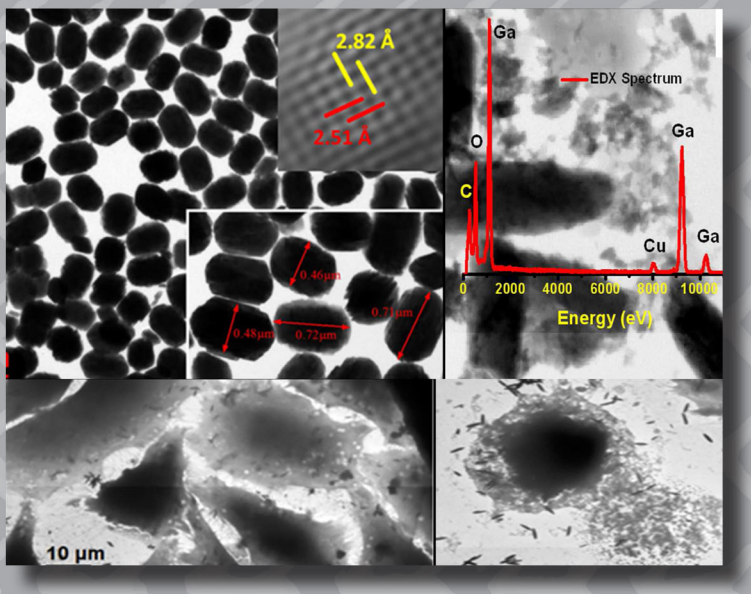
Nanomaterials in Bio-Medical Applications

A Novel approach



Edited by
Bichitra Nandi Ganguly

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The book presents new results in the areas of nanomaterials, nanoparticles, ultra-small nanoparticles, plasmonic nanoparticles and coated nanoparticles for bio-medical applications. Emphasis is placed on (1) synthetic routes (quantum dots, thermal decomposition methods), (2) characterization methods (photo-physical techniques, X-ray diffraction, electron microscopy, light scattering, positron annihilation spectroscopy) and (3) bio-medical applications (nanomaterials and nanoparticles in physiology, medicine and bio-medicine).

Keywords

Nanomaterials, Nanoparticles, Ultra-Small Nanoparticles, Plasmonic Nanoparticles, Coated Nanoparticles, Bio-Medical Applications, Quantum Dots, Thermal Decomposition Methods, Photo-Physical Characterization, X-Ray Diffraction, Electron Microscopy, Light Scattering Characterization, Positron Annihilation Spectroscopy, Capping Ligands, Surface Ligands, Passivating Agents

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Table of Contents

Preface

The nanoworld opens up a wonder world for material scientists and the bio-medicine field. *Nanomaterial* is defined as a "material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale", with *nanoscale* defined as the "length range approximately from 1-100 nanometres (nm)". This includes both *nano-objects*, which are discrete pieces of material, and *nanostructured materials*, which have internal or surface structure on the nanoscale; a nanomaterial may be a member of both these categories. Similarly, '*Nanoparticles'* are typically particles between 1-100 nm in size with a surrounding interfacial layer. The interfacial layer is an integral part of nanoscale matter, fundamentally affecting all of its properties. The interfacial layer typically consists of ions, inorganic and organic molecules. Organic molecules coating inorganic nanoparticles are known as stabilizers, capping and surface ligands, or passivating agent.

Both could be synonymous and their properties are greatly different from bulk materials. The newly emerging world in this domain also goes down to ultra small-nanoparticles and plasmonic nano particles, defining their properties which are very fascinating and drastically different from the common chemistry or physical science properties of the bulk materials. This fact exactly makes them special, as the same opens up a huge application potential.

Such properties are being utilized everywhere, because the surface atoms and the greatly enhance surface area in such particles hold many promises. Other size-dependent property changes include quantum confinement in semiconductor particles, surface plasmon resonance in some metal particles and superparamagnetism in magnetic materials. Accordingly, uses have been manifold already, for example in electro-chemistry, surface science, optics, paint industry, as catalysts in solar light harvesting agents and also in toxicology and in biomedical fields.

