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Full Length Research Paper

Detection, biological effectiveness, and characterization of nanosilver-epidermal growth factor sustained-release carrier

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A nanosilver-epidermal growth factor sustained-release carrier was synthesized with the self-assembly method and characterized by transmission electron microscopy and Ultra violet (UV) spectrophotometry. The biological activity of the sustained release carrier was determined through cytological, bacteriological, and wound-healing experiments. The results show that the nanosilver-epidermal growth factor sustained-release carrier was well dispersed, with uniform particle size and that it had good antibacterial properties that were similar to those of nanosilver. Nanosilver-epidermal growth factor sustained-release carrier is superior to epidermal growth factors in effectively promoting cell division and proliferation. The results of wound-healing experiments show a curative effect.

Key words: Epidermal growth factor, nanosilver, antibacterial, wound, cell proliferation.

INTRODUCTION

Nanoparticles have been increasingly applied in biomedical, pharmaceutical, and clinical medicine. Among them, nanosilver is widely used in clinical burn, dental, and urologic practice (Kim et al., 2007; Chekman et al., 2011; Dallas et al., 2011). In cytotoxic and animal experiments, Arorat et al. (2008), Ai et al. (2011) and Teow et al. (2011) proved that nanosilver has no toxicity, but has high antibacterial activity. Tian et al. (2007) found that local use of nanosilver wound dressing could not only accelerate healing, but also improve the appearance of scars. Madhumathi et al. (2010) and Ong et al. (2008) showed that nanosilver/chitosan dressing effectively resists *Staphylococcus*, colonic, and other bacteria and shows good hemostatic effects in treatment of burn wounds. Growth factors are a class of peptides or proteins that can regulate cell growth and differentiation and promote tissue healing (Xie et al., 2006). Epidermal growth factor (EGF) achieved good clinical results (Kiyohara et al. 1991) but its *in vivo* stability is poor; it is vulnerable to degeneration or inactivation and is easily diminished from blood circulation. Development of a stable, safe, and effective preparation has become a challenging and practical focus in pharmacy research (Değim et al., 2011).

Existing studies have demonstrated the role of controlled- or sustained-release formulations that are prepared with nanoparticles as a carrier. They can effectively protect drugs against inactivation and achieve sustained or controlled release, even targeted release, thus significantly improving the curative efficacy and reducing toxic side effects (Vaiana et al., 2011).

Anti-inflammatory nanosilver combines with EGF to create a sustained-release carrier that has both resistance to infection and sustained-release properties similar to those of EGF. The resultant carrier can promote skin damage repair and make up for the wound infection-induced low activity when EGF is used alone. Our research group has preliminarily determined the optimal particle size and complex conditions for nanosilver and EGF. In this study, we prepared a sustained-release carrier using a 20-n nanosilver particle and EGF by the self-assembly method (Janjua et al., 2011) to elucidate the characterization and biological effects of the carrier.

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MATERIALS AND METHODS

Preparation of silver nanoparticle-EGF sustained-release carrier solution

A 5,000 ppm solution of silver nanoparticles was magnetically stirred with 50 µg/mL EGF solution (10 mL) in a 5 mL sterile flask, and the solution was adjusted with HCl-Tris buffer to pH 7.0. The volume was set at 50 mL and scattered for 30 min with an ultrasonic dispersion machine. The solution was placed in a 37°C water bath overnight for 12 h to obtain a 500 ppm solution of sustained-release carrier (final concentration of silver nanoparticles, 500 ppm; final concentration of EGF, 10 µg/mL). The sustained-release solution (25 ppm) was similarly prepared (final concentration of silver nanoparticles, 25 ppm; final concentration of EGF, 10 µg/mL). Freeze-dried EGF powder (1 mg) was dissolved in sterile distilled water to prepare the EGF-alone group (10 µg/mL) and nanosilver-alone group (100 ppm). All solutions were stored in a sterile bottle in a refrigerator at 4°C for further use.

Characterization of silver nanoparticle-EGF sustained-release carrier solution

Transmission electron microscopy

The 500 ppm nanosilver particle solution and 500 ppm silver nanoparticle-EGF sustained-release carrier solution were observed with transmission electron microscopy.

