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Review Article

Nanotechnology-Present Revolutionary Biotechnology

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ABSTRACT

Nanotechnology is considered as an industrial revolution of the third millennium. Rapid development and wide applications of nanotechnology brought about a significant increase in the number of engineered nanomaterials inevitably entering our living system. Nanoparticles designed today contribute to stronger, lighter, cleaner and "smarter" surfaces and systems. Nanomaterials may in the future surround human as indispensible part of life in the form of scratchproof eyeglasses, crack-resistant paints, anti-graffiti coatings for walls, transparent sunscreens, stain repellent fabrics, self-cleaning windows and ceramic coatings for solar cells, kitchenware and interior decorations.

The nano-revolution which unfolds role of plants in bio and green synthesis of nanoparticles seem to have drawn quite an unequivocal attention with a view of synthesizing stable and safe nanoparticles used in agriculture, medical, industrial and many other fields. The issue of safety, considering environmental and ecological impacts of nanoparticles (smart dust), and standards of customer awareness are important debates. Thus considering and solving bioethical aspects lets welcome nanotechnology as an important bliss in the field of biotechnology. In this review I would make a note of nanoparticles, one pot green synthesis, their applications and bioethical issues to make nanorevolution a safe revolution which can pave way for many new fields of technology.

Key words : Nanotechnology, nano-revolution, smart dust, bioethical issues.

1. INTRODUCTION

Nanoparticles: Nanotechnology is the science that deals with matter at the scale of 1 billionth of a meter (i.e., 10^{-9} m = 1 nm), and is also the study of manipulating matter at the atomic and molecular scale. Metallic nanoparticles have different physical and chemical properties from bulk metals (e.g., lower melting points, higher specific surface areas, specific optical properties, mechanical strengths, and specific magnetizations), properties that might prove attractive in various applications.

The optical property is one of the fundamental attractions and a characteristic of a nanoparticle. For example, a 20-nm gold nanoparticle has a characteristic wine red color. A silver nanoparticle is yellowish gray. Platinum and palladium nanoparticles are black. Not surprisingly, the optical characteristics of nanoparticles have been used from

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time immemorial in sculptures and paintings even before the 4th century AD. Ancient Greeks used silver containers to store purified water and wine. In the early 1900s, families in the United States placed Silver Dollar coins into milk containers to keep it fresh. The term "Nanotechnology" was first defined in 1974 by Norio Taniguchi of the Tokyo Science University.

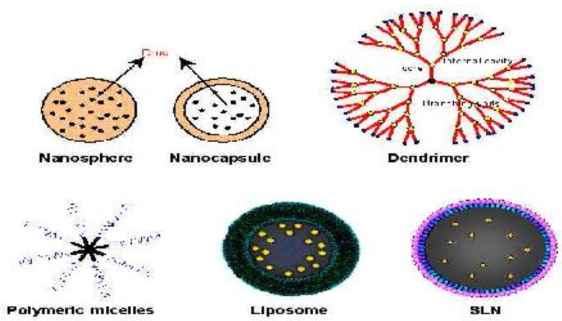


Fig 1: Types of nanoparticles

One pot green synthesis

At the present time, plant-mediated biological synthesis of nanoparticles is gaining more importance due to its simple experimental procedure and eco-friendliness. Biosynthetic processes for nanoparticles would be more useful if nanoparticles were produced extracellularly using plants or their extracts and in a controlled manner according to their size, dispersity and shape.

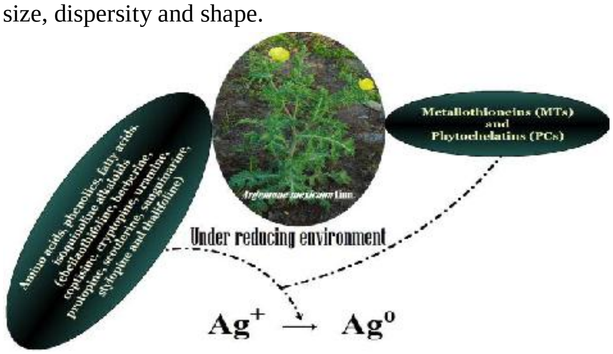


Fig 2: Schematics for the biosynthesis of AgNPs using *Argemon maxicana* leaf extract

Nanoparticles using plant extracts

Recently, there has been renewed interest in applying fundamental principles of green chemistry to produce environmentally benign nanoparticles. Bacteria, fungi, plants and seaweeds are the potential sources utilized for the synthesis of nanoparticles.

