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Dendrimers as Antibacterial Agents

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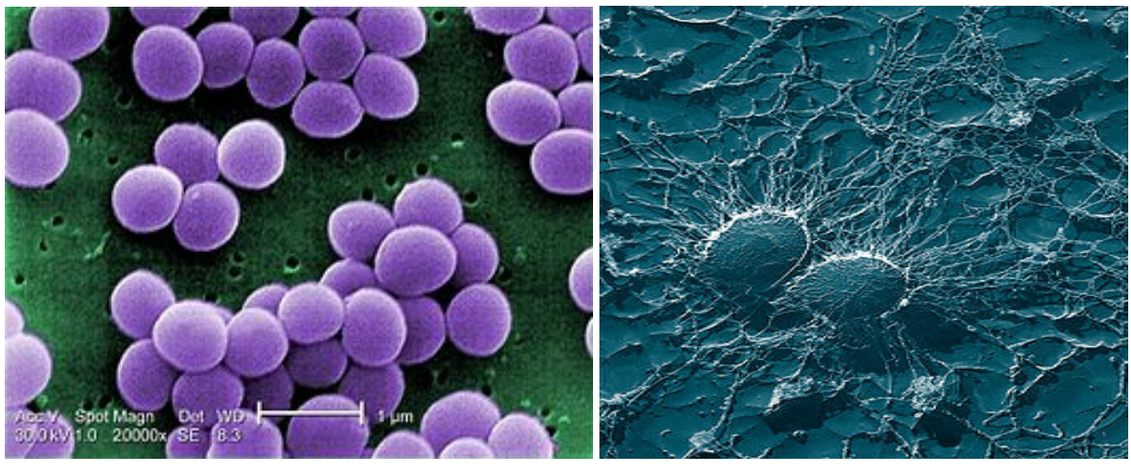
1. Introduction

No more than 100 years ago, if a person acquired a bacterial infection, the body had to clear the infection by itself or else the infection would eventually result in death. After penicillin and many other effective antibiotics were discovered, however, that changed. In the decades after penicillin was discovered in 1928, a number of powerful antibiotics were developed. They were used plentifully and often carelessly prescribed needlessly for certain bacterial infections and even for viral infections where they have no effect. Farmers found that animals fed low levels of antibiotics grow faster and are less subject to disease, so thousands of tons of antibiotics were (and still are) added to animal feed. The problem: unlike disinfectants, antibiotics generally act against a single component of a bacterium. Thus, in environments where antibiotics are present, there is great selective pressure toward bacteria that can make the relatively minor mutations needed to render them resistant. Once a single bacterium has developed resistance to an antibiotic, it can be amplified across bacterial species by quick propagation and the tendency to share antibiotic resistance genes with other bacteria. In the last decade, resistance to antibiotics even antibiotics once thought to be "last ditch" treatments has increased remarkably and is continually on the rise. Doctors are finding many once-treatable infections are now deadly (e.g. highly publicized Methicillin-resistant Staphylococcus aureus (MRSA) infections). Large pharmaceutical companies, once major sources of new antibiotics, have exhausted most "easy" targets for new antibiotics and have shifted their research and development focus to long-term, chronic diseases rather than antibiotic discovery to increase profits (Tanner, 2009).

Antibacterial and **antimicrobial are two similar concepts and sometimes they are used interchangeably; however there are some differences between them. Antibacterial:** Anything that destroys bacteria or suppresses their growth or their ability to reproduce. Heat, chemicals such as chlorine, and antibiotic drugs all have antibacterial properties. Many antibacterial products for cleaning and hand washing are sold today. Such products do not reduce the risk for symptoms of viral infectious diseases in otherwise healthy persons. This does not preclude the potential contribution of antibacterial products to reducing symptoms of bacterial diseases in the home (Dorland Medical Dictionary, 2010). The term **antibacterial** is often used synonymously with the term antibiotic(s); today, however, with increased knowledge of the causative agents of various infectious diseases, antibiotic(s) has come to denote a broader range of antimicrobial compounds, including anti-fungal and other compounds.

Antimicrobial is a substance that kills or inhibits the growth of microorganisms (Merriam-Webster Online Dictionary, 2009) such as bacteria, fungi, or protozoans. Antimicrobial drugs either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Disinfectants are antimicrobial substances used on non-living objects or outside the body (Wikipedia, 2011).



(a) (b)

Fig. 1. a) Scanning electron micrograph of S. aureus, 20,000 times enlargement, and false color added b) Bacterial cells of Staphylococcus aureus, which is one of the causal agents of mastitis in dairy cows. Its large capsule protects the organism from attack by the cow's immunological defenses (Kluytmans et al., 1997).

