REVIEW ARTICLE

Nucleic Acid Nanostructures–DNA and RNA Nanoparticles

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ABSTRACT

Nanotechnology is the field of science that encompasses the production of nanoparticles at nanoatomic scale. The nanoparticles size governs wide range of therapeutic properties. The nanostructures when formulated using deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) materials can result in enhanced hydrophobic properties, shelf life, and also may enhance targeted drug delivery. Structured or dynamic DNA nanostructures involve basic nucleotide pairing with protein backbone. The DNA nanostructures produced by bottom to top approach are currently being applied in medicine, cancer, ophthalmic drug delivery, and also for diagnosis and treatment. They involve complimentary DNA base pairing (AT/GC) for strong interaction resulting in formulation of structures with 10 to 50 mm size. RNA nanostructures are multifunctional manipulative structures synthesized using a bottom-up approach, which results in modulated physiochemical properties and enhanced stability. RNA nanoparticles are currently exploited in the field of cancer. Both DNA and RNA nanostructures are 2D/3D multidimensional models, which are novel structures in the field of nanoscience. DNA/RNA nanostructures can be identified by the Killer Killiani test, quantitative estimation, and also spectroscopic methods like UV, SDS-Page, XRD, SEM, and DLS techniques can be applied. The wide range of applications of these DNA/RNA nanostructures are currently considered as an area of interest in field of therapeutics by most of the researchers.

Keywords: Cancer, DNA, RNA, SDS-page.

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INTRODUCTION

Nanotechnology is defined as the science of skillful handling of matter on atomic, molecular, and supramolecular scale with at least one dimension sized from 1 to 100 nanometers.[1] Nanotechnology is a utilitarian asset, which escalates itself into various branches of medicine, diagnostics, tissue engineering, drug delivery, chemistry and environment, catalysis, filtration, information and communication, and many more.[2] Nanoparticles are allocated majorly into three broad types: one-dimensional nanoparticles, two-dimensional nanoparticles, and three-dimensional nanoparticles. One dimensional nanoparticle encompasses chemical and biological sensors, information storage systems, magnetic optic, and optical device, and fiber optic systems. Analogously two-dimensional systems include carbon nanotubes, and three dimensional systems embrace dendrimers, quantum dots, fullerenes (C- 60), etc.[3] Nanoparticles used for drug delivery are submicron colloidal particles of < 1 μm and are made up of biodegradable and biocompatible materials. These nanoparticles used in drug delivery systems are commanding due to their high shelf life, the molecule capacity of incorporating many drug molecules into its matrix, the compatibility of incorporation of both hydrophobic and hydrophilic substances, and its feasibility in route of administration, involving both oral route and inhalation. It also provides sustained release of the drug.[4] The DNA nanotechnology is of two types, mainly structural nanotechnology and the dynamic nanotechnology.[5] Structural DNA nanotechnology uses recurring DNA patterns to built target shapes and arrangements. These unusual recurring patterns occur by reversal attachment of DNA backbones. The combining of DNA motifs can be done by joining the ends together either by covalent bonds or hydrogen bonds. The other methods of cohesion may include edge-sharing or by parenemic interactions of two double helices.[6] DNA nanotechnology majorly uses its structural properties rather than its genetic properties and is used in the production of synthetic DNA and to incorporate those properties in structures and functions which are normally not found in natural DNA. The natural properties of DNA particles synergize the effectiveness of the particles in the field of biotechnology.[7] DNA nanotechnology has been used for characterization of protein, aggregation of enzymes, biosensing, drug delivery, and biomimetic assemblies.[8] DNA nanotechnology is known to be biocompatible and has high potency for drug delivery with

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very less toxicity. Carbon fullerenes are nowadays being used to deliver plasmids and DNA conjugates in gene therapy and gene targeting. Certain evidence of DNA nanostructures have been reported like anti-microbial property when combined with the immobile gold and silver particles with cinnamon plant extract without signs of toxicity was found in the host. These DNA particles are also known to be useful in treating cancer with low cytotoxicity and high biocompatibility. The Centers of Cancer Nanotechnology Excellence (CCNEs) have subsumed discovery and tool development for nanotechnology applications in clinical oncology. This versatile nature of DNA nanostructures led to the control of virucide action.[9]

