Acta Biomaterialia 36 (2016) 164–174

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Fluorinated methacrylamide chitosan hydrogels enhance collagen synthesis in wound healing through increased oxygen availability

Pritam S. Patil [a], Natalie Fountas-Davis [a], He Huang [b], M. Michelle Evancho-Chapman [c], Judith A. Fulton [d], Leah P. Shriver [b], Nic D. Leipzig [a,*]

[a] Department of Chemical and Biomolecular Engineering, University of Akron, OH 44325, USA
[b] Department of Chemistry, University of Akron, OH 44325, USA
[c] Summa Akron City Hospital, Akron, OH 44304, USA
[d] Akron General Medical Center, Akron, OH 44307, USA

ARTICLE INFO

Article history:
Received 16 November 2015
Received in revised form 7 March 2016
Accepted 14 March 2016
Available online 18 March 2016

Keywords:
Chitosan
Perfluorocarbons
Oxygen delivery
Metabolomics
Wound healing

ABSTRACT

In this study, methacrylamide chitosan modified with perfluorocarbon chains (MACF) is used as the base material to construct hydrogel dressings for treating dermal wounds. MACF hydrogels saturated with oxygen (+O_2) are examined for their ability to deliver and sustain oxygen, degrade in a biological environment, and promote wound healing in an animal model. The emerging technique of metabolomics is used to understand how MACF + O_2 hydrogel dressings improve wound healing. Results indicate that MACF treatment facilitates oxygen transport rate that is two orders of magnitude greater than base MAC hydrogels. MACF hydrogel dressings are next tested in an *in vivo* splinted rat excisional wound healing model. Histological analysis reveals that MACF + O_2 dressings improve re-epithelialization ($p < 0.0001$) and synthesis of collagen over controls ($p < 0.01$). Analysis of endogenous metabolites in the wounds using global metabolomics demonstrates that MACF + O_2 dressings promotes a regenerative metabolic process directed toward hydroxyproline and collagen synthesis, with confirmation of metabolite levels within this pathway. The results of this study confirm that increased oxygen delivery through the application of MACF + O_2 hydrogels enhances wound healing and metabolomics analyses provides a powerful tool to assess wound healing physiology.

Statement of Significance

This work presents the first application of a novel class of oxygen delivering biomaterials (methacrylamide chitosan modified with perfluorocarbon chains (MACF)) as a hydrogel wound dressing. This manuscript also contains strong focus on the biochemical benefits of MACF dressings on underlying mechanisms vital to successful wound healing. In this vein, this manuscript presents the application of applied metabolomics (tandem mass spectroscopy) to uncover biomaterial interactions with wound healing mechanisms. We believe the approaches described in this manuscript will be of great interest to biomedical scientists and particularly to researchers studying wound healing, metabolomics, applied biomaterials and regenerative medicine.

1. Introduction

Successful wound healing is a process of events that begins with hemostasis followed by overlapping phases of inflammation, proliferation, and reorganization, resulting in newly formed tissue [1]. Wounds with impaired healing are often mired in a vicious cycle of hypoxia and prolonged inflammation and do not proceed through the normal healing process [2]. It is well established that oxygen availability has a profound influence on repair processes [3]. During the early stages of wound healing, reduced nicotinamide adenine dinucleotide (NADH) oxidase in the presence of molecular oxygen produces superoxide radicals, which help to prevent bacterial infections [4]. Oxygen has also been shown to regulate angiogenesis and encourage cell proliferation and cell motility [5]. Further, oxygen is a prerequisite for hydroxyproline synthesis, which is used as one of the building blocks of collagen that allows

* Corresponding author at: Department of Chemical and Biomolecular Eng., 200 East Buchtel Common, The University of Akron, Akron, OH 44325-3906, USA.
E-mail address: nl21@uakron.edu (N.D. Leipzig).

http://dx.doi.org/10.1016/j.actbio.2016.03.022
1742-7061/

fibrillogenesis, increased tensile strength, and improved wound remodeling [6,7]. Although hypoxia initiates neovascularization, it cannot sustain it [7–9]. Thus, supplemental oxygen cycling is required in many cases to facilitate accelerated wound healing [8].

Several studies have shown that wound oxygenation is closely tied to the rate of wound healing [10]. Hyperbaric oxygen therapy is a clinically effective treatment for hypoxic chronic wounds, but the associated costs to build and maintain facilities, overall availability to patients, and the willingness of insurers to reimburse treatments, are major obstacles to widespread adoption of the technology [11]. Various attempts are being pursued to deliver oxygen to the wound via cheaper and less cumbersome methods [12]. One strategy is the *in situ* formation of oxygen within a biomaterial scaffold via chemical reaction or enzymatic transformation. Unfortunately, this strategy has a number of shortcomings including the possibility of systemic side-effects from the locally applied reactive chemicals or enzymes [13], local pH changes, and heating via exothermic reactions. Specific biomaterial strategies have been developed to blunt these potentially negative effects [9,12,14], or avoid their need altogether including work using methacrylamide chitosan modified with perfluorocarbons (MACF) [15,16].

