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Fabrication of ultra-small GSH-AgNCs with excellent eschar penetration and antibacterial properties for the healing of burn infection wounds

Peng Yang^{1†}, Kang Yang^{2†}, Yu liu³, Chen Li¹, Jun Tao^{1*} and Fanrong Ai^{4,5*}

Abstract

There is evidence of bioburden as a barrier to chronic burn wound healing. Compared to traditional therapy, nanotechnology has availed a revolutionary approach to therapeutic and diagnostic applications in burns. In this article, we developed the glutathione-protected Ag nanoclusters (GSH-AgNCs) to manage burn wound infection. Owing to the specific structure, the GSH-AgNCs emitted strong red fluorescence under UV excitation, quantified via both *in vivo* and *in vitro* techniques. The GSH-AgNCs showed a significant inhibition potential on the proliferation of *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *methicillin-resistant staphylococcus aureus* (*MRSA*), hiding under the eschar. Of note, with 2-6nm particle size, GSH-AgNCs are effected in renal excretion, advocating for their biomedical and pharmacological applications.

Keywords

Silver nanocluster, Burn wound healing, Antibacterial, Fluorescence, Excretion

Scheme 1. (a). Schematic diagram of the preparation of glutathione-protected nanocluster silver. (b). They application in the treatment of burns and scab wounds infected by *Staphylococcus aureus* and *in vitro* fluorescence monitoring of its renal excretion capacity.

Introduction

Skin is the largest organ in the body. It is a crucial barrier that blocks the entry of pathogens *in vivo*. Burn injury-associated impairment of the anatomical structure and function cause wounds ^[1]. Of note, the openness of burn wounds, protein coagulation, and exudation of plasma components create ideal conditions for bacterial growth. Consequently, invasive infection complications caused by sepsis readily occur, particularly when the systemic immune function is compromised ^[2,3]. An estimated

180,000 burn injury-associated deaths are reported every year, according to the World Health Organization. Sepsis and the accompanying invasive infection remain the primary causes of such burn deaths ^[4-8]. So, antibacterial treatment is essential for burn injury healing.

Although antibiotics are applied as a traditional burn infection therapy ^[9, 10], their long-term use has contributed to a rise in cases of microbial drug resistance, organ damage, allergic reaction, among other adverse side effects ^[11, 12]. A series of new approaches have been proposed as escape routes for such complications, including new antimicrobial agents (such as antimicrobial peptides, amino acid-based surfactants, etc.), antimicrobial photo- and ultrasound-therapy, and natural products such as honey ^[13-17]. Among these, nanomedicine as a novel approach has been firmly applied to manage burn infections.

Systemic antibacterial drugs can hardly land on the infected site; thus, topical antimicrobials and dressings are generally employed to enhance wound healing and resist reinfection ^[18]. Full-thickness burn wounds usually form eschars, making it difficult for topical antibacterial agents to act on deep infections^[19]. Moreover, the excellent penetrating ability of nanoclusters has received immense applications in transdermal drug delivery, brain-targeting, and tumor targeting therapy ^[20-23]. Despite unclear mechanisms underlying tissue barrier permeability, accumulating evidence insinuates that the small size of nanomaterials exerts a pivotal role in tissue penetration. Numerous reports also found that Ag nanoparticles smaller than 30nm can penetrate the deepest part of the stratum corneum ^[24-28]. Thus, we hypothesize that the GSH-AgNCs

(2-6nm in size) designed in this work could demonstrate good permeability.

The widespread application of nanotechnology in medicine is highly promising in the treatment of several diseases. Regrettably, their toxicity remains a potential limiting challenge to developing various nanotechnologies for clinical translation ^[29]. Numerous contributes to the cytotoxicity and genotoxicity of silver nanoclusters; for instance, the first toxicity reaction after *in vivo* injection with silver nanoclusters forms the protein corona. Biological response to these coronas is critical for nanotoxicology. Various proteins (i.e., human serum albumin, tubulin, ubiquitin and yeast extract proteins, etc.) are adsorbed onto the surface of silver nanoclusters ^[30]. Glutathione is a natural tripeptide with a high affinity to metal surfaces. Silver nanoclusters protected by GSH potentially acquire better biocompatibility by blocking the formation of corona ^[31].

