



Core/shell nanoparticles in biomedical applications



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ABSTRACT

Nanoparticles have several exciting applications in different areas and biomedical field is not an exception of that because of their exciting performance in bioimaging, targeted drug and gene delivery, sensors, and so on. It has been found that among several classes of nanoparticles core/shell is most promising for different biomedical applications because of several advantages over simple nanoparticles. This review highlights the development of core/shell nanoparticles-based biomedical research during approximately past two decades. Applications of different types of core/shell nanoparticles are classified in terms of five major aspects such as bioimaging, biosensor, targeted drug delivery, DNA/RNA interaction, and targeted gene delivery.

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1. Introduction

Nanotechnology is a multidisciplinary branch of science which encompasses numerous diverse fields of science and technology, ranging from biomedical, pharmaceutical, agricultural, environmental, advanced materials, chemical science, physics, electronics, information technology, and so on. The synthesis, properties, and applications of sub 100 nm materials and devices have contributed immensely to several biomedical fields such as imaging agents, drug delivery vehicle, diagnostic tools, etc. to save human life along with other areas. Biomedical engineering has bridged the gap between biology and conventional medicine by application of engineering skills in surgical diagnosis, monitoring, treatment, and therapy etc. The smaller size and high surface to volume ratio of nanoparticles are the key features which make them useful in the biomedical fields because of the development of many new properties, ease of functionalization, conjugation of biomolecules etc. In recent years, the early diagnosis of various diseases such as cancer, diabetes, stroke, Alzheimer's disease, and so on are the main focuses of biomedical field to save human life. The application of nanotechnology in this field shows further advancement in several specific areas such as drug targeting, bio-diagnostics, bioimaging, and genetic manipulation. The importance of nanotechnology in this field can be evaluated from the attention of various research groups over the last decade. The number of published papers in this field has risen sharply from a handful in the early 1990s to several thousands in the present times. Applications of new nanomaterials have opened many new possibilities for the understanding of in-depth biochemical processes, which are directly responsible for disease diagnostics and treatment. Although many techniques are still in the nascent stage of development, some are actually being employed in daily practices. The surface modification of newly developed nanomaterials is also equally important for several applications such as cellular repair, drug delivery, therapeutic applications, diagnostic aids, etc. [1,2].

1.1. Nanoparticles for biomedical applications

The advent of nanoparticles has opened up new avenues in many different fields of studies along with other nanomaterials [3]. The field of biomedical engineering has also been equally influenced. The major advantages of nanoparticles over larger sized particles are its high surface-to-volume ratio and hence higher surface energy, unique optical, [4] electronic, and excellent magnetic properties [5] and so on. The high surface area also allows it to be modified adequately so as to improve its pharmacokinetic properties, increase vascular circulation life-time, along with improving bioavailability, especially for biomedical applications. The improved properties are a boon in the field of drug delivery. The increased vascular circulation life-time increases the efficacy of the drug; the enhancement of the drug bioavailability means a lot lesser dosage could effectively work instead of bulk drugs. As mentioned before, the most important property which has attracted the attention of researchers worldwide is its ability to have better surface modifications which not only helps in targeted drug delivery but can solve the dual purpose of monitoring of drug release as well. In general, the size dependent properties of nanoparticles (mainly optical, electronic, and magnetic) have been observed to be very much useful for biomedical applications [6,7]. In biomedical applications the magnetic properties are utilized to develop drugs for targeted drug delivery systems, while optical properties are mainly utilized for diagnostic purposes [8]. The magnetic properties of nanoparticles are in general used

for drug delivery as well as MRI contrast agents in place of conventional gadolinium based contrast agents. At the same time, the optical properties are used to find as alternatives to organic dyes for imaging purposes. Other added features of nanoparticles include enhanced target specificity, and permeability across bio-membranes (semi permeable). These properties make nanoparticles an attractive drug delivery vehicle along with the possibility of monitoring drug release. Current research focuses on utilizing the electronic/opto-electric, magnetic and optical properties of these particles in signal detection transmission and amplification. At present instead of simple nanoparticles core/shell structured nanoparticles are used for several biomedical applications because of additional advantages.

Apart from its several advantages, nanoparticles are generally toxic to the living system because of its easy penetration across biomembranes and interference with basal metabolic reactions within the cell. Also these particles are easily translocated throughout the body not only through the circulatory system, but also the neural network (nerve cells). Macrophage clearance, and detoxification processes in the liver and spleen cannot always effectively reduce nanoparticle concentration in the system and hence incurable diseases such as Alzheimer's and Parkinson's may also result [9]. Apart from the toxicity of the used nanoparticles, they also tend to accumulate in the body since the body doesn't have any mechanism to eliminate them from the system or utilizing them for any metabolic pathways. These accumulations could have serious ramification not only in the immediate future but also with the passage of time that could lead to deadly diseases.

1.2. Core/shell nanoparticles

Core-shell nanoparticles have a core made of a material coated with another material on top of it. In biological applications core-shell nanoparticles have major advantages over simple nanoparticles leading to the improvement of properties such as (i) less cytotoxicity [10], (ii) increase in dispersibility, bio- and cyto-compatibility, (iii) better conjugation with other bioactive molecules, (iv) increased thermal and chemical stability and so on [11]. More elaborately, (i) when the desired nanoparticles are toxic which may cause plenty of trouble to the host tissues and organs. The coating of a benign material on top of the core makes the nanoparticles much less toxic and bio-compatible. Sometimes shell layer not only act as nontoxic layer, but also improve the core material property. In the case of semiconductor core/shell nanoparticles the shell of other materials improves the optical property and photo stability. (ii) Hydrophilicity of nanoparticles is very important to disperse them in biological systems (aqueous). The increase in bio-dispersivity, bio- and cyto- compatibility makes it a useful alternative to conventional drug delivery vehicle. The ease of synthesis also plays an important role in attracting the attention of researchers to this class of materials. When the core material is hydrophobic, coat of a hydrophilic material onto core surface in the form of core/shell nanoparticles can overcome the problem of dispersibility and bio- and cyto-compatibility. (iii) Conjugation of biomolecules onto particle surface is very important for many bio-applications. In many cases the material of interest may be difficult to conjugate with a particular type of biomolecules; in that case coating of a suitable bio-compatible material helps to solve this problem. (iv) When the core materials are susceptible to chemical or thermal change during exposure to surrounding environment, coating of an inert material generally enhances the stability of core particles. In this case core/shell nanoparticles are promising for biological applications than single nanoparticles.

1.3. Biomedical applications of core/shell nanoparticles

The core/shell nanoparticles are mainly designed for biomedical applications based on the surface chemistry, which increases its affinity to bind with drugs, receptors, ligands, etc [12,13]. This has led to the synthesis of novel nanoparticles, which in sync with the biological system, compared to bulk material. The biocompatibility and cytocompatibility increases its therapeutic value opening a whole new avenue for the synthesis of novel drug carrier with enhanced properties such as increased residence time, increased bioavailability, and reduction of dosing quantity as well as frequency along with increased specificity. As a specific example, the bio-inspired polymeric coat on hydrophobic drug can facilitate the proper release of the drug at its targeted site because of ion, temperature, and pH specific degradation of the polymer [14–16]. Core/shell nanoparticles are also extensively used for bioimaging as they have good biocompatibility compared to simple nanoparticles [17]. The contrasting ability of core/shell nanoparticles in general originates from the core material. For the core/shell nanoparticles, in general, the shell material is responsible for surface properties such as biocompatibility and conjugation of bioactive materials, because of the presence of reactive moieties on the surface. The shell thickness can be tuned to provide both adequate contrasting properties as contrast agent as well as binding of biomolecules for the purpose of targeted drug delivery, specific binding, biosensing, etc. [18]. A schematic presentation of a core/shell nanoparticle for multipurpose biomedical applications is shown in Fig. 1.

1.4. Scope of the review

The application of nanoparticles in biomedical field is an emerging research area in recent times because of their exciting performance in bioimaging, targeted drug and gene delivery, sensors and so on. It has been also observed that core/shell nanoparticles are more suitable and have shown better performance in biomedical applications than that of simple nanoparticles. Mostly the problems associated with simple nanoparticles can be solved by the use of core/shell nanoparticles. The existing published reviews show they are mainly concentrated on either

biomedical applications of general nanoparticles with a part of core/shell nanoparticles, and based on some specific application or specific nanoparticles. The development of different aspects of core/shell nanoparticles and their applications in brief has been reported recently from our research group [19].

Among several specific materials, polyethylene glycol (PEG) as a shell material of nanostructures can be used in designing of novel core/shell typed colloidal carriers for various biological and biomedical applications such as drug delivery, gene targeting, and high-throughput detection and imaging systems [20]. As targeting cancer cells was highlighted for several drugs and gene therapy, a huge variety of nanoparticles (NPs) were synthesized and studied with further up gradation of their surface design to obtain more specificity towards their target and solve several limitations of conventional drug delivery systems such as nonspecific bio-distribution and targeting, lack of water solubility, poor oral bioavailability, and low therapeutic indices. The uses of different nanoparticles including core/shell structure to overcome these limitations have been reviewed [21,22]. Several reviews recently have demonstrated the use of NPs in bioimaging which is a very recent biomedical advancement [23,24]. More reviews are also available on bioimaging where it has been shown that NP based systems are commonly used for the dual purpose of drug delivery and bioimaging [25,26]. Also some of them could be collaterally used for bio-sensing [27] and gene delivery [2]. Some articles are also reported on biomedical applications based on materials property of nanoparticles such as magnetic [28], and silica [29], and polymeric [30].

Reviewing all the contemporary pioneering research, it can be inferred that the core-shell design is the most important and widely applicable model when it comes to any field of biotechnology and biomedical science. Finally, in spite of several published articles, there is still a strong demand of an extensive review with updated literature on core/shell nanoparticles and its application in biomedical field. Hence this article has been framed to provide a complete and wholesome idea of core/shell nanoparticles used in biomedical applications except in surgical medicine. Tissue engineering has not been discussed in the article as the use of core/shell model is somewhat limited in the area, and more complicated anisotropic forms of fibers, honeycombs and

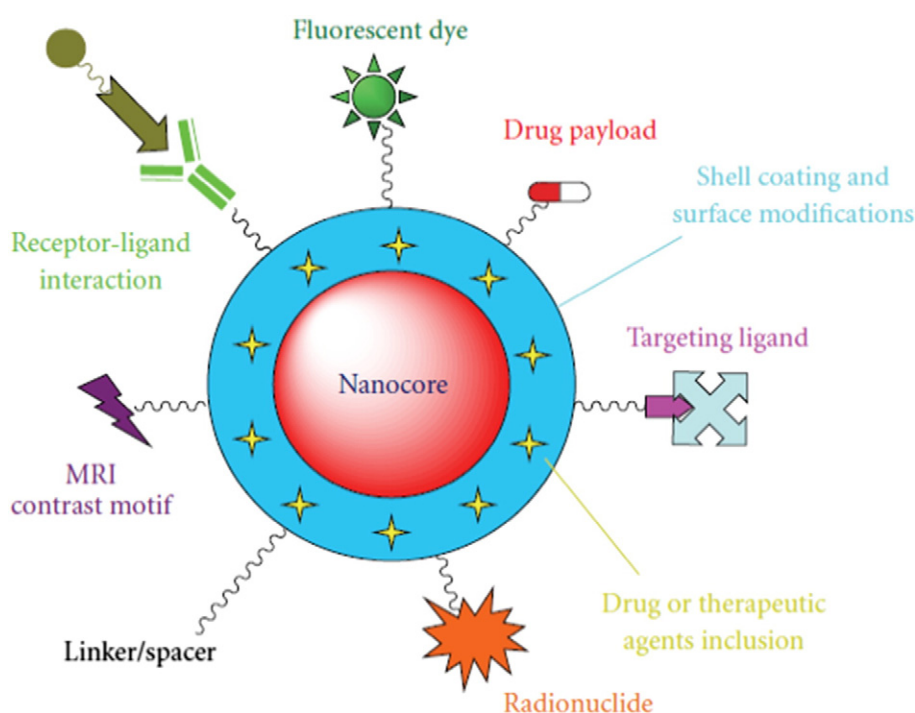


Fig. 1. Scheme of multifunctional nanoparticle for molecular imaging, drug delivery and therapy. Optionally functionalized and devised nanoparticles could be achieved for individualized diagnosis and treatments [2].

complex dendrimers are more in use. Besides this, all vital biomedical applications like bioimaging, targeted drug delivery, gene transfection, biosensor and nucleotide interactions, have been covered. Each field has been further subdivided according to their process instrumentation and different NPs according to their material structural design and function has been discussed focusing on how a slight modification in design or structure has brought about functional superiority over an earlier design. Since often comparison with earlier conventional bulk instruments has been done, the vast difference in efficiency and productivity that NPs have brought in their respective fields are highlighted.

2. Core/shell nanoparticles in bioimaging

Bioimaging technique has played a vital role in improving human health by using ‘imaging’ technique to advance diagnosis, treatment, and prevention of diseases. The development of a wide range of imaging techniques such as magnetic resonance imaging (MRI), positron emission tomography (PET), ultrasound and optical imaging are nowadays important tools for the early detection of disease, understanding basic molecular aspects of living organisms and the evaluation of medical treatment. In recent years after the development of several core/shell nanostructured materials, bioimaging techniques have been developed a lot.

2.1. Magnetic resonance imaging

Magnetic Resonance Imaging (MRI) is one of the most powerful bioimaging tools currently available, which is generally used to produce high quality images of the internal organs of the human body. It is employed to patients suffering from the following ailments: inflammation or infection in an organ, degenerative diseases, strokes, musculoskeletal disorders, tumors and other irregularities that exist in tissue or organs in the body. The MRI technique is based on the basic principles of nuclear magnetic resonance and radio frequency pulses, producing detailed digital pictures of organs, soft tissues, bone, and virtually all other internal body structures. When a specimen is placed within a homogenous, static magnetic field then some of the water molecules present in the animal body become aligned with the direction of the field. Then the molecules resonate at the resonance frequency depending on the strength of the magnetic field. It is then excited with a pulse of radio frequency which changes the net magnetization. Finally, as the field is turned off, the molecules revert back to their original lower energy state by releasing energy in the form of photons. The released photons are detected by a scanner as an electromagnetic signal and the changes induced in electromagnetic signals in the presence of linear field gradients are used to construct three dimension images of the body. To facilitate the difference between the normal and abnormal tissues, contrast agents are being injected to the patients before the imaging, which selectively highlights the abnormal cells. The various parameters affecting the contrast between different tissues are listed in Table 1.

The most commonly used contrast enhancement agents are gadolinium-based compounds. Most clinically used MRI contrast agents work through shortening the T_1 relaxation time of protons located nearby. In general, the lanthanide and transition metal series have

paramagnetic property, which makes them ideal candidates for MRI contrast agents. A magnetic nanoparticle has superparamagnetism, high coercivity, low Curie temperature, intrinsic spin structure, and high magnetic susceptibility among other properties [31], thus influencing the local magnetic environment, changing them indirectly to provide contrast enhancement by facilitating T_1 and T_2 relaxation processes. Among the large variety of metals and compounds tested, Gd (III) based contrast agents is one of the best, because of its large magnetic moment of 7.94 μ_B . Currently, commercially employed non-NP based MRI contrast agent is Gd-DTPA (diethyltriaminepentaacetic acid), where DTPA is a chelating group which binds with the Gd (III) ion. The T_1 weighted image relies upon the longitudinal relaxation of the net magnetization vector (NMV). Fat molecules present in the animal body have a large longitudinal and transverse magnetization, and appears bright on a T_1 weighted image. Conversely, water has less longitudinal magnetization and therefore has less transverse magnetization. Thus, water has low signal and appears dark. T_2 weighted image relies upon the transverse relaxation of the net magnetization vector (NMV). Thus, fat appears dark and water appears bright. T_2 contrast agent is being extensively used in MRI, as negative contrast they give darker images of the region under interest. With the development of T_1 contrast agents, giving positive images by image enhancement has opened possibility of improving the scope of bioimaging by MRI. Different nanoparticle based MRI contrast agents are classified based on imaging modality (i.e. T_1 or T_2 weighted image) applicable in obtaining the image contrast.

2.1.1. Core-shell nanoparticles based T_1 -contrast agent

The T_1 -relaxation time of all biological system is different from one another. T_1 contrast agents give positive imaging by differentiating fat from water, water in darker contrast and fat in brighter contrast. The T_1 -relaxation time is shortened in the presence of paramagnetic substances. Since Gd^{+3} (outer most shell configuration: 4f7) ion has seven unpaired electrons with a large magnetic moment, most T_1 contrast agents are Gd^{+3} based, mainly gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA) which is used as mentioned before. T_1 -weighted imaging is very sensitive to low concentrations of Gd-DTPA. Although the T_1 contrasting mechanism of inorganic nanoparticle-based agents has not been fully elucidated yet, paramagnetic ions on the nanoparticle surface seem to be responsible for the relaxation enhancement of the protons near the nanoparticle.

Nanoparticles of gadolinium oxide (Gd_2O_3) [32], gadolinium fluoride (GdF_3) [33] and sodium gadolinium phosphate ($NaGdF_4$) [34] have been investigated as possible MRI contrast agents. Kobayashi et al., [35] tried to enhance the contrasting properties of gadolinium by preparing multilayered silica-Gd core/shell nanoparticles which proved to be effective in this purpose. Water soluble Gd_2O_3 surface doped with MnO nanoparticles (1–2 nm in diameter), gave enhanced performances as proven by the in-vivo T_1 MR images of a mouse (Fig. 2) [36]. These in-vivo T_1 MR images in Fig. 2 show a high contrast enhancement in kidneys after 90 min of injection of the sample, because of the removal of nanoparticles from the organs through the kidneys, which is an important requirement for MRI contrast agents, as the nanoparticles should eventually be removed by the kidneys.

It has also been shown that gadolinium tetraazacyclododecane-tetraacetic acid (Gd-DOTA) is covalently linked with silanized nanoparticle to form Gd-DOTA, and then attached to SiO_2 coated CdS/ZnS QDs, which acts as dual imaging contrast agent for MRI and optical imaging purpose, duly demonstrated using a mouse model [37]. The surface modification of gadolinium hydroxide (LGdH) is also very important for the purpose of making it water soluble, biocompatible and acid resistance [38]. The exfoliated layers of LGdH in the aqueous colloid phase was at first incorporated with fluorescein (FS) and then encapsulated with PEG-phospholipid (PEGP) to form LGdH-FS-PEGP suspensions, which can suitably act as inorganic MRI contrast agent.

Table 1
MRI parameters: factors influencing the signal from each location [24].

Parameter	Description
T_1	Spin-lattice/longitudinal relaxation time. The T_1 relaxation time constant is a material property describing the characteristics of how this energy is given back to the surroundings.
T_2	Transverse relaxation time. T_2 relaxation time constant describes the energy transfer between adjacent protons
T_2^*	Same as T_2 , but also contains heterogeneities in the environment
ρ	Spin density: the concentration of H nuclei in the tissue

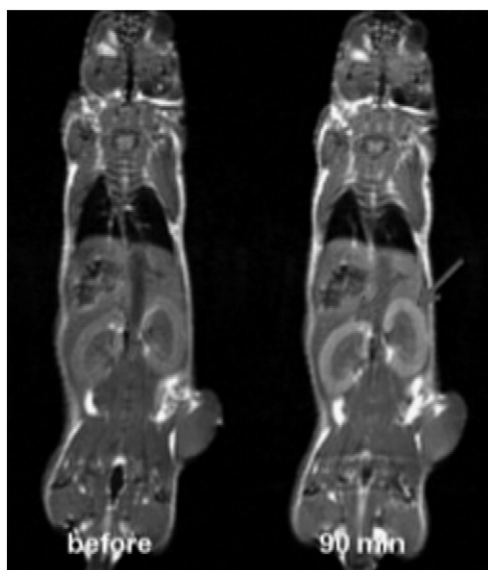


Fig. 2. 3 T in-vivo T₁ MR images of a mouse before (left) and 90 min after (right) injection of the sample solution into the tail vein of the mouse, showing a clear contrast enhancement in the kidneys (shown by arrows) [36].

Gd-based core/shell nanoparticles have shown considerable positive-contrast effects but there are some inherent problems associated with its application, as Gd itself is toxic in its ionic form. Because of the toxicity it is administered in the complex form but the risk of replacement of Gd ion by Zn and Cu, inside the body, cannot be undermined totally, since the stability of the Gd-complex is greatly influenced by the pH, temperature and concentration of surrounding ions. Since gadolinium has no biochemical cycles in the human body it accumulates inside the body and can cause harmful side-effects. Recently, various contrast agents based on oxides and chlorides of Mn (II), Fe (III), Cu (II) has been identified as potential substitute of Gd. Recently mesoporous silica-coated hollow manganese oxide (HMnO/mSiO₂) is reported as a new class of contrast agent for MRI as shown in Fig. 3.

While, Fe₃O₄ conjugated with curcumin showed contrast enhancement along with the ability to label, target, and treat mouse sarcoma-180 cells [40]. Various other Fe (III) based core/shell nanoparticles have been engineered, which can emerge as an attractive alternative to Gd based contrast agents. It has been found that iron/carbon core/shell nanoparticles have the potential for both drug delivery and as contrast agent for MRI with low toxicity and can also be bio-functionalised [41]. The magnetic core/shell nanoparticles such as Fe₃O₄/polymer/Au core/shell/shell nanoparticles [42], Fe₃O₄/Au [43–45], and FePt/

Fe₂O₃core/shell nanoparticles [46] have the potential for developing as contrast agent for MRI.

