Clindamycin-loaded polymeric nanoparticles for the effective treatment of MRSA-infected wounds

Nurhasni Hasan

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ABSTRACT

Purpose. Adhesion to the bacterial cell wall by modifying nanoparticles (NPs) physicochemical properties can improve the efficacy of antibiotic. Herein, positively charged clindamycin-releasing poly (lactic-*co*-glycolic acid)-polyethylenimine (PLGA-PEI) nanoparticles (Cly/PPNPs) and negatively charged clindamycin-releasing PLGA NPs (Cly/PNPs) were developed to treat bacterial infection. The purpose of this study was to investigate the benefit of NP-bacterial adhesion for the treatment of methicillin-resistant Staphylococcus aureus (MRSA)-infected wounds

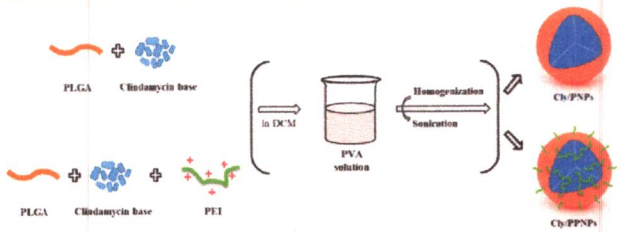
Methods. Clindamycin base was used to fabricate NPs by single oil in water (o/w) emulsification evaporation method. The *in vitro* drug release was evaluated in PBS pH 7.4 at 37°C. The *in vitro* antibacterial activity was performed against MRSA. The *in vitro* cytotoxicity study of NPs was tested to L929 mouse fibroblasts. 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. In this study, bacterial targeted-clindamycin loaded polymeric NPs were successfully developed and characterized. Both Cly/PPNPs and Cly/PNPs exhibited a sustained drug release over 2 days. The Cly/PPNPs showed the ability to bind on the surface of the bacteria, hence exhibited more potent bactericidal efficacy against MRSA as compared to the Cly/PNPs, concentration- and time-dependently. We also found that both NPs are not toxic to healthy fibroblast cells. Furthermore, Cly/PPNPs significantly accelerated wound healing and re-epithelialization in a mouse model of a MRSA-infected wounds compared to other groups.

Conclusion. These results suggest that Cly/PPNPs presented in this study could improve the efficacy of clindamycin for the treatment of MRSA-infected wounds.

RESULTS

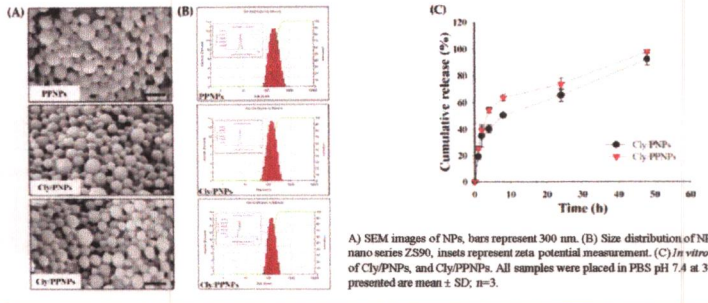
Nanoparticle preparation



Characterization of nanoparticle

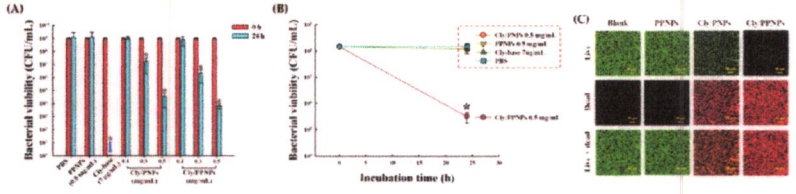
NPs	Drug Loading (% w/w)	Size (nm) DLS	Size (nm) SEM	Pdi	Zeta potential (mV)
PPNPs	Not determined	193 ± 38	184 ± 36	0.147	+17 ± 0.5
Cly/PNPs	1.43 ± 0.46	132 ± 41	141 ± 43	0.143	-16 ± 0.2
Cly/PPNPs	1.31 ± 0.26	126 ± 33	147 ± 37	0.10	+ 13 ± 0.6

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



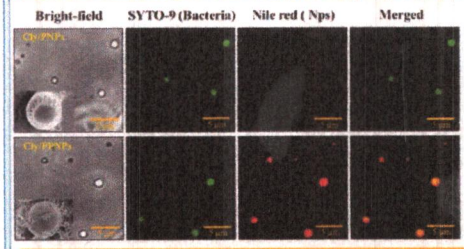
A) SEM images of NPs, bars represent 300 nm. (B) Size distribution of NPs by zetasizer nano series ZS90, insets represent zeta potential measurement. (C) *In vitro* release profile of Cly/PNPs, and Cly/PPNPs. All samples were placed in PBS pH 7.4 at 37 °C; data presented are mean ± SD; n=3.

In vitro antibacterial activity



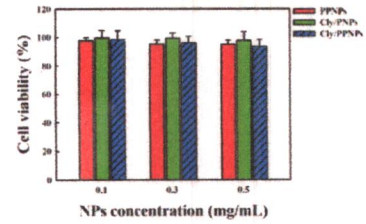
(A) The number of CFU, data shown is mean ±SD, n=3. The blue arrow at Cly-base showed no bacterial viability was found (0 CFU/mL). (B) The effect of binding followed by washing procedure on bacterial viability after treated with or without NPs. (C) Confocal microscopy images after 24 hours treatment with NPs at concentrations of 0.5 mg/mL

Adhesion of nanoparticle to MRSA



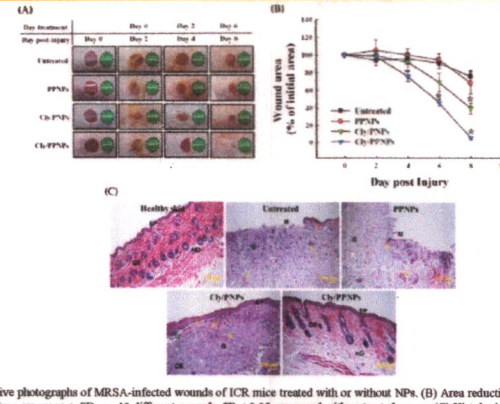
Adhesion of Cly/PNPs and Cly/PPNPs to bacteria. NPs were incubated with bacteria for 1 h and images were obtained using a confocal microscope. Bacterial membrane (green) stained with Syto-9 and NPs (red) labeled with nile red for visualization. Inset images in figures show SEM images of NPs bind on bacteria.