Particle size is an important factor influencing nanocluster distribution. Smaller-sized nanoparticles are more widely distributed than large-sized ones. In particular, reports show that 10nm diameter silver nanoparticles are enriched in several organs (e.g., liver, spleen, kidney, testis, thymus, heart, lung and brain, etc.). Larger nanoparticles are specific to blood, liver, and spleen, implicating that 2-6 nm diameter silver nanoparticles are more evenly distributed and less enriched in tissues ^[32-34]. *In vivo*, the nanoparticles continuously induce inflammation and other toxic reactions. Thus, if they do not get cleared, the long-term fate of organ affords is not clear. The renal filtration threshold of nanoparticle size is generally 5.5nm. However, there is no report on whether larger nanoparticles are metabolized by the kidney, which may be related to its rigidity^[33, 35-37].

In most cases, sepsis occurs secondary to severe infection, pneumonia, burn, and major surgery. Therefore, systemic administration must be considered for effective invasive infections prevention and treatment [2, 8]. Since pharmacokinetics after burn trauma is challenging to predict, real-time therapeutic drug monitoring (TDM) is essential for most antibiotic therapy for burns, which maintains the safe plasma concentration [38,39]. The pharmacokinetic models of NPs are considered multi-compartment models. However, its distribution and excretion are affected by various factors, posing challenges in predicting the risk of adverse effects using plasma concentration. Herein, inspired by tumor fluorescent markers, we propose a new strategy for adjusting the dosage and enhance the safety of nanomedicine through *in vivo* examination of nanocluster metabolism [40-42].

This work proposes a new approach to reduce health risks by integrating fluorescent tracer and renal excretion. It is argued that GSH-AgNCs possess an excellent broad-spectrum antibacterial activity, which potentially promotes wound healing. It introduces eschars into the bloodstream to prevent secondary sepsis and invasive infections. The unique fluorescence-exciting properties can grasp the degree of enrichment in the tissue. This justifies the excellent permeability and antibacterial effects of GSH-AgNCs. Nevertheless, excretion greatly improves the antibacterial activity and biosafety of silver nanoparticles.

Materials and methods

Materials

Silver nitrate (S116265, Aladdin, China), S-Hexylglutathione (H121370, Aladdin, China), Sodium borohydride (S817796, Macklin, China), Sodium hydroxide (S111509, Aladdin, China), LB Broth medium (HB0128, Hopebio), agar(A8190, Solarbio), Hematoxylin-Eosin/HE Staining Kit (G1120, Solarbio, China). To prepare full cell media, we constituted Dulbecco's Modified Eagle's Medium (DMEM, BI) supplemented with 10% fetal bovine serum (FBS, BI) and 1% penicillin-streptomycin. Cell Counting Kit 8 (CKK-8) and Calcein-AM/Propidium Iodide (PI) were purchased from Beyotime Biotechnology. *Staphylococcus aureus* (*S.aureus*), *Escherichia coli* (*E.coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *methicillin-resistant staphylococcus aureus* (*MRSA*) were purchased from National Center for Medical Culture Collections (CMCC). Human Skin Fibroblast (HSF) was obtained from The Second Affiliated Hospital of Nanchang University. All animal experiments were approved by the Animal Experiments Ethical Committee of Second Affiliated Hospital of Nanchang University.

Preparation of glutathione-protected Ag nanoclusters

The material synthesis process was adopted as previously described. Briefly, 125 μ L of 20 mM silver nitrate and 150 μ L of 50 mM glutathione were dissolved in 4.85 mL of water. Afterward, 50 μ L of 0.1 mM sodium borohydride dissolved in 0.2 mM sodium hydroxide was added for 5 minutes, aged for 3 h. Then, 50 μ L of boron was added. The sodium hydride solution was stirred for 15 min and aged for 6 h.

Characterization of GSH-AgNCs

The structural and size properties of the GSH-AgNCs were examined using

transmission electron microscopy JEOL 03040701(Kabushiki Kaisha, Japan). We applied the fluorescence spectrometer FS5 (Edinburgh Instruments, UK) to measure the fluorescence spectra. UV-Vis absorbance spectra were recorded with a Cary UV–Vis spectrophotometer.

