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Review Paper

THE STATE OF THE ART IN BIOMATERIALS AS NANOBIOPHARMACEUTICALS

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The development of wide spectrum of drug delivery systems is fundamental importance to change the establishment of disease diagnosis, treatment and prevention. The fusion technologies of material science and nanomedicine have emerged as a new alternative and efficient field for transporting and translocating the therapeutic molecules. Biologically active materials can be functionalized with peptides, proteins, nucleic acids and drugs, and also be used as the delivery system to cell and organs. Today nanoscience and pharmaceutical technology approaches to drug design and formulation to enhance the biological behavior and safety profile. Unique higher surface area, surface roughness, altered electron distribution, energetics and biological activity of standardized materials made a feasible role in therapeutics. Rationalized material, drug design and formulation will ensure the good clinical significance of the pharmaceutical product. This article will highlight the distinguished nanoscale biomaterials categorized by metal, non-metal, carbon, polymer, lipid, virus and miscellaneous nanostructures as nanobiopharmaceutical carrier systems and their medical/biological applications and toxicological issues in the field of biomedical nanotechnology.

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1. Introduction

The field of biomaterials has been grown and evolved in its capacity to study the molecular biology and cell biology of the implant tissue interface, used as vehicles to deliver nano and large bioactive molecules to specific tissues in order to restore normal physiological function. The strategy of using materials as delivery agents provides many opportunities related to the design of micromaterial and eventually nanomaterial, which are miniaturized constructs designed to encapsulate, target, and deliver drugs to a specific site (1). Nanotechnology can be defined as the design, characterization, production, and application of structures, devices, and systems by controlled manipulation of size and shape at the nanometer scale (atomic, molecular, and macromolecular scale) that produces structures, devices, and systems with at least one novel/superior characteristic or property (2). This has impacted the field of biomaterial in several areas, including the manipulation of surface characteristics, the production of cellular sized materials and the use of nanofabrication. Achievement of small-scale technologies has seen clinical relevance in the areas of both diagnosis and treatment with devices of BioMEMS (Biological MicroElectrical Mechanical Systems) and BioNEMS (Biological NanoElectrical Mechanical Systems) such as intraocular pressure sensors, microneedles for transdermal delivery, miniature stimulators, lab-on-a-chip, nanbiosensors, biochips, etc. Examples of such products currently in use or being developed include implantable pressure sensors (Endosure wireless AAA pressure sensor, cardio MEMS, Atlanta, GA), Catheter based flow sensors (Vermetra, Pittsburgh, PA), blood pressure sensors, blood chemistry analysis, and gene arrays (Affymetrix, Santaclara,

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CA) (3). Nanoscale drug carriers have the potential to enhance the therapeutic efficacy of drugs as they can regulate their release, improve their stability and prolong circulation time by protecting the drug from elimination by phagocytic cells or premature degradation (4). Nanosized carriers are prime factors for the delivery of highly toxic, hydrophobic therapeutic agents or both. These delivery vehicles have the potential to augment to pharmacodynamic and pharmacokinetic profiles of drug molecules, thereby enhancing the therapeutic efficacy of the pharmaceutical agents (5). Nanomedicine, the medical application of nanotechnology, promises an endless range of application from biomedical imaging to drug delivery and therapeutics (6). Nanomedicine also aims to learn from nature to understand the structure and function of biological devices and to use nature's solutions to advance science and engineering. When this nanotechnology is combined with pharmaceutical sciences, the possible applications at this frontier are widespread and unassailable like the gear of science fiction. Although several reviews are well studied on biomaterial applications in biological and pharmaceutical sciences, this present paper aimed at reviewing the different class of nanoscale biomaterial systems distinguished by metal, non-metal, carbon, polymer, lipid, virus and miscellaneous nanostructures based applications and their exploitation as "Nanobiopharmaceuticals". It also highlighted the different class of toxicological issues of different nanomaterials to the biological systems.

2. Biomaterial

Biomaterials are natural or synthetic nonviable materials introduced in a medical device, intended to interact with biological systems in order to evaluate, treat, deliver, augment, or replace any tissue, or function of body (7). Biomaterials when reduced to the size scale of nano, properties like surface-area, quantum, and optical effects, electrical and magnetic behaviors are unassailable for diagnosis and treatment of diseases. Materials that exploit these effects include quantum dots, and quantum well lasers for optoelectronics. Mechanical and electrical properties of crystalline solids are greatly affected through their interface area within material when its size reduced from micro to nanoscale. For example, most metals are made up of small crystalline grains; the boundaries between the grain slow down or arrest the propagation of defects when the material is stressed, thus giving it strength. If these grains can be made very small, or even nanoscale in size, the interface area within the material greatly increases, which enhances its strength (e.g. Nanocrystalline nickel is as strong hardened steel). Fig. 1 depicts the morphology of different class of nanomaterials as a multivalent system for biomedical nanotechnology. Studying the material properties of surfaces and interfaces is a key challenge for those working on biomaterials for health care applications (8). Table.1 shows the common biomaterials and their significance in the health care systems. Table.2 shows some examples of biomaterial based nanomedicine and delivery systems in the market.

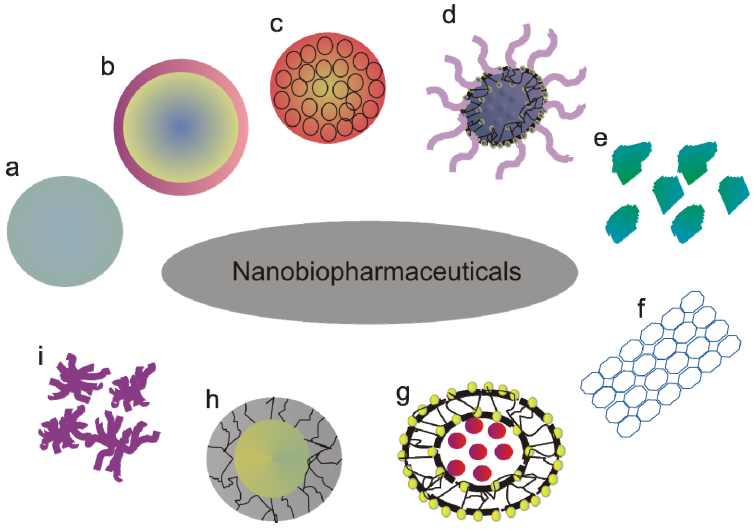

Fig.1. illustrates the different biomaterials as nanobiopharmaceuticals. (a) multifunctional nanoparticle (b) quantum dot (c) aquasomes (d) polyplexes/lipopolyplexes (e) superparamagnetic iron oxide crystals (f) carbon nanomaterial (g) liposomes (h) polymeric micelles and (i) dendrimers

