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Reduced Graphene Oxide-Based Hybrid Electrospun Nanofibers Doped with Green-Synthesized Silver Nanoparticles: An Antibacterial Wound Healing Scaffold

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Abstract

This research focuses on the development of novel antibacterial electrospun fibers composed of polyvinyl alcohol (PVA), hydrolyzed collagen (HC), and reduced graphene oxide (rGO), further modified with silver nanoparticles (AgNPs) for potential wound healing applications. Green synthesis of AgNPs, using a tea extract, was verified by a colour change from yellowish-brown to dark brown and the appearance of a UV-Vis absorbance peak at 450 nm. Scanning Electron Microscopy (SEM) revealed mostly spherical AgNPs with a primary size distribution between 10 and 50 nm. Energy Dispersive X-ray (EDX) analysis confirmed the presence of Ag atoms (a characteristic peak at 3 keV). Particle size analysis indicated an average AgNP size of 133 nm, with a particle size distribution showing a high-intensity peak at 299 nm and a lower-intensity peak at 18.39 nm, and a polydispersity index (PDI) of 0.471. SEM images of rGO-containing mats showed well-preserved fiber morphology with uniformly distributed AgNPs, in contrast to mats without rGO, where significant AgNP agglomeration covered fiber visibility. This suggests rGO's role in preventing nanoparticle aggregation and enhancing dispersion. Swelling ratio analysis demonstrated superior swelling in rGO-containing mats, reaching 390.48% after 48 hours compared to 273.6% for mats without rGO. The incorporation of rGO improves the antibacterial performance and mechanical properties of the scaffold, while green-synthesized nanoparticles further enhance the antibacterial efficacy.

Keywords: Antibacterial, electrospun nanofibers, green synthesis, reduced graphene oxide, wound healing

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Introduction

Wound healing is a complex and dynamic natural process in the body that helps to repair and restore the structure and function of damaged skin tissues through a well-coordinated series of steps (Yadav et al., 2024; Balusamy et al., 2017; Ferraz, 2025). Wound site infections are a major challenge in wound management; therefore, novel therapeutic approaches are investigated to accelerate this process through faster tissue regeneration while preventing infections.

Electrospun nanofiber-based scaffolds are highly regarded as advanced wound healing materials due to their potentiality to mimic the native extracellular matrix (ECM), providing a cell-preferred environment for attachment, proliferation, migration and differentiation (Partovi et al., 2024; Mwiiri & Daniels, 2020). Electrospinning is the most widely used nanofiber fabrication technique, which generates ultrafine fibers in the micron or sub-micron range from polymer solutions or melts in the presence of a high-voltage electric field (Yadav et al., 2024; Zhang et al., 2023; Mwiiri & Daniels, 2020). Among the wide array of biomaterials explored for electrospun scaffolds for wound healing, PVA and HC stand out for their established biocompatibility and biodegradability (Ansari & Darvishi, 2024). This natural (HC) and synthetic (PVA) duo creates a synergistic effect of their inherent merits that promotes wound healing through enhanced granulation tissue formation and angiogenesis (Zhang et al., 2023; Ansari & Darvishi, 2024). RGO has shown potent antibacterial properties owing to its ability to disrupt bacterial membranes and induce oxidative stress in microbial cells (Liu et al., 2011). Green synthesis of silver nanoparticles (AgNPs) offers an eco-friendly, sustainable, non-toxic method to incorporate antibacterial properties into scaffolds.

In this study, we fabricated a nanofibrous scaffold utilizing a novel mixture of synthetic polymeric material and bioactive compound, namely, PVA and HC. In addition, rGO was incorporated to the matrix and the scaffold was doped with green-synthesized AgNPs. Although prior studies have explored electrospinning PVA with either HC or rGO individually, the compatibility and synergistic effects of integrating all these components in a single scaffold have not been comprehensively examined. The study aims to optimize the fabrication of composite electrospun mats that not only mimic the natural extracellular matrix but also offer enhanced mechanical strength, antimicrobial activity and fast wound healing.

