Review Article

DNA-based Nanostructures as Novelty in Biomedicine

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Abstract

The application of nanocarriers in medicine and pharmaceuticals suggests a new procedure in nanotechnology terminology, nanomedicine. DNA has been significantly highlighted due to its amazing functionality and nature as a nanomaterial in biomedicine. Given that DNA is biocompatible, its use as a nanomaterial in medicine provides an excellent prospect for the rational engineering of DNA nanostructures. According to new approaches in disease treatment at gene levels, gene therapy, DNA as a nanomedicine plays an essential role in the medical sciences. In this field, researchers have published enormous documents regarding the applications of DNA and DNA-based nanostructures as drug or gene nanocarriers, DNA-based diagnostics, and DNA nano vaccines. In this review, the novelty of DNA-based nanomedicine has been considered.

Keywords: DNA-based nanostructures, Nanomedicine, Nanovaccine, Nanocarriers

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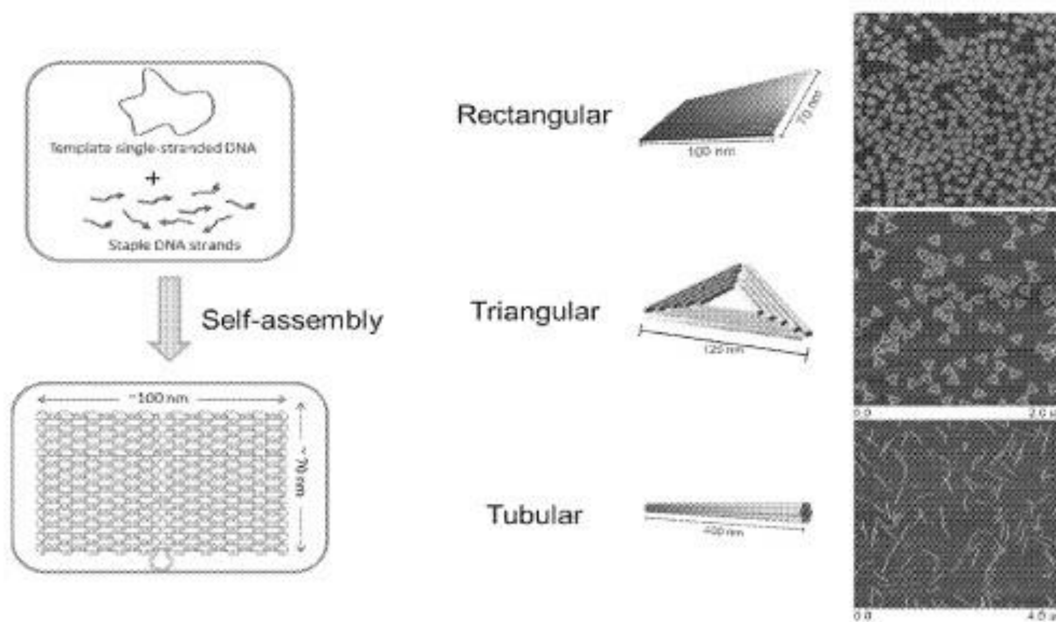
Introduction

Nanotechnology has been introduced as a new technological revolution with a highly multidisciplinary focus, which attracts a part of political and scientific communities due to its promising expansions and exciting advances[1]. This technology has led to unprecedented results in the medical field. The application of nanoscience in the medical field has created a new discipline known as nanomedicine. Nanotechnology can help diagnose, control, and treat diseases[2,3]. Nanoparticles (NPs) have represented excellent abilities for effectively transporting and delivering drugs to the action site[4]. Seeman et al. constructed DNA structures as the versatile building blocks for complex nanoscale assemblies such as cubic cages, immobile holiday junctions, and two-dimensional (2D) lattices for visualizing 3D DNA crystals. These DNA NPs may be gene and drug carriers[5,6].



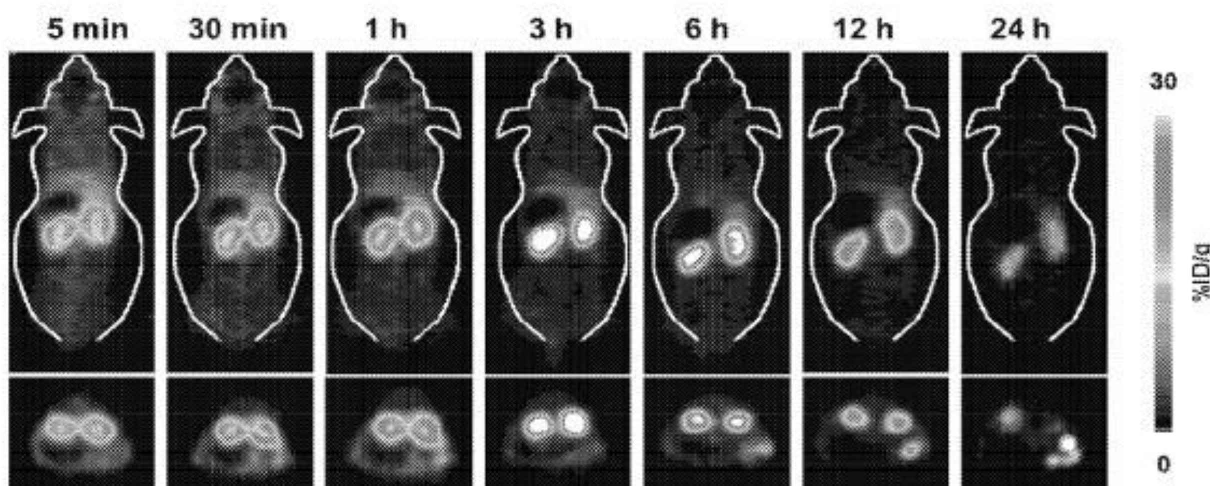


Figure 1. Formation of a DON (a) and renal accumulation of rectangular DON (Rec-DON) in acute kidney (AKI) murine models (b)[19].

DNA nanotechnology has introduced the developmental functional molecules which can be released into cells[7]. Consequently, they are classified as genetic materials with low cytotoxicity and high biocompatibility, suitable for numerous applications in the biomedical sciences field[8,9]. Its significant molecular recognition provides a basis for matching GC/AT with mechanical stiffness. The nano dimensions of replication unit and customized production with any strand length facility (in any form of 2D and 3D nanostructures) have highly non-toxic and versatile drug nanocarriers[10]. DNA-based nanomedicine encompasses a broad spectrum of diagnostics, therapeutics, and vaccination. The design of biocompatible and functional nanoassemblies with molecular accuracy and control is considered the

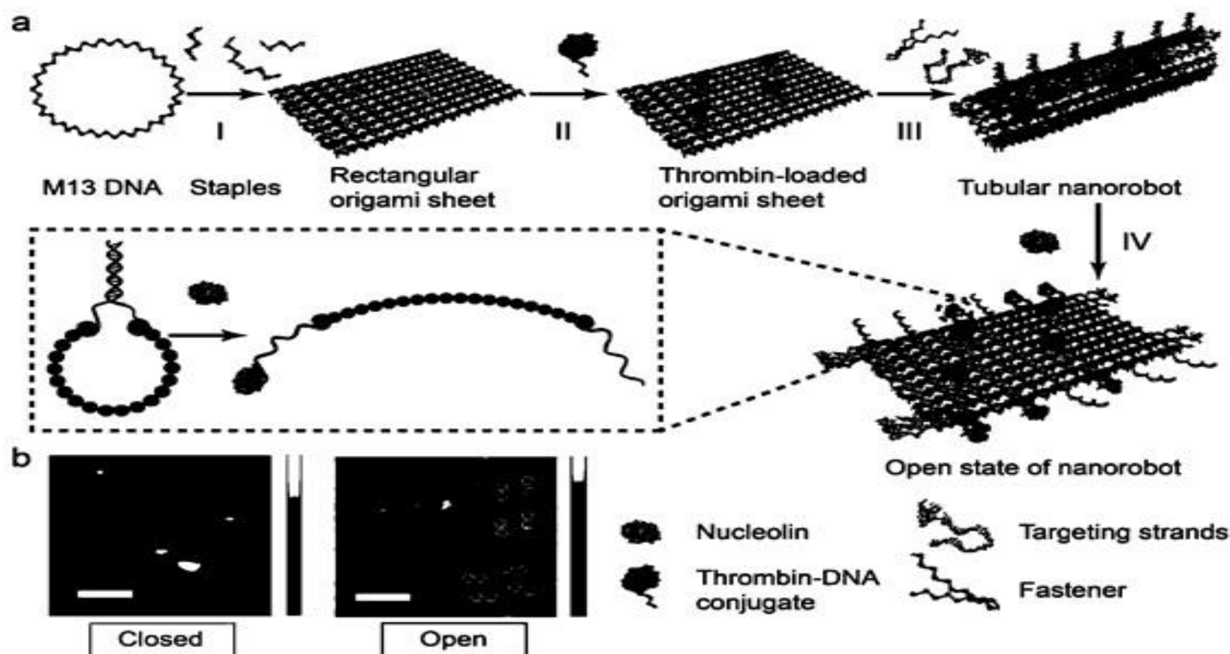


Figure 2. Schematic illustration of constructing the thrombin-loaded nanorobot by using DNA origami, as well as its reconfiguration into a rectangular DNA sheet in response to nucleolin binding (a) and closed (left) and open states (right) of the DNA nanorobots examined based on the AFM images (b)[20].

fundamental challenge in nanomedicine. Thus, designing and exploring the new materials which can meet the requirements are necessary.

DNA has been proven an excellent material with many handling options readily available from the molecular biology toolbox, making DNA nanoarchitectures a promising class of materials for nanomedicine. Most researchers have envisioned the broad potential of DNA origami nanostructures (DONs) for *in vivo* applications due to their biocompatibility and biodegradability.

Gene administration and gene therapy may be the primary utilizations of DNA in the world of nanomedicine, which involve a broad range of disease control and treatment measures such as treating cancer, central nervous system disorders (CNS), immune deficiencies, muscular dystrophy, and eye diseases. In addition, DNA as a nanomedicine can be used as a vaccine. In nano-vaccination, nucleic acids can act as a carrier and an adjuvant and stimulate the immune system and create an appropriate immune response by encoding multi-epitope polypeptides and their assemblies. Further, DNA probes are applied in several fields such as food, medical, veterinary, environment, and security for diagnosing different diseases. The use of DNA probes enables identifying microorganisms, for example, pathogen detection and quantification, when utilized in specific systems. Various techniques using DNA probes have been successful and provided quick and specific results. The present study introduced and described the recent patents on DNA nanomedicine and its applications in the fields mentioned above.

2. DNA-based Nanomedicine

2.1. DNA origami-based nanostructures (DONs)

Recently, the DNA nanotechnology field has been expanded due to its biodegradability and biocompatibility, and many studies have suggested the various abilities of DONs *in vivo*. DONs can be used as drug delivery systems for treating cancer[11-15]. Additional DNA administration systems include transportation costs for siRNA fragments[16]. Additionally, the gold NPs loaded with DNA origami can be employed for phototherapy in cancer treatment[17, 18].

The first study to assess the therapeutic influence of

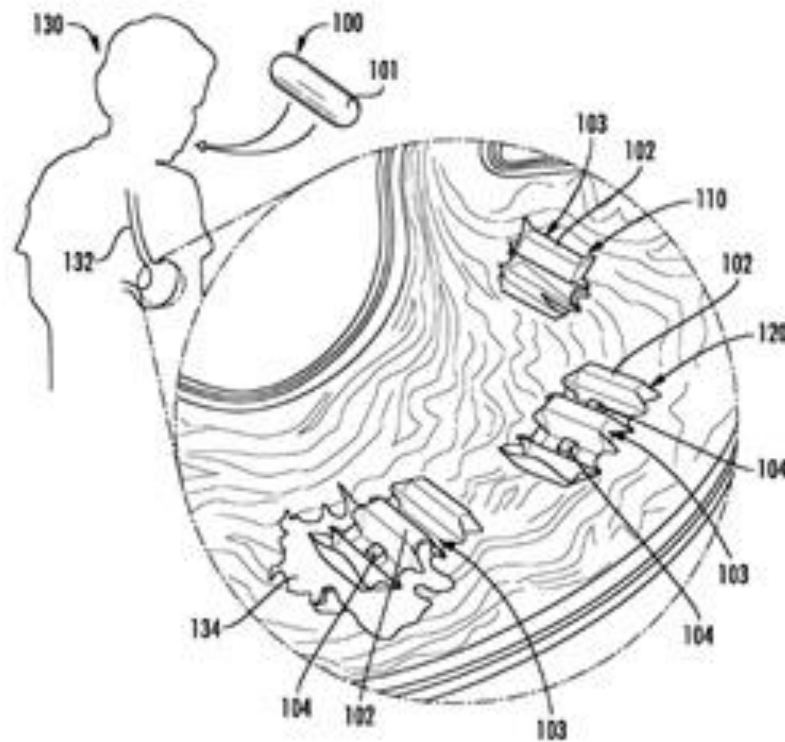


Figure 3. A magnified view of an origami robot deployed in patient's stomach[21].

