Supplementary Information (SI) for Journal of Materials Chemistry B.

Supporting Information

Enhanced Antimicrobial Protection through Surface Immobilization of Antibiotic-Loaded Peptide Multicompartment Micelles

Shabnam Tarvirdipour[a,b], S. Narjes Abdollahi[a], Joachim Köser[c], Maryame Bina[a], Cora-Ann Schoenenberger[a], Cornelia G. Palivan[a,b*]

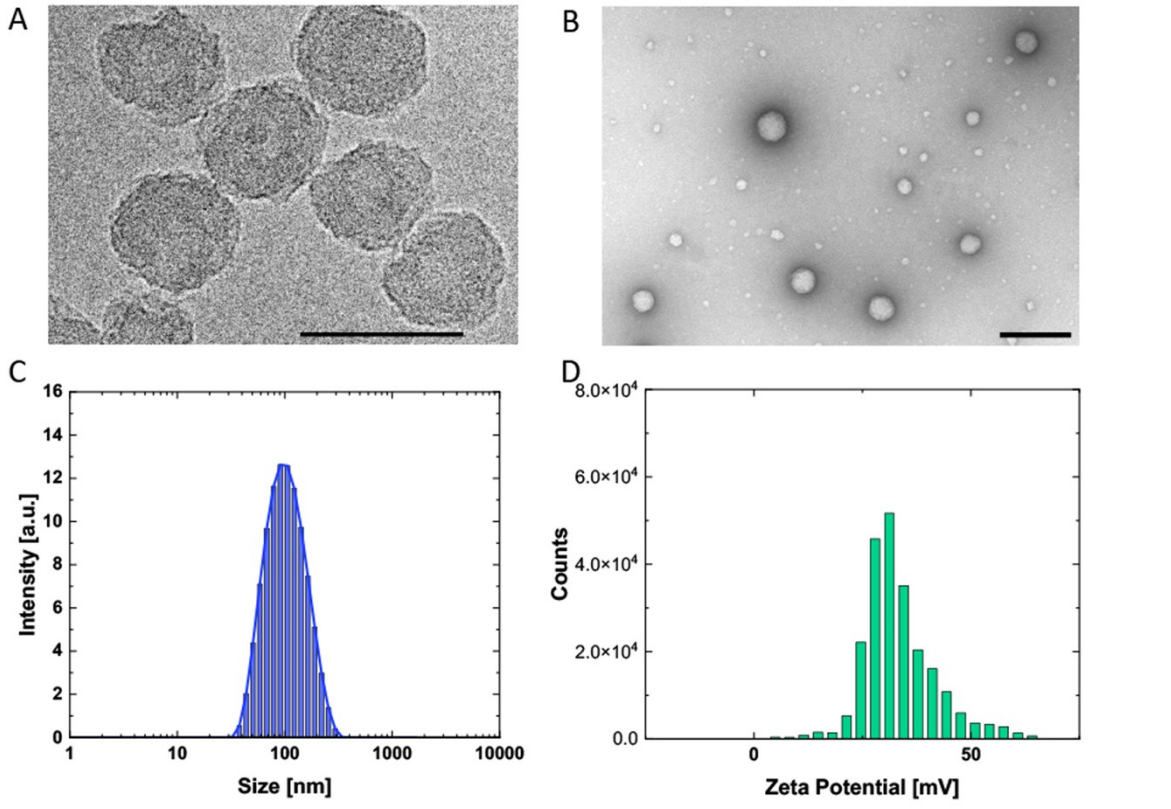

Fig. S1. A) CryoTEM image of RIF-MCMs, scale bar = 100 nm. B) Negatively stained TEM overview, C) DLS size distribution, and D) Zeta potential of unloaded MCMs, scale bar = 200 nm.

