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INTERFACE OF GOLD NANOSTRUCTURE FOR MEDICAL APPLICATIONS

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ABSTRACT

Nano gold is another name for gold nanoparticles. These nanoparticles are a fraction of the size of human hair and are less than 100 nm in diameter. Nano gold particles are so small that it they are generally found as a colloidal solution, which means that the gold nanoparticles are suspended in a liquid buffer. Therefore, nano gold, or gold nanoparticles are also called colloidal gold. Also, nano gold is generally found in a colloidal solution because gold nanoparticles are created by citrate synthesis. This process involves mixing solutions together to result in the precipitation of gold nanoparticles into solution.

Gold nanoparticles may be used in different domains, one of most important being the biomedical field. They have suitable properties for controlled drug delivery, cancer treatment, biomedical imaging, diagnosis and many others, due to their excellent compatibility with the human organism, low toxicity and tunable stability, small dimensions, and possibility to interact with a variety of substances. They also have optical properties, being able to absorb infrared light. Moreover, due to their large surface and the ability of being coated with a variety of therapeutic agents, gold nanoparticles have been showed a great potential to be used as drug delivery systems. Gold nanoparticles are intensively studied in biomedicine, and recent studies revealed the fact that they can cross the blood-brain barrier, may interact with the DNA and produce genotoxic effects. Because of their ability of producing heat, they can target and kill the tumors, being used very often in photodynamic therapy. Gold nanoparticles can be synthesized in many ways, but the most common are the biological and chemical methods, however the chemical method offers the advantage of better controlling the size and shape of the nanoparticles. In this review, we present the principal applications of gold nanoparticles in the biomedical field, like cancer treatment, amyloid-like fibrillogenesis inhibitors, transplacental treatment, the development of specific scaffolds and drug delivery systems.

LIST OF ABBREVIATION

Abbreviation	Definition
AuNPs	Gold nanoparticles
BNP	Bio nanoparticles
NPs	Nanoparticles

PH	Power Of Hydrogen
Au-Ag	White Gold
Hg	Mercury
FeO	Iron Oxide
AgNO3	Silver Nanoparticles
H[AuCl4]	Chloroauric Acid
Na3C6H5O7	Trisodium Citrate Solution
TOAB	Tetraoctylammonium Bromide Solution
NaBH4	Sodium Borohydride-Sodium Hydroxide Solution
H[AuCl4]-HCl	Chloroauric Acid-Hydrogen Chloride Solution
EN	Engineered Nanomaterials
UV	Ultraviolet
AuNDs	Gold NanoDpoles
NIR	Near Infrared Region
AuNRs	Gold Nanorods
DNA	Deoxyribonucleic Acid
PDI	Polydisperse
NH2OSO2OH	Hydroxylamine-O-Sulphuric-Acid

Abbreviation	Definition
EPFL	Ecole Polytechnique Federale de Lausanne
US	United States
HIV	Hunan Immunodeficiency Virus

CHOP	Cyclophosphamide
ROS	Reactive Oxygen Species
LDD	Lactate Dehydrogenase
MTT	Multi-Table Tournament

OBJECTIVE

The aim of this project is to improve using characteristic of gold nanoparticles in the biomedical fields, Because They have suitable properties for controlled drug delivery, cancer treatment, biomedical imaging, diagnosis and many others, due to their excellent compatibility with the human organism, low toxicity and tunable stability, small dimensions, and possibility to interact with a variety of substances. They also have optical properties, being able to absorb infrared light. Moreover, due to their large surface and the ability of being coated with a variety of therapeutic agents, gold nanoparticles have been showed a great potential to be used as drug delivery systems.

CHAPTER 1

1.1-INTRODUCTION

Nanomedicine is one of the vital and rapidly developing fields of nanotechnology that accounts for a variety of potential applications. Nanoscale structures have a size almost like many biological molecules, show completely different physical and chemical properties compared to either tiny molecules or bulk materials that exhibit a wide range of uses within the fields of medicine, imaging and diagnosis and therapy [1]. The biological activities of these structures are highly influenced by the surrounding environment that has got a significant role in the designing of these materials. Recently abundant attention has been given in controlling the shape and size of the nanostructure since all the magnetic, catalytic, electrical and optical properties of metal nanostructures are influenced by their shape and size [2]. Gold nanoparticles (AuNPs) are comparatively inert in the biological atmosphere and have several physical properties that are appropriate for many biomedical applications. The current uses of AuNPs in biomedical field includes photothermal therapy, drug delivery, photodynamic therapy], gene therapy, bio labeling], bio sensing etc. Gold nanoparticles (AuNPs) with controlled geometrical, optical, and surface chemical properties are the topic of intensive studies and applications in biology and drugs. Recent advances in synthetic chemistry make it possible to make gold nanoparticles with precise control over physicochemical and optical properties that are desired for specific clinical or biological applications. Even though gold is biologically inert and thus shows less toxicity, the comparatively low rate of clearance from circulation and tissues will result in health problems, and so, specific targeting of pathological cells and tissues should be achieved before AuNPs are used for biomedical applications.

Properties of gold nanoparticles are wine red solution that is entirely different from bulk gold which is an inert yellow solid. Gold nanoparticles exhibit varied sizes starting from one nm to eight μm and they exhibit completely different shapes like spherical, sub-octahedral, octahedral, polyhedron multiple twined, multiple twisted, irregular form gold nanoparticles have unique electric and magnetic properties.

1.2- AN OVERVIEW OF NANOPARTICLES IN MEDICAL APPLICATION

In this chapter we discuss the applications of bio nanoparticles (BNP) in biomedical and environmental fields. In the biomedical field, these nanoparticles have been investigated for antimicrobial applications, biosensing, imaging, and drug delivery. In the environmental field, nanoparticles have been investigated for applications in bioremediation of diverse contaminants, water treatment, and production of clean energy. Overall, the BNP have attracted the attention of diverse researchers because their syntheses are more environmentally friendly, produces more homogeneously distributed nanoparticles and some of them can be easily biodegradable. Although there are several studies investigating the application of BNP, these nanomaterials are still way less studied than synthetic nanoparticles, since researchers are still identifying the microbiological synthetic pathways of these BNP [3]. It is expected that with the advancement of the understanding of BNP synthesis pathways, the application of BNP will expand to many more fields than biomedical and environmental and will be potentially applied in diverse nanotechnological industries. During the last decade, there have been enormous developments in utilizing the power of nanotechnology in various fields including biomedical sciences. The most important biomedical applications of nanoparticles (NPs) are in disease diagnosis and treatment. Functionalized NPs possess unique properties as contrast agents for dual and even triple modal imaging. The potential of these new generation NPs in targeted drug delivery has revolutionized safe and effective pharmacotherapies for complex diseases. One more step ahead, theranostic NPs are equipped with dual capabilities for disease diagnosis as well as treatment. Specifically, designed NPs have also been utilized to improve the delivery and efficiency of different vaccines, including their application in cancer immunotherapy [4]. This chapter provides an overview of the biomedical applications of NPs and recent advancements in this area based on current research.

1.3-WHAT ARE NANOMATERIALS?

A nanoparticle or ultrafine particle is usually defined as a particle of matter that is between 1 and 100 nanometers in diameter. The term is sometimes used for larger particles, up to 500 nm, [citation needed] or fibers and tubes that are less than 100 nm in only two directions Nanoparticles are usually distinguished from "fine particles", sized between 100 and 2500 nm, and "coarse particles", ranging from 2500 to 10,000 nm. They are a subclass of the colloidal particles, which are usually understood to range from 1 to 1000 nm [5]. Metal particles smaller than 1 nm are usually called atom clusters instead Being much smaller than the wavelengths of visible light (400-700 nm), nanoparticles cannot be seen with ordinary optical microscopes, requiring the use of electron microscopes. For the same reason, dispersions of nanoparticles in transparent media can be transparent, whereas suspensions of larger particles usually

scatter some or all visible light incident on them. Nanoparticles also easily pass-through common filters, such as common ceramic candles, so that separation from liquids requires special nanofiltration technique [6].

1.4-THE MANOFACTURING METHODS OF NANOMATERIAL

Two basic strategies are used to produce nanoparticles: 'top-down' and 'bottom-up'. The term 'top-down' refers here to the mechanical crushing of source material using a milling process. In the 'bottom-up' strategy, structures are built up by chemical processes (Figure1.1). The selection of the respective process depends on the chemical composition and the desired features specified for the nanoparticles [7].