2.1.2. Core/shell nanoparticles based T₂-contrast agent

T₂-weighted scans are most commonly used for MRI in differentiating fat from water, with water in brighter contrast and fat in darker contrast. T₂ contrast agents permit negative contrast enhancement and also darker images of the regions of interest. Dextran-coated iron oxide nanoparticles (usually magnetite or maghemite) were among the first to be used as MRI contrast agents in the late 1970s. Since then, their popularity has increased many folds because of their ability to dramatically shorten T₂*-relaxation time in the tissue. The distribution of the magnetic nanoparticles inside the cell is directly dependent on their size which have been classified as follows: (i) micrometer-sized paramagnetic iron oxide (MPIO; several micrometers), (ii) superparamagnetic iron oxide (SPIO; 50–500 nm), and (iii) ultra-small superparamagnetic iron oxide (USPIO; less than 50 nm). SPIO [47–49] and USPIO [50] have both been extensively used as contrast agents for MRI. Reported study shows multiple SPIO core/SiO₂ core/shell nanoparticles treated with imidazolium have 7-fold clearer contrasts than that of the particles before treatments [51]. The cellular internalization of the contrast agent led to its gradual breakdown, which could be predicted on the basis of decrease in the darkness of the T₂ image with time. Presently dual contrast agent is also available which can work both in MRI and ultrasound imaging [48]. SPIO nanoparticles (mean diameter 12 nm) embedded in the microbubble composed of polyvinyl alcohol (PVA) outer layer and a poly(DL-lactide)(PLA) inner layer (shell thickness-50–70 nm) act as dual contrast agent; the structure is schematically presented in Fig. 4. The microbubble has nitrogen in its core and was synthesized using a double emulsion solvent evaporation interfacial deposition (water-in-oil-in-water emulsion) process (Fig. 5). Studies carried out in-vivo in rat liver showed enhanced images through the optimization of SPIO nanoparticles in the shell and also it had adequate echogenicity for the purpose of being used as ultrasonography (US) contrast agent. MRI images using these nanoparticles are shown in Fig. 5.

Additionally, SPIO is also commonly used for cell labeling in the present times but sufficient accumulation of SPIO is required for satisfactory images. So, it is difficult to label stem cell, since they lack phagocytic capacity, due to which SPIO uptake is restricted. Instead of SPIO nanoparticles fluorescent magnetic nanoparticle (MNP) containing rhodamine B isothiocyanate core within a silica shell can also be used [49]. Comparisons between in-vivo T₂ weighted images of rat liver showed no significant differences between MNP-labeled group and SPIO-labeled group, thus MNP-labeled group proves to be an alternative contrast agent [49]. In another work, silica coated SPIO was co-condensed with fluorescein isothiocyanate (FITC) incorporated with mesoporous silica (Mag-Dye/MSNs). It was then used to label human mesenchymal stem cells (hMSCs) through endocytosis. The cell labeling efficiency of

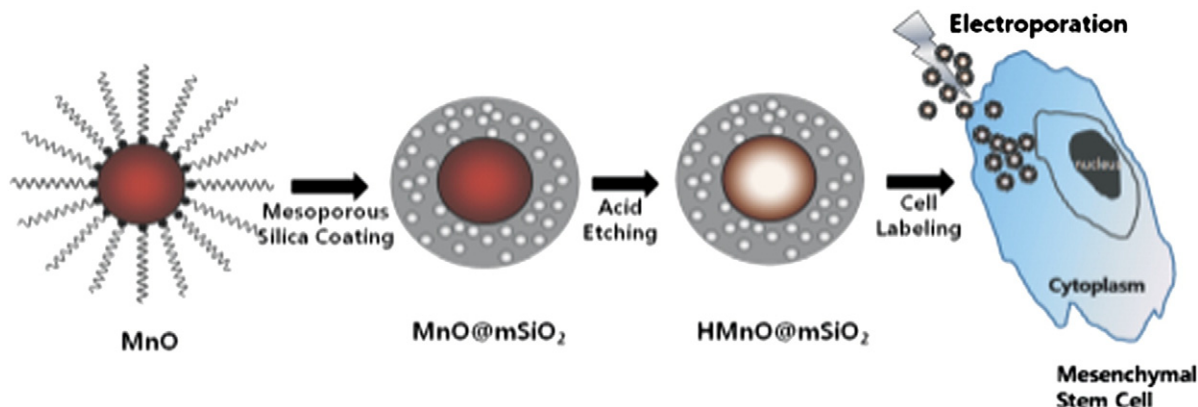


Fig. 3. Schematic illustration of the synthesis of HMnO@mSiO₂ nanoparticles and labeling of MSCs [39].

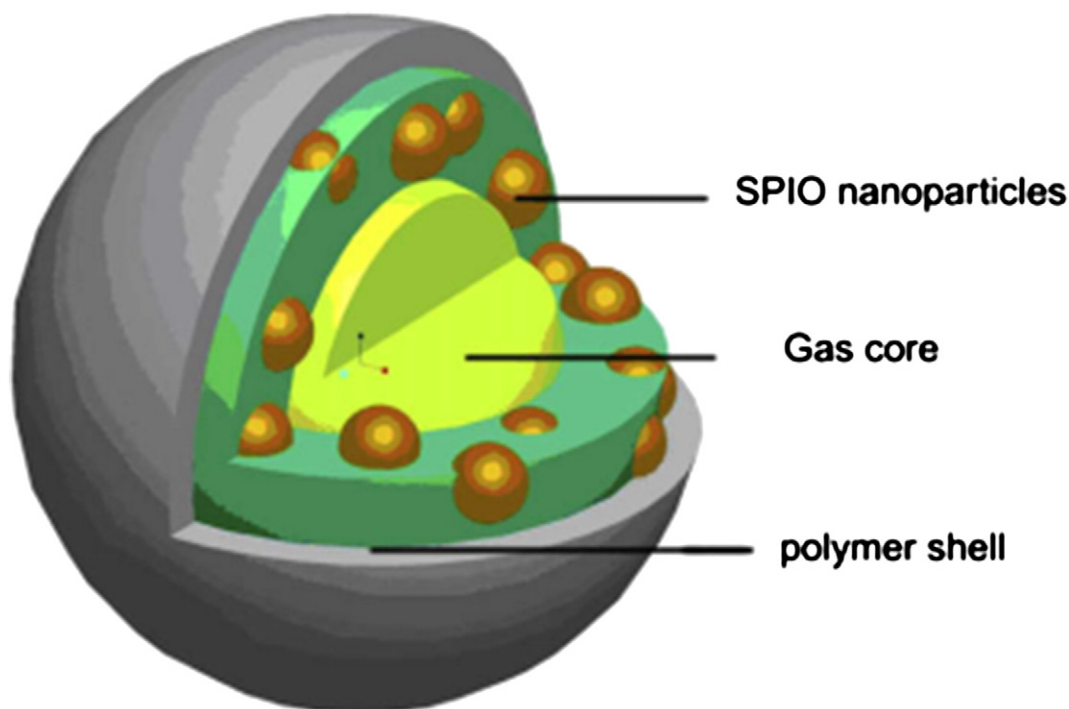


Fig. 4. The schematic diagram of the designed SPIO-inclusion encapsulated microbubble [48].

Mag-Dye/MSNs was found to be higher than SPIO/SiO₂ and it is also non-toxic, thus it can be used for stem-cell tracking agent in addition as a contrast agent for MRI [52]. Chelated gadolinium with Fe₃O₄ and encapsulated with mesoporous silica shell also showed no cytotoxicity in the short term of study [53]. The immobilization of gadolinium enhanced the transverse relaxation rate (R_2), thus led to improvement of T₂ weighted image [53]. Potential colloidal MRI contrast agent include colloidal Fe₂O₃, synthesized by laser-induced pyrolysis of Fe(CO)₅ [54], and phosphatidylethanolamine–diethylene triamine pentaacetic acid (PE–DTPA) incubated into ML (magneto liposome) coat (nanometer-sized magnetite cores encapsulated in a phospholipid bilayer), which can then immobilize up to 500 Gd³⁺ ions per ML colloid [55].

Several exciting studies have been carried out by the researchers to synthesize a single contrast agent, which can be utilized for both T₁ and T₂ weighted images. As an example, colloidal suspensions of Fe/Fe₂O₃ core/shell nanoparticles, which were capable of giving both

T₁ and T₂ weighted images [54]. Similarly, iron core (with its subsequent oxidation gives ferrite shell) with added nickel ions to form nickel ferrite shell nanoparticle (diameter of 10–15 nm); its surface treated with dopamine-PEG to make it dispersible, acting as dual mode T₁ and T₂ contrast agent [56]. The magnetic coupling between T₁ (MnFe₂O₄) and T₂ (Gd₂O(CO₃)₂) contrast agents produced a unique dual mode T₁ and T₂ contrast agent MnFe₂O₄/SiO₂/Gd₂O(CO₃)₂ with MnFe₂O₄ being the core of the core/shell nanoparticle [36].

2.2. Computed tomography (CT)

Computed tomography provides a good imaging modality for studying anatomical details as against positron emission tomography (PET) and other modalities which focus on metabolic pathways using X-ray absorption spectra as a detection signal. Gamma rays used in PET analysis have lower energy levels and lower penetration power than X-ray

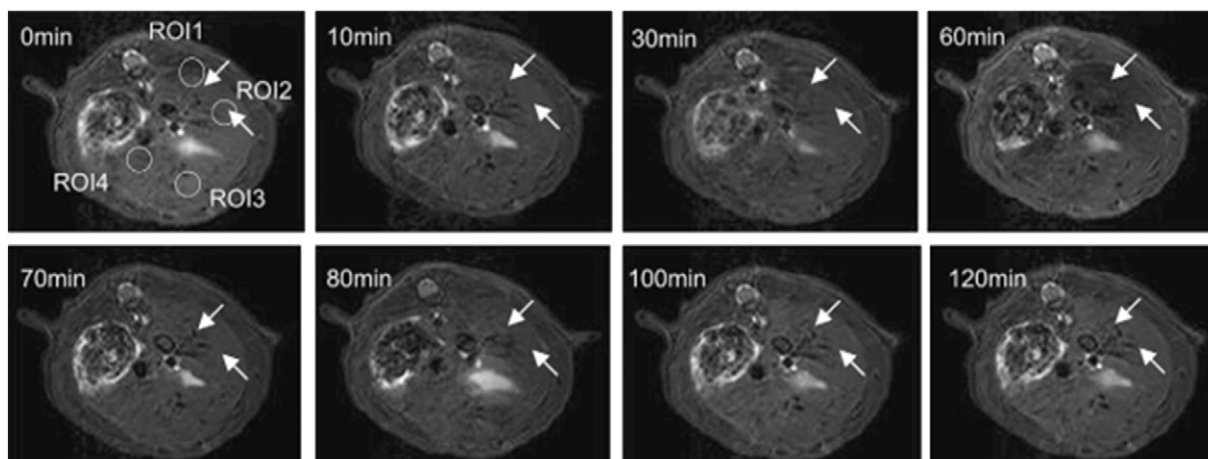


Fig. 5. Corresponding anatomical structure images from the same rat at two adjacent slice locations during SPIO-inclusion microbubble injection. The image shows that with the time lapse after injection, the T₂ signal in liver decreases at first and then increases (arrows) [48].

used in CT. Thus, high resolution CT is most constructive in tracking the site and loci of some metabolic event or analyzing its histological impact as X-ray emissions are differentially absorbed by tissues according to their X-ray attenuation coefficient, which gives a visual spectrum for image reconstruction.

To achieve high resolution, several non NP based contrasting agents based on iodine, barium, barium sulfate, etc., are in use, which selectively highlight the tissue of interest during CT analysis. Iodinated liquid contrast agents such as alcoholic iodine (1% w/v iodine in 100% ethanol or methanol) and IKI (Lugol's) iodine potassium iodide (1% w/v iodine + 2% KI) are most commonly used for imaging contrast in CT. Along with these galloxyanin-chromalum 5% (w/v) in water, 1% (w/v) phosphotungstic acid and 1–2% OsO_4 in phosphate buffer has given promising results when applied as contrast agent in CT [Metscher, B.D. 2008,]. Conventional CT contrast agents have generally high renal toxicity and suffer from low imaging time, because of rapid renal clearance. Low molecular weight nanoparticle systems comprising of gold/iron oxide core/shell nanoparticles have shown great potential to be used as CT contrasting agents, for their stability and optimum residence time in the tissues and versatility in multifunctional imaging mode. Recent developments are going on using multi-modal nanoparticles contrasting agents especially using electron dense elements such as iodine [57,58], gold [59–61] or bismuth which form well defined dispersion spectra when impinged by electromagnetic waves. Kong et al. [57], utilized pluronic F127 and an iodinated compound, lipiodol, as radiopaque nanoparticles contrast agent for in-vivo testing using micro-SPECT/CT imaging. Though each distinct modality in bioimaging can be independently exploited, contrasting agents are mostly designed for multi-contrast purposes. Especially CT is always complemented with MRI or PET for multifaceted feed which is undoubtedly more informative since together they compensate the weakness of each modality.

The core/shell particles, in general, act as dual or multicontrasting agents. These core/shell particles, not only overcome the limitations of conventional iodine based contrast agents but also provide uniform functionalizable spherical surface, which prevents particle aggregation. Hagit et al. [62] synthesized core P(MAOETIB-GMA) microparticles of 40–200 μm synthesized by suspension copolymerization of iodinated monomer 2-meth-acryloyl-oxyethyl (2,3,5-triiodobenzoate), MAOETIB, with a low concentration of the monomer glycidyl methacrylate, GMA. The formed particles are having hydrophilic surfaces; on which magnetic Fe_2O_3 was deposited to form the fully functional nanocomposite as dual contrast agent for CT and MRI (Fig. 6).

In-vivo experiments on rat's kidney reported by them showed good resolution and image sharpness in focusing a physiological phenomenon

as well as localizing its anatomical preview, which can be clearly seen in Figs. 7 and 8.

As mentioned before, iodine-based contrast agents such as iopromide, iopamidol, etc are most commonly used in CT applications. However, these agents have shortcomings such as short imaging time, toxicity and vascular permeation. So, non-iodine based nanoparticulate contrast agents are being explored to act as a possible replacement of iodinated contrast agent in the near future. Poly(acrylic acid) (PAA_{25}) stabilized NaGdF_4 or 50/50 mixtures of GdF_3 and CeF_3 nanoparticle aggregates (NPAs) were developed as contrast agents for dual modal applications in MRI and CT [63]. AuNPs are efficient CT contrast agents unless they are supplemented with biocompatible and/or bioinactive surface agents [61]. Surface modification can be done by encapsulation or entrapping Au NPs using suitable chemical agents. For example, AuNP-loaded PEGylated dendrimer capsules [61] and dendrimer-entrapped AuNPs (also complexed with diatrizoic acid) [64] have both found suitable as contrast agent for CT. Besides fabricated unlabeled AuNPs; conjugated AuNPs with 2-deoxy-D-glucose has high potential to be applied as CT contrast agent [60]. PEG coated hybrid ferric oxide nucleated and gold shell NPs have been studied as dual contrasting agent for CT and MRI showing tolerable levels of cytotoxicity (as projected by MTT assay). The intensity of CT images are directly proportional to the amount of nanoparticles used and is much better than the result given by iodine contrast agents but the T_2 weighted signal of MRI were slightly weaker, aptly shown in Fig. 9 [43].

Biocompatible, dual mode contrasting core/shell nanoparticle based agents have been reported in earlier sections. F^{18} molecules are the most common radiotracers used for dual imaging purposes in PET/CT [65]. Nanoparticles having a superparamagnetic iron core cross linked with dextran forming the corona bonded with F^{18} molecules empower them to work for PET, CT, and MRI. To overcome the problem of functionalizing F^{18} , it was prepared using N-succinimidyl 4[F^{18}] fluorobenzoate and 4-[F^{18}] fluorobenzaldehyde through nucleophilic aromatic substitution [66].

2.3. Positron emission tomography (PET)

Positron emission tomography and single photon emission tomography are radionuclide based imaging modalities frequently used in biomedical imaging for easy tissue penetration and noninvasive monitoring of body metabolism through visual reconstruction. When the radioactive atom decays, a positron is emitted and interacts with surrounding electrons. The atoms present in the tissue scatter them and quickly lose energy. Within a short distance and time, the positron

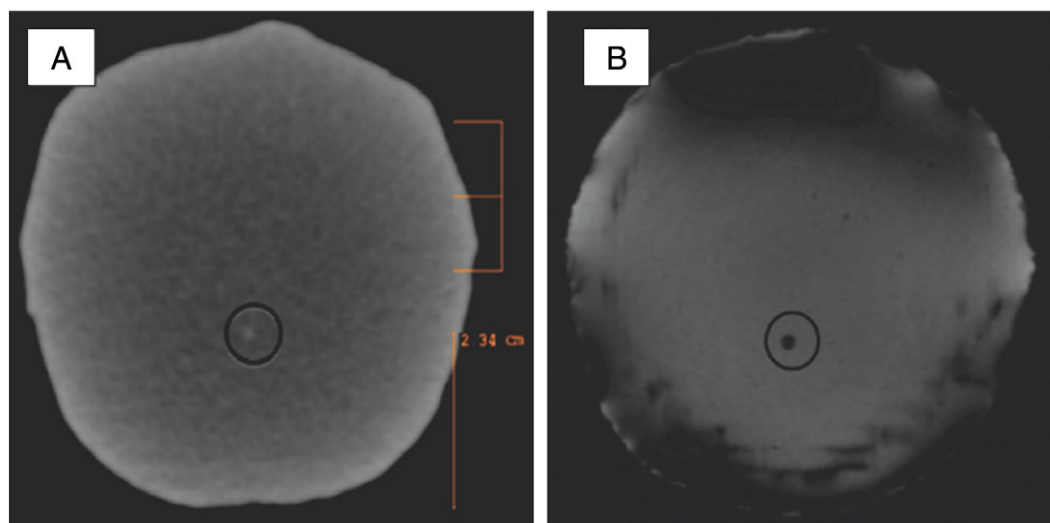


Fig. 6. In vitro CT (A) and MR (B) imaging of a dual modality $\gamma\text{-Fe}_2\text{O}_3/\text{P(MAOETIB-GMA)}$ core-shell microparticle placed in agarose gel [62].

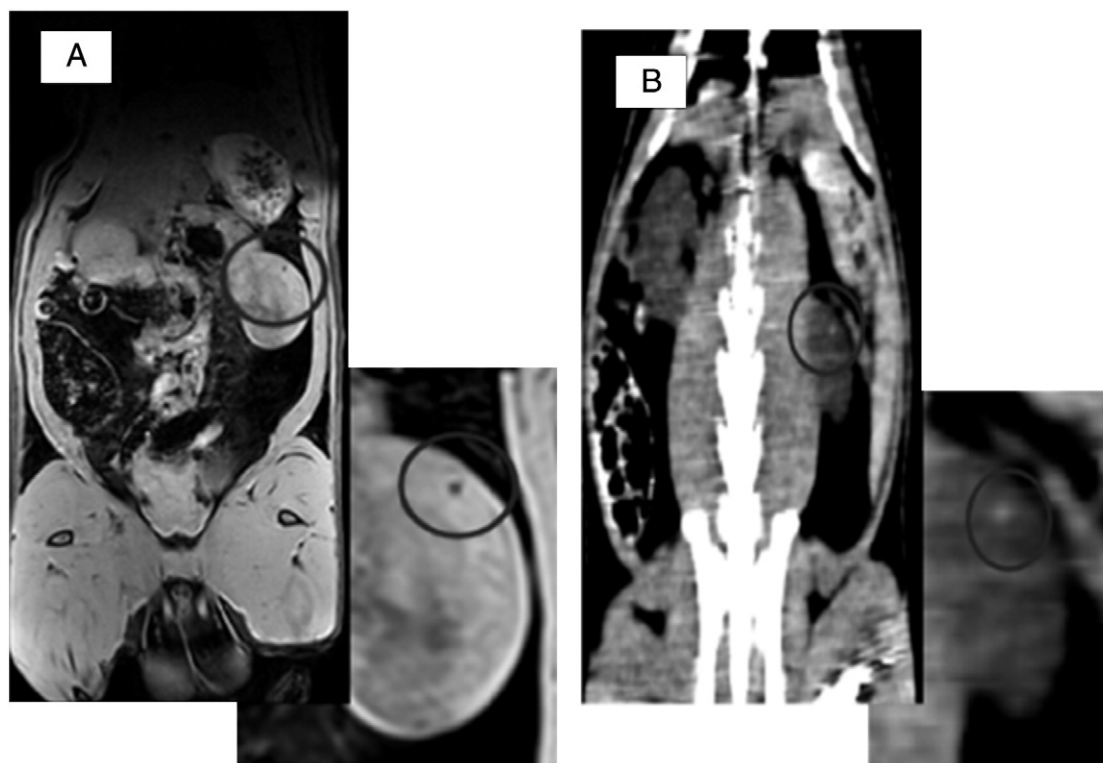


Fig. 7. MR (A) and CT (B) imaging of a dual modality $\gamma\text{-Fe}_2\text{O}_3/\text{P(MAOETIB-GMA)}$ core-shell microparticle in a rat's kidney [62].

emitted scatters in the tissue until it loses enough energy to undergo annihilation with a nearby electron, in which mass of positron and electron are converted into 2 back-to-back photons of 511 keV [67]. In PET, labeling of the distribution of annihilation sites in the body is done, which gives a fair estimation of the radiolabeled molecules that we seek to image, which is only possible due to the short distance of the decaying molecule from the annihilation site (known as positron range) [67].

