

# Nitric Oxide-releasing Alginate Dressing for *In vivo* Healing Activities in MRSA-infected wounds

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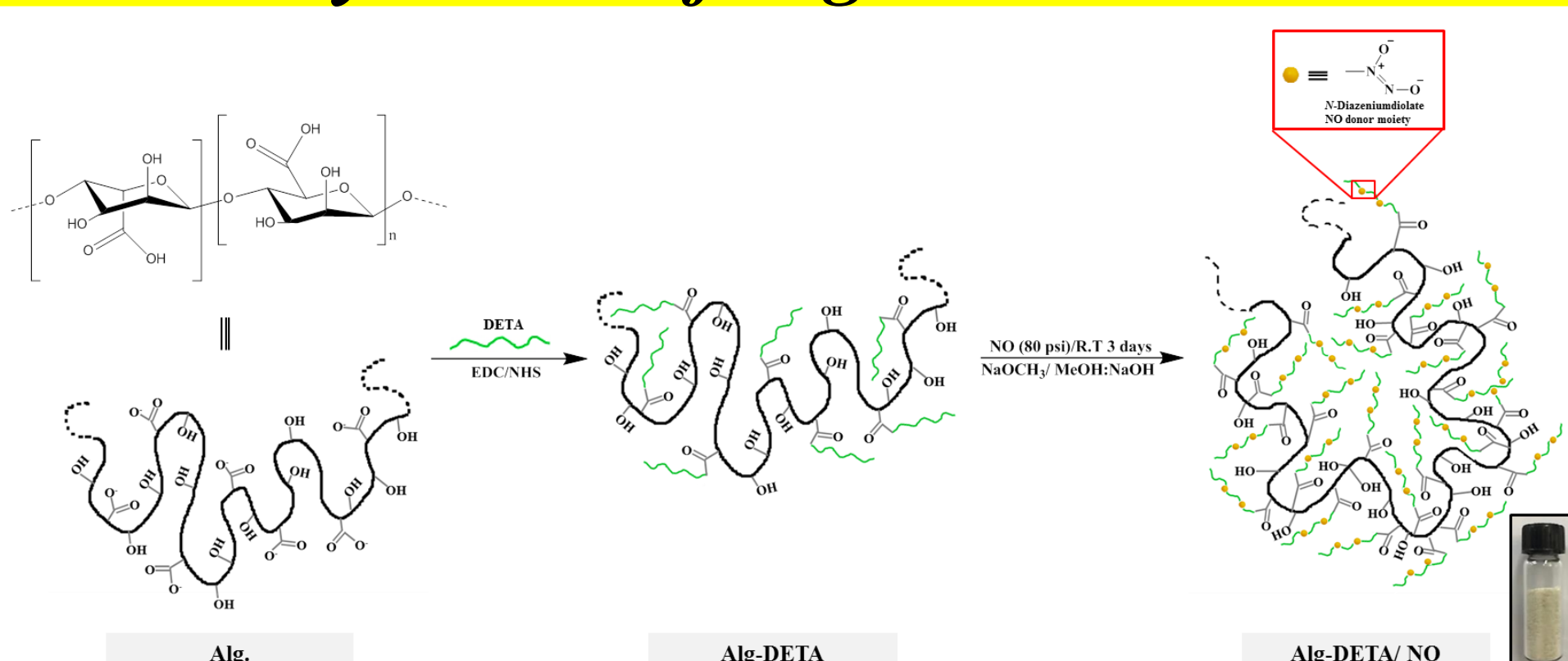
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## ABSTRACT