RNA nanotechnology is the production of particles at the nanoscale by manipulating the nanoparticles explicitly.[10] RNA particles have the potential for developing therapeutics in the treatment of cancer. RNA nanoparticles can be of great use due to the wide range of benefits. As these nanoparticles are thermodynamically stable, they can be incorporated by exploiting their chemical properties to prevent the *in vitro* and *in vivo* RNA nucleotides degradation without changes in RNA property itself, thereby decreased cost of RNA production by biological and chemical synthesis can be achieved. Also, there are various RNA structural motifs for the feasible biological synthesis of these molecules. These provide safety and specific target action in cancer and other diseases with little or no toxicity.[11]

History of DNA and RNA Nanostructures

The novelty for DNA nanoparticles was started in 1980 by Nadrian Seeman. He majorly focused on the immobile DNA junctions, which would expunge the issue of understanding the pure crystals isolation and would make their crystallographic study easier. These static cross-sections would be useful to get assembled into the crystalline structures.[12] In 1991, he constructed a cube with DNA and it was absolutely the primary nanostructure made up of DNA. In 1998, Seeman, along with Erik Winfree had developed a 2D dimensional structure, which was double-crossover tiles that were simple to research and cipher DNA. Seeman, in 1999, has developed the first nano-medicine, which could make an amendment to itself with the input thereto. In 2006, Rothemund introduced the DNA orgami initially, which he had first demonstrated about the 3D followed by 2D structures, an arrangement that allows a long strand to be folded by means of several short strands.[13]

The development of RNA nanotechnology is because of the collective efforts of assorted scientists. Peixuan in the year 1998, submitted a manuscript regarding his finding of packaging RNA dimmers, trimmers, and hexamers using re-constructed RNA fragments, which fascinated Vivian Siegel (associate edition of cell) and Benjamin Lewin (founding editor of cell). They then printed this in molecular cell and conjointly Roger Hendrix was chosen to review the finding. In the 2000s, Eric Westhof speculated that RNA kissing loop will be helpful in building the RNA structures. In 2004, MSNBC published story stating "Scientists build tiny structures of RNA", whose objective was to promote RNA Technology.[14]

Design, Optimization, and Analysis of DNA and RNA Nanostructures—Brief Outline

The nucleic nanotechnology field utilizes DNA or RNA as building materials to synthesize nanoscale devices and nanostructures.[15] The high programmability property of pairing oligonucleotides enables to synthesize 2D and 3D nanostructures up to several thousand nucleotides. The increasing complexity of designed DNA/RNA nanostructures, single-stranded DNA (ssDNA) and RNA (ssRNA) orgami structure were reported to have promising applications ranging from photonic devices to drug delivery.[16]

Available nucleic acid nanotechnology design tools include Tiamat,[17] CaDNAno,[18] vHelix,[19] Adenita,[20] MagicDNA,[21] and the CAD converters DAEDALUS[22] and PERDIX.[23] CaDNAno is frequently used to design very large structures on either a square or hexagonal lattice which requires parallel helical components. Tiamat is a lattice-free intuitive design tool that involves both DNA and RNA. MagicDNA is a Matlab based tool that is specialized in designing of large 3D structural components on 3D cubic lattice using CaDNAno parallel DNA bundles as the base unit on each edge.[22] vHelix and Adenita are DNA design templates for the commercial design platforms Maya and SAMSON. vHelix facilitates conversion of polyedral meshes to DNA sequences also enabling free-form editing available in Maya. Adenita combines the functionality of CAD converters with free-form design, allowing users to load structures from a variety of sources with additional editing tools available in the SAMSON interface. DAEDALUS and PERDIX[23] are software that facilitate conversion of meshes designed in CAD software into DNA representations. Currently, the nanotechnology field lacks universal method for assembling structures made in different design tools.[24]

Molecular simulations have proved effective in the field of nucleic acid nanotechnology, which provides in-depth information about structural characteristics, kinetics of folding pathway, conformational space, and complex nanostructures kinetics.[25] Traditional full automated simulation techniques can be applied to designed nanostructures. The study of DNA/RNA nanostructures which represent each nucleotide as a single rigid body give an idea about the interactions between nucleotides that are empirically parameterized to produce basic structural, biochemical, and thermodynamic properties of nucleic acids. The standalone simulation package provides simulation trajectory with 3D position record of all nucleotides in the simulation.

