

Nitric oxide-releasing in-situ hydrogel dressing for antibacterial and wound healing activities

Hasan Nurhasni

College of Pharmacy, Pusan National University, Busan, South Korea

Abstract

Purpose: Nitric oxide (NO) is a potent wound healing, antibacterial agent. But, NO is hard to use directly due to its physical state and poor stability. In this reason, NO donors such as s-nitrosoglutathione (GSNO) were used for developing wound healing agents. Several GSNO-containing wound formulations such as solutions, hydrogels, and wound films have been developed; however, poor stability of GSNO still remains a problem. Here we developed NO-releasing *in situ* gelling powder, which maintains GSNO stability before application and once applied on wound beds, forms a hydrogel to release NO, thus functioning as an efficient wound dressing

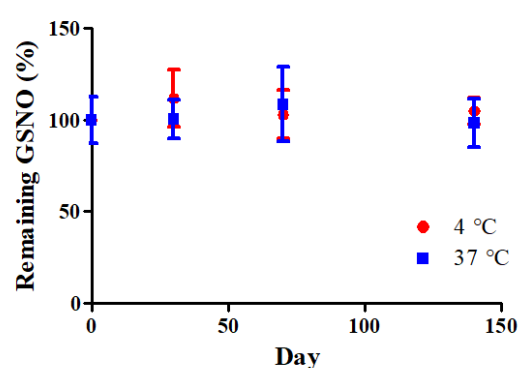
Methods: NO-releasing *in situ* hydrogel forming powder (NO/GP) was prepared by blending GSNO with alginate, pectin and PEG. Water uptake, NO release, storage stability and rheological properties were investigated in various *in vitro* condition. Antibacterial efficacy was tested against MRSA and *P. aeruginosa*. Wound healing activity was evaluated in *P. aeruginosa* challenged mice model.

Results: NO/GP powder quickly turned to hydrogel when applied in wounds with fluid absorption. After swollen, NO/GP released NO more than 12 hours. NO/GP powder remained stable more than 4 months. Yield stress that provide resistance to flow in small shear stresses was observed in NO/GP hydrogel. The NO/GP showed potent bactericidal efficacy against MRSA and *P. aeruginosa*. NO/GP exhibited significant wound healing promotion effect in *P. aeruginosa*-challenged mice model. In addition, histological examination revealed that wounds treated NO/GP facilitated collagen synthesis and tissue regeneration.

Conclusion: In this study, we successfully developed NO/GP. It showed long term storage stability in powder form and prolonged NO release profile over 12 hours after applied, followed by favorable wound healing efficacy in *P. aeruginosa*-challenged full-thickness wound. Thus, NO/GP would be a promising formulation for infectious wound treatment

