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REVIEW PAPER

Bio-functionalized doped silver nanoparticles and its antimicrobial studies

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Abstract In recent era researchers are mainly focused on silver (Ag) and silver doped nanoparticles due to its vast area of applications which are effectively acts against the microbial activity. Silver nanoparticles (AgNP's) were one of the metal nanoparticles which have high activity against various diseases causing pathogens to safeguard the environment. However, there is increasing concern related to biological impacts by the use of silver doped nanoparticles on a large scale. The synthesized silver nanoparticles were doped with other chemical reagents by using various physio-chemical methods. The stability of silver nanoparticles was governed by morphological features, capping agents and organic molecules. This review article briefs about the synthesis of silver doped nanoparticles using different methods and their antimicrobial activity.

Keywords Silver · Silver doped nanoparticles · Anti-microbial activity

1 Introduction

Nanotechnology is now creating a growing sense of excitement in life sciences, especially in the field of biomedical devices and biotechnology and also it was one of the upcoming areas with many applications in the modern medicine [1]. Nowaday's nanoparticles were acting as an intermediate between atom properties and bulk materials. These take on a major character in several application areas such as catalysis, ceramics, drug delivery, therapies and diagnostics. [2]. Silver metal was already reported as non-toxic, safe, inactive anti-bacterial agent used for several centuries, which has a capacity to kill more than 650 types of microorganisms for these above reason motivated us to propose a review about silver and its doped nanoparticles and their anti-microbial applications [3]. There are several green and Physio-chemical methods for synthesis of silver nanoparticles and by the help of some chemical process, it can be doped with various chemical reagents, ceramics, glasses, etc., which finally subjected to anti-microbial properties against several disease causing pathogens [4].

Several medical applications of silver nanoparticles were constantly elaborating due to their high bactericidal properties which coupled with low toxicity towards mammalian cells. Because of this expanding use of flatware, new methods of synthesis have been produced in order to achieve preparation through inexpensive and environmentally friendly processes; and also for doping some agents with silver nanoparticles [2].

Silver nitrate and silver sulfadiazine have been widely used for the treatment of superficial deep dermal burns of wounds and for the removal of warts [5]. The mode of action of Ag is presumed to be dependent on Ag^+ ions, which strongly inhibit bacterial growth through suppression of respiratory enzymes and electron transport components through various functions [6]. Ag in a nanometric scale less than 100 nm has different catalytical properties compared with those attributed to the bulk form of the noble metal, like surface plasmon resonance, large effective scattering cross section of individual silver nanoparticles, and strong toxicity to a wide ranges of microorganisms [7]. Green synthesized Ag nanoparticles have effective anti-larvicidal activity against *Anopheles stephensi* and *C. quinquefasciatus* [8].

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The antibacterial activity can be done for several plants by various solvent extracts which shows activity against various bacteria and fungi [9]. Green synthesis of nanoparticles is an emerging branch of nanotechnology. Ag nanoparticles are in the initial stage of antibacterial development. Researcher can find diametrically opposing points of view on the mechanism by interaction of nanoparticles with effective bactericidal agents against Methicillin resistant *Staphylococcus aureus* (MRSA) regardless of the resistance mechanisms that confer importance to these bacteria as an emergent pathogen. Besides, it is the first time that the efficacy and safety of nano silver in different sizes was determined for (MRSA) and human cells. Ag is the metal of choice in the field of biological system, living organisms and medicine. AgNp's exhibiting very strong bactericidal activity against both gram positive and gram negative bacteria including multi resistant strains. In addition, AgNp's kill bacteria at low concentrations (units of mg/L) which do not reveal acute toxic effects on human cells [10]. The synthesis of AgNp's has been of considerable interest during the past decades. A assortment of methods have been described for the synthesis of metallic nanoparticles. These include thermal decomposition, laser ablation, microwave irradiation, Sono-chemical etc., [11]. Metal nanoparticles with bactericidal activity can be immobilized and coated on to surfaces, which may find application in various fields, i.e., medical instruments and devices. Metal nanoparticles may be blended with polymers to form composites for better use of their antimicrobial action. It was found that the developed colloidal solutions of AgNp's could exist in the form of very stable aqueous dispersion up to several months and exhibited a noticeable antibacterial activity [12]. Particularly, it was realized that an advanced synthetic technique and greater stability of fine AgNp's dispersions resulted in the significant enhancement of their antibacterial activity. The use of the colloidal chemistry method for preparation of the AgNp's was difficult to obtain finely dispersed AgNp's due to the complex synthesis condition [13]. In this follow-up, we have discussed during the preparation of antimicrobial silver and silver doped nanoparticles, which can helpful for future researchers who are concentrating their research towards silver nanoparticles.