The two open-source tools to design and optimize DNA and RNA nanostructures were reported. The first tool used was oxView, a web browser-based visualization and editing platform for DNA and RNA structural design and analysis of nanostructures simulated in oxDNA/ oxRNA. This tool enabled to accommodate nanostructures with million nucleotides, which was found to be most advantageous than the other visualization tools.[26] The simulation package allows the user to load simultaneous large multiple nanostructures and edit them by addition or deletion of individual nucleotides

or entire regions, thus enabling the users to create more complex designs, which are novel from small designed units. The built-in tools like Adenita, MagicDNA, vHelix, and TacoxDNA webserver (CaDNAno, Tiamat, vHelix) can be converted to oxView format or by converting first to PDB using built-in tools and then to oxDNA using TacoxDNA (DAEDALUS, PERDIX). The visualization tool is integrated with oxDNA/ oxRNA simulations and loads long simulation trajectories quickly (including files of 10 GB) for interactive analysis and video export of nanostructure dynamics. It can also load data overlays from the analysis scripts allowing users to interactively explore features such as hydrogen bond occupancy and structure flexibility and then, use this information to redesign nanostructures based on simulation feedback using oxView.[25,26]

The standardized set of structure-agnostic geometry analysis motifs for particular DNA/RNA offer a common molecular simulation cases. The design of DNA/RNA nanostructures facilitate the simulation-guided design and lower the restricted entry into the simulation field, which is an easier tool to conduct study in-depth.

The tool may include the following:

- Mean structure and root-mean-squared fluctuation calculation to measure structure flexibility
- Quantify fraying and bond breaking by measuring hydrogen-bond occupancy during simulation
- Angle and distance measurements between respective duplex regions in a nanostructure
- To identify nanostructure motion modes by a covariance-matrix based principle component analysis tool
- Unsupervised clustering of sampled configuration

MATERIALS AND METHODS

Generally, the synthesis of nanoparticles employs subsequent methods:

- Bottom-up approach
- Top-down approach

The bottom-up technique tends to assemble primary building blocks into larger molecules that are usually a molecular self-assembly development exploitation using chemical and physical interactions.[18] The interactions embody non-valency bonds, such as, hydrogen bonds, ionic bonds, van der Waals forces, and water-mediated hydrogen bonds. The example includes the formation of quantum dots and formation of colloidal nanoparticles in epitaxial growth methods. These additionally embody physical strategies (atomic layer deposition, vacuum arc deposition, metal-organic chemical vapor deposition, sputter deposition, electric arc deposition, and molecular beam epitaxy) and chemical strategies (colloidal methods, sol-gel technique, strategies of microemulsion of oil-water, hydrothermal preparation, polyol technique, sonochemical synthesis, and electro sedimentation). Chemical strategies are preferred to the physical strategies.[27]

The top-down approach is the method of producing smaller particles from larger particles. This method involves planographic printing patterns. Scanning beam techniques offer patterns up to 20 nanometers.[28] Apart from physical and chemical strategies, there also are biological strategies that facilitate the formation of nanoparticles. The biological technique is a substitute approach for physical and chemical approaches. Green synthesis of nanoparticles involves the usage of bacteria, actinobacteria, yeasts, and molds. The intracellular and extracellular enzymes help in reduction. The foremost use of these biological processes is that they produce safe and effective particles with reduced toxicity.[29]

The fabrication of DNA nanostructures can be done by one of the following methods:

- Construction of synthetic networks with native DNA
- The incorporation of DNA onto solid surfaces
- Synthesis of metal or semiconductor nanoparticles aggregates along DNA[30]

The basic requirement for DNA nanoparticles was by synthesizing oligonucleotides primers or staple strands having cross AT/GC pairing with cccDNA strand (sometimes used is M13 viral DNA). Such DNA mixture (1:5 at 1–5 mM concentrations) when heated to 90°C and then cooled down to 20°C in presence of 20 mM $MgCl_2$, 2D and 3D nanostructures were shaped. Bacterial genomic DNA could be separated by dissolving bacteria in SDS-proteinase-K overnight, followed by extraction with phenol-chloroformisoamyl alcohol (25:24:1) and ethanol precipitation (2 volume, 99% pure) in the presence of salt (300 mM NaCl). Then, the DNA is purified by agarose gel electrophoresis followed by extraction of DNA using a DNA gel extraction kit. As DNA sequence is understood, double cross over (DX) or triple cross over (TX) can be synthesized. One can style many sticky strands or different hairpin structures between AT/GC pairing complementary staple strands that favors the DNA crystal formation varying from 10 to 50 nm.[31]

