Review

Aptamers as an Emerging Player in Biology

Aradhana Chopra[1], Ravi Shukla[2] and Tarun Kumar Sharma[2,3]*

[1]Systems Biophysics and Bionanotechnology, Physik Department and WSI/ZNN, Technische Universität München, 85748 Garching, Germany; [2]School of Applied Science, RMIT University, Melbourne, Australia; [3]Center for Biodesign and Diagnostics, Translational Health Science and Technology Institute, Gurgaon, India.

Abstract | Aptamers are single stranded nucleic acid molecules that are considered as chemical rival of antibodies. They are generated through an in vitro evolution process called SELEX. These single stranded nucleic acid aptamers can acquire various secondary and tertiary structures to bind their cognate targets with high affinity and specificity. The ease in synthesis and modification makes them an attractive candidate for a variety of applications. Since the last decade, aptamers have been regarded as an emerging player in biology. They have found applications not only in biosensors, but also in other areas such as diagnostics, biomarker discovery, drug delivery, bio-imaging and molecular therapy. In this review we have summarized recent advancements and applications of aptamers in biology.

Editor | Munir Iqbal, The Pirbright Institute, UK
Received | April 15, 2014; **Accepted** | July 3, 2014; **Published** | July 16, 2014
***Correspondence** | Sharma, T. K., Center for Biodesign and Diagnostics, Translational Health Science and Technology Institute, Gurgaon, India. and RMIT University, Melbourne, Australia; **E-mail** | tarun@thsti.res.in: Ravi Shukla: ravi.shukla@rmit.edu.au
Citation | Chopra, A. et al. (2014). Aptamers as an Emerging Player in Biology. *Aptamers and Synthetic Antibodies,* 1(1): 1-11.

Introduction

Aptamers are synthetic oligonucleotides, such as ribonucleic acid (RNA) and single-strand deoxyribonucleic acid (ssDNA) that can bind to their targets with high affinity and specificity due to specific secondary and tertiary structures. The whole idea of nucleic acids binding with a particular target emerged in the 1980s from the research on human immunodeficiency virus (HIV) and adenovirus. These viruses encode a number of small structured RNAs that bind to viral or cellular proteins with high affinity and specificity (Dollins, et al. 2008; Sullenger, et al. 1990; O'Malley, et al., 1986; Burgert, et al., 2002). Two research groups-Gold's group and Szostak's group first reported aptamers independently in 1990. The Gold lab selected RNA ligands against T4 DNA polymerase; and the Szostak lab, generated RNA ligands against various organic dyes (Ellington and Szostak, 1990; Tuerk and Gold, 1990). Both these groups employed an *in vitro* selection process called Systematic Evolution of Ligands by EXponential enrichment (SELEX) for the generation for aptamers (Figure 1). Since then, SELEX is being used as a basic technique for the generation of aptamers. Continuous efforts are being put towards improving the SELEX strategy and development of more sophisticated and faster techniques of aptamer screening such as Capillary Electrophoresis microfluidic chips (CE-Microfluidics), Cell-SELEX etc. (Weng, et al., 2012; Sefah, et al., 2010). Also, methods such as atomic force microscopy (AFM), electrophoretic mobility shift assays (EMSA), and surface plasmon resonance (SPR), have been performed in connection with SELEX (Guthold, et al., 2002; Tsai and Reed, 1998; Khati, et al., 2003; Misono, et al. 2005).

Aptamers are different from antibodies, yet they mimic properties of antibodies in a variety of diagnostic formats. As an emerging class of molecules that rivals antibodies in both therapeutic and diagnostic application, aptamers have become the focus of attention

for their novel properties, such as highly selective and specific target recognition and binding. Aptamers offer additional advantages with respect to cost, stability, ability to sustain reversible denaturation, lack of toxicity and immunogenicity between biocompatibility and ease, speed of synthesis against a variety of targets including proteins, small molecules and whole cells. These physical and chemical properties make aptamers ideal candidates for a wide variety of applications. Various applications of aptamers are portrayed in Figure 2.

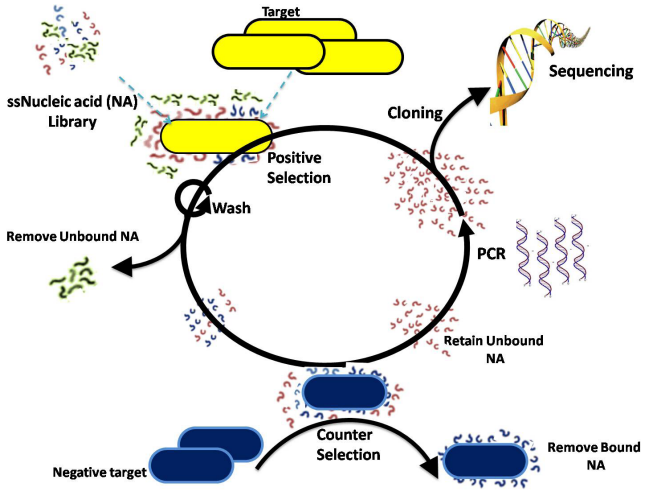

Figure 1: *Schematic illustration of SELEX process*

Aptamers have been most commonly exploited for the use as biosensors where the aptamer functions as the molecular recognition probe for the detection of a number of target molecules. A transducer that works on different principles detects the signal (Chopra et al., 2013). In addition, several nanomaterials such as gold nanoparticles, DNA hydrogels, carbon nanotubes (CNTs), quantum dots (QDs) have also been used in combination with aptamers for the development of tools for biodiagnostic applications (Han et al., 2010). Recently, the Food and Drug Administration (FDA) approved Macugen, a vascular endothelial growth factor (VEGF) specific aptamer, for the treatment of neovascular (wet) age-related macular degeneration (AMD) (Bunka and Stockley, 2006). Since then, aptamers have attracted significant attention as therapeutic agents, anti-infective agents, drug delivery vehicles; bio-imaging agents in cancer and infectious disease detection and point of care diagnostics (Ulrich, et al., 2002; Daniels, et al., 2003; Shangguan, et al., 2006; Lee, et al., 2006; Ng, et al., 2006; Bagalkot, et al., 2007; Tang, et al., 2007; Xiao et al., 2008; Sharma, et al., 2014).