3. Metal nanobiomaterials

3.1 Multifunctional metal nanoparticles

Multifunctional metal nanoparticles provide unprecedented opportunities for medical advancement like drug delivery, cellular imaging, biomedical diagnostics and therapeutics because of their small sizes and unique properties. Plasmonic nanoparticles are especially useful for several biomedical applications because of their enhanced resonant absorption and scattering properties, as well as strong Raman scattering which are essential properties for their applications in photothermal therapy (10), optical imaging (11) and Raman probe design (12). Gold nanoparticles (GNPs) are examples of extremely attractive candidates for such applications due to small sizes, ease of preparation and bioconjugation, strong absorbing and scattering properties as well as their well known biocompatibility (13). GNPs have shown the success in delivery of peptides, proteins, or nucleic acids like DNA or RNA. Non-covalent DNA-GNPs conjugate provide an effective means of delivery in mammalian 293T cells (14). GNPs could possibly be employed in delivery of diatomic therapeutic agents like singlet oxygen or nitric oxide. Singlet oxygen (O_2), a cytotoxic species involves, in photodynamic therapy of cancer (15). Nitric oxide (NO) regulates multiple cellular processes including angiogenesis, vasodilation, and the immune response (16). Properties of antibacterial and biocidal (to prevent infection in burns, traumatic wounds and diabetic ulcers) placed the silver particles or silver ions as the other important multifunctional nanoparticles. It is also used as a water disinfectant. Some of the well known silver products available in the market are silver sulfadiazine (Flammazine™, Smith and Nephew Health care Limited, Hull, Canada), silver sulfadiazine/chlorhexidine (Silverex™, Motiff Laboratories Pvt. Ltd. Kare Health Specialities, Verna, Goa), silver sulfadiazine with cerium nitrate (FlammaceriumR, Solvay, Brussels, Belgium), and silver sulfadiazine impregnated lipidocolloid wound dressing (Urgotul SSD, Urgo Laboratories, Chenova, France) (17). Marketed products of dressings containing silver nanocrystalline such as Acticoat-7 (Smith and Newphew in UK), Actisorb Silver 220 (Johnson & Johnson, New Brunswick, NJ, USA), Aquacel-Ag hydrofiber (Skillman, NJ, USA) and Silverlon (Argentum Medical, Chicago, USA). It is reported that silver products containing nanocrystalline silver kill microbes more rapidly and completely than products where silver remains in the cationic form. Recently it was reported by Elechiguerra *et al.* that silver nanoparticles in a size range 1–10 nm bind with HIV-1 in a size dependent fashion. The authors have shown that silver nanoparticles inhibit HIV-1 infection in CD4+MT-2 cells and cMAGI HIV-1 reporter cells. Depends on the size and surface modifications.

Table 1. Common biomaterials and their clinical significance (modified from ref. (9))

Biomaterial	**Clinical significance**
Metals and alloys 316L stainless steel	Fracture repair, stents, surgical instruments
CP-Ti[a], Ti-Al-V, Ti-Al-Nb	Bone replacement, joint replacement, facture repair, dental implants, pacemaker capsules
Co-Cr-Mo, Cr-Ni-Cr-Mo	Bone replacement, joint replacement, dental restoration, heart valves
Ni-Ti	Bone plates, stents, orthodontic wires
Gold Alloys	Dental restoration
Silver	Antibacterial Agents
Platinum, Pt-Ir	Electric leads
Hg-Ag-Sn amalgam	Dental restoration
Ceramics and Glasses Alumina	Joint replacement, dental implants

Zirconica	Joint replacement
Calcium phosphates	Bone repair, metal surface coatings
Bioactive glasses	Bone replacement
Porcelain	Dental restoration
Carbons	Heart valves, percutaneous devices, dental implants
Polymers Polyethylene	Joint replacement
Polypropylene	Sutures
PET[b]	Vascular prostheses, sutures
PTFE[c]	Soft tissue augmentation, vascular prostheses
Polyesters	Vascular prostheses, drug-delivery
Polyurethanes	Blood contact devices
Silicones	Soft tissue replacement, ophthalmology
Hydrogels	Ophthalmology, drug-delivery
Composites PMMA[d]-glass fillers	Dental cements
BIS-GMA[e]-quartz/silica filler	Dental restoration

[a]commercially pure titanium; [b]polyethylene terephthalate; [c]polytetrafluoroethylene; [d]polymethylmethacrylate; [e]bisphenol A-glycidyl

silver concentrations over 25µg/ml significantly inhibited HIV-1 infection. Bare silver nanoparticles showed superior effect, whereas surface modification with BSA and PVP showed moderate effect (18). Medicinal application of bare platinum nanoparticles is uncommon. Platinum nanoparticles have been used in combination with other nanoparticles, either in the form of an alloy, or core–shell or bimetallic nanoclusters. Yolk–shell nanocrystals of FePt@CoS_2 have been found to be more potent in killing HeLa cells compared to cis-platin. The IC_{50} value of FePt@CoS2 is found to be 35.5±4.7 ng of Pt/ml for HeLa cells, whereas, it is 230 ng of Pt/ml in case of cis-platin (19). Platinum nanoparticles were also used in combination with multi-walled carbon nanotubes (MWCNTs) for fabricating sensitivity-enhanced electrochemical DNA biosensor (20). This type of engineered metal bionanomaterials may ultimately lead to the development of new types of nanodrugs with improved cytotoxicity towards the malignant cells.

3.2 Quantum dots

These are nanocrystalline structures of semiconductor whose excitons are confined in all three dimensions, made from different variety of compounds such as cadmium selenide that can transform the color of light, and have been around since 1980s. Quantum dots (QDs) absorb white light and then re-emit it a couple of nanoseconds later at a specific wavelength. By varying the size and composition of quantum dots, the emission wavelength can be tuned from blue to near infrared. QDs based detection is rapid, easy and economical enabling quick point-of-care screening of cancer markers. QDs have got unique properties which make them ideal for detecting tumors (21). These structures offer new capabilities for multi color optical coding in gene expression studies, high throughput screening, and *in vivo* imaging. Highly sensitive real-time imaging with greater resolution and tracking of single receptor molecules on the surface of living cells have been made possible by QD bioconjugates (22). Nanobiopharmaceutical application of QDs is due to their high resistance to photobleaching, which enables longer time visualization of biological systems includes *in vitro* imaging of fixed cells, tissues, organelles, molecules and membranes, *in vivo* targeting of cells, tissues, organs and tumors in animal for diagnosis, therapy, and drug testing. QDs also provide a versatile nanoscale scaffold to develop multifunctional nanoparticles for siRNA delivery and imaging. RNA interference (RNAi) is a powerful technology for sequence-

specific suppression of genes, and has broad applications ranging from functional gene analysis to targeted therapy (23). Gao *et al.* have recently fine-tuned the colloidal and chemical properties of QDs for use as delivery vehicles for siRNA, resulting in highly effective and safe RNA interference, as well as fluorescence contrast (24). Future trends of QDs are disease fluorescent activated cell sorting analysis (FACS), microarrays, biosensing, drug delivery and treatment (25-27).

