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ANTIMICROBIAL, ANTIBACTERIAL, ANTIFUNGAL ACTIVITY STUDIES OF MULTIWALLED CARBON NANOTUBES: A REVIEW

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Abstract: Carbon nanotubes (CNTs) are tube – like materials that are made up of carbon with a diameter calculating on a nanometer scale. Carbon nanotubes can be described as graphite sheets that are rolled up into cylindrical shapes. CNTs can exist as either single – walled (SWCNTs) having a diameter of 1 nm or multi – walled (MWCNTs) depending upon the number of carbon layers, having a diameter ranging from 2 – 100 nm. CNTs are electrically, thermally and chemically conductive nanoparticles with unique nano – dimensions, usually strong and high aspect ratio which makes them perfect for biomedical applications. Similarly, multi – walled carbon nanotubes (MWCNTs) have received tremendous attention because of its unique electrical, optical, physical, chemical and mechanical properties which make them suitable for application in various areas such as materials science, bio – sensors, biomedical imaging, Nano – electronics, electro catalysis, water treatment, drug delivery and energy management. This review deals with the antimicrobial, antibacterial, antifungal activity of multi – walled carbon nanotubes in detail.

***Index Terms* -** Bio – sensors, Biomedical imaging Carbon nanotubes, Multi – walled carbon nanotubes, Nano electronics, Single walled carbon nanotubes.

I. INTRODUCTION

Carbon nanotubes (CNTs) are considered as a derivative of both carbon fibers and fullerene with molecules composed of 60 atoms of carbon arranged in particular muffled tubes [1]. There are two types of carbon nanotubes that are classified according to the number of carbon layers present in them. Single – walled carbon nanotubes (SWCNTs) consist of single graphene layer which usually occurs as hexagonal – packed bundles. Multi – walled carbon nanotubes (MWCNTs) comprise of two or several cylinders, each made up of graphene sheets [2]. CNTs can be obtained by three methods: Laser ablation [3], arc discharge method [4] and chemical vapour deposition (CVD) which includes high – pressure carbon monoxide method (HIPCO) [5]. MWCNTs were discovered by Iijima by Arc discharge method, used earlier for the production of carbon fibers [6, 7] (Fig. 1) [3].

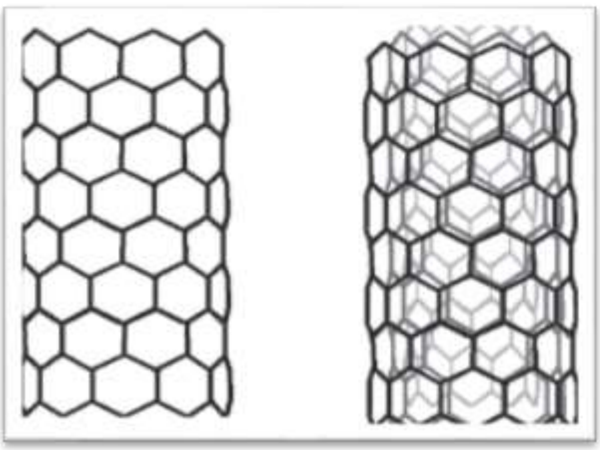

Figure 1 Single and multi – walled carbon nanotubes

Exceptional properties of CNTs such as high tensile strength, light weight, fast electron transfer kinetics, high bio – compatibility, helps in protein immobilization, large surface area, chemical inertness, large number of antimicrobial and antifungal properties can be used as protein carriers, contains exposed functional groups, etc., make them tremendously attractive in various bio – sensor applications [8–19]: tissue engineering [20], (Fig. 2) [3]

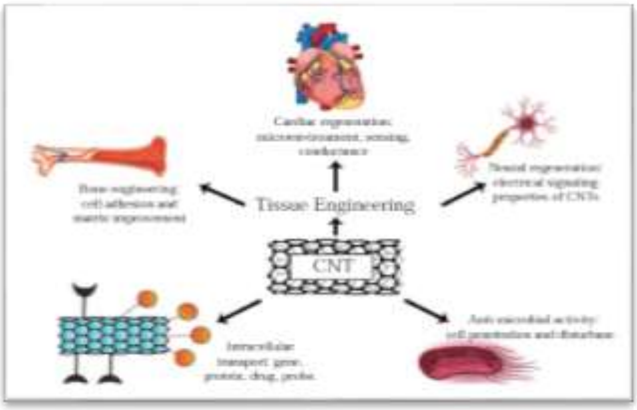

Figure 2 Schematic representation of CNTs in biomedical applications

Pharmaceutical [21], drug delivery [22]. Multiwalled carbon nanotubes also finds applications in sensors [23 – 33]. This article intends to brief a review of antimicrobial, antibacterial and antifungal activity of MWCNTs.

