Silica and ORMOSIL nanoparticles for gene delivery

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1. INTRODUCTION

Nanomedicine is an emerging new area, combining nanotechnology and medicine. One of nanomedicine main goals is the design, development and application of non-viral based methods for gene delivery and therapy. Selective gene transfer to cells, tissues or organs, aiming at clinical treatment, has been discussed throughout the last half century. Recent breakthroughs on the technology required to modify genetic material brought this goal to a foreseeable future.

Plasmids (pDNA) are widely used in clinical and non-clinical research in gene delivery, gene therapy and DNA vaccination. However, they are quite inefficient as mediators of gene expression, as these molecules are easily degraded when administered unprotected. The success of gene therapy is deeply dependent on the efficiency of the gene delivery system targeting cells, tissues or organs. It is thus important to develop new delivery vectors able to carry, target and protect the plasmid DNA.

Silica and **Or**ganically **Mo**dified **Si**lica (ORMOSIL) nanoparticles (NPs) are promising candidates as non-viral delivery vectors. Silica presents several attractive characteristics, namely high biocompatibility, nontoxicity, non-immunogenicity,

biodegradability and bioconjugation ability. The silica functionalization versatility allows hybrid NPs, with tunable hydrophilic/hydrophobic surface character, high cargo capacity, and a prolonged blood circulation time, making them even more interesting as gene carrier.

In this chapter a brief introduction to the topic of nanomaterials/NPs and their significance in the relation/application to nanomedicine will be present, followed by the description of an important process used for NPs synthesis through the bottom-up sol-gel methodology.

The main concepts of gene therapy will also be addressed. The advantages and disadvantages of different methods will be discussed, giving emphasis to the use of synthetic NPs as a method to improve the gene delivery efficiency performed by non-viral vectors.

2. Nanotechnology and nanomedicine

Nanotechnology consists on the manipulation of matter on a nanometric scale, with the aim of developing new functional material or systems, whose structures exhibit novel and/or improved properties. Due to their size, the developed materials often present very specific physical, chemical and/or biological properties. In the international units system (SI), the nano prefix refers to the multiplication of a given unit by 10^{-9}. Thus, a nanometer denotes one billionth of a meter, corresponding approximately to the length of 10 hydrogen atoms or 5 silicon atoms aligned.

Nanoscale materials may be classified in terms of the number of dimensions not confined to the nanometric scale. Nanoparticles are considered to be 0D (zero dimensions not confined to the nanomatric scale); nanotubes, nanofilaments and nanofibers, extend across one dimension, are regarded as 1D; nanofilms and nanocoatings can be characterized by a width and a length, while height is negligible, and may be regarded as 2D nanomaterials; finally, bulk materials, such as photonic crystals or metamaterials, are classified as 3D. Considering the inherent nanoscale functional components of living cells, it was inevitable for nanotechnology to meet biotechnology, giving rise to nanobiotechnology.

Nanobiotechnology is a new important field of research due to many applications in areas such as drug discovery and delivery, and pharmaceutical manufacturing. These classes of applications are often covered under the term *nanopharmaceuticals*.

Nanomedicine is a closely related field, created from the application of nanotechnology to medicine. There is an increasing optimism on its applications where significant advances in the diagnosis and treatment of disease are expected. In the last decade, a deep understanding of molecular processes underlying mechanisms of disease occurs. This knowledge changed some of the paradigms of clinical diagnosis methods, supported by the detection and monitoring at the molecular level, such as cells, DNA, messenger RNA (mRNA), peptides and proteins. Furthermore, as targets exist on a nanoscale, probes with equivalent size have been developed allowing the integration of nanotechnology and biology/medicine, answering to the increasing demand for gene profiling, high-throughput drug and disease screening without complex instrumentation or processing steps [1]. Drug delivery and gene therapy become one of the most promising applications of nanomedicine, by making use of NPs as carriers [2]. Figure 1 illustrates the relationship between nanobiotechnology and nanomedicine.

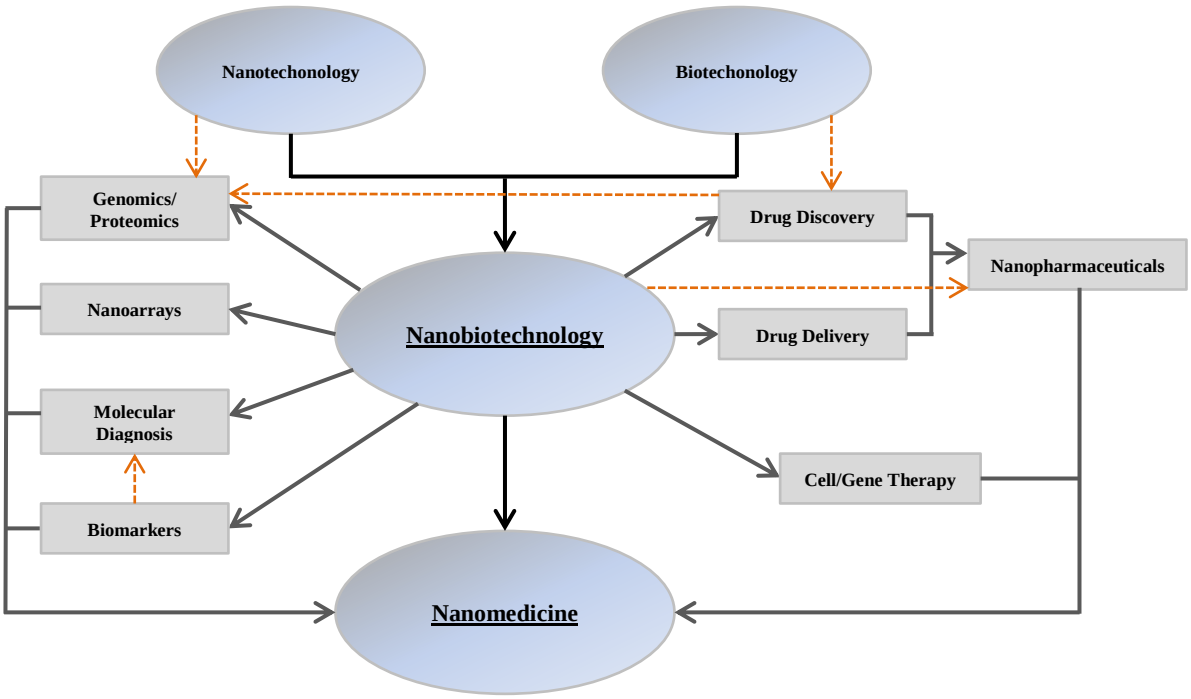


Figure 1: Nanobiotechnology and nanomedicine relationship [2].

3. Nanoparticles

Due to a wide range of attracting characteristics, NPs have a high significance in Material Science research, having attracted both the academic and industrial communities.

In addition to their outstanding surface-to-volume ratio, NPs possess unique thermal, mechanic, electrochemical, catalytic, optical, electronic and magnetic properties, depending on their size, shape and composition. Thus, instead of changing the NPs composition, one may play with the size and/or shape, to fine tune the desired property or set of properties.