In vitro cytotoxicity study



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

In vivo wound healing assay



(A) Representative photographs of MRSA-infected wounds of ICR mice treated with or without NPs. (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 = 100μm. 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 monomclear inflammatory cells and yellow arrows show neovascularization.

CONCLUSION

- ✓ In this study, clindamycin-releasing polymeric NPs with surface charge modified were successfully performed.
- ✓ 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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Pusan National University
College of Pharmacy

Bacteria-targeted clindamycin loaded polymeric nanoparticles for the effective treatment of MRSA-infected wounds

Nurhasni Hasan

Advanced Drug Delivery Laboratory
Department of Manufacturing Pharmacy,
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April 25, 2019

2019 Spring International Convention of The Pharmaceutical Society of Korea

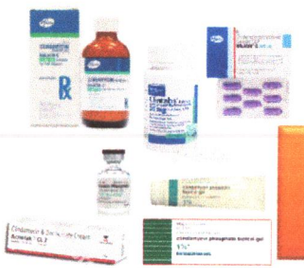
Background of the study

Bacterial infection ⇨ **MRSA-infected Wound** ⇨ Morbidity and mortality

Methicillin-resistant *Staphylococcus aureus* (MRSA)

MRSA-infected Wound ⇩ **Antibiotic**

Inefficient delivery ➔ inadequate therapeutic index and local and systemic side effects including cutaneous irritation, peeling, scaling and gut flora reduction



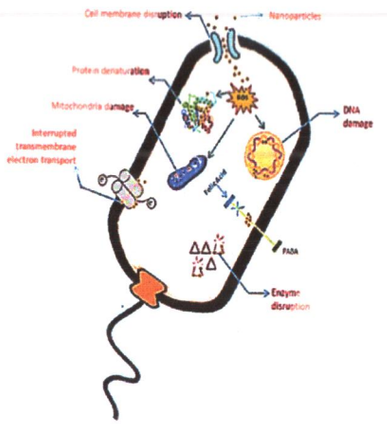
Antibiotic ⇩ **Clindamycin**

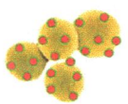
MoA : by inhibiting protein synthesis

Clindamycin ⇩ **Drug-carrier system** ⇩ **Nanoparticles** ⇩ **Clindamycin-loaded poly (lactic-*co*-glycolic acid)-polyethylenimine (PLGA-PEI) nanoparticles (Cly/PPNPs)**

Clindamycin ⇨ FDA-approved drug

Drug-carrier system ⇨
- Clindamycin/silver NPs
- (PLGA/HA) core-shell nanospheres
- SLN
- CAP NPs



