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Review Article

APTAMER: A REVIEW ON IT'S *IN VITRO* SELECTION AND DRUG DELIVERY SYSTEM

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ABSTRACT

In recent year, Aptamer has been one of the key tools in the field of advanced drug delivery systems. Aptamer are oligonucleotides or peptides that bind to a specific target molecule. In this review we summarize the major differences between the antibody and an Aptamer along with the different methodology of the *In vitro* selection of the Aptamer by using SELEX (Systematic evolution of ligands by exponential enrichment) technique. SELEX is a technique which has a based biosensor and some of the novel drug delivery system. The article referred in this review was referred from the above said source was in the range of 1990-2020 y.

Primary contents is searched from Science Direct, Springer Nature, Scopus Indexed journals. The resources are downloaded from Google Scholar, peer-reviewed published literature from scientific journals and books.

Keywords: Aptamer, SELEX, Biosensors, Novel drug delivery system, Nanoparticle, Diagnosis

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INTRODUCTION

Aptamer are single stranded folded oligonucleotides and peptide that binds to molecular protein, which target with the high affinity and specificity because of their specific three-dimensional structure [1, 2]. They can be single standard deoxyribo nuclei acid (ssDNA) or ribonucleic acid (RNA) Aptamer. RNA and ssDNA Aptamer may be differed from one another in the aspects of sequence and folding pattern, even though they bind to the same target. Aptamer have the number of unique and effective properties as compare to the antibodies which include once the Aptamer is developed can undergo amplification through Polymerase chain reaction [PCR] in order to produce larger quantity which will be having high purity. Since the Aptamer own simple chemical structure which makes them more revisable which can be further modified with required functional group as per different purpose. Finally, the stability of the Aptamer is much greater than that of antibody making the Aptamer to show their suitable application even at harsh condition i.e. High temperature and pH [3].

NeXagen and Nexstar developed the Aptamer as a therapeutic agent, which is exact analogous of antibody. NX1838 [now called Macugen] was the first Aptamer which was taken in the clinic. NX1838 acts antagonist for vascular endothelial growth factor [VEGF] nothing but angiogenesis inhibitor [4, 5]. Aptamer are similar to monoclonal antibodies which can be prepared by using SELEX [6, 7]. SELEX is the *in vitro* selection technique which is used to isolate the appropriate Aptamer for the given target. It consists of the three major steps, which include the generation of the library, binding/separation and amplification. The main aim of this review to understand the recent trends in the field of the drug delivery system using various Aptamer along with their *in vitro* selection procedure and their application.

Difference between aptamer and antibodies

Structure of aptamer

It is a single or double-stranded RNA/DNA molecule. Where DNA/RNA are double-helical or single strand molecules with bases adenine and thymine or guanine and cytosine in case of DNA. Where has in case of RNA adenine and uracil are positioned at opposite side and rest of the base pairs are similar to DNA and bonded via hydrogen bonds which is not only stable conformation of DNA and RNA there is one more stable structure is formed in guanine rich environment which consists of four-stranded motifs called a G-quadruplex [fig. 1 and table 1] in which guanines are able to associate themselves via non-covalent interactions so due to which it allow the strands of DNA/RNA to fold in stable two or three-dimensional structure maximizing the amount of favorable interaction between the nucleotide. A large amount of Aptamer occupies such a G-quadruplex structure [6].

Table 1: Difference between aptamer and antibody

S. No.	Aptamer	Antibodies	Reference
1.	They are oligonucleotide and protein	They are protein in nature	[7, 8]
2.	The shows resistance towards high temperature and they can be regenerated easily after denaturation.	Doesn't show resistance towards high temperature.	
3.	Shelf life is prolonged	Shelf life is short and limited	
4.	Viral and bacterial contamination not problematic during production	Viral or bacterial contamination possible normally produced *in vivo* due to which batch-to-batch variation may occur	
5.	Pharmacokinetic[pk] parameter can be changed on demand	Difficult to modify Pharmacokinetic[pk] parameters	
6.	Investigator determine target site of protein	Immune system determine the target site of protein	
7.	No evidence of immunogenicity	Significant immunogenicity	

