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13/05/2023

Pusan National University
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Biofilm Targeted-Nitric Oxide Releasing Polymeric Nanoparticles: Adhesion and Anti-biofilm Efficacy against MRSA Biofilm in Diabetic Wounds

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Bali, 20191025

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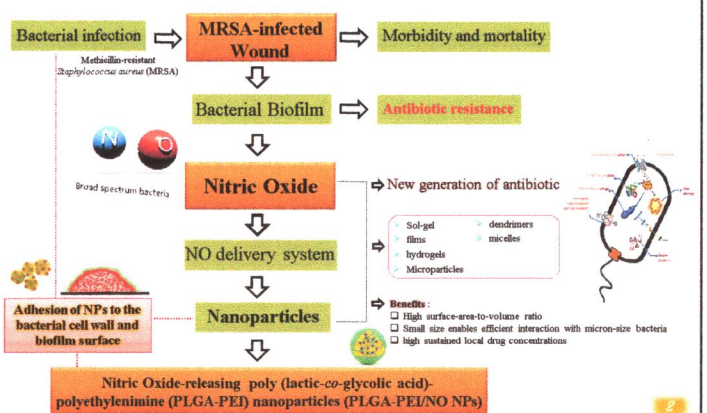
Background of the study



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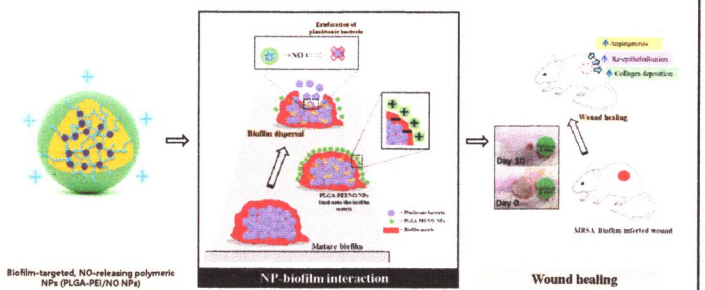
Purpose of the study

To develop NO-releasing NPs with an ability to directly bind to the biofilm matrix that provide enhanced NO availability in biofilm-infected wound areas, thereby enabling an effective treatment of the wounds.



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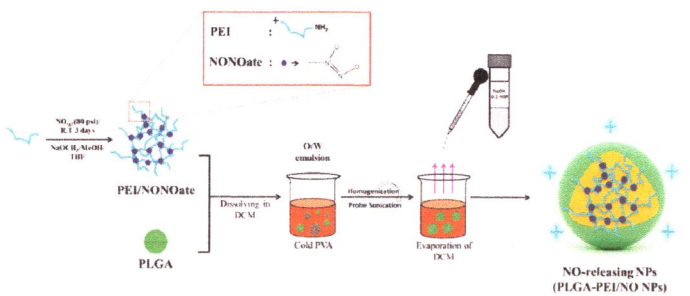
~Fabrication of surface charged PLGA-PEI/NO NPs~



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~Characterization of NPs~

NPs	Amount of loaded NO (µmoles/gNPs)	Size (nm) Zetasizer	Size (nm) qNano	PDI	Zeta Potential (mV)
PLGA NPs	N.D	252 ± 45	246 ± 49	0.133	- 24.3 ± 1.7
PLGA-PEI NPs	N.D	265 ± 11	248 ± 46	0.091	+ 34.5 ± 1.2
PLGA-PEI/NO NPs	122 ± 1	240 ± 2	247 ± 26	0.080	+ 34.6 ± 1.1

Values are expressed as mean ± SD of three different batch of particles

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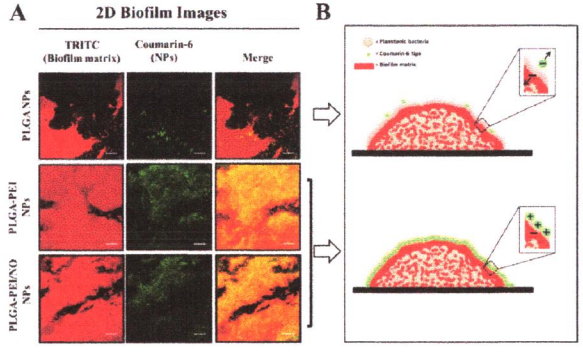
~Adhesion of NPs to biofilm matrix~



(A) 2D images showed biofilm EPS (red) stained with TRITC dye and NPs (green) stained with coumarin-6. All samples were incubated for 2 h.
(B) Schematic presentation of NP binding to the biofilm matrix.

