Research in Pharmaceutical Sciences, Oct 2007; 2(2): 59-66
Received: Feb 2007
Accepted: July 2007

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

***Review Article***

# Medical applications of aptamers

**S. Vallian[1,*] and M.R. Khazaei[1]**

[1]*Division of Genetics, Department of Biology, Faculty of Science, Isfahan University, Isfahan, I.R.Iran.*

## *Abstract*

Aptamers define a new interesting class of receptor molecules with capability of binding to potentially any kind of molecules of interest. Structurally, they are composed of nucleic acids (RNA, DNA or a mixture of both) with high specificity and affinity to amino acids, drugs, proteins and other molecules. Apatmers are isolated from complex libraries of synthetic nucleic acids against the desired target molecules. They can be chemically modified to increase their stability and availability in biological environments. These molecules have potential applications in diagnostic assays such as conventional immunoassays, and in analytical devices including biosensors. Aptamers have been recently applied as antibodies against viral antigens and several key target molecules in cellular metabolic pathways. In this article, aptamers and their application in nanomedicine have been reviewed.

***Keywords:*** Aptamer; Nanomedicine; Biosensors

Receptor-ligand interaction is the principal of many *in vitro* and *in vivo* assays with applications in medicine and industry. Designing receptors with high specificity is one of the major areas of interest in bioresearch. Aptamers introduce a new class of receptors with capability of specific binding to virtually any kind of molecules (1-3). The word 'aptamer' was taken from the Latin aptus meaning 'fitting', and by analogy with antibodies, the targets bound by aptamers were called 'apatopes' (1). These receptors are typically composed of RNA, single stranded DNA or a combination of RNA/DNA (4,5). Aptamers can be chemically modified to extend their lifetime and availability in biological environments. They can distinguish very similar molecules such as enantiomers of small molecules (6). This property will be a function of the three dimensional structure of the folded nucleic acid, which produced by a combination of Watson Crick and non-canonical intra-molecular interactions. The three dimensional structure of an aptamer is uniquely determined by the sequence of its bases (7).

Aptamers became the central dogma in investigations involving ligand receptor interactions with many applications. They are used in analysis of the natural process of nucleic acidprotein recognition, detection of the presence of target molecules in complex mixtures; and used as inhibitors of enzymes, hormones, toxins, and antibodies with potential pharmacological utilization (8). Furthermore, aptamers have been used as molecular diagnostic and therapeutic tools in a verity of diseases (4,9). Here, we review some significant developments in the field of aptamer research with emphasis on diagnostic applications.

## Aptamers as nanoprobe

Aptamers can be made against any target molecule. Their targets cover a wide range of molecular sizes from simple ions,

*Corresponding author: Dr. S. Vallian
Tel. 0098 311 7932456
Email: svallian@medinews.com