2. How do antibacterial agents work?

Most antimicrobial agents used for the treatment of bacterial infections may be categorized according to their principal mechanism of action. There are 5 major modes of action: (1) interference with cell wall synthesis, (2) inhibition of protein synthesis, (3) interference with nucleic acid synthesis, (4) inhibition of a metabolic pathway, and (5) Disruption of bacterial membrane structure. (Table 1.)(Neu, 1992). Antibacterial drugs that work by inhibiting bacterial cell wall synthesis include the b-lactams, such as the penicillins, cephalosporins, carbapenems, and monobactams, and the glycopeptides, including vancomycin and teicoplanin (McManus, 1997; Neu, 1992). β-Lactam agents inhibit synthesis of the bacterial cell wall by interfering with the enzymes required for the synthesis of the peptidoglycan layer. Vancomycin and teicoplanin also interfere with cell wall synthesis, but do so by binding to the terminal D-alanine residues of the nascent peptidoglycan chain, thereby preventing the cross-linking steps required for stable cell wall synthesis. Macrolides, aminoglycosides, tetracyclines, chloramphenicol, streptogramins, and oxazolidinones produce their antibacterial effects by inhibiting protein synthesis. Bacterial ribosomes differ in structure from their counterparts in eukaryotic cells. Antibacterial agents take advantage of these differences to selectively inhibit bacterial growth. Macrolides, aminoglycosides, and tetracyclines bind to the 30S subunit of the ribosome, whereas chloramphenicol binds to the 50S subunit. Fluoroquinolones exert their antibacterial effects by disrupting DNA synthesis and causing lethal double-strand DNA breaks during DNA replication (Drlica & Zhao, 1997), whereas sulfonamides and trimethoprim (TMP) block the pathway for folic acid

synthesis, which ultimately inhibits DNA synthesis (Petri, 2005; Yao & Moellering, 2003). The common antibacterial drug combination of TMP, a folic acid analogue, plus sulfamethoxazole (SMX) (a sulfonamide) inhibits 2 steps in the enzymatic pathway for bacterial folate synthesis. Disruption of bacterial membrane structure may be a fifth, although less well characterized, mechanism of action. It is postulated that polymyxins exert their inhibitory effects by increasing bacterial membrane permeability, causing leakage of bacterial contents (Storm et al., 1977). The cyclic lipopeptide daptomycin apparently inserts its lipid tail into the bacterial cell membrane (Carpenter & Chambers, 2004), causing membrane depolarization and eventual death of the bacterium.

1.	Interference with cell wall synthesis : - Lactams: penicillins, cephalosporins, carbapenems, monobactams - Glycopeptides: vancomycin, teicoplanin
2.	Protein synthesis inhibition - Bind to 50S ribosomal subunit: macrolides, chloramphenicol, clindamycin, quinupristin-dalfopristin, linezolid - Bind to 30S ribosomal subunit: aminoglycosides, tetracyclines - Bind to bacterial isoleucyl-tRNA synthetase: mupirocin
3.	Interference with nucleic acid synthesis - Inhibit DNA synthesis: fluoroquinolones - Inhibit RNA synthesis: rifampin
4.	Inhibition of metabolic pathway: sulfonamides, folic acid analogues
5.	Disruption of bacterial membrane structure: polymyxins, daptomycin

Table 1. Mechanisms of action of antibacterial agents

3. Methods for antibacterial activity

3.1 Disk diffusion method

Screening for antibacterial and antifungal activities are carried out using sterilized antibiotic discs (6 mm), following the procedure performance standards for Antimicrobial Disk Susceptibility Tests, outlined by the National Committee for Clinical Laboratory Standards NCCLS (Collins, 1989; Villonova, 1993).

According to Disc Diffusion Method; when a filter paper disc impregnated with a chemical is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a "zone of inhibition".

Principle; antiseptics, disinfectants and antibiotics are used in different ways to combat microbial growth. Antiseptics are used on living tissue to remove pathogens. Disinfectants are similar in use but are used on inanimate objects. Antibiotics are

substances produced by living organisms, such as *Penicillium* or *Bacillus*, that kill or inhibit the growth of other organisms, primarily bacteria. Many antibiotics are chemically altered to reduce toxicity, increase solubility, or give them some other desirable characteristic that they lack in their natural form. Other substances have been developed from plants or dyes and are used like antibiotics. A better term for these substances is antimicrobials, but the term antibiotic is widely used to mean all types of antimicrobial chemotherapy. Many conditions can affect a disc diffusion susceptibility test. When performing these tests certain things are held constant so only the size of the zone of inhibition is variable. Conditions that must be constant from test to test include the agar used, the amount of organism used, the concentration of chemical used, and incubation conditions (time, temperature, and atmosphere). The amount of organism used is standardized using a turbidity standard. This may be a visual approximation using a McFarland standard 0.5 or turbidity may be determined by using a spectrophotometer (optical density of 1.0 at 600 nm). For antibiotic susceptibility testing the antibiotic concentrations are predetermined and commercially available. Each test method has a prescribed media to be used and incubation is to be at 35-37^{o} C in ambient air for 18-24 hours. The disc diffusion method for antibiotic susceptibility testing is the Kirby-Bauer method. The agar used is Meuller-Hinton agar that is rigorously tested for composition and pH. Further the depth of the agar in the plate is a factor to be considered in the disc diffusion method. This method is well documented and standard zones of inhibition have been determined for susceptible and resistant values. There is also a zone of intermediate resistance indicating that some inhibition occurs using this antimicrobial but it may not be sufficient inhibition to eradicate the organism from the body.

