Review Article

Advanced Nanomedicine: Present Contributions and Future Expectations

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ABSTRACT

The present review highlights the development and relevance of various advanced nanocarrier systems in the field of nanomedicine for delivering drugs, contrast agents and antibodies to different sites in the body. Wherever appropriate, emphasis on targeting to tumor site is discussed to support the concepts. Several multifunctional nanoparticles developed recently and possessing unique characteristics are also discussed. The scientific contributions, technological gaps and promising opportunities presented by advanced and sophisticated but hypothetical nanodevices of future, known as 'medical nanorobots' are also described that are designed for superior targeting efficiency, controlled drug release, optimum dosing and wide range of other functional capabilities.

Keywords: Tumor targeting; Biodegradable polymers; Drug delivery; Nanomedicine; Nanoparticles; Nanorobotics.

INTRODUCTION

The Royal Society and the Royal Academy of Engineering, UK, have defined Nanoscience as "the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale"[1]. Nanotechnology, as defined by NASA website is, "the creation of functional materials, devices and systems through control of matter on the nanometer scale (1–100 nm) and exploitation of novel phenomena and properties (physical, chemical, and biological) at that length scale". Application of Nanoscience and Nanotechnology for prevention, diagnosis and treatment of diseases is known as Nanomedicine; and when the disease to be treated is cancer, the term "Nano-oncology" presents a clearer description of specific and important role it plays in the broader field of Nanomedicine. Various innovations and advances in the field of nanomedicine have improved delivery of the drugs into the body. Several

researchers have designed nanodevices that would overcome the limitations of current drug targeting systems and, perhaps, in future, would perform in accordance to the hypothetical concept of 'Magic Bullet' in future[2]. These future nanodevices or nanomachines, known as "Nanorobots", would be able to repair internal tissue damages, produce specific antibodies, carry out genetic manipulations in the cells, repair their own malfunctions and to the extreme, would replicate their own progeny to overcome and deal with a possible viral attack[3].

Extensive literature is available on the theoretical and practical aspects of nanomedicine as applied to drug delivery systems or diagnostic aids. Moghimi *et al.*, reviewed a wide range of such engineered, nanoscale materials and devices *viz.*, nanoparticles, functionalized carbon nanotubes as biological mimetics, nanomachines such as octahedron and stick cubes, nanofibers, polymeric nanoconstructs, (*e.g.*, nanoporous membranes), nanoscale microfabrication-based devices, sensors, laboratory diagnostics and nanoscale particulate technologies capable of targeting different cells and extracellular elements in the body to deliver drugs, genetic materials, and diagnostic agents specifically to these locations[4]. Engineered nanoparticles are nanosized particle collection, suspended in a gas (as an nanoaerosol), a liquid (as a colloid or nano-hydrosol) or embedded in a matrix (as a nanocomposite) with specific size, shape, chemistry and surface properties [5]. Selection of excipients to prepare nanoparticles depends upon their biocompatibility with the actives, their degradation behavior, choice of route of administration, desired release profile of the drug, and the type of biomedical application [6-8]. Colloidal drug carriers can provide site specific or targeted drug delivery combined with optimal drug release profile[9]. Numerous nanoparticle-based drug targeting systems especially those used for parenteral administration are expected to be biodegradable, easy and reasonably cheap to prepare; to have a small particle size; to possess a high loading capacity; to demonstrate prolonged circulation; and, ideally, to accumulate at the specific target sites in the body[10].

NANOSYSTEMS FOR DRUG TARGETING

Nanosystems for drug targeting may be divided into different categories depending on their constitution, targeting mechanism or functions. Some of these selective, viable, novel and promising systems are enumerated and discussed here:

(1) Lipid based nanosystems
(2) Stealth nanoparticles
(3) Multifunctional nanoparticles
  (3.1) Fluorescent Nanoparticle
  (3.2) Gold Nanoparticles
  (3.3) Quantum Dots (QDs)
  (3.4) PEBBLEs
  (3.5) MagMOON's
  (3.6) Magnetic Nanoparticles (SPION's)
  (3.7) Functionalized Carbon Nanotubes (f-CNTs)
  (3.8) Core shell nanoparticles
(4) Biomimetic nanoparticles
  (4.1) Gold Monolayer Protected Clusters (MPCs)
  (4.2) Self-Assembling Nanoparticles
    (4.2.1)Self-Assembling Peptidomimetic Nanoparticles
    (4.2.2) Self-Assembling Peptide Nanodiscs
    (4.2.3) Self-Assembling Liquid Crystal Nanoparticles (LCNP)
  (4.3) Molecular Trojan Horses (MTHs)
  (4.4) Virus as drug delivery system

(5) Medical nanorobotics
  (5.1) Nano Electro Mechanical Systems (NEMS)
  (5.2) Nanorobots
  (5.3) Rotaxanes and Catenanes (Molecular shuttle)

(1) Lipid based nanosystems

The lipid based vesicular systems have been used in last decade. There are different delivery systems related to lipid which has already been developed such as: Liposomes, Transferosomes, Ethosomes, Lipospheres, Niosomes, Cubosomes, Virosomes, Iscoms, Nano-emulsions, Cochleates and Phytosomes. Out of all these approaches liposomes have been proved to target the tumor site more efficiently. Liposomal system conjugated with ligands is found to deliver radionuclides, genes and chemotherapeutic agents to tumor site[11,12]. Northfelt *et* al., targeted liposomes bounded with covalent peptide PEG-PE anchors for tumor targeting by using doxorubicin as an anticancer drug[13]. Braqaqni *et al*., developed a topical anticancer formulation of celecoxib, by using carriers such as liposomes, transferosomes and ethosomes. It was reported that the vesicular systems were efficient in enhancing the permeation through skin[14]. The lipid based system has extensively been studied and each system possesses its own advantages and some disadvantages. Further researches lead to development of other drug delivery systems, which can serve as the ideal drug targeting system in future.

(2) Stealth nanoparticles

Targeting of nanoparticles to a specific site is primarily dependent upon their ability to prevent uptake by organs of reticulo-endothelial system (RES) *viz*., liver, lungs, spleen and to a lesser extent kidney[9]. It is well known that nanoparticles with hydrophobic and negative surfaces promote protein adsorption and activate complement system, respectively, leading to opsonization and phagocytosis, whereas, particles with hydrophilic surfaces remain in the blood circulation for longer time[4,15]. Physical adsorption or chemical grafting of PEG or its functionalized derivatives carries out surface modification of nanoparticles and is known as Pegylation[16]. The conformation and thickness of PEG layer on the surface of nanoparticles is a critical parameter for preventing the particle uptake by RES. Depending on their surface density, PEG blocks have brush like (elongated coil, high density) or mushroom-like (random coil, low density) conformations[17]. PEG surfaces with brush like configurations reduce phagocytosis as well as complement activation, whereas PEG surfaces with mushroom-like configurations are potent complement activators and favour phagocytosis. PEG with molecular weights of 5000 Da and above display maximum anti-opsonic effect. Pegylated polymeric nanoparticles which are formed by using PEG or its derivatives in combination with a biocompatible polymer such as Poly lactic acid (PLA), Poly lactide-co-glycolide (PLGA), poly butyl cyano acrylate (PBCA) and poly hexadecyl cyano acrylate has also been reported to provide targeted drug delivery. Gref *et al.*, reported nanoparticles prepared by using methoxyPEG_{2000}-PLA_{30000} moieties with hydrophobic PLA core surrounded by a hydrophilic PEG layer (18). Pegylated PLA nanoparticles have a prolonged plasma half-life (6 h) in comparison with PLA nanoparticles coated with albumin or with poloxamer F68 (2–3 min). It was observed that in comparison with non-pegylated PLA nanoparticles, pegylated nanoparticle surfaces have lower negative ς potential values due to the surface shielding by the PEG corona.