II. ANTIMICROBIAL ACTIVITY OF CARBON NANOSTRUCTURES

The resistance of microbes against antibiotics is increasing which led to severe public health problems. A vast majority of the pathogenic microbial stains are resistant against at least one antibiotic. This problem has a lead influence on the researchers to initiate the investigations of novel antimicrobial agents [34]. Studies demonstrate that carbon nanotubes can assume a strong role in antibacterial action [35 – 39]. Accumulation of microbes with CNTs causes direct contact between cells and carbon nanoparticles which thus lead to cell demise. The supposed mechanism of the antimicrobial activity of CNTs is given in Fig. 3[3]. Stronger antimicrobial activity of single walled carbon nanotubes is identified [40 – 41].

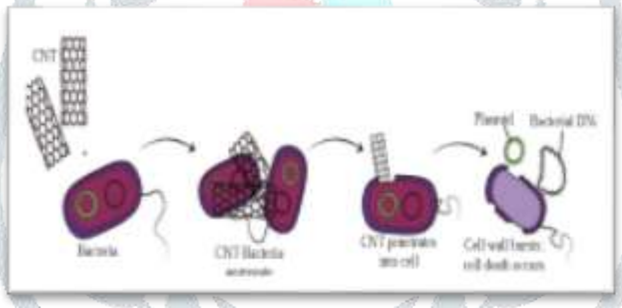

Figure 3 Supposed mechanism of CNTs' antimicrobial activity

III. ANTIMICROBIAL ACTIVITY OF MWCNTs

Multi – walled carbon nanotubes (MWCNTs) are integrated with silver nanoparticles to maximize the anti – bacterial activity and minimize the toxicity of both nanoparticles and absorbance of silver by the body. Ag – MWCNTs show an efficient antimicrobial property and less toxic effect [42]. Thomas Varghese et al investigated the antimicrobial activity of CNTs in well diffusion array against different gram – positive (*Staphylococcus aureus* and *Streptococcus klebsiella*) and gram – negative (*Proteus mirabilis, Klebsiella pneumoniae* and *Escherichia coli*) bacteria [43]. Antimicrobial activity was first reported through CNTs by Kang et al [44]. Zardini et al demonstrated the antimicrobial activity against four gram – positive and four gram – negative strains [45]. Novel aminohydrazide cross – linked chitosan filled MWCNT composites showed better antimicrobial activities than that of chitosan against *Enterococcus faecalis, Staphylococcus epidermis, Escherichia coli, Aspergillus niger, Cryptococcus neoformans* and *Candida* tropicalis [46]. Antimicrobial activity of aminosalicylhydrazide cross linked chitosan modified MWCNTs is reported by Nadia et al [47]. *S. aureus* was chosen to evaluate the quantitative test of antibacterial activity of the grafted CNTs using spread agar-plating method and cultured with a Luria-Bertani (LB) culture medium [48]. Grafted nanotubes of pyrazole and pyrazolone derivatives tested against *Staphylococcus aureus, Bacillus subtilus, and Escherichia coli* have antimicrobial activity than the neat pyrazole and pyrazolone derivatives [49].

Trimellitic anhydride isothiocyanide cross linked chitosan hydrogels modified with MWCNTs are more potent against *Bacilis subtilis, Streptococcus pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Geotrichum candidum, Candida albicans, Aspergillus fumigatus* and *Syncephalastrum racemosum* than chitosan as judged by their greater inhibition zone diameters and their lower minimum inhibitory concentration (MIC) values [49]. Phthalimido thiourea stabilizers as antimicrobial thermal stabilizers for PVC in absence or presence of MWCNTs acts as a filler [50].