This review focuses on the development of aptamer technology as an eminent player for diagnostic and therapeutic applications in biology.

Aptamers as Aptasensor

A biosensor is an analytical device for the recognition/detection of an analyte. It has two main components (i) a ligand that acts as a molecular recognition element (MRE), which produces a primary signal, upon successful binding to the target; and (ii) a transducer that acts as a detector element, which converts the signal obtained from the MRE into a detectable form. A biosensor that is based on aptamers as a recognition element is called an aptasensor. Different types of aptasensors have been developed based on the principle of detection including optics, electrochemistry, mass based detection (Chopra, et al., 2013). They detect a range of targets including proteins, small molecules, intracellular analytes, bacterial pathogens, and mammalian cells etc. (Zhu, et al., 2006; Herr, et al., 2006; Zuo, et al., 2007; Lange, et al., 2012).

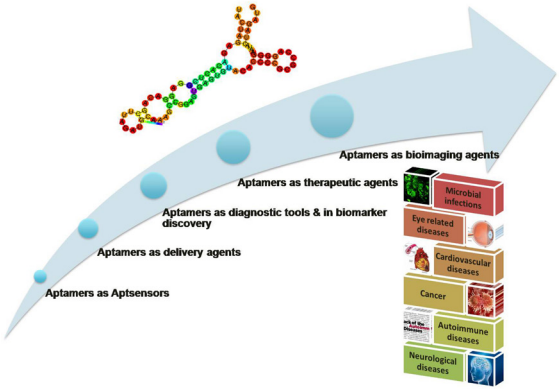

Figure 2: *Various applications of aptamer*

Aptasensors were first used in 1996 as the selective component in an optical sensor application where they were part of a model system consisting of human-neutrophil-elastase-coated beads that interact with fluorescent-tagged aptamers (Davis, et al., 1996).

The optical sensors have gained the most popularity because of their relatively high sensitivity, fast response and simple mode of operation. Different optical aptasensors are based on different working principles-colorimetry, fluorescence, chemiluminescence, Surface Plasmon Resonance (SPR), Surface Enhanced Raman Scattering (SERS), Dynamic light

scattering (DLS), ellipsometry. Many optical aptasensors for various small biomolecules have been developed, such as ATP, adenosine, cocaine, dopamine, NADP , ochratoxin A, theophylline, flavin mononucleotide, tyrosinamide, kanamycin, oxytetracycline, glucose, bisphenol A etc (Feng, et al., 2014).

Galarreta *et. al.* reported an aptasensor for ochratoxin A assisted by ellipsometry. They immobilized an aptamer for ochratoxin A on the surface of gold nanostructures. In the presence of ochratoxin A, the aptamer transformed its form from coil to its specific secondary structure that was observed as a change in ellipsometry (Galarreta, et al., 2013).

Ma *et. al.* developed an electrochemical aptasensor for detection of *Salmonella* using a *Salmonella*-specific recognition aptamer. It was based on the changes in electrochemical properties between the electrode and the electrolyte observed when *Salmonella* was bound to the aptamer linked onto the carbon electrode via gold nanoparticles. The electrochemical impedance spectrum was measured to quantify the *Salmonella*. A detection limit as low as 3 CFU/mL was obtained. This novel method is specific and fast, and it has the potential for real sample detection (Ma et al., 2014).

A lateral flow biosensor for *salmonella* detection based on aptamer mediated strand displacement amplification has been developned by Fang *et. al.* They utilized two aptamers one for magnetic bead mediated enrichment (capture) and the other was used as a reporter, which was amplified by isothermal strand displacement amplification (SDA) and detected by the biosensor. It was highly sensitive and could detect upto 10^1 CFU of *Salmonella* (Zhiyuan, et al., 2014).

Aptamer based quartz crystal microbalance (QCM) biosensors were used for the detection of immunoglobulin E (IgE), human chorionic gonadotropin and urinary protein (Zhang, et al., 2004; Luo, et al., 2006; Yao et al., 2010). The detection of the analyte was observed in terms of the frequency shifts of the QCM. This biosensor was highly specific, sensitive (detection limit up to 50pM) and could be recycled with little loss of sensitivity.

Aptamers as Diagnostic Tools and in Biomarkers Discovery

Biomarkers can be expressed in different forms, including, for example, proteins unique to specific cell types and subtypes (Henry, et al., 2012). Proteins are the most useful form of biomarkers since they reflect the genotype and phenotype of a particular disease. Alterations of proteins within the cell, as well as overexpression and down regulation of certain proteins, can have profound effects on the cell as a whole. It is quite challenging to develop a biomarker system able to provide accurate evidence that a protein is a reflection of the physiological state of the cell at some defined stage. Traditional methods employed the use of antibodies which was limited for large scale screening of biomarkers (Gold, et al., 2010). However, detection systems based on aptamers have the ability to overcome such pitfalls, making them extremely valuable as a diagnostic tool and for discovery of biomarkers. Generation of aptamers using Cell-SELEX technology has become an effective tool for molecular medicine and biomarker discovery (Sefah, et al., 2010). Aptamers selected in this manner can target overexpressed proteins on the cell surface and even detect small differences among cell- surface proteins. This capability allows aptamers to differentiate unique cellular characteristics, particularly those between cancerous and noncancerous cells based on their unique cell-surface homology.

Mi *et. al.* developed a unique approach to isolate RNA aptamers *in vivo* against tumor cells in living mice. The aptamer selected was used to identify the biomarker for hepatic colon cancer metastases. The aptamer binds to protein p68, an RNA helicase that has been shown to be unregulated in colorectal cancer (Mi, et al., 2010).

Kim *et. al.* used the cell-SELEX technique to isolate DNA aptamers against the glioblastoma brain tumour initiating cells with high affinity and specificity. Glioblastoma is classified as one of the most lethal cancers. Therefore, the selection of aptamers that can specifically bind the cancer stem cells of Glioblastoma could help discover and characterize new biomarkers for these cells (Kim, et al., 2013).