Gulyaev *et al.*, used PBCA nanoparticles to target Doxorubicin, an anti-cancer drug across BBB[19]. Lu *et al.*, developed cationic, albumin-conjugated

pegylated nanoparticles, as novel drug carrier for brain delivery[20,21]. BSA conjugated with pegylated nanoparticles (BSA-NP) containing 6-coumarin as fluorescent nanoprobes were compared qualitatively and quantitatively with cationic BSA-NP (CBSA-NP). The results showed that rat brain capillary endothelial cells (BCECs) took in much more CBSA-NP than BSA-NP and presented with lower toxicity [22]. Similarly, higher concentration of pegylated nanoparticles was observed than non-pegylated nanoparticles. An anti-cancer drug aclarubicin (ACL) was also incorporated into cationic albumin-conjugated pegylated nanoparticles (CBSA-NP-ACL), to determine their therapeutic potential in rats with intracranially implanted C6 glioma cells[23]. CBSA-NP's were shown to accumulate much more in tumor mass than un-conjugated BSA –NP's. In further researches Pegylated Immunonanoparticles, nanoparticles conjugated with target cell surface antigen specific antibodies and ligand molecules that form ligand-receptor bonds and polymeric chains in order to prevent recognition by RES were developed. Pegylated nanoparticles when attached with targeting ligands such as endogenous peptides or peptidomimetic MAbs, may allow *in-vivo* targeting to specific tissue. Olivier *et al.*, reported development of such Pegylated Immunonanoparticles (PIN) for brain targeting[24]. The targeting concept is similarly applicable to other nano-sized drug delivery systems such as pegylated immunoliposomes and pegylated immunomicelles[25]. These and many other studies have reported successful application of pegylated nanoparticles for brain targeting of different drugs including anti-cancer agents as well as therapeutic peptides and proteins.

(3) Multifunctional nanoparticles

(3.1) Fluorescent Nanoparticles

Fluorophores are glowing molecules that mark the tumor boundaries for removal by surgery. Thus, to render this technique feasible, Veiseh *et al.,* developed multifunctional fluorescent nanoparticles[26]. An MRI contrasting agent iron oxide was used as a core of around 10 nm diameter, which was then coated with poly ethylene glycol (PEG) and modified with a fluorescent molecule called Cy5.5. This fluorescent molecule gives off light at near infrared wavelengths that in turn, is capable to penetrate through several centimetres of brain tissue. This technology was used for glioblastoma tumors as they emerge rapidly and spread throughout brain[27]. Surgery is a difficult choice as these tumor cells are visually similar to normal brain tissue. This leads to either removal of normal brain cell during surgery or some of them are left behind which ultimately proliferate to form new tumors. Thus, "tailor made fluorescent nanoparticles" were used for such type of tumors[28]. Instead of using the constituents mentioned above, a different contrasting agent having selectivity for glioma tumors was used *i.e.*, chlorotoxin, a peptide derived from the venom of the giant Israeli scorpion was used as the contrasting agent due to its selective binding with tumor surface marker found in majority of glioma tumors. The final nanoparticle size (15 nm) and its composition gave it the best chance for crossing the BBB, and reaching its target. The cell culture studies clearly demonstrated uptake by glioma cells, but not healthy brain cells, and the nanoprobes were readily detectable by both MRI and near-infrared fluorescence. Near-infrared emitting nanoparticles could be a valuable tool for outlining the margins of a tumor, and helping the surgeon to avoid cutting into healthy tissue[29].

The preliminary phytochemical screening was performed for the ethyl acetate fraction of EESS.[18-20]

(3.2) Gold Nanoparticles

Gold nanoparticles are multifunctional, mono-dispersible particles formed of gold with size range between 1.5 to 10 nm[30,31]. The small size of the gold nanoparticles increases the total surface area and thus, improves drug loading efficiency and ligand conjugation. Conjugation of gold particles with target specific ligands such as transferrin, render them targetable to specific cancer sites by endocytosis or transcytosis mechanism[32]. The multifunctionality of gold nanoparticles is due to their ability to carry out two functions at a given time, *i.e.*, these can be simultaneously used as imaging agent and drug delivery systems at the target site. Gold nanoparticles have low detection levels and high optical sensitivity as a result these display colour change on aggregation due to light scattering and absorption. However, their binding affinity to nonspecific ligands in presence of high concentration of ionic species in media leads to aggregation, which restricts their use in biological assays as ,it may result in false positive effect. This effect may be reduced by using graphene like carbon nanoshells enclosing gold particles that are stable due to non-reactivity of graphene with metallic core.

Carbon shell gold nanoparticles are manufactured by chemical vapour deposition synthesis (CVD) method, which yield nanoparticles with smaller size (1.2 – 2.8 nm) and better stability[33]. The nanoparticle size may also be increased linearly, if need arise, by increasing the thickness of graphene layers. These nanoparticles prepared by CVD method yield sufficient quantities, low non-specific binding and selective surface functionalization, thus, justifying their suitability for bioanalytical applications. Gold nanoparticles offer various advantages in cancer detection as these are simple, inexpensive, less toxic, sensitive and specific for cancer cells and require simple, faster analytical techniques and instruments. Patra *et. al.*, demonstrated reduced *in-vitro* and *in-vivo* toxicity of anti-cancer drug Gemcitabine by using a combination of gold nanoparticles and Cetuximab as targeting agents[34].

(3.3) Quantum Dots

Quantum dots (QD's) are fluorescent nanocrystals with size smaller than Bohr's radius[35] or small enough to exhibit quantum confinement effect that result in their optical and electronic properties. QD's are formed of a heavy metal core formed of material such as cadmium-selenium or cadmium-telluride, followed by an intermediate, unreactive zinc sulphide shell and an outer coating of selective bioactive moiety incorporated for a specific application[36]. QD's are stable against photobleaching, display high luminescence and different colours at different particle size at a single excitation wavelength[37]. This property is useful in tumor detection and its removal from the body. QD's, when attached to tumor cells and exposed to ultraviolet rays, emit a particular coloured iridescence different from the colour of surrounding normal tissue. Their broad absorption and narrow emission spectra result in high signal-to-noise ratio with improved signal detection sensitivity. Biocompatible and biodegradable QDs have been applied for labelling cells and tissues for long-term cell trafficking, multicolour cell imaging, fluorescence energy transfer–based sensing and detection of DNA sequencing and quantification as well as RNA and protein quantification. Serum labelled with QD's has been used to carry out *in-vivo* microangiography of mouse brain. Commercial brands such as Qdot® nanocrystals of Invitrogen are available for multicolour flow cytometry experiments[38]. QD's attached with suitable bioactive moieties could also be useful in the study of reactive gliosis that occurs during injury or neurodegeneration.