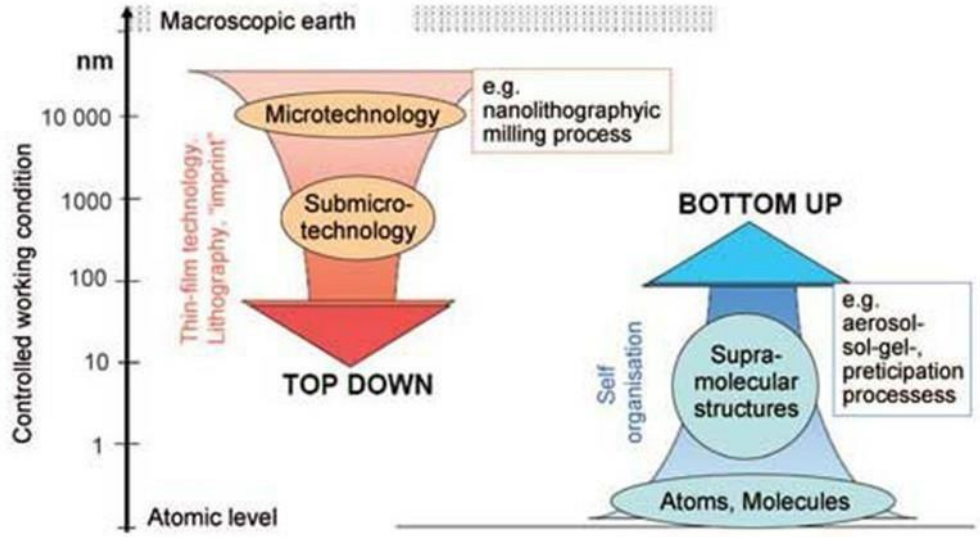

Fig (1.1) Methods of nanoparticle production: top-down and bottom-up.

1.4.1-TOP-DOWN/MECHANICAL PHYSICAL PRODUCTION PROCESSES

'Top-down' refers to mechanical-physical particle production processes based on principles of microsystem technology [8]. The traditional mechanical-physical crushing methods for producing nanoparticles involve various milling techniques (Fig 1.2)

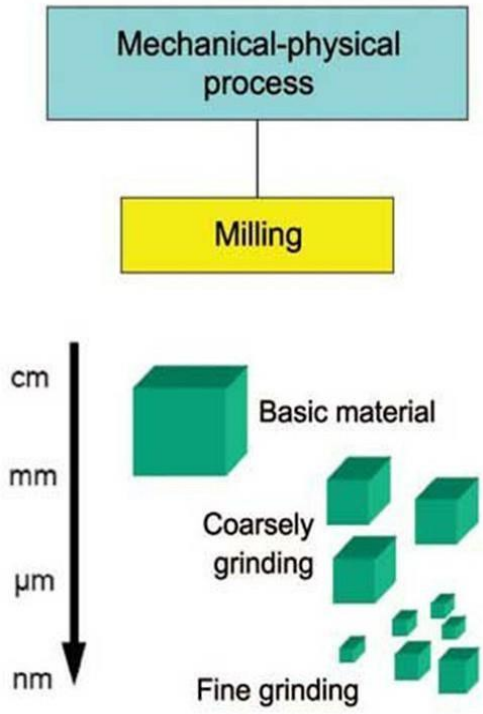

Fig (1.2) Overview of mechanical-physical nanoparticle production processes.

1.4.1.1-Milling Processes

The mechanical production approach uses milling to crush microparticles. This approach is applied in producing metallic and ceramic nanomaterials. For metallic nanoparticles, for example, traditional source materials (such as metal oxides) are pulverized using high-energy ball mills. Such mills are equipped with grinding media composed of wolfram carbide or steel.

Milling involves thermal stress and is energy intensive [9]. Lengthier processing can potentially abrade the grinding media, contaminating the particles. Purely mechanical milling can be accompanied by reactive milling: here, a chemical or chemo-physical reaction accompanies the milling process.

Compared to the chemo-physical production processes (see below), using mills to crush particles yields product powders with a relatively broad particle-size ranges. This method does not allow full control of particle shape.

1.4.2-BOTTOM-UP/CHEMO-PHYSICAL PRODUCTION PROCESSES

Bottom-up methods are based on physicochemical principles of molecular or atomic self- organization. This approach produces selected, more complex structures from atoms or molecules, better controlling sizes, shapes and size ranges [10]. It includes aerosol processes, precipitation reactions and solgel processes (Fig 1.3).

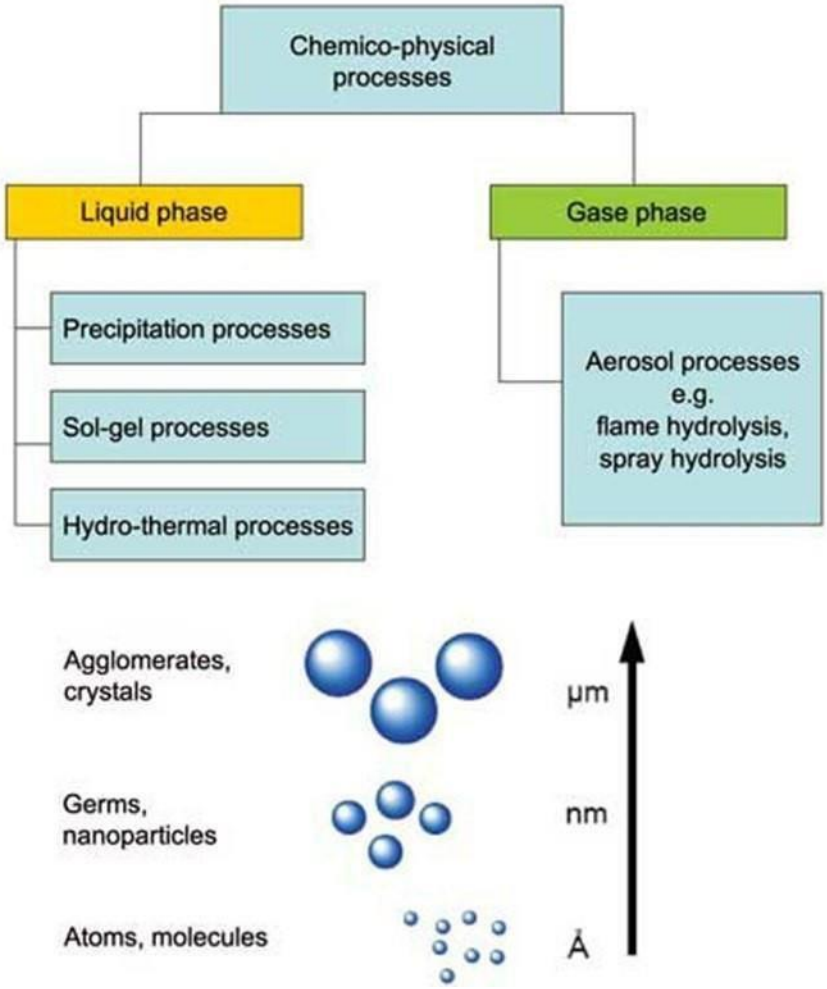

Fig (1.3) Chemo-physical processes in nanoparticle production.

1.4.2.1-Gas Phase Processes (Aerosol Processes)

Gas phase processes (aerosol processes). Gas phase processes are among the most common industrial-scale technologies for producing nanomaterials in powder or film form. Nanoparticles are created from the gas phase by producing a vapor of the product material using chemical or physical means. The production of the initial nanoparticles, which can be in a liquid or solid state, takes place via homogeneous nucleation. Depending on the process, further particle growth involves condensation (transition from gaseous into liquid aggregate state), chemical reaction(s) on the particle surface and/or coagulation processes (adhesion of two or more particles), as well as coalescence processes (particle fusion) [11]. Examples include processes in flame-plasma-laser- and hot wall reactors, yielding products such as fullerenes and carbon nanotubes:

1– In flame reactors, nanoparticles are formed by the decomposition of source molecules in the flame at relatively high temperatures (about 1200-2200°C). Flame reactors are used today for the industrial-scale production of soot, pigment-titanium dioxide and silicon dioxide particles.

2– In plasma reactors, plasma (ionized gas) provides the energy for the vaporization and for initializing the decomposition reactions.

3– In laser reactors, lasers selectively heat the gaseous source material, utilizing its absorption wavelength, and decompose it to the desired product.

4– In hot wall reactors, vaporization and condensation are applied. The source material is vaporized in an inert gas under low pressures. This removes the enriched gas phase from the hot zone. The particles created by the rapid cooling are collected on filters. Technically, hot wall reactors are used for example in producing nanoscale nickel- and iron powders.

5– The chemical gas phase deposition process is used to directly deposit nanoparticles from the gas phase onto surfaces. Here, the source material is vaporized in a vacuum and condensed on a heated surface by a chemical reaction, i.e., deposited from the gas phase into the solid final product.

