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NANOPARTICULATE DRUG DELIVERY SYSTEM USING DRUG POLYMER AND APTAMER CONJUGATION

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ABSTRACT

In ancient Greek 'Nano' means dwar. Nanotechnology is the creation and utilization of materials, devices, and systems through the control of matter on the nanometer-length scale, i.e. at the level of atoms, molecules, and supramolecular structures. These technologies have been applied to improve drug delivery and to overcome some of the problems of drug delivery for cancer treatment. The magic of nanoparticles mesmerize everyone because of their multifunctional character and they have given us hope for the recovery from this disease. Nanoparticles can be used to deliver hydrophilic drugs, hydrophobic drugs, proteins, vaccines, biological macromolecules, etc. They can be formulated by aptamer conjugation for targeted delivery to the lymphatic system, brain, arterial walls, lungs, liver, spleen, or made for long-term systemic circulation. Therefore, numerous protocols exist for synthesizing nanoparticles based on the type of drug used and the desired delivery route. In modern medicine technologies the oral administration of solid forms is the preferred route for drug delivery. Thus, in pharmaceutical applications, size, shape and morphology of the solid particles are important because they can affect the solubility as well as bioavailability of the drug particles.

Keywords: Nanoparticles, Bioavailability, Aptamer, Targeted drug delivery system

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INTRODUCTION

In the pharmaceuticals, 90% of the active ingredients are in the form of solid particles. With the development in nanotechnology, it is now possible to produce drug nanoparticles that can be utilized in a variety of innovative ways. New drug delivery pathways can now be used that can increase drug efficacy and reduce side effects. For example, in 2005, the U.S. Food and Drug Administration approved intravenously administered 130-nm albumin nanoparticles loaded with paclitaxel (Abraxane) for cancer therapy, which epitomizes the new products anticipated based on nanoparticulate systems. The new albumin/paclitaxel–nanoparticle formulation offers several advantages including elimination of toxicity because of cremophor, a solvent used in the previous formulation, and improved efficacy due to the greater dose of the drug that can be administered and delivered[1].

E.g. of some Nanoparticulate Drug Delivery Systems as given in Figure:1

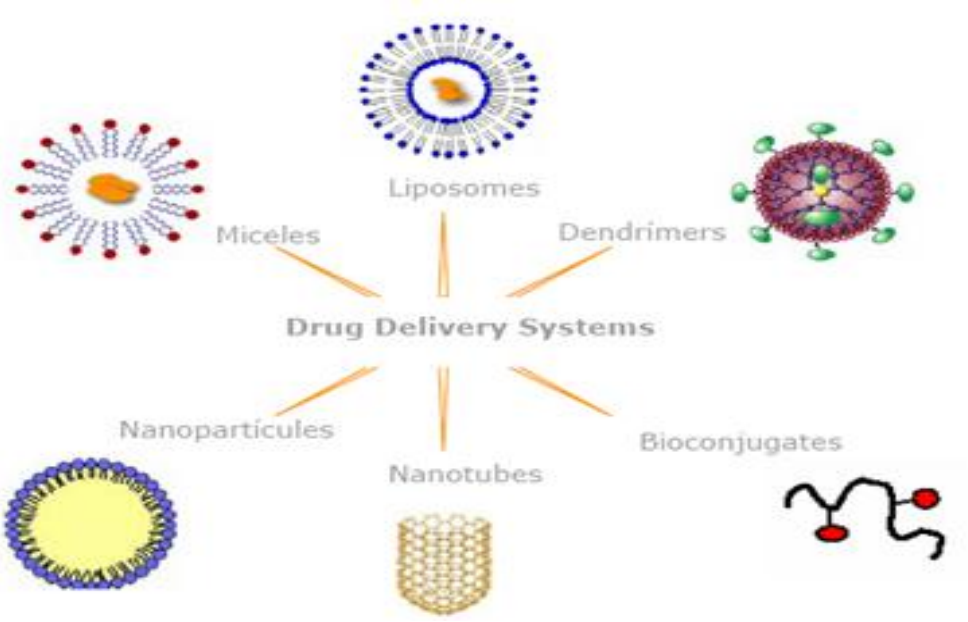


Figure: 01 Various Nanoparticulate Drug Delivery System

Liposomes:

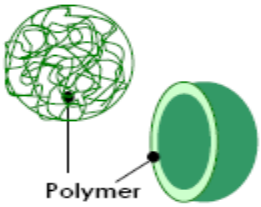
Lipid based vesicles used to entrap a drug payload and promote disease specific targeting. As Figure 02.

Figure: 02 Liposomes

There are the some examples of the liposomes product used as Nanoparticulate drug delivery system. (1) Doxil/Caelyx® (PEGlytion), Doxorubicine, 1995, (2) Ambelcet®, Amphotericin B, 1995, (3) Dauno Xome®, Daunorubicin, 1996, (4) Amphotec®, Amphotericin B, 1997, (5) Depocyt®, Cytarabine, 1999, (6) Visudyne®, Verteporfin, 2000, (7) DepoDur®, Morphone sulphate, 2004.

Nanoparticles:

Tiny particles, usually of 20-500 nm dimentions and formed from natural or synthetic polymers that are used to entrap drugs for improved drug targeting and controlled release. As Figure. 03 & 04.



