Poly(lactic-*co*-glycolic acid)-polyethylenimine nanoparticles loaded clindamycin for adhesion on bacterial surface, antibacterial efficacy and wound healing activity

Hasan Nurhasni

College of Pharmacy, Pusan National University, South Korea

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

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PURPOSE

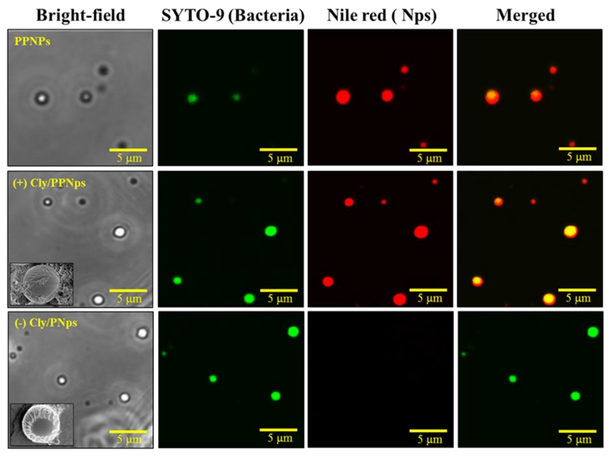
Skin bacterial infection caused by Methicillin-resistant *Staphylococcus aureus* (MRSA) has significantly increased for the past few years, recently emerged as a community-acquired (CA) organism and caused hospitalization. Positively charged clindamycin-releasing poly(lactic-*co*-glycolic acid)-polyethyleimine (PLGA-PEI) nanoparticles ((+) Cly/PPNPs), such as those prepared in this study, may exhibit potential antibacterial efficacy and wound healing activity as their unique character. The purpose of this study was to evaluate the antibacterial efficacy, adhesion to bacteria and wound healing activity of (+) Cly/PPNPs as compared to clindamycin-releasing PLGA NPs ((-) Cly/PNPs) that has negatively charged properties by nature.

METHODS

- NPs were prepared by an oil-in-water (O/W) emulsification evaporation method.
- *In vitro* antibacterial activity was performed using MRSA. ICR mouse model of an MRSA-infected wound was prepared by inoculating bacterial suspension on the surface of the wound.
- Photographs of the wounds were taken to observe the gross visual wound healing.
- Furthermore, histological analysis was performed with H&E stain to observe the skin morphology.
- The reduction of wound bacterial burden was also performed by using colony forming units (CFU) assay.