3.3 Aquasomes (carbohydrate-ceramic nanoparticles)

Aquasomes are the nanobiopharmaceutical carrier system contains the particle core composed of nanocrystalline calcium phosphate or ceramic diamond, and is covered by a polyhydroxyl oligomeric film. Aquasomes are spherical 60–300 nm particles used for drug and antigen delivery. Properties like protection and preservation of fragile biological molecules, conformational integrity, and surface exposure made it as a successful carrier system for bioactive molecules like peptide, protein, hormones, antigens and genes to specific sites (28).Three types of core materials are mainly used for producing aquasomes: tin oxide, nanocrystalline carbon ceramics (diamonds) and brushite (calcium phosphate dihydrate). Calcium phosphate is the core of interest, owing to its natural presence in the body. The brushite is unstable and converts to hydroxyapatite upon prolong storage. Hydroxyapatite seems, therefore, a better core for the preparation of aquasomes. It is widely used for the preparation of implants for drug delivery (29). Khopade *et al.* reported haemoglobin loaded aquasomes using hydroxyapatite core as potential artificial oxygen carrying system (30). Conformational integrity of aquasomes exploited as a red blood cell substitutes, vaccines for delivery of viral antigen (Epstein-Barr and Immune deficiency virus) to evoke correct antibody and as targeted system for intracellular gene therapy. Enzyme activity and sensitivity towards molecular conformation made aquasome as a novel carrier for enzymes like DNAses and pigment/dyes (31, 32).

3.4 Superparamagnetic iron oxide crystals

These entities are usually prepared by the alkaline co-precipitation of appropriate ratios of Fe^{2+} and Fe^{3+} salts in water in the presence of a suitable hydrophilic polymer such as dextran or poly(ethyleneglycol). This yields an iron core of 4–5 nm in diameter, which is hexagonally shaped and surrounded by dextran or poly(ethyleneglycol) molecules (33). Superparamagnetic iron oxide particles and ultra small superparamagnetic iron oxide particles (SPIO and USPIO) have a variety of applications in molecular and cellular imaging. Most of the recent research has concerned cellular imaging with imaging of *in vivo* macrophage activity. According to the iron oxide nanoparticle composition and size which influence their biodistribution, several clinical applications are possible: detection liver metastases, metastatic lymph nodes, inflammatory and/or degenerative diseases. Two compounds in the SPIO family are commercialized for intravenous use: Ferumoxides (Endorem® –

Table 2. Some examples of biomaterial based nanobiopharmaceutical products available in market

Company	**Product Name**	**Material**	**Indication**
*P*Sivida, Perth, Australia	Biosilicon™	Porous silicon	Drug delivery
Innovative Bioceramix,Vancouver, Canada	iRoot® and Bioaggregate®.	White hydraulic bioceramic paste, White hydraulic cement mixture	Permanent dental root canal filling and sealing
Namos, Dresden,	Namodots	Zinc sulphide particles	Laboratory use neither

Germany	ZnS:Cu-1,2 and 5 ZnS:Mn-1,2 and 5	doped with copper and manganese	medical nor pharmaceutical products
3DM, Medical technology, Cambridge, USA	PuraMatrix™	Peptide hydrogel	Tissue regeneration, cell therapies, and drug delivery
Genialab, Braunschwelg, Germany	GeniaBeads®	Hydrogel beads of chitosan	Wound healing
Organogenesis, Canton, USA	Apligraf®	Bilayered collagen gels	Dermal matrix for organogenesis
DePuy Orthopaedics, Indiana, USA	Healos®	Crosslinked collagen fibers coated with hydroxylapatite	Bone graft substitute in spinal fusions
Pfizer, New York, USA	Gelfilm®	Absorbable gelatin implant	Neurosurgery, thoracic and ocular surgery
Thermogenesis, Cordova, USA	CryoSeal®	Fibrin Sealant System	Autologous fibrin sealant
Fidia, Abano Terme, Italy	Hyalgan® and Hyalubrix®	Hyaluronan	Viscoelastic gel for surgery and wound healing
Integra, Wheaton, USA	Integra®	Chondroitin sulfate	Scaffold for dermal regeneration
Biogums, Knowsley, UK	Gelrite®	Gellan gum	A novel ophthalmic vehicle

Europe, Feridex® in the USA and Japan) and Ferucarbotran (Resovist® – Europe and Japan). In both cases, the clinical targets are liver tumours. These nanoparticles are medium-sized and coated with dextran (ferumoxides) or carboxydextran (ferucarbotran) (34). Several USPIO have been investigated in humans for several imaging applications, such as ferumoxtran-10 (dextran) (35, 36), VSOP (citrate) (37), feruglose (pegylated starch) (38) or SHU555C (carboxydextran) (39). The systemic safety of several iron oxide nanoparticles has been evaluated after injection in humans, indicating that these products have a satisfactory safety profile according to standard toxicological and pharmacological tests (40). The chemical toxicity of iron and its derivatives has been studied. Human tissues may contain iron or iron oxides in the form of haemosiderin, ferritin and transferrin. Normal liver contains approximately 0.2 mg of iron per gram and total human iron stores amount to 3500 mg. The total amount of iron oxide for diagnostic imaging (50 to 200 mg Fe) is small compared to the body's normal iron store. Chronic iron toxicity develops only after the liver iron concentration exceeds 4 mg Fe/ gram (41).

4. Non-metal nanobiomaterials

Among many structurally stable non-metallic nanobiomaterials that have been investigated for drug delivery, silica materials with defined structures and surface properties are known to be biocompatible. Silica is often the material of choice to enable the biological use of inorganic nanoparticles (42). Apart from the high stability, chemical versatility and biocompatibility silica nanoparticles are employed for many biomedical applications like artificial implants due to its osteogenic property of composites. Furthermore, silica is used to enhance the biocompatibility of several drug delivery systems, such as magnetic nanoparticles (43), biopolymers (44), and micelles (45). Mesoporous silica microspheres are potentially useful for many non-biological functions; but they are not suitable for important biotechnological and biomedical applications. For example, these materials cannot serve as efficient agents for gene transfection or carriers for intracellular drug delivery because mammalian cells cannot efficiently engulf large particles via endocytosis. Also, mesoporous silica microspheres are within the size window of bacteria and could potentially trigger acute immune response *in vivo* (46). Unique properties of mesoporous silica nanoparticles (MSN) rectified these problems by uniform tunable particle size and pore size, stable and rigid framework, high surface area and large pore volume. Cellular uptakes of molecules are often facilitated by the specific binding between species and membrane-bound receptors. Silica particles are known to have a great affinity for the head-groups of a variety of phospholipids (47). Therefore, the high affinity for adsorbing on cell surfaces leads MSN to endocytosis which is eventually studied *in vitro* by a variety of mammalian cells including cancer (HeLa, CHO, lung, PANC-1), non-cancer (neural glia, liver, endothelial), macrophages, stem cells (3TL3, mesenchymal) and others (46, 48). Impact of other non-metallic nanocomposites such as selenium and sulfur with cadmium and zinc exhibits key role in biological labeling (49). It is reported that growth of a CdS/ZnS graded shell on CdSe rods increased the quantum efficiencies (50) which has wide range of application in nanbiosensors.