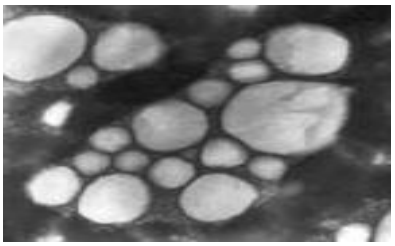


Figure: 03 Nanoparticle **Figure: 04 Nanoparticles**

There are the some examples of Nanoparticulate drug delivery systems are as under, (1) lupron®Depot, Leuprolide, 1989, (2) Sandostain®LAR, Octrotide, 1998, (3) Nutropin® Depot, Somatropin, 1999, (4) Trelstar®Depot, Triptolin, 2000, (5) Risperidal®Consta, Risperidone, 2003, (6) Abraxane® , Paclitaxel, 2004.

Micelles:

A self assembling colloidal aggregates of amphipathic molecules as polymeric block copolymers to give a polymeric micelle which occurs when the concentration reaches the critical micelle concentration. As figure 05.

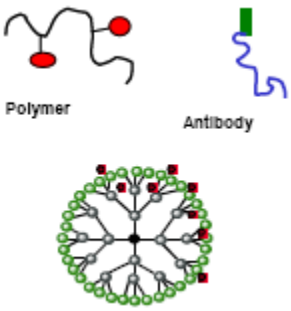


Figure: 05 Micelles

Bioconjugates:

Covalent chemical linking of an active molecule to an antibody, polymer or dendrimer t improve targeting, release or solubility. These conjugates act as prodrugs. As figure 06.

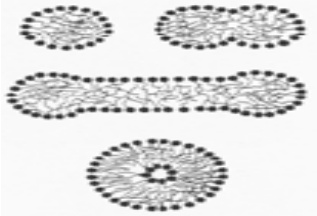


Figure: 06 Bioconjugates

For better development of the nanoparticulate systems, it is essential to understand the pharmaceutically relevant properties of nanoparticles. Some fundamental properties of nanoparticles including their size, surface area, settling velocity, magnetic and optical properties, and biological transport are brought into the perspective of drug delivery. The colloidal carriers based on biodegradable and biocompatible polymeric systems have largely influenced the controlled and targeted drug delivery concepts. It is apparent that the polymers, the building blocks of nanoparticulate composites, belong to natural or synthetic origins. Some of them have already been exploited for their biomedical applications. Nanoparticles are sub nanosized colloidal structures composed of synthetic or semi synthetic polymers[1].

Nanoparticle Size

To put the size of nanoparticles in perspective, Table 1 compares sizes of various objects. Because of the comparable size of the components in the human cells, nanoparticles are of great interest in drug delivery. It appears that nature, in making the biological systems, has extensively used nanometer scale. If one has to go hand in hand with nature in treating the diseases one needs to use the same scale, whether it is correcting a faulty gene, killing leprosy bacteria sitting inside the body cells, blocking the multiplication of viral genome, killing a cancer cell, repairing the cellular metabolism, or preventing wrinkles or other signs of aging. One cannot use a human arm to massage the hurt leg of an ant. Size matching is important in carrying out any activity. Drug delivery is aimed at influencing the biochemistry of the body[2].

Table: 01 Typical size of Various Objects

Object	Size(nm)
Carbon atom	0.1
DNA double helix (diameter)	3
Ribosomes	10
Virus	100
Bacterium	1000
Red Blood Cell	5000
Human hair (diameter)	50000
Resolutio of unaided human eyes	100000

Production of Nanoparticles

Although any particle of a size < 1-m m diameter is a nanoparticle, several national initiatives are encouraging the development of particles < 100 nm as they might exhibit some unique physical properties, and hence potentially different and useful biological properties. However, achieving sizes < 100 nm is more readily feasible with hard materials compared to drug and polymer molecules, which are soft materials. For hard materials, such as silica, metal oxides, and diamonds with melting points above 1000ºC, nanoparticles in the 1–100 nm size range have been prepared. However, for drugs that are usually soft materials with melting point below 300ºC particles in the 1–100 nm size range are more difficult to prepare. For this reason, it is a reasonable goal to aim at < 300 nm particles for drug and polymer materials. There are several success stories for pharmaceutical materials in this size range.

PREPARATION TECHNIQUES OF NANOPARTICLES

The selection of the appropriate method for the preparation of nanoparticles depends on the physicochemical characteristics of the polymer and the drug to be loaded. On the contrary, the preparation techniques largely determine the inner structure, in vitro release profile and the biological fate of these polymeric delivery systems. Two types of systems with different inner structures are possible including: (1) a matrix type system consisting of an entanglement of oligomer or polymer units (nanoparticles / Nanospheres). (2) A reservoir type of system comprised of an oily core surrounded by an embryonic polymeric shell (Nanocapsules).

