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Nanoparticles based diagnosis and treatment of diseases

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Abstract

Nanoparticles (NP) are the particles having a diameter less than 100 nm, are increasingly utilized in diverse applications, including disease diagnosis and drug carrier systems to cross organ barriers for instance the blood-brain barrier. Due to unique characteristics nanocrystals and other nanoparticles (nanobars, dendrimers, gold colloids, and nanoshells) have been receiving a lot of interest for potential use in Bioengineering, therapeutics, and drug discovery. In this review potential use of these nanoparticles and nanocrystals in a variety of significant areas has been conferred. Particular properties of these nano tools may offer new advancement in various medical applications and can serve efficiently for human beings.

Key words: Nanoparticles, Nanoemulsion, Chemical Nose, Hyperthermia, Near Infrared

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1. Introduction

A particle is defined as a tiny object that acts as a whole unit in respect of its transport and properties. Based on size it is further classified in two categories, fine particles ranges from 100 to 2500 nanometers, on the other hand, ultrafine particles, are ranged from 1 to 100 nanometers. Just like ultrafine particles, size of nanoparticles also ranges from 1 to 100 nanometers. Size-related properties of nanoparticles may or may not differ significantly from those observed in bulk or fine particles [1]. Individual molecules are usually not referred as nanoparticles. In the case of nanoclusters at least one dimension lies between 1 and 10 nanometers. Nanopowders are agglomerates of nanoparticles, nanoclusters or ultrafine particles. Nanometer-sized single-domain ultrafine particles, single crystals are often referred to as nanocrystals. Due to a wide range of potential applications in biomedical electronic and optical fields nanoparticle research is presently an area of strong scientific interest [2].

An important role is played by nanoparticles in a variety of applications. General term nanoparticle is defined as engineered structures having diameters less than 100 nm, are tools and systems prepared by chemical or physical processes or both, incorporated with particular characteristics [3]. Reason for popularity and attraction of nanoparticles for such applications is due to their unique features. One of these features is their high surface to mass ratio, it allows promotion in rate of catalytic reactions, and enhance the adsorption of other compounds allowing them to be carried and transported. A quantum phenomenon is the origin of reactivity of the surface and can make NP unpredictable. Immediately after generation, surface of nanoparticles may be modified, depending on the existence of adsorbing compounds and reactants, which may instantly change with thermodynamic conditions and changing the compounds. Therefore, NP not only has a large surface which is able to adsorb carry and bind drugs, probes and proteins but also has a surface might be more reactive chemically as compared to analogous fine particles [4].

1.1 Types of Nanoparticles

Different types of nanoparticles are as follows (Fig.1).

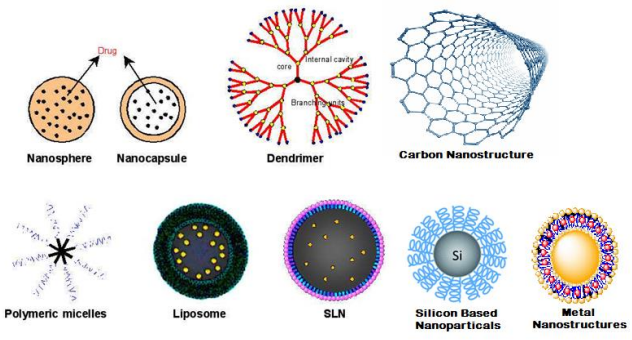

Fig.1 Types of nanoparticles

1.1.1 Liposomes

Concentric bilayer vesicles are called liposomes. In liposomes, a lipid bilayer primarily made up of synthetic or natural phospholipids, entirely encloses an aqueous volume. Liposomes are characterized on the basis of number of layers, size and charge on the surface. It provides numerous advantages with respect to biocompatibility, amphiphilic character and surface modifications making it a suitable aspirant for biotech drug delivery systems.

Since its origin liposomes have been applied efficiently in the field of biochemistry, medicine and biology. Pharmacokinetic profile of loaded drug is altered greatly, preferably in the case of proteins. Its surface modification is very easy by the attachment of polyethylene glycol components. It builds liposome as stealth particle and its circulation is enhanced in this way [5].

1.1.2 Nanosuspension and Nanocrystals

Thousands of molecules combine in crystalline state to form Nanocrystals. Pure drug crystal is enclosed with a thin protective layer of surfactants. Formulation as nanocrystals may resolve the problems such as decreased bioavailability, inappropriate absorption prototype and parenteral dosage preparation complications. Several benefits are linked with it, e.g. for steric and electrostatic surface stabilization, minute amount of surfactant is needed to be immersed in nanocrystals. Moreover, by controlling dissolution adequately slow depot release can be attained using high drug levels. It eradicates toxicity troubles linked with the delivery substances, as no carrier is required because nanoparticle made from pure drug is applied [6].

Technology based on nanocrystals can be exploited for many types of dosages. Oral administration of nanoparticles has the ability to target the gastrointestinal mucosa. For the treatment of infections as fungal mycobacterial, it can target the tissues of the MPS i.e. mononuclear phagocytic system, thus working as opt delivery system for amphotericin B, tacrolimus like drugs [7].