Cell culture and *in vitro* cytotoxicity experiment

The cytotoxicity of GSH-AgNCs was evaluated through direct culturing of HSF cells in high glucose DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. This was followed by incubation at 37°C in a humidified atmosphere of 5% CO₂ /95% air. For long-term toxicity evaluation, we assessed cell growth. Briefly, 1×10⁴ cells were seeded on a 24-well plate, and then treated with 125μM, 250μM GSH-AgNCs for 1, 3, or 7 days. Cell growth was evaluated under the microscope. Following a 5-day incubation, a life/death analysis kit was used to assess the killing effect of GSH-AgNCs on HSF cells. The dyes in the kit include calcein (AM) and propidium iodide (PI, which stains live cells (green) and dead cells (red), respectively. A fluorescence microscope was used to record cell viability.

Measurement of the concentration-toxicity relationships of GSH-AgNCs in different cell lines was taken using CCK-8 assay according to the manufacturer's protocol. Here, HSF cells were seeded onto 96-well plates overnight (1×10³ cells per well) and subsequently treated with varying concentrations of GSH-AgNCs (0, 7.8125, 15.625, 31.25, 62.5, 125, 250, 500, or 1000 μM), in triplicate for each experimental group. For the negative control group, we used a concentration of GSH-AgNCs at 0 mM, whereas medium without cells served as the blank control. Then, 10 μL CCK-8

solution was added into each well at 1, 3, and 7days post-seeding. Each group was cultured for a further 2 hours. The optical density values at 450 nm were measured using a microplate reader. An estimate of cell survival rate (%) of each cell lines treated with various concentrations of GSH-AgNCs was measured as follows: $[(\text{OD value of negative control} - \text{OD value of experimental group}) / (\text{OD value of negative control} - \text{OD value of blank control})]$.

***In vitro* antibacterial activity**

The spread plate method was adopted to evaluate *in vitro* antibacterial activity. Two Gram-negative bacterial strains, *P. aeruginosa* and *E. coli*, and two Gram-positive bacterial strains, *S. aureus* and MRSA, were used for this evaluation. We suspended GSH-AgNCs in LB broth medium at the concentrations of 0, 15.6, 62.5, 250, or 1000 mM. Then, a speed vortex mixer was used to produce dispersions. The above-mentioned microorganisms were added to the Ag nanoclusters suspensions. The positive control comprised the LB broth medium with the microorganism and without GSH-AgNCs, whereas the negative control comprised the LB broth without the microorganism. After 8 h culture at 37 °C, all the tested suspensions were diluted (1×10^5 fold) and inoculated onto nutrient agar plates. A count of CFUs on agar plates was taken after 12 h of culture. Antibacterial efficacy (%) of GSH-AgNCs at each concentration was estimated as: $[(\text{CFU count of negative control} - \text{CFU count of experimental group}) / \text{CFU count of negative control}] \times 100\%$.

Using Oxford cup, we further evaluated the transdermal antimicrobial efficiency of the GSH-AgNCs. Obtained eschar skin from rabbit infected burn wound. Afterward,

the eschar was placed on plates previously inoculated with the pathogen. Different concentrations of nanoclusters dispersions were added to each Oxford cup and placed on the above artificial eschar. After 24 h-incubation at 37°C, images of the bacterial zone of inhibition were taken using camera.

Fluorescence imaging *in vivo*

The fluorescence of the GSH-AgNCs was assessed using *In Vivo* Imaging System LB983 (BERTHOLD, USA). *In vivo* imaging was performed immediately after caudal vein injection. All the experimental Kunming mice were purchased from Jiangxi University of Chinese Traditional Medicine. The control mice were injected with the same amount of PBS. Whole-body images of GSH-AgNCs injected mice were acquired and analyzed at different time points (0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours).

