In vivo synergistic antibiofilm activity of Curcumin Silver Nanoparticles against UTI causing colistin resistance *E.coli*

Malathi S.[1,2*] and Jagathy K.[1]
1. PG and Research Department of Microbiology, Sri Akilandeswari Women's College, Wandiwash – 604408, Tamil Nadu, INDIA
2. PG and Research Department of Microbiology, Indo-American College, Cheyyar - 604407, Tamil Nadu, INDIA
*smalathi186@gmail.com

Abstract
Biofilm resilence has become a serious issue in the treatment of urinary tract infection leading to the resistance to various antibiotics. There is a rapid need to develop new antibacterial agents or amalgamation drug therapies. Silver nanoparticles (AgNPs) are used as an anti-biofilm agent for the treatment of urinary tract infections. Meanwhile curcumin, a phenolic plant extract is also acting as natural anti-biofilm agent.

The objective of this study was to analyse the amalgamation of AgNPs and curcumin nanoparticles (Cur-NPs) against gram-negative E.coli at 100 µg/mL. This disrupted 50% of established bacterial biofilms. Scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) revealed that amalgamation therapy (Cur-SNPs) was the most vigorous to destruct preformed biofilm.

Keywords: Nanoparticles, Curcumin, Biofilm, Silver, Scanning electron microscope, Confocal laser scanning microscopy (CLSM).

Introduction

Irresistible illness is the second most basic reason for death; most of these are because of bacterial-related infections. In cases concerning constant contaminations, the inability to accomplish total bacterial annihilation with anti-microbials is generally because of the switch of bacterial development mode from free-swimming planktonic cells into sessile local area organized biofilms. Bacterial biofilms are typically ensured inside an independent extracellular polymeric substance (EPS) comprising of exopolysaccharide, deoxyribonucleic corrosive (DNA) and lipid [2]. At this state, biofilms show outrageous protection from most customary anti-microbials (up to 1000-crease safe) as EPS lattice limits the infiltration of anti-toxins to arrive at microorganisms inside the biofilm through dissemination impediment or balance of antimicrobial specialists with extracellular polysaccharides [3].

Furthermore, a few anti-microbials are wasteful in pulverizing fixed stage cells and biofilm cells which regularly require low sustenance to survive. Therefore, new methodologies have arisen for decrease in passings related with bacterial contaminations utilizing numerous anti-infection treatments, which can be added substance or synergistic, or through the revelation of new medications with wide range action. This need has prompted the resurgence of silver (Ag)- based mixes because of Ag's expansive action and perhaps far lower tendency to actuate bacterial obstruction utilizing Ag in contrast with current anti-microbial treatments.

It is accepted that the likelihood of microbes getting opposition against Ag is low on the grounds that Ag+ particles at the same time follow up on various destinations inside bacterial cells. Ag based mixes are right now used to control bacterial diseases in urinary lot and urinary bladder.[7] Reducing the size of particles improves molecule take-up and accessibility at the site of contamination.

This is apparent from various examinations that exhibited predominant bactericidal movement of silver nanoparticles (AgNPs) over Ag+ against E.coli.[8] In contrast with Ag+, extra bactericidal systems are explicit to AgNPs. These incorporate the immediate connection of nanoparticles onto cell film, the arrangement of pits and higher infiltration of nanoparticles into cell dividers contrasted with Ag+. Moreover, in a new report, it was discovered that applying high Ag+ focus affected biofilm evacuation, though biofilm expulsion rate utilizing AgNPs was size-dependent [9].

The cytotoxicity of AgNPs is a worry as discoveries uncovered that AgNPs slaughtered mammalian cells as low as 2−5 µg/mL. Irrespective of the clashing cytotoxic information of AgNPs, it is expected that the base AgNPs focus to accomplish viable biofilm annihilation and will cause poisonous impacts in mammalian cells. For example, complete evacuation of biofilm was not accomplished despite the fact that 200 µg/mL 8 nm AgNPs was administered [11]. Curcumin, the dynamic compound found in turmeric, is useful as anticancer, mitigating, cancer prevention agent and antibacterial. This compound is considered nontoxic for utilization as up to 8 g/day orally. Ongoing examinations found the capacity of curcumin to repress the development of biofilm, especially in gram-negative microorganisms. Curcumin (100 µg/mL) has successfully hindered the *in vitro* arrangement of *E.coli* biofilm[12].

