Construction of antibacterial nano-silver embedded bioactive hydrogel to repair infectious skin defects

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Research Article

Keywords: Silver nanoparticles, bioderived porcine acellular dermal matrix hydrogel, infected irregular wounds, antibacterial and antioxidant properties, low toxicity

Posted Date: April 25th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1518490/v1

Abstract

Background: Hydrogels loaded with antimicrobial agents have been widely developed for the treatment of infected wound defects. However, hydrogels derived from a porcine dermal extracellular matrix (PADM), containing silver nanoparticles (AgNPs), have not yet been studied. Therefore, we investigated the therapeutic effect of a AgNP-impregnated PADM (AgNPs-PADM) hydrogel in the treatment of infected wound defects.

Methods: A AgNPs-PADM hydrogel was synthesized by embedding AgNPs into a PADM hydrogel. We examined the porosity, moisture retention, degradation, antibacterial properties, cytotoxicity, antioxidant properties, and ability of PADM and AgNPs-PADM hydrogels to treat infected wounds in animals.

Results: The PADM and AgNPs-PADM hydrogels were pH-sensitive, which made them flow dynamically under acidic conditions and become solid under neutral conditions. They also demonstrated porous network structures, satisfactory moisture retention, and slow degradation rates. Meanwhile, the AgNPs-PADM hydrogel showed a slow and sustained release of AgNPs for at least seven days without changing the size of the particles, so that they could exhibit adequate antibacterial ability, negligible toxicity, and antioxidant properties in vitro. Moreover, the AgNPs-PADM hydrogel successfully promoted the angiogenesis and wound healing of infected skin defects in vivo.

Conclusions: A AgNPs-PADM hydrogel is a promising bioderived antibacterial material for clinical infected wound dressings.

1. Background

When remedying skin nonhealing caused by wound infection, is difficult to obtain an effective bactericidal dose locally using intravenous administration, owing to the destruction of the skin barrier and blood vessels(1). Moreover, the multiplication of multidrug-resistant microorganisms increases the difficulty of treating infectious wounds(2). Current research shows that the external application of composite materials loaded with antibacterial drugs is an effective method of treatment(3). The ideal antibacterial material for external use should possess long-term resistance to a variety of bacteria, and it should also exhibit low toxicity and inflammatory response to improve blood supply and high moisture retention, thereby providing a good microenvironment for wound healing(4).

Silver nanoparticles (AgNPs) have been demonstrated to possess a broad spectrum of antibacterial effects because they can accumulate in the intima of bacteria, increase membrane permeability, and interact with sulfur-containing proteins in the bacterial cell wall, which results in bacterial wall rupture(5). Moreover, AgNPs can enter bacteria and interact with sulfur or phosphorus groups in their DNA and proteins to denature them. This induces the generation of reactive oxygen species and free radicals *via* the interaction with mercaptan groups in enzymes to activate apoptotic pathways(1). The bactericidal mechanism enables AgNPs to exert antibacterial effects on all bacteria without apparent drug resistance. However, AgNPs are toxic to normal cells, owing to their ability to penetrate cell membranes. It has been

observed that the cytotoxicity of AgNPs exhibites a dose-dependent relationship with the concentration of the NPs, and it is negatively correlated with the nanoparticles diameter(6). Therefore, AgNPs can be loaded within biomaterials and released slowly to mitigate toxicity(7). A hydrogel is a type of water-insoluble, cross-linked, fibrous network structure biomaterial, and it provides the advantages of high-water content, high porosity, and good biocompatibility. It has been used as a carrier for the sustained release of drugs; thus, it can be used to carry and slowly release silver NPs. Moreover, it also provides a moisturizing environment for the wound surface. Therefore, AgNPs containing an antibacterial hydrogel are a gel dressing developed on the theoretical basis of wet wound healing. To ensure effective antibacterial properties, it can also provide a good wet and closed environment for the wound; and thus, the drugs will not adhere to the wound or cause a secondary avulsion injury(8).

Traditional synthetic hydrogel materials include chitosan, alginate, hyaluronic acid, polyethylene glycol, and mercaptossucinic acid(9–12). These materials have particular toxicities, poor histocompatibility, and cumbersome production processes, and they cause considerable skin irritation. In addition, the synthesis of hydrogels typically requires toxic cross-linking agents, such as acrylic acid, polyvinyl alcohol, and glutaraldehyde, which may be toxic to cells and are not easily removed *in vivo*(8, 9, 13). To mitigate these limitations, many studies have focused on drug carriers in the biological field(14). It has been reported that extracellular matrix materials (ECM) possess good biocompatibility, and they can also simulate the original microenvironment for cells, provide chemical and mechanical signals, guide cell adhesion, proliferation, and differentiation, and promote the repair and regeneration of homologous tissues(11, 15). Qiu *et al.* observed that hydrogels derived from the extracellular matrix of bone can promote angiogenesis and osteogenesis(16). Farnebos *et al.* demonstrated that tendon-derived ECM hydrogels possess specific components of the extracellular matrix of tendons that promote infiltration and remodeling of host cells(17). Parmaksiz M *et al.* reported that chondrogenic ECM contained cytokines that promote the proliferation and differentiation of chondrocytes and bone marrow mesenchymal stem cells such as TGF-β1, BMP-7, and IGF, and BMSCs were enhanced in chondrogenic ECM(18). Thus, it can be observed that the extracellular matrix, as a new cell-free biomaterial, can itself become a part of regenerative tissue without degradation or requiring removal(19, 20). Our previous study demonstrated that the extracellular matrix of the skin can promote skin healing(14).

In this study, a porcine acellular dermal matrix (PADM, pig-skin extracellular matrix) hydrogel was used as a carrier to encapsulate AgNPs using a physical embedding method to cure infectious skin wounds (Scheme 1). This AgNPs-PADM composite hydrogel can reduce the cytotoxicity caused by AgNPs, and its liquid-solid transition with a change in pH makes it suitable for a variety of wound shapes. The designed AgNPs-PADM hydrogel exhibited a slow and sustained release of AgNPs, and it contributed to long-lasting antibacterial and infectious-wound healing. The porous structure provided a moist microenvironment that promoted epithelial growth within the injured area. The AgNPs-PADM composite hydrogel exhibited good antibacterial activity against Staphylococcus aureus, Enterococcus, and Enterobacter. Improving on the PADM hydrogel, the AgNPs-PADM hydrogel can reduce the inflammatory response of infected wounds and promote regenerative epithelialization, angiogenesis, and collagen

deposition in infected wounds, thereby providing enhanced wound-healing ability. Therefore, the composite AgNPs-PADM hydrogel may be a promising choice for the treatment of infectious wounds.