DONs was reported in the AKI murine model. Based on the results of PET imaging, DONs are primarily concentrated in the kidneys and poorly absorbed in the intestine and liver. Rectangular DONs (Rec-DONs) have significantly improved the excretory function of AKI kidneys through murine procedure [19]. The method includes administering a sufficient quantity of a DON to an AKI mouse to increase its excretory function. The DONs consist of a scaffold strand comprising an M13 phage genome (7249 base-pair length) and a plurality of staple strands (approximately 20-60 base-pair length in each strand) [19]. Figure 1 displays DONs (a) self-assembly and renal accumulation of Rec-DONs in AKI murine models for 24 hours (b).

The other application of DONs could be named DNA nanorobots. A DNA-based nanorobot consists of a single-stranded DNA scaffold with about 5,000-10,000 base length and DNA scaffold strands with around 20-40 bases in each staple thread. Each scaffold strand involves an unparalleled substance and is hybridized for a specific position on the scaffold strand of DNA. In this position, the pluralism of the hybridized base strands of the DNA scaffolding makes a sheet with a top and bottom surface. No more bindings, DNA strands where more bindings DNA strands can attach the leaf into an origami structure. The term "origami structure" refers to a 3D structure. In the invention, a "fastening strand" is considered an oligonucleotide that connects two DNA strands to form an origami structure/shape. For example, a plurality of fastener strands can bind ("tie") two edges of a rectangular DNA origami sheet to generate a tube shape. Thrombin is loaded on the surface of the DNA sheet structure by hybridizing the poly-T oligonucleotides conjugated to thrombin molecules with the polyA sequences developed from the DNA sheet surface.

Due to the existence of nucleolin, the tube nanocarrier can open to expose the encapsulated thrombin. Vascular occlusion can exert its effects within hours following the rapid induction of thrombus formation in tumor vessels, which leads to a treatment duration shorter than that of many other therapies and is associated with a decreased risk of resistance development. Further, vascular occlusion in tumors is

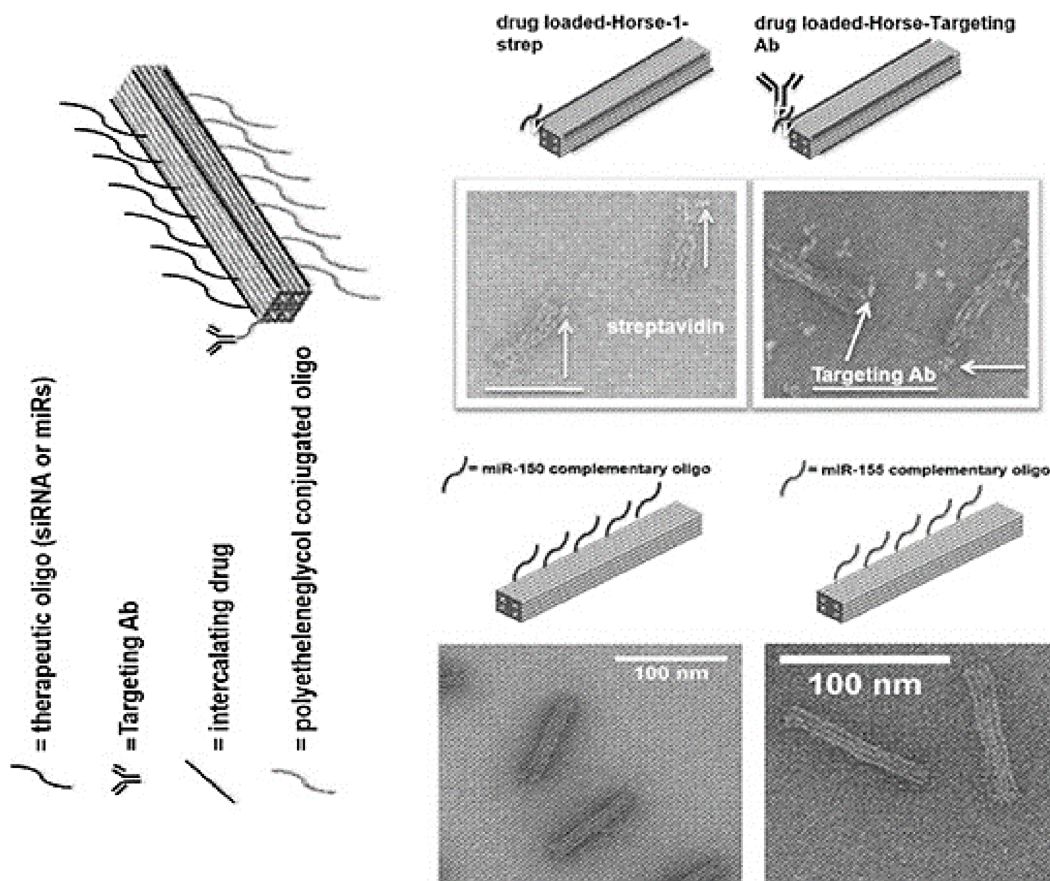


Figure 4. Design and construction of an embodiment modular DNA drug delivery device disclosed based on a DNA origami engineered rod-like nanostructure functionalized with therapeutic oligonucleotides, polyetheleneglycol (PEG)-conjugated oligonucleotides, and targeting antibody (a), and schematics and images of the drug-loaded delivery devices functionalized with streptavidin (left) and a targeting antibody (right) (b) and those functionalized with miR-150 (left) and a miR-155 (right) complementary oligonucleotides (scale bars: 100 nm)[22].

considered a strategy that can be utilized for many types of cancer since all solid tumor-feeding vessels are essentially the same. The coagulation protease, thrombin, regulates platelet aggregation by activating platelets and converting circulating fibrinogen to fibrin 13, leading to obstructive thrombosis. Given that naked thrombin is short-lived in the circulation and induces coagulation events indiscriminately, it has not been applied as an injectable therapeutic vessel occluding agent in cancer treatment. The precise delivery of sufficient quantities of the active protease solely to tumor sites in a highly controlled manner to minimize the effects of thrombin on healthy tissues is the critical challenge for its introduction as a potent anti-tumor therapy. Accordingly, DNA nanocarrier nanorobots can provide an adequate quantity of active thrombin to the tumor site[20]. Figure 2 demonstrates a schematic illustration of constructing the thrombin-loaded nanorobot using DNA origami and AFM images of closed and open states.

On the other hand, providing a therapeutic method that includes delivering an origami robot into a folded position in the gastrointestinal (GI) tract. The unfolding of DNA robot within the tract and finding a way to direct the robot to the site that requires therapy in the tract were the subjects for designing an assistant tool for this purpose. A system consisting of an origami robot encapsulated to be ingested by a patient was prepared for medical treatment. In addition, the system comprised an external system to manipulate the robot magnetically after its ingestion. Further, the

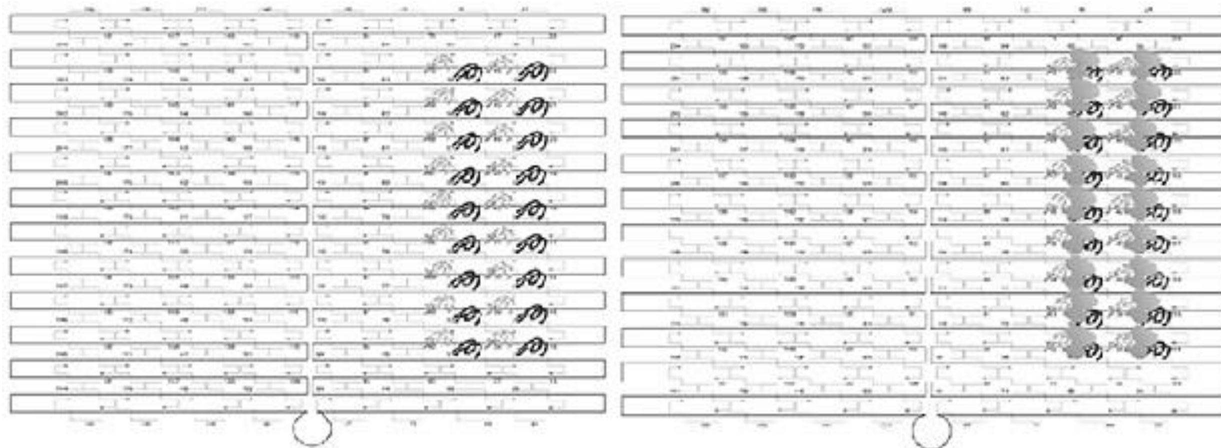


Figure 5. A schematic diagram of the distribution of two aptamers on a rectangular DNA origami structure (left) and the capture of thrombin by the rectangular DNA origami with the aptamers modified (right) [25].

method of preparing an origami robot for utilizing in the GI tract consisted of encapsulating the robot with an encapsulation material adjusted to (i) allows the robot to be engorged by a patient and (ii) release the robot from the encapsulating material in the patient's stomach. The treatment of the patients who have inadvertently or ingested a button battery is considered an example of the clinical interventions where a multifunctional miniature robot is desired. Considering the fatality of the accidents and lack of efficient interventional tools for counteracting them, the disclosure includes deploying a miniature biodegradable origami robot in the stomach, guiding the robot to a wound location where it can remove a lodged battery, and effectively administering drugs directly to the wound location, and eventually disposing of itself on-site through biodegrading or digesting. The other examples of therapies are related to wounds and other ailments in the GI tract and stomach, for which the origami robots may deliver drugs and/or provide a wound covering[21]. A schematic magnified view of an origami robot in the stomach is presented in Figure 3.

A DON was functionalized for targeted and personalized drug delivery in the disclosure. The disclosed nanostructures enter cells via the circumvent drug resistance and endolysosomal pathway in target cells. They have loaded the small molecules of a drug such as anti-metabolites and anthracyclines, and nucleic acids like siRNA, antisense oligonucleotides, and miRNA with the therapeutic and targeting antibodies against the tumor-specific antigens (e.g., anti-CD20 and anti-CD33), which can be modified for treating different cancers and finally fitted to specific patients' requirements for precision medicine[22]. The nanostructures can be constructed in minutes and functionalized with therapeutic, stabilizing, and targeting molecules for a few hours. The advantages of the disclosed nanostructures over current approved liposomal-based nanotherapeutics include the unprecedented control of molecular placement allowed for multifunctional modular nanodevices (targeted, simultaneous, and co-localized distribution), as well as tunable and faster drug release rates compared to the liposomal encapsulated anthracyclines. The other advantages are easy fabrication and molecular functionalization, scalability, biocompatibility, and stability. Figure 4 represents the design and construction of DNA drug delivery device.

The application of DON as a drug delivery system requested its construction by using rolling circle amplification technology and DNA origami, along with applying fluorescent dye DAPI with double-stranded DNA binding and cell membrane penetration properties to label and track the DONA, which realizes the real-time monitoring and simulation of loading the drug, as well as the sustained release process of the DON as a drug carrier. The method can be utilized as a new

