Biomedical applications of organically modified bioconjugated silica nanoparticles

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Abstract: Nanoparticles are one of the most important prominent areas in the field of nanotechnology, with a broad significant role in current life sciences and human healthcare applications. Among the nanoparticles silica nanoparticles are explored in many biological applications because their size dependent novel properties and functions differ drastically from those of the bulk materials. In biomedical research, silica nanoparticles act as a novel class of nanodetector or nanoseparator with unique advantages due to their size, biocompatibility, specificity and other size dependent properties. In this review, we describe the applications of silica nanoparticles in the field of biomedical nanotechnology such as highly sensitive bioanalysis systems for targeted drug delivery, clinical diagnosis and immunoassay.

Keywords: silica nanoparticle; bioanalysis; bioseparation; cellular imaging; nanomedicine; gene delivery.

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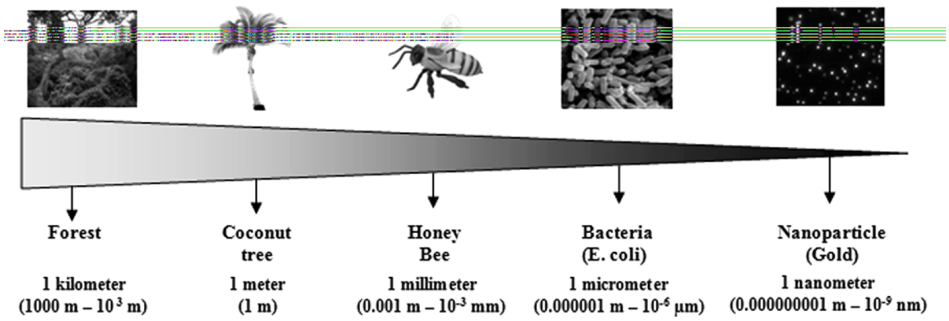
1 Introduction

Nanotechnology is one of the most challenging and rapidly expanding field in this competitive scientific world, which is an area that has seen a surge in research activity over recent years. Nanotechnology is simply defined as design, characterisation and manufacturing of products at the nanometre scale level of about 1–100 nm (Figure 1). Nanotechnology offers new solutions for the transformation of biosystems as well as provides a broad technological platform for application in bioprocessing, molecular medicine, environmental improvement, improving food and agricultural systems. In the last two decades, the research of nanotechnology has grown explosively with over three hundred thousand publications in the field of nanoscience according to Web of Science. Among these spectacular developments, a new emerging field nanobiotechnology that combines nanotechnology and biotechnology is receiving increased attentions [1]. Nanobiotechnology is the convergence of engineering and molecular biology that is leading to a new class of multifunctional devices and systems for biological and chemical analysis with better sensitivity and specificity and a higher rate of recognition [2]. In the last decade, engineered nanoparticles have become an important class of new materials with several properties that make them very attractive for commercial development. Recently, nanoparticles are used increasingly for the development of new medicines, and given the inherent nanoscale functions of the biological components of living cells it has been applied to diverse medical fields such as oncology and cardiovascular medicine. In addition, they have been highly utilised for manufacturing diverse industrial items such as cosmetics or clothes and for numerous applications in electronics, aerospace and computer industry [3]. The final result of the nanobiotechnology is to create highly sensitive systems in medical and biotechnological area for diagnosis and treatment of harmful diseases. Nanoparticles have potential role in biomedical nanotechnology and biotechnology because of their size dependent properties. Among these nanoparticles mostly silica nanoparticle has been used for bioanalysis and bioseparation process. The easy and proficient surface modifications of these nanoparticles make them to applicable in biomedical research [4]. Recent years, one problem in biomedical research has been selective identification of selective bioanalytes from complex biological matrices. To overcome the problems bioconjugated silica nanoparticles, dye encapsulated silica nanoparticles (silica nanoparticle is encapsulated by dye) and developed dye-doped nanoparticles conjugated with

biorecognition molecules are commonly used for the biomedical and biotechnological applications [5]. The properties of the silica nanoparticle differ from other nanoparticles such as gold nanoparticles and quantum dots [4] because of the excellent photostability, high signal amplification and biocompatibility [5].