(3.4) PEBBLEs

PEBBLEs or Photonic Explorers for Bioanalysis with Biologically Localized Embedding are biocompatible, highly sensitive and powerful optical nanosensors with size range of 20 nm to 200 nm, fabricated from matrices of polyacrylamide hydrogel, sol-gel silica or crosslinked decyl methacrylate, for biomedical applications. These nanosensors were developed specifically for minimally invasive real time analysis of drugs, toxins, and environmental effects on cell function in viable single cell with high spatial resolution and without any interference from cellular proteins[39]. Various advantages of nano-scale sensors *viz.*, small sample volume, high sensitivity, minimum invasiveness, better spatial resolution, improved dissipation of heat, low toxicity and lower costs. PEBBLEs are functionally flexible, as wide array of drugs and contrasting agents can be attached to their surface and delivered at a specific site in the body including brain for treatment or diagnosis of cancers. PEBBLEs can be highly localized to the cancer targets sparing any damage to the surrounding healthy tissue. PEBBLES carry a contrast agent gadolinium, a photo-catalyst for MRI. Gadolinium can destroy tumor cells when stimulated by light sourced from a fine optical fibber probe inserted into the body. Another advantage of using PEBBLEs is to prevent the development of multi drug resistance (MDR) in the cancer cells by acting on the outside of the cell, and delivering the toxic payload of oxygen which acts quickly, without giving the cancer much chance to survive and develop resistance[37]. Such nano-scale devices with multifunctional properties indicate towards their promise and potential for the development of ideal and intelligent biomedical systems that would overcome the limitations of controlled and targeted drug delivery systems. **Figure 1** depicts one such nanoplatform reported by Kopelman *et al*., for brain cancer detection, treatment, and monitoring in rats utilizing phototodynamic therapy (PDT) and magnetic resonance imaging (MRI)[40].

(3.5) MagMOONs

The fluorescent nanoparticles and PEBBLES can be further modified to form MagMOONs (Magnetically-modulated optical nanoprobes and systems) that enhances the detection sensitivity of intracellular chemical imaging. MagMOONs are nanoparticles that emit different fluxes of light in different orientation, rotate in changing magnetic fields and blink as they rotate[22,41-43]. This blinking property of MagMOONs substracts background, increasing the signal to background ratio and thus, enhance the chemical imaging detection limits of ions, dissolved gases and small molecules. MagMOONs can also be used in a wide range of highly sensitive immunoassays, micro-rheological studies in biological systems and physicochemical changes in cells or tissue[44].

(3.6) Functionalized Carbon Nanotubes (f-CNT's)

Carbon nanotubes (CNT's) or *buckminsterfullerences* (Bucky balls) are nano-sized, hollow, tubular, cage like single walled (SWCNT) or multiple walled (MWCNT) graphene sheets formed of carbon atoms, serially arranged into condensed benzene rings[45,46]. The candidature of CNT's as advanced delivery systems for anti-cancer drugs can be attributed to their ideal physicochemical properties such as high aspect ratio, ultra-light weight, tremendous strength, high thermal conductivity, flexible electronic properties, photoluminescence, and greater surface area. C_{60} is highly hydrophobic and its carboxylic acid derivatives are polar in nature and can thus, enter lipid bilayers such as phosphatidylcholine liposomes. The companion limitations such as poor aqueous

and organic solubility, aggregation (bundling), lower half-life, poor biocompatibility and tissue immunogenicity can be overcome by developing chemically modified and functionalized CNT's (*f*-CNT's). Various modification strategies such as non-covalent functionalization on the external walls, defect functionalization at the tip and the sidewalls, covalent functionalization on the external sidewalls and encapsulation of biomolecules inside CNT's have been reviewed by Pastorin[46].

Various anti-cancer drugs such as doxorubicin, Gonadotropin Releasing Hormone (GnRH), methotrexate, therapeutic proteins, DNA, siRNA and various other drugs have been studied with CNT's for their therapeutic or diagnostic applications. Experiments on rats have shown that buckyballs packed with the MRI contrast metal gadolinium can increase the sensitivity of MRI detection by at least 40-fold. This level of precision is reaching a point at which cancer cells that have spread beyond the margins of the tumor may become visible. Fatouros *et al.*, have also created a modified version of the buckyballs with a fluorescent metal atom called terbium. A glowing buckyball could guide surgeons to remove tumors with greater precision. Addition of yet another metal, lutetium, would deliver a lethal dose of radiation to the cancer cells, including those missed by the surgeon. It was also observed that water-soluble poly (ethylene glycol) (PEG) functionalized and hydroxylated endohedral tri-metallic nitride metallofullerene (Gd3N) derivatives demonstrated high relaxivities and optimal functionalities as MRI contrast agents[47]. However, the availability of insufficient, superficial and contradictory data hinders the correct evaluation of cellular uptake, release, absorption, efficacy and toxicity of drug loaded CNT's. The future studies directed towards these issues would possibly, reveal the true status of CNT's suitability as novel delivery systems for anti-cancer drugs.

(3.7) Magnetic Nanoparticles

Nanoparticles prepared by using a metal or metal oxide for its core and a biocompatible polymer for surface coat are called Magnetic nanoparticles. These nanoparticles are able to target a specific site under the influence of an externally applied magnetic field. Magnetic nanoparticles are used in Magnetic Resonance Imaging, hyperthermia/thermal ablations, drug targeting and immunoassays[48]. Various magnetic nanoparticles containing anti-cancer agents have been successfully investigated for controlled and targeted delivery. To prevent their uptake by organs of RES, magnetic nanoparticles are coated with a neutral and hydrophilic polymer such as silica, dextran, PEG and HSA. Such coatings increase the circulation half-life of the magnetic nanoparticles and extend or control the drug release for several hours to days or months. Targeting efficiency of these nanoparticles is improved by enhancing the external magnetic strength by using permanent Nd-Fe-B magnets, SPION's (superparamagnetic iron oxide nanoparticles) or by internally implanting the magnets near the target site [49,50]. Intelligent magnetic nanoparticles that may recognize and penetrate the target tumor site are developed by attaching or coating the particle surface with tumor recognizing moieties such as tumor specific antibodies, cell penetrating peptides and selective receptors. These nanoparticles are loaded with contrast agents for diagnostic applications such as MRI or with anti-cancer drugs for drug delivery applications[51]. Some magnetic nanoparticles that have been developed and commercialized for chemotherapeutic drug delivery are MagNaGel® (Alnis Biosciences, Inc.), FluidMAG® and TargetMAG-doxorubicin (Chemicell GmbH).