NPs may exhibit a wide range of geometries – from spherical to tubular, through centric, eccentric and star like – may be plain or nanostructured – core-shell or porous structure - exhibit different sizes and shell thicknesses, may be hollow, may differ in crystallinity and surface morphology, and finally may be fine-tuned relative to one or more properties.

Further, NPs can be functionalized and eventually bioconjugated with a wide range of small ligands and/or large biomolecules. Additionally, NPs enable the encapsulation and controlled release of various substances (e.g., drugs, biomolecules, cosmetics, and dyes), and may play singular performance in certain biomedical devices.

And finally, NPs can be multifunctional, i.e., capable of accomplishing multiple objectives such as imaging and therapy (*theranostics*), or performing a single advanced function through incorporation of multiple functional units [3]. For biomedical applications, an appropriate size range (~ 10 - 200 nm) and a monosized distribution are normally required for effective operation.

Due to the nanoscale associated to these materials, classical physics are often unable to explain their properties, which are easier to understand in the domain of quantum mechanics. While the use of quantum mechanics enables many interesting hypothesis, it also increases the degree of difficulty in the complete understanding of materials.

3.1. Silica Nanoparticles

NPs may be constituted by material with biological origin, like phospholipids, lipids, lactic acid, dextran, chitosan, or have an inorganic characteristic, like polymeric, carbonic, silica-based and metallic [4].

Silica is the most abundant mineral on Earth crust, and over time it was introduced in human life and latter in engineering. It is most commonly found in nature as sand or quartz, as well as in cell walls of diatoms [5]. The structural unit of silica is a tetrahedron whose base and sides have triangular shape with an oxygen atom at each vertex and a silicon atom in the center $(SiO_4)^{-4}$. These tetrahedra bond to each other by sharing their vertexes which occurs regularly or randomly. In the first case the 3D organizational diversity leads to different polymorphic forms of silica (quartz or tridymite for example). In the random case, an aperiodic 3D network forms, leading to glass formation. Silica NPs may be crystallized or amorphous.

SiO_2 NPs present several advantages when compared to organic polymers. During the SiO_2 NPs synthesis, they may be easily separated by centrifugation; they are more hydrophilic than polymers and not subject to microbial attack. Recently, different fluorescent silica (SiO_2) NPs have emerged as a particularly fascinating fluorescent probe which arouse great interest in biology and medicine [1, 6]. Highly luminescent SiO_2 NPs have been prepared for the selective targeting of a wide range of important diseases (cancer, cells, bacteria and others) and they have demonstrated advantages in terms of multiplexing capabilities, ease of functionalization and also, a greater sensitivity and photostability

Magnetic silica NPs can be used for separation and targeting techniques of trace amounts of bioanalytes in biological complex matrices. The magnetic properties can also be linked to the fluorescence, making multifunctional silica NPs, which can be useful for imaging cells, tissues and other organs as well as for the delivery of therapeutic agents to specific targets. These silica NPs present a great interest from a technological view, especially in nanomedicine for diagnosis and treatment of diseases.

SiO_2 NPs have shown a great versatility due to chemical or physical surface modification, which may increase their biocompatibility. Extensive studies about SiO_2

NPs biodistribution [7, 8] and toxicology [3, 9, 10] have shown that these NPs are well tolerated and in some cases biodegradable or excreted after performing their function. SiO_2 NPs present high and controllable mechanical and chemical stability. Their porosity can also be easily adapted in terms of the pore size and structure [11].

Due to these advantages, SiO_2 NPs are considered a great promise in several biological applications [11].Two important applications of SiO_2 NPs in nanomedicine field are: drug delivery systems and gene therapy. In drug delivery systems research, drug molecules have been loaded into SiO_2 NPs and their surface changed by introducing biorecognition entities, allowing the delivery system to target specific cells or receptors in the body [12].

Regarding gene therapy, in a pursue for more efficient DNA delivery vectors for both basic research and clinical trials, ultrafine SiO_2 NPs functionalized with amino groups, can bind efficiently to pDNA, protecting it from enzymatic attack while still transfecting *in vitro* cells [13, 14].

3.2. Silica Nanoparticles synthesis and *in situ* functionalization

Synthesis Techniques

Several methods to accurately produce silica NPs with a narrow distribution of sizes, controlled shape and morphology, surface chemistry, porosity, and homogenity, have been developed. Most of them use the bottom-up approach sol-gel methodology.

The sol-gel process has many advantages which led to its use even before the underlying scientific principles were understood. These advantages are namely, a lower processing temperature (close to room temperature), allowing minimal thermal degradation and low energy processing costs, high homogeneity, directly obtained in solution on a molecular scale, and a high purity degree which depends only on precursors purity.

Figure 2 illustrates the sol-gel process versatility.

The sol-gel chemistry comprises chemical reactions involving colloidal particles in a sol, or between alkoxide-precursors and water, in a solution, leading to a highly porous amorphous gel product, where a liquid phase (solvent, catalyst and eventually excess

reactants) may be retained. 0D, 1D, 2D, or 3D products may be obtained, depending on the set experimental conditions.

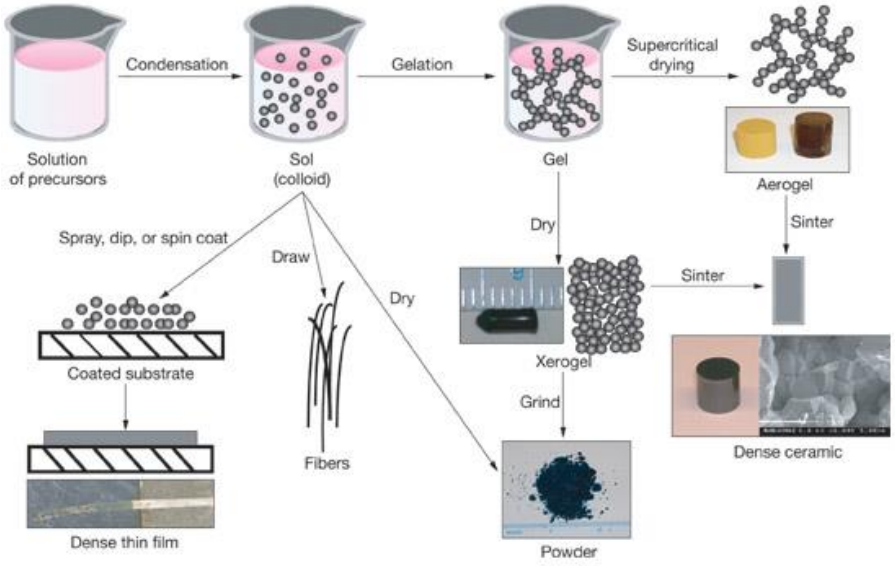


Figure 2. Sol gel versatility

The sol route

In the *sol route*, colloidal particles are formed in aqueous medium from ionic species, following colloidal chemistry principles. In the case of silica, for example, diluted silicic acid sols, containing ~ 1 nm sized NPs, will undergo rapid growth to 2 - 4 nm, at pH 2 - 3. Above pH 7, silica solubility increases so much that the particles will grow until 4 - 6 µm, by coalescence and Ostwald ripening (Figure 3).

Sodium silicate solution is another sol starter for the synthesis of SiO_2 NPs.

