Targeting Antimicrobial Drug Resistance by Amine Functionalized Metal Nanoparticles

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BY

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Chapter 8

Thesis Summary and Future Projections

The thesis entitled "**Targeting Antimicrobial Drug Resistance by Amine Functionalized Metal Nanoparticles**" has been divided into the following seven chapters:

1. General Introduction.
2. Microwave-assisted rapid synthesis and effect of organic Functionality on the antimicrobial activity of Noble metal nanoparticles.
3. Microwave-assisted rapid synthesis and molecular weight of Polyethyleneimine-dependent selective surface interaction and antimicrobial activity of functional silver nanoparticles.
4. A whole-cell fluorescence quenching-based approach for investigating Polyethyleneimine functionalized silver nanoparticles interaction with *Candida albicans*.
5. Size and zeta potential clicked germination attenuation and anti-sporangiospores activity of PEI-functionalized silver nanoparticles against COVID-19 associated Mucorales (*Rhizopus arrhizus*).
6. Synthesis of vancomycin functionalized fluorescent gold nanoparticles and selective sensing of mercury (II).
7. Making vancomycin a potent broad-spectrum antimicrobial agent using Polyaziridine-stabilized gold nanoparticles as a delivery vehicle.

Chapter 1

This section thoroughly discusses the history of nanotechnology and its application in broad fields of science as industrial such as catalysis, biomedical, energy sector, and other related sectors. Therefore, this chapter focused on the comprehensive discussion of the different synthesis methodologies, their application as antimicrobial agents, and associated modes of actions of metal and non-metal nanoparticles such as Gold, Silver, Palladium, Titanium, Copper, Zinc, Iron, and Mn.

Among all metal nanoparticles, Ag^{+} and gold nanoparticles have been used as antimicrobial agents for centuries. However, the biomedical applications of metal nanoparticles are limited because of their non-selective reactivity with biomolecules. The research community has explored the antimicrobial activity of metal nanoparticles against eukaryotic and prokaryotic pathogens in recent decades. Several organic and inorganic grafting agents are used to modify the efficacy and spectrum of activity of metal nanoparticles unfortunately the selectivity has to been achieved yet. Second, some issues are also associated with the physico-chemical stability of particular nanoparticles over time. On the other hand, the antimicrobial mechanism of metal nanoparticles against the planktonic and biofilm-forming microbes is limited. However, researchers suggested several biocidal mechanistic pathways, including antimicrobial activity by ROS generation, protein damage, membrane depolarization, DNA damage, quorum sensing inhibition, and membrane damage. However, the antimicrobial mechanisms for many types of nanoparticles have not been fully elucidated yet. Despite of the significant advancement in the biomedical field, some critical issues must be addressed before human application of metal nanoparticles. These challenges are;

1. Stability of MNPs at various physico-chemical & physiological environments.

2. Biocompatibility.
3. Nonspecific interaction of MNPs.
4. Metal resistance among microorganisms.
5. Limitations in Applicability.

Hence, the proceeding chapters are made as an effort to address the above-stated challenges to develop efficient nanotechnology-based biomedical tools, drug delivery systems, and FRET-based sensing of biological as well as non-biological environmental samples.

Chapter 2

Recently, tri-alkoxysilanes (3-APTMS and 3-GPTMS) stabilized mono-, bi- and trimetallic nanoparticles have been synthesized and successfully explored for various electrochemical sensing applications by Pandey et. al., (Pandey & Chauhan, 2012; Pandey et al., 2014 a; Pandey & Pandey, 2014 b; Pandey & Singh, 2015; Pandey & Pandey, 2016). However, some challenges were associated with synthetic protocols such as long reduction time, stability in aqueous media, and lack of biomedical applications. To overcome such challenges, certain modifications are considered i.e., the use of microwave irradiation to fasten the reduction process along with the addition of 1-vinyl 2-pyrrolidone (co-reducing agent) and ethylene glycol diacetate in the reaction mixture to prevent the evaporation of solvent during irradiation and agglomeration. Here, microwave irradiation played a significant role in enhancing the reduction rate of metal cations such as silver, gold, and palladium in the presence of reducing agents such as cyclohexanone, 3-GPTMS, and formaldehyde. Due to the penetrating characteristics of microwaves, homogenous heating of the reaction mixture allowed uniform nucleation, rapid crystal growth, and formation of nano-geometry-controlled crystallites with narrow size distribution. However, the reduction kinetics of metal cations under microwave irradiation are still to be investigated in the presence of tri-alkoxysilanes. Simultaneously, the