(3.8) Core shell nanoparticles

Nanoshells are the recently developed systems which are spherical nanoparticles with silica as a dielectric core (non conductive core) and surrounding of a thin metal shell of gold or silver (Conductive shell). The system provides tunable resonant response which helps in high optical absorption and scattering. The resonance frequency of the system has been reported to be modified systematically by changing the dimensions of core and shell layers. It has been found to be optimal within the range of (700-1300 nm) i.e from visible to the midinfrared region of spectrum. Nanoshells have appeared as a promising approach in the field of medicines for the purpose of diagnosis of disorders. The recent application includes in situ whole blood assay by using gold shell nanoparticles. This system offers high specificity and has been proved to be very efficient for analyzing the blood borne diseases. It has many advantages over the conventional systems, the approach is less time consuming and feasible in immunoassays. In the experiments, aggregation of antibody/nanoshell conjugation was observed. Immunoglobulin in saliva, serum and whole blood were detected successfully. This newly developed method is able to detect the analytes in sub-nanograms of concentration within 10-30 min [52,53].

(4) Biomimetic nanoparticles

(4.1) Gold Monolayer Protected Clusters (MPCs)

Gold Monolayer Protected Clusters (MPCs) are water-soluble, alkanethiols stabilized, gold nanoparticles in the size range of 1 – 10 nm, containing approx. 55 – 1000 gold atoms with molecular weight between 10 – 200 kDa[52]. Unlike gold colloids, gold MPCs can be dissolved, dried, isolated and redissolved in common solvents without decomposition or aggregation[53]. Water soluble and water stable gold MPCs are produced by using thiolated polar protecting ligands such as, tiopronin, glutathione, 4-mercaptobenzoic acid, 1-thio-*β*-D-glucose and *N*, *N*, *N*-trimethyl (mercaptoundecyl) ammonium (TMA). These stabilized gold MPCs are surface functionalized by one or more bioactive ligands such as a peptide sequence or a polymeric chain, to form 'Biomimetic MPCs'. These Biomimetic MPCs can effectively perform the role of an active biomolecule in the biological system, and thus, may act as a surrogate with better stability, improved performance, multiple functions and wider applications in biology, medicine and drug targeting.

In a study reported by Tkachenko *et al.*, gold nanoparticles were functionalized by attaching viral cell entry peptide, pre-conjugated to bovine serum albumin (BSA) via an ester linker[54]. These peptides functionalized gold nanoparticles reached the nucleus of HepG2 cells, the specific target site, notably, without affecting the cell viability. According to a review by Huang, "researchers are creating artificial molecular machines with tailored structures and properties, with the aim of realizing man-made active nanosystems that operate with the same efficiency and complexity as biological nanomachines. It is anticipated that in the not-too-distant future, unique applications of biological and biomimetic molecular machines will emerge in areas such as biochemical instrumentation and nanomedicine"[55].

(4.2) Self-Assembling Nanoparticles

(4.2.1) Self-Assembling Peptidomimetic Nanoparticles

Like some gold MPCs, peptidomimetic nanoparticles also involve biomimetic peptide chains to induce biological recognition property in them. Peptidomimetic nanoparticles are 'self-

assembling' *i.e.*, they can be physicochemically programmed to form spontaneously, from synthetic macromolecular polymeric chains by mimicking the weak bonding and electrostatic interaction of the natural peptides[56]. These self-assembled (or self-organizing) peptidomimetic nanoparticles possess functional specificity, optimized biological recognition and above all, unlike natural self-assembling nanostructures, can possess desired physicochemical properties such as thermodynamic stability, mechanical strength, improved bioavailability, non-immunogenicity and controlled dissolution profile. Dutta and Hoffman have reviewed various self-organizing methods for nanoparticles in terms of their application, simplicity of technique, yield and suitability of material involved[57]. Some of the reported techniques are self-organization through capillary forces, template assisted, electrostatic complexation with charged langmuir monolayers, chemically patterned substrates, optically directed, electric or magnetic field assisted and biologically assisted. The core of these nanoparticles can be metallic or polymeric and can further be pegylated or magnetically modulated to increase the circulation time or enhance the targeting efficiency. They can be conjugated with cell penetrating peptides for intracellular targeting and can be used for delivery of oligonucleotides and plasmids[58]. Such peptide-based nanostructures can be a favourable choice for molecular imaging, gene delivery, growth factor delivery, targeting of anti-cancer drugs, vaccines and other biologicals.

One useful application of self-assembling peptidomimetic nanoparticles is the delivery of protease activated prodrugs such as N-butoxycarbonyl-Ala-Ala-Asp-Leu along with the active drug such as doxorubicin, to the tumor site, where it binds with tumor cells through peptidomimetic ligands and is cleaved by legumain endopeptidase that is present in the tumor microenvironment, to another conjugate N-butoxycarbonyl - Ala-Ala-Asp-Leu-Dox[56, 59]. This endogenously formed conjugate, along with the target affinity of the nanoparticles, prevents accumulation in the normal tissue and increases the concentration of Doxorubicin at the tumor site, resulting in improved efficacy and reduced side effects.

(4.2.2) Self-Assembling Peptide Nanodiscs

Arginine (R9)-rich cationic peptides, either alone or in association with polyplexes, proteins or chemicals, confer DNA condensation and membrane translocation with dramatic improvement in stability and *in-vivo* performance of drugs[60]. By tailoring these R9 peptides with a scaffold green fluorescent protein, Vazquez, *et al.*, developed a novel category of efficient, self-assembling, regular, disc shaped, protein nanoconstructs known as *'nanodiscs'*. These nanodiscs deliver bioactive, plasmid DNA to the nucleus and promote plasmid-driven transgene expression. Further work in the field is expected to contribute for the advancement in artificial virus mediated drug and gene delivery.

(4.2.3) Self-Assembling Liquid Crystal Nanoparticles

Recently, nanoparticles of Lipid Based Self Assembled Liquid Crystalline Structures (LCNP) have emerged as alternative delivery systems to micelles, microemulsions and liposomes, owing to the improved solubilization, protection, stability, absorption and distribution properties it offers to drugs. Like SEDDS (Self Emulsifying Drug Delivery Systems) they can self-emulsify into colloidal particles, although, with greater surface area and geometrically structured surfaces. These LCNP are capable to deliver higher concentrations of drugs or therapeutic proteins with both lipophilic and

hydrophilic properties[61]. Non-lamellar LCNP are suitable for intravenous, nasal and transdermal routes of administration route. Commercial examples of LCNP are Cubosomes, Hexosomes, and Flexisomes. These nanoparticle systems have repeating, cuboidal or hexagonal internal geometric structures that increase their drug loading capacity. Cubosomes are biocompatible and bioadhesive nanoparticulate disperse systems produced by emulsification of cubic lipid phase in water[62]. Such hydrophilic-lipophilic nanoparticles may offer their potential of higher loading of amphiphilic drugs, protection and stability to control the drug release. Esposito *et al.*, have reported a simple manufacturing technique to obtain high yields of cubosomes that was otherwise a tedious process[63]. Similar other systems are Super-cooled Smectic Nanoparticles. Supercooled smectic nanoparticles are similar to LDL's in structure and composition and, thus, have been suggested to be useful for targeting of lipophilic anti-cancer drugs to brain through LDL receptors[64].

