Development of Robust Hemostatic Gauze Through Synthesis of Polymeric Composites (PVA/Chitosan) Grafted With Graphene Oxide Nanosheets/Hydroxyapatite

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**Abstract:**The development of advanced biomaterials for hemostatic and wound healing applications is crucial in modern medicine. This study explores the fabrication and characterization of electrospun nanofiber mats composed of polyvinyl alcohol (PVA) and chitosan, reinforced with graphene oxide (GO) and hydroxyapatite (HAp). Structural and chemical analyses using X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR) confirmed the successful incorporation of GO and HAp into the polymer matrix. Scanning electron microscopy (SEM) revealed a well-defined fibrous morphology, enhancing mechanical strength and surface interactions. Hemocompatibility assessments demonstrated non-toxicity and safe interaction with blood components, while drug release studies highlighted the potential for controlled therapeutic delivery. Additionally, MTT assays confirmed excellent cell viability, underscoring the composite’s suitability for tissue engineering applications. The multifunctional properties of PVA/Chitosan-GO-HAp nanofibers position them as promising candidates for next-generation hemostatic materials, offering enhanced bioactivity, mechanical integrity, and clinical adaptability.

**Keywords:** Hemostatic gauze, Polymeric composites, Graphene oxide nanosheets, Hydroxyapatite, Biomaterials

# INTRODUCTION

In recent years, significant advancements in hemostasis and wound care have been driven by the pursuit of innovative materials for medical applications. [(Harsha & Subramanian, 2022)](https://paperpile.com/c/Z3HG5j/kqxOc)Central to this effort is the development of hemostatic gauze, a critical component in managing bleeding and accelerating healing during surgical procedures and emergency situations.[(Deepika et al., 2022)](https://paperpile.com/c/Z3HG5j/aGexY). Traditional approaches to hemostasis, such as pressure dressings, sutures, and hemostatic drugs, often present limitations in terms of speed, effectiveness, and potential side effects [(Iqbal et al., 2023; Zubair et al., 2024)](https://paperpile.com/c/Z3HG5j/RrS7+UleD). These challenges underscore the pressing need for safer, more efficient alternatives that can effectively control bleeding in diverse clinical scenarios [(Jiao et al., 2023)](https://paperpile.com/c/Z3HG5j/U4RF). Advanced hemostatic materials enhance bleeding control and wound healing by ensuring biocompatibility, ease of use, and efficacy. [(Solanki et al., 2022)](https://paperpile.com/c/Z3HG5j/ZgaGT). Polymeric composites reinforced with nanoparticles offer a promising approach to improving hemostatic gauze performance [(Wang & Yang, 2023; Zheng et al., 2022)](https://paperpile.com/c/Z3HG5j/GfT5+982D). PVA and chitosan are biocompatible polymers widely used in biomedical applications.[(Chidambaram et al., 2022)](https://paperpile.com/c/Z3HG5j/Lv4eB).PVA forms hydrogels that maintain moisture and aid tissue regeneration, while chitosan provides inherent hemostatic properties. Combining these polymers creates a composite that leverages PVA’s flexibility and chitosan’s hemostatic benefits, forming an ideal matrix for integrating nanomaterials to enhance functionality [(Zhong et al., 2024)](https://paperpile.com/c/Z3HG5j/N0kI), [(Kalirajan et al., 2021)](https://paperpile.com/c/Z3HG5j/FEFR). Nanotechnology enhances hemostatic materials by improving mechanical strength, antimicrobial properties, and clotting efficiency.[(Ajay et al., 2022)](https://paperpile.com/c/Z3HG5j/qFEg) Graphene oxide (GO) reinforces polymeric composites, while hydroxyapatite (HAp) supports tissue regeneration. This study develops a PVA-chitosan-GO-HAp composite for hemostatic gauze, focusing on synthesis, characterization, and evaluation of its hemostatic and antibacterial efficacy to advance next-generation wound care materials.[(Jabin et al., 2021)](https://paperpile.com/c/Z3HG5j/6cqN8)

# MATERIALS AND METHODS

Polyvinyl Alcohol (PVA), Chitosan GO nanosheets, Calcium nitrate tetrahydrate, Diammonium hydrogen phosphate, Ammonia were purchased from Sigma-Aldrich and used without further purification.