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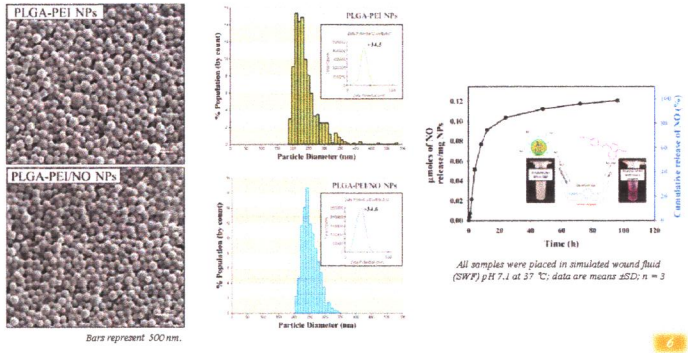
~Characterization of NPs~

Scanning Electron Microscopy (SEM) images | **Zetasizer (nano series ZS90) results** | ***In vitro* release**



Bars represent 500 nm.

All samples were placed in simulated wound fluid (SWF) pH 7.1 at 37 °C; data are means ±SD; n = 3

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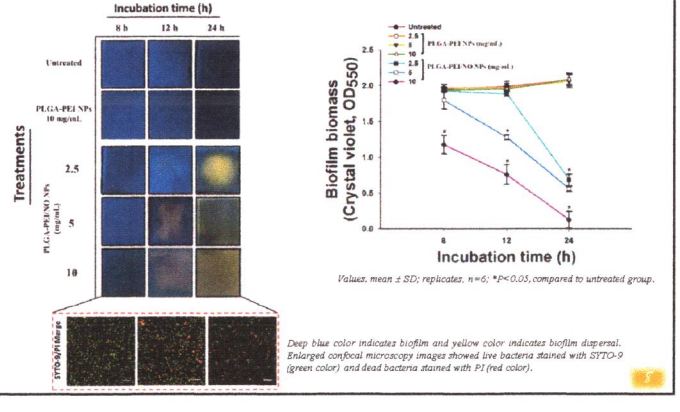
~*In vitro* anti-biofilm activity of NPs~

Crystal violet-stained biofilms on coupon | **Biofilm biomass**



*Values, mean ± SD; replicates, n=6; *P<0.05, compared to untreated group.*

Deep blue color indicates biofilm and yellow color indicates biofilm dispersal. Enlarged confocal microscopy images showed live bacteria stained with SYTO-9 (green color) and dead bacteria stained with PI (red color).

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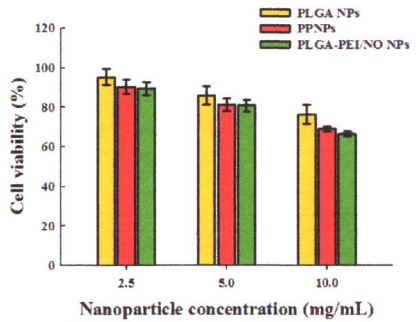
~*In vitro* cytotoxicity study~



Viability (%) of L929 mouse fibroblast cells following 24 h of exposure to different concentrations of NPs (n = 8).

