DEVELOPMENT OF A NOVEL GREEN NITRIC OXIDE-RELEASING WOUND DRESSING, CHARACTERIZATION AND IN VITRO STUDY FOR HEALING OF MSRA-INFECTED BIOFILMS AND DIABETIC FOOT ULCERS

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Introduction:

Non-healing wounds, particularly in diabetic patients, significantly affect quality of life and place a substantial burden on healthcare systems. Impaired vascularity, poor nutrition, and bacterial infections are key factors that hinder healing. With the growing threat of multidrug-resistant bacteria, traditional antibiotics are becoming less effective, underscoring the urgent need for alternative therapies. Nitric oxide (NO), a broad-spectrum antibacterial and anti-inflammatory agent, is highly promising due to its multi-targeted action and lack of resistance development. Innovative electrospun NO-releasing dressings made from biocompatible polymers like PCL, gelatin, and chitosan enhance wound healing. Green NO sources such as banana peel (rich in L-arginine, figure 1) support sustainable approaches. Additionally, hydrogel-forming powder dressings conform to irregular wound shapes and maintain optimal moisture. Combining NO donors with PDE5 inhibitors like TOP-N53 further boosts vascularization and tissue regeneration, paving the way for advanced clinical therapies.

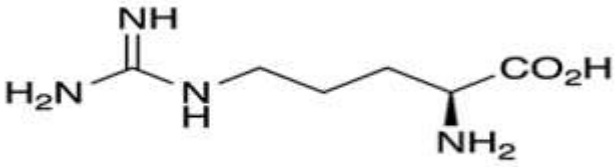

Figure 1

In this study, we present the fabrication and characterization of three biocompatible membrane blends functionalized with nitric oxide (NO) for their antimicrobial and wound-healing properties. The developed biocompatible NO-releasing films include: (1) S-Nitroso-N-acetylpenicillamine (SNAP)-encapsulated membranes, (2) L-Arginine-grafted membranes (a natural NO donor), and (3) L-Arginine-induced silver nanoparticle-impregnated membranes. These films were characterized using XRD, FTIR, SEM, and TGA techniques.

Additionally, in vitro studies were conducted to evaluate their anti-biofilm activity against MRSA-infected biofilms and their wound-healing potential in the context of diabetic foot ulcers (DFUs).

Objectives:

- To extract, isolate and purify the L-Arginine from banana peels and its spectral characterization to confirm the presence of purified tannic acid.

- To develop biocompatible nitrogen oxide (a key mediator of biofilm dispersal) releasing three films: S-Nitroso-N-acetylpenicillamine (SNAP) encapsulated, L-Arginine (natural source to release NO) grafted film and L-Arginine induced silver nano particle impregnated film and their characterization using XRD, FTIR, SEM, and TGA."

- To conduct in vitro study of the fabricated films (SNAP encapsulated, L-Arginine grafted and L-Arginine induced silver nano particle impregnated) to assess their anti-biofilm potential for MRSA-Infected Biofilms and healing efficacy for Diabetic Foot Ulcers.

Methodology:

Step 1:

Extraction of L-Arginine form waste banana peels and their characterization:

- Banana peels will be collected from the fruit Juice centres, washed, dried, and converted into air-dried powder. This material will be subjected to the extraction, isolation, and purification of L-Arginine (Arg) using high-performance liquid chromatography (HPLC). Spectral characterization will be conducted using techniques such as liquid chromatography–mass spectrometry (LC-MS) and nuclear magnetic resonance spectroscopy (NMR).

- For the insitu preparation of green silver nanoparticles, a standard procedure will be followed with slight modifications (Prem Raj, Meena, Arvind Pratap Singh, and Kiran KumarKSCST: Student Project Programme: 48th series: 2024-2025 3 Tejavath, (2020), Biosynthesis of Silver Nanoparticles Using Cucumis prophetarum Aqueous Leaf Extract and Their Antibacterial and Antiproliferative Activity Against Cancer Cell Lines, ACS Omega, 5 (10), 5520-5528, DOI: 10.1021/acsomega.0c00155).