## Preparation of Hydroxyapatite and Graphene Oxide/Hydroxyapatite Composite

Hydroxyapatite (HAp) was synthesized via a wet-chemical precipitation method using calcium nitrate tetrahydrate and diammonium hydrogen phosphate. A 1.0 M Ca(NO3)2·4H2O solution was slowly added to a 0.6 M (NH4)2HPO4 solution while maintaining the pH at 10 using ammonia.[(Balaji Ganesh S & Sugumar, 2021)](https://paperpile.com/c/Z3HG5j/2NDzO) The white precipitate formed was aged overnight, washed, and dried at 100 °C. For the GO/HAp composite, GO and HAp were mixed in a 1:1 ratio and ground for 30 minutes to ensure uniform dispersion. The mixture was then calcined at 400°C to enhance structural integrity and interaction between GO and HAp. The resulting composite exhibited improved mechanical properties, making it suitable for biomedical applications.

## Preparation of PVA/Chitosan and PVA/Chitosan-GO-HAp Solutions

A 5% (w/v) PVA solution was prepared by dissolving PVA in distilled water at 90°C with continuous stirring until fully dissolved. Simultaneously, a 2% (w/v) chitosan solution was prepared by dissolving chitosan in 1–2% acetic acid and stirring until homogeneous. These solutions were mixed in a 1:1 ratio and stirred for several hours to achieve a uniform PVA-chitosan blend. [(Tiwari & Jain, 2023)](https://paperpile.com/c/Z3HG5j/X3fHY)For the PVA/Chitosan-GO-HAp solution, a 1% (w/v) GO-HAp suspension was prepared by sonicating the composite in distilled water for 30 minutes to ensure uniform dispersion. The sonicated GO-HAp was gradually added to the PVA-chitosan blend under continuous stirring to facilitate complete integration.[(Graf et al., 2023)](https://paperpile.com/c/Z3HG5j/1KLZ2). The resulting homogeneous solution was then electrospun into nanofibrous mats, offering enhanced biocompatibility, mechanical strength, antimicrobial properties, and osteoconductivity for hemostatic and wound healing applications.

## Fabrication of electrospinning mats

Electrospun mats were fabricated using a calibrated electrospinning setup. The PVA-chitosan-GO-HAp solution was loaded into a syringe with a metal needle, mounted on a syringe pump, and extruded at a controlled rate (0.5–2 mL/h). A high voltage (15–25 kV) was applied, forming fine polymer jets collected on a grounded aluminum foil-covered collector (10–20 cm away). Rapid solvent evaporation produced nanofibers, forming a non-woven mat. The mats were dried to remove residual solvent and stored for further use, exhibiting enhanced mechanical strength, antimicrobial properties, and suitability for hemostasis and wound healing.

## Characterization

The resulting composite mats were characterized using The obtained scaffold was confirmed as by using Bruker XRD System CuKα radiation (0.154 nm) with step size 0.02◦ in a continuous scan mode ranging from 20◦ to 80◦. The FTIR spectra which was recorded using the Bruker- ALPHA II compact FT-IR spectrometer. The morphology of the samples was examined using a JEOL JSM –IT800 scanning electron microscope. Contact angle measurements were carried out using Ossila Contact Angle Goniometer.

## Hemolysis percentage

The hemolysis test, used to evaluate the toxic reaction of prepared scaffolds on blood components, involved the use of human blood mixed with Acid Citrate Dextrose (ACD). To prepare the ACD solution, 0.55 g of anhydrous citric acid, 1.67 g of trisodium citrate dihydrate, and 1.84 g of dextrose were dissolved in 100 mL of triple-distilled water and stored at 4°C. To prevent blood cell lysis, 1 mL of this ACD solution was gently mixed with 9 mL of freshly drawn human blood and stored at 4°C.Scaffolds weighing 100 mg were sterilized under UV light for 1 hour before being transferred to centrifuge tubes containing 1 mL of saline solution. These tubes were incubated at 37°C for 24 hours. After incubation, the saline was removed from the tubes, and 0.25 mL of the prepared ACD blood was added. The tubes were then incubated at 37°C for 20 minutes. Following this, 2 mL of saline was added to each tube containing the scaffold and blood samples, and the mixture was incubated for an additional hour. The tubes were then centrifuged at 750 rpm for 5 minutes using a SIGMA 3–30 KS ultra-speed centrifuge. The separated serum was carefully removed and stored in separate tubes.

The positive control for the experiment consisted of ACD human blood mixed with 2 mL of distilled water, while the negative control was ACD human blood with serum. Optical density (OD) values were measured at 545 nm. The percentage of hemolysis was calculated using the following formula:

## Percentage of hemolysis =

The calculated percentages of hemolysis were compared with ASTM standards. According to these standards, a hemolysis percentage below 5% indicates that the material is highly hemocompatible. If the percentage is within 10%, the material is considered hemocompatible. However, if the percentage exceeds 20%, the material is deemed non-hemocompatible [(Ramya et al., 2014, 2016)](https://paperpile.com/c/Z3HG5j/c5JuU+lcv10).

