

# Incorporation of nano-copper solution in wound bed preparation to facilitate wound healing: a case series

Copper plays a role in various cellular processes during wound healing. With its antibacterial and angiogenic properties, it serves as an excellent topical antiseptic agent. Wound cleansing is a crucial component of wound bed preparation to establish an optimal wound environment. A novel nanotechnology incorporating copper nanoparticles into a wound cleansing solution is used in this study to describe the efficacy of nano-copper solution in facilitating wound healing process. This evaluation includes a total of 10 patients with diverse aetiology of wounds. Each patient used the nano-copper solution as a cleansing agent and adheres to the notion of wound bed preparation. The primary outcome of wound healing is wound size reduction. The study concludes that the application of nano-copper solution improved wound healing without any adverse consequences.

In recent years, nanotechnology has been integrated into biomedical applications. Nanoparticles are efficacious in field of wound care, due their versatile therapeutic action in various stages of wound healing (Pérez et al, 2023). Their small size ranging between 1 to 100nm and large contact area increases the likelihood of bio-interaction and permeability at the wound surface (Gowda et al, 2023). Metallic based nanoparticles such as gold, silver, zinc and copper have been integrated in dressing products (Mendes et al, 2022). Copper nanoparticles (CuNPs) are widely used in dentistry (Ma et al, 2022), disinfectant industry (Alherek et al, 2023) and agriculture (Bakshi et al, 2021). Despite copper being a more accessible metal and more economic, the use of copper in wound care is relatively new with limited copper-based dressing available in the market.

Copper is essential trace element in living organisms, serving as a cofactor of enzymes necessary for cellular metabolism (Ruiz et al, 2021). Both copper insufficiency and excessive accumulation leads to a spectrum of diseases involving metabolic disorders, cardiovascular and neurodegenerative disorder (Balsano et al, 2018). In humans, the main source of copper is derived from intake of organ meat, nuts and shellfish. Copper is absorbed from the gastrointestinal tract and about 80% of it excreted through bile and faeces (Wapnir, 1998). The bioavailability of copper varies from 75% when consuming 400 mcg/day of copper through food to 12% when the diet contains 7.5 mg/day of copper (FNB: IOM, 2001).

The involvement of copper in the process of wound healing has been established through both in vivo and in vitro studies (Sandoval et al, 2022). During the inflammatory phase, the activity of neutrophils and macrophages are enhanced by Nerve Growth Factor (NGF). NGF promotes the growth of keratinocytes and the migration of fibroblasts during the proliferation and remodelling phase of wound healing (Sandoval, 2021). The activity of NGF is facilitated by the presence of copper ions (Kornblatt et al., 2016). Vascular Endothelial Growth Factor (VEGF) and angiogenin which are crucial modulators of angiogenic activity, are also regulated through copper-dependent stimulation (Sen et al, 2002).

The antimicrobial activity by copper is achieved through their adhesion to cell walls and cell membranes, leading to the release of copper ions. This in turn, disrupts enzymatic function, modifies protein structure (Xu et al, 2022) and gives rise to oxidative stress leading to cell death of susceptible microorganisms (Montero, 2019). CuNPs are shown to be effective in suppressing *Pseudomonas aeruginosa* biofilm formation (Lewis et al, 2015), as well as exhibit biocidal activity against multi-resistant organisms including MRSA, ESBL (Qiao et al, 2019) and *Acinobacter baumannii* (Al-Kadmy et al, 2023). Additionally, copper also facilitates generation of free radicals, which contribute to its virucidal effect (Merkel, 2021). Fungi, specifically *Candida Albicans* are found to be susceptible to copper ion fungicidal properties as well (Salah et al, 2021).

Prolonged exposure to high concentration

## Harikrishna K.R. Nair

MD MSC FRCPI FRCPE  
FCWCS PHD  
Head, Wound Care Unit,  
Department of Internal  
Medicine, Hospital Kuala  
Lumpur; Professor,  
Faculty of Medicine,  
Lincoln University College,  
Malaysia

## Hor Seow May

MBBS (MMMC)  
Postgraduate Diploma  
in Wound Care  
Management, Lincoln  
University College,  
Malaysia; Wound Care  
Unit, Hospital Queen  
Elizabeth II, Kota Kinabalu,  
Sabah, Malaysia

