



Role of Nanocurcumin as a Surface Modifying Agent with Excellent Preventive Effect on Device-Related CoNS Infections

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Abstract The study focused on the analysis of efficiency of curcumin and nanocurcumin (NC) against biofilm-forming methicillin-resistant clinical isolates of *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*. Antibacterial, minimum inhibitory concentration and antibiofilm activities of both curcumin and NC were carried out, and the specific changes were analyzed by transmission electron microscopy (TEM). Surface modifying effect of NC was further analyzed by coating it onto nasogastric polyvinyl chloride (PVC) tubes followed by challenging it with selected pathogens, and the results were visualized by scanning electron microscopy. Curcumin and NC were found to have antibacterial effects at a concentration of 100 and 10 $\mu\text{g mL}^{-1}$, respectively. Also 250 and 500 $\mu\text{g mL}^{-1}$ concentrations of NC were found to have 99% inhibition on biofilm formation of *S. epidermidis* and *S. haemolyticus*. TEM analysis of NC-treated coagulase-negative staphylococci (CoNS) samples showed remarkable inhibition of biofilm formation with the complete lysis of bacterial cell. NC functionalization showed excellent preventive effect on

bacterial adhesion and biofilm formation on nasogastric PVC catheters and hence has the promises to be used as alternative chemotherapeutic agents. The current study forms the first report on detailed investigation on potential of NC as an effective agent against CoNS, which is well known for biomedical infection.

Keywords Antibiofilm · MRCoNS · Nanocurcumin · Ryle's tube · Surface modification

Introduction

Curcumin is a natural polyphenolic flavonoid present in the rhizome of *Curcuma longa* L. This has been used from ancient times in Ayurvedic medicines due to its antioxidant and free radical scavenging properties [1, 2]. Curcumin has wide range of applications as anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, antidiabetic, antibacterial, antifungal, antiprotozoal, antifibrotic, antiulcer and hypocholesterolemic agent [3]. Antibacterial effects of curcumin on both Gram-positive and Gram-negative bacteria of clinical significance have already been demonstrated in various studies [4]. Also many reports are there on its antibiofilm activity against *S. aureus*, *E. coli*, *Pseudomonas* spp., *S. mutans* and *H. pylori* [5]. Apart from all these multidisciplinary beneficial effects, therapeutic use of curcumin is limited due to its poor solubility, metabolism, rapid elimination and less bioavailability [6].

Nanomised curcumin particles called nanocurcumin have been suggested to have enhanced bioactivity due to its high surface area-to-volume ratio [7]. The excellent physicochemical features of nanocurcumin have also resulted in its improved solubility, bioavailability, enhanced absorbance and reduced medicinal doses with an

Significance statement Nanocurcumin functionalization on nasogastric catheter prevented adhesion and biofilm formation by coagulase-negative staphylococci.

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extended shelf life [2]. These antibacterial and antibiofilm effects of nanocurcumin indicate its promises to be exploited for biomedical applications. Among the various biomedical materials used, Ryle's tube is a widely used nasogastric tube which is passed into the stomach via nose for nutritional support and also for medication [8]. However, these tubes implanted even for a single day can get colonized by variety of biofilm-forming bacteria including *Staphylococcus* sp. [9]. The accidental introduction of coagulase-negative staphylococci (CoNS) onto this can result in its colonization on catheter surface. Clinical challenges by CoNS, especially *S. epidermidis* and *S. haemolyticus*, are mainly due to their notorious biofilm formation and multiple antibiotic resistance. CoNS attachment to any biotic or abiotic surface can follow the typical accumulation and maturation stage of biofilm formation, which thereby increases microbial resistance to host immune system and to various antimicrobial agents [10]. So the most promising approach can be the prevention of bacterial attachment on device by modifying its surface. Hence, role of phytochemicals as surface protecting agents of medical devices to prevent device-related infections can have immense opportunities to be exploited. These have an added advantage of low resistance emergence possibility due to its multitargeted action. More specifically, with the advancement in nanotechnology, phytochemical nanoformulations can expect to have impressive application to prevent the attachment of microorganisms onto medical devices [11].

