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Physically Crosslinked-Sacran Hydrogel Films for Wound Dressing Application

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Abstract

The thin hydrogel films consisting of water-swollen polymer networks can potentially be applied for biomedical fields, such as tissue engineering, drug delivery, and wound dressing. Recently, natural polysaccharides have great attentions to be developed as wound healing and protection due to film-forming ability, biodegradability, hydrophilicity, and excellent swelling behavior. In the present study, we newly prepared and characterized a physically crosslinked-hydrogel film composed of a novel megamolecular polysaccharide sacran for wound dressing application. We successfully fabricated a physically crosslinked-sacran hydrogel film by a solvent-casting method. The thickness of a sacran hydrogel film was lower than that of a sodium alginate (Na-alginate) film. Importantly, the swollen ratio of a sacran hydrogel film in water at 24 h was 19-fold, compared to initial weight. Meanwhile, a Na-alginate hydrogel film was completely broken apart after rehydration. Moreover, a sacran hydrogel film did not show any cytotoxicity on NIH3T3 cells, a murine fibroblast cell line. The *in vivo* skin hydration study revealed that a sacran hydrogel film significantly increased the moisture content on hairless mice skin. In addition, a sacran hydrogel film, which was applied on wound site, considerably improved wound healing ability, compared to control (non-treated), probably due to not only the moisturing effect but also the anti-inflammatory effect of sacran. These results suggest that sacran has the potential properties as a basic biomaterial in a hydrogel film for wound dressing application.

Keywords: sacran, hydrogel films, polysaccharides, wound dressing

1. Introduction

The thin hydrogel films consisting of water-swollen polymer networks have attracted a lot of attentions for the last few decades because of excellent properties like stimuli-responsive behavior, macroporous structure, and molecularly imprinted polymers [1-3]. Additionally, it can potentially be applied for several biomedical applications, outstandingly in wound dressing application [4, 5]. Wound dressings are essential in wound healing therapy due to the moisturing effect to avoid not only tissue dehydration but also cell death in regeneration during repairing of dermal and epidermal tissues [6-8].

In general, the ideal properties of wound dressing materials must be biocompatible, non-irritating, non-toxic and suitable mechanical properties as well as the moisturing ability for skin [9]. Furthermore, wound dressing materials need various properties, depending on the type of wound [10]. For instance, in open wound, the dressing materials having more porous structures and swelling abilities are promising. Meanwhile, in closed wound, the durable dressing materials are important [11].

Many hydrogel films are prepared by chemical or physical crosslinking method to obtain the ideal hydrogel film properties. A highly elastic and durable polyvinyl alcohol (PVA) hydrogel films were successfully fabricated by a chemical crosslinking method using potassium persulphate as a crosslinker [12]. The similar properties also appeared in pullulan/polyvinyl alcohol (PVA) blend films which were prepared by casting the polymer solution in dimethyl sulfoxide and using glyoxal as chemical crosslinkers [13]. However, organic solvents and crosslinkers are potentially hazardous in the body [14]. Meanwhile, the physical crosslinking does not require the addition of hazardous chemical crosslinkers. The hydrogel films prepared physically with sodium alginate (Na-alginate) as a matrix agent and propylene glycol as a plasticizer were successfully developed by Aktar *et al* [15]. Moreover,

2. Materials and methods

2.1. Materials

Sacran was kindly donated by Green Science Material (Kumamoto, Japan). Na-alginate (80-120 cP) was purchased from Wako Pure Chemical Industries (Osaka, Japan). NIH3T3 cells, a murine fibroblast cell line, procured from Riken Bioresource Center (Tsukuba, Japan). Fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium (DMEM) were purchased from Nichirei (Tokyo, Japan) and Nissui Pharmaceuticals (Tokyo, Japan), respectively.

2.2. Preparation of hydrogel films

Sacran hydrogel film was prepared by a solvent-casting method referred to Okajima *et al* [16]. Briefly, sacran (0.5% (w/v)) was mixed and dissolved in 50 mL of distilled water as shown in Fig. 1. Sacran solutions were placed for 24 h at 80°C. Then, sacran solutions were poured into the polypropylene boxes (5 x 5 x 4 cm^3) and dried for 48 h at 60°C (EYELA SLI-600ND, Tokyo, Japan). After forming the films, they were heated for 2 h at 110°C (EYELA NDO-401, Tokyo, Japan) to obtain sacran hydrogel films. A Na-alginate (0.5% (w/v)) hydrogel film was also prepared as comparison with the same protocol.

2.3. Characterization of hydrogel films

2.3.1. Thickness

Thickness of hydrogel film was measured by a dial thickness apparatus (Teclock, Nagano, Japan) on three different surface areas of hydrogel films.

2.3.2. X-ray diffractometric analysis

3. Results and discussion

3.1. Preparation of hydrogel films

The solvent-casting method was used to fabricate a physically crosslinked-sacran hydrogel film (Fig. 1A). Sacran solutions were poured into the polypropylene boxes and dried at 60°C in an oven to obtain dried films. Then, the dried films were heated at 110°C for 2 h to get sacran films. Herein, the drying and heating processes were critical steps for a preparation of physically crosslinked-sacran films as Okajima *et al.* reported [16]. Namely, the drying process at 60°C was important to evaporate the solvent gradually, and then sacran molecules effectively aligned and provided an orientation of sacran molecules in films. In addition, the heating process has a critical role to form crosslinking junctions between sacran chains. Sacran has annealing temperature in the range of 70°C to 140°C and the number of crosslinking junctions is efficiently risen by increasing the annealing temperature [16]. As shown in Fig. 1B and 1C, sacran and Na-alginate hydrogel films were successfully prepared and the thickness of the films were 0.049 nm and 0.057 mm, respectively.

