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Engineering antimicrobial peptides through nanocarrier surface design and intracellular targeting for tuberculosis therapy

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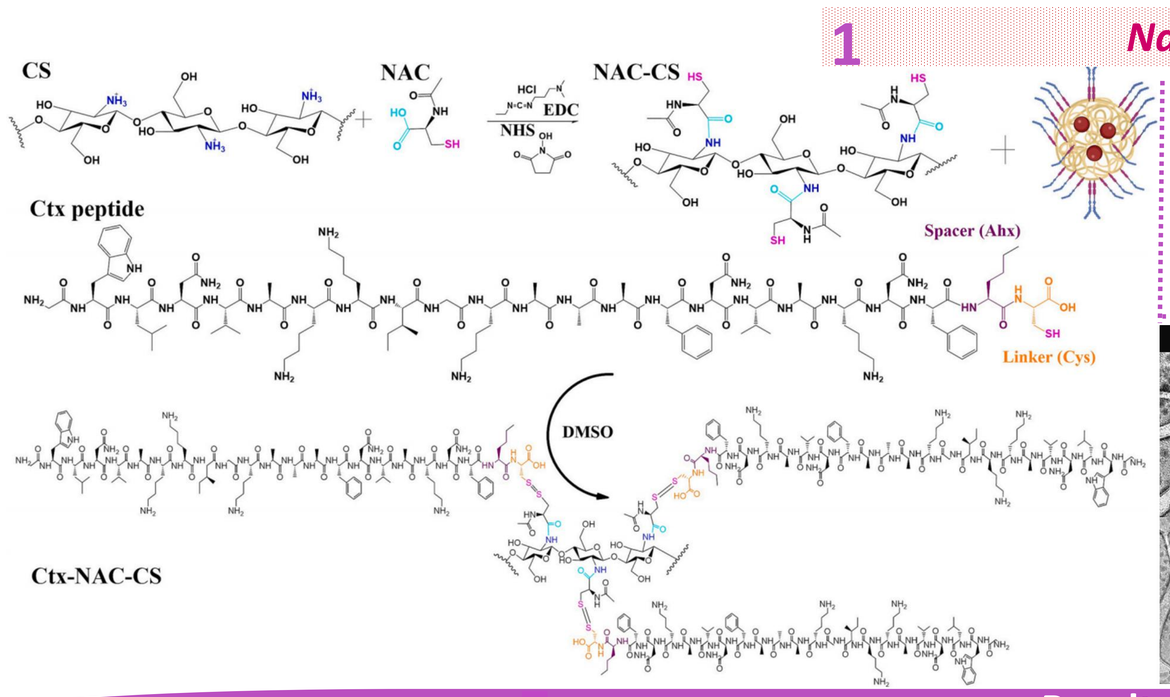
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Introduction

The emergence of multidrug-resistant *Mycobacterium tuberculosis* highlights the urgent need for therapeutic strategies capable of overcoming intracellular persistence and antibiotic failure. Although antimicrobial peptides exhibit potent antibacterial activity, their clinical translation remains limited by instability, rapid degradation, and inefficient delivery to infected macrophages. A nanotechnology-centered strategy is presented to examine how nanocarrier architecture can modulate and enhance antimicrobial peptide performance against tuberculosis.

Materials and Methods



Results and Discussions

Biological properties

Table 1. MIC values of antibiotics and NP formulations by the REMA method after 7 days of exposure.

Code	Formulation	H37RV (µg/mL)	CF 169 (µg/mL)	CF 110 (µg/mL)
INH	Isoniazid	0.5–1.0	>50	>50
RIF	Rifampicin	0.5	>25	16.29
GAT	Gatifloxacin	0.03–0.25	1.2	<0.05
MOX	Moxifloxacin	0.03–0.5	1.6	<0.05
OFL	Ofloxacin	0.71	11.5	0.3
AMK	Amicacin	0.5–1.0	0.6	0.5
CAP	Capreomycin	2.0	1.4	3.0
KAN	Kanamycin	2.0	1.5	2.8
NP1	NAC-CS	NA	NA	NA
NP2	CS@RIF[a]	<0.977	NA	NA
NP3	Ctx-NAC-CS	NA	NA	NA
NP4	NAC-CS@RIF[a]	1.47	199.05	NA
NP5	CS	NA	NA	NA
NP6	Ctx-CS	NA	NA	NA
NP7	Ctx-CS@RIF[a]	<0.977	104.34	NA
NP8	Ctx-NAC-CS@RIF[a]	<0.977	0.977	<0.977
Ctx	Ctx(Ile^{21})-Ha-Ahx-Cys	7.57	12.24	>250
Ctx wild-type	Ctx(Ile^{21})-Ha	14.0	NT	NT
NAC	*N*-acetylcysteine	NA	NA	NA

NA: no activity. NT: not tested.
CF169 strain: XDR MTB. CF110 strain: MDR MTB.
[a] MIC values based on RIF concentration. All NPs were tested based on their weight.