Material and Methods

High incidence of antibiotic resistance among bacterial pathogens and its dissemination within community demand the need for alternative therapy [10, 12]. CoNS are commensal organisms which are notorious for device-related infections. Hence, in the present study, nanostructured curcumin with 42 nm size and spherical shape was used as the anti-CoNS and antibiofilm agent [13].

Bacterial Samples and Reagents

Strong biofilm-forming, methicillin-resistant CoNS *S. epidermidis* and *S. haemolyticus* isolated from human clinical samples obtained from the Microbiology Department of MOSC Medical College, Ernakulum, Kerala, India, were used in this study. Curcumin was collected from Kancor Ingredients Ltd., Kerala, and previously characterized PVA-loaded nanocurcumin powder samples (42 nm in size) were provided by Dr. Annaraj, Department of Materials Science, Madurai Kamaraj University. Due to the nanosize, the nanocurcumin used in the study was

previously demonstrated to have a maximum absorption at 425 nm with superior water solubility than that of bulk material, which improved its suitability for antimicrobial application [13]. Both the curcumin samples selected were dissolved in methanol for further studies.

Well Diffusion Assay of Curcumin and Nanocurcumin Against CoNS

The antibacterial activity of curcumin and nanocurcumin was done using well diffusion method on Muller Hinton Agar (MHA). Wells of 6 mm size were cut on MHA plate using a gel puncture. Turbidity of CoNS cultures was compared with 0.5 McFarland standard solution, and a confluent lawn was prepared on MHA plates for each strain. Then, 50 μL of the curcumin (100, 250, 500, 750 $\mu\text{g mL}^{-1}$) and nanocurcumin (10, 50, 75, 100 $\mu\text{g mL}^{-1}$) samples was transferred onto each well. Methanol was maintained as the control. After incubation, zone of clearance around each well was measured and noted in millimeters [14].

Determination of Minimum Inhibitory Concentration of Curcumin and Nanocurcumin

MIC determination of curcumin and nanocurcumin against *S. epidermidis* and *S. haemolyticus* was performed using microbroth dilution method. Ten different concentrations of both curcumin (1 mg mL^{-1} to 1.9 $\mu\text{g mL}^{-1}$) and nanocurcumin (500 to 0.97 $\mu\text{g mL}^{-1}$) were tested. The assay was conducted in 96-well microtiter plate, and two-fold serial dilutions of test agents were prepared in MHB. Triplicate for each concentration was maintained. Turbidity of overnight CoNS broth cultures was adjusted to 0.5 McFarland standard. CoNS cultures of 100 μL were transferred to each well. After incubation at 37 °C for 24 h, MIC values were read as the lowest concentration of curcumin and nanocurcumin at which there was no visible bacterial growth or turbidity. The absorbance was also recorded at 600 nm for each well using an ELISA reader (Thermo Varioskan multimode reader), and the results were compared with bacterial control.

Antibiofilm Activity of Curcumin and Nanocurcumin Against CoNS

Curcumin and nanocurcumin were added to the growth medium at the time of inoculation, and the cells were allowed to form biofilms. Twofold serial dilutions of ten different concentrations of test agents were made in sterile 96-well tissue culture plates containing 100 μL of Tryptic Soy Broth (TSB) per well. The concentration range for curcumin used was 1–1.9 $\mu\text{g mL}^{-1}$, and that of

nanocurcumin was 500–0.97 $\mu\text{g mL}^{-1}$. These were tested against the biofilm formation by both *S. epidermidis* and *S. haemolyticus* strains. After incubation at 37 °C for 24 h, the biofilm biomass was analyzed using the crystal violet staining. Triplicates were maintained for each concentration. Bacterial control samples without test agents were also maintained as control, and biofilm of these samples was also analyzed quantitatively as per previous report [15].