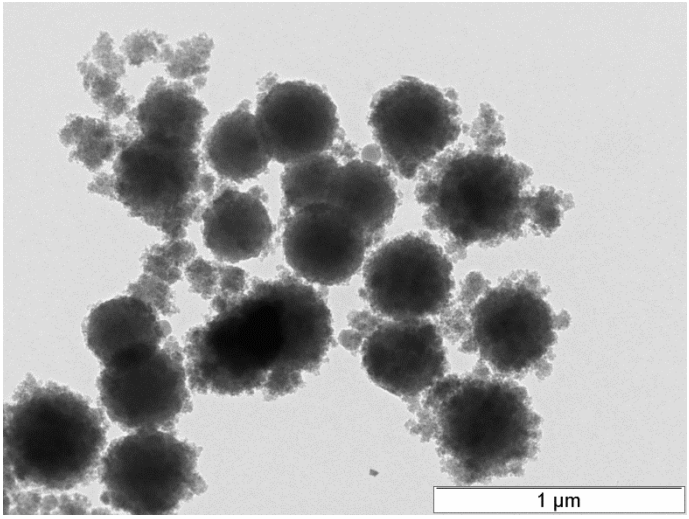


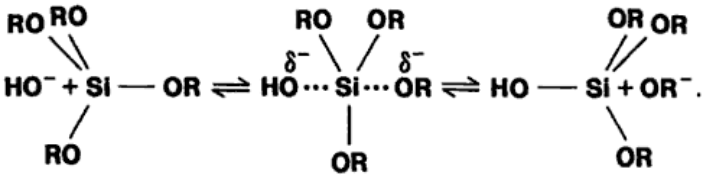
Figure 3 TEM images evidencing Ostwald ripening in SiO_2 functionalized NPs.

The solution route

The *solution route* is the most common synthesis process. Here metallic salts, metal alkoxides, or other organometallic precursors undergo hydrolysis and condensation to form a wide range of sol-gel products. Because of the hydrophobic nature of the alkyl groups, organometallic precursors and water are not miscible and the addition of a common solvent (usually an alcohol) becomes mandatory to promote miscibility between reactants.

The pH plays a critical role in all sol-gel processes. At low pH, particularly below 3 (the isoelectric point of silica is 2), complete hydrolysis produces linear or highly branched polymeric species given rise to 3D structures, with nanopore diameters < 2 nm. As the pH increases towards 7, dissolution and condensation reactions become relevant and the gel structure coarsens to some extent. Above pH 7, there is maximum NPs growth, due to increase in silica solubility, promoting depolymerization of siloxane bonds, and producing monomeric silica necessary for the aging process (Ostwald ripening coarsening mechanism).

By sol-gel methodology, NPs synthesis is always performed under basic catalyzed conditions. Water dissociates immediately at pH over 7, and a hydrolysis reaction take place by hydroxyl attack to the silicon atom, according to:



Then, a second nucleophilic attack allows the formation of the Si-O-Si network:

Hydrolysis and condensation occur simultaneously rather than sequentially; the initial condensation is quite fast, although it slows down as polymerization progresses.

The SiO_2 NPs solution route comprises two common via: the microemulsion process (or reverse emulsion) and the classical Stöber method.

In the first process a reverse-micelle or water-in-oil (w/o) microemulsion system is formed by adding water, oil and surfactant. The hydrolysis and condensation reactions will take place in confined reaction vessels, formed by the dispersed aqueous phase in the continuous oil matrix.

The nucleation and growth kinetics of silica are highly regulated in the size and size distribution of water droplets in the microemulsion system. In the last few years, several dye-dopped SiO_2 NPs have been synthesized by the w/o microemulsion technique. In this case, to increase the electrostatic attraction of the dye molecules to the negatively charged silica matrix, polar dye molecules are used to ensure successfully encapsulation into SiO_2 NPs [11].

The classical Stöber method was introduced in 1968, by Stöber and co-workers. This constitutes a method for the synthesis of fairly monodisperse SiO_2 NPs, with diameters between 50 nm and 2 μm [15]. Through the procedure described by Stöber, an alkoxide precursor such as TEOS is hydrolyzed in an ethanol mixture, under basic catalysis. This hydrolysis produces silicic acid, which then undergoes a condensation reaction to form amorphous silica NPs. The Stöber method has been extensively investigated and optimized in order to synthesize dye-doped SiO_2 NPs by covalent bonded to organic fluorescent dye molecules [11, 16, 17].

Stöber method allows an eco-friendly synthesis of particles, does not use surfactants and moreover the synthesis reactions occur at room temperature [16].

Arkhireeva and Hay [17] obtained sub-200 nm NPs by slightly modifying the Stöber method. However, synthesized SiO_2 NPs (in sub-100 nm size range) present high polydispersity and irregular shape [18-20]. Therefore, in order to obtain monosized and nanoscale particles, the classical Stöber method is slightly modified [16].

In the methods described above, the concentration of the reactants (TEOS and water), the ratio of EtOH to water ratio and pH, strongly affects the NPs size, size distribution and morphology. This feature is also affected by the nature of the surfactant, the molar ratios of water to surfactant, in the microemulsion process.

NPs prepared through the microemulsion method, exhibiting smooth surfaces and low polydispersity. On the other hand, through the Stöber method, the NPs smaller than

100 nm radii present less monodispersity, may lose spherical shape and present rough surfaces. However, for use in biomedicine, the microemulsion method is not as safe as the Stöber method. The use of surfactants in the NPs synthesis carries a higher risk of cytotoxicity.

The procedure that can be followed to synthesize SiO_2 NPs is described by Gonçalves and co-workers [21]. Figures 4 and 5 illustrate respectively the flowchart of the sol-gel procedure and the SiO_2 NPs obtained.

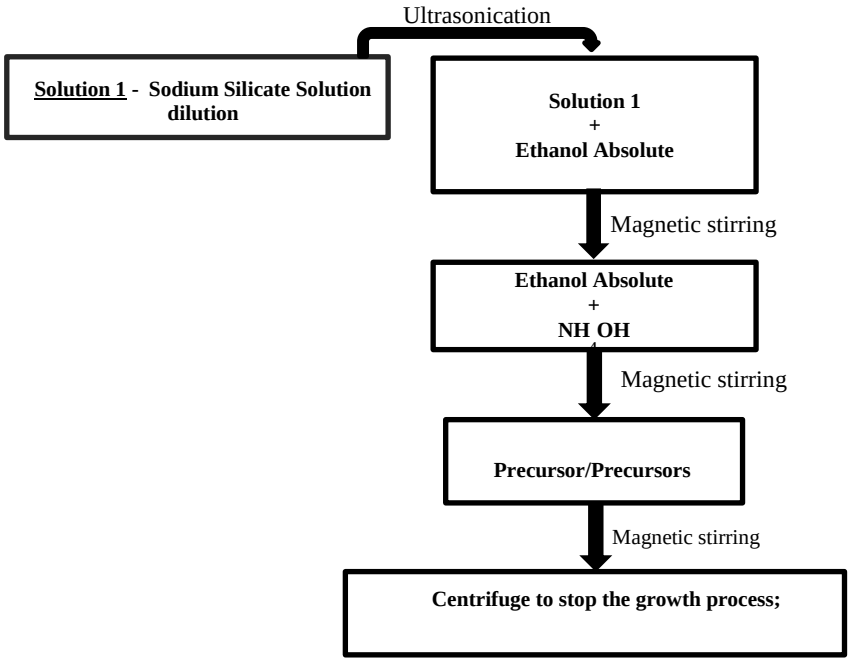


Figure 4: Stober method flowchart for the synthesis of SiO_2 NPs.