In Green synthesis nanopartiicles are synthesised by

a) use of microorganisms like fungi, yeasts (eukaryotes) or bacteria, actinomycetes (prokaryotes), b) Use of plant extracts or enzymes

c) Use of templates like DNA, membranes, viruses and diatoms.

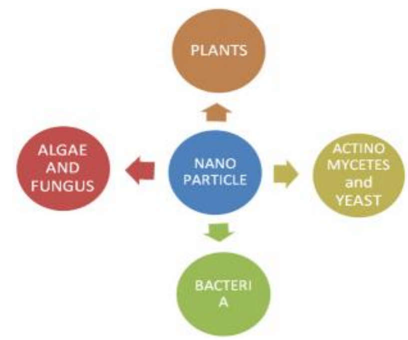


Fig 3: Different routes for biosynthesis of nanoparticles

Plant-derived compounds of therapeutic value

Recent work with regard to plant assisted reduction of metal nanoparticle and the respective role of phytochemicals showed that the main phytochemicals responsible have been identified as terpenoids, flavones,quinines, ketones, aldehydes, amides and carboxylic acids in the light of IR spectroscopic studies.

Studies indicated that the reducing phytochemicals in the neem leaf consisted mainly of terpenoids. It was found that these reducing components also served as capping and stabilizing agents in addition to reduction as revealed from FTIR studies The major chemical constituents in the extract were identified as nimbin and quercetin which are anti-pathological causes of plants. The reducing conditions of ion metals were attributed to the presence of polyphenolic antioxidant compounds found in the plant extracts such as flavonoids, and acids like 3,4-dihydroxy benzoic, p-hydroxy benzoic, caffeic, caprylic, p-coumeric, ferulic, gadoleic, lauric, sinapic, syringic and vainillic, among other compound.

Terpenes- The antibacterial activity of these compounds, oleanolic acid (OA) and ursolic acid (UA) was recently reviewed[1]. Terpenes, also referred to as isoprenoids and with oxygen, are called terpenoids. It has been shown that both acids affected peptidoglycan metabolism in Listeria monocytogenes[2], oleanolic acid cyclodextrins inhibited insoluble glucan synthesis by Streptococcus mutans and oleane-type triterpenoid, glycyrrhizin acted as a potent E. coli heat-labile enterotoxin inhibitor. Recently Ge and coworkers[3] proved the synergistic interactions of OA in combination with isoniazid, rifampicin or ethanbutol against Mycobacterium tuberculosis. Recently the Chinese group showed that diterpenes isolated from the genus Scutellaria possessed the substantial antimicrobial and antiviral activities[4].

Phenolics and polyphenols - Phenolics and polyphenols constitute a very large group of chemical compounds. Chrysin, another flavonoid abundant in propolis, also displays substantial antimicrobial activity, preferentially against Gram-positive species, e.g.Streptococcus sobrinus, Enterococcus faecalis and Micrococcus luteus. Its activity against certain oral pathogens, such as Peptostreptococcus anaerobius, Peptostreptococcus micros and Lactobacillus acidophilus creates the possibility of propolis application in the treatment of oral cavity diseases. Recently novel C(7) modified chrysin was synthesized. This modification was deliberately designed in order to enhance the antibacterial effect. The biological assays indicated that this compound is a potent inhibitor of beta-ketoacyl-acyl carrier protein synthetase (FabH) in E. coli. It should be also mentioned that quinones are strong poisons for bacterial type II topoisomerases ,gyrase and TopoIV[5] . Potent antibacterial activity is attributed to the anthraquinones. It was demonstrated that anthraquinone isolated from Cassia italica is bacteriostatic for Bacillus antracis, Corynebacterium pseudodiphtericum and P. aeruginosa, in turn hypericin and hyperforin isolated from Hypericium perforatum were active against Gram-positive species like S. aureus, S. epidermidis, E. faecalis and Bacillus subtilis[6]. Recently it was shown that the antibacterial activity of anthraquinone derivatives from Heterophyllaea postulata against S. aureus involved an increase in the level of superoxide anion and singlet molecular oxygen[7]. The antimycobacterial activity of quinones was also described.

Tannins, especially proanthocyanins, inhibit the growth of uropathogenic E. coli, S. mutans [8] as well as ruminal bacteria.