1.4.2.2-Droplet Formation Containing Particles

Particles can also be produced from droplets using centrifugal forces, compressed air, sonic waves, ultrasound, vibrations, electrostatics and other methods. The droplets are transformed into a powder either through direct pyrolysis (thermal cleavage of chemical compounds) or via direct reactions with another gas [12]. In spray pyrolysis, droplets of the source material are transported through a high-temperature field (flame, oven), which rapidly vaporizes the readily volatile components or leads to decomposition reactions. The formed particles are collected on filters.

1.4.3-LIQUID PHASE PROCESSES

The wet-chemical synthesis of nanomaterials typically takes place at lower temperatures than gas phase synthesis [13]. The most important liquid phase processes in nanomaterial production are precipitation, sol-gel- processes and hydrothermal syntheses (see Fig 1.3).

1.4.4-PRECIPITATION PROCESSES

The precipitation of solids from a metal ion containing solution is one of the most frequently employed production processes for nanomaterials. Metal oxides as well as non-oxides or metallic nanoparticles can be produced by this approach. The process is based on reactions of salts in solvents. A precipitating agent

is added to yield the desired particle precipitation, and the precipitate is filtered out and thermally post-treated. In precipitation processes, particle size and size distribution, crystallinity and morphology (shape) are determined by reaction kinetics (reaction speed). The influencing factors include, beyond the concentration of the source material, the temperature, pH value of the solution, the sequence in which the source materials are added, and mixing processes. A good size control can be achieved by using self-assembled membranes, which in turn serve as nanoreactors for particle production. Such nanoreactors include microemulsions, bubbles, micelles and liposomes [14]. They are composed of a polar group and a nonpolar hydrocarbon chain Microemulsions, for example, consist of two liquids that cannot be mixed with one another in the concentrations used, usually water and oil along with at least one tensile (substance that reduces the surface tension of liquids). In certain solvents this gives rise to small reactors in which nucleation and controlled particle growth take place. Particle size is determined by the size of the nanoreactors, and, at the same time, particle agglomeration is prevented.

Micro-emulsion processes are often used to produce nanoparticles for pharmaceutical and cosmetics applications. An additional process that is based on self-organized growth with templates and coatings is hydrothermal synthesis. Zeolites (microporous aluminum-silicon compounds) are produced from aqueous superheated solutions in autoclaves (airtight pressure chambers). The partial vaporization of the solvent creates pressure in the autoclaves (several bars), triggering chemical reactions that differ from those under standard conditions, for example by altering the solubility [15]. Nanoparticle formation and cavity shape can be controlled by adding templates. Templates are particles with bonds that enable the formation of certain forms and sizes.

1.4.5-SOL/GEL PROCESSES

Sol-gel syntheses (production of a gel from powder-shaped materials) are wet chemical processes for producing porous nanomaterials, ceramic nanostructured polymers as well as oxide nanoparticles. The synthesis takes place under relatively mild conditions and low temperatures. The term sol refers to dispersions of solid particles in the 1-100 nm size range, which are finely distributed in water or organic solvents. In sol-gel processes, material production or deposition takes place from a liquid sol state, which is converted into a solid gel state via a sol-gel transformation. The sol-gel transformation involves a three-dimensional cross-linking of the nanoparticles in the solvent, whereby the gel takes on bulk properties. A controlled heat treatment in air can transform gels into a ceramic oxide material. To start with, adding organic substances in the sol-gel process produces an organometallic compound from a solution containing an alkoxide (metallic compound of an alcohol, for example with silicon, titanium or aluminum). The pH value of the solution is adjusted with an acid or a base which, as a catalyst, also triggers the transformation of the alkoxide [16].

The subsequent reactions are hydrolysis (splitting of a chemical bond by water) followed by condensation and polymerization (reaction giving rise to many- or long-chained compounds from single-chained ones). The particles or the polymer oxide grow as the reaction continues, until a gel is formed. Due to the high porosity of the network, the particles typically have a large surface area, i.e., several hundred square

meters per gram. The course of hydrolysis and the polycondensation reaction depend on many factors: the composition of the initial solution, the type and amount of catalyst, temperature as well as the reactor- and mixing geometry. For coatings, the alkoxide initial solution of the sol-gel process can be applied on surfaces of any geometry. After the wetting, the build-up of the porous network takes place through gel formation, yielding thicknesses of 50-500 nm. Thicker layers, suitable as membranes for example, are created by repeated wetting and drying. The sol-gel process can also be used to produce fibers. In all cases, gel formation is followed by a drying step (fig1.4) illustrates the different reaction and processing steps of the sol-gel process.

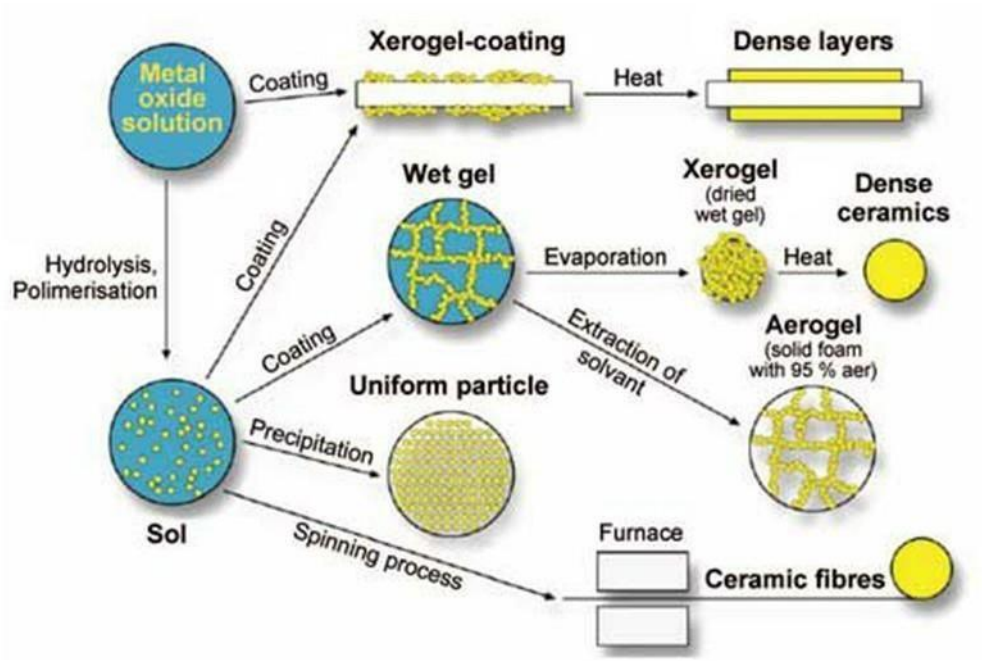

Fig (1.4) Reaction and processing steps in the sol-gel process.

1.5-GOLD PHYSICAL AND CHEMICAL PROPERTIES

1.5.1-PHYSICAL PROPERTIES OF GOLD

Gold is a soft yellow metal, with the highest ductility and malleability of any metal. Gold crystallizes in the cubic system, although crystals of gold are very rare (it is usually found as irregular plates or grains). Gold has high thermal and electrical conductivities. The only natural isotope of gold is 197Au; however, 19 isotopes-ranging from 185Au to z03Au have been produced artificially [17]. Those isotopes are radioactive, with half-lives ranging from a few seconds gold and many gold alloys are nonmagnetic. An alloy of gold and manganese is somewhat magnetic, and alloys of gold with iron, nickel, or cobalt are ferromagnetic. The equilibria of numerous binary gold alloys are described by Hansen and Anderko (1958). Except for white golds (Au-Ag), the carat golds, used mainly in jewelry, are alloys of gold, silver, and copper. The carat is used to express the proportion of gold contained: 24 carats are pure gold, 18 carats are 75% gold, and so on. Gold forms alloys with several Mercury (Hg) wets gold particles, forming

amalgams, and it is used in gold extraction operations to selectively remove gold from ground ores. Gold has a very low solubility (0.13%) in mercury. Mercury forms a solid solution with gold up to about 16% Hg. Larger contents of mercury form intermetallic com- pounds like AU3Hg and AuzHg. Molten lead is a good solvent for gold and is used as such in fire assay and in some secondary smelting operations [18].