Curcumin was additionally similarly viable in eliminating developed biofilm at a 50 µg/mL dose. Therefore, it is visualized that the utilizing AgNPs and nontoxic curcumin would upgrade the counter biofilm exercises. The current examination assessed the mix treatment utilizing curcumin

nanoparticles (Cur-NPs) and AgNPs against restraint of biofilm development and separation of set up biofilms. For this, we have created a blend of Cur-NPs and AgNPs (alluded as Cur-SNPs) with a normal size of 30 nm utilizing a dissolvable and antisolvent precipitation technique.

Material and Methods

Materials: Silver nitrate ($AgNO_3$), gallic corrosive and curcumin (immaculateness ≥ 80%) were provided by Sisco Research Laboratory. Polyvinylpyrrolidone (PVP) and pluronic F-127 were bought from Sigma Aldrich, Chennai. Deionized water was refined by invert assimilation (Milli-Q, Millipore).

Preparation of Curcumin Silver Nanoparticles: Colloids of AgNPs with normal width of 10 nm were set up by decrease of silver nitrate in water with sodium borohydride ($NaBH_4$) within the sight of sodium dodecyl sulfate (SDS) as a stabilizer. In this technique for amalgamation, curcumin is disintegrated in a natural dissolvable followed by the expansion of this arrangement into the deionized water under consistent mixing. Consequently, curcumin nanoparticles can be combined by this strategy. For the planning of Cur-SNPs, a similar strategy was utilized with a slight change in which 100 mg of re-dispersed AgNPs was added into the Cur-NPs suspension before the option of PVP [5].

Characterization of Nanoparticles: Molecule size of Cur-NPs, Cur-SNPs and AgNPs was resolved utilizing TEM. Transmission electron microscopy (TEM) was done utilizing electron magnifying lens working at 200 kV to notice the shapes and sizes of nanoparticles just as to quantify the size disseminations of particles. The presence of curcumin and silver was likewise decided utilizing Fourier transform infrared spectroscopy (FTIR) [6].

***In vitro* Biofilm Formation and Detachment Assay**: The biofilm development test was acted in 96-well microtiter plates in which the microorganisms (e.coli) were developed all the while with antimicrobial specialists (AgNPs, Cur-SNPs, or Cur-NPs). Before each examination, the freeze-dried powders of Cur-NPs, Cur-SNPs and AgNPs were resuspended in separate bacterial development media and homogenized completely utilizing a shower sonicator for 15 min. A 50 µL aliquot was set followed by the expansion of 50 µL of Cur-NPs, Cur-SNPs, or AgNPs at different focuses. The plates were hatched for 24 h without shaking at 37°C. After 24 h, the plates were exposed to staining with gem violet (CV) systems as depicted previously[1]. The biofilm destruction test was acted in 96-well microtiter plates utilizing wild sort *E.coli* bacterial strain utilizing the tissue culture plate strategy.

Qualitative Imaging of *E.coli* Biofilms: For visual perception of biofilm, tests were investigated utilizing SEM. Momentarily, biofilms were treated with various NPs containing curcumin and were flushed twice with phosphate support saline (PBS).

SEM perception tests: These were fixed utilizing 4% paraformaldehyde overnight without staining. After obsession, they were dried out through a progression of ethanol showers, dried utilizing a basic point dryer, gold covered and imaged utilizing SEM [8].

CLSM imaging: Treated examples were washed with PBS, stained with SYTO9 (Invitrogen, Australia), lastly fixed with 4% paraformaldehyde. The morphologies of biofilms were imaged utilizing an oil submersion focal point (100× target focal point and mathematical gap of 1.4). Recorded pictures were remade by Imaris and introduced as three-dimensional structures. The morphologies of biofilms were imaged utilizing an oil submersion focal point (100× target focal point. Recorded pictures were recreated and introduced as three-dimensional structures.

Cytotoxicity Evaluation using zebra fish: A 48-hour intense harmfulness (LC50) trial of Synergestic Curcumin - silver nanoparticles [CuSNP's] on grown-up male zebrafish (standard length 28.1 ± 0.2 mm weighing 0.42 ± 0.04 g; n = 110) was directed in a static water re-establishment analyse.