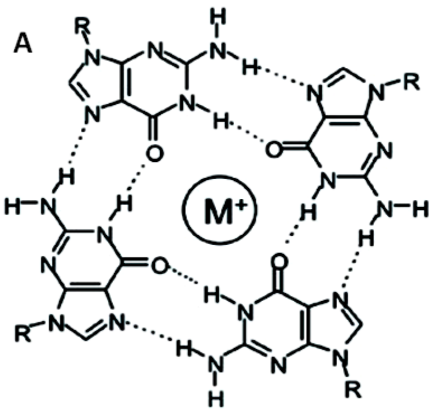

Fig. 1: [A] Planar guanine tetrad formed via Hoogsteen base-pairing. The circle in the middle represents a positive metal ion. [B] A G-quadruplex is formed by multiple stacks of these planar structures on top of each other [2]

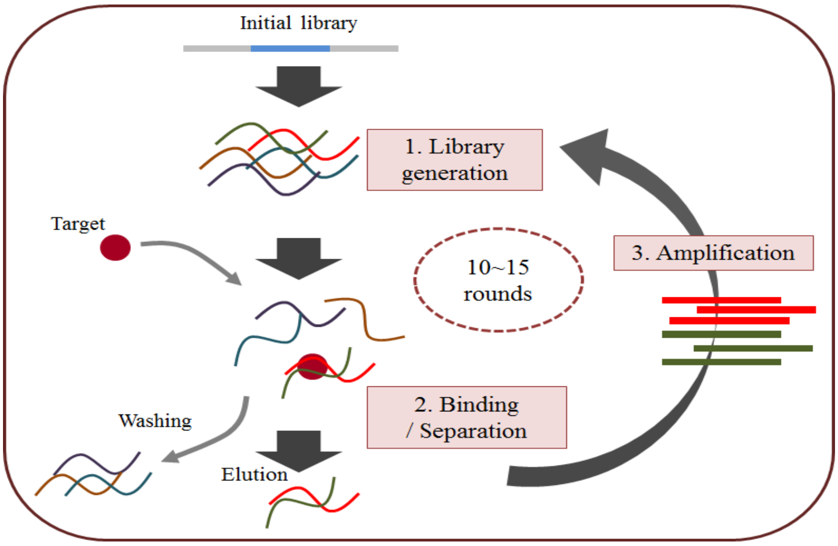

Fig. 2: Schematic representation of the SELEX *in vitro* selection of the Aptamer. (Step 1) library generation, (Step 2) binding and separation, (Step 3) amplification [3]

***In vitro* selection of aptamer**

SELEX is an *in vitro* selection technique as shown in fig. 2, which is used to isolate the Aptamer which is having high affinity towards the given target from approximately 1012-10-15 combinatorial oligonucleotide libraries [6, 7].

Different method includes

Nitrocellulose membrane filtration

Nitrocellulose membrane are used to immobilize proteins by western blots and atomic force microscopy (AFM) since it provide simple and rapid protein immobilization [8]. In 1968, nitrocellulose membrane was used by Kramlova's group to separate a protein from an RNA molecule [9]. For example, during the initial establishment of SELEX method by gold's group, the Aptamer against the T4 DNA polymerase was developed by using the nitrocellulose membrane method [10, 11].

Affinity chromatography and magnetic bead-based SELEX

It is the method in which the separation of the components from the biochemical mixture take place. It is mainly used for the purification of recombinant proteins based on specificity, such as the interaction between a receptor and a ligand or an antigen and an antibody. In this method, it generally consists of the immobilized phase, which consists of agarose-based bonds, and the beads are packed on a column for the elution process [12].

In the binding and separation steps of SELEX the principle used is affinity chromatography in which the library component, which is having the higher affinity towards the target which is being immobilized on the beads is selected (fig. 3). Several Aptamer can be developed by using this method by making an affinity column containing target immobilized beads [13]. The disadvantage of this method is that it cannot apply if the target lacks the affinity tag or functional group needed for coupling [14].