Perfluorocarbons (PFCs) were originally introduced as blood substitutes because of their impressive abilities to carry respiratory gasses, especially oxygen, in biological conditions [17]. PFCs are created by complete fluorination of hydrocarbons (replacement of hydrogen with fluorine), which generates a biologically non-reactive moiety [18]. The presence of a dense electron cloud, higher ionization potential, and greater electron affinity give rise to the highly hydrophobic and fluorophilic structure of PFCs [19]. The hydrophobic nature of PFCs is typically problematic in biologic aqueous environments if not conjugated to a more hydrophilic structure. PFCs are often combined with excipients and formed into short-lived colloidal suspensions [20]. To overcome these PFC limitations, MACF can form the basis of aqueous hydrogels that can sustain the benefit of PFCs and be used to enhance local oxygenation [16]. Chitosan is the base biopolymer of choice, because it is naturally abundant, biodegradable, and acceptable for use in many biomedical applications [21]. Chitosan is characterized as a polysaccharide polymer of β-(1,4) linked D-glucosamine that is similar in structure to hyaluronic acid (HA), a predominant constituent of the mammalian extracellular matrix (ECM) [22]. The free amino group on a primary sugar ring in chitosan is advantageous, as it allows various bioconjugate substitutions and can serve as a ligand for complex formation [23]. We have demonstrated that nucleophilic substitution targeting chitosan's primary amine groups is a straightforward way to add methacrylamide and PFCs through amide linkage. Chitosan itself acts as an antimicrobial agent against fungi, bacteria, and viruses [24], and has been shown to have beneficial hemostatic properties [25].

A plethora of FDA-approved clinical wound dressings focus on maintaining wound hydration, antimicrobial properties, and supportive ECM structure for new granulation tissue [16,26]. Many wound dressings used in standard wound care are synthetic such as polyester pads, polyurethane foams, and various synthetic blends [16]. Other materials based on collagen, alginate, cellulose, and gelatin have been formatted into dressings, and a variety of further improvements to enhance healing are being tested in preclinical studies [27]. Hydrogels have been shown to be especially beneficial for treating light to non-exudating wounds, since they provide a moist healing environment and promote the wound healing process [28]. Unfortunately, there are few dressing-based technologies available on the market to address the problem of hypoxia [29]. Thus, there exists a need for easy and affordable treatment that can provide oxygen to the wound and provide the benefits of a hydrogel dressing.

Metabolomics is the study of small molecules involved in metabolic processes in an organism [30]. Metabolomics is a powerful tool to assay end points of the genome and proteome and reveal the underlying biochemistry and physiologic state [30]. Recently, the need has been highlighted for high throughput biological techniques such as metabolomics, proteomics, and genomics to reveal new understanding of wound healing [31]. In a recent study, wound fluid samples were analyzed with proteomics and metabolomics to improve the understanding of bone defects and its connection to the biochemical mechanisms in the wound environment [32]. Another study used metabolomics profiling of diabetic and non-diabetic wounds in mice and identified key metabolites that were differentially regulated and could serve as future biomarkers [33]. Notably, only a few researchers have so far used metabolomics to understand interactions of biomaterials with biological environments [34,35].

Given the importance of oxygen in wound healing and tissue regeneration, the main objectives of this study were to create an oxygenating MACF hydrogel dressing and to evaluate this dressing in a rat excisional wound model. Metabolomics was used to reveal the physiologic state and confirm regenerative processes tied to wound treatment with MACF dressings. The MACF hydrogel studied here provides a dressing that can deliver oxygen to the wound and sustain enhanced local levels of oxygen to overcome hypoxia while providing a beneficial moist environment for enhancing reparative processes. Using MACF eliminates the need of complex oxygenating set-ups (e.g., hyperbaric oxygen, oxygen generators), colloidal suspensions, chemical reactions, and enzymatic conversions.

2. Materials and methods

2.1. Preparation of pentadecafluorooctanoyl methacrylamide chitosan hydrogels

Fluorinated and non-fluorinated methacrylamide chitosan hydrogels, MACF and MAC, respectively, were created and characterized as previously described [14]. Briefly, chitosan, average molecular weight 200,000 g/mol and degree of deacetylation 82%, (Novamatrix, Drammen, Norway) was used in a two-step reaction using methacrylic anhydride (Sigma-Aldrich, Saint Louis, MO, USA) to add methacrylate groups, followed by reaction with pentadecafluorooctanoyl chloride (Sigma-Aldrich) to obtain MACF. The resulting material was freeze-dried (Labconco, Kansas City, MO) for storage. For NMR quantification chitosan, MAC and MACFs were dissolved in 2 vol% deuterated acetic acid/D2O. Methacrylation and fluorine substitution were confirmed by 19F and 1H NMR (Varian 500 MHz). The corresponding peak areas were used to calculate% degree of substitution [15]. To form hydrogels, MACF was first dissolved in ultrapure water (18 MΩ resistance, Millipore, Billerica, MA, USA) and sterilized by autoclaving. Photoinitiator solution, 1-hydroxycyclohexyl phenyl ketone (Sigma-Aldrich) dissolved in 1-vinyl-2-pyrrolidinone (Sigma-Aldrich), was added at (0.9 mg/g) to MACF/MAC polymer solutions (2% wt/wt in ultrapure water). The resulting solution was mixed and degassed (Speed Mixer DAC 150 FVZ, Hauschild Engineering, Hamm, Germany) at 3000 RPM for 2 min at room temperature (RT). Hydrogels were then washed extensively with phosphate buffer saline (PBS, pH 7.4) to remove unreacted polymer and crosslinker.

2.2. Oxygen permeability/diffusion study

A glass chamber was designed to perform the oxygen permeability studies and fabricated by the University of Akron Glass Shop (Fig. 1A). A surgical grade membrane, Tegaderm™ (3 M, Saint Paul,