In this book, comprising of articles from different aspects of nano-materials and their application in bio-medical field has been highlighted, keeping in mind the surface conjugation properties of such nano-structures. The new developments and research in material science with an insight to use the nano-species in biomedicine is such an interface of the subject where the investigators face many challenges. The application of such materials in bio-medicine however requires a directed design providing actuation and stability in a particularly complex environment such as living organisms. Nanotoxicity and *in-vivo* clearances are some of limiting factors in the radiological tests such as PET/SPECT, etc.

As far as possible, the topics have been chosen with care, to suit the interest of the researchers both in material science and bio-medical field. We hope readers will find it interesting.

Bichitra Nandi Ganguly

SINP, KOLKATA, INDIA

June, 2018

Chapter 1

Introduction to Nano-Materials in Bio-Medical Applications: A Novel Approach

Bichitra Nandi Ganguly
Saha Institute of Nuclear Physics, Kolkata -700064, India
bichitra.ganguly@saha.ac.in

Nanoparticles (NPs) have evolved as novel and valuable functional building structures, notably being considered as one of the most relevant recent achievements in materials science. With an ever growing interaction between interface-research on nanoparticles and biomedicine, materials scientists are required to encounter new and exciting challenges both in the design and engineering of the material considering its targeted application as shown below in Figure 1.

A wide variety of nanoparticles are being used for active biological research and application [1-7], such as semiconductor quantum dots which not only initiate photon induced surface chemical reactions, but are also useful alternative fluorescent labelled compounds. Also, iron oxide NPs have been approved for the use in humans in magnetic resonance imaging (MRI) applications as contrast enhancers. A further novel use in current research is that of plasmonic nanoparticles due to their unique feature, i.e. displaying localized surface plasmon resonance bands in the UV-visible to near infrared spectral range [8].

Using well-established concepts and methods [9], while assessing the uniqueness of biomedical questions, nanoparticles have been used to develop ultrasensitive probes for the dreaded HIV infection and cancer, among others diseases [10,11]. New discoveries and directions using nanoparticles include theranostics and plasmonic photothermal therapy (PPT), depending up on understanding the destination of the nanoparticles. Once they are administered *in vivo*, it becomes a crucial aspect, that is under thorough investigation [12]. Core concepts of materials science that have led to novel and exciting applications in biomedicine are highlighted in this book.