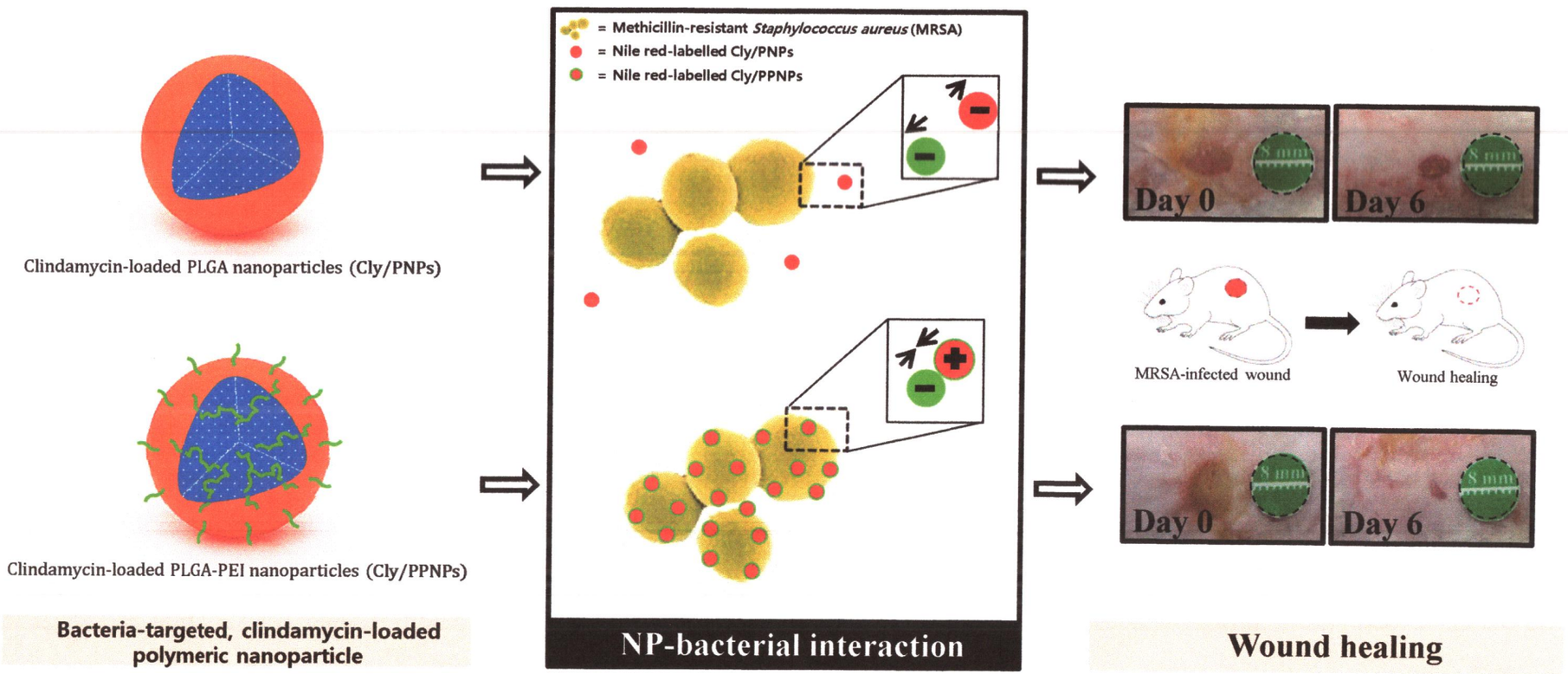


Adhesion of NPs to the bacterial cell wall

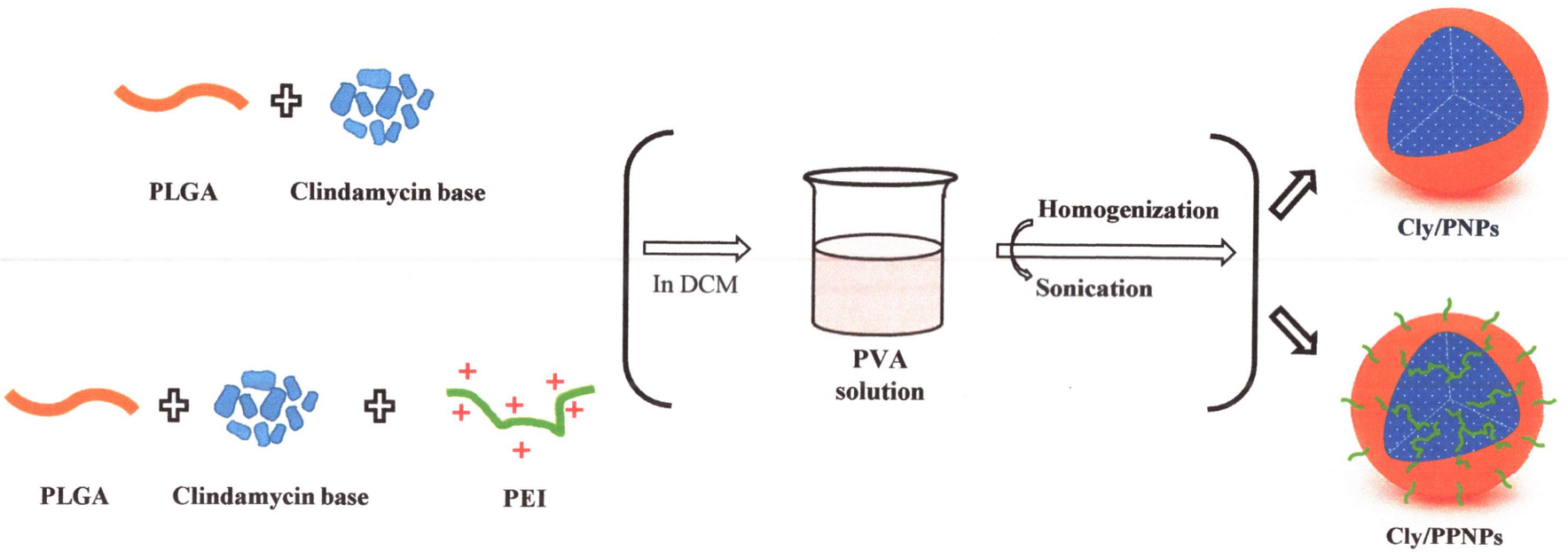
Nanoparticles ⇨ **Benefits** :
- ❑ enhanced antibiotic treatment results with fewer side effects
- ❑ diminished probability of antibiotic resistance arising from ineffective drug dosing
- ❑ high sustained local drug concentrations

Purpose of the study

To investigate the benefit of NP-bacterial adhesion for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA)-infected wounds.



~Fabrication of surface charged clindamycin NPs~



~Characterization of NPs~

NPs	Drug Loading (% w/w)	Size (nm)		Pdi	Zeta potential (mV)
		DLS	SEM		
PPNPs	Not determined	193 ± 38	184 ± 36	0.147	+17 ± 0.5
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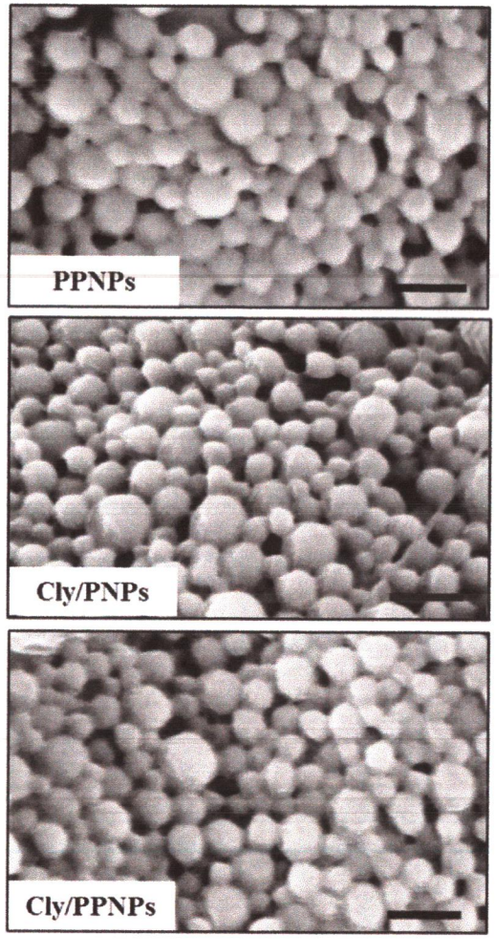
Values are expressed as mean averages ± SD of three different batch of particles

