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Advancement of Near-infrared (NIR) laser interceded surface enactment of proline functionalized graphene oxide with silver nanoparticles for proficient antibacterial, antifungal and wound recuperating therapy in nursing care in hospitals

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ABSTRACT

Medication obstruction of microscopic organisms has turned into a worldwide medical issue, as it makes the ordinary anti-toxins less effective. It is desperately expected to investigate novel antibacterial materials and create viable treatment techniques to defeat the medication obstruction of anti-infection agents. Herein, we effectively fabricated silver nanoparticles improved proline/graphene oxide nano-flakes (GO-Pro/n-Ag) as novel anti-pathogenic substance through simplistic technique. The property of the silver nanoparticles activated discharge gave amazing antibacterial and antifungal movement against the pathogenic microorganism. Upon supply of NIR, the graphene oxide based biomaterials privately increased the temperature, bringing about the high mortality of pathogenic microorganism. The GO-Pro biocomposite activated n-Ag particles discharging approach for antibacterial, antifungal enables n-Ag to be ensured by proline layout without influencing normal cells. The biocomposites provided antibacterial and antifungal movement against *S. aureus, P.aeruginosa, C. albicans and S. cerevisia*. Also, the L929 mouse fibroblast cells were utilized for cytocompatibility assessment, and the GO-Pro/n-Ag demonstrated low lethality. Likewise, the GO-Pro/n-Ag and GO-Pro/n-Ag + NIR are set up for *in vivo* tests and demonstrate incredible antibacterial property in wound model. As the fabricated GO-Pro/n-Ag biocomposite nano-flakes have the benefits of minimal effort and high anti-pathogenic activity, they may be of promising and helpful antibacterial and antifungal specialists for various biomedical applications.

1. Introduction

Bacteria and fungi are infamous and a standout among the most unsafe infectious pathogen with high death rate. They cause numerous ailments like skin disease, pneumonia, ostiomylitis, lethal interceded disorder like nourishment harming and stun disorder [1]. Victims with the balance immune systems, victims with serious consumes, and worked victims are more inclined to various pathogenic infections. Subsequently, gigantic endeavors have been paid for growing new medications and contriving new therapeutic systems for the treatment. New pathogenic microorganism medications are profoundly vital [2]. The ordinary natural medications confront difficulties, for example, low warm dependability, low effectiveness, unacceptable for painting or covering, and cause ecological contamination [3]. Moreover, microorganisms have developed and demonstrate extraordinary obstruction against a large portion of the ordinary medications accessible on market [4].

Recently, different nano-biomaterials have been broadly created as bactericidal operators rather than anti-microbial drugs, on account of their incredible physical and biochemical properties and cytocompatibility, for example, GO, n-Ag, n-ZnO, TiO_2 and ect [5–8] Among these anti-pathogenic agents, nano silver (n-Ag) based materials have pulled in the most consideration for limiting and sanitizing drug safe pathogenic organisms at low volumes, because of their little size and expansive range of anti-pathogenic action [9]. They could without much of a stretch infiltrate microbes' cells and discharge silver nanoparticles to upgrade their anti-pathogenic activity. Though, pathogenic organisms enhancement can lessen the admission of nanoparticles, which is additionally one of the key explanations behind microbial obstruction due to biofilm arrangement [10]. Thusly, it is critically required to

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discover novel and viable anti-pathogenic specialists or strategiesto beat the lack of customary anti-infection treatments.

The n-Ag particles are regularly repressed for application by simple oxidization without security, which may cause collection and loss of anti-pathogenic movement. To tackle these issues, distinctive nano-carriers (carbon-based materials, TiO_2, zeolite and silica) have been accounted for to ensure the anti-pathogenic action of n-Ag particles [11–14]. n-Ag particles could without much of a stretch consolidate with these nano-carriers through electrostatic attraction, physsorption. Graphene oxide could frame silver-consisting substances and defend n-Ag particles from collection and gradually discharge silver to eliminate microorganisms, which is more powerful than ordinary silver particles. As we known, Graphene oxide has additionally been utilized as a part of antibacterial applications due to its extraordinary physical properties. Graphene oxide -based nanomaterials have a powerful near-infrared (NIR) range ingestion capacity, and could effectively change over NIR light into warm, which could be used for photothermal medications (PTT) [15]. Although the promising properties of graphene oxide for PTT applications, the utilization of graphene oxide has been constrained by low steadiness in aqueous media because of the non-attendance of surface hydrophilic gatherings. To beat these hindrances, various sorts of natural or manufactured polymers have been used for covering graphene oxide nanosheets [16]. These qualities demonstrated a powerful urge to utilize functionalized graphene oxide as a promising substrate to covered silver nanoparticles for assist applications.

