Research Article

Shijun Nie[#], Ran Wei[#], Haoxin Zhou, Liang Zhang, Zhinan Chen, and Limin Hou^{*}

Eco-friendly synthesis of AuNPs for cutaneous wound-healing applications in nursing care after surgery

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Abstract: The current work described the preparation of gold nanoparticles (AuNPs) using the plant extract of *Impatiens balsamina* followed by evaluating their wound-healing potential. The formed NPs were studied by performing UV-visible spectroscopy, Fourier transform infrared, transmission electron microscopy and X-ray diffraction. Further, both the thermal and excision wound models were used to understand the wound-healing ability of AuNPs. It is exhibited that at a concentration of 20 mg, the AuNPs exhibited substantial decrease in excision wound within 8 days. The obtained wound-healing results indicated that the AuNPs prepared from the leaf extract of *I. balsamina* exhibited active wound-healing potential when related to traditional drugs; hence, AuNPs could have future applications in the development of dressing materials in nursing care for wound healing after surgery.

Keywords: AuNPs, polyphenols, wound healing, care

1 Introduction

Owing to their biogenic, facile, biological applications and biofabrication techniques, gold nanoparticles (AuNPs) have gained significant consideration in the recent years [1–3]. AuNPs biogenic and eco-friendly synthesis with the help of algae [4], microorganisms [5], plants [1,6] and fungi [7] was already demonstrated. Various chemical, biological and physical approaches can be utilized to fabricate NPs. Chemical and physical approaches use high temperature and pressure along with poisonous chemicals, which can also harm both the humans and environment. Thus, an environmental-friendly approach is required for the fabrication of NPs. The biofabrication of NPs with the help of microorganisms and various medically important plant extracts was previously demonstrated with considerable biological uses [2–4,6]. The application of plant extract in the fabrication of NPs is much more efficient than the utilization of microorganisms, because of the fact that microorganisms need more effort and attention, especially to isolate, identify, subculture, develop and preserve under appropriate conditions.

The fabrication of AuNPs utilizing plant extracts has been demonstrated for their variety of biomedical applications such as antiparasitic [8,9], antibacterial [10], anticancer [1,11] and antioxidant [12] activities. Similarly, green synthesized nanomaterials are reported to be widely used to reduce environmental pollution such as in waste water treatment [13–17]. Also, various nanomaterials such as metallic, bimetallic and graphene nanocomposites that are synthesized via green methods were reported to have applications in catalysis and degradation of various organic dyes and pollutants. For example, biosynthesized nanomaterials such as Ag/diatomite nanocomposite [18], Ag/Al₂O₃ NPs [19], AuNPs [20], Ag/zeolite [21], Ag NPs/clinoptilolite [22], Ag/high silica pentasil zeolite socony mobil-5 [23], Au–Ag [24], Ag/ZnO [25], AgNPs [26–30], Ag/Fe₃O₄ [31] and Ag/bone nanocomposite [32] have already been reported to exhibit good catalytic activity. Similarly, graphene composites

[#] Contributed equally for this work.

*** Corresponding author: Limin Hou**, Emergency Surgical Department, The First Affiliated Hospital of Harbin Medical University, Limin Hou, No. 199, Dongdazhi Street, Nangang District, Harbin 150001, China, e-mail: LiminHou234@outlook.com

Shijun Nie, Haoxin Zhou, Zhinan Chen: Emergency Surgical Department, The First Affiliated Hospital of Harbin Medical University, Limin Hou, No. 199, Dongdazhi Street, Nangang District, Harbin 150001, China

Ran Wei: Neurological Stroke Department, The First Affiliated Hospital of Harbin Medical University, Harbin, China