Likewise, two PVP controls (Sigma-Aldrich, Chennai) were incorporated to survey the conceivable poisonousness of the PVP covering. Zebrafish were presented to PVP fixations equalling half of the CuSNP's LC50 esteem (42 µg L^{-1}) and multiple times the CuSNP's LC50 esteem (8400 µg L^{-1}).

After 24 h of treatment, the fishes were moved to new tanks containing separate groupings of CuSNP's. The zebrafishes were not taken care of 24 hours before or during the investigation to keep up steady openness fixations, given that nanoparticles may hold fast to food and dung particles[18]. A comparative 48-hour intense harmfulness (LC50) trial of silver particles was directed with zebrafish of standard length 28.6 ± 0.3 mm and weighing 0.45 ± 0.03 g; n = 80, to contrast the poisonousness of silver nanoparticles and silver particles. The ostensible openness focuses were 13, 21, 23, 25, 29, 37 and 47 µg silver particles L^{-1} managed as silver nitrate in addition to an unexposed control gathering. The fish conduct was surveyed by a human spectator after 0, 3, 6, 12, 24, 27, 30 and 48 hours of both silver nanoparticles and Curcumin nanoparticles medicines.

Statistical Analysis: Examination was performed utilizing the SPSS Statistics 19 programming bundle. All information was gathered (n = 5) and the mean qualities and standard deviations (SD) were determined. The factual contrasts between bunches were controlled by examination of difference (ANOVA). Estimations of $p < 0.05$ were considered measurably huge. LC10 and LC50 esteems in addition to their 95% certainty spans were determined utilizing the probit examination in SPSS variant 13[4].

Results and Discussion

Characterization of Nanoparticles: Figure 1a shows the size dispersion and morphology of redispersed nanoparticles

utilizing TEM. Cu NPs showed up as groups of round particles at around 30 nm under TEM. Then, redispersed AgNPs showed up unagglomerated and round, going from 10 to 35 nm (Figure 1b), This was affirmed with molecule estimating circulation with a very much characterized populace of particles with a normal width of around 30 nm (inset of Figure 1c). During the manufacture of joined Cur-SNPs, the proportion of curcumin to silver was set at 9:1. TEM exhibited that these nanoparticles were scattered and around 30 nm in distance across.

FTIR investigation was embraced to affirm the presence of curcumin in the readied Cur-NPs and to assess the collaborations between Cur-NPs and AgNPs. Figure 2 shows the FTIR spectra for crude curcumin, Cur-NPs, Cur-SNPs and AgNPs. The pinnacles relegated for C-O-C at 1110 cm^{-1} were likewise clearly identified in both Cur-NP and Cur-SNPs, consequently showing the presence of curcumin exemplified in nanospheres[10].

Biofilm eradication Assay: AgNPs were compelling in killing set up *E.coli* biofilm. We have exhibited that the higher biofilm expulsion proficiency of AgNPs contrasted with ionized structure (Ag+) meant the presence of other bactericidal components of nanoparticles for biofilm evacuation. For example, AgNPs could be entered and scattered into biofilm grid more effectively contrasted with Ag+.

In this examination, a blend treatment utilizing AgNPs and Cur-NPs was utilized trying to give upgraded antibiofilm exercises. Curcumin is picked as a cotherapy compound since phytochemical turmeric displays antibacterial exercises against wide scopes of planktonic microorganisms[12]. The blend of Cur-NPs and AgNPs showed an added impact as they repressed biofilm arrangement more powerful than that of AgNPs or Cur-NPs alone.