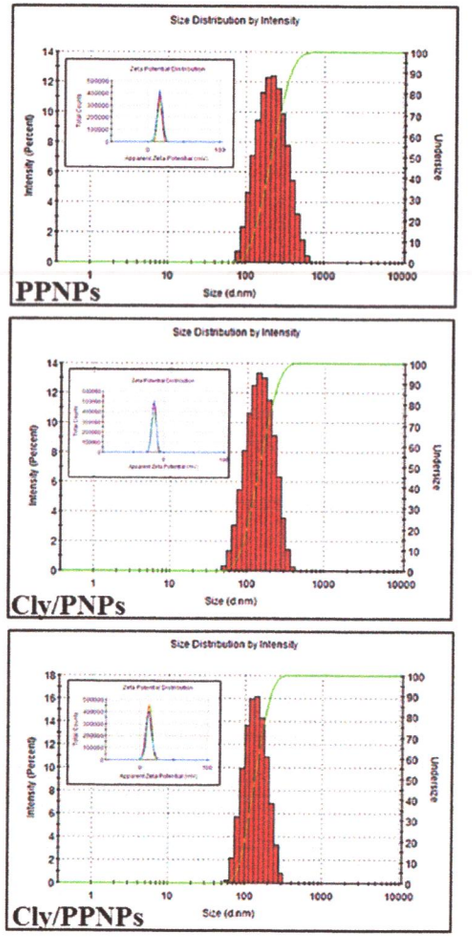
~Characterization of NPs~

Scanning Electron Microscopy (SEM) images

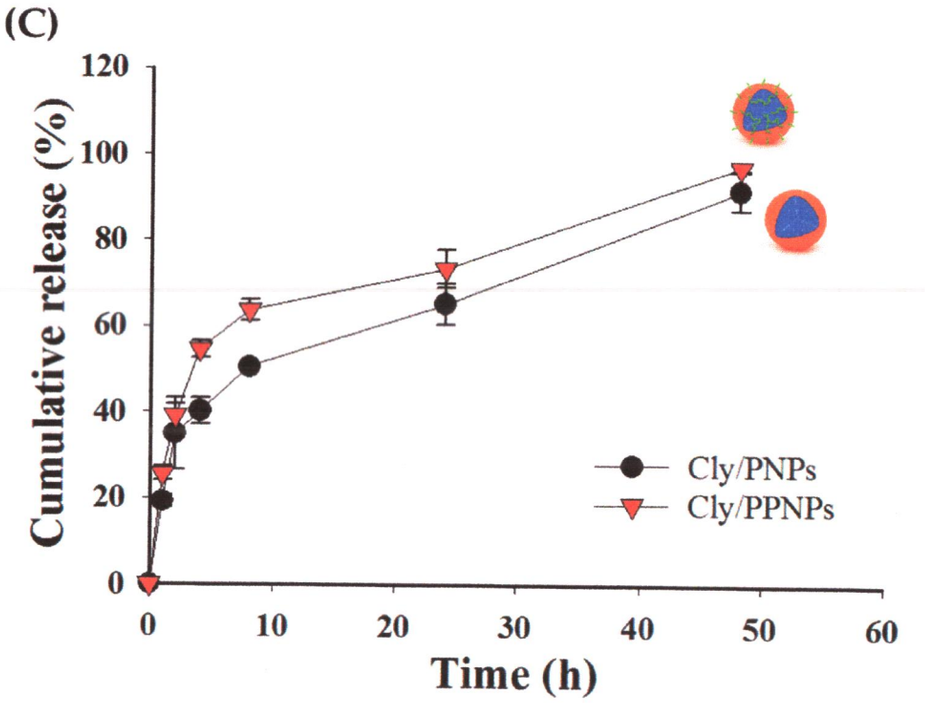


Bars represent 300 nm.