1.1.3 Solid Lipid Nanoparticles

In early 1990s solid lipid nanoparticles were constructed as an alternative to emulsions, polymeric nanoparticles and liposomes, for controlled drug delivery system to work as a colloidal carrier. Main motive for their development is the blend of advantages from different carriers systems. These have been studied for dermal, pulmonal and parenteral path ways [8].

In Solid Lipid Nanoparticles drug is usually incorporated in a solid lipid matrix. Different surfactants having accepted GRAS (Generally Recognized as Safe) status are applied to stabilize the dispersion and avoid aggregation. Cationic lipids for the matrix lipid composition have been regarded as new transfection agents. Same cationic lipids e.g. used as liposomal transfection agents, can be formulated for gene transfer in the form of solid lipid nanoparticles (SLN). Rather than the colloidal structures the composition based on cationic lipids proves to be more efficient for in vitro transfection. Hence, with distinct technological properties the range of highly effective non-viral transfection agents has been widened by using cationic SLN [9].

1.1.4 Polymeric Nanoparticles

Polymeric nanoparticles (PNPs) comprises of biodegradable polymer compared with SLN or nanosuspensions. An essential feature for probable application in drug and gene delivery, tissue engineering and new vaccination strategies is biocompatibility. Most biodegradable polymers comprise of synthetic polyesters like poly-cyanoacrylate or poly (D, L-lactide) and related. To overcome some toxicological problems of synthetic polymers latest improvements include natural polymers like sodium alginate, chitosan and gelatin. With respect to effectiveness and efficiency, polymeric nanoparticles represent an important advancement over intravenous and oral schemes of administration as practiced conventionally [10].

PNPs show many advantages in drug delivery; most important one is the enhancement in the stability of volatile pharmaceutical agents. These particles are conveniently and inexpensively manufactured in huge concentrations by a various techniques. These nanoparticles may also be designed specifically to deliver bigger concentrations of drug(s) to the targeted place [11]. Generally under the term nanoparticle, nanospheres are understood. Nanospheres are considered as uniformly dispersed matrix system. Besides of these spherical vesicular systems nanocapsules are also known, where a drug in a matrix core is surrounded by polymeric membrane. The variety of polymer and the facility to alter drug release from polymeric nanoparticles have made them supreme entrants for contraceptives, cancer therapy, targeted antibiotics and delivery of vaccines. From the perspective of polymer chemistry, in the future there will be a challenging field to produce new polymers matching hydrophilic and lipophilic characteristics for smart formulation of upcoming drugs [12].

1.1.5 Dendrimers

A distinct category of polymers is dendrimers. These are macromolecules having extensive branching. Shape and size of these particles can be controlled accurately. Either convergent or divergent step growth polymerization process is utilized to manufacture

dendrimers from their monomers. Properties of dendrimers that make them attractive drug carrier candidates are their monodispersity of size, well defined structure, stability and surface functionalization capability. Either complexation or encapsulation can be used to incorporate drug molecules into dendrimers. Dendrimers can be applied for both gene and drug delivery. They can also use in anticancer therapy and carriers for penicillin [13].

1.1.6 Silicon based Structures

Photolithography, deposition and etching techniques are used to fabricate silicon based nanoparticles. These techniques are commonly applied in the fabrication of micro-electromechanical systems and semiconductors. Frequently studied silicon based nanoparticles are silica and porous silicon usually used in drug delivery. Nanostructures include platinum-containing nanopores, calcified nanopores, nanoneedles and porous nanoparticles. The diameter and density of the pores in nanoparticles can be maintained precisely to achieve a smooth drug delivery [14]. Fabrication of porous hollow silica nanoparticles is attained in a suspension together with sacrificial nano sized templates e.g. $CaCO_3$. Precursors of silica, for example sodium silicates are introduced into the suspension. Then it is dehydrated and calcinated to generate a nucleus of the template substance, it is covered with porous silica shell. Leaving behind the porous silica shell, template is then dissolute into the wet etch bath. Drug carrier generation involves the blending the PHSNPs and molecules of drug. Then blend is dried to merge the drug particles on the silica nanoparticles porous surface. These porous hollow nanoparticles show a much more gradual release of drug as desired [15].

1.1.7 Carbon Structures

In recent much attention is received by hollow, cage-like, carbon nanotubes and fullerenes structures. Common configurations include C_{60} fullerenes, single-wall and multiwall nanotubes. Properties like geometry, size, and surface modifications have made them attractive as drug carriers. C_{60} fullerenes and SWNTs have width up to 1nm size range, about half the diameter of DNA helix [16].

MWNTs have diameters up to the tens of nanometers depending on the number of walls in the structure. Carbon nanotubes and fullerenes are usually fabricated by chemical vapor deposition (CVD), electric arc discharge (EAD), laser ablation (LA) or through combustion procedures. Surface functionalized CNTs linked to peptides may be utilized for vaccine delivery, internalized within mammalian cells. Water molecules flow through CNTs has been represented by applying molecular dynamics simulations. It provides the potential application of these particles as tiny molecule carriers. Other imitations involve their use as a tool for gene delivery. Fullerenes have also revealed drug targeting potential. Furthermore, experiments have also revealed their antioxidant and antimicrobial characteristics [17].