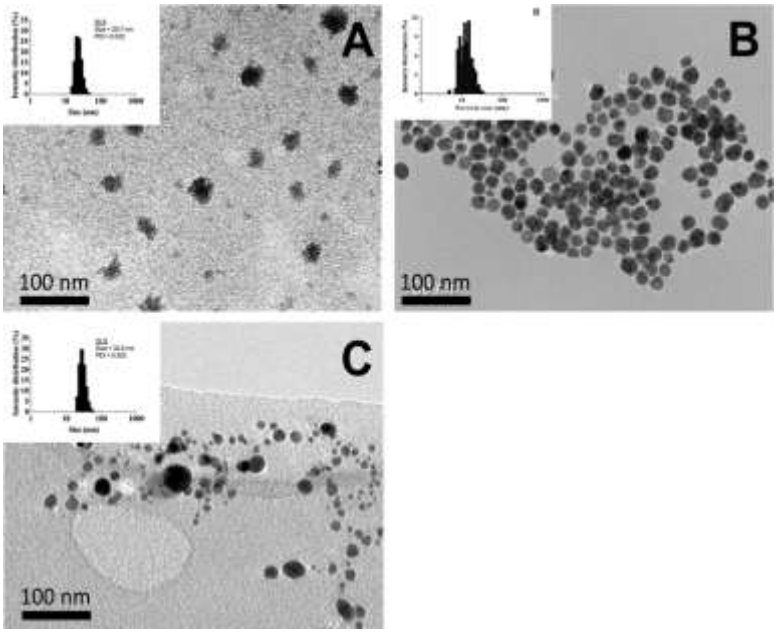

Figure 1: TEM images of (A) Cur-NPs, (B) AgNPs and (C) Cur-SNPs.

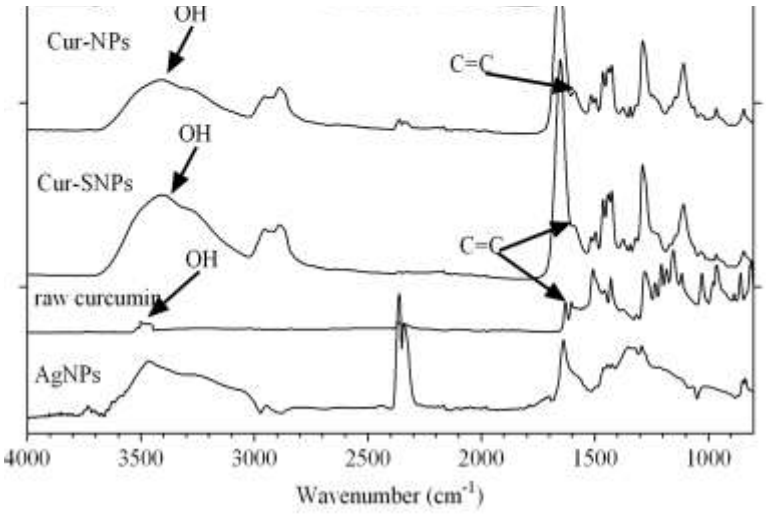

Figure 2: FTIR spectra of Cur-SNPs, AgNPs, Cur- NPs, raw curcumin

The biofilm development of *E.coli* was hindered 85% when Cur-SNPs were directed at fixations comprising 20 µg/mL curcumin and 2.5 µg/mL Ag. Restraint of biofilm arrangement was seen with Cur-SNPs comprising of 30 µg/mL curcumin and 3.75 µg/mL Ag. Numerous investigations have additionally exhibited that at any rate 100 µg/mL curcumin was needed to stop the development exercises of *E.coli* without bactericidal effect.

Moreover, this mix treatment (Cur-SNPs) likewise showed higher adequacy to completely impede the development of *E.coli* biofilm when managed with dosages containing 40 µg/mL curcumin and 5 µg/mL Ag. Strangely, at this focus no inhibitory movement on biofilm was noticed utilizing either Cur-NPs or AgNPs alone.

Visual affirmation of the impact of NP treatment on biofilm annihilation was acquired utilizing both SEM and CLSM. (Figure 4). The convergences of Cur-SNPs used to treat set up *E.coli* biofilms comprised of 400 µg/mL curcumin and 50 µg/mL Ag. To confirm that the Cur-SNPs exhibited higher viability against preformed *E.coli* biofilm, the outcomes were contrasted and those acquired utilizing medicines with AgNPs and Cur-NPs alone. The groupings of AgNPs and Cur-NPs utilized were 50 and 400 µg/mL separately.

Microcolonies of *E.coli* were still clearly seen in both Cur-NPs-and AgNPs-treated biofilms, hence affirming that the blend Cur-SNPs was more powerful in killing preformed biofilm (Figure 3). In any case, subjectively it appears that AgNPs had an irrelevant effect on *E.coli* biofilm. The weakness of *E.coli* biofilm to medicines followed the diminishing request Cur-SNPs > Cur-NP > AgNPs.

