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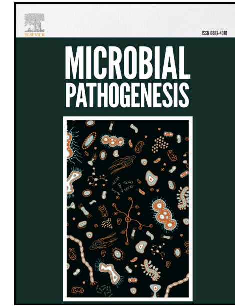

Role of BP*C@AgNPs in Bap-dependent multicellular behavior of clinically important methicillin-resistant *Staphylococcus aureus* (MRSA) biofilm adherence: A key virulence study

Zhang Xu, H.M. Manukumar, K.P. Rakesh, C.S. Karthik, H.S. Ngendra Prasad, Nanjunda Swamy, P. Mallu, Eissa Mohammed Yasser Hunnein, Hua-Li Qin

PII: S0882-4010(18)30701-0

DOI: 10.1016/j.micpath.2018.07.025

Reference: YMPAT 3065

To appear in: *Microbial Pathogenesis*

Received Date: 20 April 2018

Revised Date: 18 July 2018

Accepted Date: 20 July 2018

Please cite this article as: Xu Z, Manukumar HM, Rakesh KP, Karthik CS, Ngendra Prasad HS, Swamy N, Mallu P, Yasser Hunnein EM, Qin H-L, Role of BP*C@AgNPs in Bap-dependent multicellular behavior of clinically important methicillin-resistant *Staphylococcus aureus* (MRSA) biofilm adherence: A key virulence study, *Microbial Pathogenesis* (2018), doi: 10.1016/j.micpath.2018.07.025.

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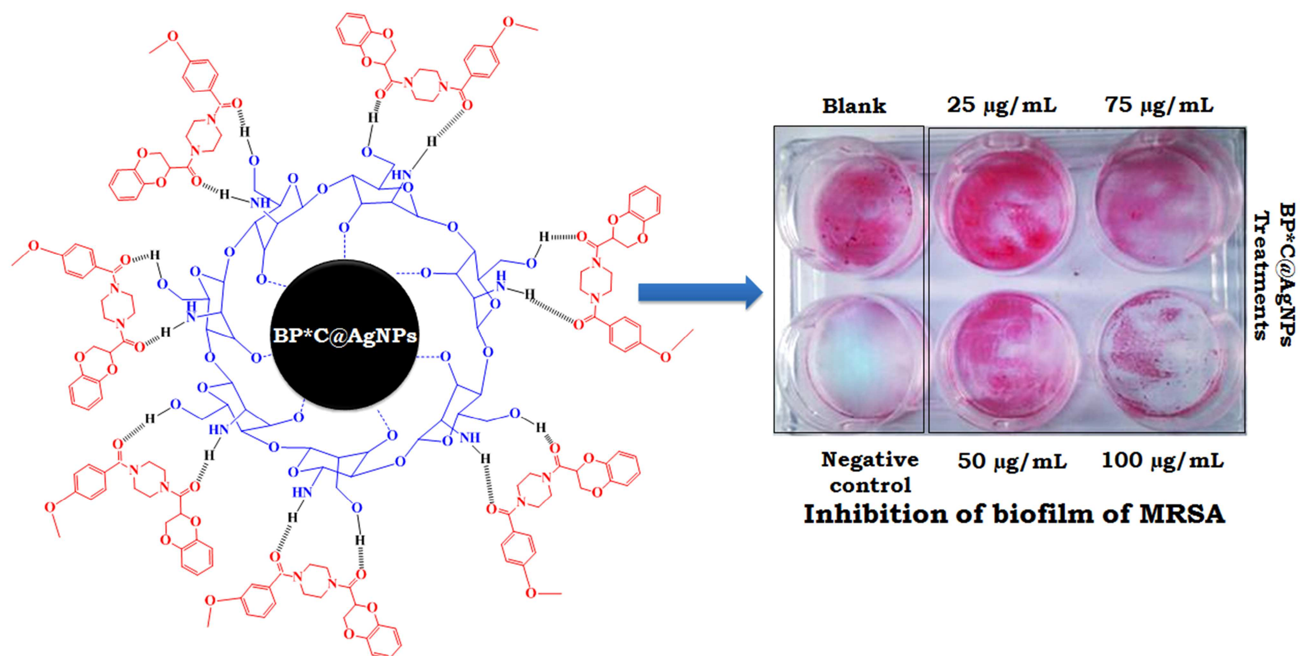
Graphical abstract



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Abstract

Bacterial adhesion is a threshold event in the formation of biofilms which leads to serious bacterial diseases. This shows that the underlining the problem is interesting and need to solve the problem of biofilm-related complications. To support this, in the present study, we first time initiated to understand the role of methicillin-resistant *Staphylococcus aureus* (MRSA) biofilm

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using previously developed benzodioxane midst piperazine decorated chitosan silver nanoparticles (BP*C@AgNPs). The BP*C@AgNPs studied for antimicrobial, anti-biofilm, biofilm adherence inhibition, the role of ions in biofilm, and an antibiotic cocktail in the treatment of biofilm was assessed. The results showed that, the significant biocidal role of BP*C@AgNPs in controlling the MRSA biofilm and interaction of biofilm protein to calcium ions were significantly decreased. This confirms that calcium ion involved in the biofilm formation and for the treatment of BP*C@AgNPs, cocktail of enzyme and antibiotic have the promising therapeutic value was observed. In future the locking of biofilm protein and its expression in presence of calcium ion was interesting, and greater application related to biofilm infection was warrantable.

Keywords: *Staphylococcus aureus*; Biofilm; Cocktail, Eradication

1. Introduction

Staphylococcus aureus is one of the important human pathogen causes numerous mild illnesses (Skin infection) to life-threatening diseases [1, 2]. An infectious agent transferred from various routes includes contaminated food, person to person, accidental blood transfer and majorly bacterial colonization on medical devices by the formation of biofilm. There are many wide ranges of medical associated devices such as endotracheal tubes, cardiac pacemakers, mechanical heart valves, orthopedic prostheses, dialysis tubes, penile prostheses and more, are not hygienically protected and thus lead to nidus for *S. aureus* and *S. epidermis* infections. This primarily affects the immune-compromised patients, leads to malfunctioning of the devices and biofilms have been implicated include common problems such as urinary tract infections, bacterial vaginosis, gingivitis, catheter infections, formation of dental plaque, and more lethal processes such as endocarditis, infections in cystic fibrosis, and infections of permanent

indwelling devices such as joint prostheses and heart valves [3-6]. The immune-compromised patients explored that, blood stream (80%) and urinary infections (90%) are originated by bacterial colonization on vascular and urinary catheter respectively [7].

