Chitosan-based nitric oxide-releasing dressing for anti-biofilm and *in vivo* healing activities in MRSA biofilm-infected wounds

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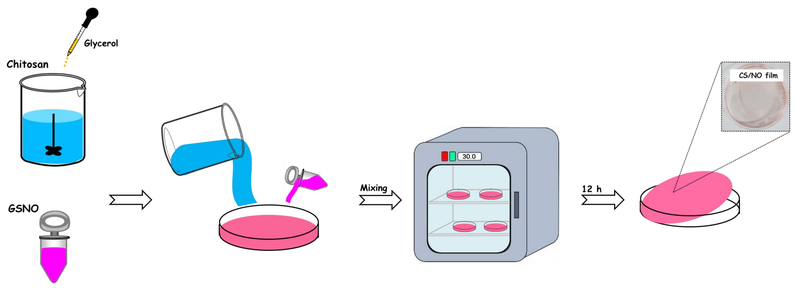
ABSTRACT

Bacterial biofilms on wounds impair the healing process and often lead to chronic wounds. Chitosan is a well-known biopolymer with antimicrobial and anti-biofilm effects. S-nitrosoglutathione (GSNO) has been identified as a promising nitric oxide (NO) donor to defend against pathogenic biofilms and enhance wound healing activities. In this study, we prepared NO-releasing chitosan film (CS/NO film) and evaluated its anti-biofilm activity and *in vivo* wound healing efficacy against methicillin-resistant *Staphylococcus aureus* (MRSA) biofilm-infected wounds in diabetic mice. The *in vitro* release study showed sustained release of NO over 3 days in simulated wound fluid. The CS/NO film significantly enhanced antibacterial activity against MRSA by >3 logs reduction in bacterial viability. Moreover, CS/NO film exhibited a 3-fold higher anti-biofilm activity than the control and CS film. In *in vivo* MRSA biofilm-infected wounds, the CS/NO film-treated group showed faster biofilm dispersal, wound size reduction, epithelialization rates, and collagen deposition than the untreated and CS film-treated groups. Therefore, the CS/NO film investigated in this study could be a promising approach for the treatment of MRSA biofilm-infected wounds.

RESULTS

Preparation of the CS/NO film

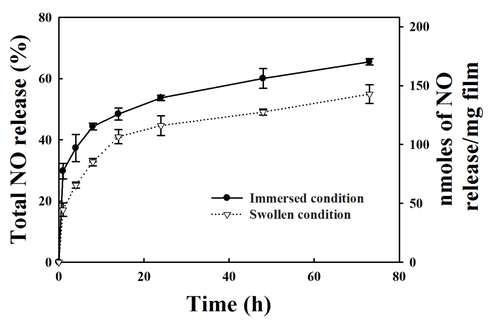


Characterization of CS and CS/NO films

Films	NO amount (μmoles/ mg films)	Thickness (μm)	Physical characteristics
CS	Not determined	68 ± 6	Transparent and homogenous surface
CS/NO	0.26 ± 0.006	69 ± 5	Reddish, transparent and homogenous surface

Values are expressed as mean ± SD of three different batches of films.