Zetasizer (nano series ZS90) results

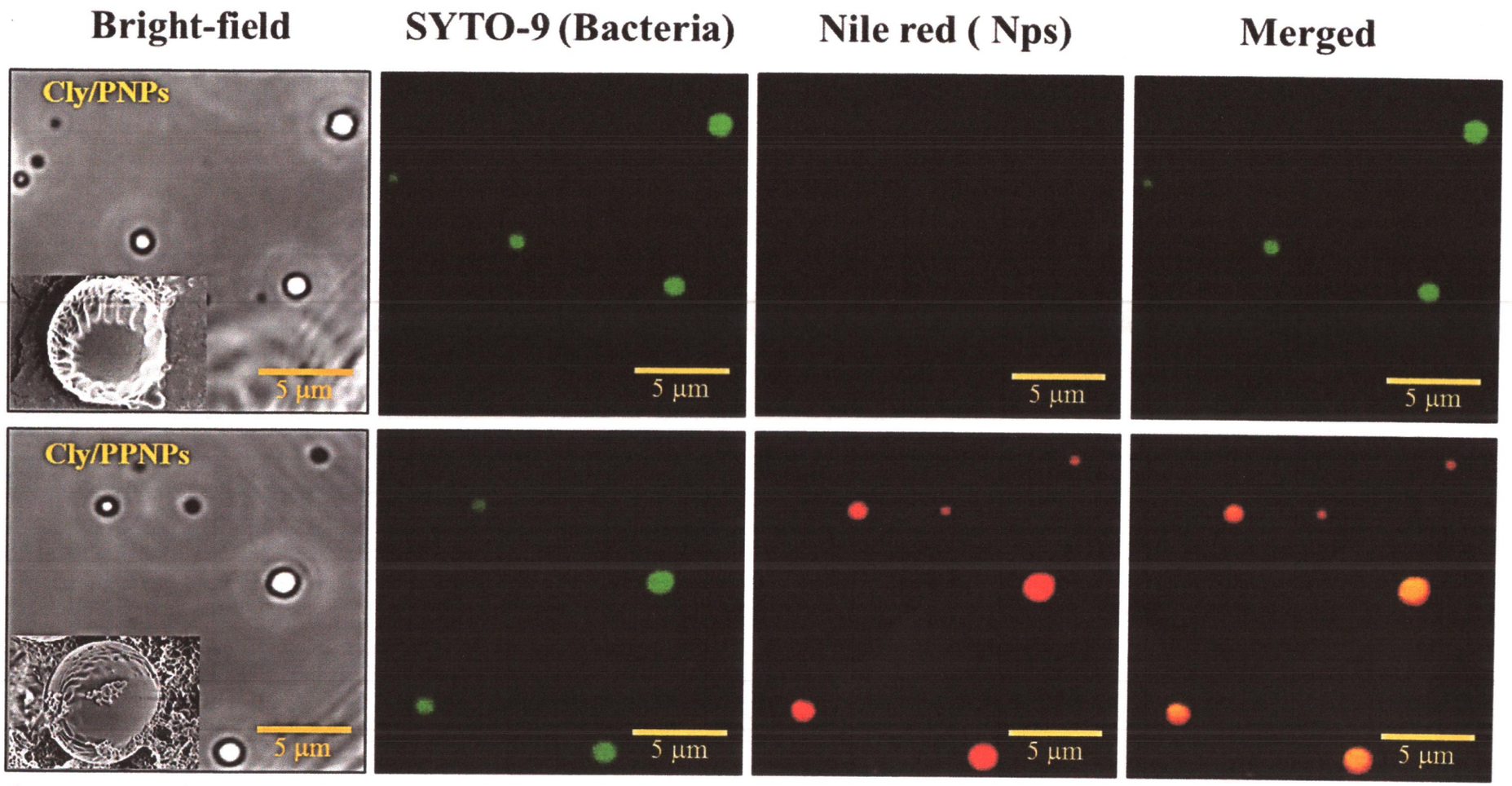


***In vitro* release**



All samples were placed in PBS pH 7.4 at 37 ℃; data are means ±SD; n = 3

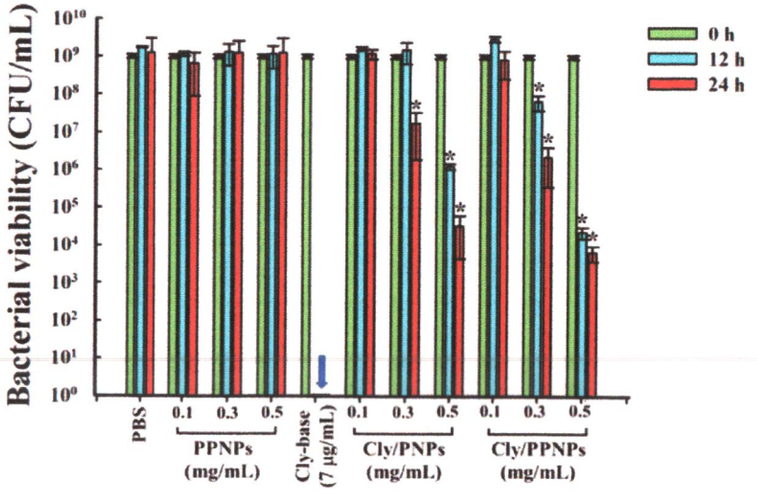
~Adhesion of NPs to bacteria~



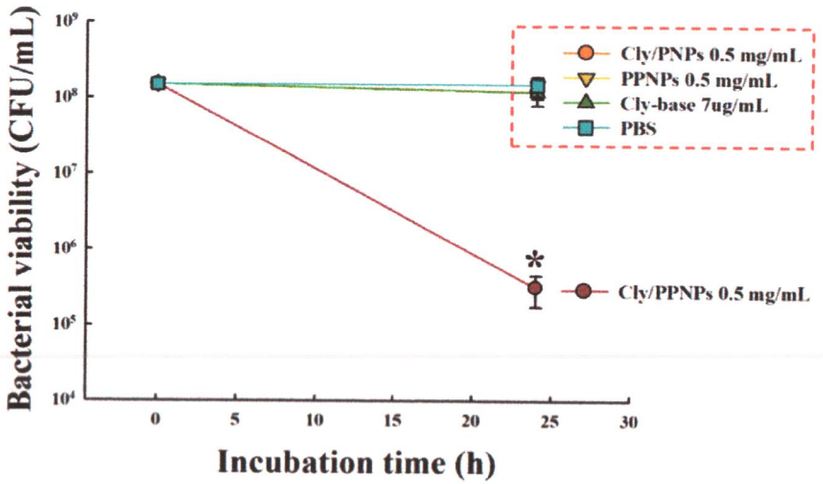
Inset images in figures show SEM images of NPs bound to bacteria.