Step 2:

Fabrication of biocompatible, green films, their characterization and assessment of nitric oxide (NO) release profile S-Nitroso-N-acetylpenicillamine (SNAP) will be synthesized using a previously reported protocol with minor adjustment (Pant, J. et al., (2017), Tunable Nitric Oxide Release from S-Nitroso-NAcetylpenicillamine Via Catalytic Copper Nanoparticles for Biomedical Applications. ACS Appl. Mater. Interfaces, 9, 15254−15264). To develop biocompatible nitrogen oxide releasing films: SNAP encapsulated (SNAP_CS), L-Arginine grafted film (Arg_CS) and L-Arginine induced silver nano particle impregnated (Arg_AgNPs_CS) biodegradable films, any one of the methods such as solvent casting can be explored.

(Borbolla-Jiménez, F. V.; Peña-Corona, S. I.; Farah, S. J.; Jiménez-Valdés, M. T.; Pineda-Pérez, E.; Romero-Montero, A.; Del Prado-Audelo, M. L.; Bernal-Chávez, S. A.; Magaña, J. J.; Leyva-Gómez, G. Films for Wound Healing Fabricated Using a Solvent Casting Technique. Pharmaceutics 2023, 15 (7), 1914. https://doi.org/10.3390/pharmaceutics15071914.

Further characterization of developed films will be carried out using several spectral techniques such as XRD, FTIR, FE-SEM and TGA. The in vitro nitric oxide (NO) release profile of the developed films will be evaluated using simulated wound fluid (SWF) based on an established protocol that mimics real wound conditions. (Jawal Said, Cornelius C. Dodoo et al, (2014), An in vitro test of the efficacy of silvercontaining wound dressings against Staphylococcus aureus and Pseudomonas aeruginosa in simulated wound fluid. International Journal of Pharmaceutics, 462 (1–2), pp 123-128).

Step 3:

In vitro studies: Antibiofilm activity, antibacterial activity and diabetic foot ulcer healing assessment. For this objective in vitro studies of different biological activities will be carried out as per the standard protocol with slight modifications. The film's efficacy in dispersing in vitro MRSA biofilm will be evaluated using the crystal violet staining method, as biofilm formation on wounds significantly impedes healing. (A. Asli, E. Brouillette, et al. (2017) Antibiofilm and antibacterial effects of specific chitosan molecules on Staphylococcus aureus isolates associated with bovine mastitis, PloS one 12, e0176988. https://doi.org/10.1371/journal.pone.0176988).

Similarly, the diabetic foot ulcer healing efficacy of the developed film will be determined following the standard procedure of the conventional 2D scratch wound healing assay using human keratinocytes cultured under hyperglycaemic conditions.

Result and Conclusion:

- Arginine (Arg) was successfully extracted, isolated, and purified using high-performance liquid chromatography (HPLC). The purity of Arg was confirmed by spectral characterization through LC-MS and ^{1}H NMR. Using the purified Arg, green synthesis of silver nanoparticles was achieved and samples were submitted for further characterization.

- S-Nitroso-N-acetylpenicillamine (SNAP) was also successfully synthesized and submitted for LC-MS and ^{1}H NMR analysis to confirm its structure. Three types of biocompatible NO-releasing films are currently under fabrication: (1) SNAP-encapsulated membranes, (2) L-Arginine-grafted membranes, and (3) L-Arginine-induced silver nanoparticle-impregnated membranes.

- Characterization of the films using XRD, FTIR, FE-SEM, and TGA is planned.
- The nitric oxide (NO) release profile will be evaluated in vitro using simulated wound fluid (SWF). In vitro biological assays will assess the films' efficacy against MRSA biofilms using the crystal violet staining method, addressing biofilm-associated wound healing delays. Additionally, the wound-healing potential of the films will be tested using a standard 2D scratch assay with human keratinocytes cultured under hyperglycaemic conditions, simulating diabetic foot ulcers.
- These studies aim to explore the feasibility of NO-releasing biocompatible membranes as multifunctional wound dressings with antimicrobial and regenerative properties.
- Overall, the developed films hold promising prospects for future development as effective antimicrobial wound dressings.

Future Scope:

- Advanced Material Characterization: Detailed Morphological Analysis: By using AFM and TEM to analyze dressing surface, porosity, and NO-agent distribution, linking structure to release kinetics and interactions with biofilms and tissue.
- Optimization of Nitric Oxide Release for Controlled and Sustained Release:
- Optimization of NO release by adjusting matrix composition, NO donor type/concentration.
- Pre-clinical In Vivo Studies:MRSA-infected Diabetic Animal Models:
- To assess the optimized NO-releasing dressing in diabetic in vivo models of MRSA-infected DFUs to evaluate wound healing, bacterial reduction, tissue regeneration, and safety in complex biological environments.