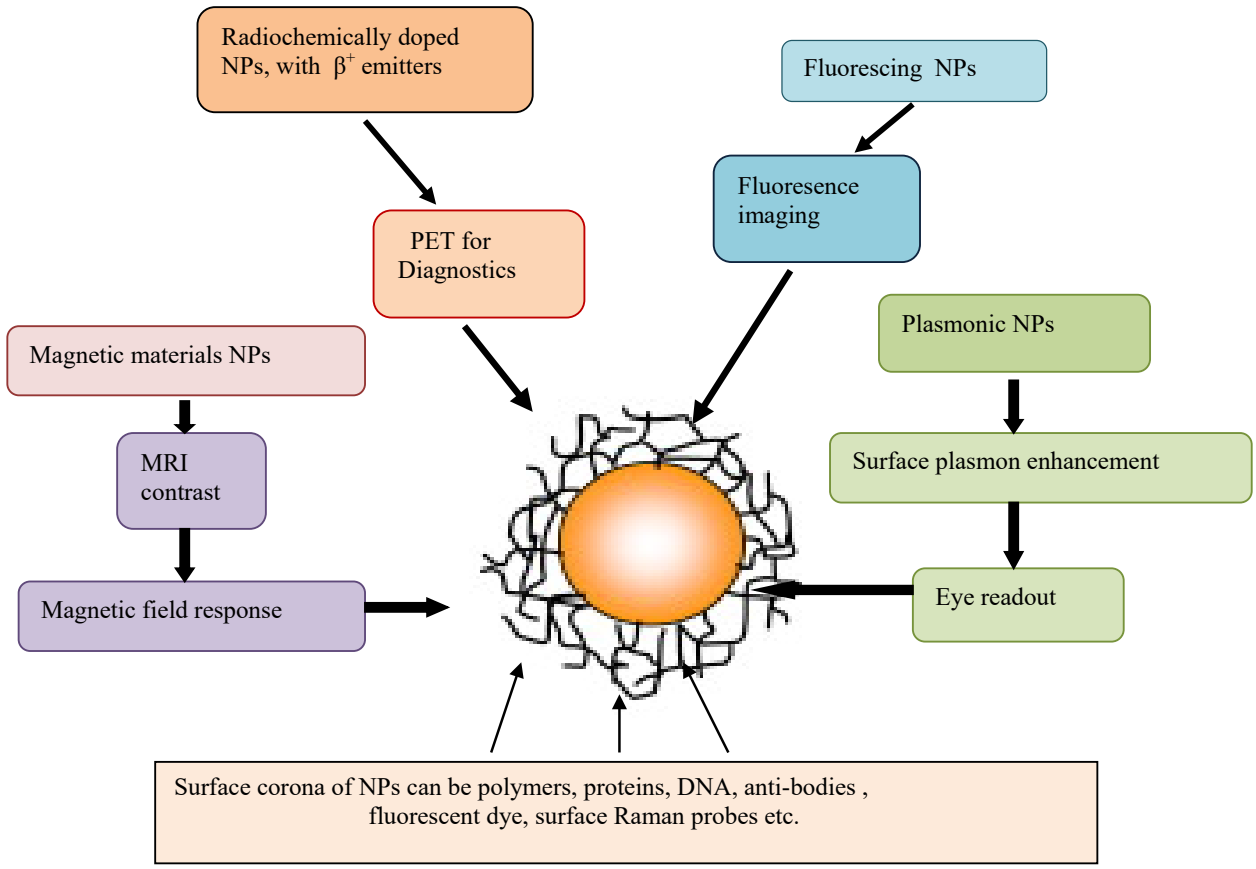

Figure 1. Functionalization, surface modification and manifold applications of nanoparticles (NPs) in biomedicine. NPs in biomedicine, are conjugated by biologically active molecules which are target specific. This new paradigm connecting nanoparticles with medical diagnostic or therapeutic use is therefore changing the point of view as well as research practices of materials scientists.

The intense interest in this specialized field (nano sized material) is due to the fact that nanotechnology involves controlled synthesis of materials where at least one dimension of the structure is less than 100 nm. This ultra-small size is comparable to naturally occurring proteins and biomolecules in the cell and is notably smaller than the typical diameter (~7 μm) of many human cells. The reduction of materials to nano-scale can frequently alter their electrical, magnetic, structural, morphological, and chemical properties enabling them to interact in unique ways with cells, biomolecules and enable their physical transport into the interior structures of cells. Nanoscale particles typically possess a larger percentage of atoms at the material's surface (see Fig. 2), which can lead to increased surface reactivity [13], and can maximize their ability to be loaded with therapeutic agents to deliver them to the target cells. As a simple illustration, one may

take note of the following: in case of ZnO nano particles, from the size effect shown, the agglomeration number (n) of the molecules in the case of each samples can be explained through simple relationship (assuming the small crystallites are roughly spherical for a minimal surface to volume ratio):

$n = 4/3\pi\, r^3\, \rho\, (N_A/M)$

where, the density of ZnO (ρ) = 5.606 gm/cm3, N_A is the Avogadro's number, molecular weight (M) = 81.389 gm/mole as shown in Table-1.

But there can be still smaller sizes of nano particles, like ultra small nano particles (USNPs), by definition, the core size of USNPs range from 1 to 3 nm with the majority of its atoms located at the surface, both the specific surface area and the number of atoms at the surface increase drastically when the core diameter decreases towards the ultra-small range (Fig. 2). For instance, more than 70% of the atoms forming a 2-nm USNP could be located on its surface.

Table-1 A simple illustration of surface occupancy of the molecules, with the decrease in size of ZnO nanoparticles.