Liang Zhang: Hyperbaric Oxygen Department, The First Affiliated Hospital of Harbin Medical University, Harbin, China

prepared via green methods such as reduced graphene oxide (RGO)/Ag [33,34], RGO/TiO₂ [35], Ag/RGO/Fe₃O₄ [36] and Ag–Cr–AC [37] were used as nanocatalysts for catalytic reduction of organic dyes. Likewise, Au/Pd [38], Au [39] and Ag/sodium borosilicate nanocomposites [40] have been used as catalysts in organic reactions. On the other hand, biomediated AgNPs were used for catalytic hydration of cyanamides [41] and cyanation of aryl and heteroaryl aldehydes [42]. Also, plant extract mediated biosynthesized NPs such as Ag/Cu NPs [43], Ag@AgCl [44], ZnO–Ag [45] and Ag/AgCl [46] are reported for the degradation of dyes and organic compounds. Similarly, Ag/Au [47], TiO₂/AgNPs [48], Fe/Pd [49], AgNPs [50] and Ag/MgO [51] have been used for photocatalytic degradation or removal of various dyes.

The activity of AuNPs depends on factors including AuNP size, concentration and type of cancer cell [3,11,52]. Because of the existence of various reducing phytochemicals [1–3], the AuNPs formed using plant extracts are poly-dispersive in nature, whereas monodispersive AuNPs are typically fabricated through citrate reduction [53,54]. Exposing NPs for quite a long period raised questions about its toxicologic effects on human health. Recent reports on NPs have shown their toxic effects and created controversy [55]. In the field of research, AuNPs are much more significant because of their self-assembled structure, non-toxic nature and enhanced drug delivery [56].

In the present work, AuNPs were prepared using the plant extract of *Impatiens balsamina* followed by evaluating their wound-healing potential. Further, two models such as thermal and excision wound models were used to understand the wound-healing ability of AuNPs.

2 Materials and methods

2.1 Materials

During the experimental process, triple distilled water of 0.2 µm (OKCHEM, China) was utilized after filtering. Sigma Aldrich, Shanghai, China supplied crystal violet stain and gold(III) chloride trihydrate (HAuCl₄ · 3H₂O; tetrachloroauric acid). The solution of WST-1VR (monosodium salt, 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2H-tetrazolium) for performing the assay of cell viability was obtained from our lab in China. Prolong antifade reagent was collected from Eugene, OR, USA. 4,6-Diamidino-2-phenylindole dihydrochloride (DAPI) and PBS (pH 7.0) were obtained from Sigma Aldrich, China. The 96-well microplate, cell culture flasks and cover slip bottom plates were utilized from our lab.

2.2 *I. balsamina* leaves extraction

Filtered triple distilled water was utilized to wash the dry leaves of *I. balsamina* followed by drying at standard room temperature (25°C). An electric grinder was utilized to fine powder the dried leaves. Filtered triple distilled water of 100 mL was taken in a flask of 250 mL. Leaf powder of 10 g was added to the flask followed by boiling the flask for about 1 h at a temperature of 70°C in a water bath. The mixture was further subjected to centrifugation for about 20 min at 2,500 rpm. Syringe filter of 0.2 µm (Sigma Aldrich, China) was utilized to filter the supernatant. The resultant was then stored at 4°C for further utilization.

2.3 Biofabrication of AuNPs

Biofabrication of AuNPs was performed by adding 1 mL *I. balsamina* plant extract to 19 mL solution of 1 mM tetrachloroauric acid. The solution was continuously stirred in a water bath at 50°C for 20 min. Then the high-speed centrifugation at 12,000 rpm for 0.5 h separates the synthesized NPs from the reaction mixture. To remove unwanted impurities from AuNPs, the centrifuged resultant was washed thrice using filtered triple distilled water. To achieve their powdered state, the extracted AuNPs are lyophilized and stored at –20°C until necessary.

