Copper Nanoparticle-based Multifunctional Scaffolds for Wound Healing

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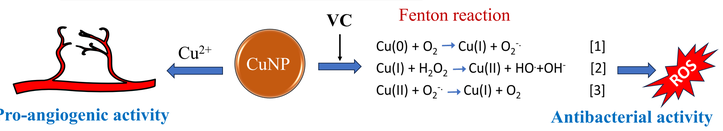
INTRODUCTION

- The pathophysiology of non-healing wounds has been associated with the colonization of multispecies bacteria as well as poor vascularization in wounds.
- Thus far, each of the above aspects have been separately investigated and an integrated approach to simultaneously addressing these issues in a cost-effective single drug delivery platform has yet to emerge.
- The objective of this study was to develop copper nanoparticle (CuNP)-based injectable scaffolds with antibacterial as well as proangiogenic properties for wound healing.
- Our strategy is based on the current understanding that (1) copper has great potential to as antimicrobial agent, while it is less harmful to the host cell because copper is an essential metal for life; (2) copper is a co-factor for many angiogenic promoters and mediators, and can switch on such molecules from the quiescent to pro-angiogenic state; (3) ascorbic acid (Vitamin C, VC) exhibits dual functions of pro-oxidative and antioxidative activities depending on its concentrations.

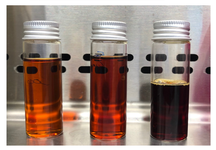
Fine tuning of the properties of CuNPs towards



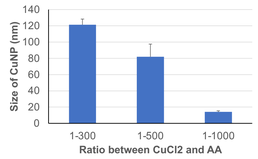
METHODS

Synthesis of CuNPs. CuNPs (20-120 nm in size) were synthesized using a green hydrothermal synthesis method. The size of CuNPs were fine-tuned by using ascorbic acid (AA) as a sacrificial agent.

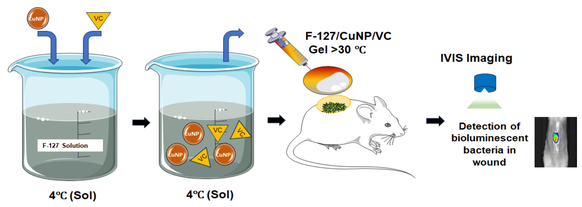
Synthesized CuNP solution



Size of CuNP determined by DLS particle size analyzer

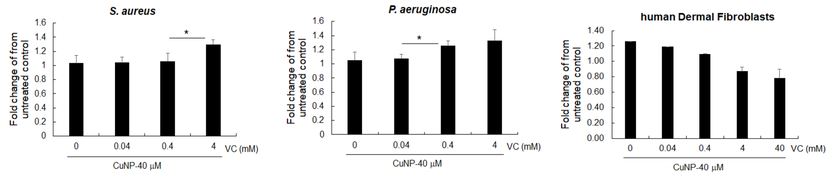


Preparation of injectable hydrogel encapsulating CuNP and VC. An injectable formulation of hydrogel scaffold was prepared by encapsulating CuNPs (80 nm in size, 100 μg/g) and VC (10%) in a thermos-reversible Pluronic F-127 hydrogel (25%), which enabled controlled release of CuNP and VC.



RESULTS

Effects of VC on CuNP-mediated Fenton reaction for ROS generation



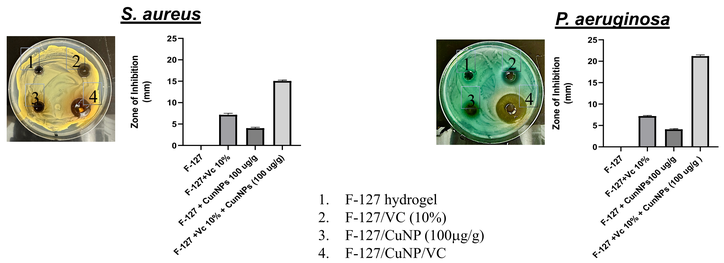
N=3 for each group. *: p<0.05

Effects of VC on the MIC of CuNP against broad spectrum bacteria

Bacterial Strains	MIC of CuNP	
	Without VC	With 10 mM VC
MSSA (ATCC 6538)	> 100 μM	20 μM
MRSA (ATCC BAA-44)	> 100 μM	10 μM
DSPA (ATCC 15692)	> 100 μM	5 μM
DRPA (ATCC BAA-2108)	> 100 μM	5 μM