Recently, Nagarkatti *et. al.* developed an aptamer based detection of biomarkers responsible for chagas disease in *Trypanosoma cruzi (T. cruzi)* infected mice. They generated RNA aptamers against excreted secreted antigens of *T. cruzi* (TESA); which were used as parasite specific ligands to develop Enzyme Linked Aptamer (ELA) assays to detect biomarkers in *T. cru-*

zi infected mice plasma. The aptamer based assay can be used as an alternate non-PCR, non-serology based method to detect *T. cruzi* infection (Nagarkatti, et al., 2014).

Aptamers not only provide a way of discovering biomarkers that help to distinguish between cancer cells and normal cells, but they can also help to differentiate the stage of carcinogenesis within one subtype of cancer. A study conducted by Ostroff *et. al.* using a DNA-based aptamer with very high specificity and affinity for protein binding revealed 44 different biomarkers able to distinguish among Stage I–III lung cancer (Ostroff, et al., 2010).

The aptamer technology can accelerate the discovery of new specific biomarkers that provide an insight to the cellular mechanisms and pathways; which would facilitate the development of highly specific and effective targeting ligands and platforms for diagnostic and therapeutic purposes.

Aptamers as Delivery Agent

The active targeting of drugs to a particular cell, tissue or disease-specific manner represents a potentially powerful and important technology with widespread applications in medicine, including the treatment of many infectious diseases and cancers. The properties of high affinity and specificity towards their targets make aptamers the molecules of choice to be used as delivery agents. Aptamers can be designed as targeting ligands, particularly when generated by cell-based SELEX, where live cells are used to select aptamers. The aptamers are selected for cell surface molecules which include the mainly cell surface proteins. The aptamers thus generated can differentiate diseased cells from the healthy one, thus enabling the selective delivery of therapeutic compounds to target cells. Zhou and coworkers demonstrated the cell specific delivery of anti-human immunodeficiency virus (anti-HIV) siRNAs through fusion to an anti-gp120 aptamer (Zhou, et al., 2008). Gp-120 is an envelope glycoprotein expressed on the surface of HIV-1-infected cells, allowing binding and internalization of the aptamer–siRNA chimeric molecules. Cells expressing HIV-1 gp120 specifically take up the chimera and dicer processes the siRNA; this releases an anti-tat/rev siRNA, which, in turn, inhibits HIV replication. Additionally Lange, et al., 2012 have also demonstrated the robust suppression of HIV using aptamer and ribozyme constructs.

Another example of aptamer mediated targeted delivery was shown by Dassie *et. al.* (2009). They conjugated an anti-PSMA RNA aptamer to a 21-mer siRNA portion, resulting in a chimera that targets polo-like kinase 1 (PLK1) and BCL2, two survival genes overexpressed in most human tumors. The aptamer portion of the chimeras selectively binds to PSMA, whereas the therapeutic siRNA portion interferes and knocks down gene expression by inhibiting the growth of cancer cells.

Recently, Lai et al conjugated a nucleolin targeting aptamer to siRNAs against two genes snail family zinc finger 2 (SLUG) and neuropilin 1 (NRP1) promote malignant transformation and activate important signaling pathways during different stages of metastasis in lung cancer. They observed that the combined treatment synergistically suppressed cancer cell invasion, growth of tumor and angiogenesis by specific targeting of cancer cells in combination with specific gene silencing (Lai, et al., 2014). Since a repertoire of siRNA is available to silence human genes, using cell-specific aptamers to target different cell types and blocking their key signaling pathways is a very promising direction for developing new therapeutic modalities (Lai, et al., 2014).

In addition, aptamers were also used as a component of polyvalent therapeutics termed as "Poly-Aptamer-Drug", which composed of multiple aptamer units and physically intercalated chemotherapy agents. Zhang *et.al.* used leukemia cell-binding aptamer and doxorubicin as a model system for the polyaptamer drug strategy. They demonstrated that the use of polyvalent aptamer is significantly more effective than its monovalent counterpart in targeting and killing leukemia cells due to enhanced binding affinity and cell internalization via multivalent effects (Zhang, et al., 2013).

Liposomes have been used in order to increase the cell membrane penetration of aptamers. This liposome nanostructure can efficiently increase cell permeability and enhance drug delivery. Another advantage of liposomes is its ability to increase the plasma residence time of aptamers from several minutes to 23 hours (Willis, et al., 1998).

Aptamer conjugated, multifunctional liposomes have been fabricated to encapsulate and deliver cisplatin to breast cancer cells (MCF-7). Here, polymeric nano-

carriers in combination with aptamer mediated targeting, can be used to selectively deliver drug payloads to cancerous cells with high efficiency and specificity (Cao, et al., 2009).

Several groups have reported the use of aptamers bound to nanoparticles such as gold and silver for targeted photothermal killing of cancerous cells (Beqa, et al. 2011; Huang, et al., 2008a; Huang, et al., 2008b; Tan, et al., 2011; Lu, et al. 2010). Also, other nanomaterials like CNTs, QDs and magnetic nanoparticles have been used extensively (Taghdisi, et al., 2011; Bruno, et al., 2013; Wahajuddin, et al., 2012; Nair, et al., 2010). The emerging integration of aptamers with nanotechnology and chemical biology is envisioned to produce more versatile target-specific molecules, stimulate further development of new diagnostic and therapeutic nanotechnologies, and provide significant potential for many research and clinical applications in the near future.