Biofilm is the thick extracellular polysaccharide produced by the *S. aureus* to protect them self, as well as escape from the immune defense machinery. Biofilms constitute of non-transient microbial cells attached to the surface and embedded within the self-synthesized extracellular polymeric matrix for growth as multicellular aggregates against various environmental stresses [8]. Biofilm-associated protein (Bap) is multi-domain, cell surface-anchored protein and plays a major role in *S. aureus* biofilm development. This protein has been reported to have four potential Ca^{2+} binding EF-hand motifs with >80% homology with the consensus sequence of the EF-hand motif (prosite accession no. PS00018) [9]. This Ca^{2+} ion is a well-known intracellular second messenger that controls many vital processes, and there is increasing evidence that it is an extracellular first messenger in complex organisms. Several of the staphylococcal surface adhesins bind Ca^{2+}, ClfA (clumping factor A) of *S. aureus*, which promotes binding to fibrinogen and fibrin, has a Ca^{2+}-binding EF-hand-like motif in the fibrinogen-binding domain [10]. Bap in *S. aureus* biofilm development was so significant, even though *ica* operon was disrupted. In *S. aureus,* cell adhesion and biofilm formation depends on the synthesis of polysaccharide intercellular adhesion (PIA) molecule, which is coded by *ica* operon comprises *ica*ADBC gene cluster [11-13].

The tissue engineering and biomedical devices related products are already at $180 billion per year worldwide and still industry continue to suffer from microbial colonization. No matter the sophistication, microbial biofilm can develop on all medical devices and implanted devices leading to 60-70% of nosocomial or hospital acquired infections. This leads to 2 million

cases in U.S., costing the health care system and if an infection develops a biofilm, it becomes even harder to treat. As the bacteria change, they become more resistant to antibiotics and the body's own host defenses [14]. In food processing and medical devices such as stainless steel surfaces and utensils are common sites for bacterial adhesion. The mature *S. aureus* biofilm is not efficiently removed by some sanitizers in food processing environment, such as sodium hypochlorite and peracetic acid due to toxic residues of sanitizers' inturn decreased their use in natural, industrial and hospital settings [15, 16].

Proteinase K is one of the highly reactive serine protease, stable at many conditions and high concentration (1 mg mL^{-1}) used for biofilm disassembly, which may lead to increased cost of treatment [11]. Thus, there is a challenging gap present to search and develop proteinase K-like an agent for biofilm dispersal by targeting Bap may be a promising approach to control staphylococci infections. Controlling of *S. aureus* is very difficult due to biofilm forming property made up of thick proteins, DNA, and polymeric exopolysaccharides that shield many devices sequentially leading to dangerous infections by preventing drugs to access the bacterium and get resistance to a many commonly used antibiotics. In this context, there is a need of active agents to control the synthesis of biofilm linked to adherence factors of bacteria. According to established reports, application of antimicrobial agent's ciprofloxacin and silver as surface modifiers or coatings efficiently reducing the bacterial colonization and biofilm development and device-associated infections [17]. Nowadays, nanotechnology became a most popular trusty area of research and sets its root by playing a major role in agrochemical, pharmaceutical, medicine and biotechnological industries. Safety and cost concern, the synthetic process for development of noble nanoparticles (NPs) replaced by green synthesis or by eco-friendly natural active agents

for synthesis of nanoscale therapeutic NPs is a challenging because there are safe in their synthetic process.

In the present investigation, we used an active antibacterial benzodioxane midst piperazine which is previously reported from the group to development of biocompatible benzodioxane midst piperazine decorated decorated chitosan silver nanoparticles (BP*C@AgNPs) against human pathogens. In this study for the first time as a nano nontoxic drug efficacy was used to study adherence inhibitory mechanism of Bap positive *S. aureus* (MRSA) using glass and polystyrene as surface models in the present research.

2. Materials and methods

2.1. Chemicals and reagents

Mueller-Hinton agar and broth were purchased from Hi-Media (Bangalore, India), Tris-buffer, HCl, and chloroform was obtained from Merck-Millipore. Polystyrene plates and tissue culture cell imaging dishes obtained from Thermo Scientific and Eppendorf, India respectively. Millipore water was used in all the experiments. All other reagents used in this study were analytical grade.

2.2. Benzodioxane midst piperazine decorated decorated chitosan silver nanoparticles (BP*C@AgNPs)

The experimental procedure briefly, 0.5 g of chitosan was dissolved in 2% acetic acid solution, also 0.5 g of silver nitrate in deionized water was prepared. Exactly above 5 mL chitosan and silver nitrate solution were mixed in the boiling tube then kept in an autoclave at 15 psi pressure, at 120 ^{o}C for 1 h. The resulting cleared yellow solution was mixed with 0.5 g of

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benzodioxane midst piperazine (BP) and sonicated for 3 h at room temperature. The resulting muddy brown colored solution indicates the formation of benzodioxane midst piperazine decorated decorated chitosan silver nanoparticles (BP*C@AgNPs) [18].

2.3. Characterization of BP*C@AgNPs

Prepared BP*C@AgNPs were vacuum dried to obtain fine powder to study size, shape, crystallinity and elemental analysis. BP*C@AgNPs were subjected to scanning electron microscope (SEM) for size, X-ray diffraction (XRD) for determination of phase purity, Fourier transform infrared (FT-IR) spectroscopy to study bonding pattern of NPs acquired between 600 to 4000 cm^{-1}. The size, distribution and surface charge of BP*C@AgNPs were studied through dynamic light scattering (DLS) and zeta potential analysis [18].

2.4. In-vitro antimicrobial efficacy of BP*C@AgNPs

2.4.1. Disc diffusion assay

The MRSA strain was subjected to agar dilution method according to Manukumar *et al.* [19] to deduce the BP*C@AgNPs minimum inhibitory concentration (MIC) using bacterial strain *Staphylococcus aureus* (96) received from Microbial Typing Culture Collection (MTCC), Chandigarh, India, as a positive control. The bacterial suspension was prepared from the overnight culture and $1x10^6$ CFU/mL cells were spread on to Mueller-Hinton agar, then sterile discs (3 mm) were placed, to which 5 µL of different serial dilutions of BP*C@AgNPs were added. Control was performed without any test sample and incubated at 37 ^oC for 24 h to examine zone of inhibition. Assay performed in triplicates and repeated thrice.

2.5. Release of cellular material

Effect of BP*C@AgNPs was analyzed by measuring cellular material (DNA) from MRSA according to the protocol of Manukumar *et al*. [20]. The experiment was carried out by inoculating log phase culture into 0.1% sterile peptone water and without samples as a control. After incubation at 37 ^{o}C (for 0, 30, 60, and 120 min), 1 mL of broth was transferred to an Eppendorf tube, centrifuged at 3,500 rpm and supernatant was measured at 260 nm using a spectrophotometer. Results were expressed in the form of optical density for the sample collected from different time interval incubated samples. Assay performed in triplicates and repeated thrice. The final amount of material released was recorded by subtracting the OD values obtained from cells without treatment and was compared with values of 0.1% Triton X-100 treatment (positive control) to get material released percentage.

2.6. Action of BP*C@AgNPs on production of MRSA biofilm

2.6.1. Inoculum preparation

The MRSA was grown in MHAB at 35 °C for 18–20 h and cells were harvested by centrifuging at 5000 rpm for 8 min at 4 °C. Wash cells thrice in sterile saline solution and re-suspend pelleted cells in sterile saline solution. Cell density adjusted to an optical density at 600 nm (OD_{600}) of 0.1 using a UV-visible spectrophotometer and viable counts of approximately 6 log CFU/mL.

