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Multifunctional core-shell nanostructures

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Introduction

Nanomedicine is a new attitude towards conventional medicine where challenges are taken over a *bottom-up* rather than *top-down* approach, medical actions are performed at a single cell level, *tailor-made* therapeutic prescription are performed and *theranosis* is promised.

Personalized nanomedicine refers to the use of nanocarriers to elaborate optimized treatment protocols tailored to each patient. While introducing thousands of times less drug into the body, nanomedicine scales down the possibility of side effects, like tissue and organs destruction, and at the same time increases the localization of pharmaceutical drugs in the diseased tissue.

Nanomedicine, the nanotechnology new costumer, is pushing up forward nanoparticles (NPs) design, where excellent review articles have recently been published [Bao, Mitragotri et al 2013, Kumar, Kumar et al 2013, Li, Zhao, et al 2013, Parveen, Misra et al 2012, Mura and Couvreur 2012, Mykhaylyk, Sobisch et al 2012, Agasti, Rana et al 2010, Coutelaud, Morosini et al 2010] (Table 4.1a, 4.1b). NPs refers to all particulate/nanocarrier with all the three dimensions falling within 1-100 nm (0-D), for nanomedicine applications the upper limit can be 200 nm, regardless of their shape or structure. A number of NPs-based products for diagnostics and therapeutics have already been approved by FDA and European for clinical applications [see Bao, Mitragotri et al 2013, Kumar, Kumar et al 2013, Parveen, Misra et al 2012, Mura and Couvreur 2012, Mykhaylyk, Sobisch et al 2012, Agasti, Rana et al 2010, Coutelaud, Morosini et al 2010 and wherein references], and even more are currently under clinical trials. Multifunctional NPs – NPs that are capable of accomplished multiple objectives such as imaging and therapy (*theranostic*) or performing a single advanced function through incorporation of multiple functional units – are a last quest in nanobiotechnology**.** Multifunctional core-shell NPs can play a significant role in a near future offering new opportunities for monitoring the response to therapy in real time.

This review focus the potential of multifunctional core-shell nanoparticles designed to integrate simultaneously several functions of clinical relevance such as: the delivery of contrast agents for different clinical imaging tools, like magnetic resonance imaging or radionuclide imaging or fluorescence imaging, the co-deliver of specific therapeutic agents such a drug or a gene and also to allow the functionalization of the external shell surface for passive targeting or active targeting or for other functionalities (Fig. 4.1). In the following sections, we briefly review NPs architecture as well as core and shell options function of the final NPs performance. We present nanoarchitectures for combining two or more image functions for multimodal imaging, integrating imaging with drug delivery for image-guided drug delivery, and combining drug delivery with thermal therapies to take advantage of synergistic effects. Current clinical trials and future perspectives concerning the application of multifunctional NPs are also focused.

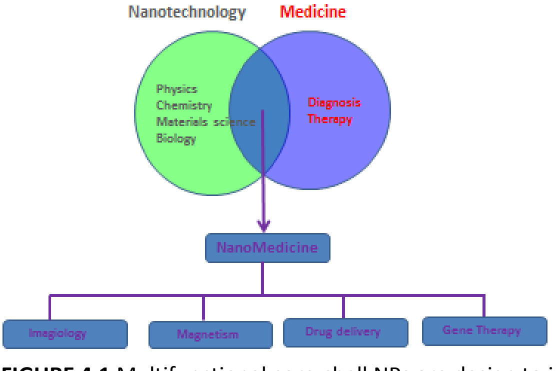


FIGURE 4.1 Multifunctional core-shell NPs are design to integrate several functions of clinical relevance.

TABLE 4.1a Selected NP-based therapeutics approved or in clinical trials.

Product	NP drug compponent	Delivery route	Indication	FDA status	Company
Doxil	PEGylated liposome/doxorubicin hydrochloride	IV	Ovarian cancer	Approved 11/17/1995 FDA050718	Ortho Biotech
Amphotec	Colloidal suspension of lipid-based amphotericin B	subcutaneous	Invasive aspergillosis	Approved 11/22/1996 FDA050729	Sequus
Estrasorb	Micellar NPs of estradiol hemihydrate	Topical emulsion	Reduction of vasomotor symptoms	Approved 10/9/2003 FDA021371	Novavax
Abraxane	Nanoparticualte albumin/paclitaxel	IV	Various cancers	Approved 1/7/2005 FDA021660	American Pharmaceutical Partners
Triglede	Nanocrystalline fenofibrate	Oral tablets	Lipid disorders	Approved 5/7/2005 FDA021350	SkyPharma PLC
Megace ES	Nanocrystal/megestrl acetate	Oral suspension	Breast cancer	Approved 7/5/2005 FDA021778	Par Pharmaceutical Companies
Combidex	Iron oxide	IV	Tumor imaging	Phase III	Advanced Magnetics
Aurimune	Colloidal gold/TNF	IV	Solid tumors	Phase II	Cytlmmune Sciences
NB-00X	Nanoemulsion droplets	Topical	Herpes labialis caused by herpes simplex I virus	Phase II	NanoBio
AuroShell	Gols-coated silica NPs	IV	Refractory head and neck cancer	Phase I	Nanospectra Biosciences
CALAA-01	Cyclodextran-containing siRNA delivery NPs	IV	Various cancers	Phase I	Calando Pharmaceuticals
Cyclosert	Cyclodextran NP	IV	Solid tumors	Phase I	Insert Therapeutics
INGN-401	Liposomal/FUS1	IV	Lung cancer	Phase I	Introgen
SGT-53	Liposome Tf antibody/p53 gene	IV	Solid tumors	Phase I	SynerGene Therapeutics

TABLE 4.1B Comparison of commonly used bioimaging techniques.

Technique	Typical NP label	Signal measured	Reso-lution	Depth	Sensitivity (moles of label detected)	Throughput	Cost	Main limitation
NIRF	QDs, dye-doped NPs, UpC NPs, SWNTs	Light (near-IV)	1-3 mm	< 1 cm	10^{-12}	high	low	Poor depth penetration
MRI	Iron oxide NPs, Gd(III)-doped NPs, NPs-based CEST and hyperpolarized probes (e.g. 129Xe)	Alterations in Magnetic field	50 μm	No limit	10^{-9}-10^{-6}	low	high	Low sensitivity, cannot follow many labels
PET	NPs incorporating radioisotopes (e.g. 18F, 11C, 64Cu, 124I)	Positron from radionuclides	1-2 mm	No limit	10^{-15}	low	high	Can detect only one radionuclide, requires radioactivity
SPECT	NPs incorporating radioisotopes (e.g. 99mTc, 111In)	γ-rays	1-2 mm	No limit	10^{-14}	low	high	Requires radioactivity
CT	Iodonated NPs, Au NPs, iron-oxide doped n-materials	X-rays	50 μm	No limit	10^{-6}	low	high	Poor resolution of soft tissues
US	μ-bubles, n-emulsions, SiO2-NPs, PS-NPs	sound	50 μm	Several cm	10^{-8}	high	low	Poor image contrast, works poorly in air-containing organs
PAI	Au-n-shells, Au-n-cages, Au-n-rods, Au-NPs, SWNTs, dye-doped NPs	sound	50 μm	< 5 cm	10^{-12}	high	low	Information processing and machines still being optimized

Nanoparticles as contrast agents for in-vivo bioimaging: current status and future perspectives. M.A. Hahn, A.K. Singh, P. Sharma, S.C. Brown, B.M. Moudgil Anal Bioanal Chem (2011) 399:3-27

Nanoparticles design

Various types of engineered core-shell NPs have been developed in outstanding efforts between academia and industry, in the last decades. Present core-shell NPs may exhibit a wide range of geometries - from spherical to tubular, through centric, eccentric and star-like - different sizes, core-shapes and shell-thicknesses, may comprise multiple cores, and may differ in crystallinity and surface morphology (Figure 4.2). NPs benchmarket demands an accurate core-shell NPs characterization which absence has been one of the main drawbacks in in-vitro/in-vivo applications. The following set of properties needs to be addressed:

i) size (actual and hydrodynamic diameter) and size distribution;
ii) shape and surface curvature;
iii) surface area and smoothness/roughness;
iv) surface charge, surface chemistry/reactivity, hydrophobicity/hydrophilicity;
v) coating thickness;
vi) chemical composition of both core and shell;
vii) crystallinity of both core and shell;
viii) porosity (size and size distribution);
ix) identification and levels of any impurities.