Systemic toxicity

Although the GSH-AgNCs could penetrate eschars into the bloodstream to prevent and treat systemic infection, it potentially causes systemic toxicity. Herein, the histopathology of organs was investigated for systemic toxicity evaluation. Briefly, seven days following a single intravenous dose of GSH-AgNCs suspension, mouse eyeballs were extracted to collect blood. Using Automated Clinical Chemistry Analyzer (AU400, Olympus), we tested the total bilirubin (ALST), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea nitrogen (BUN), and serum creatinine (SCR) to evaluate the hepatic and renal damage after GSH-AgNCs suspension inject.

The microstructure destruction of organs was evaluated via HE staining. Briefly,

organ tissues from harvested heart, liver, spleen, lung, and kidney from the above mice were fixed with 10% buffered formalin for 24 h. Tissues were then paraffin-embedded, sectioned, and subjected to HE staining following the manufacturer's protocol. Histopathological changes were examined under a light microscope.

Burn infection model

New Zealand white rabbits were purchased from the Institute of Animal Science of Nanchang University and randomly categorized into two groups. We adopted the following steps to cause burns and infections. Briefly, after removing the back hair of the rabbits, they anesthetized through the marginal ear vein of chloral hydrate at a dose of 250 mg/kg. Borrowed from previous reports, a full-thickness burn wound was made on the dorsal skin using copper coin (11mm, 92°C, 25s). The *Staphylococcus aureus* suspension (200μL, 1×10^5 cells mM⁻¹) was injected subcutaneously into the burn. The GSH-AgNCs suspension was dropped on the eschar after the wound had scabs. After that, the wound was covered with gauze and fixed with an elastic bandage. Images of the wound at the planned time points (0, 3, 7, and 10 days) were taken. The wound was cut and opened with scissors to check for infection. The surrounding gauze was removed. The antibacterial effect and tissue inflammation were evaluated using the hematoxylin-eosin staining kit.

Statistical analysis

Statistical computations were performed using one-way ANOVA between multiple groups. Data were expressed as mean $\pm$ standard deviation (SD). All statistical computations were performed using GraphPad Prism 8.0.1. The significance level was

set as $p < 0.05$.

Results and Discussion

Characterization of GSH-AgNCs

The prepared GSH-AgNCs were characterized via TEM, UV-Vis absorption, and fluorescence spectra (Figure 1). We have presented a typical TEM image of GSH-AgNCs nanoparticles in Figure 1a. GSH @ Ag NCs were spherical, well dispersed, and uniform. The particle size analysis of the nanoparticles confirmed the smaller size of GSH-AgNC, about 2.5nm. The purified GSH-AgNCs was brown in solution and demonstrated a pronounced absorption peak at 450nm (Figure 1b). The changes in product fluorescence intensity measured under excitation light with wavelengths of 400, 410, 420, 430, 440, and 450 nm are shown in Figure 1c. The emission spectrum was found at 605 nm; at this point, we noted the strongest fluorescence intensity. The excitation and emission spectra of GSH-AgNC, respectively, are presented in Figure 1d. Notably, the excitation spectrum monitored at 605 nm produced a broad excitation band from 400 to 500 nm (black line). Further, GSH-AgNCs showed a sharp excitation peak (red line) at 450nm. The synthesized cluster-shaped silver nanoparticles emitted red fluorescence visible to the naked eye under ultraviolet light, which could be explained by the formation of fluorescent AgNCs.

Based on previous findings, the characteristic absorption peaks at around 380–500 nm as found in GSH-AgNCs are for larger Ag nanoparticles [31,43]. This could be attributed to the interparticle assembly of uncapped silver nanoclusters.

Figure 1. (a). TEM image of the GSH-AgNCs. Scale bar: 10 nm. Illustration: Particle size distribution. (b). UV-vis spectroscopy of GSH-AgNCs, the inset shows the photo of GSH-AgNCs under visible light irradiation. (c). Changes in the fluorescence intensity of GSH-AgNCs measured under the action of different wavelengths of excitation light. (d). Excitation spectrum (black lines) and emission spectrum (red lines) of GSH-AgNC at 605nm and 450 nm. The inset shows a photo of GSH-AgNCs emitting red fluorescence under UV irradiation.