2 Ag doped nanoparticles and their anti-microbial studies

2.1 Ag-doped MgO layers

Necula et al. [14], reported that Ag doped MgO coatings were synthesized by in situ deposition of Ag nanoparticles during plasma electrolytic oxidation of magnesium substrate. The surface morphology studies were done by scanning electron microscope, X-ray diffraction, X-ray dispersive spectrophotometery and radio frequency discharge of optical electron microscope. The results states that coatings was found to be porous and 7 μm thick with the presence of crystalline oxide matrix of Ag nanoparticles and they also found that plasma electrolytic oxidation process has potential for synthesis of MgO-Ag nanocomposite coatings.

2.2 N-doped Multiwall CNT's with Ag nanoparticles

Castle et al., investigated the biocompatibility of Ag nanoparticles by anchoring multiwall carbon nanotubes (MWNT's) via novel chemical route without any usage of sulfur containing reagent and used three different substrates of multiwall nano tubes like AgNp's: MWNT's of pure carbon, COx-MWNTs-Ag (carboxyl functionalized) and CNx-MWNTs-Ag (nitrogen-doped). The AgNp's were synthesized without any thiol groups since it can be strongly attached to the MWNT's. The AgNP's prepared resulted an average size of 7, 10 and 12 nm in MWNTs, COx-MWNTs and CNx-MWNTs respectively which was used for the cellular preservation techniques like cellular viability using MTS assay and cellular proliferation etc., [15].

2.3 AgNP's doped poly (o-methoxyaniline)

Dawn et al. [16], proposed novel method for nano-biocomposite of silver and poly o-methoxyaniline (POMA) by adding DNA solution of POMA and $AgNO_3$ which was kept for 10 days and freeze-dried to form nanocomposite in a hybrid nature. The characterization studies were done by Transmission Electron Microscope and Field Emission Scanning Electron Microscope which confirms the hybrid structure of nanocomposites in hybrid form. The band gap energy was also evaluated and it states that higher applied voltage was due to its semiconducting property characterizing the large band gap semiconducting behavior of the nano-biocomposite [16].

2.4 Ag doped (polyvinyl alcohol) hydrogel films on poly (L-lactic acid)

Zan et al. [17] done multistep process which includes oxygen plasma treatment, UV-initiated graft polymerization, and chemical grafting methods for the preparation of Ag doped nanoparticles. The process was monitored by total reflection infrared spectroscopy and X-ray photoelectron spectroscopy. The polyvinyl alcohol and poly lactic acid has both antibacterial and adhesive properties and also plays a major role in tissue engineering process and biomedical devices.

2.5 Ag-doped ZnO nanoparticles

The ZnO nanoparticles have exhibited a strong bacterial growth inhibiting character due to significant attention towards various applications such as UV light emitting diodes, laser diodes with catalysts and in treatment of skin diseases. Sawai et al., also discussed on FWHM of the reflection peaks which have decreased after addition of the dopant cations, indicating growth of the crystalline or changes in the crystal strains. There was also a negligible shift in peaks and their FWHM obviously decreased for the samples that were doped with the different concentration of Ag compared to the undoped ZnO Np's. The crystalline size of the undoped ZnO and Ag doped ZnO with the different concentration were determined by using X-ray line broadening method using the Scherer's equation. The crystallite size of Ag doped ZnO nanoparticles have seen to decrease with the increased content of Ag [18–20].