2.6.2. Quantitative determination of biofilm production

The quantitative test was performed for determination of biofilm production using microtiter plate method (MtP). The experiment was set according to Manukumar *et al*. [21] with slight modifications, 20 μL aliquots of cell suspension was inoculated into each one of six-well

polystyrene microtiter plate containing 180 µL of trypticase soy broth (TSB) supplemented with glucose (10 g/100 mL). MtP was covered and incubated at a static condition of 37 °C for 18 h aerobically to favors greater adherence of MRSA. After each well was washed thrice with a sterile saline solution then cells were fixed with 150 µL of methanol for 20 min and dry the MtP at room temperature. The cells were stained with crystal violet (0.5%) for 15 min then discard the contents and wash thrice with 200 µL of saline solution. Dry the MtP, using 150 µL of 95% ethanol dye bound to the cells was eluted for 30 min and the absorbance at 490 nm was determined using microplate spectrophotometer. To quantify the intensity of biofilm, the mean OD was compared to the OD of negative control (only TSB medium) plus three times its standard deviation. Assay performed in triplicates and repeated thrice.

2.6.3. Bacterial cell microscopy

The scanning electron microscopy (SEM) carried out to study MRSA membrane damage and antibiofilm property by treating 1 mg/mL concentration of BP*C@AgNPs for 2 h, then cells were pelleted by centrifugation (10,000 rpm for 5 min) at 4 ^{o}C. Cells were fixed by using glutaraldehyde (2.5%) in PBS, pelleted and deposited on glass slide followed by stepwise treatment of 30% to 100% ethanol drying. After, 2 days drying under room temperature used for SEM analysis [22, 23].

2.7. Eradication of MRSA on glass surface

The overnight grown culture of MRSA was collected and washed 3 times with PBS by centrifugation at 1500 × g for 10 min. 250 µL of 0.4 OD_{600} culture was filled to form a biofilm on glass surface overnight. After 24 h, the supernatant of the dish was discarded. Each dish was washed 3 times with PBS to remove loosely bound cells and added different doses of

BP*C@AgNPs then washed with PBS thrice. The cells were stained with crystal violet, eluted and the absorbance at 490 nm was determined using microplate spectrophotometer [20, 23].

2.8. Proteinase K preparation

The enzyme proteinase K was purchased from Sigma-Aldrich (St Louis, MO, USA), which had specific activity of 30 units mg^{-1}, where one unit is defined as the amount of enzyme needed to hydrolyse urea-denatured haemoglobin to produce colour equivalent to 1.0 mM of tyrosine per min at pH 7.5 at 37 ^{o}C (colour reaction by Folin-Ciocalteu reagent). The working concentration of proteinase K was chosen as 2 $mgml^{-1}$ in most of the experiments. To inactivate the proteinase K after the prescribed experimental time, 2 mM of freshly prepared phenyl methyl sulphonyl fluoride (PMSF) was added.

2.9. Early adhesion assay

Overnight grown cultures in TSB supplemented with 0.25% glucose (TSB-glu) were diluted 1:40 in sterile TSB medium and mild proteinase K treatment (2 mg mL^{-1}) was given to the cells, to cleave and remove all surface proteins according to Shrestha *et al*. [24] with slight modifications. Then, the proteinase K-treated cells were added to 96-well microtitre plates. Untreated MRSA cells were used as a control. To evaluate the role of surface protein in initial adhesion, post proteinase K-treated cells (proteinase K was inactivated by addition of PMSF) were allowed to adhere for different time intervals that are 0, 1, 2 and 3 h at 37 ^{o}C, to aid in surface protein-mediated adherence. Adherence of cells was measured by crystal violet assay as described earlier.

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2.10. Response of Bap domain at ionic, antibiotics and BP*C@AgNPs environment

The overnight grown culture in TSB-glu was incubated with different concentrations of calcium chloride, EDTA, EGTA, BP*C@AgNPs and antibiotics (Gentamycin, Ciprofloxacin and Amoxyclav) overnight at 37 ^{o}C to know surface protein-mediated adherence effect, then adherence of cells was measured by crystal violet assay as described earlier.

2.11. Intercellular adhesion in liquid media

The assay was conducted with slight modification according to Heilmann et al. [25] in 5 ml tubes containing TSB-glucose supplemented with the appropriate concentrations of $CaCl_2$, EGTA or EDTA were inoculated with a fresh colony of MRSA strain and incubated for 24 h at 37 °C in a shaker at 200 rpm. The optical density at 600 nm (OD_{600}) of an aliquot taken from the bulk of the culture was determined before and after vigorous vortexing of the tube; the aliquots were diluted as required to obtain OD_{600} values that were less than 0.3. The measured optical density values were corrected for the dilution factor and volume proportion. The percentage of suspended cells in each tube was calculated from the ratio of the corrected OD_{600} values obtained for the culture before and after vortexing. The experiments were repeated at least three times.

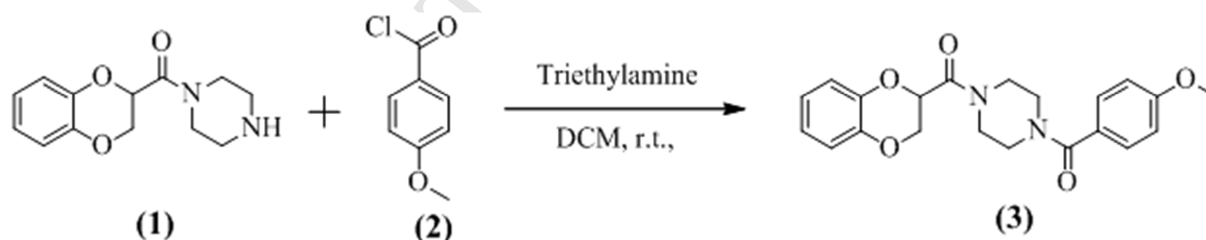
2.12. Statistical analysis

The data represented here are the mean±SD from at least 3 independent experiments. Statistical significances were analyzed using two-tailed Student's t-test.

3. Results and discussion

3.1. Synthesis and characterization of BP*C@AgNPs

The scheme for synthesis of novel benzodioxane midst piperazine (BP) compound was presented here. The BP*C@AgNPs was synthesized previously by reducing $AgNO_3$ by water soluble chitosan and then loaded antimicrobial agent BP. The UV-vis spectrum controls the formation of BP*C@AgNPs and mean while changing the color of reaction was observed visibly. The synthesized BP*C@AgNPs showed maximum absorbance at 470 nm indicates the reduction and formation of nanoparticles (NPs) having a Z average diameter of 36.6 nm, spherical and monodispersive in nature. This FT-IR analysis showing binding of chitosan (key peaks include the primary and secondary alcohols, amines and amides) and decorated BP functional groups showed interaction with silver. The co-ordination between silver and electron-rich groups such as those groups containing oxygen/nitrogen helps in the formation of stabilized Ag cluster. The XRD pattern of BP*C@AgNPs shows the nature of shape and EDS confirmed the presence of Ag in the NPs **(Fig. 1)** [18].