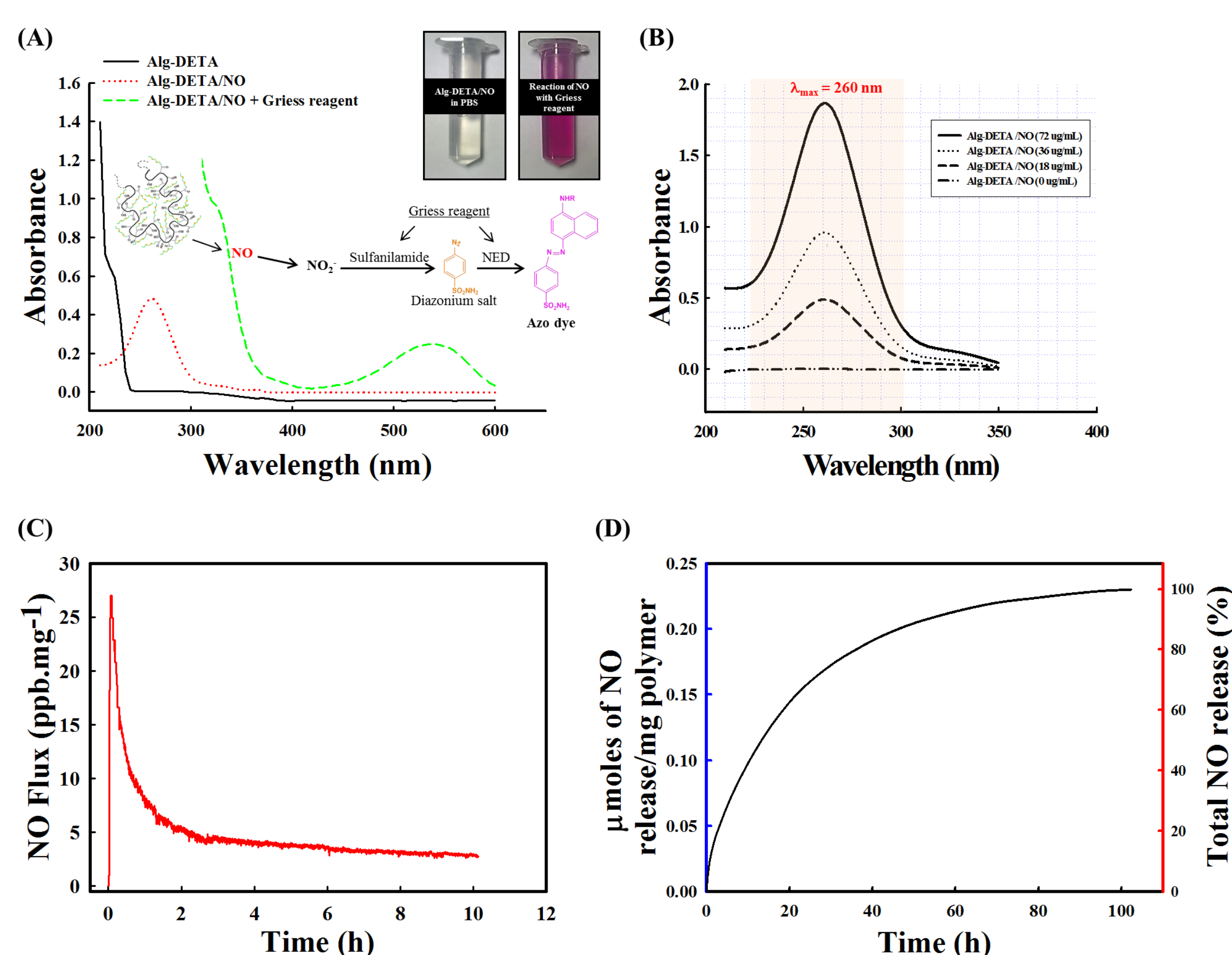
Wound infected with the pathogen resistance bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) represents a high risk in patient and can caused significant morbidity and mortality worldwide. Diazeniumdiolates, a nitric oxide (NO) donor is being investigated for its benefit in a variety of biomedical application. In this study, we synthesized NO-releasing alginate (Alg-DETA/NO) for the treatment of MRSA-infected wounds. Alg-DETA/NO exhibited a remarkable prolonged NO release profile over 4 days. Importantly, Alg-DETA/NO showed a potent antibacterial activity against MRSA with minimal toxicity to fibroblast cells. Moreover, application of Alg-DETA/NO to MRSA-infected wounds resulted in favorable wound healing with accelerated wound size reduction, reduced skin bacterial infection, and promoted angiogenesis and collagen depositions. Thus, Alg-DETA/NO present in this study could be a promising dressing for the treatment of MRSA-infected wounds.