Antimicrobial potency of polyurethane cyclodextrin co – polymerized phosphorylated multiwalled carbon nanotube – doped Ag – TiO_2 nanoparticle bionanosponge nanocomposite is reported by Anny Leudijo Taka et al [51 – 53]. MWCNTs decorated with nanoparticles such as hydroxyapatite (MWCNTs_HAp), Silver (MWCNTs_Ag) and Zinc oxide (MWCNTs_ZnO) as hybrid materials tested against five microbial species, two gram – positive (*S. aureus*, *Bacillus subtilis*), two gram – negative (*Pseudomonas aeruginosa, E. coli*) [54] and one yeast (*Candida albicans*). Higher antimicrobial activity is observed for MWCNTs_ZnO and MWCNTs_Ag due to the presence of the nanoparticles, because their action mode can disrupt bacterial cell membrane integrity, reduce cell surface hydrophobicity and downregulate the transcription of oxidative [54 – 56] stress – resistance genes in bacteria. 1,4 – dihydropyridine is synthesized in presence of agminated MWCNT and their antimicrobial properties are studied [57]. Grafting Ag Nanoparticles (NPs) onto CNT (Ag – MWCNT) antimicrobial efficiency significantly raised to 97% ± 0.5% in comparison with the percentage kill of pure Ag. Similarly, grafting of Cu NPs onto CNTs (Cu - MWCNT) exhibits antimicrobial efficiency of 75% ± 0.8% in comparison with pure Cu NPs that shows percent kill of the 52% ± 1.8% [58]

MWCNTs dispersion in *Acacia* extract and check its antimicrobial activity is done [59]. Ag - MWCNTs nanocomposites showed a higher antimicrobial capability against gram – positive than gram – negative bacteria [60]. Cephalexin – immobilized multi – walled carbon nanotubes show strong antimicrobial and anti – adhesion properties is reported by Xiaobo Qi et al [61]. MWCNTs exert a potent antimicrobial activity, even in pure form or modified with bio – compatible polymers. It can be an active antimicrobial agent and in future perspectives, it will be utilized in pharmaceutical and therapeutic techniques.

IV. ANTIBACTERIAL ACTIVITY OF MWCNTS

Antibacterial properties of MWCNTs depend on various factors such as surface functional molecules, density, diameter, length and purity of carbon nanotubes [62]. A schematic representation of antibacterial activity of CNTs is given in Fig. 10[1].

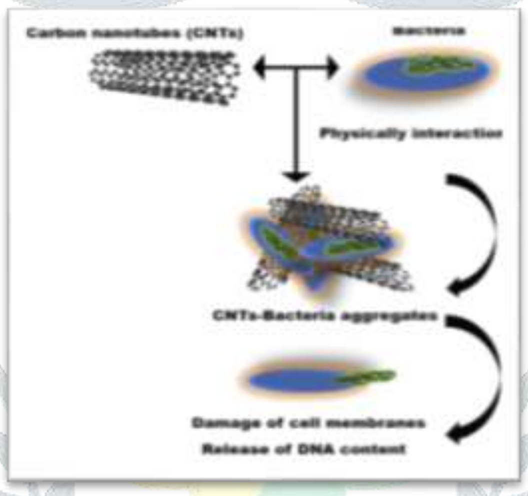

Figure 10 Schematic representation of antibacterial activity of CNTs

The antibacterial activities of Ag – NPs, MWCNTs and Ag – MWCNTs nanocomposite were initially assessed with the paper – disc diffusion method which is widely used for quick antibiotic susceptibility determinations [63]. The antibacterial activity of Ag – NPs and Ag – MWCNTs samples was more effective against *E. coli* bacteria than that of *S. aureus* bacteria [64]. This may be from the thinner thickness of the peptidoglycan layer of gram – negative *E. coli* bacteria as compared to that of the gram – positive *S. aureus* bacteria [65 – 66]. Ciprofloxacin is a well – known antibiotic and it belongs to the fluoroquinolone class. Ciprofloxacin has been widely used to treat infections caused by gram – positive and gram – negative bacteria. However, the resistance to ciprofloxacin has been growing rapidly around the world [67 – 68]. To overcome this problem, Assail et al proposed a novel type of functionalized SWCNTs with loaded ciprofloxacin [69].

The aspect ratio of MWCNTs greatly influenced the antibacterial property of MWCNTs as short tubes enables more interaction with the microorganisms as compared to the long tube MWCNTs as shorter tubes do more interaction with the cell membrane which changes the osmolality of the membrane [70]. However, the same thing gets reverse in liquid medium. In liquid medium, the short tube aggregates encompass a smaller number of cells as compared to long tube aggregates. Diameter also plays an important role as smaller diameter leads to more close interaction with the microbes. Therefore, aspect ratio of MWCNTs played an important role in determining the antibacterial property of MWCNTs [71].