The standardized methods for antiseptic and disinfectant testing are more rigorous and more difficult to reproduce in a student laboratory. Two common tests are the Phenol Coefficient Test (a comparison of the effect of the chemical and phenol on several organisms) and the Use Dilution Test (testing the chemical under actual conditions of use). A disc diffusion test can be used to approximate the Use Dilution Test. The chemical under consideration is used to saturate a filter paper disc. This disc is then used to introduce the chemical to the agar for testing. The actual zone sizes have not been standardized as in the Kirby-Bauer method, but a comparison of zone sizes for the same chemical among organisms will provide an approximate effectiveness of the chemical.

3.2 Dilution method

Screening for antibacterial and antifungal activities was carried out by preparing a broth micro dilution, following the procedure outlined in Manual of Clinical Microbial (Jones et al., 1985). The broth dilution method depends upon inoculation at a specific inoculum density of broth media (in tubes or microtitre plates) containing antibiotics at varying levels usually doubling dilutions are used and after incubation, turbidity is recorded either visually or with an automated reader, and the breakpoint concentration established. Microtitre plates or ready-to-use strips are commercially available with antibiotics ready prepared in the wells. A variation on this approach is the agar dilution method where a small volume of suspension is inoculated onto agar containing a particular concentration of antibiotic, when the inoculum has dried the plate is incubated and again examined for zones of growth.

Biological data: Standardized samples of Penicillin-g (blocking the formation of bacterial cell walls, rendering bacteria unable to multiply and spread; Ampicillin (penetrating and preventing the growth of Gram-negative bacteria); Cefotaxime (used against most Gram-negative enteric bacteria); Vancomycin (acting by interfering with the construction cell walls in bacteria), Ofloxacin (entering the bacterial cell and inhibiting DNA-gyrase, which is involved in the production of genetic material, preventing the bacteria from reproducing); Tetracyclines (exerting their antimicrobial effect the inhibition of protein synthesis; Nystatin (binding to sterols in the fungal cellular membrane altering the permeability to allow leakage of the cellular contents and destroying the fungus); Ketoconazole (inhibiting the growth of fungal organisms by interfering with the formation of the fungal cell wall) and Clotrimazole (interfering with their cell membranes and causing essential constituents of the fungal cells leakage). Mueller Hinton media, Nutrient Broth and Malt Extract Broth are purchased from Difco and yeast extracts is obtained from Oxoid.

4. Dendrimers as antibacterial or antimicrobial agents

High molecular surface functional group concentration of dendrimers can dominate antibacterial properties to the interacting molecule. If the end or surface groups of dendrimers are functionalized with biologically active antimicrobial groups, we might expect an increase in antimicrobial activity of the dendrimers depending on the high molecular antimicrobial surface functional group increase on the surface of the molecule.

The target for antimicrobials must be selected carefully. This is because of the fact that bulkier dendrimers may not be able to penetrate the cell membrane barrier and may have difficulty reaching the target site for the anticipated antimicrobial action (Chen & Cooper, 2002).

Biocides immobilized on dendrimers can be more effective if the target sites are cell walls and/ or membranes. It has been shown that small quaternary ammonium compounds exert their antimicrobial action by disrupting and disintegrating the cell membrane (Ghosh, 1988; Kourai et al., 1980; Panarin et al., 1985). Converting functional end groups of dendrimer to ammonium salts, dendrimer biocides can be synthesized. These dendrimer biocides have been shown to be more potent than their small molecule counterparts as they bear high local density active groups. Thus, dendrimer biocides may be very beneficial in terms of activity, localization in specific organs, reduced toxicity, and increased duration of action (Donaruma, 1978, 1980).

As the bacteria are negatively charged and dendrimer biocides have high positive charge density, electrostatic interactions bring them into contact with each other. Depending on the concentration of the dendrimer biocides, membrane permeability can be slightly changed or denaturized. At the end, high concentrations of dendrimer biocides can lead to complete disintegration of the bacterial membrane causing to a bactericidal effect (Neu, 1992).