5. Carbon nanobiomaterials

5.1 Carbon nanofibers

Carbon nanofibers (CNFs) are 3-150 nm diameter carbon fibers arranged as stacked cones, cups or plates. Carbon nanofibers with graphene layers wrapped into perfect cylinders are called carbon nanotubes. CNFs have moderate electrochemical properties and incorporating CNF into polymers is easier because the fibers are large. CNFs have multiple concentric nested tubes with walls angled 20º to the longitudinal axis. CNFs are similar to large diameter multi walled nano tube (MWNT), CNFs are not continuous tubes and their surfaces show steps at the termination of each tube wall. The nanofibers include PR-24 (~65 nm diameter) and the PR-19 (~130 nm diameter). The PR-19 have a chemical vapor deposition (CVD) layer with a turbostratic carbon layer parallel to the surface and these fibers may be more robust to breakage, but the electrical properties of the nanofiber are changed by the coating. The PR-24 does not have a CVD coating. There are low and high density variations between these two nanofiber types (51). Minute nanofibers of various structures and chemistries are formed through simple self association and organization of peptides and proteins (52). Several bioactive extracellular protein domains have been identified that can be incorporated as small peptides into nanofibers through simple modification of the peptides amino acid sequence. Nano fibers can be designed to present these peptide sequences at high density (53). Three dimensional (3D) macroscopic gels like solids can also present high densities of such bioactive peptides. Ji *et al.* studied the *in vivo* wound healing of diabetic ulcers using electrospun nanofibers immobilized with human epidermal growth factor (EGF) (54). Extensive application of carbon nanofibers were studied by nanoreinforcement of polymer composites (55), catalyst supports (56-58), chemical/biochemical sensing (59), neural and orthopedic implants (60), hydrogen-storage material (61) and as anode materials in lithium batteries (62).

5.2 Carbon nanotubes

The carbon nanotubes also have electrical conductivity or semiconductivity, and high thermal conductivity in the axial direction (63). The discovery of Multi-Wall Carbon Nanotubes (MWNTs) by Iijima (64) and the C60 fullerene and single wall carbon nanotubes (SWNTs) by Benning *et al.* (65) opened the possibility for a new class of smart materials based on nanoscale materials. Structural and electrical characteristics of CNTs make them promising for developing unique and revolutionary smart composite materials. In addition, unlike other smart materials, CNTs have high strength as well as high thermal and electrical conductivities, and 'therefore' can provide structural and functional capabilities simultaneously, including actuation, sensing, and generating power (66, 67). CNTs are considered ideal materials for several applications, ranging from ultra strong fibers to field emission displays. CNT have generated great interest in biology, where suitably modified CNTs can serve as vaccine delivery systems (68) or protein transporters (69). CNT as a template for presenting bioactive peptides to the immune system (70), *f*-CNT as the high propensity to cross cell membranes, which perforate and diffuse through the lipid bilayer of plasma membrane without inducing cell death (71). Dai *et al.* reports that the signal wall carbon nanotubes (SWNTs) as DNA transporters and NIR photothermal agents by receptor-mediated endocytosis (72). It demonstrates that using folic acid functionalized SWNTs to selectively kill cancer cells because of the NIR light heating effect of SWNTs. The transporting capabilities of CNTs with suitable functionalization chemistry and the intrinsic optical properties can open up exciting new venues for multifunctional nanomedicines combining drug delivery and cancer therapy. Exploitation of CNT in the field of pharmaceutical and chemical biology can be found in the literatures (73, 74).

6. Polymeric nanobiomaterials

6.1 Dendrimers

Dendrimers are highly branched class of polymeric macromolecules synthesized via divergent or convergent synthesis by a series of controlled polymerization reactions. These polymer growth starts from a central core molecule and growth occurs in an outward direction. Dendrimers are possible to synthesize amphiphilic products of hydrophobic core inside hydrophilic branching. Multiple functionalizations on the surface of dendrimers made it an ideal carrier for targeted drug delivery (therapeutic and diagnostic agents) and imaging, which are usually at 10 to 100 nm in diameter (75). A polyamidoamine dendrimer that can be synthesized by the repetitive addition of branching units to an amine core (ammonia or ethylene diamine) is an example for multiple functionalization of dendrimers. Polyamidoamine cores can function as drug reservoirs and have been studied as vehicles for delivery of drugs (76), genetic material (77), and imaging probes (78, 79). The first investigational new drug application for a dendrimer-based drug was submitted to the US FDA in June 2003, and the first clinical trial under this investigational new drug application was completed in 2004. The drug, named VivaGel (SPL7013 Gel) is a vaginal microbicide designed to prevent the transmission of sexually transmitted infections, including the human immunodeficiency virus (HIV) and genital herpes. A dendrimer can activate many receptors simultaneously as compared with small molecule, which can interact with a single receptor. Due to their size and polyvalent nature dendrimers can leads to new enhanced biological functions (80).

6.2 Polymeric Micelles

Polymeric micelles have been proposed as drug carriers due to multiple potential advantages. They can be used for solubilization, stabilization, and delivery of drugs. Drug delivery system (DDS) associated with nano-micelles could control an appropriate drug release at target sites without the removal from systemic circulation by phagocytic cells (81). These dynamic systems, which are usually below 50 nm in diameter, are used for the systemic delivery of water-insoluble drugs. Drugs or contrast agents may be trapped physically within the hydrophobic cores or can be linked covalently to component molecules of the micelle (82). Most amphiphilic

copolymers employed for polymeric micelle preparation for DDS contain either polyester or a poly (amino acid) derivative as the hydrophobic segment. Poly (lactic acid) (PLA), poly (q-caprolactone) (PCL), and poly (glycolic acid) are all biocompatible and biodegradable polyesters approved by the FDA for biomedical applications in humans. Poly (l-amino acid)s (PAA) commonly used in drug delivery include poly(aspartic acid) (PAsp), poly(glutamic acid) (PGlu), poly(L-lysine) (PLys) and poly(histidine) (PHis). Due to its biodegradability, biocompatibility and structural versatility amino-acid based copolymers are being studied extensively in the filed of drug delivery (83). Maysinger *et al.* conducted exhaustive studies to elucidate the mechanisms governing the cellular uptake of block copolymer micelles (84). Owing to its reduced toxicity and improved biocompatibility polymeric micelles are extensively used for the delivery of anticancer drugs, biomacromolecules and genes for targeted treatment.