effect of variable concentrations of 1-vinyl 2-pyrrolidone, and 3-APTMS on nanoparticle synthesis has been examined. The results demonstrated a significant influence of concentration variation on nanoparticles LSPR. Because 1-vinyl 2-pyrrolidone itself acts as a reducing agent (co-reducing agent in the presented research work), at higher concentration (400 mg/ml) along with cyclohexanone, the absorbance intensity increased drastically without any shift in SPR attributed to the complete reduction of metal cations and active role in controlling nano-geometry of silver nanoparticles. However, in the case of gold and palladium cations reduction, no significant role was observed at the given parameters.

From a biomedical application point of view, 3-GPTMS, and formaldehyde-reduced AgNPs are not suitable because of the use of a high concentration of reducing agent and hydrophobicity that leads to make it least dispersible in aqueous solution and rapid polymerization tendency. Further, the above-discussed property prevents the removal of unused reactants from suspension. Therefore, the influence of variable concentrations of 3-APTMS/cyclohexanone-mediated synthesis silver nanoparticles was established. That confirmed the significant role of certain 3-APTMS concentrations (higher conc. ≥1 M) in controlling the nano-geometry of silver nanoparticles (≤10 nm). In contrast to silver nanoparticles, AuNP stabilization required the least concentration of 3-APTMS (10 mM) in cyclohexanone-mediated synthesis. Overall, cyclohexanone-reduced AgNPs have proven a good fit for biomedical applications due to their excellent water dispersibility (hydrophilic) and antimicrobial activity. However, some challenges are associated with the use of a high concentration of 3-APTMS during synthesis. Although, 3-APTMS contains silica the biocompatibility towards different tissues is still a question that needs to be resolved to development of an effective antibacterial as well as antibiofilm platforms. Despite such shortcomings, 3-APTMS stabilized silver nanoparticles

can be applied in the surface coating of various medical equipment to remove microbial as well as viral load and further a step towards the prevention of nosocomial infections.

Though, taking into consideration of antibacterial properties of as-prepared metal nanoparticles, organic moiety-dependent rapid synthesis of noble metal mono bi- and trimetallic nanoparticles synthesis, characterization of Ag, Au, and antimicrobial evaluation against Gram-negative bacteria *Acinetobacter baumminii*. Among all, monometallic (AuNPs), bimetallic (Ag–Au and Pd–Ag), and trimetallic (Pd–Ag–Au) nanoparticles did not show any significant antimicrobial activity; on the other hand, Pd-NPs and AgNPs were shown to be potent antimicrobial agents. The AgNPs are considered for further investigation as antimicrobial agents. AgNPs were stabilized with 3-APTMS and reduced with Cyclohexanone, 3-GPTMS, and formaldehyde, which allowed the synthesis of three different types of silver nanoparticles, had spherical and less than 10 nm in size, which showed antibacterial properties against a multi-drug resistant *A. baumannii* strain. The MIC and MBC of the AgNP-1 were the lowest among the tested silver nanoparticles due to their hydrophilicity. Further, 2D and 3D fluorescence spectroscopic investigation demonstrated selective cell surface–nanoparticle interactions.

This chapter established an understanding regarding the role of various reducing agents and stabilizing agents during the reduction of metal ions along with providing a projection towards the development of selective interaction of metal nanoparticles with the microbial cell surface. As 3-APTMS stabilized AgNPs had the least selectivity and non-specific binding with cells, another stabilizing agent was required to achieve lower MIC as well as selectivity in interaction, hence proceeding chapters are continued.