The delivery of drugs into the targeted site is an important task to cure harmful diseases. During the past decades, the release of therapeutic molecules to target site by targeted and controlled delivery has created massive snags in biomedicine [6–8]. Traditionally, viral [9,10] and non-viral vectors [11,12] have been used to carry genes and it exhibited useful advantages in genetic engineering, but these vectors have some snags in the gene transfer process. For instance, at the moment of penetration of viral vector into the recipient cell may induce immunity in the recipient and create toxicity against the viral vector and targeted gene. The non-viral vectors have significant effect and merits in gene transfer process including low toxicity, and chemically based carriers are easily designed but have the drawback of lower efficiency [13]. Recently, nanomaterials mediated delivery of chemical/biological therapeutic molecules has been given a great attention due to its remarkable applications in the arena of pharmaceutical biotechnology. Nowadays, bioconjugated nanoparticles are playing a crucial role in the gene/drug delivery systems. The massive development in gene transfection process through non-viral vectors will manipulate an effective gene delivery platform in forthcoming years. Finally, nanobiotechnology can provide a great platform for scientists to model nanoparticles to refine discovery of biomarkers, molecular diagnostics, drug discovery and delivery, which could be applicable in human life care systems.

Figure 1 Size variations from kilometre to nanometre (see online version for colours)



2 Silica nanoparticles in bioassay process

The surface modified nanoparticles are conjugated with biorecognition molecules and dyes for rapid and sensitive detection of biomolecules, which are extremely prominent in biomedical nanotechnology. The properties of nanoparticles such as large surface area and other size dependent qualities differ from its original form (bulk material). Currently, there are no efficient techniques in biotechnology for rapid selection of trace elements of bioanalyte from the complex biological matrix. In order to overcome the inadequacy of the previously available separation techniques, the nanoparticles especially silica nanoparticles are selectively used for the bioseparation techniques. Silica is a well-established component that allows surface modifications with various functional groups such as carboxyl, amino, thiol, and hydroxyl (Figure 2) and is further

modified with biological materials for biomedical applications. The dye-doped silica nanoparticles are formed by water-in-oil microemulsion [1] and stober method [14]. The dye-doped silica nanoparticle is linked with biorecognition molecules like antibody and proteins and formed into a bioconjugated silica nanoparticle. The dye molecules were trapped inside the silica matrix to form the dye-doped silica nanoparticles. These dye-doped silica nanoparticles with biorecognition molecules have been used for detection of bacterial cells in a sample [1], protection and detection of nucleic acids [3,5] and imaging [14]. Moreover, bioconjugated nanoparticle is used to identify (selective identification) a single bacterial cell from a culture sample (Figure 3).