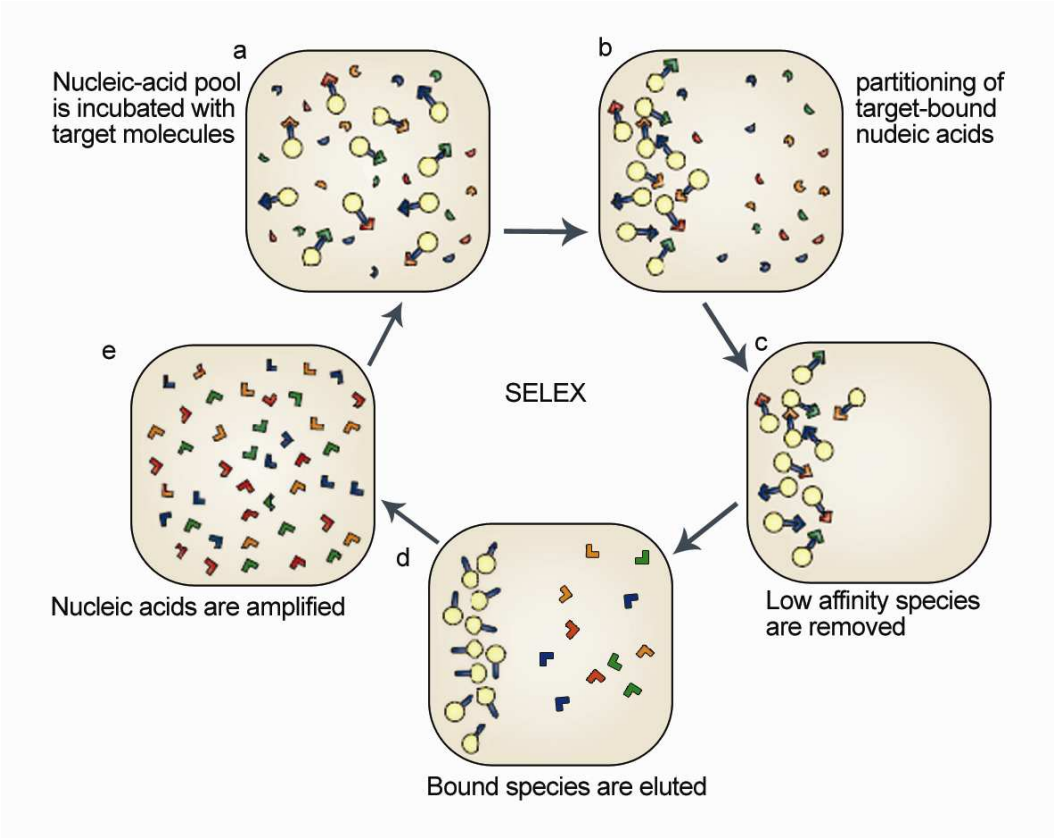


**Fig. 1.** The selective expansion of ligands by exponential enrichment, SELEX, method for isolation and purification of aptamers. An illustration of isolation of aptamers from an extremely large pool of random nucleic acids (RNA or DNA) sequences which were generated by the sequential randomized solid-phase synthesis of oligonucleotides. A-E represents the order of selection method (compiled and redrawn from refrence 27).

small molecules and peptides to complex proteins, organelles, viruses and even an entire cell (8,10,11). Also, reasonably diverse types of chemical targets, including organic and inorganic compounds and all kinds of biomolecules (e.g. saccharides, glycosides, antibiotics, vitamins, dopamine, cocaine and adenosine), can be recognized and bound by aptamers (12-14).

Aptamers against any desired molecule are made through a series of *in vitro* selection experiments called "selective expansion of ligands by exponential enrichment, SELEX". This method provides a powerful way for the identification of aptamers which bind with high affinity and specificity to target molecules (15,16). In this method, aptamers are isolated from an extremely large pool of random nucleic acid (RNA or DNA) sequences, generated by the sequential randomized solid-phase synthesis of oligonucleotides. Affinity chromatography is then used to isolate the nucleic acids with specific and selective binding characteristics to given target molecules. In fact, the molecules of interest (targets or ligands) are first immobilized on sepharose or synthetic beads and treated with a solution containing the random nucleic acids. Bound nucleic acids (aptamers) are then isolated, purified and characterized (e.g., sequenced). The selection protocol has now been reduced to an automated *in vitro* process, which enables high-throughput selection against an almost infinite set of targets (17) (Fig. 1). Having isolated, aptamers can be modified chemically by routine process, or can be amplified indirectly by cloning into an appropriate vector or directly using PCR or RT-PCR (18).

## The therapeutic potential of aptamers

Aptamers can interact tightly and specifically with their targets. This interaction may lead to different effects on the function of the target molecules. For example, binding of an aptamer to a target molecule can inhibit its binding to the cognate receptor, resulting in the alteration of its function. This property could allow aptamers to be readily used as pharmaceuticals. As instances, anti thrombin aptamers have been shown to block blood clotting (19,20), and anti-human neutrophil elastase aptamers have been used to inhibit neutrophil-mediated damage to lung tissues (21). Indeed, the first aptamer-based therapeutic agent named 'Macugen' entered clinical use for treating a form of macular degeneration (22). Moreover, aptamers have been selected against the whole Rous Sarcoma virus that efficiently inhibits the viral infection upon pre-incubation with the virus (10). Furthermore, it is conceivable that aptamers may be used in gene therapy. They can be used to inhibit the function of intracellular proteins in several genetic diseases in which abnormal proteins are causing the problem, e.g. mutant forms of p53 protein in prostate cancer (23,24). In this regard, the aptamer coding sequences can be introduced into the cells under an appropriate inducible promoter to target the p53 mutant mRNA in cytoplasm, inhibiting its translation. Moreover, aptamers have used as candidate theraputics for cardiovascular indications. Currently, there are both anticoagulant and anti thrombotic aptamers in the clinic (25). Taken together, these instances emphasize on the capability of aptamers to warrant their consideration as therapeutic agents (26).