## RESULTS

### Synthesis of Alg-DETA/NO

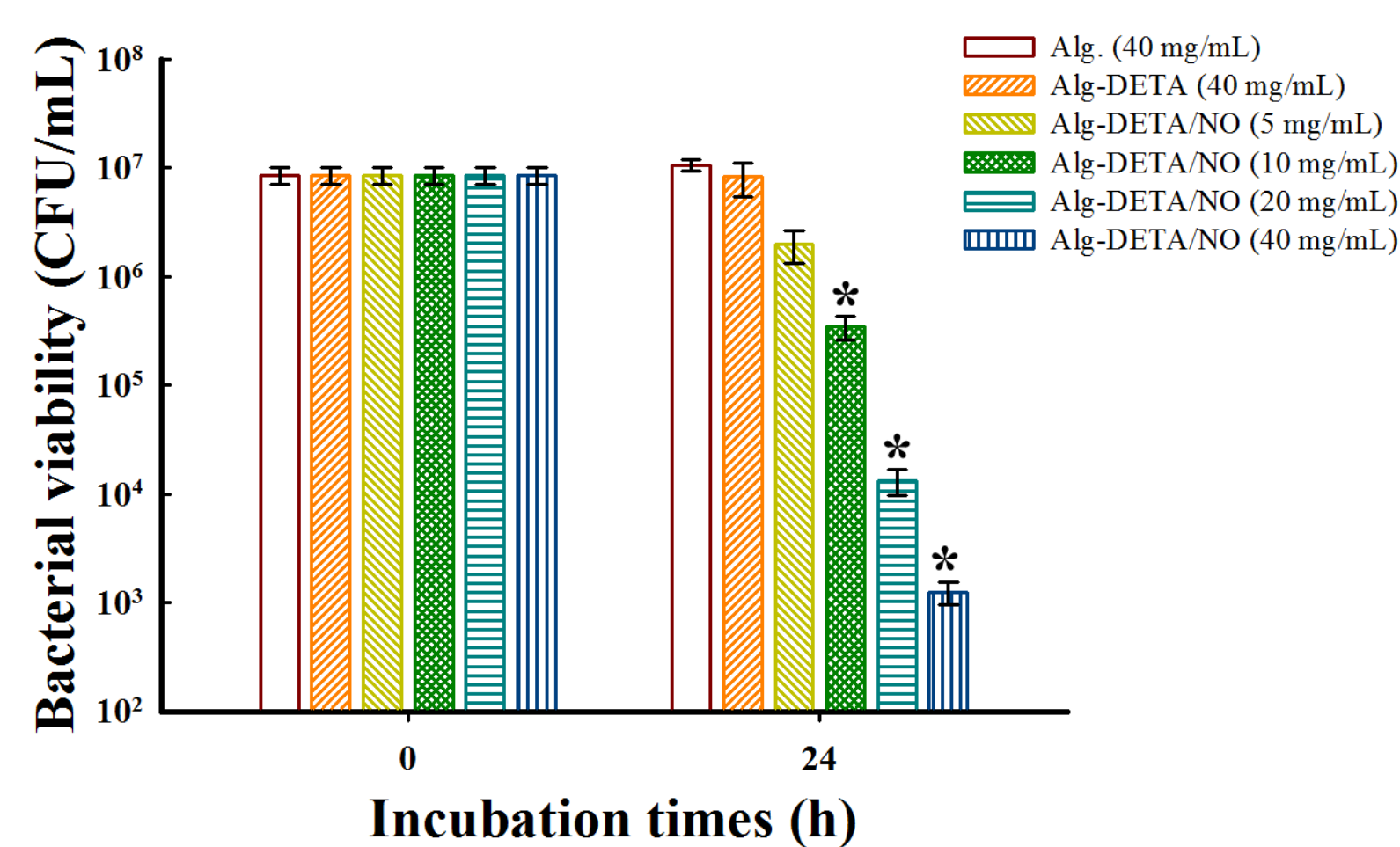


### Characterization of Alg-DETA/NO



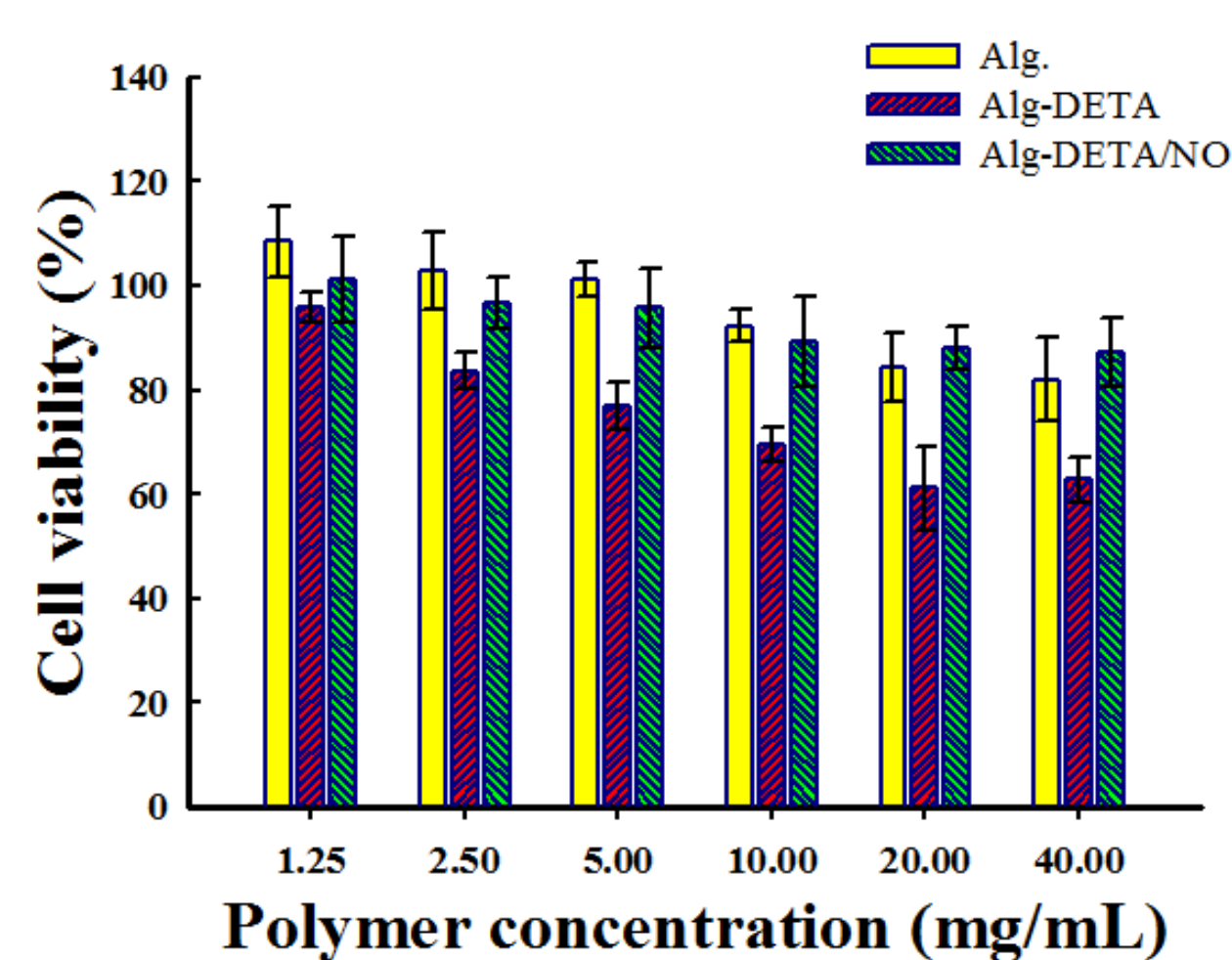
Characterization of Alg-DETA/NO. (A) UV-Vis absorption of Alg-DETA/NO (red line), Alg-DETA/NO with Griess reagent (green line) and Alg-DETA (black line). (B) UV-Vis spectra of Alg-DETA/NO in different concentration. (C) Real time NO-release for the first 10 h. (D) Plot of percentage total NO release and amount of NO release vs time measured via NOA in PBS (pH 7.4, 37°C).