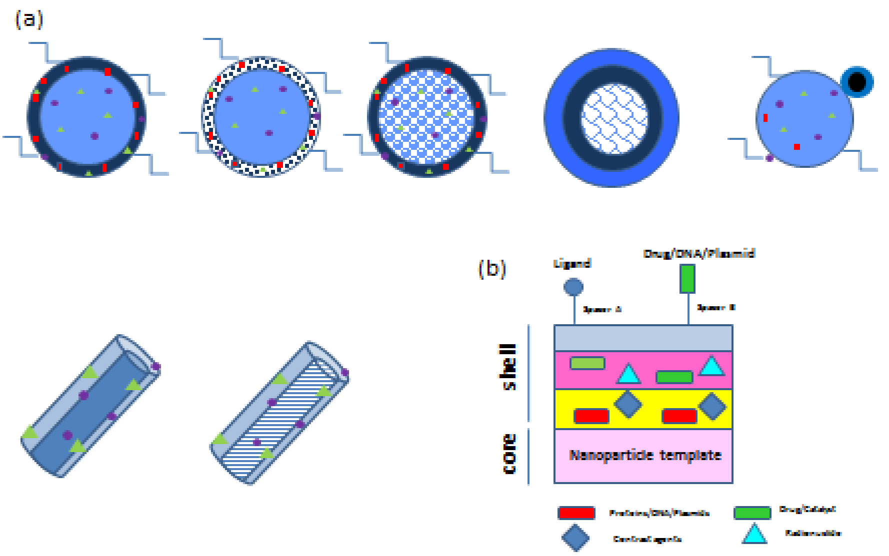


FIGURE 4.2 Core-shell NPs may exhibit a wide range of geometries (a) - from spherical to tubular, through centric, eccentric and star-like - different sizes, core-shapes and shell-thicknesses, may comprise multiple cores, may differ in crystallinity and surface morphology, alongside with being functionalized (b).

An appropriate size range is required to run a nanobiomedical system effective. The nanobiomedical system needs to be capable of targeting, entering, and providing therapy at a single cell level. The core-shell NPs ideal diameter is between 10 and less than 200 nm. Monosized distribution is required to make the process reproducible.

The properties of nanomaterials are determined mainly by the properties of their surfaces (opposed to bulk properties), due to their extraordinary high surface area to volume ratio. As a result, irregular shapes with higher surface area to volume ratio play a larger role in dictating those properties. Further, non-spherical NPs do have energetically different surface sites (surface/edge/corner) which may differently act in chemical processes. Besides, shape may be characteristic of a crystalline structure, and that is an additional reason to avoid shape distribution.
Surface roughness/smoothness may affect the contact area between a NPs and its environment (biological or other), thus reducing/increasing the number of accessible active surface sites. Chemical and structural identical NPs not surprisingly may exhibit different behavior depending on surface roughness/smoothness.
Surface hydrophobicity/hydrophilicity, which may be tuned during the synthesis processes, controls the wettability of a NP in respect to a specific environment, determining the number of accessible active surface sites.

Shell structure and particularly shell thickness may hinder the core properties. The core-shell assemblage should thus be optimized/tested according to the designed performance. Depending on the core-shell NPs application, the characterization is devised; UV-visible, NIR, fluorometry, absorption cross sections, fluorescence quantum yields, and fluorescence lifetimes for optical applications; susceptibility and relaxivity for magnetic applications and thermoelastic properties for temperature/mechanical sensoring applications, as an example. Although sheltered, the core material should be biocompatible in order to evade the immune response and avoid -rapid elimination or toxicity.
In-vivo toxicity tests are the ultimate screening to clinical use [Suh, Suslick et al 2009]. However, deciding on a set of standard toxicity assays is difficult as different toxicological tests study different toxicity facets (e.g., membrane permeability, apoptosis). Further, commercial testing kits designed for molecular toxins may not be recommended as they may interfere with core-shell NPs. Additionally, results of *in-vitro* analysis may not necessarily be valid *in-vivo*.
The last but not the least remains the lack of reproducibility in synthesis, functionalization and toxicological results of core-shell NPs. Batch-to-batch variations within the same laboratory, between laboratories and even between different protocols employed in both synthesis and decoration methodologies are frequent, often yielding the same core-shell NPs but with slightly different characteristics (e.g. size distribution, shape, number of conjugated biomolecules). These difficulties may arise from scare characterization of the NPs. Toxicological results from different laboratories where researchers may use different tests to confirm or deny toxicity may not be conclusive.
One of the great challenges in fabrication and processing of NPs is to overcome the surface energy, and to prevent the nanostructures or NPs from growth in size, driven by the reduction of overall surface energy. Missing is the discussion of all the mechanisms NPs have to face in order to keep their nanosize and nanostructure.

Core properties

The specific properties of the core materials provide distinct monitoring and therapeutic applications. It is critical to consider the purpose behind selecting a certain core material for the NPs system. For example, magnetic NPs provide monitoring and localization properties based on their magnetic susceptibility, fluorescent NPs based on light emission performance and gold NPs on plasmonic effect.

The high surface-to-volume ratio of metallic core structures yields high surface energies, promoting surface oxidation, and particles aggregation or clustering in physiological environments. Yet, passivated metal-cores (with satisfactory surface chemistry) exhibited longer plasmatic half-life and slower uptake by liver and spleen after intravenous administration [Laurent, Bridot et al 2010]. The use of a colloidal stabilizing agent, such as a surfactant or polymer, to prevent agglomeration/coagulation and to increase metal-core dispersion in various solvents and in the bloodstream is mandatory. Many of the times it is necessary to invert the surface charge of the metal-core materials through the addition of a surfactant, often followed by the addition of a stabilizer, prior to the (chemical/physical) bounding to the shell material, to increase core-shell affinity [Fortes, Li, et al 2012].

Fe_2O_3/Fe_3O_4 nanoparticles - a full range of biomedical applications

An intense research work has been devoted to iron oxide NPs, where a large number of publications have already been reported (Dave and Gao, 2009). As NPs, iron oxides can be classified based on their size, as magnetic iron oxide nanoparticles (MION, μm), superparamagnetic iron oxide (SPION, hundreds of nm), and ultra-small paramagnetic iron oxide (USPION, $<$50 nm). SPIONs with appropriate surface chemistry have been incorporated into a diversity of nanomedicine platforms for *in vivo* applications such as negative contrast agents in magnetic resonance imaging (MRI) (Figure 4.3) [Campbell, Arora et al 2011, Gonçalves, Fortes, et al 2012, Carvalho, Gonçalves et al 2013, Faria, Cruz, et al 2013, 5-8], photo thermal microscopy [Bogart, Taylor et al 2012] 9], tissue repair [10] [Chen, Wang et al 2012] immunoassay [11] [Motte, Benyettou et al 2011], detoxification of biological fluids [12] [Nikiforov, Filinova et al 2009], hyperthermia treatment [13] [Armijo, L. M.; Brandt et al 2012], guided non-viral vectors [14] [Shen, Gong et al 2012], in particular allowing for magnetic guidance in drug-delivery nanosystems [15] [Mahmoudi, Sant et al 2011].

