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Bismuth nanoparticles: antimicrobials of broad-spectrum, low cost and safety

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Outline:

Introduction

In spite of the great succes of penicillin in the 1950s, nowadays we are dealing with pathogenic bateria resistant to many common antibiotics. *Staphylococcus aureus, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* constitute the most frequent multiresistant bacteria associated with nosocomial infections. However, this phenomena is not exclusive of bacteria, resistant strains of *Candida albicans* were detected since 1980s. In the field of virology, there is a lot of viral infections whithout any treatment option. There is an urgent need to develop new alternatives to combat the multiresistant pathogens and this include antimcirobials of broad-spectrum, low cost and safety.

Over the last twenty years, the field of nanotechnology have had an amazing growth and advances. The area of nanoparticle-based medicine receives particular attention as it holds the promise to revolutionize medical treatment with more potent, less toxic, and smart therapeutics. Although questions still exist about their safty, with substantial efforts by both academia and the biopharmaceutical industry, a few nanomedicines have been successfully developed and approved for clinical use such as DOXIL nanoencapsulated by liposomal pegylation and liposomal formulations containing irinotecan, a derivative of camptothecin[1, 2] .

Although several choices of nanoparticles exhibit medicinal properties, this chapter is focused in the usefulness of bismuth nanoparticles as an alternative broad-spectrum antimicrobial agent. We describe their efectivness as bactericidal, antibiofilm, fungicidal and antiviral agent. Finally, we comment the possible toxicity of bismuth-based nanoparticles base on experiments with cell cultures and genotoxic assays.

Antimicrobial resistance among pathogen microorganisms

The increasing prevalence of resistance pathogenic bacteria to common antibiotics is one of the most important problems in modern medicine [3]. The incidence of infections by methicillin-resistant *S. aureus* increased dramatically in the past two years [4]. *Pseudomonsa aeruginosa, Escherichia coli, Mycobacterium tuberculosis, Streptococcus pneumoniae, Staphylococcus epidermidis* and *Klebsiella neumoniae* cause 60% of intrahospital infections and are the pathogenic bacteria that most commonly exhibit antibiotic resistance [5]. Several reports indicate the emerging multiresistant strains of Candida since 1980s, demonstrating that antimicrobial resistance is not exclusive to bacteria [6, 7]. Microorganisms have several mechanisms to acquire resistance against antimicrobials. They include; intrinsic resistance, excretion of bombs efflux, adaptive resistance, indifference to drug, persistence and formation of *biofilms*. The term “biofilm” refers to growth of microbes, forming highly organized communities attached to a surface through a matrix of exopolysaccharides (EPS) (Figure 17.1). Amongst other things, EPS matrix surrounding the microbial cells protects them from very high concentrations of many different antibiotic agents, often leading to chronic infections despite antibiotic treatment [8]. Cells growing within biofilms are able to evade the host immune system [9] and require frequently 1000 times more antimicrobial agent to be eliminated than planktonic cells [10].