Figure 3 shows the comparing number of joined bacterial cells (estimated as province framing units, CFU) for untreated cells (control) and tests treated with particular AgNPs, Cur-NPs and Cur-SNPs. It is obviously seen that the CFU mean microbes follows the diminishing pattern AgNPs < Cur-NPs < Cur-SNPs. For *E.coli*, the CFU for untreated control was 9.5 × 108 CFU/cm^2. The relating CFU estimations of *E.coli* after treatment with AgNPs, Cur-NPs, or Cur-SNPs was 4.0 × 107, 9.5 × 106.

Toxicity study: CuSNP's are intensely poisonous to male zebrafish with a semistatic 48-hour openness LC50 of 84 µg L^{-1} (95% CL = 74–93 µg L^{-1}) and LC10 of 57 µg L^{-1} (95% CL = 36–68 µg L^{-1}). A restricted edge was found between the LC50 24 h of 89 µg L^{-1} (95% CL = 79–100 µg L^{-1}) and the LC50 48 hour. There was no mortality in the control tank or at the three most reduced CuSNP's fixations

Following 3 hours of openness, the primary fish began passing on and following 24 hours, all fishes in the two most noteworthy test focuses were dead. Extravasations of blood were seen in the foremost ventral surface of the body, simply behind the top of the dead fish[14,15].

This was not found in dead fish presented to ionic silver. The harmful activity of CuSNP's was generally fast with indications of stress showing up inside 30 min of openness. At higher silver nanoparticle fixations (>72 µg L^{-1}), harmfulness stress signs arose, beginning with zebrafish lying on the tank base with expanded respiratory rate. From now on surface respiration occurred, lastly the fish stopped in the water section where they eventually lost balance and sank to the base. A couple of fishes showed jerky developments and round swimming not long before they lost balance. After 24 hours of openness, there were no obvious contrasts in conduct between the control and the least openness gatherings. Fish bodily fluid (dainty white expanded strings), likely discharged from the gills, was seen at the lower part of tanks presented to at any rate 89 µg L^{-1} CuSNP's. Bodily fluid emission was not seen in the control or the lower fixation openness tanks.

In another examination on zebrafish, Choi et al[3] detailed the 24-hour LC50 to be 250 mg L^{-1} for 5–20 nm silver nanoparticles, which is far less harmful than the LC50 esteem found by Griffith et al[4] and in the current examination. In Japanese medaka (*Oryzias latipes*), the 96-hour LC50 has been exhibited to be 34.6 µg L^{-1} for 50 nm uncoated silver particles.

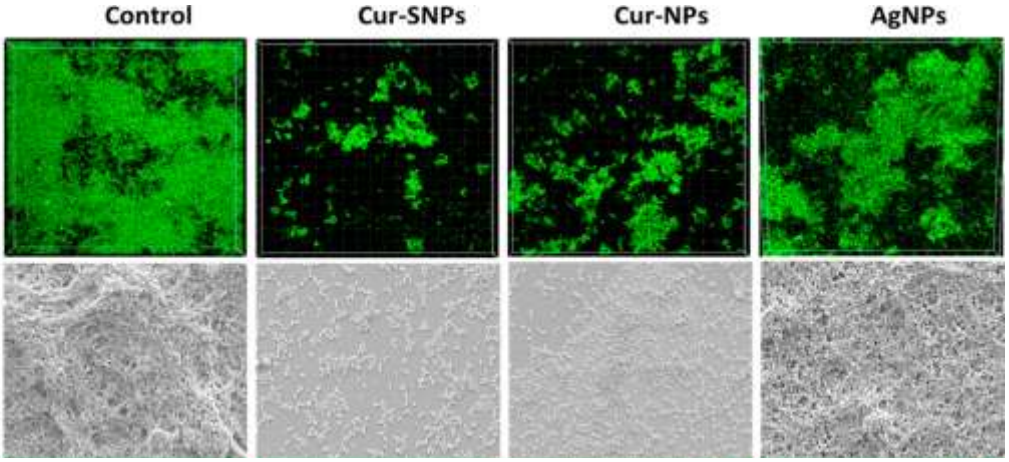

Figure 3: CLSM and SEM images of *E.coli* biofilms nontreated and treated with Cur-SNPs, Cur-NPs, or AgNPs.

