FROM SYNTHESIS TO APPLICATION: PEPTIDE-FUNCTIONALIZED NANOFORMULATIONS FOR BIOMEDICAL APPLICATIONS

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Background

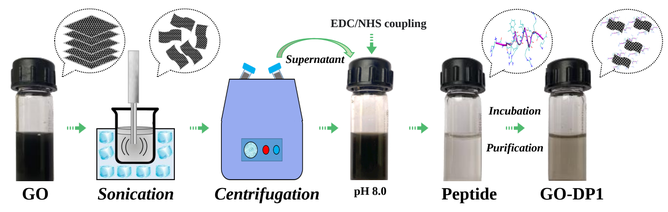
- **Healthcare-Associated Infections** (HAI) are nosocomially acquired infections that are not present or incubating at the time of admission to a hospital.
- One out of every 100 patients in acute-care hospitals, seven patients in high-income countries and 15 patients in low- and middle-income countries acquire at least one HAI during their hospital stay (WHO).
- **Need of the hour:** Development of antibacterial nanocoating's to prevent the spread of infections.
- **Antimicrobial peptides** have the potential of exhibiting broad-spectrum antibacterial and immunomodulatory activity with a low propensity of bacteria to develop resistance towards them.
- **In this study, a novel cationic peptide DP1 (RFGRFLRKILRFLKK) was covalently conjugated with nano sized graphene oxide (GO) and reduced graphene oxide (rGO) to synthesize a biotic-abiotic platforms (GO-DP1 and rGO-DP1) that are biocompatible and at the same time demonstrates significant antibacterial and antibiofilm activity**[1-3].
- These peptide based nanoformulations were explored for their ability to function as an antibacterial additive for commercial paint production (Indian Patent Office, Application No. 202211011934 A), anti-pseudomonal bandage and as an antibiofilm coating on indwelling catheters[4,5].

Objectives

- Synthesis and biophysical characterization of a stable peptide-functionalized GO/rGO nanoformulations.
- *In-vitro* biological evaluation of peptide-functionalized nanoformulations.
- Evaluation of anti-bacterial and anti-biofilm efficacy of the nanoformulations.
- Biomedical application of the nanoformulation in *P. aeruginosa* contaminated hypodermic needle model.

Methodology

Synthesis of peptide-functionalized GO nanoformulation



Evaluation of physico–chemical properties

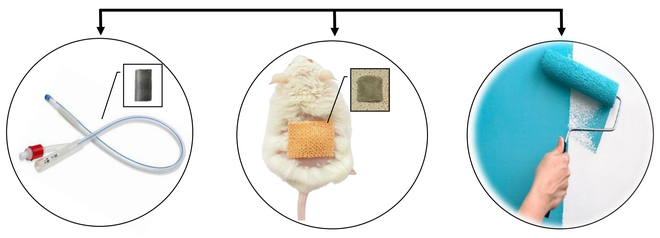
Spectroscopic	Microscopic	Wettability	Charge	Thermal stability
XRD; FT-IR; EDS	FESEM HR-TEM	WCA	Zeta potential	TGA

Evaluation of bio nano conjugates as an effective antimicrobial and antibiofilm platform

Evaluation of hemolytic toxicity against red blood cells

Evaluation of cytotoxicity by 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay

Peptide-functionalized nanoformulations based biomedical applications to control the spread of healthcare-associated infections



Results and Discussion

Characterization of GO-DP1 nanoformulation

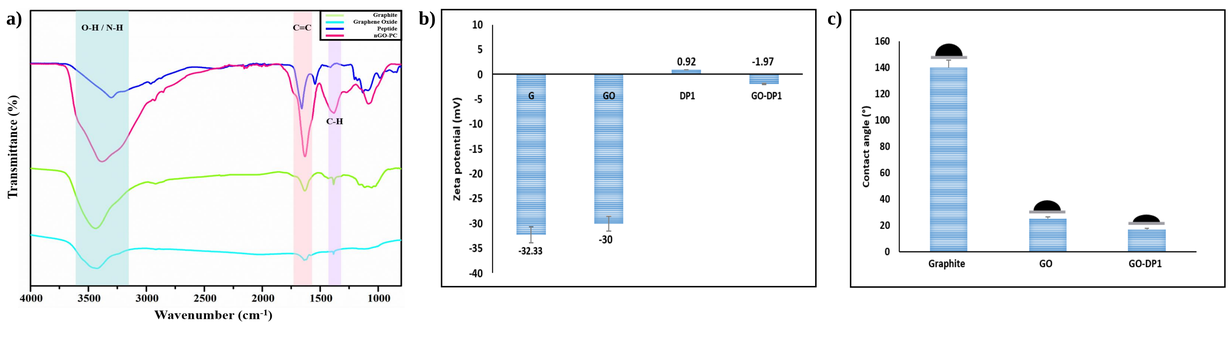


Fig. 1: Characterization of GO-DP1 nanoformulation by fourier-transform infrared spectroscopy (a), zeta potential (b) and water contact angle (c)[1].