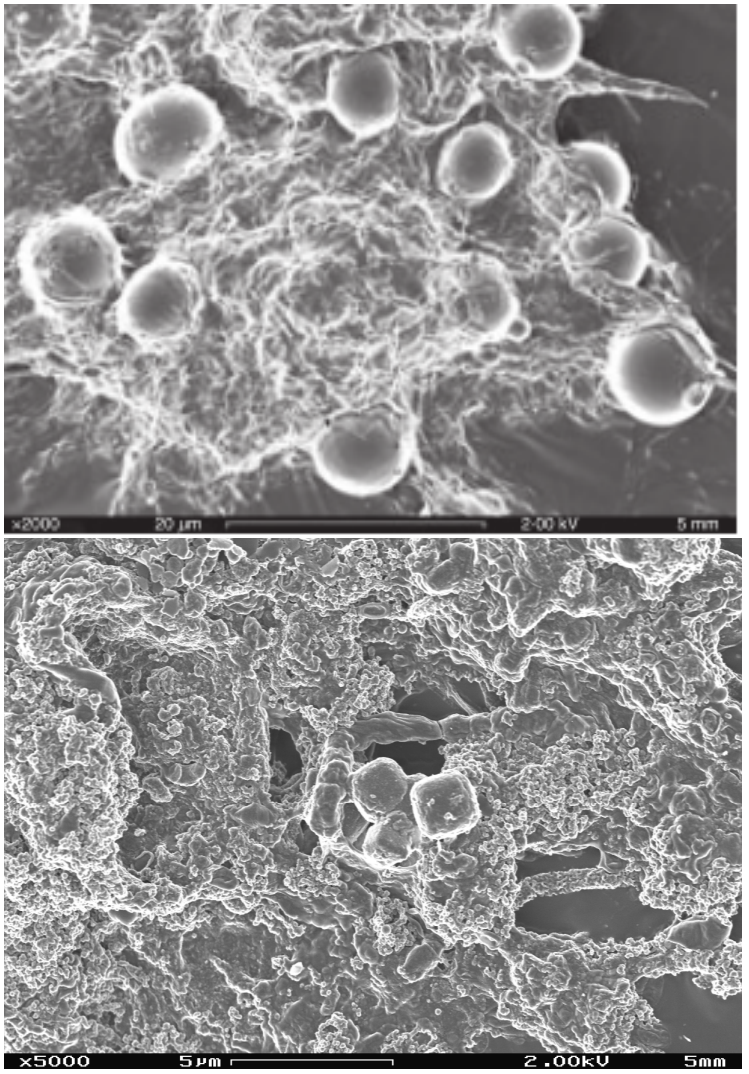

FIGURE 17.1
Scanning electron micrograph of bioaggregates from wastewater plant treatment. Adapted from Badireddy and co-workers [11] and [12]

In 2010, the American Society for Infectious Diseases, call to develop 10 new antibacterial drugs by the year 2020, showing that it is urgent to invent new drugs to fight multiresistant microorganisms. Several research groups are focusing on alternatives in plant extracts, synthetic molecules, and modifications to known antimicrobials like methicillin, first semi-synthetic penicillin. The presence of the ortho-dimethoxyphenyl group directly attached to the side-chain carbonyl group of the penicillin nucleus facilitates the β-lactamase resistance. One of the most promising strategies for overcoming microbial resistance is the use of nanoparticles.

Nanotherapeutic; a way to control multiresistant microorganisms

There are many reazons why nanoparticles inhibit the development of multiresistance by microbes. Nanoparticles prevent drug resistance because they use several mechanisms act simultaneously to inhibit the microbial growth like oxide nitric release [13], packaging several antimicrobial and anti-inflamatory drugs within same nanoparticle and controlling their realise (Figure 17.2) [14], nanoparticles have been used to target antimicrobial drugs to site of infection, letting the use of lower quantities of drugs to control the infection [15]. Metal nanoparticles with bactericidal, fungidal and antiviral activities could be employed as surface disinfectant and mixed with other materials like paint, clothes, etc.

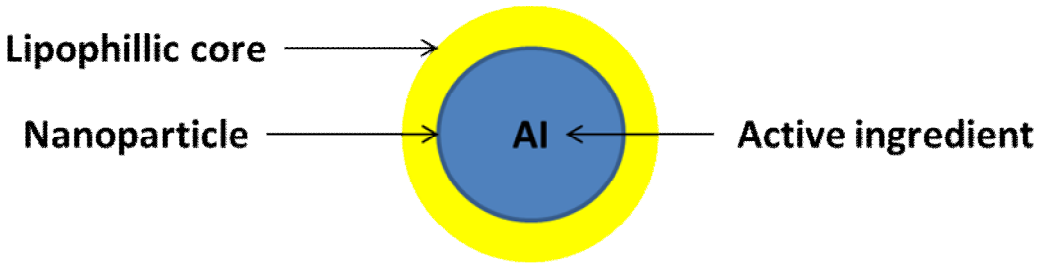

FIGURE 17.2
Diagram that ilustrate packaging of active ingredients into nanoparticles

For every plausible application, is basic the nature of nanoparticle surface which determines their interaction with the environment. These interactions could modify both nanoparticle and target; if it is a cell, it could alter cell psysiology, structure (e.g. membrane disruption including its permeability), or fundamental process like DNA division or protein synthesis [16, 17]. One kind of nanoparticle could present one or more of these action mechanisms to inactivate microbes fundamentally explaining the reasons why nanoparticles can prevent the development of drug resistance.