6.3 Polysaccharide nanobiomaterials

Natural polysaccharides, due to their outstanding merits, have received more and more attention in the field of drug delivery systems. In particular, polysaccharides seem to be the most promising materials in the preparation of nanometeric carriers. Polysaccharides are the polymers of monosaccharides. In nature, polysaccharides have various resources from algal origin (e.g. alginate), plant origin (e.g. pectin, guar gum), microbial origin (e.g. dextran, xanthan gum), and animal origin (chitosan, chondroitin) (85). Polysaccharides have a large number of reactive groups, a wide range of molecular weight (MW), varying chemical composition, which contribute to their diversity in structure and in property. The early preparation of polysaccharide nanoparticles was by means of covalent crosslinking. Among various polysaccharides, chitosan is the early one to be used to prepare nanoparticles. As a usual crosslinker, glutaraldehyde was ever used to crosslink chitosan based nanoparticles. Recently, some chitosan nanoparticles were still crosslinked by glutaradehyde (86, 87). Unfortunately, the toxicity of glutaraldehyde on cell viability limits its utility in the field of drug delivery. Compared with covalent crosslinking, ionic crosslinking has more advantages: mild preparation conditions and simple procedures. For charged polysaccharides, low MW of polyanions and polycations could act as ionic crosslinkers for polycationic and polyanionic polysaccharides, respectively. To date, the most widely used polyanion crosslinker is tripolyphosphate (TPP). Alonso *et al.* (88, 89) first reported TPP crosslinked chitosan nanoparticles in 1997. TPP is non-toxic and has multivalent anions. It can form a gel by ionic interaction between positively charged amino groups of chitosan and negatively charged counterions of TPP. From then on, TPP-chitosan nanoparticles have been widely used to deliver various drugs and macromolecules.

Recently, water-soluble chitosan derivatives were also be ionically crosslinked to prepare nanoparticles. Compared with chitosan itself, its derivatives can easily dissolve in neutral aqueous media, avoiding the potential toxicity of acids and hence protecting the bioactivity of loaded biomacromolecules. Xu *et al.* (90) synthesized water-soluble chitosan derivative, N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride by the reaction between glycidyl-trimethyl-ammonium chloride and chitosan. Polyelectrolyte polysaccharides can form polyelectrolyte complexation (PEC) with oppositely charged polymers by intermolecular electrostatic interaction. Polysaccharide-based PEC nanoparticles can be obtained by means of adjusting the MW of component polymers in a certain range. In theory, any polyelectrolyte could interact with polysaccharides to fabricate PEC nanoparticles. However, in practice, these polyelectrolytes are restricted to those water-soluble and biocompatible polymers in view of safety purpose. In this sense, chitosan is the only natural polycationic polysaccharide that satisfies the needs (91). Some polysaccharides bearing carboxylic groups on molecular chains can be crosslinked by bivalent calcium ion to form nanoparticles. You *et al.* (92) prepared Ca-crosslinked alginate nanoparticles by water-in-oil reverse microemulsion method. To examine the potency of the nanoparticles for gene delivery, green fluorescent protein-encoding plasmids were encapsulated in the nanoparticles to investigate the degree of endocytosis by NIH 3T3 cells and ensuring transfection rate. Results showed that Ca-alginate nanoparticles with an average size around 80 nm in diameter were very efficient gene carriers. Zahoor *et al.* (93) also prepared Ca-alginate nanoparticles (235.5±0 nm in size) by ion-induced gelification. Drug encapsulation efficiencies in the nanoparticles were 70–

90% for isoniazid, pyrazinamide and 80–90% for rifampicin. In addition, Kim *et al.* (94) encapsulated retinol into chitosan nanoparticles and reconstituted it into aqueous solution for cosmetic and pharmaceutical applications. Upon reviewing the promising results of polysaccharide based nanobiomaterials it evinced the versatility of nanoparticle carriers in the field of drug delivery.

7. Lipid nanobiomaterials

7.1 Liposomes

Liposomes are spherical vesicles with various targeting ligands attached to their surface allowing for their surface-attachment and accumulation in pathological areas for treatment of disease. These vesicles are form on hydration of amphiphilic phospholipids above their transition temperature, which self-associate into bilayers to encapsulate the aqueous interior. Based on their size and number of bilayers liposomes are classified into three basic types. Multilamellar vesicles consist of several lipid bilayers separated from one another by aqueous spaces, which are heterogeneous in size ranging from a few hundreds to thousands of nanometers in diameter. On the other hand, both small unilamellar vesicles (SUVs <100nm in dm) and large unilamellar vesicles (LUVs >100nm in dm) consist of a single bilayer surrounding the entrapped aqueous space. Depends on the physicochemical characteristics of the drug it can be either entrapped in the aqueous space or intercalated into the lipid bilayers of liposomes (95). Liposomes can be classified in terms of composition and mechanism of intracellular delivery into five types: conventional liposomes, pH-sensitive liposomes, cationic liposomes, immuno liposomes, and long-circulating liposomes. Due to their high degree of biocompatibility liposomes are potentially applied in the field of drug delivery. A variety of therapeutic and diagnostic agents are delivered through liposomes in order to enhance the activity and to reduce the toxicity of active ingredients. Depending upon the drug nature, materials are used in the liposomal formulation. The commonly used materials includes phospholipids, glycosphingolipids, sterols, metabolic fate of bilayer forming lipids, synthetic phospholipids, polymer bearing lipids, cationic lipids, a variety of other lipids and surfactants (96). Cationic liposomes were studied as non-viral carrier for genes (97), intracellular delivery of hydrophilic molecules (nucleic acids, messenger RNA, peptides and proteins) (98). The formation of the liposome complexes to their intracellular delivery, will lead to the design of better adapted non-viral vectors for gene therapy applications. Potential review of drug carried liposomes for disease treatment and as a gene carrier can be obtained from the literatures (99, 100).