Furthermore, some properties of aptamers make them attractive therapeutic agents compared to antibodies. Prominent among these are: i) their stability (they can be heated to 80 °C or stored in various solvents/harsh environments and they will return to their original conformation, providing a long shelf life); ii) seem to lack immunogenicity in studies in man, aptamers (whereas antibodies are significantly immunogenic precluding repeat dosing unless they are “humanized” or produced fully humane); iii) unlike antibodies, aptamers can be chemically modified to extend their lifetime in biological fluids and their availability in animals (13,27).

In the following section, several significant therapeutic applications of aptamers are briefly introduced.

## Blockade of angiogenesis and cancer by aptamers

Angiogenesis normally occurs during the body growth and wound healing that plays a central role in various disease states, including cancer and diabetic retinopathy. Aptamers selected to bind basic vascular endothelial growth factor (VEGF) have been shown to successfully inhibit VEGF binding to its receptors and inhibit *in vivo* the blood vessel growth, or angiogenesis and growth of tumors (28).

Plasminogen is the key serine protease in the fibrinolytic system and also appears to be involved in tumor invasion and metastasis. Introduction of vectors expressing aptamers against plasminogen and plasmin into cancerous cells have been resulted in successful prevention of metastasis in mice (29,30).

Recently, the first cancer therapeutic aptamer was introduced as aptamera (AS1411), which is currently in phase I trial in human (31). This aptamer forms an intracellular complex with nuclear factor-κB (NF-κB) essential modulator and nucleolin and thereby inhibits activation of NF-κB (32). Obviously the successful results of this new drug could promote the investigations toward introduction of more new therapeutic aptamers.

## Aptamers in the treatment of thrombosis

Thrombin is the key enzyme in regulation of thrombosis and haemostasis. Present anticoagulant and antithrombotic therapies rely on low molecular weight heparin and coumarin (33). Both therapies require close monitoring of the patients during the administration of the drugs to prevent sudden side effects such as systemic hemorrhage. This therapy is very unpleasant for long term application and in case of unwanted symptoms, very difficult to reverse the situation. A number of aptamers has been recently developed that function as antithrombotic agents. These molecules could bind specifically to the thrombin enzyme, inhibiting its activity. Due to the precise action of the aptamers, they have been shown to be effective in inhibiting clotting without any significant side effect. The application of aptamers as theraputic agents offers a potential effective alternative which is easier and safer comparing to the present therapies (34). Recently, an RNA aptamer targeting von Willebrand factor (VWF) has been described that can potently inhibit VWF-mediated platelet adhesion and aggregation. By targeting this important adhesion step, it was shown that the aptamer molecule can inhibit platelet aggregation in ristocetin-induced platelet aggregation assays.

## AIDS gene therapy by aptamers

In new strategies for gene therapy of AIDS, aptamers have been employed. In these new systems, vectors were used which express aptamers against the human immunodeficiency virus-1 (HIV-1) proteins, such as integrase, reverse transcriptase and nucleocapsid proteins (e.g. NCp7) (35,36). It was found that these aptamers, like Rev-decoys, were able to inhibit expression of the virus. Expression of an aptamer against HIV reverse transcriptase resulted in >75% reduction in viral replication (36). Application of these vectors *in vivo* may lead to a powerful therapy for the HIV infection.

## Diagnostic potential of aptamers

Given the high affinity and specificity for the target molecules, aptamers could have potential application in a verity of detection and diagnostic systems. For example, fluorescent labeled aptamers can be used to detect and measure serum amino acids such as phenylalanine, which is useful in diagnosis of hyperphenylalaninemia (e.g., phenylketonuria) (9). By using fluorescent labeled aptamers against surface proteins of pathogens, it would be possible to detect any infection by viruses, bacteria and even prions. Prions which are small proteins, found in the nervous system of mammals, and mutant forms of them have been found closely associated with spongiform encephalopathies (BSE, Scrapie etc.). It is thought that exposure to the mutant forms of prions may lead to infection in human. Aptamers have recently been synthesised that are capable of clear distinction between the pathogenic and non-pathogenic forms of these proteins (37,38).

Aptamers have also been used in a number of analytical methods. For example, in surface plasmon resonance (SPR) methods, aptamers are designed to detect activated 2'-5' oligoadenylate synthase and CD4. In these cases, the aptamers were used in the mobile phase and the target molecules in immobilized phase (39). Another method that is applicable to aptamer-based detection is enzyme-linked oligonucleotide assay (ELONA). The ELONA technique was first developed by the NeXstar company. This technique is basically an enzyme linked immunosorbent assay, ELISA, in which aptamers are used instead

of antibodies to detect the target molecules. So far, ELONA has been used successfully to detect some different proteins such as cytokines and Leishmania infantum H2A antigen (40,41). In another study, surface plasmon resonance was used as an efficient methodology for selecting aptamers that bind to hemagglutinin of human influenza virus. This procedure allowed monitoring and selection of target-bound aptamers specifically and simultaneously (42).