Different types of metals have been used to synthesize nanoparticles and explore their possible antimicrobial effect. Each metal can employ different mechanisms to inhibit the microbial growth (Figure 17.3). The metals most commonly use to synthesize nanoparticles for biomedical applications include silver (Ag), zinc (Zn), copper (Cu), titanium (Ti), magnesium (Mg), gold (Au) and bismuth. Inorganic colloidal nanoparticles are very small, nanostructured materials dispersed in a solvent. Base on the material of origin, nanoparticles can have several different properties such as hardness, active surface, chemical reactivity and biological activity [18]. The nanoscale materials are more andvantageous than their bulks elements, they can be applied to many fields like magnetism, electronic and medicine [13, 19, 20].

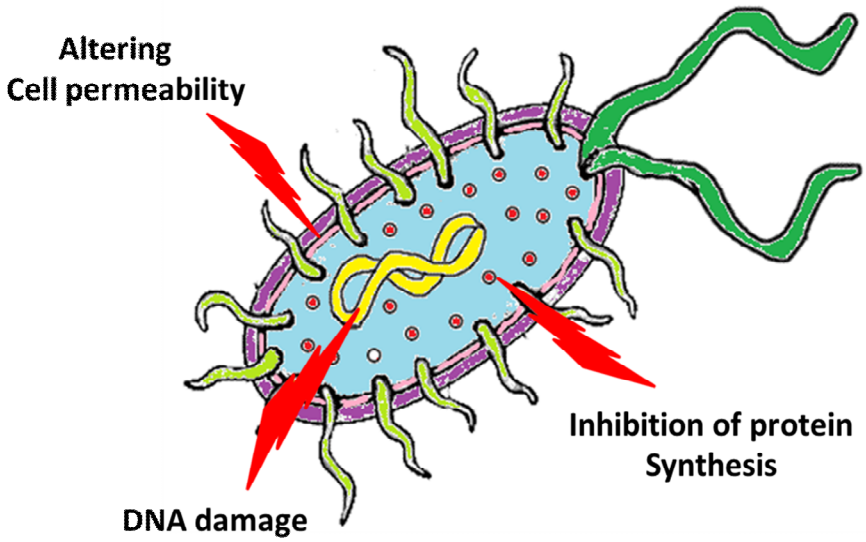

FIGURE 17.3
Main mechanisms to inhibit the microbial growth by nanoparticles

Bismuth nanoparticles as antimicrobial agents

Bismuth is a crystalline, brittle metal and constitutes the most naturally diamagnetic metal. Typically, bismuth is found as bismuthinite (bismuth sulfide), bismite (bismuth oxide) and bismuthite (bismuth carbonate) [21]. Bismuth has the property that it expands as it freezes and also has unusually high electrical resistance for a metal. Its thermal conductivity is lower than any metal except mercury [22]. Bismuth oxide is a derivative of great technological importance, and it is used in the manufacture of glass and ceramic products and also, as catalyst in the oxidation of hydrocarbons. It is widely used in applications such as microelectronics, sensor technology and optical [23, 24].

Colloidal chemistry provides opportunities to generate straightforward synthetic routes to obtain bismuth nanoparticles with well-controlled size distributions and high crystallinity. In general, commercial bismuth salts are used as precursors; also, surface modifier species and a reducing agent are added to produce the nanoparticles.
Structural characterization of the NPs is obtained by X-ray diffraction analyses of the bismuth colloids and transmission electron microscopy of high resolution (HR-TEM) [25]. This method of synthesis is the most common used to obtain metal nanoparticles; it is not expensive and scalable to industrial production. This is an important characteristic for their use in humans.