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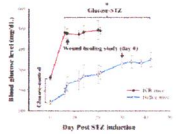
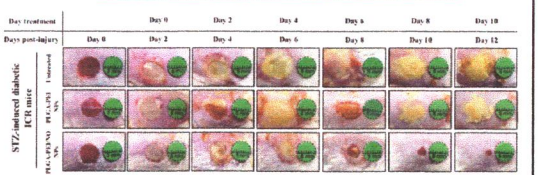
~*In vivo* wound healing assay~

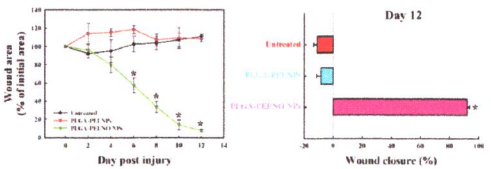


Blood glucose levels in ICR and Balb/c mice after a single i.p. injection of Streptozotocin (STZ).

Representative photographs of MRSA biofilm-infected wounds



Area reduction (%) profiles of the wounds



*Wound area reduction percentage of mice skin lesions relative to the initial 8-mm wound. Data shown are mean ± SD; n=10 different wounds; *P<0.05, compared with untreated group.*

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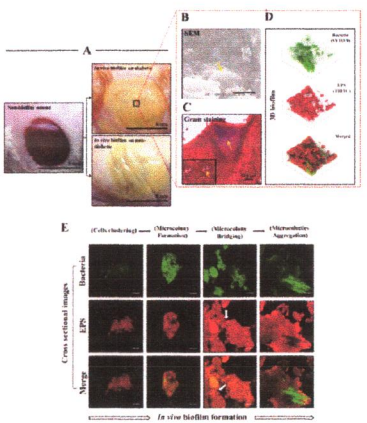
~*Characterization of MRSA biofilms on wound* ~



(A) Biofilm formation in cutaneous mouse wounds. Macroscopic appearance of normal wound without biofilm (left side) and wound colonization by biofilm (right side, day 6 post-injury).
(B) SEM images of bacterial biofilm (yellow arrows mark bacterial microcolonies enclosed in biofilm matrix).
(C) Gram staining of the wound tissue (yellow arrows mark biofilm in the wound); inset in Gram staining image shows Gram-positive cocci (MRSA).
(D) 3D biofilm visualized by confocal microscopy.
(E) Representative 3D of *in vivo* biofilm formation showing the biofilm matrix in red, the bacterial microcolonies in green, and the merged image of both stains (white arrows mark microcolonies enclosed by EPS).

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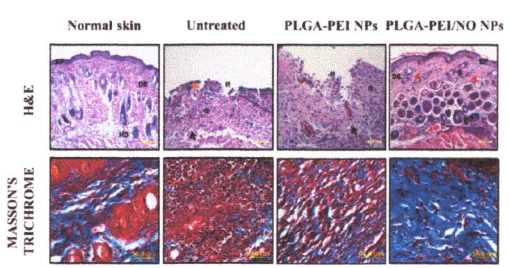
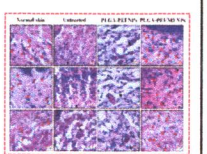
~*In vivo* wound healing assay~

Histological analyses



H&E and Masson's trichrome stain of MRSA biofilm-infected wounds in STZ-induced diabetic ICR mice at last day. Scale bar = 50 μm; EP = epidermal; DE = dermal junction; HD = hypodermis; G = granulation tissue; M = wound matrix. Orange arrows indicate early epithelialization, red arrows indicate fibroblast cells, and black arrows denote mononuclear inflammatory cells. Blue color in trichrome stained images indicates collagen arranged in parallel to the surface.



Fibroplasia in the treatment of MRSA biofilm-infected wounds. Red arrows indicate fibroblast cells, blue arrows show collagen strands, and black arrows denote mononuclear inflammatory cells. Magnification 100x. Scale bar = 10 μm

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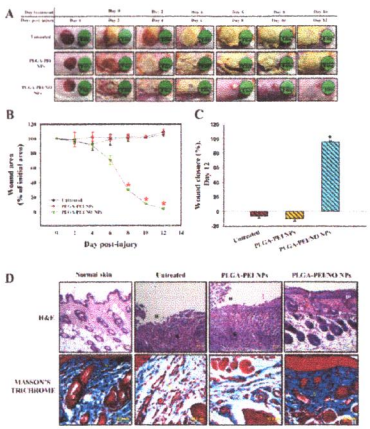
~*In vivo* wound healing assay~



Biofilm-infected wounds in STZ-induced diabetic Balb/c mice.