Chapter 3

In the previous chapter, the shortcomings of 3-APTMS stabilized metal nanoparticles were discussed in detail which led us to apply another polymeric stabilizer to achieve an excellent aqueous dispersibility and selective nano-bio interaction. Therefore, this chapter demonstrated the molecular weight of synthetic cationic polymer-dependent synthesis of silver nanoparticles and selective antimicrobial activity.

Cationic polymers, such as PEI, have intrinsic highly positive charges and have historically been employed in the materials for bioengineering. However, the demand for intelligent systems with high efficiency, bio-mimetic, and tunable features is increasing. Artificial composites that mimic biorecognition and periodic structures may propel the development of advanced materials with outstanding properties. Polyethyleneimines (PEIs) constitute a valuable class of polycations because they have repetitive structural units, a wide molecular weight range, and flexible polymeric chains, which facilitate the customization of functional composites. Specific advantageous features could be introduced by purposeful modification or functionalization, such as specificity and sensitivity, distinct geometry, biocompatibility, and long service life. Thus, PEIs have been rapidly used in a wide range of applications in the fields of biomedicine, biotechnology, and biomaterials science. In the continuation, mono-metallic and bi-metallic nanoparticles (silver and gold) were synthesized in a rapid reaction system by using microwave irradiation that allowed homogenous heat distribution in the reaction mixture and controlled nucleation process. The synthesized monometallic silver nanoparticles have demonstrated excellent antimicrobial activity as coated with variable molecular weights of cationic PEI as discussed in the result section. Mechanistically, strong electrostatic forces in PEI molecules are responsible for the steric stabilization of silver ions in the micro-domains of the PEI. Accordingly, interestingly, different molecular weights and concentrations of PEI controlled the nano-geometry as well as the zeta potential of prepared AgNPs. Higher

molecular weight PEIs such as 750 and 60kDa., allowed the formation of 20 and 3.0 nm of AgNPs respectively with highly positive zeta potential (+32 mV) suggesting a stable colloidal suspension of silver nanoparticles. However, when in a simultaneous reaction mixture, bi-metallic Ag-Au is prepared the size of particles gets reduced to 5 nm. On the other hand, lower molecular weight (1.3 kDa) allowed the formation of an average of 5 nm in size of AgNPs. Similarly, the varying concentrations of these PEI also controlled the LSPR of nanoparticles and control over the nano-geometry of AgNPs.

Further, PEI-coated AgNPs allowed selective transfusion of AgNPs across and interfered with membrane integrity by interacting with surface-expressed proteins, displaying variable antimicrobial efficiency as a function of molecular weight. High MW. of PEI-coated AgNPs (1 and 3) had a higher cationic surface charge that allowed strong interaction with the relatively negatively charged bacterial cell surface. This electrostatic interaction-induced cellular damage takes place either by pit hole formation (AgNP-1) while AgNP-2 and 3 can evade the cell barrier due to small size and destroyed internal structures by inducing reactive oxygen species. To reveal the affinity of AgNPs, towards bacterial surface-expressed proteins, an excellent experiment was performed. AgNPs quenched the fluorescence of fluorophore fluorescein by absorbing radiative energy at excitation. However, in the presence of bacterial cells, the quenching ratio was decreased gradually as a function of no. of bacterial cells, which confirmed the specific molecular interaction of AgNPs to the bacterial cell surface. The interaction of AgNPs with the cell surface (nano-bio interface) was dependent on the surface charge of nanoparticles. AgNP-1 and 3 had a strong affinity towards the cell surface in contrast to AgNP-2. Similarly, MIC and MBC values were lowest in the case of AgNP-1 and AgNP-3 (5/10 µg/mL due to the similar zeta potential values. Suggested that zeta potential (surface charge) played a significant role in the antibacterial activity of AgNPs in addition to the size of

nanoparticles. Further, fluorescence spectroscopy of AgNPs treated cells revealed selective interaction of AgNPs depending on nanoparticles' surface charge and size with bacterial cell surface proteins, which was taken as a fluorescent probe. The fluorescence of surface protein has been quenched depending on the size of AgNPs.