2.4 Characterization studies

For monitoring synthesis of AuNPs, the UV-visible (UV-Vis) spectroscopy (Shanghai, China) of range 300–800 nm was used. The synthesized AuNPs specimen was mounted on a carbon grid-copper disk, and the transmission electron microscopy (TEM; H7500, HITACHI, China) examined the morphology, size and shape. The powdered form of AuNPs was kept in double-sided taped sample stubs to determine elemental analysis using field emission scanning electron microscopy (FE-SEM; JSM-6700F; JEOL, China) equipped with energy dispersive X-ray spectroscopy (EDX). X-ray diffraction (XRD; PHILIPS X'PERT-MPD, China) was used to analyze the crystalline structure and diffraction pattern and was operated at 40 kV in Cu-Kα radiation ($\lambda = 1.540 \text{ \AA}$, 30 mA current). For the identification of phytochemical compounds associated with the synthesis of AuNPs, Fourier transform infrared (FTIR) spectroscopy (Nicolet iS10; Thermo Electron Sci Inst. LLC, WI, USA), having a scope of 500–4,000 cm^{–1} was used. Origin Pro 9.1 SRO technology (OriginLab Corp. from Northampton, MA, USA) was utilized for analyzing all the graphs (FTIR, UV-Vis and XRD).

2.5 *In vivo* wound-healing activity

2.5.1 Experimental animals

Swiss albino rats of any gender, weighing approximately 120–150 g were utilized for the *in vivo* studies. The tests were performed in agreement with globally recognized principles for animal laboratory applications and the investigational protocols are accepted by the Institution of Animal Ethical Committee. The animals were kept in cages of polypropylene under standard ecological conditions (from 20 to 25°C) and were served with regular rodent diet and surplus water.

2.5.2 Excision wound model

Rats were sedated by open-mask technique using anesthetic ether. Animals were depilated on their back and complete thickness of skin was removed in the dorsal inter-scapular area of 500 mm². Groups III, IV, V and VI were treated with AuNPs of concentration 5, 10, 15 and 20%, respectively. Group I was considered as control (untouched) and Group II was applied with regular ointment (neomycin). Proper dressing was done on the wounds, and the dressing was changed daily. While changing the dressing, wounds were examined by capturing their photographs. The progress in the wounds was observed planimetrically, and traces of the wounds were margined on graphs every alternate day. The healing of wound, i.e. the dimension of burn range on the graph paper, was expressed in “mm²”. Reduction in injury size was represented as the percentage contraction of original size of the wound.

Wound-healing percentage

$$= \frac{(\text{area of the wound at day 0} - \text{area of the wound at day } n)}{\text{area of the wound at day 0}} \times 100 \quad (1)$$

Deteriorating of skin with no raw wound behind was marked as the end point of full epithelialization, and the number of days required for the same was considered as the period of epithelialization.

2.5.3 Thermal wound model

Open-mask method was used to sedate rats using anesthetic ether followed by depilating their backs using electrical clippers. Rats are kept in a container wherein about 10% of their bodies are visible. The visible surface of

every animal was submerged for 6 s in 90°C water, which resulted in grade II burn on the 10% exposed area. In order to avoid shock, rats were treated with intraperitoneal injection consisting of 3 to 5 mL solution of saline. Wounds were then dressed appropriately and treatment was accessed per the above process for wound-healing and epithelialization period. As a standard drug, silver sulfadiazine (1%) was used.

3 Results and discussion

Several spectral findings are recorded throughout the AuNP synthesis, and a combination of reaction changes color to violet from light yellow. The characteristic shift of color is an indicator of AuNPs synthesis and may be attributed to surface plasmon resonance [57]. Such findings are well in accordance with the previous research results [10–12]. UV-Vis spectroscopic analysis verified the AuNPs fabrication. A strong peak was observed at 542 nm for 20 mL of tetrachloroauric acid solution in 5% (v/v) *I. balsamina* extract (Figure 1). The peak formed at 540 nm refers to the development of AuNPs [11,12,14] depending upon the morphology, size, shape and medium of contact [11,58].

The EDX analysis determined the elementary composition of the produced AuNPs. Figure 2 demonstrated the obtained EDX spectrum in conjunction with TEM, and the spectrum shows a strong peak for gold metal of around 2 keV. The EDX elemental peak at 2 keV was reported previously to be substantial for gold metal [11,59]. During AuNPs synthesis, the appearance of oxygen in EDX range may be attributed to stable or intermediate formation of molecules.

Figure 1: UV-Vis spectrum of AuNPs.

Figure 2: EDX spectrum of prepared AuNPs.