Capillary electrophoresis based SELEX

The capillary electrophoresis has many advantages has compared to the other SELEX method in the aspects of speed, resolution, capacity and minimal sample diffusion. In this method, ionic species are separated by their charge, frictional forces and hydrodynamic under the presence of an electric field [10]. The greatest advantage of applying this method the successful selection of the Aptamer can be achieved within very few rounds generally 2-4 rounds, in comparison of the other method. For example:-In bower's method, Aptamer for neuropeptide Y and human IgG within for rounds of the selection [15-17].

Microfluidic SELEX method

This method is mainly processed on chip. The main advantage of this SELEX it can be performed over a small scale. For example, DNA Aptamer, which is specifically bound to the neurotoxin type B is developed by using Continuous Flow Magnetic Activated Chip Based Separation [CMACS] in which within the single round the Aptamer is selected and the above this used for the development of the Aptamer is designed by the soh's group and the screening of C-reactive protein (CRP) specific Aptamer is performed by using microfluidic system and magnetic bead conjugated with CRP [18, 19].

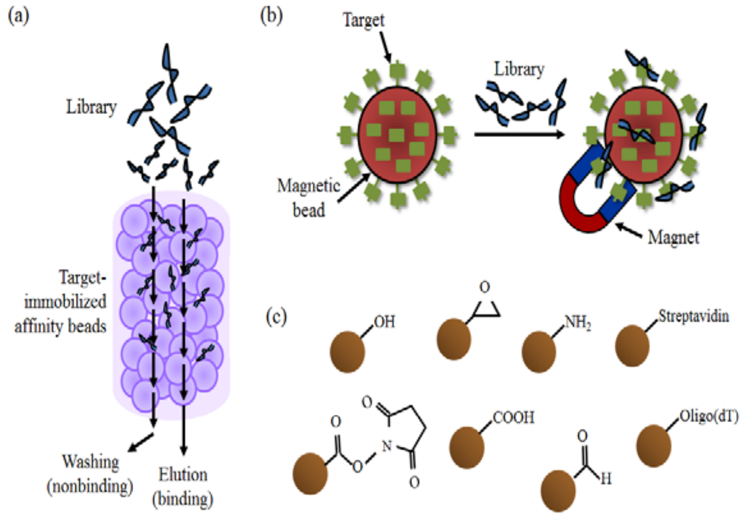

Fig. 3: A schematic representation of the steps involved in the selection of the Aptamer using affinity chromatography and magnetic-based SELEX (a) selection by using the affinity column from the library; (b) selection process by using the magnetic beads; (c) Several types of functional group-activated beads such as to syl-activated beads and epoxy-activated bead [10]

Cell SELEX

The steps involved in the cell SELEX (fig. 2 and fig. 4) are similar to that of traditional SELEX which include incubation, partitioning and amplification. The important protocol of cell SELEX it involves positive selection and negative selection. The step involved in the negative selection is very much important in order to remove the sequence which is binding to the normal cells and in order to enhance the specificity of candidate Aptamer [20].

Some modified cell SELEX methods is being developed in the past few years in order to enhance the efficiency and to enrich the Aptamer screening [21].