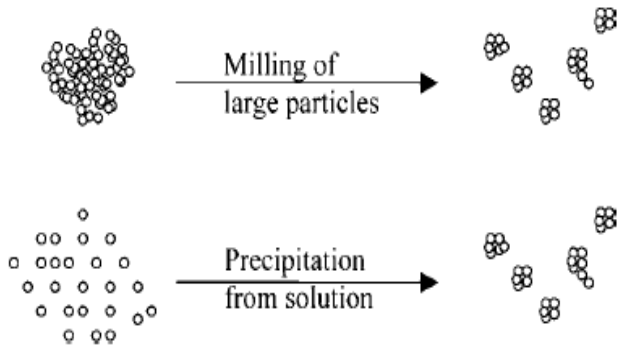


Figure: 07 Method of Nanoparticle Preparation

The drug can either be entrapped within the reservoir or the matrix or otherwise be absorbed on the surface of these particulate systems. The polymers are strictly structured to a nanometric size range particle using appropriate methodologies. These methodologies are conveniently classified as follows: (1) Amphiphilic macromolecule cross linking, a) Heat cross linking, b) Chemical cross linking. (2) Polymerization based methods, a) Polymerization of monomers in situ, b)

Emulsion (miceller) polymerization, c) Dispersion polymerization, d) Interfacial condensation polymerization, e) Interfacial complexation. (3) Polymer precipitation methods, a) Solvent extraction/evaporation, b) Solvent displacement (nanoprecipitaiton), c) Salting out. The table 02 shows the various polymers used for the preparation of nanoparticles and nanocapsules[3].

Table 02 : Polymers used for the preparation of Nanoparticles and Nanocapsules[4].

Polymer Use	Technique	Candidate Drug
Hydrophilic		
Albulin, Gelatin	Heat denaturation and cross-linking in W/O emulsion.	Hydrophilic
	Desolvation and cross-inking in aqueous medium	Hydrophilic and protein affinity
Alginate, Chitosan, Dextran	Cross linking in aqueous medium	Hydrophilic and protein affinity
	Polymer precipitation in organic solvent	Hydrophilic
Hydrophobic		
Poly(alkylcyanoacrylates)	Emulsion polymerization	Hydrophilic
	Interfacial O/W polymerization	Hydrophobic
Polyesters		
Poly (lactic acid), Poly (lactide-co-glycolate), Poly (Ɛ-caprolactone)	Solvent extraction-evaporation	Hydrophilic & Hydrophobic
	Solvent displacement	Soluble in polar solvent
	Salting out	Soluble in polar solvent

CHARACTERIZATION PARAMETERS OF NANOPARTICULATE

The nanoparticles are generally characterized for size, density, electrophoretic mobility, angle of contact and specific surface area, which are given in Table 03[4].

Particle Size

The particle size is one of the most important parameters of nanoparticles. Particle size and sizing of sub-optical particulates is a different procedure, as it involves not only procedural variability, but some of the surface associated properties may even change during sizing procedure[4].

Specific Surface Area

The specific surface area of freeze-dried nanoparticles is generally determined with the help of Sorptometer. The equation given as under can be used in the calculation of specific surface area.

$$A = 6/ \partial .d$$

Where A is the specific surface area, ∂ is the density and d, is the diameter of the particle. In the most of the cases, the measured and calculated specific surface areas fairly compare while in some cases the residual surfactant could affect deviation in measured values. The surfactant coating apparently reduces the specific surface area[4].

Table 03: Different parameters and characterization methods for Nanoparticles

Particle size and size distribution	Photon correlation spectroscopy (PCS) Laser defracrometry Transmission electron microscopy Scanning electron microscopy Atomic force microscopy Mercury porositometry
Charge determination	Laser Doppler Anemometry Zeta potentiometer
Surface Hydrophobicity	Water contact angle measurement Rose Bengal (dye) binding Hydrophobic interaction chromatography X-ray photoelectron spectroscopy
Chemical analysis of surface	Static secondary ion mass spectrometry Sorptometer
Carrier-drug interaction	Differential scanning calorimetry
Nanoparticle dispersion stability	Critical flocculation temperature (CFT)
Release profile	In vitro release characteristics under physiologic and sink conditions
Drug stability	Bioassay of drug extracted from nanoparticles Chemical analysis of drug

Electrophoretic Mobility and Surface Charge

The nature and intensity of the surface charge of nanoparticles is very important as it determines their interaction with the biological environment as well as their electrostatic interaction with bioactive compounds. The surface charge of colloidal particles in general and nanoparticles in particular can be determined by measuring the particle velocity in an electric field. Laser light scattering techniques are laser Doppler anemometry or velocimetry has become available as fast and high resolution technique for the determination of nanoparticles velocities[4].

Surface Hydrophobicity

The Hydrophobicity of nanoparticles has an important influence on the interaction of colloidal particles with the biologic environment. The Hydrophobicity and hydrophilicity collectively determine the bio fate of nanoparticles and their contents. Hydrophobicity regulates the extent and type of hydrophobic interaction of nanoparticulates with blood components[4].

Density

Freeze fracturing could successfully used in the morphological investigation of nanoparticles after of surface scanning electron microscopy, transmission electron microscopy. The interiors are continuous or some structural imperfections exists that provide an indication about the density distribution across the metrix[4].

In Vitro Release

In vitro release profile can be determined using standard dialysis, diffusion cell or recently introduced modified ultra-filtration technique. In vitro drug release from the nanoparticles can be evaluated in phosphate buffer utilizing double chamber diffusion cells on a shaker stand. A Millipore hydrophilic low protein binding membrane is placed between the two chambers. The donor chamber is filled with nanoparticulate suspension and the receptor chamber with plain buffer. The receptor compartment is assayed drug using standard procedures[4].