The emission range and deflection of the positron is directly proportional to its ground state energy level, thus indicating that the radionuclide-dependent spatial resolution of PET varies according to the ground state energy level of positron. Hence, the primary objective of PET responsive agents is to incorporate large number of radionuclides of high energy into each particle. Nanostructures having the large surface to volume ratio, not only provide the space for congregation of radionuclides which directly affect contrast and brightness of

reconstructed image but also of designated ligands for target specification. Concurrently, length and density of linear polymeric chains comprising the corona of the core/shell nanostructure (several different materials can be easily incorporated into this model of nanoparticles) can be manipulated to enhance stealth behavior in vascular channels and shielding nanoparticles from immunoresponse and coupling with proper functional groups can chelate radio-labeled ions with better radiochemical purity and in-vivo stability. Such trivial modifications significantly alter the biodistribution of the contrasting agents. As an example, the increase of chain length of PEG increased the blood circulation and decreased renal retention of a nanoparticle based contrasting agent, developed using poly(methylmethacrylate-co-methacryloxysuccinimide-graft poly(ethylene glycol)) (PMMA-co-PMASI-g-PEG) chelated to radio-isotope Cu^{64} acetate [68]. Reportedly, PEG and its derivatives have emerged as the best contender to minimize premature elimination of image contrasting particles through the mononuclear phagocytic

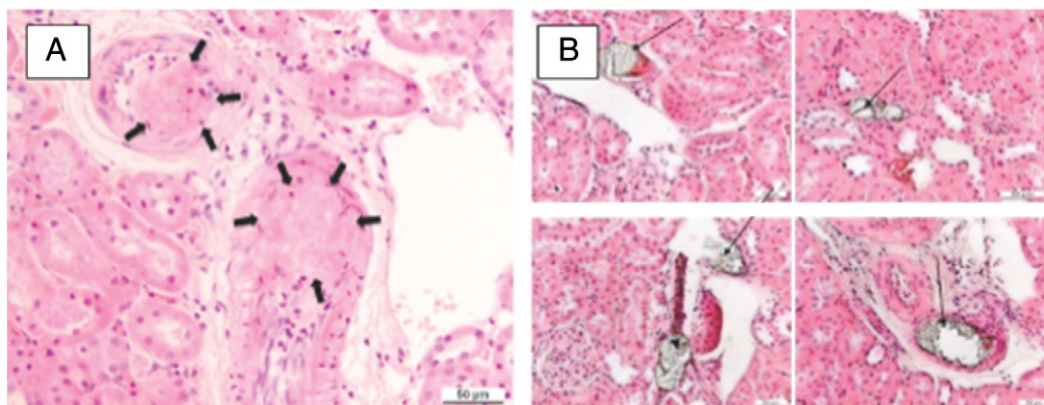


Fig. 8. Histological images of a slice containing two acutely clotted vessels (A) and four slices containing vessels of the embolized rat's kidney blocked by the $\gamma\text{-Fe}_2\text{O}_3/\text{P(MAOETIB-GMA)}$ microparticles (B) [62].

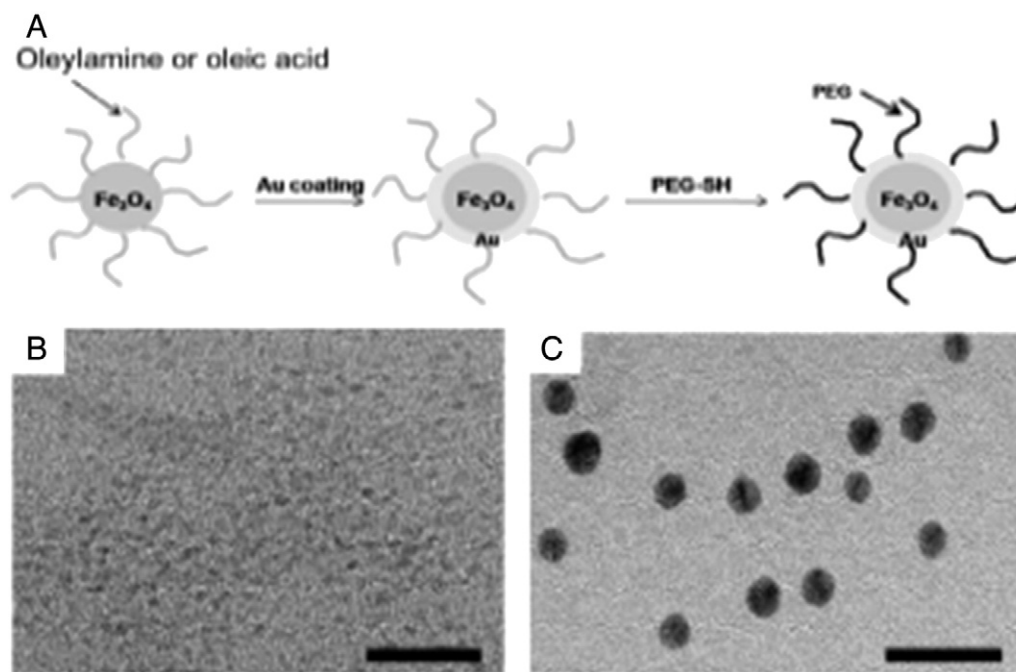


Fig. 9. (A) A synthetic scheme for preparation of PEG coated GION. TEM images of (B) SPIONs (Fe_3O_4) and (C) PEG coated GIONS. The scale bar indicates 20 nm. Antibiofouling Polymer Coated Gold@Iron Oxide Nanoparticle (GION) as a dual contrast agent for CT and MRI [43].

system, while Cu radioisotopes are commonly used for labeling PET sensitive NPs. Active ester groups of effective coupling ligands as the 1,4,7,10-tetraazacyclododecanetetraacetic acid (DOTA) may be used to chelate the Cu efficiently. Similar distinct core/shell design based PET contrasting NP with a hydrophobic core conjugated to an intermediate hydrophilic DOTA-Cu⁶⁴ functionalised layer, covered with a PEGylated outer shell have been reported independently in 2008 [69]. More reports on the importance of PEGylation for better retention in the body has been seen while studying the biodistribution and drug delivery efficiency of CSK type NPs radiolabeled with Cu⁶⁴ [70].

Various other radionuclides, such as F¹⁸, Cu⁶⁴ and Ga⁶⁸ (for PET) and I¹²⁵ and In¹¹¹ (for SPECT) have been used especially if MRI or CT imaging is coupled with PET, as PET has a distinctive advantage over MRI, since the signal strength of MRI varies non-linearly with different concentration of gadolinium [2]. Thus, PET is fully quantitative while the same cannot be true about MRI, because of its above mentioned limitations. The combination of PET and MRI is an important tool in pre-clinical and clinical applications and has the potential for commercial development [71,72]. As discussed earlier while dealing with CT imaging, multimodal imaging is a frequent and necessary practice in medicine or diagnostics, for gross localization, fine tuning and spatial resolution along with sharpness and sensitivity of reconstructed image. A basic MRI contrasting NP system with superparamagnetic iron oxide core superimposed with silica was functionalized for radiolabeling with PET sensitive Cu⁶⁴ and In¹¹¹ was found to have more imaging efficiency in comparison to commonly used MRI contrasting agent Feridex [72]. The multifunctional iron oxide encapsulated mesoporous silica nanoparticles based contrasting agents for MRI or PET with tunable surface charges can be easily taken up by stem cells. The particles thus prepared showed high biocompatibility, no short-term cytotoxicity to the cells with high cell uptake efficiency.

2.4. Optical imaging

The recent surge in medical biology and development of new tools to detect biological processes at the molecular level is intimately linked to the progress of optical imaging instruments, which gives a rapid and conclusive proof in determining them. The variety of optical imaging

instruments at our disposal has each come a long way from its earlier days specially in terms of imaging biological species as they have become more specific, less hazardous, and also now it is less arduous than ever to prepare the samples. The alarming rate of improvement that has taken place in the field of bioimaging is because of the combined contribution of electronics, computer science, chemistry, medicinal biology, and so on. However, the onus of this progress mainly lies in the preparation of novel contrast agents. The development of fluorescent nanomaterials have solved the complexities, thus making live imaging of cells and tissues at the molecular level a lot simpler and cheaper.

Optical imaging is the simplest modality of imaging that is being used in research and has been commercialized as well. It utilizes the principle of photon emission from bioluminescent and fluorescent probes. The main advantages of this technique are it can be used from visible to NIR spectrum and has good spatial resolution. Fluorophores with longer emission at the near-infrared (NIR) region are ideal as they are effective in photon reduction in living tissue. But optical imaging suffers due to very low levels of tissue penetration (0–2 cm) and fluorescent imaging is highly susceptible to noise [26]. Interestingly, even as most researchers' focus on improving the nature of the contrast agent, a group of researchers [73] improved the nature of the glass surface by coating it with Au/Ag core/shell nanostructures for enhanced fluorescence activity and improved macroscopic homogeneity.

Organic dyes such as water-soluble amine-reactive dioxaborine trimethine [74], lissamine rhodamine B sulfonyl hydrazine (LRSH) [75] and metal-organic dye molecules such as europium chelate [76], iridium (III) complexes [77], along with fluorescent proteins such as GFP [78], SYBR Green [79] are commonly used as labeling agents for biological samples. Organic fluorophore suffer from inferior photophysical properties (photo bleaching and brightness), which can be improved by covalently integrating them into the core of a core/shell nanoparticles [19]. This ensures photostability of the dye and signal improvement without any fluctuations or blinking effect. Further fluorescent nanoparticles have the ability of size dependent broad range excitations with sharp emissions in the electromagnetic spectrum where their ability can be used in multicolor studies [80]. Fluorescence-based optical core/shell imaging agents [81] are generally used to overcome limitations of

conventional organic contrasting agents which also suffer from overlapping emission spectral lines. It is imperative to limit the uptake of the contrast agent only at the preferred target site, which not only reduces the risk of toxicity but also significantly increases the imaging prowess of the contrast agent. The shell not only makes the core biocompatible but also prevents the core material from escaping into the external environment, thus reducing the toxicity risks. Core/shell nanoparticles occupy a key place in this regard as they have considerable advantages over conventional imaging agents, not only in terms of its nanosized dimension, but also because of its biodistribution, and enhanced sensitivity, made possible after conjugation with specific ligands [82]. Core/shell optical labeling agents or probes are classified into mainly four types, namely-(i) Quantum dots, (ii) C dots, (iii) Nanogels and (iv) Nanoshells. The classification is based on the different core material employed, which are responsible for the imaging contrast, while the shell itself provides no major role in providing any contrast but plays a key role in making the core biocompatible and sustainable.

2.4.1. Quantum dots

Quantum dots are semiconductor nanocrystallite substances that can confine the movement of conduction band electrons, valence band holes and excitons in all three spatial directions. They are composed of periodic groups of I–VII (CuCl), II–VI (ZnS) or III–V (GaAs) materials. Since its employment in the biological tagging started a decade ago, quantum dots have mesmerized researchers in the biomedical field. They have emerged as novel biological imaging agents with improved properties and thus can be applied where conventional dyes and fluorescent proteins have failed [83,84]. Generally, nanoparticle probes can be used in place of dyes and fluorescent proteins for immunoassay, as well as for diagnostic and therapeutic applications [85], along with biosensing in genomics and proteomics [86]. QDs can facilitate complex multicolor experiments which ordinary Au/Ag nanoparticles fail to perform. Also, QDs have broad absorption along with distinctive size, tunable and narrow emission spectra, higher photo stability and brighter contrast that make them viably alternative to conventional organic dye molecules that suffer from photo bleaching, narrow absorption and broad emission spectra [87–89]. The photoluminescent lifetime of QDs are nearly 40–50 ns, which makes imaging of live cells possible without the background autofluorescence of the cell (~1 ns) [90]. Hoshino et al., [88] found out that the capping material rather than the core metalloid contributed to the biological properties of QDs. The concept of sheathing the core with a shell has been proved to remove surface defects, increase the photoluminescent quantum efficiencies, besides also reducing the toxicity of the core material. The target of achieving proper coating of the nanoparticle for increased stability, water solubility and biocompatibility is being realized by surface coating it with silica [27,91], PEG [92,93], or silane [94,95], along with modifications using proteins [96], chitosan [97], benzaldehyde [98] and so on.

Dahan et al., [99] was among the first to demonstrate QDs as imaging probes for single-molecule tracking experiments by studying the dynamics of individual glycine receptors (GlyRs). The prominent among core/shell quantum dots investigated for bioimaging applications include CdSe/ZnS [97,100–103], CdTe/ZnS [104], CdTe/CdS [105,106], InP/ZnS [107,108], and ZnSe/ZnS [109–111] among others. Besides, ZnO polymeric core/shell QDs [112] and CdSe/CdS core-shell QDs [113], have also been found to be promising candidates for fluorescent bioimaging.

It has been found out that QDs can penetrate cells, hoard up inside them and divide along with the daughter cells at cell divisions, although the actual mechanism of its action is still being researched upon. This property has given QDs an edge over other pre-existing fluorescent probes in applications ranging from live cell labeling to early diagnosis of cancer in vivo [84,114–116]. Engineered QDs for in-vitro live cell imaging, include examples such as dihydrolipoic acid (DHLA) capped CdSe/ZnS (core/shell) QDs attached with the native protein hen egg

white lysozyme (HEWL), utilized for exploration of lysozyme fibril formation [117] while at another instance hyaluronic acid (HA)-QD conjugates [118] has been successfully employed for assessing HA derivatives as target-specific drug delivery carriers, in the treatment of chronic liver diseases. Cadmium used as the core particle in most core/shell QDs is generally quite toxic but the dosage required for in-vivo application is quite lower than its prescribed toxicity levels [119]. Jiang et al., [120] used CdSe/ZnS core/shell QDs covered with amphiphilic co-polymeric organic shell that can be used for both in-vivo and in-vitro imaging of complete animal. The multi-faceted application of core/shell QDs in cell labeling and imaging have been made possible because of suitable surface modifications, some examples of which include QD (CdSe/ZnS)-lipid conjugates for single molecule imaging [101], Ni-NTA-QD (CdTe/CdS) clusters for the targeted imaging of his-tagged fusion proteins [106], CdSe/ZnS QDs functionalized with epidermal growth factor receptor (EGFR), a known tumor marker, for the cellular imaging of cancer cell [121] and PEG coated QDs for tracking macrophage migration, responsible for playing a complex role in disease prevention [93].

The main problem associated with QDs is its high toxicity, because of the presence of heavy metals in its core, which induces or causes cell death, by leaching out to the external environment. Lovrić et al., [122] pre-treated cells with antioxidant N-acetylcysteine and BSA (bovine serum albumin) decreasing the QD induced cell death quite significantly. But, pre-treatment of cells could not possibly be a viable solution for reducing cadmium toxicity in in-vitro applications. The addition of an external shell or replacing the toxic cadmium, from the core of the core/shell QD by another metal, can go a long way in curbing the problems posed by cadmium. Kim et al., [123] used CTAB to prepare CdSe/CdS/ZnS (core/shell/shell) QDs for cell labeling in Hela cells, a human cervical cancer cell line. It was used for labeling after removing the free ligand by dialysis, which gave no significant cellular toxicity that has been associated with CTAB.

Besides the commonly used CdSe/CdS/ZnS (core/shell/shell) QDs that has found application in cell labeling, other multishell QDs used for imaging applications include CdSe/ZnSe/ZnS [124] and CdSe/Zn_{0.5}-Cd_{0.5}Se/ZnSe/ZnS [125]. Muro et al., [126] worked upon the restriction of agglomeration posed by PEG-QDs and Cys-QDs, to synthesis DHLA-sulfobetaine capped zwitterionic QDs, which are not only free from the above mentioned problems but can also be easily conjugated with biotin or streptavidin. While, non-heavy metal containing ZnS QDs have been found to have potential for in-vivo imaging [127,128], ZnTe/ZnS (core/shell) QDs [128] can also be applied for the same. Yong et al., [107] synthesized Cd-free CuInS₂/ZnS core/shell quantum dots (QDs) and employed it for imaging tumor cells in mice. It can lead to a permanent solution of producing non-toxic Cd-free QDs that can be commercially exploited for in-vivo and in-vitro imaging in the not so distant future. CdSe/ZnS/SiO₂ (core/shell/shell) nanoparticles [129–131] which gave stronger emissions with consistent fluorescence intensity than bare CdSe–ZnS nanoparticles alone, lead to improved imaging applications. Also, the silica shell makes it suitable for conjugating with bioactive agents, needed for targeted delivery to the concerned imaging site [132].

QDs have emerged as suitable candidates for multimodal imaging [90,133,134]. According to Koole et al., [133] there are four approaches for integrating the fluorescence as well as magnetic properties in a single nanoparticle. In the first approach, core contains a magnetic material over which QD shell is overgrown or QDs may even be linked to a magnetic NP. The second approach uses silica or polymeric matrix in which QDs and MNPs are incorporated. The other processes include doping of paramagnetic ions into QDs and chelating QDs with paramagnetic ions [133]. Among many, Jin & Gao have used a quantum dot core and deposited ultrathin gold shell, through layer-by-layer assembly giving rise to a multifunctional nanoparticle with both fluorescent and plasmonic activity, that has the potential to be used in multimodal imaging, since it will help in imaging with both fluorescence and scattering as well as light-triggered photothermal treatment among others, as shown in Fig. 10 [135].

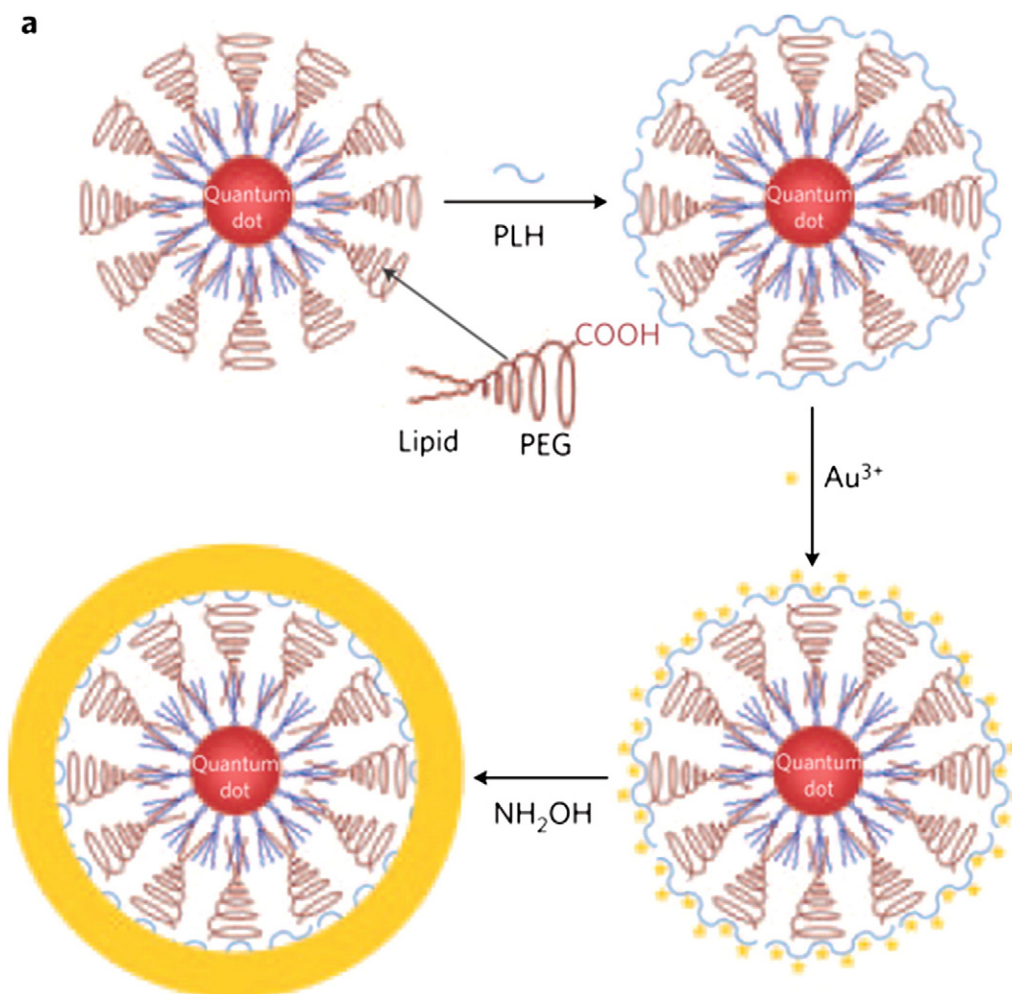


Fig. 10. Schematic of gold-shell-encapsulated quantum dots (QDs) [135].

Another very interesting result has been obtained by Kang et al., [133] where they have used QD-based fluorescence resonance energy transfer (FRET) analysis to provide information on biologically adjacent proteins in native cells that can be used to determine the gene expressions in cancer and other diseases [136]. Also, Kouskousis et al., [137] have developed a high-resolution system for nanocrystal imaging; specifically CdSe/CdS core/shell QDs based on the algorithms developed for PALM and (F) PALM.