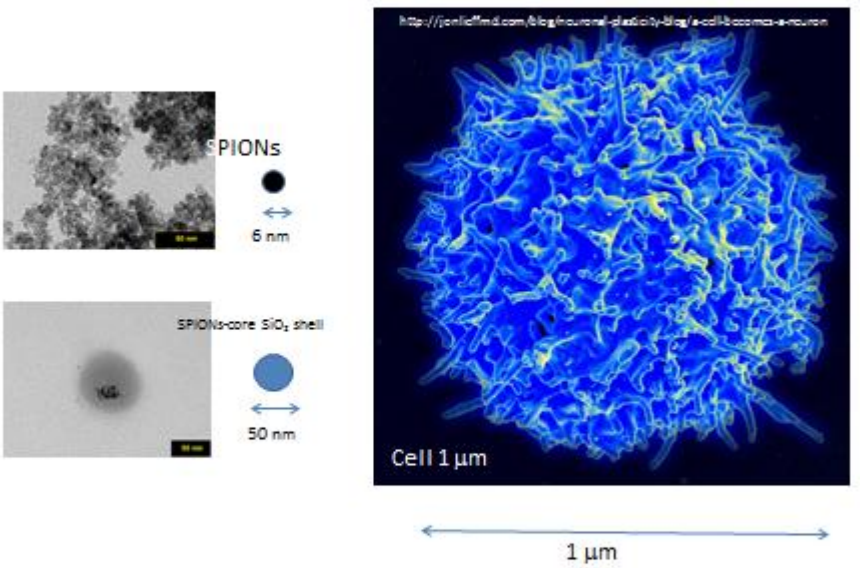


FIGURE 4.3 Silica coated SPIONs to be used *in vivo* as negative contrast agents in magnetic resonance imaging (MRI)
M. C. Gonçalves, L. M. Fortes, A. R. Pimenta, J. C. G. Pereira, R. M. Almeida, M. D. Carvalho, L. P. Ferreira, M. M. Cruz and M. Godinho, Silica/Ormosil SPIONs for biomedical applications, Current Nanoscience 9 (2013) 599-608.

SPIONs' biomedical applications require high magnetization values; narrow nanoparticles (NPs) size distributions, chemical stability, and homogeneous dispersion when in (biological) liquid media. Magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) are the most commonly used iron oxides for such applications, with a preference for magnetite because of its high saturation magnetization value [28] [Cornell and Schwertmann 2003]. A large number of publications report the development of core-shell nanoparticles with a core of superparamagnetic iron oxide nanoparticles (SIONPs), a contrast agent for magnetic resonance imaging MRI and for image guided therapy (Dave and Gao, 2009). SPIONs have been incorporated into a diversity of nanomedicine platforms [Barbosa, Finkler et al. 2013; Bonini, Berti et al. 2013; Habault, Dery et al. 2013; Hodenius, Wurth et al. 2012; Lorenzato, Cernicanu et al. 2013; Aryal, Key et al. 2013; Yan, Wu et al. 2013; Huang and Hainfeld ,2013; Habault, Dery, Leng, Lecommandoux, Le Meins, and Sandre ,2013; Martins, Corvo et al. 2013].

The synthesis of Fe_3O_4 SPIONs is not straightforward. SPIONS can be fabricated by either top-down or bottom-up approaches. Chemical routes are better suited to produce SPIONs with uniform composition and size comprising:

i) classical synthesis by co-precipitation,
ii) reactions in constrained environment,
iii) hydrothermal and high-temperature reactions,
iv) sol-gel reactions and v) polyol methods.

Coprecipitation techniques are rather complex approaches; the shape, size, size distribution and crystalline structure of NPs being strongly dependent on large number of experimental parameters. Apart from that, the easy oxidation of Fe(II) on magnetite surfaces becomes particularly important in NPs, where an extremely high surface/volume ratio is reached. Nevertheless co-precipitation methods are easy to implement and require less hazardous materials then the other procedures. Among the hydrolytic synthetic routes, the alkaline co-precipitation of ferrous and ferric salt precursors, in aqueous medium under inert atmosphere originally present by Massart et al. [Massart and Cabuil, 1987] has been the most important and widely used. Through a careful control of experimental parameters such as type of salts employed (e.g. chlorides, sulfates, nitrates), Fe^{2+}/Fe^{3+} ratio, temperature, pH, and ionic strength, *quasi*-spherical SPIONs with 8 nm [Jolivet, Froidefond et al 2004], 16.6-4.2 nm [Jolivet, Froidefond et al 2004], 2-15 nm [Jolivet, Vayssieres et al 1996, Jolivet, Tronc et al 2002, Bee, Massart et al 1995], 8-13 nm [Massart, Dubois et al 1995], 7 nm [Qu, Yang et al 1999] have been produced.

Gold nanoparticles – new unexpected properties

Among the nanocarriers, gold NPs have been actively investigated in a wide variety of biomedical applications due to its biocompatibility, non-cytotoxicity and non-immunogenicity (Figure 4.4). The ease of conjugation of gold NPs to biomolecules offers multiple modalities for biological and medical applications. Not only spherical gold NPs have been synthesized, but a wide variety of geometries /shapes can be obtained through the appropriate technique.

Gold NPs and gold colloidal suspensions exhibited a nano-property, known as surface plasmon resonance. At ~520 nm the free electrons (of the conduction band) of the surface of gold NPs collectively oscillate and scatter/absorb the incident electromagnetic wave, causing a strong absorption/scattering band. The ruby-red color of gold NPs and gold colloidal suspensions when exposed to visible light are an evidence of the phenomena.

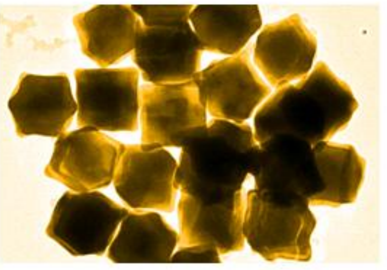


FIGURE 4.4 Gold NPs
http://www.fdbusiness.com/2013/04/gold-nanoparticles-help-detect-listeria-cheaply/

Shell properties

The use of metals as core always require a coating that inhibits surface oxidation and at the same time bring better colloidal properties to the new nanostructure. Liposomes are one of the most studied nanoplatforms for incorporation of SIONPs (Wang and Thanou, 2010 Hodenius, Wurth et al. 2012; Yan, Wu et al. 2013; Martins, Corvo et al. 2013; Lorenzato, Cernicanu et al. 2013; Huang and Hainfeld ,2013). The role of liposomes as core-shell nanoparticles is due to their quite specific structure which allows: 1) to carry into its inner compartment a diversity of SPIONs or other imaging agents 2) to load therapeutic agents either into its inner space or into its lipid bilayer 3) to be grafted into its outer surface by a diversity of molecules for targeting, for stealth properties or for other functions.
Polymer coatings through the presence of amine or carboxyl groups provide a link to functionalize these metal and QD cores with other biomolecules. Through these functional groups, molecular layers can be constructed on the core to provide biocompatibility, cell targeting, intracellular localization, biosensor diagnostics, and drug or gene delivery. Silica is another common coating material, able to be conjugated through the silanol group or the non-hydrolyzed organic group introduced *in-situ* during the synthesis procedure.