2. Methods

2.1 Materials

AgNPs (5 nm and 50 nm) were purchased from Xi'an Ruixi Biological Technology Co., Ltd (Xi'an City, China). Triton X100, sodium lauryl sulfate, and soy peptone were purchased from Sinopharm Chemical Reagent Co., Ltd (China). Agar, pepsin, and Masson staining reagents were purchased from Solarbio Science and Technology Co., Ltd (Beijing, China). Tryptone was purchased from OXOID (Shanghai, China), calcein was purchased from Yeasen Biotech Co., Ltd. (Shanghai, China), and Cell Counting Kit-8 (CCK8) was obtained from APExBIO Technology LLC (USA). Trypsin was purchased from Sigma-Aldrich (USA). The 2,2-Diphenyl-1-picrylhydrazyl(DPPH) reagent was purchased from GlpBio (USA).

2.2 Synthesis of AgNPs-PADM hydrogel

Pig skin was harvested from fresh skin tissue from adult pigs and collected from local slaughterhouses. Then, the porcine skin tissue was rinsed using sterile water for 3 h, followed by three consecutive cycles of freezing and thawing (− 80–37 ^{o}C). The tissue was cut into 1×1 cm pieces, and the subcutaneous tissue was removed with scissors, shaken at 120 rpm at a constant temperature (25 ^{o}C)). It was then treated with 1% Triton X-100 solution for 12 h, 0.1% sodium dodecyl sulfate for 6 h. It was rinsed extensively in phosphate buffered saline (PBS) and lyophilized. The sample was then ground into powder. Then, 20 mg/mL of powder was digested with pepsin powder in dilute hydrochloric acid solution (pH 2–3) for 10 min(21), and a 5 nm Ag NPs solution was added dropwise to the solution and stirred rapidly. Subsequently, the above mixture continued to digest for 2 h until the gel was translucent and viscous, and it was stored in a refrigerator at 4℃. PBS solution was added to adjust the osmotic pressure, and pre-cooled 10 M NaOH was added to adjust the pH to 7–8. After maintaining the gel at 37℃ for 20 min, and a AgNPs-PADM hydrogel was prepared.

2.3 Characterization of a AgNPs-PADM hydrogel

Cell- and nuclear-removal performances were assessed using hematoxylin and eosin (H&E) and 406-diamino-2-phenylindole (DAPI) staining. The absorption of AgNPs, PADM hydrogel, and AgNPs-PADM hydrogel was investigated using UV-Vis spectrophotometry. The particle size and distribution of the 5 nm AgNPs were observed using transmission electron microscopy (TEM) before its addition to the hydrogel and after its release from the AgNPs-PADM hydrogel. The spatial structures of the PADM and AgNPs-PADM hydrogels containing 20, 50, and 80 μg/mL of AgNPs were studied using scanning electron microscopy (SEM). Briefly, after the fixation of the hydrogels in 2.5% (w/v) glutaraldehyde for 1 h, the samples were washed three times with PBS solution and then dehydrated sequentially with 30%, 40%, 50%, 70%, 80%, 90%, 95%, and 100% CH_3CH_2OH. Subsequently, the samples were dehydrated with liquid carbon dioxide in a critical point dryer and observed *via* scanning electron microscope (SEM) (JSM-

6360LV, JEOL, Tokyo, Japan) to determine the structural characteristics of the PADM and AgNPs-PADM hydrogels. An energy-dispersive spectrometer (EDS, X-act, OXFORD, England) was used to study the composition and distribution of different elements.

The porosities of the PADM and AgNPs-PADM hydrogels were investigated by using two methods. The first is the medium-immersion method(22). First, a volume of the hydrogel was pre-frozen at -80 ^{o}C for 1 h and then placed in a freeze dryer for 12 h under negative pressure. The mass of the solid gel (W_1) was measured, and the hydrogel was immersed in Dulbecco's modified Eagle's medium (DMEM, Hyclone) until saturation. The beaker weight (W_2) of the DMEM medium and that (W_3) of the DMEM medium containing the hydrogel was measured. The hydrogel was removed, excess media was gently wiped off the gel surface with gauze, and the hydrogel was weighed after soaking (W_4). Porosity was calculated as follows: Porosity= $(W_4-W_1)/(W_3-W_2)$. For the second method, the cross-section of the sample was scanned *via* SEM, and the porosity of the hydrogel was calculated by using Image J (National Institutes of Health, USA).

2.4 Water-retention performance

The same mass of PADM and AgNPs-PADM hydrogels was placed in a 37 ^{o}C incubator, and their masses were measured at different time points until their masses no longer changed. The formula used to calculate the water-retention performance of the hydrogels is as follows: water retention rate = $W_2/W_1\times100\%$. W_2 represents the weight of the gel measured at each time point, and W_1 represents the initial weight of the hydrogel.

2.5 *In vitro* degradation performance

To measure the degradation performance under different conditions, we placed the PADM and AgNPs-PADM hydrogels in a centrifuge tube and then placed them in an incubator at 25, 37, and 42 ^{o}C and observed regularly. The dissolution of the PADM and AgNPs-PADM hydrogels was checked, the liquid dissolved in the centrifuge tube was promptly aspirated, and the remaining samples were weighed.

In addition, we prepared 500 μL of the PADM and AgNPs-PADM hydrogels into a solid state and placed them in a centrifuge tube containing 1 mL of PBS. Then, 7.5 mg of trypsin was added to the experimental group (trypsin group) at 37 ^{o}C. After the hydrogels were immersed for seven days, the two groups of solutions were changed once a day. The PBS and trypsin solutions in the centrifuge tube were removed at the same time every day, and the hydrogels were rinsed with PBS. The water on the surface of the hydrogel was wiped off, the sample was weighed, and the above solution was added in the same configuration after weighing.

2.6 Release of AgNPs from the AgNPs-PADM hydrogel

To determine the release performance of AgNPs in the AgNPs-PADM hydrogel under different conditions, we initially prepared a series of AgNP solutions, measured the absorbance values of different concentrations of AgNP solution via ultraviolet (UV) spectrophotometry, and created a standard curve.

The release of AgNPs from the AgNPs-PADM hydrogel was then measured at different temperatures. The PADM hydrogels (500 μL of the PADM and AgNPs-PADM hydrogels) were placed in a centrifuge tube, which was placed in an incubator at 25, 37, and 42 ^{o}C in particular intervals. The dissolved solution was removed for a period of time, and its optical density(OD) value at a wavelength of 400 nm was measured. The above operation was repeated until the OD value of the solution no longer increased.