1.5.2-CHEMICAL PROPERTIES OF GOLD

Gold is the most inert, or the noblest, of the metallic elements. It exhibits great stability and resistance to corrosion. Simple mineral acids, apart from selenic acid, do not dissolve gold. Hydrochloric acid in the presence of oxidants (such as nitric acid, oxygen, cupric or ferric ions, and manganese dioxide) dissolves gold. The combination of hydrochloric and nitric acids, aqua regia, vigorously attacks gold [19].

$$Au + 4HCI + HN03 \longrightarrow H\,[AuCI4] + 2H20 + NO$$

1.6-CELL ABSORPTION OF NANOMATERIALS

Biomedical applications increasingly require fully characterized new nanomaterials. There is strong evidence showing that nanomaterials not only interact with cells passively but also actively, mediating essential molecular processes for the regulation of cellular functions, but we are only starting to understand the mechanisms of those interactions. Systematic studies about cell behavior as a response to specific nanoparticle properties are scarce in the literature even when they are necessary for the rational design of medical nanodevices. Information in the literature shows that the physicochemical properties determine the bioactivity, biocompatibility, and safety of nanomaterials. The information available regarding the interaction and responses of cells to nanomaterials has not been analyzed and discussed in a single document. Hence, in this review, we present the latest advances about cellular responses to nanomaterials and integrate the available information into concrete considerations for the development of innovative, efficient, specific and, more importantly, safe biomedical nanodevices. We focus on how physicochemical nanoparticle properties (size, chemical surface, shape, charge, and topography) influence cell behavior in a first attempt to provide a practical guide for designing medical nanodevices, avoiding common experimental omissions that may lead to data misinterpretation. Finally, we emphasize the importance of the systematic study of nano–bio interactions to acquire sufficient reproducible information that allows accurate control of cell behavior based on tuning of nanomaterial properties. This information is useful to guide the design of specific nanodevices and nanomaterials to elicit desired cell responses, like targeting, drug delivery, cell attachment, differentiation, etc., or to avoid undesired side effects [20].

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1.6.1-DETERMAIN FACTORS IN NANOMATERIAL-CELL INTERACTION

Inorganic nanomaterials otherwise called nanoparticles/nanocrystals are composed of specific and unique physic-chemical properties which are factors that influence their complex interactions with cells. These include: 1- particle size and distribution.

2- surface area, charge and coatings.

3- shape/structure of particle.

4- dissolution and aggregation

5- Other properties such as magnetic, optical, electronic, thermal and mechanical make them widely used in several applications and consumer products. These properties enhance their cell permeating ability and penetration of other biological barriers into living organisms [22].

1.6.1.1-Partical Size and Distribution

Research has shown higher degree of toxicity of nanomaterials in relation to their larger bulk particles thereby leading to the assumption that nano particles are more effective in causing damage. A direct correlation between nanoparticles size, its distribution in tissues and consequent toxicity has been reported [23].

1.6.1.2-Surface Charge and Coating

Surface charge is a major determinant of nano particle dispersing features which plays an important role in binding to cell membrane, the absorption of ions and subsequent cellular uptake. Research reports enhanced toxicity due to increased surface charge of Iron Oxide (FeO) and silver nanoparticles (AgNO3) respectively while surface coatings indirectly affect aggregation and dissolution properties thereby enhancing the surface charge.

1.6.1.3-Shape and Structure of Particle

The morphology and shape of a nanoparticle are very important factors that influence their toxicity. Morphology, i.e., spheres, rods, truncated triangles, particles, cubes, wires, fields and coatings etc. affects the kinetics, transport and subsequent cellular uptake of nanoparticles. To buttress this fact, inhibition of Escherichia coli has been shown to be greater by triangular nanoplates in comparison to spherical- or rod-shaped Ag nano particles which could be due to high atom density of the triangular nano particles [24].

1.6.1.4-Dissolution and Aggregation

These properties are important in governing nanoparticles behavior and toxicity.

Since nanoparticles are not found isolated in nature, taking into perspective the added presence of other environmental stressors. Waste nanoparticles are released as aggregates and soluble ions into the environment. Dissolution and aggregation are processes that are largely influenced by size, surface properties and colloidal stability of which the latter is in turn influenced by environmental stressors which include temperature, pH, and ionic strength there by increasing exposure levels and subsequent toxicity. A study by showed silver nanoparticles exhibited high and rapid aggregation in media at high ionic strength.

1.6.2-ROUTES OF EXPOSURE, TRANSPORT AND FATE OF NANOMATERIALS

Synthesized nanomaterials are fast becoming a part of our everyday life due to our daily use of cosmetics, food packs, drugs, biosensors etc. to enhance drug delivery systems and odor- combating properties. This has spiked the rate of exposure to nanomaterials and their supposed toxic effects. It is therefore of essence to investigate and deeply understand the different routes of the body's exposure to these particles, their transport and their eventual fate and behavior in the body which influences their toxicity. Nanomaterials can be released into the environment by intention or unintentionally through manufacturing processes such as atmospheric emissions and waste streams from production industries. Environmental exposure to nanoparticles in clothes, sunscreen, cosmetics, and health care products is directly related to their usage.

Nanoparticles emitted settle on land and water and potentially contaminate ground and surface waters, soil and potentially become toxic to aquatic life and plant products. Nanoparticles intentionally released into the environment by technological applications, diffuse releases from wear and spillage also greatly increase exposure [23].

1.7-MANUFACTURE OF GOLD NANOPARTICLES AND BINDING THEM TRETMENT

1.7.1-MANUFACTURE OF GOLD NANOPARTICLES

Gold nanoparticles (AuNPs) are known to exhibit a combination of physical, chemical, optical and electronic properties. Gold nanoparticles play a multi-functional role. Hence Gold nanoparticle synthesis can be achieved by using various methods, majority of which follow the same rules that are applicable in preparation of other nanoparticles. The five commonly used methods for gold nanoparticle synthesis are [25,26]: 1- Turkevich method.

2- Brust method.

3- Perrault method.

4- Martin method.

5- Nanotech applications.

1.7.1.1-Gold Nanoparticle Synthesis Using the Turkevich Method

This method of gold nanoparticle synthesis was devised by someone known as J. Turkevich in the year 1951 and was later refined by G. Frens during the 70s. It is easily the simplest one of all gold nanoparticle synthesis methods.

It's primarily used for production of modestly mono disperse spherical gold nanoparticles (of around 10 nm to 20 nm diameter) suspended in water. It's possible to produce larger particles too, but not without compromising on the nanoparticles' shape and mono disparity [27].

This method involves reaction of tiny amounts of hot H[AuCl4] or chloroauric acid with equally little amounts of Na3C6H5O7 or trisodium citrate solution. Such a reaction leads to creation of gold nanoparticles since the citrate ion’s function both as a capping and a reducing agent. Larger nanoparticles can be obtained by adding less of trisodium citrate, as less as 0.05%.

1.7.1.2-Gold Nanoparticle Synthesis Using the Brust Method

Another important gold nanoparticle synthesis method - this one was developed by Brust and Schiffrin during the early 90s. It's actively used for production of gold nanoparticles in the organic liquids, which aren't normally miscible (like toluene) with water [27].

In this method, H[AuCl4] or chloroauric acid solution is reacted with TOAB or tetraoctylammonium bromide solution. The reaction happens in sodium borohydride (NaBH4) and toluene, which function as a reducing agent and anticoagulant respectively.

The gold nanoparticles produced by this technique are 5 nm to 6 nm in diameter.

1.7.1.3-Perrault Method of Gold Nanoparticle Synthesis

An approach developed by Perrault and Chan in the year 2009, this method involves usage of hydroquinone for reduction of H[AuCl4] or chloroauric acid inside an aqua solution consisting of gold nanoparticle seeds (of 15 nm diameter).

This seed-based technique of gold nanoparticle synthesis is quite similar to the one used in case of photographic film development. The latter involves growth of silver grains inside the film, by introduction of reduced silver on its surface. In the same way, gold nanoparticles can act with the hydroquinone for catalyzing the reduction of ionic gold on their surface.

The usage of a stabilizer like citrate helps in controlled deposition of the gold atoms on the nanoparticles [25].

1.7.1.4-Martin Method of Gold Nanoparticle Synthesis

A simple method developed by Martin and Eah in the year 2010, it leads to generation of almost mono disperse gold nanoparticles in water.

This method involves careful control over the reduction stoichiometry, by making precise adjustments to the ratio of NaBH4-NaOH ions to the H[AuCl4]-HCl ions, in a manner that it's well inside the sweet zone. It, along with the heating provides reproducible diameter in the range of 3 nm and 6 nm.

The aqueous particles retain their colloidal stability owing to the high charge obtained from the excessive ions present in the solution. They can be further coated with several hydrophilic functionalities, or combined with hydrophobic molecules, to make them applicable in non-polar solvents [26].

1.7.1.5-Gold Nanoparticle Synthesis Using Nanotech Applications

Bacterium like Bacillus licheniformis can also be used for gold nanoparticle synthesis.

Researchers have successfully produced gold nanoparticles ranging between 10 nm-100 nm using this method. The particles are normally synthesized with the help of toxic reagents, in organic solvents.