1.1.8 Metal Structures

Typically metal nanostructures are templates of the thin metal case around a silica nanoparticle core usually. Hollow nanoshells of various metals are being examined for drug delivery functions. Attributed metals include silver, gold, palladium and platinum. When embedded within polymeric transporters, these metal nanostructures can be applied as thermal release triggers, exciting by an alternating magnetic field or irradiating with infrared radiations [18].

2. Diagnosis of diseases

2.1 Diagnosis Based on Florescent Nanoparticles

For the detection of cancer biomarkers in human blood Chinese scientists have applied gold nanoparticles as ultrasensitive fluorescent probes. According to researchers, this method is so sensitive, that it outstrips existing techniques by several orders of magnitude and is also useful in direct sensing of bacterial or viral DNA. For biomedical applications gold nanoparticles are potential probes since their preparation is very easy. Further they are stable towards longer exposure to light, while other probes based on fluorescence such as organic dyes or quantum dots burn out in such conditions [19].

Two main biomarkers in the diagnosis of a variety of cancers, including lung, liver and breast cancer are alpha foetal protein (AFP) and carcinoembryonic antigen (CEA). Jicun Ren and colleagues at Shanghai Jiaotong University in China applied gold nanoparticles to detect these biomarkers. The researchers conjugated gold nanoparticles to antibodies for the measurement of biomarker levels. For example, for measurement of CEA, they prepared two types of nanoparticles, different antibody attached with each. When sample containing CEA was exposed to both of them together, the biomarker formed a dimer by attaching both types at once and total number of nanoparticles in the sample were decreased. When mixture passed under a focused laser beam the reduced number of photon bursts was detected from the gold nanoparticles. Greater the decrease observed indicates that more CEA present [20].

2.2 Diagnosis Based on the Small Organic Molecules

Researchers have established a competitive technique in which colorimetric glucose assay designed by assemblies dextran-functionalized AuNPs and concanavalin A (Con A) is utilized [21]. Con A cross-links dextran-coated nanoparticles due to multivalent bindings (shown in Fig. 2).

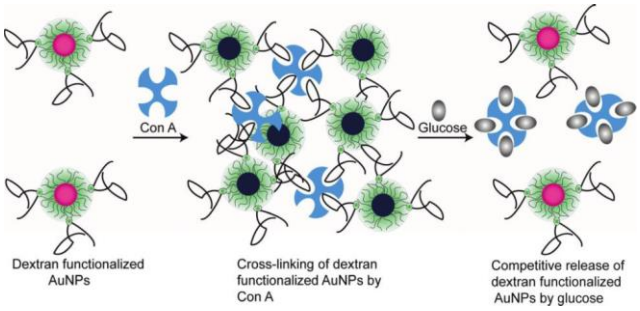

Fig.2 Colorimetric sensing of glucose via liberation of Con A assembled dextran coated gold nanoparticles Blue shift is observed when Con A cross links with dextran coated gold nanoparticles. Glucose is detected when cross links are disassembled due to replacement of nanocomposite from Con A with glucose molecules

If glucose is present in the system it will liberate the dextran-coated AuNPs will bind with Con A, competitively. UV/VIS spectrometry or wavelength-ratiometric resonance light scattering techniques are effective in monitoring these changes. A dynamic sensing range from 1−40 mM of glucose is detectable by this method [22]. This technique is applicable in identification of diabetes due to difference in blood glucose level i.e. 2-40 mM in diabetics and 3-8 mM in healthy people.

For colorimetric sensor for adrenaline, an assembly AuNPs embedded with molecularly imprinted polymers (MIP) has been applied [24]. The shrunken MIP gel shows close proximity of AuNPs if adrenaline is absent in the analyte. A blue shift in the plasmon absorption band due to the swelling of MIP gel indicates the presence of adrenaline. This technique is effective up to a significant range of concentration i.e. 5 μM to 2 mM [25].

2.3 Diagnosis of Diseases Based on the Detection of Oligonucleotides

Genetic mutation detection is one of the most critical parameter for the early diagnosis of many diseases including cancer [26]. It is directing attention toward tests on nucleic acid. Conventional fluorescent and radioactive methods for the detection of oligonucleotides include PCR, RT-PCR, and high density microarrays, Northern and Southern blots [27]. A newer technology emerged for oligonucleotide detection is AuNP-based colorimetric assay [28]. Mirkin demonstrated DNA-meditated AuNP assembly in 1996 [29]. By the production of thiolated DNA strand functionalized AuNPs characteristics of nano-probes were modified with respect to the assay requirements [30]. This disclosure has motivated wide-ranging applications for colorimetric recognition of oligonucleotides in the sample based on oligonucleotide directed AuNP aggregation and made possible the formation of structured assemblies [31]. Method utilizes AuNP probes tailored by ssDNA for detection of oligonucleotides based on colorimetry. In these probes the base sequences is intended complementary to the both ends of subjected oligonucleotides (Fig. 3).