Average grain size, (roughly spherical)	Total no. of molecules = n	Surface /volume ratio	No. of molecules in the surface
40 nm	$4x10^{24}$	0.1	$4x10^{23}$
20 nm	$2x10^{24}$	0.3	$6x10^{23}$

This increased surface/volume ratio leads to unique properties that diverge from their microscopic species or from the bulk material itself and renders the molecules to be highly surface active. As a consequence, USNPs demonstrate the variation between small molecules and conventional, larger-sized NPs, not only in terms of size, but also as regards to their physicochemical and pharmacokinetic properties [14]. A variety of physiochemical properties such as size distribution, electrostatics, surface area, general morphology and aggregation may significantly affect physiological interactions between nanomaterials and the target biological areas.

Some nanoparticles of a particular substance are thought to pose greater risks of toxicity than larger-sized particles of the same substance [15-21]. Above all, the distribution of particles within the specimen body and the accumulation of a specific type of particle in a

particular part of the body, is dependent on the particle's size and surface characteristics, that are considered critical issues.

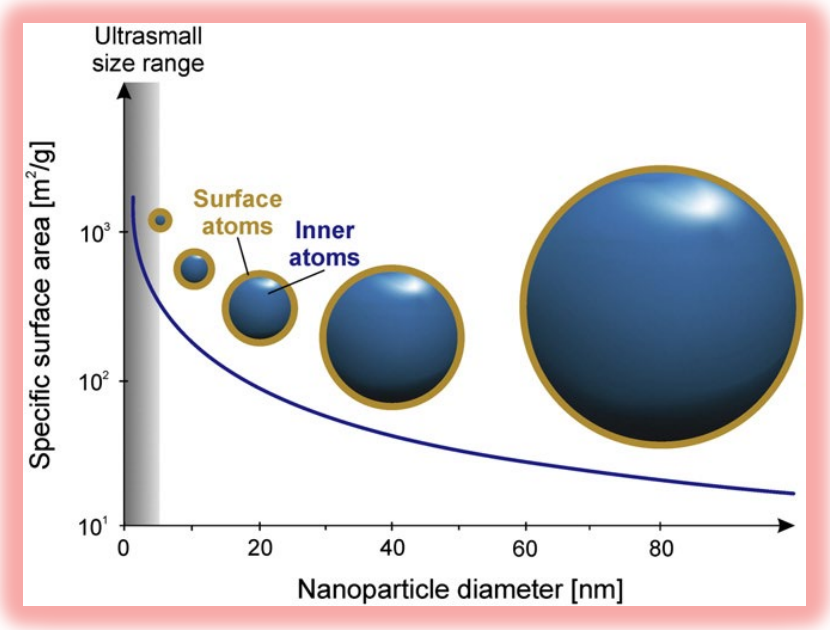

Figure 2. A simple demonstration of increase specific surface area, when the size of NP decreases, the root cause of increasing surface interaction. (ref: Nanomedicine: Nanotechnology, Biology, and Medicine 12 (2016) 1663–1701, copyright Elsevier).

Also, when the nanoparticles accumulate in specimen body system without proper excretion, it can cause continuous toxicity. The main distribution sites and target organs for nanoparticles are unknown. However, it appears that the liver and spleen may be target organs [22,23]. If nanoparticles are ingested, inhaled or absorbed through the skin, they can induce the formation of reactive oxygen species (ROS) including free radicals [24]. ROS produces oxidative stress, inflammation, and consequent damage to various biological materials such as protein, DNA, etc. Besides ROS production, other factors influencing toxicity include size, morphology, agglomeration statue, shape, chemical composition, surface structure, surface charge, aggregation and solubility [25]. As a result of their small size, nanoparticles can cross tissue junctions and even cellular membranes where they induce structural damage to the mitochondria [26,27] or invade the nucleus where they cause serious DNA mutations [28] leading to cell death [29]. The factors mentioned above can be categorized under the five characteristics of nanoparticle, which are: size; surface area; electrostatic statue of surface; morphology; and agglomeration status.