~*In vitro* antibacterial activity of NPs~

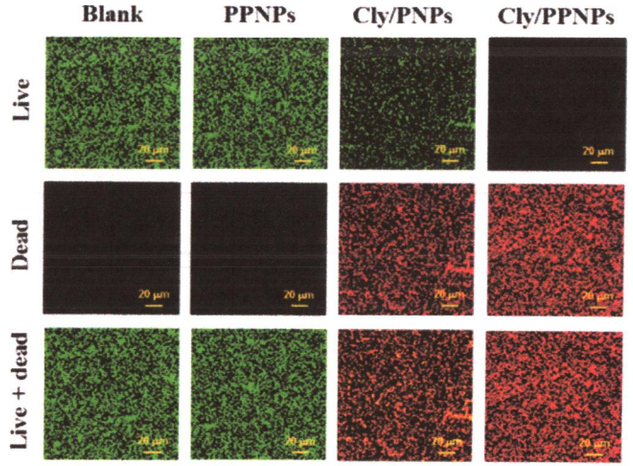
Colony forming units (CFU)



CFU with binding effect

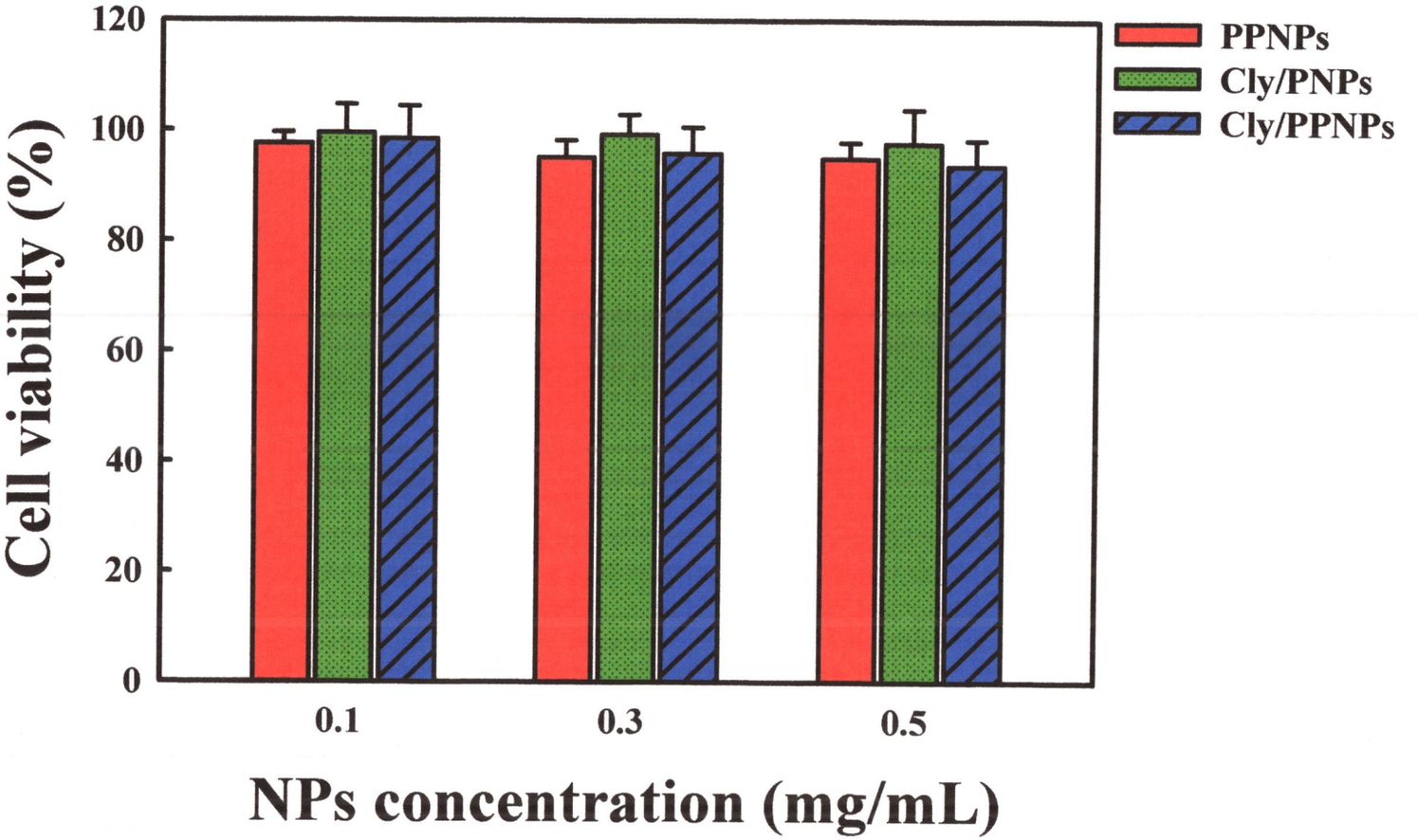


Confocal microscopy images



Syto-9 fluorescence (green) indicates the intact membrane of live bacteria, PI fluorescence (red) indicates membrane destruction and cell death. Blank is the control group (PBS alone).

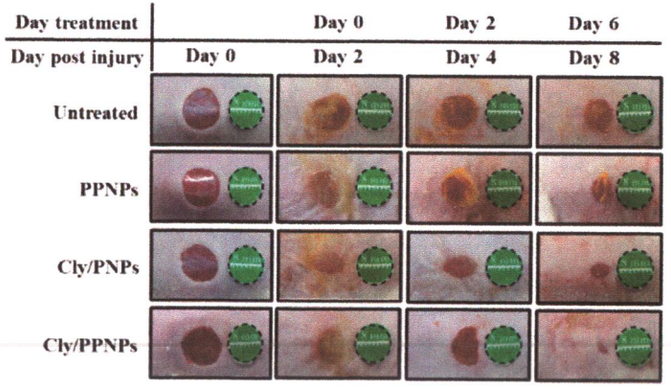
~*In vitro* cytotoxicity study~



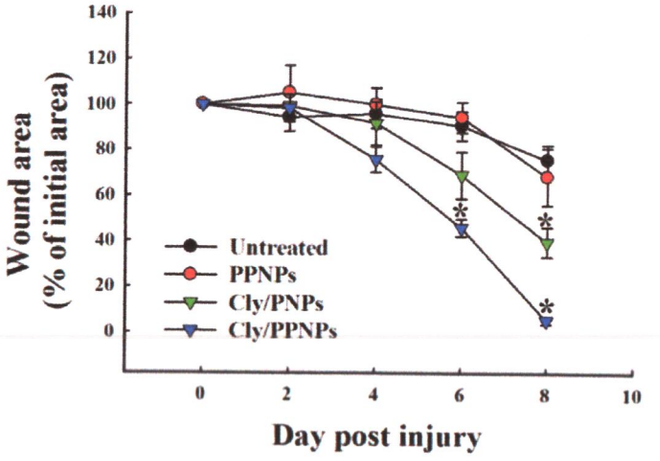
Viability (%) of L929 mouse fibroblast cells following 24 h of exposure to different concentrations of NPs (n = 8).