By adding water soluble functional end groups to dendrimers, water soluble dendrimers are obtained. When these dendrimers interacted with bacteriostatic weak water soluble or insoluble antibiotics, the antibacterial properties antibiotics can be altered, especially improved, and also clinical applications can be experimentally observed by conducting studies. Bacterial inflections remain major causes of mortality in hospitals all around the

world. Against these bacterial inflections including enteric and urinary tract, and respiratory tract, sulfonamides are widely used. They are preferred due to ease of administration and wide spectrum of anti-bacterial activity. However, the clinical use of sulfonamides is limited due to their extremely low solubility in water, rapid elimination in blood, low level of association to plasma proteins and several side effects. Microbiology studies showed that PAMAM dendrimers could increase water solubility and the anti bacterial activity of a kind of sulfonamide, Sulfamethoxazole (SMZ)(Fig. 2.) (a 4- or 8 fold increase in the antibacterial activity of SMZ in dendrimer solution compared to pure SMZ dissolved in dimethyl sulfoxide (DMSO) or 0.01 M NaOH solution) (Ma, et al., 2007). Likewise, Quinolones (Fig. 3.) which are expanding class of clinically established potent antibiotics are not freely soluble in water. Microbiological studies of the quinolones (nadifloxacin and prulifloxacin) showed that strong antimicrobial activities of nadifloxacin and prolifloxacin were still significantly increased in the presence of PAMAM dendrimers and also their water solubility increased (Cheng et al., 2007).

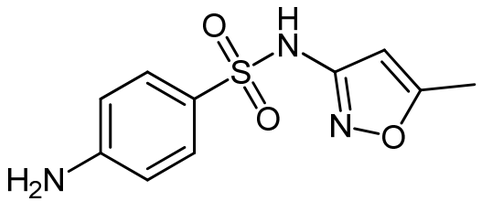


Fig. 2. Structure of Sulfamethoxazole (SMZ) 4-amino-*N*-(5-methylisoxazol-3-yl)-benzenesulfonamide

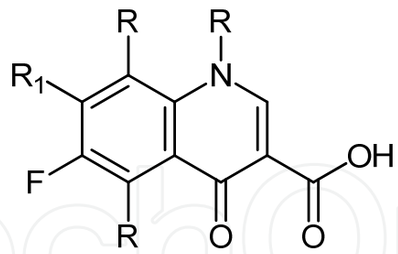


Fig. 3. Essential structure of all quinolone antibiotics: R_1 is usually piperazine; if the connection contains fluorine (F), it is a fluoroquinolone.

Highly branched dendritic structures and unique properties of dendrimers have attracted great interest in the recent studies of antimicrobial activities of dendrimers and their derivatives (Chen & Cooper, 2000). In most cases, Dendrimers have the ability of carrying biologically active agents by encapsulating them in the interior or generally, attaching them on the periphery of dendrimers. For example, most dendrimers displaying antimicrobial activities are terminated with antimicrobial agents, including ferrocene (Abd-Elzaher & Ali, 2006), quaternary ammonium (Chen et al., 2000; Chen & Cooper, 2000, 2002), boron complexes (De Queiroz, 2006), carbohydrates (Rojo, 2004), and peptides (Bernstein et al., 2003, Bourne et al., 2000; Janiszewska et al., 2006; Klajnert et al., 2006; Pini et al., 2005; Sechi et al., 2006).

Poly (amido amine) PAMAM (e.g, the generation 3 (G3) PAMAM in Fig. 4.) dendrimers are the most extensively studied dendrimers. PAMAM dendrimers with a wide variety of functional groups at the periphery are commercially available. Some of the dendrimers having terminal amino groups are shown as they are having low toxicity to eukaryotic cells (Malik et al., 2000; Roberts et al., 1996). Modification of the amino groups of the PAMAM dendrimers with poly (ethylene glycol) (PEG) or lauroly chains further improves the biocompatibility (Jevprasesphant et al., 2003a, 2003b; Luo et al., 2002). As the number of PEG or lauroyl chains increases, the cytotoxicity of PAMAM to human colon adenocarcinoma cells decreases (Jevprasesphant et al., 2003). Shielding of the positive charges of the protonated amino groups on the exterior of the dendrimer by the PEG or lauroyl chains is likely the reason for reduced cytotoxicity (Jevprasesphant et al., 2003). Due to their excellent biocompatibility, PEG-modified PAMAM dendrimers have been used as carriers of imaging agents and pharmaceuticals, including antimicrobial agents such as penicillin V and silver (Aymonier et al., 2002; Balogh et al., 2001; Bielinska et al., 1996; Svenson & Tomalia, 2005; Yang & Lopina, 2003).

