Surface charge engineering of nitric oxide-releasing polymeric nanoparticles : adhesion and anti-biofilm efficacy against wound infection associated MRSA biofilm in db/db mice

Nurhasni Hasan, RPH, M.Pharm

College of Pharmacy, Pusan National University, South Korea

CONTACT INFORMATION: nurhasni.hasan@unhas.ac.id

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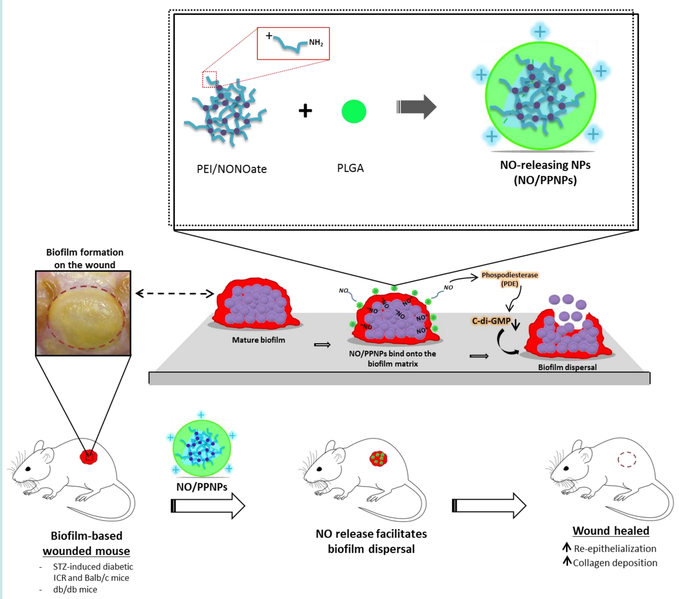
PURPOSE

Biofilm-associated wound infections has been considered a life-threatening infection that affects millions of people each year and are among the major cause of infectious disease-related mortality and morbidity worldwide. Bacterial biofilms protect bacteria from host immune responses and promote strong resistance to antibiotic treatment which leads to impaired wound healing, hospitalization and amputation particularly in chronic wound such as diabetic foot ulcer. Recently, nitric oxide (NO) has emerged as a novel agent in biofilm dispersal and accelerates wound healing. In this study, we investigated the potency of positively charge NO-releasing PLGA/PEI nanoparticles (NO/PPNPs) for adhesion on biofilm surface that elevate biofilm dispersal and wound healing efficacy.

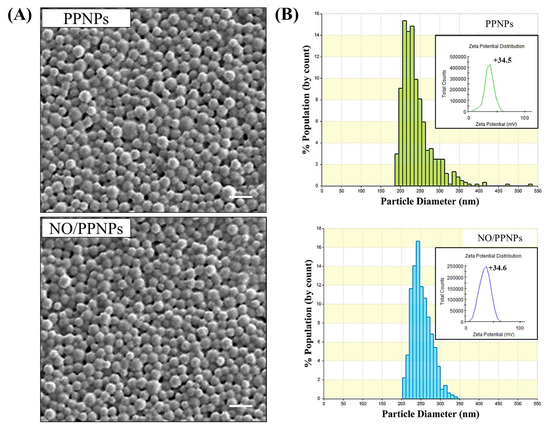
METHODS

- Poly (lactic-*co*-glycolic acid) (PLGA) were used to incorporate polyethylenimine (PEI)/NO adduct (PEI/NONOate) by an oil-in-water (O/W) emulsion evaporation method to form NO/PPNPs.
- Adhesion of NO/PPNPs on bacterial biofilm was performed in biofilm wound and characterized by 3D confocal microscopy.
- The progress of *in vivo* biofilm dispersal was evaluated by using fluorescence visualization in 3D confocal microscopy.
- Photographs of the wounds were taken to observe the gross visual wound healing. Furthermore, histological analysis was performed with H&E and Masson trichrome staining to observe the skin morphological and collagen deposition, respectively.