Surface engineering of nanoparticles

Nanoparticles are surface engineered for various purposes. On the basis of the reports in the literature, they are classified as: 1) Stearic stabilized (stealth) nanoparticles, 2) Bio-mimetic nanoparticles, 3) Antibody coated nanoparticles, 4) magnetically guided nanoparticles, 5) Bioadhesive nanoparticles[4].

THERAPEUTIC APPLICATION OF NANOPARTICLES

There are the nanoparticles with different compositions and characteristics have been formulated and investigated for various therapeutics applications. Several different types of biodegradable polymers including biopolymers (e.g. gelatin, albumin, casein, polysaccharide, lectin etc.) and synthetic polymers (polycaprolactone, polyesters, polyanhydrides, polycynoacrylates) with various drug release characteristics ranging from several hours to several months have been used to formulate sustained release nanoparticles. There are the various application of the nanoparticles as per the following list: cancer therapy, intracellular targeting, prolonged systemic circulation, vaccine adjuant, peroral absorption, ocular delivery, DNA delivery, oligonucleotide delivery and other applications like crosses blood brain barrier, improved absorption and permeation, enzyme immunoassay, radio imaging, oral delivery of peptides[5].

Introduction to Aptamer

Aptamers, derived from the Latin aptus, meaning, 'to fit', are specific RNA or DNA oligonucleotides or proteins which can adopt a vast number of three dimensional shapes. Due to this property, aptamers can be produced to bind tightly to a specific molecular target. Because an extraordinary diversity of molecular shapes exist within the universe of all possible nucleotide sequences, aptamers may be obtained for a wide array of molecular targets, including most proteins, carbohydrates, lipids, nucleotides, other small molecules or complex structures such as viruses, aptamers technique is a powerful tool in drug discovery and development. Aptamers, short stretches of DNA (figure 8 & 9) or RNA (figure 10 & 11) that can act much like antibodies,

have shown promise as targeting agents for selective nanoparticle trafficking to tumors. Their ability to recognize and bind to tumor-specific molecules is undisputed, but the strength with which aptamers bind to their molecular targets is often insufficient to act as an effective targeting agent under clinically relevant conditions [6]. Aptamers are modified oligonucleotides that are isolated by the systematic evolution of ligands by exponential enrichment (SELEX) process.

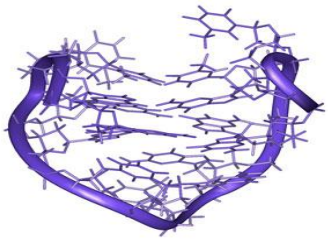


Figure: 08 DNA Aptamer

Figure: 09 DNA Aptamer

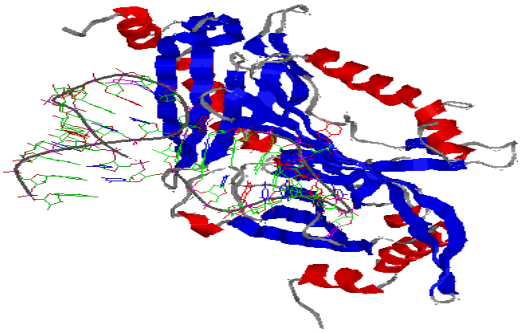


Figure: 10 RNA Aptamer

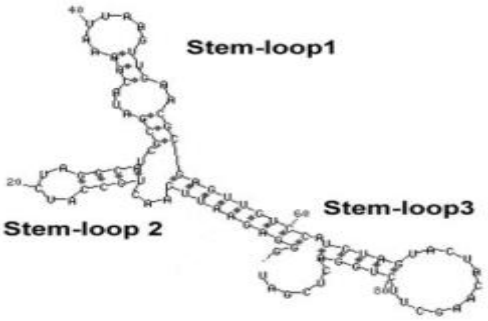


Figure: 11 RNA Aptamer

Formally, aptamers are similar in composition to natural nucleic acids but are built with 2′-modified sugars to enhance resistance to blood and tissue nucleases. Aptamers are not linear molecules that carry genetic information. Rather, they are globular molecules, as exemplified by the shape of t-RNA. Like antibodies, Aptamers most frequently function through high-affinity binding to a target protein. To disrupt not only a specific protein, but a specific interacting domain on that protein, we turn to RNA aptamer technology. We select RNA aptamers from large, highly complex RNA pools using cycles of selection and amplification (SELEX) as shown in figure 12[7].

Aptamers provide several advantages as inhibitors: Like an antibody, they can be made to order specifically for a particular protein. Like a small organic molecule, they can rapidly target a specific protein domain within cells. Like a conditional allele, they are able to exert their effect in whole organisms, but **t**hey are also targetable to specific tissues, cells, or stages of development [7].