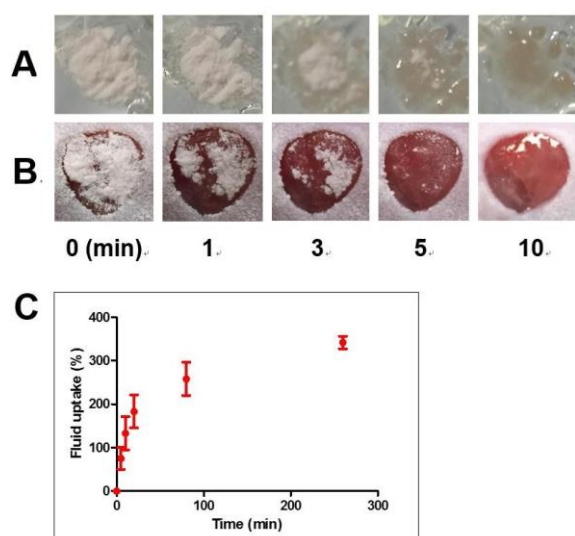
Results

Storage stability



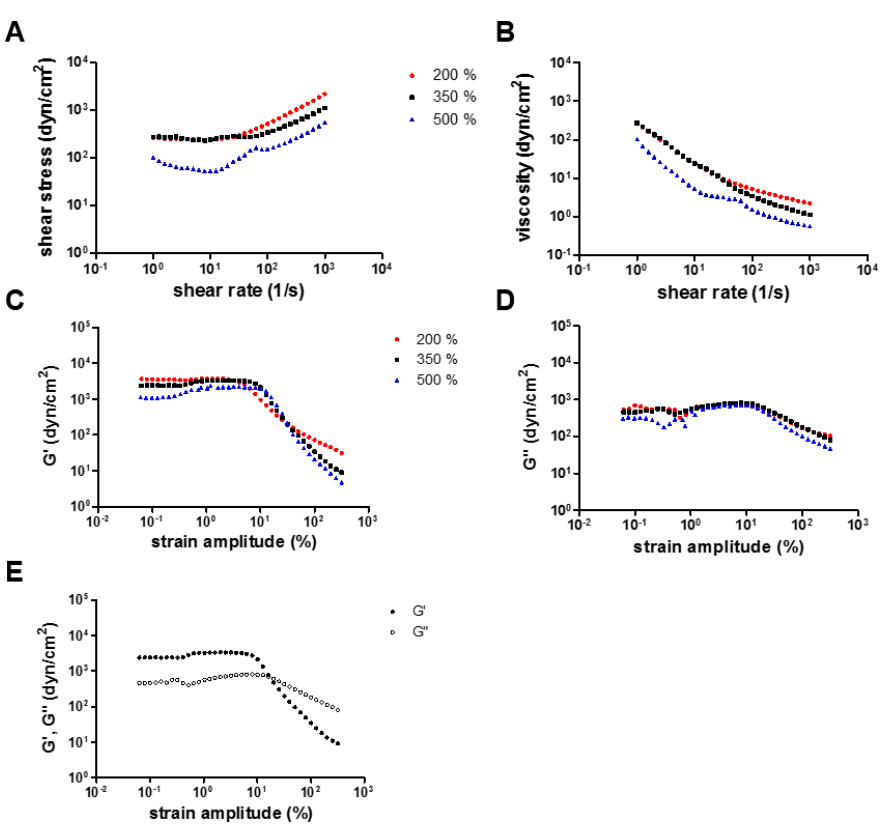
Storage stability of NO/GP in 4 °C (red) and 37 °C (blue). Significant GSNO decomposition was not detected in this period both 4 °C and 37 °C. (n = 3)

Fluid uptake ability



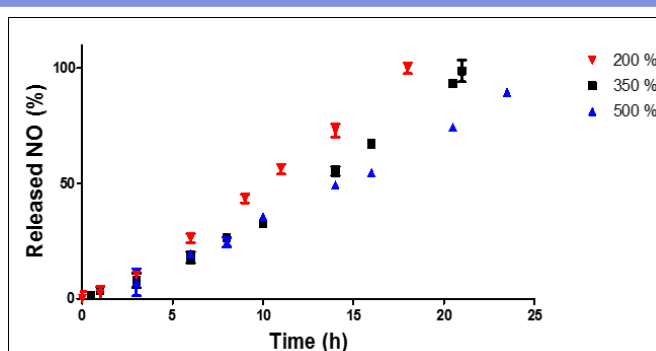
Fluid uptake ability of NO/GP. NO/GP exhibited rapid fluid absorption and turned into hydrogel within 5 minutes. Morphological change of NO/GP in vitro (A) and in vivo (B). Fluid uptake percent per initial NO/GP powder weight (C), (n = 3)

Rheological properties



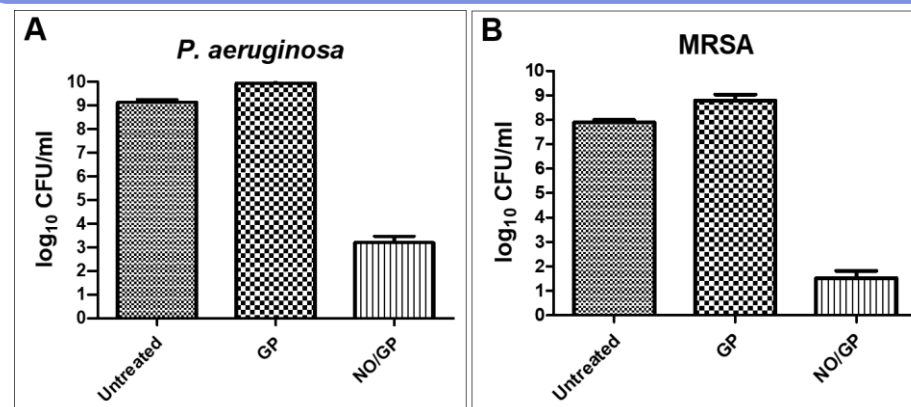
Rheological properties of NO/GP against various steady shear strains or oscillatory shear strains. Less swollen (200 %), maximum swollen (350 %) and over swollen (500 %) NO/GP hydrogel samples were examined. Yield stress was observed in all swollen conditions; it means that NO/GP hydrogel can resist to flow against small shear stress.

