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Original research article

Synthesize of Titanium dioxide nanoparticles using *Moringa oleifera* leaves and evaluation of wound healing activity

V. Sivaranjani, P. Philominathan *

PG and Research Department of Physics, AVVM Sri Pushpam College, Poondi, Thanjavur, India

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ABSTRACT

Titanium dioxide nanoparticles (TiNPs) were emerged as wound healing enhancer as well as an antibacterial agent against Gram-positive and Gram-negative bacteria due to its enhanced mechanical properties. Moreover, the green synthesized Titanium dioxide exhibited significant wound healing activity in Albino rats which was confirmed by measuring wound closure. Titanium dioxide nanoparticles were effectively synthesized using aqueous leaf extract of *Moringa oleifera* under pH and temperature dependent condition. 5 mM Titanium dioxide solution worked as a primary source for the synthesize of Titanium dioxide nanoparticles. The green synthesized TiNPs excitation was confirmed using UV–Vis spectrophotometer at 428 nm. The high-resolution Scanning Electron Microscope (SEM) results showed the spherical shape of TiNPs with a mean particle size around 100 nm. Furthermore, the wound healing activity of nano-sized particles (Control Group) when compared with micro-sized (Positive Control Group) was examined through the excision wound model by measuring wound closure and the control group revealed significant wound healing activity in Albino rats.

1. Introduction

In recent times, wounds are among major world-wide clinical problems because of morbidity associated with prolonged periods required for repair and regeneration of the injured tissue, bleeding, risk for infections and septicemias, keloids and scar formation. These risk factors are further exacerbated in patients with impaired wound healing and the usage of external wound healing medications can minimize these risk factors. Moreover, due to the increment in the proportion of aged people's population in the coming decades, the wound healing process cost in clinical aspect may likely to increase [1]. Generally, the wound infections are commonly caused by plenty of bacteria or fungi; Gram-positive (*staphylococcus*) and Gram-negative (*Pseudomonas aeruginosa*) are the most common bacteria responsible for the majority of wound infections. These bacteria can easily contaminate the surface of wounds and access the underlying tissue, thereby delaying the healing process. According to the increase in health care costs and increasing antibiotic resistances, the economic burden for the treatment of chronic wounds is rapidly growing. Considering that resistance against newly approved antibiotics developed within two years, there is an urgent need for newer generation of antibiotics to fight infections [2].

In spite of so many medical advancements, wound healing still remains as an inefficiently managed area. The process of wound healing involves four types of stages: Haemostasis, Inflammation, Proliferation and remodeling ultimately leading to scar formation. The ultimate goal of wound healing is to have a speedy recovery with minimal scaring. Nowadays, nanotechnology is emerging rapidly with the development of nanoscale materials which have potential biomedical applications, especially in fighting and preventing diseases. The new age drugs include the nanoparticles of polymers, metals, ceramics which can fight against human pathogens like bacteria and even cancer. The importance of nanoparticles having potent bactericidal activity is inevitable because of their effect against resistant strains of pathogens. Moreover, nanoparticles increase the chemical activity due to their large surface volume ratio. In recent times in fact, the metallic nanoparticles have proven that they are the most suited candidate among all the other nano particles. In particular, metal oxide nanoparticles (NPs) are known to possess strong antimicrobial activity. However, Titanium nanoparticles are one of the most important metal oxide nanoparticles compared to others and are used in glass ceramics, electrical ceramics, catalyst, solar cell sensors, electric conductors and chemical intermediates but still a limited evidence available in biological related aspects. TiO_2 have

* Corresponding author.
E-mail address: philominathan@gmail.com (P. Philominathan).

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good mechanical properties, antibacterial effect against Gram-positive and Gram-negative bacteria, cell growth and high corrosion resistance.

This investigation was aimed to study the wound healing activity of green synthesized TiO_2 nanoparticles. Although, the main phytochemicals responsible for the synthesize of nanoparticles are terpenoids, flavones, ketones, aldehydes, amides, etc., here we wish to report a facile, green and one pot synthesize method using the leaf extract of *Moringa oleifera* for synthesizing Titanium dioxide nanoparticles. As the biosynthetic route for nanoparticles has not yet been extended for the synthesize of Titanium dioxide nanoparticles and its evaluation of wound healing activity, the present study has been employed with utmost care and reported here.

