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Nitric Oxide (NO)-Releasing *in situ* Hydrogel Forming Powder as a Wound Dressing for Infected Wound Treatment

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PURPOSE

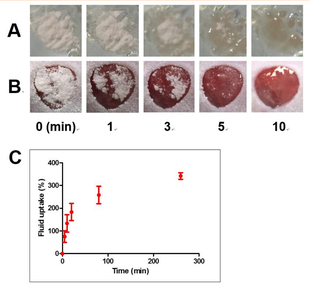
S-nitrosogluthathione (GSNO) is an endogenous nitric oxide (NO) donor with potent antibacterial and wound healing activities. Several GSNO-containing wound formulations such as solutions, hydrogels, and wound films have been developed; however, poor stability of GSNO by hydrolysis during fabrication and storage still remains a problem. Here we report on NO-releasing in situ gelling powder, which maintains GSNO stability before application and once applied on wound beds, forms a hydrogel to trigger NO release immediately, thus functioning as an efficient wound dressing.

METHODS

NO-releasing in situ hydrogel forming powder (NO/GP powder) was prepared by blending GSNO with sodium alginate, pectin and PEG. Water uptake, in vitro NO release and stability of GSNO in NO/GP was investigated in various storage and wound conditions. Bioadhesive behavior of in situ hydrogel was evaluated by measuring several rheological properties. Antibacterial efficacy was tested against two representative pathogens; methicillin-resistant Staphylococcus aureus (MRSA) and *P. aeruginosa*. The wound healing activity was evaluated in *P. aeruginosa* challenged full-thickness wound model.

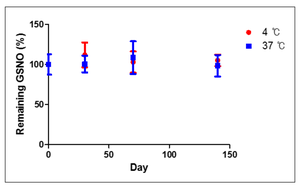
RESULTS

Fluid uptake ability



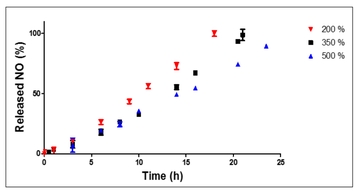
Fluid uptake ability of NO/GP. NO/GP exhibited rapid fluid absorption and turned into hydrogel within 5 minutes. Morphological change of NO/GP in vitro (A) and in vivo (B). Fluid uptake percent per initial NO/GP powder weight (C), (n = 3)

Storage stability



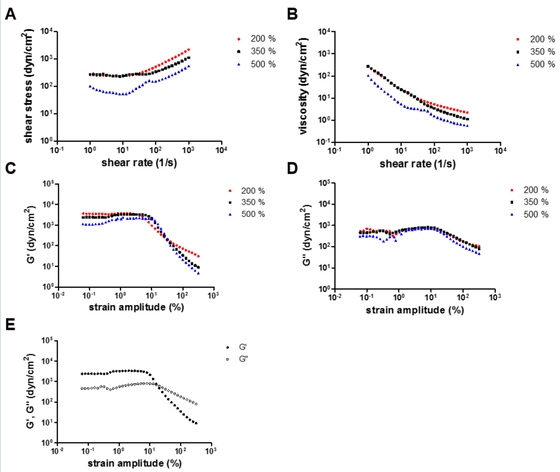
Storage stability of NO/GP in 4 ℃ (red) and 37 ℃ (blue). Significant GSNO decomposition was not detected in this period both 4 ℃ and 37 ℃. (n = 3)

NO release from NO/GP



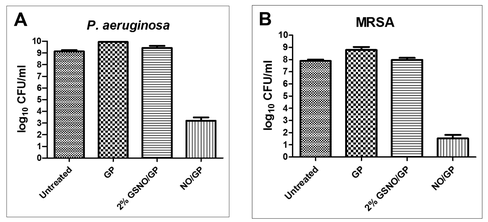
NO release from NO/GP from different swollen conditions with SWF. Less swollen (200 %), maximum swollen (350 %), over swollen (500 %) NO/GP was examined. NO release was sustained until 18, 22, and more than 24 hours in 200%, 350%, 500% swollen condition respectively. (n = 3)

Rheological properties

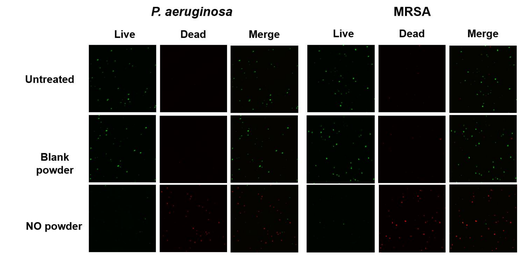


Rheological properties of NO/GP against various steady shear strains or oscillatory shear strains. Less swollen (200 %), maximum swollen (350 %) and over swollen (500 %) NO/GP hydrogel samples were examined. Yield stress was observed in all swollen conditions; it means that NO/GP hydrogel can resist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

In vitro antibacterial assay

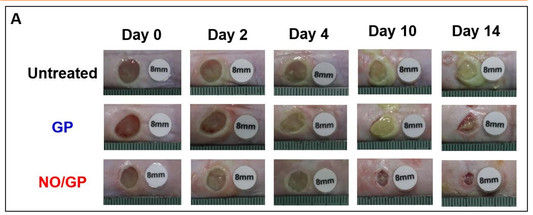


Antibacterial effect of NO/GP at maximum swollen condition in 37℃, 24-hour incubation. NO/GP exhibited more than 3 log reduction of CFU against *P. aeruginosa* and MRSA. (n = 3)

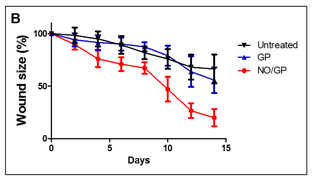


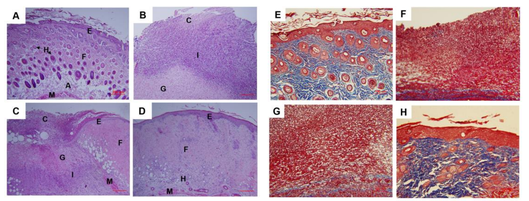
Bactericidal effect of NO/GP on *P. aeruginosa* and MRSA. Green and red fluorescence indicates live and dead bacteria respectively. NO/GP group exhibited significant bactericidal effect against *P. aeruginosa* and MRSA.

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)

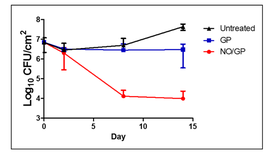


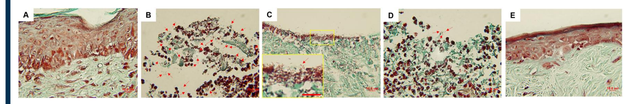


H&E staining images at 14 days after treatment initiation, normal skin (A), untreated (B), GP (C) and NO/GP (D). Masson's trichrome staining images at 14 days after treatment initiation, normal skim (E), untreated (F), GP (G) and NO/GP (H). A: adipose tissue, C: cell debris, E: epidermis, F: fibrous tissue, G: granulation tissue, H: hair follicle, I: immune cells, M: muscle. Scale bar represents 200 μm and 100 μm 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 Log_{10} CFU/cm^2). (n = 3)





Twort's gram staining at 14 days after treatment initiation. 1~2 μm, 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 μm.

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

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