7.2 Lipopolyplexes/Polyplexes

Delivery of macromolecules such as proteins and DNA to their site of action at the desired rate is challenge because of their transport through compartmental barriers (e.g., endothelium or epithelium) in the body is inefficient and/or they are readily metabolized (33). Lipopoplexes/polyplexes are the pharmaceutical carrier system designed for the site specific delivery or controlled release of such macromolecules. Spontaneous functionalization of nucleic acids and polycations or cationic liposomes (or polycations conjugated to targeting ligands or hydrophilic polymers) leads to the formation of lipopolyplexes/polyplexes and are used in transfection protocols. Composition and charge ratio of nucleic acid to that cationic lipid/polymer determines the shape, size distribution and transfection capability of these complexes. Polycations that have been used in gene transfer/therapy protocols include poly-L-lysine, linear- and branched-poly (ethylenimine), poly (amidoamine), poly-β-amino esters, and cationic cyclodextrin (101). Polyplexes have the obvious advantage of compressing macromolecules to a relatively small size. This can be crucial for gene transfer, as small particle size may be favorable for improving transfection efficacy. The addition of polycations, i.e. poly (L-lysine) and protamine, as co-polymer can markedly enhance the transfection efficiency of several types of cationic liposomes by 2–28 fold in a number of cell lines *in vitro* (102) and *in vivo* (103).

7.3 Solid lipid nanoparticles

Solid lipid particulate systems such as solid lipid nanoparticles (SLN), lipid microparticles (LM) and lipospheres have been sought as alternative carriers for therapeutic peptides, proteins and antigens. The research work developed in the area confirms that under optimized conditions they can be produced to incorporate hydrophobic or hydrophilic proteins and seem to fulfill the requirements for an optimum particulate carrier system. Proteins and antigens intended for therapeutic purposes may be incorporated or adsorbed onto SLN, and further administered by parenteral routes or by alternative routes such as oral, nasal and pulmonary (104). Since their first description by Müller *et al.* (105), SLN have attracted increasing attention as an efficient and non-toxic alternative lipophilic colloidal drug carrier prepared either with physiological lipids or lipid molecules used as common pharmaceutical excipients. Two main production techniques were then established: the high-pressure homogenisation described by Müller and Lucks (106) and the microemulsion-based technique by Gasco (107). Unlike most polymeric microsphere and nanoparticle systems, SLN production techniques do not need to employ potentially toxic organic solvents, which may also have deleterious effect on protein drugs. The solvent evaporation method is a widespread procedure for the preparation of polymeric microspheres and nanoparticles, being firstly used for SLN preparation by Sjöström and Bergenståhl (108). In another attempt to produce insulin-loaded solid lipid microparticles, Trotta *et al.* (109) used an o/w emulsion–diffusion method, obtaining an encapsulation efficiency of about 80%. The solvent displacement technique (110) was also successfully applied to the preparation of gonadorelin-containing SLN. Recently, very attractive new techniques based on supercritical fluid (SCF) technology have been studied with reference to solid lipids; the viability of using the particles from gas saturated solutions (PGSS) process to obtain spherical hydrogenated palm oil-based solid lipid microparticles, for prolonged release of hydrophilic drugs such as theophylline was demonstrated by Rodrigues *et al.* (111, 112). Extensive review of preparation techniques, physiochemical characterization, and drug delivery of SLN containing peptides, peptides, vaccines and targeted brain drug delivery can be found in the literatures (113,104).

8. Virus based nanobiomaterials

A variety of viruses have now been developed for nanotechnology applications including biomaterials, vaccines, chemical tools, imaging, and molecular electronic materials (114). There are a number of advantages for using viruses as scaffolds for designing multivalent imaging sensors. Similar to viruses in general structure are the protein cages, which are multivalent, self-assembled structures e.g. ferritin. Some examples include cowpea mosaic virus (CPMV), cowpea chlorotic mottle virus (CCMV), vault nanocapsules, hepatitis B cores, heat shock protein cages, MS2 bacteriophages, and M13 bacteriophages were used as platform technologies for diagnostic imaging (115). A significant advantage of viral platforms as opposed to other backbones is the large number of targeting molecules or peptides that can be presented within the context of a defined environment with control over the spacing and orientation of the ligands (116). In addition, the constraints of self-assembly ensure a lack of morphological polydispersity within the population of particles that can be difficult to achieve using synthetic methods. The ability to develop viruses like CPMV for nanobiotechnology purposes arose from the technology to express replicating viruses from their natural genomes. As a comovirus, the CPMV genome is encoded by two separate positive-sense RNAs, which are encapsidated in separate particles. Several studies had been published on the use of CPMV particles for vaccine applications, little was known about how the particles behaved *in vivo*, e.g. their bioavailability, biocompatibility and toxicity. The biocompatibility of CPMV *in vivo* suggested that it would be of value for use in *in vivo* imaging applications. The ability to sensitively image the vasculature during development and disease states such as tumorigenesis and atherosclerosis is of great value to reduce the risks that exist with invasive procedures used for diagnosis and treatment such as biopsy or cardiac catheterization. Thus there is tremendous interest in developing novel tools for imaging *in vivo* (117). Studies have been performed using CCMV particles for development of image-contrast agents. In these studies the CCMV coat protein was expressed in the yeast *Pichia pastoris* and cages were re-assembled from the purified coat protein. CCMV has a metal binding domain in the virus interior that was previously shown to bind to Terbium (III) (118). The particles were shown to also bind Gd^{3+} to generate paramagnetic nanoparticles with relaxivity measurements that were between 5 and 10-fold higher than those reported for Gd^{3+}-albumin or Gd^{3+}-dendrimers (119). Application of VNPs in tumor targeting was studied using canine parvovirus (CPV). A member of the family *parvoviridae* that includes the gene-delivery vehicle adeno-associated virus (AAV), CPV is a natural pathogen of dogs (120). Flenniken *et al.* reported that the heat shock protein (Hsp) cage to

encapsulate doxorubicin (DOX) and potentially release it in the acidic endosomal environment upon cell entry, the (6-maleimidocaproyl) hydrozone derivative of DOX was conjugated to interior cysteines, to create an Hsp–DOX conjugate that was designed to hydrolyze the linker at acid pH (121). These results have yielded a large variety of virus structures and capsid morphologies to work with as materials. Each class of virus is likely to have a specific utility for *in vitro* and *in vivo* applications based upon its surface characteristics, stability, and ease of modification. The ability to understand the structure, dynamics and *in vivo* behavior of virus particles promises to further their use for biomedical nanotechnology applications (117).