From a cytotoxic point of view, in-vitro cell assessment studies have reported that AgNPs are toxic to several human cell lines including human bronchial epithelial cells, human umbilical vein endothelial cells, red blood cells, human peripheral blood mononuclear cells, immortal human keratinocytes, liver cells, etc. AgNPs induce a dose-, size, and time-dependent cytotoxicity, particularly for those with sizes ≤10 nm. Furthermore, AgNPs can cross the brain-blood barrier of mice through the circulation system based on in vivo animal tests. Ag-NPs tend to accumulate in mice organs such as the liver, spleen, kidney, and brain following intravenous, intraperitoneal, and intratracheal routes of administration. In this respect, AgNPs are considered a double-edged sword that can eliminate microorganisms but induce cytotoxicity in mammalian cells.

So, PEI-coated AgNPs are taken as nanoparticles of choice because of control over the nano-geometry of particles as a function of the molecular weight of PEI. Further, the kinetics and nano-bio interface dynamics of nanoparticle adsorption on microbial cell surfaces are elucidated in the next chapter to develop an understanding of nanoparticle interaction and efficient delivery of drugs.

Chapter 4

Since nanoparticle interaction at the cell surface is considered as the first step in executing their biological influence. However, the dynamics can be different depending on the material and cell types. Therefore, during designing an effective biocidal agent it is necessary to understand

the kinetics of nanoparticle adsorption on bacterial or mammalian cell surfaces. In this row, there are few studies involving the adsorption of NPs on cell surfaces; quantitative studies involving particle adsorption kinetics have also not been previously considered. Wilhelm et al. considered NP binding on cell surfaces via a pseudo-first-order kinetic model (Wilhelm et al., 2009). Other research teams used this kinetic model to understand NP interactions with human cells (Zhang et al., 2010 & Cho et al., 2009). It should be noted that a pseudo-first-order model does not indicate the mechanism of action. Efforts are needed to develop mechanistic models for NP adsorption to bacterial cells.

Hence, this chapter focuses on understanding how silver nanoparticles' physicochemical properties affect their microbial surface adsorption and associated antimicrobial activity. When the nanoparticles are added to the microbe-containing medium, they first encounter the microbe's surface. To understand the nanoparticle interactions with the *C. albicans* cell surface, we used Polyethyleneimine-functionalized silver nanoparticles with a size of 5.6 nm having a + 18 mV zeta potential. The PEI-functionalized silver nanoparticles have shown potent biocidal activity with a 5 μg/mL MIC value.

Furthermore, FL quenching describes phenomena that are associated with a reduction in the FL intensity (Monici, 2005). These phenomena can be associated with intermolecular interactions (e.g., energy transfer, molecular rearrangements, and excited-state reactions); moreover, the quenching phenomena can exhibit dynamic (e.g., related to diffusive encounters between the quencher and the fluorophore within the excited state lifetime) or static (e.g., related to complex formation between the quencher and the fluorophore in the ground state (Monici, 2005). C. albicans exhibits an outer rigid cell wall structure covered by the cell membrane, which is composed of complex sugar moieties such as β-glucans and several anchored proteins that act as receptors for exogenous ligands. When excited with UV irradiation, these surface-anchored