However, application and clinical administration of these particles *in vivo* requires a precondition for their rapid elimination through the system. In other words, not all USNPs can *per se* be cleared renally, as their surface charge, shape and surface composition influences their pharmacokinetics in addition to their size [30-32]. The widely used term “ultra-small nanoparticles” originating from the field of material science is therefore in no way explicably justified with the pharmacological term “renal excretable nanoparticles”. In fact, their bio-distribution as well as blood clearance depends primarily on their *in vivo* hydrodynamic diameter that can be substantially larger than their *in vitro* diameter due to the unspecific adsorption of serum component including proteins and lipids. Thus, formation of a bio-molecular corona has been observed for a wide range of different NP platforms. As a result, many nano-sized objects are scavenged by the mononuclear phagocyte system (MPS) and adequate surface modifications need to be made to counter this issue and to render NPs more suitable for bio-medical applications.

The reactivity (and potential risks) posed by inorganic nanoparticles in biological environments depends on their physical and chemical state. Biological fluids such as blood, mucus, cell culture media, and others contain a large variety of substances that can interact with and modify nanoparticles. Control of interactions between nanoparticles and bio-systems is essential for the effective utilization of these materials in biomedicine. But an useful surface coronation can lead to a beneficial effect. Such as a wide variety of nanoparticle surface structures including small molecules like folic acid, giant sugars and peptides have been developed and suggested for imaging, sensing, and drug delivery applications [33-35] to cancer cells.

Nanoparticles exhibit unique physical properties, but only with proper bio-conjugation and with an appropriate hydrodynamic size, they may be accepted into bio-molecular and cellular systems. These features make them attractive materials for therapeutic and diagnostic applications (see for example Fig.3). However, utilization of these materials in biomedicine requires controlled interactions with bio-macromolecules. For example, specifically designed nanoparticle monolayer structures can impart enhanced cellular internalization ability, noncytotoxicity and improved payload binding capacity, which is therefore necessary for effective intracellular delivery. Similarly, surface functionality can be tuned to provide the selective or specific recognition required for bio-sensing. These bio-sensors are becoming increasingly important in the diagnostic field which will eventually translate into micro-devices, where actually their functional basis relies on molecular level recognition.

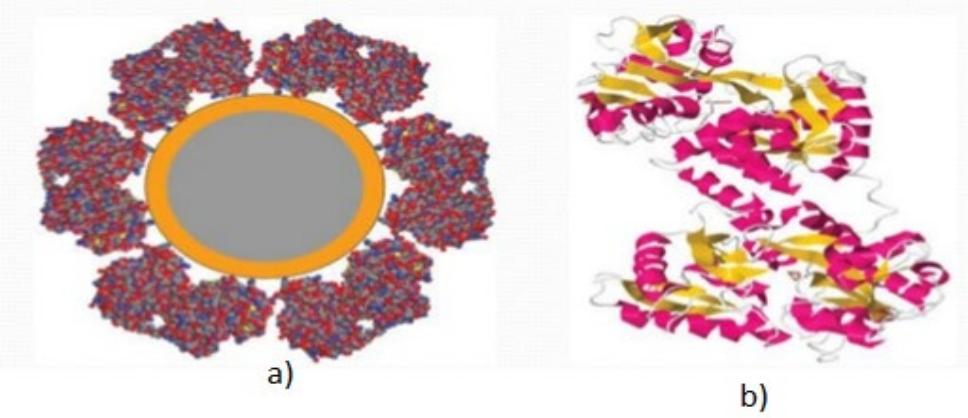

Figure 3. Nano particle with protein corona: cartoon showing: a) polymer coated nanoparticle coronated with protein molecules, b) the protein macromolecule.

As for example, biomolecules such as proteins present in biofluids, entering into contact with the NPs surface, can be adsorbed forming the so-called protein corona. There can be other molecules like giant sugar molecules or other alike soluble polymeric molecules, which may also help to perform the same function [36-38]. The bio-corona may play a key role in the *in vivo* biological fate establishing the bio-identity of the NPs. In the case of nanoparticle protein corona (Fig. 3), the outcome of the absorption of proteins onto the inorganic surface, is one of the most significant alterations. This coating provides a "biological identity" to nanoparticles in those biologically relevant fluids (e.g. commonly used cell culture medium supplemented with serum) [39,40] and determines their interaction within cells, immune systems and other components of biological systems.