Bismuth compounds are most commonly used for treating gastrointestinal disorders. Although elemental bismuth exhibits antimicrobial activity, it does so only at relatively high (order of millimolar) concentrations due to its limited water solubility. However, solubility is increased upon chelation and

bismuth´s antimicrobial properties are manifested at much lower (order of micromolar) concentrations with bismuth dimercaptopropanol (BisBAL) being highly effective against several bacteria [26]. However, BisBAL's long-term efficacy might be limited as it is readily consumed upon contact with microorganisms. For this reason, we have been exploring BisBAL in its nanoparticulate form with the idea that its slow dissolution would enable it to act as an antimicrobial agent for extended time period [27]. Bismuth compounds remain important components of stomach remedies, such as Pepto-Bismol (bismuth subsalicylate, BSS)[28] and De-Nol (colloidal bismuth subcitrate, CBS), and derivatives of CBS, such as ranitidine bismuth citrate (RBC), are currently under development [22]. The diversity of bismuth compounds in medicine extends to the treatment of syphilis[29] and tumors[30], and in radioisotope therapies[31]. Recently, bismuth nanoparticles had been employed in diagnosis of biomolecules as well as antimicrobial agents of broad-spectrum [32-38]. It has been reported that bismuth nanoparticles (Bis-NPs) can inhibit bacterial growth at concentrations lower than 1 mM [32]. Bismuth oxide nanoparticles exhibited antifungal activity since 2 mM, showing better results than commercial antifungals [33]. The most interesting is that Bis-NPs interfere with biofilm formation of *S. mutans*, main etiological agent of caries. Early results indicate that Bis-NPs inhibit rotavirus replication, probably due to inactivation of capsid proteins (unpublished data). BisBAL significantly reduced extracellular polymeric substances (EPS) expression by *Brevundimonas diminuta* in suspended cultures at levels just below the minimum inhibitory concentration [39]. Bismuth-containing nanoparticles in combination with X-ray treatment also have potential in treating drug-resistant bacteria [40]. Since X-rays can easily penetrate human tissues, this bactericidal strategy has the potential to be used in effectively killing deeply buried MDR bacteria in vivo. Recently, it was reported that Bis-NPs inhibited *Helicobacter pylori* growth altering their Krebs cycling, nucleotide and amino acid metabolism [41].

In summary, Bis-NPs present bactericidal, fungicidal and antiviral activity. Base on bismuth subsalicylate is used to treat stomach ailments; we hypothesize that Bis-NPs will be not toxic towards human cells, until the moment there are not reports indicating secondary effects of bismuth nanoparticles. When monkey kidney cells were expose for 24 hours at a final concentration of 2 mM of Bis-NPs, no cytotoxic effect was detected [33]. Bismuth nanoparticles constitute a promising approach to fight infectious diseases, but more testing is necessary to ensure their safe use in humans. Silver nanoparticles show an important antimicrobial activity, but several reports indicate that they may present important toxic effects [16, 17, 42]. Genotoxic effects of Bismuth (III) oxide nanoparticles (BONPs) were investigated on the root cells of Allium cepa by Allium and Comet assay. Results indicate that BONPs exhibit genotoxic activity in A. cepa root meristematic cells [43]. More studies about the possible cytotoxity of bismuth nanoparticles are needed to detect any secondary effect in humans.

These results permit us to be excited about a new kind of antimicrobial of broad-spectrum, effective and low cost. Bismuth nanoparticles could be used as surface disinfectant; hospitals, pediatric and geriatric clinics, food and pharmaceutical industries, laboratories and in general all locations were microbial contamination needs to be counteracted. One interesting alternative is to adhere as a film within valves, catheter, prosthesis, and dental implants. Silver nanoparticles have been incorporated to several items; however Bis-NPs would have the advantage of being more effective and lower toxic side-effects.

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