Aptamers as Therapeutic Agent

In the 1980s, it was discovered that HIV and adenovirus contain several small RNA sequences or regions that can specifically bind to viral or cellular proteins with high affinity and inhibit viral replication. Functional studies indicated that such viral RNA-protein interactions could be exploited as competitive anti-viral therapeutics (Dollins et al., 2008; Sullenger, et al., 1990; O'Malley, et al., 1986; Burgert, et al., 2002). With the development of SELEX in the 1990s, several aptamers were quickly developed against important clinical targets such as vonWillebrand Factor (vWF), Platelet-derived growth factor (PDGF), E-selectin, Vascular endothelial growth factor (VEGF), Nuclear factor κB (NFκB), tenascin-C and Prostate Specific Membrane Antigen (PSMA) etc. The aptamers were generated with a mindset to function as novel anti-coagulants and therapeutics in case of cardiovascular diseases, angiogenesis, diabetes and cancer (Bunka, et al., 2006; Ni, et al., 2011). Some of the therapeutic applications of aptamer are described below.

Aptamers in Microbial Infections

Vivekananda *et. al.* generated DNA aptamers AT-27, AT-33, AT-36 and AT-49 that specifically inhibit the cytotoxic activity of α-toxin from *Staphylococcus aureus* using SELEX process. They demonstrated that the aptamers produced inhibit α-toxin-induced cytolysis and transcriptional activation of the inflammatory cytokines TNF-α and IL-17 in human Jurkat T cells. This cell line model was selected for testing because it was reported to be sensitive to α-toxin-induced cell death. Their results show a promising therapeutic potential; however it still need to be seen how these aptamers fair in animal models and later in clinical trials (Vivekananda, et al., 2014).

Aptamers in Eye Related Diseases

Pegaptanib (brand name Macugen), is an anti-vascular endothelial growth factor (VEGF) RNA aptamer and is the first nucleic acid aptamer approved by the US FDA. It was approved in December 2004 as a anti-angiogenic therapeutic agent for neovascular (wet) age-related macular degeneration (AMD), a disease of the eye which does not cause total blindness, but a loss of central vision, leaving only the side vision intact (Bunka and Stockley, 2006).Other aptamers developed against clinically important targets are currently undergoing clinical trials, and hopefully in future there would be more aptamer based therapeutics. ARC1905 is an RNA aptamer selected to bind complement component 5 (C5), an important protein in AMD. The complement system is part of the innate immune system that is based on a number of proteins. When the system is triggered, a cytokine cascade is started which recruits inflammatory cells and activates the membrane attack complex (MAC). Inhibition of C5 by ARC1905 potentially inhibits the inflammation and angiogenic components of AMD (Biesecker, et al., 1999). ARC1905 is administered by intravitreal injection and is currently in Phase I clinical trials by Ophthotech.

Aptamers in Cardiovascular Diseases

Thrombin is a serine protease and a key activator of several proteins in the coagulation cascade. Nu172 is a DNA aptamer that selectively binds and inhibits thrombin. It is administered intravenously during acute cardiovascular surgical procedures to prevent formation of blood clots. This aptamer is in Phase II clinical trials for anticoagulation in heart disease treatments (Waters, et al., 2009).

The von Willebrand factor (vWF) plays an important role in the clotting cascade by recruiting platelets to damaged arteries. ARC1779 is a DNA aptamer selected to bind to a region of vWF and blocks bind-

ing to platelet membrane glycoprotein Ib receptors, thus inducing antithrombotic effects (Diener, et al., 2009). It provides a potential therapeutic benefit in acute coronary syndromes and von Willebrand disease. Currently ARC1779 is in Phase II clinical trials for thrombotic microangiopathies and in patients with carotid artery disease undergoing carotid endarterectomy.

Aptamers in Cancer

AS1411 is a 26 nucleotide in length G-rich DNA aptamer considered to be the first anticancer aptamer. AS1411 targets nucleolin, a nuclear matrix protein which can be found on the surface of cancer cells. It has been demonstrated to be effective in many different preclinical cancers such as human breast cancer, lung cancer, pancreatic cancer, and acute myelogenous leukemia (Bates, et al., 2009; Girvan, et al., 2006; Ritchie, et al., 2007; Ritchie, et al., 2007; Soundararajan, et al., 2008). It is currently in Phase II clinical trials for acute myeloid leukemia by Antisoma Research.

NOX-A12 is a 45-nt L-RNA aptamer, based on L-ribose, selected against stromal cell-derived factor-1 (SDF-1), which binds to chemokine involved in tumor metastasis, angiogenesis, cell homing and tissue regeneration. Inhibition of the SDF-1 by NOX-A12 may open up a direction in the treatment of several types of cancer. It is developed by NOXXON Pharma AG (Berlin, Germany) and is currently in phase I clinical trials for the treatment of lymphoma, multiple myeloma and hematopoietic stem cell transplantation (Nolte, et al., 1996; Sayyed, et al., 2009).

Aptamers in Autoimmune Diseases

Ishiguro *et. al.* generated an aptamer Apt21-2 against human Interleukin-17 (IL-17), that also binds mouse IL-17 and blocks the interaction between IL-17A and its receptor. IL-17 is produced by the CD4+ T cell subset called Th17 cells, which are involved in host defense, inflammation, and autoimmune disorders. They observed that Apt21-2 inhibited the activity of IL-17 interms of production of IL-6 and IL-17 induced cytokine in mouse and human fibroblast cells. Also, it prevented the development of autoimmunity seen in different RA and EAE mouse models of autoimmunity, therby elucidating its its broad therapeutic potential in autoimmune diseases (Ishiguro, et al., 2011).

Aptamers in Neurological Diseases

Diseases of the nervous system are difficult to identify and treat because of the difficulty to access the nervous system. Alzeheimer's disease (AD) is one such example. The exact causes of AD remain unknown. The deposition of β-amyloid (Aβ) protein seems to disrupt the functional connectivity of neurons in the brain. Aptamers provide viable options for treatment- as small size allows for deep tissue penetration and crossing of the blood brain barrier. An aptamer that targets the β-secretase (BACE1) protein is designed to inhibit the formation of Aβ for treatment of AD Aβ is created by the cleavage of β-amyloid precursor protein by BACE1. The anti-BACE1 aptamer may help to elaborate on the cause of AD and provide new treatment options, but it still needs to undergo further testing (Rentmeister, et al., 2006).

