Antimicrobial Nanocomposite Coating for Rapid Intervention of Catheter-Associated Urinary Tract Infections

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Materials and methods

Spectrochem provided reagent grade chloroform ($CHCl_3$), dichloromethane (DCM), ethanol (EtOH), tert-butanol (t-BuOH), and methanol (MeOH) (India). Isopropanol (IPA) of HPLC quality was obtained from Spectrochem. Sigma Aldrich provided dimethyl sulphoxide (DMSO) and poly(2-ethyl-2-oxazoline). Wherever solvents needed to be dried, it was done. Sigma Aldrich provided the 1-hexadecane. The chemicals were employed in the process directly. Nuclear magnetic resonance (NMR) spectra of the compounds were recorded in deuterated solvents in a Bruker AMX-400 spectrometer. A local hardware store provided silicone sheets with a thickness of 0.7 mm. Medical grade

silicone catheters were purchased. The bacterial strains, *E. coli* ATCC 25922, *E. coli* CFT073, MRSA ATCC 33591, *E. faecium* ATCC 19634, *K. pneumoniae* ATCC 700603 were obtained from American Type Culture Collection (ATCC, Rockville, Maryland). The bacterial strains, *E. coli* MTCC 443, *E. coli* MTCC 448, *S. epidermidis* MTCC 3615 were obtained from Microbial Type Culture Collection and Gene Bank (MTCC, Chandigarh). The bacterial strains, *E. coli* R3336, *E. coli* R250, *E. coli* 4806, *P. aeruginosa* R590, *K. pneumoniae* R3934, were obtained from the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India. Fungal strains (*C. albicans* AB226 and *C. albicans* AB399) were obtained from Anthem Biosciences, Bangalore, India. As a solid growth medium, nutrient agar was used for both Gram-negative and Gram-positive bacteria. YPD agar was used for fungi-related experiments. All of experiments involving evaluation of antimicrobial activity against pathogenic bacteria and fungi, were approved, and permitted by the Institutional Bio-safety Committee of Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR) (JNC/IBSC/2020/JH– 12) constituted under the national laws as per the Recombinant DNA safety Guidelines, 1990 issued by Department of Biotechnology (DBT) by Govt. of India (GoI). Human urine was collected from a healthy donor with informed consent following the ethical committee protocols permitted by the Institutional Ethics Committee (IEC) of Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR) (JNC/IEC/M3-2022/JH-001) constituted as directed by Indian Council of Medical Research (ICMR), by Govt. of India (GoI). The *in-vivo* animal experiments for biocompatibility studies of catheters were done by following the appropriate protocols, approved, and permitted by the Institutional Animal Ethics Committee (IAEC) of Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR) (201/Go/ReBi/S/2000/CPCSEA) constituted under the national laws of the Committee

for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) by Govt. of India (GoI). Two groups (n=4) of BALB/c mice (male, 20-25 g), bred in the institute animal facility, were used for this study. The 96-well plates and 6-well plates were obtained from Tarsons (India). X-ray diffraction studies were done in Rigaku XRD instrument. SEM and EDX were performed in Zeiss Gemini 500 FESEM comprising an EDX unit. Confocal studies were performed in Zeiss LSM 800. Thermogravimetric analysis (TGA) was performed on the Perkin Elmer STA 6000. FACS studies were performed on BD FACS Aria III. Electron paramagnetic resonance studies were done on JEOL JES-X320. Membrane permeability and depolarization was measured in Tecan Spark microplate reader.

***N*-hexadecyl-1-bromoethanamide** FT-IR ($\boldsymbol{\nu}$): 3252 cm^{-1} (amide N–H str.), 2925 cm^{-1}(–CH_2- assym. str.), 2850 cm^{-1} (–CH_2– sym. str.), 1679 cm^{-1} (Amide I, C=O str.), 1565 cm^{-1}(Amide II, N– H ben.), 1469 cm^{-1} (–CH_2– scissor); ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 0.87 (t, terminal–CH_3, 3H), 1.310 (m, –$(CH_2)_{13}$–, 26H), 1.550 (q, –$CH_2(CH_2)_{13}$–, 2H), 3.278 (t, –$CONHCH_2$–,2H), 3.880 (s, –$COCH_2Br$, 2H), 6.572 (br s, amide –NHCO, 2H).