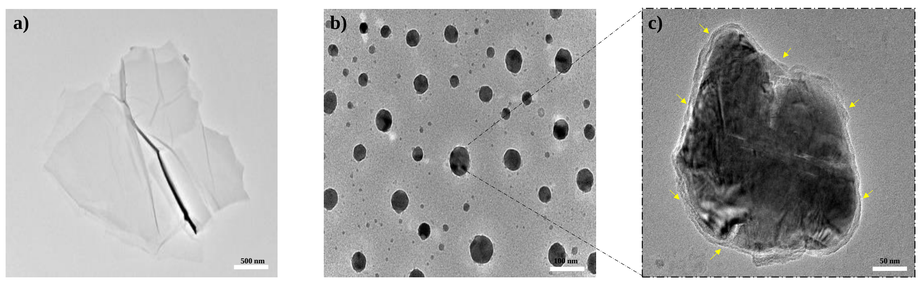


Fig. 2: High-resolution transmission electron micrographs of graphene oxide (d) and GO-DP1 nanoformulation (b,c)[1].

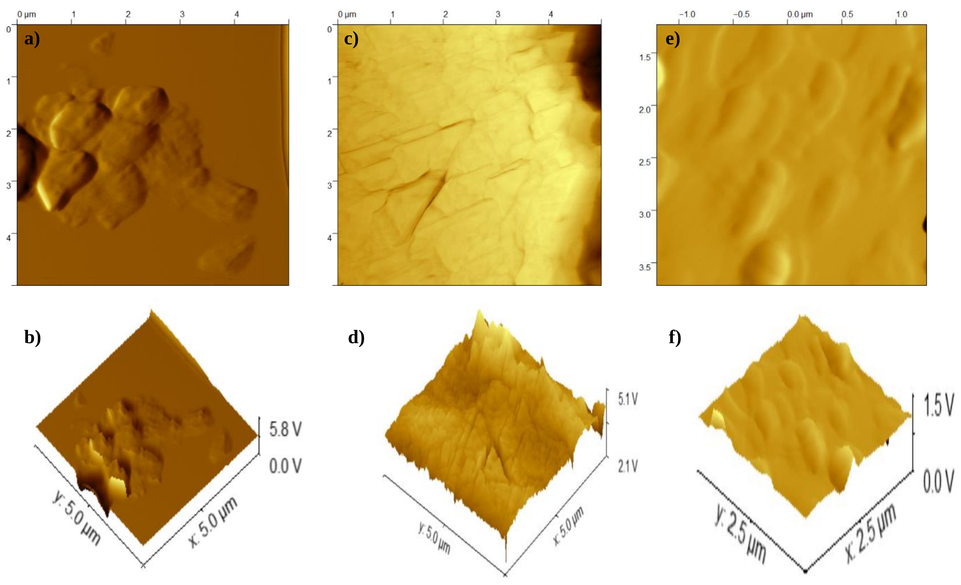


Fig. 3: AFM micrographs and 3D topology of graphite (a-b), GO (c-d) and GO-DP1 (e-f)[1].

Biomedical applicability of GO-DP1 nanoformulation as an antibiofilm coating

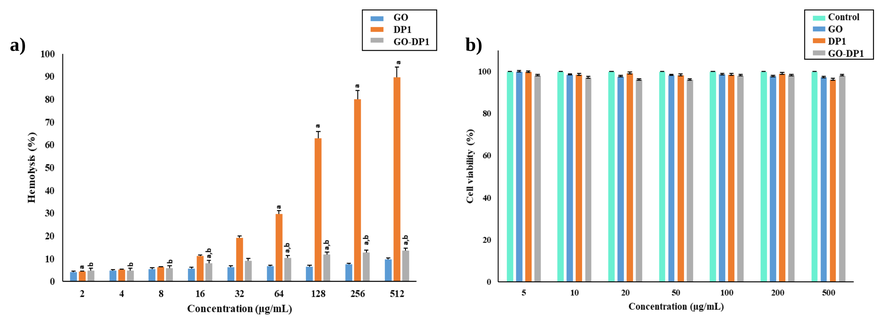


Fig. 4: *In-vitro* biocompatibility assessment via determination of hemolytic activity (a) and cell viability towards 3T3-L1(b) at different concentrations. Values represent mean ± SD (n = 6)[1].