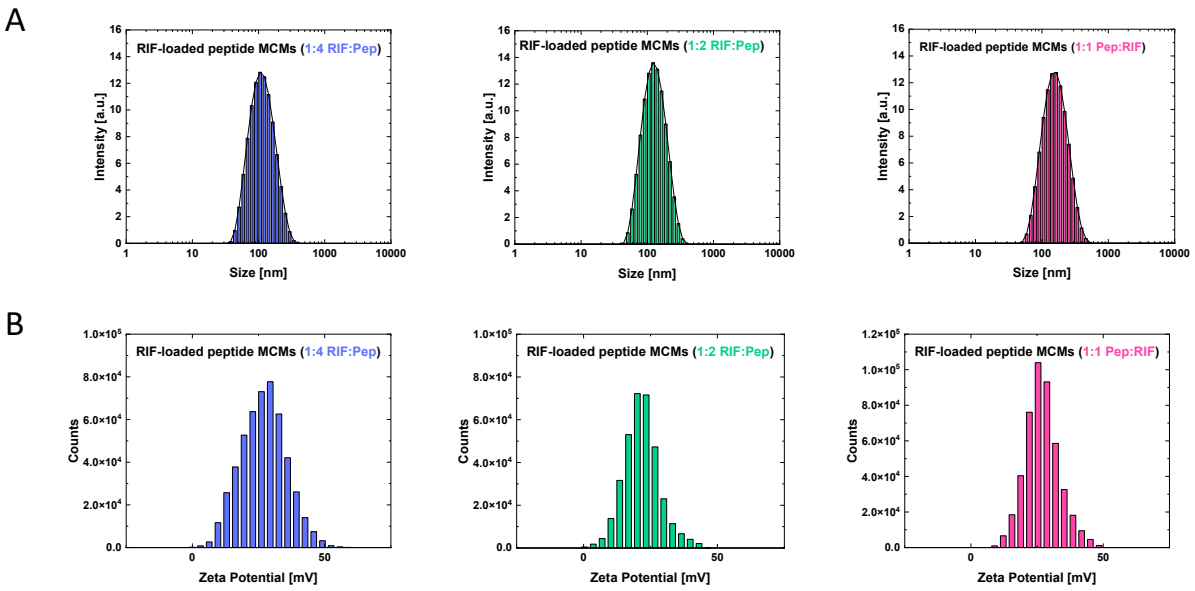

Fig. S2. (A) Size distribution and (B) zeta potential distribution of RIF-MCMs formed at different mass ratios of rifampicin to peptide (1:4, 1:2, and 1:1 RIF:peptide).

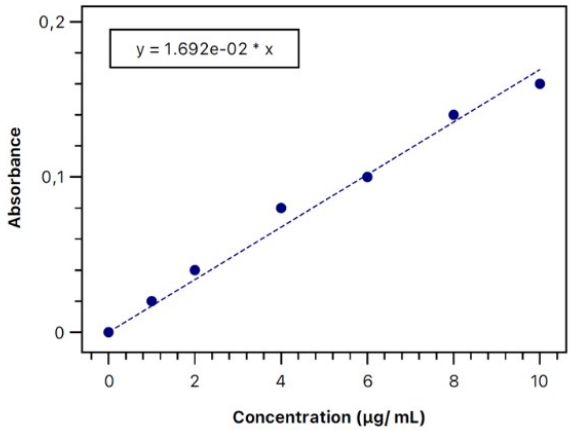

Fig. S3. Standard curve of free RIF in dimethyl sulfoxide (DMSO).

Table S1. Concentration of RIF-MCMs determined by NTA and calculated encapsulation efficiencies (EEs).

RIF-loaded MCMs	Concentration (particles/mL)*10^9	EE (%)
1:4 RIF:Pep	12.6 ± 0.9	46
1:2 RIF:Pep	9.8 ± 1.6	84
1:1 RIF:Pep	4.4 ± 0.6	67

