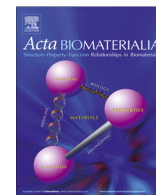
Acta Biomaterialia xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

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



# Alkylation of human hair keratin for tunable hydrogel erosion and drug delivery in tissue engineering applications

Sangheon Han [a], Trevor R. Ham [a,b], Salma Haque [a], Jessica L. Sparks [a], Justin M. Saul [a,*]

[a] Department of Chemical, Paper, and Biomedical Engineering, Miami University, 650 E. High Street, Oxford, OH 45056, USA
[b] Department of Biomedical Engineering, University of Akron, Auburn Science and Engineering Center 275, West Tower, Akron, OH 44325, USA

ARTICLE INFO

Article history:
Received 16 December 2014
Received in revised form 8 May 2015
Accepted 12 May 2015
Available online xxxx

Keywords:
Recombinant human BMP-2
Recombinant human IGF-1
Antibiotic
Ciprofloxacin
Regenerative medicine

ABSTRACT

Polymeric biomaterials that provide a matrix for cell attachment and proliferation while achieving delivery of therapeutic agents are an important component of tissue engineering and regenerative medicine strategies. Keratins are a class of proteins that have received attention for numerous tissue engineering applications because, like other natural polymers, they promote favorable cell interactions and have non-toxic degradation products. Keratins can be extracted from various sources including human hair, and they are characterized by a high percentage of cysteine residues. Thiol groups on reductively extracted keratin (kerateine) form disulfide bonds, providing a more stable cross-linked hydrogel network than oxidatively extracted keratin (keratose) that cannot form disulfide crosslinks. We hypothesized that an iodoacetamide alkylation (or "capping") of cysteine thiol groups on the kerateine form of keratin could be used as a simple method to modulate the levels of disulfide crosslinking in keratin hydrogels, providing tunable rates of gel erosion and therapeutic agent release. After alkylation, the alkylated kerateines still formed hydrogels and the alkylation led to changes in the mechanical and visco-elastic properties of the materials consistent with loss of disulfide crosslinking. The alkylated kerateines did not lead to toxicity in MC3T3-E1 pre-osteoblasts. These cells adhered to keratin at levels comparable to fibronectin and greater than collagen. Alkylated kerateine gels eroded more rapidly than non-alkylated kerateine and this control over erosion led to tunable rates of delivery of rhBMP-2, rhIGF-1, and ciprofloxacin. These results demonstrate that alkylation of kerateine cysteine residues provides a cell-compatible approach to tune rates of hydrogel erosion and therapeutic agent release within the context of a naturally-derived polymeric system.

© 2015 Published by Elsevier Ltd.

## 1. Introduction

Polymeric materials are a key component in tissue engineering/regenerative medicine (TERM) approaches to promote tissue healing and regeneration. Materials used for these applications typically must have minimal toxicity, elicit minimal immune/inflammatory response, have appropriate mechanical properties, and promote favorable cellular responses (attachment and/or infiltration and/or proliferation) [1,2]. It is generally held that materials should degrade in a manner inversely proportional to the rate of tissue regeneration such that the material provides a cell-support matrix but degrades to avoid impeding tissue regeneration [3]. These materials are also widely used to achieve controlled release of various exogenous therapeutic agents ranging from antibiotics that prevent local infection to growth factors that promote tissue formation or healing [4,5]. The two general classes of polymeric materials used in TERM applications are natural and synthetic polymers.

Synthetic polymers have the advantage of tunable degradation or controlled release of therapeutic agents via modifications of the polymer backbone constituents, side-chains, and molecular weight [6–8]. The main drawback to the use of synthetics revolves around poor cell attachment, which requires modifications with peptide groups, use of natural-synthetic hybrids, or electrospinning techniques to provide surface topography [9–12]. Conversely, natural protein polymers such as collagen and fibrin have favorable biological interactions because they promote cell attachment and have amino acids as their degradation products [13]. However, the ability to achieve tunable rates of degradation or release of therapeutic agents can be a challenge for natural proteinaceous polymers.