RNA particles can be prepared by using a bottom-up self-programmable approach. This represents an important way by which biological techniques and biomacromolecules can be successfully incorporated into nanotechnology. The self-assemblies are chiefly of two categories: templated and non templated assemblies. Templated assemblies include the interaction of RNAs with one another with the help of external force, structure, or spatial constraint, and phi29 pRNA hexameric ring formation are all under this category. Non-template assembly involves the construction of a bigger structure in the absence of external influence. Such as ligation, chemical conjugation, covalent linkages, loop/loop interactions of RNA, such as, the HIV kissing loop and phi29 pRNA dimer or trimer construction. RNA nanostructures can be constructed by using the following approaches:

- The use of assembly mechanisms of RNA nanoparticles that form distinctive multimers *in vivo*. Samples of this technique are the retrovirus kissing loops, which allow genomic RNA dimerization, the pRNA of the bacteriophage phi29 DNA packaging motor are arranged into dimers and hexamers with hand-in-hand interactions between two right and left interlocking loops.
- The second approach is the use of the DNA nanotechnology principles for preparing RNA nanostructures. Although the

fundamentals of DNA and RNA are somewhat different, the fundamental principles of DNA nanotechnology can be applied in RNA nanotechnology.

- The third maneuver is to use computational methods in RNA nanoparticle construction. This approach helps in design of novel RNA nanoparticles. An example is construction of cubic RNA-based scaffolds, where RNA sequence designs were computed to obstruct kinetic traps.
- The known RNA structure or with far-formed functions as building blocks in RNA nanoparticles.[30,31]

Several strategies are used for RNA nanoparticles construction borrowing RNA properties in loop/loop interactions. The first method is on the basis of structural characteristics on the pRNA of bacteriophage pRNA packaging motor, which utilizes hexameric ring to gear the machine. The pRNA has been re-engineered to form dimmers, trimers, tetramers, hexamers, arrays via hand-in-hand or foot-to-foot interactions between two interacting loops. The second method is the RNA "architectonics," where structural modules for bends or stack can be encoded within artificial RNA sequences for constructing RNA self-assembly shapes. Such as RNA filaments, molecular puzzle units called tectosquares and tRNA antiprisms. The third method is the arrangement of 3WJ (three-way junction) and 4WJ that are utilized from known RNA structures or motifs to be used as the cornerstone in nanoparticle preparation. Some examples include the tetramer assembly of L-shaped tecto RNAs guided by RNA-structural motif, 3WJ-motif (from 23S rRNA) to assemble a t-shaped arrangement of three helices, and tRNA motifs consisting of 4- and 5-WJ to fold L-shaped tertiary structures.[31] The fourth method is to assemble non-natural functional RNAs with outlined 3D structures using non-natural ribozyme ligase by employing molecular design of RNA based on the *in vitro* selection technique. Conformational switch of RNA nanostructures may also be constructed using a peptide-binding RNA structural motif. The palindrome sequence can also be used at the 5' or 3' end of RNA as one of the methods in construction of RNA nanoparticles, unlike the sticky ends. The molecule will automatically assemble via self-annealing of the palindrome sequence just after *in vitro* transcription or chemical synthesis, before purification. This technique is beneficial for the creation of bundles, especially for designing 3D branches. Since every 11-nucleotides of the A-form RNA generates one helical turn of 360°, the angle for RNA fiber extension is controllable by differing the number of nucleotides in the palindrome sequence.[32,33]

CHARACTERIZATION OF DNA AND RNA NANOPARTICLES

Quantitative Estimation of DNA and RNA

Requirements

Ferric chloride, glacial acetic acid, concentrated sulphuric acid, diphenylamine, ethium bromide, electrophoresis apparatus, NaOH solution, and perchloric acid.