Ultraviolet visible spectrophotometry

The 25 ppm sustained-release carrier solution was centrifuged at 20,000 rpm for 2 h and the supernatant was then collected for detection. Double distilled water served as a reference sample adjusted to “A0.000.” The test sample was placed into the cuvette followed by the ordered measurement of the EGF-alone group (10 µg/mL), nanosilver-alone group (100 ppm), nanosilver-EGF sustained-release carrier group (25 ppm), and sustained-release supernatant group.

Cell proliferation experiments with nanosilver-EGF sustained release carrier

Skin fibroblast cell line KMST6 was resuscitated and added to culture medium to obtain a triturated cell suspension. The cell suspension at 5×10^5/mL was subpackaged and cultured in a 75 mL culture flask at 37°C in a 5% CO_2 incubator until passages of 4 to 8. The suspension was then seeded onto a 96-well cell culture plate with 100 µL in each hole at 37°C in saturated humidity of 5% CO_2 for 24 h. The culture medium was discarded and the sample was added to the EGF (10 mg/L) group, nanosilver solution (500 ppm) group, nanosilver-EGF (500 ppm) group, nanosilver-EGF combination group, and control group. Each group was set at eight double holes; there were four repeated plates in each group with 100 µL of solution in each hole. All samples were cultured at 37°C in a 5% CO_2 incubator, and one culture plate was taken out at 12, 24, 36, and 48 h for an MTT colorimetric test. The cell growth curve was plotted.

Antibacterial test with nanosilver-EGF sustained-release carrier

Five pathogenic microorganisms, namely *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (10102), *Candida albicans*, and *Streptococcus pneumoniae* were incubated with the culture medium (including nutrient broth medium and agarose medium) at 4°C. Each bacterial species was repeatedly incubated on five plates; the concentration of bacterial suspension was estimated turbidimetrically and inoculated onto petri dishes at concentrations of 5×10^5 to 5×10^6 cfu/mL. The bacterial suspension was smeared onto the surface of a nutrient agar plate, and the petri dishes were dried at room temperature. The sterile, dried filter paper (5 mm diameter) was collected and added to 5 µL of reagents to prepare antibacterial slices. Samples were cultured for 24 h in a 37°C incubator. The diameter of the antibacterial ring was measured with compasses and a caliper; measurements were repeated three times.

Wound healing experiments with nanosilver-EGF sustained-release carrier

One wound was made on each side of the spinal cord in 15 rats which were intramuscularly injected with gentamicin at 5 mg/kg (equivalent to the plasma concentration in adults) once daily for 3 days for systemic anti-infective treatment. All wounds were randomly assigned to the nanosilver-EGF sustained-release carrier group (NanoAg-EGF), nanosilver group (NanoAg), EGF-alone group, nanosilver-EGF combination group (NanoAg+EGF), or saline control group. The two wounds were treated by different means once daily. The drugs infiltrated the whole wound by sterile syringe infusion, and each wound received 0.25 mL of drug per treatment. Wounds were photographed on days 3, 7, and 12 and at the time of wound healing, and the non-healing area was calculated using a computer image analysis system (CAD software). The healing rate was calculated as follows: Healing rate = (initial wound area – nonhealing area) / initial wound area × 100%.

Statistical analysis

Measurement data are expressed as mean ± standard deviation and were analyzed using SPSS 13.0 software. Differences among groups were compared using analysis of variance. Pairwise comparison was performed with the LSD test.

RESULTS AND DISCUSSION

Characterization of nanosilver-EGF sustained-release carrier with transmission electron microscopy

A transmission electron microscopic image of nanosilver at 500 ppm is shown in Figure 1. The silver nanoparticles were spherical with uniform distribution, showing no agglomeration or growth, and the particle size was about 15 to 25 nm. A transmission electron microscopic image of the nanosilver-EGF sustained release carrier is shown in Figure 2. Lightly stained EGF covered the surface of the spherical silver nanoparticles and formed a nebula-like shadow, which was surrounded by silver nanoparticles.