# RESULTS

## XRD ANALYSIS

X-ray diffraction (XRD) analysis is a crucial technique in material science for determining the crystalline structure and phase composition of materials. In the context of developing hemostatic gauze using polymeric composites (PVA/Chitosan) grafted with graphene oxide nanosheets and hydroxyapatite, XRD can provide invaluable insights. It allows for phase identification, confirming the presence of graphene oxide, hydroxyapatite, PVA, and chitosan, and detecting any new phases formed due to interactions between these materials. XRD also reveals the crystal structure of these phases, such as lattice parameters, essential for understanding their physical properties. Additionally, it quantifies the degree of crystallinity in the composite materials, which is crucial since changes in crystallinity can impact mechanical strength and thermal stability—properties vital for the hemostatic application. Furthermore, XRD patterns can elucidate the interaction and compatibility of graphene oxide and hydroxyapatite with the PVA and chitosan matrices, helping to optimize the synthesis process for better dispersion and component compatibility. Characteristic peaks of PVA and chitosan (CS), along with hydroxyapatite (HAp), were observed in the XRD patterns, confirming the successful formation of PVA/CS-GO-HAp composite nanofibers [(Sathiyavimal et al., 2019)](https://paperpile.com/c/Z3HG5j/aUZS).

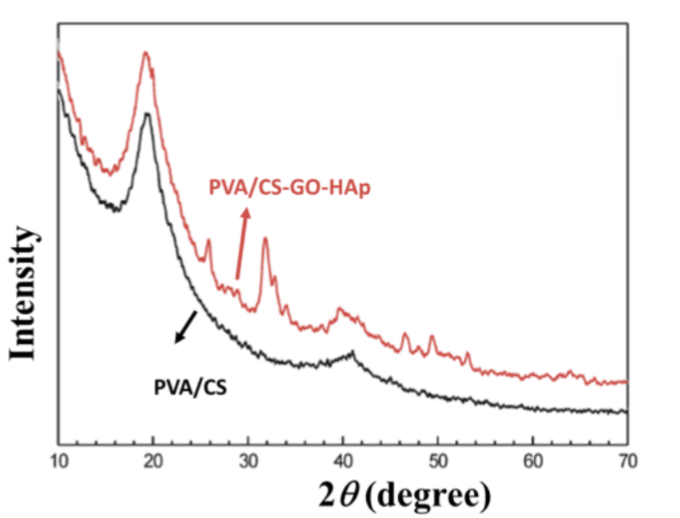


Fig 1: XRD analysis

## FTIR ANALYSIS

Fourier-transform infrared (FTIR) spectroscopy was used to analyze the chemical structure of electrospun PVA/Chitosan and PVA/Chitosan-GO-HAp mats, confirming the incorporation of graphene oxide (GO) and hydroxyapatite (HAp). The FTIR spectrum of PVA/Chitosan mats showed characteristic absorption bands, including O-H stretching (~3300 cm⁻¹), C-H stretching (~2900 cm⁻¹), and chitosan’s amide I and II bands (~1650 cm⁻¹ and ~1550 cm⁻¹). In PVA/Chitosan-GO-HAp mats, additional peaks indicated GO and HAp presence, such as C=O stretching (~1720 cm⁻¹) and C-O-C stretching (~1220 cm⁻¹) from GO, along with phosphate peaks (~1040 cm⁻¹ and ~560 cm⁻¹) confirming HAp incorporation. These findings validate the composite structure and its potential for enhanced hemostatic performance [(Ahmed, 2020)](https://paperpile.com/c/Z3HG5j/1Hhq).