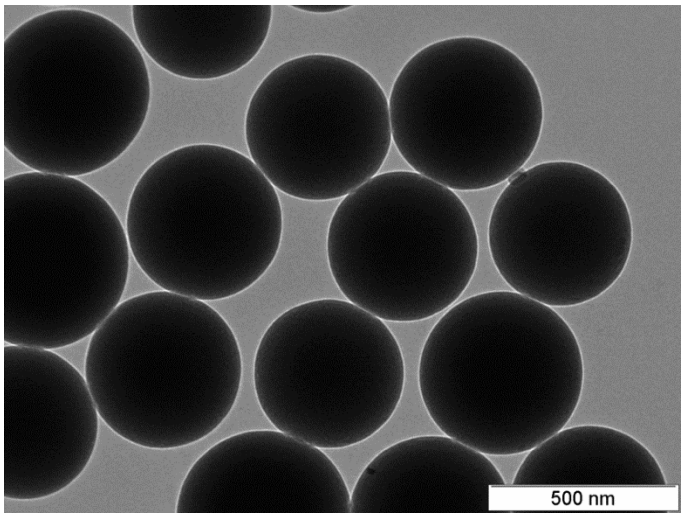


Figure 5: TEM images of SiO_2 NPs synthesized through the Stober method.

Based in the classical two-dimensional model described by LaMer et al. [22], which was used as a model for qualitative interpretation in monodisperse NPs synthesis, Huang et al. [23] recently proposed a procedure to produce monodisperse spherical SiO_2 NPs with sizes ranging between 30–100 nm. The strategy is based on an effective selection of reaction conditions for the Stöber method, and relies on a modification of the conceptual classical LaMer model of nucleation and particle growth.

Based on these principles, Gonçalves and co-workers used the procedure shown in Figure 5 to obtain monodisperse SiO_2 NPs.

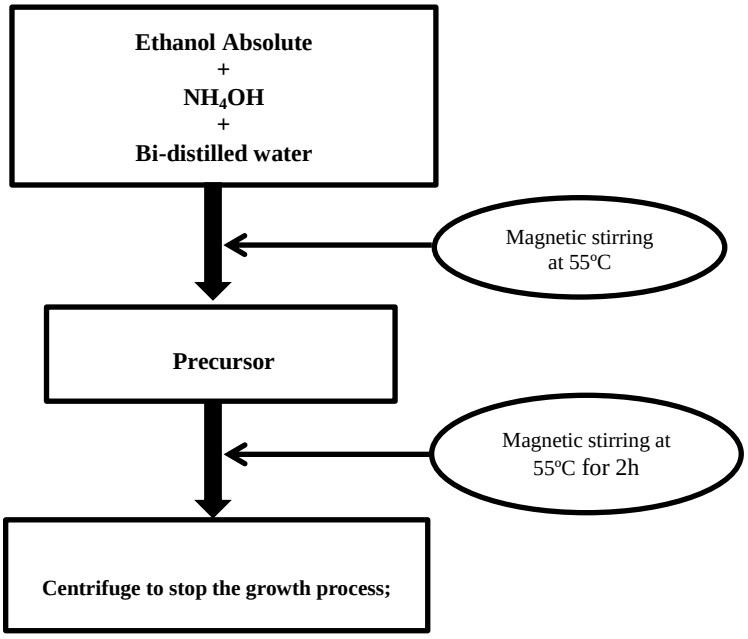
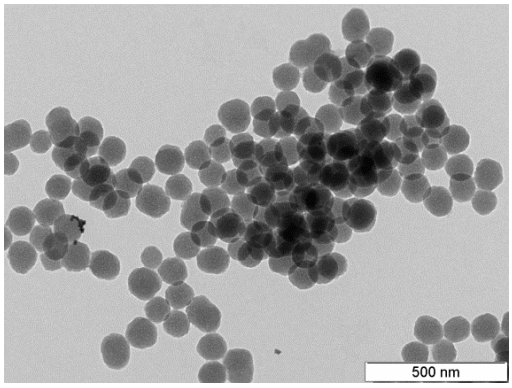
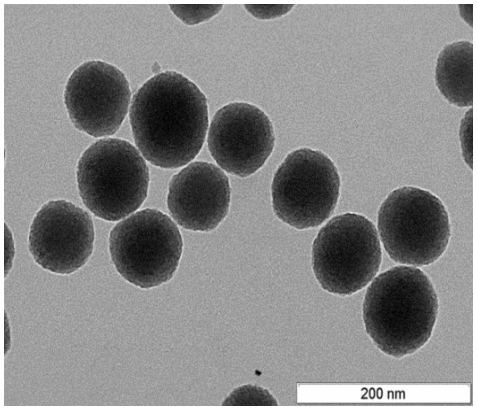


Figure 6: LaMer method flowchart for the synthesis of SiO_2 NPs.

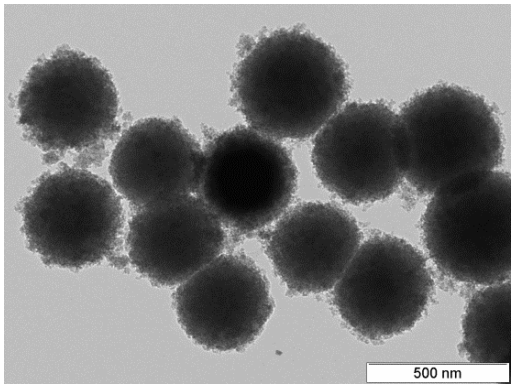
(a)



(b)


200 nm

(c)


500 nm

(d)

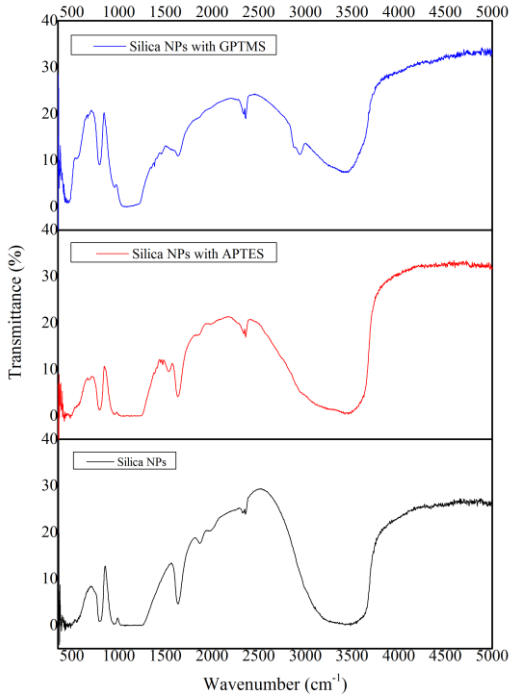

Silica NPs with GPTMS
Silica NPs with APTES
Silica NPs
Transmittance (%)
Wavenumber (cm-1)
500 1000 1500 2000 2500 3000 3500 4000 4500 5000
0 10 20 30 40

Figure 7: TEM images of silica NPs synthesized by LaMer method: (a) inorganic SiO_2, (b) *in situ* amine-functionalized silica NPs and (c) *in situ* GPTMS-functionlized SiO_2 NPs. (d) FTIR of the same NPs.