### In vitro antibacterial activity



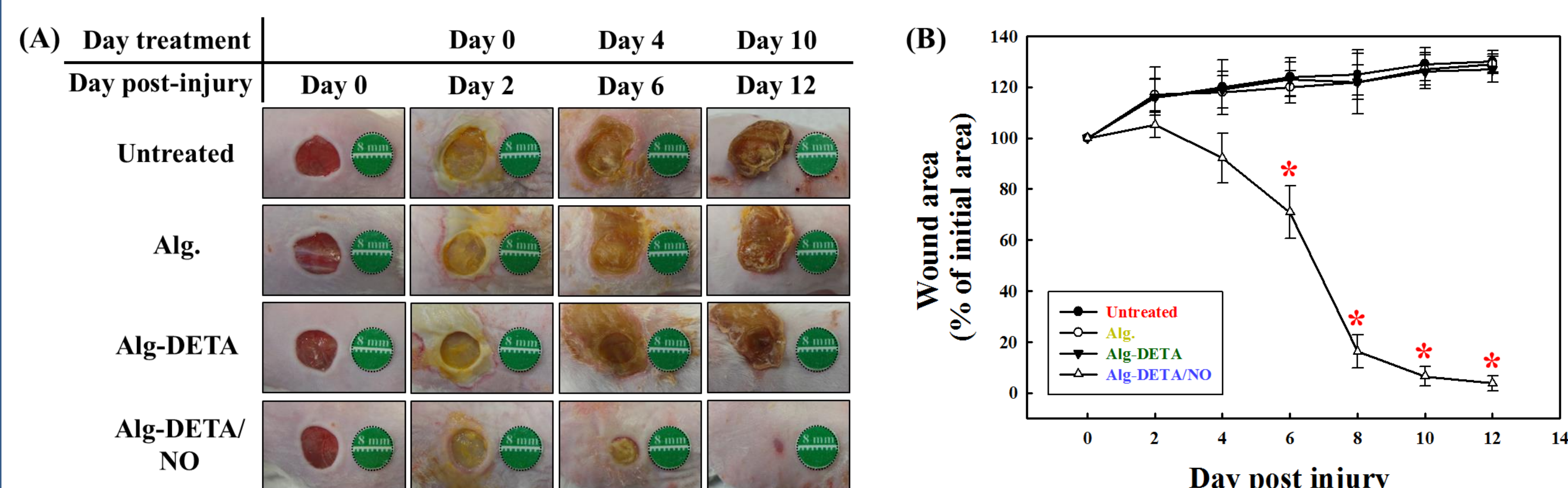
Antibacterial activity of alginate, Alg-DETA and Alg-DETA/NO against MRSA. Data shown are mean  $\pm$  SD, n = 3.