Beside quantum dots, quantum rods have also found widespread application in bioimaging, few of which are prominent enough to be mentioned here. Quantum rods can be used for both in-vivo and in-vitro optical imaging [138]. The fluorescent properties of elongated core/shell CdSe-based nanocrystals [139] and silica coated CdSe/CdS/ZnS quantum rods (QRs) are used as probes for in-vitro and in-vivo optical bioimaging of different cell lines (Panc 1 and RAW) [3].

2.4.2. C-Dots

QDs offer numerous benefits but their application has been limited owing to inherent problems like disposal and toxicity associated with heavy metals within its core (i.e., cadmium, lead, zinc), which can leach into solution and cause harm to both cells in culture and animals. Until detailed toxicological studies have been undertaken to ascertain the potential side effects of in-vivo QD usage, it is not appropriate for application in biological and particularly clinical purpose. C-dots are a new class of non-toxic, highly fluorescent core/shell dye/silica nanoparticles with narrow size distributions and enhanced photostability which offer a feasible alternative to quantum dots [17,140]. They provide ease of

bioconjugation, are easy to synthesize, cheaper and thus can be used in a broad range of imaging applications [141]. Wang et al., [142] prepared luminescent non-water soluble C-dots by coordinating organosilane as solvents for its synthesis in under a minute. C-dots are also used for real-time imaging of tumor metastasis in mice [143]. The results showed in Fig. 11 illustrates the fact that fluorescent C-dots can be used for in-vitro imaging particularly in cancer biology.

Further development of C-dots was done by coating it with a neutral organic compound, which prevent adsorption of serum proteins and at the same time facilitated efficient urinary excretion, thus providing a promising platform for this material to be adapted for clinical use [144]. Herz et al., [145] used several dyes including Cy5, Alexa Fluor 700, DY730, Alexa Fluor 750, and DY780 incorporating it in 9–14 nm diameter core/shell silica particle for studying dye structure–optical property correlations. They reported that for Cy5, that brightness is enhanced in C-dots and also for all dyes photobleaching is slower in C-dot than free dyes.

2.4.3. Nanogels

Core/shell nanogels are composed of a metal core and a hydrophilic shell such as PEG, poly (N-isopropylacrylamide-co-acrylic acid, etc. Wu et al., [146] constructed hybrid nanogels by coating the Ag–Au bimetallic NP core with a thermo-responsive nonlinear poly(ethylene glycol) (PEG)-based hydrogel as shell. They then loaded the nanogel with anti-cancer drug temozolomide and used it for drug delivery as well as fluorescence imaging of mouse melanoma cells (B16F10 cell-line). The drug release can be induced by both the heat generated by external NIR

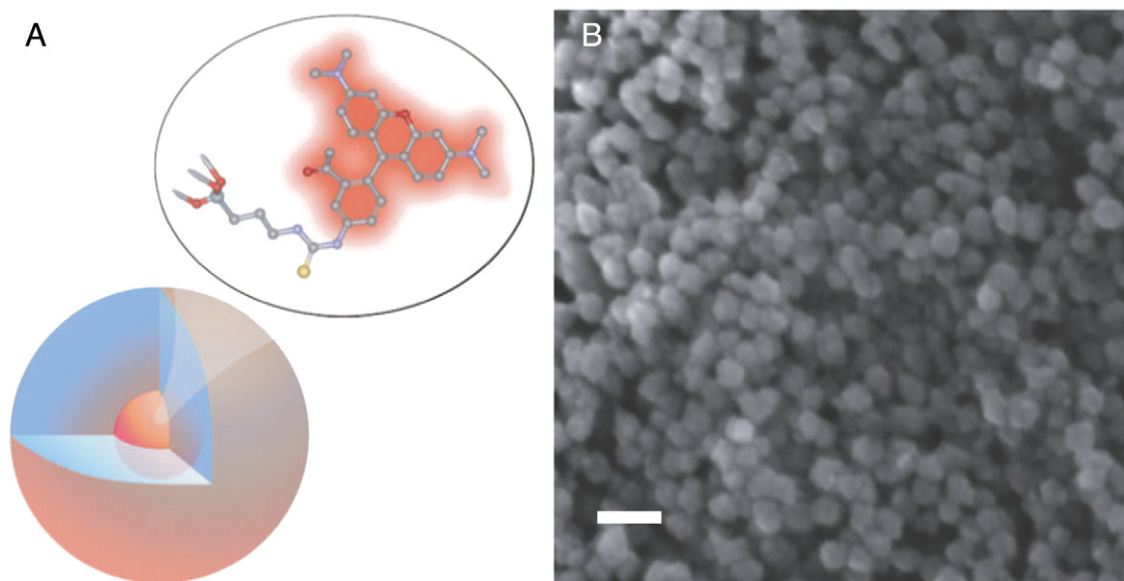


Fig. 11. C dot core/shell fluorescent silica nanoparticles, shown in schematic form (A) with the covalently bound TRITC dye within the particle core (B) Scanning electron micrographs of 30-nm-diameter C-dot particles. Scale bar of 50 nm [143].

irradiation and the temperature increase of local environmental media. In one of their works Wu and his fellow workers [147] developed core-shell structured hybrid nanogels (40–80 nm) composed of a Ag nanoparticle as a core and smart gel of poly(N-isopropylacrylamide-co-acrylic acid) as shell which can overcome cellular barriers to enter the intracellular region and light up the mouse melanoma cells, including the nuclear regions. The pH-responsive hybrid nanogels exhibit not only a high drug loading capacity but also a pH-controllable drug releasing behavior, clearly visualized in Fig. 12 [146].

2.4.4. Nanoshells

Nanoshells are composite nanoparticles that contain a dielectric core surrounded by a thin metallic shell usually gold. These nanoparticles have a shell with dimensions of a few nanometer and exhibit unusual optical properties, which can be used in applications as diverse as biomedicine and surface enhanced Raman spectroscopy. Gold nanoshell possess unique and tunable optical properties, in particular, strong optical responses in the NIR, desirable for biological applications, which were predicted using Monte Carlo models by Lin et al., [148]. Even

with a small concentration of nanoshell a considerable change in reflectance was observed, which makes it suitable for optical diagnostics [148]. The silica shells can mediate a variety of surface reactions which helps it to easily conjugate with various biomolecules. This property has been utilized for biosensing, bioimaging and cellular tagging in molecular biology [29]. Khanadeev et al., [149] first labeled pig embryo kidney (SPEV) cells with primary phage antibodies and then used nanoshells tagged with secondary rabbit antiphage antibodies to visualize it under dark-phase microscope. These particles are also effective substrates for surface-enhanced Raman scattering (SERS). The core/shell nanoparticle of this particular class has also been found suitable in targeted drug delivery, cancer therapy, and as contrast agent in drug delivery [150,151].

Nanoshell of silica/gold was used recently first time for dual purposes of imaging and photo thermal cancer therapy for ovarian cancer cells (in-vitro). It detected and destroyed drug resistant ovarian cancer cell OVCAR3 that over expresses HER2, a known clinically relevant cancer biomarker [152]. A schematic representation is shown in Fig. 13.

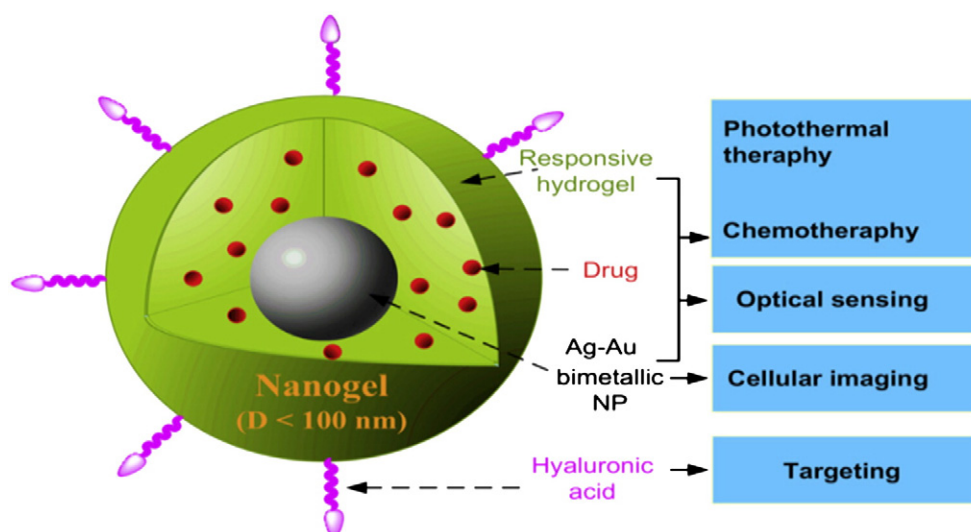


Fig. 12. Schematic illustration of multifunctional core/shell hybrid nanogels [146].

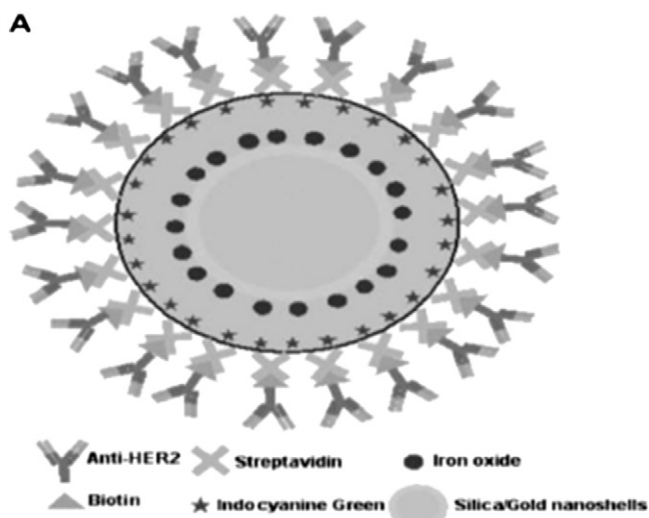


Fig. 13. (A) Schematic representation of anti-HER2-conjugated nanoshell contrast agents [152].

Loo et al., [153] employed gold nanoshell for imaging and photothermal cancer therapy of breast carcinoma cells that over expresses HER2. While in the later years, Gobin et al., [154] designed PEG/Au (core/shell) nanoshell that possessed both absorption and scattering properties in the NIR to provide optical contrast for improved diagnostic imaging and, at higher light intensity, rapid heating for photothermal therapy. Mullner et al., [155] worked with organic-inorganic hybrid network, produced via RAFT polymerization. They verified the potential of these stabilized and fluorescent nanoparticles as biocompatible carriers for intracellular delivery via in vitro experiments on lung cancer cells (A549) [155]. A comparative study among nanoshells and nanorods proved the latter has better photoabsorbing nanoparticles as they have better absorption and scattering co-efficients per micron, which is nearly an order higher than nanoshells [156].

The enhancement of pre-existing technologies and the development of new ones has facilitated the development of multimodal contrast agent [24,27,157]. MRI and optical imaging represents a complementary imaging pair with potential commercialization for clinical applications and when used in tandem can overcome shortcomings of optical fluorescence imaging such as limited tissue penetration and low 3-D spatial resolution. The luminescent hybrid nanoparticles with a paramagnetic Gd_2O_3 core/polysiloxane shell were applied as contrast agents for both in vivo fluorescence and magnetic resonance imaging, which can freely circulate in the blood vessels without undesirable accumulation in lungs and liver [158]. While in another study, luminescent silicon quantum dots (SiQDs) conjugated with superparamagnetic iron (III) oxide was used for in-vivo prostate cancer tumor model [159]. In another development protein annexin A5-conjugated nanoparticle, consisting of a QD encapsulated by paramagnetic micelles was successfully applied for the detection of apoptosis with the help of both fluorescence microscopy and MRI [160]. Saha and co-workers worked with glucose-functionalized $\gamma-Fe_2O_3$ -Au and $\gamma-Fe_2O_3$ -Ag for plasmon-based optical detection of protein as well as magnetic separation applications in COS-7 cell line. TAT peptide- and oleylamine-functionalized $\gamma-Fe_2O_3$ -QDs are used for fluorescence-based cell imaging and magnetic cell separation [161]. Polymers [162] and rare earth upconversion nanophors [115] have also find its application in bioimaging purposes. In fact Li et al., [163] has gone a step further in producing hybrid lanthanide nanoparticles coated with paramagnetic shell which are perfectly capable of both MRI and optical imaging, thus having the potential of being developed as a tool for detection as well as diagnosis in the near future. Also dual contrast agent, namely Fe_2O_3 /poly(2-methacryloyloxyethyl (2,3,5-triiodobenzoate)) core-shell nanoparticles for X-ray and MRI has been synthesized by Galperin, and Margel, [164].

3. Biosensor

A 'biosensor' is an analytical device for the analysis or sensing of biological samples by converting a biological response into an electrical signal. It is essentially a biocompatible diagnostic device able to respond to a signal generated because of some biochemical reaction (e.g., enzyme-substrate reactions) or bimolecular interactions (antigen-antibody, receptor-ligand, nucleic acid-protein, nucleic acid-nucleic acid, metal-macromolecule) convert the signal into electronic mode such that it can be quantified and discretized and generate amplified, comprehensible and feasible output.

A successful biosensor possesses at least a highly specific and stable biocatalyst, capable of analyzing on the basis of a reaction independent of physical parameters as agitation, pH and temperature, giving an accurate, precise, reproducible and linear response over the required range without dilution or concentration. The main components of a biosensor are; (i) the receptor (where the bioreaction converts the substrate to product), (ii) the transducer (which converts the observed signal to an electrical signal), (iii) amplifier (which can increase the signal response), (iv) processing and (v) display units [8]. The receptor of a biosensor is naturally a biological substance, designed to mimic a metabolic function. Most commonly used receptors are designed to contain an enzyme which detects its substrate in vitro or in vivo. Others include immobile antigen/antibody, nucleic acid, cell organelles (contain non-specific enzymes) or whole cells (microbes). Conventional biosensors used semi permeable membranes or polymeric matrices for immobilization of these receptors. Designing of bio-responsive shell over the core/shell nanoparticles will give larger surface for interaction, at the same time with the reduction in particles size sensitivity of the sensor phenomenally increases.

Though biosensors are diverse in nature they are grouped as calorimetric, potentiometric, optical, optoelectric, piezoelectric, and amperometric based on their transducing system. However, the most promising of these types are undoubtedly the piezoelectric, amperometric, and optical types. Also these biosensors are highly sensitive and easier to design as electronics; fluorescence, and crystallography are currently the most sought after subjects of research.

Nanoparticles have been found to play a critical role in augmenting the quality of these four types of biosensors because of their inherent magnetic, electro-sensitive, and optical properties. FET based biosensors have been until recently considered the best in terms of resolution, accuracy, and response time. Based on the property measured by the transducer element of the nanoparticles-based biosensors, these can be of (i) Piezoelectric, (ii) Amperometric, and (iii) Optical sensors; which are discussed in the following sections.

3.1. Piezoelectric biosensor

Piezoelectric crystals (e.g. quartz) oscillate with a specific resonant frequency (f) under the influence of an electric field. A change in resonant frequency occurs with a change in mass at the crystal surface, and may be due to molecules adsorbed or desorbed from the surface of the crystal obeying the relationship;

$$\Delta f = \frac{Kf^2 \Delta m}{A} \quad (1)$$

Where f is the change in resonant frequency (Hz), m is the change in mass of adsorbed material (g), K is a constant for the particular crystal dependent on such factors as its density and cut, and A is the adsorbing surface area (cm^2). This frequency change is easily detected by simple biological-sensing element, for example, formaldehyde dehydrogenase on a quartz crystal [165]. The major drawback of these devices is the interference from atmospheric humidity and the difficulty in using them for the determination of material in solution.

A piezoelectric core/shell nanoparticles system on the other hand can easily eliminate these problems. In fact they offer an attractive transduction mechanism and bio-recognition event with advantages such as solid-state construction, chemical inertness, and cost-effectivity. Once the variations of crystal frequency are amplified by such transduction system, the signal easily comes within the range of detection of quartz–crystal microbalance (QCM) which works on this principle and similar techniques. Metallic elements like iron, silicon, gallium and noble metals like gold find eminence in these nanoparticles. The frequency enhancement in the presence of nanoparticles is because of three reasons: (i) The nanoparticles bind and hence help concentrating the analyte molecules on the surface of the quartz crystal. (ii) The nanoparticles themselves form a substantial mass accumulating on the crystal surface. Hence the resultant frequency generated (which is directly proportional to accumulated mass) is high. (iii) Nanoparticles and nanostructures themselves possess some inherent piezoelectricity due to their crystal structure which is related to a phenomenon called Surface Optical Phonon Resonance (SOPR). Such sharp signal enzyme based sensor of core/shell Fe oxide/Au nanoparticles were obtained by coating pre-synthesized iron oxide nanoparticles with gold shells and tested for detection of volatile organic compounds using QCM by Wang et al., [166]. The nanoparticles were deposited as thin films on the crystal surface and the resultant change in frequency was correlated with thickness change. The device was then used to detect hexane, toluene and xylene based on the absorption potential of the Fe/Au nanoparticles towards these compounds. Shown in Fig. 14, is the set of the QCM response profiles, along with the sensor sensitivity (see insert), for the sorption of different vapor molecules {hexane (blue), toluene (green), and p-xylene (red)} obtained by their experiments.

For thin film Au shell constructs, 1,9-nonandithiol (NDT) or 11-mercaptoundecanoic acid (MUA) were used as mediators. The results show that higher sensitivity is shown in the case of NDT-mediated thin film than that of MUA-mediated thin film, reflecting partially the differences in dielectric medium, molecular structure, and more importantly film thickness between the two variants of nanoconstructs thus formed. The elemental compositions of the nanoparticles were so chosen simply to have a large adsorption capacity for the volatile gases and ability to form ‘thin films’ on the quartz surface.

In a more complex biosensing endeavor, similar $\text{Fe}_3\text{O}_4/\text{Au}$ core shell nanocomposites probes have been devised to detect DNA point mutation in aqueous solutions of DNA, using DNA ligase enzyme immobilized on the Au surface. While the Au particle provided the LSPR associated amplification of signal, the iron core provided target specificity and better conjugation with other bioactive molecules. For reproducibility and further signal strength probe surface was functionalized with

desthiobiotin which complexes with free avidin. The entire metastable conjugate complex accumulated on a crystal surface and induced piezoelectric response [167]. This shows that multifunctional piezoelectric biosensors which can be tuned to have other signaling pathways like magnetic and optical also exist.

As mentioned earlier, advanced and sharper piezoelectric signal generation is achievable by utilizing the SOPR mode. These genres of devices use an instrument devised by thermolysis of FeO and Pt over Zn core to form FePt/ZnO core/shell nanostructured biodetector showed advanced characteristics like discrete piezo-electric property, along with semiconductor property, both whose intensity could be adjusted according to practical situations, thus optimizing cost and reproducibility [168]. The nanostructures used and synthesized for the purpose of obtaining piezoelectricity need very accurate designing of its crystal structure. Though most elements used in their construction is not commonly piezoelectric in bulk state, the lattice structure development during synthesis is paid much attention to, such that at the nanocrystalline state high surface area is generated and piezoelectric properties are obtained through a vibrational phenomenon of SOPR [166]. Semiconductor type materials like phosphides and arsenides of gallium and elements of same group in the periodic table have been used to experimentally justify the structure–function relationship between piezoelectricity and nanocrystal structure [115]. GaP/GaPO₄ nanoparticles developed on such models have shown high peak resolution and strong signal output in piezoelectric SO phonon vibrational mode. It was noted that surface optical phonon mode can be clearly observed only when microcrystals are about one order of magnitude smaller than the wavelength of an incident radiation [115,169,170]. This limitation of design scale makes piezoelectric sensing a supplementary mode to other more refined sensing techniques discussed hereafter.

3.2. Amperometric biosensors

Amperometric biosensors work by the production of current when a potential is applied between two electrodes. They can be used to detect a wide range of biochemical reactions since most of them redox reactions or involve transfer of free electrons in unsteady state. Detection of these electrons requires mediators which transfer the electrons to the electrode. The electric current produced is proportional to the analyte concentration and independent of both the enzyme and electrochemical kinetics.

Compounds like ferrocenes represent a commonly used family of ‘mediators’. Glucose oxidase mediated detection of glucose is the most common biochemical reaction studied by such sensors. Hence we will concentrate on this biochemical process to illustrate the technique of nanoparticles mediated sensing, suitably shown in Fig. 15.

Fig. 15 shows glucose will be oxidized at the working electrode surface by the glucose oxidase enzyme. This reaction causes the mediator (ferrocenes) to be reduced. At a fixed potential applied between the two electrodes, the mediator is oxidized in generating a signal response. The anodic current was produced due to the release of electrons directly proportional to the glucose concentration in the solution. Nanoparticles based sensors can be effectively used in determination of the various analytes instead of the bulky and complex electrode/electrolyte probe systems. Uses of nanostructures increase the sensitivity and limit of detection when compared with conventional techniques. Nanostructures based amperometric sensors normally do not require any pretreatment of the sample and extra chemicals, unlike a number of alternative sensing systems. Moreover, core/shell based catalysts could improve the catalytic activity and exhibit higher activity and stability in electrocatalysis for the special electron–interaction between core and shell composites. Core/shell nanoparticles can be used in designing of highly selective, stable, fast response and portable devices [171]. For example, microchips using core/shell nano-composites can be used especially in in-situ diagnostic kits, where simply the core can act as the electrode/transducer system and shell immobilized with sensing molecules can

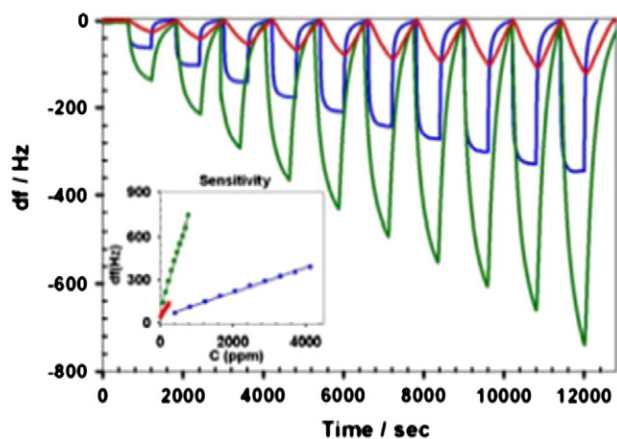


Fig. 14. QCM response profile of different volatile organic compounds as detected by piezoelectric sensor.