## Aptamer Microarrays

Human body is made of many different kinds of cells, each of which is suited to its particular function as a part of the whole. Every cell type has a unique molecular signature or proteome, the protein complement expressed by the genome. DNA microarrays (DNA chips) are revolutionizing biology and medicine by expanding our analysis from the study of gene expression to the study of genome wide gene transcription (43). However, DNA microarrays are limited in studying the proteome, due to: first, mRNA levels may not accurately reflect the corresponding level of a given protein; second, post-translational modification of proteins also regulates protein function. Using aptamers a new generation of DNA microarrays called aptamer microarrays (aptamer chips) is under development, which use both RNA and DNA oligoprobes (44,45). As in the DNA chips, each different target molecule in the cell extract will be localized to a different site on an aptamer microarray surface by its specific affinity to an aptamer probe, which is coated onto an array site of a glass slide. These microarrays could potentially quantify 102 to 105 different proteins in a cell (46). Furthermore, because of their high specificity, aptamers are capable of detecting differences in the type and level of post-translational protein modifications, and even the presence of mutant proteins (47) (Fig. 2).

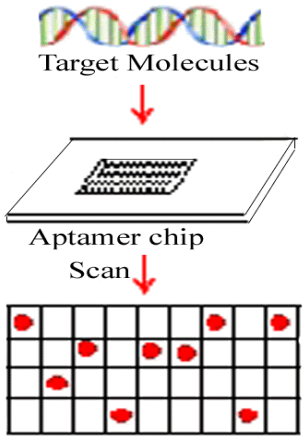


**Fig. 2.** A schematic representation of aptamer microarray (chips). As illustrated, each different target molecule (e.g. DNA) can be localized to a different site on an aptamer microarray surface by its specific affinity to an aptamer probe. The probe is coated onto an array site of a glass slide. The bond target molecules will then be easily quantified using a scanner linked to the computer with the appropriate software.

Aptamers seem to be more advantageous than antibodies in microarrays. First, aptamers could be regenerated multiple times without loss of sensitivity, while the antibody microarrays suffere irreversible damage. Second, the small size of aptamer provides a greater surface density of receptors (48). Furthermore, since aptamers can be selected for specific binding to small molecules, aptamer microarrays could also be useful for detecting small molecule ligands, such as cellular metabolites. By development of this technology, it may eventually prove possiblity to build an instrument that could simultaneously analyze metabolites, proteins, and nucleic acids. This instrument would also allow the digitization and study of the concentrations of all molecules involved in virtually all biochemical or signal transduction pathways. With the increasing applications

of proteomic strategies for cancer diagnosis, the detection of cancer related proteins, especially tumor onco-proteins could be facilitated by aptamer chips (49,50).

The application of aptamer microarrays to analyze and study the proteome has opened a new window on the etiology, early detection, diagnosis, and treatment of many cancers. It would be also possible to identify cancer-causing infections and environmental carcinogens. Moreover, the aptamer technology provided an alternative monitoring method, by which the protein changes in the blood could be recorded to trace the onset of carcinogenesis in the high-risk people. Such a test is even less invasive than the biopsy that is necessary for a DNA microarray analysis, which detects the messenger RNAs manufactured in tumor cells. Aptamer microarrays have been constructed for detection of many cancers such as the breast cancer. These microarrays are capable of detecting the disease-causing variants of the BRCA1 or BRCA2 proteins (49,50). Also aptamers have been used to detect biomarkers that are over expressed on cancer cell membranes (e.g., prostate-specific membrane antigen, PSMA) (51).

## Concluding remarks and future prospects

The studies performed on the application of aptamers clearly show that the aptamer technology is rapidly growing into a powerful technology. The ability of aptamers to mimic many monoclonal antibodies could result in the development of commercial kits for rapid diagnostic applications, especially in the cancer area. Moreover, advances in the understanding of the superstructure of ribonucleic acids would help in designing more efficient aptamer molecules to detect and deliver bio-molecules into cells. The generation of specific anti-viral aptamers against critical regions of the disease-associated viruses (e.g, the HR2 region of HIV gp41) is underway. Indeed, the rapid advances in the field of aptamers could result in the introduction of a new world of micro-target therapy, and could revolutionize therapeutic strategies such as gene therapy and anti-viral therapy.