Scheme for the synthesis of compound BP

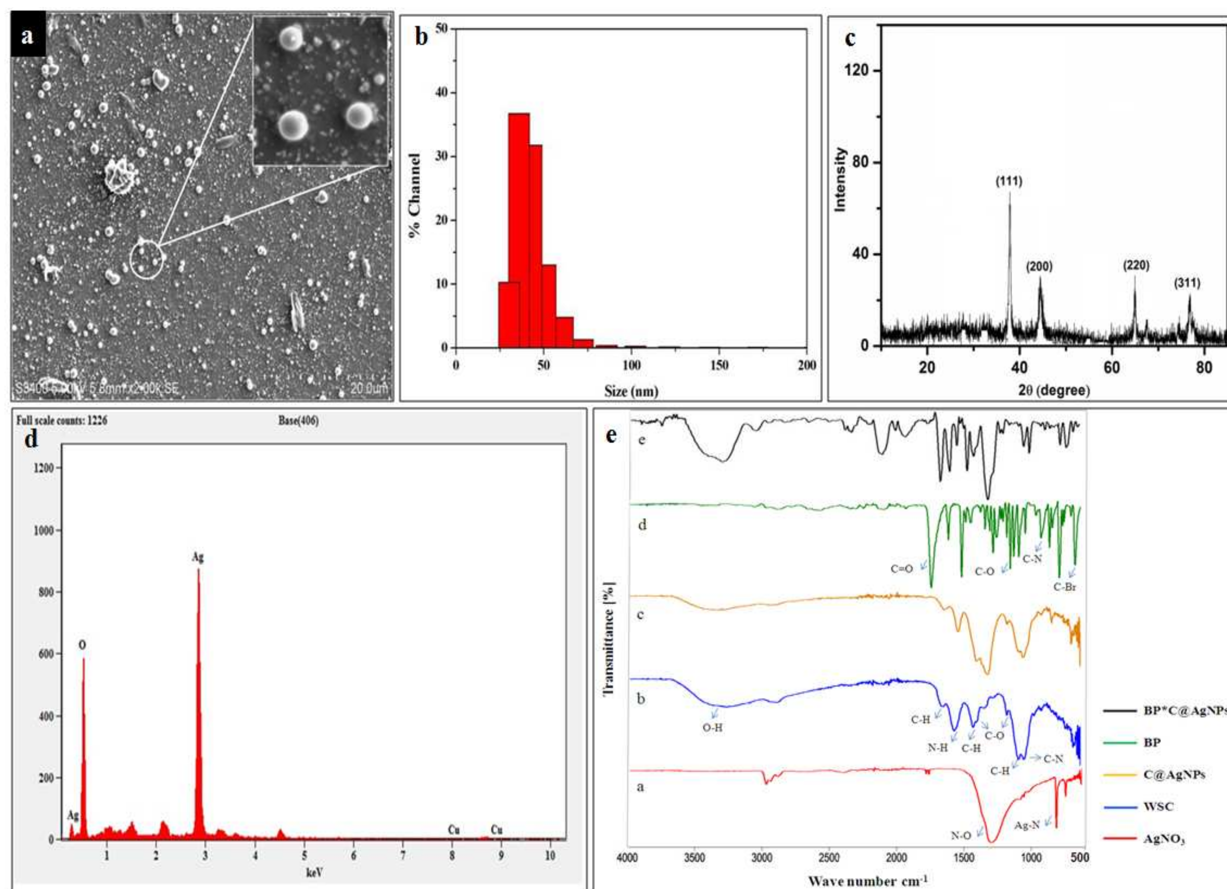


Figure 1: Characterization of synthesized BP*C@AgNPs. The 'a' depicts SEM, 'b' DLS, 'c' XRD,'d' EDS, and 'e' FT-IR.

3.2. Antibacterial activity: Zone of inhibition (ZOI)

The well diffusion method was studied by loading different concentration of synthesized BP*C@AgNPs to plate containing Bap positive methicillin-resistant *Staphylococcus aureus* (Bap-MRSA) and showed dose dependent bacterial inhibition compared to negative control. As expected, the BP*C@AgNPs exhibited considerable inhibition zone against Bap-MRSA **(Fig. 2)** [18]. The growth analysis exhibited the MIC of 100 µg/mL for BP*C@AgNPs and strong inhibition of Bap-MRSA was found at 150 min. The effective inhibition observed was dependent on particle size, shape and importantly capping and loaded agent has an important role in

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verifying bactericidal activity of silver nanoparticles. This result was compared to the standard antibiotic in the previous report. Hence, the active agent BP decorated against Bap-MRSA has advantage over other chemically produced nanoparticles [26-29].

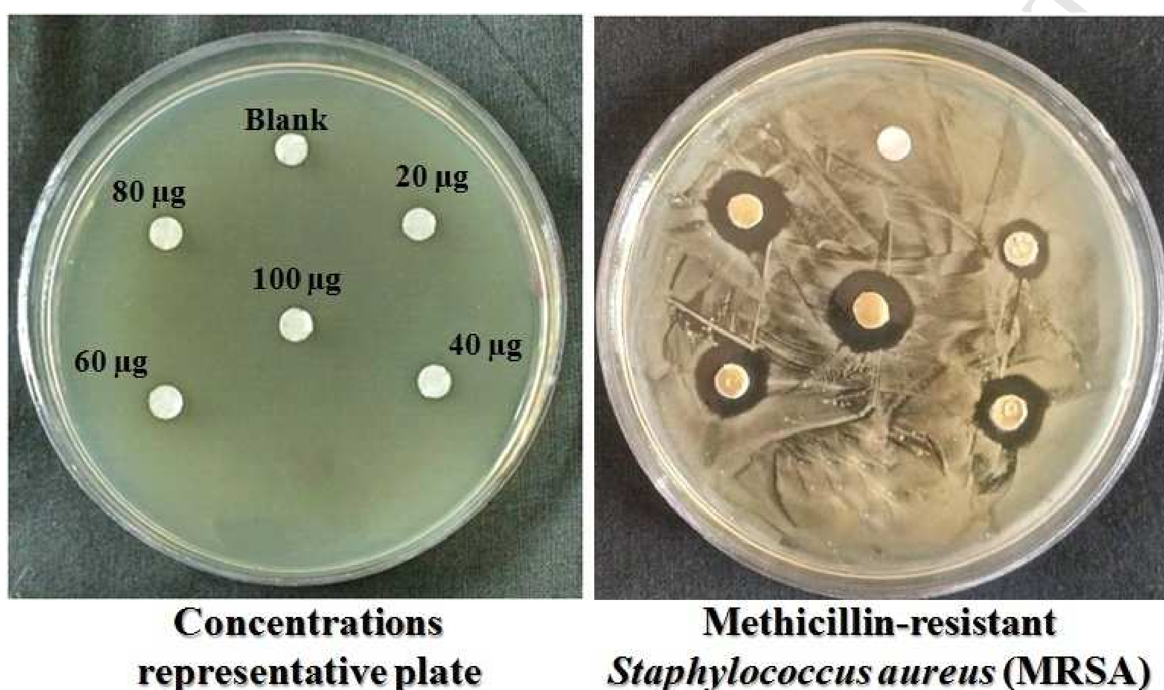

Figure 2: Antibacterial activity of BP*C@AgNPs against Bap-MRSA.