***N*-Methyl branched PEI:** FT-IR ($\boldsymbol{\nu}$): 2947 and 2785 cm^{-1} (C-H str), 1463 cm^{-1}(C-H bend), 1030 cm^{-1} (C-N str); ^{1}H NMR (400 MHz, $CDCl_3$, δ): 2.250 (s, 3H, –$N(CH_3)$-), 2.426-2.605 (m, 4H, -$N(CH_2CH_2)$-).

***N*-hexadecyl, *N*-Methyl PEI:** FT-IR (ν): 3200-3450 cm^{-1}(amide N–H str.), 2935 cm^{-1}(–CH_2– assym. str.), 2865 cm^{-1}(–CH_2– sym. str.), 1680 cm^{-1}(amide I, C=O str.), 1556 cm^{-1}(amide II, N–H ben.),1475 cm^{-1}(–CH_2– scissor); ^{1}H-NMR (400 MHz, $CDCl_3$, δ): 0.856 (t, terminal –CH_3, 3H), 1.233 (–$CH_3(CH_2)_{13}CH_2$–,26H), 1.510 ($CH_3(CH_2)_{13}CH_2CH_2$–, 2H), 3.186 ($(CH_3)N^+(CH_2CH_2)_2(CH_2CONH)$–, 3H), 3.480-3.585 (-$(CH_3)N^+(CH_2CH_2)_2(CH_2CONH)$–,4H), 3.835 (–$CH_2CONHCH_2CH_2$–, 2H), 4.475 (-$(CH_3)N^+(CH_2CH_2)_2(CH_2CONH)$–, 2H), 8.320 (–$CH_2CONHCH_2CH_2$–, 1H)

