

<https://helda.helsinki.fi>

Application of aptamers in diagnostics, drug-delivery and imaging

Chandola, Chetan

2016-09

Chandola , C , Kalme , S , Casteleijn , M G , Urtili , A & Neerathilingam , M 2016 , ' Application of aptamers in diagnostics, drug-delivery and imaging ' , Journal of Biosciences , vol. 41 , no. 3 , pp. 535-561 . <https://doi.org/10.1007/s12038-016-9632-y>

<http://hdl.handle.net/10138/166773>

<https://doi.org/10.1007/s12038-016-9632-y>

publishedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

Application of aptamers in diagnostics, drug-delivery and imaging

CHETAN CHANDOLA¹, SHEETAL KALME¹, MARCO G CASTELEIJN², ARTO URTTI² and MUNIASAMY NEERATHILINGAM^{1,*}

¹Center for Cellular and Molecular Platforms, NCBS-TIFR, Bangalore 560 065, India

²Centre for Drug Research, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland

*Corresponding author (Email, mneerathilingam@gmail.com)

Aptamers are small, single-stranded oligonucleotides (DNA or RNA) that bind to their target with high specificity and affinity. Although aptamers are analogous to antibodies for a wide range of target recognition and variety of applications, they have significant advantages over antibodies. Since aptamers have recently emerged as a class of biomolecules with an application in a wide array of fields, we need to summarize the latest developments herein. In this review we will discuss about the latest developments in using aptamers in diagnostics, drug delivery and imaging. We begin with diagnostics, discussing the application of aptamers for the detection of infective agents itself, antigens/toxins (bacteria), biomarkers (cancer), or a combination. The ease of conjugation and labelling of aptamers makes them a potential tool for diagnostics. Also, due to the reduced off-target effects of aptamers, their use as a potential drug delivery tool is emerging rapidly. Hence, we discuss their use in targeted delivery in conjugation with siRNAs, nanoparticles, liposomes, drugs and antibodies. Finally, we discuss about the conjugation strategies applicable for RNA and DNA aptamers for imaging. Their stability and self-assembly after heating makes them superior over protein-based binding molecules in terms of labelling and conjugation strategies.

[Chandola C, Kalme S, Casteleijn MG, Urtti A and Neerathilingam M 2016 Application of aptamers in diagnostics, drug-delivery and imaging. *J. Biosci.* **41** 535–561]

1. Introduction

Aptamers are small, single-stranded oligonucleotides (DNA or RNA), which specifically bind to their target with high affinity. The term ‘aptamers’ comes from the Latin word *aptus* (fitting) and the Greek word *meros* (part). The DNA and RNA aptamers can bind to the same target with different folding patterns and sequences. Aptamers are selected from a pool of a random oligonucleotide library ($>10^{15}$ random oligonucleotides) by iterative rounds of affinity purification and amplification by a process called Systematic Evolution of Ligands by Exponential enrichment (SELEX) (figure 1). This concept of nucleic acid binding to their targets like a protein came into the light when small RNA structures were found to bind with viral or cellular proteins at high affinity and specificity during an

investigation on human immunodeficiency virus (HIV) and adenovirus (Song *et al.* 2012). Initial studies on aptamers started with the report of SELEX method in 1990 by both Gold’s and Szostak’s groups, which was more than two decades ago (Ellington and Szostak 1990; Tuerk and Gold 1990). SELEX is a basic technique for the selection of aptamers *in vitro* against various targets, from small biomolecules to larger biomolecules like proteins and even cells. The interaction between an aptamer and its target involves different intermolecular interactions including van der Waal’s forces, electrostatic interactions between charged groups, three-dimensional shape, stacking and hydrogen bonds. Aptamers can fold into a variety of secondary and tertiary structural elements, like stem loops, pseudoknots, kinks, and bulges, which can recognize multiple surfaces as target binding sites. Due to the ability of

Keywords. Aptamer; cancer; conjugation-methods; diagnostics; drug-delivery; imaging

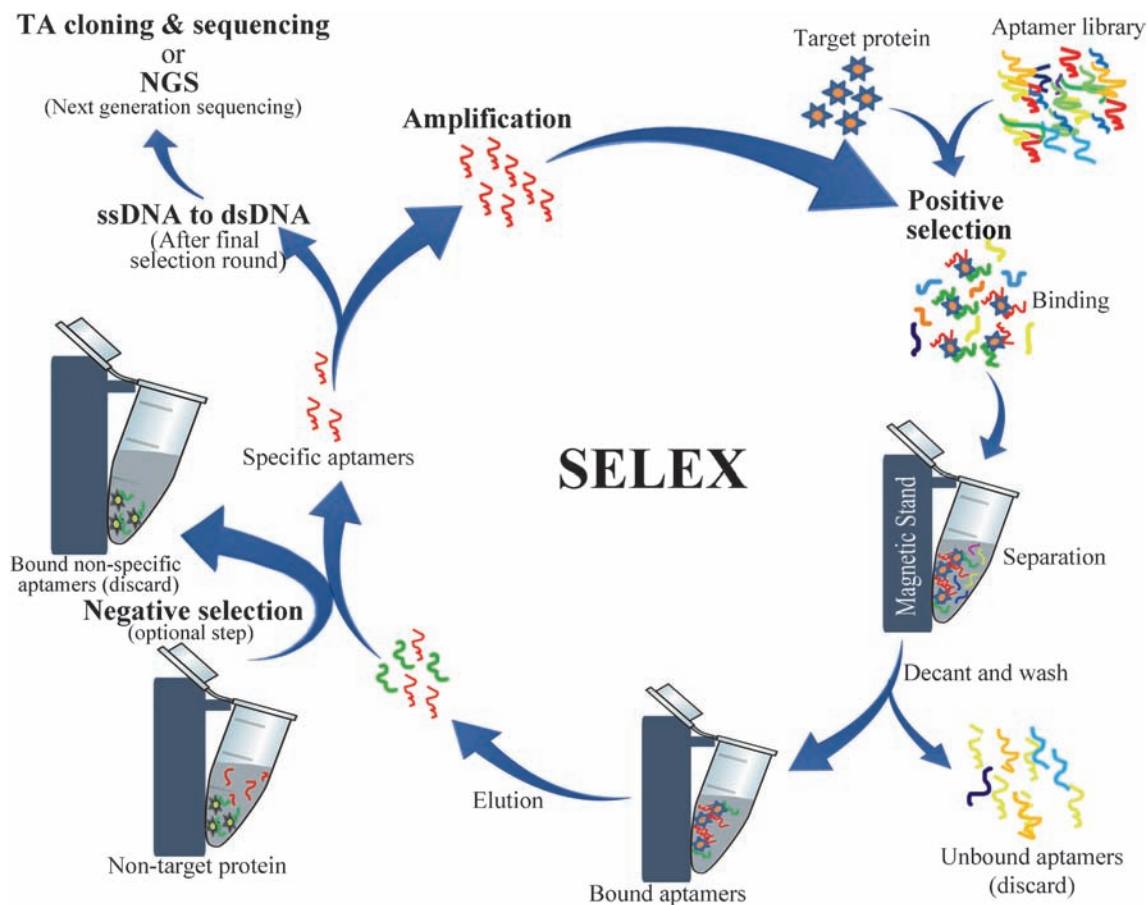


Figure 1. Diagrammatic representation of systematic evolution of ligands with exponential enrichment (SELEX).

aptamers to recognize targets and binding specificity, they are often compared to antibodies. However, unlike antibodies, aptamers can be selected under non-physiological conditions like extremely high or low temperature, or pHs. Aptamers can be readily chemically synthesized with minimal batch-to-batch variations. Moreover, they are easily selected, more stable to heat and withstand organic solvents, and show low immunogenicity. Compared to antibodies, the smaller size of aptamers makes them easier to be synthesized in large quantities, and can be easily introduced with a wide range of chemical modifications, which help them to retain their binding and inhibitory behavior after immobilization on a carrier material or after delivery into animals. They are $1/10^{\text{th}}$ the molecular weight of antibodies and yet provide complex folded tertiary structures with sufficient recognition surface areas to rival and even surpass antibodies. All these properties provide a potential market for aptamers in the field of biomedical applications.

Aptamers have been studied as a biomaterial for numerous applications like diagnostics and in the development of new drugs, drug delivery systems and imaging. Today at

least a dozen distinct aptamer products are being tested in several dozen trials. The first aptamer approved by Food and Drug Administration (FDA) for use in humans is Pfizer's Macugen that is administered locally to treat age related macular degeneration (AMD) by targeting vascular endothelial growth factor (VEGF) (Ruckman *et al.* 1998); however, monoclonal antibodies that are more effective clinically for this purpose have been developed (Fong and Lai 2013). The most advanced aptamer in cancer setting is AS1411, a 26-mer guanosine rich oligonucleotide against human tumor, formerly known as Agro100 (Teng *et al.* 2007). In the recent years, the aptamer technology field has evolved significantly, and is regarded as a more effective technology as the number of studies in the application of aptamers is rapidly increasing.

In this article we emphasize on applications of aptamer in the field of (a) diagnostics, (b) drug delivery and (c) imaging. This era is steering towards translational research where there is a need to move the research findings from bench-to-bedside, i.e., for clinical outcomes with commercial viability. Aptamers, because of their wide variety of

applications and the ability to undergo easy modifications are a good example to be exploited. Diagnostics, drug delivery and imaging are gaining attraction in the field of translational research because of their huge impact towards diagnosing and treating a disease. Hence, in this review article we have focused on the latest developments in the application of aptamers and how it has affected the above mentioned three fields, describing the advantages and the challenges ahead.

We begin with the role of aptamers in diagnostics where they have been a tradition in this application. As known, signal generated by a conformational change induced by the presence of a ligand is ideal for the creation of sensors for which aptamers are ideal candidates. The ease of conjugation and labelling of aptamers allows them to be combined with other advanced technologies like flow cytometry, microfluidic cell separation, endogenous nucleic acid analysis, nanoparticle based sensing and, thus, positioning them as a potential tool for diagnostics. We will cover the application of aptamers for detection of pathogenesis involving, bacteria, bacterial toxins, viruses, parasites and cancer. Then we will move towards the application of aptamers as drug delivery agents. Off-target delivery of drugs during therapeutic treatment of various diseases, especially in chemotherapy for cancer, is an important problem to be overcome. Here, we have discussed that how aptamers, due to their small size and high specificity, have been used for targeted drug delivery. They are either bound with drugs alone, or used in conjugation with nanoparticles, liposomes and antibodies for increased delivery of various therapeutic payloads. Finally, we shed light on the role of aptamers in imaging where they have been utilized in clinical and molecular imaging. We discussed how aptamers have been used in positron emission tomography (PET) scans, magnetic resonance imaging (MRI), CT, ultrasound and nuclear imaging. Also, we briefly discuss about the conjugation strategies of fluorescent probes with aptamers.

2. Aptamers in diagnostics

Key for the effective treatment of any disease is an early and accurate diagnosis. Early diagnosis is only possible with sensitive methods involving acute detecting agents such as aptamers. Aptamers have been used for the detection of bacterial, parasitic and viral mediated diseases along with cancer (figure 2). In the field of diagnostics, aptamers are applied for the detection of infective agents itself, the detection of the antigens/toxins (bacteria), or biomarkers (cancer), or a combination. Some existing diagnostic methods are in need of high-end machinery and skilled people, which increases the cost. Currently used methods in diagnosis may take long due to the isolation of infective agents and subsequent cultivation for detection. Also, in some cases

enrichment of causative agents is required for proper diagnosis because of its low titer. In order to address these points and simplify the process, aptamers can be used. In the following sections we discuss the use of aptamers as a diagnostic tool for detection of various diseases including cancer and other diseases caused by bacteria, parasites and viruses.

2.1 Bacteria

Increasing bacterial infections have triggered an unmet demand for the development of rapid and sensitive point of care diagnostic tests. In case of *Staphylococcus aureus*, infection-detection combined with culturing takes days while other methods are less cost effective. A new ultra-sensitive and non-polymerase chain reaction (PCR)-based method has been developed which measures the resonance light-scattering signal of aptamer-conjugated gold nanoparticles to detect a single cell of *S. aureus* cell within 1.5 h (Chang *et al.* 2013). A sandwich luminescent bioassay was developed for the detection of *Salmonella typhimurium* and *S. aureus* simultaneously using an aptasensor with magnetic nanoparticles (MNPs). In this bioassay two different aptamers were used, and both were immobilized on magnetic nanoparticles to capture the bacteria; furthermore, these two aptamers were modified with nanoparticles making them dual color labels, and allowed to bind the captured bacteria. The luminescent signal generated by this assay showed a linear correlation with *S. typhimurium* and *S. aureus* concentrations. Detection limits for this method was found to be 5 and 8 cfu/ mL for *S. typhimurium* and *S. aureus*, respectively. This assay is highly sensitive and selective for these two different types of bacteria (Duan *et al.* 2012). Zelada-Guillén and coworkers reported label free detection of *S. aureus* in real-time. A biosensor was fabricated with aptamers against *S. aureus* and single-walled carbon nanotubes (SWCNTs) acting as potentiometric transducers. The aptamers were attached to SWCNTs in two ways: (i) drop-casted pyrenil modified aptamers were non-covalently adsorbed to the external walls of the SWCNTs, and (ii) amine-modified aptamers were covalently bound to carboxylic groups at the ends of the SWCNTs which were introduced by oxidation. Both approaches indicated a linear correlation between potential, as a function of decade, and bacteria concentration. This biosensor was tested on pig skin as a substitute for human skin (Zelada-Guillén *et al.* 2012). Recently the dos Santos team used previously screened aptamers for *S. aureus* to evaluate its use in diagnostics by scintigraphy. The aptamers were directly radiolabeled with technetium-99m (^{99m}Tc) and injected in three groups of Swiss mice. The first two groups were infected with *S. aureus* and *C. albicans* individually, whereas the third group was induced for aseptic inflammation. Aptamers were

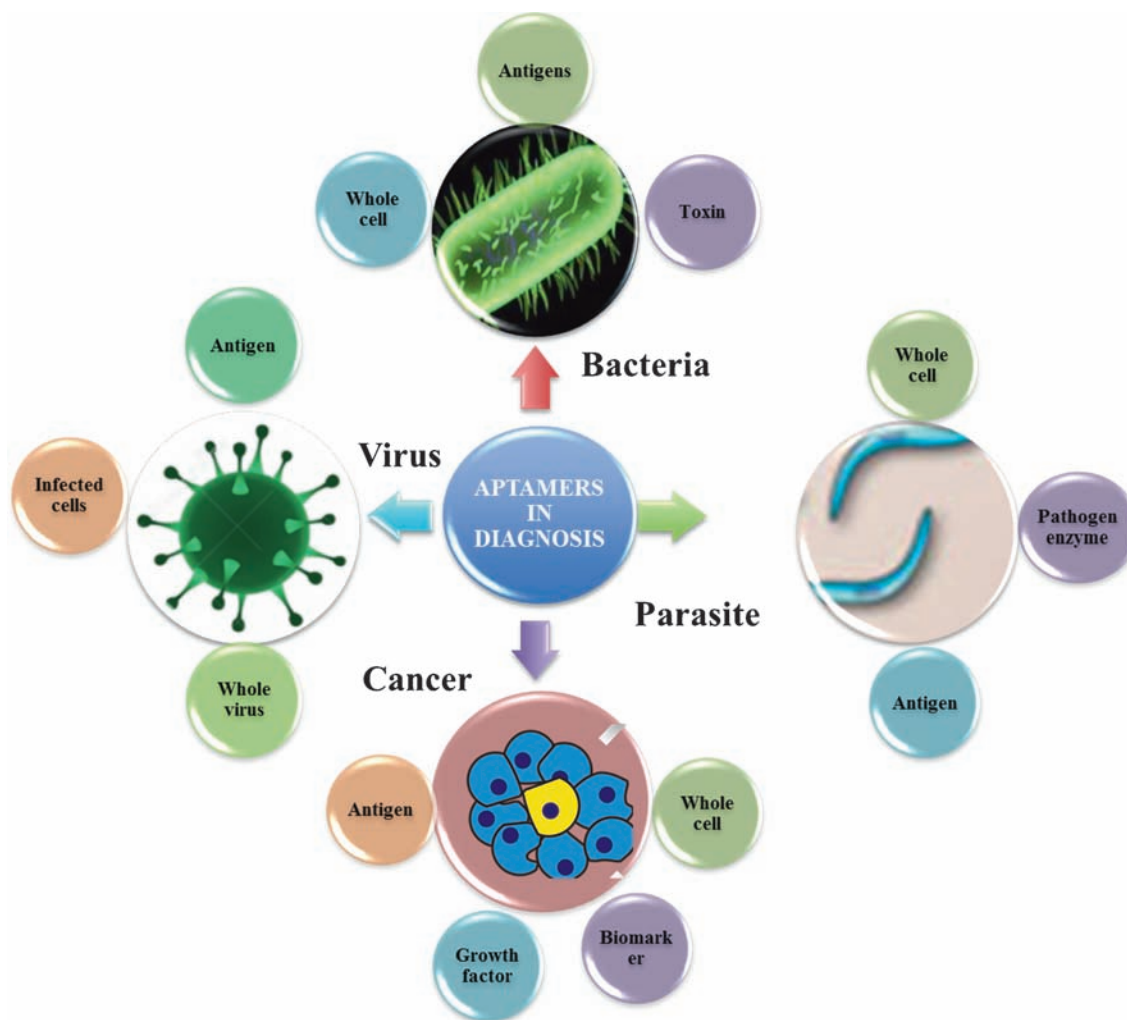


Figure 2. Application of aptamers in field of diagnosis.

able to detect infection foci caused by *S. aureus* and *C. albicans* with a target/non-target ratio of 4.0 ± 0.5 and 2.0 ± 0.4 , respectively. The ratio for aseptic inflammations was 1.2 ± 0.2 . These results show the potential of ^{99m}Tc labeled aptamers for the diagnosis of bacterial infections by scintigraphy (dos Santos *et al.* 2015). Another report states development of sensor based on graphene bound to interdigital gold electrodes (IDE) connected to a series of electrode piezoelectric quartz crystals (SPQC) for the specific detection of *S. aureus*. *S. aureus* aptamers were connected to graphene via π - π stacking of DNA bases. The aptamers fall from the graphene surface in the presence of *S. aureus* resulting in a change in the electric parameters and altering the oscillator frequency of the SPQC. This sensor can detect *S. aureus* rapidly and specifically within 60 min and can be used for clinical diagnosis (Lian *et al.* 2015).