3.3. Mesoporous silica nanoparticles

Mesoporous SiO_2 NPs present a very large surface area from controllable pore size and volume which is an important advantage. This characteristic allows the loading of large amounts of drug payloads while behave like non-viral vectors.

The synthesis of these NPs is done by modifying the Stöber method, adding surfactants, micelle forming type materials, which behave like templates, that will be latter chemically or thermally removed [24-26] (Figure 8). Pores size of 10 and 300 Å have been reported along with different porous structured nanophases, replica of the surfactant template (Figure 9). During the synthesis of mesoporous SiO_2 NPs, fine synthetic control will results in high surface areas with well controlled particle sizes and shapes increasing the interest of research for these NPs [24-28].

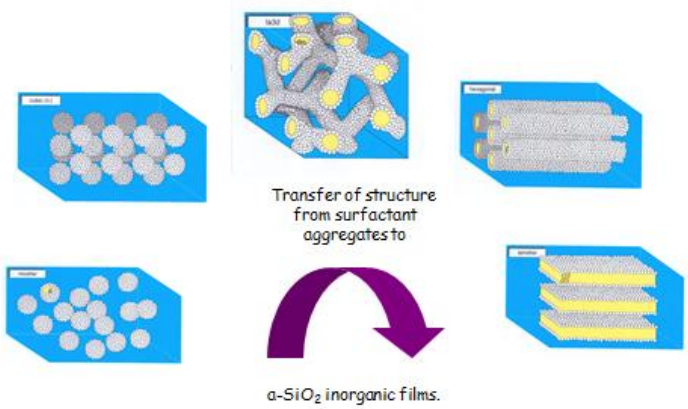


Figure 8 Surfactant as template in nanostructured silica NPs.

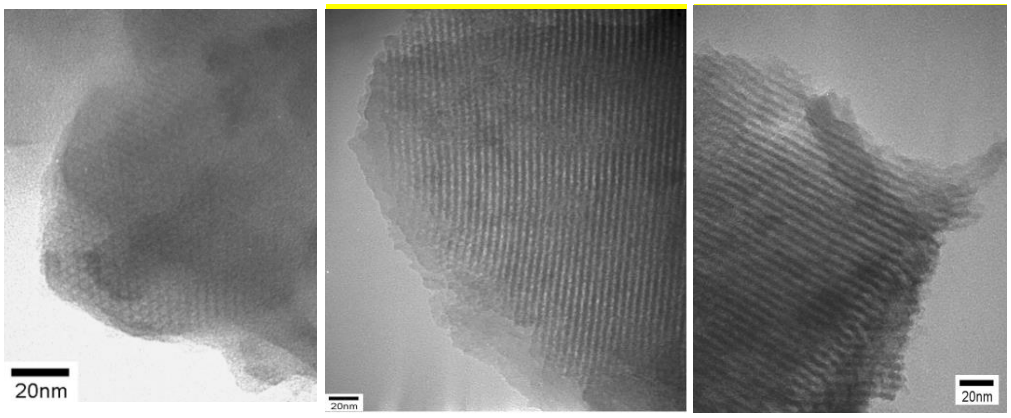
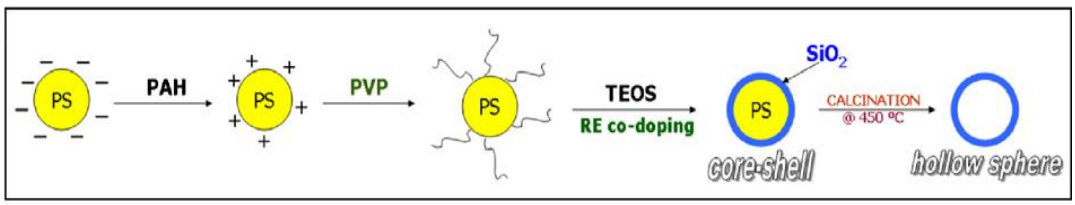


Figure 9. TEM images of nanostructured silica NPs: (a) cubic domain, (b) biphasic I1+HI, (c) L□.

3.4. Hollow-sphere silica nanoparticles

Hollow-sphere NPs can be created through the condensation of alkoxysilanes onto polymer based templates, metal organic frameworks or other nanomaterials. Lately the template will be removed by chemical etching or thermal degradation (Figure 10). Hollow-sphere NPs are capable of carrying large amounts of payload or fill their cores with other desirable materials such as polymers, gold or silver [29-34] along with the gene delivery performance.



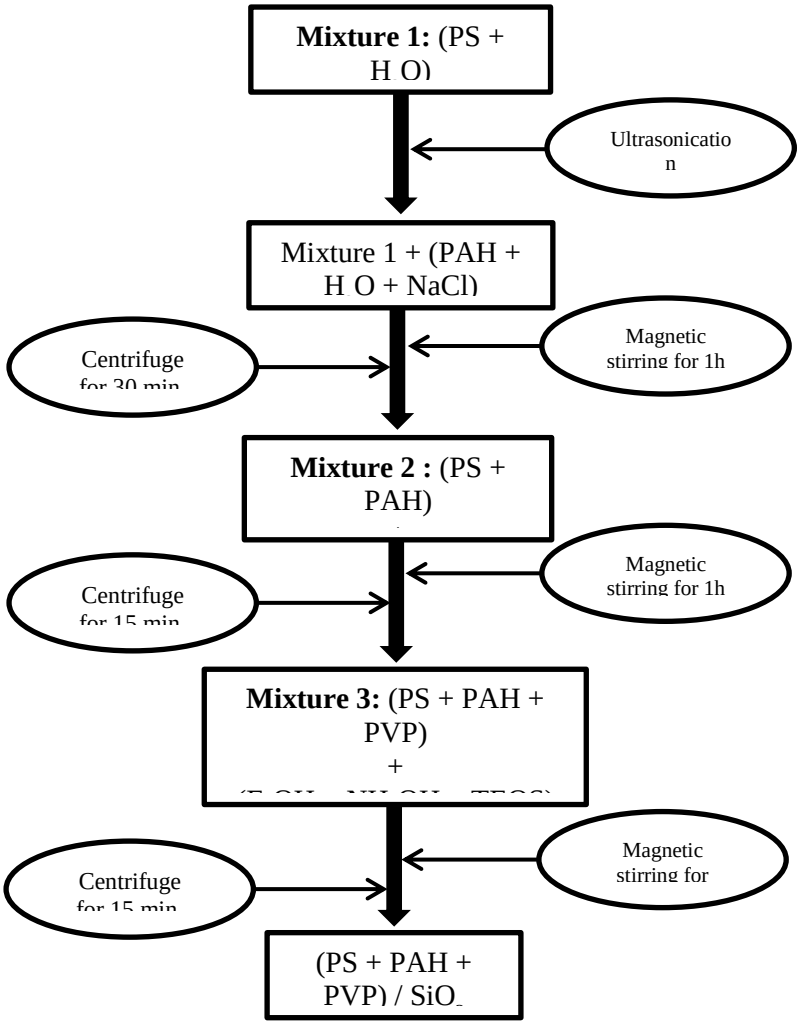


Figure 10. Scheme of hollow-sphere synthesis.