Figure 2 The various functional groups on silica surface

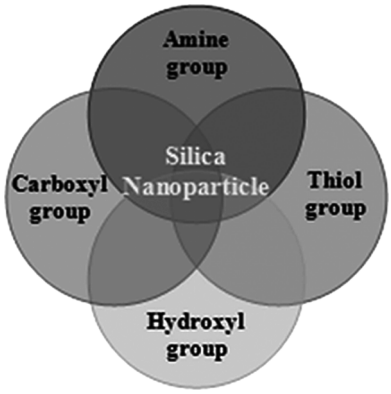
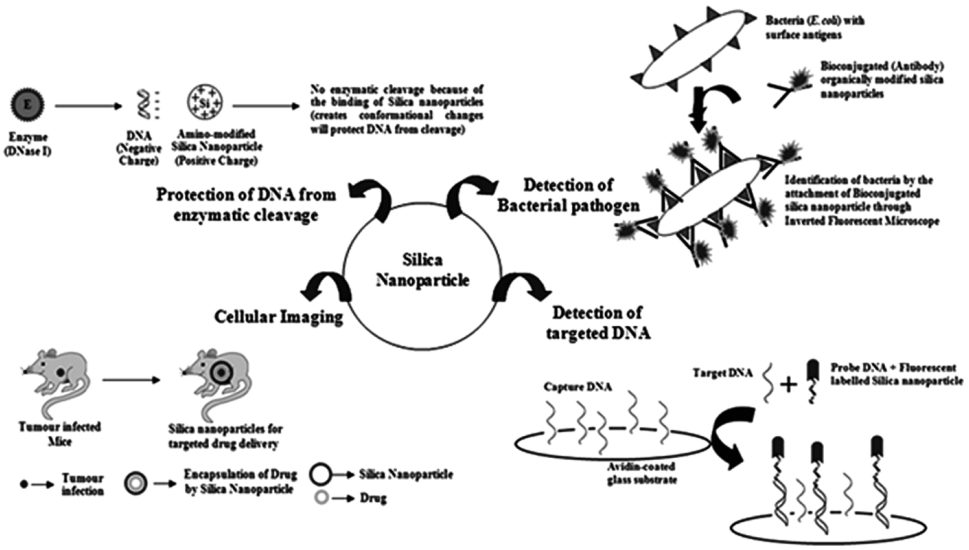


Figure 3 Applications of organically modified silica nanoparticles in the biomedicine: detection of bacterial pathogen, detection of targeted DNA, targeted drug delivery and Protection of DNA from enzymatic cleavage



Fluorescent-based bioassay has been developed for hasty and accurate determination of a single bacterial cell *E. coli* O157:H7 from ground beef sample within 20 min

by using the bioconjugated silica nanoparticles. The monoclonal antibody against *E. coli* is immobilised onto the silica nanoparticle and the surface of the nanoparticle is encapsulated by the dye (RuBpy) molecules [1]. A similar work has reported the detection of pathogenic microorganism *Mycobacterium tuberculosis* by using fluorescent silica nanoparticle. Here the anti-*Mycobacterium tuberculosis* antibody was used as primary antibody to recognise *M. tuberculosis*. The antibody of the *M. tuberculosis* labelled with (RuBpy) doped silica nanoparticles is used to generate fluorescent signal for microscopic examination. *M. tuberculosis* could be detected in both mixed bacterial samples and sputum samples. This total assay time including sample pre-treatment is 4 h for identification of *M. tuberculosis* [15]. Comparing with conventional fluorescent dyes, the use of fluorescent nanoparticles as label in immunofluorescence microscopy offers advantages of higher luminescence and higher photostability. This type of bioassay method is vital for food and water safety, clinical diagnosis, and to thwart the bioterrorism. In addition, by replacing the antibody of other bacteria, the bioassay technique by dye-doped and fluorescent silica nanoparticle has the potential to develop a universal method for detecting a wide variety of pathogenic bacteria in biomedical and biotechnological research. These techniques have proved that bioconjugated dye-doped nanoparticles are good candidates for rapid identification of pathogenic microorganism from a culture sample.

3 Bio safeguard

Another interesting application of silica nanoparticle in molecular biology is the protection of DNA from enzymatic cleavage and ultra sound damage. A novel method has been developed to protect DNA from cleavage by using bioconjugated amino-modified silica nanoparticles (Figure 3). Silica nanoparticles have efficient properties to protect Green Fluorescent Protein (GFP) plasmid DNA from enzymatic cleavage (DNaseI). Amino-modified silica nanoparticles have positive charge and it can serve as a foundation for an effective enrichment of negatively charged DNA strands onto the positively charged amino-modified silica nanoparticle surfaces. There could be some possibilities in the protection of DNA from enzymatic cleavage in that the positive charge on the amine group (NH_2) of the nanoparticles keeps Mg^{2+} away from the positively charged nanoparticles. It can control the enzymatic cleavage and the Mg^{2+} is vital one for the DNA protection process. Another possibility is the binding of nanoparticles to the DNA, which results in the structural modification of DNA due to size variation and firmly fixed DNA on to the nanoparticles. The structural changes could protect the DNA from cleavage [16]. The protection method resembles the enzymatic cleavage that protects the DNA from ultrasound damage. It has been demonstrated that poly-L-lysine starch nanoparticles have been used for the protection of DNA from ultrasound damage. The GFP plasmid DNA is treated with ultrasound 120 W for diverse times (10, 20, 30 min) and the results proved that the poly-L-lysine alone could not protect the DNA; combined with starch nanoparticles the complex protects the GFP plasmid DNA from the ultrasound [17]. The silica nanoparticle is an efficient way to protect DNA strands and it has significant effects in separation, detection of nucleic acids and in gene therapy studies.