Along with early detection of a disease, it is also important to find the causative agent of the disease which helps in deciding the correct treatment plan. Here, one bottleneck is the detection limit, which is sometimes below the level of the infection dose required by the pathogen to onset a disease or because the diagnostic method is not adept to detect low levels of pathogens. Salmonellosis is one of the major food borne diseases caused by *Salmonella enterica serovar typhimurium*. Enrichment of *S. typhimurium* cells in the sample is important, which can be carried out by concentrating *S. typhimurium* using specific aptamers, followed by direct detection using qPCR. Similarly, DNA and RNA aptamers were selected against several species of Salmonella with varying dissociation constants (table 1).

Escherichia coli (*E. coli*) O157:H7 is an enterohemorrhagic *E. coli* strain, which secretes exotoxins known as

Table 1. Aptamers selected against *Salmonella* species

Bacteria	Aptamer	Kd	Application	Reference
<i>S. typhimurium</i>	DNA	1.73±0.54 μ M		Dwivedi <i>et al.</i> 2013
<i>S. typhimurium</i>	RNA	20 nM	Aptamer-immobilized ELISA and aptamer-linked precipitation experiments	Han and Lee 2013
<i>S. typhimurium</i>	DNA	3.0 nmol		Moon <i>et al.</i> 2013
<i>S. typhimurium</i>	DNA	6.33±0.58 nM	Fluorescent bioassay	Duan <i>et al.</i> 2013
<i>S. paratyphi A</i>	DNA	47±3 nM	Biosensor based on SWNTs and DNAzyme-labeled aptamer detection probes	Yang <i>et al.</i> 2013

verotoxins (shiga like toxins). Currently available detection methods for this strain are time consuming as very low dose of infection causes the disease and pathogen numbers have to be increased to reach the detection level. A subtractive cell-SELEX method was employed by Lee *et al.* (2012) for selective and specific aptamer detection of *E. coli* O157:H7 related to food borne diseases. In this method the RNA aptamer library was allowed to bind with *E. coli* K12, and unbound aptamer library was further used for the selection against *E. coli* O157:H7. The specificity of the aptamers was tested using aptamer-immobilized ELISA, real-time PCR analysis, and an aptamer-linked precipitation test. These tests revealed the specificity of aptamers towards lipopolysaccharide O antigen on O157:H7. Therefore, authors suggest its application as a diagnostic ligand for the detection of *E. coli* O157:H7 (Lee *et al.* 2012). When cell SELEX is used, most of the time it is not possible to recognize the target for the selected aptamers simply because the targets for different aptamers on the cell surface could be the same or different. Thus many aptamers can be selected towards a cell with high affinity and specificity. Taking advantage of this effect by combining aptamers against same bacteria to increase the sensitivity, Kim *et al.* (2014) screened aptamers against *E. coli* using cell SELEX and compared the sensitivity of biosensors with a single aptamer and a cocktail of aptamers. A mixture of fluorescence-tagged aptamers showed an enhanced signal compared to the single aptamer. This was further evaluated by immobilizing the single aptamer and the mixture of aptamers on gold electrodes resulting in a detection limit of approximately 18 times more than that of the single aptamer. This strategy was also suggested for the detection of other bacteria, cancer cells, and tissues (Kim *et al.* 2014).

Burkholderia pseudomallei is a causative agent of melioidosis. The symptoms may include pain in chest, bones/joints, cough, skin infections, lung nodules and pneumonia which are noticed after high exposure to the bacteria. Lack of intensive antimicrobial treatment for a prolonged period may cause death. Hence, the need of an improved diagnostic reagent is vital for early detection. For this purpose, high

affinity aptamers were selected against recombinantly expressed *B. pseudomallei* specific proteins: a putative oxidoreductase BPSL2748 and secretion pathway proteins (BipD and BopE type III), which are considered to be potential targets for applications in diagnostic assays for *B. pseudomallei* (Gnanam *et al.* 2008).

Bacterial toxins are major components of virulence factors of pathogenic bacteria and thus detecting them is an imperative for the diagnosis of a bacterial disease. For example, toxin A (TOA) is one of the lethal enterotoxins produced by *Clostridium difficile* and causes antibiotic-associated diarrhea and pseudomembranous colitis in humans. Presently available laboratory tests for the diagnosis of *C. difficile* infection are by detecting bacteria itself and TOA or Toxin B (TOB is another potent cytotoxin of *C. difficile*) in stool samples. However, the microbiological culture method is insensitive and time consuming, the cytotoxic assay is costly and requires expertise. Additionally, an enzyme immunoassay can give false positive results due to cross-reacting antibodies, and polymerase chain reaction (PCR)-based tests requires pre-treatment of sample, where small amounts of free DNA can affect the results. Here, aptamer biosensors for the detection of TOA in clinical samples provide a better alternative. Luo *et al.* (2014) developed a biosensor based on the conformational change of an aptamer for quantitative analysis of TOA in stool samples. This biosensor has thiolated ssDNA as a capture probe immobilized on a Nafion–thionine–gold nanoparticle modified screen-printed electrode (SPE), which is hybridized with a horseradish peroxidase (HRP)-labeled aptamer for detection. In the presence of TOA, there is a conformational change in the aptamer and HRP labeled aptamer is released from the complex in turn lowering the response current. However, the biosensor was not effectively able to differentiate between TOA and TOB, which directs towards finding specific aptamers in the future (Luo *et al.* 2014).

Tuberculosis (TB) is a fatal disease spread worldwide. Available diagnostic tests for TB are time consuming, expensive, or have low sensitivity, whereas aptamers with their ability to be highly sensitive and specific can be apt as an

alternative diagnostic tool. Rotherham *et al.* (2012) selected aptamers against the 10-kDa culture filtrate protein (CFP-10) and the 6-kDa early secreted antigen target (ESAT-6), which are potent T-cell antigens recognized in over 70% of TB patients. In total six aptamers were selected against these targets, which had Kd values in the nanomolar (nM) range. The study suggested that aptamers are feasible for diagnosis of active TB in human biological samples obtained from patients with and without active TB (Rotherham *et al.* 2012). Similarly, Tang *et al.* (2014) selected aptamers for CFP-10 and ESAT-6, which were able to detect these proteins in serum. The authors suggest that these aptamers have a broad range of application, as they were able to detect CFP10 and ESAT6 in active pulmonary TB patients, extrapulmonary TB patients and healthy donors by using an enzyme-linked oligonucleotide assay (Tang *et al.* 2014). Zhu and coworkers developed a sandwich ELISA based on an anti-MPT64 antibody aptamer for the diagnosis of TB. They used a combination of immunization and genetic engineering to obtain an anti-MPT64 antibody, which was further used as a target for SELEX. This method can be used for the serological diagnosis of pulmonary TB, both in sputum smear positive- and negative-patients, with a lower detection limit of 2.5 mg/L (Zhu *et al.* 2012a). Surface plasmon resonance-based SELEX (SPR-SELEX) was used to select two RNA aptamers against EsxG protein with nM affinity. This protein is a secreted substrate of the ESX-3 secretion system. The ESX-3 secretion system is one of the complexes of mycobacteria, which help the bacteria to grow in low iron environments such as macrophages. Thus, specific aptamers towards the EsxG protein can be used as molecular probes for TB diagnosis (Ngubane *et al.* 2014).

2.2 Parasites

Trypanosoma cruzi causes Chagas disease, estimating about 6 million to 7 million people to be infected worldwide. Nearly 30% of chronically infected people with Chagas disease develop cardiac alterations and up to 10% develop digestive, neurological or mixed alterations which may require specific treatment. The parasite has to invade the host cells in order to cause the disease. This invasion is most likely supported by the cell surface macromolecules laminin, thrombospondin, heparan sulfate, and fibronectin. Therefore, these molecules were used as targets for selection of aptamers by Ulrich *et al.* (2002). The selected nuclease resistant RNA aptamers showed specific binding affinities in the 40-400 nM range and they were shown to inhibit *T. cruzi* invasion in LLC-MK2 monkey kidney cells *in vitro* (Ulrich *et al.* 2002). The currently available detection method of *T. cruzi* in humans is PCR based and is only sensitive at the acute stage of the disease when parasite numbers are high in the blood. Thus, to improve the PCR

based method Nagarkatti *et al.* (2012) utilized a whole cell SELEX method for the selection of serum stable RNA aptamers against living *T. cruzi* trypomastigotes. The high affinity aptamer Apt68 was found to be specific towards *T. cruzi* trypomastigotes and it captured living parasites when biotinylated and immobilized on solid phase. Apt68 was applied in collecting parasites from parasite-spiked blood at low concentrations to facilitate the PCR based assay for detection of *T. cruzi* (Nagarkatti *et al.* 2012). A combinatorial selection method was used to select RNA aptamers against *Trypanosoma* using living *Trypanosoma brucei* parasites, which causes African trypanosomiasis. The selected aptamers showed specificity towards a 42 kDa protein present in the flagellar pocket of the parasite (Homann and Göringer 1999). *T. brucei* escapes the host immune system by antigenic variations in surface proteins known as variant surface glycoproteins. Loriger *et al.* (2003) targeted these variant surface glycoproteins and selected a serum stable RNA aptamer with sub-nanomolar affinity. Bound with an antigenic side group the aptamer was able to direct antibodies towards the surface of the parasite *in vitro* (Loriger *et al.* 2003). Furthermore, Homann *et al.* (2006) selected a 2'-NH(2)-modified RNA aptamer towards living trypanosomes with an affinity at nanomolar range with a half-life of more than 30 hours in human serum (Homann *et al.* 2006).

Leishmaniasis caused by *Leishmania* is another important disease which severely affects millions of people worldwide. Martín *et al.* (2013) used *L. infantum* KMP-11, LiH2A and LiH3 proteins from *Leishmania* as targets. The two selected aptamers had different secondary structures, but were able to recognize the same peptides located on the side of the LiH2A. These aptamers were able to detect the parasite on ELONA platforms and were able to purify o LiH2A from complex mixtures (Martín *et al.* 2013). Kinetoplastid membrane protein-11 (KMP-11) is a widely distributed, conserved kinetoplastid and a major cell membrane component of kinetoplastid parasites like *Leishmania infantum*. DNA aptamers were selected against KMP-11 bound to colloidal gold during SELEX processes intended for diagnostic purpose. The use of colloidal gold added a higher mass to the protein in order to be purified by centrifugation during the partition phase of the SELEX process (Moreno *et al.* 2003).

The aptamer's secondary structure plays an important role in determining its specificity and high affinity towards the target. Aptamers selected against lactate dehydrogenase of *Plasmodium falciparum*, a causative agent of malaria, had a dissociation constant of 42 nM. The crystal structure of the protein-aptamer complex revealed two aptamers binding one tetramer of the protein, where both of them displayed a distinct hairpin loop structure involved in the binding with a *Plasmodium* lactate dehydrogenase loop that is absent in human lactate dehydrogenase. Furthermore, gold nanoparticles were

conjugated to this aptamer for a colorimetric application to detect malaria (Cheung *et al.* 2013).

2.3 Viruses

The usual methods for the detection of viruses are time consuming so a need for more efficient, specific and faster technique is evident. Aptamers are a good solution in all these aspects. For example, Parekh *et al.* (2010) used cell SELEX to select aptamers against *Vaccinia* virus, since it is closely related to smallpox virus *Variola*. The selected aptamer was highly specific towards virus-encoded hemagglutinin expressed on the surface of infected cells. Thus, aptamers can be generated against a given specific infectious agent like virus and can also be used for diagnostic purposes (Parekh *et al.* 2010). For early detection of hepatitis C virus (HCV) from patient's sera a protein chip-based assay was developed. Here, RNA aptamers specific for HCV core antigen were immobilized on a sol-gel based material to form a biosensor, and then patient sera along with Cy3-labeled human antibody was applied for the detection of HCV infection. The RNA aptamers showed stronger signals with the infected sera compared to negative controls (Lee *et al.* 2007). Furthermore, a functional ssDNA aptamer specific towards HCV envelope surface glycoprotein E2 was screened by cell surface-SELEX. The selected ZE2 aptamer showed highest specificity and affinity to E2-positive cells from patient sera when tested with the E2 antigen-aptamer assay, and assays for HCV RNA quantification or HCV antibody detection. Sandwich ELISA was used for virus capture assays using ZE2, indicating that ZE2 aptamer can also bind to HCV and thus can be used for capture and diagnosis of HCV (Chen *et al.* 2009a). After treatment against HCV it is important to monitor efficacies of potential anti-HCV drugs, for example by quantification of HCV infectious units. The conventional foci counting method is laborious and time-consuming, while other methods including quantitative reverse transcriptase-PCR (RT-PCR) and immunological measurement of core proteins are not ideal either. The viral RNA, which is measured by real-time RT-PCR cannot correlate with the infectivity of a HCV sample, moreover, immunological measurement of core proteins also fails since core proteins exist in both infectious and noninfectious HCV particles. A recently developed assay called 'Enzyme Linked Apto-Sorbent Assay (ELASA) for the quantification of infectious HCV particles uses aptamers against the HCV E2 envelope protein (Park *et al.* 2013). ELASA utilizes two different aptamers recognizing two different regions of HCV E2, where one capture aptamer is attached directly to the surface of a microtiter plate well and binds to E2 proteins on the virus surface, while the other detection aptamer is biotinylated. In the presences of HCV the biotinylated aptamer will react with streptavidin conjugated with HRP correlating proportionally to the amounts of HCV E2. This assay can also

monitor the activities of anti-HCV drugs. Liu and coworkers were the first to report a RNA aptamer, which could bind to a surface antigen (HBsAg) of Hepatitis B virus (HBV) for the diagnosis of the virus. They selected aptamer HBs-A22, which binds specifically to the HBsAg expressing hepatoma cell line HepG2.2.15, but did not bind to HBsAg-devoid HepG2 cells (Liu *et al.* 2010).

Avian influenza viruses (AIV) have caused devastating outbreaks worldwide with high mortality rates in both poultry and humans. To contain spreading of the disease, quick and sensitive diagnosis of H5N1 infections is essential. Currently available laboratory methods use conventional virus cultures followed by serological differentiation, RT-PCR and real-time RT-PCR, which are time consuming and demand technical expertise as described earlier. As an alternative an aptamer hydrogel based quartz crystal microbalance (QCM) biosensor was developed by Wang and Li (2013) for the detection of AIV H5N1. Specifically selected aptamer against AIV H5N1 surface protein was hybridized with ssDNA to form the cross linker in the polymer hydrogel. Furthermore, the hydrogel was immobilized on the gold surface of the QCM sensor, where the hydrogel is in a shrunken state in the absence of the H5N1 virus due to the crosslinking between the aptamer and ssDNA in the polymer network. Presence of H5N1 virus causes the dissolution of the linkage between the aptamer and ssDNA resulting in subsequent swelling of the hydrogel. The swelling of the hydrogel is observed in terms of a decreased frequency by the QCM sensor. The hydrogel QCM aptasensor has lowered the detection time twofold when compared with the anti-H5 antibody coated QCM immunosensor, which takes an hour (Wang and Li 2013). The influenza virus acquires new antigenicity with every outbreak, leading to the emergence of new virus strains, for which new diagnostic tools must be developed rapidly to detect novel influenza strains. Shiratori *et al.* (2014) developed DNA aptamers that bind to HA1 proteins of multiple influenza A virus subtypes. A novel aptamer-based assay for evaluating the binding properties of the selected aptamers was developed. A microtiter plate well coated with viral protein and flooded with HA1 specific biotinylated aptamers is followed by the addition of streptavidin-HRP. In this approach H5N1, H1N1, and H3N2 subtypes of influenza A virus were detected (Shiratori *et al.* 2014). Bruno and coworkers screened DNA aptamers against various virulent arboviruses like Chikungunya, Crimean-Congo hemorrhagic fever (CCHF), dengue, tick borne encephalitis and West Nile viruses using recombinant envelope proteins or synthetic peptides and/or whole inactivated viruses. They showed the diagnostic utility of some aptamers for arbovirus detection on the surface of magnetic micro-beads, lateral flow chromatographic assays and in fluorescent-based sandwich assay (Bruno *et al.* 2012). Park *et al.* (2011) screened an aptamers against Hemagglutinin (HA) which is present in the virus envelope. Antiviral efficacy of the selected RNA aptamer HAS15-5, binding to HA1, was

determined by a hemagglutination inhibition assay using egg allantoic fluids harboring the virus. This lead to blocking and inhibition of viral HA, and thus can further be developed as a diagnostic tool as well as a novel antiviral agent against type H5 avian influenza virus (Park *et al.* 2011).

Drug-resistant variants of HIV-1 are also known to be resistant to anti RT-RNA aptamers. Li *et al.* (2008) isolated two aptamers against Mutant 3 (M3) from the multidrug-resistant HIV-1 RT panel. These aptamers were able to bind a new RNA-binding epitope on the surface of HIV-1 RT. Using these aptamers, M3 and wild type HIV-1 RTs could be distinguished using an aptamer based microarray which can help in determining HIV-1 drug resistance. By probing protein conformation as a correlation to drug resistance an additional and useful measure has been introduced (Li *et al.* 2008).