High-Resolution TEM Analysis of Nanocurcumin-Treated CoNS

S. epidermidis and *S. haemolyticus* cultures grown in TSB were treated with 99% biofilm-inhibiting concentration of nanocurcumin (250 and 500 $\mu\text{g mL}^{-1}$) for 16 h at 37 °C. Following the treatment, pellets were washed twice with phosphate-buffered saline and electron microscopic analysis was conducted for both treated and control bacterial samples. Samples were coated onto carbon-coated copper grid and were imaged using JEOL JEM 2100 high-resolution TEM.

Surface Functionalization of Nanocurcumin onto Nasogastric PVC Tube

The nasogastric PVC tube (Ryle's tube) was cut into $1 \times 1 \text{ cm}^2$ pieces aseptically, and these pieces were completely immersed in nanocurcumin solution at concentrations 250 and 500 $\mu\text{g mL}^{-1}$ for overnight at room temperature in 6-well microtiter plate. After incubation, the catheter pieces were taken and allowed to dry at 40 °C for 3 h. These 250 and 500 $\mu\text{g mL}^{-1}$ nanocurcumin-coated catheter pieces were then incubated with 3 mL of *S. epidermidis* and *S. haemolyticus* cultures, respectively, for 24 h at 37 °C. Another control set was maintained which contained uncoated catheter pieces and bacteria. Both nanocurcumin-coated catheter and uncoated catheters were treated with bacteria. These were further subjected to SEM (JEOL JSM 6390) analysis.

Results and Discussion

Antibacterial Activity of Curcumin and Nanocurcumin Using Well Diffusion Against CoNS

Both the curcumin and nanocurcumin showed good antimicrobial activity against the tested pathogens *S. epidermidis* and *S. haemolyticus*. For both the isolates, the zone of inhibition was observed to be 10 $\mu\text{g mL}^{-1}$ for nanocurcumin and 100 $\mu\text{g mL}^{-1}$ for curcumin. The anti-

CoNS activity demonstrated for both test agents was concentration dependent as it increased with their concentration (Fig. 1). No zone was observed for the methanol, which was used as control (Table 1). In this study, well diffusion analysis was conducted using different concentrations of curcumin and nanocurcumin to analyze its anti-CoNS effect. Antibacterial activities for curcumin and nanocurcumin were observed at a concentration of 100 and 10 $\mu\text{g mL}^{-1}$, respectively, against both the CoNS isolates tested. Among the various nanosized particles, silver nanoparticles are considered as one of the most effective antibacterial agents. Interestingly, the antibacterial concentration of nanocurcumin noted in this study is totally comparable with the anti-CoNS concentration of silver nanoparticles of a previous study [16].

Minimum Inhibitory Concentration Analysis

MIC determination of curcumin and nanocurcumin against *S. epidermidis* and *S. haemolyticus* was done using microbroth dilution method. MIC values were visually recorded as the lowest concentration which showed no turbidity, and these data were also in accordance with the OD value measured at 600 nm. The MIC value of curcumin against *S. epidermidis* was 31.25 $\mu\text{g mL}^{-1}$, and for *S. haemolyticus* it was 62.5 $\mu\text{g mL}^{-1}$. Very remarkably, nanocurcumin at low concentration was found to have inhibitory effect toward these CoNS when compared to curcumin. The MIC values for nanocurcumin against both the *S. epidermidis* and *S. haemolyticus* clinical isolates were found to be 7.8 $\mu\text{g mL}^{-1}$.