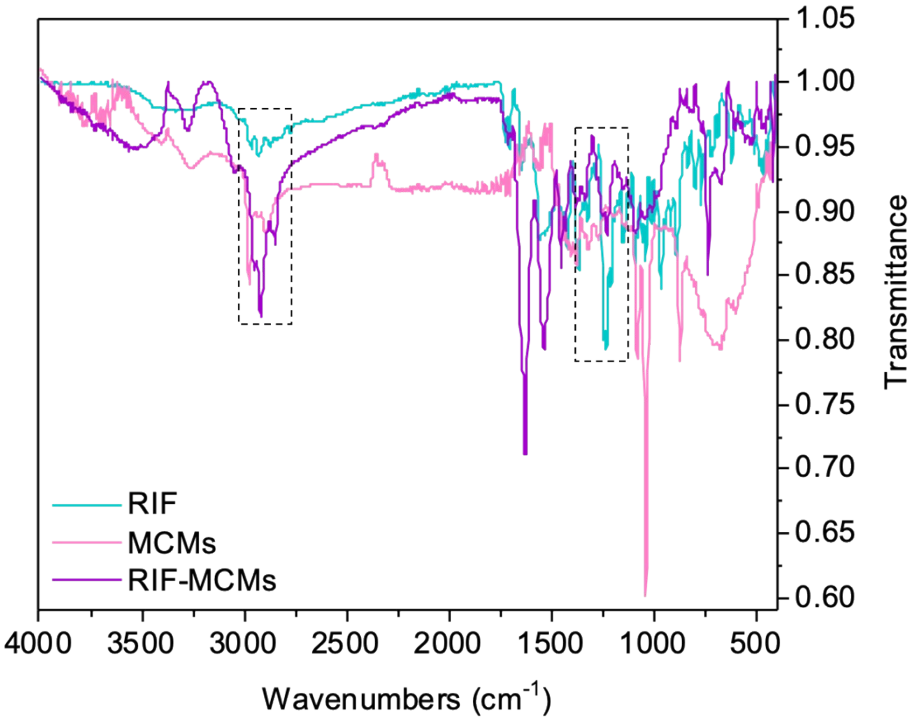

Fig. S4. FTIR spectra of RIF, MCMs and RIF-MCMs (1:2 RIF:Pep).

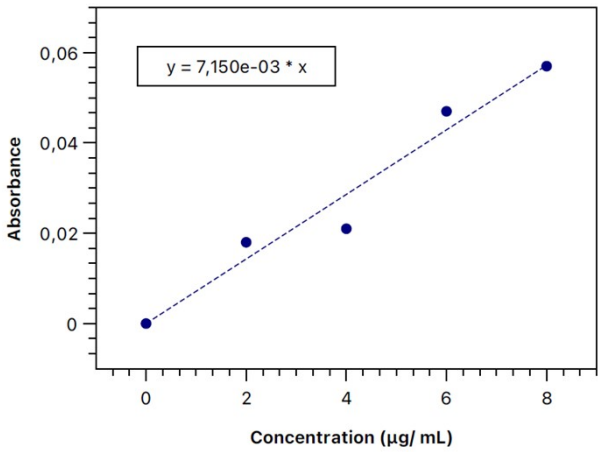

Fig. S5. Standard curve of free RIF in HEPES buffer (25 mM HEPES, pH 7.4, containing 150 mM NaCl and 0.5% ascorbic acid).