(A) Representative photographs of healing in STZ-induced diabetic Balb/c mice with MRSA biofilm challenge treated with or without PLGA-PEI/NO NPs.
(B) Wound area reduction percentage of mice skin lesions relative to the initial 8-mm wound. Data shown are mean ± SD; n = 10, different wounds; *P < 0.05, compared with untreated group.
(C) Wound closure percentage of Balb/c mice skin lesions at the last day relative to the initial 8-mm wound.
(D) Histological analyses (H&E and Masson's trichrome staining) of biofilm-infected wounds in STZ-induced diabetic Balb/c mice (at day 12). Scale bar = 50 μm; Ep = epidermal; DE = dermal junction; HD = hypodermis; G = granulation tissue; M = wound matrix. The orange arrows indicate early epithelialization, red arrows indicate fibroblast cells, and black arrows denote mononuclear inflammatory cells. Blue color in the trichrome stain indicates collagen arranged in parallel to the surface.

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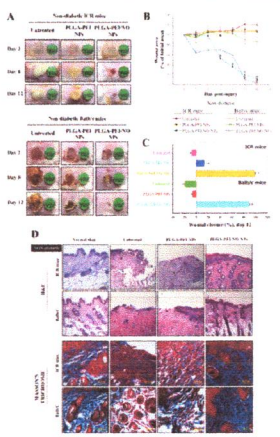
~*In vivo* wound healing assay~



Biofilm-infected wounds in non-diabetic ICR and Balb/c mice.

(A) Representative photographs of healing in non-diabetic ICR (up) and Balb/c (down) mice with MRSA biofilm challenge treated with or without PLGA-PEI/NO NPs.
(B) Wound area reduction percentage of mice skin lesions relative to the initial 8-mm wound. Data shown are mean ± SD, n = 10 different wounds, *P < 0.05 compared with untreated group.
(C) Wound closure percentage of ICR and Balb/c mice skin lesions at last day relative to the initial 8-mm wound.
(D) Histological analyses (H&E and Masson's trichrome staining) of biofilm-based wound challenged in non-diabetic mice (day 12). Scale bar = 50 μm. Ep = epidermal, DE = dermal junction, HD = hypodermis, G = granulation tissue and M= wound matrix. The arrow colored with orange arrows indicate early epithelialization. Red arrows indicate fibroblast cells and black arrows denote mononuclear inflammatory cells. Blue color on the trichrome staining indicates collagen arranged parallel to the surface.

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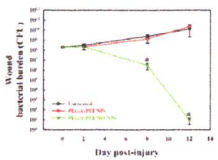
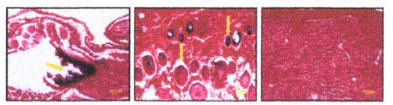
~Bacterial burden in the wound~

Colony forming units (CFU)

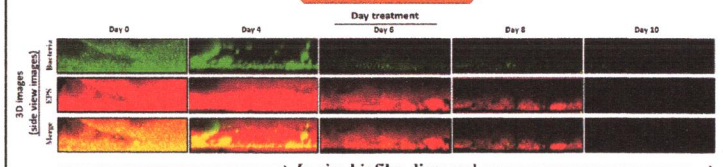


Gram staining of the skin tissue



Purple color spots marked with arrows represent MRSA colonies. At day 2, 8, and 12 after biofilm infection, wounds were swabbed and the bacterial burden was examined.

3D *in vivo* biofilm dispersal



3D in vivo biofilm dispersal in biopsy samples collected on day 0 and on treatment days 4, 6, 8, and 10, analyzed by confocal microscopy (side view images).