3.3. Cellular content leakage

The bacterial cells are metabolically highly active and cytoplasmic membrane is very delicate in nature. Thus, any active molecule having potential interaction with the cytoplasmic membrane leads to damage in membrane anatomical structure and release of potassium ions, DNA and other cellular materials. The treatment of BP*C@AgNPs in at the concentration of MIC induced a different degree of membrane damage with time was determined. The cytoplasmic membrane of Bap-MRSA was effectively disintegrated upon interaction of BP*C@AgNPs by forming pore on the cell membrane in a dose dependent manner and achieved

average damage at the concentration of 200 and 500 µg/ml at 150 min compared to positive control 0.1% Triton X-100 **(Fig. 3)**. The Gram-positive Bap-MRSA do not contain a pore on its cell surface and any stress or damage leads to a destruction or formation of pore on the cell surface inturn leakage of cellular materials such as protein, nucleic acids and lipid [20].

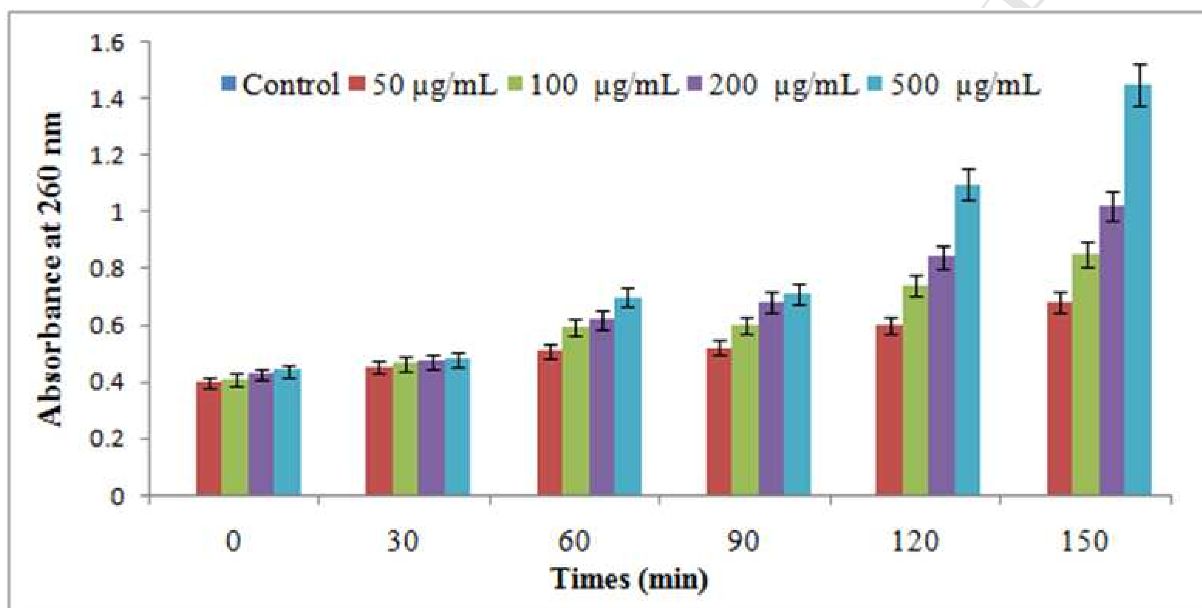

Figure 3: Cellular content release analysis to show the membrane pore formation by BP*C@AgNPs.

3.4. Bap-MRSA adhesion

The six-well polystyrene microtiter plate was examined for bacterial adherence using crystal violet method and showed BP*C@AgNPs efficiently inhibited the growth of the bacteria. As dose increased (50 to 500 µg/ml concentration), the inhibition is also elevated by supporting to BP*C@AgNPs. The promising action of BP*C@AgNPs was representing at lower dose of 100 µg/ml concentration compared to positive control **(Fig. 4A)**. The percentage of inhibition of biofilm at 52.60% and 73.79% at 200 and 500 µg/mL concentrations respectively compared to

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positive control. There are many proteins reported in Staphylococcal infection and development of a biofilm. The biofilm was composed of macromolecules such as exopolysaccharides, proteins, and DNA.

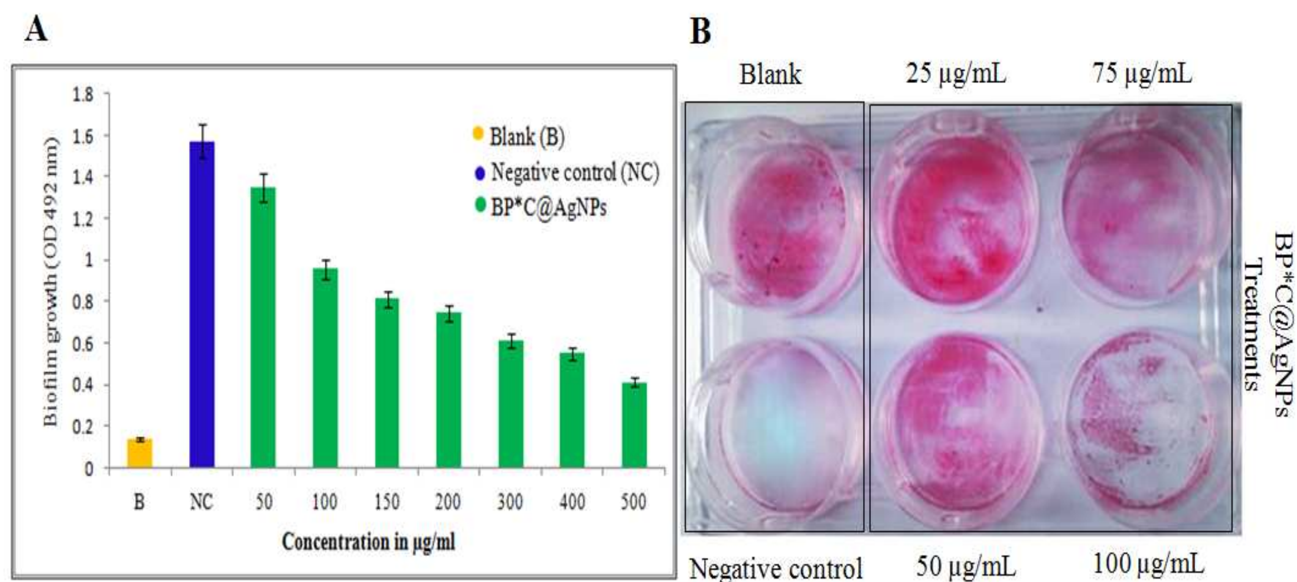

Figure 4: Effect of BP*C@AgNPs in adhesion of MRSA. The ‘A’ indicates the quantitative and ‘B’ shows qualitative representation of biofilm formation.

The important exopolysaccharides is a polymer of poly-*N*-acetyl-β-(1-6)-glucosamine, called as polysaccharide intercellular adhesin (PIA) or poly-*N*-acetylglucosamine (PNAG). Which is depends on synthesized enzymes encoded by the *icaADBC* operon. Even though PIA/PNAG part of biofilm matrix, it is not important but existence of proteins takes responsibility for mediating cell to cell interaction by forming biofilm and multicellular behavior. Bap is one of large cell-wall associated proteins used to mediate intial attachment to abiotic surfaces and intercellular adhesion [30, 31]. There is report on Bap has only been described in mastitis-derived staphylococci but here we strongly reporting Bap-MRSA for the

first time and used as a model organism and the inhibition at minimum concentration of BP*C@AgNPs was considered further experiments.