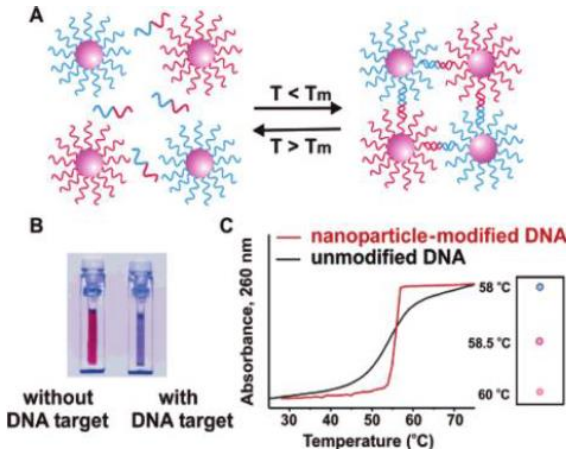

Fig.3 Oligonucleotide AuNPs aggregation in existence of complementary DNA target (A) causing red to blue shift in color of subjected solution (B)

Presence of intended oligonucleotides is detected by the color change due to AuNP aggregation as a result of DNA strand hybridization. Colorimetric detection of minute quantities (subpicomolar) of oligonucleotides is possible due to precise base-coupling of DNA strands and high absorption of AuNPs at specified wavelength [34].

2.4 Diagnosis by Detection of Proteins

Numerous diseases such as cancer are often linked with the irregular protein concentrations or occurrence of certain biomarker proteins. AuNPs are also effective for colorimetric recognition of proteins. Huge variety of carbohydrate functionalized AuNPs is being discovered for the colorimetric recognition of carbohydrate binding proteins. Example is the identification of Recinus communis agglutinin (RCA120) in which the aggregation of AuNPs functionalized by β-D-lactopyranoside are utilized [35].

This technique is useful in quantitative analysis of lectin because the concentration of protein is proportional to the extent of colloidal aggregation. With this system greater sensitivity of detection (1 ppm), has been achieved. Afterwards detection range of lectin was controlled by varying the moiety of Lac on the surface of particles [36].

AuNPs can cross-link Dithiols, this ability is utilized for colorimetric detection of proteases by producing assemblies of AuNP, with the help of dithiol functionalized peptides. Thrombin and lethal factor specific substrates, C-terminal and N-terminal cysteinyl derivatives of peptide have been designed [37]. At first Peptides were processed with the analytes for their assay. Later, the solution was incorporated with citrate-stabilized AuNPs (12 nm). Intact peptides in the absence of subjected proteases analytes cause aggregation of nanoparticle, whereas AuNPs are not bridged

by protease-cleaved peptides. Afterward, this approach was additionally simplified by utilizing AuNPs loaded with Fmoc-protected peptides bearing an anchor cysteine [33]. Peptide ligands are cleaved due to the occurrence of thermolysin in the sample, blue-to-red color variation with increasing sensitivity occurs due to the dispersion of AuNPs in the solution (Fig. 4).

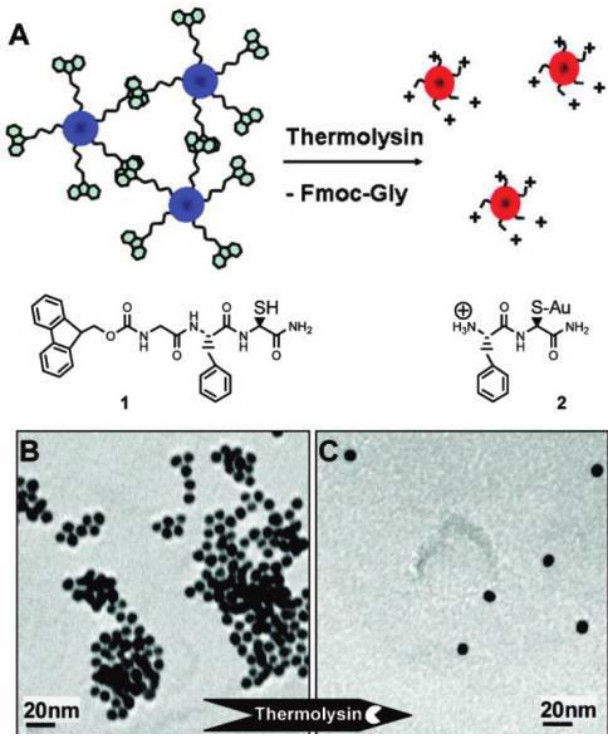

Fig.4 Schematic images of thermolysin activated scattering of AuNPs (A) Images recorded by TEM before (B) Images recorded by TEM after (C) adding thermolysin and formation of image II

By using DNA-mediated AuNP assemblies based on the enzymatic cleavage of DNA molecules, a real-time screening technique based on colorimetric detection for activity of endonuclease was developed. Exploiting the colorimetric inhibition assay, immediate determination of the effectiveness of endonuclease suppressors was studied. In the same way, enzyme-activated assembly/disassembly of AuNP based technique was utilized for the detection of β-lactamase, kinase, amino-peptidase and phosphatases together with the determination of their functioning. By applying AuNP probes, researchers have revealed a colorimetric sensor for the identification of conformational changes of protein [38].