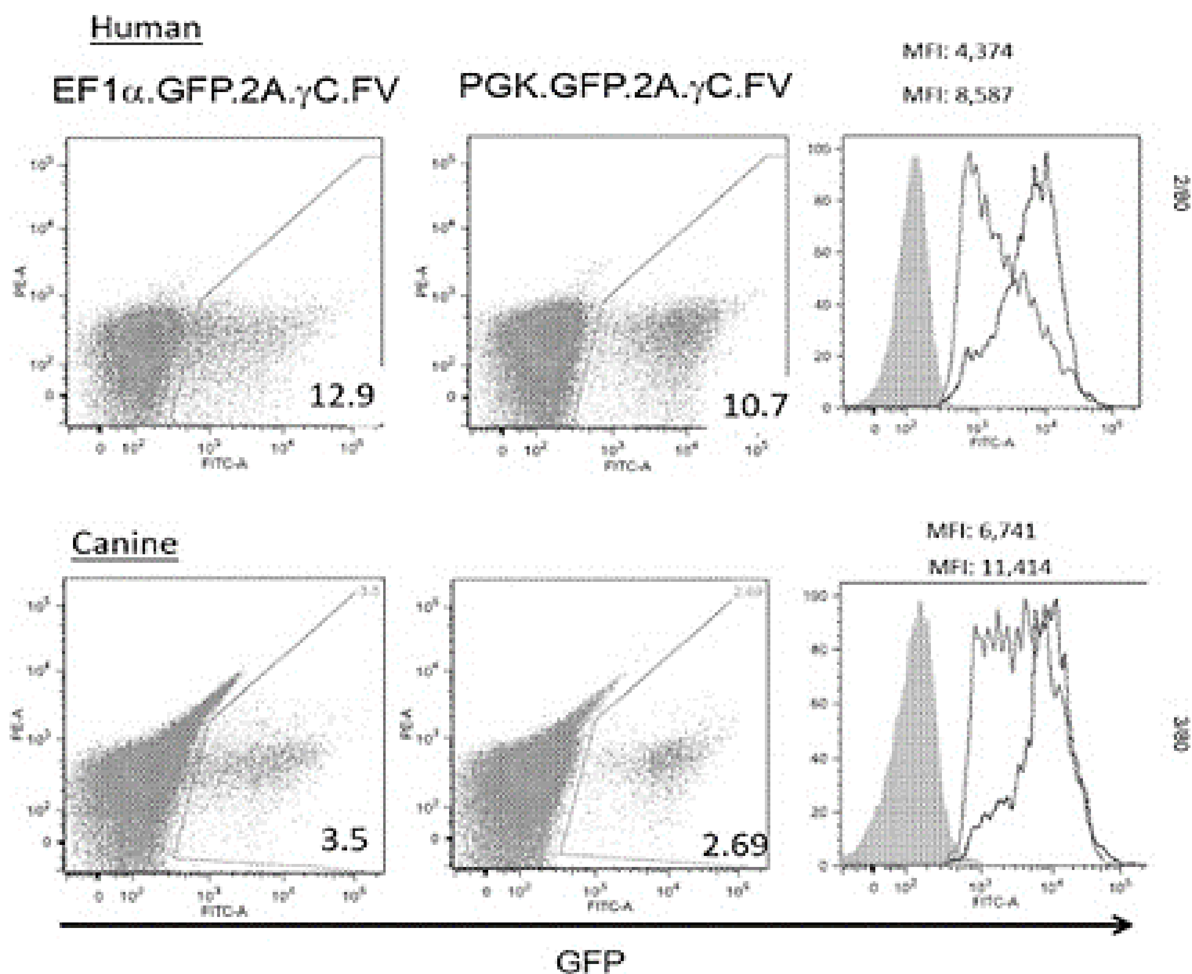


Figure 6. Increased transgene expression in CD34+ cells transduced with *PGK.GFP.2A.yC.FV*. Human and canine CD34+ cells enriched from mobilized peripheral blood and steady-state bone marrow were transduced with *PGK.GFP.2A.yC.FV* or *EF1a.GFP.2A.yC.FV* at an MOI of 10. GFP expression was measured by flow cytometry at impost transduction, and the fraction of GFP+ cells and mean fluorescence intensity (MFI) of the GFP+ fraction was shown for each vector (Filled gray histograms were from untransduced cells)[30].

fluorescent quantitative analysis for biomolecular detection such as tumor early diagnosis, postoperative monitoring and evaluation, and cell imaging. A type of DAPI is used for releasing or embedding analog DNA nanometer paper folding structure as a pharmaceutical carrier, characterized, and promising results were achieved. Based on the result, a long single-stranded DNA can be prepared as a support chain using DNA rolling circle amplification, advanced paper folding after using staple chain and achieving 2D nanometer paper folding structure of DNA, the fluorescent dye having the strong binding ability in the cases of DNA double chain accommodates by the cause rear fluorescence intensity to be enhanced. Nanometer paper folding composite structure of DNA was degraded after dyestuff release lead to the stability of fluorescence intensity reduce procedure monitoring nanometer paper folding structure of DNA and introduce its drug loading having antineoplastic attribute as drug carrier slowly-releasing procedure[23].

DNA paper folding was employed for solving the inadequate technical issue of the common drug delivery approach. This approach is considered as a precise targeted detection method with different usages. The targeted nanocarrier consists of biofacies, is nontoxic, and high. Further, the significant benefits of the drug-loading rate could precisely target neoplastic cells and dramatically enhance the antitumor impact of anthracene ring antitumor

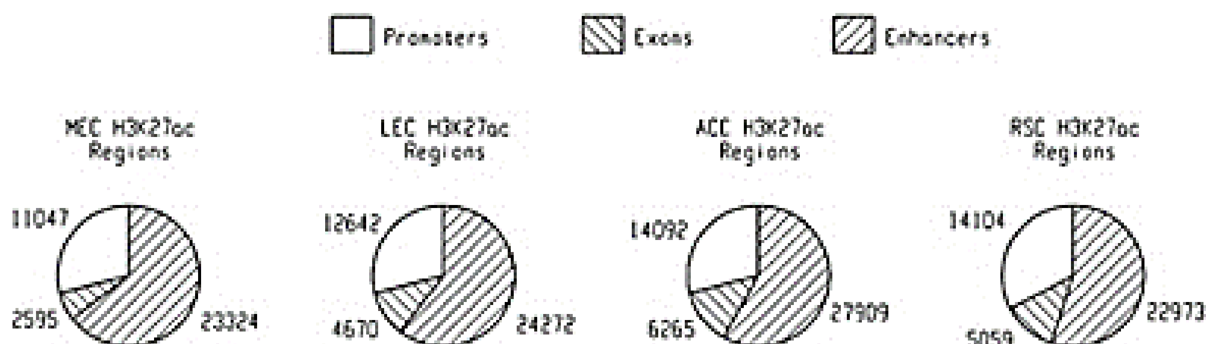


Figure 7. The pie charts of the proportions (and numbers) of distinct active genomic elements identified by *H3K27ac ChIP*-seq of the 4 cortical sub-regions (These numbers are roughly similar to those found by *ChIP*-seq of other organs [31]).

medicinal. It was recognized as a precise biological treatment for chemotherapy and tumor applications according to the precise detection of DNA paper folding targeted nanocarrier arts. The invention was related to a construction method of accurate identification of targeted nanocarrier based on DNA paper folding arts and its application, which belonged to the pharmaceutical technology field. The carrier consisted of scaffold DNA, staple single-stranded DNA, special staple single-stranded DNA, and aptamer.

Furthermore, the ratio scope of scaffold DNA and amount of the material of staple single-stranded DNA were 1: (5-10) :(5-10), and the ratio scope of the amount of aptamer and the material of paper folding were 1: (1-32). While conducting load medicine, the ratio scope of carrier and amount of the material of antineoplastic adriamycin was 1: (12500-50000). In addition, the suitable medicine time ranged 2-6 hours. Further, modifying DNA carrier of the achievable target head of the present invention accurately improved the biological agent of carrier targeting and target head induction. After loading the drug, its release can be delayed, which reduces the toxic side effect of anthracene ring antitumor medicinal and strengthens the antitumor action on the tumor cell. Finally, the drug-loading system plays a chemotherapy effect and biological therapy[24].

DONs as the anticoagulant nanocomposites were applied onto the nanostructure surface by hybridizing thrombin aptamer via the aptamer's type, site design, and density. Additionally, site distribution was regulated, thrombin activity was efficiently and stably prohibited, and quickly performed detoxification. Hence, the anticoagulant nanocomposite structure of DNA possesses a broad application perspective and an excellent market value. Firstly, the invention prepared an anticoagulant nanocomposite structure of DNA consisting of an antithrombin aptamer and DNA nanostructure, where the DNA nanostructure was constructed by self-assembling a template chain of DNA, an auxiliary folding chain of DNA, as well as a capture chain of DNA. The antithrombin aptamer was combined and hybridized onto the nanostructure surface of the DNA via a capture DNA chain, and the combination may be damaged competitively by a sequence of detoxification which is supplementary with the aptamer. Hence, the anticoagulation function of the nanocomposite structure of DNA was reversed. In the invention, in the long-term scientific research and practice process, the inventor intensively studied the advantages and disadvantages of coagulation treatment in the prior art, provided a nanostructure assembled creatively by a DNA template, an auxiliary folding DNA chain, and a capture DNA chain in order to improve the stability and long circulation of the antithrombin aptamer in a living body, integrated the antithrombin aptamer skillfully by designing the capture DNA chain and realized the accurate positioning of the antithrombin aptamer on the surface of a DNA self-assembly nanostructure through DNA

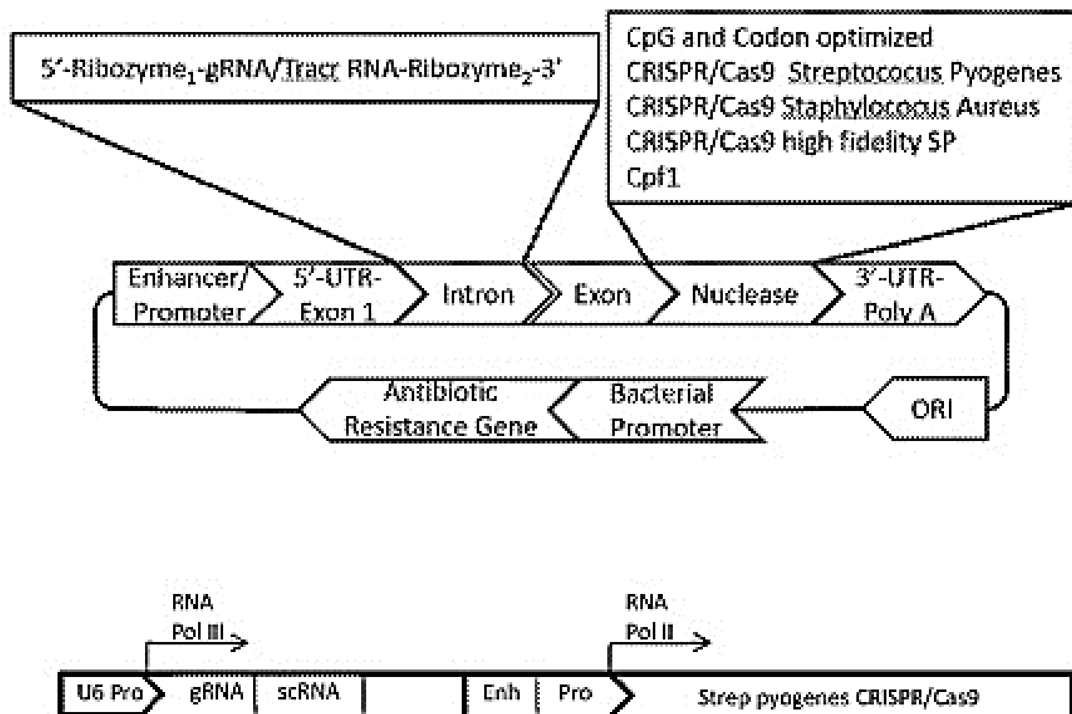


Figure 8. Pictorial diagrams showing exemplary HBV gene editing expression plasmids of the invention[34].

hybridization. In addition, it formed a nucleic acid aptamer array by adjusting the type, the number of sites, distribution density, and relative distance of the aptamer distributed on the DNA nanostructure, which resulted in combining thrombin functional sites effectively, inhibiting thrombin activity, and achieving the anticoagulation effect. Further, the design of the aptamer hybridized with the captured DNA strand could utilize the strand complementary with the aptamer to compete and combine from the surface of the nanostructure and could realize reversible response and rapid detoxification by destroying the single strand of the aptamer[25]. Figure 5 shows a schematic diagram of the distribution of two aptamers on a rectangular DNA origami structure and the capture of thrombin by the rectangular DNA origami.

2.2. Gene delivery

Gene delivery has been introduced as a process in which foreign DNA can be shifted to host cells in the cases of applications like gene treatment or genetic research. Gene treatment is related to DNA, which can act as a pharmaceutical agent to treat many diseases[26-30] such as brain tumors[29], ocular diseases[28], cancer[27].

For *In-vivo* gene therapy of the immune deficiencies, the preparation methods and systems could enhance the kinetics of T cell modification and extension in immune deficit subjects obtained by the former art. These methods and systems use a foamy viral vector including a human phosphoglycerate kinase (*PGK*) enhancer as an *EF1a* enhancer for explaining therapeutic genes like *FancA* for *FA* or *yC* for SCI D. The use of the *PGK* promoter could be beneficially combined with mobilization such as with the combination of granulocyte-colony-stimulating factor (*G-CSF*) and *AMD3100* before FV vector administration. This addition enhanced FV vector transduction of HSPCs, which generally reside in the bone marrow stoma. These conditions markedly increased both kinetics and clonal diversity of

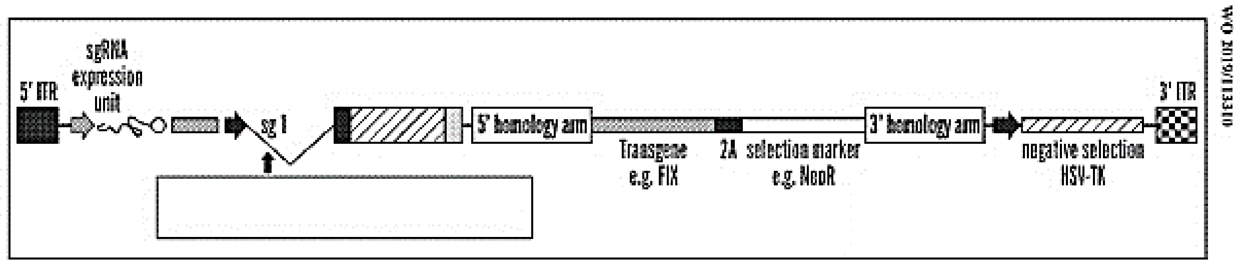


Figure 9. A schematic illustration of a ceDNA vector under the present disclosure [35].

lymphocyte reconstitution and were correlated with more robust thymopoiesis (Figure 6). These significant enhancements can result in continuous efforts to bring genetic therapies to patients needing immune system reconstitution due to primary or secondary immune deficiencies[30].