Procedure

Quantitative estimation of DNA and RNA:

- Killer-Killani test: Add 1 mL of glacial acetic acid containing a ferric chloride solution. Then, add 1 mL of concentrated sulphuric acid by the walls of the test tube. A brown ring at the interface indicates the presence of nucleic acid.[34]
- Diphenylamine (DPA) test: Add DPA reagent (1-gram DPA + 50 mL glacial acetic acid + 25 mL concentrated sulphuric acid) to the sample. Put this on a boiling water bath for few minutes. If blue color is present, confirm the presence of DNA.[34]
- Agarose gel electrophoresis: It is used for the sequence of DNA nanoparticles by using 0.7% agarose. Mix the sample with ethium bromide and then, with agarose gel, which is kept in electrophoresis chamber. Apply current to it. View it under ultraviolet light after stopping the current, when dye reaches its end point.[34]
- Orcinol method: 2.0 mL RNA standard solutions (RNA was dissolved in 5% RNA dilutions containing 100 to 500 mg RNA/mL with 5% $HClO_4$) were taken and 2.0 mL of 5% $HClO_4$ as a blank in different test tubes. 3 mL of orcinol reagent (100 mg of ferric chloride in 100 mL concentrated HCl, and then 3.5 mL of 6% solution of orcinol was prepared in alcohol) to all test tubes and mix appropriately. Place the test tubes in boiling water bath for 20 minutes. After cooling, 7.0 mL of n-butanol was added to each test tube, and A665 was measured against blank. A graph was plotted between A665 *vs.* the amount of RNA and the amount of RNA was determined from the standard curve.[35]
- SDS-PAGE: This technique is used for identification and segregation of nucleic acid. The nucleic acids are added with tris-borate buffer and incubated at 15 to 20 minutes at room temperature. This mixture is then placed on non-denaturizing polyacrylamide gel slab. The bands are then visualized with TCP-20. It is illuminated at 254 nm using UV illuminator, and the image was photographed.[36-38] Gel analysis insinuates the successful formation of RNA nanoparticles. Discrete RNA bands are formed for each nanoparticle.[36]
- Dynamic light scattering (DLS) technique: The DLS technique is used to determine particle size, reaction between macromolecules, and physical and chemical treatments. This is based on the principle where the light scattering is of macromolecules are measured with the help of the Brownian movement. It can measure particle sizes from 1 to 1,000 nm.[39]
- X-ray diffraction (XRD): It analyzes the sizes and substructures of nanoparticles.[38] X-ray crystallography is used for determination of 3D structure of macromolecules at the atomic scale.[39] The principle involved in X-ray crystallography is X-ray diffraction of the electrons in the macromolecules. Electron density is then calculated, which provides the localization of each atom in the molecule and

hence, the coordinates can be determined in 3D space.[40,41]

- Fourier infrared spectroscopy: It determines the covalent bonding between the nucleotides and thus, helps to understand the binding between the base pairs and the functional moieties. The study of covalent interactions is necessary to understand the stability also it gives the idea about the agglomeration of particles.[42]
- Quantitative estimation of purity: Prepare the standard solution by dissolving nucleic acids in 5 mM NaOH solution and then add 1 N perchloric acid and put it on flame for 15 minutes. Measure absorbance at 260 and 280 nm using a spectrophotometer. A graph is then plotted. A small volume of DNA/RNA is taken and extracted on a test tube, which is then added with perchloric acid. The solution is diluted so that 0.002 to 0.25 mole of sample was present. Take 2 mL of DNA sample and then 4 mL of DPA reagent was added. The mixture was incubated at 25 to 30°C for 15 to 17 hours. The absorbance was measured at 260 nm after calibrating the instrument with blank tube and standard solution. As the concentration of DNA/RNA was increased UV absorption is also increased, which indicates absorbance is directly proportional to DNA/RNA concentration (Figure 1).[34]
- UV-vis spectroscopy: The UV spectrum is recorded after the incubation period to understand the formation of nanoparticles by recording the characteristic SPR peak at λ_{max}. The color change[43] of the solution indicates the formation of nanoparticles that can be studied using UV spectrum.
- Scanning electron microscopy or transmission electron microscopy (SEM/TEM) studies: The SEM/TEM helps to determine the size, shape, and morphology. Also, the particle's distributions can be clearly studied using SEM/TEM microscopy.[43]