Each CoNS isolate showed different concentration sensitivity towards test agents. When compared with curcumin, 8 times reduction in MIC value was observed for nanocurcumin for *S. haemolyticus* and 4 times reduction for *S. epidermidis*. The difference in MIC values observed can be due to intrinsic difference in cell wall and biofilm composition of selected CoNS isolates. In a previous work, aqueous suspension of PVA-loaded nanocurcumin at a concentration of 200 $\mu\text{g mL}^{-1}$ was found to have greater sensitivity to Gram-positive *S. aureus* and *B. subtilis* [13]. Another study also supported the antistaphylococcal activity of curcumin nanoparticles with an MIC of 250 $\mu\text{g mL}^{-1}$ [17]. When compared with previous reports, the concentration of NC needed to inhibit MRCoNS was much lower. In a study to investigate the antiMRSA effect of curcumin–PLGA nanoparticles, in vivo efficiency in rats was observed even with 10 $\mu\text{g mL}^{-1}$ of nanoparticle administration. The study also demonstrated the nontoxicity of curcumin nanoformulations on normal cells, in which 75% of the cells were survived when treated with these nanoparticles [18].

Fig. 1 Well diffusion analysis of curcumin and nanocurcumin. Antibacterial activity of curcumin against **a** *S. epidermidis*, **b** *S. haemolyticus* and antibacterial activity of nanocurcumin against, **c** *S. epidermidis*, **d** *S. haemolyticus* (C in each plate represented—control)

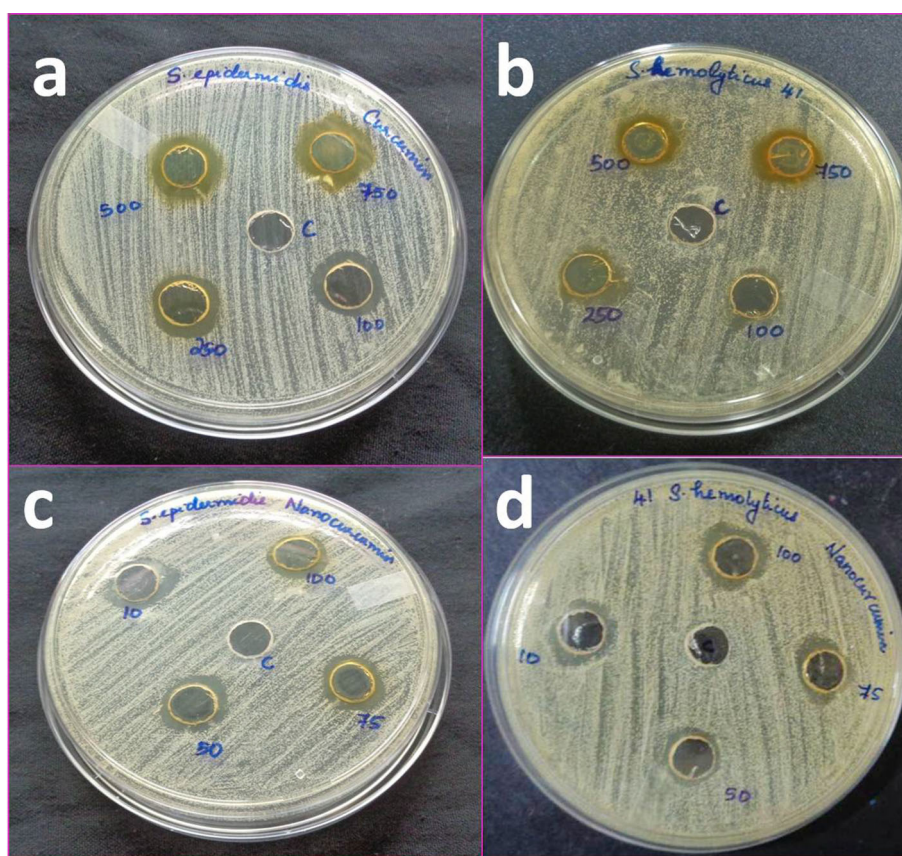


Table 1 Antibacterial activity of curcumin and nanocurcumin against *S. epidermidis* and *S. haemolyticus*