4 Detection of nucleic acids

The important application of silica nanoparticle is the ultrasensitive detection of DNA. Sensitive DNA detection is enormously important in molecular and clinical diagnostics, gene therapy, and a variety of biomedical studies. Recently, great efforts have been made to develop new techniques in biotechnology to improve the sensitivity and selectivity for gene analysis. The DNA hybridisation is one of the most widely used methods for DNA detection, offering excellent selectivity by the DNA base pair coupled with optical detection. Usually, fluorescent dye molecules such as organic dyes are utilised to amplify signals and also dyes have some disadvantages like low signal amplification and poor photostability. Most of the fluorophores like organic dyes suffered seriously by photobleaching, resulting in irreproducible signals for bioanalysis. Several of nano metals such as silica nanoparticles, luminescent particles, metal nanoparticles, nanotubes, nanocages, [18–22] semiconductor nanocrystals and quantum dots [23,24] have strong and photostable fluorescent signal and are used as signalling probes for bioassay and bioanalysis process. Recently fluorescent labelled gold and silver nanoparticles have been used to detect the target DNA. This gold and silver based detection is a complicated process, and it causes reduced reproducibility. For better sensitivity and reproducibility the dye-doped silica nanoparticles have been developed and used for bioanalysis process. Some workers have reported that a novel ultrasensitive DNA detection method to detect target DNA by using fluorescent bioconjugated silica nanoparticles (Figure 3). First, they reported a biotinylated capture DNA is immobilised on an avidin-coated glass substrate. The target DNA and probe DNA nanoparticle complex (NP-DNA) added to the glass substrate for hybridisation. The detection of the target DNA is done by monitoring fluorescence signals of the NP-DNA conjugates left on the glass surface after thorough washing steps and with proper excitation. Moreover the study concluded with the 0.8 fm (femtomolar) concentration detection limits in DNA analysis to detect the target DNA has been achieved by using bioconjugated silica nanoparticles based sandwich assay [25]. The sensitivity and reproducibility properties of nanoparticles are useful for the further development of immunosensor and other bioanalytical applications.

5 Cellular imaging

The semiconductor quantum dots, light emitting nanoparticles and organically modified biocompatible nanoparticles are emerging as a new class of fluorescent labels for bioimaging in biomedicine [26]. Currently, the development of biocompatible nanoparticles for cellular imaging and targeted therapy is a considerable research area in nanomedicine. Cancer nanotechnology is currently under extreme development for applications in cancer cell imaging, molecular diagnosis and targeted therapy. Metal nanoparticles such as gold, dye-doped silica nanoparticle and quantum dot have more importance and potential application in cell imaging and immunoassay methods to detect cancerous cells [20,23,24,27–29]. Nanoparticles are being designed for a number of cancer applications. Recent advances in the nanotechnology have led to multifunctional nanoparticles that can act as probes for molecular and cellular imaging, it promises novel approaches for earlier detection, diagnosis, screening and deterrence of cancer disease. Nowadays diagnosis of cancer relying on flow cytometry using single dye-labelled antibodies is an important area in cancer nanotechnology. Silica nanoparticle is a