Current diagnostic tools for the isolation of viruses during an outbreak rely on serotyping. Several aptamers were selected against the recombinant HA protein from swine Influenza A virus (IAV) H3 cluster IV for a more rapid approach. From the selected aptamers the HA68 aptamer was able to bind and detect H3N2v isolated from recent human cases. This aptamer can be used as a subtype-specific aptamer against Swine H3N2 IAVs for fast detection and virus sero-typing (Wongphatcharachai *et al.* 2013). Dengue fever is one of the prominent arthropod-borne viral diseases and it can be detected by ELISA-based and reverse transcriptase polymerase chain reaction methods. A biosensor for the detection of the Dengue virus genome was developed by Fletcher and co-workers which consists of three modules: (a) detection of target the nucleic acid, (b) signal transduction and (c) signal detection module. In the detection module the target-of-interest is detected by a stem/loop linker oligonucleotide. The loop portion is complementary to the target-of-interest, while one of the strands of the stem duplex (i.e. the trigger) is complementary to a portion of the aptamer within the transducer module. The signal transducer comprises an *EcoRI* inhibitory-aptamer complex. In the signal detection module an oligonucleotide like molecular break-light (MBL) is used. It consists of a stem/loop-forming unit with a 5' fluorophore and 3' quencher with an *EcoRI* recognition sequence in the stem duplex. When the dengue virus genome is present the linker interacts with the signal transducer module and liberates the restriction endonuclease *EcoRI* from aptamer-mediated inhibition. The active *EcoRI* molecule then cleaves multiple signaling molecules by dissociation of the fluorophore and the quencher resulting in a detectable signal. In this modular system the linker can be changed for the target-of-interest, e.g. for the detection of the four Dengue genome serotypes (DEN1-DEN4) without modifying the signal transduction module (Fletcher *et al.* 2010). Dengue virus enters the host cell through receptor-mediated endocytosis with the help of envelope (E) protein, cell surface receptors. The 395 residue E ectodomain has three domains, namely EDI, EDII and EDIII (residues 295–394), and the EDIII is possibly involved in host cell receptor

binding. A thioaptamers targeting this EDIII protein of dengue-2 virus was developed by Gandham *et al.* (2014). This aptamer DENTA-1 was able to bound DENV-2 EDIII which is adjacent to a known neutralizing antibody binding site with a kd of 154 nM. Thus, can be used as potential candidate for diagnosis as well as for antiviral drug discovery (Gandham *et al.* 2014).

DNA aptamers can also be used as a replacement of antibodies to characterize the subtle conformational changes in therapeutic proteins which might occur during pasteurization step of manufacturing. Early detection of these subtle conformational changes is important for safety, stability and efficacy of the therapeutic protein. It has been previously shown that heat treatment does not affect the protein activity; however, the resulting conformational changes cannot be detected by antibodies. To address this issue, Zichel *et al.* (2012) developed a panel of aptamers for the detection of conformational changes in thrombin after heat treatment. These aptamers were also used in both ELISA and label-free platform type assay to characterize different thrombin products (Zichel *et al.* 2012).

2.4 Cancer

Biomarkers for specific diseases are an important tool for the timely and effective management of pathological conditions, including determination of susceptibility, diagnosis, and monitoring efficacy of preventive or therapeutic strategies. Early detection of cancer is crucial in oncology. In this aspect, aptamers play a crucial role in screening against particular cancer cell biomarkers. When biomarkers and efficient tools like specific aptamers with high affinity are merged, an effective tool to detect their interaction using a sensor is needed, e.g. an aptasensor. Thus, numerous aptasensors were developed recently which facilitate the detection of various cancers at an early stage. In this section, we discuss about the aptamer mediated diagnostic tools for cancer detection, categorized according to the cancer subtype:

2.4.1 Breast cancer detection: Human mucin-1 (MUC1) and vascular endothelial growth factor-165 (VEGF-165) are important biomarkers for the diagnosis of breast cancer. Elevated levels of MUC1 in breast cancer is associated with its recurrence and increased lymph node metastases (Mukhopadhyay *et al.* 2011), while overexpression of VEGF is related to increased mortality in lymph node-negative and lymph node-positive breast cancer patients (Ghosh *et al.* 2008). Many aptasensors have been developed using these two biomarkers for the assessment of breast cancer. Previously discovered aptamers for MUC1 and VEGF165 were used by Zhao *et al.* (2012a) for the development of so called 'signal-on' electrochemical aptasensors. This aptasensor has a Fc-labeled cDNA-

fragment, immobilized on a gold electrode, with a complementary binding site for MUC1 and VEGF165 aptamers. In absence of MUC1 and VEGF165 biomarkers the aptamers are hybridized with the cDNA-fragment to form a long double strand that keeps Fc-molecules away from the electrode surface, resulting in no signal. In the presence of only one of these biomarkers, one of the aptamers will be detached from the cDNA-fragment, thus, reducing the distance between electrode surface and the Fc-molecule, resulting in a minor signal. However, in the presence of MUC1 and VEGF165, both, the aptamers bind to their respective targets, triggering the reduction of distance between the electrode and the Fc-molecule due to stem loop formation by the ss-cDNA-fragment, resulting in a stronger signal. The method of using two tumor markers over only one can be expanded to other types of tumors by changing the aptamers for their respective targets (Zhao *et al.* 2012a).

Another sensing platform developed by Zhao *et al.* (2012) uses aptamers and RNA polymerase-based amplification for the detection of Michigan cancer foundation-7 (MCF-7) breast cancer cells. In this assay, a biotinylated aptamer specific to MUC1 was immobilized on a microtiter plate well using streptavidin that captures tumor cells (i.e. recognition probe). To this, a complex oligonucleotide, composed of a MUC1 aptamer region and single-stranded T7 RNA polymerase promoter (ssPr), is added to form a sandwich structure. A template probe comprised of complimentary ssPr and a template sequence hybridizes with the ssPr region of the recognition probe forming a double-stranded T7 RNA polymerase promoter site (dsPr). Finally, T7 RNA polymerase can attach and generate multiple copies of RNA strands which in turn are detected by the addition of SYBR Green II dye. The cell concentration is determined by the fluorescence intensity with 500 cells/mL limit of detection (LOD) (Zhao *et al.* 2012b).

Furthermore, a signal-on fluorescence aptasensor was developed for label-free detection of MCF-7 breast cancer cells. This sensor uses a MUC1 specific aptamer which binds MCF-7 cells and is then removed by centrifugation. A signal probe complementary to anti-MUC1 aptamer is added to the supernatant along with terbium (III) (Tb^{3+}), as ssDNA subsequently enhances the emission of Tb^{3+} . However, the lack of MCF-7 cells results in hybridization of the signal probe with aptamer forming ds-DNA. The ds-DNA cannot increase the emission intensity of Tb^{3+} compared to ssDNA. This signal-on aptasensor has a LOD of 70 cells/mL (Cai *et al.* 2015).

A label-free and electrical detection of vascular VEGF using anti-VEGF aptamer-modified Si nanowire field-effect transistors (SiNW-FETs) was developed for breast cancer diagnosis. SiNW-FET biosensors were prepared by immobilization of anti-VEGF aptamers on the surface of modified single-crystalline SiNWs (n-type and p-type). Since the

VEGF molecules are positively charged under experimental conditions at pH 5.4 (the iso-electric point of VEGF is 8.5), they are effectively screened against RNA aptamers due to their negative charge. The resulting charge depletion in the nanowire channel and the decrease in conductance in case of the p-type Si nanowire biosensors were observed. However, this positive gate voltage by VEGF had the opposite effect on the device in case of n-type Si nanowire biosensors. The LOD of the VEGF biosensor was ~ 1 nM for n-type and 100 pM for p-type SiNW-FETs (Lee *et al.* 2009). In addition, for detection of breast cancer, a new quantitative immunohistochemistry (QIHC) microfluidic polydimethylsiloxane (PDMS) platform was developed with aptamer as a reporting probe specific to the Fc region of IgG antibodies. The microfluidic PDMS platform harbors breast cancer cells in paraffin block-slides to form a micro-reaction chamber. Primary antibodies specific to breast cancer marker proteins were loaded followed by the aptamer. The marker proteins were quantified by real time amplification of the aptamers using quantitative nucleic acid sequence based amplification, resulting in detection of cancer proportional to the concentration of different primary antibodies for marker proteins. This microfluidic platform was also used for aptamer-assisted IHC for breast cancer cell lines and showed good correlation with conventional IHC (Yeong Won *et al.* 2013).

Another electrochemical technique based on the aptamer-cell-aptamer sandwich architecture for the detection of MCF-7 human breast cancer cells was based on immobilized MUC1 aptamer on a gold electrode. In the presence of target cells and after incubation with the HRP-labeled MUC1 aptamer, the electrochemical response is catalysed by HRP, with the help of thionine, an electron mediator. The LOD for this technique was 100 cells/mL (Zhu *et al.* 2013a).

A selective electrochemical diagnosis of breast cancer was developed by Zhu and coworkers based on a hydrazine-Au nanoparticle-aptamer bioconjugate (Hyd-AuNP-Apt), where the aptamer is specific toward human epidermal growth factor receptor 2 (HER2), which is overexpressed in breast cancers. Here, hydrazine, capable of reducing silver ions to silver metal is attached to AuNPs and aptamer to form Hyd-AuNP-Apt. The sensor probe has a monoclonal anti-HER2 antibody immobilized on electrode, which is the nanocomposite surface with self-assembled DPB (2, 5-bis (2-thienyl)-1H-pyrrole-1-(p-benzoic acid)) and AuNPs attached to indium tin oxide coated glass. During the detection of HER2-overexpressing SK-BR-3 breast cancer cells, a sandwich is formed between the sensor probe, HER2 expressing cells and Hyd-AuNP-Apt, which is then treated with silver nitrate solution where silver ions are reduced by hydrazine and deposits onto the breast cancer cells. This deposited silver on breast cancer cells can be observed under a microscope and analysed using square wave stripping voltammetry to determine the

amount of HER2 cells with a LOD of 26 cells/mL in human serum samples (Zhu *et al.* 2013b).

2.4.2 Cervical cancer: In case of cervical cancer, most of the carcinogenesis is caused by human papillomavirus (HPV) infection, which may or may not lead to carcinogenesis and, hence, there is need to find biomarkers that differentiates the two. For this purpose, Graham and Zarbl screened a panel of aptamers against cell surface epitopes of human cervical cancer using a HeLa cell line for whole cell SELEX and isogenic HeLa cells for negative selection. The aptamers had a high affinity for epitopes specific to the cell surface of non-tumorigenic revertants derived from the human cervical cancer HeLa cell line, but did not bind to the HPV transformed cervical cancer cell line. This indicated that the aptamers are specific for epitopes that were lost during HPV induced transformation of the cervical cells and can be used to identify new biomarkers that are related to carcinogenesis and for diagnosis (Graham and Zarbl 2012).

2.4.3 Burkitt lymphoma detection: Burkitt lymphoma diagnosis is challenging due to its morphologic overlap with diffuse large B cell lymphoma and the person's level of experience performing diagnosis (Rane *et al.* 2014). Shi *et al.* (2010) were the first to report molecular engineered aptamers with signaling molecules for fluorescence imaging of specific tumor cells in a mouse, for which, a known aptamer, TD05, specific against Ramos cells – a human Burkitt's lymphoma cell line – was used. The specificity of the TD05 aptamer was tested via labelling with Cy5 dye. Cy5-TD05 aptamer along with control Cy5-Sgc8a and Cy5 dye alone were injected into mice harboring a Ramos tumor. The authors observed that Cy5-TD05 accumulated in the Ramos tumor while Cy5 dye and the control probe Cy5-Sgc8a were gradually cleared from mice (Shi *et al.* 2010). For visualized detection of Ramos tumor cells a chemiluminescence imaging array (CLIA) strategy along with a combination of tumor cell aptamer recognition and amplification of bio-bar-code nanotechnology with rolling circle amplification (RCA) for signal amplification was used. The aptamers TD05 and TE02 bind specifically to Ramos cells and were used in the CLIA. Biotinylated TE02 was attached to a streptavidin-coated 96-well microtiter plate as a capture aptamer, and thiol-TD05 was immobilized on the AuNPs along with circular DNA templates as bio-barcodes hybridized with primer for recognition of cells and to avoid cross-reactions. Upon addition of Ramos cells on the CLIA a sandwich assay format is formed to give a AuNP-TD05aptamer-cell-TE02 aptamer complex. When Klenow DNA polymerase and dNTPs are added to this complex a RCA is initiated on the AuNPs which produces a long ssDNA with tandem-repeat sequences, which bind to biotinylated detection probes. This biotinylated probe further binds to streptavidin-HRP to which luminol-H₂O₂-PIP is

added for 10 min and visual detection is performed by observing the chemiluminescence intensity. Quantitative analysis was done by using analysis software for reading the optical intensity, and 3 σ was used to detect LOD at 163 Ramos cells/mL. Even with the complexity of the method, the authors claim it to be an operationally robust, easy and cost-effective strategy (Bi *et al.* 2013).

2.4.4 Multiplexed cancer cell detection: Chen *et al.* (2009b) developed an aptamer-conjugated fluorescence resonance energy transfer (FRET)-nanoparticles (NPs) assay for the detection of simultaneous multiplexed monitoring of cancer cells. They used the sgc8 aptamer for CEM cells (human acute lymphoblastic leukemia cells) conjugated to a NP (FAM-R6G), the TD05 aptamer against Ramos cells (human Burkitt's lymphoma cells) coupled with a NP (FAM-R6G-ROX), and the T1 aptamer for Toledo cells (human diffuse large cell lymphoma) coupled to a NP (FAM). These conjugated aptamers when added to cancer cells were able to bind specifically to the respective cells (Chen *et al.* 2009b).

Viraka Nellore *et al.* (2015) reported for the first time an efficient capture and accurate identification of multiple types of circulating tumor cells (CTCs) from infected blood using aptamer-modified porous graphene oxide membranes. They used Cy2-modified S6 aptamer that binds specifically to HER2 biomarker for breast cancer CTCs, Cy5-modified A9 aptamer that binds to prostate-specific membrane antigen (PSMA), a biomarker for prostate cancer CTCs, and the Alexa Fluor 488 dye modified YJ-1 aptamer specific towards CEA biomarker for colon cancer CTCs. These three aptamers were conjugated to porous graphene oxide membranes, where graphene oxide is an efficient quencher and, thus, when dye conjugated aptamers are attached to graphene oxide, fluorescence from dye gets quenched by graphene oxide. In principle, when the dye-conjugated aptamer binds to targeted cancer cells the distance between the dye and graphene oxide increases resulting in fluorescence. This graphene oxide membrane conjugated with aptamers was able to capture multiple types of tumor cells selectively and simultaneously from infected blood and was also able to identify captured cancer cells via multicolor fluorescence imaging (Viraka Nellore *et al.* 2015).

2.4.5 Cancer detection using common biomarkers: Many cell surface cancer biomarkers are common in a variety of cancers, e.g., nucleolin, carcinoembryonic antigen (CEA), HER-2, epithelial cell adhesion molecule (EpCAM) etc. Taking advantage of this information many aptamers have been developed against these biomarkers, which serve as a diagnostic tool for the generic detection of these cancer types. We have discussed some of these recently developed aptamers and aptasensors below.

The cancer biomarker nucleolin is overexpressed on the plasma membrane of tumor cells as compared to normal cells. To detect cancer cells Feng *et al.* (2011) used aptamer AS1411, specific for nucleolin, to develop a label-free detection method based on a graphene electrochemical-aptasensor, which was utilized in the phase II clinical trial. Perylene tetracarboxylic acid (PTCA) was used to functionalize chemically converted graphene, and the NH₂-modified aptamer strand was linked to it by a carbodiimide-mediated wet-chemistry approach. These aptamer-PTCA nanocomposites were used as nanoscale anchorage substrates to capture tumor cells on the electrode surface. Electrochemical impedance spectroscopy (EIS) was then used to monitor change in electron transfer resistance. Addition of AS1411 complementary DNA (cDNA) hybridized with the aptamer and disrupted aptamer-tumor cell binding, regenerating the sensors. The LOD for HeLa cells was calculated to be 794 cells/mL at 3 σ (Feng *et al.* 2011).

Highly glycosylated CEA is another over-expressed protein in a large number of tumors. An electrochemical aptamer biosensor was designed by Shu and coworkers based on the signal amplification of AuNPs for the detection of a CEA. In this biosensor CEA is sandwiched between an Au electrode with thiol-terminated CEA aptamer-1 and the AuNPs with thiol-terminated CEA aptamer-2 and 6-ferrocenyl hexanethiol (Fe). The concentration of CEA was determined by the detection of Fe by differential pulse voltammetry with a LOD of 0.5 ng/mL (Shu *et al.* 2013). Recently, a new method was developed for radiolabelling aptamers with ^{99m}Tc where the two aptamers - Apt3 and Apt3-amine - selected against the well characterized human tumor associated antigen CEA were used. In binding assay the radiolabeled aptamer showed high specificity and affinity to T84 cells making the aptamer a promising agent for the identification of CEA present in tumor cells (Correa *et al.* 2014). One more method was developed for the detection of CEA by capillary electrophoresis (CE) which is based on the combination of graphene oxide (GO) and quantum dots labeled with aptamer (QD-aptamer). Addition of CEA to the QD-aptamer forms QD-aptamer-CEA complex. Since the CE peaks for the QD-aptamer are close to the QD-aptamer-CEA, to reduce this, GO is first added to the QD-aptamer, which quenches its fluorescence. The QD-aptamer-CEA complex could not bind to GO stably, hence, gets separated by CE, which in turn improves the fluorescence. The LOD for this method was approximately 5 pg/mL (Zhou *et al.* 2014).

A label-free and turn-on aptamer strategy for cancer cell detection was developed by Yin and colleagues. This strategy involves two probes; a signal probe (S-Probe) tailored with DNA template with dark silver nanoclusters (Ag-NCs) and a link sequence complementary to the arm segment of the recognition probe (R-Probe). The R-Probe includes a

central target-specific aptamer sequence at the 3'-end, a guanine-rich DNA sequence and an arm segment at the 5'-end forming a hairpin structure due to hybridization of the arm segment with its complementary part of the aptamer sequence in absence of target cells. In the presence of target cancer cells, aptamer binds to the protein receptors on the cancer cells enforcing conformational changes in R-Probe and causing the arm segment dissociation, followed by hybridization between the arm segment and the link sequence in the S-Probe. This brings S-Probe templated dark Ag-NCs close to the guanine-rich DNA sequences of the R-probe resulting in enhanced fluorescence. In particular, this strategy was used for the detection of CCRF-CEM cancer cells using sgc8c aptamer and, in general, it has the potential for being a simple, sensitive, universal and specific cancer cell detection method (Yin *et al.* 2013).