Trypsin is a commercial food-grade enzyme with increased proteolytic activity, used to break down collagen(23).The AgNPs-release performances of the PADM and AgNPs-PADM hydrogels in trypsin-containing PBS and PBS alone were also measured. A group of 500 μL solid samples prepared from the PADM and AgNPs-PADM hydrogels were placed in centrifuge tubes, and trypsin (7.5 mg) and PBS solution (1 mL) were added to the tubes. Another set of centrifuge tubes containing two samples only were added with PBS solution (1 mL) respectively. A 1 ml solution was taken every day, and its OD value was measured at a wavelength of 400 nm. Then, 1 ml of the corresponding solution was added to the centrifugal tube after measurement, and the above operation was repeated until the OD value of the removed solution no longer increased.

Finally, we measured the release of AgNPs from the PADM and AgNPs-PADM hydrogels using an ultrasonic crushing instrument. The solid samples prepared by using 500 μL of the PADM and AgNPs-PADM hydrogels were cut into small pieces and placed in a centrifuge tube. Deionized water (400 μL) was added, and an ultrasonic shatterer was used to vibrate the samples at frequencies of 25 Hz and 30 Hz. The samples were vibrated for 30 s and rested for 20 s in a cycle to prevent an excessively high solution temperature. After 24 cycles, the samples were centrifuged at 4000 g for 3 min, and then 200 μL of the supernatant was removed. The OD value was measured at a wavelength of 400 nm. The supernatant was added to the sample after the test, and the above operations were repeated until the OD value of the solution did not increase.

2.7 Antibacterial properties *in vitro*

The *in vitro* antibacterial potential of the AgNPs-PADM hydrogel was investigated using the Oxford cup method (OCM) and colony count method (CCM). Gram-negative bacteria (Escherichia coli) and gram-positive bacteria (Staphylococcus aureus and Enterococcus faecalis) were used for the OCM experiments. First, single colonies of Escherichia coli, Enterococcus, and Staphylococcus aureus were added to the test tubes containing the medium, and the tubes were placed on a shaking table at 150 rpm and 37 ^{o}C overnight. Bacteria in the logarithmic growth stage were selected for the experiment. An appropriate number of bacteria were selected, and their OD value was adjusted to 0.1 (the number of bacteria was 0–1.5x10^8/L). The number of bacteria was diluted to 10^5/L. Then, 1/10 of the culture-medium volume was added to the uncoagulated culture medium containing Agar, the medium was fully mixed and added to a Petri dish. After cooling, the PADM and AgNPs-PADM hydrogels were added. The Petri dish was placed in an incubator at 37 ^{o}C. After culturing for 12 h, the diameter of the bacteriostatic zone around the samples was measured using a scale. Each experiment was repeated thrice.

Subsequently, we selected Staphylococcus aureus for CCM detection. First, the PADM and AgNPs-PADM hydrogels were cut into 3 mm pieces and sterilized for 2 h under UV light. Then, 2 mL of Staphylococcus aureus at a concentration of 1x10^8/mL was put into a test tube. The test tube was placed on a shaking table at 150 rpm and 37 ^{o}C to reproduce the bacteria. Bacterial suspension (100 μL) and EP tubes were centrifuged at 2, 4, and 12 h. After discarding the supernatant, the bacteria were suspended in normal saline.20ul bacterial suspension was evenly coated on nutrient agar plates, and bacteria were counted after 24 h of culture at 37 ^{o}C.

The hydrogels prepared with 80 g/mL AgNPs was placed in a centrifuge tube, and ultrasonic shock at a working frequency of 25 Hz was carried out for 30s and then for 20s. The AgNP release solution (100 μL) at 60, 100, and 140 min was obtained and added to an Oxford cup that was prepared in advance. The Oxford cup was prepared as follows. First, an appropriate amount of Escherichia coli solution was used, its OD value was adjusted to 0.1, and it was diluted to 10^6/L. Then, 1/10 of the volume of the culture medium was mixed into the unsolidified Agar medium. The culture medium was poured into the Petri dish, and after it cooled, the Oxford cup was placed, and an appropriate amount of the unsolidified Agar medium was added again. After the upper medium solidified, the prepared AgNPs release solution was added, and the Oxford cup was removed 12 h later to observe the bacterial-removal status at the bottom.

2.8 Cytotoxicity test

The hydrogel (100 μL) was added to a 24-well plate and sterilized using irradiation (25 kGy γ radiation). HeLa cervical epithelioid carcinoma cells were seeded at 4x10^{4} per well in blank Petri dishes and PADM and AgNPs-PADM hydrogels(24). DMEM (Hyclone) was added to 10% (v/v) fetal bovine serum, 100 μg/mL penicillin, and 100 μg/mL streptomycin. Cells were cultured in a humid environment containing 5% carbon dioxide at 37 ^{o}C After 48 h of co-culture, the cells were stained with a calcium lutein staining kit and observed under a 515 nm fluorescence microscope.

Cytotoxicity was investigated by using CCK8. PADM and AgNPs-PADM hydrogels were immersed in DMEM (hydrogel volume/medium volume 1:5). The supernatant was collected as a 100% leaching solution and then mixed with a particular proportion of DMEM medium to obtain culture solutions containing 0%, 20%, 50%, and 100% leaching solutions. For further experiments, HeLa cells were seeded in 96-well plates at a density of 2000 cells/well. After culturing at 37 ^{o}C for 24 h, the supernatant medium was removed from each well, and 100 μL of each medium containing different proportions of leaching solution were added to the DMEM medium without the leaching solution as the control group. Then, 10 μL CCK8 reagent was added to each well on the day 1, day 3 and day 5. Meanwhile, the cells were incubated at 37 ^{o}C for 1 h. Absorbance at 450 nm was measured using a spectrophotometer.

2.9 Oxidation resistance

The free-radical scavenging capacities of the PADM and AgNPs-PADM hydrogels were measured using the DPPH free-radical scavenging assay (9), in which the clearance rate of ascorbic acid was used as the reference standard. Then, 80 μg/ mL ascorbic acid and PADM and AgNPs-PADM hydrogels were added to

0.4 mM DPPH anhydrous ethanol solution, and the solution was placed away from light for 10 min. An appropriate amount of supernatant was obtained from each sample, and its absorbance at 517 nm was measured. The content of the test sample in the anhydrous ethanol solution containing DPPH was adjusted until the absorbance of the DPPH anhydrous ethanol solution corresponding to the AgNPs-PADM hydrogel sample did not change. The scavenging ability of radicals in the samples at a particular concentration was calculated using the following formula: Inhibition (%) $=(C_1-C_2)/C_1*100\%$. C_1 represents the difference between the maximum and minimum absorbance values of the DPPH solution containing ascorbic acid, and C_2 represents the difference between the absorbance value of the DPPH solution at this sample concentration and that of the DPPH solution alone.

