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Synthesis of a chitosan derivative soluble at neutral pH and gellable by freeze–thawing, and its application in wound care

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ABSTRACT

Conventional chitosan hydrogels exhibit an acidic nature and contain unfavorable additives because (i) chitosan is soluble only in acidic solutions and (ii) toxic chemicals or proteins of non-human origin that serve as antigens are necessary for preparing chitosan hydrogels. These characteristics of the chitosan hydrogels limit their possibilities as wound dressings. In this study, a chitosan–gluconic acid conjugate is developed, soluble in an aqueous solution at neutral pH and gellable by freeze–thawing (cryogelation) without using additives. The viability of L929 fibroblasts cultured in the presence of the chitosan derivative for 24 h was >96%. The degradation rate of the corresponding chitosan cryogels by lysozyme was tunable via the derivative concentration in the gels. The gels had low cellular adhesiveness. The gels promoted the accumulation of inflammatory cells such as polymorphonuclear leukocytes, which have the potential to release chemical mediators effective for wound healing, in full-thickness skin wounds in rats and accelerated the healing of the wounds. These results demonstrate that cryogels are promising for wound care.

1. Introduction

Moist wound healing refers to the provision of a moist environment to exposed tissue in the wound as opposed to traditional dry wound healing, which allows or encourages those tissues to dehydrate and dry out [1]. Compared with dry healing, moist healing results in increased healing rates and reduced infection, owing to the complex mechanisms, e.g., prevention of scab formation and drying of exudates containing nutrients, oxygen, growth factors and leucocytes [2,3]. Hydrogels are suitable as wound dressings for moist healing owing to their hyperhydrous structure. Various inert polymers (poly(methacrylates), polyvinylpyrrolidone, poly(vinyl alcohol) (PVA), poly(ethylene glycol), carboxymethylcellulose and alginate) have been used as hydrogel dressing materials [3–5].

Chitosan is an aminopolysaccharide present in fungal cell walls and exoskeletons of arthropods such as insects and crabs [6]. The polysaccharide has already been proposed as a biomedical material because of various characteristics, such as biocompatibility, non-toxicity and biodegradability [7,8]. In particular, antimicrobial and hemostatic activities of chitosan are promising for wound care [9]. In addition, chitosan has been reported to promote granulation and organization of wounds by the enhancement of the functions of inflammatory cells, such as polymorphonuclear leukocytes (PMN) and macrophages [2,10]. Thus, hydrogel wound dressings composed of chitosan have the potential to greatly promote wound repair owing to the inherent biological properties of the polysaccharide, compared with the inert hydrogels described above. However, conventional chitosan hydrogels exhibit an acidic nature and contain unfavorable additives, because (i) chitosan is soluble only in acidic solutions, owing to the rigid crystalline structure associated with intra- and/or intermolecular hydrogen bonding [8] and (ii) toxic chemicals (crosslinkers, polymerization initiators and salts) and/or proteins of non-human origin which serve as antigens are necessary for preparing chitosan gels [6,11–16]. These characteristics of the chitosan gels limit their possibilities as wound dressings.

In this study, a chitosan derivative was developed, soluble at neutral pH and gellable by freeze–thawing (cryogelation). The chitosan cryogels, exhibiting neutral pH and containing no additives, can eliminate the drawbacks of traditional chitosan gels. In addition, the gels would enable growth factors that accelerate wound healing to be incorporated with less denaturation, compared with the traditional chitosan gels. Although chitosan derivatives soluble at neutral pH and gellable without use of unfavorable additives have been reported, they show thermoreversible gelling behavior (i.e., a gel at body temperature and a sol at lower temperature) [17,18]. This characteristic is unsuitable for wound dressings, as the corresponding gels would dissolve at wound sites on the body surface, because hydrogel temperature depends on the external temperature (lower than body temperature in most situations).

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