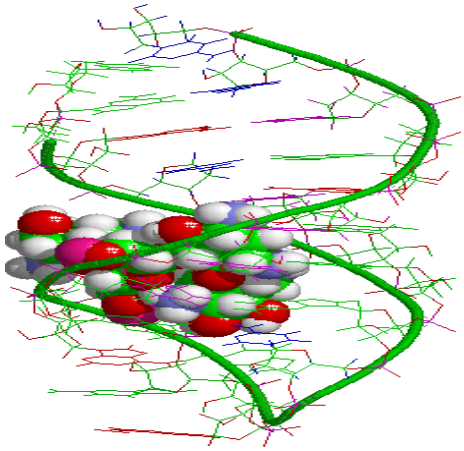


Figure: 12 Aptamer Selex

As shown in figure. 13, an archetypal escort aptamer. (a) A high-affinity aptamer is identified by the SELEX process. 2′-F pyrimidines are incorporated during selections. (b) The aptamer is truncated to minimal size and is now a synthetic molecule. (c) The escort aptamer as an in vivo diagnostic agent as shown in figure 13. Further nuclease stabilization is achieved: only two positions remain 2′-OH (underlined); the remainder are 2′-F pyrimidine and 2′-OCH3 purine (bold). Chemical synthesis adds a 3′-3′ exo-nuclease cap and a primary amine (or thiol, etc.) for desired modifications. For in vivo imaging, a radiometal chelator is conjugated to the 5′ amine and 99mTc is incorporated.

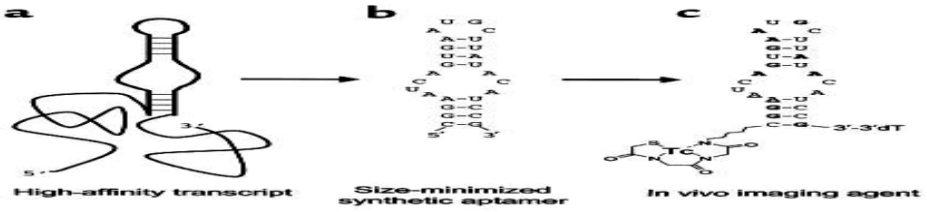


Figure: 13 Aptamer isolation: the selex process

Introduction to bioconjugation

Bioconjugation involves the linking of two or more molecules to form a novel complex having the combined properties of its individual components.

Natural and synthetic compounds with their individual activities can be chemically combined to create unique drug carriers, which possess carefully engineered characteristics. Essentially, proteins (anti target or target specific) are used in bioconjugation technique as one of the handles, which are conjugated to other molecules or carriers capable of being detected by target site(s) as shown in figure 14. It has now been recognized that delivery of many of the new therapeutic biotechnology products, recombinant proteins, oligonucliotites and their analogues can potentially benefit by the production from enzymatic degradation, decreased uptake by

reticuloendothelial system(RES) rich organs, or from reduction of other unwanted manifestation of biological recognition. This can be achieved through their conjugation with macromolecular or carrier system [8].

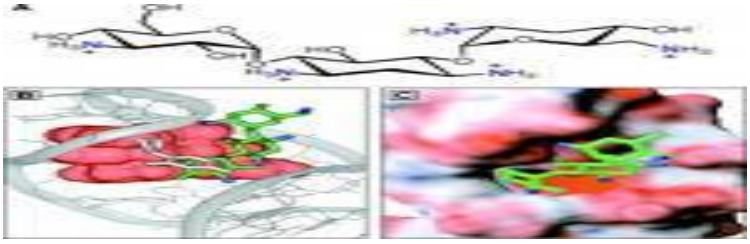


Figure: 14 Drug polymer Bioconjugation

Introduction to bioconjugation techniques

Modification and conjugation techniques are dependent upon two inter related chemical reactions; the reactive functional groups present on the various cross-linking or derivatizing agent and the functional groups present on the target macromolecules or delivery system to be modified. It is the aim that determines the type of conjugation system to be implemented. The labeling, tagging, cross linking or targeting of small ligands, peptides, proteins, carbohydrates, nucleic acid, oligonucliotides, and lipids with targeted molecules (pr delivery device) can be accomplished by using various covalent and noncovalent conjugation techniques. Whether it be tagging proteins to target molecules to render them chromogenic or fluorescent, labeling molecules with bio-specific ligands for subsequent affinity interaction or cross linking two or more moieties to create site specific active conjugates, the choice of cross linking and derivatizing reagents in pivotal. The endogenous or exogenous ligands can be conjugated with either the drug or drug bearing delivery systems using various non-covalent or covalent techniques [9]. Aptamers are small (i.e., 40 to 100 bases), synthetic oligonucleotides that can specifically recognize and bind to virtually any kind of target, including ions, whole cells, drugs, toxins, low-molecular-weight ligands, peptides, and proteins. An aptamers affinity depends on its target type. Aptamers against small molecules have affinities in the micro molar range (e.g., 2.8 mM for dopamine and ATP 6 mM for adenosine 5 triphosphate). Aptamers have shown affinities in the nanomolar and sub-nanomolar range against some proteins, such as vascular endothelial growth factor with an affinity of 100 pM, and keratinocyte growth factor, 1 pM. High affinity often means high specificity for aptamers. They can discriminate between targets using suitable structural differences such as the presence of a hydroxyl group or a methyl group. Furthermore, they can distinguish between enzymes with similar catalytic function, such as a-thrombin and G-thrombin[10].

Origin of Aptamer

Aptamers emerged from the experimental efforts of three independent groups that first published their work in 1990. Gerald F. Joyce's group at the Scripps Research Institute (La Jolla, CA) was looking for new enzymatic activity of RNA. The researchers used in vitro mutation, selection, and amplification to isolate the RNA with enzymatic functionality. This approach became the basis for the current in vitro selection of aptamers. Larry Gold's group at the University of Colorado (Denver) was trying to identify the sequences of T4 DNA polymerase in vitro. Their library was based on the natural structure, but included a randomized eight-nucleotide sequence. The group named the patented process (U.S. Patent 5,270,163) of in vitro selection "Systematic Evolution of Ligands by Exponential Enrichment (SELEX). The SELEX process could identify the most selective aptamer for an enzyme.