proficient molecule conjugated with dye molecules and targeting ligands for efficient imaging of cancer cells (Figure 2). Though this combination may not lead to high signal output, it should hamper the detection of targeted molecules, particularly when the probes have weak affinity or when the receptor is expressed in low concentration on target cancerous cell surface. To resolve these difficulties novel dye-doped silica nanoparticles have been developed for sensitive and rapid detection of cancer cells. The nanoparticles are approximately 60 nm in size and it could be encapsulated by thousands of individual dye molecules within the silica matrix. The dye-labelled biotinylated aptamer is immobilised on nanoparticle surface and it could be used for the identification of cancerous cells through flow cytometry [28]. Recent reports showed the effective labelling of lung cancer cells (A549) (*in vitro* bioimaging) and rat brain tissue (*in vivo* bioimaging) using transactivator of transcription peptide (TAT) conjugated with fluorescent isothiocyanate (FITC) doped silica nanoparticle. Here, the A-549 cells are incubated for 2 h with TAT-FSNP complex (fluorescent silica nanoparticle) and then view the *in vitro* labelling of A-549 cells and TAT-FSNP under laser scanning confocal microscope [30]. Another parallel study shows the *in vivo* bioimaging by using the TAT-FSNPs that the TAT-FSNPs penetrate intra-arterially through the right common carotid artery (CCA) that supplies blood to the right part of the brain of a Sprague–Dawley rat. After, the whole brain is sliced into four pieces and imaged with a fluorescence microscope, and the fluorescent images confirmed the efficacy of the TAT-FSNP based in vivo bioimaging [31].

6 Non-viral gene delivery system

Efficient and secure gene delivery system by using non-viral vectors is one of the important areas in the field of nanobiotechnology. One of the reasons for genetic disorder is the absence or the damage of specific genes. The treatment of genetic disease by delivering of specific designed therapeutic genes into the targeted region is a significant improvement in disease therapy. Some of the quantum dots, chitosan nanoparticles and silica nanoparticles have been used for the proficient non-viral delivery of therapeutic drugs, proteins and genes [32–34]. Recent research showed that organically modified silica (ORMOSIL) nanoparticles as a non-viral vector for proficient factor in vivo gene delivery [35]. Here the intraventricular injection of ORMOSIL and plasmid of enhanced green fluorescent protein-N2 (pEGFP-N2) nanoparticles in the mouse brain resulted in the effective transfection and expression of enhanced green fluorescent protein (EGFP) in neuronal-like cells in periventricular brain regions and the SVZ (Subventricular Zone). In addition, transfection with ORMOSIL-FGFR1 (fibroblast growth factor receptor 1) nanoparticles resulted in the modulation of the replication cycle of the stem progenitor cells in the SVZ. These reports provide the groundwork for the use of ORMOSIL nanoparticle formulations for *in vivo* gene transfer into the CNS (central nervous system) and have the potential to provide a safe and efficient mechanism for *in vivo* gene therapy applications [35]. The use of organically modified silica nanoparticle in *in vivo* gene transfer has the potential to provide a safe and proficient process for *in vivo* gene therapy applications.

7 Discussion

This review represents the application of organically modified silica nanoparticles in a variety of *in vivo* and *in vitro* bioassays in the field of biomedicine. The excellent photostability, compatibility, easy bioconjugation and flexible nature make the silica nanoparticle a powerful tool in bioseparation and bioanalysis. They have also found human healthcare for example the diagnosis of harmful disease with proper bioassay technique and efficient delivery of therapeutic drugs in the biomedical field. However, applications of silica nanoparticles in medical research and clinical practice will require further development. According to these studies the cytotoxicity of silica NPs, which must be fully investigated before considering the intravenous injection of silica NPs with drugs, genes and dyes into diseased tissues and organs of the human. For drug delivery, there are barriers to controlling the interaction of NPs within the body before this technology it can be effectively translated into practical therapeutic regimens. Methods of targeting NPs to specific sites of the body while avoiding capture by organs, such as the liver and spleen, must also be addressed. However, currently few data available on silica nanoparticle toxicity exist. On the one hand, high dosage of silica nanoparticle is more toxic to normal human fibroblast cells than to cancer cells. At high dosages, amorphous silica nanoparticles may retard cell proliferation, damage the cell membrane, and possibly induce cell apoptosis/necrosis [36]. The polymorphic forms of silica are shown to induce red blood cells (RBC) haemolysis by interacting with the cell membrane of mammalian red blood cells [37].