9. Miscellaneous nanostructures as nanobiopharmaceuticals

Depends upon the nanostructures the properties of the biomaterial differs and exploited for various applications in the field of biomedical sciences. Nanoshell particles represent one of the most interesting areas of material science because of their unique combined and tailored properties for several applications (nonlinear optics, catalysis, and surface-enhanced Raman scattering (SERS). Metal nanoshells are a new class of nanoparticles consisting of dielectric cores with metal shells. Silica is a good candidate to prepare core–shell nanoparticles and silica nanoparticles emerge particularly as a suitable matrix due to their surface functionality. Distinct property of gold nanoshells (GNSs) due to the localized surface plasmon resonance (LSPR) in the near-infrared (NIR) region of the spectrum, it is used as an effective signal transduction through tissue and whole blood. GNS SAMs were used as a novel optical biosensor for real-time detection of streptavidin–biotin interactions in diluted human whole blood within short assay time, without any sample purification/separation (122). Ring-shaped nanostructures are also particularly attractive for biosensing applications due to their ability to contain high volumes of molecules and provide uniform electric fields inside the cavity (123). Despite of unique surface plasmon resonance rod shaped nanostructures have wide range of application in molecular diagnostics and photothermal therapy. Especially the gold nanorod with an intense surface plasmon band affords absorption, fluorescence, and light scattering in the near infrared region. Near-IR inducing two-photon luminescence made it a viable role as multimodal agents in tumor imaging and photothermal therapy. Gold nanorod stabilized by hexadecyltrimethyl ammonium bromide (CTAB) induces strong cytotoxic effect, which limits the bioscience application of gold nanorod. To rectify this, surface nanorod with phosphatidylcholine (PC), i.e., CTAB was extracted from the nanorod solution into the chloroform phase containing phosphatidylcholine (124). Niidome *et al.* reported that PEG-modified gold nanorod has nearly neutral surface and little cytotoxicity *in vitro* (125).

Self assembly of nanoparticle into nanoclusters has recently been suggested to realize conventional delivery methods at the tumor site as well as in individual cancer cell. Formation of primary nanoclusters by incorporating nanoparticles into a carrier vehicle such as liposomes or viruses, which subsequently provide the selective delivery of nanoclusters to the cancer cells, followed by the formation of secondary nanoclusters as the carrier vehicle within the cell (126). Self assembled nanoclusters shows potential for nanodiagnostics of many cellular events at the nanoscale level, such as cell metabolism, intracellular motion, and protein-protein interactions. Synergistic effects of overlapping thermal and bubble formation phenomena around nanoparticles in nanoclusters can also amplify local heat release, which is very important for photothermal therapeutic applications. There has been significant progress in the surface functionalization of gold nanoparticles in the form of monolayer protected gold clusters (MPCs) or mixed monolayer protected clusters (MMPCs) for anti-angiogenic activity. Rotello and his co-workers reported that proteins adsorbed onto the surface of MPCs or MMPCs loose their structural integrity (127). This tends to denature and inhibition of the function of those proteins that are known to induce angiogenesis. In that studies anionically functionalized amphiphilic MMPCs were shown to inhibit chymotrypsin through a two-stage mechanism featuring fast reversible inhibition followed by a slower irreversible process.

Nanocapsules are other promising particulate nanostructures for drug delivery of several pharmaceutical ingredients due to the benefit of common coat layers made up of crosslinked proteins or interfacial polymers, especially acrylic acid derivatives. Coat layers consisting entirely or partially of proteins are of special interest, as they can be designed to be biocompatible and degradable. Ranging in size between 50nm and 240nm, wherein a coat layer separates an inner space from the exterior medium. This property distinguishes nanocapsules from nanospheres; the

latter have a uniform cross-section. Proteins used in building up are structure-forming, but may also be activity-bearing. Such particles are suitable for the inclusion of foreign substances and in binding other components to the surface. Owing to the natural variety of employable proteins, the surface properties are highly variable and can be adapted to meet various requirements (128). These nanocapsules are comprised of branched or hyperbranched polymers and copolymers and have a core-shell structure forming a pocket volume appropriate for complexing and retaining enzymes and other bioactive molecules (129). Sun *et al.* reported the biological application of nanocapsule by optical trapping to position a single carbachol-loaded capsule adjacent to a single Chinese hamster ovary (CHO) cell that has been transfected with muscarinic acetylcholine (M1) receptors, released the carbachol from the capsule with a single 3-ns N_2 laser pulse, and then monitored the subsequent intracellular signaling triggered by the binding of carbachol to the M1 receptors (130). Bionanocapsules (BNCs) is another type of nanocapsules of recombinant yeast-derived hepatitis B virus surface antigen particles, which have been used as a recombinant hepatitis B vaccine for the last 20 years throughout the world, suggesting that BNCs are a safe DDS. If BNCs can be specifically delivered to brain tumors, BNCs might be promising carriers of anti-tumor drugs to brain tumors and be effective for the treatment of brain tumors. Tsutsui *et al.* developed pre-S1 peptide ligand on the surface of BNCs for binding to the human hepatocytes. Therefore, BNCs are not delivered to other tissues, such as the brain. The hybrid BNCs were efficiently delivered to glioma cells but not to the normal glial cells. Moreover, they confirmed the specific delivery of the hybrid BNCs to brain tumors in an *in vivo* brain tumor model. These results suggest that this new approach using BNCs is a promising system for brain tumor-targeted drug delivery (131).

Further nanostructures such as nanowires/nanopillars have been under significant exploitation from semi conducting application to cell transfection, recently Kuo *et al.* reported the aminothiol modified gold nanowires have been used as vectors for the delivery of plasmid DNA into two different types of mammalian cells: 3T3 and HeLa. It was measured that positively charged gold nanowires with a diameter of 200 nm and a length around 5 μm were capable of carrying 1 pg of plasmid DNA per nanowire into cells. Compared with other transfection reagents, the gold nanowires exhibited the highest transfection efficiency while almost no cytotoxicity was observed. In addition, there has been shown that individual nanowires can be visualized with sub-micrometer resolution, which may allow the use of functionalized multi-segment nanowires as local probes for the investigation of the microenvironment inside cells (132). Biomaterial based nanobiopharmaceutical carriers may overcome pharmaceutical issues like solubility, stability, bioavailability and clinical issues for the drug and minimize drug induced side effects. But there could be significant toxicity issues associated with the nanoscale biomaterials themselves, which requires resolution. A number of toxicological reports have demonstrated that exposure to nanotechnology derived particles pose serious risks to biological systems (133). Nanocarrier systems may induce cytotoxicity and/or genotoxicity, whereas their antigenicity is still not well understood. Nanocarrier may alter the physicochemical properties of xenobiotics resulting in pharmaceutical changes in stability, solubility, and pharmacokinetic disposition. In particular, nanocarriers may reduce toxicity of hydrophobic cancer drugs that are solubilized. Nano regulation is still undergoing major changes to encompass environmental, health, and safety issues (134). The fundamental concerns are that NPs are very small, and therefore present a very large surface area relative to the particle volume, or that the surface of manufactured NPs may be reactive. There is at least some speculation that the ability of NPs to generate reactive oxygen species (ROS) at the surface of the NPs, when adjacent to cell membranes, might initiate inflammation reactions or immune responses (135). Table.3 shows a different kind of biological/medical applications of nanobiomaterials and their reported toxicological issues.