CoNS species (zone in mm)	Curcumin concentrations ($\mu\text{g mL}^{-1}$)				Nanocurcumin concentrations ($\mu\text{g mL}^{-1}$)				Methanol (C)
	750	500	250	100	100	75	50	10	
<i>S. epidermidis</i>	16	14	13	13	15	14	14	14	0
<i>S. haemolyticus</i>	15	13	12	10	16	15	10	10	0

Biofilm Inhibition Activity of Curcumin and Nanocurcumin

The curcumin and nanocurcumin showed dose-dependent inhibition of biofilm formation in CoNS. More specifically, curcumin showed 99% biofilm inhibition at $500 \mu\text{g mL}^{-1}$ for *S. epidermidis* and at 1 mg mL^{-1} for *S. haemolyticus*, and for nanocurcumin, it was of $250 \mu\text{g mL}^{-1}$ for *S. epidermidis* and $500 \mu\text{g mL}^{-1}$ for *S. haemolyticus*. Here also, biofilm reduction for nanocurcumin was observed at half of the concentration of curcumin against the same CoNS isolates. CoNS strains cultured without test agents were quantified for biofilm formation and have resulted in $\text{OD} > 0.12$ which confirmed the ability of selected isolates to form biofilm on microtitre plates. The comparison of biofilm and antibiofilm quantification results thus

confirmed the superior performance of nanocurcumin over curcumin as antibiofilm agent.

Several research studies proposed the potential of curcumin as antibiofilm agent. Total eradication of *E. faecalis* biofilm on tooth surface has been reported by the use of 3% curcumin treatment [19]. Another study also demonstrated biofilm inhibition capacity of curcumin on uropathogens by interfering with their quorum sensing systems [20]. Studies on biofilm inhibition by nanocurcumin are very limited, and the current result is the first report on in-depth analysis of nanocurcumin as anti-CoNS biofilm agent. The present study showed a 99% reduction in CoNS biofilm on polystyrene microtitre plates when treated with NC. The NC concentration needed was only two times lesser than curcumin. A recent study showed 50% biofilm reduction among *P. aeruginosa* and *S. aureus* with $100 \mu\text{g mL}^{-1}$

silver–curcumin nanoparticle synergistic combination. But when treated alone with curcumin nanoparticle, about $500 \mu\text{g mL}^{-1}$ was required for 50% reduction [21]. However, in the present study, 99% biofilm inhibition was observed with $500 \mu\text{g mL}^{-1}$ concentration of NC for *S. haemolyticus* and $250 \mu\text{g mL}^{-1}$ for *S. epidermidis*. This result proposes the nanocurcumin as an effective nanomedicine against CoNS biofilm formation.

Transmission Electron Microscopic Analysis of Nanocurcumin-Treated CoNS Isolates

Since nanocurcumin was found to have superior efficiency in biofilm prevention than curcumin, further analysis was done using nanocurcumin only. Here, *S. epidermidis* and *S. haemolyticus* were treated with nanocurcumin at a concentration of 99% biofilm inhibition (250 and $500 \mu\text{g mL}^{-1}$) in a 6-well microtitre plate. The changes in the ultrastructure of cells following exposure to nanocurcumin were confirmed by TEM. Here, TEM images of *S. epidermidis* and *S. haemolyticus* from control samples showed intact cells embedded in extracellular matrix. Also proliferating cells with well-defined cell membrane and dividing septa were seen for both the isolates. Nanocurcumin-treated samples showed complete bacterial cell lysis. Remarkably, nanocurcumin infiltration onto cell membrane was also observed. Thus, TEM results confirmed the antibiofilm and antimicrobial activity of nanocurcumin against CoNS (Fig. 2).