### Key words

- Nano-copper solution
- Wound bed preparation
- Case series
- Wound healing

### Declarations

The authors declare that this study was carried out without any commercial or financial affiliations that could be seen as a possible conflict of interest.

of copper may cause cytotoxicity through the activation of reactive oxygen species (Karlsson et al. 2008). Zhou et al evaluated wound healing in vivo using different concentrations of CuNPs (10, 20, 50, 100 and 200µg/ml) and observe enhanced wound healing with no cytotoxicity. Considering the small size of CuNPs, any surplus particles are eliminated through the gastrointestinal system and efficiently metabolised within the body to ensure equilibrium is maintained. There is little evidence regarding the systemic effects of CuNPs through topical absorption, as most toxicology studies on CuNPs have focused on inhalation and ingestion route (ATSDR, 2022). However, copper has been used safely for decades as food additives, in intrauterine devices and dental amalgam. Copper based nanomaterials with maximum concentration of 1% are declared safe by Scientific Committee on Consumer Safety (2021) to be used on dermal and oral surface.

Given copper's essential role in wound healing and its antimicrobial properties, it

demonstrates great potential as a topical antiseptic agent. Regular cleansing with topical antiseptics has been an integral part of routine wound care. The integration of CuNPs in solution may aid the goal in wound bed preparation through its action as antimicrobial cleansing solution. Rinsing or soaking the wound will help in reducing microbial load and loosen devitalised tissue, facilitating debridement process (Rodeheaver and Ratliff, 2018). As slough, necrotic tissue, foreign bodies and excessive exudate can promote a favourable environment for wound infection and biofilm formation (Murphy et al, 2019). Ohsumi et al (1988) observed that the presence of copper ion (Cu<sup>2+</sup>) resulted in disruption of the plasma membrane's permeability barrier in *Saccharomyces cerevisiae* within a span of 2 minutes. There is also reduction of 99% of *Escherichia coli* and *Staphylococcus aureus* at 10 minutes of exposure to CuNP composites (Banerjee et al, 2023).

All wounds contain some degree of bacterial contamination, but this does not impact

Table 1. Results.

Patient	Age (year)	Gender	Wound diagnosis	Co-morbidis	Wound dimension at initial assessment (cm <sup>2</sup> )	Wound dimension at endpoint (cm <sup>2</sup> )	Total percentage of wound size reduction	Duration of treatment (weeks)
1	60	Male	Left leg necrotising fasciitis	-Type II DM -Hypertension	60.8	6	90%	12
2	54	Male	Right DFU	-Type II DM	40.5	3	93%	6
3	58	Female	Right leg abscess	-Type II DM -Hypertension	35	5.8	77%	6
4	48	Male	Sacral PI stage 4	-IHD -Inflammatory myositis	11	0	100%	6
5	69	Male	Right peripheral arterial ulcer	-Hypertension -Dyslipidemia	19.3	4.5	77%	8
6	52	Male	Right DFU	-Type II DM	60	2.5	96%	8
7	59	Female	Left foot necrotising fasciitis	-Type II DM -Hypertension -Dyslipidemia	112	38	66%	8
8	41	Female	Right DFU	-Type II DM	42	6.3	85%	10
9	72	Male	Sacral PI stage 4	-Type II DM -Hypertension	162	50	69%	12
10	60	Male	Right DFU	-Type II DM -IHD	18	0	100%	10

Figure 1. Case 1; 60-year-old, male, diabetic and hypertensive, Post wound debridement for left leg necrotising fasciitis;  
**a.** Intial assessment  
**b.** Week 6; **c.** Week 12.



Figure 1a



Figure 1b



Figure 1c

Figure 2. Case 2; 54-year-old, male with diabetes, Done surgical debridement for infected right DFU;  
**a.** Intial assessment  
**b.** Week 3; **c.** Week 6.



Figure 2a



Figure 2b



Figure 2c

Figure 3. Case 3; 58-year-old, female, diabetic and hypertensive, Underwent incision and drainage for right leg abscess;  
**a.** Intial assessment  
**b.** Week 3; **c.** Week 6.



Figure 3a



Figure 3b



Figure 3c

Figure 4. Case 4; 48-year-old, male with sacral pressure injury grade 4, Developed pressure injury due to reduced immobility from inflammatory myositis;  
**a.** Intial assessment  
**b.** Week 3; **c.** Week 6.