	DNA nanostructures	RNA nanostructures
Building elements	A G T C 4 nucleic bases	A G U C 4 nucleic bases
Building blocks	Medium sized DNA Long DNA ssDNA RNA	RNA motifs DNA
Building block manufacturing	Chemical synthesis	Enzymatic synthesis Chemical synthesis
Partners orientation	Anti parallel	Anti parallel
Interactions governing 2D structures	Nucleotide base pairing	Nucleotide base pairing

Figure 1: Elements of DNA and RNA nanostructures

APPLICATIONS

Nucleic acid nanostructures involving both DNA and RNA nanoparticles have wide range of applications (Figure 2), which are described below in detail.

DNA nanotechnology encompassing DNA 2D arrays, 3D nanostructures, and DNA nanomechanical devices has shown great promise in biomedical field. It is multifaceted involving protein characterization, enzyme aggregation, biosensing, drug delivery, and biomimetic assembly.[35]

DNA-nanotechnology in medicine: The nanotechnology can be employed in treatment, detection, and management of diseases is called "nano-medicine." DNA structures are feasible

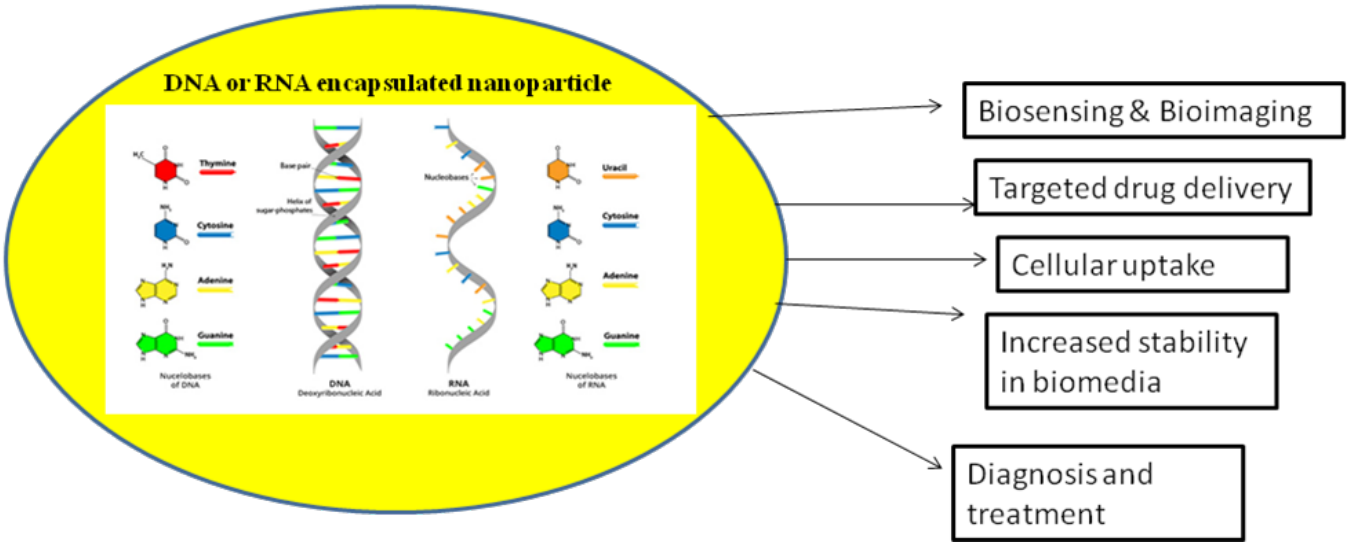

Figure 2: Applications of nucleic acid nanostructures

to carry drug with the assistance of their convoluted assembly together with immobile holiday junctions, cubic cages, and 2D lattices. Though this application of nanotechnology looks to be new, it started in the 1960s with the outline of the use of liposomes in drug delivery and DNA transfection. These structures have larger biocompatibility with no toxicity. The application of nanoparticles in medicine is diverse.