Although distinct from classic Creutzfeldt–Jakob disease (CJD), Variant Creutzfeldt–Jakob disease (vCJD) is thought to be closely related to mad cow disease and is caused by deposition of an insoluble form of prion protein (PrP) in the brain. It is currently unknown what causes the structural change from the soluble form to the insoluble, infectious form. Since PrP binds to nucleic acids, it makes it difficult to obtain a functional aptamer. However , two aptamers have been developed which may be developed as therapeutics for vCJD due to their recognition of insoluble PrP over soluble PrP *in vitro* (Proske, et al., 2002; Rhie, et al., 2003).

Aptamers as Bioimaging Agent

The features of aptamers also make them a favorable candidate to be used as bioimaging agents. Aptamers can be synthesized with specific functional groups on 5'or 3' termini for site-specific conjugation to imaging agents without significantly affecting their binding affinity.

The first reports of using aptamers for imaging were in 1997 to identify sites of inflammation. Using a rat model, Charlton and colleagues used radiolabelled NX21909, a DNA aptamer which targets elastase and compared its benefits with a clinically proven, radiolabelled IgG-based imaging agent (Charlton, et al., 1997). Radiolabelled NX21909 was able to specifically accumulate at the site of inflammation and achieve

a high target to background ratio.

Vamira *et. al.* developed a technique for the for diagnostic imaging of ovarian cancer cells (SKOV-3) with high HER2 expression. HER2 is a member of epidermal growth factor receptor (EGFR) family. HER2 expression is increased to about 30% in several types of human tumors, including breast, head, neck, prostate, and ovary. HER2-targeted RNA aptamer was radiolabeled with 99mTc and assessed for its stability, its cell-specific binding in SKOV-3 ovarian cell lines and its biodistribution in SKOV-3 tumor-bearing mice. They observed that the use of modified RNA aptamers would further improve the
the overall stability and be more promising for *in vivo* applications (Kambiz, et al., 2013).

DNA aptamer A9 and A10 targeted to prostrate specific membrane antigen (PSMA) have been used in combination with QDs and super-paramagnetic iron oxide NPs for the recognition of cell receptors as well as optical imaging or MRI of the cancerous cells (Bagalkot, et al., 2007; Wang, et al., 2008; Zhang, et al., 2013). Kim *et. al.* developed a multifunctional aptamer nanoparticle based system for targeted computed tomography (CT) imaging and therapy of prostate cancer. They used an RNA aptamer for PSMA conjugated to GNPs for imaging. They observed that aptamer-conjugated GNPs showed more than 4-fold greater CT intensity for a targeted LNCaP cells than that of a nontargeted PC3 cells. Also, this system when loaded with doxorubicin was more potent against targeted LNCaP cells than against nontargeted PC3 cells thereby signifying its potential as a therapeutic agent also (Kim et al., 2010).

In vivo studies regarding imaging of tumors inside mice have also been made possible with the help of aptamers. TD05, an aptamer that specifically binds to Ramos cells (Human Burkitt's lymphoma cell line) was modified with fluorophores, successfully exhibiting aptamer fluorescence in an area overlapping with the site of the tumor in the mice (Tang, et al., 2007). Recently, Shi *et al.* developed a strategy to incorporate locked nucleic acids in the aptamer design of TD05 aptamer against Ramos cells to enhance their stability and the detection efficiency in serum. This approach would not only extend the imaging window *in vivo* but also facilitate the clinical usage of aptamers (Shi, et al., 2014).

According to recent report, an activatable aptamer based on sgc8 DNA aptamer which targets a cell membrane protein on CCRF-CEM cancer cells (Human T cell lymphoblast-like cell line) was used as an imaging probe (Shi et al., 2011). This strategy exploits the property where the aptamer is able to adopt a new conformation upon binding to its target. The optical signal is quenched when it is unbound, but once the aptamer binds its target a conformational change occurs, thereby resulting in fluorescence.

Bioimaging using aptamer technology provides a new direction for imaging of biomolecules. It offers rapid uptake by the target molecules, faster clearance of the residual aptamers and superior tumour imaging. In addition, it reduces the toxicity to normal tissues, which was often observed when radiolabeled antibodies were used.

Conclusions

Aptamer technology is evolving rapidly and is becoming more accessible by the introduction of new developments including for example improvisation and automation of the SELEX method making the selection process faster and more reproducible. In addition, the inherent properties of aptamer and the ease of modification make it the molecule of interest for a variety of applications. The advancements in the fields of nanotechnology, chemical biology and RNA interference have taken the aptamer technology to a new horizon with a plethora of applications in different spheres of life. In this review, we have tried to give a glimpse of the applications of aptamers in the field of biology. Aptamers have truly emerged as a strong player in form of biology including but not limited to sensing, diagnostics and therapeutics. More recently, the aptamer technology is being used extensively for biomarkers discovery and targeted cancer diagnosis in order to find solutions to some of the major problems faced today.

References

- Akira Ishiguro, Taishin Akiyama, Hironori Adachi, Jun-Ichiro Inoue and Yoshikazu Nakamura. Therapeutic potential of anti–interleukin-17A aptamer: Suppression of interleukin-17A signalling and attenuation of autoimmunity in two mouse models. *Arthritis & Rheumatism*, 2011, 63, 455–466.