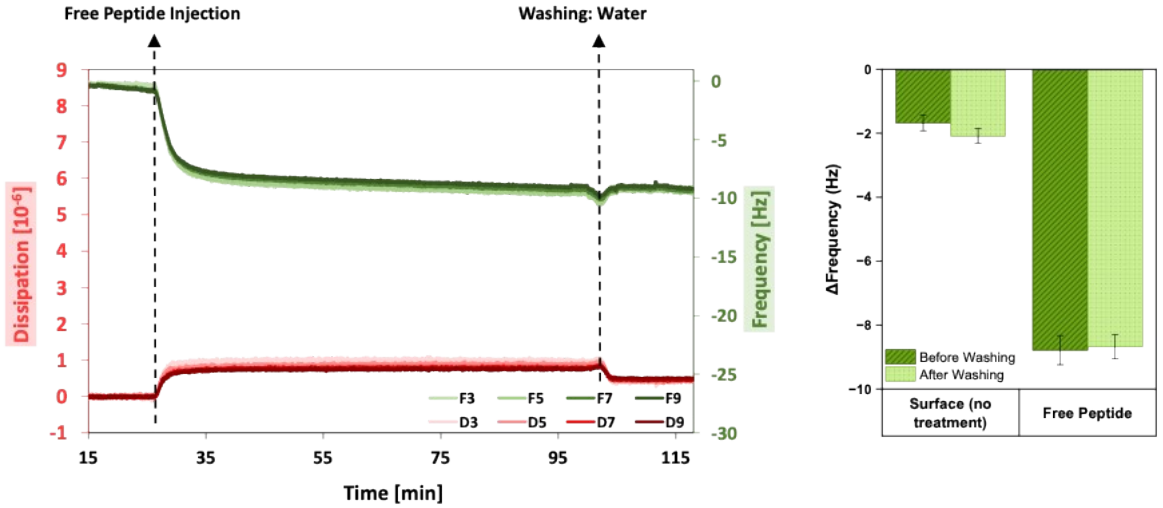

Fig. S6. QCM-D profiles for the deposition of free peptide, followed by a washing step with water at room temperature. The frequency variations were recorded for the 3rd, 5th, 7th, and 9th harmonics. The right panel displays the frequency shifts associated with the binding of free peptide to the solid support, before and after the washing step.