Peptides and proteins are increasingly used in clinical practice, and the preparation method of nanoparticles has been developed (Zhao et al., 2008; Zheng et al., 2006) so that they may serve as carriers of peptide and protein drugs. The use of biodegradable polymers or inorganic nanoparticles as carriers of peptides and proteins, thus achieving a sustained-release effect, is a

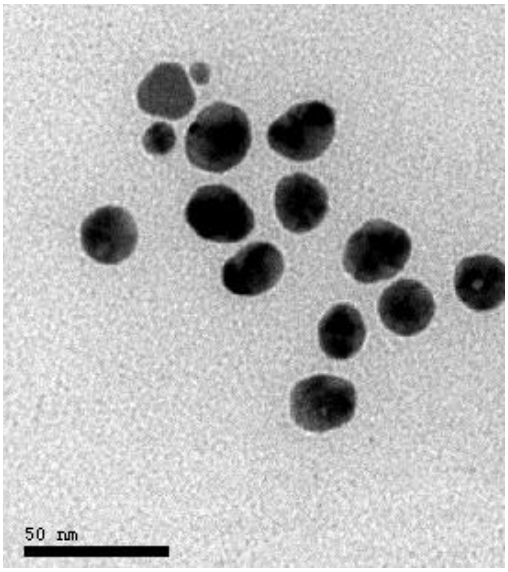

Figure 1. Transmission electron microscopic photos of silver nanoparticles (×500,000).

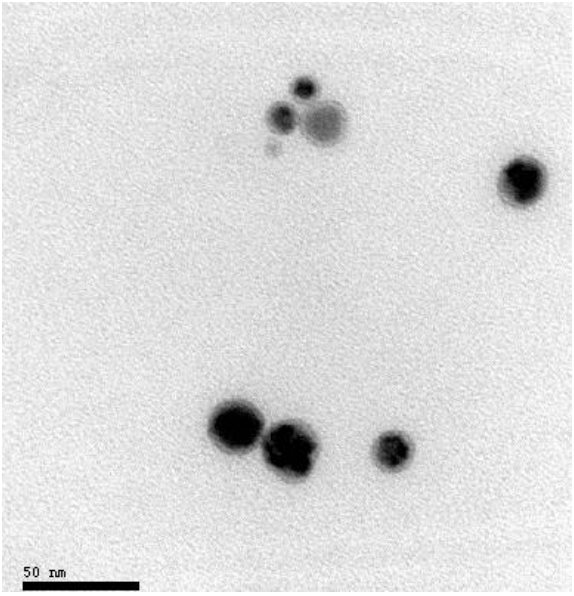

Figure 2. Transmission electron microscopic photos of nanosilver-EGF complex (×500,000).

current research focus (Tan et al., 2010; Ana et al., 2011; Harde et al., 2011).

In this study, transmission electron microscopy characterization analysis showed that the obtained silver nanoparticles were evenly distributed and close to spherical in shape, and there was no agglomeration or growth. Transmission electron microscopic images of the nanosilver-EGF sustained-release carrier displayed that the carrier was well dispersed in the solution with no reunion or growth and that the lightly dyed EGF adhered to the surface of the spherical silver nanoparticles, forming a nebula-like shadow surrounded by silver nanoparticles. This is objective evidence of EGF adhesion on the silver nanoparticles.

UV-VIS characterization

The UV visible absorption spectra of nanosilver solution, EGF solution, and nanosilver-EGF sustained release solution are shown in Figure 3. The first absorption peak in curve 4 was exactly the same as that in curve 1, indicating that free EGF was present in the nanosilver-EGF sustained-release solution. The second absorption peak of curve 4 shifted to the right compared with the absorption peak in curves 2 and 3, with a 5 nm gap; that is to say, the UV visible absorption peak of the nanosilver-EGF sustained-release carrier was 5 nm away from that of the silver nanoparticles. This evidence suggests that the EGF acting with nanosilver in the nanosilver-EGF sustained-release solution produced a nanosilver-EGF complex. According to the MieTheory (Papoff et al., 2011; Bhandari et al., 2011), the plasma absorption peak gradually red-shifts with increasing nanoparticle size. When the size of silver nanoparticles increased, the plasma absorption peak red-shifted, which is strong evidence for nanosilver adhesion on EGF. EGF effectively adsorbed to the surface of silver nanoparticles, indicating that the nanosilver-EGF sustained-release carrier was successfully prepared. This is consistent with a previously described outcome (Tsai et al., 2011) showing that silver-nanometer particles can act with proteins, resulting in the alteration of its spectrum.