The phytochemicals present in *Bryophyllum sp*. (xerophytes), *Cyprus sp.* (mesophytes) and *Hydrilla sp.* (hydrophytes) were studied for their role in the synthesis of silver nanoparticle. The xerophytes were found to contain emodin, an anthraquinone which could undergo redial tautomerization leading to the formation of silver nanoparticles. The mesophytes studied contained three types of benzoquinones, namely, cyperoquinone, dietchequinone and remirin. It was suggested that gentle warming followed by subsequent incubation resulted in the activation of quinones leading to particle size reduction. Catechol and protocatechaldehyde were reported in the hydrophyte studied along with other phytochemicals. It was reported that catechol under alkaline conditions gets transformed into protocatechaldehyde and finally into protocatecheuic acid. Both these processes liberated hydrogen and it was suggested that it played a role in the synthesis of the nanoparticles. The size of the nanoparticles synthesized using xerophytes, mesophytes and hydrophytes were in the range of 2- 5nm[9]. The leaf buds of R. mucronata extract have no antibacterial activity, but it plays a major role after the biosynthesis of AgNPs.

Experimental procedures involved in the synthesis of silver nanoparticles using plant extract

leaves were boiled in 100ml of distilled water contained in the conical flask. The resulting filterate (12ml) was taken and treated with 88ml of aqueous 1 mM AgNO3 solution and incubated in dark condition, at room temperature. Appearance of brownish yellow coloured solution indicates the formation of AgNPs [10].

Nanoparticle synthesis-factors affecting the synthesis

Smetana et al. observed that silver ions eroded from high-surface area silver powders prepared by SMAD method interacted and destroyed bacterial cells. In the same study, a second preparation of silver nanoparticles using water-soluble ligands was used to obtain silver nanoparticles with higher surface area to improve their antibacterial efficacy. This preparation lowered the toxicity.

It is clear that synthesis techniques can affect considerably the properties of the nano-objects. The synthesis techniques can be categorized into top-down and bottom-up strategies. The top-down techniques work with the material in its bulk form, and the size reduction to the nanoscale is made via specialized ablations (*e.g.* lithography, thermal decomposition, laser ablation). In the case of nanoparticle (NPs) synthesis, bottom-up (or self-assembly) procedures involve a homogeneous system wherein catalysts (*e.g*. reducing agents, enzymes) are producing nanostructures affected by catalyst properties, reaction media and conditions (*e.g.* solvents, stabilizers, temperature). Indeed, NP physicochemical properties, surface and morphological characteristics will influence their fate, activity, transport and toxicity

BioNPs can be classified into organic, inorganic, and hybrid as recently reviewed[11,12,13]. BioNPs can be synthesized from carbohydrates, lipids, DNA, proteins and also complex molecular mixtures. Inorganic bioNPs can be classified into oxides and metallic. The synthesis of inorganic bioNPs is carried out by unspecific reducing agents present in the medium and/or as the result of triggering the SOS system in the cell to reduce toxicity.

In the case of hybrid bioNPs, the synthesis can be driven by molecular precursors in the presence of biological templates (*e.g.* DNA, proteins). The advantage of using biological templates is the huge diversity of tridimensional biostructures available as templates that can be used to create NPs with many different characteristics and properties.

Besides, the potency of the antibacterial effects corresponds to the size of the nanoparticle. The smaller particles have higher antibacterial activities due to the equivalent silver mass content. It was observed with increase in reaction time to 9 h and 13 h, the size of the nanoparticles was increased to 25±3 nmand40±5 nm, respectively.The effect of temperature on nanoparticle formation was investigated and it was reported that polydisperse particles with a size range of 5-300nm was obtained at lower temperature while a higher temperature supported the formation of smaller and spherical particles. While fungi and bacteria require a comparatively longer incubation time for the reduction of metal ions, water soluble phytochemicals do it in a much lesser time. The influence of different factors on the formation of nanoparticles in freshly brewed tea extracts has been investigated. The mean particle size and number of nanoparticles increase with decreasing temperature.