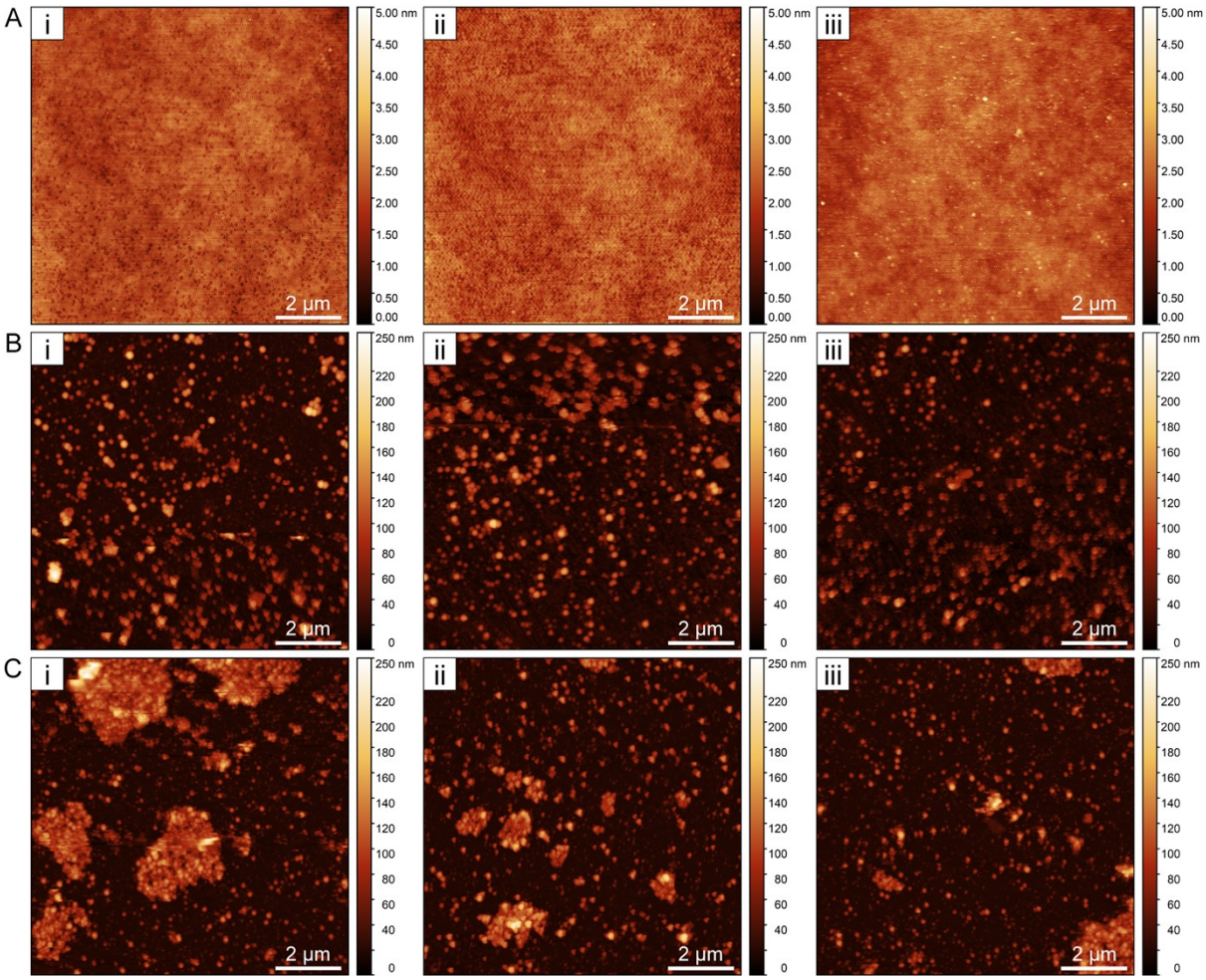

Fig. S7. AFM topography of bare glass substrate (Ai-iii), and unloaded MCMs (Bi-iii) and RIF-MCMs (Ci-iii) immobilized on a glass surface. For each sample, three randomly selected areas were recorded.

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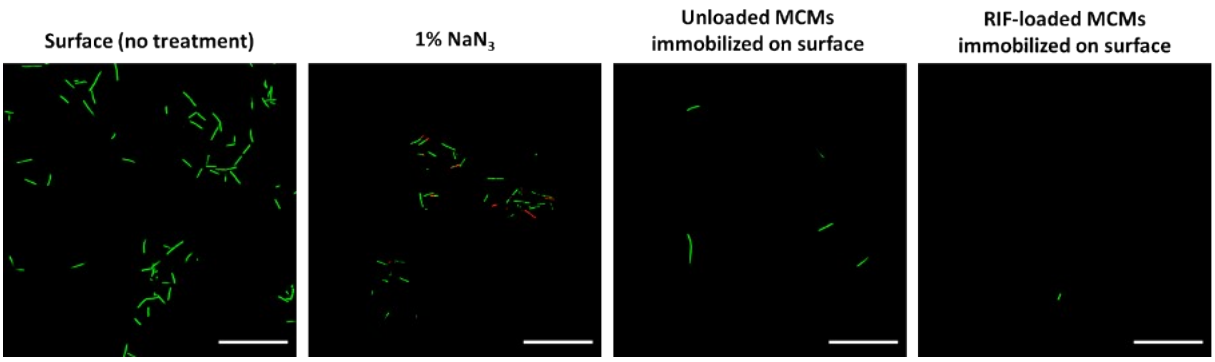

Fig. S8. CLSM micrographs of *Bacillus subtilis*-incubated surfaces. Substrates were immersed in a bacterial suspension (10^7 CFU mL^{-1}) and incubated for 4 h at 37 °C. Bacteria were stained with Syto 9 (green, live cells) and PI (red, dead cells). Scale bar = 50 µm.