proteins fluoresce due to the aromatic amino acid tyrosine, tryptophan, and phenylalanine and act as a binding site for PEI-f-Ag-NPs. The fluorescence quenching data were correlated with the time-dependent kill kinetics of the cell; the results indicate that cellular architecture was destabilized as treatment time increased from 5–240 min. To better understand the interaction of PEI-f-Ag-NPs with molecules other than proteins, the cells were stained with Evans blue and calcofluor white for 15 min and then washed with water; 2D and 3D fluorescence spectra were obtained in the presence of PEI-f-Ag-NPs at various times. Calcofluor white (CFW) is a fluorescent blue-colored dye that is used for diagnosing fungal onychomycosis, which binds to the 1–3 and 1–4, β-linkage of chitin and cellulose in fungal, plant, and algal cell walls. The excitation wavelength for CFW is 380 nm; the emission maxima are between 440 and 475 nm (Shetty et al., 2019). The results showed that PEI-f-Ag-NPs quenched the fluorescence of CFW within 5 min; however, complete quenching was recorded at 120 min of incubation.

The function of electrostatic interactions between the cell surface and charged nanoparticles has previously been described for cellular uptake (Farquhar, 1978; Ghinea and Simionescu, 1985; Mutsaers and Papadimitriou, 1988). Cationic liposomes were noted to bind more efficiently than anionic and neutral ones (Lee et al., 1993; Miller et al., 1998; Chenevier et al., 2000). Cationic ferritin particles were noted to uniformly adsorb to the plasma membrane of fixed mammalian cells; this phenomenon was attributed to the many large anionic domains on the cell surface (Farquhar, 1978; Mutsaers and Papadimitriou, 1988). These previous findings, strongly support our approach to studying the inter-molecular interactions at the nano-bio interface. Our present study was dedicated to the qualitative investigation of functionalized silver nanoparticle binding on living system surface biomolecules.

Additional studies were carried out to investigate the specific binding of PEI-f-Ag-NPs with the protein BSA and selected aromatic amino acids, tryptophan, and tyrosine residues. The FL

emission of the BSA-PEI-f-Ag-NP system was measured in phosphate buffer at neutral pH and room temperature. The BSA was incubated with functionalized silver nanoparticles at various times and concentrations similar to *C. albicans cells*. Thus, the study showed that PEI-f-Ag-NPs interacted strongly with proteins and cell wall polysaccharide components within 60 min of treatment. The binding constant ($Kb = 1.0 \times 10^5\ M^{-1}$) and the number of binding sites ($n = 1.01$) were high for the cell surface proteins. BSA is comprised of 607 amino acids (66 kDa), with 24 tyrosine residues and two tryptophan residues (Ray et al., 2009). Tyrosine and tryptophan residues exhibit intrinsic fluorescence; the tryptophan emission dominates the UV fluorescence spectra for BSA (Ray et al., 2009). The results indicated that the silver nanoparticles bound and quenched FL of BSA with increasing contact time and PEI-f-Ag-NP concentration were similar to *C. albicans* cells with a comparable dynamic. The characteristic emission band at 340 nm decreased as an increment in concentration of PEI-f-Ag-NPs; this correlation suggested a strong interaction between nanoparticles and BSA. Mariam et al. performed a similar study in which 10 mg/mL BSA and 90–812 mL AgNPs were used (Mariam et al., 2011). In another study, Huang et al. described the interaction between gold nanoparticles and BSA (Huang et al., 2014). The quenching ratio was studied; with an increase in incubation time and concentration, the binding of BSA on nanoparticles achieved a steady state; this result indicated the saturation of vacant binding sites on silver nanoparticles. Additional studies are underway to estimate the number of bindings of BSA molecules on the surface of a single PEI-functionalized silver nanoparticle.

The interaction of PEI-f-Ag-NPs with tryptophan and tyrosine residues was investigated in a more detailed study as a standard fluorophore. Tryptophan and tyrosine exhibit intrinsic emission upon excitation on 280 and 270 nm, respectively. Figure 4.7 shows the FL emission spectra and quenching ratio of tryptophan and tyrosine recorded with various PEI-f-Ag-NPs