2.5 Chemical Nose Approach Diagnosis Based on the Detection of Proteins, Pathogens and Cancerous Cells

Recently a protein sensor has been developed using chemical nose approach [39]. Six cationic and an anionic poly (p-phenylene ethynylene) polymer AuNPs were used to generate prototype sensor array. Electrostatic binding of AuNPs with polymer causes fluorescence inhibition of the polymeric material. This fluorescence quenching is due to energy transmission as shown in Fig.5a. Fluorescence of the polymer is recovered via competitive binding of protein analytes by disrupting the polymer and AuNPs complexes, resulting in restoration of fluorescence. A pattern of fluorescence like fingerprints is obtained for all the targeted proteins (Fig.5b) due to the differential interactions of nanoparticles. Proteins are distinguished using linear discriminate analysis (LDA). A green fluorescent protein assembly of AuNP was applied on undiluted human serum to identify proteins at 500 nM (Fig.5c) by employing the same principle [40].

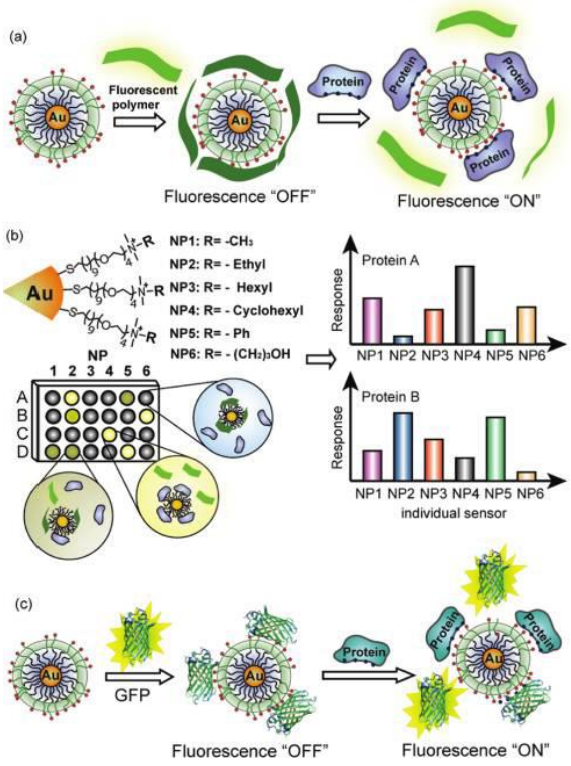

Fig.5 Schematic diagram showing "chemical nose" approach for gold nanoparticle-fluorescent polymer composites (a) Fluorescence of quenched polymer is restored due to competitive binding between protein and polymer resulting due to liberation of polymer (b) Finger print responses due to the assembly for individual proteins with an array of nanoparticles (c) competitive assembling between nanoparticle GFP complexes and protein leading to fluorescence restoration

Similar AuNP-conjugated polymer approach was applied to sense bacteria. For the generation of sensor, one anionic PPE polymer and three cationic AuNPs were utilized. Existence of bacteria in analyte interrupts the previously fluorescence inhibited assemblies and fluorescence of polymer is restored. Due to the discrete response prototypes, the array of sensors was able to sense 12 bacteria including both Gram-negative and Gram-positive species, in addition three dissimilar strains of Escherichia coli were also identified (Fig.6) [41].

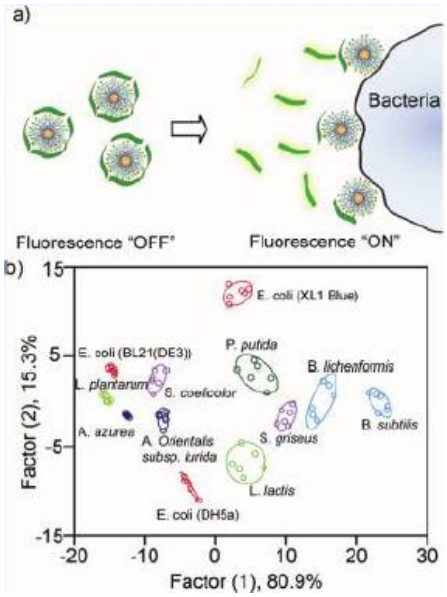

Fig.6 Fluorescence based identification of microbes (a) Polymer show fluorescence when liberated from nanoparticles surface due to replacement with bacteria and (b) Illustration of bacterial presence utilizing first two factors showing fluorescence response prototypes in the form of canonical score plot obtained with NP-PPE composites

For quick and efficient discrimination between cancerous, metastatic and normal cells AuNP-assembled polymer structures were utilized [43]. LDA fluorescence responses were proficient to distinguish (1) different cell types (2) isogenic, cancerous, normal and metastatic epithelial cells of murine and (3) normal, metastatic and cancerous breast cells of humans (Fig. 7).