One of the most promising applications of nanobiotechnology is to delivering of therapeutic molecules. The polyethylene glycol and hydrogels as non-viral based gene/drug carrier materials have been used as delivery systems in pharmaceutical biotechnology [9,12]. Polyethylene glycol (PEG) with dextran acts as a non-viral gene vehicle for transfer of plasmid DNA containing the coding sequence of LacZ and an SV40 promoter. Herein, the PEG is used to modify the surface functional groups of the dextran for compatible binding of plasmid DNA and dextran. The cationised dextran is actively bound with the negatively charged plasmid DNA. The modification of cationised dextran with PEG could easily transfer the plasmid DNA to the targeted tumour cells. The plasmid DNA in the absence of PEG with cationised dextran alone did not show any gene expression due to electrostatic interaction with cationised dextran and negatively charged blood cells and also other cellular matrix prevents the plasmid DNA to reach the tumour cells [9]. Other than PEG, hydrogels are also used for the release of drug into the targeted tumour infected region [12]. Konishi et al. [12] have been reported the in vivo release of anti tumour drugs cisplatin (CDDP) and adriamycin (ADM) by using non-toxic and biodegradable material gelatin hydrogel. Here, the degradation of hydrogel results in the release of CDDP and ADM into the tumour. The CDDP was strongly linked to the hydrogels due to the presence of carboxyl groups and it controls the initial release of CDDP is 5–30% by the strong affinity between the CDDP and carboxyl groups in hydrogel. But the initial release of ADM 60–80% was achieved due to the weak interaction between the ADM and hydrogel. The weak electrostatic interaction between the amine residues (NH_2) of ADM and COOH group of gelatin results in the slow delivery of ADM from the gelatin. The similar results have been reported by Bouuhadir et al. [38] and both of these results suggested that the increased amount of water molecules could affect the nature of gelatin and finally it turns to flexible [38]. The flexibility of the gelatin could affect the mobility of the gelatine molecules by

increased amount of cross linking agent glutaraldehyde [12,38]. On the other hand, organically modified silica nanoparticles have been successfully used for *in vivo* gene delivery [37] and bioimaging of human lung carcinoma (A-549) cells with TAT peptides [31]. During this short period, these silica nanoparticles have been successfully integrated with the biological systems and in future it will be created a remarkable application in nanomedicine.

8 Conclusion

Nanobiotechnology and nanomedicine are in infancy stage in the widespread ocean of nanoscience and nanotechnology, which are having significant effects in biomedical research in 21st century. The long-standing expectations in biomedical research are expanded from diagnosis of detrimental diseases to delivery of drugs and genes. Some researchers in the realm of nanobiotechnology believe that novel nanoparticles could potentially overthrow the traditional notions about the harmful diseases and health to usher in a form of medicine based on identification and prevention instead of treatment. The silica nanoparticles are likely to be a keystone of the inventive biomedical devices to be used for drug delivery, development of bioprobes for diagnosis and treatment for diseases and also for detection of nucleic acids were discussed. Moreover, like silica nanoparticle many novel nanoparticles and nanodevices are expected to be used with a huge benefit on human health. Therefore we concluded that the growth of nanobiotechnology and nanomedicine provides revolutionary opportunities for early diagnosis of diseases, other therapeutic processes for developing human health and physical abilities, enabling precise efficient and easiest therapy to the patients. The function and properties of silica nanoparticle will provide a graceful opening for scientist and engineers to initiate and create new and amazing applications in the pool of nanobiotechnology.

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