In this work, we fabricate a novel anti-pathogenic microorganism agent to enormously enhance antibacterial properties by PTT synergistic impact. Graphene oxide was picked as the substrates with amazing PT change capacities to successfully create proline functionalized graphene oxide (GO-Pro) nano-flaks. At that point, silver nanoparticles with anti-pathogenic properties were decorated on the exterior of GO-Pro/n-Ag nano-flaks. The PT execution of GO-Pro/n-Ag nano-flaks is additionally enhanced attributable to the PT change capacity of the n-Ag and the diminishment of the GO in the arrangement procedure of n-Ag particles. Additionally, the inhibitory impacts of GO-Pro/n-Ag nano-flaks on pathogenic microorganism were assessed, demonstrating altogether improvement by NIR laser illumination. The as-fabricated GO-Pro/n-Ag nano-flaks are exhibited to have great cytocompatibility. Furthermore, the *in vivo* wound disease of was likewise researched. The information displayed that injury disease was demonstrated quick mending. The present information emphatically recommended that GO-Pro/n-Ag nano-flaks can possibly serve for PTT against pathogenic bacteria and fungi.

2. Materials and Method

The chemicals used were GO (99%), proline (99%), KOH (99%) and Ag(NO3) (99%). All chemical purchased from Sigma Aldrich china, analytical grade. Graphene oxide was prepared from graphite using a modified Hummers strategy [17, 18]. A fluid graphene oxide suspension was vigorous stirring for 3 h. Afterward, proline functionalized graphene oxide biocomposite was fabricated through probe sonicated of 0.08 g of proline, 0.02 g of graphene oxide, and 0.1 g of fluid potassium hydroxide solution for 6 h. Subsequently, proline functionalized graphene oxide biocomposite suspension was gathered by centrifugation and washed with water a few times to evacuate abundance starting materials and contaminations.

An ethanol suspension of GO-Pro biocomposite (2 mL) was vivaciously sonicated for 1 h. At that point, Ag(NO3) arrangement (0.05 g in 5 mL ethanol) of prepared using ultrasonicated, 0.1 mL of N-[3-(Trimethoxysilyl)propyl)] ethane-1,2-diamine was added to frame complex arrangement. The as-formed complex arrangement was dropwise included into the GO-Pro biocomposite arrangement and kept sonicated for 2 h, afterward the GO-Pro/n-Ag nano-flakes were acquired. At the point when the solution chilled off to 37 °C, it was centrifuged and rinsed with DD a few times, and after that the GO-Pro/n-Ag biocomposite nano-flakes dried in hot air oven at 40 °C overnight.

For the whole NIR laser (808 nm) presentation part of the investigation, all pathogenic microorganism suspension set in the microtiter plate. 1 mL of pathogenic microorganism suspension was uncovered with fabricated samples and NIR light as for various introduction times interim changes from 1 to 6 min for improving the best outcomes. After the presentation therapy, the feasible pathogenic microorganisms were checked by customary standard micro-dilution methods.

2.1. Characterization

2.1.1. Surface and Functional Characterization

The surface of fabricated samples was described by a transmission electron microscopy (TEM, TF20) and atomic force microscopy (AFM) tests were performed by a Veeco Multimode Microscope (Veeco Instruments Inc). The Fourier change infrared (FT-IR) spectra were recorded utilizing a Bruker Tensor27 spectrometer. Raman spectra were procured at room temperature by utilizing a laser confocal Raman spectrometer (Renishaw-2000).

2.1.2. L929 Cell Viability

Cells were developed in Dulbecco's Modified Eagle Medium (Hi Media Laboratories) supplemented with 10% fetal bovine serum, streptomycin (100 U mL^{-1}) and penicillin (100 U mL^{-1}). The medium was revived each days. Cells were hatched in a humidified atmosphere with CO_2 at 37 °C. The samples were sterilized in an autoclave at 80 °C for120 min and then aliquot into 96-well cell growth plates.

To assess the cytocompatibility of the novel GO-Pro/n-Ag nano-flakes, fibroblast cells were grown on the plate. The fabricated samples were added to the cell culture solution during 1, 4, 7 days. Cells were presented to the arranged supernatants amid 1, 4, 7 days and estimating the cell viability, MTT strategy was used [19].

2.1.3. In vivo Wounds Model

To assess the antibacterial impact of fabricated GO-Pro/n-Ag *in vivo*, the injuries display was manufactured. The four gatherings of 16 male rats with wounds (four rats for each gathering) were separated into control (Group A), GO (Group B), NIR, GO-Pro/n-Ag (Group C), and GO-Pro/n-Ag + NIR (Group D). The rats in four distinct gatherings with various bandages on their injuries were detected. The bandages were changed with one-day interims subsequent to taking the photo. The NIR and GO-Pro/n-Ag + NIR were lighted by NIR for 120 s subsequent to changing the wraps. Following 21 days, all rats were relinquished, the tissues of the injuries were collected, and the quantity of microscopic organisms was measured at every day of the remedial procedure. Aliquots of weakened homogenized intestinal tissues were set on agar. The developed settlements were meant for investigation. Every single creature method was as per the rules of the Institutional Animal Care and Use Committee.

3. Statistical Analysis

According to the methodology used in each test, one-way ANOVA followed by the Tukey test for multiple comparisons was used.

4. Results and Discussion

4.1. Surface Characterization

Characteristic TEM pictures of the GO and GO-Pro/n-Ag biocomposite appear in Fig.1. As can be seen in Fig.1a, graphene oxide displayed a thin, visible, layer-like morphology with collapsing nature, apparently because of the way that mostly conjugated structures of graphene oxide [19]. Clearly, n-Ag particles with uniform distribution are effectively developed on the surfaces of the GO-Pro nano-sheets