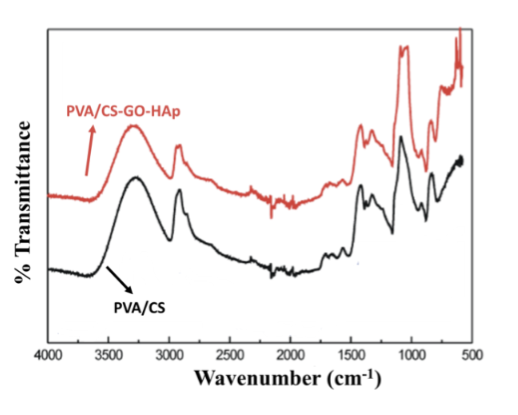


Fig 2: XRD analysis

## SEM ANALYSIS

Scanning electron microscopy (SEM) analysis of polyvinyl alcohol (PVA) and chitosan (CS) nanofibers revealed a smooth surface texture, characteristic of homogeneous fiber formation. In contrast, nanofibers incorporating graphene oxide (GO) and hydroxyapatite (HAp) exhibited a markedly different morphology under SEM, displaying small bead-like structures dispersed within the fibers and a roughened surface texture. Notably, the incorporation of GO and HAp led to a reduction in the diameter of the nanofibers, indicating a potential influence on fiber size during the fabrication process. These findings underscore the distinct structural changes induced by the addition of GO and HAp to PVA/CS nanofibers, highlighting their role in altering both surface characteristics and fiber dimensions in nanofibrous materials [(Sanchez-Alvarado et al., 2018)](https://paperpile.com/c/Z3HG5j/5qXx).

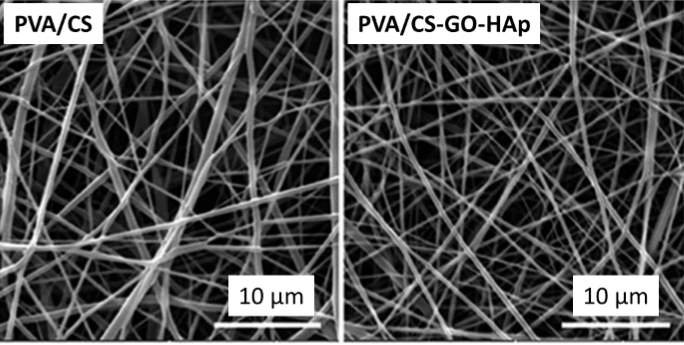


Fig 3: SEM

## HEMOCOMPATIBILITY

The non-toxic and non-immunogenic properties of PVA/CS nanofibers, particularly when enhanced with GO and HAp, render them highly suitable for interacting with blood components without triggering adverse reactions, as confirmed by hemocompatibility assays. This characteristic is crucial for biomedical applications where materials must interface with blood without causing harm or immune responses. The ability of these nanofibers to maintain hemocompatibility underscores their potential for use in various medical devices and therapies, promising safer interactions with biological systems in clinical settings [(Kamoun et al., 2021)](https://paperpile.com/c/Z3HG5j/yQJ0).

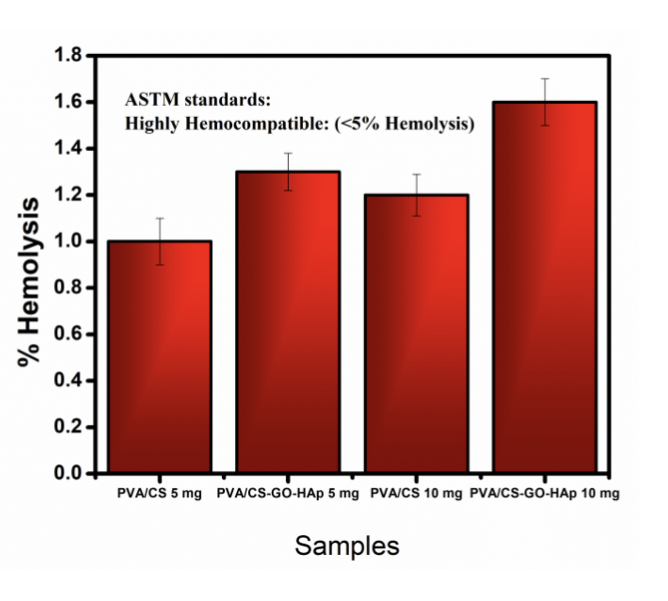


Fig 4: HEMOCOMPATIBILITY

## DRUG RELEASE

Incorporating graphene oxide (GO) and hydroxyapatite (HAp) nanoparticles into the PVA/CS matrix has been found to significantly increase the surface area available for drug loading, thereby enhancing the rates of drug release in drug delivery studies. This enhancement is attributed to the additional surface area provided by GO and HAp, which facilitates greater interaction with the drug molecules. Furthermore, the functional groups present on GO and HAp nanoparticles are believed to interact with clotrimazole, a common drug, potentially weakening the interactions between the drug and the polymer matrix(Rafi et al., 2024). This interaction phenomenon further contributes to the accelerated release of clotrimazole from the nanofibers. These findings underscore the role of nanomaterials in modifying drug release kinetics and highlight their potential in optimizing drug delivery systems for enhanced therapeutic efficacy [(Hoseini-Ghahfarokhi et al., 2020; Muthukumar et al., 2023)](https://paperpile.com/c/Z3HG5j/k5Pb+VFOP).