Proliferation and *in vitro* viability

HSF cells gradually proliferated within 7 days and covered the entire culture dish over time (Figure 2a). There was almost no difference in the average cell number of the control group without cluster silver. Notably, cells incubated with different concentrations of GSH-AgNCs were all alive (Figure 2b). After 5 days of culture, the live cells (green) and propidium iodide (PI) were paired with calcein-AM (Cal-AM). After staining the dead cells (red), both the 125 μ M and 250 μ M GSH-AgNCs

experimental groups survived, slightly less than the average cell number of the control group. It was suggested that GSH potentially reduced the toxicity of silver ions, thereby promoting the proliferation of HSF cells.

Furthermore, CCK-8 analysis was undertaken to quantitatively analyze the toxicity of GSH-Ag NCs to LSF cell survival. We used different concentrations of GSH-Ag NCs and HSF for 1, 3, and 7 days of co-cultivation to validate the results of the cck8 experiment (Figure 2c). Of note, the toxicity of GSH-Ag NCs to cells was dose-dependent. Compared to the control group, the survival rate of cells below 250 μ M was not affected.

Figure 2. (a). Fluorescence inverted microscopy of the images of 125 μ M, 250 μ M GSH-AgNC, and HSF incubated together for 1, 3, and 7 days. Scale bar: 200 μ m. (b). Live/dead fluorescent staining images of HSF cells incubated with different GSH-AgNCs after 5 days. Live cells are labeled with green fluorescence, while dead cells are labeled with red fluorescence. Scale bar: 200 μ m. (c) The results of CCK-8 after co-culturing HSF cells with different concentrations of GSH-AgNCs for 1, 3, and 7 days. (*P<0.05).

Collectively, GSH-AgNCs concentration lower than 250 μ M demonstrated satisfactory biocompatibility, which is beneficial to the growth and proliferation of HSF cells, thus, has potential biomedical applications.

***In vivo* biocompatibility**

Silver nanoclusters display intense colors due to the collective oscillation of conduction electrons as they interact with light. Owing to their fluorescent properties, metal nanocluster materials received wide application in tumor imaging, microRNA detection, and DNA base detection [49-51]. Here, the synthesized silver nanoparticles had been tested for their *in vitro* fluorescence capabilities previously. The real-time fluorescence *in vivo* performance of GSH-AgNCs was investigated on Kunming mice (Figure 3a). Similarly, it demonstrated good fluorescence imaging capabilities *in vivo*, thus can be applied as a dynamic biological probe. We observed the *in vivo* fluorescence signal after injection (Figure 3a). The signal of nanoparticles was further attenuated over time, which was not due to the fluorescence quenching of GSH-AgNCs but because they were excreted. After 24 h post-injection, most GSH-AgNCs had been cleared out. The accumulation site of residual nanoclusters confirmed that GSH-AgNCs is renal excretion type, consistent with the theoretical pharmacokinetics of 2-6nm diameter nanocluster. Results of major liver and kidney function tests (including ALT, AST, ALP, BUN, SCR, TBIL) after intravenous injection of GSH-Ag NCs suspension are presented in Figure 3b. Compared to the control group, the experimental group had no significant difference in various indicators on the 7th day. This implied that GSH-AgNCs nanoparticles did not influence the normal liver and kidney basic physiological

functions of the mice post injection. H&E-stained tissue sections from the main organs (heart, liver, spleen, lung, and kidney) of km mice 7 days after a single intravenous injection of GSH-Ag NCs suspension are depicted in Figure 3c. Mice in the control group received 200 μ L of normal saline intravenously but showed no obvious pathological changes.

Figure 3. (a). Fluorescence images at different times point after injection of GSH-AgNCs. (b). Evaluation of the liver function (ALT, AST, TBIL, ALP)) and kidney function of km mice injected with GSH @ Ag NCs (BUN, SCR); mice in the control group received an intravenous injection of 200 μ L of normal saline. (c). Seven days after a single intravenous injection of GSH-Ag NCs suspension, H&E-stained tissue (heart, liver, spleen, lung, and kidney) sections were taken from km mice. Scale bar: 200 μ m. (n = 3).