(4.3) Molecular Trojan Horses (MTH)

Another advanced drug targeting system that is being studied is 'Molecular Trojan Horses' (MTH). These are peptidomimetic, receptor specific monoclonal antibodies (MAb's) that bind to endogenous peptide receptors and are taken across BBB by receptor mediated transport (RMT)[65,66]. In order to transport human specific MTH across BBB, the murine MAb's to the human insulin receptor (HIRMAb's) are genetically engineered or humanized to prevent immunologic reaction[67]. The genetically engineered, expressed and purified recombinant fusion protein of HISMAb is fused with non-transportable therapeutic protein such as neurotrophin to enable transport across BBB and provide neuroprotection. MTH can also be designed to deliver non-viral plasmid DNA across BBB by formulating 'Trojan Horse Liposomes' (THL)[68]. These tailored liposomes are also reported to transport therapeutic and antisense RNA genes across BBB for the treatment of brain cancer in animal models[69]. More studies are being carried out to establish the potential of MTH as delivery systems for brain targeting. Selected reviews on brain targeting by Partridge offer detailed discussion on MTH for interested readers[66,69,70].

(4.4) Virus like Particles as Drug Delivery System

In the recent researches viruses has been explored as the delivery system for anticancer agents. The surface of virus can be modified chemically or genetically to provide targeted drug delivery. Singh *et al.*, examined canine parvovirus (CPV) produced by expression of the CPV-VP2 capsid protein in a baculovirus expression system which were attatched with small molecules for targeting to tumor cells[71]. Ogris *et al.*, synthesized surface-shielded DNA delivery systems that can target gene expression into distant tumor tissues. Repeated systemic application of Tf–PEG–PEI/DNA complexes encoding Tumor Necrosis Factor alpha (TNF-α) into tumor-bearing mice induced tumor necrosis and inhibition of tumor growth in three murine tumor models of different tissue origin (Neuro2a, M-3 or B16 melanoma)[72]. Schiller and Hidesheim conducted the preclinical studies along with clinical trials of Human Papilloma Viruses (HPVs) as vaccine for reduction in cervical cancer incidence. From the trials it was concluded that there was effective prevention of incidence of cervical cancers. In addition, prophylactic HPV vaccines could potentially decrease the incidence of some other cancers with lower prevalence and/or weaker association with HPV infection, such as anal, vulvar and tonsillar cancers[73].

(5) Medical Nanorobotics

(5.1) Nano-Electromechanical Systems (NEMS)

Biomedically inspired Nanorobotics or NEMS research involves design, prototyping, fabrication, programmable assembly and applications of robots with overall dimensions below a few micrometers in all the three spatial directions. Vision of nanosystems as factories using nanomachines to build complex products, including additional nanomachines was first proposed by the physicist Richard Feynmen, the field being known as 'Molecular Manufacturing' [74]. The medical importance and capabilities of molecular manufacturing in nanotechnology, termed as 'Molecular Nanotechnology' was presented in detail by researcher and nanoscientist K.E. Drexler in his book titled "Engines of Creation" published in 1986[75]. Mechanically and forcefully guided, non-biological chemical synthesis of such nanosystems known as 'Mechanosynthesis' or 'Mechanosynthetic Chemistry' is quite different from the conventional chemistry and follow eutectic (ordered and controlled) machine phase chemistry instead of solution phase chemistry[76,77]. In machine-phase systems, most atomic paths and all reactive moieties follow nominal and controlled trajectories during complex motion in extended region. The controlled manufacturing process involves vacuum instead of solution as reaction environment and the product size is greater than 10^{10} atoms instead of general 10-100 atoms.

(5.2) Nanorobots

Various artificial, nanorobotic mechanical devices such as pharmacytes[78], microbivores[79], clottocytes[80], chromallocytes [79] and vasculoids have been proposed by researchers with different biomedical applications including drug targeting to a specific site in the body including 'Hard Targets' such as brain and several tumors[81]. Although, presently, there is no synthetic process, no advanced technology and no infrastructural facilities known to construct such systems, the advances and current pace of research in the field of nanotechnology would deliver promising results in the future. Experts in the field agree that it is feasible to design 'nanobots' using molecular tools and use them to detect, identify and destroy cancer cells inside the body[82]. What actually needed is more and more input from the scientific fraternity in this 'less conventional' but promising field of molecular nanotechnology. In the field of advance nanotechnology, researchers are trying hard to develop highly sophisticated techniques, one out of these approaches include creation of nanofactories based on integration of biomolecules with polymer surface. The naofactory would have extensive capability to produce multifunctional nanoparticles with high sensitivity and specificity. These systems can serve as nanosensors, actuators and reactors. Polymers, biomolecules and nanoparticles when combined with the polymeric surface can create wonders in the form of multifunctional structural devices. Atomic force microscopy (AFM) is the foundation behind the creation of nanofactories[83]. The approach exhibit the importance through imaging, manipulation and detection at nanoscale. AFM serve as an ideal technique for characterization of nanoparticles along with microscopy of a single molecule. It offers high resolution (lateral~1nm, vertical resolution~0.1 nm). It can provide imaging even in the fluid environment. AFM is having the capability to investigate the mechanical and chemical forces exerted on the tip of microscope. It plays extensive role in the detection of nanoparticles by using electric or magnetic force microscopy. The system uses special mode (magnetic force microscope) to detect the magnetic nanoparticles through the tip

deflection of microscope. The technique detects and serves as the indispensable part for the nanoparticles detection within biomolecules and polymeric surfaces[84].

(5.3) Rotaxanes and Catenanes (Molecular Shuttles)

Rotaxanes are the class of molecules consisting of macrocycles which encircles the large linear components[85]. According to IUPAC these are defined as molecules in which a ring encloses another rod-like molecule having end-groups too large to pass through the ring opening; the rod-like molecule is thus held in position without covalent bonding. Despite the presence of covalent bonds between axes and rings, rotaxanes are stable entities, because high free activation energy is required to withdraw a ring from the axis of a rotaxane. The system can be covalently linked together in many ways to create oligomeric or polymeric species, called polyrotaxanes. The rotaxanes have been studied to behave as molecular shuttles because of the freely sliding macrocycle over the axis of the ring. The macrocycle can be controlled by applying and varying light, pH and temperature[86]. Catenanes are also the closely related compounds in which a chain is formed by the interlocking of macrocycles. These molecular shuttles have recently been studied to control the drug release and to enhance the ability of drugs to enter through various membranes[87]. It is a novel approach to achieve cell selective chemotherapy, new cellular –transport rotaxanes containing cancer cell-surface recognition elements were synthesized. It has been fascinating the investigators to promote selective chemotherapy to reduce side effects of anticancer therapy[88].