2. CHARACTERIZATION

After silver nanoparticles were synthesized, they were characterised by UV-Visible spectrophotometer and transmission electron microscope (TEM). Formation of AgNPs was established by X-ray diffraction, transmission electron microscopy and UV-Visible spectroscopic techniques. The spectra were recorded from 300-600 nm at a resolution of 0.1 nm. Synthesis of silver nanoparticles from plant extract shows a maximum absorbance at 430 nm which increases as a function of reaction time. Shape and size was determined by TEM and XRD. The results recorded from UV–vis spectrum, scanning electron microscopy (SEM), X-ray diffraction (XRD) and energy dispersive spectroscopy (EDS) support the biosynthesis and characterization of silver nanoparticles. The morphology of AgNPs was also determined using atomic force microscopy (AFM).Raman spectroscopy is a widely used tool to characterize material composition, sample temperature, and strain from analysis of the material-specific phonon mode energies. The calculated spectra clearly reflect the wellknown dependence of nanoparticle optical properties, viz. the resonance wavelength, the extinction cross-section, and the ratio of scattering to absorption, on the nanoparticle dimensions. Use of surface-enhanced Raman spectroscopy (SERS) in new material characterization, concept development and in identifying their applications has been thoroughly reviewed.

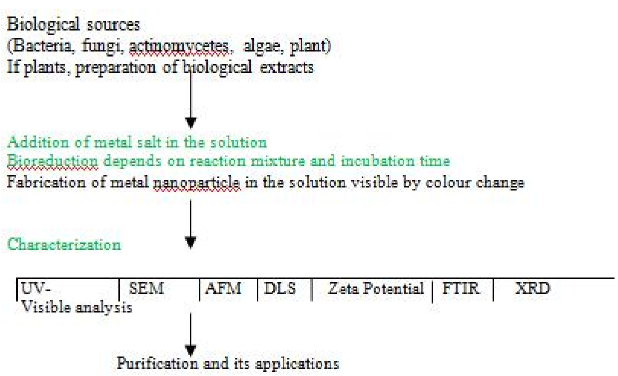


Fig 5: Flow chart of the synthesis of nanoparticles

3. NANOPARTICLES –APPLICATIONS

NPs are characterized by a very large surface area to volume ratio. Copper, zinc, magnesium but especially silver and gold NPs display antibacterial activity and are used for various healthcare, hygiene and personal care purposes and also in water-treatment.

Main fields of nanotechnology applications range from catalysis, micro- and nano-electronics (semiconductors, single electrons transistors), non-linear optic devices, photo-electrochemistry to biomedicine, diagnostics, foods and environment, chemical analysis.

Nanotechnology is used in various areas, such as health care, consumer products, ICT, food and feed, environmental health, and agriculture.

The addition of silver nanoparticles to socks kills the bacteria associated with foot odor. Nano-silver is also used in washing machines because of its antimicrobial activity. The widest and best known use of silver in medicine is in combination with sulfadiazine, where it becomes a topical antibacterial agent for the treatment of burns.

Antimicrobial activity

The antibacterial activity of silver nanoparticles is estimated by the zone of inhibition. Nano-silver is an effective killing agent against a broad spectrum of Gram negative and Gram-positive bacteria, including antibiotic-resistant strains.

Fig 6: General methodology of synthesis of nanoparticles

Anti fungicidal

Nanosilver is a much effective and a fast-acting fungicide against a broad spectrum of common fungi including genera such as Aspergillus, Candida and Saccharomyces. The silver nanoparticles that are produced extracellularly from *Fusarium oxysporum* can be used in several of materials like clothes which are sterile and thus used in hospitals to prevent or to minimize the infection with pathogenic bacteria like Staphylococcus aureus.

Antiviral

Rather, nanoparticle are also used in biological detection, controlled drug delivery, optical filters, sensor design etc. Silver nanoparticles (diameter 5-20 nm, average diameter 10 nm) inhibit HIV-1 virus replication.

Medicine

Silver nanoparticles are used in bone cements that are used as artificial joint replacements. Polymethyl methacrylate loaded with nanosilver is being considered as bone cement. The plasmonic properties of nanosilver also make it an excellent candidate for bioimaging as they, contrary to commonly used fluorescent dyes, do not undergo photobleaching and can be used to monitor dynamic events over an extended period of time.

Agriculture

Nanoscale science and nanotechnologies are envisioned to have the potential to revolutionize agriculture and food systems [14].

Cancer therapy

Nanosilver can be utilized for detecting various abnormalities and diseases in the human body including cancer [15,16].Silver nanoparticles were synthesized using common medicinally promising weed, *A. mexicana* and its application on cervical cancer cell line SiHa was studied with an approach to suggest a herb based nano-fortified cure for cancer having no and/or least side effect[17]. Chinese scientists have used gold nanoparticles as ultrasensitive fluorescent probes to detect cancer biomarkers in human blood.

Stem cell therapy

Nanoparticles may prove effective tools for improving stem cell therapy, new research suggests.