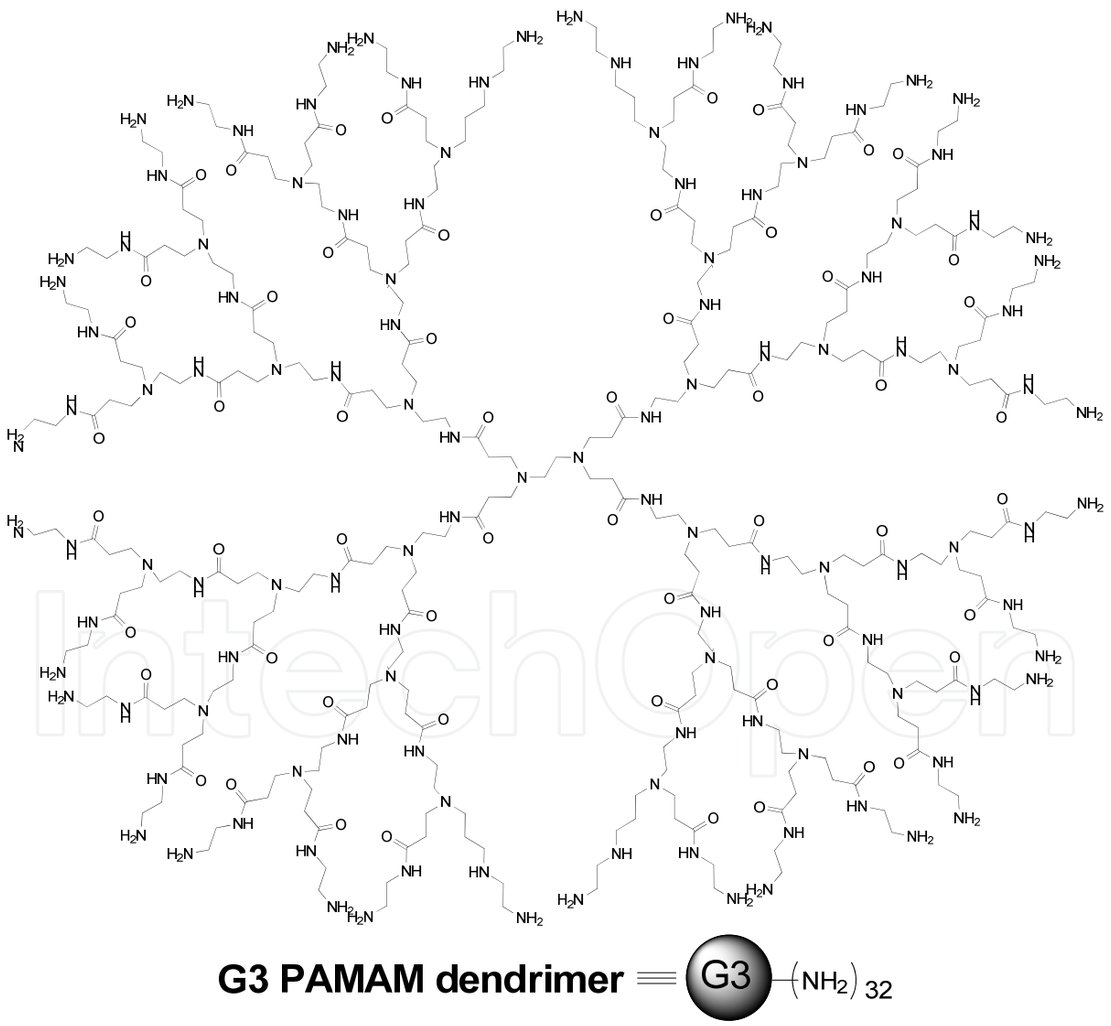


Fig. 4. Structure of the G3 PAMAM dendrimer possessing ~32 amino groups at the periphery.

Even if mostly PAMAM dendrimers stated as the carriers or the modifiers of the antibacterial agents, it has been shown that PAMAM dendrimers themselves show antibacterial properties and they are highly toxic to some bacteria. They are also suggested PAMAM dendrimers as the antimicrobial agents because of the fact that PEG-coated PAMAM derivatives exhibited low toxicity to human corneal epithelial cells (Calabretta et al., 2007). Epidemiologic studies performed in Europe countries report an increase in infectious diseases and bacterial resistance to antibiotics. This has resulted in intensive world wide search for natural linear antimicrobial peptides and their derivatives. Antimicrobial peptides are 10 to 50 amino-acid-long peptide antibiotics that have been recently discovered in many living organism including humans. These peptides are an important part of the innate defense system. They are an important part of the innate defense system, possessing a high potency and broad spectrum of activity against prokaryotic cells with only a minor impact on eukaryotic cells. Such properties raised some hope that natural antimicrobial peptides and their synthetic analogs may be adaptable for use in vivo as new generation antibiotics (Lequin et al., 2003; Papo & Shai, 2003; Powers & Hancock, 2003).

Active structures of linear peptides can be modeled by application of dendrimer chemistry. Dendrimers have nanoscopic dimensions having surface functional groups. Dendrimers can be synthesized by using organic chemistry methods and they can be indicated as the well suited compounds for biotechnological and biochemical applications. This is because of the fact that they can have multivalent nature, unambiguous composition, reliability and versatility in their synthesis. Eventually, antibacterial properties between two structurally different class of molecules- linear peptides and dendrimers can be transferred to attribute active conformation of amphiplic dendrimeric peptides (Janiszewska et al., 2007).