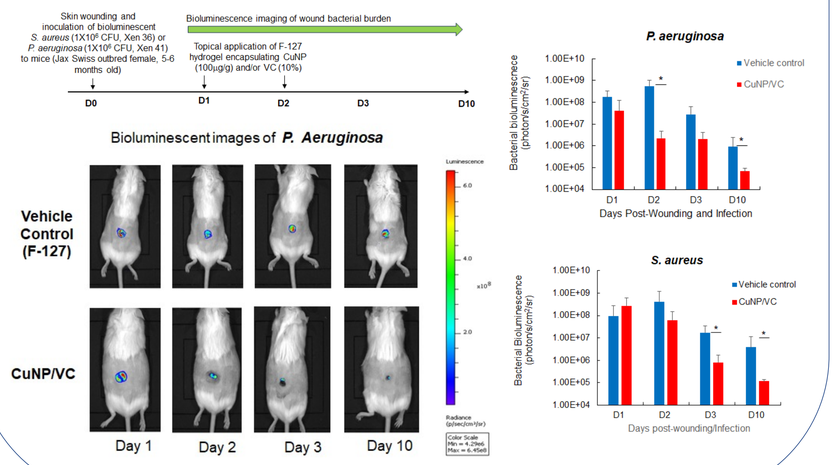
MSSA: Methicillin-susceptible *S. Aureus*
MRSA: Methicillin-resistant *S. Aureus*
DSPA: Drug-susceptible *P. aeruginosa*
DRPA: Drug-resistant *P. aeruginosa*

Antibacterial effect of CuNP/VC hydrogel –*In vitro* zone inhibition assay



1. F-127 hydrogel
2. F-127/VC (10%)
3. F-127/CuNP (100μg/g)
4. F-127/CuNP/VC

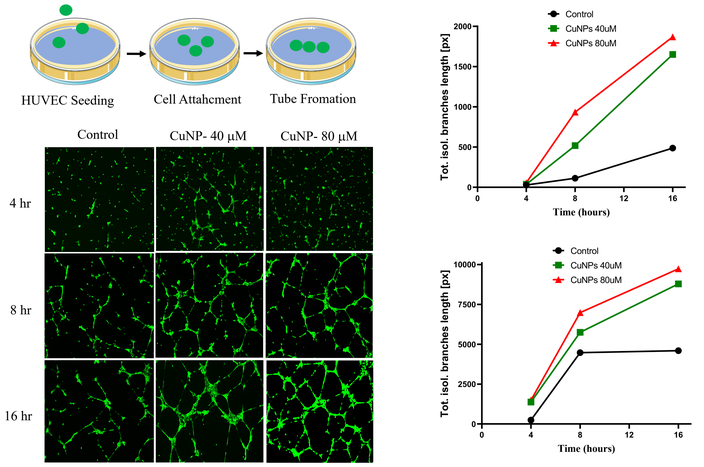
Antibacterial effect of CuNP/VC hydrogel –*In vivo* mouse skin wound infection



N=4-6 mice for each group. *: p<0.05

Effects of CuNP on angiogenesis- *In vitro* tube formation assay

In vitro endothelial cell tube formation assay



DISCUSSION

- In this study, CuNPs (20-120 nm in size) were synthesized using a hydrothermal synthesis method and the antibacterial and proangiogenic capabilities of CuNPs were fine-tuned by optimizing the dose of vitamin C that reacts with CuNPs.
- The antibacterial activity of CuNPs was significantly enhanced when combined with higher concentrations of vitamin, which synergistically enhanced a Fenton reaction for reactive oxygen species (ROS) generation in both gram-positive and gram-negative bacteria, while hDFs were less susceptible to it.
- The CuNPs also exhibited pro-angiogenic activity via dose-dependent manner.
- Our results support the feasibility of CuNP-based multifunctional hydrogel scaffold that facilitates the eradication of bacterial pathogens as well as proangiogenic response for wound healing.