concentrations. The fluorescence intensity of tryptophan and tyrosine was noted to decrease as the silver nanoparticle concentration was increased. Silver nanoparticles were able to interact with tryptophan and tyrosine, thus quenching the fluorescence intensity. PEI-f-Ag-NPs exhibit strong interactions with tyrosine compared to tryptophan residues, indicating a strong probability for interactions with tyrosine-rich proteins. The presented work conclusively demonstrated a complex formation between cell surface anchored proteins and functionalized silver nanoparticles and quenched the fluorescence of protein with a saturation kinetic model. At this point, it is obvious to study, whether; functionalized nanoparticles are binding on specialized cell surface domains or binding uniformly. Depending on the type of cells, the distribution and nature of surface biomolecules varies. Thus, it is interesting to note the cell and functionalizing agent-specific binding kinetics of nanoparticle interaction with cells.

This chapter provides an approach to qualitatively understand the molecular-level interactions of metal nanoparticles and biomolecules by exploiting cell proteins as a molecular probe. Understanding the interaction of NPs and surface proteins will facilitate the development of intelligent antimicrobial nanomaterials with enhanced biocidal properties against many microorganisms.

Chapter 5

Chapters 2, 3, and 4 established the fundamental understanding of synthetic protocol optimization and nano-bio interaction dynamics of PEI-coated AgNPs. Further, the previous chapters addressed the antibacterial and antifungal activity of as-produced silver nanoparticles however, the broad-spectrum impact of these PEI-coated AgNPs was achieved by evaluating their anti-sporangiospores activity against *R. arrhizus* spores. The SARS-CoV-2 infections in Indian people have been associated with a mucormycosis fungal infection caused by the

filamentous fungi *Rhizopus arrhizus*. The sporangiospores of *R. arrhizus* are omnipresent in the environment and cause infection through inhalation or ingesting contaminated air and foods. Therefore, the anti-sporangiospores activity of Polyethyleneimine functionalized silver nanoparticles (PEI-f-Ag-NPs) with variable size and surface charge as a function of the molecular weight of PEI was explored. The results showed that both PEI-f-AgNP-1 and PEI-f-AgNP-2 potentially attenuated the germination and reduced the viability of sporangiospores. Furthermore, the results showed that the minimum inhibitory concentration (MIC) values of PEI-f-AgNP-1 and PEI-f-AgNP-2 (1.65 and 6.50 μg/mL, respectively) depended on the nanoparticle size and surface ζ potentials.

Similarly, the sporangiospores germination inhibition at MIC values was recorded, showing 97.33 % and 94 % germination inhibition by PEI-f-AgNP-1 and 2 within 24 hours, respectively. The confocal laser scanning microscopy, SEM-EDS, and confocal Raman spectroscopy investigation of PEI-f-Ag-NPs treated sporangiospores confirmed size and surface charge-dependent killing dynamics in sporangiospores. These results are significant and can be translated into the development of air filter technology. Sporangiospores of *R. arrhizus* are omnipresent and infect immunocompromised individuals as attributed to the COVID-19 pandemic in SARS-CoV-2 infected patients. Hence, the design and development of PEI-f-AgNPs coated air filters could prevent the patients from inhaling sporangiospores by contact-dependent neutralization of spores.

Chapter 6

The above chapters described the synthesis and antimicrobial activity of amine-functionalized metal nanoparticles, especially silver and gold. The output of the work strongly suggested the broad-spectrum application of as-prepared nanoparticles and their versatile conjugation with

other molecules to achieve selectivity in sensing as well as drug delivery. Taking this into consideration, this chapter is designed as an application of vancomycin functionalized gold nanoparticles for environmental monitoring of mercury ions. Therefore, Polyethyleneimine mediated the controlled and rapid synthesis of vancomycin-functionalized fluorescent AuNPs achieved for detecting Hg^{2+} in environmental samples. The size of fluorescent Au-NPs (~7 nm) and the zeta potential value (~38 mV) suggested high fluorescence and stability. The physicochemical properties of the fluorescent PEI-f-AuNPs@Van were determined using X-ray photoelectron spectroscopy, UV–vis spectroscopy, X-ray diffraction, transmission microscopy, and photoluminescence spectroscopy. The synthesized fluorescent AuNPs demonstrated a strong binding affinity for Hg^{2+}, associated with a low detection limit of 0.988 nM and the capability for detecting Hg^{2+} in environmental samples with high selectivity and sensitivity. This study indicates possibilities for developing low-cost, ultra-sensitive, and straightforward detection methods for Hg^{2+}.