TEM indicated morphological characteristics, shape and size of particles. The TEM images produced via synthesized AuNPs are shown in Figure 3a and b. TEM images reveal that the AuNPs synthesized were mostly oval, and spherical in shape, ranging in size of approximately 25–30 nm. TEM images also displayed the poly-dispersed existence of the AuNPs synthesized, which can occur due to a combination of one or more reduction agents present in the leaf extract of *I. balsamina*. Similar findings have been reported previously [11,59,60]. A unified compound from the plant extract can be used to form monodispersed AuNPs. Per our knowledge, we are the first to document the synthesis of AuNPs using *I. balsamina* extract from the leaf as a reduction agent.

Figure 4 displays the XRD pattern of the formed AuNPs. The XRD pattern shows four peaks corresponding to (311), (220), (200) and (111) planes dependent on AuNPs face-centered cubic configuration with diffraction peaks of 2θ degrees = 77.70° , 64.71° , 44.31° and 38.14° , respectively. XRD examination has verified the crystalline structure of AuNPs. AuNPs that are synthesized with extracts of

different plants such as *Hibiscus sabdariffa*, *Benincasa hispida* and *Rhus chinensis* [11,59,60] also reported, a similar pattern of peaks; this suggests that the above results are in concordance with earlier studies.

FTIR studied the role of biomolecules during AuNPs synthesis. Figure 5 displays the spectrum obtained from FTIR examination. FTIR peaks of the *I. balsamina* leaf extract (897 , $3,427$, $1,391$, $1,632$, and $1,328\text{ cm}^{-1}$) and the AuNPs synthesized ($1,062$, $1,381$, $1,638$, and $3,442\text{ cm}^{-1}$) indicated shifting. The peak-to-peak comparison helps determining the participation of functional molecular groups that interacted during the process of reduction. The peaks found at $3,442$ and $3,427\text{ cm}^{-1}$ represent the O–H stretching vibrations and were noticed to be formed because of the presence of organic molecules such as phenols and alcohols. The $1,638$ and $1,632\text{ cm}^{-1}$ peaks refer to the N–H functionalities of 1° amines. *I. balsamina* extract peak obtained at $1,328\text{ cm}^{-1}$ may refer to N–O symmetric stretching vibrations allotted to nitro compound that vanished in fabricated AuNPs. The obtained peaks at $1,162$ and $1,117\text{ cm}^{-1}$ refer to C–N functional group formed

Figure 3: TEM images of AuNPs with high (a) and low magnification (b).

Figure 4: XRD pattern of AuNPs.

because of the existence of aliphatic amine functional groups. The peak obtained at 897 cm^{-1} corresponding to C–H bond has vanished because of the functional groups present in aromatic compounds. Related observations have been recorded at bands $3,400$ and $3,224\text{ cm}^{-1}$, suggesting the presence of O–H hydroxyl group in the fabrication of AuNPs [11,59]. The results indicate that during AuNPs synthesis, functional groups are responsible in the process of stabilizing and minimizing. These results are in agreement with the previous literature where enzymes were used for synthesis and surface stabilization or functionalization of NPs [61,62].

3.1 Stability of the formulations

We did not observe any evidence of impact on the storage at different temperatures, physical instability or any indication on the formation of objectionable odor as well as phase separation.

Figure 5: FTIR spectrum of AuNPs and plant extract.

3.2 Pharmacological evaluation of wound-healing activity

3.2.1 Excision wound model

During the treatment period, the rate of reduction of the unhealed area was indicated by the factor called wound contraction. The medication's efficacy is higher when the reduction is greater. It can be observed that in the case of rats treated with various concentrations of AuNPs, wound healing took place much faster, which took around 8 days for complete healing. Whereas in the control group, the animals that did not receive any treatment showed the least wound-healing rate. Even though the area of unhealed wound was lesser than the control group for neomycin ointment, the injury failed to recover even post day 8. In the group of animals treated with AuNPs, the injury contraction rate was substantially lower ($p < 0.05$) than that in the animal's group of control (Table 1).

The assessment of the epithelialization duration showed that groups treated with AuNPs needed a maximum healing and epithelialization duration of about 9 days, while the standard and control groups took around 10 and 12 days accordingly (Figure 6).