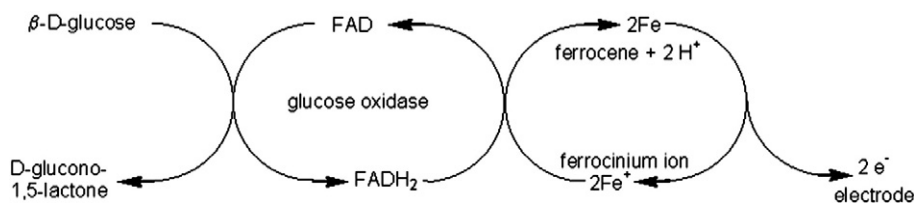


Fig. 15. Biochemical reaction of enzyme mediated glucose oxidation.

act as the analyte probe. Most of the unique properties of NPs such as magnetic, optical, or semiconductor find relevance in these ranges of biosensors since electron release, transport, and multiplicity, is the basic criteria for such sensors. The amperometric response of nanoparticle-based biosensors ranges within a few seconds. The most important feature is the 'noise' reduction. The signal to noise ratio of these biosensors ranges from 3–6 as against the very low values obtained for macro systems. Good reproducibility and long term stability are the added advantages. Such nanochips have been developed for the detection of H_2O_2 , glucose, uric acid and many other metabolic substrates. They commonly contain a core metal transducer nanoparticles coated with an insulating polyaromatic shell for conjugating with ligands and enzymes for ambient charge transport efficiency. In one method, enzyme HRP (Horse Radish Peroxidase) was immobilized on a polyaniline nanofilm by electrostatic attachment, onto an Au core. The Au nucleus embedded into the redox polymer acted as an effective charge migration channel and augmented the biocatalytic activity of the enzyme [163]. Poly(propyleneimine) coated dendrimeric gold nanoparticles functionalized with myoglobin as a protein based multi-shelled sensors [172], three-layered Au-modified poly pyrrole coated Ag nanocomposite as dopamine sensor [173], glucose sensor of Au/polyaniline/AgCl matrix type design [174] are some prototypic examples of different varieties of electron responsive biosensors designed on similar basis. In another record, instead of Au, polyaniline was electropolymerised over silica template to show that catalytic reduction of HRP was enhanced over polymer films in the presence of silica against non-silica counterparts [175]. These biosensor models typically represent the group of NP sensors built with an organic polymeric shell (whatever the core might be) since such polymers are good at conjugating with the probe molecules and easily form stable immobilization template and hence modulate the biosensor industry as the most sought after design construct.

Magnetic core of iron compounds are often used in sensors whose location and movement needs to be restricted by external fields. For example if nanosensor particles are released in bulk, in the analyte carrier medium in vivo (like into blood stream) given some time to interact with a certain biomolecule present in very low concentrations (as a biomarker), it also needs to be accumulated and channeled out of the stream at a later period for further diagnosis. Besides the biosensor constructs, however biocompatible they may be, is cytotoxicity upon accumulation in any region of the body for prolonged periods. Thus controlled dispersion of the NP sensors out of the system is a vital issue in certain cases, and in here magnetic NPs find major application. Their circulation and dissipation is most lucid to control externally and so in vivo biosensing devices mostly comprise oxides, selenides and sulfides of Fe, Cd and Ni. Different functionalizing molecules immobilized on ferric oxide core and SiO_2 shell NPs have been used in clinical diagnostics [176]. Hydroquinone biosensor determining hydroquinone concentration in compost extracts with immobilized laccase enzyme on the surface of modified magnetic Fe_3O_4/SiO_2 core/shell nanoparticles show that the use of the magnetic property is not restricted to in vivo clinical diagnosis only [177].

In novel design architecture nanosensor aggregates were supported on a conventional electrode. Nanoelectrode arrays synthesized by self-assembling of $CoFe_2O_4/Au$ core/shell magnetic nanoparticles were demonstrated as a potential electro responsive biosensor embedded on an

Au electrode surface by application of an external magnetic field. The nanowires were visualized by atomic force microscopy showing diameters around 40 nm and a length increase from 0.57 to 1.53 μm when the time intervals allowed for the self-assembling process ranged from 15 to 120 min. The conducting nanowires caused an increase of the electrode surface area yielding a 6.5-fold increase in signal strength after 120 min [178]. A slightly different microsphere structured Fe_3O_4 /chitosan NP was developed for detection of H_2O_2 by hemoglobin which was similarly attached to carbon glassy electrode surface [179].

Ferromagnetic cores are uniquely used in sensors meant for in vivo cell labeling as removal of the particles or often particle attached cells are an absolute necessity in these deep tissue diagnosis methods. Cell labeling is done using specific biomarker molecules which bind to specific target ligand counterparts expressed on the surface of corresponding cell types. Different types of cancerous or defective cells mostly have a widely studied set of marker molecules and are often identified through them using ferromagnetic core type amperometric nanosensors. Derivatization of super paramagnetic iron oxide nanoparticles with targeting ligands lactoferrin (Lf) or ceruloplasmin (Cp) (acting as the cellular markers) and their targeting to surface receptors expressed on human fibroblasts surface without being internalized by the cell was shown by Gupta and Gupta [180] (Fig. 16).

Recently, another intricate design has been reported of a fully integrated core-shell nanoparticle system responsive to glucose comprising a self-assembled glucose oxidase and an osmium molecular wire on core/shell Au nanoparticles $[Au/(PAH-Os)_4]$. The osmium wiring increases the interaction surface of the shell which due to Raman scattering property launches contactless optical signal thus amplifying the output signal in addition to the amperometric transduction of Au core [181]. An outer coating with functional organic molecules is often necessary in electron sensitive sensors to offer advantages including steric and/or electrostatic repulsion between the NPs, which can increase water solubility and stability in biological environment, in vitro and buffer against pH change and ionic interference in vivo [51].

Amperometric response of Au has been augmented by association with Pd in a novel technique for the development of nonenzymatic glucose sensor. The Au/Pd nanoparticles having a flower-shaped structure have been seen to have greater absorbance quotient and due to positively charged Pd surface, glucose oxidation is facilitated. Ionic liquid $[P(C_6)_3C_{14}][Tf_2N]$ functionalized on the shell surface provided a cross-bridging agent forming a matrix which could be immobilized on C-electrodes forming a high sensitivity, reproducibility amperometric sensor with signal to noise ratio of 3, at an even 0.1–1 mM analyte range [17,182]. These purely metallic nanosensors have recently been given much impetus due to their fine designing, smaller shape, construction efficiency and multimodal signal amplification.

Rapid functionalization of Au nanoparticles based on ligand-exchange process at citrate-capped gold nanoparticles, with homocysteine acting as the incoming ligand and glutathione as the moderator providing two dimensional nucleation and growth of ligand-exchange domains has been developed for the design of biosensors by Stobiecka et al. The fine tuning achieved due to the presence of the moderator brings down the lower detection limit of the system and the SERS effect of Au provides the signal amplification forming an optoelectric sensor [183].

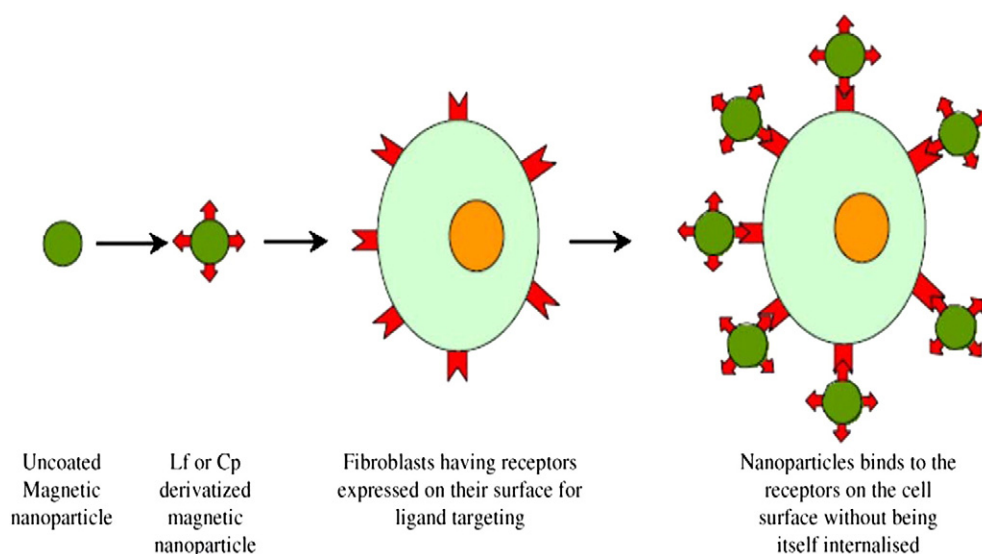
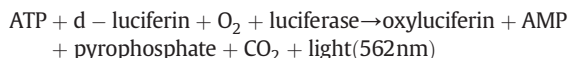


Fig. 16. General scheme of developing a composite nanoparticles system for sensing specific biomolecule [180].

3.3. Optical biosensors

The advancement in the area of optical biosensors has been increasing continuously during the last decade because of the development of several new nanomaterials. In general there are two main ways of developing optical biosensors. These involve determining changes in light absorption between the reactants and products of a reaction or measuring the light output by luminescent process. Many optical biosensors are based on the phenomenon of surface plasmon resonance (SPR) techniques. The light emission can be measured using spectroscopy or indirectly by fluorescent markers. Example of a luminescent process commonly monitored by an optical biosensor is oxidation of luciferin (source: firefly or bacteria).



Nanoparticle based biosensors can utilize external fluorescent markers similar to the FET sensors. Fluorescent semiconductor nanocrystals (NCs) also called Quantum dots (QDs) consisting of Cd, Se Zn and other elements of the same period in the periodic table are potent fluorescent labels, and do not suffer the setback of photobleaching as fluorescent dyes do. Also their emission colors are tunable from the visible to the NIR region by size variation. Fluorescent resonant energy transfer or FRET sensing commonly uses QDs [184]. Noble metals like Au and Ag are an alternative to semiconductor based NPs because of their better surface functionalization capacity. Often magnetic particles supplement both QDs and noble metal types by forming the easily exploitable core element [27]. In an experiment, controlled synthesis of cobalt ferrite superparamagnetic nanoparticles covered with a gold shell; the Au shell served as a versatile platform for linkage of protein nucleic acid (PNA) oligomers which could hybridize with complementary DNA and detect single nucleotide polymorphisms. The optical signal was given by fluorescent molecule Rhodamine 6G intercalated in the probe. Presence of a magnetic core provided easy dispersibility while the Au shell provides an optimized platform for chemical functionalisation and protects the magnetic core against oxidation [185]. Like fluorescent molecules, chromogenic compounds or their derivatives coated Au or Ag nanoparticles may act as optical probes [186]. Hamer et al., [187] reported a protocol for designing polyallylamine-chlorophyllide (PAH-Chl) derivatized spherical Au and Ag nanoparticles as a sensitive optical probe. PAH-Chl is important molecule in electron transfer systems and photosystems and has an absorption maximum at 650 nm. The

quantum efficiency of the chromophore was seen to increase many folds when electrostatically assembled with the metal seed, shown in Fig. 17.

Inorganic nanoparticles have found use as sensory instruments for their characteristic optical properties especially Dipole Plasmon Resonance (DPR) and Surface Enhanced Plasmon Resonance (SERS). These features are very prominent for metallic nanoparticles such as Au and Ag which is widely used in most nanoparticle based biosensors.

When a small spherical metallic nanoparticle is irradiated by light the oscillating electric field causes the conduction electrons to oscillate coherently. The oscillation frequency is determined by four factors: the density of the electrons, the effective electron mass and the shape and size of the charge distribution. This collective oscillation of the electrons is DPR. The DPR spectra show variation in peak height, width and position with respect to size, curvature and presence of other molecules in solution.

On the other hand, Surface Enhanced Raman Scattering, (SERS), is a surface sensitive phenomenon that results in the further amplification of Raman scattering by molecules adsorbed on surfaces, especially, on rough metal ones. The intensity of the Raman signal for adsorbates increases, depending on particular surfaces because of an enhancement in the electric field induced by the surface. Metallic nanoparticles especially Au, Ag respond sharply to SERS signals because of their surface chemistry, large surface to volume ratio and electric susceptibility and hence these noble metals are widely used in optical biosensors. Here the analyte molecules react with the catalyst functionalized on the NP surfaces and (1) generate free electrons and (2) cause absorption or desorption on the NP surface, both triggering a sharp change in native SERS pattern. Notably these particles are also good electron transporters; they make an ideal amperometric as well as optical, dual signal amplification mediators some of whose functioning have been discussed in the earlier section. It must be mentioned here that single mode optical sensing or amperometric sensing is not always preferred due to the common problem of background scattering from cell organelles. Using both these modes together create natural confirmatory signals for one another.

Combination of amperometric and optical transducer systems has led to the development of a hybrid generation of biosensors called opto-electric sensors. The wired-enzyme glucose oxidase (GOD) based biosensor designed by Scodeller and co-workers [181], mentioned earlier shows how nanoparticles-based biosensors can be versatile in the signal detection and transduction mechanism.

The osmium- GOD multi-layer conjugate shell illustrated Resonant Raman scattering when reduced in the presence of glucose. The Au

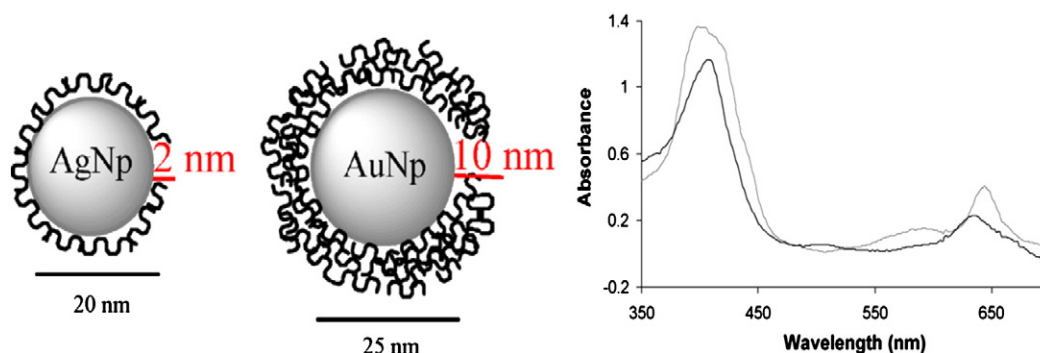


Fig. 17. PAH-Chl functionalized Ag and Au nanoparticles (left); Absorbance spectra in water of AuNP/MPS/PAH-Chl and PAH-Chl (right). The background spectra of the PAH and the MPS have been subtracted, to evaluate the surface plasmon effect on the chromophore signal [187].

core also sends SERS signal in response to change in electron density as the reaction proceeds, producing a loud signal even for a very low reaction rate thus making the instrument highly sensitive, accurate and specific with almost negligible noise. SERS based optical sensors can be specific enough to detect single nucleotide addition or deletion in *in vivo* gene analysis. Differentiation between strands, slight variation in morphology and concentration change has been shown to be picked up by these sensors quite effectively [186].

The resolution and sensitivity of nanostructured opto-electric sensors has been optimized further by associating it with magnetic nanoparticles. In a simple model, designed for *in vitro* DNA probing, a three layer composite structure with a gold surface, Fe_3O_4 inner shell, and silica core was synthesized by electrostatic assembling of negatively charged superparamagnetic, water-soluble Fe_3O_4 nanoparticles on amino-modified SiO_2 templates followed subsequent nucleation on Au seeds. The magnetic susceptibility of these sensors made them easily dispersible and negated the possibility of accumulation induced toxicity [188]. The plasmonic and magnetic responses can be tuned by controlling the shell thickness [189]. Another approach was followed to develop sensors showing reversible multicolor photochromism using nanocapsules of Titania shell and Ag core [190]. Photostability and dispersion stability could both be controlled by such sensor.

Au/Ag core-shell nanoparticles labeled by monoclonal antibodies onto silica/polymer shell are a lucrative option for immunosensor chips. These are fast replacing macromolecular matrix based ELISA, RIA kits. Mouse polyclonal antibody against hepatitis B surface antigen (mouse anti-HBsAgPAb), covalently cross-linked on silicon chips modified by a self-assembled monolayer of (3-amino-propyl) trimethoxysilane via glutaraldehyde (GA) activation and a corresponding probe of mouse monoclonal anti-hepatitis B antibody immobilized on Au nanoparticles integrated together could be potentially used as a high resolution, high specificity and effectively reproducible immunosensor. The inherent SERS signal strength of the nanoparticles produces the necessary amplification required in the output signal [191].

4. Targeted drug delivery

After the 1950s, with the advancement in the field of genetics and biotechnology, and a general impetus to hygiene consciousness, life expectancy suddenly crossed 77–90 years in many developed countries and most of the known pathogenic diseases were deemed curable. However, new virus-influenced physiological syndromes and nonpathogenic disorders now became statistically significant to be the focus of medical science. Today, ‘cancer’ and ‘AIDS’ evoke the same dreaded feeling as ‘plague’ and ‘leprosy’ would elicit in the 13th century Europe. However, though diseases have changed their form and manner, medicines and drugs have still remained the easiest and conventionally the most secure remedy or relief to all diseases.

Most recently, because of different breakthrough discoveries in medical science and engineering, drugs have become more and more customized and efficient to specifically address a physiological discrepancy in a more localized fashion. Most of the drugs till date are dependent on the circulatory system of the body to be channeled into their site of action, with the penalty of heavy dilution rate, reduced efficacy, and consequent increased side effects. These drawbacks sometimes surpass the benevolence of the drug itself. As an example, in chemotherapy normal cells heavily suffer from collateral damage along with the cancer cells. Research is now focused on more sophisticated modes of drug delivery which can be designed to channel itself discrepantly and confined to its site of action with a high level of specificity, evading the immune system till its purpose is served. This brings us to the realm of “targeted drug delivery” where the medication is concentrated on specific tissues, while reducing their relative concentration in others.

Conventional targeted drug delivery can be categorized as (i) active and (ii) passive targeting. In active targeting, antibodies are used which being inherently specific, needs no external targeting facility. The more important and difficult technology is the second approach of passive or EPR-dependent drug targeting which do involve immunoglobulins. The Enhanced Permeability and Retention Effect (EPR) is most commonly seen in tumor cells (being dysfunctional endothelial cells) which exist as cell aggregates with abnormal lymphatic–blood vascular dynamics and cell morphology. Owing to their high growth rate these cells trigger production of new blood vessels and subsequently become dependent on the neovasculature for their oxygen and nutrient supply creating a local micro-environment. Liposomes and similar sized particles such as nanoparticles can easily accumulate in these tissues and are retained more than in normal tissues. Hence EPR forms the principle of the modern targeted drugs and NPs become a core area of rationale designing of these drugs [21]. Targeting mechanisms are diverse but mainly based on biomarkers and their ligand–receptor interaction with corresponding tags present on the drug carriers. Macro systems of delivery become complex and non-specific, due to difficulty of combining so many types of materials (drug, adjuvant, carrier, targeting and other molecules) and therefore have low efficacy. Also drug carrying capacity and payload delivery remains low and slightly erratic.

Core/shell type of nanoparticles serve as crucial carrier of targeted drugs of the new era. The core material is coated with a suitable shell for the purpose of making the core/shell NP biocompatible and with improved pharmacokinetic property inside the body than earlier carriers. The drug is encapsulated or attached to the surface of the core/shell NP; the higher surface area leads to increased drug loading capacity. The surface modification is also done so that the nanocarrier only releases its load in the desired place of interest, under the influence of pH, temperature, additives (including drug molecules), etc., [192]. The controlled and targeted drug delivery can be monitored externally by fluorescent labeling of the nanoparticles or using inherent magnetic/

optical/semiconductor properties of the nanomaterials. We now further exemplify the use of nanoparticles in targeted drug delivery.

4.1. Nanoparticle based targeted drug delivery

Most targeted drug delivery research is concentrated in areas of cancer therapy and treatment of static tumors since the medication involved here, has a very low therapeutic index (ratio of therapeutic efficacy to side effects) leading to frequent relapse or severe deterioration in health [193]. So localizing the actions of these drugs to disorder of affected cells only is of utmost importance. After cancer therapy, diabetes and cardiovascular diseases are next in line in terms of current medical importance; all being diseases originating from malfunctioning of certain tissue regions, or cells. For example, diabetes is related to malfunctioning of pancreatic β cells. Hence, targeting these cells specifically for drug therapy, would provide a precise and permanent cure, and may root out the problem once and for all. Most of these cells are identified using biomarkers (i.e. a substance used as an indicator of a biologic state) which may be proteins, nucleic acids, carbohydrates, lipids, small molecules and so on. Complementary ligand tags conjugated to drug carrier locates the biomarkers thereby achieving quite precise targeting efficiency. In absence of markers, the drug is directed by external monitoring, ligand mediation or environmentally induced mechanisms.