Curcumin can bind to the membrane proteins which can result in cell wall disruption and interference in electron transport chain. The thick peptidoglycan layer of Gram-positive bacteria can interact more with the curcumin and thereby result in cell disruption [22]. Since it is a phenolic compound, higher penetration into the cells with altered membrane permeability and inactivation of cellular enzymes can occur which may ultimately lead to complete cell disruption and cell death. Confocal microscopy and SEM analysis on 24-h treatment with $100 \mu\text{M}$ curcumin against *S. aureus* and *E. coli* previously demonstrated bacterial cell membrane damage and cell death with leakage of cell material and bursting of cells [23]. Higher activity of nanocurcumin on Gram-positive bacteria than Gram-negative bacteria was also demonstrated in a recent study [24]. Similar results have been observed in the present study, where TEM analysis confirmed the complete cell lysis among 24-h NC-treated biofilm-forming methicillin-resistant *S. epidermidis* and *S. haemolyticus*. This result confirmed that NC can have broad range of antibacterial effects which can be replaced for resistance suspecting antimicrobials.

Surface Functionalization of Nanocurcumin onto Nasogastric PVC Tube

Efficiency of nanocurcumin in preventing CoNS biofilm formation on PVC catheter was analyzed using SEM. After 24 h of incubation, thick matured biofilm formation was

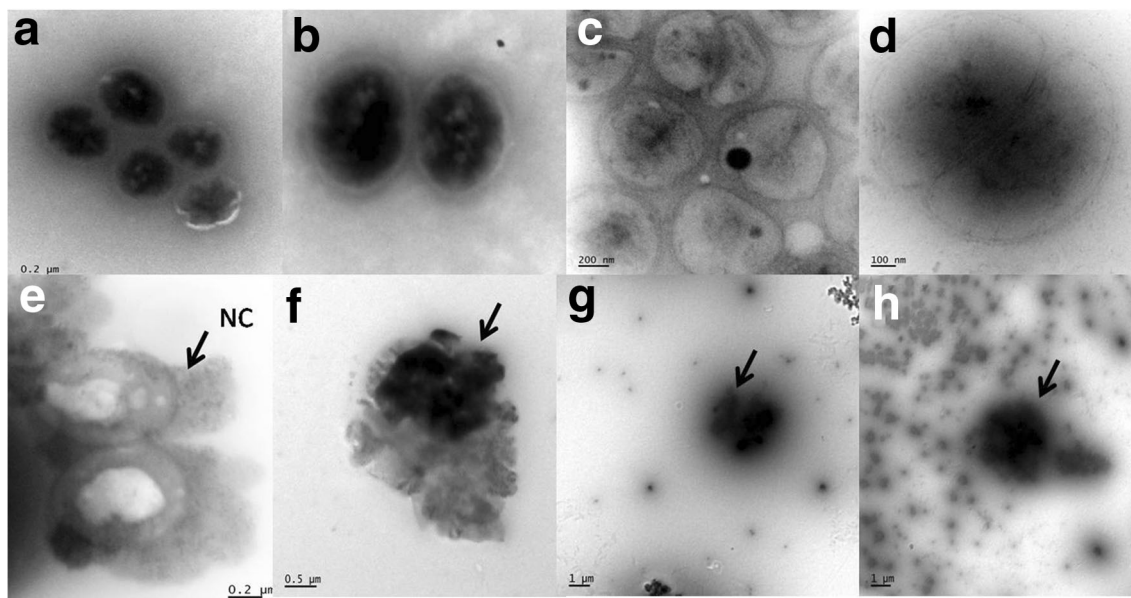
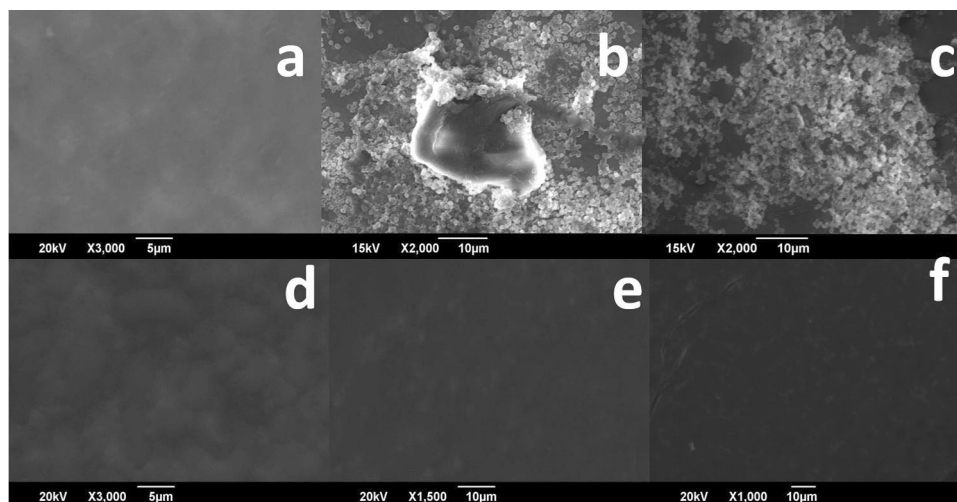