Liposome dual character

Liposomes are nanoparticles, with a vesicle structure (Figure 4.5), spontaneously formed when phospholipids are suspended in water in a definite range of molar ratios. The phospholipids become organized in bilayers that surround an aqueous core. They can also form an onion like structure with concentric bilayers entrapping water between them surrounding an inner water core. They also can be formed with several vesicles inside. The final structure of a liposome NPs is a function of the mixture of phospholipids that form the bilayers, of the method of preparation and of the composition of the water medium. Liposomes made with natural phospholipids are identical to biological membranes, consequently they are totally biocompatible. Medicines and other molecules or nanostructures, like contrast agents for imaging can be encapsulated into liposomes, being located there according with their lipophilic ties. Strongly lipophilic molecules or nanostructures are completely entrapped in the lipid bilayer, strongly hydrophilic molecules or nanostructures are sequestered in the aqueous compartment, and molecules or nanostructures partially hidophobic/hidrophilic are located partially buried between the lipid and partially exposed to the aqueous phases

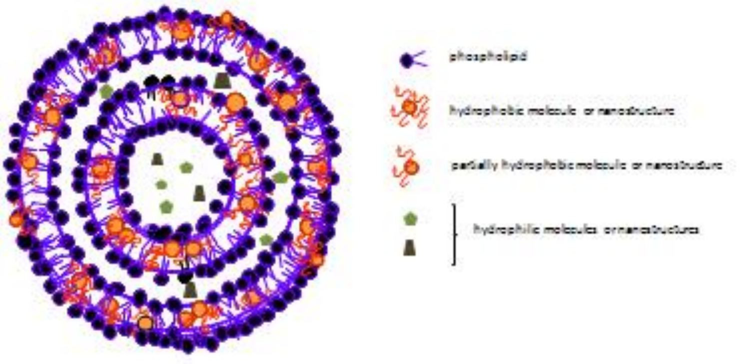


FIGURE 4.5 Liposomes are NPs, with a vesicle structure, spontaneously formed when phospholipids are suspended in water in a definite range of molar ratios. The phospholipids become organized in bilayers that surround an aqueous core. They can also form an onion like structure with concentric bilayers entrapping water between them surrounding an inner water core.

Liposomes were first identified in the 60´s by (Bangham and Horne,1965) and were initially used in research related with biomembranes (Bangham, Standish et al. 1965; Bangham and Papahadj,1966; Bangham,1972). But the technology of liposomes did considerable progress during the last four decades (Gregoriadis,2008; Gregoriadis ,1995). The success of liposomes as drug delivery systems with several medicines loaded in liposomes in clinical use for years (Barenholz ,2012; Chang and Yeh ,2012) and several other in clinical studies, (Barenholz and Peer,2012; Cern, Golbraikh et al. 2012) bring this class of nanoparticles a focus of attention for many clinical purposes including teranostics purposes (Emanuel, Rosenfeld et al. 2012). Furthermore a number of different molecules can be covalently linked to outer surface of liposomes, for active targeting or for other functionalities. A review the diversity of reactions was published by (Algar, Prasuhn et al. 2011).
Liposomes have also been used to obviate the tendency of SPION to aggregate when suspended in water, due to the absence of electrostatic and/or steric stabilization which is of special concern for in vivo applications. The term magnetoliposome was first mentioned by (Decuyper and Joniau ,1988) for small liposomes coating each IONP by a phospholipidic bilayer, without an internal aqueous compartment. Since then an increasing number of publications focus on the development of magnetoliposomes obtained by the incorporation of IONPs previously stabilized with different coatings (polymers, lipids, surfactants) and incorporated into the inner aqueous space of the liposomes or into the bilayer of the liposomes, according with the hydrophilic or hydrophobic coating of the IONPs (Al-Jamal and Kostarelos ,2011); (Soenen, Vande Velde et al. 2011).
Other works focus the development of liposomes for imaging purposes (Mandal, Bhattacharjee et al. 2013;Hansen, van Emmerik et al. 2013;Fattahi, Laurent et al. 2011;Chang and Yeh, 2012).

Polymer diversity

A diversity of polymers has been used to build nanoparticles for drug delivery purposes. Polymeric nanoparticles are obtained by different processes based on two main approaches: polymerization reactions or the use of preformed polymers These include polymeric micelles, capsules, colloids, dendrimers, and others. The term polymeric nanoparticle encloses nanospheres and nanocapsules. The polymers extensively used are poly(D,L-lactic acid) (PLA), poly(D,L-lactic-co-glycolic acid) (PLGA), poly (ε-caprolactone) (PCL) and their copolymers diblocked or multiblocked with poly(ethylene glycol) (PEG) polycyanocrylate (PACA), chitosan, gelatin and sodium alginate. A number of recent publications focus the development multifunctional polymeric nanoparticles for drug delivery (Wang, Jiang et al. 2013) and (Lehner, Wang et al. 2013). Several recent publications focus the development of magnetic core-shell polymeric nanoparticles (Chertok, David et al. 2010; Aryal, Key et al. 2013; Wang, Jiang, Li, Tang, Wei, and Mai ,2013; Lv, Ding et al. 2013; Yan, Lv et al. 2013; Kim, Vitol et al. 2013)

Silica versatility

Silica-based biomaterials have unique structural characteristics, with a 3-D amorphous silica network, where surface silanol groups render silica a high hydrophilic character, a high biocompatibility and the further possibility of functionalization and/or bioconjugation [Mykhaylyk, Sobisch et al 2012]. Organically modified silica (ORMOSIL) [Schmidt 1985] is an alternative material for biomedical applications with even better and more versatile properties than silica; the presence of non-hydrolysable organic groups in the alkoxysilane precursors behave like glass modifier and reduce the degree of the silica network cross-linking. In addition, ORMOSIL surfaces will be populated both with silanol and organic groups, allowing an easier chemical conjugation/decoration of biomolecules at the ORMOSIL surfaces and/or the load with either hydrophilic or hydrophobic drugs/dyes. A tunable wettability, by a judicious choice of the ratio of hydrophilic to hydrophobic sol-gel precursor monomers, a tailor-made porosity (size and shape) and a shell hardness/complacency making ORMOSIL a very competitive material. Mammalian cells take up and internalize easily silica/ORMOSIL-coated NPs without any cytotoxic effects [Kumar, Roy, 2010], opening the door to their use in health science.
Silica may also be mesoporous nanostructured, with pores ranging between 2 and 10 nm in diameter, by the use of templating agents, such as lyotropic liquid crystalline phases of surfactants (Figure 4.6) [Gonçalves, Attard 2003, Ariga, Vinu et al 2011]. Nanostructured mesoporous silica material is an ideal candidate for host-guest nanosystems to confine drugs or biologically active molecules into their mesopores [Vallet-Regí, 2009]. Nanostructured mesoporous silica have a high pore volume, an homogeneous size ordered pore network, an high surface area, all together allowing the hostage of a large amount of drugs, fine control of the drug load and release kinetics, and high potential for drug adsorption through the interaction between the drug and the pore walls. The organic functionalization of mesoporous silica walls revealed to be the main factor governing release kinetics, and it may also influence molecule adsorption by promoting host-guest interactions.