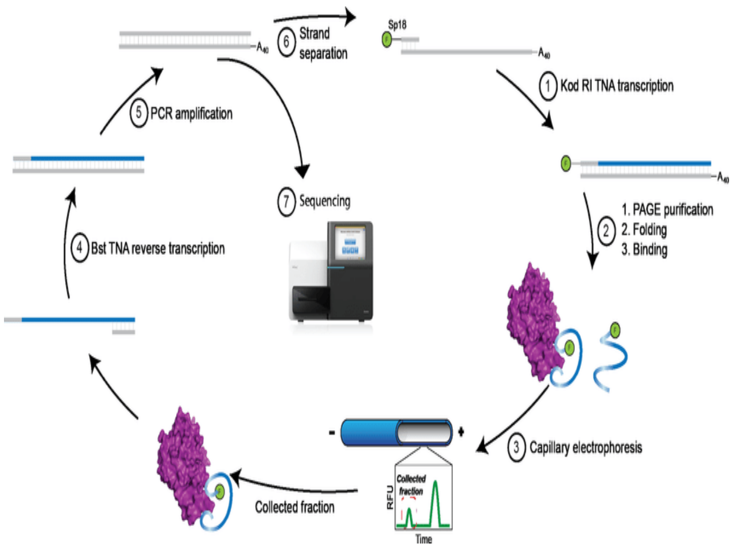

Fig. 4: Schematic representation of the steps involved in the capillary electrophoresis based SELEX method for the selection of the Aptamer [10, 20]

Modified cell SELEX method

a) Fluorescence activated cell sorting SELEX [FACS-SELEX]

In this method, a cytometry device is used to separate the cell [target] which is bound to Aptamer from the unbound Aptamer, which is based upon the principle of fluorescence and scattering. Mayer *et al.* developed this technique in which the isolation and identification of the bound Aptamer to the target is carried out by using FACS device [22].

b) Cell internalization SELEX

The major advantage of this method which is being stated by many studies that in this method the Aptamer is not only bound to the cell surface, but it is also intracellular transported [23, 24].

c) 3D cell SELEX

This method is the combination of the three-dimensional [3D] cell culture and cell SELEX method, which is used for the development of the appropriate Aptamer against the target molecule. In this method 3D cell culture is used since it provide or mimics the natural cellular environment in which the cell grows; therefore, it provides physiologically suitable environment which will be helpful for improving the research and drug discovery process. The 3D cell structure is developed from the two-dimensional cell cultures with the help of magnetic levitation technology [MLT] [25].

d) Ligand gaided selection [LIGs]

In this method the Aptamer is developed against the epitopes of interest which is expressed over the target cells [26].

e) Cross over SELEX

This method was developed by the Hicke's laboratory; the main aim of this method is to develop the Aptamer with enhanced efficiency and this method also avoid the generation of the Aptamer against biomarker or molecule expressed on the target cells[27].

Other method based SELEX

AFM [Atomic field microscope], Electrophoretic mobility shift assays (ESMA) Surface Plasmon resonance (SPP) these are the other methods that have been performed with SELEX [28-30]. The major advantage of this method is to reduce the number of selection rounds. However, the effectiveness of this method is no clearly demonstrated [31].

Aptamer based sensor

The sensor which is present over the Aptamer and act as a recognition site is called aptasensors [32], can be developed by the various technique and methodology.

Electrochemical biosensors

The main advantage of the electrochemical Aptasensors is, its high sensitivity, compatibility with novel microfabrication technologies, inherent miniaturization and low cost. There are a various technique that are used for the fabrication of the electrochemical Aptasensors some of them are ETS (Electrochemical Impedance Spectroscopy), Potentiometry with ISEs (Ion Selective Electrodes), ECL(Electrogenerated Chemiluminescence), CV (Cyclic Voltammetry) and DPV(Differential Pulse Voltammetry) [33-38].

Optical biosensors

Fluorescence-based APTA sensors

This optical biosensor is commonly based on the consolidation of a fluorophore or a nanoparticle. In this method for the fluorescence detection the Aptamer is labeled with both quencher and fluorophore for example the cocaine specific Aptamer was able to detect the target by using FRET [Fluorescence Quenching/Fluorescence Resonance Energy Transfer] signal between fluorescence and DABCYL moiety [A Quencher] [39].

Colorimetric based APTA sensors

In this method the colour changing novel reagent such as AUNPs or other polymer are used which is called as colorimeter [40].

Apart for the above sensor there are various other sensors that have been exploited in the combination with the various types of the analytical equipment such as those used for SAW [Surface Ascoustic Wave], QCM [Quartz Crystal Microbalance] and microchannel cantilever sensor [41-43].