The other application of the DNA-based nanostructures was as a delivery system including a nucleic acid make where the made nucleic acid includes (i) an enhancer which mainly conducts gene expression into the entorhinal cortex cells, (ii) an enhancer, and (iii) a remedial gene where promoter and enhancer, as well as therapeutic gene, are functionally connected, stated therapeutic gene and enhancer are heterologous and stated delivery system is determined to be appropriate for delivering the nucleic acid made for the brain of a mammal. The invention is considered a method for treating or preventing Alzheimer's disease by administering a delivery vehicle or a composition mentioned above to a subject in need thereof. In another aspect, a method of driving entorhinal cortex-specific gene expression in a mammalian subject includes administering a delivery vehicle or a composition described before. The present application discloses enhancer sequences that can specifically drive gene expression in the epithelial cells (ECs), particularly in certain sub-sets of EC cells, including even EC layer II neurons. These enhancer sequences can be used in gene therapy to drive the expression of therapeutic genes, specifically in EC layer II cells, targeting AD at its source. The use of these enhancer sequences to drive cell-type-specific gene expression enables the expression of a therapeutic gene in EC layer II cells, even if the therapeutic gene is toxic if expressed more widely since the expression of the gene is limited to the desired EC layer II neurons by placing the therapeutic gene under the control of an appropriate enhancer. The delivery vehicles described herein include nucleic acid constructs in which a therapeutic gene is controlled by a promoter and an enhancer that drives EC-specific gene expression. The delivery vehicles can be used in gene therapy to prevent AD or treat AD at an early stage before appearing any symptoms, which results in arresting its development before damaging brain function irreparably. Thus, the disclosed enhancers can open up a wholly new avenue for AD prevention and treatment. The sequence IDs are available in document[31]. Figure 7 shows pie charts indicating the proportions (and numbers) of distinct active genomic elements identified by *H3K27ac ChIP*-seq of the 4 cortical sub-regions.

The other embodiment of DNA-based nanostructures was suggested as a “localizable” liver gene treatment procedure that can decrease the escape of the gene vectors from the liver like minimizing the waste of vector by systemic dilution, which can confine the inappropriate immune reactions. Here, the invention provides a two-inflatable balloon catheter, which may construct a finite included volume and coextending blood vessels to increase the virus local concentration and enhance the perception of vectors in the close tissue, leading to a reduction in vector loss. Electrodes in adjoining blood vessels were utilized to produce electroporation in the tissue area, which was among the electrodes rectifying this localization of delivery and enhancing the perception of the vector. At the same time, the involved volumes seemed to be against the purpose of remedying large quantities of tissue. Generally, a pair of catheters is inserted into a venous access site for hepatic vein catheterization. The

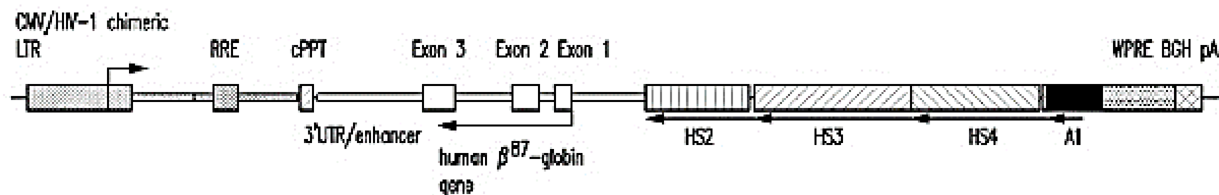


Figure 10. Depicts a recombinant vector including an expression cassette following one non-limiting embodiment of the presently disclosed subject matter, where "*RRE*" indicates "Rev Response Element". *cPPT* represents "Central Polypurine tract". [36] reported hypersensitive site-4 (*HS4*), and an area of DNase I hypersensitive site-2 (*HS2*).

medical professional may visualize the hepatic vein using ultrasound or x-ray (fluoroscopy) guidance to advance the catheter into coextending liver blood vessels. A pair of inflated balloons flanking an active delivery section of the catheter may secure the location and positioning of the active delivery section in the catheter while containing the vector volumes. Viral vectors were then injected through the pair of catheters to pass outward through holes in the active delivery section of the catheters to define a gene delivery area. In addition, an electrical charge was delivered to create a voltage between electrodes of the pair of catheters and an electric field commensurate with the gene delivery area, which results in improving the transduction rate of the viral vectors into the hepatic cells and improving the conversion of the hepatocytes with smaller vector doses. The invention provided a gene therapy delivery system including a first balloon catheter by providing a distal end having the first and second inflatable balloon spaced apart along the distal end to define an intervening catheter section and at least one passageway through a delivery lumen of the intervening catheter section for delivering a gene vector. Further, a second balloon catheter provided a distal end having a first and second inflatable balloon spaced apart along the distal end to define an intervening catheter section and at least one passageway through a delivery lumen of the intervening catheter section for delivering a gene vector. Furthermore, it included a first electrode extending along the first balloon catheter, a second electrode extending along the second balloon catheter, and a power supply by providing a voltage across the first and second balloon catheters[32].

The other application of DNA-based nanostructures was revealed approaches and compositions, which can play a role in the therapy and prohibition of hearing loss made by genetic alteration of the *LOXHDl* gene or the *TMPRSS3* gene. The approaches and compositions used adeno-associated viral (AAV) vector gene delivery of *LOXHDl* or *TRMPSS3* in the inner ear to restore the activity of the *LOXHDl* gene or the *TMPRSS3* gene, respectively, which can result in increasing restitute hearing and hair cell durability in the patients suffering from hearing loss. The invention described a method for treating or preventing hearing loss, such as administering an effective expression vector to a subject in need, including nucleic acid sequences having at least 90% sequence identity together. The nucleic acid sequences were operatively linked to a promoter. In some embodiments, the nucleic acid sequence had at least 75, 80, 85, 90, 95, 96, 97, 98, or 99% sequence identity to the other nucleic acid sequences. In some embodiments, the expression vector was selected from an adeno-associated viral vector, an adenoviral vector, a herpes simplex viral vector, a vaccinia viral vector, a helper-dependent adenoviral vector, or a lentiviral vector. In some embodiments, the vector was an adeno-associated viral vector selected from AAV2, AAV2/*Anc80*, AAV5, AAV6, AAV6.2, AAV7, AAV8, AAV9, AAV*rh8*, AAVrhlO, AAV*rh39*, AAV*rh43*, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8 or *Anc80*. In some embodiments, the adeno-associated viral vector was AAV2 or *Anc80*, while the promoter was selected from any hair cell promoter derived from the expression of an operably linked nucleic acid sequence at early development in some other embodiments,

Figure 11. An embodiment of the expression cassettes of the disclosure[38].

which maintain expression throughout the life such as *TMPRSS3*, human cytomegalovirus (HCMV), cytomegalovirus/chicken beta-actin (*CBA*), and *Myo7a* or *Pou4f3* promoters. In some others, the expression vector was administered into the subject's inner ear, for example, by injection[33].

The other embodiment provided a gene delivery approach and a CRISPR-expressing plasmid DNA targeted to hepatocytes infected using the hepatitis B virus. In addition, it provided the approaches of conducting the formation of CRISPR apparatus like cells to certify the improved specificity to target recognition for improving, altering and treating chronic infection of HBV. Moreover, the CpG quantity of the expressing plasmid DNA was minimized for increasing gene expression and reducing inflammation. The invention was based on the detection. A polynucleotide coding of a clustered interspaced short palindromic replicated (CRISPR)/CRISPR-associated (Cas) CRISPR-Cas system was delivered by utilizing a lipid envelope planned for targeting hepatocytes infected with HBV, leading to a decrease in viral nucleic acid. Accordingly, the invention provided an inactivating hepatitis B virus (HBV) nucleic acid in a host cell. The method included transfecting a host cell containing HBV viral nucleic acid with a polycistronic expression cassette, where the expression cassette includes an RNA polymerase III promoter sequence, a 5'-untranslated region comprising two or more complexes, each of which encompasses a guide RNA (gRNA) sequence and a scaffold RNA (scRNA) sequence, and a 3'-untranslated region sequence, where CpG content of all sequences was minimized to reduce immunostimulatory response but maintained maximal gRNA and CRISPR nuclease expression. In addition, each gRNA sequence can hybridize to a target sequence of hepatitis B viral nucleic acid. In various embodiments, once the host cell is transfected with the polycistronic expression cassette, the method includes incubating the cell to promote the expression of the polycistronic expression cassette, leading to the inactivation of HBV nucleic acid in the host cell. The method may be performed in vitro or in vivo, and the host cell may be a human cell such as a hepatocyte. While performing in vivo, the vector may be administered in a lipid envelope by encapsulating the vector, such as a lipid nanoparticle (LNP). In various embodiments, the LNP includes one or more cationic lipids of the ssPalm class but is not limited to ssPalmM, ssPalmE-P4C2, ssPalmE-Paz4-C2, and any combination. In various embodiments, the LNP has a ratio of cationic lipid to plasmid DNA of 4:1 to 16:1 such as 8:1 or 12:1 [34]. Figure 8 shows a schematic diagram showing the invention's exemplary HBV gene editing expression plasmids.

The other report of DNA-based nanostructures was a non-viral capsid-free DNA vector possessing covalently-closed ends or a “ceDNA vector” to edit a gene. The ceDNA vectors included an expression cassette, which was flanked with two ITR subsequences in which the expression cassette coded a gene for modifying the molecule. Many ceDNA vectors could have cis-regulatory factors such as regulatory switches. In addition, there were some approaches and cell lines in the case of dependable gene editing by utilizing the ceDNA vectors. The ceDNA vectors described in this study are capsid-free, linear duplex DNA molecules formed from a continuous strand of complementary DNA with covalently-closed ends including linear, continuous, and non-encapsulated structure, which consisted of a 5' inverted terminal repeat (ITR) and 3' ITR sequence,

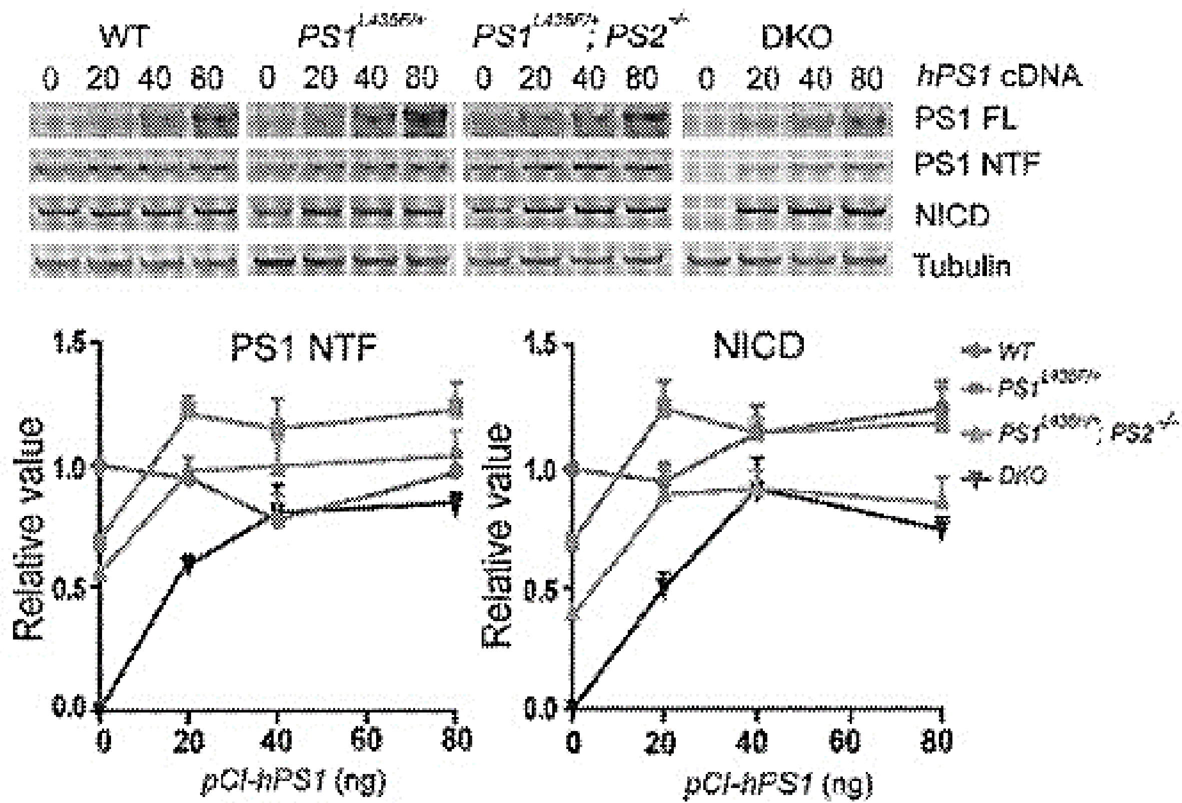


Figure 12. Restoring impaired g-secretase activity by WT *hPSl*. Increasing amounts of pCI-*hPSl* plasmid DNA, as indicated, are transfected into MEFs of varying genotypes. Western analysis showed that *PS1 NTF* and *NICD* are restored in various PS mutant *MEFs*. Heterozygous L435F KI cells are labeled as KI/+ or PS1L435F/+. N=3 independent experiments. Data represent mean ± SEM. *P<0.05; **P<0.01; ***P<0.001 (one-way ANOVA with Tukey's posthoc analysis)[39].