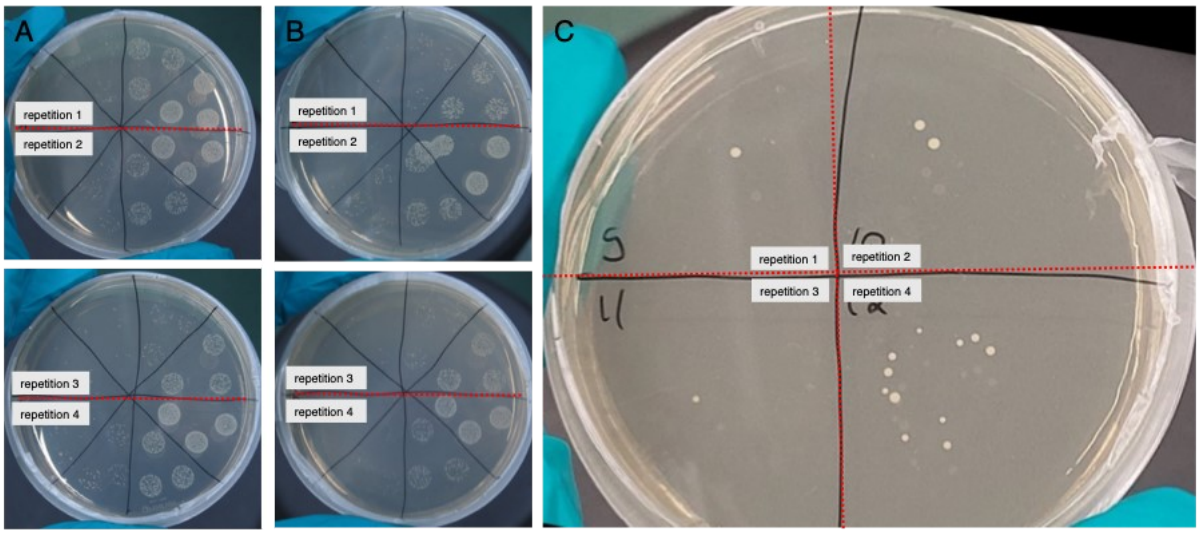

Fig. S9. Image of agar plates showing a dilution series of *S. aureus* bacteria retrieved from: (A) untreated, (B) MCM-modified, and (C) RIF-MCM modified surfaces using antimicrobial assays in accordance with *ISO 22196* standards. For untreated and MCM-modified surfaces, 10 µl aliquots were plated in triplicates for each dilution, with the highest dilution displayed on the left side of each plate. For RIF-MCM-modified surfaces, single 100 µl aliquots were plated.

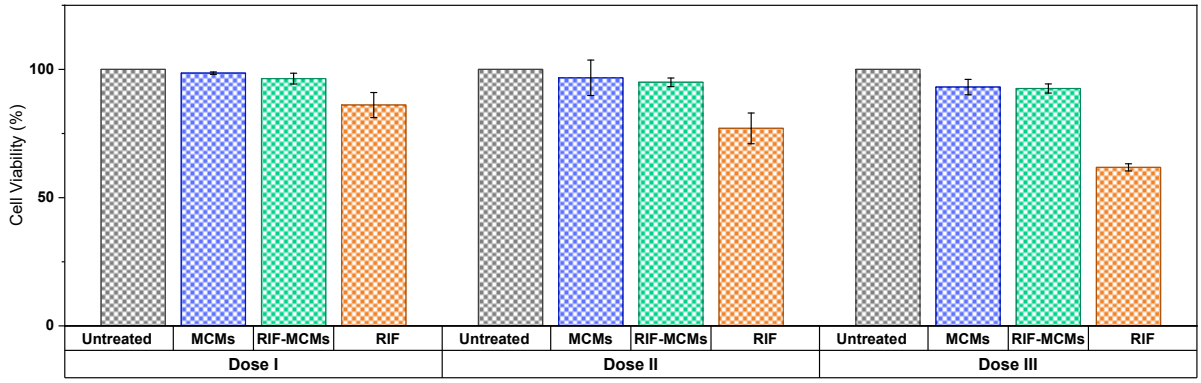

Fig. S10. Effect of MCMs and RIF on human cell viability. The proliferation of A549 cells after 48 h of treatment with Dose I, Dose II, and Dose III at 37 °C was assessed using MTS assays. For RIF-MCMs, dose I corresponds to the concentration applied for full surface coverage with RIF-MCMs, dose II is double this concentration, and dose III triple. For unloaded MCMs, doses I to III have peptide concentrations equivalent to those used for RIF-MCM treatment. Doses I, II and III of free RIF correspond to the amount of RIF entrapped in the respective doses of RIF-MCMs. Control cells were incubated correspondingly but without treatment. Error bars represent standard deviation of triplicate measurements.