- Bagalkot V, Zhang L, Levy-Nissenbaum E, et al: Quantum dot-aptamer conjugates for synchronous cancer imaging, therapy, energy transfer. *Nano Lett* 2007, 7, 3065-3070 2007.
- Bates PJ, Laber DA, Miller DM, Thomas SD, Trent JO. Discovery and development of the Grich oligonucleotide AS1411 as a novel treatment for cancer. *Exp. Mol. Pathol.* 2009, 86, 151–164.
- Beqa, L.; Fan, Z.; Singh, A.K.; Senapati, D.; Ray, P.C. Gold nano-popcorn attached SWCNT hybrid nanomaterial for targeted diagnosis and photothermal therapy of human breast cancer cells. *ACS Appl. Mater. Interfaces.* 2011, 3, 3316-3324.
- Biesecker G, Dihel L, Enney K, Bendele RA. Derivation of RNA aptamer inhibitors of human complement C5. *Immunopharmacol.* 1999, 42, 219–230.
- Bruno, J.G.; Miner, J.C. Therapeutic nucleic acid- 3'—Conjugates. *U.S. Patent Nos*. 7,910,297, 8,318,920, and 8,389,710, 5 March 2013.
- Bunka, D.H.; Stockley, P.G. Aptamers come of age—At last. *Nat. Rev. Microbiol.* 2006, 4, 588–596.
- Burgert, H.G.; Ruzsics, Z.; Obermeier, S.; Hilgen dorf, A.; Windheim, M.; Elsing, A. Subversion of host defense mechanisms by adenoviruses. *Curr. Top. Microbiol. Immunol.* 2002, 269, 273–318.
- Cao, Z.H. et. al. Reversible cell-specific drug delivery with aptamer functionalized liposomes. *Angew. Chem. Int. Ed.* 2009, 48,6494–6498.
- Charlton J, Sennello J, Smith D. In vivo imaging of inflammation using an aptamer inhibitor of human neutrophil elastase. *Chem. Biol,* 1997, 4,809–816.
- Chopra A, Sharma T.K., Lambadi P, Pathania P., Navani N.K. Aptamer-Nanoparticles Based Nanobiosensor for Bacterial Detection. *Nanotechnology Series* Chapter 2 (Volume 10), 2013.
- Cuiling Zhang, Xinghu Ji, Yuan Zhang, Guohua Zhou, Xianliang Ke, Hanzhong Wang, Philip Tinnefeld, Zhike He. One-Pot Synthesized Aptamer-Functionalized CdTe:Zn2+ Quantum Dots for Tumor-Targeted Fluorescence Imaging in Vitro and in Vivo. *Anal. Chem.* 2013, 85, 5843–5849.
- Daniels, D.A.; Chen, H.; Hicke, B.J.; Swiderek, K.M.; Gold, L. A tenascin-C aptamer identified by tumor cell SELEX: systematic evolution of ligands by exponential enrichment. *Proc. Natl. Acad. Sci. USA.* 2003, 100, 15416–15421.
- Dassie, J.P. et. al. (2009) Systemic administration of optimized aptamer-siRNA chimeras promotes regression of PSMA-expressing tumors. *Nat. Biotechnol.* 27, 839–849.
- Diener JL, Lagasse H.A. Daniel, Duerschmied D, Merhi Y, Tanguay JF, Hutabarat R, Gilbert J, Wagner DD, Schaub R. Inhibition of von Willebrand factor-mediated platelet activation and thrombosis by the anti-von Willebrand factor A1-domain aptamer ARC1779. *J. Thromb. Haemost.* 2009, 7,1155–1162.
- Dollins, C.M.; Nair, S.; Sullenger, B.A. Aptamers in immunotherapy. *Hum. Gene. Ther.* 2008, 19,443–450.
- Ellington, A.D.; Szostak, J.W. In vitro selection of RNA molecules that bind specific ligands.*Nature* 1990, 346, 818–822.
- Feng C.,Dai S., Wang L. Optical aptasensors for quantitative detection of small biomolecules: A review, *Biosen. Bioelec.* 2014, 59, 64–74.
- Galarreta, B.C.,Tabatabaei, M.,Guieu,V., Peyrin, E., Labarthet, F.L. Anal.*Bioanal. Chem.,* 2013, 405,1613–1621.
- Girvan AC, Teng Y, Casson LK, Thomas SD, Juliger S, Ball MW, Klein JB, Pierce WM Jr. Barve SS, Bates PJ. AGRO100 inhibits activation of nuclear factor-kappaB (NF-kappaB) by forming a complex with NF-kappaB essential modulator (NEMO) and nucleolin. *Mol. CancerTher.* 2006, 5, 1790–1799.
- Guthold, M.; Cubicciotti, R.; Superfine, R.; Taylor, R.M. Novel methodology to detect, isolate, amplify and characterize single aptamer molecules with desirable target-binding properties. *Biophys. J.* 2002, 82, 797.
- Han, K.; Liang, Z.; Zhou, N. Design strategies for aptamer-based biosensors. *Sensors* 2010, 10, 4541–4557.
- Herr J.K., Smith J.E., Medley C.D., Shangguan D., Tan W. Aptamer-Conjugated Nanoparticles for Selective Collection and Detection of Cancer Cells. *Anal. Chem.,* 2006, 78, 2918–2924.
- Huang, Y.F.; Chang, H.T.; Tan, W. Cancer cell targeting using multiple aptamers conjugated on nanorods. *Anal. Chem.* 2008, 80, 567-572.
- Huang, Y.F.; Sefah, K.; Bamrungsap, S.; Chang, H.T.; Tan, W. Selective photothermal therapy for mixed cancer cells using aptamer-conjugated nanorods. *Langmuir* 2008, 24, 11860-11865.
- Hui Shi, Xiaoxiao He, Wensi Cui, Kemin Wang-Ke Deng, Duo Li, Fengzhou Xu. Locked nucleic

acid/DNA chimeric aptamer probe for tumor diagnosis with improved serum stability and extended imaging window in vivo. *Anal. Chimica Acta,* 2014, 812, 138–144.