Materials

Polyvinyl Alcohol (99% hydrolyzed, MW 146 000-186 000 g/mol), Hydrolysed collagen type 1 and 3 powder (Doctor's Best dietary supplement), Glutaraldehyde was supplied from a local supplier, Silver nitrate 99.5% (Johnson Matthey PLC, UK), Gentamicin Injection BP 80 mg/ 2mL (Belco Pharma, India). Tea leaves (Alagalla State, Kandy), syringe pump (Harvard apparatus 11 plus), needle (25G), bacterial strains *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923) purchased from MRI,Sri Lanka, Mueller Hinton Agar from Sisco Research Laboratories (SRL), India, Nutrient Agar, from HiMedia Laboratories Pvt.Ltd, India.

Methodology

Green synthesis of AgNPs was performed by reacting 100 mL of 10 mM silver nitrate solution with 10 mL of tea extract at room temperature for 24 h. The resulting nanoparticles were separated by centrifugation. A series of PVA solutions was prepared with concentrations of 6%,8% and 10% PVA and 5% HC. The mixture was stirred for 1 hour, and 0.2 g of rGO was added and stirred overnight to obtain a well-dispersed homogenous solution. The prepared nanofiber mats were crosslinked with 50 mL of acetone 5 mL of glutaraldehyde and HCl. To optimize the electrospinning parameters for nanofiber fabrication, a series of

experiments was conducted, and continuous fiber formation without any visible dripping was obtained with the solution containing 8% PVA, 5% HC, and 1% rGO. The optimum voltage, tip-to-collector distance, and flow rate were identified as 20 kV, 12 cm, and 2 mL $hr^{-1,}$ respectively. The prepared nanofibers were crosslinked by dipping in a solution containing 25 µL glutaraldehyde, 0.5 mL HCl, and 50 mL of acetone for 1 h. The crosslinked samples were then washed with DI water three times, followed by air drying. An adsorption procedure was used to load AgNPs into the electrospun nanofiber mat. For that, an aqueous solution of 1 % AgNPs was prepared, and the electrospun nanofiber mats were dipped in the AgNPs solution for 30 minutes. The samples were then dried for 15 minutes in an oven at 50 °C. For the characterization of nanofiber mats, four types of samples were used.

Table 1. *Different electrospun sample types*

Sample type	Sample Name
8% PVA +5% HC+ 1% rGO dipped in 1% AgNPs solution	PVA/HC/rGO+AgNPs
8% PVA 5% HC No rGO dipped in 1% AgNPs solution	PVA/HC+AgNPs
8% PVA 5% HC 1% rGO	PAV/HC/rGO
8% PVA 5% HC No rGO	PVA/HC

Characterization of the physicochemical properties of green-synthesized AgNPs and electrospun nanofibers

The formation of AgNPs by the reduction of Ag^{+} ions was monitored by checking the absorbance from 200–650 nm in a double-beam UV–VIS spectrophotometer (Hitachi U-2910 UV-VIS Spectrophotometer). The shape, particle size, and morphology of the synthesized AgNPs and the electrospun nanofibers were evaluated by the Scanning Electron Microscope (Hitachi SU6600). Particle size and size distributions of the AgNPs were determined using the particle size analyzer (Malvern Zetasizer Ver. 7.03). EDX analysis was used to study the composition of the green-synthesized AgNPs and their distribution on the nanofiber mats after doping.

The gravimetric method was used to measure the swelling ratio of the electrospun mats. The crosslinked nanofiber mats containing AgNPs (Samples PVA/HC/rGO+AgNPs and PVA/HC+AgNPs) were cut into samples of 1 cm x 1 cm, and their dry mass was measured. Next, they were immersed in 1 ml of the phosphate-buffered saline (PBS; pH=7.4) in a 24-well plate separately. The samples were incubated at 37°C for a period of 3,6,9,12,24, and 48 h. The swelling ratio was measured according to the following equation where, W_d represents the dry weight of the nanofiber mat, W_w is the weight of the nanofiber mats after swelling.

$$Swelling\ Ratio\% = \frac{Ww - Wd}{Wd} \times 100\ \%$$

To check the antimicrobial activity of AgNP-modified nanofiber mats, a disc diffusion method was carried out. For this, two different strains of bacteria were used, namely, *E. coli* and *S. aureus*. For the disk diffusion test, Mueller-Hinton Agar (MHA) plates were prepared. The subcultured bacterial colonies were suspended in saline solution, and the turbidity was adjusted to 0.5 McFarland Standard. Each bacterial strain was spread on MHA plates using a cotton swab. The nanofiber mats, along with the positive control (gentamicin, 500 ppm), were placed over the bacterial spread plates, followed by incubation overnight at 37 °C. The observed zones of inhibition in each sample were measured in mm.