DRUG POLYMER APTAMER CONJUGATION IN NANOPARTICULATE DRUG DELIVERY SYSTEM

Type of aptamer conjugation

Aptamers are oligonucleic acid or peptide molecules that bind a specific target molecule. Aptamers are usually created by selecting them from a large random sequence pool, but natural aptamers also exist in riboswitches. Aptamers can be used for both basic research and clinical purposes as macromolecular drugs. Aptamers can be combined with ribozymes to self-cleave in the presence of their target molecule. These compound molecules have additional research, industrial and clinical applications. More specifically, aptamers can be classified as: 1) DNA or RNA aptamers. They consist of (usually short) strands of oligonucleotides. (2) Peptide aptamers. They consist of a short variable peptide domain, attached at both ends to a protein scaffold[11].

(1) RNA and DNA Aptamers

Aptamers are nucleic acid species that have been engineered through repeated rounds of in vitro selection or equivalently, SELEX (systematic evolution of ligands by exponential enrichment) to bind to various molecular targets such as small molecules, proteins, nucleic acids, and even cells, tissues and organisms. Aptamers offer the utility for biotechnological and therapeutic applications as they offer molecular recognition properties that rival that of the commonly used biomolecule, antibodies. In addition to their discriminate recognition, aptamers offer advantages over antibodies as they can be engineered completely in a test tube, are readily produced by chemical synthesis, possess desirable storage properties, and elicit little or no immunogenicity in therapeutic applications[12].

(2) Peptide Aptamers

Peptide aptamers are proteins that are designed to interfere with other protein interactions inside cells. They consist of a variable peptide loop attached at both ends to a protein scaffold. This double structural constraint greatly increases the binding affinity of the peptide aptamer to levels comparable to an antibody's (nanomolar range).The variable loop length is typically comprised of 10 to 20 amino acids, and the scaffold may be any proteins which have good solubility and compacity properties. Currently, the bacterial protein Thioredoxin-A is the most used scaffold protein, the variable loop being inserted within the reducing active site, which is a -Cys-Gly-Pro-Cys- loop in the wild protein, the two Cysteines lateral chains being able to form a disulfide bridge.Peptide aptamer selection can be made using different systems, but the most used is currently the yeast two-hybrid system. Selection of Ligand Regulated Peptide Aptamers (LiRPAs) has been demonstrated. By displaying 7 amino acid peptides from a novel scaffold protein based on the trimeric FKBP-rapamycin-FRB structure, interaction between the randomized peptide and target molecule can be controlled by the small molecule Rapamycin or non-immunosuppressive analogs[13-14].

How are Aptamers produced?

Aptamers are generally produced through an in vitro evolutionary process called "systematic evolution of ligands by exponential enrichment" (SELEX). The method is an iterative process based on selection and amplification of the anticipated tight binding aptamer. The start library for selection of aptamers contains single stranded DNA oligonucleotides with a central region of randomized sequences (up to 10^{15} different sequences) which are flanked by constant regions for subsequent transcription, reverse transcription and DNA amplification. The start library is amplified by PCR and transcribed to an RNA start pool by T7 transcription[15]. Target specific RNA is selected from the pool by allowing the pool to interact with the target molecule, only tight binding RNA molecules with high affinity are removed from the reaction cycle, the tight binding RNA molecules are reverse transcribed to cDNA and amplified to double stranded DNA by PCR. These enriched binding sequences are transcribed back to RNA which is the source for the next selection and amplification cycle. Such selection cycles are usually repeated 5-12 times in order to obtain only sequences with highest binding affinities against the target molecule. In addition to high specificity, aptamer have very high affinities to their targets. Typically aptamers generated against proteins have affinities in the picomolar to low nanomolar range[16].

Why Nanoparticulate drug delivery system?

In ancient Greek 'Nano' means dwar. Nanotechnology is the creation and utilization of materials, devices, and systems through the control of matter on the nanometer-length scale, i.e. at the level of atoms, molecules, and supramolecular structures. These technologies have been applied to improve drug delivery and to overcome some of the problems of drug delivery for cancer treatment. Several nano-biotechnologies mostly based on nanoparticles, have been used to facilitate drug delivery in cancer[17-18-19-20]. The magic of nanoparticles mesmerize everyone because of their multifunctional character and they have given us hope for the recovery from this disease. Although we are practicing better drug delivery paths into the body, we ultimately seek more accurate protocols to eradicate cancer from our society. Nanoparticles have a further advantage over larger microparticles, because they are better suited for intravenous (i.v.) delivery. The smallest capillaries in the body are 5–6 mm in diameter. The size of particles being distributed into the bloodstream must be significantly smaller than 5 mm, without forming aggregates, to ensure that the particles do not form an embolism. Nanoparticles can be used to deliver hydrophilic drugs, hydrophobic drugs, proteins, vaccines, biological macromolecules, etc. They can be formulated for targeted delivery to the lymphatic system, brain, arterial walls, lungs, liver, spleen, or made for long-term systemic circulation. Therefore, numerous protocols exist for synthesizing nanoparticles based on the type of drug used and the desired delivery route. Once a protocol is chosen, the parameters must be tailored to create the best possible characteristics for the nanoparticles [21-22-23]. In modern medicine technologies the oral administration of solid forms is the preferred route for drug delivery. Thus, in pharmaceutical applications, size, shape and morphology of the solid particles are important because they can affect the solubility as well as bioavailability of the drug particles. Since the bioavailability of orally applied drugs depends on the rates of dissolution and absorption, methods to increase such rates are often essential to reach significant levels (concentrations) in the blood[24]. A very suitable way to increase the rate of dissolution is the reduction of the particle size. Particle design, in particular the design of micron, submicron, or nanoparticles, is thus critical. There are several methods for the production of drug particles of decreased sizes such as pulverization of large particles using a ball or jet mill, solidification of emulsions by in-water drying methods, spray freezing, spray drying and supercritical antisolvent technique (SAS), etc. These methods are reviewed here with a focus on the production of micro/nano-sized drug particles with or without water soluble materials. Such particles are used in oral, pulmonary and transdermal drug delivery of water insoluble or poorly water soluble drugs[25-26-27].