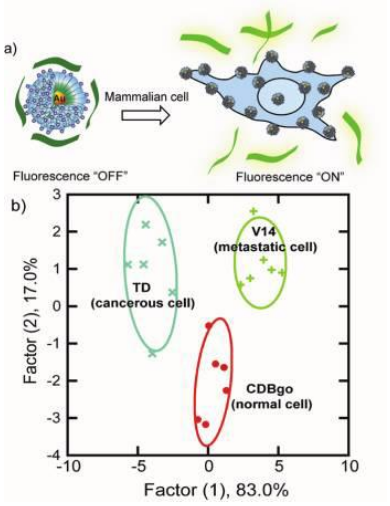

Fig.7 (a) Illustration of restoration of quenched fluorescence of polymer when nanoparticle protein composite was exposed to mammalian cells (b) Graphical description with score plot for different types of mammalian cells based on fluorescence of gold nanoparticle assembly with fluorescent polymer

2.6 Diagnosis by Nanoparticle Modified Electrodes

Nanocomposite gels (AuNPs/chitosan) has been utilized for electrochemical based detection of proliferation, apoptosis, and adhesion of cells/tissues on the surface of electrodes. Cells fixed on glassy carbon electrode have displayed voltammetric response (irreversible) and increased resistance for electron transfer with 8.7×102 cells/mL LOD. Effectiveness of Methotrexate (Antitumor drug) was observed through by using this approach from K562 leukemia cells fixed on the surface of AuNPs tailored with porous cellulose membrane [45]. Similarly, adriamycin anticancer drug was tested for cytotoxic effect by immobilizing pancreatic cancerous cells on a AuNPs and carbon paste merged electrode. Recently, for the targeted recognition of cancerous cells an electro catalytic platform/sensor has developed. In this system, species on the surface of cells are identified using AuNPs conjugated with antibodies (Fig.8), catalytic hydrogen reduction is utilized for cell detection [46].

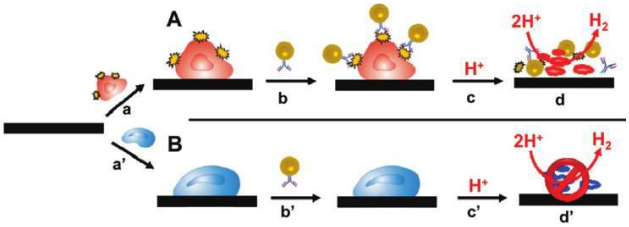

Fig.8 Schematic diagram showing detection of (A) Cancerous tissues by electrochemical response because of hydrogen reduction at immobilized gold nanoparticles on surface of electrode (B) Normal cells showing no electrochemical response due to absence of hydrogen reduction activity

3. Treatment of diseases using nanoparticles

3.1 Targeted Drug delivery

Main concern in drug delivery is the precise release of the drug to the targeted tissue or cells. Drug targeting system must be capable of controlling the outcome of a drug penetrating the body. At the start of the 20th century Paul Ehrlich suggested the design so called “magic bullet”, in which the drug is accurately targeted to the exact place of action. Today’s delivery technologies are far away from this design. To achieve this goal nanotechnology offers here a new way in delivering the drug at the right time to the right place [12]. By bring a primary changes in manufacturing in the upcoming years nanotechnology is considered to have a massive influence on Life Sciences, including diagnostics, nutraceuticals, drug delivery or biomaterials production [47]. A comparison of targeted and normal drug delivery is shown in Fig.9.

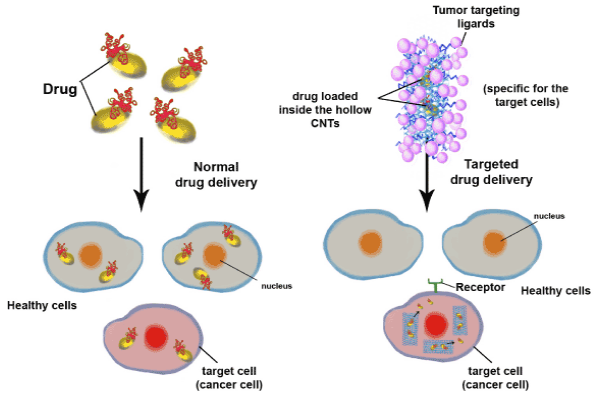

Fig.9 Comparison of targeted and normal drug deliveries

Targeting is the process in which drug-loaded system is directed precisely to the diseased cells. Mainly two major mechanisms can be addressed for drug release to targeted cells: (i) passive targeting and (ii) active targeting. The preferential absorption of chemotherapeutic agents in tumor tissue due to increased vascular permeability in comparison with healthy tissues is the example of passive targeting [48]. An approach of active targeting includes the attachment of ligands on the surface of drug carriers that are specifically identified by receptors on the exterior faces of the targeted cells. In view of the fact the higher selectivity can be found in ligand–receptor interactions, this could permit a more specific targeting of concerned site [49]. However, multiple obstacles are encountered in passive targeting with nanoparticles, on the way to their target; including mucosal barriers, uncontrolled release of drug due to nonspecific uptake and delivery. Consequently, two central features of nanoparticle drug delivery must be: [48]

a) Targeting the damaged/unhealthy tissue selectively with nanoparticles (suitable sized selectively bound or surface functionalized particles provide improved drug delivery and reduced toxicity)
b) Release of the drug at specific time (to prevent nonspecific toxicity the drug must not diffuse out of the particle while it is still in the circulatory system, and must remain encapsulated until the particle binds to the target).