EpCAM is overexpressed in most adenocarcinomas, cancer stem cell and metastases CTCs (Bellone *et al.* 2009). Song and coworkers screened DNA aptamers that selectively bind human recombinant EpCAM protein and these aptamers were able to detect live human cancer cells derived from breast, colorectal, and gastric cancers expressing EpCAM. One of the selected SYL3 aptamer was optimized in length to improve its binding ability which was also able to detect target cancer cells from mixed cells in cell media (Song *et al.* 2013).

A single-step single aptamer-based surface-enhanced fluorescent optical sensor was developed for the detection of a biomarker of cancer angiogenesis i.e., VEGF165. The aptasensor uses a combination of an aptamer-target interaction and nanoplasmonic-fluorophore interaction for target recognition and for signal enhancement respectively. When clinical samples of serum and saliva were tested, the aptasensor results for VEGF165 were in agreement with the limits of the ELISA kit (Cho *et al.* 2013).

2.4.6 Circulating tumor cell detection: For the detection, diagnosis, and monitoring of CTCs a method based on aptamer-functionalized hydrogels and restriction endonucleases was proposed by Li and coworkers. CCRF-CEM cells (CL-119, human T lymphocytic leukemia cell line) were used as a model cell line for this assay method. Here, hydrogel functionalized with aptamer was able to capture target cancer cells with a density over 1000 cells/mm. The hydrogel coating was regenerated by treatment with restriction endonucleases due to sequence-specific hydrolysis of the aptamer. Moreover, 98% of the released cells were viable with release efficiency reaching 99% (Li *et al.* 2013).

2.4.7 Metastatic hepatocellular carcinoma detection: Hepatocellular carcinoma (HCC) has the tendency for metastasis and, hence, to arrest it, an early prediction of metastasis is necessary. To address this issue Wang *et al.* selected aptamers against HCC

cell line HCCLM9 with high metastatic potential and used MHCC97-L cell line with low metastatic potential as a control. When labeled with FITC, selected aptamer LY-1 was able to detect HCC cells in simulated peripheral blood environment. They also successfully used biotinylated LY-1 aptamer to capture CTCs using streptavidin coated magnetic beads in peripheral blood environment (Wang *et al.* 2013).

2.4.8 Colon cancer metastasis at lymph node: Colon cancer leads to metastasis most of the time. Utmost the tumor progression to metastasis is through entry of tumor cells into the circulatory system like lymphatic channels. Li *et al.* (2015a) developed DNA aptamers that specifically bind to SW620 colon cancer cells derived from metastatic site-lymph node. Selected DNA aptamer XL-33-1 was truncated to 45 nt and showed specificity towards SW620 with the binding target proposed to be a membrane protein on the cell surface. To specifically recognize lymph node tissue with colon cancer metastasis XL-33-1 aptamer was FAM-labeled which showed bright green fluorescence after incubating with the colon cancer tissue with metastasis in regional lymph nodes, and lymph node tissue with colon cancer metastasis (Li *et al.* 2015a).

2.4.9 Gastric carcinoma detection: Human gastric carcinoma is the most common malignant cancer due to lack of premature state detection methods. To overcome this, aptamers were selected against human gastric cancer cell line SGC7901 for high affinity and specificity using cell-SELEX. The specificity of selected S1a and S4a aptamers labeled with Cy5- fluorophore at the 5' end and the quencher group BHQ2 at 3' was verified by detecting clinical samples of human gastric tumor tissues and normal human gastric tissues, where tumor tissue gave bright red fluorescence with negligible signal from normal tissue. Furthermore, for the CTCs capture of SGC7901 cells a fluorescent-magnetic multifunctional nanospheres (FMNS) were prepared by mixing Nano- γ -Fe₂O₃ particles, QDs and ethanol-dehydrated nanospheres which were conjugated with streptavidin and subsequently, selected S4a biotinylated aptamers were functionalized with the streptavidin-FMNS. This assay showed a capture efficiency of 93% for SGC7901 cells compared to 7% of control cells. The aptamers also showed potential for *in vivo* testing as confirmed by the *in vivo* study in mice (Ding *et al.* 2015).

2.4.10 Lung cancer detection: Late diagnosis of lung cancer has become a major cause of cancer death since in the advanced stage the curative treatment is no longer possible (Detterbeck *et al.* 2013). Hence, there is an urgent need to develop tools for diagnosis of this cancer at an early stage. In this regard, Kunii *et al.* (2011) selected aptamers against small cell lung cancer adherent SBC3 cell line. The aptamers were evaluated and identified by fluorescent confocal

microscopy and flow cytometry. The selected aptamers preferentially bound to intercellular junctions of SBC3 cells with the ability to be used as a detection probe (Kunii *et al.* 2011).

In another study, Zhao and coworkers performed cell-SELEX to obtain four aptamer probes against a subtype of non-small cell lung cancer (NSCLC), i.e., adenocarcinoma. They used A549 cell line (NSCLC, adenocarcinoma) for the positive selection and HLAMP (NSCLC, large cell carcinoma) cell line for counter selection by cell-SELEX. When the aptamers were tested with clinical tissue sections representing various subtypes of lung cancer, three of them showed a significantly stronger fluorescence with adenocarcinoma tissue sections compared to other lung cancer sections (Zhao *et al.* 2009). Hence, these aptamers have paved a way for early detection and treatment of adenocarcinoma subtype of NSCLC.

2.4.11 Leukemia detection: Sefah *et al.* (2009) selected aptamers against acute myeloid leukemia (AML) cells using cell-SELEX. One of the selected aptamers, KH1C12, showed selectivity towards AML cell line (HL60) compared to control cell lines K562 and NB4, and was able to recognize the target cells within a complex mixture of normal bone marrow aspirates (Sefah *et al.* 2009).

2.4.12 Prostate cancer detection: Prostate cancer occurrence has been increasing recently. The early diagnosis of this cancer involves a prostate biopsy test, a microscopic examining process, but is not used in all cases as it may cause acute pain for patients. Other tests, digital rectal examination (DRE), blood test for prostate specific antigen (PSA) and trans rectal ultrasonography (TRUS), are prone to errors due to the indirect process. To overcome these limitations Min and coworkers designed a dual-aptamer probe, conjugating A10 RNA aptamer to DUP-1 peptide aptamer for detection by an electrochemical impedance spectroscopy (EIS) sensor. Biotin-A10 aptamer with 2'-F modified pyrimidines for the PSMA (+) cell line and a biotin-DUP-1 peptide aptamer for the PSMA (-) cell line were immobilized to streptavidin to form a dual-aptamer probe. These aptamers were conjugated to a Au electrode, to which, samples containing prostate cancer cells were added. Due to difficulty of charge transfer through physical blocking by bound cells to the aptamer probe on the electrode surface, impedance increases. The authors expect this sensitive electrochemical approach to be used for preliminary diagnosis of prostate cancer with an advantage of being a non-painful biopsy test (Min *et al.* 2010).

2.4.13 Glioblastoma detection: Glioblastoma is the most common and malignant brain tumor with short survival and fatal outcome even after treatment, and is associated with morphologic, genetic, and gene-expression heterogeneity (Olar and Aldape 2012). Two aptamers named GBM128 and GBM131 were selected against cultured human glioblastoma

cell line U118-MG using cell-SELEX. These aptamers were also able to differentiate between clinical glioma tissues and normal brain tissues. The binding affinity and selectivity of these two aptamers were also retained in biological environment and, thus, can be used for early detection of glioblastoma (Kang *et al.* 2012).

3. Aptamers for drug delivery

There are various methods for delivering drugs or biomolecules in cells for the therapeutic purposes. Historically, payloads have been delivered to the target cells using liposomes, microspheres, nanofibers, antibodies or peptides. However, these drug delivery carriers have their own limitations, most commonly non-specificity, or sometimes lower tissue penetration in case of antibodies and peptides. In this regard, aptamers have emerged as a new class of drug delivery tools that carry very high specificity, lacking immunogenicity, and have higher tissue penetration, which are imperative prerequisites for a good drug delivery vehicle. Also, less challenging process of industrial synthesis gives them an advantage over antibodies or peptides in terms of being more economical to synthesize. Aptamers are easy to synthesize, and it takes a short time, i.e., 3 months on average to obtain a novel aptamer through the process of SELEX (Sefah *et al.* 2010), with less batch to batch variability than existing tools.

There are some challenges in using aptamers for therapeutic purposes as well. Aptamers are susceptible to nuclease degradation, especially RNA aptamers, because the nucleases are present in serum. However, this problem can be overcome by chemical modifications in the sugar unit of the phospho-sugar backbone of aptamers either post-selectively or during the synthesis step of SELEX. Modifications include replacement of the 2'-OH group of ribose sugar with 2'-fluoro, 2'-O-methyl or locked nucleic acids (LNAs) (Behlke. 2008). Also, the phosphate backbone of nucleotides can be replaced with phosphorothioate (Green *et al.* 1995) or boranophosphate (Keefe and Cload 2008) to reduce the nuclease affinity. Additionally, binding PEG (polyethylene glycol) to the aptamers can increase the half-life of the aptamer in physiological environment (Tucker *et al.* 1999). In the last decade there has been a significant growth in the efforts towards using aptamers as a tool for drug delivery, using various payloads. Below, we have discussed some significant works where aptamers have been used with different conjugates, or alone, to increase their efficiency for targeted delivery (figure 3). A table (table 2) shows recent work from the last five years where aptamers have been used as a drug delivery tool.

3.1 siRNA and other oligonucleotide conjugated aptamers

The use of oligos such as siRNAs, miRNAs, shRNA as an effective tool to inhibit the expression of complementary RNA transcripts is being used as a new class of therapeutics for diseases including cancer and HIV. However, the successful intracellular delivery of the oligos to a specific cell type is one of the stumbling blocks towards their use as a therapeutic agent. Also, their anionic charge is an impediment to their entry into the cells, and hence, various transporters, viz., liposomes, cationic nanoparticles, antibodies and cholesterol conjugates are used for their trans-membrane movement.

Aptamers have been used with oligos recently for their specific delivery. The advantage of using aptamers with oligos is their relative ease of synthesis because they can be attached to the aptamer by extension of the RNA aptamer, thus, circumventing the need of conjugation or purification steps. Such constructs are called as aptamer-siRNA chimeras (AsiCs) as they represent the combination of two noncoding nucleic acids with different functions. The first si-RNA-aptamer conjugates were produced by Ellington *et al.* (Chu *et al.* 2006) and Sullenger *et al.* (McNamara *et al.* 2006) using two different approaches. Since then, many works have been done showing the role of aptamer-siRNA conjugates as therapeutic agents.

In one such example, an aptamer against CTLA4 surface antigen in both tumor associated T-cells and tumor cells helped in the successful internalization of siRNA against STAT3 gene resulting in an inhibition of tumor growth and metastasis (Herrmann *et al.* 2014). Also, AS1411 aptamer has been used along with PEGylated liposomes to target nucleolin protein for the delivery of siRNA targeting BRAF gene to prevent melanoma. This *in vitro* study was extended to *in vivo*, where A375 tumor xenograft mice showed inhibition of melanoma growth on using siRNA conjugated AS1411 aptamer (Li *et al.* 2014b).

Controlled delivery of a drug to target cells has been a challenge since long. To overcome this problem, a group used an anticancer drug, doxorubicin (Dox), bound nanocarriers capped with a programmable DNA hybrid. This DNA hybrid consisted of an aptamer against nucleolin and an antisense oligonucleotide for miR-21 both of which are overexpressed in cancer cells. Here, after the nanocarriers undergo aptamer mediated endocytosis, the overexpressed endogenous miR-21 unlocks the nanocarriers by competitive hybridization with the DNA hybrid leading to a sustained lethality of cancer cells with maximum therapeutic efficacy and minimal side effects (Zhang *et al.* 2014b).

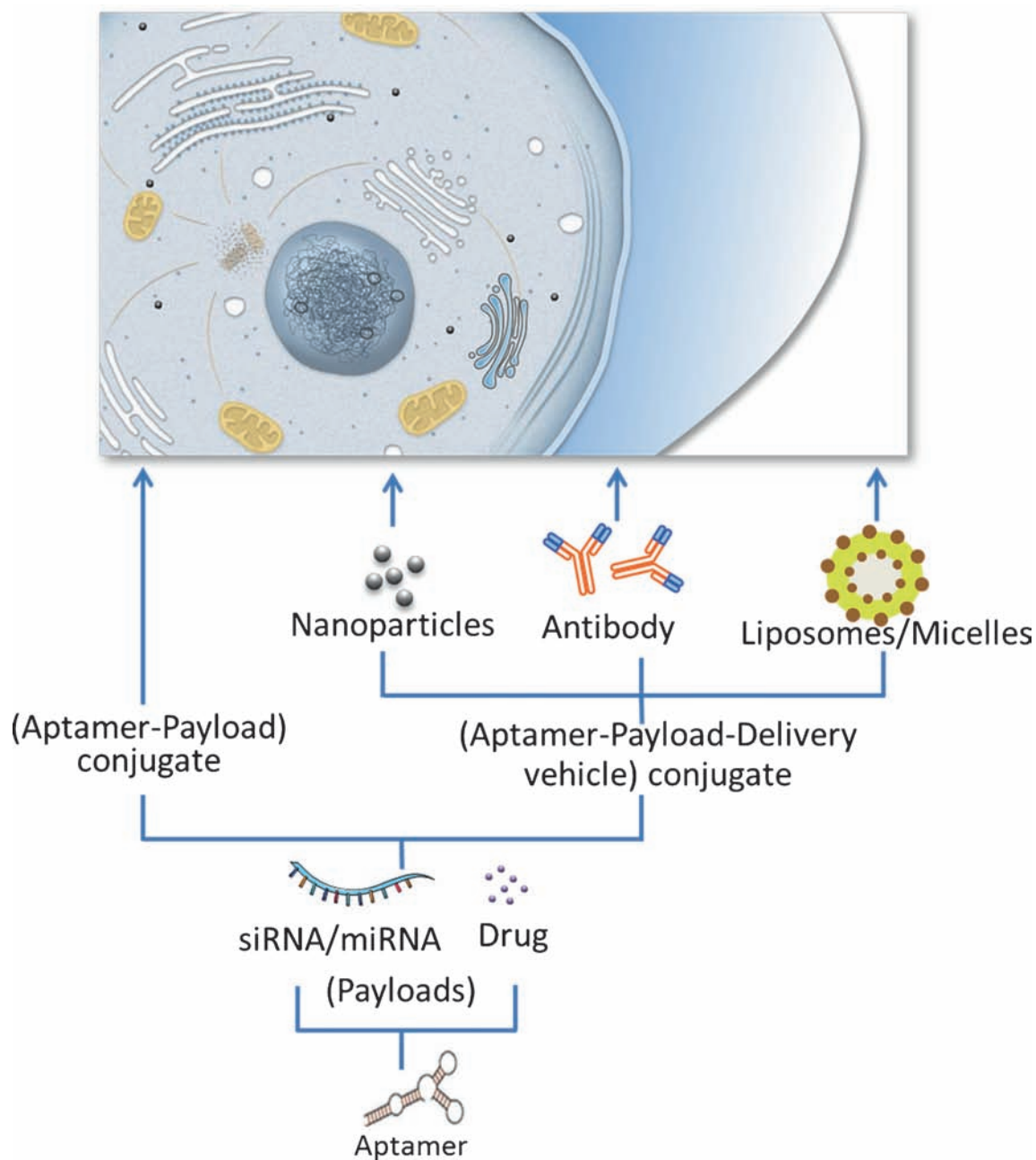


Figure 3. Aptamers are either used alone or in conjugation with nanoparticles, liposomes/micelles and antibodies to increase their specificity for targeted delivery of various payloads, viz. drugs, siRNAs or miRNAs.

In addition to cancer, siRNA-aptamer conjugates have also been used in countering AIDS. A chimera of DNA aptamer and siRNA has been used to efficiently knock down the expression of exogenously expressed HIV1 protease gene (HIV-PR) in CD4⁺ T cells (Zhu *et al.* 2012b). In another study, a group was successfully able to conjugate and deliver a siRNA conjugated to a RNA aptamer into the

HIV1 infected cells. They tagged an aptamer against gp120, a protein that is present on HIV-1 envelope and helps in the entry of HIV-1 into the CD4 cells, to the si-RNA. This gp120 aptamer-siRNA conjugate acted as a dual function chimera, where both the aptamer and the siRNA had potent anti-HIV activity that prevented HIV-1 infectivity (Zhou *et al.* 2011).

3.2 Nanoparticle conjugated aptamers

Use of aptamers along with nanoparticles has seen a substantial growth in the last decade. Aptamer functionalized nanoparticles have been used in recent years for better targeting and increased delivery of drugs for various diseases, especially cancer. As a result of multivalent binding, aptamer-nanoparticle conjugates have higher binding affinities to targeted cells as compared to free aptamers (up to 1,70,000 fold) (Zhou *et al.* 2015).

During a targeted delivery, the aptamer binds to a specific cell surface protein, thereby increasing the specificity of the therapy. Thus, the drug is delivered only to the specific set of cells bearing the aptamer specific target protein, increasing the potency of localized delivery. Therefore, in contrast to a systemic therapy, the local concentration of the drug can be increased while side-effects to the healthy cells can be reduced.

Drug release, in addition to specific targeting by a delivery vehicle, is an important aspect of drug therapeutics. To this aim, a group used aptamers along with nanotechnology in the field of cancer therapeutics. This group synthesized a hollow gold nanosphere (HAuNS) that was bound with a biomarker specific aptamer and the drug doxorubicin for internalization and cytotoxicity in tumor cells respectively. This conjugate (Apt-HAuNS-Dox) was stable at the biological pH 7.4 but at the low pH 5.0 in lysosomes, after internalization, aptamer releases Dox that selectively kills tumor cells (Zhao *et al.* 2013). On the same principle, aptamer-functionalized calcium carbonate (CaCO_3) nanostructure (Apt-CCN) resulted in pH dependent release of drug doxorubicin, i.e., at low pH on entering lysosomes of target cells (Zhou *et al.* 2015). In addition, photodynamic therapy (PDT) (Shiao *et al.* 2014), miRNA (Zhang *et al.* 2014b) and ATP (Mo *et al.* 2014) have also been used as an alternative for the controlled release of a drug to the cancer cells.

