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pH sensitive methacrylated chitosan hydrogels with tunable physical and chemical properties

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ABSTRACT

The pH of cutaneous wounds is dynamic and correlates with the stage of the wound healing process. The inflammation stage is acidic, granulation shifts to a progressively alkali pH, and the remodeling phase returns skin to its pre-injury pH. By taking the advantage of this pH difference, stage-specific wound treatments can be developed to respond to these environmental cues using pH sensitive hydrogels. To obtain tunable physical and chemical properties, chitosan was first methacrylated and then crosslinked through three polymerization methods step growth by a thiol-ene photoclick reaction, chain growth by UV polymerization, and mixed mode in which both step growth and chain growth mechanisms were used. pH sensitive methacrylated chitosan (MAC) hydrogels were synthesized and confirmed through ^{1}H NMR. The resulting hydrogels exhibited adjustable mechanical properties, swelling ratios, and pH sensitivities without affecting degradation behavior or *in vitro* cell responses. Cytocompatibility studies were performed using NIH/3T3 fibroblasts. Cell proliferation or adhesion was suppressed when seeded on the hydrogel surfaces compared to tissue culture plastic (TCP), yet no measureable cell death was observed. The responsivity of these gels to changing pH environments may prove useful as stage-responsive wound dressings.

1. Introduction

Wound healing can be divided into four stages: hemostasis, inflammation, proliferation, and remodeling [1]. Generally, hemostasis occurs shortly after injury with inflammation following immediately and persisting on the order of days for acute wounds. Next, epithelial cells and fibroblasts migrate to the wound site to synthesize collagen and regenerate tissues during the proliferation stage [2]. During the final stage – remodeling – connective tissue is generated and collagen fibers are reconstructed, which determines the extent of scarring [1,2]. Inappropriate treatment may lead to infection or hematoma formation and result in impaired tissue repair and chronic wounds [3]. Approximately 2.4–4.5 million Americans have chronic lower extremity ulcers which results in costs of $20 billion annually [4,5].

Current efforts have mostly focused on cellular behavior such as inflammation and infiltration [6–8]; however, the pH of the wound site is dynamic during the healing process and is often neglected when designing wound dressings. The surface of normal skin is commonly below pH 5, which is essential for pathogen prevention and enzymatic activity [9]. Once injured, the internal tissue having a pH of 7.4 will be exposed, which provides a favorable environment for bacterial multiplication. Cellular products released during the inflammation stage of wound healing decrease the pH, which favors antibiotic activity and can reduce bacterial colonization [10–12]. The shift from the inflammatory stage (pH 5.7 ± 0.5 standard deviation (SD)) to the proliferative stage (7.6 ± 0.2 SD) results in a gradual pH increase [11]. This process is illustrated in Fig. 1. For acute wounds, the healing process transitions to the remodeling stage and the pH returns to that of normal skin. In chronic wounds, the pH remains alkali [11].

Hydrogels are often used as bioactive wound dressings. Passive wound dressings are designed to staunch blood flow and prevent infection [1]. Bioactive wound dressings can release growth factors, antibacterial agents, corticosteroids, and other drugs designed to aid in chronic wound healing [1]. By taking advantage of pH changes during wound healing, 'smart wound' dressings could be created using pH sensitive hydrogels, which could yield stage-specific treatment and significantly accelerate wound healing.

Chitosan is formed by chitin deacetylation and consists of β-(1-4)-linked D-glucosamine and *N*-acetyl-D-glucosamine [13].

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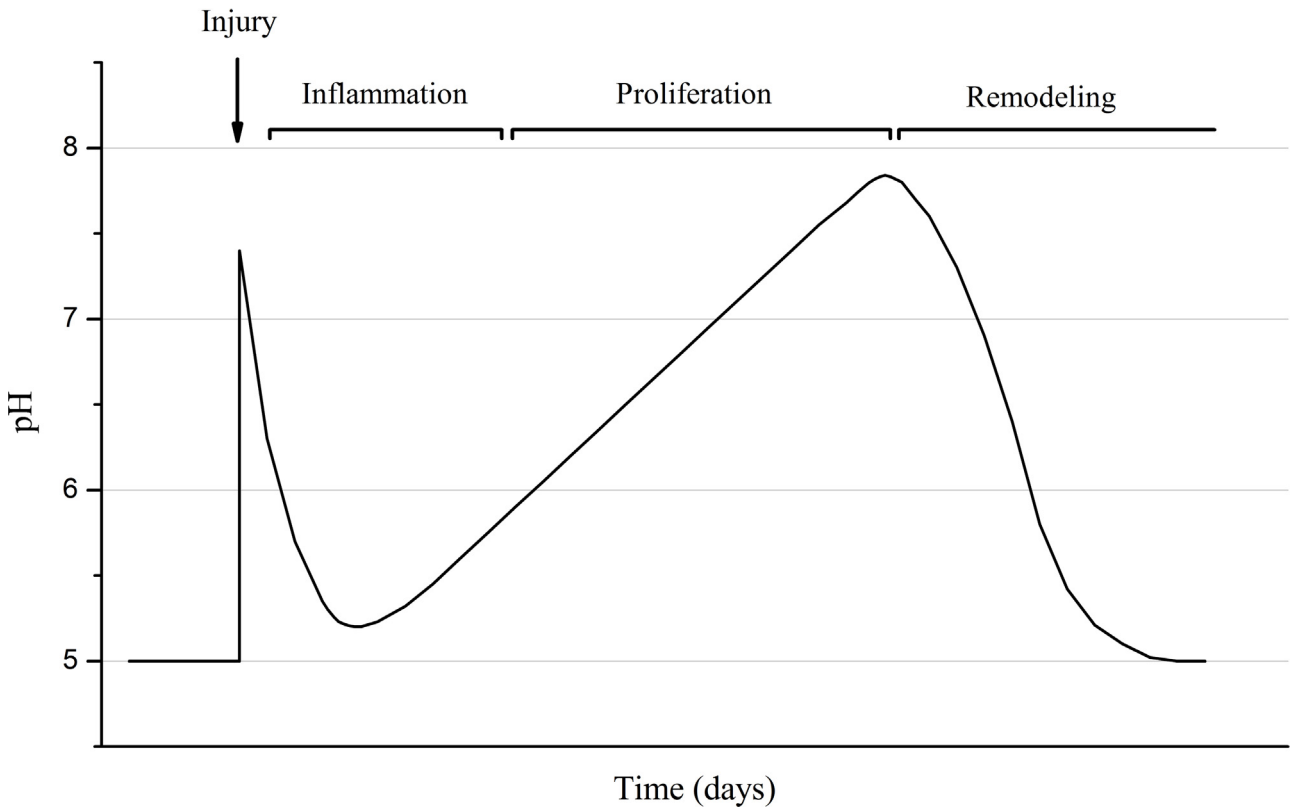