NO release from NO/GP

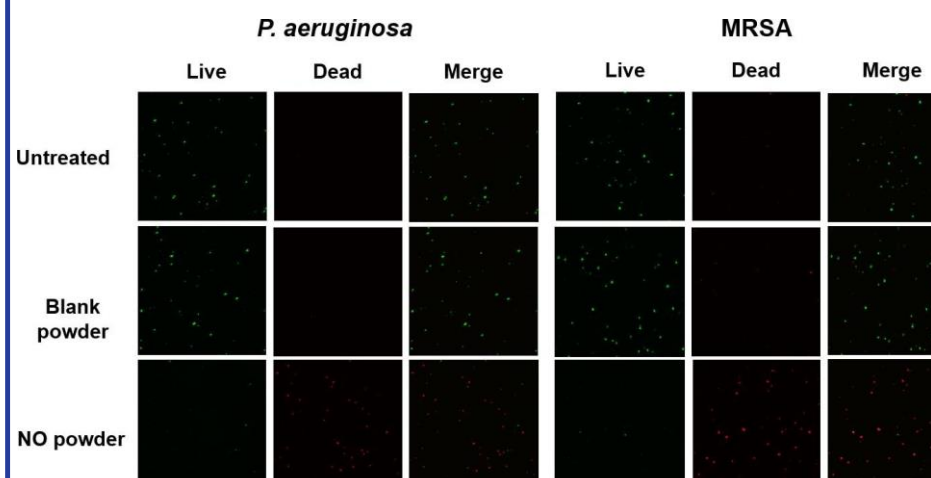


NO release from NO/GP from different swollen conditions with SWF. Less swollen (200 %), maximum swollen (350 %), over swollen (500 %) NO/GP was examined. NO release was sustained until 18, 22, and more than 24 hours in 200%, 350%, 500% swollen condition respectively. (n = 3)

In vitro antibacterial assay

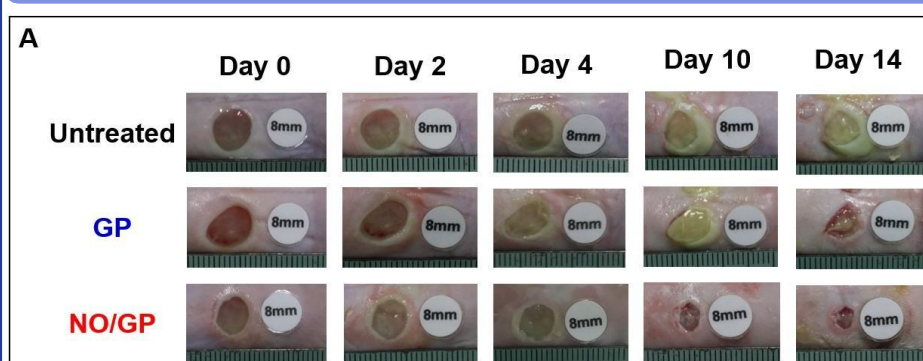


Antibacterial effect of NO/GP at maximum swollen condition in 37°C, 24-hour incubation. NO/GP exhibited more than 3 log reduction of CFU against *P. aeruginosa* and MRSA. (n = 3)

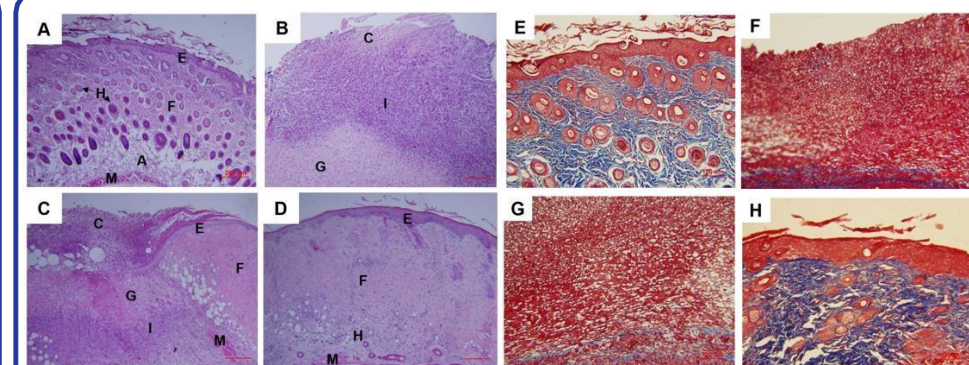
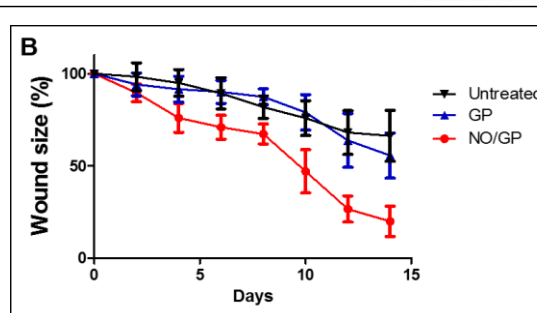


Bactericidal effect of NO/GP on *P. aeruginosa* and MRSA. Green and red fluorescence indicates live and dead bacteria respectively. NO/GP group exhibited significant bactericidal effect against *P. aeruginosa* and MRSA.