MWCNTs can show its antibacterial property by various ways: change in the integrity of the cell membrane by forming close association with the membrane surfaces [72] and ROS generation which ultimately leads to cell death and DNA damage [73 – 74]. However, the exact mechanism behind the antibacterial property is yet to be understood.

Thirdly, impurity associated with the MWCNTs can also be an integral part of its antibacterial property. Low pH facilitates more protonation of functional group associated with the polymer [75]. In – vitro antibacterial activity of nanocomposites, i.e., chitosan, its derivatives against both gram positive and gram negative bacteria was assessed by agar diffusion technique [46]. The orientation and aspect ratio of MWCNTs greatly affects its antibacterial property [76 – 79]. Chitosan and their modified composites along with carbon nanotubes were found to be effective bacterial disinfectors [80].

MWCNT – epsilon – polylysines have improved antibacterial activities against all the three bacteria, *E. coli, P. aeruginosa* and *S. aureus* when compared to MWCNTs [81]. MWCNT – ZnO demonstrated excellent photo inactivation of *E. coli* under UV – visible light illumination. This activity is attributed to charge transfer through Zn – O – C bonds formed between the oxygen atoms of the carboxylic functional groups of the CNT and the Zn atoms of the ZnO film [82].

Engel et al have reported the antibacterial activity of SWCNTs in combination with iron oxides against *E. coli* bacteria [83]. MWCNTs is inducing the generation of reactive oxygen species as all fullerene compounds has a closed cage like structure and conjugation happens through π – electron configuration [84]. Membrane filters covered with carbon tubes were used to investigate the antibacterial activity against a number of bacteria namely *E. coli, Salmonella* species, *Klebsiella* and *Shigella flexineri* [74, 85]. Bhaduri and his co – workers have demonstrated the antibacterial activity of SWCNT modified with Ag – Fe_3O_4 [86]. Ranaga and Magadzu have demonstrated that by using MWCNTs doped with cyclodextrin and silver have shown enhanced antibacterial activity [87]. Zhu et al investigated the antibacterial activity of MWCNTs functionalized with carboxyl group [88]. A nanocomposite of polyaniline/graphene/carbon nanotubes have been reported for antibacterial activity against *S. aureus* and *E. coli* [89 – 90]. Kang et al demonstrated that MWCNTs were more toxic when they were uncapped, de – bundled and dispersed in the solution [44]. Vecitis et al described MWCNT filters to treat drinking water through removal and inactivation of virus and bacteria [91]. The antibacterial capacity of Ag – NPs decorated on MWCNTs synthesized through *Camellia sinensis* extract in an organic solvent – free medium displayed a superior activity by inhibiting the growth of gram negative (*E. coli* and *Klebsiella*) and gram positive (*S. aureus* and *E. faecalis*) [92].

V. ANTIFUNGAL ACTIVITY OF CNTs

Rathore et al have demonstrated the antifungal activities in – vitro by agar well diffusion technique. Derivatives of chitosan are used along with the MWCNTs in order to demonstrate the antifungal activity against fungi namely *A. niger, C. trobicalis* and *C. neoformans* [93]. The nanocomposites showed a great potential in which polymer chitosan inhibit the germination of spores, elongation of germ tube and radial growth [94]. Chitosan promotes morphogenesis of the cell wall which restricts the growth of fungi. Chitosan combines with the DNA after entering into the cell walls as they are small in size and it was revealed by microscopic examination. After conjugation with the DNA, the transcription and translation process also gets inhibited which will ultimately affect the production of enzymes and proteins which are necessary for the growth of fungal hyphae [95]. Polymer derivative with MWCNT showed unique antifungal activities than parent chitosan. MWCNTs have potential to show electrostatic interaction with the cell membrane and changes its permeability. Zari et al [96] carried out a study to show the antifungal effect against various fungi viz *A. niger, A. fumigatus, C. albicans, P. chrysogenum, S. cerevisiae, F. culmorum, M. canis, T. mentagrophytes, T. rubrum* and *P. lilacinum*. SWCNTs dispersed with tetra – arylbimesityl derivative activated with by adding carboxy group were used for the demonstration of antifungal activity against *E. coli, S. aureus* and *C.* albicans [97].