Table 2. Antitubercular activity, cytotoxicity in mammalian cells, and selectivity indices for peptides. All values are expressed in micromolar (µm).

AMPs	$H_{37}Rv$	IC_{50} MRC-5	IC_{50} J774A.1	SI MRC-5	SI J774A.1
DC05	12.46 ± 0.06	>250	115.18 ± 8.49	>20.05	9.24 ± 0.68
DC08	37 ± 2.07	>250	229.78 ± 4.08	>6.76	6.21 ± 0.36
DC09	49 ± 2.89	>250	236.77 ± 4.57	>5.10	4.83 ± 0.30
DC10	24 ± 1.72	>250	211.08 ± 3.63	>10.41	8.79 ± 0.65
CPP	>50	>250	205.9 ± 6.5	n.d	≤ 4.12

Table 3. Antimycobacterial activity of MSN-based nanoplatforms.

MIC of MSN formulations		
Nanoplatform	MIC values (Mtb $H_{37}Rv$)[a]	MIC values (Mtb $H_{37}Rv$)[b]
MSN	N.A.	–
MSN-CPP	N.A.	–
MSN@DC05	205 µg/mL	7.74 µg/mL
MSN-CPP@DC05	284.66 µg/mL	10.75 µg/mL

a MIC values calculated based on the total nanoparticle mass required to inhibit *M. tuberculosis* H37Rv. **b** MIC values calculated based on the effective peptide content encapsulated within each formulation. **N.A.** not active under the tested conditions.

Figure 1. Confocal analysis of internalization of FITC-NPs in macrophages after incubation for 24 h.

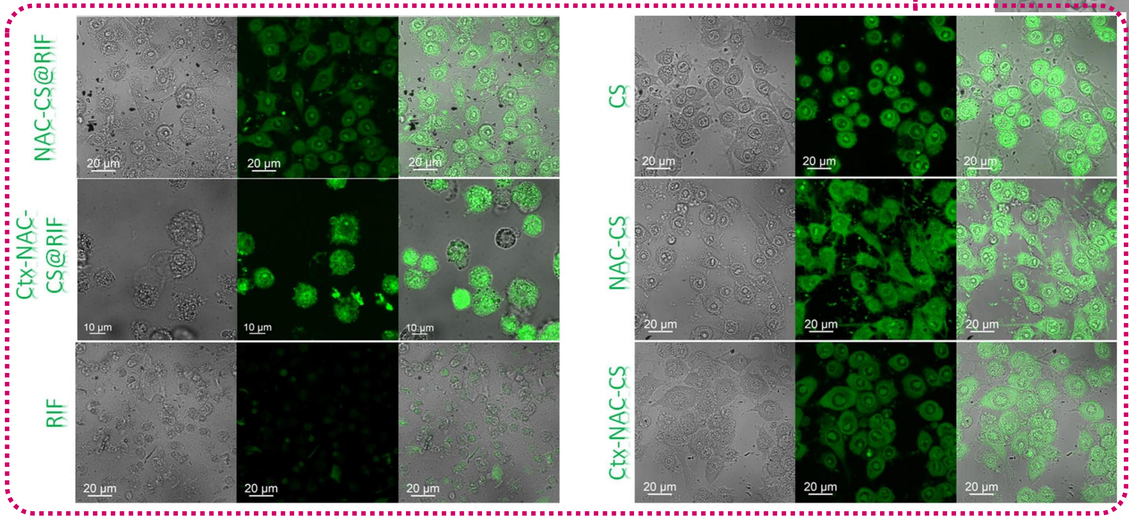


Figure 2. Confocal analysis of internalization of FITC-NPs in macrophages after incubation for 24 h.