In the excision injury, as contrasted with the control group, the individual animal group administrated with AuNPs displayed significant reduction in healing the wound. Full healing was achieved in animals administered AuNPs within 8 days, while 12 and 10 days were taken, respectively, for control and standard groups. Re-epithelialization was achieved within 2 weeks in the burn wound in AuNPs-treated animals, whereas it required 3 weeks for re-epithelialization in the control group animals.

3.2.2 Thermal wound model

Table 2 demonstrated the development of healing the injury caused by ointments of AuNPs-administered groups (20, 15,

Table 1: Effect of AuNPs on wound contraction percentage in excision wound model

Groups	Percentage of wound contraction		
	Day 4	Day 6	Day 8
Control	49.23 ± 0.16	72.57 ± 0.16	73.36 ± 1.003
Neomycin 1%	50.69 ± 0.17	78.77 ± 0.18	81.23 ± 0.23
AuNPs 5%	51.58 ± 0.24	77.19 ± 0.15	99.19 ± 0.66
AuNPs 10%	57.63 ± 0.22	84.56 ± 0.16	98.26 ± 0.68
AuNPs 15%	59.67 ± 0.31	86.41 ± 0.15	99.04 ± 0.29
AuNPs 20%	60.37 ± 0.23	85.47 ± 0.16	99.89 ± 0.45

Figure 6: Effect of AuNPs on period of epithelialization in excision wound model.

Table 2: Effect of AuNPs on wound contraction in thermal wound model

Treatment (mg/g)	Percentage of wound healing				
	12th day	10th day	8th day	6th day	4th day
Sulfadiazine %	96.98 ± 0.18	85.78 ± 0.19	78.02 ± 0.10	47.92 ± 0.12	34.85 ± 0.19
Control	86.04 ± 0.09	56.94 ± 0.10	35.06 ± 0.40	25.16 ± 0.15	17.34 ± 0.11
AuNPs 20%	99.57 ± 0.17	98.44 ± 0.39	88.05 ± 0.13	77.89 ± 0.13	51.04 ± 0.14
AuNPs 15%	98.92 ± 0.11	98.02 ± 0.13	85.83 ± 0.18	76.69 ± 0.14	48.97 ± 0.25
AuNPs 10%	98.77 ± 0.14	97.81 ± 0.16	85.90 ± 0.13	75.85 ± 0.18	47.98 ± 0.12
AuNPs 5%	98.62 ± 0.12	95.40 ± 0.20	84.66 ± 0.10	74.59 ± 0.12	46.87 ± 0.13

10, and 5% w/w), animals groups treated with silver sulfadiazine (standard drug) as well as the control group animals. It is found that the capacity of AuNP ointment to contract injury is considerably higher in various concentrations compared to the control group animals (i.e., untreated group). From the 4th day onward, the animal group treated with 20% AuNPs ointment displayed considerable wound contraction, equivalent to that of the animal group treated with standard drug. When 10% AuNPs ointment was

administered to the animal group, they displayed considerable wound healing from the 8th day onward, reaching 100% with 13 days of closing time. It was noticed in the epithelialization phase that the group of animals administered with AuNPs ointments displayed a considerable reduction. The group of animals treated with AuNPs took about 13 days to re-epithelialize, whereas the control group took around 3 weeks. For full epithelialization, the standard animal group only took around 15 days (Figure 7).

Figure 7: Effect of AuNPs on period of epithelialization in thermal wound model.

4 Conclusions

The current work described the preparation of AuNPs with the help of *I. balsamina* plant extract followed by evaluating their wound-healing potential. TEM images confirmed the formation of 30 nm AuNPs. Furthermore, wound-healing studies exhibited that at a concentration of 20 mg, AuNPs exhibited substantial decrease in excision wound within 8 days. The obtained wound-healing results indicated that the AuNPs prepared from leaf extract of *I. balsamina* exhibited active wound-healing potential when related to traditional drugs; hence, AuNPs could have future applications in the development of dressing materials in nursing care for wound healing after surgery.

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