of OMA macromer

The OMA macromer was prepared by reacting OA with 2-aminoethyl methacrylate (AEMA, Sigma, St. Louis, MO, USA) [1,12]. To prepare OMA at a theoretical methacrylation of 45%, OA (10 g) was dissolved in a buffer solution (1w/v %, pH 6.5) of 50 mM 2-morpholinoethanesulfonic acid (MES, Sigma) containing 0.5 M NaCl. N-hydroxysuccinimide (NHS, 2.65 g; Sigma) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC, 8.75 g; Sigma) (molar ratio of NHS:EDC = 1:2) were added to the mixture to activate the carboxylic acid groups of the alginate. After 5 min, AEMA (3.8 g) (molar ratio of NHS:EDC:AEMA = 1:2:1) was added to the product, and the reaction was maintained in the dark at RT for 24 h. The reaction mixture was precipitated with the addition of excess of acetone, dried under reduced pressure, and rehydrated to a 1w/v % solution in diH_2O for further purification. The OMA was purified by dialysis against diH_2O (MWCO 3500; Spectrum Laboratories Inc.) for 3 days, treated with activated charcoal (0.5 mg/100 ml, 50–200 mesh, Fisher, Pittsburgh, PA) for 30 min, filtered (0.22 μm filter), and lyophilized.

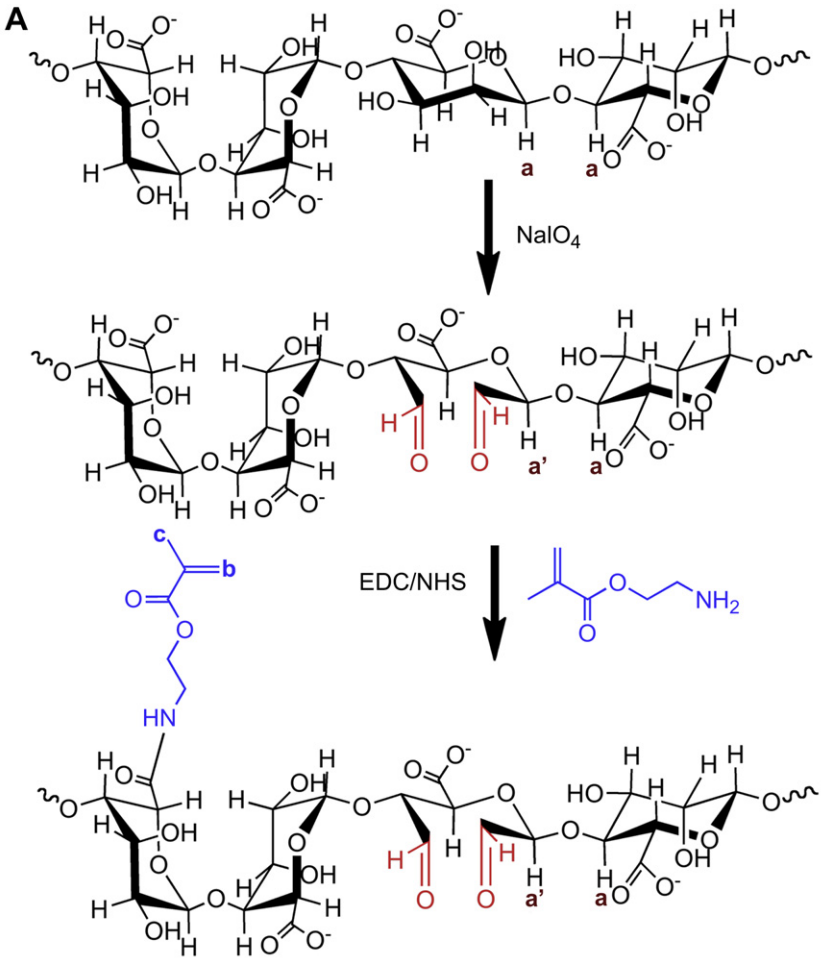

Fig. 1. (A) Schematic illustration for preparation of the OMA and ^{1}H NMR spectra of (B) OAs and (C) OMAs with various degrees of oxidation in D_2O.