1.7.2-GOLD NANOPARTICLES CANCER TREATMENT

It's no news that cancer is not an easy illness to treat. Many a times, the residual cancer cells can be found in the body even after the removal of tumours. At other times, a tumor can't be removed completely owing to the manner in which the cancer cells attach themselves to a vital organ. Hence, there was always the need for an effective method of detection and destruction of the cancer cells. Gold nanoparticles have provided great hope in this field and are being effectively used for destruction of cancer cells. How the gold nanoparticles cancer treatment works is that many gold nanoparticles are clustered inside a cancer cell and then the area is blasted using an infrared laser pulse. The laser pulse causes the fluid around the cluster to reach a temperature high enough for it to evaporate. This leads to destruction of the cancer cells, causing no harm to the healthy cells [28].

Please note that the nearby cells also remain unharmed by this process as the high temperature (because of the laser pulse) stays confined inside the nano-bubble surrounding the cluster. There are various ways in which the gold nanoparticles can be clustered inside the cells, however, a method that's quite commonly used for this purpose is known as receptor- mediated endocytosis. In this method, the foreign objects (gold nanoparticles) attach themselves to the receptors on the outside areas of the cells (on their outer membranes).

The gold nanoparticles cancer treatment can only be effective if the nanoparticles are made to attach themselves to the cancer cells more easily than getting attached to the healthy cells. Else, the laser pulse may damage even the healthy tissues. To achieve this, the nanoparticles are delivered in the form of an antibody that attaches itself only to a particular type of cancer cell. Furthermore, the antibody attaches to the receptors located on the cell membranes.

It was found that the gold nanoparticles had to be of a particular optimum size to prevent their elimination from the cells. Any particles lesser than10 nm in diameter can get quickly cleared out. And any that are greater than 100 nm in diameter face trouble getting internalized into the cells. The scientists discovered that the gold nanoparticles that worked best for such gold nanoparticles cancer treatment were around 60 nm in size [28].

These antibodies coated gold nanoparticles can find their way into the cancer cells much more easily compared to the healthy cells. Please note that the average cluster size in case of cancer cells is normally around 300 nm, while it is around 64 nm in healthy cells.

As the tumours normally have leaky vascular systems, it works as a great natural advantage for this process. Hence, the gold particles spread rapidly and get incorporated through the entire cancerous region as soon as they are injected intravenously into the body area closer to the cancer. It was found that at least 24 hours' time (after the injection) is needed for the gold nanoparticles to form clusters inside the cells.

1.8-APPLICATIONS OF GOLD NANOPARTICLES IN THE MEDICAL

1- Application in biomedical [29].

2- Cytochemical labels and other applications.

3- Photothermal cancer cell therapy in near infrared region using Anti-epidermal growth factor receptor antibody conjugated gold nanorods.

4- Gold nanoparticles in biosensor applications.

5- Bioconjugation of GNP.

6- Application of gold nanoparticles for immunosensors.

7- Citrate and transfer in (Amines, Oligonucleotides, Peptides, Antibodies, Lipids).

8- Gold nanoparticles in diagnostics.

9- Gold nanoparticles in therapy.

1.9-AN OVERVIEW OF THE STUDY OF NANOMATERIALS AND THEIR APPLICATION

Nanomaterials are cornerstones of nanoscience and nanotechnology. Nanostructure science and technology is a broad and interdisciplinary area of research and development activity that has been growing explosively worldwide in the past few years. It has the potential for revolutionizing the ways in which materials and products are created and the range and nature of functionalities that can be accessed. It is already having a significant commercial impact, which will assuredly increase in the future [30].

Nanoscale materials are defined as a set of substances where at least one dimension is less than approximately 100 nanometers. A nanometer is one millionth of a millimeter - approximately 100,000 times smaller than the diameter of a human hair. Nanomaterials are of interest because at this scale unique optical, magnetic, electrical, and other properties emerge. These emergent properties have the potential for great impacts in electronics, medicine, and other fields. Some nanomaterials occur naturally, but of particular interest are engineered nanomaterials (EN), which are designed for, and already being used in many commercial products and processes. They can be found in such things as sunscreens, cosmetics, sporting goods, stain-resistant clothing, tires, electronics, as well as many other everyday items, and are used in medicine for purposes of diagnosis, imaging and drug delivery [31].

Engineered nanomaterials are resources designed at the molecular (nanometer) level to take advantage of their small size and novel properties which are generally not seen in their conventional, bulk counterparts. The two main reasons why materials at the nano scale can have different properties are increased relative

surface area and new quantum effects. Nanomaterials have a much greater surface area to volume ratio than their conventional forms, which can lead to greater chemical reactivity and affect their strength. Also at the nano scale, quantum effects can become much more important in determining the materials properties and characteristics, leading to novel optical, electrical and magnetic behaviors. Nanomaterials are already in commercial use, with some having been available for several years or decades. The range of commercial products available today is very broad, including stain-resistant and wrinkle-free textiles, cosmetics, sunscreens, electronics, paints and varnishes. Nanocoating and nanocomposites are finding uses in diverse consumer products, such as windows, sports equipment, bicycles and automobiles. There are novel Ultraviolet (UV)-blocking coatings on glass bottles which protect beverages from damage by sunlight, and longer-lasting tennis balls using butyl-rubber/nano-clay composites. Nanoscale titanium dioxide, for instance, is finding applications in cosmetics, sunblock creams and self-cleaning windows, and nanoscale silica is being used as filler in a range of products, including cosmetics and dental fillings [32].

CHAPTER 2

2.1-INTRODUCTION

Recent studies have shown the significant roles of nanomaterials in the progress of nanoscience and nanotechnology. In comparison to either small molecules or bulk materials, nanoscale structures express various physical and chemical properties. Nanoparticles possess particular intrinsic reactivity because of increased surface area, so an appropriate choice of materials for the manufacture of nanoparticle-based therapeutics would be made. The surface functionalities and depending on the particle size, shape and state of aggregation, the interaction will occur between nanomaterials and biological systems in various ways depending on the cell type, employing different uptake routes or targeting different organelles. Among the different types of nanomaterials, metal nanoparticles especially AuNPs have attracted tremendous interests from different fields of science, due to their features: high X-ray absorption coefficient, ease of synthetic manipulation, enabling precise control over the particle's physio-chemical properties, strong binding affinity to thiols, disulfides and amines, unique tunable optical and distinct electronic properties. Since the early twentieth century, various investigations have been conducted on the existence of anisotropic AuNPs [32]. The structural, optical, electronic, magnetic, and catalytic properties of anisotropic AuNPs are different from, and most often superior to spherical gold nanoparticles. Based on dimensions, AuNPs can be divided into three parts:

1-one dimensional AuNPs: nanorods, nanowires, nanotubes and nanobelts.

2-Two dimensional AuNPs: gold nanoplates such as stars, pentagons, squares/ rectangles, dimpled nanoplates, hexagons, truncated triangles.

3-three dimensional AuNPs: gold nano tadpoles, gold nano dumbbells (AuNDs), branched AuNPs such as nano pods, nano stars and gold nano dendrites.

The anisotropy of these non-spherical, hollow, and nano shell AuNP structures is the source of plasmon absorption in the visible region as well as in the near infrared (NIR) region, which is especially sensitive to the AuNP shape. This property has given rise to medical applications such as diagnostics and therapy. Spherical or quasi-spherical gold nanoparticles have received the most attention because of the ease of synthesizing such structures. The transversal band and the longitudinal band occur in the visible region and in the near infrared region (NIR) respectively. The absorption in the NIR region produces maximal penetration of light in tissues. This makes the Gold Nanorods (AuNRs) suitable for in vivo applications [33].

2.2-RESULTS OF APPLICATION OF GOLD PARTICLES

Gold nanoparticles may be used in different domains, one of most important being the biomedical field. They have suitable properties for controlled drug delivery, cancer treatment, biomedical imaging, diagnosis and many others, due to their excellent compatibility with the human organism, low toxicity and tunable stability, small dimensions, and possibility to interact with a variety of substances. They also have optical properties, being able to absorb infrared light. Moreover, due to their large surface and the ability of being coated with a variety of therapeutic agents, gold nanoparticles have been showed a great potential to be used as drug delivery systems. Gold nanoparticles are intensively studied in biomedicine, and recent studies revealed the fact that they can cross the blood-brain barrier, may interact with the Deoxyribonucleic Acid (DNA) and produce genotoxic effects. Because of their ability of producing heat, they can target and kill the tumors, being used very often in photodynamic therapy. Gold nanoparticles can be synthesized in many ways, but the most common are the biological and chemical methods, however the chemical method offers the advantage of better controlling the size and shape of the nanoparticles. In this review, we present the principal applications of gold nanoparticles in the biomedical field, like cancer treatment, amyloid-like fibrillogenic inhibitors, transplacental treatment, the development of specific scaffolds and drug delivery systems [34].