There is a significant global need for new antibacterial and alternative mechanisms of action given the rise in resistance among bacteria (MacDougall & Polk, 2005; Mah & O'Toole, 2005). Of the various known antibacterial agent classes, amphiphilic compounds act through perturbation and disruption of the prokaryotic membrane (Denyer, 1995). It has been hypothesized that amphiphilic anionic dendrimers may exhibit antibacterial activity with minimal eukaryotic cell cytotoxicity, since dendrimers with terminal anionic charges are generally noncytotoxic and have low toxicity in zebrafish whole animal development studies (Heiden, 2007). On the other hand, cationic dendrimers, some of which have antibacterial properties if the positive charge is properly shielded (Chen & Cooper, 2002), have repeatedly shown cytotoxicity against a variety of eukaryotic cell lines (Gurdag et al., 2006; Hong et al, 2004).

Apart from the cytotoxicity studies about the antimicrobial activities of the dendrimer integrated molecules, dendrimer studies also have come to a major interesting point in terms of many oral care products. Most of these products, especially toothpastes include Triclosan (TCN) (Fig. 5.) in order to prevent the bacterial growth called dental plague on the surface of the teeth. PAMAM dendrimers are of interest because they preferred in strategy in formulation to increase the delivery efficiency of antibacterial. TCN has broad spectrum antimicrobial activity against many types of Gram positive and Gram-negative non-sporulating bacteria, some fungi and yeasts (Brading & Marsh, 2003). For an antibacterial to be effective when delivered from an oral care product it should be efficiently retained and

subsequently released at the site of interest. Dendrimers are good candidates for delivery systems as they can be modified by the addition of molecules to their surface groups. Active molecules either can be encapsulated or conjugated to surface groups. The surface groups also can be modified to enable specific targeting of the dendrimer carrier (Sampathkumar & Yarema, 2005). TCN is not an ionic molecule, itself. In theory, cationic dendrimers may have intrinsic mucoadhesive properties for use in the oral cavity, as mucin (which covers oral epithelia) is negatively charged (Hao & Heng, 2003), thus creating an electrostatic attraction between mucus and dendrimer. An agent such as TCN, when encapsulated in the dendrimer architecture may then be slowly released into the oral cavity, potentially increasing efficacy (Gardiner et al., 2008).

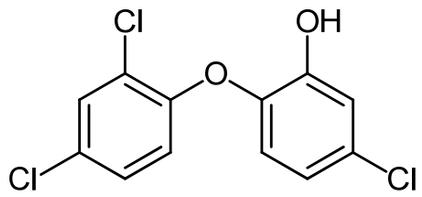


Fig. 5. Structure of Triclosan

Metal containing dendritic nanoparticles called as the Metallodendrimers. They are incorporated with metal atoms. Silver has known since ancient times as a very effective antimicrobial agent. Silver containing compounds and materials have been routinely used to prevent attack of a broad spectrum of microorganisms on prostheses (Gosheger et al., 2004), catheters (Semuel & Guggenbichler, 2004), vascular grafts (Strathmann & Wingender, 2004), human skin (Lee & Jeong, 2005), also used in medicine to reduce infection in burnt treatment (Parikh et al., 2005; Ulkur et al., 2005), arthroplasty (Alt, 2004). However they exhibit low toxicity to mammalian cells. The antibacterial activity of silver nanoparticles was tested against *Bacillus subtilis* and *Staphylococcus aureus* bacteria at different concentrations by using the diffusion disc technique. The results have showed that the antibacterial activity increases with the increase of concentration of the active agent (Mahapatra & Karak, 2008). An example demonstration of Nano-scaled silver interaction with bacteria cell can be shown from Fig. 6.

The scope of Metallodendrimers has been developing by the interest of the antibacterial studies conducted by the application of them to different type of materials as in interaction. Silver complexes of dendrimers have a broad spectrum of preventing variety of microorganism attacks and so they are preferred as antibacterial study materials. For example, it has been shown that Dendrimers have been used as vehicle to develop the antimicrobial properties of textile fabrics by modifying (PAMAM) G3 dendrimer to provide antimicrobial properties. By accomplishing this, metal nanoparticles $AgNO_3$-PAMAM (G3) complex as well as a MesoSilver-PAMAM (G3) complex has been formed and applied to Cotton/Nylon blend fabric. SEM analysis has shown that Dispersion of the silver nanoparticles onto the fabric (see Fig. 7. and Fig. 8.) was well and treated fabric against *Staphylo-coccus aureus* exhibited significantly biocide activities for each type of modified dendrimers (Ghosh et al., 2010).