In an early work chitosan/B-lactoglobulin core/shell nanoparticles were successfully prepared as nutraceutical carriers for nutrient delivery increasing permeability of the molecules, increasing gastric residence time and providing the environmental stability (that it lacked in normal food processing). This encapsulated nutrient system was deemed safe for oral administration [194]. Except for such exceptions, however most NP based drug delivery research is focused on cancer therapy as mentioned earlier. The material comprising the nanoparticles plays a significant role on the working mechanism.

4.1.1. Metal and metal oxide based core

Under this category two types are most important in drug delivery carriers: (i) magnetic materials such as iron and its oxides and sulfides, and (ii) noble metals such as gold and silver. The first kind of materials, i.e. the magnetic are mostly used as core material, because their properties are utilizable with or without surface exposure and except for Au, which is sometimes used as a shell material for its efficient surface functionalization of different ligands. Most inorganic nanomaterials remain foreign and toxic to the living system. Sometimes other metaloxide NPs TiO_2 , MnO , ZnO have also been examined for drug delivery.

Magnetic nanoparticles such as ferrites are most appealing for the purpose of controlled drug administration because of their biocompatibility and superparamagnetic behavior, facilitating the channeling of the drug towards specific target cells, by externally controlling its path using a magnetic field [195]. Such magnetic core NPs have been investigated to be potential drug carriers in cultured cell lines and in vitro systems simulating human physiological systems. Bare Fe NPs however are toxic due to their easy oxidizability, induction of free radicals in blood-stream and tendency to aggregate leading to thromboses formation. Structural stabilization of these ferrite NPs may be achieved by casing it with a noble metal shell, additionally providing platform for better surface chemistry between the drug and the carrier. Gold coating shows good adsorption for amine-groups in anticancer drugs such as doxorubicin, and also reduces particle aggregation by steric hindrance [196].

These type of carriers distribute the drug in adsorbed form and release them to the target sites in response to ionic, pH stimuli or externally controlled mechanism (magnetic or thermal). Magnetic core/mesoporous silica shell (MFeCMS) structures have been reported for in-vitro drug carrier application (Fig. 18) [197]. While Fe/Au core/shell nanocomposites [198], $\text{Fe}_3\text{O}_4/\text{CaCO}_3/\text{PMMA}$ [199], Au/poly(L-aspartate-doxorubicin)-b-poly(ethylene glycol) copolymer [200] may also find similar biomedical applications in the

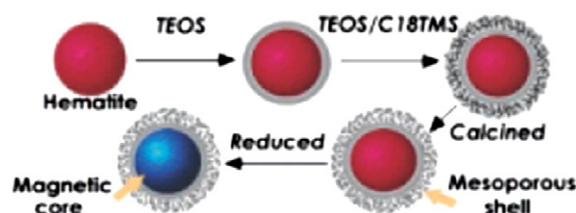


Fig. 18. Schematics of Fe/silica core/shell mesoporous nanoparticles synthesis route [197].

near future. Mesoporous silica containing free silane groups in the pore-walls or polymers as N-isopoly-acrylamide (NIPA) or folate-chitosans have been investigated other functionalizable shell components. As NIPA is a thermosensitive compound, provides an added advantage of hyperthermia treatment [201]. The Lower Critical Solution Temperature (LCST), the temperature at which thermosensitive polymers experience a phase shift from hydrophilic to hydrophobic state (for NIPA, it is 305 K) can be manipulated to release the entrapped drug under suitable conditions. The magnetic core was prepared by coprecipitating method, coupled with silane groups by catalyst hydrolysis and electrophilic substitution, and finally polymerized with NIPA [201]. Later follow-up showed an encapsulation efficiency of 72% and a higher release rate of doxorubicin at 314 K than at 310 K to prostate cancer cells JHU31 showing temperature sensitivity. Furthermore, NP uptakes by the cells were found to be dose and time dependent with saturation at 500 $\mu\text{L}/\text{mL}$ within 4 h. These carrier NPs were also modified for MRI contrasting agents [202]. As an example, immobilized ssDNA on MnO nanoparticle were effectively used for trimodal applications as diagnostic agent as well as contrast agent for MR and optical imaging in Caki-1 (human kidney cancer) cells [203]. Integrin $\alpha\text{v}\beta 3$ is expressed in both activated endothelial cells as well as some tumor cells, thus proving to be a promising imaging target for angiogenic activity. But PET and SPECT are the only bioimaging tools that has been successfully used in patients to produce images for pre-clinical studies using the integrin $\alpha\text{v}\beta 3$ specific tracers [^{18}F]galacto-RGD and [$^{99\text{m}}\text{Tc}$]NC100692 [204].

In general, the use of metal NPs is a cost effective drug delivery system especially because of the simplicity and stability of the drug-carrier nanoconjugate, high efficacy and target identification, ease of its circulation, traceability, and most importantly they are easy to manipulate from synthesis to disposal. However, the disadvantage is their toxicity to the body, and sometimes for more sensitive tissues delicate handling is required. Also for remote organs and tissues externally controlling the process of drug targeting via magnetic or optically sensitive NPs is very difficult and often outside the range of monitoring system. Finally, hydrophilic drugs cannot always circulate in the body as bare, adsorbed molecules without being noticed by other hydrophilic molecules and having unwanted or premature interactions with them. So other organic compounds and more delicate systems of nanoparticles have also been researched upon, as highlighted in the next sections.

4.1.2. Organic polymeric core nanoparticles

A much conventional mode of transport is however, the encapsulated form, using biocompatible, polymeric polysaccharides, lipids, amino groups or other synthetic structures mimicking the liposomal model as in gene transfection systems. The models basically consist of an insoluble lipophilic layer on the outside acting as a molecular barrier confining the drug in its inner hydrophilic environment and protecting it till it reaches its site of release, where conformational change is induced to break the barrier. These carriers bear the possibility of intravenous introduction, with low side-effects, facilitating extravasation to tumor regions, and are easily permeable across membranes.

In-vivo applications, however sometimes illicit phenomenal cytotoxicity (adding sometimes to the inherent cytotoxicity of the drug itself), mainly because of the presence of surfactant molecules used during the preparation of NPs [205]. An in-vitro (BGC23 and H22 cell lines) and

subsequent in-vivo study showed that PEG/PCL (polyethylene glycol and polycaprolactone) NP capsulated cisplatin delivered intratumorally, with encapsulation efficiency of more than 75%, was much less cytotoxic and yet superior in antitumor effect in contrast to administration of free cisplatin. Biocompatible PEG coating also helped the drug conjugate to evade endo-reticular scavenging, until drug release at targeted tumor regions [206].

There have been several works using LMWSC (Low Molecular Weight Water Soluble Chitosan) as carriers of anticancer drugs. Limitations to using such polysaccharides are their low solubility in water, especially as molecular weight increases and blocking of reactive amine groups in the presence of salts. The high surface area allows a wide platform for binding specific and modifiable ligands for targeting and localizing. The loading efficiency of drugs not only increases in the presence of hydrophobic core, but also decreases the sudden bursting and release of the drugs from the core/shell NP, because of increased stability. Incorporation of hydrophilic groups of MPEG (Methoxy Polythethylene Glycol) and a hydrophobic core of cholesterol (stabilizing the hydrophobic drug conjugation) on LMWSC to provide better drug efficacy of paclitaxel as observed on CT26 cells implanted on mouse models (Fig. 19) [207]. Besides, PLGA/polymeric liposome [118], polystyrene/PBCA [poly(butyl-2-cyanoacrylate)] [208] core/shell nanoparticle has also found applications in targeted drug.

Lectins have been used in some studies as a non-immunogenic highly specific targeting and drug adsorbing moiety (oligosaccharides that inhibit lectin induced agglutination in the body easily identify lectin bound drugs). Lectin-decorated nanoparticles with a hydrophobic poly(caprolactone) (PCL) core and a hydrophilic dextran (Dex) shell corona showed cellular tolerance of 70% even at a concentration as high as 660 $\mu\text{g/mL}$ [209].

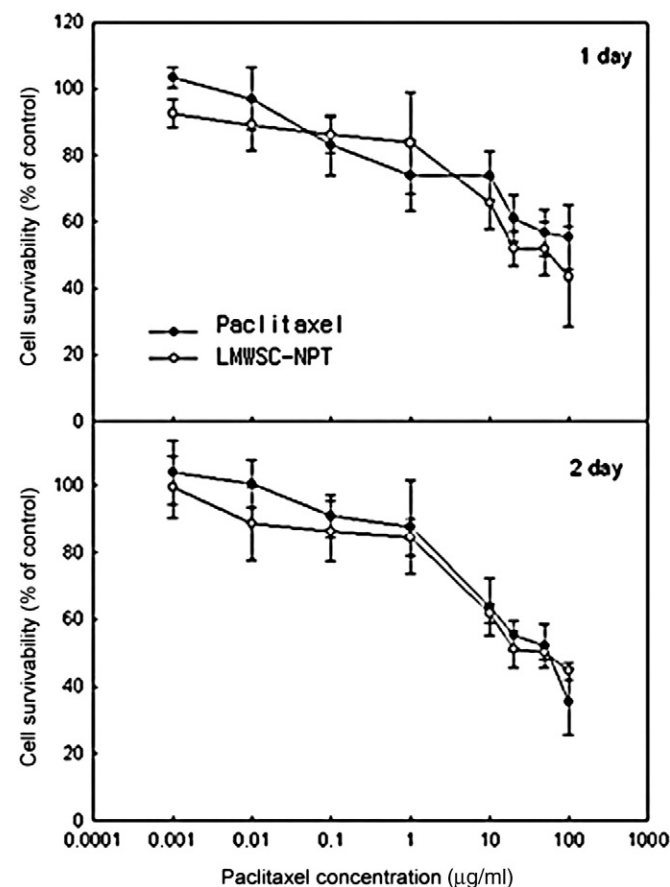


Fig. 19. Cell mortality rate of CT26 cancer cells in presence of a nanoparticle based drug delivery (chitosan microencapsulated) system in comparison to loose drug (Paclitaxel is antitumor drug) [207].

Folate-PEG coated cationic modified chitosan/cholesterol nanocomposites developed around the same time, is an example of ligand mediated targeting as a contrast to the more simpler stimuli sensitive targeting mentioned above. Here chitosan was modified using lysine providing steric stabilization and amphiphilicity, PEG coating prolonged the circulation time of the NP and Folate acted as the targeting moiety. This structure showed better encapsulation efficiency, slow controlled release of drug, and high infliction rate in MCF-7 (cancerous cells) only, as compared to normal cells, as supported through NMR, DLS and TEM [118]. Moreover, the work explained how such simple pH sensitive systems could be manipulated to deliver and release the drug at certain tissues only; the inflamed or tumor forming regions which become more acidic than the normal counterparts. In general monolayer protected silica/Au core/shell NPs have been investigated by several independent researchers to be an appealing mode for imaging and monitoring the pharmacodynamics of drug delivery. NIR thermal therapy coupled with photonics based imaging also requires AuNPs [153,210].

4.1.3. Nanohydrogel particles with core/shell morphology

Polymeric gels commonly termed as hydrogels have network like three dimensional structures, cross linked together either by physical or chemical cross-linking. They have a tendency to imbibe water and hence respond to changes in their microenvironment by morphological deformation such as bending, shrinking, swelling, or ionic and colorimetric alterations. The use of these versatile highly bio compatible structures for drug delivery has been extensively studied and reported in past reports [15]. Nanometer sized hydrogels in the form of a core/shell type structures have some unique properties, because of that these materials have numerous applications ranging from sensor to controlled release of active agents such as drugs [211]. Nanohydrogels with core/shell morphology include materials such as acrylonitrile/acrylamide [212], PMMA/PEI [213], 98% N-iso-propylmethacrylamide (NIPMAm)/97.5%NIPMAm, 2% BIS (N, N'-methylenebis(acrylamide)), and 0.5% APMA (N-(3-aminopropyl) methacrylamide hydrochloride) [214], poly(lactide-co-glycolide fumarate) (PLGF)/poly(lactide-co-ethylene oxide fumarate) (PLEOF), and poly(lactide-fumarate) (PLAF)/poly(lactide-co-ethylene oxide fumarate) (PLEOF) [215] and so on. The hydrogel nanocomposites can be classified according to incorporated materials and are receptive to pH and surrounding environments. For example, the effects of both pH sensitivity and magnetic field have been studied extensively as non-toxic drug-carriers using acrylonitrile/acrylamide core/shell nanoparticles. The polymeric gel imparts pH sensitive release modality, while the ferrite core provides for field directed targeting and disposal after use [212]. Also in an exclusive study [216], hydrogel nanoparticles synthesized with a N-isopropylacrylamide based core (365 nm), 3-(acrylamidopropyl)-trimethyl ammonium chloride (APTMAcI) shell (167 nm) and functionalized with a charged based bait (acrylic acid) were developed to simultaneously conduct size exclusion and affinity chromatography in a solution sequestering specific molecules present in very low concentrations. The objective of studying pH sensitivity and magnetic field effect was to collect several biomarker molecules present in blood plasma which are difficult to detect normally because of their very low concentrations with respect to the high amounts of proteins such as serum albumin. The model biomarker Platelet Derived Growth Factor (PDGF) was sequestered by the NPs in detectable range of ELISA tests.

Hydrophobic cored PMMA/PEI core/shell dendritic NPs containing amines such as lactate, aspartate (Fig. 20), supposedly offer good surface conjugation for drugs such as Ibuprofen with a high loading efficiency of about 23% [213]. The drug release efficiency is important in developing drug delivery systems as drug retention capacity. Naproxen and Trimethoprim were used as model drugs in synthesis of 2-Acrylamido-2-methylpropane sulfonic acid (AMPS) and 3-(acrylamidopropyl) trimethyl ammonium chloride (APTMAcI) shell forming charged monomers onto acrylonitrile (AN) core coupled with ferrites, for the synthesis of p(AN-co-AMPS) and p(AN-co-APTMAcI) respectively, which would

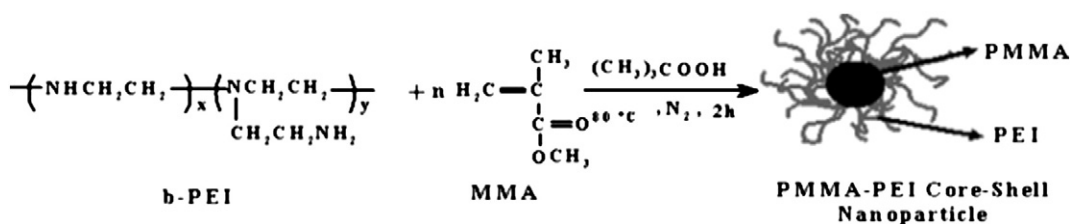


Fig. 20. Construction of PEI/PMMA NPs by free radical polymerization [213].

be dual responsive against pH and magnetic field, studied in oil-in-water emulsion system [212]. Chemical alterations were made to exchange the hydrophobic nitrile group in the core with hydrophilic amidoxime group and together with the sulfonic acid in the AMPS impart a high degree of hydrophilicity to the system necessary for keeping the drug functionally intact.

In a promising investigation in the field of molecular biology, such hydrogels were used in a gene silencing experiment. The poly N-isopropylmethacrylamide cross linked to N,N'-methylene (bisacrylamide) nanogel of about 100 nm diameter, was functionalized with a 12 amino acid peptide recognition tag, and carried Si RNAs targeting EGFR (Epidermal Growth Factor receptor) in absence of whose expression, cancer cells would become more susceptible to taxane-therapy (drugs like Paclitaxel, Docetaxel). Such gene silencing mechanisms are highly efficient if perfectly targeted and delivered in curbing tumor formation and metastasis [217].

In an earlier review Torchillin [218], noted that lipid core micelles with phospholipid chains are a suitable choice for carrying hydrophilic drugs and is often coupled with hydrophilic moieties like PEI to form amphiphilic micelles with stable retention and loading efficiency. Also double shell nanoparticles Au/oleic acid/N-isopropylacrylamide and acrylic acid core/shell/shell has been used as drug delivery vehicle [211]. Here it must be mentioned that drugs generally need to be hydrophilic for easy circulation within the vascular system (in water soluble form) and slightly hydrophobic to facilitate transmembrane diffusion and permeability. Hence, drug formulation and targeting requires relatively varying hydrophobic and hydrophilic duality in the systems, specifically altering in conformation and ionic nature at different stages in its pathway in vivo.

Though different types of core-shell NPs have been used in different designs the basic principle of protecting the drug until tissue specific release has been realized everywhere. The individual models may be compared on their efficacy, loading and releasing efficiency, biocompatibility and target specificity.

5. Interaction of nanoparticles with DNA and RNA

The interaction of nanoparticles with human cell has been a topic of profound interest among researchers, as they are thought to hold the key for future developments in the fields of biondiagnostic and therapeutics, among other fields. The range of nanoparticles between 50 and 200 nm has been deemed most effective for uptake in cells and this has opened new avenues of applications [219]. Gold nanoparticles are most commonly used for the detection of DNA and spectroscopic and electrophoretic technique has been applied to evaluate the interaction of Au with calf thymus DNA [220]. The use of gold nanoparticle has been prevalent as they can easily be synthesized in relatively pure and monodispersed form. Sun et al., [221] studied the effect of pH on the assembly of ss-DNA functionalised Au nanoparticles. Since the isoelectric point (IP) of ss-DNA is between pH 4 and 4.5, they are negatively charged above this pH and are easily conjugated with Au nanoparticle. They used this Au-ssDNA assembly for single-base mismatch detection and successfully applied it to detect 12 point mutations derived from human p53 gene. This methodology is unique in the sense that it neither requires complex DNA modifications nor signal amplifications; however

the only limitation is that it requires two individual reactions for comparison between a wild type sequence and a mutant sequence. A thermodynamically stable complex aggregate of DNA-Au nanoparticles are also reported [222], which can be prepared by two sharp and co-operative melting transitions, unlike the single sharp transition that is observed during dehybridization of ssDNA. The complex aggregate of DNA-Au has significant advantage in having two DNA binding domains, whereas the ssDNA has only one DNA binding domain, and also it can be prepared by different methodologies [222]. Han et al., [223] prepared DNA bound with trimethylammonium-modified mixed monolayer protected clusters (MMPCs), to protect the DNA from DNase 1 digestion. Then they studied the stability of Au-bound DNA toward biological (DNase 1), physical (sonication), and chemical agents (hydroxyl radicals) and concluded that DNA binding to positively charged capped gold nanoparticles provided adequate protection against enzymatic and physical degradation [223]. Similar reported studies have been found using silver nanoparticle/oligonucleotide conjugates based upon DNA with triple cyclic disulfide-anchoring groups, which has shown to withstand NaCl concentration up to 1.0 M [224].

The core/shell nanoparticles can also be used for inhibition of DNA hybridization [225]. It has been found that surface modification of Ag/Pt core/shell nanoparticles by thiol-modified oligonucleotides successfully reduces the nonspecific interaction between DNA and nanoparticles, by increasing the Pt particle size. Similarly, gold coated magnetic NPs (Co and Fe₃O₄) core/shell nanoparticles and their interaction with thiolated DNA was also studied [226]. Reduction of Au salt over pre formed magnetic cores resulted in composite type NPs. Core/shell NPs with Co gave Co-Au alloy type deposition shell; while in Fe₃O₄ core NP has distinct magnetite and gold phases. In general, functionalized magnetic NPs with Au shell facilitates thiol mediated conjugation of DNA on nanoparticle's surface. Mesoporous silica nanoparticle containing Fe₃O₄ inner core and silica shell has been prepared to study the DNA adsorption and desorption process and it has the added advantage of separation by the application of external magnetic field (Fig. 21) [227].

The schematic provided in Fig. 16, amply illustrates the advantages of using Fe₃O₄/silica core/shell mesoporous nanoparticle for the adsorption and desorption study of DNA over using normal core/shell nanoparticles for the same purpose. Localized surface plasmon resonance (LSPR) is well known to be excited on nanostructured noble metals, such as gold, platinum, and copper. Au/silica core/shell nanoparticles

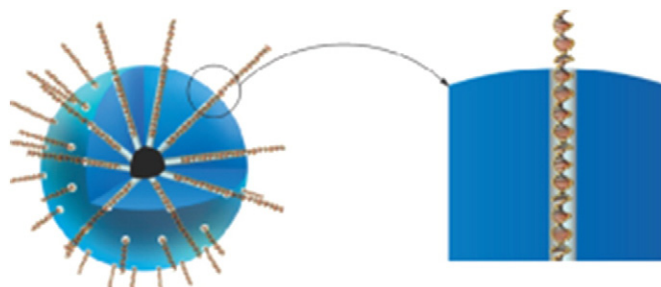


Fig. 21. Structure of M-MSN after DNA adsorption [227].