### In vitro cytotoxicity study



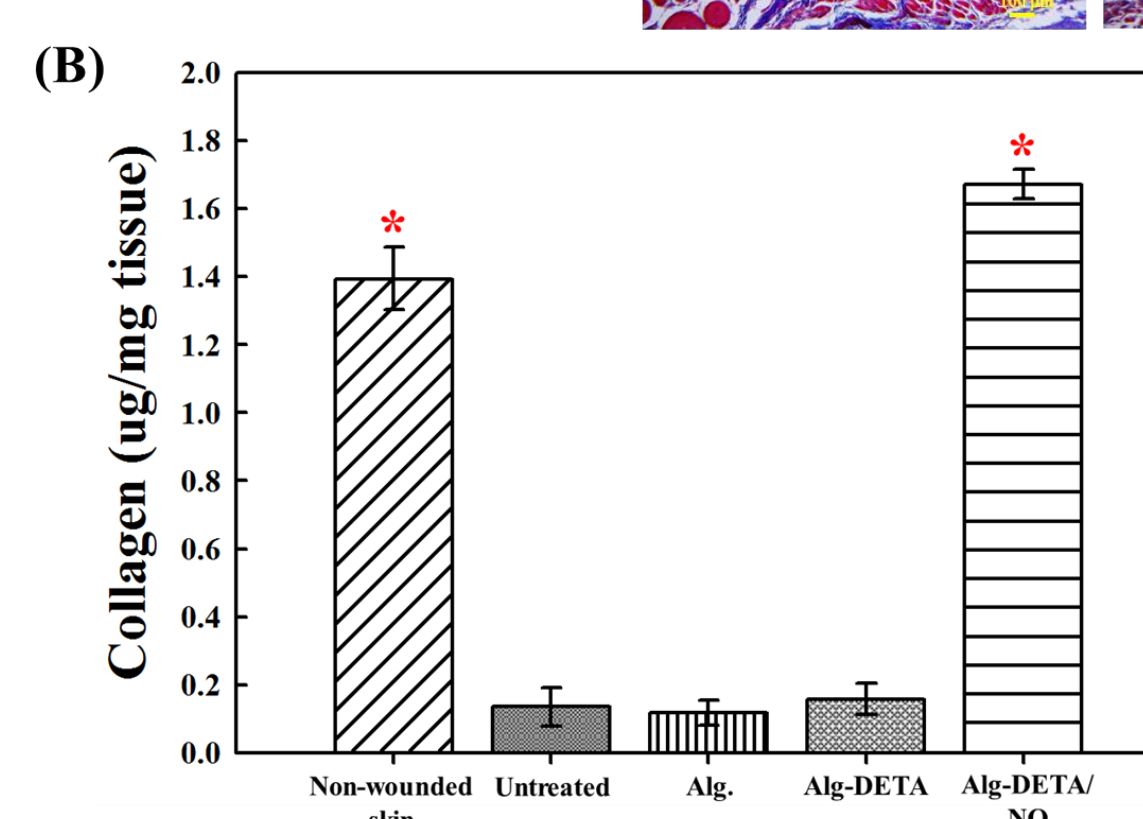
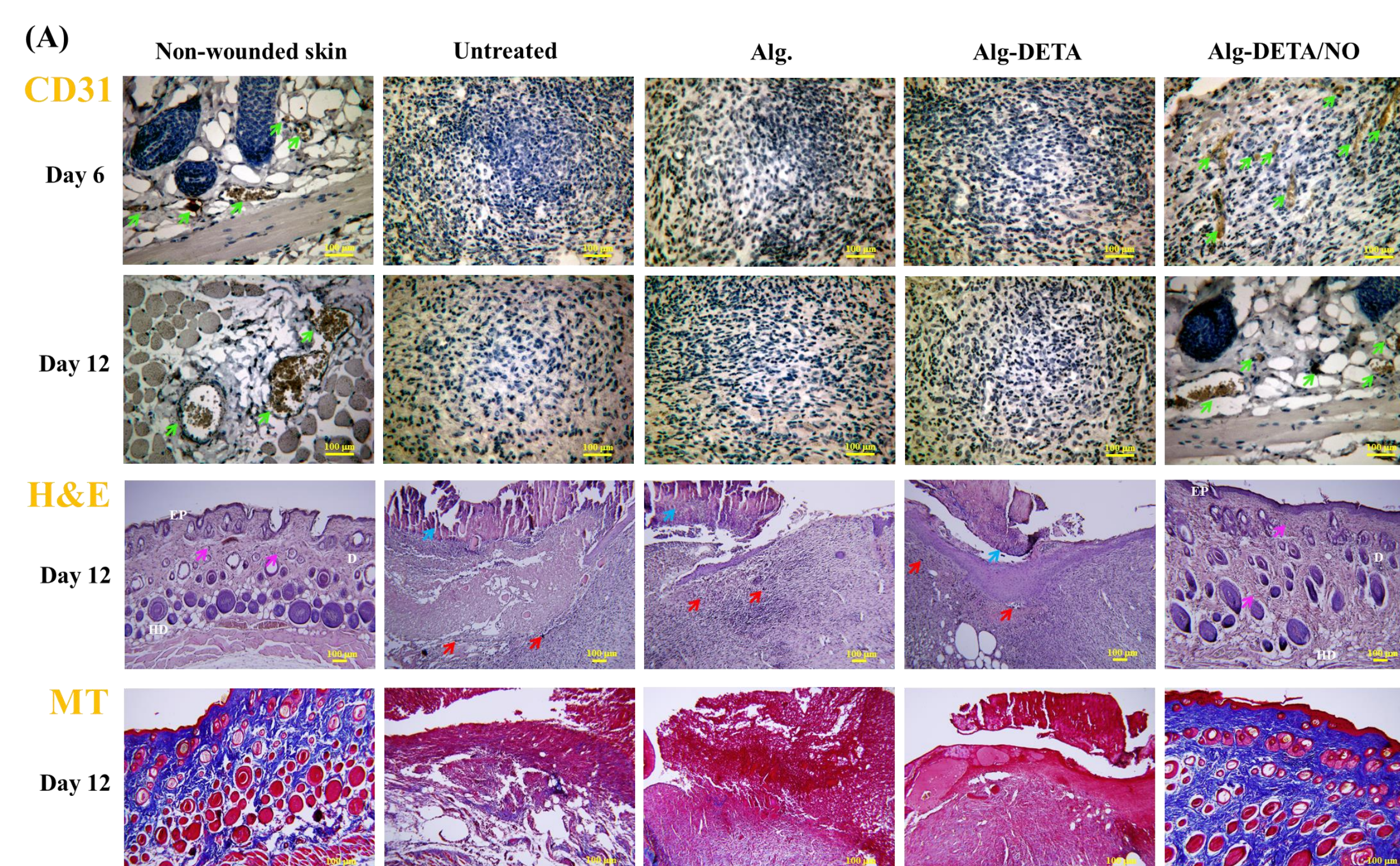
Viability (%) of L929 mouse fibroblast cells after 24 h exposure to alginate, Alg-DETA and Alg-DETA/NO at different concentrations (n = 8)