They offer great assistance to fight bacterial infections. The employment of silver nanoparticles in the treatment of bacterial infections was found in ancient Chinese and Indian *Ayurvedic* medicines, which were documented in 1,000 BC. These particles act by initially attaching to the membrane affecting the porosity and respiration, followed by disrupting the DNA then by finally releasing the silver ions, which bind to the cysteine inflicting the bacteria to die. The releasing of free radicals causes toxicity effects like inflammation, oxidative stress, membranes, and DNA for bactericidal action. But due to the drug resistance, alternative pathways methods are allowed for these treatments. Silver and gold nanoparticles with cinnamon plant extract show potent antibacterial activities and also cure multidrug resistance. These, when combined with DNA nanotubes, immobilized with nanostructures, were found to have potent antibacterial activity without causing toxicity in the host. These nanoparticles also have been used to treat viral diseases impeding virus penetration and elimination.[36] DNA delivery using non-viral ways is useful as it is less toxic, great biocompatibility, and adaptability.[37]

Uses of DNA Nanoparticles to Cure Cancer

DNA nanostructures have the efficiency to transport toxic drugs to tumor affecting sites without causing harm to normal cells.[37] DNA strands can be combined with nanostructures like nanosheets, nanotubes, nanowires, and gold particles, iron oxides and quantum dots show great efficacy in the timely identification and treatment of cancer. DNA orgami plays prominent role in drug delivery for cancer therapy and biosensing for cancer diagnosis. They can be helpful to exert therapeutic effects along with specific location of cancer cells. The use of icosahedral, tetrahedral, and square has been used for *in vivo* and *in vitro* applications. These structures were used for effective drug delivery of doxorubicin for breast cancer. The tetrahedral structure was found to be effective for drug-resistant cells. The tetrahedral structures were shown to protect singe strands against nuclease degradation and also to increase *in vivo* circulation half time of siRNA and to deliver cytosine-guanosine-phosphate to elicit immune response. Aptamer DNA-nanotrain against folic acid was most effective of all with reduced side effects of doxo in mouse xenograft tumor model. Different square, triangle DNA orgami were also used to check the effectiveness of these structures in drug delivery of doxorubicin drug in mice.[44]

DNA Nanoparticles for Ophthalmic Drug Delivery

The DNA nanotechnology is used in ophthalmic drug delivery. The eye drops ordinarily used as ophthalmics has the disadvantage of short survival time of the drug on eye surface. As a result, these administrations require high doses, frequent administration with minimum bioavailability. To beat these, DNA nanotechnology is used as being harmless, biocompatible, higher time on the eye surface with good biocompatibility. The lipid-modified DNA strands form uniform nanoparticle micelles, which adhere to the corneal surface with extended amount of time. The practicality was demonstrated in several *ex vivo* experiments and a person's *in vivo* animal model.[45]

DNA Nanostructures for Biosensing

DNA based biosensors are cost-effective and sensitive, and have the potential to be used as diagnostic tools.[45] This specific DNA sequence is essential for identification of pathogenic and genetic diseases.[46] The nanoscale sizes of these DNA nanostructures provide large volume to surface ratio, hence, produce large signal modifications on target binding. The DNA based biosensors depend on specific recognition events between a substrate and target analyte. Nucleic acid-based biosensors involve hybridization of DNA or RNA strand to its complement or a complementary region in stem-loop. Such stem-loop configurations help in global environmental changes, such as, temperature by using fluorophore-quencher pair on the ends of the strands. These DNA biosensors can also be used for measuring pH changes and sequence-sequence conformational changes.[47]

DNA Nanotechnology Uses in MicroRNA Detection and Diagnosis

The DNA nanotechnology has shown great scope in the creation of novel microRNA that can be employed in lab-based biosensing and disease based diagnostics. MicroRNAs play essential roles in numerous biological processes, including embryogenesis, organ development, proliferation, differentiation, apoptosis, homeostasis, and metabolism. They can be collected in saliva, tears, blood, and alternative bodily secretions. These can be found in early stages of some of the diseases.[43] They have deregulated levels in diverse diseases like cancer, muscle degenerative diseases, diabetes, and viral infections. For example, in sporadic Alzheimer's disease, these changes are from 0.6 to 1.25-fold.[48] Therefore, reliable and sensitive detection methodology will be the pre-requisite for using microRNAs as new generation diagnostic biomarkers. DNA nanostructures, such as, DNA orgami, DNA tetrahedra, DNA devices, and other assemblies are used for microRNA detection.[49]