***In vitro* antibacterial activity**

The microbial load reduces burn wound contraction, and eventually, death may occur. Silver and associated preparations have been applied as antimicrobials for thousands of years ^[46]. Though the mechanism of antibacterial activity of AgNCs remains elusive, theories are hypothesizing that they (I) react with thiol moieties of enzymes and proteins, (II) produces free radicals, (III) causing membrane structure damage ^[47,48]. It is worth mentioning that the specific surface area of silver nanoclusters exerts a crucial role in the adsorption and destruction of proteins. Herein, the antibacterial property of GSH-AgNCs was quantitatively assessed against both Gram-positive and Gram-negative bacteria using CFU count. The data are presented in Figure 4a, 4c. For a similar antibacterial effect, the Gram-negative species required a larger GSH-AgNCs exposure concentration than the Gram-positive species. Excellent antibacterial effect against both Gram-negative and Gram-positive bacteria was recorded at concentrations as low as 62.5 μ M.

Eschar formation in the infected area of burns is an important factor in antibacterial treatment failure ^[7]. Referring to the upper schematic diagram (Figure 4b), adding different concentrations of GSH-AgNCs to each Oxford cup enhance the penetration of nanoclusters of small particles into skin eschar at the bottom. Eventually, the *Staphylococcus aureus* pre-inoculated on the plate die.

The image of the bacterial inhibition zone taken after adding cluster silver to the Oxford cup followed by 24hr incubation at 37°C is shown in Figure 4b. GSH-Ag NCs penetrated the eschar, exerting an antibacterial effect. The variation in the area of the inhibition zone with the concentration of GSH-AgNCs shows that it can penetrate the

eschar, killing the bacteria in the wound and blood at the bottom of the eschar. We speculate that this may be due to the small size of clustered silver nanoparticles, and the naturally occurring small peptide GSH can act as a protective shell, so GSH-AgNCs show excellent permeability in the eschar tissue.

Figure 4. (a). Antibacterial efficiency of GSH-AgNCs against different bacteria using the Coated plate method. (b). The pictures and simple schematic diagrams of GSH-AgNCs permeating the skin eschar to form a zone of inhibition. (c). Histogram representation of the bacterial survival rate results.

Treatment of infected burn wounds

After introducing a burn on the shaved back of New Zealand white rabbit using a preheated brass block (92°C) for 25 seconds, *Staphylococcus aureus* was injected subcutaneously into the wound. Two days later, the wound crusted. An abscess occurred after cutting the wound with scissors. This meant that a closed full-thickness burn wound infection model was successfully established.

As shown in the schematic diagram of Figure 5a, to evaluate the effects of the GSH-AgNCs on the closing burn wound *in vivo*, a full-thickness burn wound infection model was used in the rabbit. The subsequent wound repair processes of the treated burn infection wounds were tracked over 10 days. The wounds were either dressed with PBS (blank) or treated with GSH-AgNCs. Significant differences in wound healing were examined during the entire process. The visual observations of burn wounds treated with GSH-AgNCs at different post-operation time points are presented in Figure 5b. Notably, wounds treated with GSH-AgNCs showed a significantly higher wound healing rate than blank groups tested. Such wounds appeared to be fully recovered at day 10. On the contrary, we reported a significant delay in the closure of wounds in the blank group. Pus was discharged from the wounds, suggesting the development of severe skin infection. These observations were validated through HE staining (Figure 5c). The GSH-AgNCs treated wounds had more capillaries and fibroblasts. After 10 days, no obvious inflammation occurred, and the healing outcome was better. Nevertheless, there were several neutrophils and necrotic nuclei under the infection wound in the control groups. Taken together, significantly inhibited inflammation, less necrotic tissue, more regenerated skin appendages, and well-wound closure are better healing outcomes in GSH-AgNCs treated burn infection wounds.

Figure 5. (a). Schematic diagram of the closed full-thickness burn wound infection modeling and GSH-AgNCs treatment process. (b). Wound healing efficacy of GSH-AgNCs in a burning infection wound model in rabbits; red circles indicate pus spots. (c). H&E staining of the sampled wound tissues after 10 days post-treatment. Scale bar: 200μm.