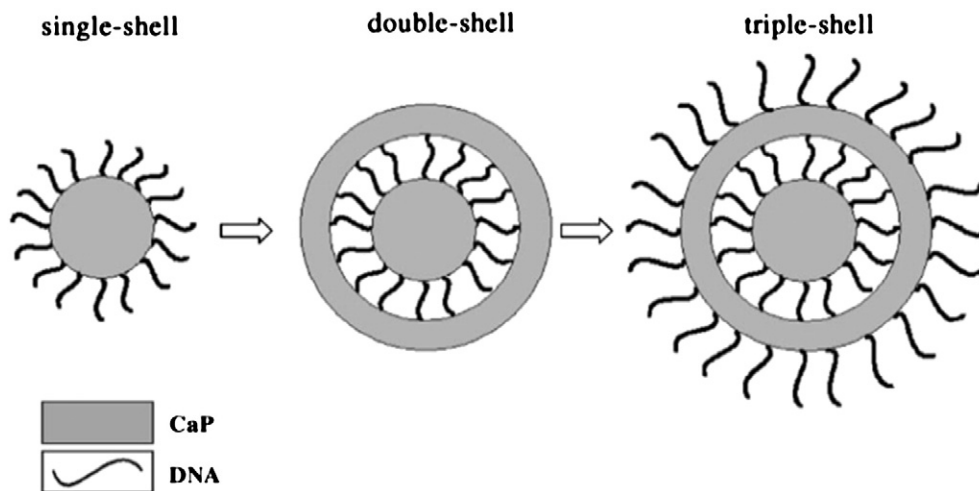


Fig. 22. Schematic representation of three types of Ca phosphate/DNA nanoparticles [233].

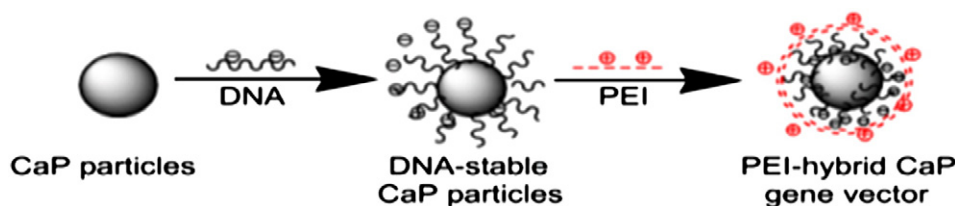


Fig. 23. Formation illustration of PEI-hybrid multi-shell calcium phosphate gene particles [104].

can be used for the optical detection of specific DNA–DNA and PNA–DNA hybridization reaction [186]. The LSPR label-free biochip constructed with a core/shell structured nanoparticle can detect very low amounts of DNA and it is cheaper than conventional SPR apparatus. Fluorescein isothiocyanate (FITC)/silica core/shell (C-dot) was taken and its surface modification was done using ssDNA oligomers tagged with Cy5 fluorophores. It was then applied in the quantification of DNA cleavage by heavy metals (Pb^{+2} , Cd^{+2} , and Hg^{+2}). This is particularly effective since it does not require separations or treatments for the

analysis of cleaved fragments, thus saving considerable time and labor [228]. Along with these CdSe/ZnS core/shell NPs with the attachment of Au nanocrystals (one to seven) [229], and dendritic polyphenylene-amino acids water soluble core/shell nanoparticles [230] have been complexed with DNA and its effect studied.

Nanoparticles conjugation has not been limited only to DNA but RNA also falls under its ambit. Su et al., [231] developed poly-(β -amino ester) (PBAE)-phospholipid bilayer core/shell nanoparticles for the purpose of developing a delivery vessel for mRNA-based vaccines. The PBAE aided

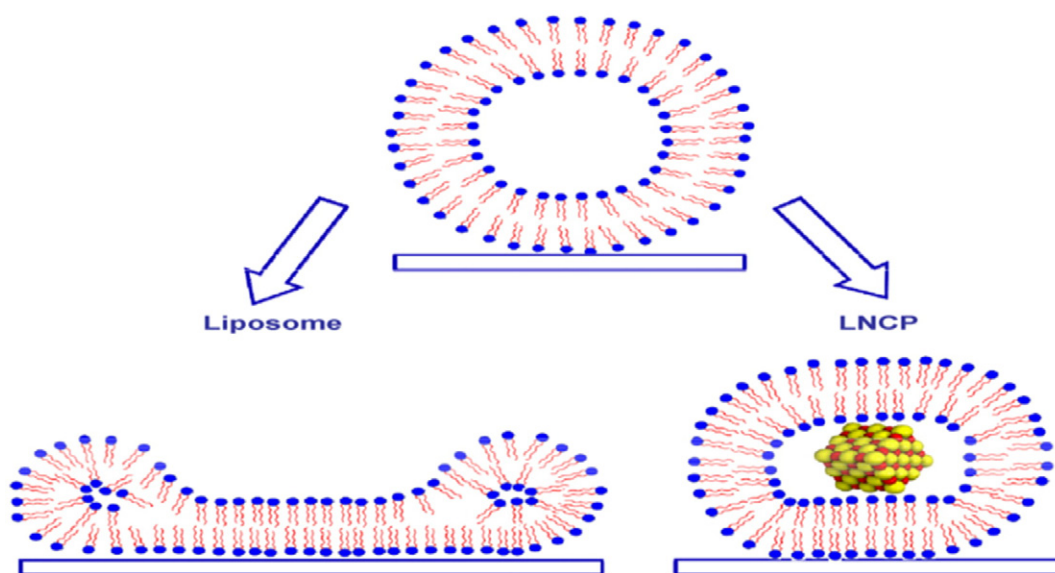


Fig. 24. Schematic model of DDAB liposomes and the LNCP adsorbed on the mica surface [234].

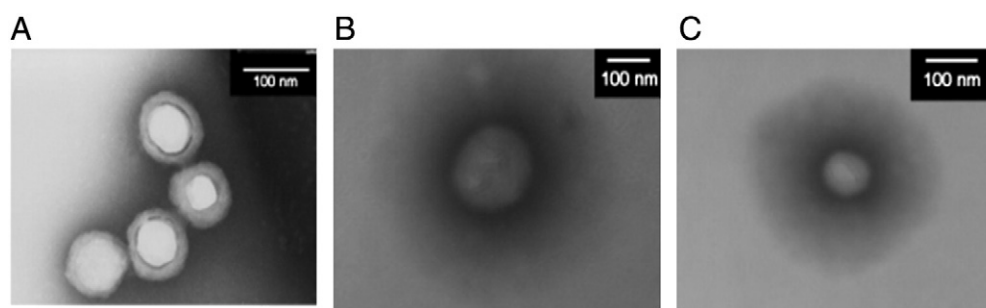


Fig. 25. TEM micrographs of PMMA-PEI core-shell nanoparticles: (A) NP1, (B) NP1 at a high magnification, and (C) NP5 at a high magnification [235].

in endosome disruption while the phospholipid bilayer minimized the polycation core. In-vitro studies were carried on in DC2.4 cells after the loading of mRNA by adsorption on this nanoparticles surface. Polymeric micelles (PEI-SA) were prepared by modification of Polyethylenimine (PEI) by grafting of stearic acid (SA) onto it. Then si RNA was bound on the nanoparticles surface and studies in Huh-7 cells indicated that bound si RNA to be less degradable and less toxic compared to free si RNA. This has then been used for co-delivering doxorubicin and vascular endothelial growth factor in the cells [232].

6. Targeted gene delivery

Gene is essentially the functional component of the DNA and incorporation of a DNA sequence into another both in-vitro and in-vivo is a common technique of genetic engineering. The oldest technique of gene transfer from one living cell to another was by using viral vector, plasmids, and other inherently infectious cells or their components — a process termed as ‘transduction’. Over the years non-viral gene transfer methods in prokaryotic cells were developed known as ‘transformation’. So, basically gene delivery can occur via two vectors, viral and non-viral. The non-viral vectors have added advantages over the viral

vectors, by being easier in production, storage and inducing less cytotoxic responses. Such safer non-viral methods when modified and implemented on the more complicated and advanced eukaryotes with the potential of application in medical treatment of a wide variety of diseases (by specifically incorporating or replacing desirable sequences) formed the basis of ‘gene transfection’. Typically gene transfection is done through chemical methods (using polyethylene glycol) electroporation, through liposomes or co-precipitation (with Ca-phosphate) techniques. Nanoparticle based systems as the carriers of DNA into the cell are the most recent tools developed in this field. The first approach is a direct approach to transfection, where DNA-coupled inert nanoparticles is “shot” directly into the target cell’s nucleus (Gene-Gun method) while the second approach uses magnetic nanoparticles to target and deliver DNA into cells, where the cargo is released (magneto-infection).

6.1. Principle of gene transfection

The basic pathway of gene insertion into the host DNA involves overcoming the natural tendency of the cell in discarding and disintegrating the foreign gene by its natural barriers. The external gene must be

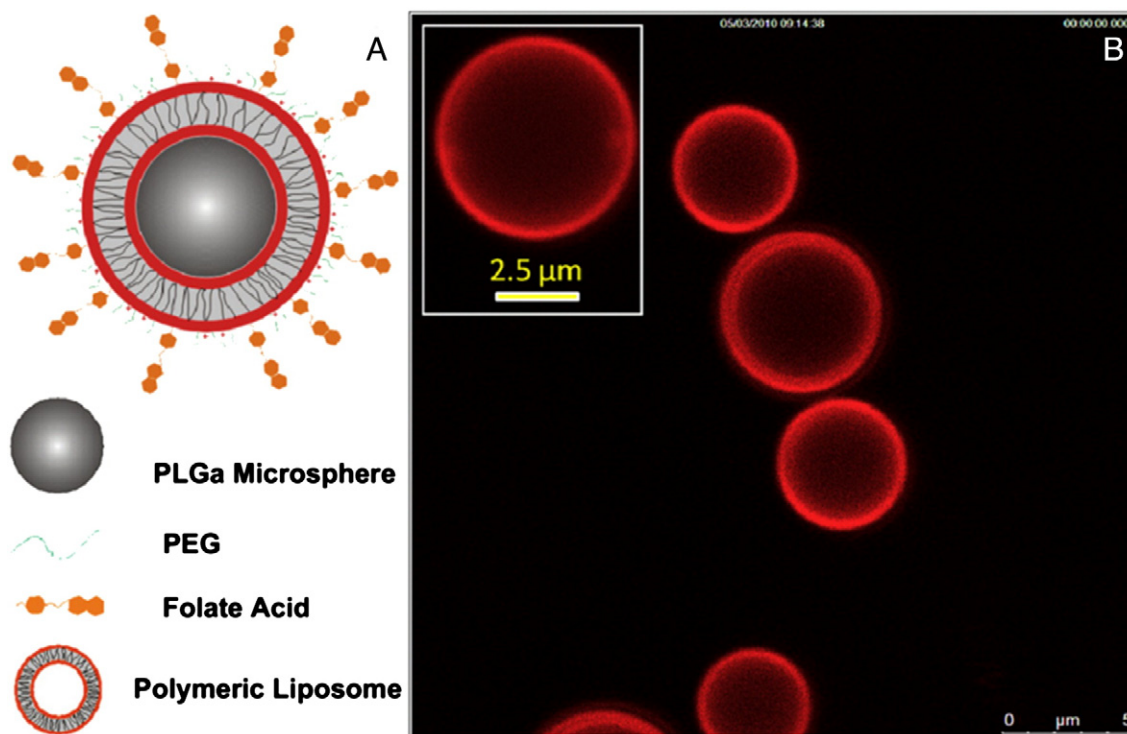


Fig. 26. (A) Schematic showing the structure of a PLGA/polymeric liposome core/shell particle. (B) Confocal fluorescence images of the Texas Red-labeled lipid shell, confirming the postulated core/shell structure [118].

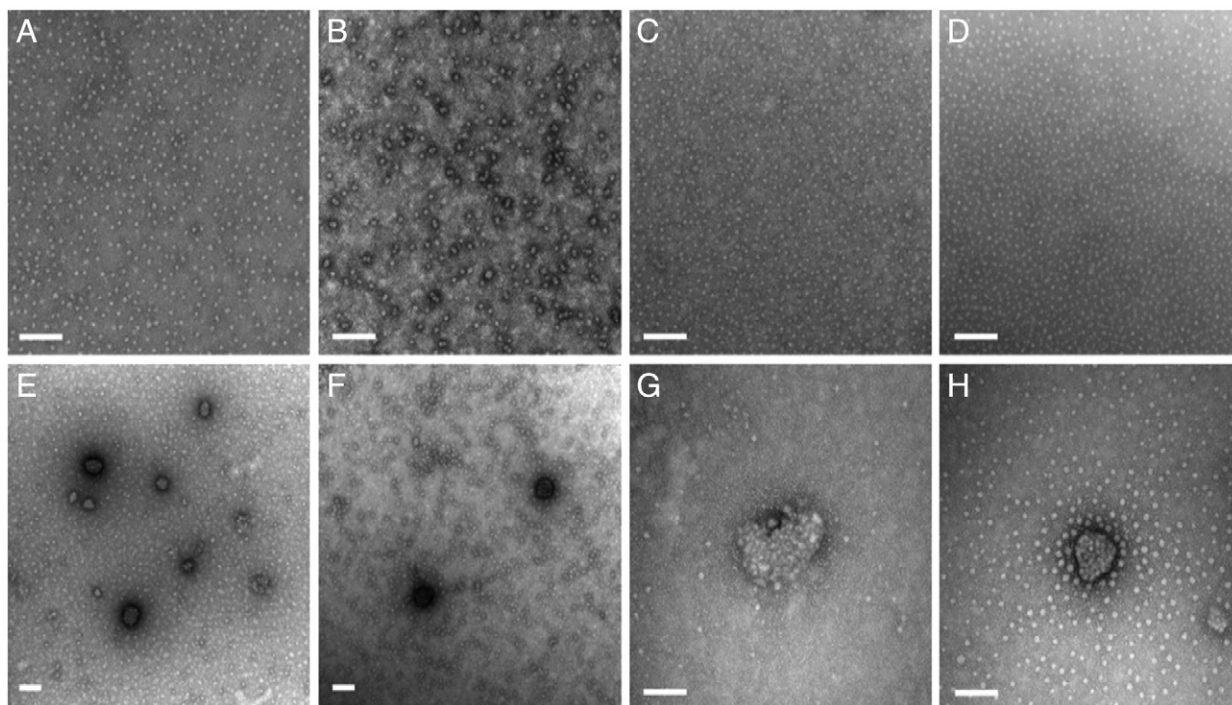


Fig. 27. TEM images of cSCKs/ODN and cSCK/pDNA complexes at different N/P ratios. (A) cSCK-pa 100/ODN, N/P 6:1; (B) cSCK-pa 50-ta 50/ODN, N/P 20:1; (C) cSCK-ta 100/ODN, N/P 20:1; (D) cSCK-pa 70-ca 30/ODN, N/P 30:1; (E) cSCK-pa 100/pEGFP, N/P 6:1; (F) cSCK-pa 50-ta 50/pEGFP, N/P 20:1; (G) cSCK-ta 100/pEGFP, N/P 20:1; (H) cSCK-pa 70-ca 30/pEGFP, N/P 30:1. The bar represents 100 nm [237].

protected from endonucleases and lysosomal secretions and thus is coated by an inert material. The complex is then permeabilized through the cell membrane by making the latter porous (poration process) or endocytosis. Once in the cytoplasm, the gene must be protected from lysosomal attack and channelized into the nuclear membrane. After delivery into the nucleus, the DNA must be free to attach to the host DNA specifically at the target site. Because of this extremely complex pathway gene transfection efficiency is generally very low. Viral methods though immunogenic or pathogenic are still the most widely used methods for gene transfer. Nanoparticles based systems therefore must be much meticulously designed to match the efficiency rate of the conventional transduction methods.

There are mainly four categories of core/shell nanoparticles developed for gene transfection in recent years based on the shell material for it is evident from the above discussion that protection of contained nucleotides and smooth circulation to target site is possible only by proper surface coating. All the compounds discussed below are basically chemicals known to conventionally interact with DNA long before the concept of nanoparticles existed.

6.2. Calcium-phosphate/DNA core/shell nanoparticles

Ca-Phosphate core type nanoparticles modified with DNA shell were the simplest NP based gene transfection agents developed [21]. Ca-phosphate is commonly used as co-precipitation salt for permeabilization of cell membrane and is an easy choice over silicates or magnetic NPs for their biocompatibility and biodegradability. However, using simple Ca phosphate core nanostructures coated by naked DNA core/shell NP, shows very low transfection efficiency even if the dose of DNA is high. In a typical experiment with Ca-P spherical nanoparticles carrying fluorophore protein coding plasmid DNA, pcDNA3-EGFP of about 10–20 nm size on typical cell lines HeLa, LTK, T-HUVEC showed that transfection efficiency could be increased many folds if the DNA was immobilized between alternate layers of Ca–P forming a multi-shell structure. For colloidal stabilization another DNA coat could be provided forming a “triple shell” of alternate DNA and Ca–P layer. Thus Ca–P/DNA structures with multiple layers are simplest core multi-shell types available without much variation in component materials, as illustrated in Fig. 22 [233].

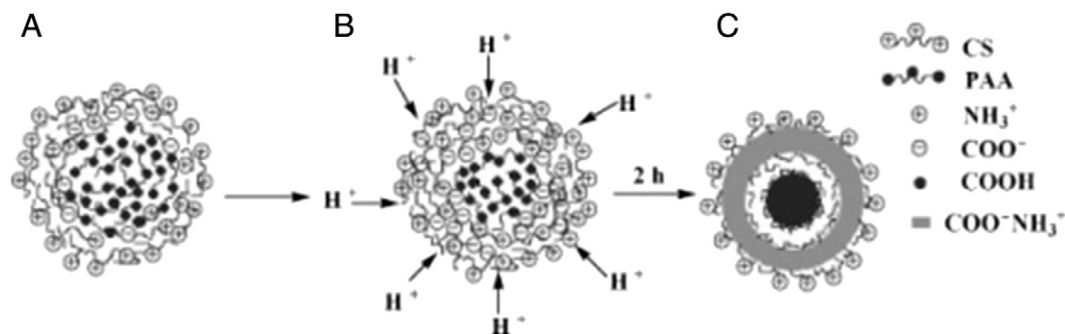


Fig. 28. Schematic illustration of formation mechanism for core-shell CSPAA nanoparticles. Structure illustration of CS-PAA nanoparticles (A) after PAA solution was immediately dropped into CS solution; (B) after incubation for a few minutes; (C) after incubation for 2 h [194].

Table 2

Classification of core/shell NPs based on different core materials and their applications.