In another work, EpCAM targeted aptamers conjugated with Dox loaded PEG-PLGA polymericosomes were used against adenocarcinoma cell lines successfully. Since EpCAM is a cancer stem cell marker, this conjugate can improve the specificity to EpCAM positive cancer cells (Alibolandi *et al.* 2015). The overexpression of EpCAM has been capitalized for the targeting and delivery of many other drug-aptamer-nanoparticle conjugates for cancer therapeutics, e.g., retinoblastoma (Subramanian *et al.* 2012) and hepatocellular carcinoma (Pilapong *et al.* 2014). Also, a targeting supported by aptamers along with nanoparticles has been utilized for the nuclear uptake of an anticancer drug, specifically for the treatment of multidrug resistant cancers (Qiu *et al.* 2015).

In some studies, success has been observed also at the *in vivo* level. In one such study, Balb/c mice were injected with CD26 cells for colon cancer tumor progression. After a

sizeable tumor formation they were treated with epirubicin loaded 5TR1 aptamer functionalized super paramagnetic iron oxide nanoparticles (SPION), which was able to significantly reduce the tumor growth *in vivo* (Jalalian *et al.* 2013). Similarly, Yu *et al.* were also able to show selective drug delivery in LNCaP xenograft mouse model. They used a doxorubicin conjugated PSMA aptamer functionalized thermally cross-linked superparamagnetic iron oxide nanoparticles (TCL-SPIONs). The advantage of this Dox-Apt-hybrid-TCL-SPION conjugate was that super paramagnetic iron oxide nanoparticles facilitated the MRI of the target tumor tissue while Dox conjugated aptamer resulted in tumor cytotoxicity *in vivo* (Yu *et al.* 2011).

3.3 Micelles and liposomes conjugated aptamers

Though there has been significant development in the field of nanotechnology in the last decade, some challenges in their clinical application still persist. Firstly, nanoparticles do not have very satisfactory physico-chemical properties, mainly because of tedious preparation process, low drug loading, and poor stability. Secondly, although NPs reach the target cells by enhanced permeability and retention (EPR) effect, their non-specific uptake by the mononuclear phagocytic cells of liver, spleen and lungs may cause severe side effects.

Among all nanoparticle based drug delivery systems, liposomes are the most suitable to be used for clinical purpose. They are biocompatible, stable, easy to synthesize, have a low batch-to-batch variation, stable, high drug payload and could carry one or more therapeutic molecules. Liposomes have earlier been used in cancer cell targeting with chemotherapeutic payloads based on the principle of enhanced permeability and retention. However, this principle has a drawback that liposomes cannot differentiate between normal and diseased cells, and can be taken up by non-target cells causing serious side effects. Hence, aptamers have been used to functionalize liposomes for the targeted delivery of drugs to the diseased cells causing higher cytotoxicity and minimize the off-target side effects.

In one of the studies, doxorubicin loaded AS1411 aptamer (Ap) functionalized pluronic F127/cyclodextrin linked polymer composite micelles (CM) were used and studied both *in vitro* and *in vivo*. *In vitro*, CM-Ap showed higher cellular uptake and reduced proliferation due to the nucleolin-mediated endocytosis as compared to the control. In the *in vivo* study involving MCF-7 tumor bearing mice the CM-Ap showed extended circulation time in blood, improved antitumor activity, decreased cardiotoxicity and better accumulation in tumor. Thus, aptamer-conjugated composite micelles could be used as potential anticancer targeting vehicles (Li *et al.* 2015b).

Table 2. List of aptamer mediated drug delivery systems for therapeutic purposes

a. siRNA and other oligonucleotide conjugated aptamers				
Aptamer type	Aptamer target	Cargo	Vehicle	siRNA/miRNA target
RNA	gp120	siRNA	None	tat/rev
RNA	CTLA4	siRNA	None	STAT3
DNA	Nucleolin	siRNA	PEGylated Liposome	BRAF
DNA	Nucleolin	anti miR-21 and Doxorubicin	Mesoporous silica coated quantum dots (MSQD)	miR-21
DNA	CD4	siRNA	None	HIV1 protease
DNA	MUC1	let-7i miRNA	None	Cyclin D1, cyclin D2, Dicer1, PGRMC1
RNA	PSMA	miR-15a & miR-16-1	PAMAM-PEG	Bcl-2, Cyclin D1, Wnt3a
RNA	gp120	siRNA	None	tat/rev
b. Nanoparticle conjugated aptamers				
Aptamer type	Aptamer target	Cargo	Vehicle	-
RNA	CD30	Doxorubicin	Gold nanosphere (AuNS)	-
DNA	PTK7	Doxorubicin	Calcium carbonate nanostructure (CCN)	-
DNA	Nucleolin	Doxorubicin	UCNP-MOF nanocomposites	-
DNA	PTK7	Doxorubicin	Nanoparticle (Gold)/Nanorod (Gold-Silver) assembly	-
DNA	HER2	Doxorubicin	Mesoporous silica-carbon nanoparticles	-
RNA	EpCAM	Nutlin-3a	PLGA nanoparticle	-
DNA	EpCAM	Doxorubicin	CMC-MNPs (Carboxymethyl cellulose-modified magnetic nanoparticles)	-
RNA	EpCAM	Doxorubicin	PEG-PLGA nanopolymersomes	-
DNA	Nucleolin	Doxorubicin	Au NP	-
DNA	MUC1	SN38	Chitosan NP	-
DNA	PTK7	Doxorubicin	Gold coated Fe ₃ O ₄ nanoroses	-
RNA	EpCAM	Curcumin	PLGA-Lecithin-PEG NPs	-
DNA	Nucleolin	Doxorubicin	pPEGMA-PCL-pPEGMA	-
DNA	Nucleolin	Doxorubicin	Single walled carbon nanotubes	-
DNA	Mucin-1	Epirubicin	Super paramagnetic iron oxide nanoparticles (SPION)	-
RNA	PSMA	Doxorubicin	Super paramagnetic iron oxide nanoparticles (SPION)	-
c. Micelles/liposome conjugated aptamers				
Aptamer type	Aptamer target	Cargo	Vehicle	-
DNA	Nucleolin	Doxorubicin	Composite micelle (Pluronic F127 and beta-CD-PELA)	-
RNA	CD44	None	PEGylated liposome	-
DNA	Nucleolin	Doxorubicin	Liposome	-
RNA	PSMA	Doxorubicin	H40-PLA-PEG micelle	-
d. Drug only conjugated aptamers				
Aptamer type	Aptamer target	Cargo	Vehicle	-
DNA	Unknown	Doxorubicin	None	-
RNA	EGFR	Gemcitabine	None	-
DNA	HER2	Doxorubicin	None	-
DNA	Unknown	Doxorubicin	None	-
DNA	MUC1	Doxorubicin	None	-
RNA	EpCAM	Doxorubicin	None	-
e. Antibody conjugated aptamer				
Aptamer type	Aptamer target	Cargo	Vehicle	-
DNA	HER2	Doxorubicin	Anti-HER2 Ab	-

Table 2. (continued)

a. siRNA and other oligonucleotide conjugated aptamers				
Aptamer type	Study type	Model	Disease	Reference
RNA	<i>In vitro</i>	CEM, PBMC	HIV-1	Zhou <i>et al.</i> 2011
RNA	<i>In vitro/ In vivo</i>	CD4+, CD8+ T cells/ Mouse	Cancer	Herrmann <i>et al.</i> 2014
DNA	<i>In vitro/ In vivo</i>	A375 cells/ Mouse	Melanoma	Li <i>et al.</i> 2014b
DNA	<i>In vitro</i>	HeLa	Cancer	Zhang <i>et al.</i> 2014b
DNA	<i>In vitro</i>	CD4+ T cells	HIV-1	Zhu <i>et al.</i> 2012b
DNA	<i>In vitro</i>	OVCAR-3	Ovarian cancer	Liu <i>et al.</i> 2012a
RNA	<i>In vitro</i>	LNCaP	Prostate cancer	Wu <i>et al.</i> 2011
RNA	<i>In vivo</i>	Humanized mice Rag2 ^{-/-} γc ^{-/-}	HIV-1	Neff <i>et al.</i> 2011
b. Nanoparticle conjugated aptamers				
Aptamer type	Study type	Model	Disease	Reference
RNA	<i>In vitro</i>	HDLM2, KMH2, L-428, L-540, SUDHL-1, Kapas 299	Cancer	Zhao <i>et al.</i> 2013
DNA	<i>In vitro</i>	CEM	Cancer	Zhou <i>et al.</i> 2015
DNA	<i>In vitro</i>	MCF-7	Cancer	Deng <i>et al.</i> 2015
DNA	<i>In vitro</i>	CEM	Cancer	Qiu <i>et al.</i> 2015
DNA	<i>In vitro/ In vivo</i>	SK-BR-3 cells/ Mouse	Breast cancer	Wang <i>et al.</i> 2015
RNA	<i>In vitro</i>	MCF7, SKOVK3 & ZR751	Cancer	Das <i>et al.</i> 2015
DNA	<i>In vitro</i>	HepG2	Hepatocellular carcinoma	Pilapong <i>et al.</i> 2014
RNA	<i>In vitro</i>	MCF-7	Cancer	Alibolandi <i>et al.</i> 2015
DNA	<i>In vitro</i>	HeLa, MCF-7	Cancer	Shiao <i>et al.</i> 2014
DNA	<i>In vitro</i>	HT29	Colon cancer	Sayari <i>et al.</i> 2014
DNA	<i>In vitro</i>	CEM	Cancer	Li <i>et al.</i> 2014a
RNA	<i>In vitro</i>	HT29	Colon cancer	Li <i>et al.</i> 2014c
DNA	<i>In vitro</i>	MCF-7, PANC-1	Cancer	Lale <i>et al.</i> 2014
DNA	<i>In vitro/ In vivo</i>	PC3 cells/ Mouse	Cancer	Zhang <i>et al.</i> 2014a
DNA	<i>In vitro/ In vivo</i>	C26 cells/Mouse	Colon cancer	Jalalian <i>et al.</i> 2013
RNA	<i>In vitro/ In vivo</i>	LNCaP cells/ Mouse	Prostate cancer	Yu <i>et al.</i> 2011
c. Micelles/liposome conjugated aptamers				
Aptamer type	Study type	Model	Disease	Reference
DNA	<i>In vitro/ In vivo</i>	MCF-7 cells/ Mouse	Cancer	Li <i>et al.</i> 2015b
RNA	<i>In vitro</i>	A549, MDA-MB-231	Cancer	Alshaer <i>et al.</i> 2015
DNA	<i>In vitro/ In vivo</i>	MCF-7 cells/ Mouse	Cancer	Xing <i>et al.</i> 2013b
RNA	<i>In vitro/ In vivo</i>	CWR22Rv1 cells/ Mouse	Prostate cancer	Xu <i>et al.</i> 2013a
d. Drug only conjugated aptamers				
Aptamer type	Study type	Model	Disease	Reference
DNA	<i>In vitro</i>	HCT116	Cancer	Li <i>et al.</i> 2014d
RNA	<i>In vitro</i>	MiaPaCa-2	Pancreatic cancer	Ray <i>et al.</i> 2012
DNA	<i>In vitro</i>	SK-BR-3	Breast cancer	Liu <i>et al.</i> 2012b
DNA	<i>In vitro/ In vivo</i>	LH86 cells/ Mouse	Liver cancer	Meng <i>et al.</i> 2012
DNA	<i>In vitro</i>	A549	Cancer	Hu <i>et al.</i> 2012
RNA	<i>In vitro</i>	Y79, WERI-Rb1	Retinoblastoma	Subramanian <i>et al.</i> 2012
e. Antibody conjugated aptamer				
Aptamer type	Study type	Model	Disease	Reference
DNA	<i>In vitro</i>	SK-BR-3	Cancer	Kang and Hah 2014

In another work, CD44 specific RNA aptamer, Apt1, was bound to PEGylated liposomes using the thiol-maleimide click reaction. It was observed that the binding affinity of Apt1-liposome conjugate increased as compared to the free Apt1 to CD44, and also, Apt1-liposome had higher sensitivity and selectivity compared to the blank liposomes. In conclusion, anti-CD44 aptamer liposome conjugate (Apt1-lip) could be used as a potential system for specific drug delivery in cancer cells (Alshaer *et al.* 2015).

The majority of work in aptamer functionalized liposome drug delivery has been done *in vitro*. However, a recent work has confirmed the efficient role of liposomes in *in vivo* as well. In this study, AS1411 aptamer functionalized liposomes carrying doxorubicin payload increased cellular internalization and cytotoxicity to MCF-7 breast cancer cells as compared to non-functionalized liposomes. Also, in the xenograft MCF-7 breast tumors in athymic nude mice, the aptamer functionalized liposomes showed enhanced tumor penetration and cytotoxicity (Xing *et al.* 2013a). Similar work, where aptamer functionalized liposomes or micelles were used for specific drug delivery *in vivo* has been performed by others as well (Baek *et al.* 2014).

3.4 Aptamer–drug conjugates

There are several anthracycline based drugs, viz., doxorubicin, daunorubicin and epirubicin that are currently being used for the treatment of cancer. However, a side effect caused by the off-target binding of these drugs is a cause of major concern. To overcome this problem aptamer-drug conjugates have been developed recently using covalent or non-covalent interactions. Compared to the drug alone these aptamer-drug conjugates are easy to make and have significantly higher specificity and cytotoxicity enabling localized targeting of the drug. Dox causes cytotoxicity by its ability to intercalate between the GC or CG base pairs in the DNA. The same concept is used to bind Dox to the oligonucleotide aptamers, and then its delivery in the cells.

In spite of the advantages of the apt-drug conjugates, there are limitations. However, work is in progress to alleviate these limitations. First, the weakly stable noncovalent aptamer-drug conjugates can be stabilized by using covalent interaction, where, the drug is bound covalently to the aptamer through a functional linker moiety. Secondly, the short half-life of aptamer-drug conjugates *in vivo* may be enhanced by the addition of PEG to the aptamer. In addition, the PEGylation of the aptamer also enhances their stability and reduces their toxic accumulation in non-target tissues (Taghdisi *et al.* 2013; Tan *et al.* 2011). Lastly, aptamers are known to have a very poor drug payload capacity because of its very simple structure, which can be overcome to some extent by synthesizing polyvalent aptamers (Zhang *et al.* 2013).

Using aptamers alone along with cancer drugs directly may help in reducing off-target effects of anti-cancer drugs and increase their potency. For this purpose a conjugate of modified aptamer TLS11a-GC with an antitumor drug doxorubicin was prepared which displayed both high potency and target specificity to liver cancer cells. Here, the non-specific uptake of membrane permeable drug Dox was prevented by its conjugation with the target specific aptamer (Meng *et al.* 2012).

In a similar example, a conjugate of RNA aptamer against EpCAM with Doxorubicin was used to deliver the chemotherapeutic drug to the retinoblastoma cells thereby preventing their proliferation (Subramanian *et al.* 2012). EpCAM, used in this study, is overexpressed in most of the solid cancers and is a cancer stem cell (CSC) marker (Shigdar *et al.* 2011). In this study, EpCAM aptamer (EpDT3-apt) conjugated with doxorubicin was able to bind specifically and internalize the nucleus of retinoblastoma cell lines Y-71 and WERI-Rb1 but not to the control Muller glial cells. The presence of EpCAM in various cancers also allows the EpCAM aptamer-Dox conjugate to be used as a therapeutic agent against all the cancers overexpressing EpCAM, viz., breast cancer, pancreatic cancer, colorectal cancer and liver cancer.

CD44 belongs to proteoglycan family of transmembrane glycoproteins and a primary receptor for hyaluronic acid which in turn plays an important role in tumor growth and metastasis. The hyaluronic acid binding domain (HABD) is conserved among the CD44 splicing variants. Hence, Somasunderam *et al.* (2010) specifically selected thioaptamer binding to the CD44's HABD with 180–295 nM affinities. These thioaptamers specifically bound to CD44 positive human ovarian cancer cell lines when compared with the CD44 negative NIH3T3 cell line. The selected thioaptamers can further be developed as a targeting or imaging agent for the delivery of therapeutic payloads for cancer tissues (Somasunderam *et al.* 2010).

3.5 Aptamer–antibody conjugate

It is known that a dimer of antibodies that recognize adjacent and non-overlapping epitopes of the same target antigen have enhanced receptor binding (Kang and Hah 2014). This increase in affinity occurs due to the increase in k_{on} value and decrease in k_{off} value, which ultimately leads to the increase in dissociation constant (K_d) value for the dimer as compared to either of the two antibodies alone. In the past both antibody homodimer, and aptamer homodimer and heterodimer have been made (Kang and Hah 2014). However, in an attempt to increase the affinities of antibodies or aptamers to their target molecule, the antibody-aptamer pin-cer or heterodimer was made.

Since aptamers are advantageous over antibodies due to their high binding affinity and the ability to undergo numerous chemical modifications they can be used instead of antibodies to form aptamer-antibody dimers. Kang and Hah (2014) worked in this direction and called these dimers as antibody-aptamer pincers (AAP). AAP has a higher affinity with sustained or possibly higher specificity for its target molecule. The group also observed drug delivery along with higher cytotoxicity in cancer cells. To use AAP as a platform for therapeutic delivery the anti-HER2 aptamer and anti-HER2 monoclonal antibody conjugate was loaded with doxorubicin, and the resulting AAP-HER2-Dox conjugate showed an approximately 3- and 6- fold higher cytotoxicity than drug alone and antibody alone, respectively (Kang and Hah, 2014). Thus, the conjugation of the aptamer and the antibody can significantly improve the affinities of the resulting AAPs towards their target molecules and the drug loaded AAP may be used as a platform for targeted drug delivery against many malignancies.

4. Aptamers in imaging

Traditional imaging techniques primarily rely on morphological evidence, to example X-rays in CAT scans, radio-labeled compounds (e.g. fluorodeoxyglucose) in PET, MRI, and sonography. These techniques are used routinely in the clinic, for example in cancer detection strategies.

