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A Type of Metal-Tannin Nanoparticles: Featuring Oxidative Antibacterial Effects Applied in Wound Healing

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Abstract. The number of people who die annually due to wound infections globally is not insignificant, hence there is an urgent need to find an inexpensive and efficient antibacterial and anti-inflammatory therapy. Metal-tannin complexes combine the advantages of coordination polymers and high metal content, and their simple processing, good biocompatibility, as well as the antibacterial and anti-inflammatory properties brought about by polyphenolic substances, have attracted widespread attention. Here, we utilize the sol-gel chemistry principles of formaldehyde-assisted metal-tannin crosslinking to synthesize Metal-TA nanoparticles for wound treatment. These nanoparticles release high concentrations of metal ions in weakly alkaline environments, and under stimulation from high levels of reactive oxygen species (ROS) in the wound, undergo a Fenton-like reaction to produce •OH, thereby exerting antibacterial effects on the wound.

Keywords: Wound Healing; Metal-Polyphenol Coordination Complex; Fenton-like Reaction; Antibacterial and Anti-inflammatory.

1. Introduction

Wounds are primarily caused by trauma, surgery, and diseases including diabetes and vascular disorders. They can have profound adverse effects on the quality of life of patients. Inflammation, infection, and accompanying pain are the most common complications of wounds, which can delay wound healing and potentially lead to the formation of chronic wounds. [1-3] Persistent polymicrobial infections can lead to excessive wound inflammation, unnecessary local tissue damage, and chronic, non-healing wounds, [4] severe infections can also lead to the overproduction of reactive oxygen species (ROS) within uncontrolled inflammation.[5]They exacerbate local tissue damage, leading to chronic inflammation. Furthermore, they can also result in infection-related mortality, which is not uncommon globally. Therefore, reducing the burden of infection-related deaths is an urgent global public health priority. [6] Traditional treatment methods for infected wounds include antibiotic therapy, dressing changes, and negative pressure wound therapy (NPWT). However, these measures are costly and not always ideal. [7] Therefore, there is an urgent need to explore a green and non-toxic therapy to address the healing of infected wounds.

Fe^{3+} will be reduced to Fe^{2+} by tannic acid (TA), thereby undergoing a Fenton reaction with hydrogen peroxide (H_2O_2) generated at the affected site, producing toxic hydroxyl radicals (•OH).[8] This not only achieves bactericidal effects but also stimulates cell proliferation, collagen deposition, and promotes angiogenesis. This is because it can stimulate bone marrow-derived stem cells to produce vascular endothelial growth factor (VEGF).[9, 10] The Cu-based Fenton-like reaction can occur at higher pH values and can be regulated by the same reaction intermediates as the classical Fenton reaction. [11-14] Therefore, the addition of copper ions is necessary. However, an excess of copper ions possesses certain cytotoxicity as it can directly bind with lipid components in the tricarboxylic acid (TCA) cycle. [15]This leads to the aggregation of lipidated proteins and subsequent loss of iron-sulfur cluster proteins, resulting in protein toxicity stress and ultimately leading to cell death. Therefore, the dosage and targeting of copper ions are crucial.

Combining two compatible metal ions, such as copper and iron ions, in appropriate proportions can achieve the highest Cu^{+}/Cu^{2+} and Fe^{2+}/Fe^{3+} ratios, greatly enhancing the catalytic performance of the catalyst. Nanoparticles (NPs) were synthesized with different copper-iron ratios (Cu_xFe_{1-x}, where x=1, 0.7, 0.3, and 0, denoted as CNPs, 7FNPs, 3FNPs, and FNPs respectively), to investigate their ability to generate hydroxyl radicals. It is worth noting that in the ESR experiments, nanoparticles composed purely of copper generate the highest amount of hydroxyl radicals under pH 7.4 conditions.[16]

In this study, we report a type of nanoparticles capable of consuming ROS at wound sites under neutral conditions, generating •OH, thereby achieving bactericidal effects. In this research, we demonstrated that Cu-TA particles exhibit a stronger ability to generate •OH compared to nanoparticles with other ratios, and tannic acid possesses antibacterial and anti-inflammatory effects. We envision this as a highly desirable approach for wound healing therapy.

2. Materials and Methods

2.1. Main reagents

Ethanol absolute (analytically pure,Macklin), Ammonium hydroxide (25%, Xilong Scientific), Formaldehyde solution (37%, Xilong Scientific), $CuCl_2·2H_2O$ (Macklin), $FeCl_2·4H_2O$ (Macklin), $FeCl_3·6H_2O$ (Macklin), DMPO (Macklin), ferrozine (Sigma), Tannic acid (Macklin).

2.2. Synthesis of metal-phenolic coordination spheres

F127 is added to a mixture of water and ethanol, followed by the addition of 1 mL of 25 wt% ammonia solution and stirred for 1 hour. Tannic acid is then added to dissolve completely, followed by the addition of 0.76 mL of formaldehyde solution, and stirred overnight. Subsequently, a solution of $CuCl_2·2H_2O$ or $FeCl_2·4H_2O$ is added to the above solution. After stirring for 24 hours, dialysis is performed.

2.3. Characterizations

Scanning electron microscopy (SEM) images were taken with a field-emission scanning electron microscope (Hitachi SU-8000). The UV−vis absorption spectra were acquired using a Shimadzu UV-1780 spectrophotometer. Particle Size Analyzer (Bettersize 2600LD) was used to determine the size and zeta potential of metal-TA.

2.4. OH Generation Activity.

DMPO was used as a trapping agent to clarify the generation of •OH at different pH values, which can be recognized with a characteristic 1:2:2:1 of hydroxyl radical by ESR (Bruker EMX PLUS).