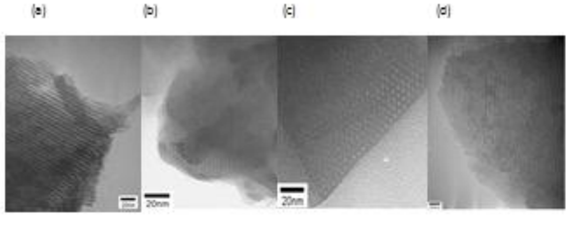


FIGURE 4.6 Mesoporous nanostructured silica
M.C. Gonçalves and George S. Attard, 'Nanostructured Mesoporous Silica Films', Rev. Adv. Mater. Sci. 4, No 2. (2003) 147-154.

Regarding silica as coating material, the two major approaches include the reverse microemulsion [Mackenzie and Bescher 2007] and the sol-gel methods [Arkhireeva and Hay 2003]. In reverse microemulsion, aqueous solution disperses in the organic phase (in the interior of the self-assembly reverse micelles) and forms a number of monodisperse nano-droplets. The confined nanoreactor environment within the reverse micelle has been shown to yield highly monodisperse nanoparticles and increase the incorporation of non-bonded non-polar molecules, which are often difficult to incorporate into the hydrophilic silica matrix [Buining and Liz-Marzán 1996]. The principal advantage of using reverse microemulsion is that particle shape and corresponding size distributions can be readily controlled by adjusting the molar ratio of water to surfactant, aging time, and reactant concentration. However the reverse microemulsion synthesis often have low yields and the use of surfactants and potentially toxic organic solvents demands extensive washing before any biological application, to avoid disruption or lyses of biomembranes by the surfactant molecules, rendering the process slow, expensive and low eco-friendly [22].

Alternatively Stober [Ohulchanskyy and Roy 2010] developed a mild synthetic protocol for growing monodisperse spherical silica nanoparticles based on the sol-gel of silicon alkoxides. Stober's method involves the hydrolysis and condensation of tetraethoxysilane (TEOS) in ethanol solution in the presence of water with ammonia as a catalyst, to create monodisperse, spherical, electrostatically-stabilized particles. The Stober method is a promising method for producing surfactant free silica coatings, yet, the final particles size remain in the hundreds of nanometers to microns regime, which are too large to some of the biologic studies.

Multifunctional NPs platforms for nanomedicine - selected examples

Medical imaging plays an important role in disease detection, prognosis, follow-up and treatment planning. Major medical imaging techniques include X-ray computed tomography (CT), magnetic resonance imaging (MRI), ultrasound imaging, positron emission tomography (PET), single-photon emission CT (SPECT), optical imaging, and photo acoustic imaging. Biomedical imaging is rolling toward the development of bimodal and multimodal imaging agents, following a number of relevant developments on combined imaging equipment for clinical and pre-clinical diagnostic such as the combination of CT and MRI and of PET and MRI and more recently the combination SPECT and MRI.

The development of multifunctional NPs has greatly expanded the outlook of nanomedicine with advanced imaging and therapeutic platforms. Contrast agents for MRI, optical imaging, photo acoustic imaging and on other imaging modalities are discussed by (Bao, Mitragotri et al. 2013) with a focus on the intrinsic quantum mechanical properties of inorganic NPs.

An overview of the variety of nanomaterials under investigation for diagnosis, imaging, and therapy of cancer is reported in the review of (Nazir, Hussain et al. 2013) with a special focus on inorganic NPs suitable for exploitation in different imaging modalities, their capability for thermotherapy and photodynamic therapy. These authors present a summary of some of the successfully approved and commercialized nanomedicines for the treatment and detection of cancers and many others at the various stages of clinical trials. They also discuss the effective modification and functionalization of these nanoprobes to provide further control of the localization, biodistribution, biocompatibility, and efficacy of nanomaterial systems *in vivo*.

Engineering NPs with more than one type of contrast agent in the same NPs, combining multimodal detectability consequently multiple components, it is a challenge with impact on imaging, molecular diagnostics, and therapeutics. However, combining multiple components on a nanometer scale to

create new imaging modalities unavailable from individual components has proven challenging (Jin, Jia et al. 2010).
Before the clinical translation, basic research aimed to gain deeper understandings on imaging agents, particularly on the connections between their imaging capability and their physicochemical microenvironments within nanocarriers aspects that will have a key role in developing robust nanotherapeutic platforms with high-performance imaging capability (Luk, Fang, and Zhang, 2012).

Optical imaging

Hollow spheres are utilized for the encapsulation and controlled released of various substances (e.g. drugs, biomolecules, cosmetics, dyes and inks), as confined reaction vessels, in catalysis and removal of pollutants as light fillers, acoustic insulation materials, low dielectric constant materials and photonic band gap materials [Moghimi, Hunter, et al 2005, Lammers, Hennink, et al 2008, Torchilin, 2006, Bechet, Couleaud et al 2008, Jiang, Gnanasammandhan et al 2010, [ISO/TS 27687, 2008], Caruso, Caruso et al 1998, Fortes, Réfega, 2012] (Figure 4.7). In nanomedicine silica hollow spheres can be used as host for Er^{3+} enhanced photoluminescence (PL) in visible range and Yb^{3+} to Er^{3+} enhanced energy transfer phenomena [Armes, Yuan et al 2010, Fortes, Réfega, 2012] as non-invasive in vivo imaging [Caruso 2000, Son, Bai et al 2007, Hahn, Singh 2011]. Up-conversion (UpC) effect (which converts long-wavelength excitation light into short-wavelength emitted light) based on rare-earth (RE) doped materials [Lim, Smith et al 2002] is a promising phenomenon, since the exciting source (in the NIR) falls within the therapeutic window (600-1300 nm) [Gasser, Brechet et al 2004] , allowing maximum penetration depth, minimizing photo-damage and reducing tissue auto-fluorescence [Shankar, Krishna et al 1999]. Er/Yb co-doped silica hollow spheres takes advantage of the high capacity of this platforms to ferry cargo and loads onto them both imaging and therapeutic functions. Applications such as drug-delivery and bio-label targeting can be combined into bio-imaging nanosystems creating multifunctional platforms for theranostic [Son, Bai et al 2007, Hahn, Singh 2011, Lim, Smith et al 2002]. When the matrix is silica, the advantages of biocompatibility and biodegradability [Gasser, Brechet et al 2004] combine with silica optical transparency, allowing the PL emitted light to cross the silica matrix efficiently [Shankar, Krishna et al 1999, Artemyev, Woggon et al 2001]. Among the RE, Er^{3+} PL at 1.54 μm is of particular interest because it corresponds to the minimum absorption loss in silica-based matrices [Lammers, Hennink et al 2008].

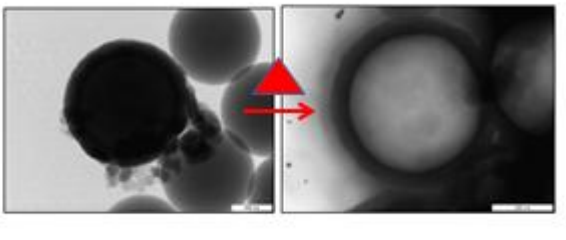


FIGURE 4.7 Silica hollow spheres can be used as host for Er^{3+} enhanced photoluminescence (PL) in visible range and Yb^{3+} to Er^{3+} enhanced energy transfer phenomena
Luís M. Fortes, Yigang Li, Ricardo Réfega, M. Clara Gonçalves, Up-conversion in rare earth-doped silica hollow spheres, Optical Materials 34 8 (2012) 1440-1446.