RESULTS

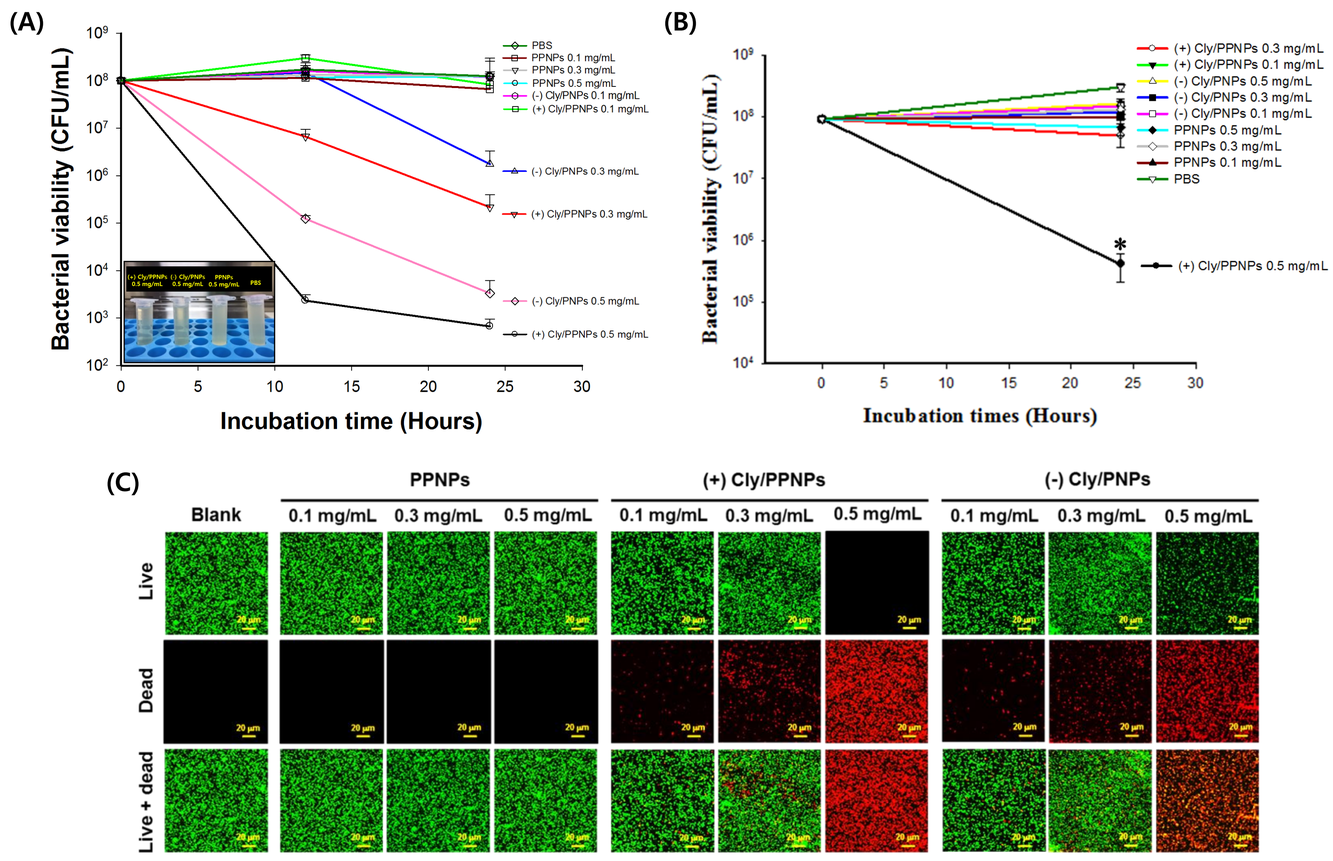
Nanoparticles adhesion to the bacteria



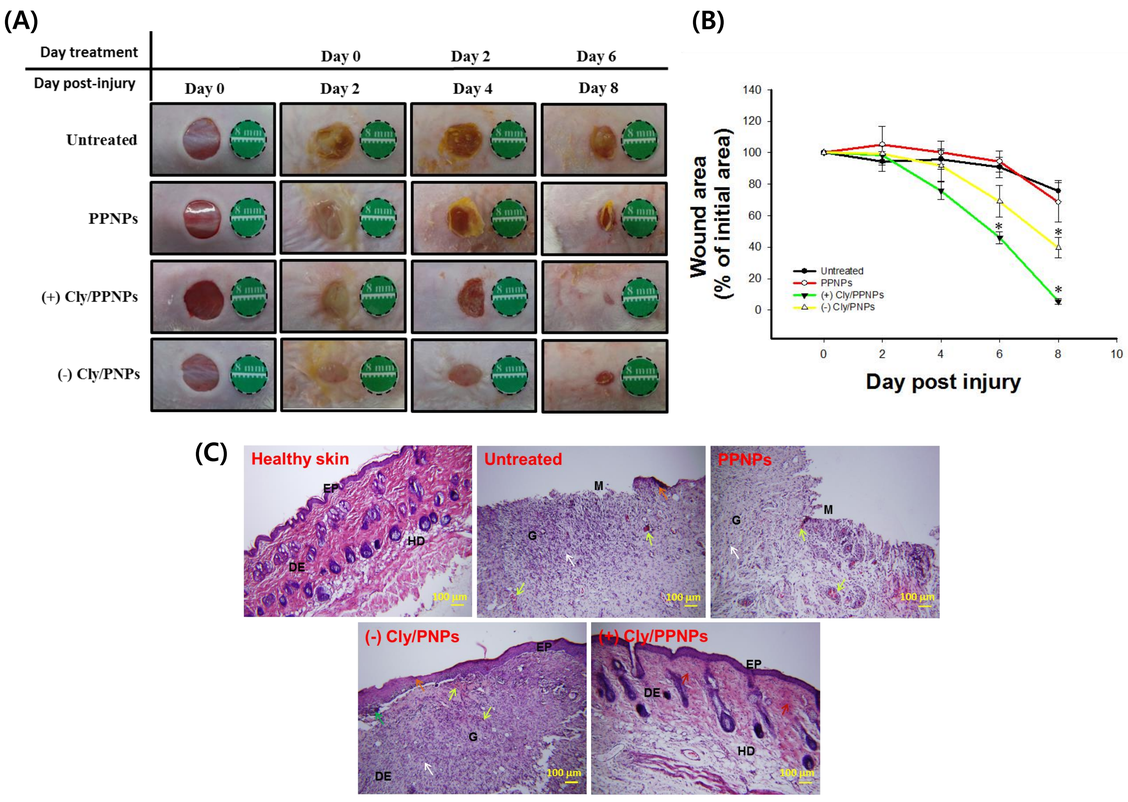
Nanoparticles were incubated with bacteria for 1 h and images were obtained using a confocal microscope. Bacterial membrane (green) stained with Syto-9 and nanoparticles (red) labeled with nile red for visualization. Inset figures show SEM images of nanoparticles bind on the bacteria.