2.6 Titanium dioxide doped Ag nanoparticles

TiO_2 was a well known semiconductor due to its optoelectronic properties and chemical stability. TiO_2 films have possessed high photosensitivity hence they become very promising material for integrated optical devices [21]. The Ag/TiO_2 thin films were successfully prepared by the sol gel technology. In addition of silver affects the crystallinity and the optical properties of TiO_2: Ag thin films. The prepared sol solutions have found to be very stable in time. The comparison studies were performed for TiO_2 and TiO_2 doped Ag thin films depending on the annealing temperature and the gas ambient. The XRD analysis revealed that the Ag is crystallized in metallic state and Ag nanoparticles are successfully formed in titanium dioxide matrix without indication of existence of AgOx phases. Interestingly, it has been shown that the Ag incorporation induces an anatase to rutile transformation only for the films, treated in air. The TiO_2: Ag films annealed in nitrogen ambient are polycrystalline with XRD lines attributed to anatase TiO_2 and to cubic Ag. The difference in the crystallite sizes of Ag nanoparticles have been found depending on the ambient thermal treatment. Eckhardt et al., reported that TiO_2 nanofiber incorporated with hydroxyapatite and Ag nanoparticles are doped and introduced for tissue engineering process The synthesized nanofiber were characterized by various state of art techniques like; SEM, XRD, TEM, EDS and XPS analyses. AgNP's having diameter of 5 nm in size investigated by TEM. The surface analyses of nanofiber were investigated by XPS which indicated the presence of AgNP's on the surfaces of nanofiber [22, 23].

2.7 Ag doped silica and Fiber-reinforced nanocomposites

Ji et al. [24], reported that Ag doped silica nanocomposite which has been synthesized by sol gel technique combined with thiol stabilizer 3-mercaptopropyl trimethoxysilane. The freshly prepared sol of Ag-doped silica nanocomposite was investigated by TEM. Ag ions in a solution can be easily reduced to silver atoms by a reducing agent, such as $NaBH_4$. The formed silver atoms will aggregate to form particles and precipitate if an appropriate measure is not taken to prevent the aggregation of the particles. Nganga et al. [25], on Ag polysaccharide doped on fiber reinforcement composites (FRC) which was successfully reinforcement in several cranial reconstructions and characterized by laser scanning microscopy and scanning electron microscope. It was subjected for antimicrobial properties against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The Ag doped FRC shows good antimicrobial property against bacterial strains.

2.8 Polyelectrolyte thin films doped with biosynthesized Ag nanoparticles and its antimicrobial activity

Multifunctional polyelectrolyte thin films were loaded and delivered to therapeutic drugs. The multilayer thin films were assembled by the electrostatic adsorption of polyallyamine hydrochloride (PAH) and dextran sulfate (DS). The AgNp's biosynthesized from *Hybanthus enneaspermus* leaf extract act as reducing agent were successfully incorporated into the film. The biosynthesized AgNp's showed excellent antimicrobial activity against the range of enteropathogens, which could be significantly enhanced with commercial antibiotics. The leaf extract *H. enneaspermus* extract act as a reducing agent and the process resulted narrow size particles of 60–80 nm in suspension which was significantly influenced by reaction pH. This prepared AgNp's showed excellent antibacterial properties against enteropathogens such as *E. coli, S. aureus, P. vulgaris* and *B. cereus*. In this article Jaganathan et al., reported the synthesis of silver nanoparticles by leaf extract of *Hybanthus enneaspermus*. This extract act as a reducing agent to form silver nanoparticles and the prepared silver nanoparticles was doped with polyelectrolyte film, which was characterized by TEM, XRD. After entrapping silver with film, it was checked for antimicrobial property Fig. 1 against enteropathogens which showed excellent antimicrobial activity [26, 27].