2.3-CONCLUSIONS

A Solution in complex media with a high ionic strength, a very low or no cytotoxic effect as well as coating with biomolecules of interest. In this study, a new method for synthesizing bigger size AuNPs between 60 and 150 nm through direct reduction of gold precursor using Hydroxylamine-O-Sulphuric-Acid (NH2OSO2OH) as weak reducing agent at room temperature was performed. The obtained nanoparticles were not polydisperse (PdI < 0.17) and have size-dependent optical properties [35]. In contrast, we show here that the citrate reduction method is not successful in producing AuNPs with sizes more than 40−50 nm. Therefore, the above method represents an extension of the approaches described by Turkevich, Frens, and others to produce AuNPs with a diameter above 50 nm without increasing polydispersity of the colloidal solution. Finally, bioconjugation of the obtained Au NPs with proteins make them very attractive for applications in biology, a concept to be investigated in future work [36].

2.4-CASE STUDY

According to a study of new nanotechnology, groups of gold atoms can detect and kill cancer cells that usually remain after surgery to remove a tumor. This approach has not yet been applied to only a small number of mice, but researchers are designing clinical trials to test treatment in humans in the next two years, which could dramatically increase the likelihood of cancer patients recovering, especially in cases where fully surgical removal of the tumor is not possible. When surgeons operate on cancer patients, they do their best to remove all sick cells. Because any cell left behind, it may grow again, causing a new tumor, or it may spread to another part of the body. Oncologists usually follow the surgery with radiotherapy or chemotherapy, to increase the chances of eliminating any remaining tumor cells. However, this traditional approach to fighting cancer is not guaranteed. In recent years, doctors and scientists have researched nanotechnology to help with treatment. Over the past decade, there has been one pioneering approach in this area, by researchers at Rice University in Houston, Texas. In addition to other groups, it has been shown that groups of gold atoms known as nanoparticles can be an effective weapon in fighting cancer cells [37].

Solid cancerous tumors usually have vascular and powerful blood vessels. As a result, when gold particles are injected into the bloodstream, they leak through openings in the blood vessels and collect around the tumor. These cells also swallow nanoparticles to clean up their surroundings. Once these molecules are inside the cells, they behave like Trojans. When the researchers shed light the infrared laser rays on the gold particles, centimeters of tissue were penetrated and the molecules heated, which in turn killed the cancer cells. "Unfortunately, the strategy for heating nanoparticles has two problems," says Dmitry Lapotco, a former physicist at Rice University and head of the laser science division at Masimo, a nanotechnology company in Irvine California.

First, some of the gold particles are located inside and around normal cells, so healthy tissues can be damaged when the laser targets cancer cells.

The second: The lasers, which are usually used to heat molecules, release continuous beams of infrared light, and heat spreads far beyond cancer cells, into normal tissues.

And in cases where the growth of tumors in and around vital tissues, such as nerves or arterial walls, any secondary damage to healthy tissues can weaken them or make them in a serious condition. Treatment, they started with mice whose cells were implanted with human squamous cell carcinoma, which are cancerous cells common in head and neck tumors and are considered to be difficult human tumors to treat in the traditional way. The surface of the squamous cells, and thus molecules accumulate, forming groups of dozens of them, in and around cancer cells. Instead of releasing continuous laser beams, the researchers released only pulses of ultra-short infrared radiation.

As expected, this modification prevented the heat from spreading to the surrounding healthy tissues. But this method has a more important effect as it causes temperatures to rise further where large clusters of gold particles are found, which leads to the evaporation of neighboring water molecules, and the formation of nanoparticles that rapidly expand and burst and rupture the cancer cells. "The key is that groups of nanoparticles produce nanoparticles inside cancer cells without healthy tissue," he says.

CHAPTER 3

3.1-INTRODUCTION

Nanoparticles (NPs) have unique properties that may be useful in a diverse range of applications, and consequently they have attracted significant interest. Particularly in the biomedical field, the use of nano vaccines and nano drugs are being intensively researched. Nevertheless, our knowledge about the biocompatibility and risks of exposure to nanomaterials is limited. Exposure to nanomaterials for humans may be accidental, for example occupational exposure, or intentional, for example in the use of nano enabled consumer products. There are an increasing number of studies that demonstrate adverse effects of nanomaterials in invitro cellular systems, but it is unclear whether the available data can be reliably extrapolated to predict the adverse effects of nanotechnology for humans [38]. Hence, there is an urgent need to understand the molecular mechanisms of nanoparticles to biological system interaction. In a biological medium, NPs may interact with biomolecules such as proteins, nucleic acids, lipids and even biological metabolites due to their nano size and large surface to mass ratio. Of particular importance is the adsorption of proteins on the nanoparticle surface [39].

3.2-STUDY THE PROPERTIES OF CELL PROTEINS, THEIR INTERACTION WITH NANOMATERIALS, AND THEIR ENGINEERING DESIGN

Proteins are large, complex molecules that have a variety of functions in the body and are essential to good health. Like fats and carbohydrates, proteins are long polymer chains. They are made from amino acids and are used by organisms to build structures, facilitate chemical processes and give an animal locomotion. Given the small size of NPs, it is quite likely that they can encounter different types of cells and also translocate across membrane barriers in an organism. NPs less than 100 nm in diameter can enter cells, less than 40 nm can enter the cell nucleus and below 35 nm can cross the blood–brain barrier Uptake of NP can occur via phagocytosis, micropinocytosis or endocytosis. This is of particular importance when considering NPs that have a propensity to dissolve after reaching the acidic lysosomal compartments of the cell, thus contributing to cellular toxicity [40]. To understand the fate of NPs in the biological context it is imperative to systematically analyses the intricate factors involved in uptake of these novel materials. Protein adsorption, physical characteristics of the NPs and the properties of interacting cells may influence NP uptake. Kinetics of uptake of the same nanomaterial has been shown to differ with different cell types. Adsorption of proteins on the NP surface can take place almost instantly [41]. This is particularly important when looking at differential binding of physiologically active proteins on the NP. Several in vitro studies have explored cellular uptake of NP in the presence of serum proteins. Emerging protein design strategies are enabling the creation of diverse, self-assembling supramolecular structures with precision on the atomic scale. The design possibilities include various types of architectures: finite cages or shells, essentially unbounded two-dimensional and three- dimensional arrays (i.e., crystals), and linear or tubular filaments. In nature, structures of those types are generally symmetric, and, accordingly, symmetry provides a powerful guide for developing new design approaches. Recent design studies have produced numerous protein assemblies in close agreement with geometric specifications. For certain

design approaches, a complete list of allowable symmetry combinations that can be used for construction has been articulated, opening a path to a rich diversity of geometrically defined protein materials. Future challenges include improving and elaborating on current strategies and endowing designed protein nanomaterials with properties useful in nanomedicine and material science applications [42].

3.3-DIRECT APPLICATION TO PATIENTS USING GOLD NANOPARTICLES

Despite many decades of technological development, the healthcare potential of gold nanoparticles is only coming into focus now. This largely reflects recent developments which have allowed scientists to synthesize gold nanoparticles of various shapes and sizes, consistently and at scale. These nanoparticles are also stable, easily functionalized and, critically, biocompatible, meaning they can potentially be used in the body as therapeutic agents. In combination, these properties mean that gold nanoparticle-based technologies have enormous potential in the healthcare sector. We are already seeing a wide range of possible applications in diagnostic testing, medical treatments and procedures, such as drug delivery, gene therapy, tumor detection and radiotherapy dose enhancement. Innovative applications for gold nanoparticles regularly make headlines in scientific journals and the mainstream press too. Polish scientists, for example, recently patented a formula for artificial saliva that includes gold nanoparticles. The saliva will help patients who have salivary secretion disorder, a condition that means they are unable to swallow, eat and speak. Professor Halina Car from the Medical University of Bialystok said the formula combines at least four properties of natural saliva, while improving lubricity, boosting anti-microbial properties and maintaining hygiene by preventing dental plaque development. In the United States (US), scientists from the University of Missouri have developed a method of cross-linking gold nanoparticles with collagen gels to treat ageing or damaged skin. Collagen gels are used as soft tissue fillers, to reconstruct soft tissue that has been damaged as a result of age, disease or trauma. The gold nanoparticles, which exhibit high surface reactivity, antioxidant and antimicrobial behaviors, are used to improve the collagen's resistance to degradation. And in Switzerland, scientists have pioneered a revolutionary approach in the use of gold nanoparticles that could lead to the development of a new generation of broad-spectrum antiviral drugs. Researchers at the Ecole Polytechnique Federale de Lausanne (EPFL) in Switzerland created gold nanoparticles that can attract viruses and destroy them [45]. When injected into the body the nanoparticles mimic human cells and 'trick' the viruses into binding with them to infect them. This deforms the viruses and opens them, rendering them harmless. Broad- spectrum antiviral drugs have the potential to cure many deadly viruses that are currently untreatable, from Hunan Immunodeficiency Virus (HIV) to Ebola. They could also help curb the rise of antimicrobial resistance arising from the over-use of antibiotics.