Nanosilver-EGF sustained-release carrier promoted cell proliferation

Growth of fibroblast cell culture

Under light microscopy, the number of fibroblasts was increased, the distribution was dense in the whole field of vision, and the cell shape was mostly spindle. Hematoxylin-eosin staining showed pink-stained cytoplasm and blue-stained nuclei. Cells were fusiform-shaped with several processes or star-shaped and flat. Their outlines were clear and their nuclei were oval. There were no significant differences in the morphology of the treated cells (Figure 4).

Nanosilver-EGF sustained-release carrier promoted cell proliferation

The absorbance value in each group was detected at 12, 24, 36, and 48 h (Figure 5). There was no significant difference in the absorbance value of human fibroblasts at 12 h (0.180 ± 0.011 versus 0.186 ± 0.009; $P > 0.05$).

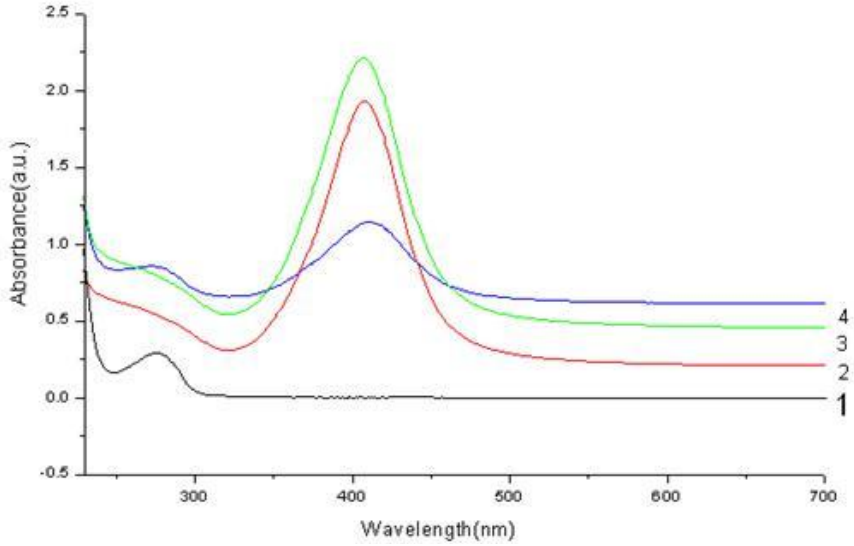

Figure 3. UV absorption spectrum of nanosilver-EGF complex. (1) Absorption spectrum of complex supernatant, (2, 3) absorption spectrum of nanosilver-alone group (100 and 500 ppm, respectively), (4) absorption spectrum of nanosilver-EGF sustained-release group (25 ppm).

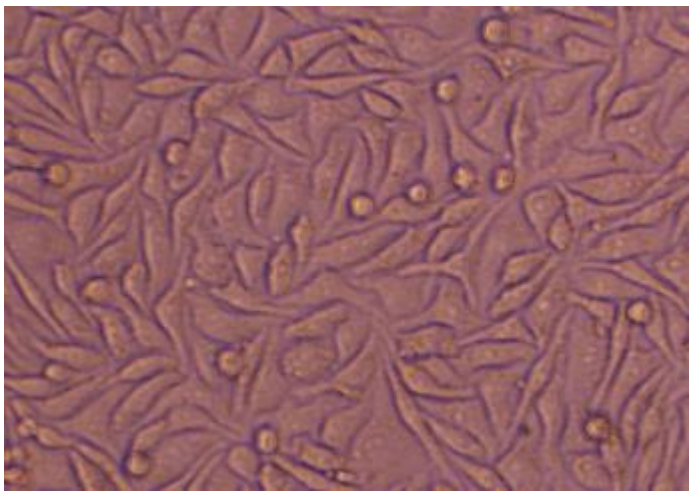

Figure 4. Light microscopic photo of human dermal fibroblasts at 36 h after hematoxylin-eosin staining in the nanosilver-EGF complex group.

Cell proliferation was apparent as time went by. Cell proliferation in groups containing EGF (that is, NanoAg-EGF, NanoAg+EGF, and EGF groups) was significantly apparent, compared with that in the nanosilver and control groups ($P < 0.05$). At 36 and 48 h, cell proliferation in the sustained-release carrier group was the most obvious. Absorbance values were 0.359 ± 0.027 and 0.467 ± 0.026, respectively, which were significantly greater than those in the combined group (0.324 ± 0.022 and 0.359 ± 0.027) and EGF group (0.316 ± 0.019 and 0.357 ± 0.016; $P < 0.05$). This is evidence that cell proliferation was faster and more stable after 24 h in the sustained-release carrier group. Accordingly, we speculate that the nanosilver-EGF sustained-release carrier can greatly promote cell proliferation and that this ability is closely attributed to the sustained-release effect of silver nanoparticles on EGF.