Multifunctional nanomaterials with unique magnetic and luminescent properties have broad potential in biological applications. (Yang, Zhao et al. 2013) describe the development of multifunctional core-shell Fe_3O_4@SiO_2 NPs with the ability to target inflammatory endothelial cells via VCAM-1, magnetism, and fluorescence imaging, with efficient magnetic resonance imaging contrast characteristics. Superparamagnetic iron oxide and fluorescein isothiocyanate (FITC) were loaded successfully inside the NP core and the silica shell, respectively, creating VCAM-1-targeted Fe_3O_4@SiO_2(FITC) NPs that were characterized by scanning electron microscopy, transmission electron microscopy, fluorescence spectrometry, zeta potential assay, and fluorescence microscopy.
Focusing on the need of noninvasive and sensitive tumor diagnosis NIR fluorescent probes, which activate their fluorescence following interaction with functional biomolecules, are desirable. Ref1007 developed a probe with a self-assembling polymer micelle, encapsulating various quantities of NIR dye (IC7-1) conjugated anti-HER2 single chain antibodies to the micelle surface and examined the probe's capacity to detect HER2 in cells and in vivo and the results presented that this core-shell NPs would be a useful NIR probe that is applicable for use in noninvasive *in vivo* optical imaging for specific detection of target biomolecules expressed in tumors.
The development of novel core-shell α-($NaYbF_4$:0.5% Tm^{3+})/CaF_2 NPs with efficient NIR_{in}/NIR_{out} UpC for high contrast and deep bioimaging and their applications for high-contrast in vitro and deep tissue bioimaging are reported by (Chen, Shen et al. 2012). Whole-body imaging of a BALB/c mouse, intravenously injected with an aqueous dispersion of that core-shell NPs (700 pmol/kg), showed a signal to back ground ration about 10-fold higher than that previously reported for *in vivo* imaging by these UpC NPs. The retention of the NIR_{in}-NIR_{out} ($NaYbF_4$:Tm^{3+})/CaF_2 NPs on a synthetic scaffold surrounding a rat femoral bone under centimeter-deep soft tissues was successfully visualized, demonstrating potential of these NPs for image-guided tissue engineering applications. Also UpC PL from a ($NaYbF_4$:Tm^{3+})/CaF_2 NPs suspension was imaged through a 3.2-cm pork tissue, with a high contrast against the background. The authors conclude that the observed capabilities of these engineered NIR_{in}-NIR_{out} UpC NPs, provide promise for their wide application in biomedical imaging.

Plasmonic effect

Gold NPs and gold-based multifunctional core-shell NPs are the subject of intensive studies and biomedical applications. Applications of engineered gold-based NPs and nanocomposites in analytical and theranostic by using plasmonic properties and a diversity of optical techniques are reviewed by (Khlebtsov, Bogatyrev et al. 2013), specifically bioimaging of bacterial, mammalian, and plant cells; photodynamic treatment of pathogenic bacteria; and photothermal therapy of xenografted tumors. In addition to recently published reports, the authors discuss new data on dot immunoassay diagnostics of mycobacteria, multiplexed immunoelectron microscopy analysis of Azospirillum brasilense, materno-embryonic transfer of gold NPs in pregnant rats, and combined photodynamic and photothermal treatment of rat xenografted tumors with gold nanorods covered by a mesoporous silica shell doped with hematoporphyrin.
Plasmonic gold-shell-magnetic core star shape NPs developed for the early detection of circulating tumor cells are reported by [Zen Fan Mol Pharmaceutics 2013, 10, 857], using magnetic/plasmonic NPs with the surface conjugated with SK-BR-3 breast cancer S6 aptamer able to perform magnetic separation of cancer cells from whole blood sample by fluorescence imaging, followed by separation and phototermal destruction.
The development of a theranostic plasmonic shell−magnetic core star shape NPs for the targeted isolation of rare tumor cells from the whole blood sample, followed by diagnosis and photothermal destruction is reported by (Fan, Senapati et al. 2013). These authors demonstrate that the plasmonic

star shape NPs developed are capable of isolating rare cancer cells from whole blood samples, followed by imaging and photothermal destruction, using the SK-BR-3 human breast cancer cell line, which overexpresses the epidermal growth factor receptor HER2/c-erb-2/Neu (HER-2) on the cell surface pointing to improve early detection of cancer in personalized medicine

The development of a multifunctional plasmonic shell–magnetic core nanotechnology-driven approach for the targeted diagnosis, isolation, and photothermal destruction of cancer cells is reported by (Fan, Shelton et al. 2012) with experimental data showing that aptamer-conjugated plasmonic/magnetic NPs can be used for targeted imaging and magnetic separation of a particular kind of cell from a mixture of different cancer cells. A targeted photothermal experiment resulted in selective irreparable cellular damage to most of the cancer cells. The authors showed that the aptamer conjugated magnetic/plasmonic NPs-based photothermal destruction of cancer cells is highly selective. They discuss the possible mechanism and operating principle for the targeted imaging, separation, and photothermal destruction using magnetic/plasmonic nanotechnology.

Magnetic performance

A summary on different core materials based on ferrite and ferrite doped magnetic NPs and on shell coating is reported by Karimi et al. 2013 (Karimi, Karimi et al. 2013) with an emphasis on suitable magnetic core-shell NPs with chemical/biological functionalization to be used in nanomedicine. A comparison of different properties of shell materials such as dextran, polyethylene glycol, chitosan and silica to improve the performances of magnetic materials is performed. Their advantages and disadvantages and properties such as bio-adhesive, charge, functional groups, increase in blood circulation time are focused.

Superparamagnetic NPs with Fe_3O_4/Fe_2O_3 core and inorganic and organically modified silica as shell were produced through a non-reverse emulsion method. The superparamagnetic nanostructures exhibited several magnetic cores and a highly porous external shell. No significant modification of the magnetic properties of the core iron oxide NPs were detected, conferring the core-shell nanostructures strong magnetic behavior and making them appropriate to biomedical applications [Gonçalves, Fortes et al 2013] (Faria, Cruz et al. 2013), (Martins, Corvo, Marcelino, Marinho, Feio, and Carvalho ,2013), (Carvalho, Gonçalves et al. 2013) (Figure 4.8).

FIGURE 4.8 Core-shell SPIONS-silica as MRI negative contrast agents
A. Carvalho, M.Clara Gonçalves, unpublished work.