CONCLUSION

Scientists throughout the world are striving to develop an ideal delivery system to achieve drug targeting to a specific site in optimized and controlled manner. Probability to target drugs to the tumor site more efficiently can further be developed by novel techniques in genomics and proteomics. Research taken up to discover with new technologies can reveal newer vistas in cancer targeting. Medical nanorobotic systems, though, not feasible presently, would definitely benefit drug targeting in the later years by development and implementation of tools that are more sophisticated, facilities and devices. Nevertheless, these later years are not too far, when promising approaches of the future would revolutionize the controlled and targeted drug delivery; and would serve the purpose of dedicated researchers all over the world, who are constantly striving to relieve the humankind from the agony of diseases.

REFERENCES

1. Park B. Current and Future Applications of Nanotechnology. In: Hester RE, Harrison RM, editors. Nanotechnology: Consequences for Human Health and the Environment. Cambridge: The Royal Society of Chemistry, UK; 2007. p. 1-18.
2. Bae YH. Drug targeting and tumor heterogeneity. J Control Rel. 2009; 133:2-3.
3. Freitas RA. Current status of nanomedicine and medical nanorobotics. JCTN. 2005; 2:1-25.
4. Moghimi SM, Hunter AC, Murray JC. Nanomedicine: current status and future prospects. FASEB J. 2005 Mar; 19(3):311-30.
5. Approaches to safe nanotechnology: An information exchange with NIOSH. 1.1 ed. USA: Center for Disease Control and Prevention, National Institute for Occupational Safety and Health; 2006.
6. Jaeghere FD, Doelker E, Gurny R. Nanoparticles. I ed. Mathiowitz E, editor. New york, USA: John Wiley & Sons, Inc; 1999.
7. Kreuter J. Physicochemical characterisation of polyacrylic nanoparticles. Int J Pharm. 1983; 14:43 - 58.

8. Magenheim B, Benita S. Nanoparticles characterisation: a comprehensive physicochemical approach. S T P Pharma science. 1991; 1:221.
9. Kreuter J. Colloidal drug delivery systems. Swarbrick J, editor. New York: Marcel Dekker; 1994.
10. Gref R, Minamitake Y, Perracchia MT, Trubetskoy V, Torchilin VP, Langer R. Biodegradable long circulating polymeric nanospheres. Science. 1994; 263(5153):1600-3.
11. Allen TM, Cullis PR. Drug delivery System: Entering the main stream. Science. 2004; 303:1818-22.
12. Kreuter J, Ramge P, Petrov V, Hamm S, Gelperina SE, Engelhardt B. Direct evidence that polysorbate-80-coated poly(butylcyanoacrylate) nanoparticles deliver drugs to the CNS via specific mechanisms requiring prior binding of drug to the nanoparticles Pharm Res. 2003; 20:409-16.
13. Northfelt DW, Martin FJ, Working P, Volberding PA, Russell J, Newman M, et al. Doxorubicin encapsulated in liposomes containing surface-bound polyethylene glycol: pharmacokinetics, tumor localization, and safety in patients with AIDS-related Kaposi's sarcoma. J Clin Pharmacol. 1996; 36:55-63.
14. Braqaqni M, Mennini N, Maestrelli F, Mura P. Comparative study of liposomes, transfersomes and ethosomes as carriers for improving topical delivery of celecoxib. Drug deliv. 2012; 19:354-61.
15. Kreuter J, Ramge P, Petrov V, Hamm S, Gelperina SE, Engelhardt B, et al. Direct evidence that polysorbate-80-coated poly(butylcyanoacrylate) nanoparticles deliver drugs to the CNS via specific mechanisms requiring prior binding of drug to the nanoparticles. Pharm Res. 2003; 20(3):409-16.
16. Brigger I, Dubernet C, Couvreur P. Nanoparticles in cancer therapy and diagnosis. Adv Drug Deliv Rev. 2002; 54(5):631-51.
17. Olivier JC. Drug transport to brain with targeted nanoparticle. ASENT. 2005; 2:108-19.
18. Gref R, Luck M, Quellec P, Marchand M, Dellacherie E, Harnisch S. 'Stealth' corona-core nanoparticles surface modified by polyethylene glycol (PEG): influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption. Colloids Surfaces B: Biointerfaces. 2000; 18:301-13.
19. Gulyaev AE, Gelperina SE, Skidan IN, Antropov AS, Kivman GY, Kreuter J. Significant transport of doxorubicin into the brain with polysorbate 80-coated nanoparticles. Pharm Res. 1999; 16:1564-9.
20. Lu W, Zhang Y, Tan YZ, Hu KL, Jiang XG, Fu SK. Cationic albumin conjugated pegylated nanoparticles as novel drug carrier for brain delivery. J Control Release. 2005; 107(3):428-48.
21. Xu F, Lu W, Wu H, Fan L, Gao X, Jiang X. Brain delivery and systemic effect of cationic albumin conjugated PLGA nanoparticles. J Drug Target. 2009; 17(6):423-34.
22. Gail Roberts T, Anker JN, Kopelman R. Magnetically modulated optical nanoprobes (MagMOONs) for detection and measurement of biologically important ions against the natural background fluorescence of intracellular environments. J Magn Magn Mater. 2005; 293:715-24.
23. Remaut K, Lucas B, Raemdonck K, Braeckmans K, Demeester J, De Smedt SC. Protection of oligonucleotides against enzymatic degradation by pegylated and nonpegylated branched polyethyleneimine. Biomacromolecules. 2007; 8(4):1333-40. Epub 2007 Mar 15.
24. Olivier JC, Huertas R, Lee HJ, Calon F, Pardridge WM. Synthesis of Pegylated Immunonanoparticles. Pharm Res. 2002; 19(8):1137-43.
25. Yang T, Choi MK, Cui FD, Kim JS, Chung SJ, Shim CK, et al. Preparation and evaluation of paclitaxel-loaded PEGylated immunoliposome. J Control Release. 2007; 120(3):169-77. Epub 2007 May 17.
26. Veiseh O, Sun C, Gunn J, Kohler N, Gabikian P, Lee D, et al. Optical and MRI multifunctional nanoprobe for targeting gliomas. Nano Lett. 2005; 5(6):1003-8.