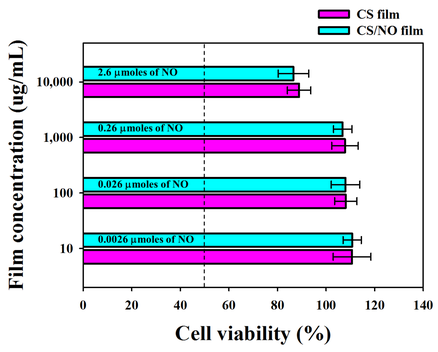
In vitro release



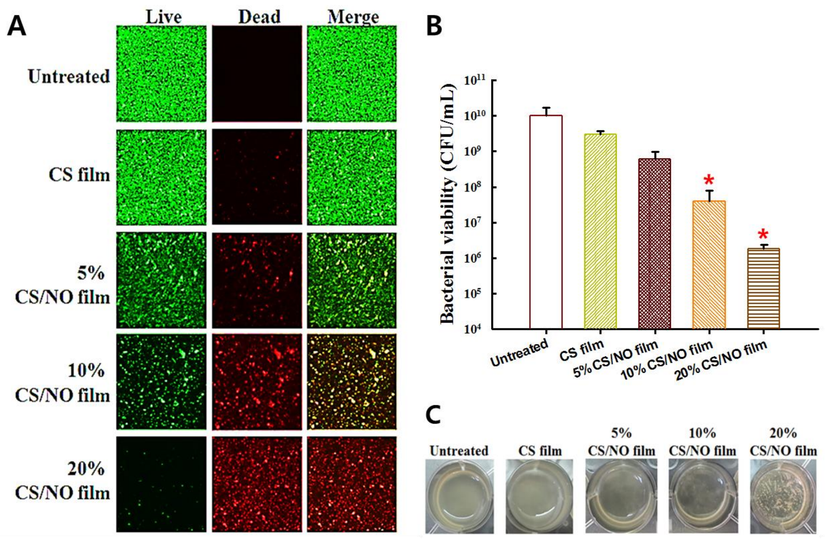
In vitro NO release from the 20% CS/NO film in simulated wound fluid (SWF). The CS/NO films were placed in SWF (pH 7.0) under the swollen and immersed conditions at 37°C. Values are expressed as mean ± SD (n = 3).

In vitro cytotoxicity study

Viability (%) of L929 mouse fibroblast cells after 24 h exposure to CS film and CS/NO film at various concentration (n = 8).

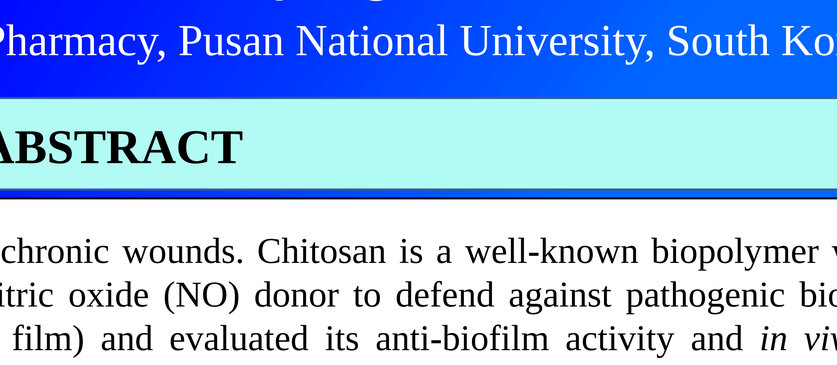


In vitro antibacterial activity



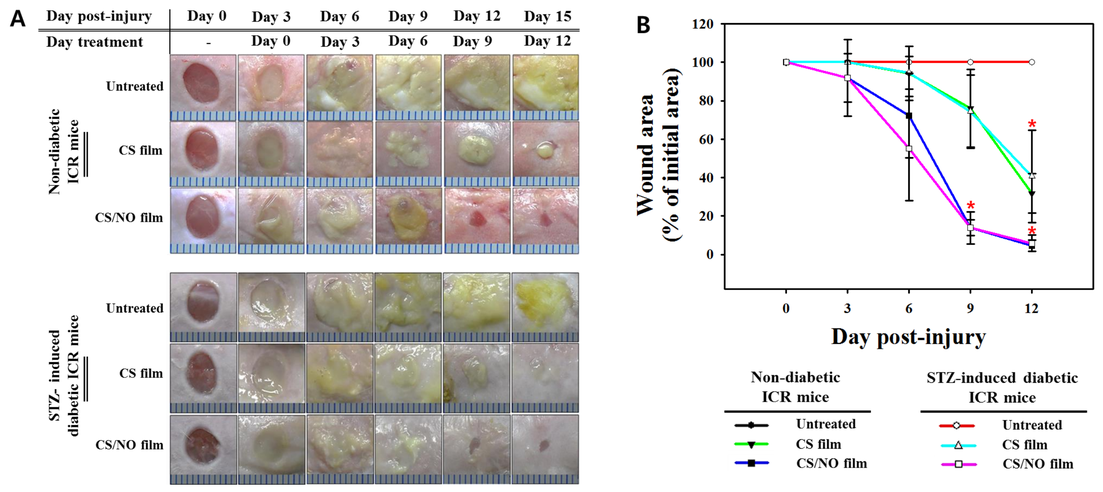
In vitro antibacterial activities of the CS film and CS/NO film against MRSA. (A) Confocal microscopy images of MRSA biofilm after 24 h treatment with the CS/NO film at different concentrations. (B) Bacterial viability (CFU measurement). Values are expressed as mean ± SD (n = 3); *P < 0.05 compared with the untreated group. (C) Macroscopic images of bacterial density in TSB medium.