- J. Vivekananda, C. Salgado, J. Nancy, N. Millenbaugh. DNA aptamers as a novel approach to neutralize Staphylococcus aureus alpha-toxin. *Biochem. Biophys. Res. Comm.*2014, 444 , 433–438.
- K. Sefah, D. Shangguan, X. Xiong, M. B. O'Donoghue, and W. Tan, "Development of DNA aptamers using Cell-SELEX," *Nature Protocols*, 2010, 5, 1169–1185.
- KA Davis et. al., "Use of a High Affinity DNA Ligand in Flow Cytometry," *Nucl. Acid Res.* 1996, 24,702–706.
- Kambiz Varmira , Seyed Jalal Hosseinimehr , Zohreh Noaparast, Seyed Mohammad Abedi. AHER2-targeted RNA aptamer molecule labeled with 99mTc for single-photon imaging in malignant tumors. *Nuclear Med. Biol.*2013, 40, 980– 986
- Khati, M.; Schüman, M.; Ibrahim, J.; Sattentau, Q.; Gordon, S.; James, W. Neutralization of infectivity of diverse R5 clinical isolates of human immunodeficiency virus type 1 by gp120-binding 2′ F-RNA aptamers. *J. Virol.* 2003, 77, 12692–12698.
- Kim D, Jeong YY and Jon S: A drug-loaded aptamer-gold nanoparticle bioconjugate for combined CT imaging and therapy of prostate cancer. ACS Nano 2010, 6, 3689-3696.
- Kim Y, Wu Q, Hamerlik P, Hitomi M, Sloan AE, et. al. (2013) Aptamer identification of brain tumor- initiating cells. *Cancer Res*,2013, 73, 4923-4936.
- L. Gold, D. Ayers, J. Bertino et. al., "Aptamer-based multiplexed proteomic technology for biomarker discovery," *PLoS ONE*, 2010, 5, e15004.
- Lange MJ, Sharma TK, Whatley AS, Landon LA, Tempesta MA, Johnson MC, Burke DH. *Mol Ther.* 2012, 20, 2304-2314.
- Lee, J.F.; Stovall, G.M. and Ellington, A.D. Aptamer therapeutics advance. *Curr. Opin. Chem. Biol.* 2006, 10, 282–289.
- Lu, W.; Singh, A.K.; Khan, S.A.; Senapati, D.; Yu, H.; Ray, P.C. Gold nano-popcorn-based targeted diagnosis, nanotherapy treatment, and in situ monitoring of photothermal therapy response of prostate cancer cells using surface-enhanced Raman spectroscopy. *J. Am. Chem. Soc.*, 2010, 132, 18103-18114.
- Luo, Y.; Chen, M.; Wen, Q.; Zhao, M.; Zhang, B.; Li, X.; Fu, W. Rapid and simultaneous quantification of 4 urinary proteins by piezoelectric quartz crystal microbalance immunosensor array. *Clin. Chem.* 2006, 52, 2273-2280.
- Ma X, Jiang X, Jia F, Yu Y, Chen J, Wang Z. An aptamer-based electrochemical biosensor for the detection of Salmonella. *J. Microbiol. Method.* 2014, 98, 94–98.
- Mi J, Liu Y, Rabbani Z N, Yang Z, Urban J H, Sullenger B.A., Clary B.M. In vivo selection of tumor-targeting RNA motifs. *Nat Chem Biol.* 2010, 6, 1: 22–24.
- Misono, T.S.; Kumar, P.K. Selection of RNA aptamers against human influenza virus hemagglutinin using surface plasmon resonance. *Anal. Biochem.* 2005, 342, 312–317.
- N. L. Henry and D. F. Hayes, "Cancer biomarkers," *Mol. Oncol.*, 2012, 6, 140–146,.
- Nagarkatti R, de Araujo FF, Gupta C, Debrabant A. Aptamer Based, Non-PCR, Non-Serological Detection of Chagas Disease Biomarkers in Trypanosoma cruzi Infected Mice. *PLoS Negl Trop Dis*, 2014, 8, e2650.
- Nair, B.G.; Nagaoka, Y.; Morimoto, H.; Yoshida, Y.; Maekawa, T.; Kumar, D.S. Aptamer conjugated magnetic nanoparticles as nanosurgeons. *Nanotechnology*, 2010, 21, 455102.
- Ng, E.W.M.; Shima, D.T.; Calias, P.; Cunningham, E.T.; Guyer, D.R. and Adamis, A.P. Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease, *Nat. Rev. Drug Discov.* 2006, 5, 123–132.
- Ni X, Castanares M, Mukherjee A, Lupold S.E. Nucleic acid aptamers: clinical applications and promising new horizons. *Curr Med Chem.* 2011; 27, 4206–4214.
- Nolte A, Klussmann S, Bald R, et. al. Mirror-design of L-oligonucleotide ligands binding to Larginine. *Nat Biotechnol.* 1996, 14,1116–1119.
- O'Malley, R.P.; Mariano, T.M.; Siekierka, J.;Mathews, M.B. A mechanism for the control ofprotein synthesis by adenovirus VA RNAI. *Cell* 1986, 44, 391–400.
- Proske D, Gilch S, Wopfner F, Schätzl HM, Winnacker EL, Famulok M. Prion-protein-specific aptamer reduces PrPSc formation. *Chembiochem.* 2002, 2, 717-725.
- R. M. Ostroff, W. L. Bigbee, W. Franklin et. al., "Unlocking biomarker discovery: large scale application of aptamer proteomic technology for early detection of lung cancer," *PLoS ONE*, 2010