Nanobiosensors

Nanotechnology based biosensors can be used in the aquaculture industry for microbe controlSliver nanoparticles are even able to kill methicilin resistant Staphylococcus aureus. Tracking nanosensors such as “Smart fish” are being developed which may be fitted with sensors and locators that relay data about fish health and geographical location to a central computer.

DNA Nano vaccines

Use of nanoparticle carriers like chitosan and poly-lactide-coglycolide acid (PLGA) of vaccine antigens, together with mild inflammatory inducers, one may achieve a high level of protection to fish and shellfish not only against bacterial diseases, but also from certain viral diseases with vaccine-induced side effects. Further, the mass vaccination of fish can be done using nanocapsules containing nano-particles.

Smart drug delivery

With the help of a smart delivery system that posesses multifunctional characteristics, such as pre-programmed, time controlled monitoring, the effect of the delivery of probiotics, hormones, chemicals, vaccines and labile pharmaceuticals[18] is possible. These particles have been widely used in various biomedical applications and drug delivery systems due to their inert nature, stability, high dispersity, non-cytotoxicity and biocompatibility.

Disease diagnosis

Nanosensors are also becoming available to detect pathogens. It is now possible to detect single virus particles using electrical nanosensors.

Water filtration and remediation

The use of silver NPs in water purification to remove low concentrations of halogenated compounds like pesticides and heavy metals is reported, with several products being commercially available. Nanofiltration methods are also suggested over traditional micron scale filtration to greatly improved removal of foreign substances from water. Antibacterial, antifouling, and high dye absorption properties of our functional nanomaterial are exceptionally promising for the development of high efficiency and low cost water purification technologies (water filters).

$AgNo_3$ + Protein from *Rhizopus oryzae* ⟶ Protein⟶
coated AgNPs   Ag NP

Insitu synthesis of silver nanoparticle on silica support by protein extract for effective water disinfection

A ceramic water filter (CWF) is a simple device that can eliminate water-borne pathogens. Zero Valent Iron nanoparticles (nZVI) filtration unit is very effective for water treatment as compared to classical filtration unit.

Gene delivery

The development of new carrier systems for gene delivery represents an enabling technology for treating many genetic disorders. Effective fish feed Production of more effective fish feed for aquaculture species is very important. Nanoformulations of feed help to maintain better consistency and flavor[19].

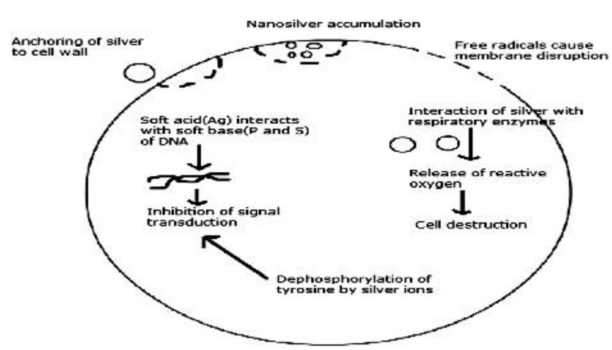
Packaging

Nanopackaging can be made from natural nanoscale polymers, such as cellulose and starch, or chitosan particles , and are therefore likely to be biodegradable, unlike some conventional plastics. For fish health in aquaculture, nanotechnological applications include antibacterial surfaces in the aquaculture system, nanodelivery of veterinary products in fish food using porous nanostructures, and nanosensors for detecting pathogens in the water.

4. NANOPARTICLES - MECHANISM OF ACTION

With respect to the microbes, the silver nanoparticles get attached to the cell wall, thereby disturbing the permeability of cell wall and cellular respiration. The nanoparticles may also penetrate deep inside the cell wall, thus causing cellular damage by interacting with phosphorus and sulfur containing compounds, such as DNA and protein, present inside the cell.

Bacteria has a characteristic peptidoglycane layer by which it is differentiated into gram positive and gram negative bacteria. Based on studies having shown that silver nanoparticles anchor to and penetrate the cell wall of Gram-negative bacteria, it is reasonable to suggest that the resultant structural change in the cell membrane could cause an increase in cell permeability, leading to an uncontrolled transport through the cytoplasmic membrane and ultimately cell death. It has also been proposed, that the antibacterial mechanism of silver nanoparticles is related to the formation of free radicals and subsequent free radical-induced membrane damage. Recently, evidence has been obtained suggesting that silver nanoparticles may modulate the phosphotyrosine profile of putative bacterial peptides that could affect cellular signaling and therefore inhibit the growth of bacteria.