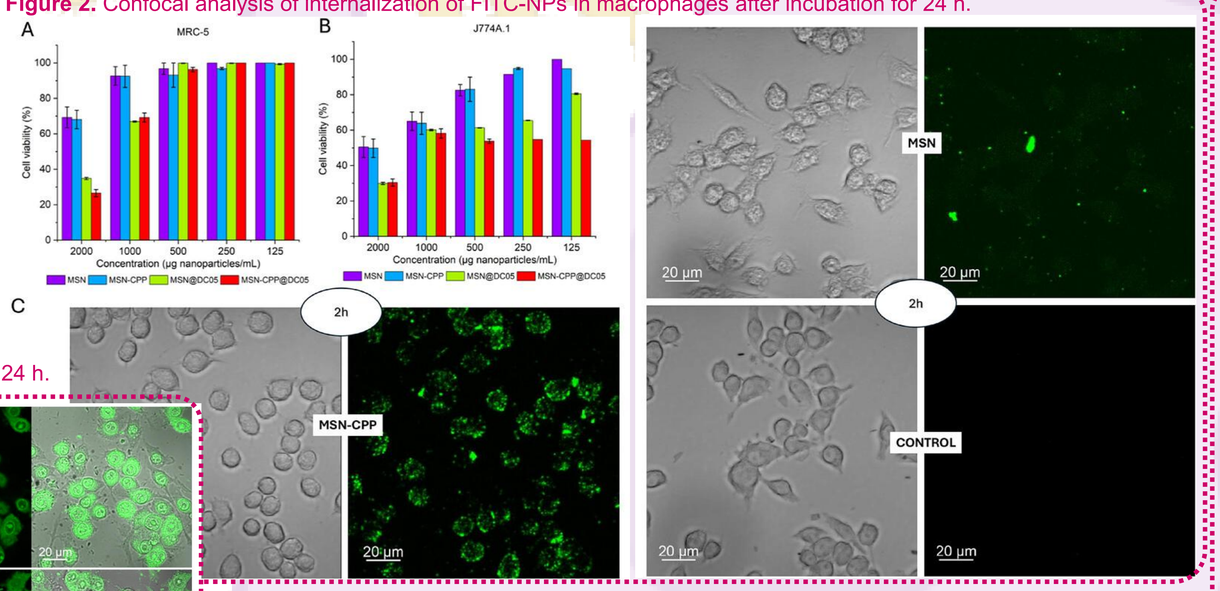
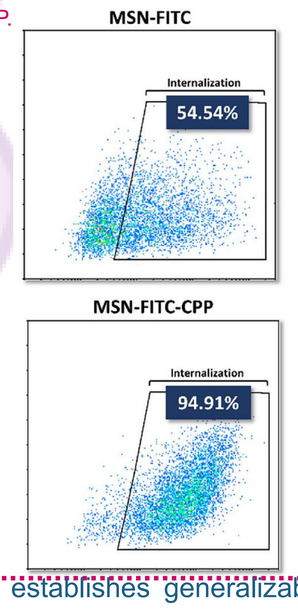


Figure 3. Representative flow cytometry dot plots showing untreated cells (control) and cells exposed to MSN-FITC or MSN-FITC-CPP.



Conclusions

By integrating peptide engineering with advanced nanomaterial design, this work establishes generalizable principles for next-generation peptide-based therapeutics targeting intracellular pathogens and provides a strategic foundation for precision antimicrobial interventions against resistant tuberculosis.

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References

1. Primo, L. M. D. G., Roque-Borda, C. A., Canales, C. S. C., Caruso, I. P., de Lourenço, I. O., Colturato, V. M. M., ... & Pavan, F. R. (2024). Antimicrobial peptides grafted onto the surface of N-acetylcysteine-chitosan nanoparticles can revitalize drugs against clinical isolates of Mycobacterium tuberculosis. Carbohydrate Polymers, 323, 121449.2. Roque-Borda, C.A. et al. Chitosan and HPMCAS double-coating as protective systems for alginate microparticles loaded with Ctx(Ile21)-Ha antimicrobial peptide to prevent intestinal infections. Biomaterials 2023, 293, 121978.
2. Carnero Canales, C. S., Roque-Borda, C. A., Negri, A. C. C., Delgado, O. R., Catarin Nunes, L. O., Resende, F. A., ... & Pavan, F. R. (2026). Dual-Peptide Nanoplatform: Mesoporous Silica Nanoparticles Functionalized With a Cell-Penetrating Peptide and Loaded With Rationally Designed Antimicrobial Peptides for Tuberculosis Therapy. Advanced Healthcare Materials, 15(21), e04285.