Dispersion of organic/ inorganic hybrid materials could be utilized to form regular thin film coatings with antibacterial effects by using dendritic-polymer templates. The antibacterial activity of the coating films based on the hyper branched core/shell type hybrids and closely associated with the silver ions release of the films. The molecular architecture of the core/shell type hybrids used as hosts for silver nanoparticles can provide an improved control of the silver ion release and therefore an adjustment of the antibacterial affect specific to its application. This can be succeeded by the utility of the controllable generation structure of dendrimer nanoparticles (Gladitz et al., 2009). It has been shown that dendrimer films effectively inhibited the colonization of the Gramnegative bacteria *Pseudomonas aeruginos*a (strain PAO1) and, to a lesser extent, the Gram-positive bacteria *Staphylococcus aureu*s (SA). Moreover, The antibacterial activity of the films was maintained even after storage of the samples in Phosphate Buffered Saline (PBS) for up to 30 days (Wang et al., 2011).

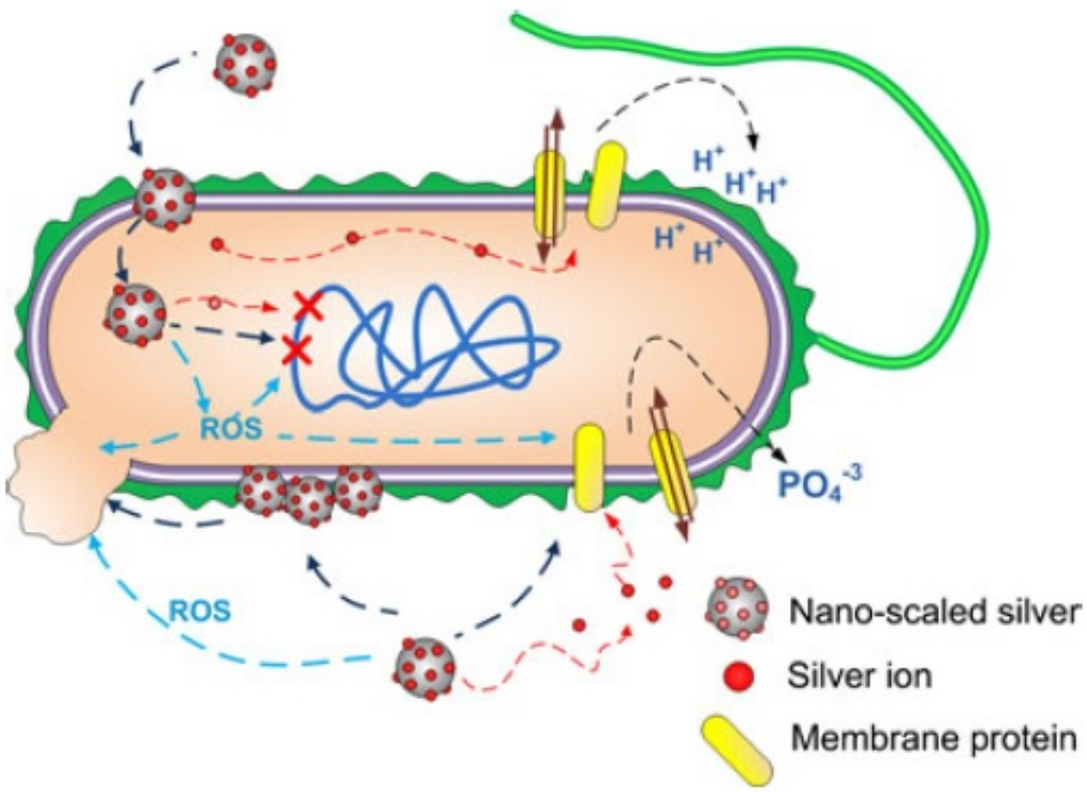


Fig. 6. Diagram summarizing nano-scaled silver interaction with bacterial cells. Nano-scaled silver may (1) release silver ions and generate reactive oxygen species (ROS); (2) interact with membrane proteins affecting their correct function; (3) accumulate in the cell membrane affecting membrane permeability; and (4) enter into the cell where it can generate ROS, release silver ions, and affect DNA. Generated ROS may also affect DNA, cell membrane, and membrane proteins, and silver ion release will likely affect DNA and membrane proteins (Marambio-Jones & Hoek, 2010).