* Corresponding author at: Department of Chemical, Paper, and Biomedical Engineering, 650 East High Street, Engineering Building, Room 064L, Miami University, Oxford, OH 45056, USA. Tel.: +1 (513) 529 0769 (O); fax: +1 (513) 529 0761.
E-mail address: sauljm@MiamiOH.edu (J.M. Saul).

http://dx.doi.org/10.1016/j.actbio.2015.05.013
1742-7061/© 2015 Published by Elsevier Ltd.

Please cite this article in press as: S. Han et al., Alkylation of human hair keratin for tunable hydrogel erosion and drug delivery in tissue engineering applications, Acta Biomater. (2015), http://dx.doi.org/10.1016/j.actbio.2015.05.013

UNCORRECTED PROOF

Keratins are a class of intermediate filament proteins that can be derived from various sources including human hair, the source of keratin described in this report. Keratins used without modification or delivery of therapeutic agents have been described for biomedical applications including skin [14], hemostasis [15], bone [16], and nerve [17,18]. We and others have also reported the use of keratins to promote delivery of therapeutics including model drugs [19], antibiotics [20,21], and recombinant human bone morphogenetic protein 2 (rhBMP-2) [22–24].

An intriguing characteristic of keratin proteins that might be exploited for controlled erosion and release of therapeutics is the presence of a relatively high percentage of cysteine residues (reported between 7 and 20 mol%) compared to other proteins [25,26]. Two well-known extraction methods have been developed that lead to physiochemically different forms of keratin. Oxidatively extracted keratin (keratose; KOS) yields a form in which thiol groups on cysteine residues are "capped" with sulfonic acid groups that prevent covalent disulfide cross-linking. Keratose can be formed into hydrogels, but these gels form by chain entanglement and lack covalent disulfide crosslinking. Reductively extracted keratin (kerateine; KTN) yields a form that has chain entanglements and free thiol groups that can spontaneously form covalent disulfide crosslinks. Different levels of disulfide bonding between keratose and kerateine have previously been shown to lead to different rates of hydrogel erosion with KOS erosion occurring in days to weeks and KTN persisting for months [27,28]. It has also previously observed that rates of therapeutic agent release correlate with the rates of keratin hydrogel erosion [21,22]. We hypothesized that thiol groups on KTN could simply be alkylated ("capped") to modulate disulfide crosslinks, which in turn, would lead to tunable rates of gel erosion and therapeutic delivery.

The approach described in this report uses iodoacetamide as the alkylating agent for keratin cysteine residues [29], as shown in Fig. 1A. After assessing levels of alkylation, the effects of the modification on the hydrogel network, mechanical properties, and rate of erosion were also investigated. Cell viability and adhesion were then studied in order to determine if the alkylation had effects on the biological response to the modified kerateines. Lastly, the ability to control the rates of release was assessed for three different therapeutic agents: ciprofloxacin, recombinant human insulin-like growth factor 1 (rhIGF-1), and recombinant human bone morphogenetic protein 2 (rhBMP-2). These molecules have differing physiochemical properties, the most notable being molecular weights of 300 Da (ciprofloxacin), 7 kDa (rhIGF-1), and 26 kDa (rhBMP-2). These agents were selected to assess controlled release due in part to their relevance to tissue engineering. Ciprofloxacin is a quinolone antibiotic that has been used for treatment of Gram-positive and Gram-negative infections in burn wound healing [30–32]. IGF-1 affects multiple types of cells and has been used for regenerative treatments including myocardial infarction [33], nerve defects [34], cartilage [35], and periodontal diseases [36]. BMP-2 belongs to the TGF-β family, is involved in cartilage and bone formation [37], and is used clinically for spinal fusion and dental applications (Infuse/Inductos®, Medtronic) [38–40].