where the 5' ITR and 3' ITR could have the same symmetrical three-dimensional organization concerning each other (i.e. symmetrical or substantially symmetrical), or the 5' ITR and 3' ITR can have a different three-dimensional organization for each other, i.e., asymmetrical ITRs. Further, the ITRs could be from the same or different serotypes. In some embodiments, a ceDNA vector for gene editing could include ITR sequences that had an asymmetrical three-dimensional spatial organization in such a way that the structure had the same shape in geometrical space or the same A, C-C' and B-B' loops in 3D space. They are the same or are mirror images to each other. In such an embodiment, a symmetrical ITR pair or substantially symmetrical ITR pair could be modified ITRs (e.g. mod-ITRs) in the same manner without having wild-type ITRs. A mod-ITR pair could have the same sequence that had more modifications from wild-type ITR and was considered reverse or inverted complements. In alternative embodiments, a modified ITR pair was substantially symmetrical. In other words, the modified ITR pair can have a different sequence but with corresponding or the same symmetrical three-dimensional shape. In some embodiments, one ITR could be from one AAV serotype, and another ITR could be from a different AAV serotype. Accordingly, some aspects of the technology are related to a ceDNA vector for gene editing, including ITR sequences selected from any of (i) at least one WT ITR and modified AAV inverted terminal repeat (ITR) (e.g. asymmetric modified ITRs), (ii) two modified ITRs where the mod-ITR pair had a different three-dimensional spatial organization for each other (e.g. asymmetric modified ITRs), (iii) symmetrical or substantially symmetrical WT-WT ITR pair, where

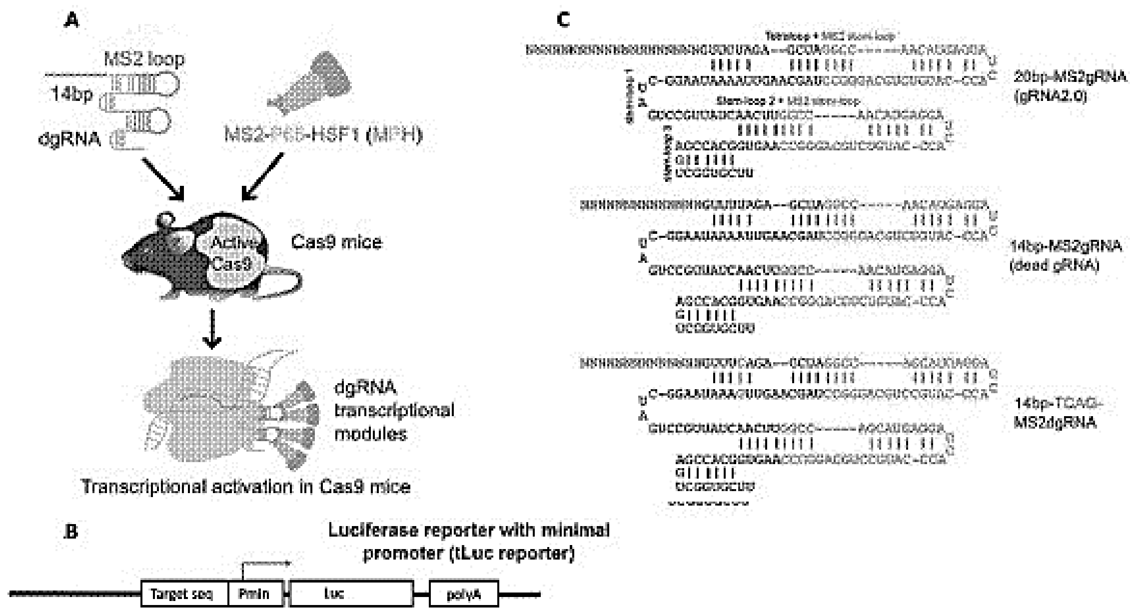


Figure 13. A) A schematic representation of how sgRNAs work, including a truncated l4-bp gRNA (dgRNA) and MS2 loops, which are introduced with the *MS2-P65-HSF1* (MPH) transcriptional activation complex into Cas9-expressing mice for *TGA*, B) The luciferase reporter (*tLuc*) including a dgRNA binding site (Target seq) followed by a minimal promoter (*Pmin*), a luciferase expression cassette (Luc), and a polyA termination signal, and C) Modified gRNAs. MS2gRNA (or gRNA 2.0) including a wild type 20-bp gRNA and stem-loops for MPH binding (MS2dgRNA or dead gRNA includes a truncated l4-bp MS2gRNA which can recruit MPH to activate gene expression without inducing *Cas9*-mediated double-stranded breaks) (The *MS2*dgRNA (designated dgRNA) includes a l4-bp *MS2*dgRNA with modifications which enhance *TGA*)[40].

each WT-ITR had the same three-dimensional spatial organization, or (iv) symmetrical or substantially symmetrical modified ITR pair, where each mod-ITR had the same three-dimensional spatial organization, as displayed in Figure 9. The ceDNA vectors disclosed could be produced in eukaryotic cells, thus devoid of prokaryotic DNA modifications and bacterial endotoxin contamination in insect cells[35].

Particularly, applying an enhancer of blocking insulators and specified insulators had a barrier for insulator activity. Additionally, the disclosed subject matter prepared expression cassettes containing one or sometimes more insulators and could express a globin gene such as a human b globin gene [36]. Further, some vectors containing expression cassettes, vectors, or cells transduced having expression cassettes were prepared. Further, expression cassettes were used for therapizing hemoglobinopathies such as sickle cell anemia and b-thalassemia (Figure 10). Furthermore, a cassette expression containing a functional portion or globin gene was prepared. Therefore, the operation was connected to a b-globin locus control region (LCR), consisting of Dnase I hypersensitive site-3 (HS3).

Gene treatment vectors such as encoding g-sarcoglycan and recombinant adeno-associated virus (AAV) vectors for releasing these vectors encoding g-sarcoglycan to the muscle for reducing or preventing fibrosis could enhance muscle function. This technology could increase muscular force and muscle endurance and treat a g- sarcoglycanopathy in a mammalian issue suffering from muscular dystrophy. In addition, the disclosure provides therapies and approaches by using gene therapy vectors to deliver g-sarcoglycan to address the gene defect observed in limb-girdle dystrophy type 2C (LGMD2C). On the one hand, a method was provided for one or more treating g-sarcoglycanopathy, increasing muscular force, muscle endurance, and/or muscle mass, as well as reducing fibrosis, contraction-induced injury, fatty infiltration, and central nucleation in a subject in need

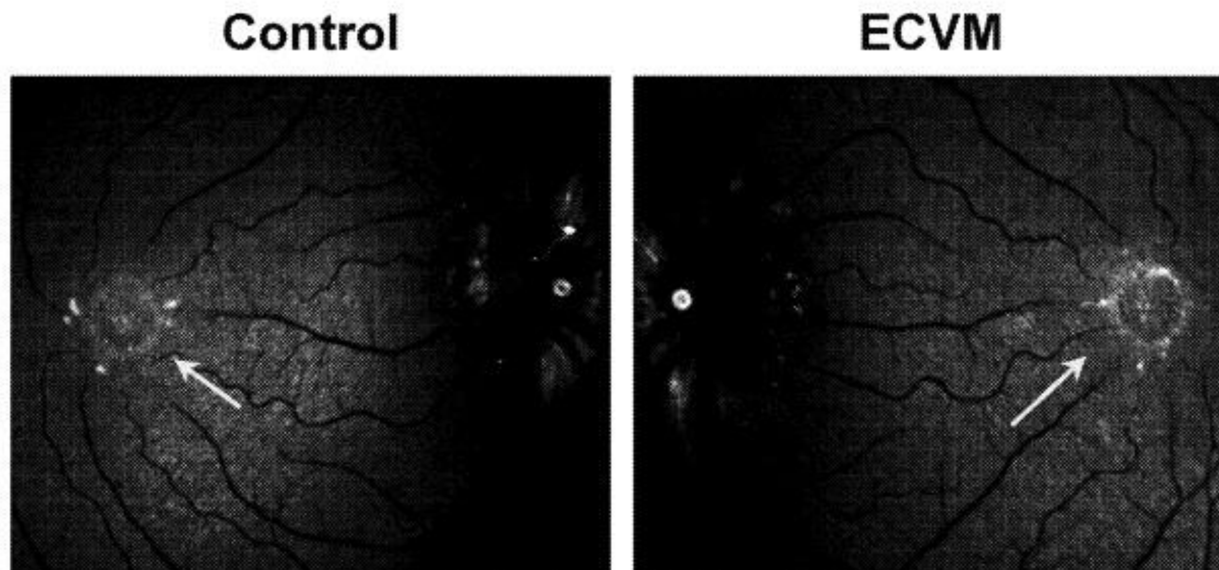


Figure 14. A comparison of *GFP* signal in the fovea (arrows) of rhesus monkey eyes injected intravitreally with an AAV8-CMV-*EGFP* (1e12 vg/dose) on both eyes[41].

treating muscular dystrophy, reducing degenerating fibers or necrotic fibers and inflammation, elevating creatine kinase levels, treating myofiber atrophy and hypertrophy, and/or decreasing dystrophic calcification in a subject suffering from muscular dystrophy. The methods consist of administering a therapeutically effective amount of a recombinant adeno-associated virus (AAV) vector to the subject, in which the rAAV vector encompassing a gene expression cassette including a polynucleotide sequence encoding g-sarcoglycan under the transcriptional control of a promoter, called cassette flanked by one or more AAV inverted terminal repeats. On the other hand, it was a recombinant AAV (rAAV) vector consisting of a polynucleotide sequence encoding g-sarcoglycan under the transcriptional control. In some embodiments, the polynucleotide sequence encoding g-sarcoglycan encoded a protein that retained a g-sarcoglycan activity[37].

Gene therapy of CNS degeneration could be applied for preventing, delaying, treating, inhibiting the decadence of the central nervous system. Remarkably, the inventors revealed many embodiments and approaches which correspond to a recombinant gene for treatment vector, including E3 ubiquitin-protein ligase (*PARK2*), Parkinson protein 2, protein deglycase DJ-1 (*DJ-1*), PTEN-induced putative kinase 1 (*PINK1*), alpha-synuclein (*SCNA*), Leucine-rich repeat kinase 2 (LRRK2), proto-oncogene c-Rel (*c-Rel*), synaptic vesicular amine transporter (*VMAT2*), glucocerebrosidase (*GBA*), Ubiquitin-like modifier-activating enzyme (*ATG7*) genes, as well as functional segment or variant. The disclosure provided a method of inhibiting degeneration or death of a dopaminergic neuron comprising a mutation in a gene associated with Parkinson's Disease (PD). In this regard, the method consisted of contacting the neuron with a recombinant gene therapy vector, including a polynucleotide encoding as a wild-type protein expressed by a wild-type version of the mutated gene or a functional variant or fragment. Following the contact with the recombinant gene therapy vector, the neuron expressed the wild-type protein or a functional variant or fragment. In some embodiments, the *PARK2*, *PINK1*, *LRRK2*, *SCNA*, *c-Rel*, *ATG7*, VMAT2, or GBA protein consisted of the amino acid sequences. In some embodiments, the gene is *PARK2*, and the wild-type PARK2 protein includes amino acid sequences. In some, the polynucleotide comprised a sequence having at least 70 to 99% identity to a PARK2,

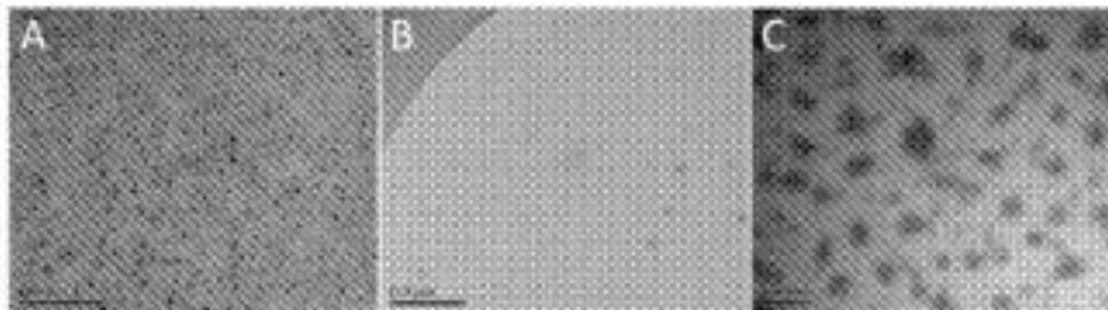


Figure 15. TEM that viroid rough surface genomic medicine prepared by the present invention transmits nanocarrier PEI/DNA@DOX altogether Image, wherein A is DOX NPs, B PEI/DNA, C are PEI/DNA@DOX[42].