The first issue is resolved by surface functionalizing the nanoparticles with recognition elements towards receptors present on the diseased tissue. For this purpose conjugated antibodies or short chain variable fragments can help in selective binding to the surface of specific cell's, and with suitably adjusted binding affinities their endocytosis will be enhanced [50].

Multilayered nanoparticles can be designed to tackle the second issue, where each layer will hold one drug from the brew, and will be sequentially released in agreement with the appropriate timing of combination therapy. Presently researches have shown that combination therapy is more efficient than conventional therapies [51].

Nanoparticles can help in drug bioavailability by improving the uptake of weakly soluble drugs by a specific tissue. Numerous anti-cancer drugs have been effectively designed using nanomaterials; examples include doxorubicin 5-fluorouracil, paclitaxel, and dexamethasone. As a glucocorticoid specific for intracellular functioning, Polylactic acid (PLA) and Polylactic/glycolic acid (PLGA) based nanomaterials were prepared for the encapsulation of dexamethasone. Dexamethasone is a chemotherapeutic agent that has anti-inflammatory anti-proliferative effects. It attaches to the cytoplasmic receptors followed by the transport of drug-receptor complex to the core of cell into the nucleus. It results in the expression of specific genes that are responsible for cell proliferations [52].

NP-based drug delivery is practicable both in hydrophilic and hydrophobic states administrated through different routes, including oral, inhalation and vascular. For Targeted drug delivery, numerous approaches are presently being investigated for enhanced site-specific delivery using dendrimers, liposomes, polymeric micelles, ceramic NPs, proteins and iron oxide [3].

3.1.1 Nanoparticles target ovarian cancer

According to a group of researchers from MIT and the Lankenau Institute ovarian tumor growth can be effectively suppressed using nano particles bearing a killer gene. This discovery could open new horizons for the remedies of ovarian cancers, which is causing enormous deaths around the world [53].

Ovarian cancer is one of the most deadly forms of the disease because it is usually diagnosed at a relatively late stage. In nanoparticles based treatment, a gene responsible for the generation of diphtheria toxin, having the ability to kill cells by interrupting their capability to produce proteins, can be selectively transferred to cancerous cells. Normally this toxin is produced by the bacterium Corynebacterium diphtheriae. No better conventional therapies are available for treatment of tumors at later stages, mostly cancer regenerates even after therapy. Scientists have found that nanotechnology based gene therapy treatment in most of the cases is more effective, than the usual chemotherapy combination of paclitaxel and cisplatin [54].

Moreover, there is no risk of toxic effects like chemotherapy because the gene is specific to perform it functions in ovarian cells only and dormant in all other cells. For further surety the nanoparticles are directly injected to the peritoneal cavity, which encloses abdominal organs such as the liver, spleen, stomach, uterus and ovaries. Ovarian cancer firstly spread throughout the peritoneal cavity and

direct injection into the peritoneal space exactly targets the therapy to the ovaries and close by tissues where tumors may have extend. Positively charged nanoparticles have been made using biodegradable polymers poly(beta-amino esters). Spontaneous assembly of polymers and DNA takes place to construct nanoparticles. When inserted into or close to the cancerous tissues polymer-DNA nanoparticle can deliver functional DNA [55].

3.2 Stem cell therapy

In a study it has been reported that nanoparticles were efficiently utilized by chemical engineers for the improvement of the stem cells ability to stimulate reconstruction of injured vascular tissue and decrease susceptibility of muscles towards degeneration. Therefore, cells can be recurred by these reinforcing genes, having the capability to endorse growth in the targeted tissues. Normally, these remedial genes are transported to stem cells through viral vectors [56].

Research's has shown that nanoparticles could be twice as probably to stick to the interface of two immiscible liquids, than formerly thought. A range of new possibilities has been opened in this way for the uses of nanoparticles in living cells, high-tech foams, polymer composites, paints and gels [57].

3.3 Anthrax Vaccine uses nanoparticles to produce immunity

Recently in tests it has found that nano-vaccine against anthrax is more useful and easier to govern than the usual vaccine. Researchers were able to activate a strong immune response by treating the nose tissues with a suspension of water, alcohol, soybean oil and surfactant emulsified to generate droplets of just 200 to 300 nanometers in size, so called "nanoemulsion". It would acquire about 265 droplets aligned side by side equivalent the thickness of a human hair. The particles of oil are sufficiently small to transmit a key anthrax protein inside the nasal membranes, allowing initiation of protective immune response by the reaction of protein with immune-system cells [12]. When it encounters the microbes, immune system is prepared to promptly fight off infection. It not only eliminates the requirement for needles, another advantage of nanoemulsion anthrax vaccine is that these are easy to store and applicable where refrigeration is unavailable. Dealing with any future attack in which a terrorist might spread anthrax microbes, an effective and easy to manage vaccine would be a valuable tool for health authorities. The researchers say if it proves effective in humans, a nasal nanoemulsion based anthrax vaccine along with antibiotics could be given easily to people even after they are exposed to anthrax attack. In some diseases, vaccines given after exposure are used to enhance the pace of the immune response [9].