Figure 4a



Figure 4b



Figure 4c

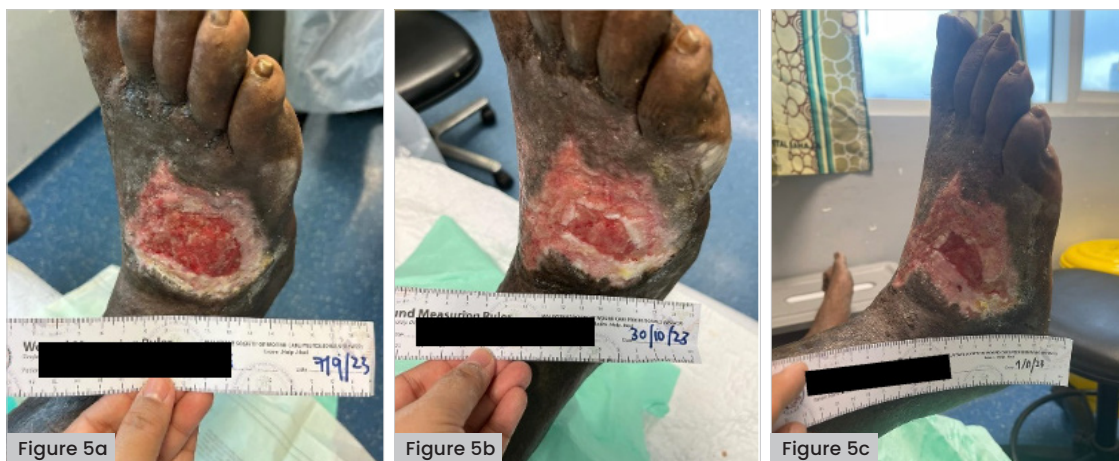


Figure 5. Case 5; 62-year-old, male with hypertension, dyslipidaemia and peripheral arterial disease, Non-healing right foot ulcer. Ankle brachial index of 0.5 with monophasic Doppler waveform; a. Initial assessment b. Week 4; c. Week 8.

the course of wound healing (Edwards et al 2004, IWII 2022). However, when colonisation advances to wound infection, wound healing will be delayed. Davies et al (2007) associates wound with microbial load more than  $1 \times 10^4$  CFU/g to have this detrimental effect. Not all infection elicits symptoms or signs, thus, routine cleansing plays a crucial role in preventing and reducing microbial burden. Moreover, an expert panel concurred that proactive approach in preventive medicine need to be implemented in prevention of infection (Nair et al, 2023).

The objective of this study is to assess the clinical effectiveness of using nano-copper solution on wound bed preparation to enhance wound healing through re-epithelisation.

### Methodology

This study was conducted in Wound Care Unit of Hospital Kuala Lumpur. Patients are selected randomly among the individuals who are regularly followed-up in the outpatient wound care clinic. The observation encompassed various size and types of wounds, including diabetic foot ulcers (n=4), surgical wounds (n=3), pressure injuries (n=2) and peripheral arterial ulcer (n=1). The study examines the wound healing progress over the period of 6 to 12 weeks using nano-copper solution containing 0.2% ionised copper (CuHeal++TM) as a wound cleanser.

Each subject received the same standard of care and the principle of wound bed preparation were followed. The wound and peri-wound skin were cleansed using a solution containing nano-copper before applying gauze soaked with nano-copper solution on the wound for a period of 5 to 10 minutes. Then, debridement of wound is performed if indicated. Wound is cleansed again with nano-copper solution after debridement to remove any remnants of debrided tissue. Lastly, appropriate an wound dressing is applied based on wound assessment with

TIME framework. The frequency of dressing changes differs depending on the condition of the wound and type of secondary dressing provided. However, all patients were subjected to the same procedure and documentation for subsequent follow-up. Data regarding the subjects' demographic and their co-existing medical conditions are gathered along with the evaluation of wound progress. Consent had been obtained for all data and images collected during the period of study.