As a result, detailed knowledge of the nanoparticle protein corona has emerged as a crucial aspect in understanding their bio-distribution and reactivity of nanoparticles in organisms, for the safe design of the engineered nanoparticles. Differential protein adsorption can potentially lead, for example, to different organ distribution by interacting with different tissue specific receptors [41-43].

Thus through appropriate surface conjugation, these nanomaterials can acquire the ability to selectively target particular types of cells or to pass through physiological barriers and penetrate deep into tumour sites, which has been explained below through illustrations.

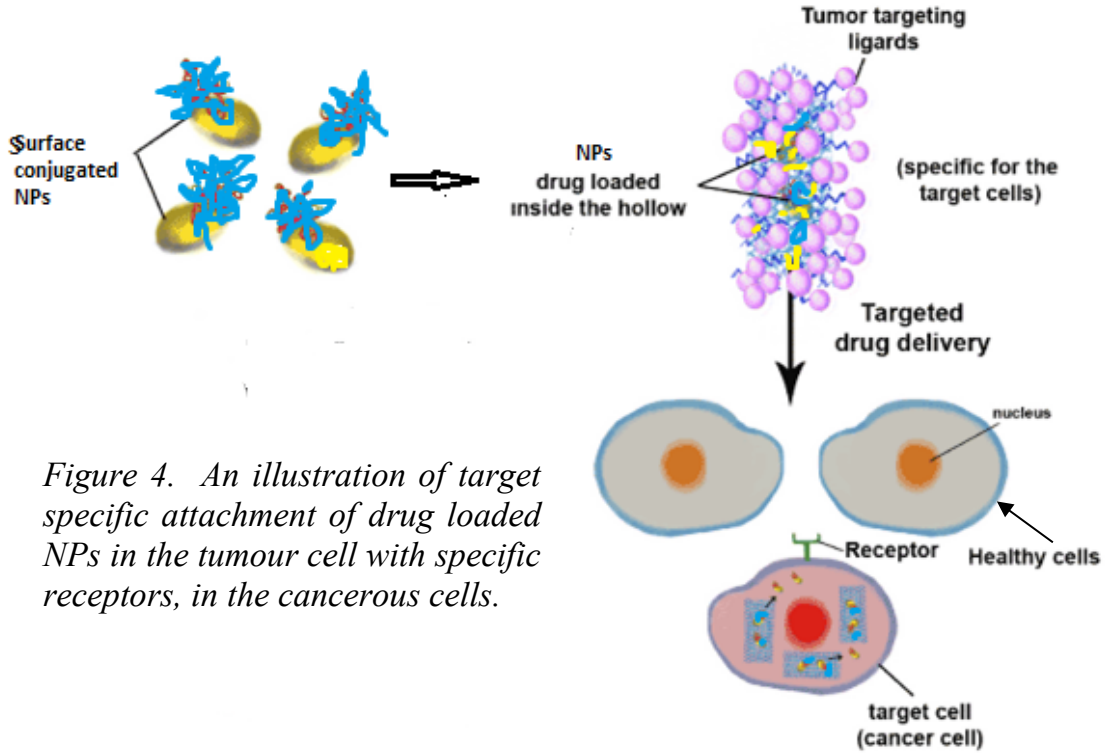

Figure 4. An illustration of target specific attachment of drug loaded NPs in the tumour cell with specific receptors, in the cancerous cells.

It is due to nano-material conjugated surface scaffold, specific pharmacokinetic properties and good tissue penetration, renal excretable USNPs or only some NPs qualify for special purposes for which other NPs with long retention time in the body are not suitable. Such classification include first and foremost their diagnostic application as molecular imaging agents since this requires fast and specific accumulation at target sites within a few hours.

Combined with rapid excretion from non-targeted tissues, this situation allows faster imaging after injection and results in high signal-to-background ratios [44,45]. semiconductor QDs [46-50] and fluorescent dye labeled USNPs [50,-52] have been developed as optical imaging probes. Additionally, some of these ultrasmall nanoparticulate probes have been utilized as intra-operative visualization tools during image-guided surgical and interventional procedures [53,54]. Depending on the attached or incorporated radiolabel, USNPs can be either applied for SPECT [52,55,56] or positron annihilation tomography/PET [57-60] imaging as found in the literature.