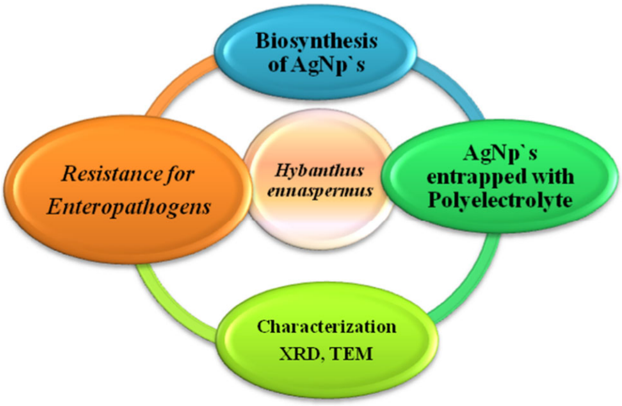

Fig. 1 Ag entrapped with polyelectrolyte film and its anti-microbial activity

2.9 Ag doped hydroxyapatite nanoparticles synthesis and its antimicrobial activity

The synthesize of Ag doped hydroxyapatite nanorods was done by using modified sol gel method at low temperature of 100 °C Fig. 2 with various concentration of substrate. Characterization studies were done through XRD which states that fully crystalline size of 25 nm and in TEM studies revealed that the prepared nanorods were crystalline size which ranges from 110–180 nm at all the concentrations. These all three different concentration was subjected to antimicrobial activity against *E. coli* (MTCC 2345) and *S. aureus* (MTCC 737). Antimicrobial activity was observed for all the three silver doping concentrations with the highest activity terms of the zone of inhibition and the percentage reduction in the number of colonies. The formation of silver nanoparticles and silver doped with hydroxyapatite nanoparticles to play a role in antibacterial and antifungal activity. The Ag doped nano crystalline hydroxyapatite powder was synthesized by 100 °C and synthesized material was undergone characterization with the help of XRD, TEM and FT-IR. The characterized material was interacted with gram positive and gram negative microorganisms for its antimicrobial activity and it showed positive results for the same [28, 29].

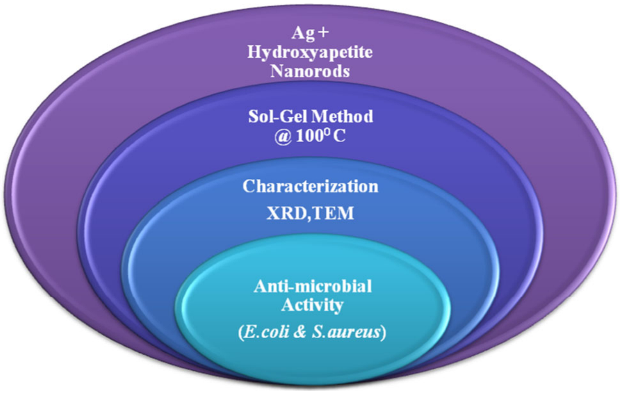

Fig. 2 Ag doped with hydroxyapatite and its anti-microbial activity

2.10 Ag doped organic–inorganic hybrid coatings and its antimicrobial activity

Marini et al., have prepared silver doped organic and inorganic hybrid coatings of tetra ethoxysilane and tri ethoxysilane terminated poly (ethylene glycol) block polyethylene by the sol–gel process and the prepared thin layer coated films was subjected to antimicrobial activity against Gram-negative (*Escherichia coli* ATCC 25922) and Gram-positive (*Staphylococcus aureus* ATCC 6538) bacteria. The highest antibacterial activity was gained for samples with an organic inorganic ratio of 80:20 and 5 % weight silver salt. It was then subjected to coating of a high level performance of antibacterial activity of the coated films which was done by continuous washing of the samples in warm water or by immersion in physiological saline solution at 37 °C for 3 days [30].

2.11 Ag doped with zirconium titanium phosphate and its antibacterial activity

Biswal et al. [31], stated that Ag doped ZTP's are very efficient materials due to their best antibacterial performance against *E.coli.* These material was incorporated each other by ion-exchange process at 65 °C. The Characterization studies were done by XRD, UV–Vis spectroscopy, FTIR, XPS, SEM–EDS, TEM, and AAS measurement that it shows 99 % activity of inhibition over 12 h of contact Fig. 3. Hence they stated that ZTP entrapped with Ag has high antibacterial activity.