Folic acid conjugated on the surface of $FePt@Fe_2O_3$-PEG NPs loaded with the chemotherapy drug, doxorubicin (DOX) via hydrophobic physical adsorption, were developed by Liu et al 2013 (Liu, Yang et al. 2013) for targeting to folate receptor (FR)-positive tumour cells, targeted intracellular drug delivery

and selective cancer cell killing acting as a multifunctional theranostic nanoplatform in imaging guided cancer therapy.
The use of superparamagnetic core shell multifunctional NPs, as novel drug delivery vehicles, to be guided with the help of an external magnetic field to its target is discussed by (Wahajuddin and Arora ,2012), stressing the need of monitoring the burst release effect of SPIONs. The authors focus the need of an adequate shell matrix controlling the drug release rate and incorporating the SPIONs within a polymer matrix that allows retention of their magnetic properties, thus enabling them to be guided by an external magnetic field. The matrix would regulate the release of drug as desired. Guidance by external magnets enables a third targeting mechanism, giving them an advantage when all three mechanisms work in unison. Carcinomas near the body surface, like squamous cell carcinoma, malignant melanoma, Kaposi's sarcoma, and breast carcinoma, are expected to benefit the most from SPION-based therapy. Development of transdermal patches containing magnetic circuitry using supermagnets capable of generating highly penetrating localized magnetic fields could result in enhanced and efficient accumulation of drugs carrying SPIONs at desired sites (Wahajuddin and Arora ,2012). This would be particularly beneficial for ambulatory patients and could also increase patient compliance with treatment. Additionally, the complex dosing regimen according to the individual needs of the patient can be carried out by modulation of the magnetic field strength used.
Iron oxide and gold coupled core-shell NPs with defined structural characteristics (e.g., size, shell thickness, and core-shell separation) and physical properties (e.g., electronic, magnetic, optical, thermal, and acoustic) were developed by (Jin, Jia, Huang, O'Donnell, and Gao ,2010). The reported NIR responsive magnetic-gold core-shell nanostructures were obtained by creating a gap between the core and shell, as the core and shell of our particles are spatially separated with a dielectric polymer layer. The resulting NPs show highly integrated properties including electronic, magnetic, optical, acoustic, and thermal responses, which allow multimodality imaging. Additionally the surface of the particles will also allow conjugation with targeting ligands to develop all-in-one nanostructures for non-invasive imaging, molecular diagnosis, and hyperthermia-based treatment of complex diseases.

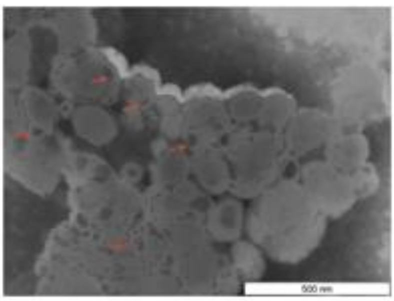
Lipossome carriers

The development of nanotheranostics using lipid- and polymer-based formulations, with a particular focus on their applications in cancer research are reviewed by (Luk, Fang et al. 2012)[with an emphasis on recent advances in nanotechnology aimed to combine therapeutic molecules with multimodal imaging agents for magnetic resonance imaging, radionuclide imaging, or fluorescence imaging and with a primary focus on platforms using liposomes and polymers. Liposomes and polymer-based NPs are both established drug delivery platforms for cancer treatment. Combine both therapeutics and imaging into a single carrier, results in a number of novel theranostic platforms engineering lipid and polymer based nanotherapeutic platforms with multiple imaging modalities incorporated into increasingly sophisticated architectures (Luk, Fang, and Zhang, 2012), not only oncological applications but for other fields like cardiology where nanotherapeutic platforms will find numerous potential applications.
Magnetoliposomes assume a special place among the diversity of paramagnetic core-shell iron oxide NPs used as contrast agents to improve MRI sensitivity. Several works focus the effect of the NPs on magnetic properties [(Soenen, Vande Velde et al. 2011; Al-Jamal and Kostarelos,2011; Skouras, Mourtas et al. 2011; Bothun, Lelis et al. 2011; Jokerst and Gambhir,2011; Plassat, Wilhelm et al. 2011; Faria, Cruz et al. 2013), (Carvalho, Gonçalves et al. 2013)]. Other works focus the increasingly relevant role of magnetolipossomes in the targeting of MRI contrast agents (Martins, Corvo, Marcelino, Marinho, Feio, and Carvalho ,2013), (Bothun, Lelis et al. 2011), (Fattahi, Laurent et al. 2011), (Habault,

Dery, Leng, Lecommandoux, Le Meins, and Sandre, 2013), (Lorenzato, Cernicanu, Meyre, Germain, Pottier, Levy, de Senneville, Bos, Moonen, and Smirnov ,2013) and for the co-delivery of medicines and imaging agents (Bonini, Berti, and Baglioni ,2013), (Yan, Wu, Feng, Chen, Liu, Hao, Yang, Zhang, Lin, Xu, and Liu ,2013), (Aryal, Key, Stigliano, Ananta, Zhong, and Decuzzi ,2013), (Bao, Mitragotri, and Tong ,2013), (Guo, Zhang et al. 2013), (Hodenius, Wurth, Jayapaul, Wong, Lammers, Gatjens, Arns, Mertens, Slabu, Ivanova, Bornemann, De Cuyper, Resch-Genger, and Kiessling ,2012), (Duncan and Gaspar ,2011), (Jokerst and Gambhir 2011) (van Bochove, Paulis et al. 2011)

The magnetic properties of long circulating magnetoliposomes sterically stabilized by PEG (PEGylated) were first studied by ((Martina, Fortin et al. 2005) that rank them among efficient MR T2 contrast agents. More recently (Skouras, Mourtas, Markoutsa, De Goltstein, Wallon, Catoen, and Antimisiaris ,2011) demonstrated that the magnetic properties of PEGylated magnetoliposomes are dependent on the mol% of PEG lipid. PCA applied to FTIR data can successfully differentiate magnetoliposomes from the empty liposomes as reported by Marta et al. 2013.

Long circulating magnetoliposomes designed for the load of PEG-coated SPION and for passive targeting to liver ischemia-reperfusion injuries were developed by Martins et al. 2013 (Figure 4.9), the authors demonstrated that the passive targeting of the optimized magnetoliposomes improved the sensitivity of MRI to visualize inflamed tissues.



TEM image of magnetoliposomes. Red arrows point to SPIONs.

FIGURE 4.9 Long circulating magnetolipossomes
M. R. Faria, M. Margarida Cruz, M. C. Gonçalves, Alexandra Carvalho, Gabriel Feio and M. B. F. Martins, Synthesis and Characterization of Magnetoliposomes for MRI contrast enhancement, International Journal of Pharmaceutics V446, 1-2, 25 March (2013) 183-190.

A review on the design and multifunctional properties of lipid bilayer coated presented by (Ramishetti and Huang ,2012) evidences how lipid bilayers are now being utilized as excellent carriers for drug-loaded and solid core NPs such as iron oxide, mesoporous silica and calcium phosphate and polycation-based solid NPs with a focus on their design as well as their multifunctional role in cancer therapy are discussed.

The state of the art of magnetic resonance imaging (MRI)-guided nanorobotic systems associated to drug delivery (nanorobotic-MRI DDS) addressing the novel concept of guiding core shell NPs in the human vasculature for drug delivery purposes using an MRI scanner is reviewed by (Vartholomeos, Fruchard et al. 2011). The authors discuss the potentiality of these platforms, to perform diagnostic, curative, and reconstructive treatments in the human body at the cellular and subcellular levels in a controllable manner. The concept of an MRI-guided nanorobotic system is based on the use of an MRI

scanner to induce the required external driving forces to propel magnetic multifunctional core-shell NPs to a specific target.
Some perspectives on the successful realization of the nanorobotic-MRI DDS are expected to produce a significant increase in therapeutic efficiency and a decrease in side effects on healthy tissue (Vartholomeos, Fruchard, Ferreira, and Mavroidis ,2011).
Polymeric core-shell NPs produced by a non-emulsion technique, were fabricated to carry iron oxide within the shell and the chemotherapeutic agent, temozolomide (TMZ) are described by (Bernal, LaRiviere et al. 2013) and results on the endocytosis-mediated uptake by glioma cells using intracranial delivery through rodent brain and tracked in vivo by standard MR imaging were demonstrated (Bernal, LaRiviere et al. 2013) and reduction of the growth of glioma xenografts and extend survival of tumour bearing animals was rerported.