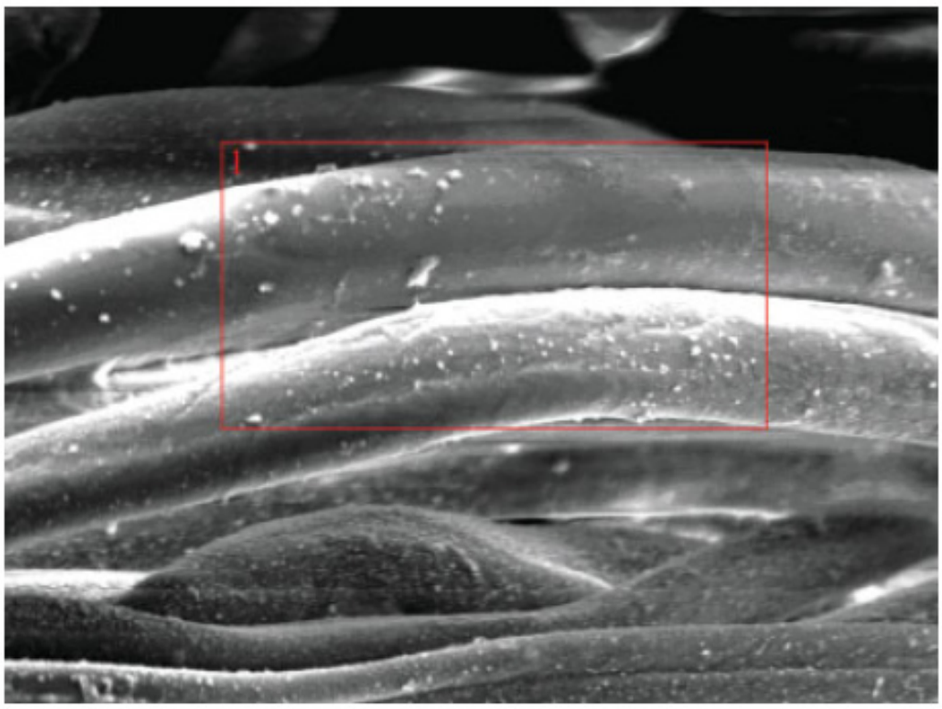


Fig. 7. SEM image of the fabric treated with silver/ dendrimers complex at 1200 magnification (10.0 kV and 10 mm distance) (Ghosh et al., 2010)

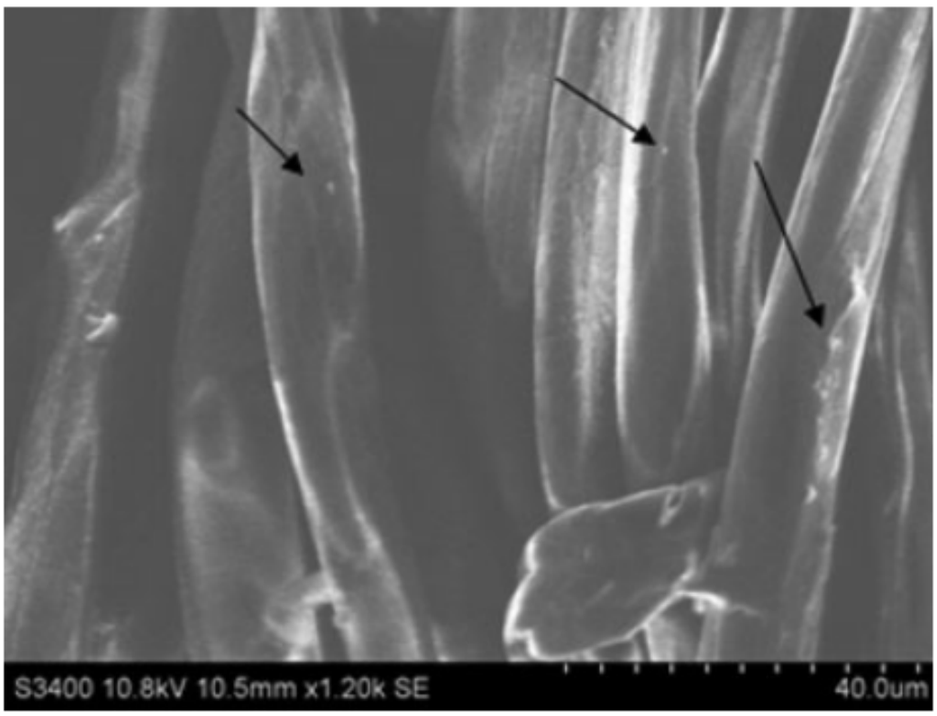


Fig. 8. SEM image of MesoSilver–dendrimer composite treated-fabric (1200 mag) (Ghosh et al., 2010)

5. Conclusion

It is clear that bacteria will continue to develop resistance to currently available antibacterial drugs by either new mutations or the exchange of genetic information, that is, putting old resistance genes into new hosts. In many healthcare facilities around the world, bacterial pathogens that Express multiple resistance mechanisms are becoming the norm, complicating treatment and increasing both human morbidity and financial costs. Prudent use of antibacterial drugs using the appropriate drug at the appropriate dosage and for the appropriate duration is one important means of reducing the selective pressure that helps resistant organisms emerge. The other vital aspect of controlling the spread of multidrug resistant organisms is providing sufficient personnel and resources for infection control in all healthcare facilities. New antibacterial agents with different mechanisms of action are also needed. It is difficult to outsmart organisms that have had several billion years to learn how to adapt to hostile environments, such as those containing antimicrobial agents. Yet, with sufficient efforts to use antimicrobial agents wisely, thereby preventing the emergence of resistant organisms, and strict attention to infection control guidelines to contain the spread of resistant organisms when they develop, we should be able to stay at least 1 step ahead of the next resistant plague.

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