3.4 Hyperthermia/drug delivery

A recent nanotechnology application of hyperthermia is to inject magnetic NPs to desired tissues and heat them inside the cancerous cells by applying magnetic field. Major challenge in hyperthermia is the accurate transportation of a nanoparticles thermo seeds to the target tissue with minimal side effects. Polymer coated magnetic nanoparticles specifically functionalized is probable solution for targeted hyperthermia [58].

Many obstacles can be overcome in magnetic hyperthermia by using Polymer-coated magnetic NPs such as reticulo-endothelial permeability, heat damage to normal tissues and the level of toxicity. Composite MNPs loaded with drug propose a scheme of achieving various processes simultaneously e.g. controlled drug release, drug targeting and hyperthermia of tumor cells. Several limitations of traditional cancer therapy are thus overcome. Controlled release of drug can maintained if the NPs polymer shell is receptive to stimuli such as temperature and pH [59].

For treating malignant tumors hyperthermia method is recognized as a valuable therapeutic tool, leading to possibly kill the tumor cells not by drugs instead using nanoscale heaters, these small nanoheaters necessarily roast tumor cells to fatality [60]. Three general subclasses magnetically mediated hyperthermia (MMH) has evolved: direct injection hyperthermia (DIH), arterial embolization hyperthermia (AEH) and intracellular hyperthermia (IH). In Direct injection hyperthermia, the particles are inoculated directly into the tumor cells while in arterial embolization hyperthermia; magnetic particles are delivered to the tumor cell through arterial pathway. In intracellular hyperthermia approach, magnetic nanoparticles are tailored to assist the cellular uptake by the cancerous cells. Example includes magnetite NPs conjugated with antibody-liposomes (immuno-liposomes). These can proficiently deliver magnetic nanoparticles to targeted cells due to specificity of antibodies for tumoral antigens at their surface [61].

Diseased cells preferably cancerous cells are more susceptible to increase in temperature normal cells; consequently, intracellular hyperthermia techniques are developed and suggested utilizing magnetic NPs. In these methods nanoparticles are accumulated at the cancerous parts of body and heated remotely applying magnetic field to 42°C to 45°C required hyperthermic temperatures [62]. Magnetic NPs tend to aggregate in the cancerous cells due to unorganized vasculature of the tissues, thus successfully heating the malignant cells and surrounding healthy tissue remained intact [63]. Additionally, if they are focused by a changing magnetic field, production of heat takes place due to hysteresis loss of magnetic field [64]. When temperature

gets higher than 43°C cancer cells are destroyed by apoptosis while normal cells can still stay alive at the same temperature [65]. Smart-polymers are also receiving much attention because they able to respond any change in surrounding environment, for instance variation in pH and temperature. At temperature value which is known as lower critical solution temperature (LCST) thermally responsive polymers experiences a coil-globule transition in aqueous solution [66]. Most studied polymer exhibiting LCST around 32°C the thermo-responsive material is poly (N-isopropylacryl amide) (PNIPAM) [67]. Phase transition effect is illustrated in Fig.10.

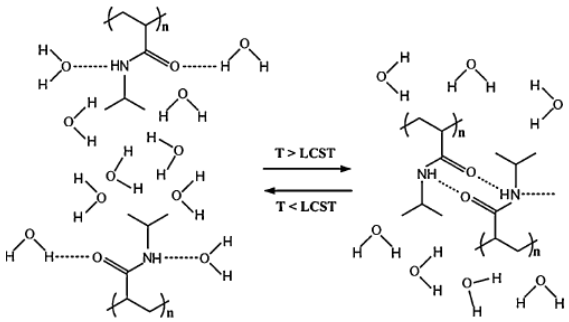

Fig.10 Temperature variation effect on phase change of PNIPAM

It can be observed that the basis of the "smart" behavior is due to the entropic gain as water molecules linked with the amide groups are liberated when the temperature is raised above the critical point as shown in Fig.11 [67].

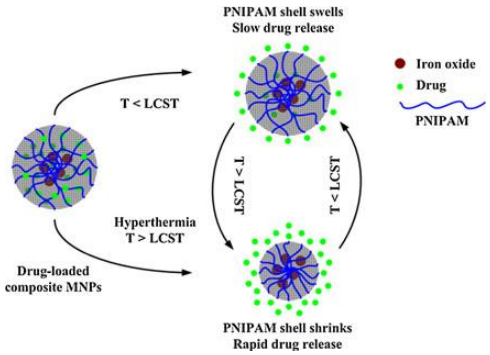

Fig.11 Representation of drug discharge lowers and higher LCST of polymer from PNIPAM coated magnetic nanoparticle

3.5 NIR-based photothermal therapy

A great attention has been paid to noninvasive photothermal therapy recently for the selective healing of tumors. The combination of magnetic resonance imaging (MRI) diagnosis, targeted drug delivery and NIR photothermal ablation would deeply enhance the treatment efficiency and concurrently reduces the damage to healthy tissues and cells. This therapeutic technique uses the large absorption cross section of nanomaterials in the near infrared (NIR) region as basic principle [68]. Due to weak absorption by healthy tissues, NIR radiations are capable of penetrating the skin without causing significant harm to normal tissues, consequently, can be utilized to selectively kill cancer cells by the antibody-conjugated nanomaterials, including gold or gold-nanoshell magnetic Nanoparticles [69].

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