DNA vectors

One focus in nanobiotechnology is the development and use of nonviral vectors for safe and efficient gene delivery. Eco-friendly inorganic and organically modified silica NPs were prepared through a non-reverse micro-emulsion technique and successfully complexed to DNA plasmids, at different ratios (**Figure 4.10**) [Colaço, Gonçalves et al 2013].

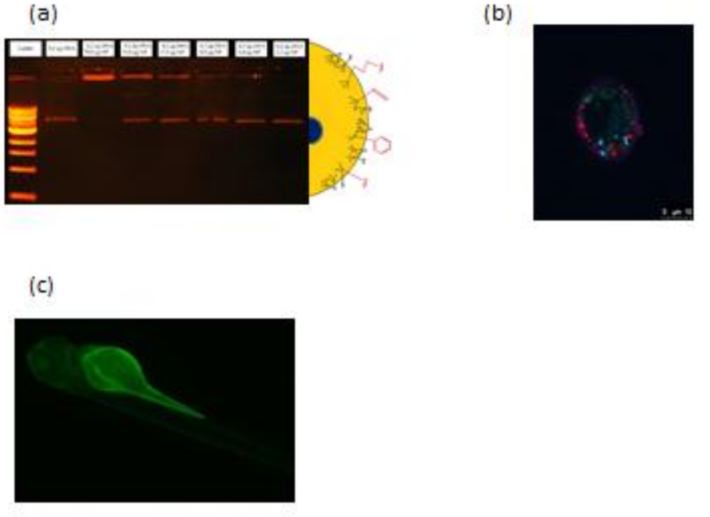


FIGURE 4.10 Ormosil nanoparticles as nonviral vectors for safe and efficient gene delivery
Rui Colaço, M. Clara Gonçalves, L. Fortes, Lídia M.D.Gonçalves, António J. Almeida, M. Bárbara Martins, Preparation and chemical characterization of eco-friendly ORMOSIL nanoparticles of potential application in DNA gene therapy Current Nanoscience 9 1 (2013) 168-172 (5); and J. C. Matos, A.R. Soares, I. Domingues, G. A. Monteiro and M. C. Gonçalves unpublished results.

The use of external magnets for nonviral gene vectors that facilitate the introduction of plasmids into the nucleus with a performance improved when compared with the routinely available standard technologies is also reported. SPION-induced hyperthermia has also been utilized for localized killing of cancerous cells (Wahajuddin and Arora, 2012).

Targeting

The possibility of selective delivery of VCAM-1-targeted Fe_3O_4@SiO_2(FITC) NPs to sites of inflammation and their accumulation or uptake by targeted cells give them high potential in vascular magnetic resonance imaging for clinical diagnosis of cardiovascular disease, eg, atherosclerosis and thrombosis is discussed by the authors (Yang, Zhao, Li, Xu, Li, Wu, Miyoshi, and Liu, 2013).

Photothermal ablation is a minimally invasive approach, which typically involves delivery of photothermal sensitizers to targeted tissues. (Guo, Zhang et al. 2013) demonstrated that the use of gold shell–iron oxide core hybrid NPs (Fe_3O_4/Au) for both imaging and laser irradiation. They demonstrated that the core-shell NPs are up taken by pancreatic cancer cells, permitting magnetic resonance imaging (MRI) of sensitizer delivery and photothermal ablation with NIR laser irradiation. An exposure to these NPs and subsequent laser irradiation led to significant reductions in pancreatic cancer cell proliferation.

A review from (Sah, Thoma et al. 2013) focuses on the fundamentals and strategies that are used to develop diverse functional PLGA nanoparticulate carriers with a focus on recent research trends with multifunctional PLGA core-shell hybrid NPs that provide temporal drug delivery, enable imaging and drug targeting in a single utility, and achieve synergistic therapeutic outcomes to develop highly effective nanoparticulate carriers that can deliver a spectrum of chemotherapeutic, diagnostic, and imaging agents for various applications.

The development of a surface engineered magnetic core-shell NPs-based drug delivery system and PLGA core-shell NPs designed for aerosol therapy of lung diseases are reported by (Verma, Crosbie-Staunton et al. 2013). These authors developed NPs by coating of Fe_3O_4 magnetic NPs (MNPs) with poly(lactic-co-glycolic acid) (PLGA). The polymeric shell of these engineered NPs was loaded with a potential anti-cancer drug quercetin and their suitability for targeting lung cancer cells via nebulization was evaluated. The quercitin loaded PLGA-MNPs were applied to the human lung carcinoma cell line A549 following a single round of nebulization. The drug-loaded PLGA-MNPs significantly reduced the number of viable A549 cells, which was comparable when applied either by nebulization or by direct pipetting.

New magnetic-based core-shell NPs (MBCSP) were developed by (Wadajkar, Bhavsar et al. 2012) to target skin cancer cells while delivering chemotherapeutic drugs in a controlled fashion. MBCSP consist of a thermoresponsive shell of poly(N-isopropylacrylamide-acrylamide-allylamine) and a core of poly(lacticco- glycolic acid) (PLGA) embedded with magnetite NPs. To target melanoma cancer cells, MBCSP were conjugated with Gly-Arg-Gly-Asp-Ser (GRGDS) peptides that specifically bind to receptors of melanoma cell. MBCSP consist of unique multifunctional and controlled drug delivery characteristics. Specially, they can provide dual drug release mechanisms (a sustained release of drugs through degradation of PLGA core and a controlled release in response to changes in temperature via thermo-responsive polymer shell), and dual targeting mechanisms (magnetic localization and receptor-mediated targeting). The particles exhibited excellent cytocompatibility to healthy cells and efficient uptake by the targeted cancer cells. Moreover, the particles displayed a high potential as imaging probes for MRI and optical imaging modalities. Finally, the tumour-targeting capabilities of GRGDS-conjugated NPs are promising, as they are effectively recruited by static magnets to the tumor site in melanoma mice models.

Over the past decade, positron emitter labeled NPs have been widely used in and substantially improved for a range of diagnostic biomedical research. A variety of NPs has been engineered and explored for diagnostic and therapeutic potential in various diseases. A review of (Liu and Welch, 2012) summarizes the major applications of NPs labeled with positron emitters for cardiovascular imaging, lung diagnosis and tumour theranostics taking into account that for personalized medicine and

translational research, a major challenge in the field will be to develop disease specific nanoprobes with facile and robust radiolabeling strategies and that provide imaging stability, enhanced sensitivity for disease early stage detection, optimized in vivo pharmacokinetics for reduced non-specific organ uptake, and table improved targeting for elevated efficacy.
The examples presented in this review focus on NPs labeled with PET isotopes for cardiovascular, pulmonary and tumour imaging, as well as for pharmacokinetic evaluation. So far, significant progress has been achieved in NPs structure/design, *in vitro* trafficking, and *in vivo* fate mapping by using PET. More effort will be necessary to achieve development of approved biocompatible and biodegradable NPs for personalized medicine and translational research.

Final Remarks

The research innovations in the field of design and concept of multifunctional core-shell nanoparticles reached a level that allows the identification of some critical parameters.
The core materials can be improved, for example magnetic nanoparticles biocompatible, biodegradable, and have improved magnetic characteristics needed to be developed.
The shell materials can be focussed to maximize their functionality in vivo to allow the implementation of real-time feedback control of the targeting process.
The assemblage of a diversity of functions, forming complex systems in one nanoparticle, need to be refined.
Although a number of biomedical systems are in the borderline of prototyping.

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