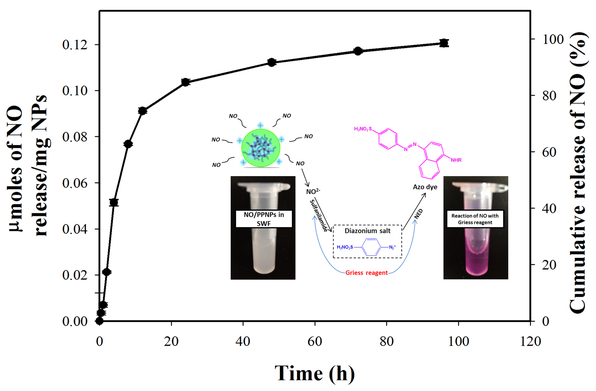
RESULTS

Characterization of nanoparticles



(A) SEM images of PPNPs and NO/PPNPs, scale bar = 500 nm.
(B) Size distribution of PPNPs and NO/PPNPs from Q-Nano particle analyzer (IZON®), insets show zeta potential distribution.

In vitro drug release

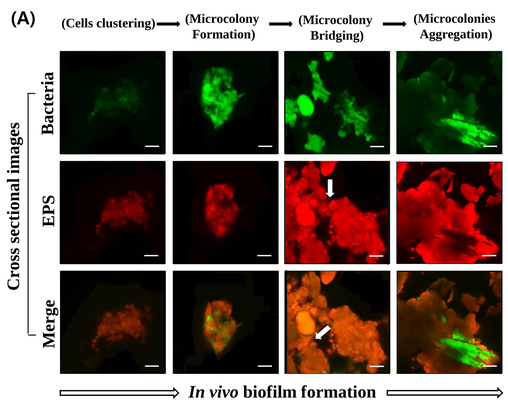


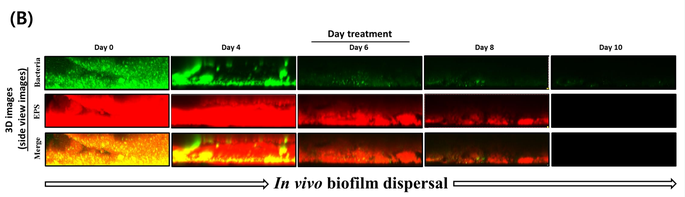
In vitro release profile of NO/PPNPs. All samples were placed in simulated wound fluid (SWF, pH 7.1) 37°C; data presented are mean ± S.D, n=3.

Size and zeta potential of nanoparticles

NPs	Amount of loaded NO (µmole/g Nps)	Size (nm)		Zeta potential (mV)
		DLS	qNano	
PLGA NPs	N.D	252 ± 45	246 ± 49	- 24.3 ± 1.7
PPNPs	N.D	265 ± 11	248 ± 46	+ 34.5 ± 1.2
NO/PPNPs	122 ± 1	240 ± 2	247 ± 26	+ 34.6 ± 1.1