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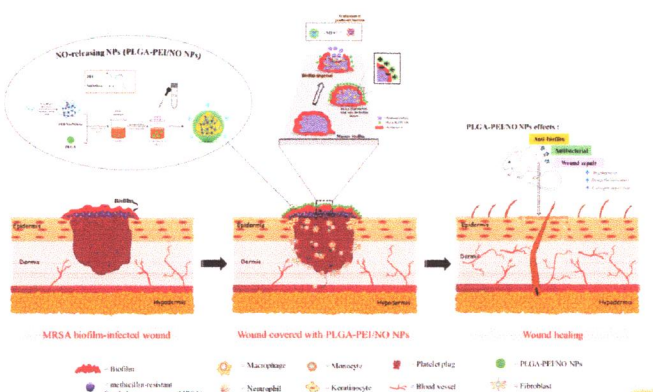
~Illustration of mechanisms of action of PLGA-PEI/NO NPs in MRSA biofilm-infected wound healing~



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Conclusion

- ✓ In this study, nitric oxide has been successfully incorporated into the polymeric nanoparticles (PLGA-PE/NO NPs).
- ✓ PLGA-PEI/NO NPs effectively bind to the surface of the biofilm matrix, causing strong biofilm dispersal *in vitro* and *in vivo*.
- ✓ PLGA-PEI/NO NPs can effectively treat MRSA biofilm-infected wound in diabetic mice model by clearing the infection and promoting wound healing.
- ✓ The novel biofilm-binding, NO-releasing NPs represent a promising technology for the development of high-efficacy treatments for biofilm-infected chronic wounds.

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References

- Edwards, R.; Harding, K.G. Bacteria and wound healing. *Current opinion in infectious diseases* **2004**, *17*, 91-96.
- Food, U.; Administration, D. Guidance for industry. Acute bacterial skin and skin structure infections: developing drugs for treatment. *Food and Drug Administration, Silver Spring, MD. http://www. fda. gov/downloads/Drugs/. /Guidances/ucm071185. pdf* **2013**.
- Andersson, H.; Lindholm, C.; Fossum, B. MRSA–global threat and personal disaster: patients' experiences. *International nursing review* **2011**, *58*, 47-53.
- Huh, A.J.; Kwon, Y.J. "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. *Journal of controlled release* **2011**, *156*, 128-145.
- N. Hasan; Cao, J.; Choi, M.; Kim, I.; Lee, B.L.; Jung, Y.; Yoo, J.-W. Nitric oxide-releasing poly (lactic-co-glycolic acid)-polyethylenimine nanoparticles for prolonged nitric oxide release, antibacterial efficacy, and in vivo wound healing activity. *International journal of nanomedicine* **2015**, *10*, 3065.
- N. Hasan, J. Cao, J. Lee, M. Naeem, S.P. Hlaing, J. Kim, Y. Jung, B.-L. Lee, J.-W. Yoo, PEI/NONOates-doped PLGA nanoparticles for eradicating methicillin-resistant Staphylococcus aureus biofilm in diabetic wounds via binding to the biofilm matrix,
- N. Hasan, J. Cao, J. Lee, S.P. Hlaing, M.A. Oshi, M. Naeem, M.-H. Ki, B.L. Lee, Y. Jung, J.-W. Yoo, Bacteria-Targeted Clindamycin Loaded Polymeric Nanoparticles: Effect of Surface Charge on Nanoparticle Adhesion to MRSA, Antibacterial Activity, and Wound Healing, Pharmaceutics 11 (2019) 236.
- S.P. Hlaing, J. Kim, J. Lee, N. Hasan, J. Cao, M. Naeem, E.H. Lee, J.H. Shin, Y. Jung, B.-L. Lee, S-Nitrosoglutathione loaded poly (lactic-co-glycolic acid) microparticles for prolonged nitric oxide release and enhanced healing of methicillin-resistant Staphylococcus aureus-infected wounds, Eur. J. Pharm. Biopharm. 132 (2018) 94–102.

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Thank you for your kind attention

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