3.5. Eradication of biofilm by BP*C@AgNPs

The formation of the Bap-MRSA biofilm on inert solid surfaces was clearly observed **(Fig. 4B)**. In the present study assessed a biofilm formed on the tested surfaces by crystal violet method and results showed that, maximum eradication of biofilm by BP*C@AgNPs observed at the concentration of 500 µg/mL with an inhibition rate of 50.36% on cover slip compared to positive control **(Fig. 5A)**. The subsequent application of BP*C@AgNPs at different concentrations represents statistically significant and consistency effect of BP*C@AgNPs on eradicating the Bap-MRSA on cover slip **(Fig. 5B)**. The biofilms are resistant material to host defense machineries, antibiotic treatment and inturn cause biofilm related complications leading to increased mortality in the globe [9]. The SEM image showing clumping of bacteria due to interaction of bacterial Bap (control) and after treatment of 200 µg/mL of BP*C@AgNPs overnight inhibited a bacterial Bap interact each other effectively **(Fig. 5B).**

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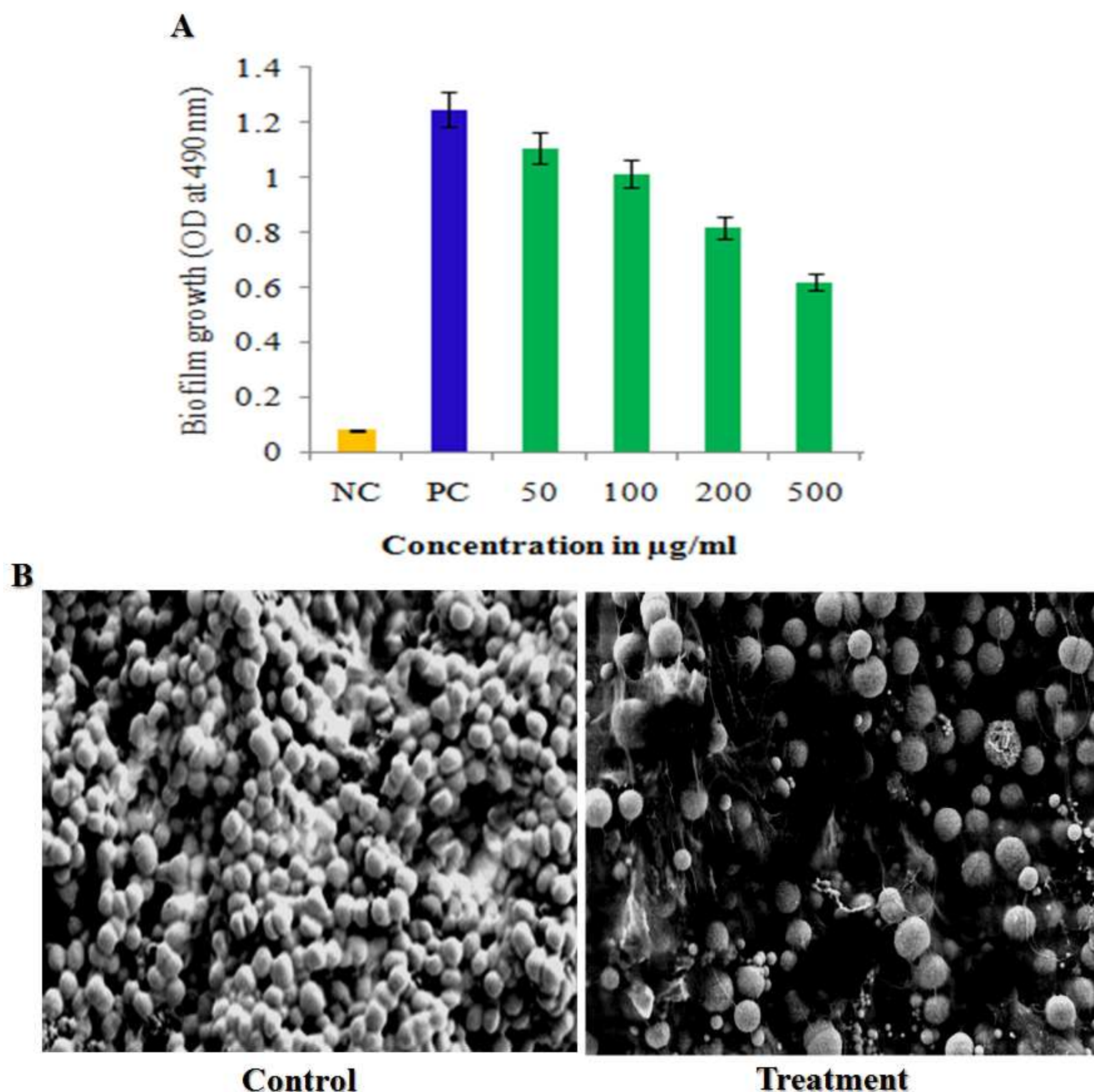

Figure 5: The MRSA Biofilm eradication efficacy and biofilm inhibition confirmed by SEM.

3.6. Effect of Ca^{2+} - BP*C@AgNPs on biofilm and clumping of Bap-MRSA

The used motif search at prosite for amino acid sequence of Bap and PS00018 definition surprisingly revealed the presence of four potential calcium binding sites in Bap sequence with similarity of ≥80% containing loop of consensus EF-hand motif [32]. The addition of Ca^{2+} to

TSB medium (already having 7.36±0.8 mM of Ca^{2+} intrinsic concentration) might be complex intraction of Ca^{2+} with essential nutrients or which might be helps in biofilm formation [33]. To reveal this possibility, minimal medium was used to observe the influence of Ca^{2+}, EGTA, EDTA and BP*C@AgNPs on biofilm formation of Bap-MRSA. The **figure 6A** shows that, week biofilm forming property after 10 mM concentration of Ca^{2+}. At the 10 mM Ca^{2+} concentration shows distinguishing phenotypic action of Bap-MRSA on biofilm does not altering the growth of planktonic cell growth **(Fig. 6B)**. The influence of tested EGTA, EDTA and BP*C@AgNPs showed different effects on clumping of bacterial cells. The EDTA at the concentration 25 mM showed clear solution compared to EGTA, BP*C@AgNPs at 50 mM and 500 µg/ml concentrations respectively **(Fig. 6A&B)**. This indicates chelating action may also influence the intercellular interaction and biofilm formation [34].