27. Quarta A, Di Corato R, Manna L, Ragusa A, Pellegrino T. Fluorescent-magnetic hybrid nanostructures: preparation, properties, and applications in biology. IEEE Trans Nanobioscience. 2007 Dec; 6(4):298-308.
28. Jain KK. Use of nanoparticles for drug delivery in glioblastoma multiforme. Expert Rev Neurother. 2007; 7(4):363-72.
29. Broaddus WC, Gillies GT, Kucharczyk J. Minimally invasive procedures. Advances in image-guided delivery of drug and cell therapies into the central nervous system. Neuroimaging Clin N Am. 2001 Nov:727-35.
30. Lu Y, Liu J. Catalyst-functionalized nanomaterials. Wiley Interdiscipl Rev Nanomed Nanobiotechnol. 2009; 1:35-46.
31. Huang X, Jain PK, El-Sayed IH, El-Sayed MA. Gold nanoparticles: interesting optical properties and recent applications in cancer diagnostics and therapy. Nanomed. 2007; 2(5):681-93.
32. Sonvico F, Dubernet C, Colombo P, Couvreur P. Metallic colloid nanotechnology, applications in diagnosis and therapeutics. Curr Pharm Des. 2005; 11(16):2095-105.
33. Chopra N, Bachas LG, Knecht MR. Fabrication and Biofunctionalization of Carbon-Encapsulated Au Nanoparticles. Chem Mater. 2009; 21:1176-8.
34. Patra CR, Bhattacharya R, Wang E, Katarya A, Lau JS, Dutta S, et al. Targeted delivery of gemcitabine to pancreatic adenocarcinoma using cetuximab as a targeting agent. Cancer Res. 2008; 68:1970–8.
35. Akerman ME, Chan WCW, Laakkonen P, Bhatia SN, Ruoslahti E. Nanocrystal targeting in-vivo. Proc Natl Acad Sci U S A. 2002; 99(20):12617-21.
36. Silva GA. Neuroscience nanotechnology: progress, opportunities and challanges. Nature Reviews: Neuroscience. 2006; 7:65-74.
37. Nie S, Xing Y, Kim GJ, Simons JW. Nanotechnology applications in cancer. Annu Rev Biomed Eng. 2007; 9:257-88.
38. Zhang Y-Z, Jaron S, Buller G, Godfrey WL. Use of Qdot® nanocrystal primary antibody conjugates in flow cytometry. Journal [serial on the Internet]. 2008 Date: Available from: www.invitrogen.com/qdotinflow.
39. NCI. Nanotechnology tackles brain cancer. Journal [serial on the Internet]. 2005 Date; 2007(October 10): Available from: www.cancer.gov.
40. Kopelman R, Philbert M, Koo YE, Moffat BA, Reddy GR, McConville P, et al. Multifunctional Nanoparticle Platforms for In Vivo MRI Enhancement and Photodynamic Therapy of a Rat Brain Cancer. J Magn Magn Mater. 2005; 293:404-10.
41. Anker JN, Behrend CJ, Huang H, Kopelman R. Magnetically-modulated optical nanoprobes (MagMOONs) and systems. J Magn Magn Mater. 2005; 293:655-62.
42. Anker JN, Behrend CJ, Huang H, Kopelman R. Magnetically-modulated optical nanoprobes (MagMOONs) and systems. J Magn Magn Mater. 2005; 293:655-62.
43. Gail Roberts T, Anker JN, Kopelman R. Magnetically modulated optical nanoprobes (MagMOONs) for detection and measurement of biologically important ions against the natural background fluorescence of intracellular environments. J Magn Magn Mater. 2005; 293:715-24.
44. Behrend CJ, Anker JN, McNaughton BH, Kopelman R. Microrheology with modulated optical nanoprobes (MOONs). J Magn Magn Mater. 2005; 293:663-70.
45. Rodriguez-Manzo J, Terrones M, Terrones H, Kroto H, Sun L, Banhart F. In situ nucleation of carbon nanotubes by the injection of carbon atoms into metal particles. Nat Nanotechnol. 2007 May; 2(5):307-11. Epub 2007 Apr 29.
46. Pastorin G. Crucial Functionalizations of Carbon Nanotubes for Improved Drug Delivery: A Valuable Option? Pharm Res. 2009; 26(4):746-69.
47. Zhang J, Fatouros P, Shu C, Reid J, Owens L, Cai T, et al. High Relaxivity Trimetallic Nitride (Gd3N) Metallofullerene MRI Contrast Agents with Optimized Functionality. Bioconjugate Chemistry. 2010; 21(4):610-5.
48. Brazel CS. Magnetothermally-responsive Nanomaterials: Combining Magnetic Nanostructures and Thermally-Sensitive

Polymers for Triggered Drug Release. Pharm Res. 2009; 26(3):644-56.
49. Pathak AP. Magnetic resonance susceptibility based perfusion imaging of tumors using iron oxide nanoparticles. Interdiscipl Rev Nanomed Nanobiotechnol. 2009; 1:84-97.
50. Gossuin Y, Gillis P, Hocq A, Vuong QL, Roch A. Magnetic resonance relaxation properties of superparamagnetic particles. WIREs Nanomedicine and Nanobiotechnology. 2009; 1:299-310.
51. Arruebo M, Fernández-Pacheco R, Ibarra MR, Santamaría J. Magnetic nanoparticles for drug delivery. Nanotoday. 2007; 2(3):22-32.
52. Cliffel DE, Turner BN, Huffman BJ. Nanoparticle-based biologic mimetics. Wiley Interdiscipl Rev Nanomed Nanobiotechnol. 2009; 1:47-59.
53. Templeton AC, Wuelfing WP, Murray RW. Monolayer protected cluster molecules. Acc Chem Res. 2000; 33:27-36.
54. Tkachenko AG, Xie H, Coleman D, Glomm W, Ryan J. Multifunctional gold nanoparticle-peptide complexes for nuclear targeting. J Am Chem Soc. 2003; 125(16):4700–1.
55. Huang TJ, Juluri BK. Biological and biomimetic molecular machines. Nanomed. 2008; 3(1):107-24.
56. Jabbari E. Targeted delivery with peptidomimetic conjugated self-assembled nanoparticles. Pharm Res. 2009 Mar; 26(3):612-30. Epub 2008 Dec 17.
57. Dutta J, Hofmann H. Self organization of colloidal nanoparticles. Nalwa HS, editor. USA: American Scientific Publishers; 2003.
58. Juliano RL, Alam R, Dixit V, Min Kang H. Cell-targeting and cell-penetrating peptides for delivery of therapeutic and imaging agents. WIREs Nanomed Nanobiotechnol. 2009; 1:324-35.
59. Wu W, Luo Y, Sun C, Liu Y, Kuo P, Varga J, et al. Targeting cell-impermeable prodrug activation to tumor microenvironment eradicates multiple drug-resistant neoplasms. Cancer Res. 2006; 66(2):970-80.
60. Vazquez E, Roldán M, Diez-Gil C, Unzueta U, Domingo-Espín J, Cedano J, et al. Protein nanodisk assembling and intracellular trafficking powered by an arginine-rich (R9) peptide. Nanomedicine (Lond). 2010; 5(2):259-68.
61. Tiberg F. Improving Drug Delivery by use of Lipid Self-assembly Particle Structures – Beyond Liposomes and Emulsions. Camurus®; 2005.
62. Larsson K. Aqueous dispersion of cubic lipid-water phases. Curr Opin Colloid Interface Sci. 2000; 5:64-9.
63. Esposito E, Cortesi R, Drechsler M, Paccamiccio L, Mariani P, Contado C, et al. Cubosome Dispersions as Delivery Systems for Percutaneous Administration of Indomethacin. Int J Pharm. 2005:1-11.
64. Kuntsche J, Westesen K, Drechsler M, Koch M, Bunjes H. Supercooled smectic nanoparticles: a potential novel carrier system for poorly water soluble drugs. Pharm Res. 2004; 21:1834
65. Pardridge WM, Kang YS, Buciak JL, Yang J. Human insulin receptor monoclonal antibody undergoes high affinity binding to human brain capillaries in vitro and rapid transcytosis through the blood–brain barrier in vivo in the primate. Pharm Res. 1995; 12:807–16.
66. Pardridge WM. Molecular Trojan horses for blood-brain barrier drug delivery. Curr Opin Pharmacol. 2006:494-500.
67. Coloma MJ, Lee HJ, Kurihara A, Landaw EM, Boado RJ, Morrison SL, et al. Transport across the primate blood–brain barrier of a genetically engineered chimeric monoclonal antibody to the human insulin receptor. Pharm Res. 2000; 17:266–74.
68. Shi N, Pardridge WM. Noninvasive gene targeting to the brain. Proc Natl Acad Sci USA. 2000; 97:7567–72.
69. Pardridge WM. Drug and gene targeting to the brain with molecular trojan horses. Nat Rev Drug Discov. 2002:131-9.
70. Pardridge WM. Blood-brain barrier drug targeting: the future of brain drug development. Mol Interv. 2003; 51:90-105.
71. Singh P, Destito G, Schneemann A, Manchester M. Canine parvovirus-like particles, a novel nanomaterial for tumor targeting. J Nanobiotechnology. 2006; 4:1-15.