In vivo wound healing study



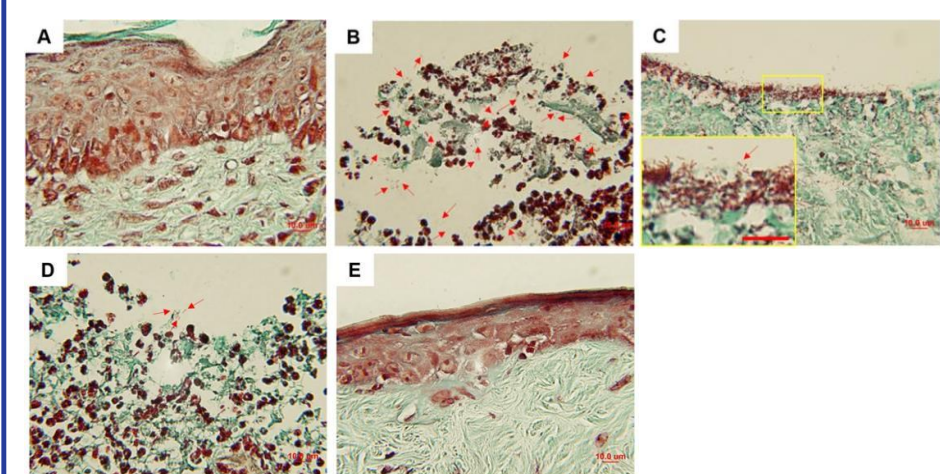
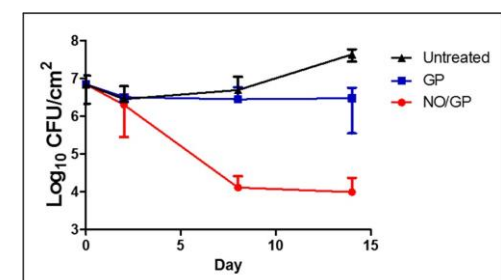
Wound healing effect of NO/GP in *P. aeruginosa* challenged full-thickness wound mice model. NO/GP group exhibited significant wound size reduction. Macroscopic images of wound site (A), wound size reduction profiles (B). (n=4)



H&E staining images at 14 days after treatment initiation, normal skin (A), untreated (B), GP (C) and NO/GP (D). Masson's trichrome staining images at 14 days after treatment initiation, normal skin (E), untreated (F), GP (G) and NO/GP (H). A: adipose tissue, C: cell debris, E: epidermis, F: fibrous tissue, G: granulation tissue, H: hair follicle, I: immune cells, M: muscle. Scale bar represents 200 μ m and 100 μ m in H&E and Masson's trichrome staining images respectively.

In vivo antibacterial study

Bacteria quantification at the wound site. NO/GP group exhibited bacterial reduction up to similar level of *P. aeruginosa* of normal skin (3.8 Log₁₀ CFU/cm²). (n = 3)



Twort's gram staining at 14 days after treatment initiation. 1-2 μ m, rod shaped red dots are *P. aeruginosa* (red arrows). Normal skin (A), untreated (B), on the outer layer of untreated group (C), GP (D) and NO/GP (E). Green color: cytoplasm, red color: nuclei or gram-negative bacteria, dark blue: gram-positive bacteria. Scale bar represents 10 μ m.

Conclusion

In this study, we successfully developed NO-releasing *in situ* hydrogel forming powder (NO/GP). Storage stability of GSNO and immediate NO triggering was achieved by *in situ* hydrogel forming powder system. NO/GP exhibited a prolonged NO release profile over 12 hours, followed by favorable wound healing, antibacterial efficacy in *P. aeruginosa*-challenged full-thickness wound. Thus, NO/GP could be a promising NO-releasing wound formulation for the treatment of infectious wounds.

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2016R1D1A3B03931302) and by a grant from the Korean Healthcare Technology R&D Project, Ministry for Health and Welfare Affairs, Republic of Korea (HI15C2558)