Different drug delivery system

Liposome based nanostructure for APTAMER directed delivery

Nanomaterial based drug delivery have special attention due to its physical, chemical and biological properties, especially in many targeted drug delivery systems have been successfully used for cancer therapy. Among them, the liposome based drug delivery system is one of the most successfully established technologies. Liposomes are a spherical structure with lipid bilayer. Liposomes surface can be passivated with ligand such as Polyethylene Glycol (PEG), which extends their systematic circulation time and exhibit preferential accumulation at the tumor site. Due to the excellent properties of Aptamer which are correlated with the liposomes, therefore it makes the Aptamer ideal for the preparation of multifunctional target specific liposomes.

The first Aptamer conjugated multifunctional liposomes, nanostructure for potential targeted drug delivery system was accomplished by using sgc8 Aptamer which has a high binding affinity (kd=0.8) towards leukemia CEM-CCRF cells. By using the sgc8 Aptamer a therapeutic liposome drug delivery system is developed by covalently linking the sgc8 Aptamer to the liposomes by using PEG spacer [44, 45].

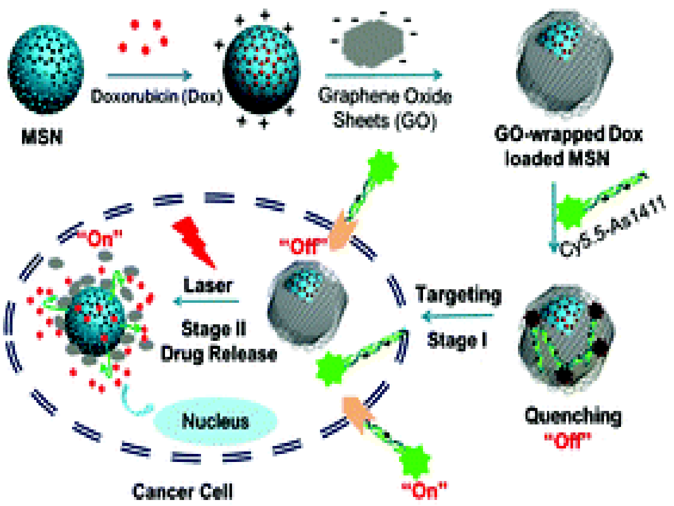

Fig. 5: Schematic illustration of acy5,5-AS1411 Aptamer targeting photoresponsive drug delivery system [26, 27]

An APTAMER targeting photoresponsive drug delivery system

It is developed by using the assembly of acy 5,5-AS1411 (fig. 5). Aptamer by non covalently conjugating over the surface of graphene oxide, which is being wrapped with doxorubicin(DOX) loaded mesoporous silica nanoparticles (MSN-DOX@GO-apt) for the light-mediated drug release and the Aptamer targeting the cancer therapy. It consists of ON and OFF switch is controlled by Aptamer targeting and light triggering. The graphene oxide(GO) prevent the leaking of the loaded DOX in the absences of laser irradiation and ensures the DOX release in response to laser irradiation. If the GO wrapping falls off upon laser irradiation, the off-on photoresponsive drug delivery is activated, thus including chemotherapy. As the result with increase in the laser power, the synergism of chemotherapy and phototherapy in a single MSN-DOX@GO-Apt platform led to much more effective cancer cell killing than monotherapy [46].

APTAMER functionalized PEG-PLGA nanoparticle for enhanced anti-glioma drug delivery

The above-mentioned drug delivery system is developed by using the AS11411 which specifically bind to nucleolin and targeting ligand in order to facilitate anti-glioma delivery of paclitaxel (ptx). Here the Aptamer is conjugated via an EDC/NHS technique over the surface of PEG-PLGA and resultant conjugation is confirmed by urea PAGE and XPS. As the result, the obtained Ap-ptx-NP should be uniformly round, particle size at 156.0±54.8 nm and zeta potential at-32.93±3.1 mV [47].