Fig. 2 TEM analysis of nanocurcumin-treated samples. **a, b** Untreated *S. epidermidis*. **c, d** Untreated *S. haemolyticus*. Extracellular matrix-embedded cells can be seen for both CoNS isolates. Dividing cells with intact septa are also visualized. **e, f** NC-treated *S. epidermidis*. NC demonstrates the nanocurcumin infiltration and

accumulation onto the cell membrane leading to cell lysis. **g, h** Nanocurcumin-treated *S. haemolyticus* showing complete cell lysis

Fig. 3 SEM analysis of the nanocurcumin functionalization on nasogastric PVC tube. **a** Uncoated catheter. **b**, **c** Biofilm formation of *S. epidermidis* and *S. haemolyticus* on the uncoated catheter, respectively. **d** Nanocurcumin-coated catheter. **e**, **f** Nanocurcumin-coated catheter incubated with *S. epidermidis* and *S. haemolyticus*, respectively



observed on the control catheter treated with both *S. epidermidis* and *S. haemolyticus*. But on the nanocurcumin-treated catheter pieces, there was no bacterial attachment or accumulation. This confirmed the efficiency of nanocurcumin in preventing attachment and biofilm formation of CoNS onto these nasogastric PVC tubes, confirming its therapeutic potential (Fig. 3). Application and modification of various medical devices with nanocurcumin have been recently reported. Studies have also reported efficacy of nanocurcumin-coated surgical bandages in inhibiting *E. coli*, *S. aureus* and *S. pyogenes* [25]. Good antimicrobial action was demonstrated for nanocurcumin impregnated gelatin cellulose fibers against *E. coli* and *S. aureus* [26]. Usage of nanocurcumin as a surface modifying agent on nasogastric PVC tube is a novel approach. On SEM analysis, both the CoNS isolates showed thick biofilm formation onto the catheter. In the nanocurcumin-treated catheter samples, no such attachment or cell accumulation was observed. Interestingly, lower concentration of NC prevented both the attachment and accumulation of CoNS onto the catheter pieces. Thus, from these data, the authors can confirm the effectiveness of nanocurcumin as a surface functionalizing agent. SEM and TEM results of nanocurcumin-treated samples confirmed its role as a potent antibacterial and antibiofilm agent which highlights the importance of the current study.

Conclusion

The results presented in the present study suggest curcumin and nanocurcumin to have excellent antibacterial and antibiofilm activity against strong biofilm-forming methicillin-resistant *S. epidermidis* and *S. haemolyticus*. Electron microscopic analysis showed the cell lysis efficacy of nanocurcumin. Also, nanocurcumin proved to be an

excellent surface modifying agent in limiting CoNS colonization on nasogastric PVC tubes. Such antibiotic alternate strategy can have immense promises in the current scenario of the antibiotic resistance crisis caused by the MRCoNS. Therefore, nanocurcumin with its easy availability, low cytotoxicity and high efficacy could be an ideal candidate to be used in chemopreventive and medical implant modified therapies against bacterial pathogens.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest to publish this manuscript.

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