Chapter 7

This chapter concluded with the synthesis of vancomycin functionalized, PEI-stabilized gold nanoparticles to explore antimicrobial activity against *C. albicans, C. tropicalis, E. coli*, and *P. aeruginosa*. The synthesized PEI-AuNP@Van have shown potent antimicrobial activity within 4 hours of treatment against all tested microbial strains with a lower MIC value as *C. albicans* (4.8 µg/ml), *C. tropicalis* (1.2 µg/ml), *E. coli* (2.5 µg/ml), and *P. aeruginosa* (4.81 µg/ml) respectively. Further, PEI-AuNP@Van treated cells of *C. albicans* and *C. tropicalis* have demonstrated blue emission after laser irradiation suggested, PEI-AuNP@Van were internalized inside cells. Unlike *Candida* spp., PEI-AuNP@Van is adsorbed on the surface of *E. coli* and *P. aeruginosa* cells. Viability assay confirmed that 16.3 % of *C. albicans*, 91.8 % of *C. tropicalis*, 12 % of *E. coli*, and 85.8 % of the *P. aeruginosa* population were killed after

4 h of treatment. Antifungal mechanism demonstrated that PEI-AuNP@Van exposed cells of strains mentioned above get accumulated endogenous ROS in *C. albicans*, *C. tropicalis*, *E. coli*, and *P. aeruginosa* as 11.38 %, 25.02%, 30.4 %, and 36.0 %, respectively. Accordingly, the Phosphatidylcholine externalization assay confirmed apoptosis-like behavior in all exposed strains. Raman spectroscopy and TEM analysis confirmed the mode of action of PEI-AuNP@Van against all exposed strains. The *C. albicans* and *C. tropicalis* killing was associated with ROS-induced apoptosis and cell wall damage; however, *E. coli* and *P. aeruginosa* exhibited ROS-induced apoptosis and deterioration in peptidoglycan sheet by vancomycin. Overall, designing and exploring antibacterial drugs against fungi and drug-resistant bacterial strains using an efficient delivery system like gold nanoparticles could be a potential weapon to tackle emerging antimicrobial drug resistance.

Future Projections

So far, the proposed objectives have been completed, and the following contributions made;

(i) 3-APTMS stabilized rapid, and nano-geometry controlled synthesis of mono-, bi-, and tri-metallic nanoparticles of gold, silver, and palladium.

(ii) Procedure optimization and molecular weight of PEI-dependent, rapid, and nano-geometry controlled synthesis of gold and silver nanoparticles.

(iii) Efficient antimicrobial activity of gold and silver nanoparticles with low MIC value recorded against a broad spectrum of microbes.

(iv) Interaction between microbial cell surface biomolecules and PEI stabilized nanoparticles, studied at a molecular level along with plausible antimicrobial mechanism.

(v) Successful conjugation and delivery of vancomycin achieved against a broad-spectrum pathogenic microorganism.

Since, the research work allowed us to control the nano-geometry along with stability and a way forward to conjugate other molecules on gold nanoparticles, these designed and developed nanoparticles can be employed for a wide range of applications such as sensing of metal & biomolecules and as a drug delivery tool against deadly diseases like cancer and intracellular bacterial infections.

Further, these findings can be translated into the designing and development of biocompatible nano-formulated bandages, topical ointments, facemasks, air filters, and nano-coating material for medical catheters against planktonic as well as biofilm-forming

microorganisms. However, a thorough investigation is still needed to explore the application of synthesized nanoparticles in catalysis, and nanomaterial-based sensing of diverse biological and non-biological analytes.