2.10 *In vivo* experiment

The rats used in this study were purchased from the Zhejiang Academy of Medical Sciences. They were six months old and weighed 200–300 g. They were reared in separate cages, fed standard feed and tap water, and the cages were maintained at a controlled temperature of 25°C and humidity of 55%, alternating day and night for 12 h. According to the National Institute of Health publication No. 18–23, 1985, which is typically referenced for the care and use of laboratory animals, great care should be taken with rats. The rats were anesthetized with ketamine (30.0 mg/kg). Their dorsal hair was removed, and their skin was rinsed with ethanol (70%). Three 1 cm square skin wounds were created on the midline side with a scalpel and tweezers, and 30 μL Staphylococcus aureus (bacterial concentration: 1x10^8 CFU/L) was dropped into each wound. Then, 2 h was given to allow the bacteria to fully infiltrate the wound. The wounds were divided into three groups. Group I was treated as the control group and did not receive any treatment (naked wound); group II was treated with PADM gel; group III was treated with the AgNPs-PADM hydrogel and covered with gauze, and the PADM and AgNPs-PADM hydrogels were replaced with new ones on the 3rd, 5th, 7th, 9th, and 11th days.

Reduction in the wound area (wound contraction) was used as an indicator of the therapeutic outcome. Wound contractions were recorded on day 0, 7, and 14. The wound healing area was expressed as a percentage. The wound-shrinkage percentage was estimated using the following formula: wound-healing ratio (%) $=(C_1-C_2)/C_1x100\%$, where C_1 represents the initial wound area, and C_2 represents the wound area at each measurement time.

On the 7th and 14th days, half of the rats in group I, in group II and group III were randomly anesthetized and sacrificed, and non-wound skin approximately 5 mm around the wound was biopsied. Skin tissue was immobilized in buffered formalin (4%) for 2–3 days before tissue processing and paraffin embedding. Tissue sections with a thickness of 5 μm were made using a sectioning mechanism and stained with hematoxylin and eosin (H&E). The neovascularization, epidermis, scarring, and granulation tissue were observed and photographed.

Masson's trichrome staining can be used to dye early collagen in light blue and mature collagen in dark blue to assess wound healing. On the 7th and 14th days, the paraffin sections of the wound were stained with Masson's trichrome staining, and the collagen content in the tissue sections was analyzed under a

microscope. Masson's trichrome stain was designed to distinguish smooth muscle cells from collagen. Additionally, paraffin sections were stained using immunohistochemistry to measure the expression of CD68 and CD34 in the samples to evaluate the changes in macrophage content and tissue micro-vessel content during wound healing, respectively.

- **2.11 Statistical analysis**

Statistical analysis was performed using GraphPad Prism 8 (GraphPad Software Inc., USA). The results are expressed as mean ± standard deviation. A one-way ANOVA and two-way ANOVA were used to determine whether there was a significant difference in the results, and a P-value ≤ 0.05 was considered acceptable.

3. Results

3.1 Characterization of the AgNPs-PADM hydrogel

When AgNPs were added to the acidic PADM hydrogel solution, the hydrogel color changed from white to pale yellow. After proper agitation and adjustment of the pH value, a hydrogel was formed, and the distribution of AgNPs was uniform (Fig. 1A, B). H&E and DAPI staining of acellular pig skin tissue showed that the cells in pig skin were completely removed, and the extracellular structure of pig skin remained intact, which is consistent with our previous experimental results (Fig. 1C, D)(14). The UV spectrum analysis of the pure AgNPs (5 nm) solution exhibited a single peak at an absorption wavelength of 400 nm, which indicates that the AgNPs (5 nm) were uniformly distributed without aggregation, which is consistent with previous results(11). The UV spectrum of liquid AgNPs-PADM hydrogels demonstrated that the AgNPs-PADM hydrogels also produced a single peak at ~ 400 nm, which confirmed that the AgNPs (5 nm) were distributed in the PADM hydrogels without agglomeration (Fig. 1E). We also measured the UV spectrum of AgNPs (50 nm) and the corresponding AgNPs-PADM hydrogels. The results revealed that the AgNPs (50 nm) exhibited a single peak at ~ 400 nm (Fig. 1F). However, the AgNPs-PADM (50 nm) hydrogels did not exhibit a peak at ~ 400 nm (Fig. 1F), which demonstrates that AgNPs (50 nm) may accumulate in the PADM-hydrogel. Transmission electron microscopy showed that the size of the AgNPs before being added to the hydrogels and after being released from the AgNPs-PADM hydrogels was not significantly different for the 5 nm particles (Fig. 1G). However, the size of the AgNPs (50 nm) significantly increased (Fig. 1H). It can be observed that the physical properties of 5 nm AgNPs do not change significantly before and after the reaction, and their distribution is more uniform than that of the 50 nm AgNPs. Moreover, smaller AgNPs have a larger surface area; therefore, it is easier for these particles to be in direct contact with bacteria, and the sterilization effect is better(1). Therefore, 5 nm AgNPs were selected for subsequent experiments.

3.2 The fiber structure and porosity of the AgNPs-PADM hydrogel

The SEM images of the PADM and AgNPs-PADM hydrogels are shown in Fig. 2A. The AgNPs-PADM hydrogels were prepared using 20, 50, and 80 ug/mL AgNP solutions. Compared with the PADM hydrogel, all the AgNPs-PADM hydrogels formed a mesh fiber structure, which is consistent with the results of the extracellular matrix hydrogels and extracellular tendon hydrogels(16, 17). These results indicated that extracellular collagen may be reassembled into a new collagen-like fiber structure after pepsin digestion. The porosities of the PADM and AgNPs-PADM hydrogels prepared using the three different concentrations of AgNPs were 21.53 ± 1.49%, 20.79 ± 1.72%, 20.13 ± 1.51%, and 20 ± 1.38%, respectively. These results indicate that the concentration of AgNPs had little effect on the hydrogel porosity (Fig. 2B, C). The EDS analysis results demonstrated that the presence of Ag could not be detected in the blank hydrogel group, but Ag could be detected in the AgNPs-PADM hydrogels (80 μg/mL AgNPs concentration) (Fig. 2D). Moreover, Ag was uniformly distributed in the AgNPs-PADM hydrogel (Fig. 2E), which is consistent with the UV observation results. Therefore, these results indicate that the PADM hydrogel may be a good dispersion medium for AgNPs.