Antibacterial activity

(A) The number of CFU, data shown is mean ± SD, n=3.
(B) The effect of binding followed with washing procedure on bacterial viability after treated with or without PPNPs, (+) Cly/PPNPs, and (-) Cly/PNPs.
(C) Confocal microscopy images after 24 hours of treatment with nanoparticles at different concentrations. Syto-9 fluorescence (green) indicates intact membrane of healthy bacteria, PI fluorescence (red) indicates membrane destruction and cell death. Blank is control group (buffer alone). Bars represent 20 μm.



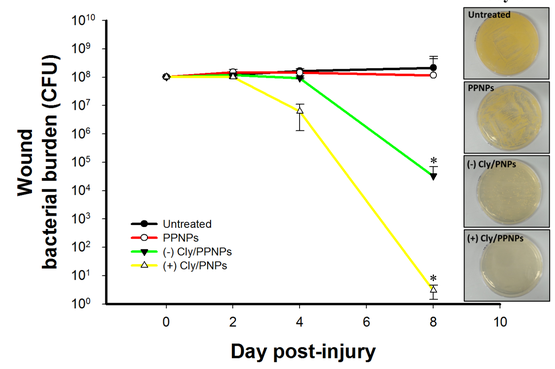
In vivo wound healing assay



(A) Representative photographs of MRSA-infected wounds of ICR mice treated with or without PPNPs, (+) Cly/PPNPs, and (-) Cly/PNPs. (B) Area reduction (%) profiles of the wounds. Values are mean ± SD, n=10 different wounds, *p < 0.05 compared with untreated group.

(C) Histological analysis (H&E staining) of MRSA-infected wounds of ICR mice at day 8. Scale bar = 100 μm. Ep = epidermal, DE = dermal junction, HD = hypodermis, G = granulation tissue and M= wound matrix. The arrow colored with orange arrows indicate early epithelialization. Green arrow shows skin crust, red arrows indicate fibroblast cells, white arrows denote mononuclear inflammatory cells and yellow arrows show neovascularization.

Viability of bacteria on the wound



Inset shows photographs of bacterial cultures from the MRSA infected wound-skin tissues (10^6 CFU) at day 8 post injury in four treatment group (PBS, PPNPs, (+) Cly/PPNPs and (-) Cly/PNPs).

CONCLUSION

✓The (+) Cly/PPNPs investigated in this study showed a potent *in vitro* and *in vivo* antibacterial activity, followed by favorable wound healing efficacy in an MRSA-infection wound of ICR mouse model.

✓Thus, could be a promising approach for treating wounds and various MRSA skin infections.

ACKNOWLEDGMENTS

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