Fig. 1. pH change during wound healing process. Reprinted with permission of Springer [11].

Chitosan is the most common naturally occurring cationic polymer [14]. As a cationic hydrogel, chitosan wound dressings can promote binding of negatively charged proteoglycans and glycosaminoglycans, which can further promote adsorption of bioactive molecules [15,16]. This cationic nature has also been reported to limit bacterial metabolism through electrostatic stacking preventing mass transport of nutrients [17]. Chitosan has a $pK_a \sim 6.5$, which confers pH sensitivity yielding a swollen gel or dissolved polymer in acidic conditions and a stable material at alkali pHs [18]. Hydrogels that swell under acidic conditions at the early stages of wound healing may lead to enhanced cell infiltration and proliferation and increased oxygen permeability.

Chitosan wound dressings are known to alter macrophage properties [19], stimulate cell proliferation [20], and exhibit hemostatic effects [14]. Although chitosan is not native to the human body, its structure is similar to hyaluronic acid, which is one of the main components of the extracellular matrix and plays an important role in cutaneous wound healing [13,21,22]. Chitosan is enzymatically degradable and cell-adhesive [23]. It can be degraded by several human enzymes, particularly lysozyme, which decomposes the β-(1-4)-glycosidic bonds in the chitosan backbone [24–26]. The degradation product, *N*-acetyl-β-D-glucosamine, is known to increase fibroblast proliferation and activates hyaluronic acid synthesis, thus aiding the healing process [1].

In this work, we present a method for functionalizing chitosan with methacrylic anhydride such that the polymer can be photo crosslinked through chain-growth, step-growth, or a combination of both mechanisms referred to as mixed mode (Fig. 2) [27]. MAC based hydrogels have been reported to be biocompatible [6,23]. For the chain growth mechanism, photopolymerization activating the carbon–carbon double bond was achieved using Irgacure (I2959) after degassing the polymer mixture [28]. I2959 acts as an initiator to create a free radical under UV light. The free radical further reacts with carbon–carbon double bonds to form the hydrogel network [27]. Gelation via step growth polymerization involves a thiol-ene photoclick reaction, which is different from Michael-type addition in that both photoinitiator and thiol groups are required [29]. The UV activated photoinitiator first abstracts a proton from the thiol group to generate a thiyl radical, which reacts with an alkene group, further forming a carbon-centered radical [30,31]. In this reaction, chain growth gelation is largely quenched by oxygen [32]. For mixed-mode polymerization, both the step-growth and chain-growth mechanisms were used to form the gel by introducing the thiol crosslinker and removing oxygen. Since chain growth gelation can still occur in the presence of oxygen [27], there is a possibility that both mechanisms are occurring in the step growth gel. Chemically crosslinking naturally occurring polymers has been done on gellan gum [33,34], hyalyronic acid [35], and alginate [36]. Thiol-ene chemistry has been employed on hyaluronic acid [37], dextran [37], and polyethylene glycol [38]. Here, we have fabricated chitosan based hydrogels, taking advantage of their pH sensitivity for potential application as a wound dressing.

We hypothesized that the crosslinking density would change with different cross-linking mechanisms and, therefore, the mechanical and pH responsive swelling properties could be tuned. It has been shown that lower stiffness materials generally result in successful dermal implants [19]. The aim of this study was to synthesize pH sensitive hydrogels with various mechanical and pH-responsive properties to exam their potential application value as a wound dressing. The expectation is to create a "smart" hydrogel that may have applications in wound healing.

2. Experimental

2.1. Materials

Chitosan (50–100 cps, 0.5% in 0.5% acetic acid, 87% degree of deacetylation) was obtained from Tokyo Chemical Industry.