Interestingly, Navarro et al[12] assessed that 1% of the silver in a carbonate-covered nanosilver suspension was having free silver particles. This underlines the significance of assessing the disintegration of metal nanoparticles.

Conclusion

All in all, the blend treatment of Cur-NPs and AgNPs was compelling in killing set up develop biofilm and hindered biofilm arrangement.

Acknowledgement

We thank Akilandeswari college for Electron microscopy and FTIR analysis.

References

1. Bar-Ilan O., Albrecht R.M., Fako V.E. and Furgeson D.Y., Toxicity assessments of multisized gold and silver nanoparticles in zebrafish embryos, *Small*, **5(16)**, 1897-910 **(2009)**

2. Catauro M.I., Raucci M.G., De Gaetano F. and Marotta A., Antibacterial and bioactive silver-containing Na 2 O· CaO· 2SiO 2 glass prepared by sol–gel method, *Journal of Materials Science: Materials in Medicine*, **15(7)**, 831-7 **(2004)**

3. Basniwal R.K., Buttar H.S., Jain V.K. and Jain N., Curcumin nanoparticles: preparation, characterization, and antimicrobial study, *Journal of Agricultural and Food Chemistry*, **59(5)**, 2056-61**(2011)**

4. Hu P., Huang P. and Chen M.W., Curcumin reduces Streptococcus mutans biofilm formation by inhibiting sortase A activity, *Archives of Oral Biology*, **58(10)**, 1343-8 **(2013)**

5. Hussain S.M., Hess K.L., Gearhart J., Geiss K.T. and Schlager J., *In vitro* toxicity of nanoparticles in BRL 3A rat liver cells, *Toxicology In Vitro*, **19**, 975−983 **(2014)**

6. Jones K.E., Patel N.G. and Levy M.A., Global trends in emerging infectious diseases, *Nature*, **451**, 990−993 **(2008)**

7. Kim H.R., Kim M.J., Lee S.Y., Oh S.M. and Chung K.H., Genotoxic effects of silver nanoparticles stimulated by oxidative stress in human normal bronchial epithelial (BEAS 2B) cells, *Mutation of Research and Genetical Toxicology Environment Mutagen*., **726**, 129−135 **(2011)**

8. Kora A.J. and Arunachalam J., Assessment of antibacterial activity of silver nanoparticles on *E.coli* and its mechanism of action, *World Journal of Microbiology and Biotechnology*, **27**, 1209−1216 **(2011)**

9. Laban G., Nies L.F., Turco R.F., Bickham J.W. and Sepúlveda M.S., The effects of silver nanoparticles on fathead minnow (Pimephalespromelas) embryos, *Ecotoxicology*, **19(1)**, 185-95 **(2010)**

10. Loo C.Y., Young P.M. and Cavaliere R., Silver nanoparticles enhance *E.coli* PAO1 biofilm detachment, *Drug Dev. Ind. Pharm*., **40**, 719−729 **(2014)**

11. Mun S.H., Joung D.K., Kim Y.S., Kang O.H., Kim S.B., Seo Y.S., Kim Y.C., Lee D.S., Shin D.W., Kweon K.T. and Kwon D.Y., Synergistic antibacterial effect of curcumin against *e.coli*, *Phytomedicine*, **20**, 714−718 **(2013)**

12. Packiavathy I.A., Priya S., Pandian S.K. and Ravi A.V., Inhibition of biofilm development of uropathogens by curcumin–an anti-quorum sensing agent from *Curcuma longa*, *Food Chemistry*, **148**, 453-60 **(2014)**

13. Raffi M., Hussain F., Bhatti T.M. and Akhter J.I., Antibacterial characterization of silver nanoparticles against *E. Coli* ATCC15224, *Journal of Material Science and Technology*, **24**, 192−196 **(2008)**

14. Simoes M., Antimicrobial strategies effective against infectious bacterial biofilms, *Current Medical Chemistry*, **18**, 2129−2145 **(2011)**

15. Sondi I. and Salopek-Sondi B., Silver nanoparticles as antimicrobial agent: a case study on E. Coli as a model for Gram-negative bacteria, *Journal of Colloid Interface Science*, **275**, 177−182 **(2004)**.

(Received 12th June 2021, accepted 19th August 2021)