Wang et al [98] studied SWCNTs, MWCNTs, graphene oxide (GO), reduced graphene oxide (rGO), fullerene (C60) and activated carbon against two important plant pathogenic fungi, *F. graminearum* and *F. poae*. The strongest antifungal activity was observed for SWCNTs, followed by MWCNTs, GO and rGO, where C60 and AC exhibited no noteworthy antifungal activity. Grafted nanotubes of pyrazole and pyrazolone derivatives against fungi *Aspergillus niger* and *Candida albicans* are reported.

Amphotericin B – doped conjugate CNTs (CNT – AMB) demonstrated very good activity against several AMBD – resistant *Candida* strains [95]. Branched TiO_2/CNTs revealed good photocatalytic behaviour in the reduction of *C. albicans* growth; this activity is due to the generation of electron – hole pairs under visible light excitation [99]. Modified MWCNTs by functional groups in their surfaces (OH, COOH and NH_2) exhibited an increased inhibition of the elongation and germination of *Fusarium graminearum* spores by an accumulation of MWCNTs around the spores, which became an unfavourable factor for spore growth and development [100]. Shakoor et al [101] reported that the pre – exposure of nematodes to MWCNTs increased the colony formation of *C. albicans* in their bodies and suppressed innate immunity by decreasing the expression of certain antimicrobial genes. H.Z. Zardini et al [96] studied the antifungal activity of MWCNTs against *Aspergillus niger, Aspergillus fumigatus, Candida albicans, Penicillium chrysogenum, Saccharomyces cerevisiae, Fusarium culmorum, Microsporum canis, Trichophytan mentagrophytes, Trichophytan rubrum* and *Penicillium lilacinum* by Radial Diffusion Assay (RDA) and Minimal Inhibitory Concentration (MIC) methods. Fungal spores are reproductive structures which plays an important role in the dissemination of diseases. Studies show that the average length of normal spores is about 68.5 µm, and was reduced to 54.5, 28.3, 27.4 and 29.5 µm after being treated with MWCNTs [102]. Fungal pathogens can generate asexual conidia to initiate the infection cycle. The specialized reproductive structures can survive for extended periods even with little or no nutrients. Extensive exposure of microorganisms to various antibiotics has developed strong resistance which is insurmountable and extremely widespread, impelling the production of multi drug – resistant pathogens [103 – 105]. Studies show that the effects of o – MWCNTs were experimented on the vegetative biomass of seedlings and results that wheat seedlings germinated and developed on media with different concentrations of o – MWCNTs (10, 20, 40, 80 and 160 lg/ml) exhibited a consecutive increase [106 – 108]. Some studies show that mechanism of CNT dispersion can either be ionic or steric depending on the surfactant, but the exact mechanism of action has not been concluded yet [109 – 110]. Antifungal roles of NPs against notorious fungal pathogen *Botrytis cinerea* have been evaluated by Yi Hao et al [111].

VI. SCOPE FOR FUTURE RESEARCH

Even though carbon nanotubes emerged about three decades ago, much progress has been achieved within this short time interval. Most antibacterial carbon nanotubes are still under research. Progress in carbon nanotechnology may well lead to better insights into antimicrobial, antibacterial and antifungal activities. Especially, further research on the applications of multi – walled carbon nanotubes in antimicrobial, antibacterial and antifungal activities can be done on various other microorganisms for the benefit of human health.

VII. CONCLUSION

Carbon nanotubes are rolling cylindrical structures. Multi – walled carbon nanotubes have improved electrical conductivity, large surface area, better chemical consistency and mechanical strength. Carbon nanotubes act as a carrier for the effective delivery of biomolecules like antibiotics, proteins, DNA, RNA, immune – active compounds and lectin. Today, antibiotic resistance has emerged as a strong health concern worldwide. To eradicate this problem, synthesis and application of new antimicrobial, antibacterial, antifungal materials are necessary and required. Emerging nanotechnology has provided a suitable platform to resolve the problem of resistance by the use of antimicrobial nanomaterials, identified as nanomaterials with antibacterial properties to contest infections by antibiotic – resistant bacteria. This review paper has focused on antimicrobial, antibacterial and antifungal activity of multi – walled carbon nanotubes in detail. Therefore, further research should be focused on investigating more efficient MWCNTs based devices for the betterment of human health.

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