In the cell proliferation experiments, cell proliferation was promoted to varying degrees in the different groups, but no significant statistical difference was found. The reason may be because the biological effects on promotion of cell proliferation remained in the initial phase within a short duration of EGF action. Cell protein and DNA and RNA synthesis was significantly increased,

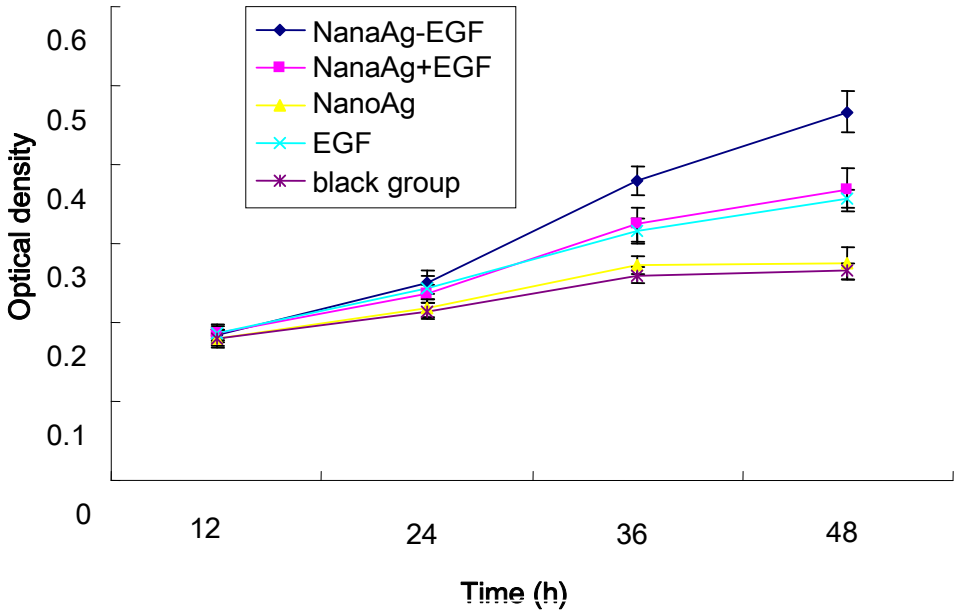

Figure 5. Light microscopic photo of human dermal fibroblasts at 36 hours after hematoxylin-eosin staining in the nanosilver-EGF complex group.

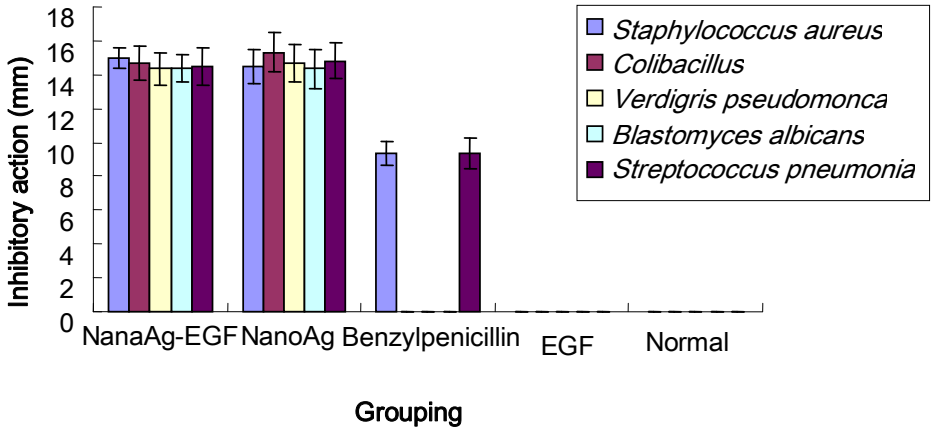

Figure 6. Experimental group's inhibitory action on five pathogenic microorganisms. (1) NanoAg-EGF group, (2) NanoAg group, (3) Benzylpenicillin group, (4) EGF group, (5) Normal control group.