APPLICATION OF APTAMER CONJUGATION IN NANOPARTICULATE DRUG DELIVERY

Therapeutic Application

The systematic evolution of ligands by exponential enrichment (SELEX) is a combinatorial oligonucleotide library-based in vitro selection approach in which DNA or RNA molecules are selected by their ability to bind their targets with high affinity and specificity, comparable to those of antibodies. Nucleic acids with high affinity for their targets have been selected against a wide variety of compounds, from small molecules, such as ATP, to membrane proteins and even whole organisms. Recently, the use of the SELEX technique was extended to isolate oligonucleotide ligands, also known as aptamers, for a wide range of proteins of importance for therapy and diagnostics, such as growth factors and cell surface antigens [28]. The number of aptamers generated as inhibitors of various target proteins has increased following automatization of the SELEX process. Their diagnostic and therapeutic efficacy can be enhanced by introducing chemical modifications into the oligonucleotides to provide resistance against enzymatic degradation in body fluids. Several aptamers are currently being tested in preclinical and clinical trials, and aptamers are in the process of becoming a new class of therapeutic agents. Recently, the anti-VEGF aptamer pegaptanib received FDA approval for treatment of human ocular vascular disease [29].

Nanoparticle-aptamer bioconjugates for targeted antineoplastic drug delivery.

Targeted drug delivery technologies can provide physicians with new approaches to treat and manage patients with cancer. Nucleic acid ligands (aptamers) are a novel class of targeting molecules that can be used in a similar manner to antibodies. Beyond use as drugs themselves, aptamers have the potential to serve as targeting ligands to deliver drugs, imaging agents, or other bioactive agents to the intended site of action. Bioconjugates of nanoparticles and aptamers can selectively bind and be taken up by cancer cells. Aptamers are isolated through a process of in vitro selection, also referred to as systematic evolution of ligands by exponential enrichment (SELEX). These aptamers often interact with antigens that are over expressed exclusively, or preferentially, on cancer cells or in the cancer microenvironment. As novel drug delivery vehicles, nanoparticle-aptamer bioconjugates may be developed to target a myriad of diseases including many cancers by delivering a variety of therapeutic agents specifically to the site of interest[30]. The first in vivo study of antineoplastic drug delivery by a bioconjugate employed nanoparticle encapsulating docetaxel and aptamers that bind certain prostate cancer cells. In this

study using a xenograft murine model of prostate cancer, these bioconjugates were shown to significantly improve tumor reduction after intratumoral injection compared with all controls. Furthermore, the docetaxel-loaded nanoparticle-aptamer bioconjugates demonstrated reduced toxicity in terms of acute bodyweight loss compared with the controls. In vitro, the efficacy of the docetaxel-loaded Nanoparticle-aptamer bioconjugates was shown to be due to intracellular delivery of the drug to the cancer cells, and the bioconjugates without the drug had no cytotoxicity. Nanoparticle-aptamer bioconjugates may prove to be useful not only for management of cancer but also various other indications. New aptamers, multivalent targeting strategies, and multimodal treatments such as simultaneous radio- and chemotherapy may further increase the efficacy of these bioconjugates and facilitate their clinical translation for therapeutic and diagnostic application[31]. Aptamer: Toxin Conjugates that Specifically Target Prostate Tumor Cells **.**RNA aptamer:gelonin conjugates to target and specifically destroy cells over expressing the known cancer biomarker prostate-specific membrane antigen (PSMA). Aptamer: toxin conjugates have an IC50 of 27 nmol/L and display an increased potency of at least 600-fold relative to cells that do not express PSMA. The aptamer not only promotes uptake into target cells but also decreases the toxicity of gelonin in non-target cells. These results validate the notion that "escort aptamers" may be useful for the treatment of specific tumors expressing unique antigen targets [32-33-34].