Conclusions

This study developed a silver nanocluster through glutathione capping, functioning as an antibacterial drug with promising potential for eschar penetration, fluorescent tracing, and renal excretion for burn infection wound healing. Of note, small particle size and surface chemistry offer GSH-AgNCs an excellent permeability; therefore, it

kills the bacteria under the eschar and prevents secondary sepsis upon entry into the systemic circulation. *In vitro* and *in vivo* toxicity analyses show that GSH-AgNCs are less toxic; they undergo renal excretion and get cleared within 24 hours. Also, they maintain fluorescence excitation with a certain intensity in the body for a given time, thus can be adopted as a fluorescent quantitative method to assess the distribution of nanocluster *in vivo* and regulate the amount of dose to avoid accumulated drug toxicity event. GSH-AgNCs treated wounds demonstrate accelerated wound healing evidenced by faster wound closure, Infection suppression and inflammation inhibition compares to untreated wounds after 10 days. However, future work should focus on the toxic dose effect of GSH-AgNCs on different organs, and establish a model for evaluating *in vivo* fluorescence intensity and the number of GSH-AgNCs. This would pass the reference for the transdermal treatment system.

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Availability of data and materials

All the generated or analyzed data during this study are included in this manuscript.

Ethics approval

The animal study was reviewed and approved by Ethical Committee of Laboratory Animal Science Department, Nanchang University.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' contributions

PY and KY contributed to conception and design, data acquisition, analysis, interpretation, and drafting of the manuscript. YL and CL contributed to data acquisition and drafting of the manuscript. JT contributed to interpretation and critical revision of the manuscript. FA and JT contributed to conception and design, interpretation, and critical revision of the manuscript. All authors gave final approval and agreed to be accountable for all aspects of the work.

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Figures

Figure 1

(a). TEM image of the GSH-AgNCs. Scale bar: 10 nm. Illustration: Particle size distribution. (b). UV-vis spectroscopy of GSH-AgNCs, the inset shows the photo of GSH-AgNCs under visible light irradiation. (c). Changes in the fluorescence intensity of GSH-AgNCs measured under the action of different wavelengths of excitation light. (d). Excitation spectrum (black lines) and emission spectrum (red lines) of GSH-AgNC at 605nm and 450 nm. The inset shows a photo of GSH-AgNCs emitting red fluorescence under UV irradiation.

Figure 2

(a). Fluorescence inverted microscopy of the images of 125 μ M, 250 μ M GSH-AgNC, and HSF incubated together for 1, 3, and 7 days. Scale bar: 200 μ m. (b). Live/dead fluorescent staining images of HSF cells incubated with different GSH-AgNCs after 5 days. Live cells are labeled with green fluorescence, while dead cells are labeled with red fluorescence. Scale bar: 200 μ m. (c) The results of CCK-8 after co-culturing HSF cells with different concentrations of GSH-AgNCs for 1, 3, and 7 days. (*P<0.05).

Figure 3

(a). Fluorescence images at different times point after injection of GSH-AgNCs. (b). Evaluation of the liver function (ALT, AST, TBIL, ALP)) and kidney function of km mice injected with GSH @ Ag NCs (BUN, SCR); mice in the control group received an intravenous injection of 200 μL of normal saline. (c). Seven days after a single intravenous injection of GSH-Ag NCs suspension, H&E-stained tissue (heart, liver, spleen, lung, and kidney) sections were taken from km mice. Scale bar: 200 μm . (n = 3).

Figure 4

(a). Antibacterial efficiency of GSH-AgNCs against different bacteria using the Coated plate method. (b). The pictures and simple schematic diagrams of GSH-AgNCs permeating the skin eschar to form a zone of inhibition. (c). Histogram representation of the bacterial survival rate results.

Figure 5

(a). Schematic diagram of the closed full-thickness burn wound infection modeling and GSH-AgNCs treatment process. (b). Wound healing efficacy of GSH-AgNCs in a burning infection wound model in rabbits; red circles indicate pus spots. (c). H&E staining of the sampled wound tissues after 10 days post-treatment. Scale bar: 200 μ m.