## 2. Materials and methods

### 2.1. Preparation of modified kerateines and characterization of thiol content

Alpha fractions of kerateine (KTN) and keratose (KOS) were obtained as lyophilized, sterile (via 2 MRad gamma irradiation) powders from KeraNetics, LLC (Winston-Salem, NC). KTN was alkylated by adding iodoacetamide (Sigma, St. Louis, MO) at 0, 0.4, 0.9 or 2.5-fold molar ratios of keratin cysteine residues in deionized water at pH 9.6 for 1 h at room temperature. Table 1 shows the amounts of kerateine, water, and iodoacetamide used for preparation of the materials reported below. In each case, the reaction proceeded for 1 h at room temperature. The resulting alkylated kerateine was dialyzed in 3500 Da MWCO dialysis tubing (Spectrum Labs, Houston, TX) against deionized water at pH = 7 with 3 changes of dialysis buffer over the course of 3 days. After dialysis, the kerateine solutions were frozen at −80 °C and lyophilized to obtain the final powder form of alkylated kerateine, which we refer to as modified kerateine (MKTN).

The free thiol content was determined by Ellman's assay [41]. In brief, keratin solutions (KOS, KTN, or MKTN) were prepared in water at 1 mg/mL with the concentration confirmed by a modified Lowry method (DC protein assay; Bio-Rad, Hercules, CA). 2 μL of 100 mM Ellman's reagent in DMSO (Sigma, St. Louis, MO) was added to the keratin solution. The resulting mixture was allowed to react for 10 min at room temperature and the absorbance was read on a Biotek Synergy HT microwell plate reader (Winooski, VT) at 420 nm and compared to a standard curve of known amounts of cysteine. The free thiol content was used to identify the various formulations (e.g., 7% free thiol is referred to below as 7% S-S MKTN).

### 2.2. Formation of keratin (KOS, KTN, or MKTN) hydrogels

All keratin gels (KOS, KTN, or MKTN) described for Sections 2.3–2.6, 2.10 and 2.11 were fabricated at 15% (w/v). In a typical preparation, 150 mg of keratin (KOS, KTN, or MKTNs) was placed in a 15 mL conical tube. 1 mL of water or water containing therapeutic agents (see Section 2.11 below) was added. The mixtures were vigorously mixed by vortex and manual agitation. The mixtures were then briefly centrifuged. Typically, the resulting mixtures of keratin were packed into a 1 mL syringe, and known volumes (and masses) were placed into 1.5 mL tubes (Sections 2.3, 2.10 and 2.11), Sylgard molds (Sections 2.4–2.5), or wells of 48-well plates (Section 2.6), depending on the experiments described below. Then, the mixtures were allowed to incubate overnight at 37 °C to gel.

### 2.3. Scanning electron microscopy of keratin materials

We used Scanning Electron Microscopy (SEM) to visualize the porous architecture of keratin materials. Keratin hydrogels (KOS, KTN, or MKTNs) were fabricated as described in Section 2.2. After loading into a syringe, approximately 400 μL of keratin was placed into a 1.5 mL tube and allowed to gel overnight at 37 °C. The keratin gels were then frozen at −80 °C overnight and placed on a Labconco freeze drier (Kansas City, MO) for at least 24 h. The resulting samples were cut horizontally with a scalpel blade to expose the internal structure, mounted on an SEM stub, immediately sputter coated for 30 s at 45 mV (Desk II, Denton Vacuum, Moorsetown, NJ), and imaged at 5.0 kV with a Zeiss Supra 35 (Carl Zeiss Microscopy, Thornwood, NY).

### 2.4. Swelling and compression testing for characterization of network structure of keratin hydrogels

15% (w/v) keratin gels (KOS, KTN, or MKTNs) were prepared as described in Section 2.2. After placing into the 1 mL syringe, the keratin was injected into a 5 mm diameter by 5 mm high mold (made of Sylgard 184) and allowed to incubate overnight at 37 °C to gel. The swelling ratio, Q, was determined as described previously by others [42]. In brief, the keratin gel cylinders were weighed after gelation (dry mass, $M_d$) and placed into phosphate buffered saline until they reached an equilibrium swelling point (swelling mass, $M_s$). The swelling time was found to be 2 h by a pilot experiment, and we noted that keratose gels began to erode

Download English Version:

https://daneshyari.com/en/article/6483565

Download Persian Version:

https://daneshyari.com/article/6483565

Daneshyari.com