but no quantitative change in cell number was found. When fibroblasts are treated with EGF, the cell cycle duration is about 10 h and DNA synthesis starts at 8 h and becomes active. After 24 h of cell culture, the number of cells increased in the NanoAg-EGF, EGF, and NanoAg+EGF groups with significant differences, compared with the nanosilver and control groups. This is evidence that EGF promoted cell proliferation. After 36 h of cell culture, the number of cells in the NanoAg-EGF group was significantly higher than that in the EGF and NanoAg+EGF groups, suggesting that the concentration of sustained-release carrier was better than that of EGF and explaining the cell proliferation at 36 and 48 h.

Antibacterial test of nanosilver-EGF sustained-release carrier

The inhibitory action on five pathogenic microorganisms in each treatment group was compared. As shown in Figure 6, the NanoAg-EGF and NanoAg groups showed good antibacterial properties on five pathogenic microorganisms, and there was no statistically significant difference in antimicrobial resistance to the five pathogenic microorganisms between the two groups ($P > 0.05$). In the positive-control benzylpenicillin group, antibacterial activity against only *S. aureus* and *Str. pneumoniae* was weak and was significantly lower than

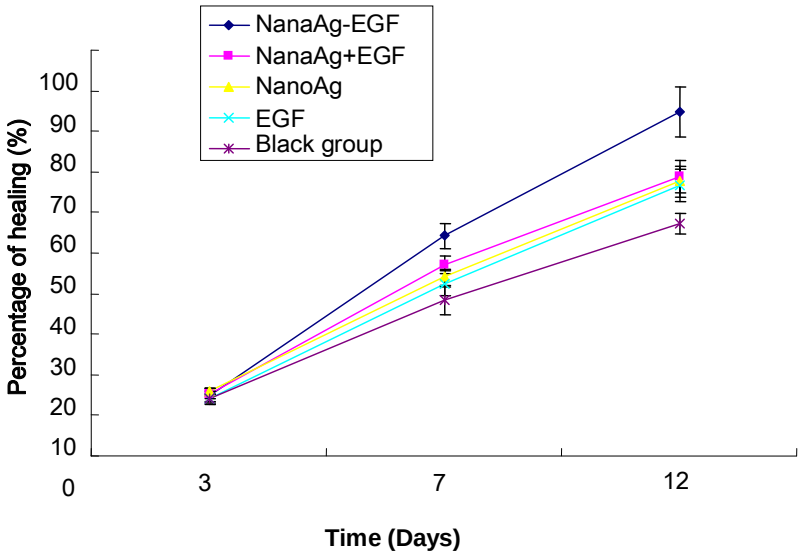

Figure 7. Comparison of wound-healing rate in each group at post-traumatic 3, 7, and 12 days.

that in the NanoAg-EGF and NanoAg groups ($P < 0.05$). In the normal saline and EGF groups, there was no antibacterial effect on the five pathogenic microorganisms.

The antibacterial effect of the nanosilver-EGF sustained-release carrier was determined. Both the NanoAg-EGF and nanosilver groups showed strong inhibitory actions on the five pathogenic organisms, namely *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans*, and *Str. pneumoniae*, while the EGF-alone and saline control groups showed no inhibitory effect. In the positive-control group, benzylpenicillin sodium was only resistant to *S. aureus* and *Klebsiella pneumoniae*, and the antibacterial effect was significantly lower than that in the nanosilver and NanoAg-EGF groups. There was no significant difference between the nanosilver and NanoAg-EGF groups. The present experiments not only validate the antibacterial effect of nanosilver, but also confirm that nanosilver has a good inhibitory effect on *S. aureus* and *P. aeruginosa*, which readily demonstrate drug resistance.

Nanosilver-EGF sustained-release carrier promoted wound healing

Morphological observation

The wounds in rats of the NanoAg-EGF group were cleaner than those in the other groups, with less leakage, a mental status that was close to that of normal rats, a normal diet, vigorous activity, and no hair removal. Wound healing in the other groups was relatively poor or even difficult, with more secretions and surrounding swelling, leading to formation of chronic ulcers.