Alternatively, molecular imaging uses specific molecular probes to discriminate specific biological events (Massoud *et al.* 2003). The advances of molecular imaging are: (i) the ability to detect diseases at an earlier stage, (ii) measure and characterize its pathogenesis, and (iii) use them in *in vitro* and *in vivo* assays. By using specific molecular probes particular biological processes or targets can be visualized (Wang and Farokhzad 2014). Aptamers are a very interesting class of molecular probes due to their ability to spontaneously fold into 3D scaffolds with similar binding recognition as antibodies. The great advantages of aptamers over antibodies are due to stability and relative ease of chemical modification. Another advantage is that they can be produced synthetically. Their smaller size than antibodies gives them the added advantages of higher tissue penetration and increased systemic clearance. These advantages are beneficial for diagnostics as well (Cruz-Aguado and Penner 2008).

We will highlight a few examples of molecular probes for traditional clinical imaging purposes and then briefly discuss general conjugation strategies applicable for RNA and DNA aptamers intended for imaging.

4.1 MR imaging

Traditional gadolinium-based magnetic resonance (MR) contrast agents are mostly used to provide non-invasive

two- and three-dimensional images of living specimens. Recently the need for smart contrast agents that induce detectable MRI responses in the presence of specific targets are under investigation (Lauffer 1987; Caravan *et al.* 1999). Since the first responsive Ca^{2+} -chelator for gadolinium, that induced changes in the longitudinal relaxation time of water protons (referred to as T_1) by Meade *et al.* (Li *et al.* 2002), only a few other systems have been reported (Xu and Lu, 2011). SPIONs have advantages over traditional gadolinium-based MR contrast agents due to their lower toxicity, stronger enhancement of proton relaxation, and lower detection limit. However, they are still less widely used clinically than gadolinium compounds.

Both SPIONs and responsive chelator systems for gadolinium have been combined with aptamer technology. The A10 RNA aptamer (Farokhzad *et al.* 2004) was conjugated to a TLC-SPION using standard coupling chemistry (Wang *et al.* 2008). The complex was aimed to target PSMA in prostate cancer lymph node disease. In addition, the aptamer-TLC-SPION-MR contrast agent was loaded with doxorubicin for targeted delivery. An example of a responsive system by Xu and Lu (2011) was via a NHS coupled DNA aptamer against adenosine and to gadolinium-tetraazacyclododecane tetraacetic acid (DOTA-Gd). The MRI agent increased the T_1 responds on binding of the aptamer to its target, thus reducing the intensity of the signal upon binding. In a follow-up study the authors demonstrated an opposite effect and increase in MRI signal upon binding with uranyl cations via a 39E DNAzyme-based smart MRI contrast agent (Xu *et al.* 2013b).

4.2 CT imaging

The A10 aptamer, targeted against PSMA (Farokhzad *et al.* 2004), was conjugated to gold nanoparticles (Kim *et al.* 2010). A thiol-modified capture probe (5'-HS-CC-(A)₁₀-TCGTCGTCGTCGTCGTCGTCG-3') was covalently bound to 13 nm gold particles. The A10 aptamer was then hybridized to the capture probe. Due to the high atomic number, gold nanoparticles can act as CT imaging contrast agents (Kim *et al.* 2007; Popovtzer *et al.* 2008).

Kim *et al.* (2010) demonstrated that the A10 aptamer-nanoparticles bind specifically to PSMA-expressing prostate cancer cells and the feasibility of these particles to be used as molecular probes for the detection of PSMA-expressing cancer cells by CT imaging. In another recent example, Kuo *et al.* (2014) conjugated the AS1411 aptamer to europium-doped gadolinium oxide nanoparticles to target nucleolin, which is highly expressed in CL1-5 lung cancer cells. Due to their fluorescent nature, these particles can be used as a visual marker, but also act as contrast agents in MR and CT due their excellent T_1 contrast and strong CT signal.

4.3 Ultrasound imaging

Ultrasound is a commonly used clinical imaging technique. Advances to overcome the low resolution have resulted in the use of micro- and nano-bubbles to increase the contrast (Gramiak and Shah 1968; Ferrara *et al.* 2007). In addition, this technique has been utilized in sonodynamic therapy, ultrasound-mediated chemotherapy, ultrasound-mediated gene delivery and anti-vascular ultrasound therapy (Wood and Sehgal 2015). Specific biomarkers have been targeted via antibodies or small peptides (Chen *et al.* 2015; Huang *et al.* 2015), and recently aptamers (Wang *et al.* 2011b).

In order to overcome immunogenic side effects due to biotin-avidin conjugations an easier method for functionalize bubbles was developed by Wang *et al.* (2011b). Disulfide sgc8c aptamer was conjugated to PEG-maleimide in a lipid mixture of 1,2-Distearoyl-sn-glycero-3-phosphocholine DSPC (95%), PEG2000-1,2-Distearoyl-sn-glycero-3-phosphoethanolamine (DSPE; 2.5%), and the maleimide-functionalized PEG-DSPE. Nano-bubbles were generated by mechanical activation and the whole formulation took 2 hours. The sgc8c aptamer was linked to an imaging agent for the specific targeting of T-cell, human acute lymphoblastic leukemia (CCRF-CEM cells). Due to the strong binding of the aptamer to CEM cells and excellent hydrodynamic stability, the nanoparticles also exhibited superior behavior in local accumulation under flow conditions. Micro-bubbles can also act as a stimulus-responsive contrast agent. For the detection of thrombosis, or malignant blood clot formation, and *in vivo* clot activity Nakatsuka *et al.* (2012) developed aptamer-thrombin and polymer-DNA coated microbubbles where the aptamer can bind to thrombin and the DNA of the polymer-DNA coating. Microbubble shell components were prepared so that they would respond to thrombin. Poly(acrylic acid) (PAA) was coupled to the amine of DSPE via carbodiimide-mediated amidation. Subsequently two amine terminated DNA strands were attached. A DNA strand, the thrombin aptamer crosslinking strand (TACS) contained a portion complementary to the polymer-DNA to induce crosslinking, as well as two flanking 15-base DNA aptamer sequences. Both strands each possessed a half maximal inhibitory concentration (IC₅₀) in the order of 25 nM for thrombin. Nine of the fifteen aptamer bases are left unbound at the ends of the crosslinking strand so that the binding of thrombin resulted in the complete displacement of the TACS from the polymer-DNA strands. Therefore, upon binding to thrombin the aptamer was displaced from the complex to generate an ultrasound signal. No signal was observed in normal blood conditions.

4.4 Nuclear imaging

Radionuclide-based imaging techniques, such as SPECT and PET, have excellent tissue penetration capability. Especially, pre-targeting and immunotargeting of drug carriers in

combination of SPECT/CT have great potential (Lehtinen *et al.* 2012). Several radioisotopes are in use, such as ^{99m}Tc, ¹¹¹In, and ¹²⁵I for SPECT, ¹¹C, ¹⁸F, ⁷⁶Br, and ⁶⁴Cu for PET (Younes *et al.* 2002; Perkins and Missailidis. 2007; Lucignani 2006). A radiolabeled aptamer (NX21909-^{99m}Tc) against neutrophil elastase to identify inflammation was the first report of such an imaging probe (Charlton *et al.* 1997). Here the authors conjugated a peptide-N₃S₁-type Tc-binding via the amine linker of the NX21909 aptamer by NHS-ester reaction in dry DMSO/5% triethylamine. Compared to IgG probes the target-to-background ratio was increased 4-fold due to more rapid clearance of unbound aptamers.

Also, in the case of a radiolabeled aptamer against the extracellular matrix protein tenascin-C the rapid clearance from the blood-stream gave a 50:1 tumor to blood ratio. The TTA1 aptamer was developed in a SELEX against tumor cells and purified tenascin-C, after initial discovery of an aptamer to U251 glioblastoma cells (Daniels *et al.* 2003; Hicke *et al.* 2001). TTA1 aptamer was bioconjugated with technetium chelator MAG₂-uccinimidyl ester (Hilger *et al.* 1999) through the 5' amine in dimethylformamide (Winnard *et al.* 2008). MAG₂ was either labeled with ^{99m}Tc, ¹¹¹In or ³²P.

Other examples are ^{99m}Tc-labeled aptamer against MUC1 protein (a tumor biomarker) (Da Pieve *et al.* 2009), the AS1411 aptamer targeted against nucleolin (a cobalt-ferrite nanoparticle) labeled with ⁶⁷Ga (Hwang *et al.* 2010), and the aptamer HYNIC against tyrosine kinase-7 labeled with ^{99m}Tc to image acute lymphoblastic leukemia CCRF-CEM cells (Calzada *et al.* 2014).

4.5 Fluorescence imaging

Fluorophores can be labeled to the 5'- or 3'-prime end or in the middle of aptamers by use of phosphoramidite chemistry. Fluorescence of this nature has been widely applied in cancer imaging (Wu *et al.* 2015). Fluorescently tagged RNA aptamer has been applied in the detection of amyloid plaques in both *ex vivo* human Alzheimer's disease brain tissue and *in vivo* APP/PS1 transgenic mice. Imaging probes were introduced in the anti-Aβ RNA aptamer, β55, by transcription of the DNA template with T7 RNA polymerase by either using biotin-labeled uracil, for *ex vivo* studies, or fluorescein-labeled uracil directly.

Alternatively, displacement of a fluorescently labeled oligo from the aptamer by the target can be detected by fluorescence polarization (FP/FA) (Cruz-Aguado and Penner 2008). Here, a small ssDNA aptamer of 36 bases bound specifically to the small molecule ochratoxin A and levels as low as 5 nM could be detected with the FP displacement assay. Fluorescence polarization or anisotropy analysis has also been applied to map aptamer-protein interactions. Zhang *et al.* (2011) studied human α-thrombin and aptamer TA29 to identify 3 thymines and the 3'-end of the aptamer as the closest contact groups.

4.6 Labelling, conjugation and ligation

Due to their synthetic nature, chemical modification of aptamers is relatively easy. For therapeutic purposes a durable performance of aptamers *in vivo* and prolonged half-lives are required (Wang et al. 2011a, b). For imaging purposes, most aptamers are conjugated or hybridized to DNA/RNA with imaging moieties. Typically, the architecture of the bioconjugate has to be adjusted to the application for which it is developed (Canalle *et al.* 2010).

In a recent review by Paredes *et al.* (2011) strategies are discussed in great detail for RNA labelling, conjugation and ligation. Summarizing, imaging labels or the introduction of moieties for later labelling can be introduced via internal handles. In addition, synthetic chemistry, enzymatic incorporation and labelling or conjugation chemistry can be applied to modify RNA. Many strategies presented can be applied to DNA as well. Incorporation of labels via solid-phase RNA synthesis has been applied for incorporation of ³²P (Hilger *et al.* 1999; Paredes *et al.* 2011; Winnard *et al.* 2008) and by use of phosphoramidites for the synthesis of RNA with modifications at the 5'-end (Paredes *et al.* 2011). In post-synthetic labelling and conjugation, four general chemical strategies based on reactive species can be identified, viz. periodate chemistry (Paredes *et al.* 2011), amine chemistry, thiol chemistry, and click chemistry (Canalle *et al.* 2010; Paredes *et al.* 2011). In enzymatic incorporation of labels and reactive tags T4 polynucleotide kinase, terminal transferase, DNA polymerase I, T7 RNA polymerase, Poly (A) polymerase, and T4 RNA ligase have been used (Paredes *et al.* 2011). In chemical ligations native phosphate linkage by cyanogen bromide or a water-soluble carbodiimide and the subsequent reaction with a hydroxyl group has been utilized (Paredes *et al.* 2011). DNA splits and peptides have been used via these methods. Both protein and nucleic acid enzymes can be used to ligate two strands of RNA, or DNA. Most commonly used is T4 DNA ligase. Alternatively, T4 RNA ligase 1, T4 RNA ligase 2, and deoxyribozymes (DNAzyme) have been used for ligation and fluorescent labelling of RNA (Paredes *et al.* 2011).

5. Conclusion

Various aptamers have been selected to detect a wide range of targets; however, a significant number of them have not been used for diagnostic applications. These aptamers need further development to be used in biosensors to increase the efficiency and their ability to detect targets in clinical samples. Mostly the problem lies in clinical sample preparation, which is tedious, time consuming, costly and needs expertise. So aptamers must be tuned to detect the antigen at low concentration and in presence of serum impurities. Biosensors involving aptamers should have high range of sensitivity concerning the signal amplification. Some aptamers need to be more specific towards their target, mostly in case of cancer detection *in vivo*,

where they have shown nonspecific signals when tested in mice. Aptamers have already proven to be a better candidate for target binding than its other counterparts and, hence, will maintain its position in diagnosis. To do so, the developed biosensors or diagnostic tests involving aptamers should be tested with human samples and should be taken from research labs to diagnostic labs. In case of drug delivery, aptamers have emerged as a new set of biomolecules that are efficient in highly specific delivery of a payload to the target cells both *in vitro* and *in vivo*. Their sturdiness and ability to undergo a range of chemical modifications gives an advantage of binding aptamers with various payloads. Also, aptamer binding and delivery to the cells can be increased by conjugation with nanoparticles, liposomes and even antibodies. Until now, an overwhelming majority of the work for targeted delivery using aptamers has been done in cancer with a very limited number of attempts on other diseases like HIV, AMD and few others. This leaves a wide possibility of using aptamers for targeted delivery of drugs in a myriad range of diseases as well. Also, a push has to be given towards taking the studies from *in vitro* to *in vivo* and clinical level, as relatively large amount of work, at present, is limited to the targeted delivery of aptamers at *in vitro* level only. Coming towards imaging applications, aptamers due to their small size, specific binding, and stability have great potential for clinical and molecular imaging. Their stability and self-assembly after heating makes them superior over protein-based binding molecules, such as antibodies (Daugherty and Mersny 2006) or even affibodies (Feldwisch 2010) in terms of labelling and conjugation strategies.

We expect that in the near future more combinations of diagnostics, drug-delivery and imaging aptamer based tools to be combined into integrated methods to detect, visualize and treat (infectious) diseases *in vivo* at the same time.

Acknowledgement

We would like to acknowledge contribution of Lakshmi Narayanan Gopalan for preparation of a figure in the article. The work was supported by the Indo-Finland research grant, funded by Department of Biotechnology (Government of India) and Academy of Finland (Government of Finland). None of the authors have any competing interests.

References

- Alibolandi M, Ramezani M, Sadeghi F, Abnous K and Hadizadeh F 2015 Epithelial cell adhesion molecule aptamer conjugated PEG-PLGA nanopolymerosomes for targeted delivery of doxorubicin to human breast adenocarcinoma cell line *in vitro*. *Int. J. Pharm.* **479** 241–251