PINK1, *LRRK2*, *SCNA*, *c-Rel*, *ATG7*, *VMAT2*, or *GBA* polynucleotide sequences [38]. Figure 11 displays a schematic expression of cassettes in the invention.

DNA-based gene therapy is utilized for treating subjects having Alzheimer's disease (AD) and many neurodegenerative diseases, conditions, or disorders. Treating some neurodegenerative diseases, conditions, or disorders and managing for a subject are used for therapizing a polynucleotide encoding presenilin 2 (*PS2*) protein/a presenilin 1 (*PS1*) where the subject possesses many mutations in at least one allele of *PSEN2*/*PSEN1*, which encoded a preferable negative PS2 or PS1 protein isoform. Mutations in the presenilin genes- *PSEN1* and *PSEN2*- are highly penetrant and account for ~90% of all mutations identified in familial AD (FAD), highlighting their importance in the pathogenesis of AD. More than 260 distinct mutations in *PSEN1* were reported, dominantly inherited, and mostly missense mutations. Pathogenic *PSEN1* mutations act in cis to impair mutant PS1 function and act in trans to inhibit wild-type Presenilin-l (*PS1*) function. It was known that dominant-negative mutations in the *PSEN1* and *PSEN2* genes are associated with early-onset familial Alzheimer's disease. It was generally believed that the PS1 and presenilin-2 (PS2) proteins were a part of the g-secretase complex and that mutations in the *PSEN1* and *PSEN2* genes contributed to the accumulation of amyloid-beta (Ab) protein in Alzheimer's disease patients. Thus, the present disclosure provides methods for effective gene therapy based on *PSEN1* to express PS1 and/or *PSEN2* to express PS2 for Alzheimer's disease and other neurodegenerative dementia, representing a significant breakthrough in this disease area [39]. Figure 12 shows the results of restoring impaired g-secretase activity by WT *hPSl*.

Using modified guide ribonucleic acid (gRNA) molecules like "dead" gRNA molecule (dgRNA) for targeted gene activation was the other DNA-based strategies for activating transcription for many purposes like a gene, the expression of which decreased or eliminated in disease. Using disclosed gRNA molecules and targeted gene activation (TGA) approaches enhances gene explanation without utilizing double-strand bread of DNA. In these approaches, at least one component of CRISPR, namely gRNA or *Cas9*, is an inactivated (dead) state such as a dead *Cas9*. It is worth noting that a dead gRNA consists of just around 14 or 15 bp of the supplementary target sequence or can have both. For example, a gRNA included the structure A-B-C-D-E where A is the 5'-end, and E is the 3'-end. For example, the gRNA could include the first region (e.g., A, in A-B-C-D-E), which included a tetra-loop backbone sequence with at least 90, 95, 98, 99, or 100% sequence identity the sequence GTTTTAGAGCTA or GUUUUAGAGCUA. The second region (e.g., B, in A-B-C-D-E) was linked to the first and third regions between regions A and C and included a modified MS2-binding loop sequence. The third region (e.g., C in A-B-C-D-E) was linked to

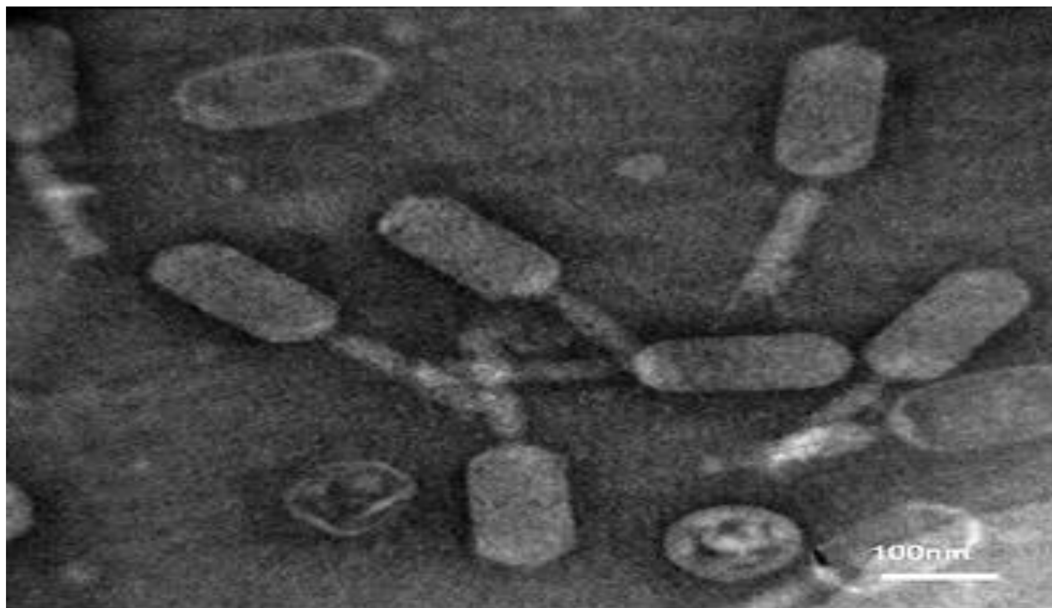


Figure 16. Electron micrographs of the family siphoviridae phage serogroups A, which is negatively stained with 2% uranyl acetate (pH 4-4.5), 150 kV, voltage, and 100nm scale bar [45].

the second and fourth region (e.g., between the two regions B and D). It included a stem-loop 1 and 2 backbone sequence comprising at least 90 to 100% sequence identity to the sequence TAGCAAGTTAAAATAAGGCTAGTCCGTTATC AACTT or UAGCAAGUUAAAAUAAGGCUAGUCCGUUAU CAACUU. The fourth region (e.g., D in A-B- C-D-E) linked to the third and fifth regions (e.g., between the C and E) and included the modified MS2-binding loop sequence. The fifth region (e.g., E in A-B-C-D-E) is at the 3'-end of the gRNA, was linked to the fourth region, and included a stem-loop 3 backbone sequence including at least 90 to 100% sequence identity to AAGTGGCACCGAGTCGGTGCTT or AAGUGGCACCGAGUCGGUGCUU. The modified MS2-binding loop sequences of the gRNA included at least two nucleotide changes to the native MS2-binding loop sequence GGCCAACATGAGGATCACCCATGTCTGCAGG GCC or GGCCAACAUGAGGAUCACCCAUGUCUGCAG GGCC, which increased the GC content and/or shortened repetitive content of the modified MS2-binding loop sequence relative to the native MS2-binding loop sequence[40]. Figure 13 shows a schematic representation of how sgRNAs works.

The strategic intravitreal assignment of gene treatment vectors was the other application of DNA-based procedure that it could be employed with only and together with utilizing low electric current stimulation used among the eye *in vivo* for improving the delivery of gene treatment vectors, consisting of therapeutic adeno-associated viral vector fabricates managed in the vitreous. Therapeutic adeno-associated viral was an icosahedral non-enveloped DNA virus, which is resistant and thermostable for mild proteolytic nonionic detergents and digestion. In a neutral environment, it possessed a total net negative charge introduced as a model gene treatment vector in the case of testing intraocular implementation and evaluating gene treatment expression. By considering the mentioned approaches, the inventors were expanded and evaluated a new non-invasive method of using electric current, namely electric-current vector mobility (ECVM) having intravitreal vector injection. This approach indicated that it could dramatically enhance the transduction efficiency for AAV vectors in mouse retina of wild type (WT). Thus, the present invention was directed for methods of treating a disease or disordering the eye, including the administration of an expression vector capable of

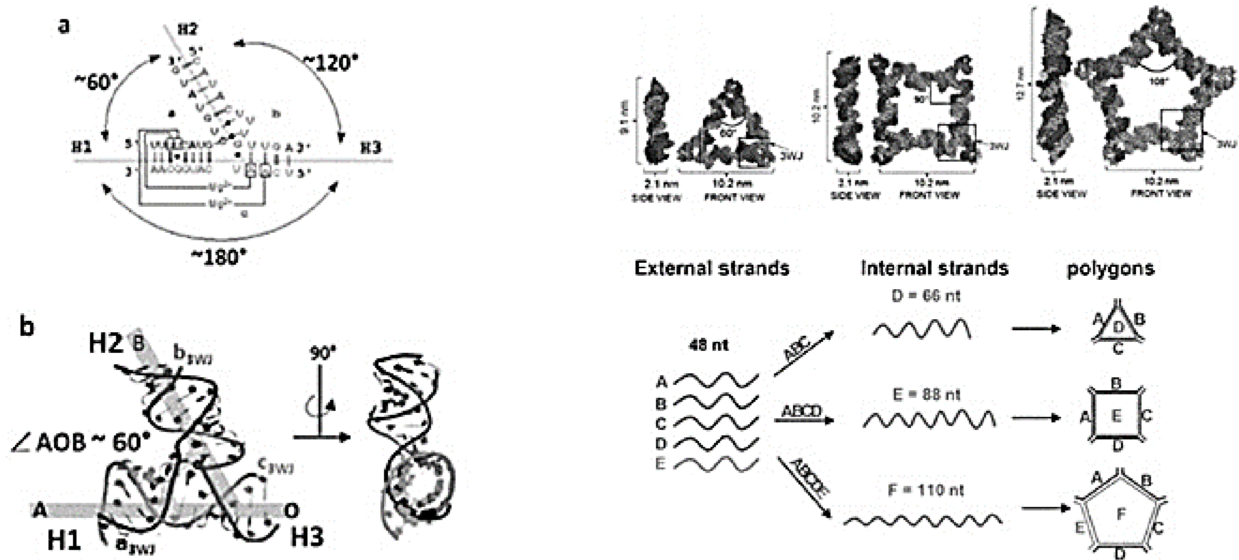


Figure 17. The details of the structural features of the RNA 3WJ motif: (a) Secondary structure of 3WJ motif with base pairs annotated using Leontis-Westhof nomenclature. (b) The tertiary structure of the 3WJ motif with an indication of the ∠AOB˜60° angle formed between H1 and H2. The ∠AOB Angle corresponds to the inner angles of polygons. Figs. 18c and 18d show the design of RNA nanostructure polygons and assembly properties. (c) 3D modeled structures of polygons with 3WJ motif located at vertices, where inner angle corresponds to ∠AOB 60°, and (d) the increasing length of the internal strand stretches the 3WJ ∠AOB where the nanoparticles are assembled along with an increasing number of external 'short' strands [51].

modulating a target gene or gene product in a therapeutically effective amount to a subject by administering the expression vector by intravitreal injection alone or in conjunction with the application of an electric current to the injected eye. In these methods, the gene therapy vector can be considered any suitable expression vector for influencing gene therapy in the eye of a mammal, such as retroviruses, adenoviruses, and/or neurotropic viruses. These administration methods could be applied to encapsulated vectors such as nanoparticles, modified DNA, or vectors (e.g., PEGylation as a modification) and applied to the administration of naked DNA plasmids to the eye. These methods enhanced the delivery of gene therapy vectors into and through tissues such as tissues of the eye. More specifically, the methods described utilized the application of an electric current to actively deliver a gene therapy expression vector such as an AAV vector into a mammalian eye. The methods focused on the effective delivery of expression vectors to maximize gene delivery, protein expression, and distribution in the eye, especially beyond the inner limiting membrane (ILM). Figure 14 shows a comparison of GFP signal in the fovea (arrows) in rhesus monkey eyes injected intravitreally with an AAV8-CMV-*EGFP* (1e12 vg/dose) on both eyes. One eye (ECVM) received ECVM (850 pA/20min) following the intravitreal injection, while another eye received no ECVM (control)eye within the photoreceptors, bipolar cells, amacrine cells, retinal ganglion cells, and/or retinal pigment epithelial (RPE)[41].