### Discussion

Two subjects achieved complete wound epithelisation, whereas four subjects' wound area achieved near complete closure. Patient no 7 and no 9 exhibit a lower percentage of wound size reduction in comparison to the others, possibly due to the substantial initial wound dimension ( $>100\text{cm}^2$ ) prior to commencement of treatment. However, all subjects (n=10) exhibited healthy, viable and moist wound bed at the end of study. The findings of the study align with multiple case reports on the use of copper-impregnated wound dressings to promote wound healing in challenging cases such as hard-to-heal wounds, diabetic foot ulcers, and infected wounds. (Melamed et al, 2023; Lu et al, 2022). Several studies have documented the role of CuNPs in promoting fibroblast proliferation and keratinocyte migration, both of which are crucial for wound repair (Wang et al, 2021, Kornblatt et al, 2016). Comparatively to a more widely used topical antimicrobial, silver, it has been established that dressing containing copper significantly promote angiogenesis and upregulates proangiogenic factors (Borkow et al, 2010).

During the period of study, none of the patient indicated signs of wound infection which supported the efficacy of nano-copper solution in reducing microbial load. Nair et al (2023) utilised fluorescence imaging to

identify presence of bacterial on wound and they observed a decrease in bacterial load following wound cleansing with CuNPs based solution. Copper induces damage to bacterial cells mainly by the production of reactive oxygen species. On top of it, the inclusion of copper-containing nanoparticles induces a more potent antimicrobial action even at low concentration (Ma et al, 2022).

Not only effective as a wound cleanser, the nano-copper solution also proved useful to prevent wound infection and aid re-epithelisation. Ideally topical antiseptic should be able to reduce microbial load, promote wound healing, be non-toxic and not cause resistance (Bigliardi et al, 2017). The nano-copper solution applied topically, which is also odourless, colorless and sting-free is well tolerated by all subjects. No local or systemic side effects were reported despite the extended duration of usage up to 12 weeks in some subjects.

Undeniably, copper is indispensable in various human physiological function especially in context of wound healing. As an important mineral, the Recommended Dietary Allowance (RDA) for adults is 900µg/day. Copper deficiency is linked to reduction of collagen and elastin synthesis resulting in poor wound healing (Kornblatt, 2016). Additional research should be undertaken to explore the full potential of CuNPs in tissue repair and regeneration, other than as topical antimicrobial agent.

### Limitations

This study has a limited sample size of 10, which is not representative of the general population. Furthermore, there was no specific delineation of patient selection criteria and the duration of study for each subjects varies depending on wound condition.

### Conclusion

This case series concluded that the use of nano-copper solution in wound cleansing has synergistic effects that are of both antimicrobial and enhance wound healing without any adverse outcome. ●

### References

- Agency for Toxic Substances and Disease Registry (ATSDR). 2022. Toxicological Profile for Copper (Draft for Public Comment). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.
- Alherek, M. and Basu, O.D. (2023), Impact of low levels of silver, zinc and copper nanoparticles on bacterial removal and potential synergy in water treatment applications. *J Chem Technol Biotechnol*, 98: 1137–1146. <https://doi.org/10.1002/jctb.7318>
- Al-Kadmy, I. M. S., Aziz, S. N., Rheima, A. M., Abid, S. A., Suhail, A., Hamzah, I. H., Naji, E. N., Besinis, A., & Hetta, H.