- Alshaer W, Hillaireau H, Vergnaud J, Ismail S and Fattal E 2015 Functionalizing liposomes with anti-CD44 aptamer for selective targeting of cancer cells. *Bioconjug. Chem.* **26** 1307–1313
- Baek SE, Lee KH, Park YS, Oh D-K, Oh S, Kim K-S, et al. 2014 RNA aptamer-conjugated liposome as an efficient anticancer drug delivery vehicle targeting cancer cells in vivo. *J. Control. Release* **196** 234–242
- Behlke MA 2008 Chemical modification of siRNAs for in vivo use. *Oligonucleotides* **18** 305–319
- Bellone S, Siegel ER, Cocco E, Cargnelutti M, Silasi D-A, Azodi M, et al. 2009 Overexpression of epithelial cell adhesion molecule in primary, metastatic, and recurrent/chemotherapy-resistant epithelial ovarian cancer: implications for epithelial cell adhesion molecule-specific immunotherapy. *Int. J. Gynecol. Cancer* **19** 860–866
- Bi S, Ji B, Zhang Z and Zhang S 2013 A chemiluminescence imaging array for the detection of cancer cells by dual-aptamer recognition and bio-bar-code nanoprobe-based rolling circle amplification. *Chem. Commun. (Camb.)* **49** 3452–3454
- Bruno JG, Carrillo MP, Richarte AM, Phillips T, Andrews C and Lee JS 2012 Development, screening, and analysis of DNA aptamer libraries potentially useful for diagnosis and passive immunity of arboviruses. *BMC Res. Notes* **5** 633
- Cai S, Li G, Zhang X, Xia Y, Chen M, Wu D, et al. 2015 A signal-on fluorescent aptasensor based on single-stranded DNA-sensitized luminescence of terbium (III) for label-free detection of breast cancer cells. *Talanta* **138** 225–230
- Calzada V, Fernández M, González J, Moreno M, Chabalgoity A, Cerecetto H, Cabral P and Quinn T 2014 Aptamer-HYNIC-^{99m}Tc: a molecular imaging agent of PTK7. *Nucl. Med. Biol.* **41** 618
- Canalle LA, Löwik DWPM and van Hest JCM 2010 Polypeptide-polymer bioconjugates. *Chem. Soc. Rev.* **39** 329–353
- Caravan P, Ellison JJ, McMurry TJ and Lauffer RB 1999 Gadolinium(III) chelates as MRI contrast agents: structure, dynamics, and applications. *Chem. Rev.* **99** 2293–2352
- Chang Y-C, Yang C-Y, Sun R-L, Cheng Y-F, Kao W-C and Yang P-C 2013 Rapid single cell detection of *Staphylococcus aureus* by aptamer-conjugated gold nanoparticles. *Sci. Rep.* **3** 1863
- Charlton J, Sennello J and Smith D 1997 In vivo imaging of inflammation using an aptamer inhibitor of human neutrophil elastase. *Chem. Biol.* **4** 809–816
- Chen F, Hu Y, Li D, Chen H, Zhang XL 2009a CS-SELEX generates high-affinity ssDNA aptamers as molecular probes for hepatitis C virus envelope glycoprotein E2. *PLoS One* **4**
- Chen X, Estevez M-C, Zhu Z, Huang Y-F, Chen Y, Wang L, et al. 2009b Using aptamer-conjugated fluorescence resonance energy transfer nanoparticles for multiplexed cancer cell monitoring. **81** 7009–7014
- Chen Q, Luo L and White HS 2015 Electrochemical generation of a hydrogen bubble at a recessed platinum nanopore electrode. *Langmuir* **31** 4573–4581
- Cheung Y-W, Kwok J, Law AWL, Watt RM, Kotaka M and Tanner JA 2013 Structural basis for discriminatory recognition of *Plasmodium lactate* dehydrogenase by a DNA aptamer. *Proc. Natl. Acad. Sci. U. S. A.* **110** 15967–15972
- Cho H, Yeh E, Sinha R, Laurence TA, Bearinger JP, Lee LP 2013 Single-step nanoplasmonic VEGF165 aptasensor for early cancer diagnosis. **6** 7607–7614
- Chu TC, Twu KY, Ellington AD and Levy M 2006 Aptamer mediated siRNA delivery. *Nucleic Acids Res.* **34** 1–6
- Correa CR, De Barros ALB, Ferreira CDA, De Goes AM, Cardoso VN and De Andrade ASR 2014 Aptamers directly radiolabeled with technetium-99m as a potential agent capable of identifying carcinoembryonic antigen (CEA) in tumor cells T84. *Bioorg. Med. Chem. Lett.* **24** 1998–2001
- Cruz-Aguado JA and Penner G 2008 Fluorescence polarization based displacement assay for the determination of small molecules with aptamers. *Anal. Chem.* **80** 8853–8855
- Da Pieve C, Perkins AC and Missailidis S 2009 Anti-MUC1 aptamers: radiolabelling with ^{99m}Tc and biodistribution in MCF-7 tumour-bearing mice. *Nucl. Med. Biol.* **36** 703–710
- Daniels DA, Chen H, Hicke BJ, Swiderek KM and Gold L 2003 A tenascin-C aptamer identified by tumor cell SELEX: systematic evolution of ligands by exponential enrichment. *Proc. Natl. Acad. Sci. USA* **100** 15416–15421
- Das M, Duan W and Sahoo SK 2015 Multifunctional nanoparticle-EpCAM aptamer bioconjugates: a paradigm for targeted drug delivery and imaging in cancer therapy. *Nanomed. Nanotechnol. Biol. Med.* **11** 379–389
- Daugherty AL, Mrsny RJ 2006 Formulation and delivery issues for monoclonal antibody therapeutics. *Adv. Drug. Del. Rev.* **58**(5–6) 686–706
- Deng K, Hou Z, Li X, Li C, Zhang Y, Deng X, et al. 2015 Aptamer-mediated up-conversion core/MOF shell nanocomposites for targeted drug delivery and cell imaging. *Sci. Rep.* **5** 7851
- Detterbeck FC, Mazzone PJ, Naidich DP, Bach PB 2013 Screening for lung cancer: diagnosis and management of lung cancer, 3rd ed: American college of chest physicians evidence-based clinical practice guidelines. *Chest* **143**
- Ding F, Guo S, Xie M, Luo W, Yuan C, Huang W, et al. 2015 Diagnostic applications of gastric carcinoma cell aptamers in vitro and in vivo. *Talanta* **134** 30–36
- dos Santos SR, Rodrigues Corrêa C, Branco de Barros AL, Serakides R, Fernandes SO, Cardoso VN, et al. 2015 Identification of *Staphylococcus aureus* infection by aptamers directly radiolabeled with technetium-99m. *Nucl. Med. Biol.* **42** 292–298
- Duan N, Wu S, Zhu C, Ma X, Wang Z, Yu Y, et al. 2012 Dual-color upconversion fluorescence and aptamer-functionalized magnetic nanoparticles-based bioassay for the simultaneous detection of *Salmonella Typhimurium* and *Staphylococcus aureus*. *Anal. Chim. Acta* **723** 1–6
- Duan N, Wu S, Chen X, Huang Y, Xia Y, Ma X, et al. 2013 Selection and characterization of aptamers against *salmonella typhimurium* using whole-bacterium systemic evolution of ligands by exponential enrichment (SELEX). *J. Agric. Food Chem.* **61** 3229–3234
- Dwivedi HP, Smiley RD and Jaykus LA 2013 Selection of DNA aptamers for capture and detection of *Salmonella Typhimurium* using a whole-cell SELEX approach in conjunction with cell sorting. *Appl. Microbiol. Biotechnol.* **97** 3677–3686
- Ellington AD and Szostak JW 1990 In vitro selection of RNA molecules that bind specific ligands. *Nature.* **346** 818–822
- Farokhzad OC, Jon S, Khademhosseini A, Tran TT, Lavan DA, Langer R 2004 Nanoparticle-aptamer bioconjugates: a new approach for targeting prostate cancer cells nanoparticle-aptamer

- bioconjugates: a new approach for targeting prostate cancer cells. *Biomaterials* 7668–7672. doi:10.1158/0008-5472.CAN-04-2550
- Feldwisch J, Tolmachev V, Lendel C, Herne N, Sjöberg A, Larsson B, Rosik D, Lindqvist E, Fant G, Höidén-Guthenberg I, Galli J, Jonasson P, Abrahmsén L 2010 Design of an optimized scaffold for affibody molecules. *J. Mol. Biol.* **398**(2) 232–247
- Feng L, Chen Y, Ren J and Qu X 2011 A graphene functionalized electrochemical aptasensor for selective label-free detection of cancer cells. *Biomaterials* **32** 2930–2937
- Ferrara K, Pollard R and Borden M 2007 Ultrasound microbubble contrast agents: fundamentals and application to gene and drug delivery. *Annu. Rev. Biomed. Eng.* **9** 415–447
- Fletcher SJ, Phillips LW, Milligan AS and Rodda SJ 2010 Toward specific detection of Dengue virus serotypes using a novel modular biosensor. *Biosens. Bioelectron.* **26** 1696–1700
- Fong AHC and Lai TYY 2013 Long-term effectiveness of ranibizumab for age-related macular degeneration and diabetic macular edema. *Clin. Interv. Aging* **8** 467–483
- Gandham SH, Volk DE, Lokesh GLR, Neerathilingam M and Gorenstein DG 2014 Thioaptamers targeting dengue virus type-2 envelope protein domain III. *Biochem. Biophys. Res. Commun.* **453** 309–315
- Ghosh S, Sullivan CA, Zerkowski MP, Molinaro AM, Rimm DL, Camp RL, *et al.* 2008 High levels of vascular endothelial growth factor and its receptors (VEGFR-1, VEGFR-2, neuropilin-1) are associated with worse outcome in breast cancer. *Hum. Pathol.* **39** 1835–1843
- Gnanam AJ, Hall B, Shen X, Piasecki S, Vernados A, Galyov EE, *et al.* 2008 Development of aptamers specific for potential diagnostic targets in *Burkholderia pseudomallei*. *Trans. R. Soc. Trop. Med. Hyg.* **102**
- Graham JC and Zarbl H 2012 Use of cell-SELEX to generate DNA aptamers as molecular probes of HPV-associated cervical cancer cells. *PLoS One* **7** 1–9
- Gramiak R and Shah PM 1968 Echocardiography of the aortic root. *Investig. Radiol.* **3** 356–366
- Green LS, Jellinek D, Bell C, Beebe LA, Feistner BD, Gill SC, *et al.* 1995 Nuclease-resistant nucleic acid ligands to vascular permeability factor/vascular endothelial growth factor. *Chem. Biol.* **2** 683–695
- Han SR and Lee SW 2013 In vitro selection of RNA aptamer specific to *Salmonella Typhimurium*. *J. Microbiol. Biotechnol.* **23** 878–884
- Herrmann A, Priceman SJ, Kujawski M, Xin H, Cherryholmes GA, Zhang W, *et al.* 2014 CTLA4 aptamer delivers STAT3 siRNA to tumor-associated and malignant T cells. *J. Clin. Invest.* **124** 2977–2987
- Hicke BJ, Marion C, Chang YF, Gould T, Lynott CK, Parma D, *et al.* 2001 Tenascin-C aptamers are generated using tumor cells and purified protein. *J. Biol. Chem.* **276** 48644–48654
- Hilger CS, Willis MC, Wolters M and Pieken WA 1999 Tc-99m-labeling of modified RNA. *Nucleosides Nucleotides* **18** 1479–1481
- Homann M and Göringer HU 1999 Combinatorial selection of high affinity RNA ligands to live African trypanosomes. *Nucleic Acids Res.* **27** 2006–2014
- Homann M, Lorger M, Engstler M, Zacharias M and Göringer HU 2006 Serum-stable RNA aptamers to an invariant surface domain of live African trypanosomes. *Comb. Chem. High Throughput Screen* **9** 491–499
- Hu Y, Duan J, Zhan Q, Wang F, Lu X and Da Yang X 2012 Novel mucl1 aptamer selectively delivers cytotoxic agent to cancer cells in vitro. *PLoS One* **7** e31970
- Huang H-Y, Liu H-L, Hsu P-H, Chiang C-S, Tsai C-H, Chi H-S, *et al.* 2015 A multitheragnostic nanobubble system to induce blood-brain barrier disruption with magnetically guided focused ultrasound. *Adv. Mater.* **27** 655–661
- Hwang DW, Ko HY, Lee JH, Kang H, Ryu SH, Song IC, *et al.* 2010 A nucleolin-targeted multimodal nanoparticle imaging probe for tracking cancer cells using an aptamer. *J. Nucl. Med.* **51** 98–105
- Jalalian SH, Taghdisi SM, Hamedani NS, Kalat SAM, Lavaee P, ZandKarimi M, *et al.* 2013 Epirubicin loaded super paramagnetic iron oxide nanoparticle-aptamer bioconjugate for combined colon cancer therapy and imaging in vivo. *Eur. J. Pharm. Sci.* **50** 191–197
- Kang S and Hah SS 2014 Improved ligand binding by antibody-aptamer pincers. *Bioconjug. Chem.* **25** 1421–1427
- Kang D, Wang J, Zhang W, Song Y, Li X, Zou Y, *et al.* 2012 Selection of DNA aptamers against glioblastoma cells with high affinity and specificity. *PLoS One* **7** 1–9
- Keefe AD and Cload ST 2008 SELEX with modified nucleotides. *Curr. Opin. Chem. Biol.* **12** 448–456
- Kim D, Park S, Jae HL, Yong YJ and Jon S 2007 Antibiofouling polymer-coated gold nanoparticles as a contrast agent for in vivo X-ray computed tomography imaging. *J. Am. Chem. Soc.* **129** 7661–7665
- Kim D, Jeong YY and Jon S 2010 A drug-loaded aptamer-gold nanoparticle bioconjugate for combined CT imaging and therapy of prostate cancer. *ACS Nano* **4** 3689–3696
- Kim YS, Chung J, Song MY, Jung J and Kim BC 2014 Aptamer cocktails: enhancement of sensing signals compared to single use of aptamers for detection of bacteria. *Biosens. Bioelectron.* **54** 195–198
- Kunii T, Ogura S, Mie M and Kobatake E 2011 Selection of DNA aptamers recognizing small cell lung cancer using living cell-SELEX. *Analyst* **136** 1310–1312
- Kuo T, Lai W, Li C, Wun Y, Chang H, Chen J, *et al.* 2014 AS1411 aptamer-conjugated Gd₂O₃:Eu nanoparticles for target-specific computed tomography/magnetic resonance/fluorescence molecular imaging. *Nano Res.* **7** 658–669
- Lale S V, Aswathy RG, Aravind A, Kumar DS, Koul V 2014 AS1411 aptamer and folic acid functionalized pH-responsive ATRP fabricated pPEGMA – PCL – pPEGMA polymeric nanoparticles for targeted drug delivery in cancer therapy. *BioMacromolecules* **ASAP**
- Lauffer RB 1987 Paramagnetic metal complexes as water proton relaxation agents for NMR imaging: theory and design. *Chem. Rev.* **87** 901–927
- Lee S, Kim YS, Jo M, Jin M, Lee DK and Kim S 2007 Chip-based detection of hepatitis C virus using RNA aptamers that specifically bind to HCV core antigen. *Biochem. Biophys. Res. Commun.* **358** 47–52
- Lee HS, Kim KS, Kim CJ, Hahn SK and Jo MH 2009 Electrical detection of VEGFs for cancer diagnoses using anti-vascular endothelial growth factor aptamer-modified Si nanowire FETs. *Biosens. Bioelectron.* **24** 1801–1805

- Lee YJ, Han SR, Maeng JS, Cho YJ and Lee SW 2012 In vitro selection of Escherichia coli O157:H7-specific RNA aptamer. *Biochem. Biophys. Res. Commun.* **417** 414–420
- Lehtinen J, Raki M, Bergström KA, Uutela P, Lehtinen K, Hiltunen A, et al. 2012 Pre-targeting and direct immunotargeting of liposomal drug carriers to ovarian carcinoma. *PLoS One.* **7** 1–10
- Li W-H, Parigi G, Fragai M, Luchinat C and Meade TJ 2002 Mechanistic studies of a calcium-dependent MRI contrast agent. *Inorg. Chem.* **41** 4018–4024
- Li N, Wang Y, Pothukuchy A, Syrett A, Husain N, Gopalakrishna S, et al. 2008 Aptamers that recognize drug-resistant HIV-1 reverse transcriptase. *Nucleic Acids Res.* **36** 6739–6751
- Li S, Chen N, Zhang Z and Wang Y 2013 Endonuclease-responsive aptamer-functionalized hydrogel coating for sequential catch and release of cancer cells. *Biomaterials* **34** 460–469
- Li C, Chen T, Ocoy I, Zhu G, Yasun E, You M, et al. 2014a Gold-coated Fe₃O₄ nanoroses with five unique functions for cancer cell targeting, imaging, and therapy. *Adv. Funct. Mater.* **24** 1772–1780
- Li L, Hou J, Liu X, Guo Y, Wu Y, Zhang L, et al. 2014b Nucleolin-targeting liposomes guided by aptamer AS1411 for the delivery of siRNA for the treatment of malignant melanomas. *Biomaterials* **35** 3840–3850
- Li L, Xiang D, Shigdar S, Yang W, Li Q, Lin J, et al. 2014c Epithelial cell adhesion molecule aptamer functionalized PLGA-lecithin-curcumin-PEG nanoparticles for targeted drug delivery to human colorectal adenocarcinoma cells. *Int. J. Nanomedicine* **9** 1083–1096
- Li W, Chen H, Yu M and Fang J 2014d Targeted delivery of doxorubicin using a colorectal cancer-specific ssDNA aptamer. *Anat. Rec.* **2288** 2280–2288
- Li X, An Y, Jin J, Zhu Z, Hao L, Liu L, et al. 2015a Evolution of DNA aptamers through in vitro metastatic-cell-based SELEX for metastatic cancer recognition and imaging. *Anal. Chem.* doi:10.1021/acs.analchem.5b00637
- Li X, Yu Y, Ji Q and Qiu L 2015b Targeted delivery of anticancer drugs by aptamer AS1411 mediated Pluronic F127/cyclodextrin-linked polymer composite micelles. *Nanomedicine* **11** 175–184
- Lian Y, He F, Wang H and Tong F 2015 A new aptamer/graphene interdigitated gold electrode piezoelectric sensor for rapid and specific detection of Staphylococcus aureus. *Biosens. Bioelectron* **65** 314–319
- Liu J, Yang Y, Hu B, Ma ZY, Huang HP, Yu Y, et al. 2010 Development of HBsAg-binding aptamers that bind HepG2.2.15 cells via HBV surface antigen. *Viol. Sin.* **25** 27–35
- Liu N, Zhou C, Zhao J and Chen Y 2012a Reversal of paclitaxel resistance in epithelial ovarian carcinoma cells by a MUC1 aptamer-let-7i chimera. *Cancer Investig.* **30** 577–582
- Liu Z, Duan J-H, Song Y-M, Ma J, Wang F-D, Lu X, et al. 2012b Novel HER2 aptamer selectively delivers cytotoxic drug to HER2-positive breast cancer cells in vitro. *J. Transl. Med.* **10** 148
- Lorger M, Engstler M, Homann M, Göringer HU 2003 Targeting the variable surface of African trypanosomes with variant surface glycoprotein-specific, serum-stable RNA aptamers. **2** 84–94
- Lucignani G 2006 Aptamers and in-beam PET for advanced diagnosis and therapy optimisation. *Eur. J. Nucl. Med. Mol. Imaging* **33** 1095–1097
- Luo P, Liu Y, Xia Y, Xu H and Xie G 2014 Aptamer biosensor for sensitive detection of toxin A of Clostridium difficile using gold nanoparticles synthesized by Bacillus stearothermophilus. *Biosens. Bioelectron* **54** 217–221
- Martín ME, García-Hernández M, García-Recio EM, Gómez-Chacón GF, Sánchez-López M and González VM 2013 DNA aptamers selectively target Leishmania infantum H2A protein. *PLoS One* **8** 1–11
- Massoud TF, Massoud TF, Gambhir SS, Gambhir SS 2003 Molecular imaging in living subjects: seeing fundamental biological processes in a new light. *Genes Dev.* **17** 545–580
- McNamara JO, Andrechek ER, Wang Y, Viles KD, Rempel RE, Gilboa E, et al. 2006 Cell type-specific delivery of siRNAs with aptamer-siRNA chimeras. *Nat. Biotechnol.* **24** 1005–1015
- Meng L, Yang L, Zhao X, Zhang L, Zhu H, Liu C, et al. 2012 Targeted delivery of chemotherapy agents using a liver cancer-specific aptamer. *PLoS One* **7** e33434
- Min K, Song K-M, Cho M, Chun Y-S, Shim Y-B, Ku JK, et al. 2010 Simultaneous electrochemical detection of both PSMA (+) and PSMA (-) prostate cancer cells using an RNA/peptide dual-aptamer probe. *Chem. Commun. (Camb.)* **46** 5566–5568
- Mo R, Jiang T, DiSanto R, Tai W and Gu Z 2014 ATP-triggered anticancer drug delivery. *Nat. Commun.* **5** 1–10
- Moon J, Kim G, Lee S and Park S 2013 Identification of salmonella typhimurium-specific DNA aptamers developed using whole-cell SELEX and FACS analysis. *J. Microbiol. Methods.* **95** 162–166
- Moreno M, Rincón E, Piñeiro D, Fernández G, Domingo A, Jiménez-Ruiz A, et al. 2003 Selection of aptamers against KMP-11 using colloidal gold during the SELEX process. *Biochem. Biophys. Res. Commun.* **308** 214–218
- Mukhopadhyay P, Chakraborty S, Ponnusamy MP, Lakshmanan I, Jain M and Batra SK 2011 Mucins in the pathogenesis of breast cancer: implications in diagnosis, prognosis and therapy. *Biochim. Biophys. Acta* **1815** 224–240
- Nagarkatti R, Bist V, Sun S, Fortes de Araujo F, Nakhasi HL, Debrabant A 2012 Development of an aptamer-based concentration method for the detection of trypanosoma cruzi in blood. *PLoS One* **7**(8) e43533
- Nakatsuka MA, Mattrey RF, Esener SC, Cha JN and Goodwin AP 2012 Aptamer-crosslinked microbubbles: smart contrast agents for thrombin-activated ultrasound imaging. *Adv. Mater.* **24** 6010–6016
- Neff CP, Zhou J, Remling L, Kuruvilla J, Zhang J, Li H, et al. 2011 An aptamer-siRNA chimera suppresses HIV-1 viral loads and protects from helper CD4+ T cell decline in humanized mice. *Sci Translational Medicine* **3** 1–20
- Ngubane NA, Gresh L, Pym A, Rubin EJ and Khati M 2014 Selection of RNA aptamers against the M. tuberculosis EsxG protein using surface plasmon resonance-based SELEX. *Biochem. Biophys. Res. Commun.* **449** 114–119
- Olar A and Aldape KD 2012 Using the molecular classification of glioblastoma to inform personalized treatment. *Changes* **29** 997–1003