(a) (b)

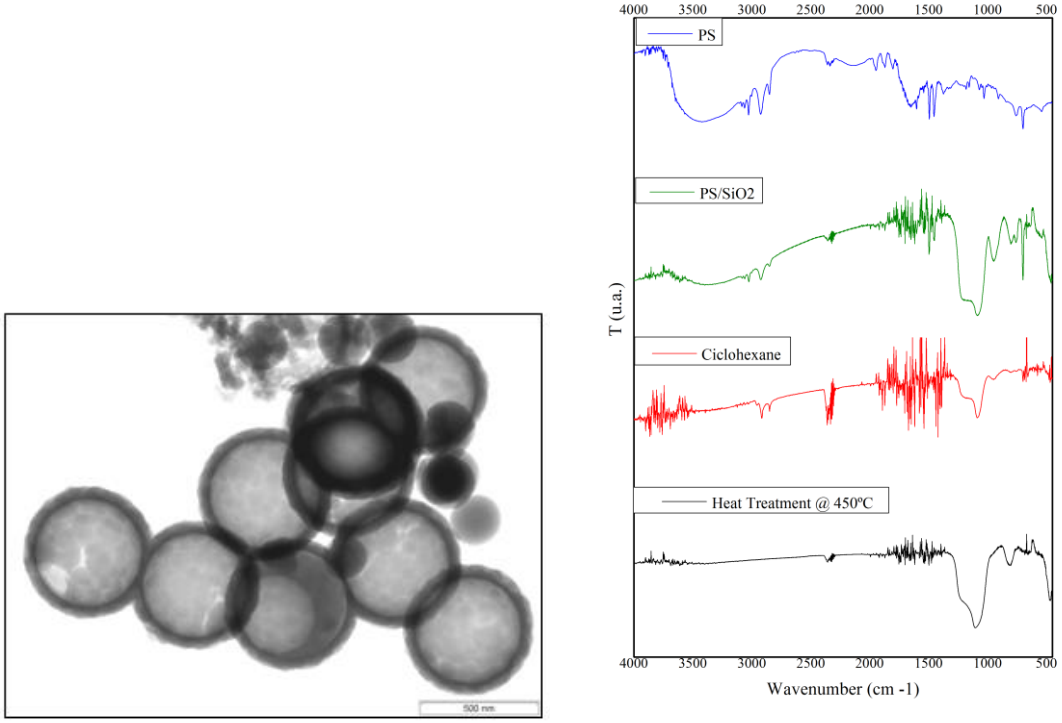
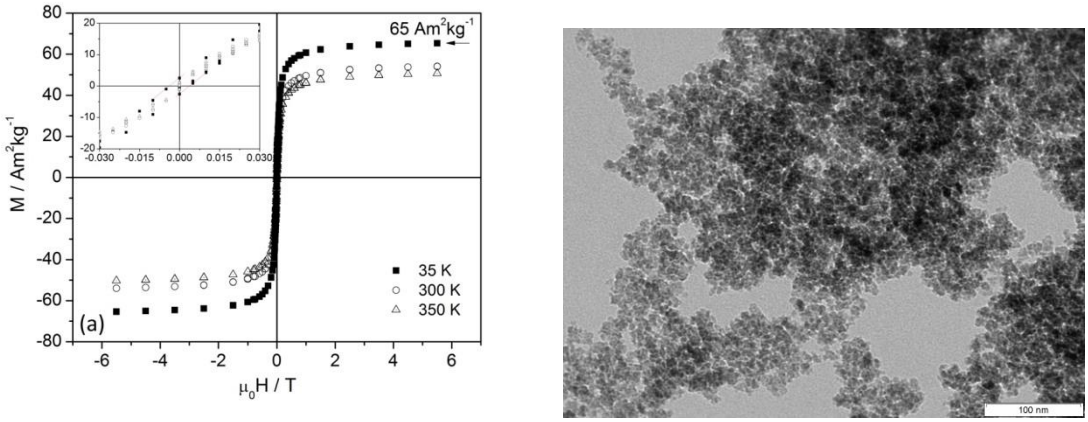


Figure 11. Hollow SiO_2 NPs: (a) TEM image, (b) FTIR of NPs in all stages of the synthesis process.

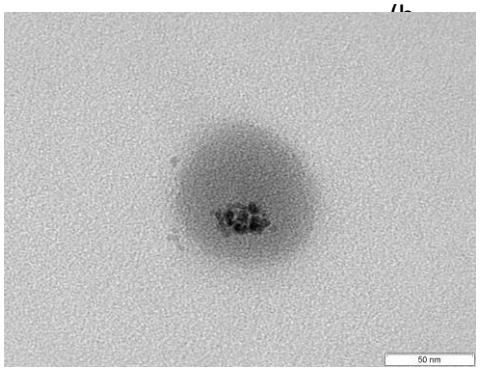
3.5. Core-shell silica nanoparticles

Other materials can also be used as the core template in the core-shell particles synthesis. Gonçalves and co-workers [35] described the synthesis of ORMOSIL core-shell NPs for biomedical purposes. They used two different materials as core, iron oxide and SiO_2 and as shell they used amino-, methyl-, vinyl- and phenyl- functionalized ORMOSIL which grew successfully around the cores.

Core-shell nanoparticles have a great potential in the future on biomedical applications, since these NPs constitute a scaffold to create multi-functional NPs, applicable to several fields; theranosis (therapeutics and diagnostics) [35] and gene delivery performance are some of the possibilities.



(a) (b)



(c)

Figure 12. Fe_2O_3 superparamagnetic core, in core-shell silica NPs (a, b); (c) core-shell NPs.

(a) (b)

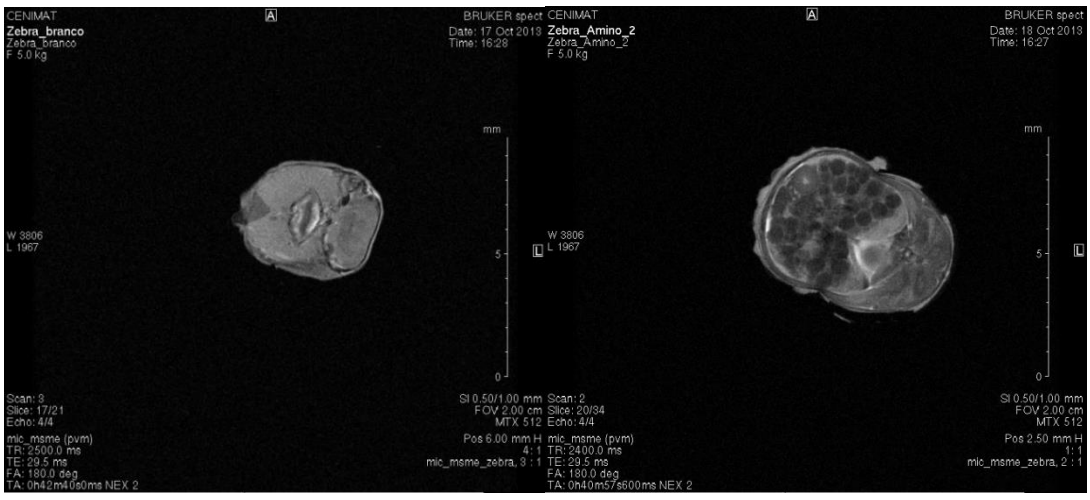


Figure 13. MRI image of non-contrasted (blank) zebrafish (a) and MRI image of contrasted zebrafish with core-shell NPs (b).