Single-photon emission computed tomography (SPECT) is a nuclear medicine tomographic imaging technique using gamma rays. It is very similar to conventional

nuclear medicine planar imaging using a gamma camera. However, it is able to provide true 3D information.

PET on the other hand is based on recording the emission of the positrons by radioactive isotopes within the nanoparticles under study [61]. This technique offers the advantages of a definitely large sensitivity and space-resolving capability. Moreover, provided that an adequate radio-activation reaction is available, PET can in principle be used to follow the bio-distribution of any type of nanoparticles. It has been demonstrated the different bio-distribution of aluminum oxide nanoparticles, as a function of core size, in an elegant application of PET imaging [62]. By radioactive labelling of oxygen atoms in the aluminum oxide nanoparticles, the bioaccumulation of the nanoparticles in different organs could be studied *in vivo*. As noted in that study, the decay time of the radioactive isotope is relatively short, in the range of 1 hour. However, radioactive labelling cannot be used for bio-distribution studies comprising longer periods of time such as days or weeks for dynamic studies on biological pathway of the NPs, here again fast renal clearance of the drug is a foremost requirement.

Summary and conclusions

The intervention of material science in to biomedicine through the surface activity of nano materials have opened a new paradigm, which is under intensive investigation. Researchers have proposed for a variety of medical applications within the last decade, in particular for diagnosis and therapy of cancer, due to the unique properties of these materials. As it stands now, the majority of commercial nanoparticle applications in medicine are geared towards drug delivery. In biosciences, nanoparticles are replacing organic dyes in the applications that require high photo-stability as well as high multiplexing capabilities. There are some developments in directing and remotely controlling the functions of nano-probes, for example driving magnetic nanoparticles to the tumour and then making them either to release the drug load or just heating them in order to destroy the surrounding tissue. The major trend in further development of nanomaterials is to make them multifunctional and controllable by external signals or by local environment thus essentially turning them into nano-devices.

However, of late, boosting research has been devoted to USNPs as these materials display properties, such as size as well as physicochemical and pharmacokinetic characteristics, which are at the interface between molecules and larger particles. Through this introductory approach, a comprehensive overview of the current scenario of preparation, surface modification, characterization and biomedical applications of the most thoroughly investigated and emerging inorganic nano-material platforms has been outlined. Of particular importance while in application, is their potential systemic

clearance via the renal pathway once they possess appropriate surface characteristics and their size is below the kidney filtration threshold. As not all USNPs can *per se* be cleared renally, the pharmacological term “renal excretable nanoparticles” can be used to recommend and to differentiate them in the future from standard NPs as too large for renal elimination. Although a tremendous amount of outstanding research has been published on this particular subset of nanomaterials, a wide variety of issues remain unexplored, unclear or unexplained.

This concerns in particular the interactions of NPs within individual cells including intracellular trafficking and precise targeting of certain cellular organelles e.g. mitochondria. As mentioned before, future research should furthermore shed light on the extent to which ligand-mediated targeting contributes to total NP accumulation at the pathological site. Here primarily a scalable, controlled and reproducible synthesis procedure resulting in defined, highly mono-disperse and uniform products under physiological conditions is an essential prerequisite. This problem commonly faced also for bigger NPs get exacerbated in the ultra-small range where even small differences in size and shape have a tremendous impact on blood circulation time, bio-distribution and elimination of NPs.

Another major issue is related to the characterization: the available techniques to characterize NPs are often not suitable for USNPs, due to instrumental limit of detection. Moreover, a lack of a precise surface characterization, once again lead to deeper consequences for NPs resulting in inconsistent outcomes *in-vivo* and *in-vitro* tests. Perfectly reproducible NPs samples are therefore a challenging goal and in order to achieve this objective, it will be necessary to radically improve the synthesis methods as well as find alternative characterization protocols and methods. Thus, many critical studies addressing the pros and cons of these materials have to be conducted in the future to deploy their full medical potential and to increase the clinical impact of *renal excretable nanodiagnostics and nanotherapeutics.* As of today, there remains immense dearth of proper and precise application of such materials through radiological processes such as theranostics, PET, MRI, etc.

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