Viroid gene-drug transmit nanocarriers were the other way for the anti-tumor agent fabrication, which possessed different benefits like high transfection efficiency, low bio-toxicity, and strong and simple treatment ability of medicine. All viroid gene-drug transmits nanocarrier was nanoparticles of medicament by self-assembly drug, which could be fabricated by electrostatic cationic polymer, and gene was stoutly mixed for forming nanocomplex and recycling electrostatic force, where the nanoparticle of medicament was mixed onto the surface of nanocomplex. The nanocarrier had a viroid rough

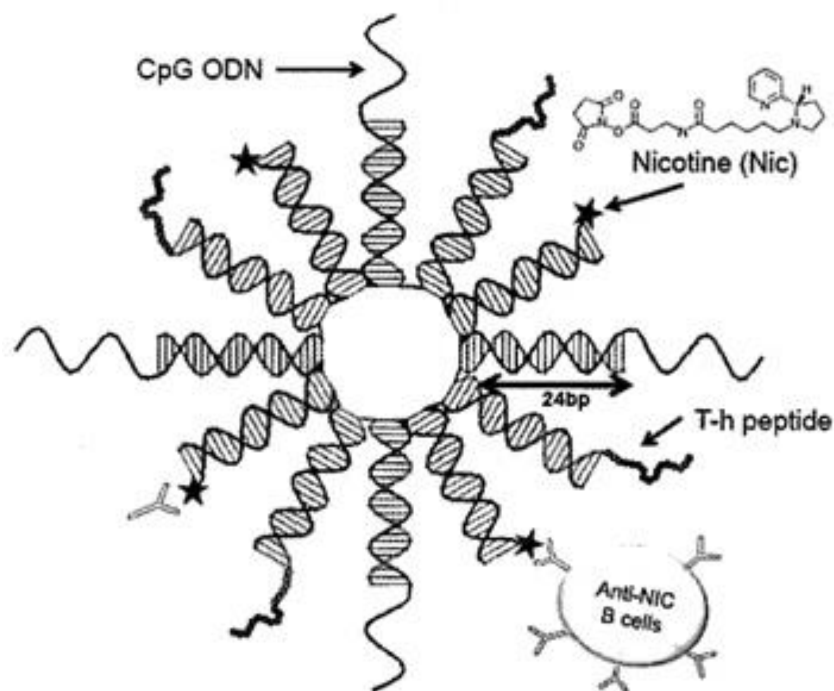


Figure 18. A 12-arm DNA-nicotine nanostructure incorporating CpG motif and T-helper peptides[52].

surface of a gene. In addition, the drug could be formed while attached. In this study, the drug was adriamycin, the gene was milt DNA or *p53* gene which is about 140-150 nm where viroid gene-drug transmitted the size of nanocarrier altogether, and the whole negative potential was presented in the body. While being prepared, three steps were taken. The first was medicament nanoparticle by drug self-assembly. In the second step, gene and cationic polymer were compounded to be formed by nanocomplex and electrostatic force. During Step 3, medicament nanoparticle was compounded in nanocomplex surface using electrostatic force. The viroid gene-drug of the invention transmits nanocarrier in gene therapy and/or chemotherapeutic agent altogether. In the application where the present invention prepares the genomic medicine with viroid surface transmitted carrier selection altogether was the poly- second of low molecular weight Alkene imines, toxicity was low and good biocompatibility. DOX nanoparticles increased by the surface that electrostatic force was compounded in PEI/DNA. The surface roughness of PEI/DNA nanocomplex was added so that the carrier was easier to carry out cell endocytic and improved transfection efficiency.

Meanwhile, the total transmitting of genes and drugs could be implemented in the transport system prepared by the present invention, and gene therapy was realized in cancer treatment with the synergistic chemotherapy treatment. The preparation method of the present invention was simple, and the low transfection efficiency of toxicity was high, the benefit of drug and gene could be improved with rate, and the fragmentation effect to tumor cell increased more [42]. Figure 15 displays the TEM of the viroid rough surface in genomic medicine.

DNA aptamers of multidrug-resistant colon cancer cells were the other embodiments of DNA-based nanostructures. This strategy could be applied as a type of single-stranded DNA particularly bounded with multidrug resistor colon cancer cell prepared aptamer LA1. The use of the single-stranded DNA aptamers provided into multidrug resistance targeted therapy of colon cancer. The aptamers possessed

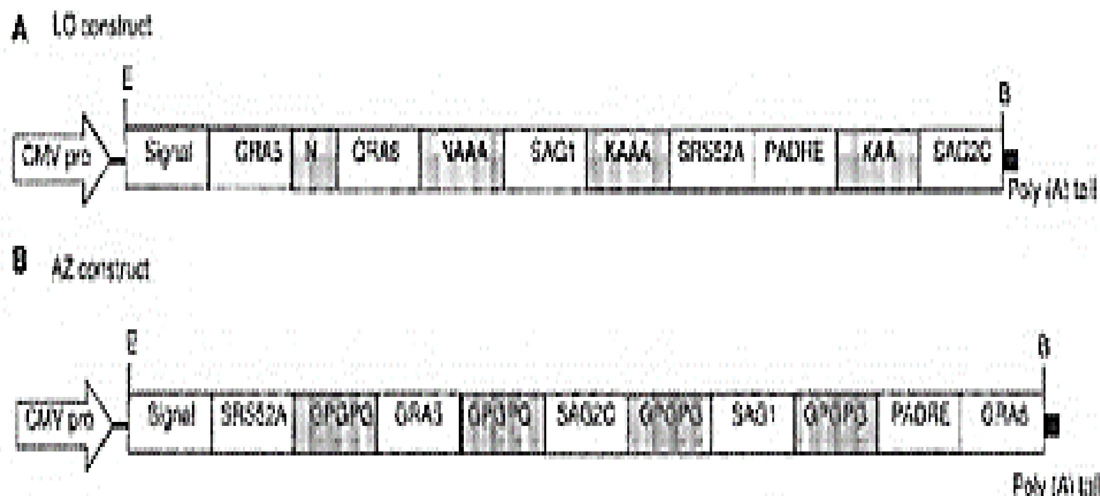


Figure 19. A schematic diagram of the DNA vaccine constructs including A and B as the orientation of the HLA-A*11:01-restricted CD8+ T cell epitopes and PADRE in the synthetic gene with 2 different types of spacers called LO and AZ for N/KAAA and GPGPG linker, respectively[53].

similar matches with the attributes of the acceptor, which they were distinct from other targeted molecules due to high specificity and affinity by a target cell, simple screening technology, small physical toxicity of aptamers, excellent usage value, a kind of medium of impressive neoplasm targeted therapy, and low cost. The application invention further provided the aptamer mentioned above LA1 in tumor cell-specific identification. LA1 was used as template, PCR amplification FITC mark aptamer, and then by flow cytometry assay from outside different tumor cells and normal person. The affinity size of all hemocytes indicated LA1 and LoVo/MDR multidrug resistance colon cancer cell had the highest affinity, which hardly combined with the hemocytes of normal human peripheral Thus, this aptamer can be used as a mediation nanocarrier targeting multidrug resistance as a kind strategy of a colon cancer cell. Further, it offered a kind of application with aptamer LA1 in target nanocarrier. The present invention focused on hair existing LA1 which could promote medicament-carried nanocarrier to enter target cell while enhancing target nanocarrier to the distribution of multidrug resistance colon cancer tissue. Thus, the aptamer of the present invention was prepared into the target nanocarrier by targeting multidrug resistance colon cancer for multiple medicines. The targeted therapy of resistance colon cancer as a new therapy approach was provided for treating resistance post-colon cancer, which had good face bed application prospect[43].

DNA-based composition for CNS gene therapy was the other application utilizing four distinct kinds of aqueous nanometer carriers, including one nucleic acid assembled at least in the identical formulation in the case of nasal administration targeting the CNS for gene treatment. In this treatment, the compositions of the central nervous system could be managed as an intratracheal or intranasal spray in the case of aspiration delivery of the brain and other aerosol systems. Further, the invention incorporated a plasmid of the CRISPR/*Cas9* system together with another nucleic acid. Furthermore, it provided the incorporation of a recombinant plasmid by encoding a protein. The invention provided a composition for gene therapy of the central nervous system comprising at least one adsorbed or encapsulated nucleic acid and non-viral carriers with an average droplet/particle diameter in the range of 0.001 to 1.0 micrometer in the first object, while the invention provided a method of obtaining composition for gene therapy of the central nervous system in the second object, in which non-

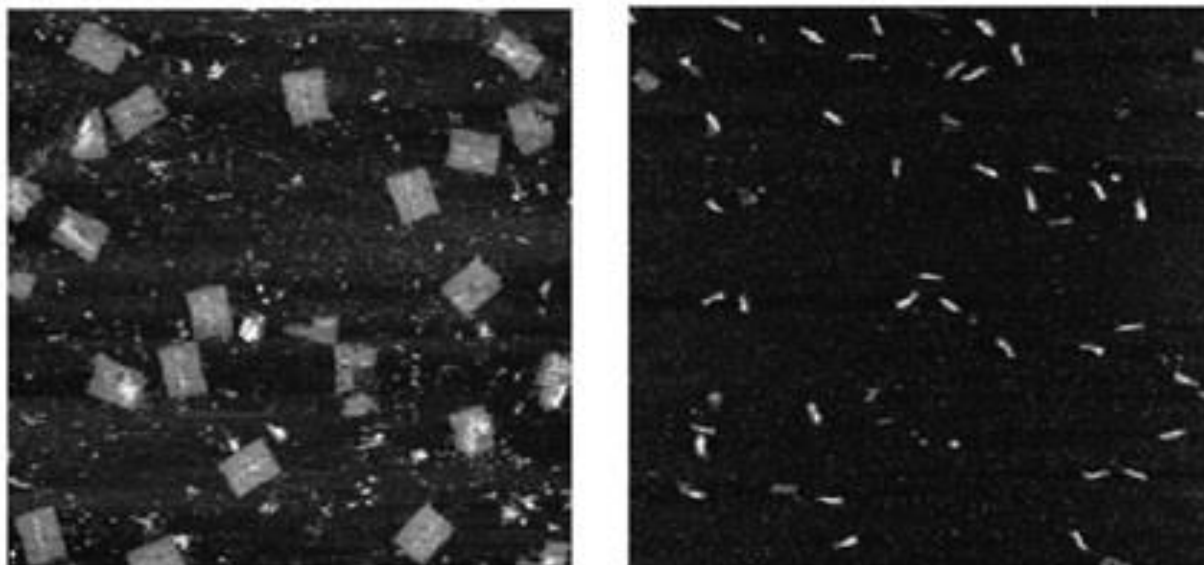


Figure 20. The atomic force microscope morphology of rectangle lamella DNA nanostructure (left) and atomic force microscope morphology of tubular DNA nanovaccine (right)[54].

viral carriers were prepared in nine steps [44]. In the third object, the present invention provided a method for obtaining gene therapy of the central nervous system for producing non-viral carriers, including solid lipid nanostructures and nanostructured lipid carriers containing the adsorbed nucleic acids. It included seven steps. In a fourth object, the present invention provided the use of the gene therapy composition of the central nervous system in preparing a medicament for treating diseases caused by deficiencies or genetic abnormalities such as lysosomal deposition diseases.

On the other hand, DNA-based vectors encapsulated within a phage capsid could be used for methicillin-resistant staphylococcal aureus (MRSA) infections. Controlling wound infections is considered one of the main problems in burn wounds due to increased strains of MDR (multidrug resistance) such as MRSA, while the infection is the main cause of mortality in burn patients. According to increased MDR, the return of infections in the burn wounds, and the side effects of drugs currently used in burn patients, such as silver sulfadiazine, its application on a wide surface can be toxic for the presence of silver. The product need which has an antimicrobial activity with a minimum dose on MRSA induced wound healing in the shortest time and the product of a natural source and had no side effects for burn patients. This invention suggested lytic bacteriophages and their products, such as endolysin, as a solution. Bacteriophages are viruses that infect and kill bacteria by a specific lytic activity without destroying the natural flora of the human body and affecting eukaryotic cells, which were not reported unlike antibiotics resistance, and can eliminate MDR bacteria. In this study, a part of the bacteriophage, endolysin, was used because whole bacteriophages can transfer antibiotic-resistant and pathogenic genes via transduction. It is recommended to use nanocarrier technology in the presence of bacteriophages and their products in nanomedicine to treat resistant bacterial infections. In this method, the endolysin gene technique is displayed as inner entrapment, and endolysin as outer entrapment carries on bacteriophages as nanocarrier. The purpose of manufacturing this nanocarrier is designing and defining nanobioparticles of bacteriophages with some features including i) active targeting that nanobioparticles of bacteriophages specifically lysis of MRSA and no effect on other normal flora bacteria, ii) passive targeting which defined some features such as nano-scale for nanocarrier penetrates to the depth of