In vitro anti-biofilm activity



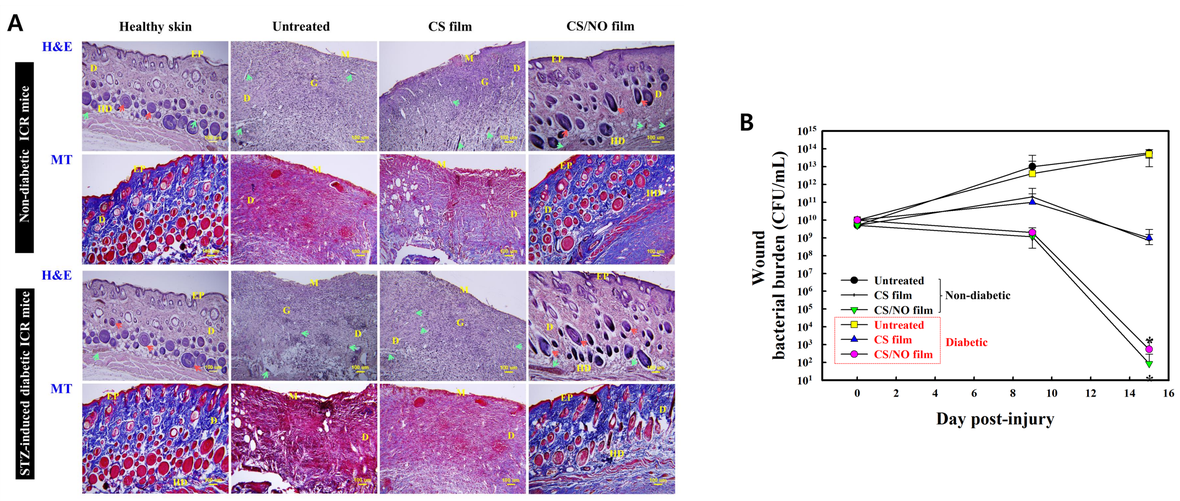
In vitro anti-biofilm activity of the CS and CS/NO films. MRSA biofilms were grown in polyurethane coupon for up to 24 h in the presence/absence of CS or CS/NO. (A) Stained biofilms on coupon by crystal violet. (B) Biofilm biomass by crystal violet staining (OD_{550}). Values are mean ± SD (n = 3); *P < 0.05 compared with the untreated group.

In vivo wound healing assay



MRSA biofilm-infected wounds in non-diabetic and STZ-induced diabetic ICR mice. (A) Representative photographs of healing in non-diabetic (upper panel) and STZ-induced diabetic ICR mice (lower panel) with MRSA biofilm challenge treated with or without the CS/NO film. (B) Wound area reduction percentage of mice skin lesions relative to the initial 6-mm wound. Data shown are mean ± SD (n = 6), different wounds; *P < 0.05, compared with untreated group.

Histological analyses and wound bacterial burden



(A) Histological analyses (H&E and MT staining) of MRSA biofilm-infected wounds in non-diabetic and STZ-induced diabetic ICR mice at day 15 post-injury (magnification 10×, scale bar = 100 μm); EP = epidermis; D = dermis; HD = hypodermis; M = wound matrix; G = granulation tissue. Orange arrows denote hair follicles and green arrows indicate blood vessles (capillaries and neovascularization). Blue colors in the MT staining images indicate collagen arranged parallel to the surface. (B) Bacterial viability (CFU/mL) on MRSA biofilm-infected wounds in non-diabetic and STZ-induced diabetic ICR mice. At day 9 and 15 post-injury, skin lesion tissues with biofilm were homogenized in sterile PBS and cultured onto TSB agar at 37°C overnight.

- ✓ In this study, chitosan-based nitric oxide-releasing dressing (CS/NO film) were successfully prepared.
- ✓ In the *in vitro* antibacterial and anti-biofilm studies, the CS/NO film significantly decreased the bacterial viability and biofilm biomass of MRSA, followed by favorable wound healing efficacy in an MRSA biofilm-infection wound of STZ-induced diabetic ICR mice model.
- ✓ Thus, could be a promising approach for treating wounds and various MRSA skin infections.

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