RNA's application in nano-medicine is diverse, and it encompasses cell recognition and attachment to the identification, targeted delivery through receptor endocytosis, intracellular control and computation through silencing gene, nuclear membrane penetration, and blood-brain barrier passing.[46] These nanoparticles also have the ability to catalyze biological reactions, sensing and communicating responses, and attachment to specific targets for therapeutic drug delivery.[47]

- Small interfering RNA (siRNA) interferes with gene expression by cleavage of protein called RNA-induced silencing complex (RISC). It specifically suppresses the

expression of target protein, whose mRNA includes a sequence similar to the sense strand of siRNA.[48] These siRNAs have gained potential due to their ability to inhibit specific genes in many genetic diseases. They have been designed to target dominant oncogenes, viral oncogenes involved in carcinogenesis, or malfunctionally regulated oncogenes. The silencing of critical cancer-associated target proteins by siRNAs has resulted in significant anti-proliferative and/or apoptotic effects.[49]

- Ribozyme is an RNA molecule that has enzymatic activity. They have notable RNA therapeutic capability of regulating gene function by impeding and breaking down RNA, such as, mRNA or the viral genome RNA containing a sequence complementary to the ribozyme or catalytic center.
- The RNA aptamer is a family of oligonucleotides with functions similar to that of antibodies as they can identify specific ligands through the formation of binding pockets.[48] Aptamer based therapeutics have been developed to treat cancer by targeting adherence factors, immune system modulators, receptor tyrosine kinases, and modulators of cell growth. Several other aptamers have also been developed to treat or diagnose neurological diseases, like multiple sclerosis, stroke, Alzheimer's disease, Parkinson's disease, variant Creutzfeldt-Jacob's disease, and neuronal cell death.[50]

Riboswitches are RNA components that attach small molecules and regulate gene expression based on organism's requirement. As a biological control mechanism, riboswitches can identify metabolites, trigger premature termination of mRNA transcription, obstruct ribosomes from translating mRNAs, cleave mRNAs, and even trigger mRNA destruction. Therefore, RNA switches can be recreated to construct a new generation of controllers regulated by drug-like molecules to tune the expression levels of targeted genes *in vivo*. Such RNA-based gene-control machines hold great scope in future gene therapies by providing nanoscale cis-acting modulation.[51]

FUTURE ASPECTS

The DNA nanotechnology has become an interdisciplinary research field with scientists from computer science, chemistry, materials science, biology, and physics come together to tackle problems. Though the field of DNA nanotechnology is progressing rapidly, there are still barricades that are to be overcome. The high cost of DNA and a high error rate of self-assembly are impeding the development of this technology. However, these can be overcome by further research, which scientists are already working on. Apart from DNA nano-medicine and therapeutics, the DNA nanotechnology also has others applications in molecular and cellular biophysics.[52] DNA nanostructures have impact on studies in single-molecule biophysics both as aids for imaging and as tools for constraining multiple macromolecules. Biomimetic systems made from DNA nanoparticles,[53] which can generate an artificial cell in which most of the functional nature is provided by DNA, which is, however, a long term challenge. It can also be applied in energy transfer and photonics, DNA computing, contraception.[54] There are wide variety of materials, such as, DNA transistors, capacitors, and optoelectronics.

RNA nanotechnology therapeutics is under clinical investigation for a range of diseases from genetic disorders to HIV infection to various cancers. The emerging drugs include ribozymes, aptamers, and small interfering RNA, which demonstrate the versatility of RNA. However, RNA drugs need efficient vehicle transport for the targeted drugs and these structures, highly unstable, immunogenic.[55] These have become major reasons of hindrance for the clinical trial progress of RNA therapeutics. The siRNAs require will require some chemical modifications and synthetic or natural carriers have to be used for drug delivery.[56] Like siRNAs, ribozymes and aptamers face similar challenges of delivery and off-drug toxicity. Modifications and required research have to be done for further development in the field of RNA nanotechnology.[57,58] Even though there are factors that hinder the growth, further research is going on, and RNA technology will grow to have further applications in the future.

DNA and RNA nanotechnology can also be directed into new and different directions with increasing interest and scientific research. They have promising and bright future not only in the field of therapeutics but also in the field of physics, chemistry, and molecular and cellular biophysics.

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