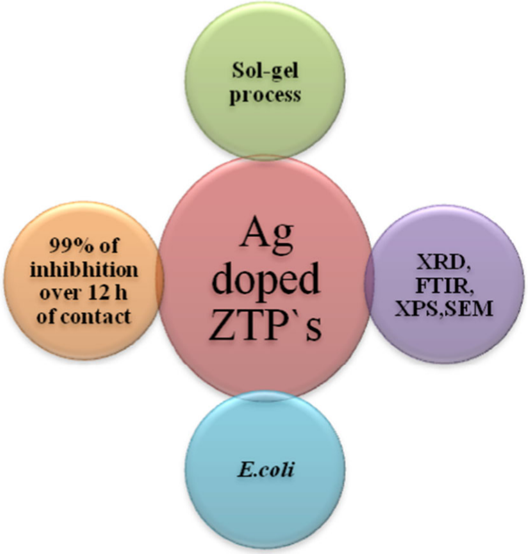

Fig. 3 Ag doped with zirconium titanium phosphate and its antibacterial activity

2.12 Ag doped Zinc orthotitanate nanoparticles synthesis and its antimicrobial activity

Latesh et al. [32], processed the synthesis of Ag and Zn orthotitanate nanoparticle at 650 °C by thermal combustion Fig. 4. The synthesized products were characterized by XRD, FESEM and TEM analysis and characterization states that size of nanoparticle was 100 nm. The antimicrobial activity of Zinc orthotitanate and Ag doped Zinc orthotitanate has been carried out against *Bacillus subtilis* NCIM 6633 in Mueller–Hinton (MH) medium. The MBC was found to be 2.0 and 0.5 mg/mL^{-1} in case of Zinc orthotitanate and Ag doped Zinc orthotitanate indicating enhancement of antimicrobial activity due to Ag doping.

2.13 Ag loaded calcium phosphate and its antimicrobial activity

Swanprateeb et al. [33], prepared nanosilver loaded calcium phosphate by single step co-conversion method at low temperature and it was further characterized by several characterization techniques. Antimicrobial activity was done for two selected samples *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The cytotoxic potential of the same was done by MTT assay which was observed for both samples at 24 and 48 h showed good antimicrobial activity.

2.14 Ag doped ZnO nanoparticles for its antimicrobial activity

The synthesis of Ag doped ZnO designer nanoparticles of solvothermal technique at 150 °C with autogenous pressure for 16 h by using methanol as solvent. The characterization and morphology studies were done and synthesized doped nanoparticle was subjected to undergo antimicrobial activities, which showed good zone of inhibition. The antibacterial activities of Ag doped ZnO nanoparticles were compared with silver content and crystal size by evaluating the zone of inhibition against the microorganism's *B. subtilis* and *S. aureus*. It was observed that bacterial susceptibility improved considerably with increase in the Ag content and decrease in the crystallite size [34].

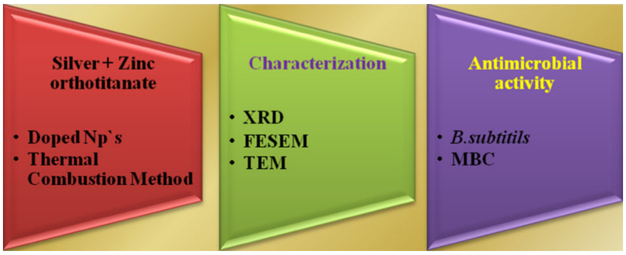

Fig. 4 Ag doped with zinc orthotitanate and its anti-microbial activity

2.15 AgNP's doped lysozyme enzyme and its antibacterial activity

Eby et al. [35], reported synthesis of the AgNP's and colloidal suspensions of lysozyme was deposited into stainless surface on blades. Blade coatings also exhibited antimicrobial activity against a range of bacterial species. In particular, coated blades demonstrated potent bactericidal activity, reducing cell viability by at least 3 logs within 1.5 h for *Klebsiella pneumoniae*, *Bacillus anthracis* Sterne, and *Bacillus subtilis* and within 3 h for *Staphylococcus aureus* and *Acinetobacter baylyi*. The results confirmed that complex antimicrobial coatings can be created using facile methods for silver nanoparticle synthesis and electro deposition.

