microorganisms

MDPI

Supplementary Material

Cysteine-Capped Hydrogels Incorporating Copper as Effective Antimicrobial Materials against Methicillin-Resistant *Staphylococcus aureus*

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1. Materials and Methods

1.1 TUNEL Assay.

To examine the cytotoxicities of hydrogels, dorsal skin of ICR mice was topically applied with a hydrogel with or without cooper for 24 h. Skin was excised, immersed and fixed in 10% formalin. Death cells in a 3 μm thick skin section was examined using a TUNEL assay kit (R&D systems) [1]. Briefly, biotinylated nucleotide was incorporated at the 3′-OH DNA ends of the fragmented DNA in dead cells. Horseradish-peroxidase-labeled streptavidin was bound to biotinylated nucleotides, which were detected using diaminobenzidine as a substrate to produce a dark brown reaction. To quantify the dead cells, a total of at least 3 random visual fields (150 μm x 150 μm) in a skin section was counted to quantify the dead cells.

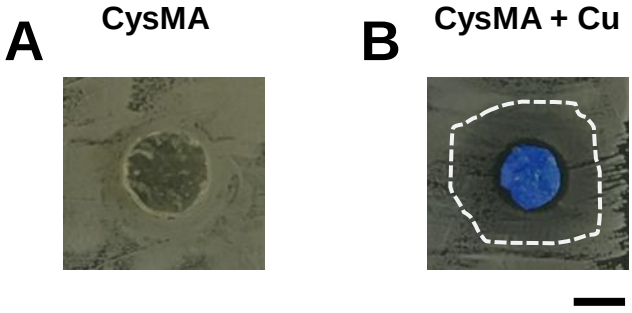

Figure S1. Anti-MRSA activity of copper release from hydrogels. The inhibition zone (dashed circle) tests were used to detect the antibacterial activities of hydrogels without (A, CysMA) or with (B, CysMA + Cu) copper ions on agar plates spread with MRSA252. Bar = 0.5 cm.

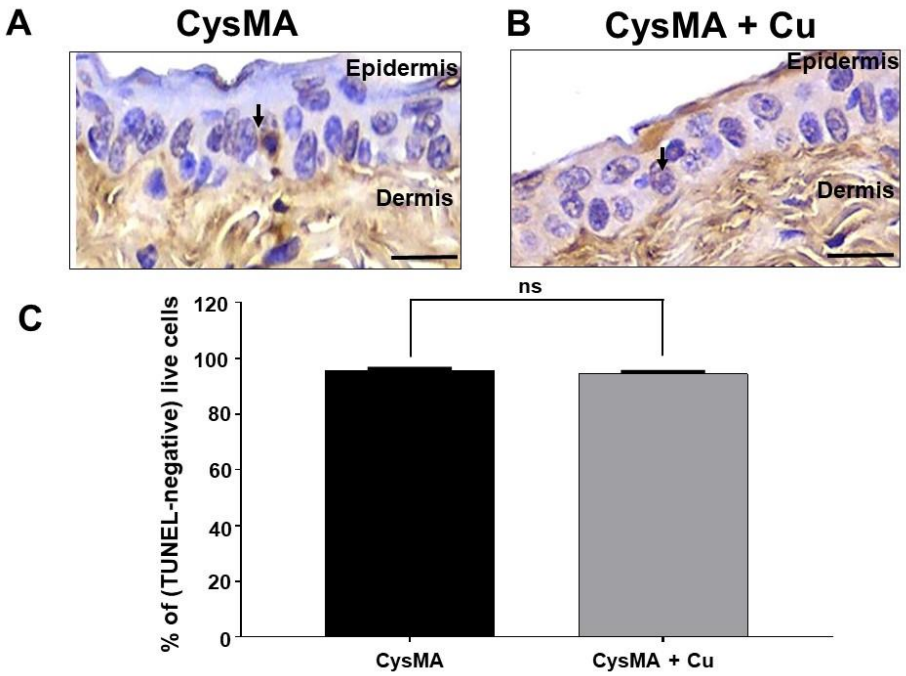

Figure S2. No significant cytotoxicities of hydrogels. (A) Skin histology (TUNEL staining) of mice 24 h after topical application of a hydrogel with (CysMA + Cu) or without (CysMA) cooper. Nuclei of live cells was stained with hematoxylin (blue stains). Arrows indicate the dead cells detected by diaminobenzidine (brown stains). Bars = 30 μm. (B) The percentages of (TUNEL-negative) live cells in skin applied with a hydrogel with or without cooper were quantified. Data are the mean ± SD of three independent experiments. ns = not significant.

References

1. Traisaeng, S.; Herr, D.R.; Kao, H.-J.; Chuang, T.-H.; Huang, C.-M. A Derivative of Butyric Acid, the Fermentation Metabolite of Staphylococcus epidermidis, Inhibits the Growth of a Staphylococcus aureus Strain Isolated from Atopic Dermatitis Patients. *Toxins* **2019**, *11*, 311, doi:10.3390/toxins11060311.