Core/shell (metal or metal alloy as core)	Surface modification/ligands	Application	Ref
Fe/CNP	Poly(acrylic acid)(PAA), polyvinylpyrrolidone (PVP), poly(2-acetoxyethyl methacrylate) (PAEMA), poly-N-hydroxyethylacrylamide (PHEA)	MRI	[41,249]
Au/PEG		Optical imaging, drug delivery	[146]
Fe/ γ -Fe ₂ O ₃	Dopamine, PEG-600, dextran,	MRI	[54,250]
Ag/poly(N-isopropylacrylamide-co-acrylic acid)		Optical imaging, drug delivery	[147]
Au/polyaniline	Enzyme HRP	Amperometric sensor	[163]
Au/polypropyleneimine	Myoglobin	Amperometric sensor	[172]
Ag/Au-polypyrrole	Dopamine receptors	Amperometric sensor	[173]
Au/Pd	[P(C ₆) ₅ C ₁₄][Tf ₂ N]	Amperometric sensor	[182]
Au/citrate	Homocysteine, glutathione	Amperometric sensor	[183]
Au/CoFe	PNA oligomers	Optical sensors	[185]
Au/polyallylamine-chlorophyllide	Cofactors	Optical sensors	[187]
Fe/Au	Assorted ligands and bioconjugation molecules	Drug delivery	[198]
Fe/N-isopoly-acrylamide	Assorted ligands and bioconjugation molecules	Drug delivery and MRI imaging	[201,202]
Au/PEG-amino acid	Radioactive iodine and other targeting molecules	Drug targeting	[39]
Au/Os	Enzymes glucose oxidase and cofactors	Amperometric sensor	[181]
Ag/titanium	Chromophores and enzymes	Optical sensors	[190]
Au/oleic acid/N-isopropylacrylamide	Stabilizer molecules and target receptors	Hydrophilic drug carriers	[211]
Au-ssDNA	Assorted ligands	Detect mutations in human p53 gene	[221]
FeCo/graphite		MRI, optical imaging	[251]
Co(Fe)/Au	Possible attachment of organic molecules with thiol-terminations	MRI, medical labeling	[252]
FePt/ZnO		Piezoelectric sensor	[168]
Au/Ag + silica/polymer dual conjugate	Monoclonal antibodies onto Au/Ag NPs ligated to polymer surface using trimethoxysilane	Immunosensor chip	[191]
Core/shell (oxide core)	Surface modifications/ligands	Application	Ref
Fe ₃ O ₄ /SiO ₂	Fluorescein isothiocyanate (FITC) dye, chelated Gd(III)	MRI	[51–53]
Fe ₃ O ₄ /poly(allylamine hydrochloride) (PAH)/Au		MRI	[42]
Fe ₃ O ₄ /chitosan (CS) or oleic acid (OL) entrapped curcumin		MRI, optical imaging and drug delivery vehicle	[40]
γ -Fe ₂ O ₃ /polymers (PEG; D-glucuronic acid, PEI, PEGPEI)		Potential application in MRI	[253,254]
γ -Fe ₂ O ₃ /poly(2-methacryloyloxyethyl (2,3,5-triiodobenzoate))		MRI, X-ray	[164]
γ -Fe ₂ O ₃ /SiO ₂	PEG, amino acid, FITC	MRI, biolabeling	[18,255–257]
SiO ₂ /Au	Dye-functionalized monomer 1-pyrenebutyl acrylate and a trimethoxysilane-carrying one, (3-acryloxypropyl)-trimethoxysilane, antibody conjugation	Optical imaging, drug delivery	[153–155]
SiO ₂ /NaYF ₄		Optical imaging	[206]
Fe ₃ O ₄ /CaCO ₃ /PMMA	Assorted ligands and bioconjugation molecules	Drug delivery	[199,254]
MnO	PEG, D-glucuronic acid, lactobionic acid	Potential application in MRI	[254]
MnO/SiO ₂		MRI, cell labeling	[39,166,167]
Fe oxide or Fe ₃ O ₄ /Au	DNA ligase enzyme/desthiobiotin	Piezometric sensor	[166,167]
SiO ₂ /polyaniline	Enzyme HRP	Piezometric and optical sensor	[175]
Fe ₃ O ₄ /SiO ₂	Assorted biomarkers	Amperometric sensor	[176]
CoFe ₂ O ₄ /Au	Biomarkers and enzymes	Amperometric sensors	[178]
Fe ₃ O ₄ /chitosan	Hemoglobin for H ₂ O ₂ detection	Amperometric sensors	[179]
Fe ₃ O ₄ /silica/Au	Enzymes/nucleotides	Optoelectric sensors	[188,189]
MnO/ssDNA	Biomarkers, assorted molecules	MRI imaging, drug delivery and tumor sensor	[203]
Core/shell (semiconductor core or shell)	Surface modifications/ligands	Application	Ref
CdSe/CdS/SiO ₂		Biolabeling	[129]
GaP/GaPO ₄		Piezoelectric sensor	[115]
Core/shell (lanthanide/lanthanide oxide core)	Surface modifications/ligands	Application	Ref
β -NaGdF ₄ : Yb ³⁺ /Tm ³⁺	PVP octylamine-PAA	MRI, optical imaging, biolabeling	[34,258,259]
α -NaGdF ₄ :Yb ³⁺ :Er ³⁺ /NaGdF ₄	PEG-phospholipid	MRI	[260]
NaYF ₄ /NaGdF ₄		MRI	[261]
NaGdF ₄ : Tm ³⁺ /Er ³⁺ /Yb ³⁺	Azelaic acid	MRI, optical imaging	[262]
KGdF ₄ : Yb ³⁺ , Er ³⁺ , Ho ³⁺ , Tm ³⁺		Optical-magnetic dual modal nanoprobes	[263]
Gd ₂ O ₃ /D-glucuronic acid	D-glucuronic acid	MRI	[32]
GdF ₃ /citrate	Citrate, 2-aminoethyl phosphate (AEP)	MRI	[33]
GdF ₃ /LaF ₃			
Gd ₂ O ₃	PEG, D-glucuronic acid, lactobionic acid	MRI	[32]
Gd ₂ O ₃ /MnO	Lactobionic acid	MRI	[36]
Gd ₂ (OH) ₅ NO ₃ ·nH ₂ O	PEG	MRI	[38]
Gd ₂ O ₃ /SiO ₂	Poly(2-methacryloyloxyethyl phosphorylcholine), poly(lactic-co-glycolic acid) (PLGA).		[264,265]

(continued on next page)

Table 2 (continued)

Core/shell (lanthanide/lanthanide oxide core)	Surface modifications/ligands	Application	Ref
NaYF ₄ /Si-DTTA (3-aminopropyl (trimethoxysilyl)diethylenetriamine tetraacetic acid) loaded Gd (III)		MRI	[163]
Gd/SiO ₂	Multi-layered silica, PEI, 3-hydroxypicolinate	MRI, biolabeling	[35,37,266,267]
Gd-DTPA/SiO ₂			
Core/shell (organic core)	Surface modifications/ligands	Application	Ref
Fe ₃ O ₄ embedded in poly(DL-lactide) (PLA)/polyvinyl alcohol (PVA)		MRI, ultrasound	[48]
Cyanine dye/SiO ₂		Optical imaging	[268]
TRITC dye/SiO ₂		Optical imaging	[143]
Rhodamine B (RB) or (6G) dye/SiO ₂		Optical imaging	[140,269]
Cy5 dye/SiO ₂	PEG	Optical imaging	[145,270]
Alexa Fluor 700 (or 750) dye/SiO ₂		Optical imaging	[145,270]
DY730 (or 780)/SiO ₂		Optical imaging	[145,270]
Fluorescein isothiocyanate dye/SiO ₂		Cell labeling, Optical imaging	[271]
Coumarin 7 dye/SiO ₂		Optical imaging	[272]
Chitosan/B-lactoglobulin			[194]
PEG/PCL	Biomarkers	Drug delivery	[206]
Cholesterol/chitosan	MPEG	Drug delivery	[207]
PLGA/PEG	Folate and assorted biomarkers and stabilizers	Drug targeting	[118]
Polystyrene/polybutyl-2-cyanoacrylate	Assorted biomarkers and stabilizers	Drug targeting	[208]
Polycaprolactone/dextran	Lectin, biomarkers	Drug delivery	[209]
Chitosan/cholesterol	Folate, PEG	Drug delivery	[273]
Ferrite impregnated acrylonitrile/acrylamide	Assorted biomarkers and stabilizers	Drug delivery	[212]
PMMA/PEI	Assorted biomarkers and stabilizers	Drug delivery	[213]
PLGF-PLAF/PLEOF	Assorted biomarkers and stabilizers	Drug delivery	[215]
Polystyrene/polybutyl-2-cyanoacrylate	Thioflavin receptors	Drug delivery	[208]
PMMA/PEI	Lactate, aspartate and biomarkers	Drug delivery	[213]
N-isopropylmethacrylamide/ N,N'-methylene (bisacrylamide)	Peptide recognition tag, EGFR	Gene silencing and drug targeting	[217]
PEG/chitosin	Gene insert and associated targeting molecules	Gene transfection	[243]
PEG/polyglycerol	Gene insert and associated targeting molecules	Gene transfection	[237]
Hyperbranched polyglycerol/PEI	Gene insert and associated targeting molecules	Gene transfection	[239]
PEG/PBLG	Gene insert and associated targeting molecules	Gene transfection	[240]
L-aspartate/PEI	Gene insert and associated targeting molecules	Gene transfection	[238]
PLGA/folate coated PEG- cholesterol	Gene insert and associated targeting molecules	Gene transfection	[118]
DNA-PMMA/chitosan	Gene insert and associated targeting molecules	Gene transfection	[243]
Histidine and lysine oligopeptide/DNA	Gene insert and associated targeting molecules	Gene transfection	[244]
Oligopeptide/DNA-PEG	Gene insert and associated targeting molecules	Gene transfection	[245]
Polylysine/PELGE	Gene insert and associated targeting molecules	Gene transfection	[246]
PAEA128-b-PS40/DNA	Gene insert and associated targeting molecules	Gene transfection	[237]
PGLA/DNA functionalized glycol/chitosan	Folate and other biomarkers	Gene transfection	[247,248]
Chitosan/polyacrylamide	Gene insert and associated targeting molecules	Gene transfection	[111]

Fig. 23, schematically shows the composite structures of CaP/DNA core/multi-shell nanoparticles, which has increased transfection efficiency, nearly comparable to polyfect (a conventionally used gene carrier) and storage stability of 2 weeks at 277 K. Similar hybrid Ca-P based nanoparticles were also developed with the DNA 'sandwiched' between distinct Ca-P core and polymeric polyethylenimine (PEI) shell [104]. Experiments showed that the sizes of the particles are dependent on PEI concentration with agglomerates being formed because of inter-particular cross-bridging which is detrimental to the internalization capacity of the NPs [104].

After the attachment of DNA to the CaP particles, PEI adsorbs outside the wall of the DNA layer to give rise to hybrid multi-shell NP (as shown in Fig. 23) which has found application in gene delivery. Lipid coated Ca phosphate NPs (LPCP) which closely resembles the liposome structure (Fig. 24), have been recently studied for effective pDNA delivery in standard cell lines with high storage stability without loss of efficiency [234].

6.3. Organic polyethylenimine and other cationic polymeric shell

Cationic polymers (usually branched and linear polyethylenimine (PEI), copolymers of PEI, poly(L-lysine) and its copolymers, chitosan and dendrimers etc.) are in general widely used as shell, because easy adsorption of DNA by electrostatic interaction protects it from degradation and enhances transport across cell membrane by endocytosis—all

attributed to their "proton-sponge" effect, though their molecular weight and particle size greatly influences the transfection efficiency. These cationic polymers are easily synthesized and engineered to cater to the specific requirements of gene delivery [225]. Grossly, PEI with molecular weight higher than 25 kDa are toxic and non-biodegradable but providing another polymer as core and complexing with DNA (Fig. 25), decreases the cytotoxicity to about three times [235]. PEI is quite often used as a control to evaluate the gene expression of other non-viral vectors, because it gives higher gene transfer efficiency. Cytotoxicity studies as shown by MTT and bioluminescence assays, coupled with the primary objective of enhancing transfection efficiency has led to experiments by grafting PEI with other polymers such as chitosan [236], polyglycerol [237], α , β -poly(L-aspartate-graft-PEI) [238], hyperbranched polyglycerol [239] and polymethylate/PEI core/shell NPs [235].

Along with PEI, poly(ethylene-glycol) (PEG) has also been used in conjugation with poly(γ -benzyl-L-glutamate) PEG/PBLG core/shell NP [240] and transfer in (Tf)-PEG-modified liposome [241] for the purpose of gene therapy. Since gene therapy is frequently associated with cancer therapy, tissue specific targeting compounds are frequently inserted into the gene NP complex where cationic polymers are commonly sought after. This is because, these core/shell NPs when used in-vivo as in chemotherapy, inherently have the hydrophobicity associated stability and high target localization (mimicking the liposomal behavior).

But it must be modified for better penetration and retention rates along with circulation life, which is grossly dependent on its size and surface charge. Subsequently positively charged shelled NPs must be neutralized for in-vivo applications. As reported by Tong et al., [242] shell zeta potential of ± 10 mV and particle size of 50–200 nm is ideal for evading the immune system of the body till admission into the target tumor cell. Recently in a novel study, cationic PLGA/folate coated PEGylated polymeric liposome core/shell nanoparticles (PLGA/FPL NPs) self-assembled from a hydrophobic PLGA core and a hydrophilic folate coated PEGylated lipid shell was designed for targeted co-delivery of drug and gene. Hydrophobic drugs were included into the core and the positively charged shell of the drug-loaded nanoparticle was integrated with DNA increasing chemo sensitivity of cancer cells at a gene level, at the same time targeting delivery of drug to the cancer tissue enhancing its bioavailability and reducing the toxicity [118]. A schematic of the drug carrier and its confocal fluorescence images of the Texas Red-labeled lipid shell as shown in Fig. 26.

As shown in Fig. 20, the PLGA microsphere was coated with polymeric liposome and modified by the addition of PEG and folate acid. Besides PEI, poly(methyl methacrylate) (PMMA) cores surrounded by acid-modified chitosan shells were synthesized and plasmid DNA was complexed with it. This plasmid DNA-PMMA/chitosin complex was used to investigate zeta potential measurements, which showed that this assembly could be a potential gene carrier [243].

6.4. Polypeptide shell

Amphiphilic and cationic polypeptides and compounds naturally found in the body have been used alternatively for the creation of core/shell NP for the application of targeted gene delivery. In one study, amphiphilic oligopeptides/DNA core/shell NP comprising alanine, histidine and lysine oligopeptides (AK27,AK32) were combined in different ratios to form NP micelle transfection vectors with a natural hydrophobic block (due to amphiphilic nature) protecting the transgene, having very high transfection efficiency and 30% less cytotoxicity than PEI on testing on HepG2 and 4T1 cell line. The cationic domain successfully intercalated with the oligonucleotides and showed strong proton-sponge effect [244]. However such self-assembling polyion complex micellar (PIC) NPs have low solubility in blood stream. Attaching a hydrophilic segment of polyethylene glycol has shown to increase the solubilization potential of such vectors when intravenously administered into the body in an in-vivo tumor turnover study [245]. Similar results were also observed using biodegradable monomethoxy (polyethylene glycol)-poly(lactide-co-glycolide)-monomethoxy (polyethylene glycol) (PELGE) on polylysine core as vectors in HepaG2 and HeLa cell cultures [246]. Prevention of DNA fragmentation during double emulsification phase of developing the DNA-PIC conjugate is a difficulty commonly faced while dealing with this category of vectors. It is however conclusively proved that both charge ratio and charge distribution ratio of all the ions constituting the nanocomposite vector play the most important role in effective gene transfection.

Polymeric shell cross linked to form knedel-like cationic micellar structures (CSKs) can be constructed from block copolymers as another important modification over the spherical NPs which offer good packaging of the nucleotides mimicking the histone-DNA model of nucleosomes. Such an amphiphilic block copolymer, poly(acrylamidoethylamine) 128-b-polystyrene40 (PAEA128-b-PS40), was synthesized, micellized in water and shell-crosslinked using a diacid-derivatized crosslinker, to give cationic shell-crosslinked nanoparticles (cSCKs) with a mean hydrodynamic diameter of about 14 nm and reported to transfect HeLa cells with a transfection efficiency of 27% as against 12% transfection efficiency of polyfect under identical experimental environment [239]. They also reported a facile method of imparting cationic nature to neutral or anionic shell material (Fig. 27). The transfection efficiency of cSCKs increases in the presence of tertiary amines while decreased when carboxylates are added [237].

6.5. Polysaccharide shell

Polysaccharide based NPs however are also a biocompatible, stable and effective gene delivery agent. PGLA/DNA functionalized glycol/chitosan comprising core/shell NP has been used for transdermal gene delivery for studying functional aspects of the immune system by expression of a reporter transgene. Chitosan and its derivatives form a very stable protective shell preventing enzymatic degradation of the nucleotide and evades immunogenic response and rigid enough to be used in 'gene gun' technique [247]. In a folate responsive tumor cell targeted gene transfection experiment, histidine modified chitosan (of molecular weight 15 kDa) was used to increase nuclear uptake of the particles. Here too, charge ratio was an important factor of transmembrane permeation while degree of quaternization of the polysaccharide influenced the transfection efficiency [248]. An earlier work showed coacervation technique as best suited for developing polysaccharide based NPs without addition of surfactants and organic solvents. Chitosan/Polyacrylamide complexed with nucleic acid synthesized by this method forms micro-structures whose properties like size distribution efficiency, solubility, stability in solution was characterized. Studies showed small size of these NPs can be achieved by increasing solution temperature and using low molecular weight chitosan (as shown in Fig. 28), while external crosslinking agents increase stability [194].

7. Summary of the reported studies

We have attempted to present the overview of biomedical applications of core/shell nanoparticles in this review. Owing to the fact there are several types of core/shell nanoparticles are involved, several possibilities are there to structure the article. This section aims to provide a summary of all core/shell particles based on the type of materials in a tabular form (Table 2).

8. Concluding remarks

Diagnosis of diseases is very important in health care, which in turn not only enhance the effectiveness of medical treatment but also save human life where early diagnosis is crucial. However, in many cases early diagnosis needs sophisticated biomedical instruments or improved techniques. The application of nanoparticles in the realm of biomedical engineering has ushered in a new era for the development for novel contrast agent and drug delivery vehicle, which has the potential to revolutionize in the area of health care. The idealistic concept of a single platform for drug delivery to its monitoring of drug-release seems to be feasible in the near future, because of the recent advances in the application of novel nanomaterials in this field. The versatility of nanoparticles has been applied in various studies related to disease diagnostics, early detection studies, and better contrast agents for improved imaging techniques. The development of new drug delivery vehicles has not only reduced the payload of the drugs but has also improved the efficacy of the drug in the system because of improved bio- and cyto-compatibility along with increased circulation time. Thus, the advent of nanoparticles has influenced all the spheres pertaining to medical biotechnology and biomedical engineering, improving and enhancing the already existing techniques along with the experimentation of new and advanced techniques for drug delivery and its monitoring. In this article, two general fields of applications namely diagnosis (analytical- biosensor/nucleotide interactions or visual-bioimaging) and transportation (drug delivery and gene transfection) are discussed. The review clearly shows, except bioimaging, that most other areas of bio-application are concentrated on nanoparticles of noble metals or magnetic materials. Quantum dots and other semiconductor types are quite common in bioimaging and cell signaling but less in biosensors, drug or gene delivery. Iron and its compounds are extensively used as the core particles. However, gold particles seem to be the most versatile and 'all-rounder' element being useful both in core and shell, for its high optical and amperometric

signaling capacity, good surface functionalization of several ligands and general inertness. It has been the most convenient nanomaterial for biosensors where a strong signal transduction and display system played a major role. Silica is often used to provide structural integrity and additional inertness finding a unique place in all applications.

Organic biomaterials, such as peptides, chitosan or polymers such as PEI, PMMA etc. become more important in drug/gene delivery systems over the diagnostic applications. This may be attributed to the fact that though biocompatibility and stability of the nanoparticle system is essential for every aspect of biomedical engineering, it becomes more complex and challenging in case of delivery systems. Here the carrier needs to be translocated deeper into the body, often into live cells, and infuse the payload systematically, without itself getting unwantedly or prematurely disrupted. The literature in this area illustrates the extent of feasibility in the usage and constraints of these biocompatible polymers as novel drug and gene carriers. In general, this type of work requires specific and highly accurate interaction of a vast number of molecules which will synchronize the translocation, delivery and not to mention the excretion of the nanoparticles. Because of the importance of these particular types of nanoparticles in this specific area there is a huge diversity of literature coming up almost every day in nanoparticles synthesis, modification of ligands, mechanism of action and so on.

It is also important to note that biomedical applications of nanoparticles would not have been possible without the presence of core/shell structure. Different types of core/shell structures have been presented in our discussion. Single layered/multilayered/layered layer template design has been used in bioimaging, biosensors and DNA interactions while matrix embedded, hollow core/shell templates find more useful in encapsulation of drug and gene. Because of the toxicity and instability of pure nanoparticles, different shell materials are designed to coat on the core surface in the form of core/shell structure. Apart from few commonly used materials such as gold, silver and mesoporous silica, most of the nanoparticles are used organic polymers or biomolecules such as amino acids, cholesterol, complex polysaccharides and their derivatives as a shell layer. In addition, enzymes or reactants, probe molecules, targeting ligands, oligonucleotides and a vast array of stabilizers, mediators, and translocators are immobilized onto the shell as required. Thus surface modifications and interactions are the most complex process and unique part to engineered nanoparticles.

The size and shape of the particles are also important parameter sensor applications. Most of the nanoparticles have a direct size–property relationship, hence its tunability, stability, specificity and signaling properties depends on it. For example, the exact crystal lattice structure and size deeply affect the working of piezoelectric sensors. For Au-based nanosensors, the LSPR pattern can be used for amplification signal using core/shell nanoparticles for signaling of bioactive molecules.

This review clearly shows that the field of biosensors and targeted drug delivery has received the highest impetus by incorporating core/shell nanoparticles in design strategy covering 93% of the research articles found through Scopus and SciFinder. Bioimaging, which often comes concurrently with both biosensor and drug delivery system has the potential to mobilize many kinds of inorganic and organic nanomaterials, in contrast to others where a restriction of choice of nanomaterials is common. Works limited to only bioimaging area is only 39%, but due to its versatility and diversity in material and implementation, it becomes statistically most significant part of our discussion. Gene transfection and other specific areas such as cell labeling and DNA/RNA interactions, require a great deal of molecular engineering. Apart from that, there is endless scope for further research to develop many new materials. There is also limited study on toxicity of nanoparticles on human body for in vivo applications of nanoparticles, which has to be diligently undertaken before these can be used for in-vivo applications.

The current thrust in the development of new drug delivery vehicles along with better contrast agents has provided interesting alternatives to the existing methods. The development of improved contrasting

agents by incorporating the same equipments will not only reduce the establishment cost but would also dramatically enhance the efficiency of these instruments in disease monitoring. The development of many new nanoparticles with improved properties has opened the door to its application in various fields. The time is ripe for researchers to apply these novel nanoparticles for the improvement of the health care system ranging from early disease diagnostics to targeted and controlled drug delivery. The need of the hour dictates various research groups from varied backgrounds to collaborate and share their knowledge in this field so as to realize the dream where a single platform could do everything related to disease detection to its treatment. Although, it might sound a bit unrealistic at this time but the development of multi-modal contrast agents is a good indicator as to where this research might be leading. The multi-modal system currently lacks the efficiency to be used in real life applications but it has definitely opened up the idea of a single platform for disease detection. Similarly, the various drug delivery platforms have been tinkering with the idea of a targeted drug delivery, its release and its monitoring abilities. To an extent researchers have been successful in doing targeted and selected drug release based on parameters like pH, temperature, etc. But an ideal platform with selective and sensitive drug delivery system is yet to be achieved, which can be applied for different systems. In the modern era, average human life span is increasing, however at the same time the better quality of human life is also very much essential and it depends to a large extent directly or indirectly on the advancement of this area. Despite significant advances in the area of laboratory-based applications core/shell nanoparticles in the biomedical field for diagnosis of diseases and drug delivery; clinical trial or real applications are in the infancy stage. So there is a huge future scope for the research to bring this technology for the end use.

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