Results and Discussion

Characterisation of the physicochemical properties of green-synthesized AgNPs

To characterize the optical properties of green-synthesized silver nanoparticles, UV-VIS spectrometry was used. The initial colour of the solution was yellowish brown, and it gradually turned to dark brown with the formation of AgNPs. The appearance of UV–vis absorbance peaks at 450 nm verified the formation of AgNPs. (Kartini et al., 2020; Menon et al., 2017; Sreelekha et al., 2021) The conversion of AgNPs was periodically recorded by UV–VIS spectra every 30 minutes for 2 hours. (Figure 1).

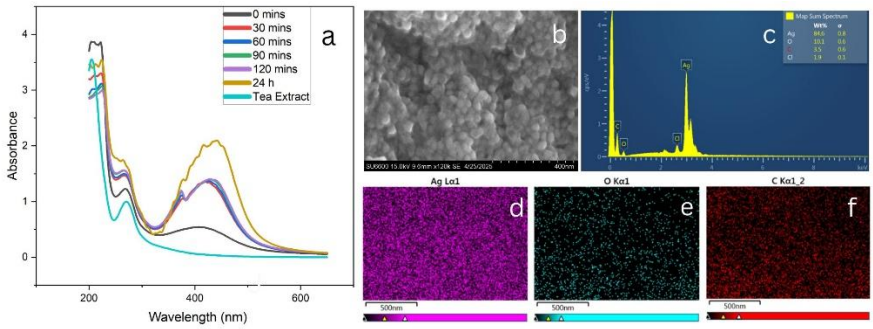

Figure 1: *UV-VIS absorbance spectra of AgNPs and the tea extract over time (a) SEM image of AgNPs (scale bar = 500 nm) (b) EDX spectrum of AgNPs (c) Elemental mapping of the constituent elements (d-f) Elemental mapping indicating the presence of O, Ag, and C.*

The SEM images (Figure 1 b) confirm the formation of nanoparticles, mostly spherical, with the particle size distribution in the range from 10 to 50 nm. The EDX profile shown in Figure 1(c) depicts a strong signal for the presence of Ag atoms. The other peaks observed were C and O, which could be due to the remaining biomolecules from the tea extract. This indicates that Ag is the predominant element in the product obtained after green synthesis. For Ag nanocrystals, the typical absorption peak, which is at 3 keV, is visible in the green synthesized nanoparticle EDX spectra.

The particle size analysis results revealed that the average size of the AgNPs was 133 nm. The size distribution showed one small and one large peak. The peak with the highest intensity had an average particle size of 299 nm, while the peak with lower intensity consisted of an average particle size of 18.39 nm with a polydispersity index (PDI) of 0.471, which confirmed that tea extract can be utilized and further optimized to produce Ag NPs.

Characterisation of the morphology and physicochemical properties of the nanofiber mats

To evaluate the morphology of the electrospun mats before and after crosslinking, Scanning Electron Microscopy was used.

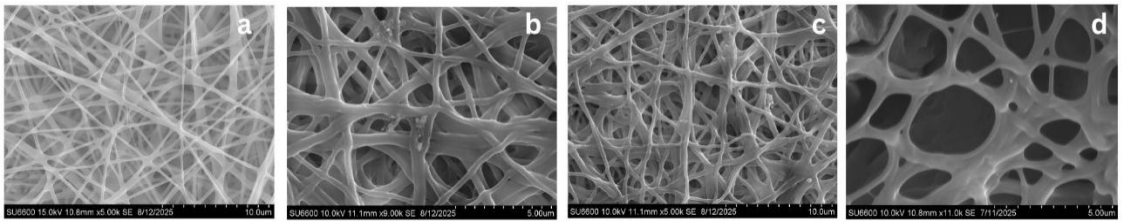

Figure 2: *(a) SEM image of sample PVA/HC before crosslinking (b) SEM image of sample PVA/HC/rGO before crosslinking (c) SEM image of sample PVA/HC after crosslinking (d)SEM image of sample PVA/HC/rGO after crosslinking*