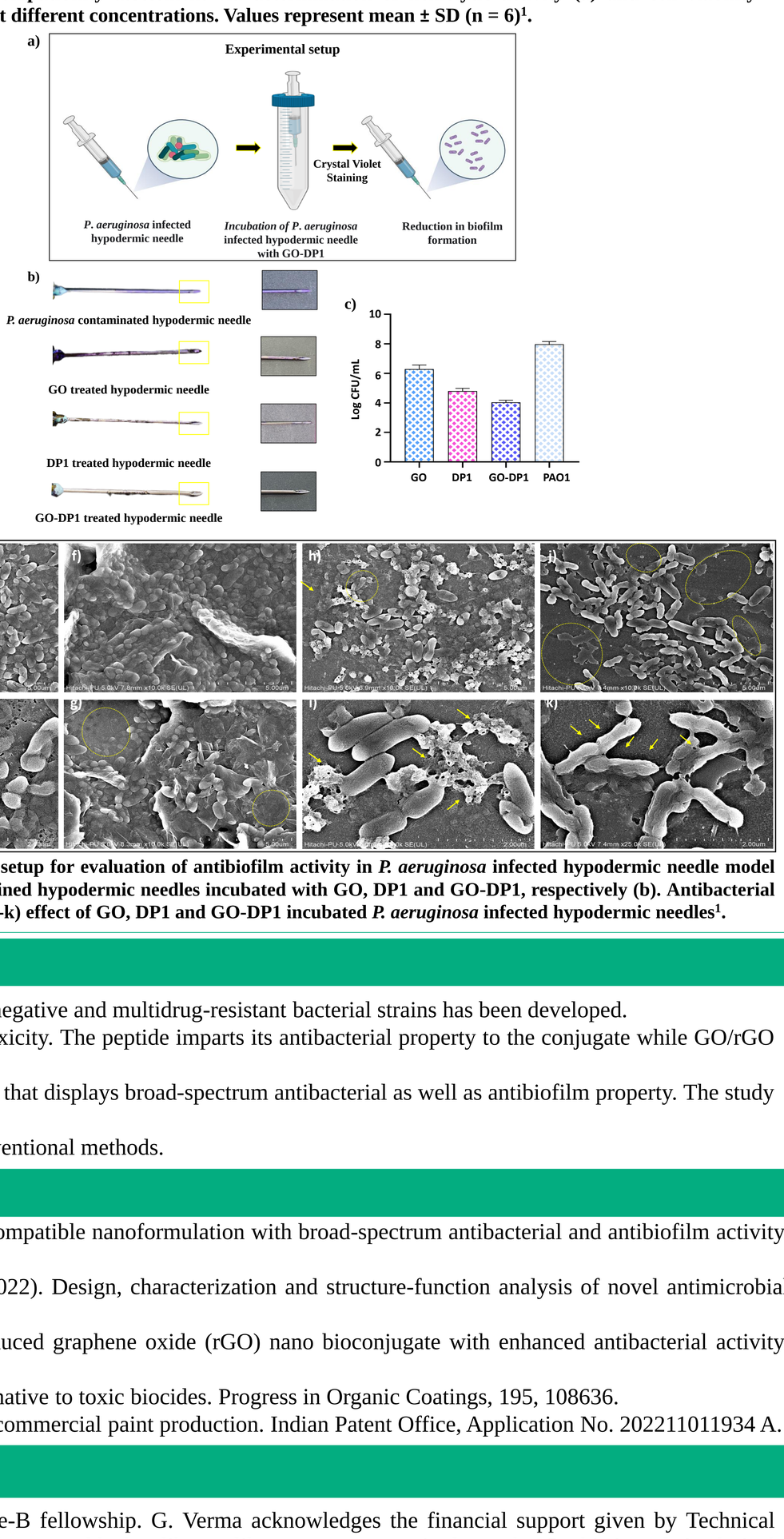


Fig. 5: Experimental setup for evaluation of antibiofilm activity in *P. aeruginosa* infected hypodermic needle model (a). Crystal violet-stained hypodermic needles incubated with GO, DP1 and GO-DP1, respectively (b). Antibacterial (c) and antibiofilm (d-k) effect of GO, DP1 and GO-DP1 incubated *P. aeruginosa* infected hypodermic needles[1].

Conclusion

- Biocompatible nanoformulations of GO-DP1 and rGO-DP1 with superior antibacterial properties against Gram-positive, Gram-negative and multidrug-resistant bacterial strains has been developed.
- Peptide DP1 and graphitic materials (GO and rGO) complimented each other in terms of antibacterial activity and hemolytic toxicity. The peptide imparts its antibacterial property to the conjugate while GO/rGO contributes by lowering the hemolytic toxicity.
- The synergy between the two materials led to the development of a one-of-a-kind non-hemolytic, antibacterial nanoformulations that displays broad-spectrum antibacterial as well as antibiofilm property. The study reports biomedical applicability of GO-DP1 as an antibiofilm nanocoating in *P. aeruginosa* infected hypodermic needles.
- Overall, the nanoformulations constitute highly promising system to combat bacterial infections that are difficult to treat by conventional methods.

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