10. Perspective of nanobiopharmaceuticals

Advancement of nanoscience and technology has generated a number of novel applications in the field of health care system. Pharmaceutical nanotechnology can address many important health crisis by using nanoscale-structured materials and simple nanobiodevices. Biomaterial based nanobiopharmaceuticals are the valuable tool for both diagnosis and therapy. Our view is that the enhanced stability, tenability, and biocompatibility of materials will ultimately lead to the development of effective carriers for *in vivo* drug delivery, molecular imaging, biosensing, and cellular characteristics. Researchers at Rice University have constructed the “nanocar” composed of four C60 molecules (the wheels), connected by organic molecules (the chassis), the nanocar measures

Table 3. Nanobiomaterials: Medical/biological applications and toxicological issues

Nanobiomaterials	Medical/biological applications	Toxicological issues
Metal nanobiomaterials		
Multifunctional metal nanoparticles	Photothermal therapy (10), contrast agents for tumor imaging (11), delivery of biomolecules, (proteins, peptides, nucleic acid) therapeutics, biosensor, etc. (14, 15)	Nanocopper cause pathological damage to the mice liver, kidney, and the spleen (136), nanoscale TiO_2 dots produced transient inflammatory and cell injury effects at 24 h post exposure in rats, dose dependant Ni inhalation caused increased lung weight and degeneration of alveolar macrophages in rats, inhalation of ultra fine cadmium oxide (40nm) cause multifocal alveolar inflammation in rats (135)
Quantum dots	Optical coding in gene expression studies, high throughput screening, *in vivo* imaging (33)	Bare CdTe QDs directly prepared in aqueous phase are severely toxic to K562 cells (137), Cd^{2+} ions and the surface chemistry of QDs were responsible for the cytotoxicity of QDs
Aquasomes (carbohydrate-ceramic nanoparticles)	Delivery of xenobiotics and antigens (33)	CeO_2 nanoparticles cause ROS on A549 cells and decrease cell viability, membrane damage due to increased production of MDA and LDH (indicators of lipid peroxidation) (138)
Superparamagnetic iron oxide crystals	*In vivo* and *in vitro* diagnostic procedures (33)	USPIOs with cross linking agent epichlorohydrin, is classified as a carcinogenic, mutagenic and reprotoxic substance (40)
Non-metal nanobiomaterials		
MSN	Intracellular controlled release delivery (46-48)	Silica nanoparticles below 100 nm induce cytotoxicity (139)
Selenium nanocomposites	High photoconducting, xerography, nano-building block for nanomedicine (133)	High dose of selenium nanocomposite pronounced oxidative stress, greater liver injury, and prominent retardation of growth, inhibiting superoxide dismutase activities (140)
Carbon nanobiomaterials		
Carbon nanofibers	Biopolymer composites (55), biocatalysts (56-58), chemical/biochemical sensing (59), neural and orthopedic implants (60)	Exposure of human keratinocytes to insoluble carbon nanofibers was associated with oxidative stress and apoptosis (33)
Carbon nanotube (MWNTs, SWNTs and carbon nanohorns CNHs)	Synthetic transmembrane pores, antimicrobial surface coatings, nanowire biosensors (134), delivery of drugs to specific cells, carriers to	MWNTs with metal trace impurities release the reactive oxygen species (ROS) and decrease the mitochondrial membrane potential on A549 cells (134), Pharyngeal aspiration of SWCNTs caused

	improve controlled drug release, and adjuvants for vaccine delivery, diagnostic, and potential nanofluidic drug delivery devices (74)	increased inflammation and cell damage (135)
Polymer nanobiomaterials		
Dendrimers	Delivery of therapeutic (chemotherapy, photodynamic therapy, boron neutron capture therapy) and diagnostic agents (molecular probes, X-ray and MRI contrast agents), site selective catalyst (33, 140)	Amine terminated dendrimers exhibits cytotoxicity and haematotoxicity, where primary amines are more toxic than secondary or tertiary amines. As with PAMAM, PPI dendrimers exhibit a generation-dependent haemolytic effect on blood cells, with high generation dendrimers having the most haemolytic activity (141)
Polymeric micelles	Systemic delivery of water-insoluble xenobiotics (33)	Differential gene expression after delivery of micelles-carrying cisplatin has been reported in certain cells which resulted in induced cell death via apoptosis or necrosis (135)
Polysccharide nanobiomaterials	Biocompatible, biodegradable, highly stable, safe, non-toxic for variety of drug delivery (hemoglobin, anti-cancer agents, vitamins, lipids, fatty acids, amino acids, etc.) (91)	Polycationic formulations have been described to affect cell proliferation, differentiation, and pro-apoptotic genes in human epithelial cells (142)
Lipid nanobiomaterials		
Liposomes	Specific delivery of xenobiotics (33)	PEG-grafted liposome infusion was described to trigger non-IgE-mediated signs of hypersensitivity (135)
Lipopolyplexes/polyplexes	Transfection, gene therapy, gene transfer	Limited efficiency of delivery and gene expression (as compared to viral vectors), toxicity at higher concentrations, potentially adverse interactions with negatively charged macromolecules present in serum and on cell surfaces, and impaired ability to reach tissues beyond the vasculature unless directly injected into the tissue (143)
SLNPs	Targeted brain drug delivery, retinal gene therapy (144)	Higher amounts of negatively charged SLNPs (20 μg/mL) or positively charged SLNPs significantly increased the cortical cerebrovascular volume pointing out a BBB disruption (113)
Virus nanobiomaterials	Tissue-specific targeting and imaging agents *in vivo* (117)	Triggering B-lymphocytes to produce virus-specific antibodies, nanotoxicology is not yet been evaluated detaily (117)

just 3-by-4 nm. This provides support that materials can be moved around in a controlled manner at the nanoscale level using fullerene based technology. Nanocars can be applied to targets and further enhance drug delivery systems (145). The artificial mechanical red blood cell or "respirocyte" (medical nanorobotics) is a blood borne spherical 1- micron diamondoid 1000-atmosphere pressure vessel with active pumping powered by endogenous serum glucose, able to deliver 236 times more oxygen to the tissues per unit volume than natural red cells and to manage carbonic acidity (146). "Microbivore" an artificial mechanical white cell of microscopic size, has its primary function to destroy microbiological pathogens found in the human bloodstream using a digest and discharge protocol (147). If the efforts of pharmaceutical nanobiotechnology directed towards the nanomedicine, then the obstacle for delivering peptides and protein medications of limited bioavailability, inadequate stability, immunogenicity and limited permeability can be eradicate. Thus, introduction of nanotechnologies into pharmaceutical sciences and material will tremendously extend their everlasting functions in the nearest future.

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