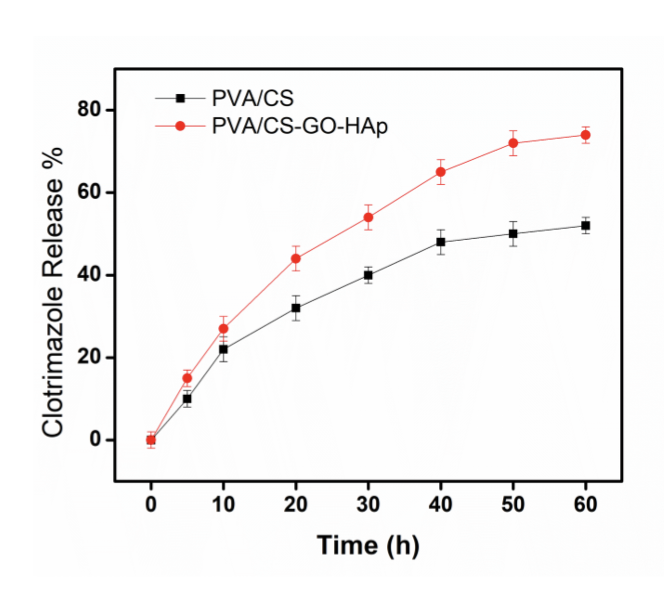


Fig 5: DRUG RELEASE

## MTT ASSAY

In the MTT assay, composite nanofiber membranes comprising polyvinyl alcohol (PVA), chitosan (CS), graphene oxide (GO), and hydroxyapatite (HAp) exhibited notable enhancement in cell viability, with viability levels exceeding 80% by day 3 of testing. This finding underscores the favorable environment these membranes create for cell proliferation and survival. The synergistic incorporation of GO and HAp nanoparticles within the PVA/CS matrix likely contributes to these positive outcomes by providing structural support and bioactive cues that promote cellular adhesion and growth (Tuluwengjiang et al., 2024). Such results are pivotal for biomedical applications where scaffolds or membranes must support cell growth while maintaining biocompatibility. The observed high cell viability suggests that these composite nanofiber membranes hold promise for tissue engineering and regenerative medicine applications, where fostering robust cell behavior is critical for successful clinical outcomes [(Dang et al., 2022)](https://paperpile.com/c/Z3HG5j/MUgz).

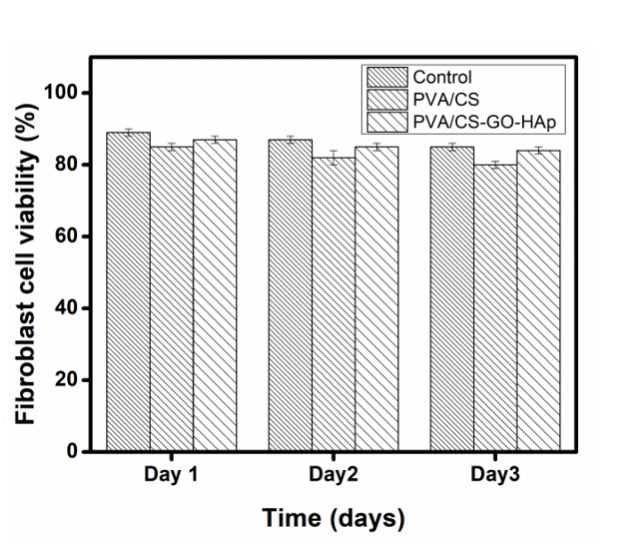


Fig 6: MTT ASSAY

# CONCLUSION

The analysis of PVA/Chitosan nanofiber composites reinforced with graphene oxide (GO) and hydroxyapatite (HAp) highlights their biomedical potential, particularly in hemostatic and drug delivery applications. X-ray diffraction (XRD) confirmed the structural integration of GO and HAp, while Fourier-transform infrared spectroscopy (FTIR) validated their chemical incorporation. Scanning electron microscopy (SEM) revealed enhanced fiber morphology, contributing to improved mechanical and bioactive properties. Hemocompatibility assays demonstrated non-toxicity and blood compatibility, essential for biomedical use. The increased surface area provided by GO and HAp significantly improved drug release kinetics. Additionally, MTT assays confirmed excellent cell viability, indicating the composite’s suitability for tissue engineering. These findings emphasize the composite’s multifunctionality, combining mechanical robustness, bioactivity, and biocompatibility, making it a promising candidate for advanced wound healing and regenerative medicine applications.

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