3.4-TYPE OF CLINICAL TRIAL

Clinical trials vary considerably in size, duration, and design. These factors play a major part in the interpretation of trial results. The most informative clinical trial design is the 'double- blind randomized comparison', in which some patients receive the new medicine while others receive an alternative

treatment. The alternative treatment, sometimes called the 'control', may be A placebo–an inactive 'dummy' treatment. An active comparator–generally a well-established treatment for the illness being studied. Participants are allocated to each study group by chance. The trial is set up so that while the study is going on, neither the doctor nor the patient knows who is receiving which treatment. A trial setup like this is said to be 'double-blinded'. Double-blinding reduces the potential for bias in the results. In such trials, the results are presented in terms of the difference between the group receiving the new medicine and the group receiving the control treatment:

Where the comparison is against a placebo, this difference is a measure of the real effect of the new medicine. Where the comparison is with an active comparator, the difference gives insight into how the new medicine compares with current medical practice. In both cases, two aspects of the difference are likely to be reported:

Size: This is often reported as the actual difference recorded in a particular trial together with a '95% confidence interval'. This is the range within which we can be 95% sure that the true difference would lie for the population. Although you may detect a statistical significance, it may not be clinically relevant. Generally speaking, the larger this difference, the more likely it is to be clinically relevant (to increase survival by a year is of more clinical relevance than to increase it by a day).

Statistical significance: Because some individuals respond better than others to treatment, there is always a risk that the difference between groups seen in a clinical trial may have arisen by chance. For example, if all the inherently good responders were randomized to one group, and the bad responders to the other. Statisticians can calculate how likely it is for this scenario to have occurred in a particular clinical trial and they express their result as a 'pvalue'.

A p-value of 0.05 means that there is a 5% or 1 in 20 chance that the difference happened by chance. It is conventionally taken as the threshold for accepting results as 'statistically significant'. It is important to realize that the word 'significant' used in this sense says nothing about the medical importance of the results. it merely offers reassurance that the result is unlikely to be accidental. For example, a one-meter increase in a six-minute walk distance might, in a large enough trial, be shown to be statistically significant (i.e., unlikely to have arisen by chance) but it would never be regarded by a heart-failure patient or his doctor as being of any clinical value.

3.5-RESULTS FROM CLINICAL TRIALS

When pharmaceutical companies conduct clinical trials, medical details of the patients taking part (but not their identities) are collected in a computer database together with the results of any measurements made. Statistical analyses are then conducted to formally assess the outcomes of the trial. Analyses of clinical trial results cover three areas of interest: Demographic and baseline information (Efficacy, Safety).

3.5.1-EFFICACY

This part of the analysis is based on pre-defined 'endpoints. These are specific measurements related to the illness in question. Endpoints are specified in advance in the trial protocol (the document which describes in detail how the trial will be performed). Endpoints in general can be categorized as: 'Hard' endpoints those that take the form of numerical facts with intrinsic clinical importance. For example, how long the patient survived or what proportion of patients recovered from an infection [44].

Soft endpoints those which are potentially influenced by the measurement process or with questionable reproducibility. For example, a quality-of-life questionnaire or the description of the patient's mood at a given moment. In order to be analyzed statistically, soft endpoints have to be converted into a numerical format. This process can be controversial as it often relies on subjective data and is potentially open to inconsistencies.

'Surrogate' endpoints those that are not in themselves part of the patient's experience of the illness but may be closely related to it. For example, the results of laboratory tests.

In general, hard endpoints are preferable to soft and surrogate endpoints. Soft and surrogate endpoints need to be assessed carefully in the light of how well they represent the illness being studied. Choosing which endpoints to use depends heavily on the nature of the illness being studied. Cancer, for instance, offers obvious hard endpoints in the form of survival, whereas an evaluation of depression must inevitably involve softer endpoints. Other illnesses, such as diabetes, are associated with well-established surrogate endpoints such as blood sugar levels.

3.5.2-SAFETY

Whenever the doctor conducting a clinical trial sees a patient, he or she is asked if the patient has experienced anything untoward. Information on these 'adverse events' is collected and later analyzed to give insight into possible a causal relation with the medicine studied. If such a causal relation is established, the adverse event becomes an 'adverse reaction' or side effect. Particular attention is paid to 'serious' adverse reactions–those which are life- threatening or associated with death, hospitalization, or birth abnormalities [45].

3.6-APPROACH TO RESPONSE TO CONVENTIONAL CHEMOTHERAPY AND NANOMEDICINE THERAPY

The use of systemic chemotherapy for the treatment of hematological malignancies and solid tumours relies on three established pillars: cytotoxic chemotherapy, targeted treatments and immunotherapy. In combining the use of these pillars, large steps forward have been taken in oncology. Over the last decade, chemotherapy has advanced, becoming 'smarter' and less likely to adversely affect healthy tissues. Such advances provide the real possibility of improvements in efficacy with acceptable tolerability profiles.

Taken together, this results in improved patient outcomes. The use of monotherapy in the treatment of cancer is unusual, with most agents administered as part of a combination regimen. Thus, it is common to combine a cytotoxic-chemotherapeutic agent with a targeted agent, such as a monoclonal antibody. In the past, the efficacy of cytotoxic combination regimens has been disappointing, with little benefit observed with intensification of dose and schedule, but an increase in the occurrence and severity of adverse events [46]. For example, although there is statistically significant evidence that high-dose chemotherapy in conjunction with subsequent autologous stem cell transplant improves event free survival in women with metastatic breast cancer over conventional chemotherapy, this comes at a cost of greater toxicity and no statistically significant improvement in overall survival .Combination chemotherapy regimens, such as CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone) for lymphoma, have become established as standard of care, however patient mortality remains high .Thus, there is a continued need for the development of more effective chemotherapeutics with acceptable tolerability profiles [47].

Nanomedicine is an emerging form of therapy that focuses on alternative drug delivery and improvement of the treatment efficacy while reducing detrimental side effects to normal tissues. Cancer drug resistance is a complicated process that involves multiple mechanisms. Here we discuss the major forms of drug resistance and the new possibilities that nanomedicines offer to overcome these treatment obstacles. Novel nanomedicines that have a high ability for flexible, fast drug design and production based on tumor genetic profiles can be created making drug selection for personal patient treatment much more intensive and effective. This review aims to demonstrate the advantage of the young medical science field of nanomedicine for overcoming cancer drug resistance. With the advanced design and alternative mechanisms of drug delivery known for different nanodrugs including liposomes, polymer conjugates, micelles, dendrimers, carbon-based, and metallic nanoparticles, overcoming various forms of multi-drug resistance looks promising and opens new horizons for cancer treatment [48].

CHAPTER 4

4.1-INTRODUCTION

The field of nanomedicine encompasses the utilization of nanoparticles for diagnostic and therapeutic purposes. In general, nanoparticles are used as delivery vehicles for imaging and therapeutic agents, e.g., small molecules, proteins, peptides, and nucleic acids. Numerous materials have been employed to construct such nanoparticles, including lipids metal ,silicon and silica ,polymers ,proteins ,and carbon .To date, a plethora of nano-based drugs has been designed to treat various diseases such as neurological disorders, diabetes, cancer, infectious diseases, and allergy .Accordingly, many nanotherapeutics have made it to clinical trials and several have gained regulatory approval .The major categories of nanomedicines that are clinically approved are lipid, polymer, and protein-based particles. For instance, liposomal drugs are available on the USA, European or Chinese markets, while over 20 are enrolled in clinical trials [49]. Nanocarriers can protect the payload from degradation and enable sustained and controlled drug release. Furthermore, nanoparticles have the potential to decrease clearance and improve

accumulation of drugs in diseased tissue, thereby increasing therapeutic efficacy and reducing side effects. The body contains several barriers that must be overcome for a drug to reach an intended location. These barriers include, immunological clearance, renal clearance, enzymatic and mechanical degradation, vascular endothelium, the extracellular matrix, the cell membrane, the lysosome, and membrane pumps. In essence, the properties of nanoparticles can be optimized to overcome such obstacles. In particular, nanoplatforms enable a multifaceted approach, where various materials and compartments can be combined to efficiently combat the challenges of targeted delivery. For example, the multistage vector, which sequentially releases cargo, is well equipped to handle the various in vivo conditions and compartments that are encountered upon systemic injection. Furthermore, certain nanoparticles have unique electrical and optical properties that can be employed for therapeutic purposes. For instance, metal nanoparticles combined with external energy can be used to thermally ablate diseased tissue. As an illustration, gold nanoparticles can be heated with infrared light and radio waves, while iron oxide particles can generate heat when placed in a magnetic field. Taken together, these advantages suggest that nanoparticles could be effectively used to combat several diseases. Since the field of nanomedicine displays great promise, it is imperative to also develop safety tests that can accurately predict the potential toxicity of nanotherapeutics [50]. Especially since nanoparticles exhibit distinct and unique properties that cannot be predicted from analyzing the bulk material, suitable assays for evaluation of nanoparticle toxicity should be taken into practice. Nevertheless, it may not be necessary to establish stricter guidelines for the approval of nanoparticles, as compared to small molecule drugs. Rather, the methods for assessing safety may in certain cases be different. Moreover, as nanoparticles are solely defined by size criteria and encompass a large quantity of particles with different composition and morphology, general statements regarding the safety or toxicity of nano-sized objects are impossible to make.