2.16 Ag doped di-phenylalanine hydrogels and its antibacterial property

Paladini et al. [36], introduced AgNP's into fibrillar structure of self-assembling aromatic di-phenylalanine derivatives modified with aromatic groups such as 9-fluorenylmethoxycarbonyl is stated to act as a antibacterial wound dressings. The SEM–EDX characterization was carried out to check out the identification and size distribution of silver nanoparticles. The antibacterial potential of the treated flax was demonstrated through microbiological tests on *Staphylococcus aureus* and also it plays an important role in wound dressings.

2.17 Ag entrapped with polystyrene nanocomposites and its anti-microbial activity

Palomba et al. [37], synthesized Ag nanoparticles based on doping polymers and they dissolve Ag in polystyrene solution to form solid at temperature of 180 °C. The antimicrobial characteristic of polystyrene based material has been accurately evaluated toward *Escherichia coli* (*E. coli*).

2.18 Ag doped TiO_2 and its anti-microbial activity

Silver doped titanium oxide was prepared using a sol gel technique and the morphology studies were evaluated by AFM. The antimicrobial activity was evaluated by disk diffusion method and the MIC values were obtained for nanosized Ag doped TiO_2 solutions against the test microorganisms *Escherichia coli, Staphylococcus aureus, Candida albicans, Bacillus subtilis, and Salmonella* *typhimurium* [38]. The antimicrobial activity was observed against all tested microorganisms at a very low concentration. Pure Ag doped titanium nanoparticles were prepared using sol gel method. Samples were characterized by TEM, X-ray diffraction, N_2 adsorption measurement, AAS, UV–Vis spectroscopy and further evaluated for antibacterial activity by MIC method and MBC method. The results showed very good antibacterial activity for gram positive *Staphylococcus aureus* and gram negative *Escherichia coli* and *Klebsiella pneumonia* [39]. There is a report on TiO_2 nanoparticles entrapped with hydroxyapatite which was further doped in AgNP's. the synthesized nanoparticles were in the size of 5 nm and surface studies was done by XPS which confirms that AgNP's was incorporated on surface of nanofiber. Further it was tested for antimicrobial activity for two microorganisms like *E. coli and S. aureus* results stated that antimicrobial tests have indicated that the prepared nanofiber do posses high bactericidal effect. Accordingly, these results strongly recommend the use of obtained nanofiber mats as future implant materials [40].

2.19 Ag nanoparticles doped collagen and keratin materials

Carmen et al. [40], reported the preparation of Ag nanoparticles with polyurethanes doped in the chromium compounds. It was mainly characterized by UV–Vis, Zeta sizer Nano equipment, TEM. The antibacterial and antifungal property was carried out by using AAS, antibiogram method for *Aspergillus, Penicillium, Trichoderma, Candida species* and inhibitory minimal concentration for *Staphylococcus aureus* (ATCC), *Pseudomonas aeruginosa* (ATTC), *Escherichia coli* (ATCC), *Acinetobacter spp* and *E. Enterobacter spp*.

2.20 Ag nanoparticles doped textile and its antimicrobial activity

The preparation of three textiles and doping with commercial Ag nanoparticles with silica spheres were carried out. All the three textiles doped with Ag nanoparticles were active against selected microbial strains. MIC value for antimicrobial activity showed that Ag nanoparticle doped in textile silica spheres was active and also these Ag doped nanoparticles was plays a more important role in dye degradation industries [41].

2.21 Ag doped mullite ceramic against pathogenic bacteria

Ag doped mullite ceramic disks against bioflim producing pathogens of *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus mutans and Pseudomonas aeruginosa.* Ag mullite disks showed high potential for controlling pathogens [42].