Although the mechanisms behind the activity of nano-scaled silver on bacteria are not yet fully elucidated, the three most common mechanisms of toxicity proposed to date are: (1) uptake of free silver ions followed by disruption of ATP production and DNA replication, (2) silver nanoparticle and silver ion generation of ROS, and (3) silver nanoparticle direct damage to cell membranes. The various observed and hypothesized interactions between silver nanomaterials and bacteria cells are conceptually illustrated in Fig.

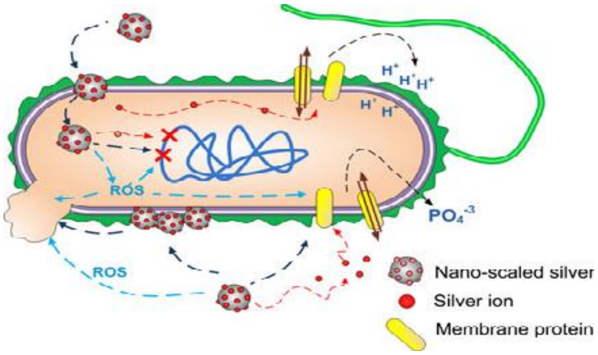


Fig 6: Diagram summarizing nano-scaled silver interaction with bacterial cells. Nano-scaled silver may (1) release silver ions and generate ROS; (2) interact with membrane proteins affecting their correct function; (3) accumulate in the cell membrane affecting membrane permeability; and (4) enter into 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.

In eukaryotic cells particularly in the mitochondria, where exists an important concentration of $H^{+.}$ Silver nanoparticles have been reported to dissolve generating silver ions on reactions of silver nanoparticles with H2O2. Asharani mechanism for silver nanoparticle oxidative dissolution.

$$2Ag + H_2O_2 + 2H^+ \longrightarrow 2Ag^+ + 2H_2O \quad E^0 = 0.17\ V$$

Similarly, Oxidative dissolution of silver nanoparticles in the presence of oxygen has been reported by Choi et al.,2008,

$$4Ag + O_2 + 2H_2O \longleftrightarrow 4Ag^+ + 4OH^-$$

As Ag^+ has high affinity with thiol groups present in the cysteine residues of those proteins, it interacts with respiratory and transport proteins . Silver inhibits the uptake of phosphate and causes the efflux of intracellular phosphate , it has been reported that Ag^+ increases DNA mutation frequencies during polymerase chain reactions. Hwang et al. observed that Ag^+ induced the same effect in bioluminescence bacteria sensitive to membrane protein damage and slightly less effect in a strain sensitive to superoxides compared to silver nanoparticles. In other research, bacterial activity of activated carbon fiber supported silver was attributed to the synergistic action of silver ions, superoxides, and hydrogen peroxide. Studies done in eukaryotic cells suggest that silver nanoparticles inhibit the antioxidant defense by interacting directly with GSH, binding GSH reductase or other GSH maintenance enzymes. This could decrease the GSH/GSSG ratio and, subsequently, increase ROS in the cell. In bacterial cells, silver ions would likely induce the generation of ROS by impairing the respiratory chain enzymes through direct interactions with thiol groups in these enzymes or the superoxideradical scavenging enzymes such as superoxide dismutases. Bactericidal activity of silver ions loaded in nanoporous materials such as zeolites has also been related to the generation of ROS. Using ROSdepletion, i.e., the formation of irregular-shaped pits the outer membrane and change in membrane permeability by the progressive release of LPS molecules and membrane proteins. This may be fairly general for gram-negative bacteria.

Bioethics

The 'Grey-goo' scenario and the concerns about 'post-humanism' are also discussed by bioethicists. There are further concerns about justice, intellectual property rights, accountability, and the probability of military and security misuse. ethics that should underpin global use of emerging technologies such as nanotechnology as forms of planetary therapeutics. It has been argued here that nanotechnology may be particularly important ethically, for instance, as a mechanism for improving upon photosynthesis and engineering it into human structures for localised production of carbonneutral hydrogen based-fuel and carbohydrate-based food and fertilizer.

5. CONCLUSIONS

Despite potential benefits of nanotechnology, there are potential ethical issues which need desirable solutions. According to the emphasis of the United Nations Educational, Scientific and Cultural Organization (UNESCO) on the nanoethics in recent years early assessment of ethical, legal and social implications of nanotechnologies will create opportunities to develop a normative framework in this field.

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