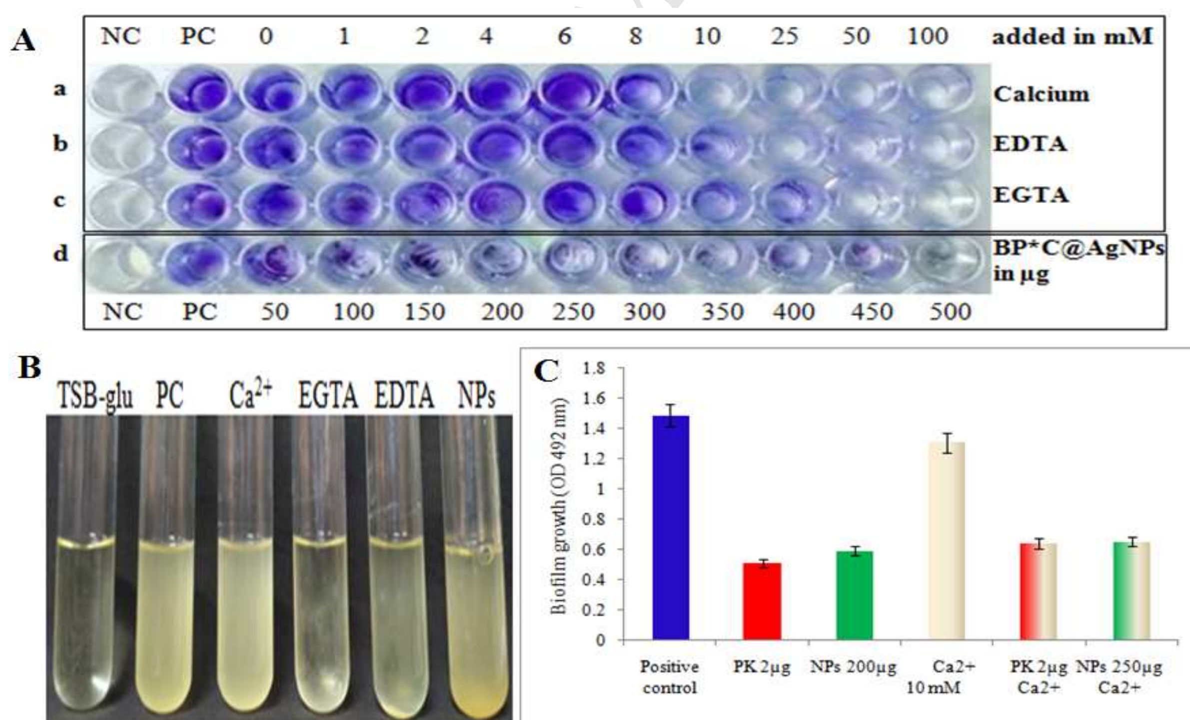

Figure 6: Effect of different effectors on MRSA biofilm and comparative role of proteinase K and BP*C@AgNPs.

3.7. Interaction of BP*C@AgNPs on Bap mediated biofilm and zero effect of Ca^{2+} binding EF-hand domains

To reveal the action of BP*C@AgNPs on Bap-MRSA biofilm, comparison study carried out with proteinase K on biofilm and showed effective inhibition of biofilm growth was observed **(Fig. 6C)**. The inhibited hampering the early adhesion of Bap-MRSA to micortitre plate and active adherence property was restored after addition of PMSF was elucidated for BP*C@AgNPs with comparison to proteinase K. The BP*C@AgNPs treated adherence property of Bap-MRSA increased with time as compared to the proteinase K and significant results obtained compared to untreated PMSF cells **(Fig. 7A&B)**.

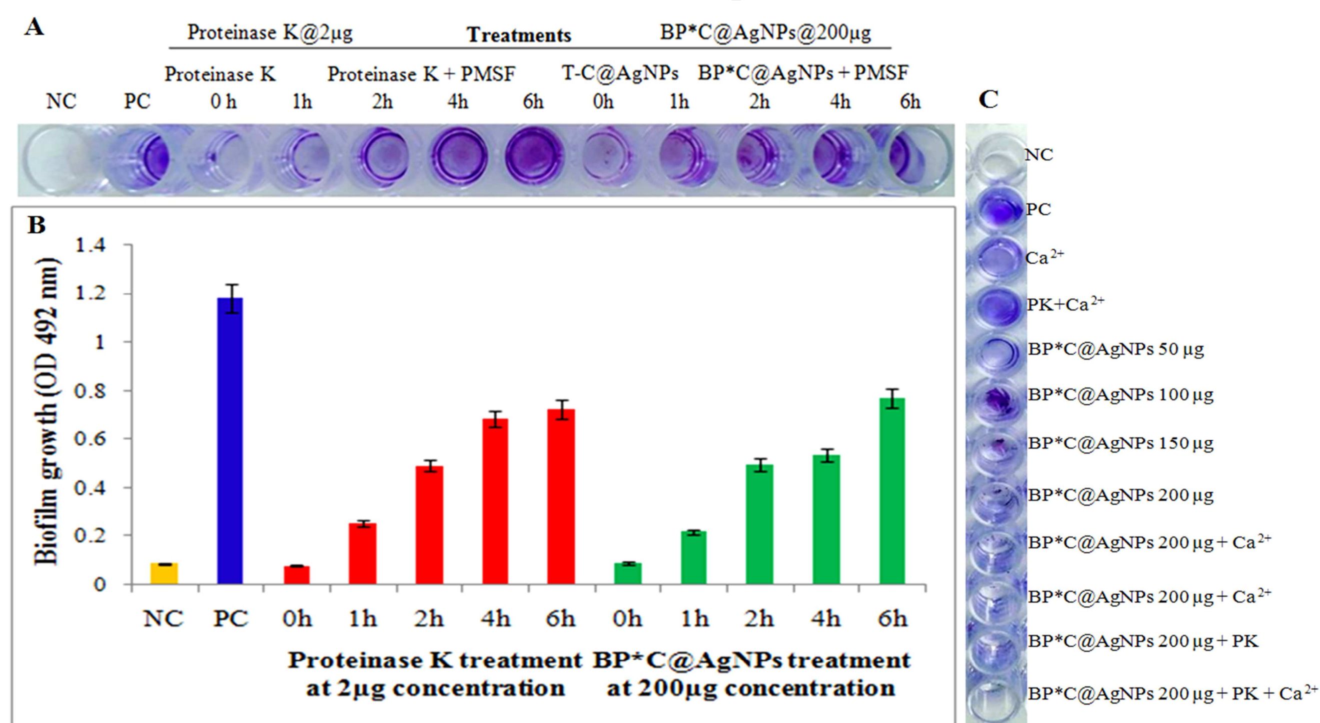

Figure 7: Role and interaction of BP*C@AgNPs in presence and absence of proteinase K and Ca^{2+} in MRSA biofilm.

We evaluated whether Ca^{2+} binding to Bap confer resistance to BP*C@AgNPs mediated degradation of biofilm, experiment significantly showed inhibition of biofilm formation of Bap-MRSA. To correlate the Bap degradation, proteinase K and BP*C@AgNPs were treated in the presence of Ca^{2+} (10 mM) and demonstrated, even Ca^{2+} binding to Bap do not confer the protection against Bap by BP*C@AgNPs **(Fig. 7C)**.