3.3 The water storage performance, degradation and release behavior of hydrogels

The porosities of the PADM and AgNPs-PADM hydrogels containing different concentrations of AgNPs were measured using the immersion method. It was observed that the porosities of the two hydrogels were similar at 20 ± 0.4% and 19 ± 0.2%, respectively (Fig. 3A). There was no statistical significance in the porosity difference (P 0.05), which is consistent with the results in Fig. 2B-C. A porous network structure can better permeate oxygen, absorb exudate, and provide a scaffold for cell attachment, which is also conducive to wound healing(9, 25).

The ability of the PADM and AgNPs-PADM hydrogels to retain water is shown in Fig. 3B. At 37 ^{o}C, both hydrogels lose all internal water after approximately 30 h, and the results were not significantly different for each hydrogel. The addition of AgNPs did not change the water-storage performance of the PADM hydrogels, which can maintain the wet state of the wound for a long time and promote wound healing(8). Moreover, this also guarantees the water-based medium required for the uniform dispersion of AgNPs. Wound defects typically lead to fluid leakage from the wound, and the hydrogels absorb part of the seepage when losing water at body temperature, which is also beneficial for wound healing(26).

The degradation properties of the PADM and AgNPs-PADM hydrogels at different temperatures were carefully tested. As shown in Fig. 3C, the degradation rates of the PADM and AgNPs-PADM hydrogels increased with increasing temperature. Compared with the 2 days required to degrade the blank and AgNPs-PADM hydrogels to 0.46 g and 0.54 g at 25 ^{o}C and 37 ^{o}C, respectively, the hydrogels were almost completely degraded at a temperature of 42 ^{o}C for two days, and the degradation rate was significantly accelerated. The degradation time required for the hydrogel was longer at room temperature, and the degradation increased significantly with an increase in temperature.

Trypsin was used to test the degradation performance of the PADM and AgNPs-PADM hydrogels. The degradation rates at various times are shown in Fig. 3D. It was observed that the degradation rates of the PADM and AgNPs-PADM hydrogels in the PBS environment were 24.59 ± 2.755% and 20.67 ± 2.510% on day 4, respectively. In the trypsin solution, the degradation rates were significantly increased (PADM groups: 45.31 ± 2.198% vs. 24.59 ± 2.755%, $P < 0.05$, AgNPs-PADM groups: 39.55 ± 1.269% vs. 20.67 ± 2.510%, $P < 0.05$). On day 8, there was no significant difference in the degradation rate between the PADM and AgNPs-PADM hydrogel groups in PBS and trypsin alone (trypsin groups: 76.43 ± 0.1044% vs. 72.13 ± 1.227%, $P > 0.05$). PBS groups: 34.07 ± 2.416% vs. 31.21 ± 2.829%, $P > 0.05$). The degradation behaviors are similar, which may be because the interaction of AgNPs and PADM hydrogels are primarily for adsorption, and AgNPs have little effect on the degradation performance of enzymes(27). However, compared with the pure PBS solution groups, the degradation rate of the hydrogels in trypsin solution was significantly higher. This may be because the decellularized pig skin particles were first decomposed into various collagen fibers during gel preparation, and the PADM hydrogel contained particular protein components that can be degraded by trypsin(28). These results suggest that during tissue damage, various proteases that are released locally may degrade other protein components in the hydrogel and promote the release of AgNPs.

We tested the AgNPs release rate of the AgNPs-PADM hydrogel at 25, 37, and 42 ^{o}C. As shown in Fig. 3E, the AgNPs-PADM hydrogel continuously released AgNPs for up to 60 h at 42 ^{o}C. The release rate was at its fastest in the first 10 h, and it then gradually slowed until it stopped at ~ 60 h. This corresponds to the complete dissolution of the AgNPs-PADM hydrogel. The AgNPs release rate of the AgNPs-PADM hydrogel was slower at 37 and 25 ^{o}C compared with that at 42 ^{o}C. After one week, the amount of AgNPs released was 6.306 ± 0.8149% and 2.835 ± 0.2675% of that released at 42 ^{o}C, respectively, which is consistent with the degradation rate of the AgNPs-PADM hydrogel in Fig. 3C.

The AgNPs release behavior of the AgNPs-PADM hydrogel was investigated in PBS and trypsin solutions. As shown in Fig. 3F, the AgNPs-PADM hydrogels soaked in trypsin solution exhibited a sustained release of AgNPs for eight days. The amount of AgNPs was 22.19 ± 0.4058% of the total. The release rate of AgNPs in PBS solution was significantly slower than that in trypsin solution, and the release time of the AgNPs was more than eight days. The release rate was fast on day 3, and it then slowed. The amount of AgNPs released on day 8 was 6.693 ± 0.4163% of the total amount. These results are also consistent with those shown in Fig. 3D.

We also measured the release of AgNPs from the AgNPs-PADM hydrogel using the ultrasonic oscillation method. As shown in Fig. 3G, the released AgNPs increased with increasing oscillation time. Compared with the results of the 25 Hz ultrasonic shock group, a 30 Hz ultrasonic shock significantly accelerated the release of AgNPs, and the maximum release value was measured at approximately 60 min, which is similar to the result of the 5 Hz ultrasonic shock group measured at approximately 140 min, i.e., 10.69 ± 0.1398% of the total AgNPs in the AgNPs-PADM hydrogel. The AgNPs-PADM hydrogel group exhibited the highest release content of AgNPs measured by dissolution at 42 ^{o}C, which was compared with the

maximum release value of AgNPs measured in the trypsin and ultrasonic shock groups (Fig. 3G), which indicates that there were still some AgNPs remaining in the undecomposed hydrogels. This may be the reason that the fibrous network structure inside the hydrogel is dense, and the AgNPs are not easily released.

As shown in Fig. 3H, there is no significant difference in the wavelength of the maximum absorption peak for hydrogels with 5 nm and 50 nm AgNPs (Fig. 3H). The absorption peak of the liquid AgNPs-PADM hydrogel was slightly wider than that of the pure AgNP solution (Fig. 1E) in the UV absorption spectrum, thereby indicating that the AgNPs were uniformly dispersed in the PADM hydrogel, and there was no agglomeration phenomenon. If agglomeration occurs, the particle size of the AgNPs increases, which reduces the bactericidal effect(1).