4.2-OUTPUTS OF SIDE EFFECTS

There are certain categories of nanoparticles that have frequently been reported to have cytotoxic effects. For example, carbon-based nanoparticles have displayed toxicity in multiple in vitroand in vivo assays, although conflicting results exist. In particular, carbon nanotubes have been shown to induce mesothelioma, thereby mimicking the toxicity of asbestos, a naturally occurring carcinogenic mineral fiber [51]. Accordingly, the harmful effects of carbon nanotubes may not be a consequence of the actual material, but rather the shape, demonstrating that the safety of nanoparticles is highly dependent on particle morphology. Likewise, the size of nanoparticles may also be a determining factor for biocompatibility. For instance, gold nanoparticles with a diameter of 1.4 nm were found to be toxic, while the same particles with a diameter of 15 nm did not display toxicity. In addition to gold nanoparticles, other metal-based particles have also demonstrated cytotoxic effects. For instance, several studies have revealed cytotoxic effects of silver nanoparticles. Moreover, iron oxide particles have also been found to exhibit harmful characteristics both in vitro and in vivo, mainly due to the generation of reactive oxygen species (ROS). The hazardous properties of certain nanoparticles do not necessarily hinder them from being used for medical purposes [52]. Although a nanoparticle that simultaneously displays biocompatibility and ability to deliver drugs to a tissue of interest is usually considered optimal, harmful nanoparticles could also be utilized. Namely, the toxic properties of nanoparticles could be directly

harnessed to ablate diseased tissue, thereby eliminating the need for a drug component. Nevertheless, this approach would require selective targeting of nanoparticles, in order to spare healthy tissue from damage. Additionally, the harmful effects of pristine nanoparticles can be reduced using several approaches, such as surface modification. As an illustration, the addition of hydroxyl groups to gadolinium fullerene particles prevented the generation of ROS, thereby reducing toxicity Similarly, a polymer coating on the surface of iron oxide nanoparticles dramatically improved cell viability [53].

4.3-ENGINEERING GUIDANCE FOR NANOMOLECULAR THERAPY

Molecular engineering is an emerging field of study concerned with the design and testing of molecular properties, behavior and interactions in order to assemble better materials, systems, and processes for specific functions. This approach, in which observable properties of a macroscopic system are influenced by direct alteration of a molecular structure, Molecular engineering is highly interdisciplinary by nature, encompassing aspects of chemical engineering, materials science, bioengineering, electrical engineering, physics, mechanical engineering, and chemistry. There is also considerable overlap with nanotechnology, in that both are concerned with the behavior of materials on the scale of nanometers or smaller [54]. Given the highly fundamental nature of molecular interactions, there are a plethora of potential application areas, limited perhaps only by one's imagination and the laws of physics. However, some of the early successes of molecular engineering have come in the fields of immunotherapy, synthetic biology, and printable electronics. Molecular engineering is a dynamic and evolving field with complex target problems; breakthroughs require sophisticated and creative engineers who are conversant across disciplines. A rational engineering methodology that is based on molecular principles is in contrast to the widespread trial-and-error approaches common throughout engineering disciplines. Rather than relying on well-described but poorly understood empirical correlations between the makeup of a system and its properties, a molecular design approach seeks to manipulate system properties directly using an understanding of their chemical and physical origins. This often gives rise to fundamentally new materials and systems, which are required to address outstanding needs in numerous fields, from energy to healthcare to electronics [55]. Additionally, with the increased sophistication of technology, trial-and-error approaches are often costly and difficult, as it may be difficult to account for all relevant dependencies among variables in a complex system. Molecular engineering efforts may include computational tools, experimental methods, or a combination of both Molecular engineers utilize sophisticated tools and instruments to make and analyze the interactions of molecules and the surfaces of materials at the molecular and nanoscale. The complexity of molecules being introduced at the surface is increasing, and the techniques used to analyze surface characteristics at the molecular level are ever changing and improving. Meantime, advancements in high performance computing have greatly expanded the use of computer simulation in the study of molecular scale systems.

4.4-THE INTEREST AND RISK RATIO

Gold nanoparticles have a good safety profile and are often used as a non-toxic control in many studies [56]. Bulk gold is well known to be safe and chemically inert, and gold-based compounds have been used in the clinic as anti-inflammatory agents to treat diseases such as rheumatoid arthritis:

1- furthermore, radioactive gold microparticles have been effectively used in local radioisotope cancer therapy.

2- Nanoscale gold particles show great potential as photothermal therapy agents and as imaging agents in living systems.

3- In most of these imaging and therapeutic applications, the gold particles are ~5 nm or larger. At sizes larger than ~5 nm, the gold is treated as chemically inert, similar to the bulk material.

Like pharmaceutical drugs which have different functional groups within their chemical structure that affect their reactivity, the safety and efficacy of nanoparticles is dependent on the nanoparticle's surface. Surface capping agents such as antifouling molecules, recognition molecules, or steric stabilizers affect the nanoparticle's fate and transport within biological and environmental systems [57].

4- As with any complex system, each nanoparticle should be studied for in vivo use with a wide variety of tests to gain more information because nanoparticle toxicity can vary depending on the size, shape and surface as well as dose.

5-A number of assays can be used to predict nanoparticle safety with the most common measuring the impact of nanoparticle exposure on cells. Some common assays are the Lactate Dehydrogenase (LDH) assay and the Multi-Table Tournament (MTT) assay which is considered the “gold standard” for cytotoxicity.

6- Environmentally, gold nanoparticles have shown to be benign when released into the ecosystem. In exposure studies, gold nanoparticles were found to accumulate in filter feeders, but no harmful effects were observed.

7- Gold nanoparticles have also been shown to be non-toxic to zebrafish (an excellent in vivo model which has been used to assess environmental toxicity due its high degree of homology to the human genome).

CHAPTER 5 CONCLUSION AND REFERENCE

5.1-CONCLUSION

Gold nanoparticles have, in some ways, revolutionized the field of medicine because of its widespread applications in targeted drug delivery, imaging, diagnosis and therapeutics due to their extremely small size, high surface area, stability, non-cytotoxicity and tunable optical, physical and chemical properties [58]. Functionalized gold nanoparticles with various biomolecules such as proteins, DNA, amino acids and carboxylic acids have been used in cancer therapy and provide excellent drug delivery system. Targeted delivery and programmed release of therapeutic drugs to the specific site is achieved by using gold nanoparticles because they can bear high drug load and release it to the specific site through various administration routes and can interact with cancerous cell. Side effects of

conventional drugs have been minimized by conjugation with gold nanoparticles and they increase the quality life of patients [59].

AuNPs have multiple attributes that make them potent tools for the use in bio nanotechnology. The wide range of surface functionality and bioconjugates coupled with the outstanding physical properties of AuNPs make these systems valuable for imaging applications. Moreover, the creation of highly sensitive and selective diagnostic system for target analytes can be achieved by engineering their surface monolayer. AuNP-based delivery vectors have also shown promise in therapeutics with their high surface loading of drug and gene as well as the controllable release of the payloads. Taken together, AuNPs are incredibly versatile materials for next-generation biomedical applications [60].

This review describes recent advances in nanomaterials fabrication that have led to the synthesis of high aspect ratio particles on nanometer length scales. The elongated structure of these materials often results in inherent chemical, electrical, magnetic, and optical anisotropy that can be exploited for interactions with cells and biomolecules in fundamentally new ways. We briefly describe the synthetic procedures that have been developed to fabricate nanorods, nanowires, and nanotubes. We summarize literature reports that describe the use of high aspect ratio nanoparticles for biological sensing, separations, and gene delivery. We emphasize the recent discovery of single nanowire field-effect transistors that may revolutionize biological sensing and yield extremely low detection limits. Separations technology with chemically modifiable nanotube membranes and with magnetic nanowires that can be tailored to selectively interact with molecules of interest is also described. Other areas of biotechnology that have been improved by the integration of high aspect ratio nanoparticles are also described [61].

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