- F. (2023). Anti-capsular activity of CuO nanoparticles against *Acinetobacter baumannii* produce efflux pump. *Microbial Pathogenesis*, 181, 106184. <https://doi.org/10.1016/j.micpath.2023.106184>
- Banerjee, A., Aremu, B. R., Dehghandokht, S., Salama, R., Zhou, H., Lackie, S. M., Seifi, M., Kennepohl, P., & Trant, J. F. (2023). Lethal weapon II: A nano-copper/tetraalkylphosphonium ionic liquid composite material with potent antibacterial activity. *RSC Sustainability*. <https://doi.org/10.1039/d3su00203a>.
- B.H.J. Gowda, S. Mohanto, A. Singh, A. Bhunia, M.A. Abdelgawad, S. Ghosh, M.J. Ansari, S. Pramanik. Nanoparticle-based therapeutic approaches for wound healing: a review of the state-of-the-art. *Materials Today Chemistry* 2023, 27, 101319. <https://doi.org/10.1016/j.mtchem.2022.101319>
- Bakshi, M., & Kumar, A. (2021). Copper-based nanoparticles in the soil-plant environment: Assessing their applications, interactions, fate and toxicity. *Chemosphere*, 281, 130940. <https://doi.org/10.1016/j.chemosphere.2021.130940>
- Balsano, C., Porcu, C., & Sideri, S. (2018). Is copper a new target to counteract the progression of chronic diseases?. *Metallomics : Integrated Biometal Science*, 10(12), 1712–1722. <https://doi.org/10.1039/c8mt00219c>
- Bigliardi PL, Alsagoff SAL, El-Kafrawi HY et al (2017). Povidone iodine in wound healing: A review of current concepts and practices. *Int J Surg* 44: 260–68
- Borkow, G., Gabbay, J., Dardik, R., Eidelman, A. I., Lavie, Y., Grunfeld, Y., Ikher, S., Huszar, M., Zatzoff, R. C., & Marikovsky, M. (2010). Molecular mechanisms of enhanced wound healing by copper oxide-impregnated dressings. *Wound Repair and Regeneration*:18(2), 266–275. <https://doi.org/10.1111/j.1524.475X.2010.00573.x>
- Davies CE, Hill KE, Newcombe RG, et al. A prospective study of the microbiology of chronic venous leg ulcers to reevaluate the clinical predictive value of tissue biopsies and swabs. *Wound Repair Regen* 2007;15:17–22
- Edwards R, Harding KG (2004) Bacteria and wound healing. *Curr Opin Infect Dis* 17: 91–6
- Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington, DC: National Academies Press; 2001.
- International Wound Infection Institute (IWII) Wound Infection in Clinical Practice. *Wounds International*. 2022.
- Kornblatt A.P, Nicoletti V.G., Travaglia A. The neglected role of copper ions in wound healing. *J. Inorg. Biochem.* 2016;161:1–8. doi: 10.1016/j.jinorgbio.2016.02.012.
- Lewis Oscar F, Mubarak Ali D, Nithya C., Priyanka R., Gopinath V., Alharbi N.S., Thajuddin N. One pot synthesis and anti-biofilm potential of copper nanoparticles (CuNPs) against clinical strains of *Pseudomonas aeruginosa*. *Biofouling*. 2015;31:379–391. doi: 10.1080/08927014.2015.1048686.
- Lu, W., Rao, A., Orpallo, A., Gawlik, S., & Haight, J. (2022). Use of Copper Nanoparticles to Reduce Bioburden in the Treatment of Diabetic Foot Ulcers. *Eplasty*, 22, QA4.
- Ma, X., Zhou, S., Xu, X., & Du, Q. (2022). Copper-containing nanoparticles: Mechanism of antimicrobial effect and application in dentistry—a narrative review. *Frontiers in surgery*, 9, 905892. <https://doi.org/10.3389/fsurg.2022.905892>
- Mendes, C., Thirupathi, A., Corrêa, M. E. A. B., Gu, Y., & Silveira, P. C. L. (2022). The Use of Metallic Nanoparticles in Wound Healing: New Perspectives. *International Journal of Molecular Sciences*, 23(23), 15376. <https://doi.org/10.3390/ijms232315376>
- Melamed, E., & Borkow, G. (2023). Continuum of care in hard-to-heal wounds by copper dressings: a case series. *Journal of Wound Care*, 32(12), 788–796. <https://doi.org/10.12968/jowc.2023.32.12.788>
- Merkel P, Long S, McInerney GM, Sotiriou GA. Antiviral Activity of Silver, Copper Oxide and Zinc Oxide Nanoparticle Coatings against SARS-CoV-2. *Nanomaterials*. 2021; 11(5):1312. <https://doi.org/10.3390/nano11051312>
- Montero, D. A., Arellano, C., Pardo, M., Vera, R., Gálvez, R.,