3.4 Bactericidal ability of the AgNPs-PADM hydrogel

The antibacterial activities of the PADM and AgNPs-PADM hydrogels were determined using the modified Oxford cup method. The results showed that the antibacterial effects of hydrogels with 20 μg/mL and 50 μg/mL AgNPs were not significantly different to those of the PADM hydrogels. Hydrogels with 80 μg/mL AgNPs exhibited a marked antibacterial effect. Hence, we chose AgNPs-PADM hydrogels with a AgNPs concentration of 80 μg/mL as the sample for subsequent experiments (Fig. 4A). The inhibition area diameters of the AgNPs-PADM hydrogels against Staphylococcus aureus, Enterococcus faecalis, and Escherichia coli were 1.707 ± 1.3149 mm, 1.317 ± 0.187 mm, and 2.363 ± 0.062 mm, respectively. The results indicated that the antibacterial effect of the AgNPs-PADM hydrogels were higher than that of the PADM hydrogels ($p < 0.05$, Fig. 4B). Moreover, AgNPs-PADM hydrogels exhibited better antibacterial effects on gram-negative bacteria than gram-positive bacteria. The AgNPs-PADM hydrogels may have a lower resistance to the cell membranes of gram-negative bacteria than the thicker layer of peptidoglycan cell walls of gram-positive bacteria(29). In addition, we stored the acidic AgNPs-PADM hydrogel in a refrigerator at 4 ^{o}C for several days and then adjusted its pH value to neutrality to prepare the hydrogels for the inhibition-zone experiment. This effect was not significantly different from the bacteriostatic effect on the first day (Fig. 4C).

3.5 AgNPs-PADM hydrogel ability to kill planktonic bacteria

The ability of the PADM and AgNPs-PADM hydrogels to kill planktonic bacteria was investigated using the dilution coating method, as shown in Fig. 5A. Compared with the PADM hydrogel groups, the bacterial counts of Staphylococcus aureus in the AgNPs-PADM hydrogel groups at 2, 4, and 12 h were relatively lower, and this difference was most prominent at 12 h. The bacterial counts for the PADM and AgNPs-PADM hydrogel groups at 12 h were 852.7 ± 6.498 and 205.3 ± 14.53, respectively (Fig. 5B). These results also confirmed the antibacterial effect of the AgNPs-PADM hydrogel in the Oxford cup experiment, that is, the hydrogels impregnated with AgNPs has a strong antibacterial potential against a variety of bacteria. In addition, we added 100 μL of the AgNPs release solution that was obtained at 60, 100, and 140 min under ultrasonic vibration to the prepared Oxford cup and performed the sterilization test again. The

results showed that AgNPs were released at 60, 100, and 140 min, and all solutions completely killed E. coli in the medium in the wells (Fig. 5C).

3.6 Cytotoxicity, cell proliferation, and antioxidant activity of the AgNPs-PADM hydrogel

We evaluated the cytotoxicity of the PADM and AgNPs-PADM hydrogels using calcein method. After the hydrogels were co-cultured with HeLa cells for 48 h, the results of the calcein staining demonstrated that the number and density of viable cells on the PADM and AgNPs-PADM hydrogels increased significantly compared with the cells on the blank group. Additionally, good cell morphology was observed. Thus, the PADM and AgNPs-PADM hydrogels did not produce observable cytotoxicity to HeLa cells, but they could promote cell growth (Fig. 6A). In addition, we analyzed the cytotoxicity of the soaking solution by measuring the cellular metabolic activity of the PADM and AgNP-PADM hydrogels using the CCK-8 method. As shown in Fig. 6B, there was no significant difference between the groups on day 1 ($p > 0.05$). On the 3rd day, the number of cells in the PADM hydrogel groups increased significantly, and the number of cells in the 100% AgNPs-PADM hydrogel soaking-solution groups decreased compared to the PADM groups. On the 5th day, the metabolic number of cells in the PADM groups and the 25%, 50%, and 100% AgNPs-PADM hydrogel soaking-solution groups increased ($p > 0.05$); however, the cell-survival rate did not decrease. Thus, based on these results, the PADM and AgNPs-PADM hydrogels did not clearly exhibit toxicity to HeLa cells. This indicates that the PADM hydrogel embedded with AgNPs can reduce the toxicity of AgNPs to cells, which also proves that a bioderived hydrogel is an appropriate biomaterial for the growth of recipient cells and wound healing.

The antioxidant capacity of the samples was measured using the DPPH method, and ascorbic acid was used as the control. The free radical scavenging capacities of ascorbic acid and PADM and AgNPs-PADM hydrogels increased in a dose-dependent manner (Fig. 6C). In the concentration range of 50–250 μg/mL, ascorbic acid possessed the highest scavenging ability against DPPH free radicals, and its IC50 was the lowest (50 μg/mL). This was followed by the AgNPs-PADM hydrogel with an IC50 of 125 μg/mL, and the PADM hydrogel also had the ability to scavenge part of the free-radical activity (Fig. 6D).

3.7 Repair of infected gap in rats and reduction of local inflammatory response

The experiments demonstrated that the AgNPs-PADM hydrogel can significantly promote the healing of infected wounds in rats. After only seven days of wound treatment, the wounds in the AgNPs-PADM hydrogel groups contracted significantly. The wound shrinkage in the AgNPs-PADM hydrogel groups was 94.53 ± 0.8090%, that in the PADM hydrogel groups was 83.37 ± 0.6489%, and that in the control group was 70.33 ± 0.6064%. On the 14th day, the wounds of the blank and PADM hydrogel groups healed similarly, whereas the wounds of the AgNPs-PADM hydrogel groups healed completely (Fig. 7A).

We used H&E staining to assess wound inflammation and fibroblast development(30). The results are shown in Fig. 7B. The AgNPs-PADM hydrogel groups demonstrated clear continuous epithelial tissue with less inflammatory cell infiltration, whereas the blank and PADM hydrogel groups exhibited less epithelial tissue generation and clear inflammatory-cell infiltration. On day 14, well-developed hair follicles were observed in the AgNPs-PADM hydrogel group, whereas a small amount of granulation tissue was still present in the blank group. These results indicate that the AgNPs-PADM hydrogel groups can accelerate wound healing and repair tissue structures(26).

Collagen deposition was assessed using Masson's trichrome staining (Fig. 7C), and we observed that on day 7, slightly more collagen was produced in infected wounds in the AgNP-PADM hydrogel groups than in the blank and PADM hydrogel groups. On day 14, collagen production in infected wounds in all groups was significantly increased, and the AgNPs-PADM hydrogel groups exhibited mature collagen deposition and good growth. As an important component of the extracellular matrix, collagen plays an active role in promoting wound healing(31). Collagen is formed and cross-linked at the wound site to produce collagen fibers, thereby enhancing the strength of the wound.