~In vivo wound healing assay~

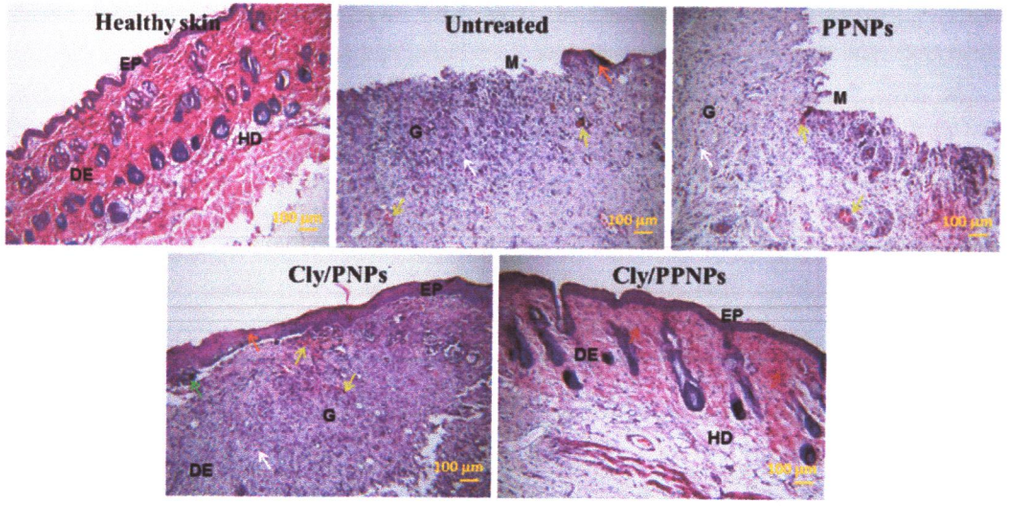
Representative photographs of MRSA-infected wounds



Area reduction (%) profiles of the wounds

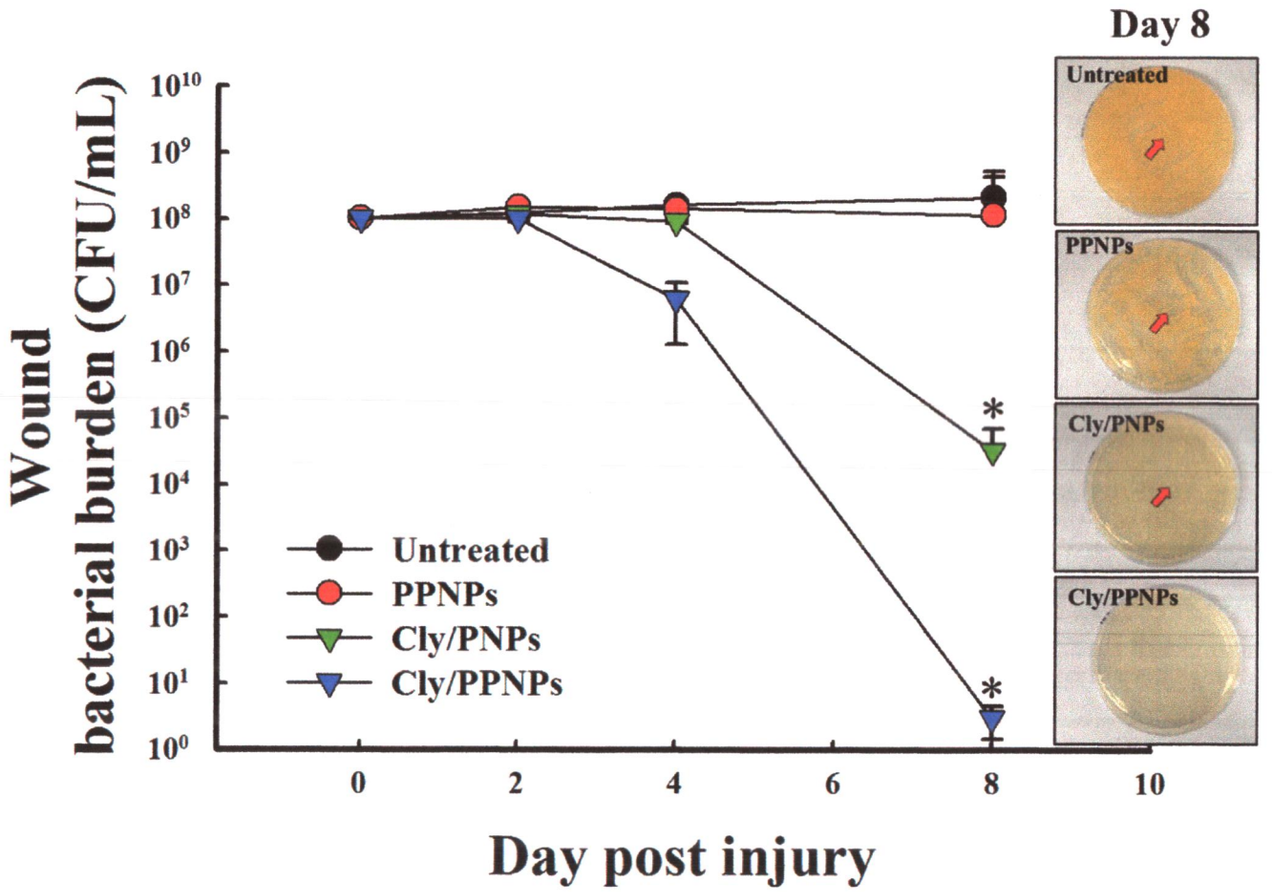


Histological analysis



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 orange arrows indicate early epithelialization. The green arrow shows skin crust, red arrows indicate fibroblast cells, white arrows denote mononuclear inflammatory cells and yellow arrows show neovascularization.