3.6. *In situ* functionalization

Several attempts have been made to develop procedures to modify the NPs surface chemistry (functionalization procedures), incorporating a variety of functional (organic) groups within the silica matrix, in order to increase their biocompatibility, improve its resistance to enzymatic action and internalization efficiency [16, 36] alongside with gene targeting. The aminosilane commonly used is the (3-Aminopropyl) triethoxysilane (APTES). This alkoxide precursor is used for the *in situ* surface functionalization of polymeric vectors for gene delivery. The amino group will electrostatically interact with proteins, enhancing their adsorption [37-39]. Studies carried out recently, have shown that, among the commonly used alkoxyde precursors, APTES is the one promoting plasmid DNA interactions more efficiently [16, 40-42].

It has been shown that SiO_2 NPs, functionalized with amino groups, bind and protect pDNA from enzymatic digestion allowing cell transfection *in vitro* [13, 14, 21, 43]. SiO_2 NPs functionalized with non-hydrolyzed organic groups, also known as ORMOSIL NPs, have advantages over SiO_2 NPs, since the presence of non-hydrolyzed organic groups allows tuning the surface hydrophilic/hydrophobic character and enhances bio-conjugation.

The ORMOSIL NPs can be easily loaded with biomolecules such as proteins and drugs, among others [44, 45].

4. Gene therapy

Gene therapy is based on a process, conceptually simple and attractive, which consists on the transfer of one or more functional genes into a cell, tissue or organ (*in vivo*, *in situ* or *ex vivo*) for the treatment of genetic or acquired diseases. The list includes genetic diseases, infections, cancer and degenerative diseases among others.

The first gene therapy clinical trial occurred in the early 1970's, it was observed that DNA and RNA of the tumor viruses were successfully delivered as genetic information to the genomes of mammalian cells [46]. Despite the large number of failures throughout history, in 2006 the confidence in gene therapy increased, when a melanoma treatment using a retroviral formulation was successful [46]. In early 2015,

approximately 2100 clinical trials had been conducted or had been approved being the majority based on viral vectors for gene delivery [47].

Over the last decades, several new methods aiming at the delivery of genes to mammalian cells have been developed, followed by an increasing interest on the possibility of using them for gene therapy and DNA vaccination. The success of gene therapy is strongly dependent of the efficacy of the transfection processes, that is, the ability of DNA to reach the nucleus of the target cell in sufficient amounts to be effective, i.e. able to properly express the gene of interest. It is thus necessary to identify the target cell types and the appropriate DNA sequences to use and adopt suitable methods to reach these objectives. Then, an efficient transportation of the therapeutic gene(s) to the nucleus of the target cells can be carried out by either viral or non-viral vectors. Examples of viral vectors include the modified retrovirus and adenovirus, and they are generally more efficient than the non-viral vectors, since the transportation of the genes to the nuclei of the target cells is more successful. However, these viral systems raise several safety concerns which are absent in the case of genes inserted in non-viral vectors such as plasmids. Plasmids present a great potential as safe vectors. Also, they can be produced in large scales with high reproducibility, thus lowering the associated costs and they are passive to be stored at room temperature [48]. This kind of delivery system has the potential to provide nucleic acid-based therapeutic where the products should have the ability of being easily and repeatedly administrated to the patients with a reduced immune response. These features make them similar to the traditional pharmaceuticals. However, non-viral methods typically produce low levels of transfection and expression of the gene when compared with viral methods. Nevertheless, some recent developments in vector technology have yielded molecules and techniques with efficiencies that approach those of viruses [49].

The injection of naked DNA, is the simplest method of non-viral transfection although is many times inefficient. The main physical methods used to enhance the gene delivery are the electroporation and the “gene gun” or particle bombardment. The electroporation method consists in short high voltage pulses allow transient pores in the plasma membranes allowing of DNA entrance into cells. In the “gene gun” method,

DNA is coated onto gold particles and loaded into a device which generates a force to achieve penetration of the DNA into the cells.

Non-viral gene carriers normally include organic polymers and liposomes [50, 51]. The former are those with more potential for gene delivery because they are easily prepared and present a reduced risk of immune response. Lipoplexes (lipid nucleic acid complexes), cationic polymers and solid lipid NPs, have shown promise as a delivery vector in gene therapy [52-54]. However, cationic molecule based systems present low gene transfer efficacy and toxicity associated with inflammation. Several attempts have been made to decrease the cytotoxicity of cationic non-viral systems [55]. However, an ideal method with high transfection efficiency and relatively safety *in vitro* and *in vivo* has not yet been found. Inorganic NPs present high biocompatibility, lack of toxicity and stable chemical and physical properties, therefore these are relevant promises as gene carriers [56]. Roy et al. [57] used SiO_2 NPs as gene carriers in pDNA transfection studies. Several attempts have been made to develop procedures for modifying the carriers surface (functionalization procedures), in order to increase their biocompatibility and resistance to enzymatic action and internalization efficiency [16, 36].

The NPs should be stable enough to interact with the cell membrane while still allowing the release of the DNA from the complex. The particles in a colloidal dispersion, sometimes adhere to each other, resulting in aggregates that will successively increase their size. When the particles present mutual repulsive forces, these will be stable. Positively charged particles aggregate and are quickly precipitated above their critical flocculation concentration. To avoid this precipitation, it is important to take into account the buffer used, the pH of the final dispersion and its temperature. The size of the delivery systems is also an important parameter to be considered. Some processes will influence the diameter of the particles/complexes and their net charge stability. They are the sonication and the filtration or dialysis. The nanometer size ranges, around the 10 – 1000 nm, of these systems are an important feature, since their sub-micron size and consequently, their sub-cellular size, allows the deep penetration of the NPs into the tissues by fine capillaries, being normally taken up efficiently also by the cells.

There are several physiological barriers that must be overcome to achieve the desired gene expression. For an efficient gene delivery and transfection, internalization must occur without disruption of the gene intended to be expressed. Internalization through the cell membrane must occur, followed by endosomal escape, going through the cytoplasm until getting in the nucleus. However, during this process some bottlenecks may exist which will limit the transfection efficiency. The internalization mechanisms are also largely dependent on the cell type, the vector used and the physical-chemical parameters of the complex determined by the synthesis or production process. Other physiological constraints that can influence the expressed protein levels is the nucleases role in the pDNA degradation after administration and during the traffic to the cell nucleus [57, 58]. It has already been shown that the majority of pDNA administrated in animal models is degraded by a population of exo/endonucleases that are constitutively present in plasma and cytosol of the receptor cells [64, 65]. Thus, the use of vectors that encapsulate the DNA is essential to avoid its degradation.