2. Materials and methods

2.1. Preparation of Titanium nanostructures

For the preparation of aqueous leaf extract (ALE) solution of *M. oleifera*, 10 g of *M. oleifera* powder dissolved in 100 ml of deionized water, boiled at 60 °C for 10 min to kill the pathogens in ALE solution. After cooling, ALE solution was filtered using Whatman No. 1 filter paper. Titanium dioxide nanoparticles were synthesized by adding 10 ml of filtered ALE solution to 90 ml of 5 mM Titanium dioxide solution (pH 1.5) in an Erlenmeyer flask under stirring at 50 °C. After 5 h, the developed dark brown colour confirmed the formation of Titanium dioxide nanoparticles (TiNPs) [3]. Finally, well-formed Titanium dioxide nanoparticles were acquired by centrifugation at 10,000 rpm for 15 min and thus separated Titanium dioxide nanoparticles (TiNPs) were dried and used for further analytical techniques.

3. Characterization techniques

3.1. UV–Vis spectral analysis

Colour change in the reaction mixtures is the evidence for TiNPs formation. The reduction of pure titanium ions was monitored by measuring the UV–Vis spectrum of the reaction medium after 5 h. UV–Vis spectral analysis was carried out by UV–Vis spectrophotometer (Perkin Elmer Lamda 35)in the range 200–800 nm.

3.2. SEM analysis of Titanium nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using VEGA3 TESCAN equipment. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. Extra solution was removed using a blotting paper and then the films on the SEM grid were allowed to dry under a mercury lamp for 5 min.

3.3. Excision and treatment on animals

The wound healing activity was examined upon the Male albino rats of Wistar strain with weight of 160–1100 g and these animals were purchased from Indian Institute of Science, Bangalore. The rats were housed in spacious polypropylene cages bedded with rice husk and the room was well ventilated maintained under standard experimental conditions (temperature 27 ± 2 °C and 12 h light/dark cycle) throughout the experimental periods. All the animals were fed with standard pellet diet and water ad libitum. These animals were acclimatized to the environment for one weak prior to experimental use. The food composition to animal was taken by the following ratios, crude protein (22.3%), crude oil (4.01%), crude fibre (4.02%) and sand silical (1.02%).

The wound site of the animals were produced by excision wound model [4]. First, the animals were anesthetized with diethyl ether and the hairs on the skin of the back, shaved with sterilized razor blades. Then the dorsal fur was shaved and full thickness of the excision wound of 1.5 cm in width and 0.2 cm depth was created in animals. After completion of wounding process, the wound part was well washed with normal saline solution. Animals bearing partial thickness wound were distributed into various groups such as Group I, II and III where Group I (Negative control group) is treated with gel alone to serve as self immunity, Group II (control group) animals are treated with gel based ointment (TiNPs + gel). The standard drug sulfadiazine purchased from medical store was treated on Group III (positive control) (sulfadiazine + gel) animals. The very purpose of selecting a standard drug is to ascertain the size of particles which play a crucial role in wound healing activity, rather than other medicinal and ethical aspects. In this directions, sulfadiazine was chosen as standard drug which contains micro-sized particles. It is found to be a good antibiotic for the treatment and prevention of local infections particularly, in animals. Each group has six animals.

3.4. Preparation of gel based ointment

The gel based ointment was prepared using Titanium nanoparticles (TiNPs) by the following procedure: 0.5 g of TiNPs was dispersed in gel with mild stirring and is allowed to swell for 5 min to obtain 0.5% of medicated gel.

3.5. Wound measurement

The measurement of the wound area was carried out from the day of the excision of the wound and at every three (3) days interval until the epithelisation of the wound was completed. The area of the wound contraction was measured in different treated and control groups on 3rd, 6th, 9th and 12th day. Wound contraction which contributes to wound closure was studied by tracing of the site. A meter ruler was placed over the wound and that estimate provides scale calculation. The percentage of wound closure was calculated as follows using the initial and final area drawn on glass slides during the experiments [5].

$$\%\text{of WH} = \frac{WA_0 \times WA_n}{WA_0} \times 100$$

where, WH – wound healing; WA_0 – wound area on day 0; WA_n – wound area on day n; and n = 3, 6, 9 and 12th day.

4. Results and discussion

4.1. Titanium nanoparticles

The colour change due to the reaction of TiO_2 with *M. oleifera* is displayed in Fig. 1 and the colour changes observed in the vessels is due to the reduction of titanium ions. This same type of reaction (i.e. colour formation) was found in earlier reported article Shankar et al. [3]. It is well known that the Titanium nanoparticles exhibit brown colour in aqueous solution due to the excitation of surface plasmon vibrations. The appearances of brown colour in the reaction vessels suggest the formation of Titanium nanoparticles.

4.2. UV–Vis spectral analysis

Titanium nanoparticles were characterized using UV–Vis spectroscopy to find the structural properties of TiNPs as depicted in Fig. 2. It can be seen that the spectra was recorded as a function of reaction time (5 h) of TiNPs. From this analysis it is observed that