PVA and hydrolyzed collagen (HC) produce uniform, straight, and bead-free fibers because the solution's viscosity and surface tension are perfectly balanced. This balance promotes a stable jet formation, which is crucial for creating consistent nanofibers. However, the addition of reduced graphene oxide (rGO) significantly alters this process. The rGO nanosheets increase the solution's viscosity and conductivity, leading to thicker, wavy fibers. This is because rGO acts as a physical filler, disrupting the smooth elongation of the polymer jet and causing a less-uniform fiber morphology. When the fiber mats are treated with glutaraldehyde crosslinking, the resulting effect depends on the presence of rGO. In PVA/HC mats, crosslinking causes the fibers to contract and aggregate, drastically reducing porosity. Conversely, in rGO-containing mats, the porosity is maintained because the rigid, planar rGO nanosheets act as physical spacers, preventing the fibers from collapsing. This preservation of porosity is beneficial for applications like wound healing, as it allows for sustained permeability and cell infiltration while the material retains its mechanical integrity.

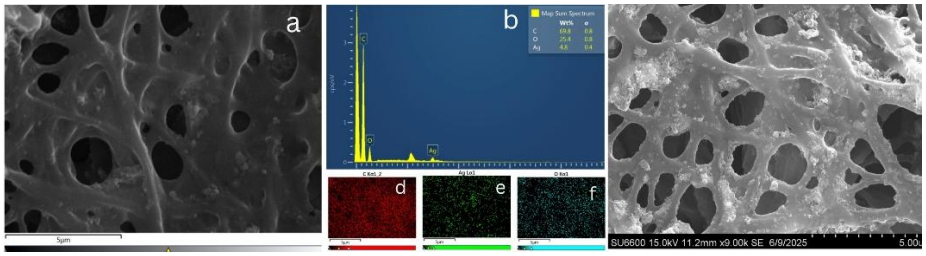

Figure 3: *SEM images of sample PVA/HC/rGO+AgNP (a,c) EDX spectrum of sample PVA/HC/rGO+AgNPs, (b) Elemental mapping indicating the distribution of C, Ag, and O, respectively. (d-f)*

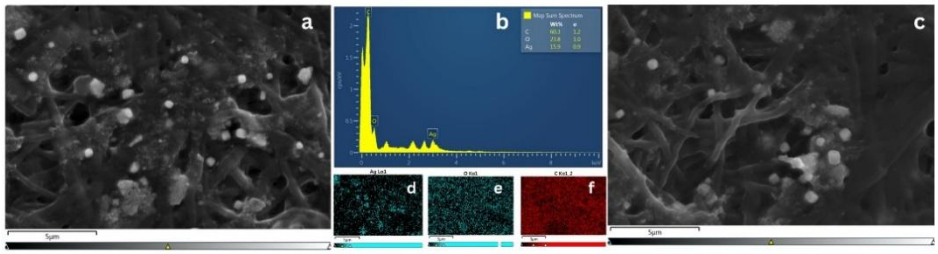

Figure 4: *SEM images of sample PVA/HC+AgNP (a,c) EDX spectrum of sample PVA/HC+AgNPs, (b) Elemental mapping indicating the distribution of C, Ag, and O, respectively. (d-f)*

Fibers were still visible in the SEM images of rGO containing fiber mats doped with AgNPs (Figure 3 a,c) opposed to those without rGO (Figure 4 a,c) where the visibility of the fibers was hindered by the agglomeration of AgNPs on the fiber mat surface. This is due to the presence of rGO, which can prevent the aggregation of nanoparticles and improve their distribution more uniformly throughout the fiber matrix. This observation is consistent with the results reported in the literature on nanoparticle-incorporated rGO nanofibers. (Tan et al., 2020; Wang et al., 2020)

Swelling ratio of the nanofiber mats

The swelling ratio of nanofiber mats with and without rGO, at different time points, are shown in Figure 5. The results showed that the nanofiber mats with rGO showed superior swelling compared to those without rGO at all time points, indicating the presence of rGO showed a noticeable effect on the membrane swelling.

