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Enhancing effect of γ-cyclodextrin on wound dressing properties of sacran hydrogel film

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ABSTRACT

A wound dressing is one of the essential approaches for preventing further harm to cutaneous wounds as well as promoting wound healing. Therefore, to achieve ideal wound healing, the development of advanced dressing materials is necessary. Recently, we revealed that a novel megamolecular polysaccharide, sacran, has potential properties as a biomaterial in a physically cross-linked hydrogel film (HGF) for wound dressing application. In this study, to enhance the wound-healing properties of sacran hydrogel film (Sac-HGF) further, we fabricated and characterized novel Sac-HGFs containing cyclodextrins (CyDs). The sacran/α-CyD film (Sac/α-CyD-HGF) and sacran/γ-CyD HGF (Sac/γ-CyD-HGF), but not sacran/β-CyD HGF (Sac/β-CyD-HGF), were well prepared without surface roughness. Powder X-ray diffraction (XRD) patterns of the Sac/γ-CyD-HGFs showed a totally amorphous state compared to that shown by Sac/α-CyD-HGFs. Furthermore, the addition of γ-CyD to Sac-HGFs significantly increased the swelling ratio, porosity, and moisture content of the HGFs, compared to those of the Sac-HGF without CyDs. The Sac/γ-CyD-HGFs were not cytotoxic against NIH3T3 cells, a murine fibroblast cell line. Notably, the Sac/γ-CyD-HGFs significantly improved wound healing in mice, compared to that achieved with the Sac-HGF without γ-CyD. These results suggest that γ-CyD has the potential to promote the wound healing ability of Sac-HGF.

1. Introduction

Cutaneous wound healing, a natural biological reaction to skin injury, is a complex and intricate process [1,2]. Currently, the healing process is considered to consist of four stages. The hemostasis stage starts immediately after wound infliction, via platelet aggregation and fibrin clot formation. Then, the inflammatory stage can be identified by the presence of neutrophils for wound debridement as well as macrophages that release cytokines at the wound site. In the proliferative stage, fibroblasts penetrate the wound and deposit a new extracellular matrix to begin the re-epithelialization process. Finally, the synthesis of collagens and myofibroblasts facilitates further tissue remodeling processes [3,4]. All the stages must occur in the correct sequence and timespan to ensure complete healing [5].

A wound dressing plays a pivotal role in maintaining and producing a moist environment around the wound, which promotes cutaneous wound healing [6,7]. The moist environment provided by wound dressing materials is closely associated with the migration of epidermal cells in the re-epithelialization process and the formation of scar tissue in the inflammatory stage, in addition to the avoidance of tissue dehydration [8–10]. Presently, various hydrogel films (HGFs) are used as wound-dressing materials [11,12]. In particular, natural polymer-based hydrogels have attracted considerable attention because of their excellent swelling ability in aqueous solutions, biodegradability at the wound site, and hydrophilicity, which is important in drug delivery systems [13,14]. For example, konjac glucomannan films showed good biocompatibility and efficiently accelerated wound repair [15]. Additionally, the alginate films ionically crosslinked with calcium chloride ($CaCl_2$) had an impressive surface appearance and mechanical properties [16]. However, organic solvents and crosslinkers used often are potentially hazardous to the body [17]. Therefore, the physical crosslinking method, which does not require the addition