We also examined the inflammatory response(32) and angiogenesis(33) during wound repair. The results of the immunohistochemical staining are demonstrated in Fig. 8. We observed that on the 7th day after injury, the expression of CD34 in the PADM and AgNPs-PADM hydrogel groups was significantly increased compared with that of the blank group (Fig. 8A, red arrow). These results suggest that the PADM hydrogel itself can promote the formation of wound blood vessels, which may be related to its retention of extracellular matrix components(34). On the 14th day after injury, the expression of CD34 in all groups decreased compared with that on the 7th day after injury, which may be related to the gradual accumulation of collagen in the granulation tissue in the wound(35). On the 7th day after injury, all groups demonstrated different degrees of inflammatory response based on the expression level of CD68 (Fig. 8B, black arrow). However, on the 14th day after injury, the expression level of CD68 decreased significantly, which indicates that the inflammatory response was alleviated. On the 7th and 14th days after injury, CD68 expression in the AgNPs-PADM hydrogel groups was significantly lower than that in the blank and PADM hydrogel groups. This may be owing to the presence of AgNPs in the hydrogels, which possess particular anti-inflammatory effects, reduce the inflammatory response of infected wounds, and significantly accelerate the wound-healing process. In conclusion, the AgNPs-PADM hydrogel accelerated wound healing by reducing the local inflammatory response, promoting microvascular formation, and accelerating collagen deposition.

4 Discussion

In this study, we synthesized PADM hydrogels containing AgNPs with diameters of 5 and 50 nm to determine whether PADM hydrogels influence the size and stability of AgNPs of different diameters. It is well known that the sterilization effect of AgNPs is negatively correlated with their size, and that maintaining the size and stability of AgNPs is conducive to maintaining effective sterilization. For example, Rao *et al.* encapsulated AgNPs (diameter: 50 nm) inside surface-layer proteins through

electrostatic interactions and hydrogen bonding to maintain the stability of nano-silver and stagger its release(36). Using ultraviolet spectra, which are sensitive to the size of AgNPs (37), it was observed that 50-nm-diameter AgNPs shifted in the PADM hydrogel and release solution, whereas 5-nm-diameter AgNPs maintained stability. This indicated that the PADM hydrogel destabilized AgNPs (diameter: 50 nm). This might be attributable to the desired acidic conditions during the preparation of the PADM hydrogel. These conditions disintegrated the stable layer of sodium citrate, which maintained a uniform distribution of AgNPs(38). Sodium hydroxide, which was later added, provided a large number of hydroxide ions that affected the positively charged silver ions(24). The nano-silver particles fused or separated, resulting in a change in size. This suggests that small-diameter AgNPs (diameter: 5 nm) are more suitable for loading onto the PADM hydrogel.

Additionally, when the acidic AgNPs-PADM hydrogel was placed in a refrigerator at 4°C, it could be stored for a week and maintained its flow dynamics. This was consistent with the results of Farnebo *et al.*, who found that the tendon extracellular matrix hydrogel could be preserved under acidic conditions without altering its properties(17). In addition, the AgNPs-PADM hydrogel maintained its antibacterial effect after being adjusted to neutral pH. This suggested that the hydrogel maintains the homogeneous dispersion of AgNPs (diameter: 5 nm), thereby preserving their potent antimicrobial activity. The neutralized AgNPs-PADM hydrogel could then be plasticized to form different shapes and converted into a solid hydrogel within 10–15 min, which is beneficial for healing irregular infection wounds.

AgNP-embedded hydrogels are synthesized in situ on the surface of the hydrogel by chemical reduction (39, 40), resulting in only one antibacterial agent being carried. Physical embedding was used to add antimicrobial agents to the PADM hydrogel. The PADM hydrogel not only carries AgNPs but also other special antimicrobial agents to enhance its antibacterial effects(14, 41). This compensates for the lack of antibacterial ability of AgNPs against certain bacteria.

As biologically derived collagen is the main component of the PADM hydrogel, it can be digested into solution by pepsin. When neutralized to isoelectric points by alkali, it can additionally be reconstituted into collagen fiber networks. The reason may be because the storage (G') and loss moduli (G") of the ECM hydrogel increased after the temperature was increased from 10 to 37 ^{o}C (42). We observed that the recombinant collagen fiber network, the hydrogel, was susceptible to increased temperature, and the degradation rate could be significantly accelerated by high temperatures. Once tissue damage and infection of the microenvironment cause a local inflammatory response, secondary heat can be released through the bloodstream, increasing temperature(43). Therefore, the accelerated degradation of the AgNPs-PADM hydrogel in the infected state may contribute to the release of AgNPs, thereby improving the infected state. It is also recommended that high-temperature environments should be avoided as much as possible in clinical use. The polymerization structure of PADM and the triple helix structure of collagen provide stability against the degradation of enzymes other than collagenase(27). However, we found that the degradation of recombinant collagen fibers could be accelerated by the presence of proteases, such as matrix metalloproteinases(44), proteolytic enzymes, and collagenases(45, 46). The release of necrotic cells, inflammation-mediated cells, and fibroblasts during wound healing may

accelerate the release of AgNPs to some extent, which is conducive to maintaining the sterile state of the wound(47). Simultaneously, it leads to a decrease in its mechanical strength; therefore, photochemical and chemical cross-linking methods have been developed to reduce the enzyme degradation rate(48).

It is worth noting that ECM hydrogels can maximize the retention of low-molecular-weight peptides and growth factors present in natural ECM(49). ECM hydrogel degradation products, such as oligopeptide and oligosaccharide derivatives (42), as well as vascular endothelial growth factors (50), can promote angiogenesis. Based on the immunohistochemical staining (CD34) of rat wound sections, we found that the PADM and AgNPs-PADM hydrogels significantly increased intraconal capillary production compared to the blank group and accelerated the growth of granulation tissue, promoting wound healing. Various studies have shown that ECM derived from homologous tissue is more conducive to cell growth and promotes the production of original extracellular matrix components(51–53); however, the specific components and mechanisms of this phenomenon are not yet fully understood.

The antibacterial effect of AgNPs, which reduces the viability of human keratinocytes(54), is usually accompanied by cytotoxicity even when AgNPs are coated with sodium citrate. The significant increase in AgNP dose-dependent reactive oxygen species is a primary cause of cytotoxicity(55). Antioxidants can minimize the cytotoxicity of AgNPs(56). Interestingly, we found that both PADM and AgNPs-PADM were antioxidants. This may be due to the large number of negatively charged amino acids in collagen-rich PADM and AgNPs-PADM hydrogels, such as proline (23), which can combine with positively charged DPPH to scavenge oxygen free radicals (9). It is well known that cells in the infected wound release many reactive oxygen species. This can disrupt the oxidation–antioxidant balance at the cellular level, thereby aggravating tissue damage(57). The antioxidant properties of the AgNPs-PADM hydrogel are beneficial for reducing the inflammatory response of wounds and the toxicity of AgNPs to normal cells, thereby accelerating wound healing(58).