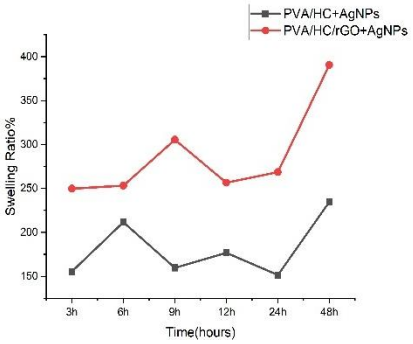

Figure 5: *Swelling ratio (SR%) of nanofiber mats*

The swelling ratios recorded after 48 hours were 390.48% and 273.6% for the mats with and without rGO, respectively. This significant increment in SR% could be attributed to the increased hydrogen bonding between the water molecules in the PBS solution and the remaining functional groups of rGO. This good swelling property of rGO nanofibers could maintain a better wound-healing environment by absorbing wound extrude.

Antibacterial activity of the nanofiber mats

Inhibition zones recorded for each sample type are displayed in Figure 6, and their average values are given in Table 2.

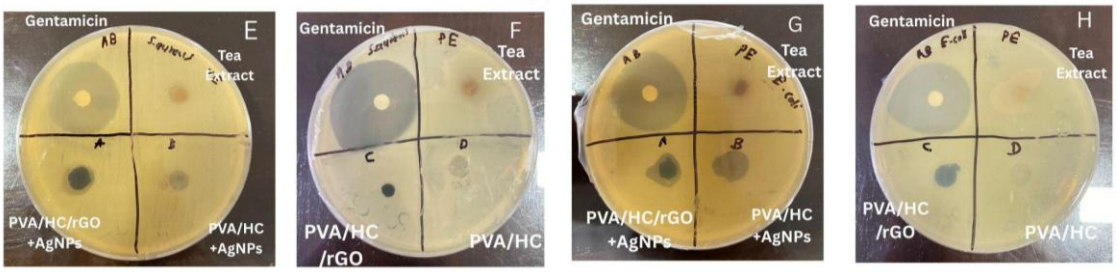

Figure 6: *Inhibition zones of nanofiber mats (E, F) Bacterial Strain S.aureus (G, H) Bacterial Strain E.coli"*

Table 2: *Average diameter of the zone of inhibition*

Sample type	Average diameter of the inhibition zone in mm	
	E.coli	***S.aureus***
Gentamicin	29.0 ± 0.0	28.0 ±1.0
Tea Extract	07.5 ± 0.7	07.7 ± 0.6
PVA/HC/rGO+AgNPs	14.3 ± 1.5	12.3 ± 2.5
PVA/HC +AgNPs	12.0 ± 1.0	09.00 ± 1.0
PVA/HC/rGO	No Inhibition zone	No Inhibitory Zone
PVA/HC	No Inhibitory Zone	No inhibitory zone

The PVA/HC/rGO+AgNPs containing sample has the highest average diameter for the inhibition zone against both Gram-positive *S. aureus* and Gram-negative *E. Coli*. This shows the synergic antibacterial property of AgNPs and rGO. Even though rGO does not diffuse into the agar medium in a short duration of time, which could be the reason for not having an inhibition zone for the PAV/HC/rGO sample, the presence of rGO may enhance the adsorption of AgNPs to the electrospun mat. AgNPs are well-known bactericides known for their broad-spectrum and long-lasting antibacterial activity. However, their effectiveness is often hindered by their tendency to aggregate due to high surface energy, especially as particle size decreases. The rGO matrix in the nanofiber mat prevents this agglomeration, maintains a larger surface area, ensuring a sustained and controlled release of silver ions, thereby prolonging the antibacterial effect.(Tan et al., 2020; Wang et al., 2020)

Conclusion

In conclusion, this research successfully established the optimal parameters for electrospinning novel composite fiber mats composed of PVA, HC and rGO. The nanoscale fibers had a random arrangement and dimensions mimicking the extracellular matrix. The resulting fiber mats exhibited favorable mechanical properties, notably high extensibility attributed to PVA. The incorporation of reduced graphene oxide has increased the swelling ratio and antibacterial property of the nanofiber mats. Future studies will focus on evaluating their degradability, mechanical properties, and biocompatibility to fully evaluate their potential for wound healing applications. This comprehensive characterization lays a strong foundation for the development of advanced biomaterials with tailored properties for wound healing and tissue regeneration studies.

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