3.8. Dispersive property of BP*C@AgNPs and efficacy of antibiotic cocktail against on Bap-MRSA biofilm

To understand the role of BP*C@AgNPs on Bap proteins, different concentration were tested for matured biofilm of MRSA and showed 49.46 % biofilm dispersal at the concentration of 200 µg/ml and highest dispersal property 62.97 % obtained at 500 µg/mL. In the other hand proteinase K clearly shows that increased concentration never achieved the significant biofilm dispersal action **(Fig. 8)**. By addressing this approach is economical, the BP*C@AgNPs gained an attention to use in place of proteinase K was pointouted. The important four extracellular enzymes such as two cysteine proteases (scpA and sspB), metalloprotease aureolysin, (aur) and seven serine proteases secreted by *S. aureus* [35]. Even though these enzymes play an important role in biofilm detachment, their exact role is still unknown. Among these, serine protease showed dominant role in biofilm detachment [36, 37]. The antibiotics (Amoxyclav, Amphicillin, Chloramphenicol, Ciprofloxacin, Gentamicin, Kanamycin, Linezolid, Oxacillin, Rifampicin, Streptomycin and Tetracycline) efficacy increased significantly when the biofilm was treated with MIC of BP*C@AgNPs. The combination effect showed more impact against MRSA compared to antibiotics alone. The dose of antibiotics increased 5 times and observed only 20±2% reduction in the biofilm formation. Consistently on the other hand cocktail increased

average of 25±1% in biofilm reduction. The study strongly denotes BP*C@AgNPs acting as anti-biofilm agent and can be used for dispersion of biofilm [33].

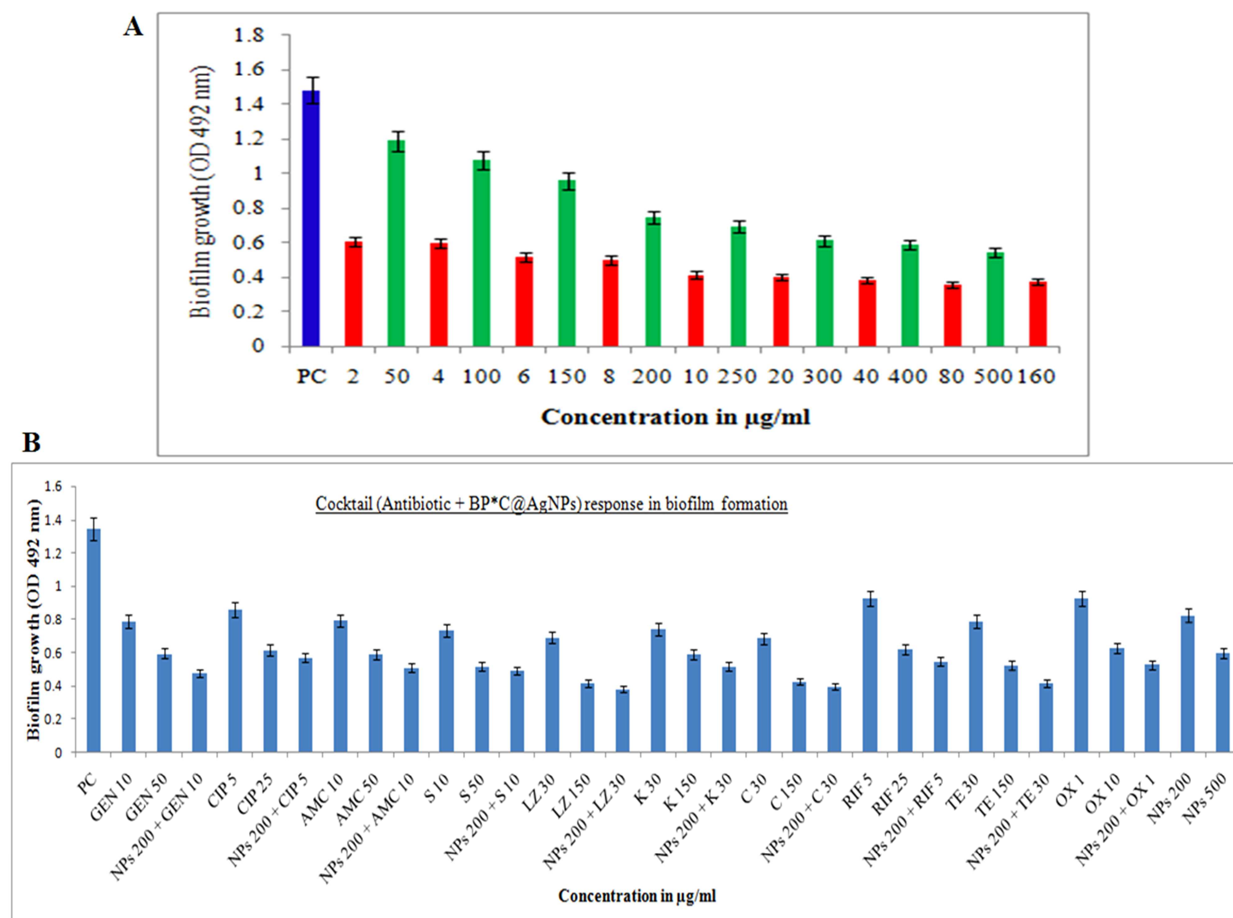

Figure 8: Understanding the dispersive property and cocktail effect of BP*C@AgNPs against MRSA biofilm formation.

The established fact says that, biofilm is extremely resistant to antibiotics compared to planktonic cells [38]. It is due to physical or genetical background influence the enhancing the biofilm to resist antibiotics. Assessed synergestic effect of BP*C@AgNPs-antibiotic mediated killing indicates cells in biofilm exposed to bulk liquid inturn dissolve antimicrobial agents. Generally, biofilm limiting the antibiotics to diffuse thereby reduce its efficacy [39, 40].

Potentially BP*C@AgNPs enables antibiotic to penetrate deep into biofilm by maximizing diffusion distance and killing the cells efficiently. Numerous reports have been addressed to neutralize or detect the expelled toxins of pathogens [41, 42]. Number of natural sources having rich phytochemical with antioxidant property, and other chemical compounds synthesized were help full in the control of microbial and other diseases, was also reported and expect to the future healthy life [43-60]. In this regard, utilization of the natural source with less toxic nature to management of the pathogenesis in the host is the best way in medicine can be hypothesized.

4. Conclusion

With these present investigations, we conclude MRSA adhesion is one of the key players in the cause of clinically related infections which leads to dangerous diseases. The previously developed nanoparticle (BP*C@AgNP) was used to study the further role in controlling notorious pathogen MRSA biofilm. The study highlights the interaction of biofilm protein with calcium ion in a dose dependent manner was underlined by nanoparticle approach and pointed that, the tuning of nanoparticle decoration definitely prove the therapeutic efficacy. In future, the role of active molecule and cocktail of potent antibiotic treatment will have the chance to overcome the drug resistant MRSA and its burden of public health was warranted.

Competing financial interests

The authors declare no competing financial interest.

Acknowledgments

The author greatly acknowledges the National Natural Science Foundation of China (Grant No. 21772150), Wuhan University of Technology and University of Hajjah, Yemen for

financial support. We also thank Anand, A. P. for providing reference bacterial strain used in the present study and SJCE, Mysore for providing instrumentation facility.

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Highlights

[1] Synthesized BP*C@AgNPs acting as an antibiofilm candidate.

[2] The biofilm of MRSA is Ca^{2+} and extracellular polysaccharide dependent.

[3] Ruled out the cocktail of treatment beneficiary for the drug resistant pathogens.

[4] Regulation of biofilm dependent protein in the Ca^{2+} presence, appreciate the future application in antibiofilm therapy.

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