## REFERENCES

1. Ellington AD, Szostak JW. *In vitro* selection of RNA molecules that bind specific ligands. Nature. 1990;346:818-822.
2. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science. 1990;249:505-510.
3. Nimjee R, Sullenger M. Aptamers: an emerging class of therapeutics. Ann Rev Med. 2005;56:555-583.
4. Gewirtz AM. Oligonucleotide therapeutics: clothing the emperor. Curr Opin Mol Ther. 1999;1:297-306.
5. Feigon J, Dieckmann T, Smith FW. Aptamer structures from A to zeta. Chem Biol. 1996;3: 611-617.
6. Jenison RD. High-resolution molecular discrimination by RNA. Science.1994;263:425-459.
7. Fredriksson S, Gullberg M, Jarvius J, Olsson C,Pietras K, Gustafsdottir SM, et al. Protein detection using proximity-dependent DNA ligation assays. Nat Biotech. 2002;20:473-477.
8. Murphy MB, Fuller ST, Richardson PM, Doyle SA. An improved method for the in vitro evolution of aptamers and applications in protein detection and purification. Nuc Acids Res. 2003;31:e110.
9. Osborne SE, Matsumura I, Ellington AD. Aptamers as therapeutic and diagnostic reagents: problems and prospects. Curr Opin Chem. 1997;1:5-9.
10. But K, Denk C, Fitscher B, Crnkovic-Mertens I, Ullmann A, Schroder CH, et al. Peptide aptamers targeting the hepatitis B virus core protein: a new class of molecules with antiviral activity. Oncogene. 2001;20:6579-6586.
11. Shangguan D, Meng L, Cao ZC, Xiao Z, Fang X, Li Y, et al. Identification of liver cancer-specific aptamers using whole live cells. Anal Chem. 2008;1;80:721-728.

12. Mannironi C. *In vitro* selection of dopamine RNA ligands. Biochemistry. 1997;36:9726-9734.
13. Baker L, Wood D, Heeger P. An electronic aptamer-based small molecule sensor for the rapid, label-free detection of cocaine in adulterated samples and biological fluids. J Am Chem Soc. 2006;128:3138-139.
14. Zayats H, Gill M, Willner M. Label-free and reagent less aptamer-based sensors for small molecules. J Am Chem Soc. 2006;128:13666-13667.
15. Golden MC, Collins BD, Willis MC, Koch TH. Diagnostic potential of Photo SELEX-evolved ssDNA aptamers. J Biotechnol. 2000;81:167-178.
16. Shamah SM, Healy JM, Cload ST. Complex target SELEX. Acc Chem Res. 2008;41:130-138.
17. Ellington AD, Cox JC, Lee JF, Collett JR. Automated *in vitro* selection and microarray applications for functional RNA sequences In: Gesteland RF, Cech TR Atkins JF, editors. The RNA world. 3rd ed. New York: Cold Spring Harbor; 2006. p. 683-719
18. Kaur S. Affinity selection and mass spectrometry-based strategies to identify lead compounds in combinatorial libraries. J Protein Chem. 1997;16:505-511.
19. Reyderman L, Stavchansky S. Pharmaco-kinetics and biodistribution of a nucleotide-based thrombin inhibitor in rats. Pharm Res. 1998;15:904-910.
20. Müller J, Wulffen B, Pötzsch B, Mayer G. Multidomain targeting generates a high-affinity thrombin-inhibiting bivalent aptamer.Chem-BioChem. 2007;8:2223-2226.
21. Bless NM. Protective effects of an aptamer inhibitor of neutrophil elastase in lung inflammatory injury. Curr Biol. 1997;7:877-80.
22. Schachat AP. New treatments for age-related macular degeneration. Ophthalmology. 2005; 112:531-532.
23. Green LS. Inhibitory DNA ligands to platelet-derived growth factor B-chain. Biochemistry. 1996;35:14413-14424.
24. Floege J. Novel approach to specific growth factor inhibition *in vivo*: antagonism of platelet-derived growth factor in glomerulo-nephritis by aptamers. Am J Path. 1999; 154:169-179.
25. Keefe AD, Schaub RG. Aptamers as candidate therapeutics for cardiovascular indications. Curr Opin Pharmacol. 2008;8:147-152.
26. Kulbachinskiy AV. Methods for selection of aptamers to protein targets. Biochemistry (Mosc). 2007;72:1505-1518.
27. Bunka DHJ, Stockley PG. Aptamers come of age at last. Nat Rev Microb. 2006;4:588-596.
28. Hasegawa H, Sode K, Ikebukuro K. Selection of DNA aptamers against VEGF (165) using a protein competitor and the aptamer blotting method. Biotechnol Lett. 2008;30:829-834.
29. Lupold SE, Hicke BJ, Lin Y, Coffey DS. Identification and characterization of nuclease-stabilized RNA molecules that bind human prostate cancer cells via the prostate-specific membrane antigen. Cancer Res. 2002;62:4029-4033.
30. Zhai G, Iskandar M, Barilla K. Romaniuk. Characterization of RNA aptamer binding by the Wilms' tumor suppressor protein WT1. Biochemistry. 2001;40:2032-2040.
31. Ireson L, Kelland R. Discovery and develop-ment of anticancer aptamers. Mol Cancer Ther. 2006;5:2957-2962.
32. Girvan T, Casson T, Jüliger B. AGRO100 inhibits activation of nuclear factor-kappaB (NF-kappaB) by forming a complex with NF-kappaB essential modulator (NEMO) and nucleolin. Mol. Cancer Ther. 2006;5:1790-1799.
33. Nobile V. Inhibition of human angiogenin by DNA aptamers: nuclear colocalization of an angiogenin-inhibitor complex. Biochemistry. 1998;37:6857-6863.
34. Haung J, Moore J, Soffer S, Kim E, Rowe D, Manley CA, et al. Highly specific anti-angiogenic therapy is effective in suppressing growth of experimental Wilms tumors. J Pediatr Surg. 2001;36:357-361.
35. Symensma TL. RNA aptamers selected to bind human immunodeficiency virus type 1 rev *in vitro* are rev responsive *in vivo*. J Virology. 1996;70:79-87.
36. Jing NIon selective folding of loop domains in a potent anti-HIV oligonucleotide. Biochemistry. 1997;36:2498-2505.
37. Weiss S. RNA aptamers specifically interact with the prion protein PrP. J Virology. 1997;71:8790-8797.
38. Gilch S, Kehler C, Schatzl HM. Peptide aptamers expressed in the secretory pathway interfere with cellular PrPSc formation. J Mol Biol. 2007;371:362-373.
39. Kraus E, James W, Barclay AN. Novel RNA ligands able to bind CD4 antigen and inhibit CD4+ T lymphocyte function. J Immunol. 1998;160:5209-5212.