- Paredes E, Evans M and Das SR 2011 RNA labeling, conjugation and ligation. *Methods* **54** 251–259
- Parekh P, Tang Z, Turner PC, Moyer RW and Tan W 2010 Aptamers recognizing glycosylated hemagglutinin expressed on the surface of vaccinia virus-infected cells. *Anal. Chem.* **82** 8642–8649
- Park SY, Kim S, Yoon H, Kim K-B, Kalme SS, Oh S, *et al.* 2011 Selection of an antiviral RNA aptamer against hemagglutinin of the subtype H5 avian influenza virus. *Nucleic Acid Ther.* **21** 395–402
- Park JH, Jee MH, Kwon OS, Keum SJ and Jang SK 2013 Infectivity of hepatitis C virus correlates with the amount of envelope protein E2: Development of a new aptamer-based assay system suitable for measuring the infectious titer of HCV. *Virology* **439** 13–22
- Perkins AC and Missailidis S 2007 Radiolabelled aptamers for tumour imaging and therapy. *Q. J. Nucl. Med. Mol. Imaging* **51** 292–296
- Pilapong C, Sitthichai S, Thongtem S and Thongtem T 2014 Smart magnetic nanoparticle-aptamer probe for targeted imaging and treatment of hepatocellular carcinoma. *Int. J. Pharm.* **473** 469–474
- Popovtzer R, Agrawal A, Kotov NA, Popovtzer A, Balter J, Carey TE, *et al.* 2008 Targeted gold nanoparticles enable molecular CT imaging of cancer. *Nano Lett.* **8** 4593–4596
- Qiu L, Chen T, Öçsoy I, Yasun E, Wu C, Zhu G, *et al.* 2015 A cell-targeted, size-photocontrollable, nuclear-uptake nanodrug delivery system for drug-resistant cancer therapy. *Nano Lett.* **15** 457–463
- Rane SU, Shet T, Sridhar E, Bhele S, Gaikwad V, Agale S, *et al.* 2014 Interobserver variation is a significant limitation in the diagnosis of Burkitt lymphoma. *Indian J Med Paediatr Oncol.* **35** 44–53
- Ray P, Cheek MA, Sharaf ML, Li N, Ellington AD, Sullenger BA, *et al.* 2012 Aptamer-mediated delivery of chemotherapy to pancreatic cancer cells. *Nucleic Acid Ther.* **22** 295–305
- Rotherham LS, Maserumule C, Dheda K, Theron J and Khati M 2012 Selection and application of ssDNA aptamers to detect active TB from sputum samples. *PLoS One* **7** 1–11
- Ruckman J, Green LS, Beeson J, Waugh S, Gillette WL, Henninger DD, *et al.* 1998 2'-fluoropyrimidine RNA-based aptamers to the 165-amino acid form of vascular endothelial growth factor (VEGF165). *J. Biol. Chem.* **273** 20556–20567
- Sayari E, Dinarvand M, Amini M, Azhdarzadeh M, Mollarazi E, Ghasemi Z, *et al.* 2014 MUC1 aptamer conjugated to chitosan nanoparticles, an efficient targeted carrier designed for anticancer SN38 delivery. *Int. J. Pharm.* **473** 304–315
- Sefah K, Tang Z, Shangguan D, Chen H, Lopez-colon D, Li Y, *et al.* 2009 Molecular recognition of acute myeloid leukemia using aptamers. *Brain* **23** 235–244
- Sefah K, Shangguan D, Xiong X, O'Donoghue MB and Tan W 2010 Development of DNA aptamers using Cell-SELEX. *Nat. Protoc* **5** 1169–1185
- Shi H, Tang Z, Kim Y, Nie H, Huang YF, He X, *et al.* 2010 In vivo fluorescence imaging of tumors using molecular aptamers generated by cell-SELEX. *Chem. Asian. J.* **5** 2209–2213
- Shiao Y, Chiu H, Wu P and Huang Y 2014 Aptamer-functionalized gold nanoparticles as photoresponsive nanoplatform for co-drug delivery. *ACS Appl. Mater. Interfaces* **6** 21832–21841
- Shigdar S, Lin J, Yu Y, Pastuovic M, Wei M and Duan W 2011 RNA aptamer against a cancer stem cell marker epithelial cell adhesion molecule. *Cancer Sci.* **102** 991–998
- Shiratori I, Akitomi J, Boltz DA, Horii K, Furuichi M and Waga I 2014 Selection of DNA aptamers that bind to influenza A viruses with high affinity and broad subtype specificity. *Biochem. Biophys. Res. Commun.* **443** 37–41
- Shu H, Wen W, Xiong H, Zhang X and Wang S 2013 Novel electrochemical aptamer biosensor based on gold nanoparticles signal amplification for the detection of carcinoembryonic antigen. *Electrochem. Commun.* **37** 15–19
- Somasunderam A, Thiviyathan V, Tanaka T, Li X, Neerathilingam M, Lokesh GLR, *et al.* 2010 Combinatorial selection of DNA thioaptamers targeted to the HA binding domain of human CD44. *Biochemistry* **49** 9106–9112
- Song KM, Lee S and Ban C 2012 Aptamers and their biological applications. *Sensors* **12** 612–631
- Song Y, Zhu Z, An Y, Zhang W, Zhang H, Liu D, *et al.* 2013 Selection of DNA aptamers against epithelial cell adhesion molecule for cancer cell imaging and circulating tumor cell capture. *Anal. Chem.* **85** 4141–4149
- Subramanian N, Raghunathan V, Kanwar JR, Kanwar RK, Elchuri SV, Khetan V, *et al.* 2012 Target-specific delivery of doxorubicin to retinoblastoma using epithelial cell adhesion molecule aptamer. *Mol. Vis.* **18** 2783–2795
- Taghdisi SM, Danesh NM, Sarreshtehdar Emrani A, Tabrizian K, Zandkarimi M, Ramezani M, *et al.* 2013 Targeted delivery of Epirubicin to cancer cells by PEGylated A10 aptamer. *J. Drug Target.* **21** 739–744
- Tan L, Neoh KG, Kang E-T, Choe WS and Su X 2011 PEGylated anti-MUC1 aptamer-doxorubicin complex for targeted drug delivery to MCF7 breast cancer cells. *Macromol. Biosci.* **11** 1331–1335
- Tang XL, Zhou YX, Wu SM, Pan Q, Xia B and Zhang XL 2014 CFP10 and ESAT6 aptamers as effective Mycobacterial antigen diagnostic reagents. *J. Infect.* **69** 569–580
- Teng Y, Girvan AC, Casson LK, Pierce WM, Qian M, Thomas SD, *et al.* 2007 AS1411 alters the localization of a complex containing protein arginine methyltransferase 5 and nucleolin. *Cancer Res.* **67** 10491–10500
- Tucker CE, Chen LS, Judkins MB, Farmer JA, Gill SC and Drolet DW 1999 Detection and plasma pharmacokinetics of an anti-vascular endothelial growth factor oligonucleotide-aptamer (NX1838) in rhesus monkeys. *J. Chromatogr. B Biomed. Sci. Appl.* **732** 203–212
- Tuerk C and Gold L 1990 Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* **249** 505–510
- Ulrich H, Magdesian MH, Alves MJM and Colli W 2002 In vitro selection of RNA aptamers that bind to cell adhesion receptors of *Trypanosoma cruzi* and inhibit cell invasion. *J. Biol. Chem.* **277** 20756–20762
- Viraka Nellore BP, Kanchanapally R, Pramanik A, Sinha SS, Chavva SR, Hamme A, *et al.* 2015 Aptamer-conjugated graphene oxide membranes for highly efficient capture and accurate identification of multiple types of circulating tumor cells. *Bioconjug. Chem.* **26** 235–242

- Wang AZ and Farokhzad OC 2014 Current progress of aptamer-based molecular imaging. *J. Nucl. Med.* **55** 353–356
- Wang R and Li Y 2013 Hydrogel based QCM aptasensor for detection of avian influenza virus. *Biosens. Bioelectron.* **42** 148–155
- Wang AZ, Bagalkot V, Vasilliou CC, Gu F, Alexis F, Zhang L, Shaikh M, Yuet K, et al. 2008 Superparamagnetic iron oxide nanoparticle–aptamer bioconjugates for combined prostate cancer imaging and therapy. *Chem Med Chem.* **3** 1311–1315
- Wang RE, Wu H, Niu Y and Cai J 2011a Improving the stability of aptamers by chemical modification. *Curr. Med. Chem.* **18** 4126–4138
- Wang CH, Huang YF and Yeh CK 2011b Aptamer-conjugated nanobubbles for targeted ultrasound molecular imaging. *Langmuir* **27** 6971–6976
- Wang FB, Rong Y, Fang M, Yuan JP, Peng CW, Liu SP, et al. 2013 Recognition and capture of metastatic hepatocellular carcinoma cells using aptamer-conjugated quantum dots and magnetic particles. *Biomaterials* **34** 3816–3827
- Wang K, Yao H, Meng Y, Wang Y, Yan X and Huang R 2015 Specific aptamer-conjugated mesoporous silica-carbon nanoparticles for HER2-targeted chemo-photothermal combined therapy. *Acta Biomater* **16** 196–205
- Winnard PT, Pathak AP, Dhara S, Cho SY, Raman V and Pomper MG 2008 Molecular imaging of metastatic potential. *J. Nucl. Med.* **49** 96S–112S
- Wongphatcharachai M, Wang P, Enomoto S, Webby RJ, Gramer MR, Amonsin A, et al. 2013 Neutralizing DNA aptamers against swine influenza H3N2 viruses. *J. Clin. Microbiol.* **51** 46–54
- Wood AKW and Sehgal CM 2015 A review of low-intensity ultrasound for cancer therapy. *Ultrasound Med. Biol.* **41** 905–928
- Wu X, Ding B, Gao J, Wang H, Fan W, Wang X, et al. 2011 Second-generation aptamer-conjugated PSMA-targeted delivery system for prostate cancer therapy. *Int. J. Nanomedicine.* **6** 1747–1756
- Wu X, Chen J, Wu M and Zhao JX 2015 Aptamers: active targeting ligands for cancer diagnosis and therapy. *Theranostics.* **5** 322–344
- Xing H, Tang L, Yang X, Hwang K, Wang W, Yin Q, et al. 2013a Selective delivery of an anticancer drug with aptamer-functionalized liposomes to breast cancer cells in vitro and in vivo. *J. Mater. Chem. B Mater. Biol. Med.* **1** 5288–5297
- Xing H, Tang L, Yang X, Hwang K, Wang W, Yin Q, et al. 2013b Selective delivery of an anticancer drug with aptamer-functionalized liposomes to breast cancer cells in vitro and in vivo. *J. Mater. Chem. B.* **1** 5288
- Xu W and Lu Y 2011 A smart magnetic resonance imaging contrast agent responsive to adenosine based on a DNA aptamer-conjugated gadolinium complex. *Chem. Commun.* **47** 4998
- Xu W, Siddiqui IA, Nihal M, Pilla S, Rosenthal K, Mukhtar H, et al. 2013a Aptamer-conjugated and doxorubicin-loaded unimolecular micelles for targeted therapy of prostate cancer. *Biomaterials.* **34** 5244–5253
- Xu W, Xing H and Lu Y 2013b A smart T1-weighted MRI contrast agent for uranyl cations based on a DNzyme-gadolinium conjugate. *Analyst* **138** 6266–6269
- Yang M, Peng Z, Ning Y, Chen Y, Zhou Q and Deng L 2013 Highly specific and cost-efficient detection of Salmonella Paratyphi A combining aptamers with single-walled carbon nanotubes. *Sensors (Basel)* **13** 6865–6881
- Yeong Won J, Choi JW and Min J 2013 Micro-fluidic chip platform for the characterization of breast cancer cells using aptamer-assisted immunohistochemistry. *Biosens. Bioelectron.* **40** 161–166
- Yin J, He X, Wang K, Xu F, Shangguan J, He D, et al. 2013 Label-free and turn-on aptamer strategy for cancer cells detection based on a DNA-silver nanocluster fluorescence upon recognition-induced hybridization. *Anal. Chem.* **85** 12011–12019
- Younes CK, Boisgard R and Tavitian B 2002 Labelled oligonucleotides as radiopharmaceuticals: pitfalls, problems and perspectives. *Curr. Pharm. Des.* **8** 1451–1466
- Yu MK, Kim D, Lee IH, So JS, Jeong YY and Jon S 2011 Image-guided prostate cancer therapy using aptamer-functionalized thermally cross-linked superparamagnetic iron oxide nanoparticles. *Small* **7** 2241–2249
- Zelada-Guillén GA, Sebastián-Avila JL, Blondeau P, Riu J and Rius FX 2012 Label-free detection of Staphylococcus aureus in skin using real-time potentiometric biosensors based on carbon nanotubes and aptamers. *Biosens. Bioelectron* **31** 226–232
- Zhang D, Lu M and Wang H 2011 Fluorescence anisotropy analysis for mapping aptamer-protein interaction at the single nucleotide level. *J. Am. Chem. Soc.* **133** 9188–9191
- Zhang Z, Ali MM, Eckert MA, Kang D-K, Chen YY, Sender LS, et al. 2013 A polyvalent aptamer system for targeted drug delivery. *Biomaterials* **34** 9728–9735
- Zhang H, Hou L, Jiao X, Yandan J 2014a In vitro and in vivo evaluation of antitumor drug-loaded aptamer targeted single-walled carbon nanotubes system. *Curr. Pharm. Biotechnol.* **14**(13) 1105–1117. doi:10.2174/1389201015666140408123710
- Zhang P, Cheng F, Zhou R, Cao J, Li J, Burda C, et al. 2014a DNA-hybrid-gated multifunctional mesoporous silica nanocarriers for dual-targeted and MicroRNA-responsive controlled drug delivery. *Angew. Chem.* **126** 2403–2407
- Zhao Z, Xu L, Shi X, Tan W, Fang X and Shangguan D 2009 Recognition of subtype non-small cell lung cancer by DNA aptamers selected from living cells. *Analyst.* **134** 1808–1814
- Zhao J, He X, Bo B, Liu X, Yin Y and Li G 2012a A ‘signal-on’ electrochemical aptasensor for simultaneous detection of two tumor markers. *Biosens. Bioelectron* **34** 249–252
- Zhao J, Zhang L, Chen C, Jiang J and Yu R 2012b A novel sensing platform using aptamer and RNA polymerase-based amplification for detection of cancer cells. *Anal. Chim. Acta.* **745** 106–111
- Zhao N, You J, Zeng Z, Li C and Zu Y 2013 An ultra pH-sensitive and aptamer-equipped nanoscale drug-delivery system for selective killing of tumor cells. *Small* **9** 3477–3484
- Zhou J, Li H, Zhang J, Piotr S, Rossi J 2011 Development of cell-type specific anti-HIV gp120 aptamers for siRNA delivery. *J. Vis. Exp.* 1–9. doi:10.3791/2954
- Zhou Z-M, Zhou J, Chen J, Yu R-N, Zhang M-Z, Song J-T, et al. 2014 Carcino-embryonic antigen detection based on fluorescence resonance energy transfer between quantum dots and graphene oxide. *Biosens. Bioelectron.* **59** 397–403

- Zhou C, Chen T, Wu C, Zhu G, Qiu L, Cui C, Hou W and Tan W 2015 Aptamer-CaCO₃ nanostructures: a facile, pH-responsive, specific platform for targeted anticancer theranostics. *Chem. Asian. J.* **10** 1199–1216
- Zhu C, Liu J, Ling Y, Yang H, Liu Z, Zheng R, *et al.* 2012a Evaluation of the clinical value of ELISA based on MPT64 antibody aptamer for serological diagnosis of pulmonary tuberculosis. *BMC Infect. Dis.* **12** 96
- Zhu Q, Shibata T, Kabashima T and Kai M 2012b Inhibition of HIV-1 protease expression in T cells owing to DNA aptamer-mediated specific delivery of siRNA. *Eur. J. Med. Chem.* **56** 396–399
- Zhu X, Yang J, Liu M, Wu Y, Shen Z and Li G 2013a Sensitive detection of human breast cancer cells based on aptamer-cell-aptamer sandwich architecture. *Anal. Chim. Acta.* **764** 59–63
- Zhu Y, Chandra P and Shim YB 2013b Ultrasensitive and selective electrochemical diagnosis of breast cancer based on a hydrazine-Au nanoparticle-aptamer bioconjugate. *Anal. Chim.* **85** 1058–1064
- Zichel R, Chearwae W, Pandey GS, Golding B, Sauna ZE 2012 Aptamers as a sensitive tool to detect subtle modifications in therapeutic proteins. *PLoS One* **7**

MS received 05 April 2016; accepted 13 July 2016

Corresponding editor: INDRANIL DASGUPTA