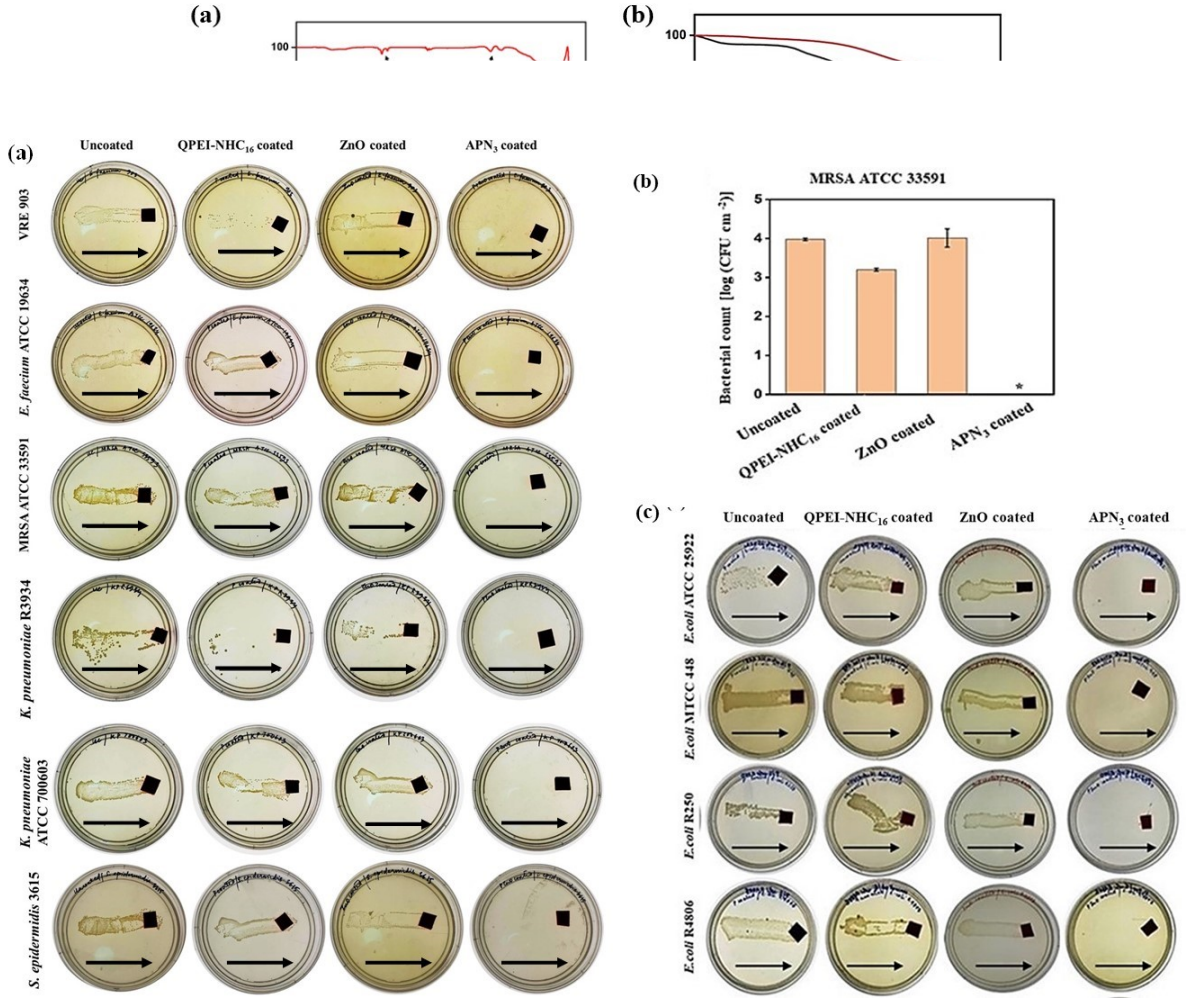

Fig. S2 (a) Antibacterial activity of coated silicone surfaces against drug-resistant Gram-positive and Gram-negative bacteria, including clinical isolates. Antibacterial activity of uncoated silicone surface, and surfaces coated with QPEI-NHC_{16}, ZnO and **APN_3** respectively; (b) Reduction in bacterial count for MRSA ATCC33591; (c) Potent killing of bacteria by optimized coating **APN_3** against key UTI-causing multi-drug resistant Gram-negative bacteria *E. coli* strains. An asterisk (*) indicates bacterial count of < 50 CFU cm^{-2}.

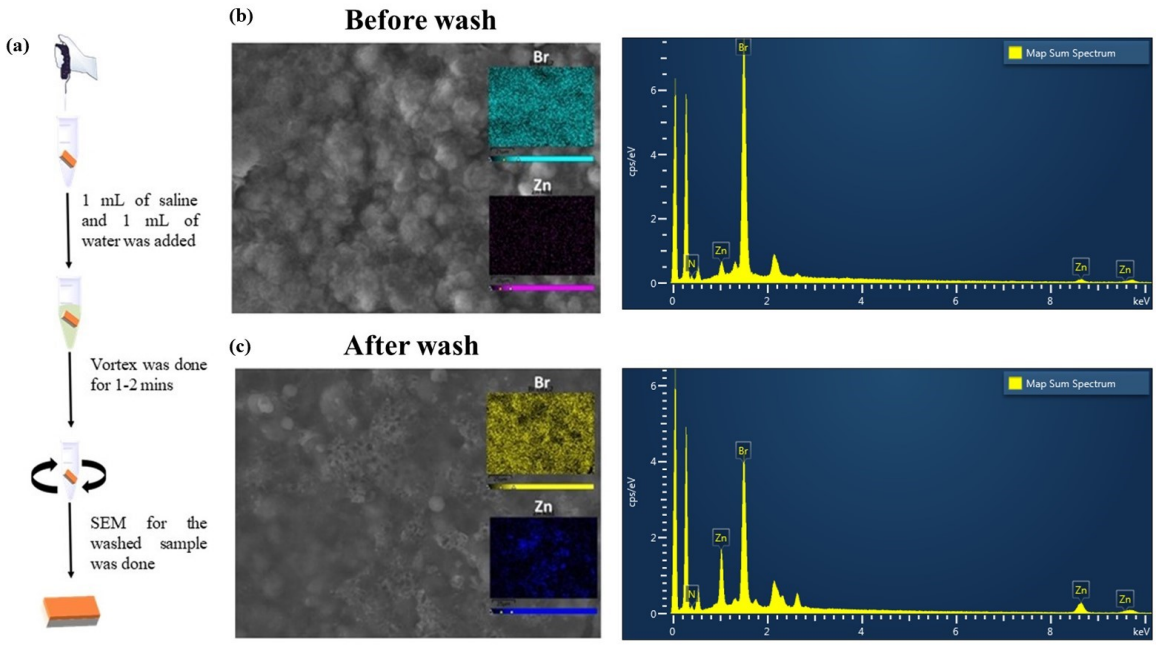

Fig. S3 (a) Schematic representation of multiple washing of the APN_3 coated surfaces in saline; (b) Scanning electron microscopy image of APN_3 coated silicone surface before washing. The inset image represents colour mapping of Bromine (blue) and Zinc (purple) after energy dispersive X-ray analysis. Scale bar= 250 mm; (c) Scanning electron microscopy image of APN_3 coated silicone surface after washing. The inset image represents colour mapping of Bromine (blue) and Zinc (purple) after energy dispersive X-ray analysis. Scale bar= 250 mm.