~Wound bacterial burden~



Wounds were swabbed, and bacterial burden was examined. Inset shows the bacterial growth after plating of swab samples on TSB agar at day 8 post injury.

Conclusion

- ✓ In this study, clindamycin-releasing polymeric NPs with surface charge modified were successfully performed.
- ✓ 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.

References

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Juho Lee
Shwe Phyu Hlaing
Junhwan Bae
Jihyun Kim
Hasan Elbanna.

PUSAN NATIONAL UNIVERSITY

Thank you
for your kind attention

감사합니다

Young Scientists Session 1, 2

Apr. 25th 14:30-16:20, Jade

Research regions:

P3. Pathology · Physiology

P4. Biochemistry · Molecular Biology

P9. Pharmaceutics · Physical Pharmacy

Chairperson (Reviewer)	TBA
YSS1-1 (P3-05) 14:30~14:35	The interplay between slow-cycling chemoresistant cancer cells and fibroblasts in tumor microenvironment mediates tumor recurrence in lung Jaebeom Cho (College of Pharmacy, Seoul National University)
YSS1-2 (P3-13) 14:35~14:40	Potassium usnate, shows inhibitory activity in invasion and metastasis on colorectal cancer: implication of clinical application of a water soluble form of usnic acid in anticancer therapy Yi Yang (College of Pharmacy, Sunchon National University)
YSS1-3 (P3-21) 14:40~14:45	Different context for shear stress mechanotransduction between left and right atrial muscle Anh Qui Le (College of Pharmacy, Chungnam National University)
YSS1-4 (P3-24) 14:45~14:50	Micro-environmental regulation of in vivo adipogenesis by adipose tissue macrophages Yoonkeun Cho (College of pharmacy, Seoul National University)
YSS1-5 (P4-07) 14:50~14:55	CD36 mediates fatty acid uptake and oxidation via activation of AMPK in breast cancer cells Jones Gyamfi (College of Pharmacy, Yonsei Institute of Pharmaceutical Sciences, Yonsei University)
YSS1-6 (P4-13) 14:55~15:00	FoxO6-mediated IL-1β induces insulin resistance and age-related inflammation via the TF/PAR2 pathway Sugyeong Ha (Aging Tissue Bank, College of Pharmacy, Pusan National University)
YSS1-7 (P4-15) 15:00~15:05	Interleukin-32θ inhibited macrophage-regulated breast cancer progression by targeting CCL18 signaling Thu-Huyen Pham (Department of Bioscience and Biotechnology, Konkuk University)
YSS1-8 (P4-22) 15:05~15:10	Aspirin suppresses prion infection Trang H.T. Trinh (College of Pharmacy and Institute of Pharmaceutical Science and Technology, Hanyang University)
YSS1-9 (P4-23) 15:10~15:15	S6K1 is imported into mitochondria and regulates mitochondrial gene transcription Hwamok Oh (Research Center for Epigenome Regulation, School of Pharmacy, Sungkyunkwan University)
YSS1-10 (P4-37) 15:15~15:20	Nrf2 and NF-kB signaling pathway contribute to gamma-irradiated fibroin protein-mediated inhibition of H2O2-induced inflammation in skin Hyun-Ji Park (Department of Medical Engineering & Science, Konyang University)
Coffee Break (15:20~15:30)	

YSS2-1 (P4-46) 15:30~15:35	The role of chromatin modifying enzymes in stage-specific transcriptional activation during cardiac lineage commitment **Ki Hong Nam** (School of Pharmacy, Sungkyunkwan University)
YSS2-2 (P4-55) 15:35~15:40	NMR studies on the inhibitors of IL-33/ST2 interaction **Jeongwoo Park** (College of Pharmacy, Korea University)
YSS2-3 (P9-20) 15:40~15:45	Colloidal systems of iron(II) sulfate for the therapy of colon cancer **Sungyun Kim** (College of Pharmacy, Kangwon National University)
YSS2-4 (P9-26) 15:45~15:50	Clindamycin-loaded polymeric nanoparticles for the effective treatment of MRSA-infected wounds **Nurhasni Hasan** (College of Pharmacy, Pusan National University)
YSS2-5 (P9-34) 15:50~15:55	Intestinal transport mechanism and enhanced antitumor efficacy of oral metronomic oxaliplatin-loaded **Saurav Kumar Jha** (College of Pharmacy, Mokpo National University)
YSS2-6 (P9-36) 15:55~16:00	Design and optimization of metformin hollow-core floating tablet for gastric retention and controlled release **Hyun Wook Huh** (College of Pharmacy, Chungnam National University)
YSS2-7 (P9-39) 16:00~16:05	A supersaturated self-microemulsifying drug delivery system for improving intestinal lymphatic absorption of saquinavir **Dohyun Kim** (College of Pharmacy, Chung-Ang University)
YSS2-8 (P9-42) 16:05~16:10	Preparation and characterization of eutectic mixture of pirfenidone and N-acetylcysteine **Jae-gon Song** (College of pharmacy, Chungbuk National University)
YSS2-9 (P9-48) 16:10~16:15	Development of physiologically-based pharmacokinetic model for the short chain perfluoroalkyl acid: Case of perfluoropentanoic acid for risk **Go-Wun Choi** (College of Pharmacy, CHA University)
YSS2-10 (P9-56) 16:15~16:20	Tumor microenvironment enzyme triggered imaging and consequent release of peptide therapeutics **Juhan Suh** (College of pharmacy, Seoul National University)