- Cifuentes, M., Berasain, M. A., Gómez, M., Ramírez, C., & Vidal, R. M. (2019). Antimicrobial properties of a novel copper-based composite coating with potential for use in healthcare facilities. *Antimicrobial Resistance and Infection Control*, 8, 3. <https://doi.org/10.1186/s13756-018-0456-4>
- Murphy, C., Atkin, L., Dissemond, J., Hurlow, J., Tan, Y. K., Apelqvist, J., James, G., Salles, N., Wu, J., Tachi, M., & Wolcott, R. (2019). Defying hard-to-heal wounds with an early antibiofilm intervention strategy: 'wound hygiene'. *Journal of Wound Care*, 28(12), 818–822. <https://doi.org/10.12968/jowc.2019.28.12.818>
- Nair HKR et al (2023) International Consensus Document: Use of wound antiseptics in practice. *Wounds International*. Available online at [www.woundsinternational.com](http://www.woundsinternational.com)
- Nair, H., Tang, P. L., Abdul Rashid, R., & M Kamal, N. A. (2023). Effectiveness of nano-copper solution to reduce wound bioburden in chronic wounds: a case series. *Wounds APAC 2023*, Vol 6 Issue 3, page 37-43.
- Ohsumi, Y., Kitamoto, K., & Anraku, Y. (1988). Changes induced in the permeability barrier of the yeast plasma membrane by cupric ion. *Journal of Bacteriology*, 170(6), 2676–2682. <https://doi.org/10.1128/jb.170.6.2676-2682.1988>
- Peiyuan Wang, Lianli Peng, Jinyan Lin, Yang Li, Qiang Luo, Suhua Jiang, Haina Tian, Yun Zhang, Xiaolong Liu, Jingfeng Liu. Enzyme hybrid virus-like hollow mesoporous CuO adhesive hydrogel spray through glucose-activated cascade reaction to efficiently promote diabetic wound healing, *Chemical Engineering Journal*, Volume 415, 2021, 128901, ISSN 1385-8947. <https://doi.org/10.1016/j.cej.2021.128901>.
- Pérez Díaz, Mario & Prado-Prone, Gina & Díaz, Arisbet & González Torres, Maykel & Silva-Bermudez, Phaedra & Sanchez, Roberto. (2023). Nanoparticle and nanomaterial involvement during the wound healing process: an update in the field. *Journal of Nanoparticle Research*. 25. 10.1007/s11051-023-05675-9.
- Rodeheaver GT, Ratliff CR (2018) Wound cleansing, wound irrigation, wound disinfection. In: Krasner DL, van Rijswijk L (eds.) *Chronic Wound Care: The Essentials e-Book*. HMP: Malvern, PA pp47–62
- Ruiz, L. M., Libedinsky, A., & Elorza, A. A. (2021). Role of Copper on Mitochondrial Function and Metabolism. *Frontiers in Molecular Biosciences*, 8, 711227. <https://doi.org/10.3389/fmolb.2021.711227>
- Salah, I., Parkin, I. P., & Allan, E. (2021). Copper as an antimicrobial agent: recent advances. *RSC Advances*, 11(30), 18179–18186. <https://doi.org/10.1039/d1ra02149d>
- Sandoval, C., Ríos, G., Sepúlveda, N., Salvo, J., Souza-Mello, V., & Fariás, J. (2022). Effectiveness of Copper Nanoparticles in Wound Healing Process Using In Vivo and In Vitro Studies: A Systematic Review. *Pharmaceutics*, 14(9), 1838. <https://doi.org/10.3390/pharmaceutics14091838>
- Sandoval, Cristian & Salvo, Jessica. (2021). Role of copper nanoparticles in wound healing for chronic wounds: Literature review. *Burns & Trauma*. 10. 10.1093/burnst/tkab047.
- Bernauer, U., Bodin, L., Chaudhry, Q., Coenraads, P., Dusinska, M., Gaffet, Panteri, E., Rouselle, C., Stepnik, M., Wijnhoven, S., De Jong, W., Von Goetz, N., & Bernauer, J. (2021, December 30). SCCS Opinion on Copper (nano) and Colloidal Copper (nano) - Final Opinion. <https://doi.org/10.2875/407340>
- Sen, Chandan & Khanna, Savita & Venojärvi, Mika & Trikha, Prashant & Ellison, E & Hunt, Thomas & Roy, Sashwati. (2002). Copper-Induced Vascular Endothelial Growth Factor Expression and Wound Healing. *American Journal of Physiology. Heart and Circulatory Physiology*. 282. H1821-7. 10.1152/ajpheart.01015.2001.
- Wapnir R. A. (1998). Copper absorption and bioavailability. *The American Journal of Clinical Nutrition*, 67(5 Suppl), 1054S–1060S. <https://doi.org/10.1093/ajcn/67.5.1054S>
- Zhou W, Zi L, Cen Y, You C, Tian M. Copper Sulfide nanoparticles-incorporated hyaluronic acid injectable hydrogel with enhanced angiogenesis to promote wound healing. *Front Bioeng Biotechnol*. 2020;8:417