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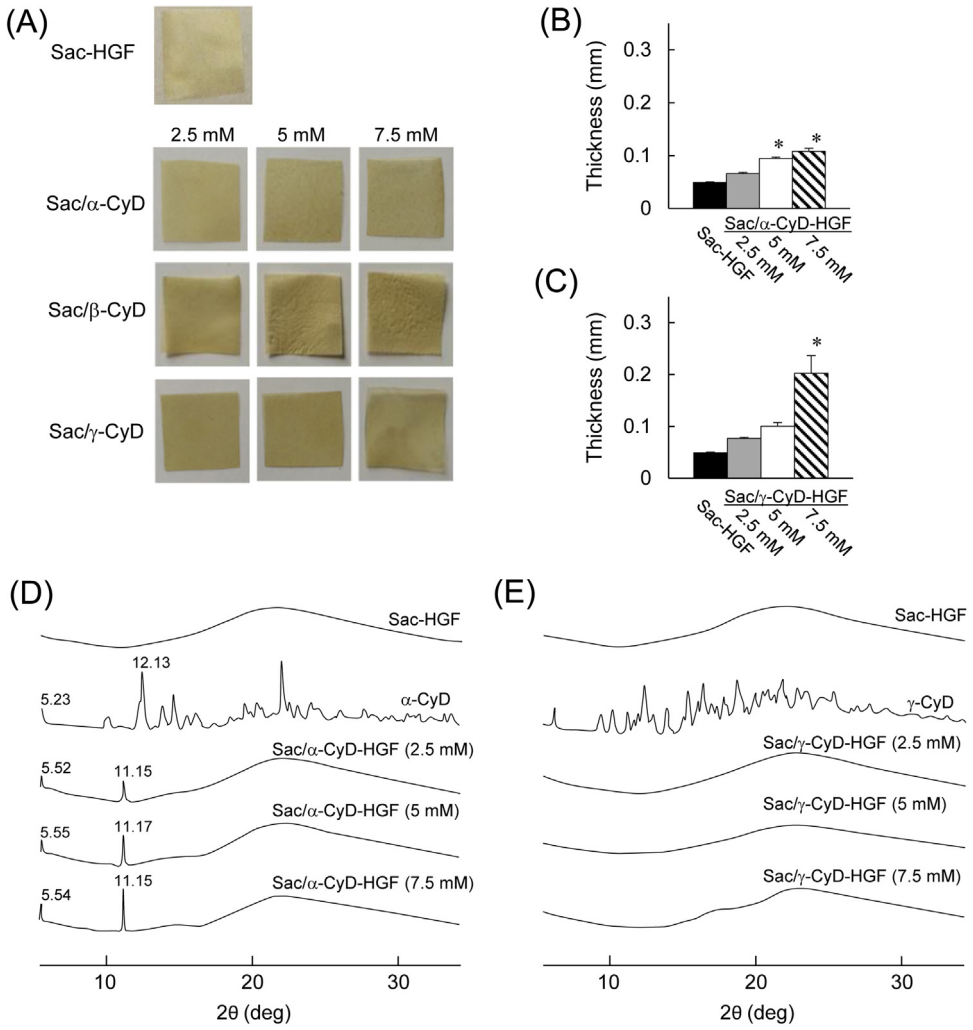

Fig. 1. Appearance, thickness, and crystallinity of sacran/cyclodextrin-hydrogel films (Sac/CyDs-HGFs) (A) Appearance of Sac/CyDs-HGFs. (B and C) Thickness of Sac/α-CyD-HGFs and Sac/γ-CyD-HGFs. Values are mean ± standard error of the mean (S.E.M.) of six experiments; $^{*}p < 0.05$ compared to sacran. (D and E) X-ray diffraction (XRD) patterns of Sac/α-CyD-HGFs and Sac/γ-CyD-HGFs.

of hazardous chemical crosslinkers, is expected to be a safe option for the preparation of HGFs.

Sacran, a novel megamolecular polysaccharide derived from the cyanobacterium *Apanothece sacrum*, has a very high molecular weight that exceeds 10^7 g/mol and water-superabsorbent capacity that is 6-fold higher than that of hyaluronan [18]. Additionally, sacran has a film-forming ability by the water-casting method and shows a self-assembled in-plane orientation [19]. Recently, we demonstrated that sacran provides anti-inflammatory activity by ameliorating the skin barrier function not only in various inflammatory models but also in patients with atopic dermatitis (AD) [20,21]. Based on this background, we previously prepared a physically crosslinked sacran HGF (Sac-HGF) and revealed its potential as a novel wound dressing material [22]. However, some properties of the Sac-HGFs require improvement such as the porosity, swelling capability, and skin hydration ability to enhance the potential of Sac-HGF for wound dressing application.

Cyclodextrins (CyDs), which are cyclic (α-1,4)-linked oligosaccharides of α-D-glucopyranose, are commonly used as pharmaceutical excipients for numerous purposes, including the enhancement of drug bioavailability and inclusion complex ability [23]. Recently, CyDs have been utilized as functionalized excipients in hydrogel formulations. For example, Demir et al. reported that β-CyD enhanced the swelling ability of a hydrogel composed of β-CyD urethane-methacrylate monomer, poly(ethylene glycol) diacrylate, and 2-hydroxyethyl methacrylate by the formation of a porous fractured surface [24]. In addition, we also reported that α-CyD and γ-CyD provided porous structures and improved the swelling ability of the insulin/α-CyD and insulin/γ-CyD polypseudorotaxane hydrogels, respectively [25].

Considering these observations, we hypothesized that CyDs might provide porous structures in Sac-HGFs, resulting in the maintenance of a moist environment in the wounded skin area. Therefore, in this study, we prepared novel physically cross-linked Sac-HGFs containing CyDs and examined their physicochemical properties, cytotoxicity, skin hydration, and wound healing ability.

2. Materials and methods

2.1. Materials

The sacran used in this study was kindly provided by Green Science Material (Kumamoto, Japan) while α-CyD, β-CyD, and γ-CyD were a kind gift from Nihon Shokuhin Kako (Tokyo, Japan). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Nissui Pharmaceuticals (Tokyo, Japan) and Nichirei (Tokyo, Japan), respectively.