Nanosilver-EGF sustained-release carrier promoted wound healing in animal experiments

The statistical data of the wound-healing rate in each group are shown in Figure 7. The wound-healing rate at 3 days after treatment in each group ranged from 14.105 ± 1.098% to 15.814 ± 1.518%, with no significant difference between groups ($P > 0.05$) at 7 and 12 days. The healing rates in the NanoAg-EGF group were 54.19 ± 3.1137% and 84.933 ± 6.147%, respectively, which were significantly higher than those in the other four groups ($P < 0.05$). The wound-healing duration in the NanoAg-EGF group was 14.75 ± 1.603 days, which was significantly shorter than that of the other four groups (combination group, 17.25 ± 1.422 days; EGF group, 20.167 ± 1.697 days; nanosilver group, 17.083 ± 1.505 days; and control group, 20.333 ± 1.303 days; $P < 0.05$). Thus, the wound-healing rate and duration were the highest and shortest, respectively, in the NanoAg-EGF group.

Many measures are used to improve the wound-healing duration and quality, such as infection control, active removal of necrotic tissue, correction of metabolism, and application of exogenous growth factors. Wound healing is a key issue in plastic surgery and related research, and how to speed wound healing is an expected issue in clinical research (Zhou et al., 2004). In this study, there was no significant difference in the wound-healing rate of each group at 3 days after surgery, indicating that EGF was ineffective in the promotion of wound healing in the early inflammatory stage. Even if anti-infection measures are performed in a timely manner, wound edema and acute infection occur in the post-traumatic 2 to 3 days. Because of the dramatic change in the surrounding environment, cells on the wound surface are still in the shock stage and growth

factors are not available. In addition, a certain amount of time is required to upregulate the exogenous EGF receptor; thus, no promotion of wound healing was found in the NanoAg-EGF group at 3 days.

The healing rate reached a peak in the NanoAg-EGF group 7 days after the injury, with significant differences compared with the other groups; the differences were most significant with time. The wound-healing duration in the NanoAg-EGF group was 4 to 5 days shorter than that in the saline group and 2 to 3 days shorter than that in the combination group. Although the combination group showed a better ability to promote wound healing at all time points, there was no significant difference compared with the EGF-alone and nanosilver groups. Therefore, we speculate that the combination of silver nanoparticles with EGF cannot lead to a qualitative change in wound healing.

In the NanoAg-EGF group, the healing rate was significantly higher than that in the other groups at 7 days, suggesting that our new formulations can avoid wound hydrolysis, induce a sustained and steady release of EGF, and protect factors from wound hydrolysis and bacterial destruction before the adherent growth factor detaches from silver nanoparticles. When the amount of growth factors on the wound surface decreases, the adherent growth factor gradually becomes free from the nanosilver and then binds with receptors that can repair cells and promote cell proliferation. Therefore, the wounds maintain a relatively high concentration of growth factors, and wound healing is accelerated.

Conclusion

All our experimental findings confirm that the herein described nanosilver-EGF sustained-release solution can disperse well, that EGF adheres to the surface of silver nanoparticles, and that growth factor activity and antimicrobial resistance coexist and can effectively promote wound healing. Further studies are required to conclusively determine the clinical application and significance of these results.

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REFERENCES

Ai J, Biazar E, Jafarpour M, Montazeri M, Majdi A, Aminifard S, Zafari M, Akbari HR, Rad HG (2011). Nanotoxicology and nanoparticle safety in biomedical designs. Int. J .NanoMed. 6:1117-1127.

Ana DP, Diego D, Maria S A, Alicia R G(2011). Lipid nanoparticles as vehicles for macromolecules: nucleic acids and peptides. Recent Pat Drug Deliv. Formul.5(3):214-226.

Arorat S, Jain J, Rajwade J M, Paknikar KM (2008). Cellular responses induced by silver nanoparticles: *In vitro* studies. Toxicol. Lett.179(2):93-100

Bhandari A, Hamre B, Frette Ø, Stamnes K, Stamnes JJ (2011). Modeling optical properties of human skin using Mie theory for particles with different size distributions and refractive indices. Opt Express.19(15):14549-14567.

Chekman IS, Ulberg ZR, Gorchakova N O, Nebesna TY, Gruzina T G, Priskoka AO, Doroshenko AM, Simonov PV (2011). The prospects of medical application of metal-based nanoparticles and nanomaterials. Lik Sprava.(1-2):3-21.