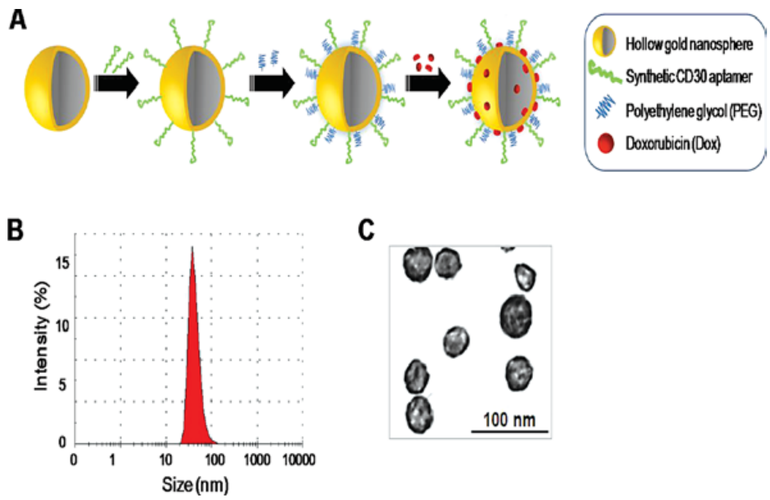

Fig. 6: Schematic representation for the development of the Apt-HAuNS-DOX (a) Aptamer and PEG are conjugated over the surface of HAuNS via covalent S-Au bond followed by loading with doxorubicin by a charge force (b) indicates the peak size of formed Apt-HAuNS-DOX 42 nm measurement which is carried out by dynamic light scattering method (c) Transmission electronmicrograph of the Apt-HAuNS-Dox with scale bar [50]

APTAMER–equipped and doxorubicin-loaded hollow gold Nanospheres drug delivery system for selective killing tumor cells

For selective targeting of tumor cells, the surface of HAuNS is chemical conjugated with 39-mer RNA Aptamer specific CD30 [48], which is diagnostic marker for Hodgkin's lymphoma and anaplastic large cell lymphoma [49]. To enhance biostability, surface modification of the Apt-HAuNS was subsequently performed using polyethylene glycol (PEG) then DOX was loaded through charge force [50]. Dox loaded into Apt-HAuNS was monitored by quantifying residue-free Dox in the reaction with a UV-Vis absorption assay, which indicated that Aptamer conjugation had no effect on Dox loading efficiency. The resultant conjugate (i.e. Apt-HAuNS-DOX) is stable under normal biological condition (pH 7.4), ultrasensitive towards pH changes and also rapidly release about 80% of the loaded DOX with in the period of 2 h which happens at the pH of 5.0 is the condition which can be observed in cell lysosome. The functional assay using the cell mixture (containing both normal with lymphoma tumor cells) is carried out, and the results show that the conjugate of Apt-HAuNS-DOX selectively kills the tumor cells but doesn't show any effect over the growth of any normal cells in the cell mixture. As the result, the Apt-HAuNS-DOX can be used as a selective treatment for cancer i.e. lymphoma tumor cells (fig. 6) [48].

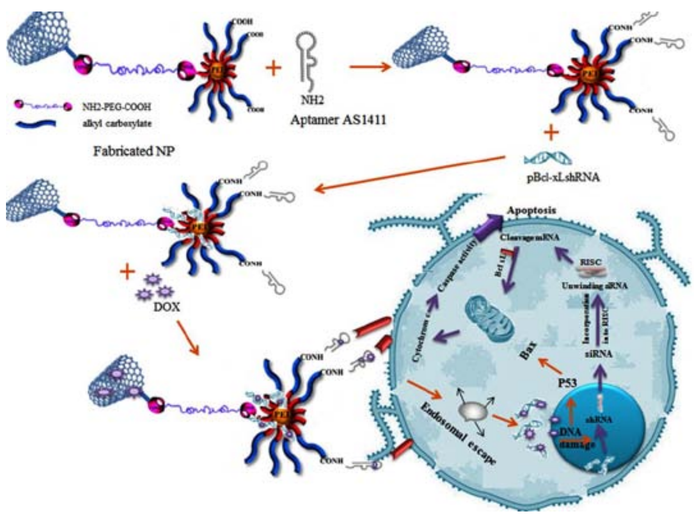

Fig. 7: Schematic illustration of polyethylamine functionalized carbon nanotube tagged with AS1411 APTAMER and intercalation of DOX with Pbcl-xL-shRNA-SWNCT-PEG-10-10%PEI-Apt [66]