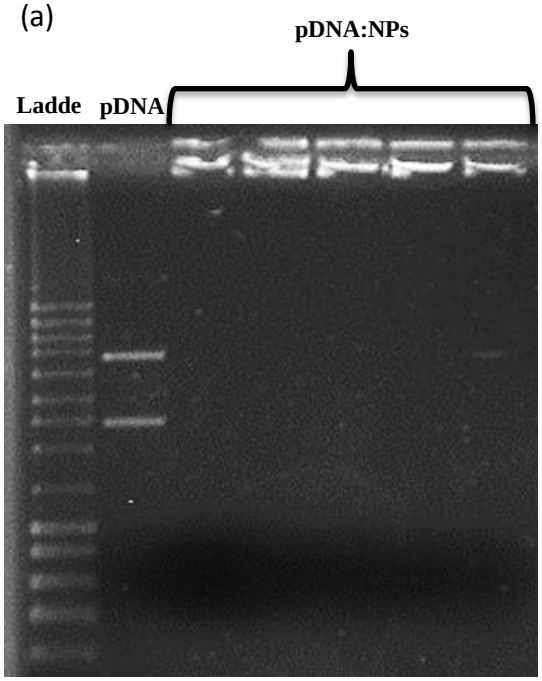
4.1. Silica and ORMOSIL nanoparticles for gene therapy

An ideal gene delivery system should present some important features such as biodegradability, non-toxicity, non-immunogenicity, stability and target-specificity, as already mentioned. It should also assist and improve gene expression, should protect the genetic material from degradation in the extracellular environment and should be able to be used to treat genetic diseases with systemic administration of therapy. An ideal method with both high transfection efficiency and relative safety *in vitro* and *in vivo* has not been found.

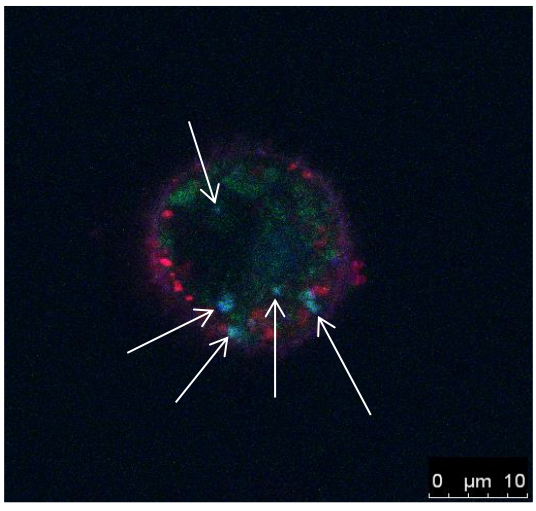
Inorganic NPs such as those made of silica are a promising solution to gene delivery system, since they present some of the pretended characteristics such as biocompatibility, lack of toxicity and stability as already referred [56]. Several studies have been successful performed using silica NPs as a system for gene delivery [41, 59]. Furthermore, several efforts have been made to modify the surface of various polymer vectors in order to improve their biocompatibility, increasing their circulation time, internalization efficiency and improve the linking to the genes. A common functionalization procedure uses silane compounds with terminal functional groups

that will interact electrostatically or covalently with the molecule that is intended to be delivered. One common chemical used for surface functionalization is the APTES. Aminopropyltriethoxylane (APTES) is a precursor that is currently used in the functionalization of the surface of various polymeric vectors used for gene and drug delivery. The amine molecule increase the adsorption of proteins to the surface through electrostatic interactions [37-39]. Studies carried out recently, shown that APTES, from the commonly used precursors, is actually the one which promotes more efficient pDNA interactions [16, 21, 40-42], stronger than an alkylthiol [60]. APTES can also dissolve in both polar and non-polar solvents, and has high solubility in cell membranes [61, 62]. Gonçalves and co-workers have shown that amino ORMOSIL NPs efficiently bind to the pDNA forming complexes that have been successful internalized by Chinese hamster ovary cells [21]. SiO_2 NPs functionalized with APTES are promising candidates to be used as carriers in gene therapy.

(a)



(b)



(c)

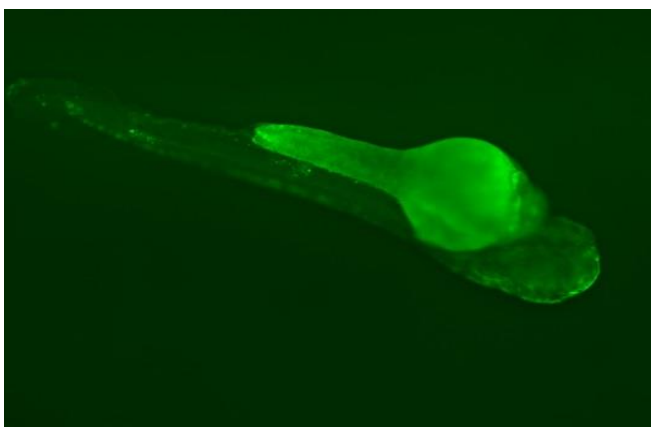


Figure 14. Efficient complexation between pDNA and ORMOSIL NPs were proved by agarose gel electrophoresis (a); fluorescence labeled ORMOSIL NPs were successfully internalized by Chinese hamster ovary cells and transfected (b); fluorescence labeled ORMOSIL NPs were internalized by zebrafish *larvae* evidencing no cytotoxicity (b).

5. CONCLUSIONS

Over the last decades a large number of viral and non-viral systems for gene delivery have been developed and studied. Both types present advantages but still drawbacks. Gene delivery through viral-based systems, present a long-term expression, effectiveness and expression of the therapeutic genes, having a high transfection rate. However, they also present some limiting disadvantages: immunogenicity, toxicity, the risk associated with virus handling and the limitation on the production of large batches. The non-viral methods are supported by higher biosafety, simpler techniques, can effectively target cells and tissues. However, their biggest disadvantage for clinical use is their low transfection efficiency.

Silica and ORMOSIL NPs are considered to be a good scaffold for multi-functional biomedical applications. They present a large surface area which could allow them to be loaded with one or more types of drugs to be used in drug delivery, or even by a fluorophore, for real time imaging and diagnosis. By surface functionalization with specific groups, SiO_2 NPs can be conjugated with several types of biomolecules, such as specific linkers, fluorescent labels, antibodies or DNA, giving them simultaneous therapeutic and diagnostic (theranosis) capabilities. Therefore, many studies have shown that SiO_2 NPs or ORMOSIL NPs are promising candidates to be used as non-viral carriers in gene therapy. These NPs present many unique properties, such as a high biocompatibility, nontoxicity and a high capability to control their size, shape and cargo. It has also already been shown that these NPs bind efficiently to plasmid DNA and are capable to protect it from enzymatic degradation. Thus, these are all promising characteristics for non-viral vectors which are likely to be used in gene therapy.

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