2.5. Relative Ratios of Dissolved Cu^{+}/Cu^{2+} and Fe^{2+}/Fe^{3+} from Different NPs under the same pH Conditions.

The NPs (CNPs, 7FNPs, 3FNPs and FNPs) are dispersed in buffered solutions with pH 7. The ratio of low oxidation states to high oxidation states of metal ions in the solution is analyzed using ferrozine, which selectively reacts with reduced copper and iron ions to form complexes. The absorbance of the formed complexes at specific wavelengths is then utilized to quantify the ratio of metal ions in different oxidation states.

3. Results and Discussion

The formation of metal-phenolic resin coordination polymers occurs in weakly alkaline ethanol/water solvent, utilizing formaldehyde to pre-crosslink tannic acid (TA), followed by coordination between the abundant phenolic hydroxyl groups on the polyphenol and the metal ions to form polymers that are spherical in shape. Taking Cu-TA NPs as an example, it can be observed through SEM that it forms metal-phenolic resin coordination spheres with a diameter of approximately 200 nm (Figure a). This result is also confirmed by particle size analyzer measurements (Figure b).

ESR data confirms that under the same pH conditions (pH 7), CNPs nanoparticles exhibit a better ability to generate hydroxyl radicals (•OH) compared to other nanoparticles (Figure c), thereby enhancing the antibacterial capability of the nanoparticles at the wound site while reducing toxicity to surrounding normal skin cells.

We investigated the mutual conversion of Cu ions and Fe ions in different oxidation states in the class Fenton-like reaction under the same pH conditions (pH 7) for nanoparticles with varying metal compositions (CNPs, 7FNPs, 3FNPs, and FNPs). As shown in Figure d, during the class Fenton-like reaction, the ratio of dissolved Cu^+/Cu^{2+} is significantly higher than that of dissolved Fe^{2+}/Fe^{3+}, indicating a notably higher efficiency of Cu^{2+} to Cu^+ conversion compared to Fe^{2+} to Fe^{3+}under these conditions, with CNPs exhibiting the most significant effect. This result highlights the outstanding performance of CNPs in enhancing the efficiency of the class Fenton-like reaction, thereby enhancing the antibacterial capability of the nanoparticles at the wound site.

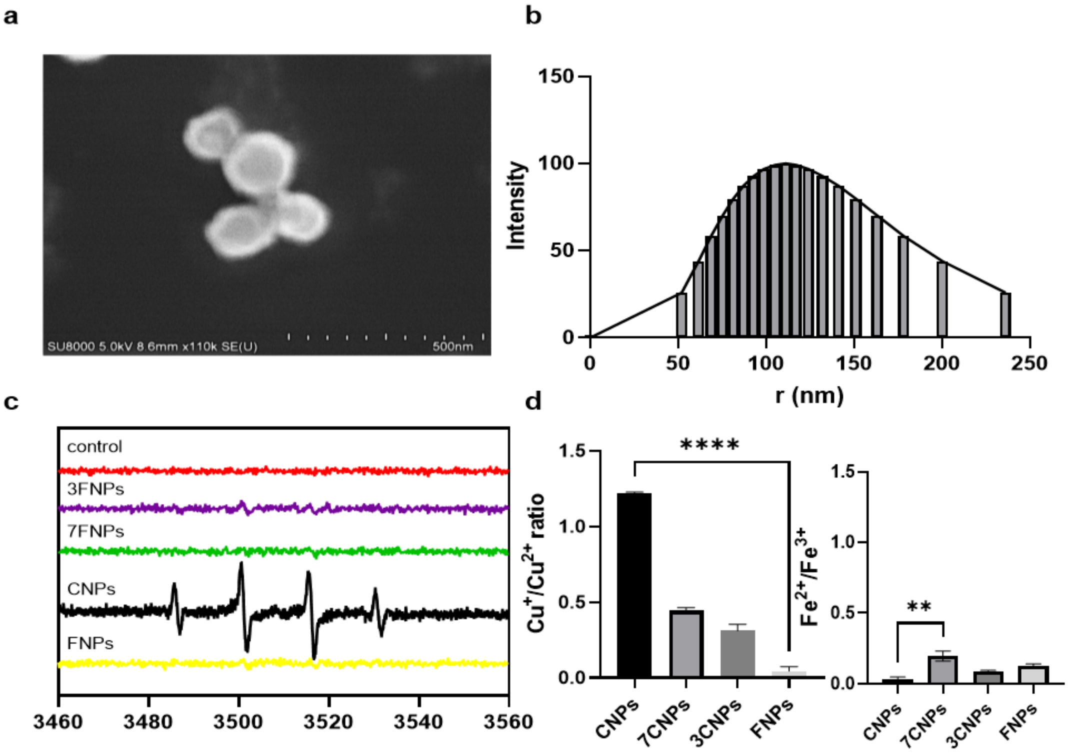

Figure 1. (a) SEM images of Cu-TA. (b) Hydrodynamic diameter of CNPs NPs in water.(c) ESR of CNPs, 7FNPs, 3FNPs, and FNPs NPs.(d) Ratios of the reduced-state Cu and Feions at different pH (normalized by the ratio of CNPs or FNPs NPs).

4. Conclusion

In summary, we synthesized Cu-TA NPs using the aldehyde crosslinking method. The specific enhancement of its oxidation and antibacterial capabilities by the high Cu^+/Cu^{2+} ratio precipitated by Cu-TA NPs in weakly alkaline environments helps to reduce cytotoxicity. Simultaneously, the free tannic acid exhibits anti-inflammatory and antibacterial effects, promoting wound healing. Therefore, this study may provide a new approach for dynamic wound treatment, and Cu-TA nanoparticles could emerge as a promising alternative therapy for infectious diseases.

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