In vivo biofilm formation and dispersal

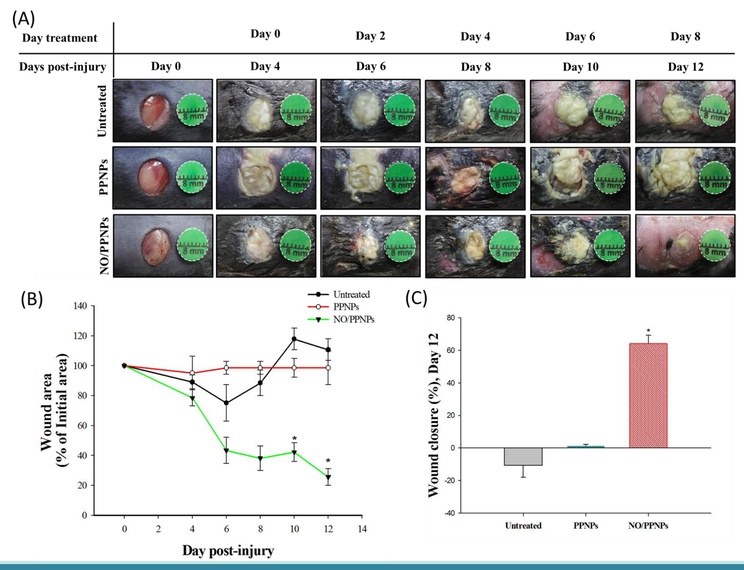


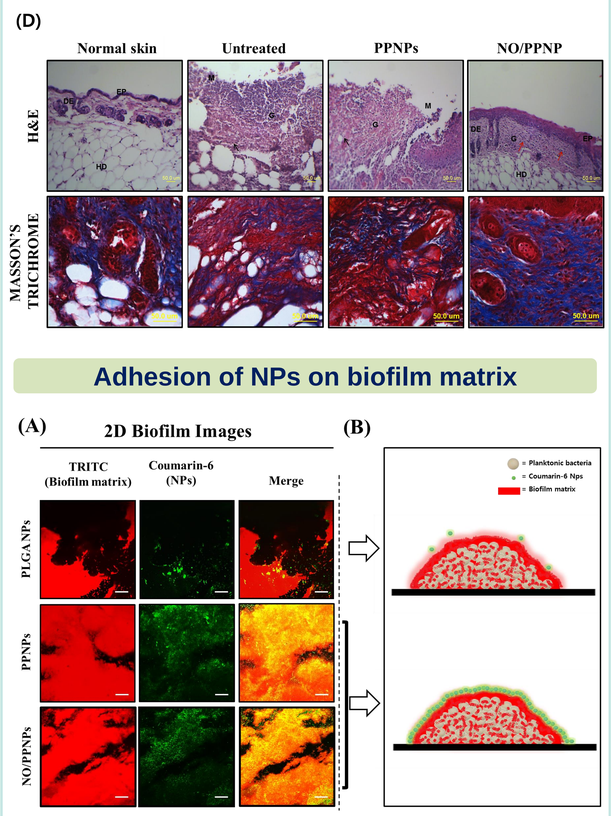


(A)3D *In vivo* biofilm dispersal (side view images) by confocal microscopy from biopsy day 0 treatments to the day 10.
(B)Representative 3D *in vivo* biofilm formation renderings of the biofilm matrix in red, the bacterial micro colonies in green and the merge of both (white arrows represent micro colonies bridging by EPS).

In vivo wound healing assay

Biofilm-based wound challenged in db/db mice.
(A)Representative photographs of healing with Methicillin-resistant *Staphylococcus aureus* (MRSA) biofilm challenge treated with or without NO/PPNPs.
(B)Area reduction (%) profiles of the wounds. Data shown are mean ± S.D., n=10 different wounds, *P<0.05 compared with untreated group.
(C)Wound closure percentage of DB/DB mice skin lesions at last day relative to the initial 8-mm wound.
(D)Histological analysis (H&E staining and trichrome staining) of biofilm-based wound challenged (day 12) .
Scale bar = 50 µm.





Adhesion of NPs on biofilm matrix

(A) 2D images that show biofilm EPS (red) were stained with TRITC and NPs (green) labeled with Coumarin-6 for visualization. All samples were incubated for 2 h. (B) Illustrations of Nps binding to the biofilm matrix.

CONCLUSION

- ✓The binding of positively charged NO/PPNPs on biofilm matrix showed potent anti-biofilm activity, followed by favorable wound healing efficacy in a biofilm-challenged diabetic mouse model.
- ✓Thus, the NO-releasing polymeric nanoparticles investigated in this study could be a promising approach for the treatment of biofilm-challenged chronic wounds and various skin infections.

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