72. Ogris M, Wagner E. Targeting tumors with non-viral gene delivery systems. therapeutic focus. 2002; 7:479-85.
73. Schiller JT, Hidesheim A. Developing HPV virus-like particle vaccines to prevent cervical cancer: a progress report. J CLIN Virol. 2000; 19:67-74.
74. Feynman R. There's plenty of room at the bottom. Science. 1991; 254:1300-1.
75. Drexler KE. Engines of Creation: The Coming Era of Nanotechnology. 1986.
76. Drexler KE. Molecular engineering: an approach to the development of general capabilities for molecular manipulation. Proc Natl Acad Sci USA. 1981; 78(9):5275-8.
77. Drexler KE. Nanosystems: Molecular Machinery, Manufacturing and Computation. New York: John Wiley & Sons; 1992.
78. Freitas RA. Pharmacytes: an ideal vehicle for targeted drug delivery. J Nanosci Nanotechnol. 2006; 6(9-10):2769-75.
79. Freitas RA. The Ideal Gene Delivery Vector: Chromallocytes, Cell Repair Nanorobots for Chromosome Replacement Therapy. The Journal of Evolution and Technology. 2007; 16(1):1-97.
80. Freitas RA. Clottocytes: artificial mechanical platelets. Foresight Inc; 2000.
81. Freitas RA. Nanomedicine, Volume I: Basic Capabilities. Georgetown, TX: Landes Bioscience; 1999.
82. Jain KK. Advances in the field of nanooncology. BMC Medicine. 2010; 8:83.
83. Agarwal G. Atomic force microscopy for nanotechnology 2nd US-Korea Nano Forum,; 2005 February 17; Los Angeles, USA. 2005. p. 1.
84. Manyes SG, Guell AG. Nanomechanics of silicon surfaces with atomic force microscopy: An insight to the first stages of plastic deformation. J CHEM PHYS. 2005; 123:1-7.
85. Blackburn A. Rotaxanes as Molecular Machines – The Work of Professor David Leigh. ChemNZ. 2010; 10:129-35.
86. Deska M, Kozlowaska J, Sliva W. Rotaxanes and pseudorotaxanes with threads containing viologen units. ARKIVOC. 2013; 12:66-100.
87. Sauvage JP, Buchecker CD, editors. Molecular catenanes, rotaxanes and knots. 2nd ed. Weinheim: Wiley-VCH; 1999.
88. Nobufumi O, inventor Anti-cancer agent. US patent US20100137419 A1. 2010 Jun 3, 2010.

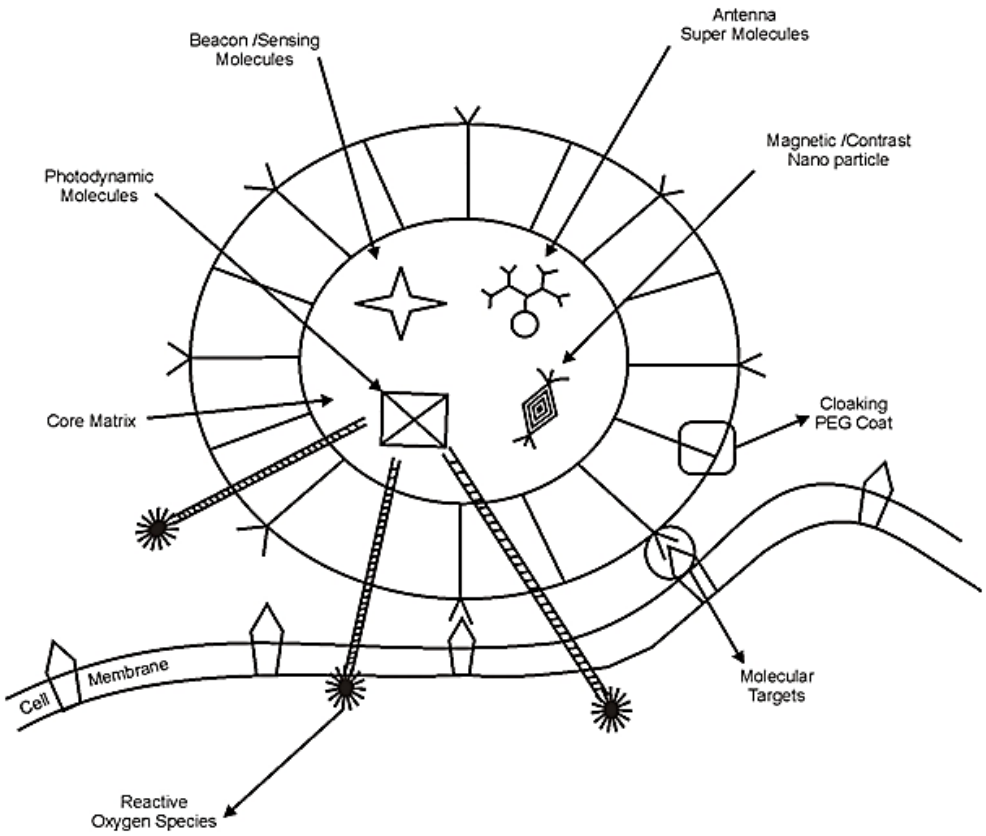

Figure.1. Schematic representation of an intelligent nanomedical device

[Adopted and modified from reference[40]]