2.22 Eu-Ag co doped TiO_2 surface and anti-bacterial studies

Photo catalytic property of titanium oxide is well known for its antimicrobial activity. The Eu-Ag co-doped with TiO_2 was done by sol gel method. The prepared material was checked for its antimicrobial property against disease causing pathogens like *Pseudomonas aeruginosa*. The results stated that photocatalytic activity was response to the antibacterial activity [43].

2.23 (Au, Ag): TiO_2 nanocomposites with antimicrobial activity

Titanium oxide doped with nanometals like Ag and Au by sonochemical reduction method were processed. The antimicrobial activity of the resulting samples was compared by the measurement of colony numbers. The initial antimicrobial activity of the metal (Au, Ag) doped TiO_2 was higher than that of the TiO_2. The prepared nanocomposites were kept at 4 °C for prolong time and the aged samples showed special antimicrobial activity [44].

2.24 Ag doped with Faujasite zeolites

FAU zeolites were prepared by ion exchange method and it was doped with Silver. The synthesized material was characterized by XRD, SEM, FT-IR, and XPS. The antimicrobial evaluations of Ag zeolites was studied by using some pathogens *E. coli*, *B. subtilis* and yeasts such as *S. cerevisiae, C. albicans* as sensitive indicator strains. The zeolites showed good antimicrobial properties, in particular antibacterial activity. AgNP's also plays a major role in many wound healing properties and also Faujasite doped AgNP's shows good antimicrobial property was reported [45].

2.25 Ag doped calcium phosphate cements and antimicrobial activity

They have checked the activity of Ag outsourced from the cements was processed against the growth of both *S. aureus* and *S. epidermidis*; the cement exhibited excellent antibacterial properties and also showed an increased 30 % compressive strength [46].

2.26 Ag doped yttria-stabilized zirconia ceramics and antimicrobial activity

Ag nanoparticles were entrapped into yttria-stabilized zirconia (YSZ) ceramic for eliminating microorganisms. The biocompatibility of Ag incorporated into YSZ was evaluated by MTT assay and characterized by SEM and

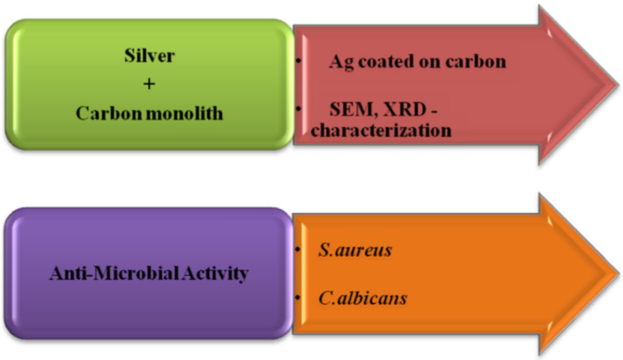

Fig. 5 Ag nanoparticles entrapped with carbon monolith and its antimicrobial activity

(ICP-MS). The results state that it can inhibit the growth of *E. coli, S. mitis* and *C. albicans* [47].

2.27 Ag doped carbon monolith and antimicrobial activity

Carbon monolith and doped silver nanoparticle was prepared and the Ag coatings were confirmed by SEM and power XRD. The antimicrobial activity of the carbon monolith with a silver coating was detected (Fig. 5) using standard microbiology methods. The carbon monolith samples with Ag coating showed good antimicrobial activity against *E. coli, S. aureus* and *C. albicans* [48].

2.28 Ag doped phosphate-based glasses and antimicrobial activity

Phosphate based glasses (PBG) doped with Ag were investigated for their antimicrobial activities by the method of disk diffusion assays against disease causing pathogens *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Methicillin-resistant Staphylococcus aureus* and *Candida albicans*. The PBG3 and 5 mol.% Ag were having good bactericidal activity for *S. aureus* and *E. coli*. And it significantly decreased the growth rate of *C. albicans* [49].

3 Conclusion

In this review we have stated that silver doped nanoparticles also have efficiency like silver nanoparticles to kill or resist some pathogenic organisms by its antimicrobial property. This review will be fruitful for those researchers who were planning to carry out their research focus towards antimicrobial assays using Ag and Ag doped nanoparticles in future.

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Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this article.

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