5 Conclusions

In summary, as a treatment for infected skin defects, AgNPs-PADM hydrogels were prepared by embedding AgNPs into a PADM hydrogel, and their performance was tested in vivo and in vitro. AgNPs were uniformly distributed in the PADM hydrogel and slowly released at body temperature without changing particle size. The AgNPs-PADM hydrogel exhibited significant antibacterial and antioxidant properties, increased nontoxicity, and the ability to promote angiogenesis in vivo. Because of this, the AgNPs-PADM hydrogel successfully treated infected skin defects in rats, demonstrating its potential for clinical applications.

Abbreviations

AgNPs: silver nanoparticles; PADM: porcine acellular dermal matrix; AgNPs-PADM: silver nanoparticle-impregnated porcine acellular dermal matrix; ECM: extracellular matrix; CCK8: Cell Counting Kit-8; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; H&E: hematoxylin and eosin staining; DAPI: 406-diamino-2-phenylindole

staining ; TEM: transmission electron microscopy; SEM: scanning electron microscopy; EDS: energy-dispersive spectrometer; OD: optical density; OCM: Oxford cup method ; CCM: colony count method.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

The authors thank Mr.Zhangsen Yu from the School of Basic Medicine of Shaoxing University and Mr. Danhua Tao from the Affiliated Central Hospital of Shaoxing University for their technical support.

Authors' contributions

QD prepared the hydrogel and was a major contributor in writing the manuscript. DZ was responsible for the testing of materials. LK performed the animal experiments. SC and JY were responsible for histological examinations. JL performed all the bacteria-related experiments. LL assisted in data processing and paper writing. BW and BF were responsible for the design and process management of the entire research. All authors read and approved the final manuscript.

Funding

This work was supported by the Natural Science Foundation of Zhejiang Province (grant number LBY22H180007, LBY22H270004), and the Medical and Health Science and Technology Plan Project of Zhejiang Province (grant number 2020KY990, 2022KY416).

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Scheme

Scheme 1 is available in supplementary section.

Figures

Figure 1

(A, B) Preparation of AgNPs-PADM hydrogels. (C, D) H&E staining and DAPI staining of the extracellular matrix of porcine skin were performed, and the results showed that the cells were completely cleared. (E, F) shows that the AgNP (5 nm) solution, AgNP (50 nm) solution, and AgNPs (5 nm)-PADM hydrogels exhibited maximum absorption peaks at 400 nm in the UV-Vis spectra; however, there was no obvious single peak in the AgNPs (50 nm)-PADM-hydrogels. (G, F) shows the change in the size and diameter of AgNPs (5 nm and 50 nm) before and after release.

Figure 2

(A) The SEM images of PADM hydrogels (PADM) and AgNPs-PADM hydrogels (AgNPs-PADM) with 20, 50, and 80 μg/ml AgNPs solutions. The PADM and AgNPs-PADM hydrogels exhibited a three-dimensional reticulated fiber structure. Aa, Ab, Ac, and Ad are magnified by 5000x. Ae, Af, Ag, and Ah are magnified by 10000x. (B) Porosity of PADM and AgNPs-PADM hydrogels with 20, 50, and 80 μg/ml AgNPs solutions calculated using Image J; Be1, Bf1, Bg1, and Bh1 correspond to Ae, Af, Ag, and Ah, respectively. (C) The porosity between PADM and AgNPs-PADM hydrogels with 20, 50, and 80 μg/mL AgNPs solutions. (D) EDS analysis of the PADM and AgNPs-PADM hydrogels with 80 μg/mL AgNPs solutions. (E) The element mapping of AgNPs-PADM hydrogels with 80 μg/mL AgNPs solutions.

Figure 3

(A) The porosity of the PADM and AgNPs-PADM hydrogels containing 20, 50, and 80 μg/mL AgNPs. The results exhibited no significant difference in porosity between the PADM and AgNPs-PADM hydrogels containing different concentrations of AgNPs. (B) There was no significant difference in the water-retention performance between the PADM and AgNPs-PADM hydrogels with 80 μg/mL AgNPs . (C) The solution curves of the PADM and AgNPs-PADM hydrogels with 80 μg/mL AgNPs at 25, 37, and 42 ^{o}C. (D) The degradation curves of the PADM and AgNPs-PADM hydrogels with 80 μg/mL AgNPs in the presence or absence of trypsin. (E) The release curves of the PADM and AgNPs-PADM hydrogels with 80 μg/mL AgNPs at 25, 37, and 42 ^{o}C. (F) The release curve of the PADM and AgNPs-PADM hydrogels with 80 μg/mL AgNPs in the presence or absence of trypsin. (G) The release curve of the PADM and AgNPs-PADM hydrogels with 80 μg/mL AgNPs under ultrasonic oscillation. (H) UV-visible spectra of AgNPs (5 nm and 50 nm) released from the AgNPs-PADM hydrogels. All values are represented as the mean ± standard deviation.

Figure 4

(A) The bactericidal effect of AgNPs-PADM hydrogels with 20, 50, and 80 μg/mL on Enterococcus faecalis. (B) Bacteriostatic effect of AgNPs-PADM hydrogels with 80 μg/mL AgNPs on Staphylococcus aureus, Enterococcus faecalis, and Enterococcus Escherichia coli. (C) The bacteriostatic effect of AgNPs-PADM hydrogels with 80 μg/mL AgNPs after 3, 6, and 9 days of storage at 4 ^{o}C was demonstrated, and the results showed that the bacteriostatic effect of the AgNPs-PADM hydrogels were not significantly different from that on the first day.

Figure 5

(A-B) The ability of PADM and AgNPs-PADM hydrogels to kill planktonic bacteria via the dilution-plate method. (C) The bactericidal effect of releasing the AgNPs solution under ultrasonic shock conditions.

Figure 6

(A, B) The cell viability of HeLa cells after 48 h of co-culture with PADM and AgNPs-PADM hydrogels. (C) The CCK-8 method was used to detect the effects of PADM and AgNPs-PADM hydrogel immersion on cell viability. (D) The ability of ascorbic acid, PADM, and AgNPs-PADM hydrogels to scavenge free radicals. (E) IC50 values of ascorbic acid, PADM, and AgNPs-PADM hydrogel groups.

Figure 7

(A) The infected wound healing in the PADM and AgNPs-PADM hydrogel groups on days 7 and 14. (B) H&E staining results and (C) Masson staining results of wounds in the PADM and AgNPs-PADM hydrogel groups on day 7 and 14.

Figure 8

The expression of (A) CD34 and (B) CD68 in the wounds of the PADM and AgNPs-PADM hydrogel groups on days 7 and 14. The results show that the expression of CD34 (red arrow) in the wounds of the AgNPs-PADM hydrogel group was significantly increased, and the expression of CD68 (black arrow) was significantly reduced compared with those of the blank and PADM hydrogel groups.

Supplementary Files

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