Table 2: Application and therapeutically uses of the aptamer

Nature	Ligand/target	Aptamer	Diseases
	PSMA [Prostate-specific membrane antigen]	RNA Aptamer	Cancer [52]
RNA	T-cell factor 1	Aptamer number 5	Colon cancer [53]
	Prostate specific membrane antigen[PSMA]	ssDNA-A9 PSMA	Cancer [54]
	Cancer stem cell surface marker	EpCAM[epithelial cell adhesion molecule]RNA Aptamer	Cancer [55]
	WT1	RNA Aptamer22	Wilm's tumor [56]
	HIV-1 susceptible cells	CCR5 RNA Aptamer	Inhibit HIV-1 infectivity [57]
	β-catenin	RNA Aptamer	Colon cancer [58]
	L-selectin	RNA Aptamer [10th round 4 °c]	Inflammation [59]
	Coagulation factor IX	RNA Aptamer	Acute coronary syndrome [60]
	AFP[fetal protein]	AFP Aptamer	Heptocellular carcinoma [61]
	ErbB2	RNA Aptamer	Breast cancer [62]
	CCRF-CEM leukemia cells	Sgc8c	Leukemia [63]
DNA	Prostate specific membrane antigen (PSMA+) cancer cells	DNA Aptamer	Cancer [64]
	MUC 1[MUCIN 1]	Aptamer guided DNA tetrahedron	Breast cancer [65]
	LNCaP cells	ssDNA Aptamer	Prostate cancer [66]
	0mpc	DNA Aptamer	Food brone disease [67]

Polyethylamine functionalized carbon nanotube tagged with as Aptamer for combination gene and drug delivery into human gastric cell

This drug delivery system was developed for the synergistic cancer cell death. This can be achieved by the target delivery system which consists of BcL-xL-specific sh RNA and along with very low level of DOX content, which parallelly activates an intrinsic apoptotic pathway. Which consists of modified branched polyethylenimine was grafted to carboxylated single walled carbon nanotube (SWNCT) through polyethylene glycol (PEG) which serves as vehicle for sh RNA delivery and the resultant conjugate i.e. (SWNCT and PEG-PEI) was attached to AS1411 Aptamer (fig. 7) through covalent attachment. The final vehicle was obtained after the inhalation of DOX with the pBcL-xL-shRNA-SWNCT-PEG-10-10%PEG-Apt. As the result, the combination of shRNA mediated gene silencing along with chemotherapeutic agent is a valuable and safe approach for antitumor activity [51].

Application and therapeutically uses of the aptamers

Aptamer in recent years shown an extensively important role in the field of research and development, some of them include proteomic technology for the early detection of the lung cancer [68], the detection of the pathogenic microorganism with the help of Aptamer based sensors [69], also the identification technology for the rapid analysis of mycotoxins [70] (table 2). From past few years Aptamer are also being used for the purification and immobilization of enzyme [71] and it is also used to control the water pollution by detecting the low molecular weight pollutants in water sources [72].

CONCLUSION

In this review, the structures of an Aptamer, the difference between the Aptamer and antibody along with different drug delivery have been presented. Since the Aptamer have a wide range of advantage as compare to antibody, for example, Aptamer can withstand at high temperature and pH, whereas the antibody cannot be able to withstand at high temperature and pH; therefore, Aptamer is an ideal substitute for an antibody. With the help of the SELEX technique, we can isolate the ideal Aptamer for a target through *in vitro* selection method. Since Aptamer are non-immunogenic and no toxic material which can be used in the diagnosis and treatment of the various disease in their initial stages itself. The quick degradation of the Aptamer in the biological media due to interaction with the biomolecule is one of the limitations of the Aptamer, which have to be further investigated to overcome the existing limitation on Aptamer.

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AUTHORS CONTRIBUTIONS

We declare that this work was reviewed by the all authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

CONFLICT OF INTERESTS

No conflict of interest associated with this work.

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