e15003.
- Rentmeister A, Bill A, Wahle T, Walter J, Famulok M. RNA aptamers selectively modulate protein recruitment to the cytoplasmic domain of beta-secretase BACE1 in vitro. *RNA.* 2006, 12,1650-60.
- Rhie A, Kirby L, Sayer N, Wellesley R, Disterer P, Sylvester I, Gill A, Hope J, James W, Tahiri-Alaoui AJ. Characterization of 2'-fluoro-RNA aptamers that bind preferentially to disease-associated conformations of prion protein and inhibit conversion. *Biol Chem.* 2003, 10, 39697-705.
- Ritchie C, Doran B, Shah K, Rowlinson-Busza G, Courtenay-Luck N, Jones D. Combination of the aptamer AS1411 with paclitaxel or Ara-C produces synergistic inhibition of cancer cell growth. *AACR.* 2007.
- Sayyed SG, Hagele H, Kulkarni OP, et. al. Podocytes produce homeostatic chemokine stromal cell-derived factor-1/CXCL12, which contributes to glomerulosclerosis, podocyte loss and albuminuria in a mouse model of type 2 diabetes. *Diabetologia.* 2009, 52, 2445–2454.
- Shangguan, D.; Li, Y.; Tang, Z.; Cao, Z.H.; Chen, H.W.; Mallikratchy, P.; Sefah, K.; Yang, C.J. and Tan, W. Aptamers evolved from live cells as effective molecular probes for cancer study. *Proc. Natl. Acad. Sci. USA.* 2006, 103, 11838–11843.
- Shi H, He X, Wang K, Wu X, Ye X, Guo Q, Tan W, Qing Z, Yang X, Zhou B. Activatable aptamer probe for contrast-enhanced in vivo cancer imaging based on cell membrane protein triggered conformation alteration. *Proc. Natl. Acad. Sci. U S A.* 2011; 108, 3900–3905.
- Soundararajan S, Chen W, Spicer EK, Courtenay-Luck N, Fernandes DJ. The nucleolin targeting aptamer AS1411 destabilizes Bcl-2 messenger RNA in human breast cancer cells. *Cancer Res.* 2008, 68, 2358–2365.
- Sullenger, B.A.; Gallardo, H.F.; Ungers, G.E.;Gilboa, E. Overexpression of TAR sequences renders cells resistant to human immunodeficiency virus replication. *Cell* 1990, 63, 601–608.
- T.K. Sharma, R. Shukla, Nanoscience and Aptamer Technology for Point of Care Diagnostics. *Nanosci Technol* . 2014, 1, 1-2
- T.K. Sharma, R. Shukla, Nucleic Acid Aptamers as an Emerging Diagnostic Tool for Animal Pathogens. *Adv. in Animal Vet. Sci.* 2014, 2, 50–55
- Taghdisi, S.M.; Lavaee, P.; Ramezani, M.; Abnous, K. Reversible targeting and controlled release delivery of daunorubicin to cancer cells by aptamer-wrapped carbon nanotubes. *Eur. J. Pharm. Biopharm.* 2011, 77, 200-206.
- Tan, W.; Wang, H.; Chen, Y.; Zhang, X.; Zhu, H.; Yang, C.; Yang, R.; Liu, C. Molecular aptamers for drug delivery. *Trends Biotechnol.,* 2011, 9, 634-640.
- Tang, Z.; Shangguan, D.; Wang, K.; Shi, H.; Sefah, K.; Mallikratchy, P.; Chen, H.W.; Li,Y. and Tan, W. Selection of aptamers for molecular recognition and characterization of cancer cells. *Anal. Chem.* 2007, 79, 4900–4907.
- Tsai, R.Y.; Reed, R.R. Identification of DNA
- Tuerk, C.; Gold, L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* 1990, 249, 505–510.
- Ulrich, H.; Magdesian, M.H.; Alves, M.J.M. and Colli, W. In Vitro Selection of RNA Aptamers That Bind to Cell Adhesion Receptors of Trypanosoma cruzi and Inhibit Cell Invasion. *J. Biol. Chem.* 2002, 277, 20756–20762.
- Wahajuddin, S.A.; Arora, S. Superparamagnetic iron oxide nanoparticles: Magnetic nanoplatforms as drug carriers. Int. *J. Nanomed.* 2012, 7, 3445-3471.
- Wang AZ, Bagalkot V, Vasilliou CC, et al: Superparamagnetic iron oxide nanoparticle-aptamer bioconjugates for combined prostate cancer imaging and therapy. *Chem Med Chem,* 2008, 3, 1311-1315.
- Waters EK, R.J. Schaub RG, Kurz JC. Effect of NU172 and bivalirudin on ecarin clotting time inhuman plasma and whole blood. *J. Thromb. Haemost.* 2009; (Supplement 2) Abstract PPWE-168.
- Wei-Yun Lai, Wei-Ya Wang , Yi-Chung Chang, Cheng-Ju Chang, Pan-Chyr Yang, Konan Peck Synergistic inhibition of lung cancer cell invasion, tumor growth and angiogenesis using aptamer siRNA chimeras. *Biomaterials,* 2014, 35, 2905-2914.
- Weng,C.H.; Huang C.J.; Lee G.B. Screening of Aptamers on Microfluidic Systems for Clinical Applications. *Sensors* 2012, 12, 9514-9529.
- Willis, M.C. et. al. Liposome anchored vascular endothelial growth factor aptamers. *Bioconjug. Chem.* 1998, 9, 573–582.
- Xiao, Z.Y.; Shangguan, D.H.; Cao, Z.H.; Fang, X.H. and Tan, W.H. Cell-specific internalization

study of an aptamer from whole cell selection. *Chem. Eur. J.* 2008, 14, 1769–1775.

- Yao c, Zhu T , i Qi Y, Zhao Y, Xia H, Fu W.Development of a Quartz Crystal Microbalance Biosensor with Aptamers as Bio-recognition Element. *Sensors* 2010, 10, 5859-5871.
- Zhang Z, Monsur Ali M, Eckert M.A., Kang D, Chen Y.Y. , Sender L.S., Fruman D.A., Zhao W. A polyvalent aptamer system for targeted drug delivery. *Biomaterials* 34 2013, 9728-9735.
- Zhang, B.; Mao, Q.; Zhang, X.; Jiang, T.; Chen, M.; Yu, F.; Fu, W. A novel piezoelectric quartz micro-array immunosensor based on self-assembled monolayer for determination of human chorionicgonadotropin. *Biosens. Bioelec.* 2004, 19,711-720.
- Zhiyuan Fang, Wei Wu, Xuew. Lateral flow biosensor for DNA extraction-free detection of salmonella based on aptamer mediated strand displacement amplification. *Biosen. Bioelec.* 2014, 56, 192–197.
- Zhou, J. et. al. (2008) Novel dual inhibitory function aptamer-siRNA delivery system for HIV-1 therapy. *Mol. Ther.* 16, 1481–1489.
- Zhu H, Suter J.D, White I. M .Aptamer Based Microsphere Biosensor for Thrombin Detection. *Sensors* 2006, 6, 785-795.
- Zuo X, Song S, Zhang J, Pan D, Wang L, Fan C..A Target-Responsive Electrochemical Aptamer Switch (TREAS) for Reagentless Detection of Nanomolar ATP. *J. Am. Chem. Soc.*, 2007, 129, 1042–1043.