Cancer Cell Targeting Using Multiple Aptamers Conjugated On Nanorods (Nr)

Molecular recognition toward specific cells is a key issue for effective disease, such as cancer, diagnosis and therapy. Although many molecular probes such as aptamers and antibodies can recognize the unique molecular signatures of cancer cells, some of these probes only have relatively weak binding affinities. This results in poor signaling and hinders cell targeting. Here, we use Au-Ag nanorods (NRs) as a nanoplatform for multivalent binding by multiple aptamers on the rod to increase both the signal and binding strengths of these aptamers in cancer cell recognition. Up to 80 fluorophore-labeled aptamers can be attached on a 12 nm x 56 nm NR, resulting in a much stronger fluorescence signal than that of an individual dye-labeled aptamer probe[35]. The molecular assembly of aptamers on the NR surfaces also significantly improves the binding affinity with cancer cells through simultaneous multivalent interactions with the cell membrane receptors. This leads to an affinity at least 26-fold higher than the intrinsic affinity of the original aptamer probes. As determined by flow cytometric measurements, an enhancement in fluorescence signal in excess of 300-fold is obtained for the NR-aptamer-labeled cells

compared with those labeled by individual aptamer probes. Therefore, the molecular assembly of aptamers clearly shows potential applications for the elucidation of cells with low density of binding sites, or with relatively weak binding probes, and can thus greatly improve our ability to perform cellular imaging and targeting. This is an excellent example of using nanomaterials to develop advanced molecular binders with greatly improved properties for cellular studies[36].

Reversed Activity of Aptamer

Mechanism Of The Aptamer-Antidote Pair

The aptamer is stabilized by a 3'-3'-linked deoxythymidine at the 3'-end and by 2'-fluoro-2'-deoxy modifications at every pyrimidine residue. It carries a cholesterol-modification at the 5'-end to increase the plasma residence time. The aptamer binds to activated factor IX and prevents the proteolytic cleavage of factor X (left). In the presence of the antidote (blue sequence), the aptamer is released from factor IXa (right). Together with activated factor VIII (VIIIa), factor IXa catalyzes the cleavage of factor X to yield activated factor X (Xa), which is required for the blood clotting cascade: with factor Va, Xa cleaves prothrombin to yield thrombin, which catalyzes cleavage of fibrinogen to yield fibrin. Cross-linked fibrin forms the clot as shown in figure 15[36].

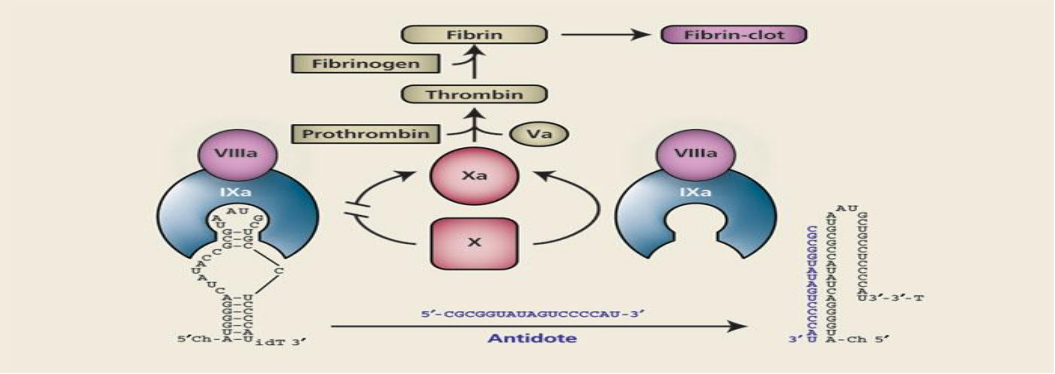


Figure: 15 Mechanisms of the Aptamer-Antidote Pair

Aptamer activity can be reversed by an antidote.

Two aptamer–antidote designs have been described in figure 16. (a) The classic antisense oligonucleotide as an antidote was added to a protein–aptamer complex as illustrated by the anti-Factor IXa aptamer 9.3t. When the antidote was added, the anticoagulating effect of aptamer 9.3t was abolished and clotting parameters returned to normal.6 (b) An aptamer-'caged' antidote chimera was designed to allow for light-triggered deactivation of a thrombin [37].

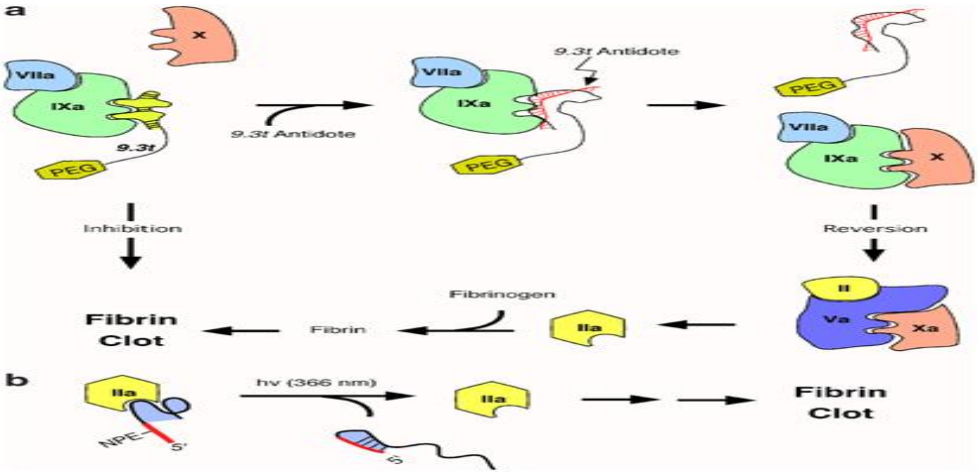


Figure: 16 Reversed Aptamer Activity

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