Değim Z, Celebi N, Alemdaroğlu C, Deveci M, Öztürk S, Özoğul C(2011). Evaluation of chitosan gel containing liposome-loaded epidermal growth factor on burn wound healing. Int Wound J.8(4): 343-354.

Dallas P, Sharma VK, Zboril R (2011). Silver polymeric nanocomposites as advanced antimicrobial agents: classification, synthetic paths, applications, and perspectives. Adv. Colloid Interface Sci.166(1-2):119-135.

Harde H, Das M, Jain S (2011). Solid lipid nanoparticles: an oral bioavailability enhancer vehicle. Expert. Opin Drug Deliv. 8(11):1407-1424.

Janjua M, Nudurupati S, Singh P, Aubry N (2011). Electric field-induced self-assembly of micro- and nanoparticles of various shapes at two-fluid interfaces. Electrophoresis, 32(5): 518-526.

Kiyohara Y, Komada F, Iwakawa S, Hirai M, Fuwa T, Fuwa T, Okumura K (1991). Improvement in wound healing by epidermal growth factor (EGF) ointment. II. Effect of protease inhibitor, nafamostat, on stabilization and efficacy of EGF in burn. J. Pharmacobiodyn, 14(1):47-52.

Kim JS, Kuk E, Yu KN, Kim SH, Park S J, Lee HJ, Kim SH, Park YK, Hwang CY, Kim YK, Lee SY, Jeong DH, Cho MH (2007). Antimicrobial effects of silver nanoparticles. NanoMed. 3(1):95-101.

Madhumathi K, Kumar P T S, Abhilash S, Sreeja V, Tamura H, Manzoor K, Nair SV, Jayakumar R (2010). Development of novel chitin/nanosilver composite scaffolds for wound dressing applications. J. Mater. Sci .Mate Med. 21(2):807-813.

Ong SY, Wu JSM, Moochhala, MH, Tan J, Lu (2008). Development of a chitosan-based wound dressing with improved hemostatic and antimicrobial properties[J]. Biomates. 29(32):4323-4332.

Papoff F, Hourahine B (2011). Geometrical Mie theory for resonances in nanoparticles of any shape. Opt Express, 19.(22):21432-21444.

Teow Y, Asharani PV, Hande MP, Valiyaveettil S (2011). Health impact and safety of engineered nanomaterials. Chem Commun. Camb. 47(25):7025-7038.

Tan ML, Choong PF, Dass CR (2010). Recent developments in liposomes, microparticles and nanoparticles for protein and peptide drug delivery. Peptides, 31(1):184-93.

Tsai DH, Davila-Morris M, DelRio FW, Guha S, Zachariah MR, Hackley VA (2011). Quantitative determination of competitive molecular adsorption on gold nanoparticles using attenuated total reflectance-Fourier transform infrared spectroscopy. Langmuir, 27(15): 9302-13.

Tian J, Wong K Y, Ho C, Lok C, Yu W, Che C, Chiu J, Tam PKH (2007). Topical delivery of silver nanoparticles promotes wound healing. Chem. Med. Chem. 2(1): 129-136.

Vaiana CA, Leonard MK, Drummy LF, Singh KM, Bubulya A, Vaia RA, Naik RR, Kadakia MP (2011). Epidermal growth factor: layered silicate nanocomposites for tissue regeneration. Biomacromole.12(9):3139-46.

Xie HQ, Zhou J D, Luo C Q, He QY (2006). Construction of the eukaryotic expression plasmid containing human epidermal growth factor gene with signal peptide. J Chin. Phys. 8(2): 189-191.

Zhou JD , Chen DJ, Chen Y, LI P, Li GF, He QY, Chen TF, Zhu J, Peng H, Xia K, Luo CQ (2004). Human VEGF121 gene transfected adult dermal fibroblasts *in vitro*. Chin. J. Exp. Surg.10, 21(12):1539-1541.

Zhao Yan-zhong, Huang Yan-yan, Chen Yu-xiang, Zhu Shai-hong, Wang Guo-hui, Zhou Jian-da, Huang Dong, Zhou Ke-chao (2008). Preparation and biological savety evaluation of silicon

nanoparticles for gene therapy. Chin. J. Nonferrous Metals, p. 5.
Zheng M, Wang Z S, Zhu Y W (2006). Preparation of silver nanoparticle via active template under ultrasonic. J. Central South Univ. p.6