3.2. Characterization of hydrogel films

Generally, it is important for hydrogel films to have an amorphous state due to a high thermodynamic activity [17, 18]. To investigate the crystalline or amorphous state of a sacran hydrogel film, we analyzed the X-ray diffraction pattern of the hydrogel films. As shown in Fig. 2A, the X-ray diffraction patterns of sacran and Na-alginate hydrogel films were amorphous patterns. These results suggest that both sacran and Na-alginate hydrogel films have a high thermodynamic activity.

We next examined the thermal behavior of hydrogel films by DSC (Fig. 2B). The endothermic peaks of sacran and Na-alginate hydrogel films were found around 100ºC derived from dehydration process. The degradation peak of sacran hydrogel film at 260ºC

4. Conclusions

In the present study, we successfully fabricated a physically crosslinked-sacran hydrogel films by a solvent-casting method. Importantly, the sacran hydrogel films considerably improved wound healing ability, probably due to not only the moisturing effect but also the anti-inflammatory effect of sacran. These results suggest that sacran has the potential properties as a basic biomaterial in a hydrogel film for wound dressing application.

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Conflict of interest

The authors have no conflict of interest directly relevant to the content of this article.

References

[1] S. Naficy, G.M. Spinks, G.G. Wallace, ACS Appl Mat Int, 6 (2014) 4109-4114.

[2] J. Sun, M.A. Memon, W. Bai, L. Xiao, B. Zhang, Y. Jin, Y. Huang, J. Geng, Adv Func Materials, 25 (2015) 4334-4343.

[3] K. Zhao, T. Chen, B. Lin, W. Cui, B. Kan, N. Yang, X. Zhou, X. Zhang, J. Wei, Reactive Func Polymers, 87 (2015) 7-14.

[4] S.K. Bajpai, V. Pathak, B. Soni, Int J Biol Macromol, 79 (2015) 76-85.

[5] R. Pereira, A. Carvalho, D.C. Vaz, M.H. Gil, A. Mendes, P. Bartolo, Int J Biol Macromol, 52 (2013) 221-230.

[6] A.A. Adamian, S.V. Dobysh, L.E. Kilimchuk, I.N. Shandurenko, I.A. Chekmareva, Khirurgiia, (2004) 10-14.

[7] G.D. Mogoşanu, A.M. Grumezescu, Int J Pharm, 463 (2014) 127-136.

[8] G.D. Winter, Nature, 193 (1962) 293-294.

[9] M. Shojaee, F. Navaee, S. Jalili-Firoozinezhad, R. Faturechi, M. Majidi, S. Bonakdar, Mater Sci Eng C Mater Biol Appl, 48 (2015) 158-164.

[10] J.S. Boateng, K.H. Matthews, H.N. Stevens, G.M. Eccleston, J Pharm Sci, 97 (2008) 2892-2923.

[11] V. Leung, R. Hartwell, H. Yang, A. Ghahary, F. Ko, J Fiber Bioeng Inform, 4 (2011) 1-14.

[12] A. Gupta, R. Kumar, N.K. Upadhyay, P. Surekha, P.K. Roy, J App Polymer Sci, 111 (2009) 1400-1408.

[13] N. Teramoto, M. Saitoh, J. Kuroiwa, M. Shibata, R. Yosomiya, JAppl Polym Sci, 82 (2001) 2273-2280.

[14] Y.-L. Luo, Q.-B. Wei, F. Xu, Y.-S. Chen, L.-H. Fan, C.-H. Zhang, Mat Chem Physics, 118 (2009) 329-336.

[15] B. Aktar, M. Erdal, O. Sagirli, S. Güngör, Y. Özsoy, Polymers, 6 (2014) 1350.

[16] M.K. Okajima, R. Mishima, K. Amornwachirabodee, T. Mitsumata, K. Okeyoshi, T. Kaneko, RSC Advances, 5 (2015) 86723-86729.

[17] F. Bigucci, A. Abruzzo, B. Saladini, M.C. Gallucci, T. Cerchiara, B. Luppi, Carbohyd Polymers, 130 (2015) 32-40.

[18] N.-T. Nguyen, J.-H. Liu, Eur Polym J, 49 (2013) 4201-4211.

Figure legends

Figure 1. Preparation of a sacran hydrogel film. Sacran solution (0.5% (w/v)) was poured into the polypropylene box, and dried for 48 h at 60°C. Then, the sample was heated for 2 h at 110°C to obtain sacran hydrogel film. (B) Appearance of sacran and Na-alginate hydrogel films. (C) Thickness of sacran and Na-alginate hydrogel films. Thickness of the film was measured by a dial thickness apparatus. Each value represents the mean±S.E. of 6 experiments.

Figure 2. Physicochemical properties of a sacran hydrogel film. (A) powder X-ray diffraction patterns of sacran and Na-alginate hydrogel films. (B) DSC thermographs of sacran and Na-alginate hydrogel films. (C) Swollen ratios of sacran and Na-alginate hydrogel films. The hydrogel films (1.5 x 1.5 cm^2) were immersed into water and weighted (W_t) at 24 h. The swollen ratio was determined by comparing W_t and initial weight (W_o). Each value represents the mean±S.E. of 6 experiments. (D) SEM analysis of a sacran hydrogel film.

Figure 3. Cytotoxicity of a sacran hydrogel film in NIH3T3 cells. NIH3T3 cells were incubated with the hydrogel films for 4 h. Then, the cell viability was measured by a WST-1 method. Each value represents the mean±S.E. of 3 experiments.