40. Guthrie JW, Hamula CLA, Zhang H, Le XC. Assays for cytokines using aptamers. Methods. 2006;38:324-330.
41. Ramos P, Soto A, Martin S. A DNA aptamer population specifically detects Leishmania H2A antigen. Lab Invest. 2007;87:409-416.
42. Misono TS, Kumar PKR Selection of RNA aptamers against human influenza virus hemagglutinine using surface plasmon resonance. Anal Biochem. 2005;342:312-317.
43. Vallian S, Khazaey MR DNA microchips technology and its application in molecular diagnosis of cancer and genetic diseases. Tashkhis Azmayeshgahi. 2002;8:10-16.
44. Brody EN, Willis MC, Smith JD, Jayasena S, Zichi D, Gold L. The use of aptamers in large arrays for molecular diagnostics. Mol Diagn. 1999;4:381-388.
45. Lee M, Walt DR A fiber-optic microarray biosensor using aptamers as receptors. Anal. Biochem. 2000;282:142-146.
46. Cho EJ, Collett JR, Szafranska AE, Ellington AD. Optimization of aptamer microarray technology for multiple protein targets. Anal Chimi Acta. 2006;564:82-90.
47. Li L. Detection of protein biomarkers using RNA aptamer microarrays and enzymatically amplified surface plasmon resonance imaging. Anal Chem. 2007;79:1082-1088.
48. Petersen L, Prohaska W. An aptamer-based quartz crystal protein biosensor. Anal Chem. 2002;74:4488-4495.
49. Walter G,Bussow K, Lueking A,Glokler J. High-throughput protein arrays: prospects for molecular diagnostics. Trends Mol Med. 2002;8:250-253.
50. Elenbaas B. The MDM2 oncoprotein binds specifically to RNA through its ring finger domain. Mol Med. 1996;2:439-451.
51. Farokhzad C, Teply S, Kantoff J. Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy *in vivo*. Proc Nat Acad Sci USA. 2006;103:6315-6320.