### In vivo wound healing assay



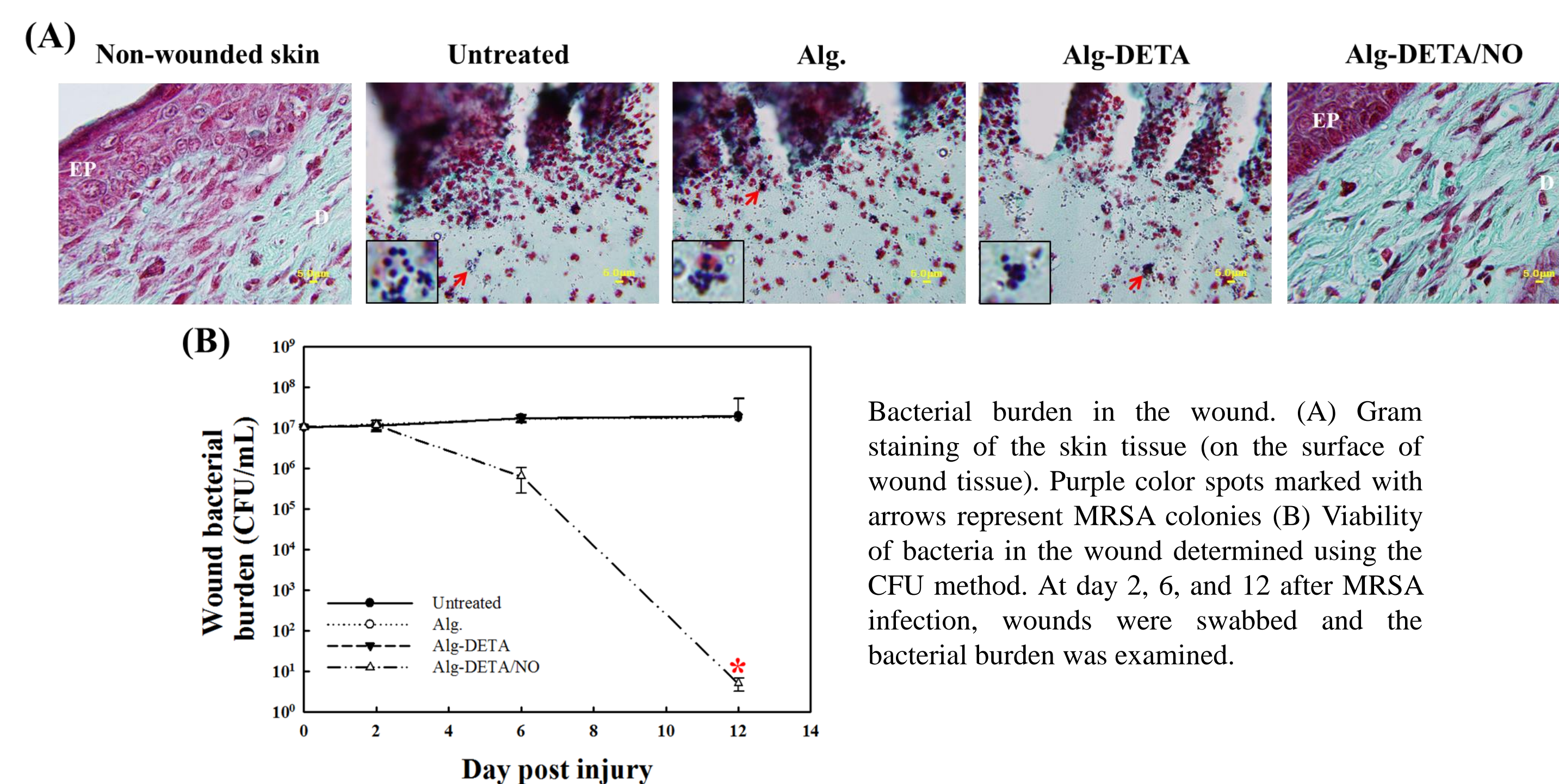
MRSA-infected wounds in ICR mice. (A) Representative photographs of healing in MRSA-infected wounds treated with or without Alg-DETA/NO. (B) Wound area reduction percentage of mice skin lesions relative to the initial 8-mm wound. Data shown are mean  $\pm$  SD; n=10, different wounds; \*P<0.05, compared with untreated group.

### Histological analyses



(A) Immunohistochemistry (CD-31) and histological (H&E and MT stain) analysis of MRSA-infected wounds in ICR mice. Scale bar = 100  $\mu$ m; Ep = epidermal; D = dermal junction; HD = hypodermis; G = granulation tissue; M = wound matrix. Green arrows indicate blood vessels, blue arrows indicate early epithelialization, pink arrows indicate fibroblast cells, and red arrows denote mononuclear inflammatory cells. Blue color in trichrome stained images indicates collagen arranged in parallel to the surface. (B) Collagen amount in wound tissue at day 12 post-injury (data presented as mean  $\pm$  SD)

### Reduction of wound bacterial burden



Bacterial burden in the wound. (A) Gram staining of the skin tissue (on the surface of wound tissue). Purple color spots marked with arrows represent MRSA colonies (B) Viability of bacteria in the wound determined using the CFU method. At day 2, 6, and 12 after MRSA infection, wounds were swabbed and the bacterial burden was examined.

## CONCLUSION

- ✓ NO-releasing functionalized alginate has been successfully synthesized and characterized.
- ✓ The Alg-DETA/NO exhibited a remarkably prolonged NO release profile over a period of 4 days.
- ✓ The Alg-DETA/NO investigated in this study showed a potent *in vitro* and *in vivo* antibacterial activity, followed by favorable wound healing efficacy in MRSA-infected wounds of ICR mouse.
- ✓ Thus, could be a promising approach for treating wounds and various MRSA skin infections.

## ACKNOWLEDGMENTS

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