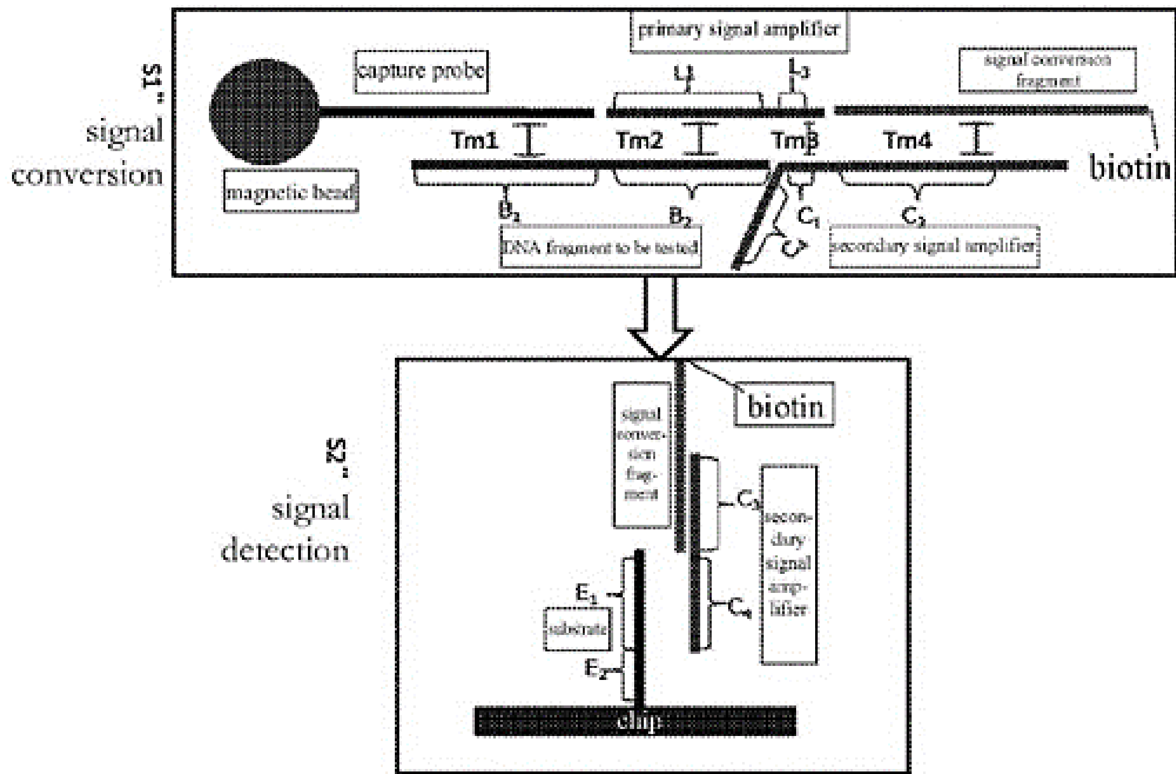


Figure 21. A schematic diagram of a method for detecting a DNA sample, where Tm1 is a temperature at which binding base pairs of the capture probe and the partial nucleotide sequence Ef of the DNA fragment to be tested are separated, Tm2 is a temperature at which binding base pairs of the nucleotide sequence Li of the mediator and the partial nucleotide sequence B2 of the DNA fragment to be tested are separated, Tm3 is a temperature at which binding base pairs of the nucleotide sequence L2 of the mediator and the partial nucleotide sequence C of the terminal signal are separated, Tm4 is a temperature at which binding base pairs of the partial nucleotide sequence C3 of the terminal signal and the signal conversion fragment are separated, among which the numerical value of Tm3 the lowest compared to Tml, Tm2, Tm3, and Tm4 [61].

the tissue by reducing the effective dose, treatment time, and appropriate surface charge which caused nanocarrier not to have a toxic effect on eukaryotic cells. Designing and manufacturing phage nanobioparticles based on phage display technique was the optimal presence of bacteriophages in the field of nanomedicine and medicine for treating bacterial infections. This technique eliminated cells and genetic engineering, which consumed a lot of time and money and produced a low yield of recombinant proteins. Finally, manufacturing phage nanobioparticles aimed to create a smart candidate with a highly specific for treating infections caused by MDR as MRSA, reducing the effective dose without any side effects, and large-scale production, which is economically affordable[45]. Figure 16 shows the electron micrographs of the family in siphoviridae phage serogroups A.

2.3. DNA nanovaccines

Vaccination plays an effective role in prohibiting many health promotions and diseases [46]. Many kinds of vaccines have been licensed in production and distribution in recent years. In this regard, scholars tried to fabricate enhanced vaccines and follow some procedures, although different issues should be solved[47]. Currently, nanotechnology is suggested in the cases of medical science, which solves different medical issues. Nanotechnology has helped scholars improve these issues about common vaccines [48] (e.g., a new nanovaccine antigen adjuvant and delivery system invented for overcoming oral Salmonella vaccine) [49]. In addition, mucoadhesive NPs included

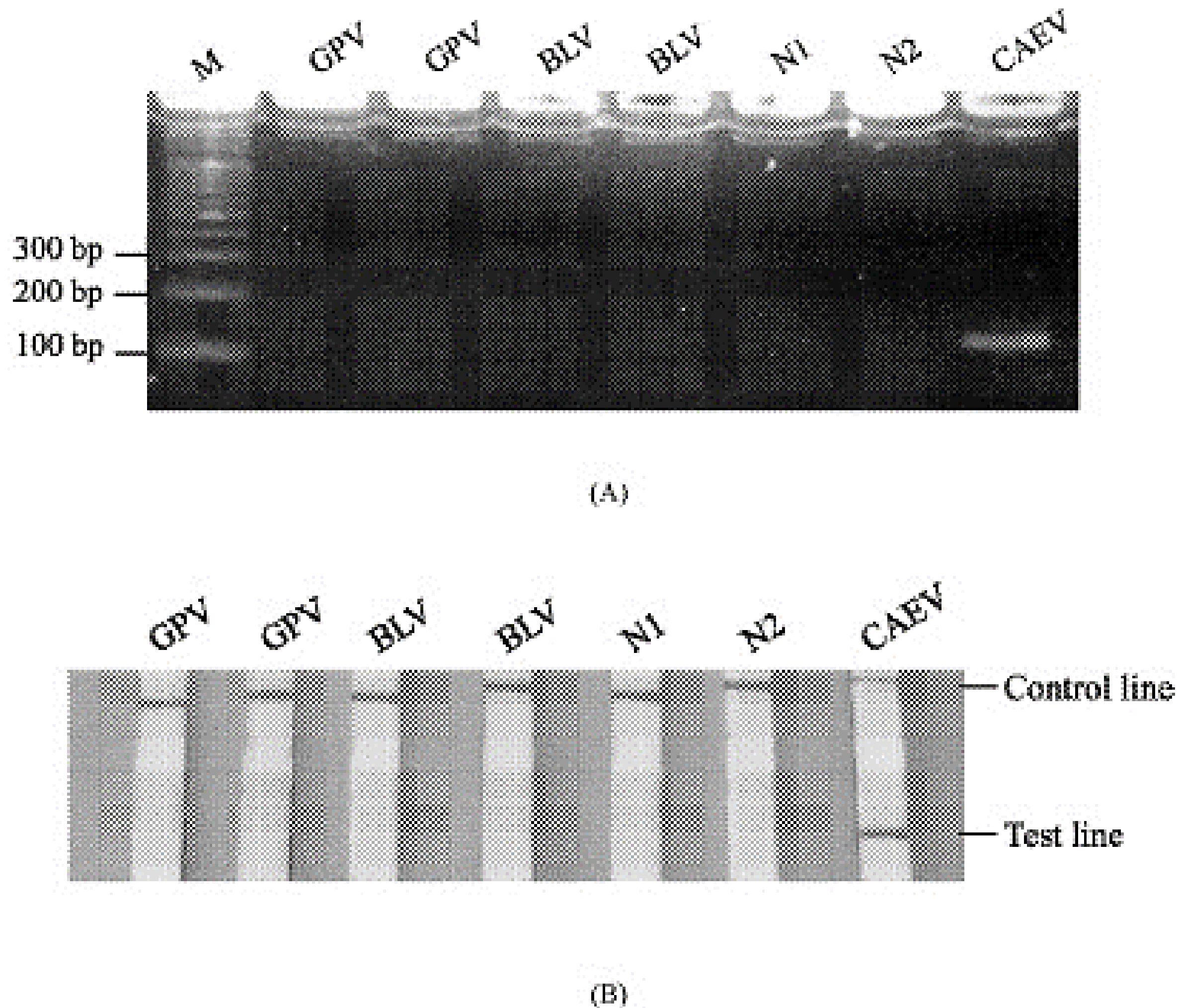


Figure 22. (A) the results visualized by AGE, (B) those visualized by LFD. N1: total DNA extracted CAEV-negative goat. N2: negative control (DNase-free water)[62].

an inactivated influenza A virus (IAV) antigen and chitosan. The NP can decline nasal shedding of an inactivated influenza A virus [50]. NPs are individually appropriate in vaccines managed for pigs to limit or treat the infection of IAV.

Using an artificial RNA nanostructure comprising multiple external strands of RNA, each of which included about 40-50 nucleotides was one of the compositions employed for prophylactic and treatments. One internal strand of RNA comprised more than 50 nucleotides, and the internal and external strands were assembled to form a triangle nanostructure, square nanostructure, polygon nanostructure, and pRNA three-way junction (3WJ) motif at each vertex of the nanostructure. Such nanostructure can be provided in a composition together with an adjuvant for use in inducing the production of high affinity neutralizing antibodies or inhibitory antibodies, production of cytokines, as well as an immune response in a subject or a combination. The nanostructure included one or more immunostimulatory motifs or adjuvants. One or more immunostimulatory motifs or adjuvants were selected from one or more immunostimulatory RNAs (isRNA) CpG oligodeoxyribonucleotide (CpG) motifs where there are one or more CpG motifs. The nanostructure could also induce an immune response in which the immune response increased cytokine at least tenfold compared to the adjuvant, antigen, and/or targeting ligand provided from the RNA nanostructure

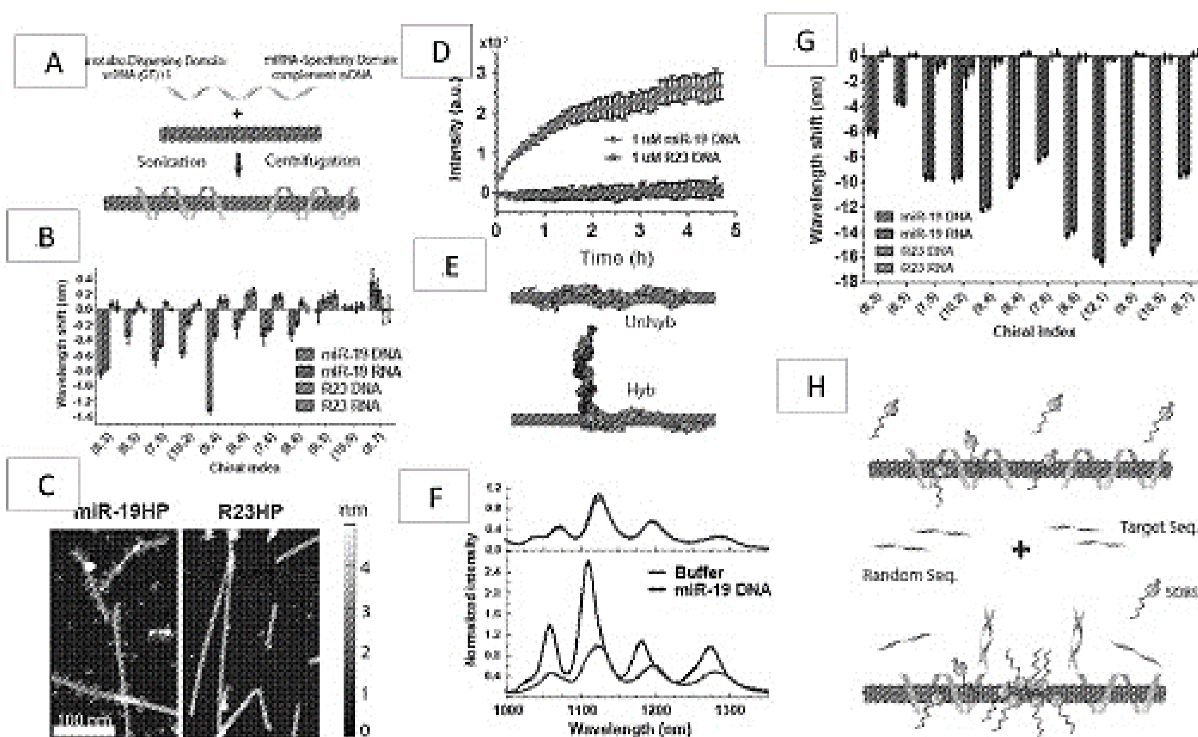


Figure 23. (A). A schematic plan for the construction of the SWCNT sensing platform based on an illustrative embodiment of the invention, B) wavelength shifts from miR-19 and R23 sequences, C) atomic force microscopy (AFM) images of a sensor complex upon incubation with non-complementary (R23HP) or complementary (miR-19HP) hairpin DNA, D) fluorescence restoration in the -presence of 1 µM miR-19 DNA vs. 1 µM I123, F) a spectrum from 730 nm excitation with and without target miR-19 in buffer only conditions in the top panel, and the presence of 0.2% SDBS in the bottom panel, G) wavelength shifts from miR-19 and R23 (+presence of surfactant), H) a graphic representation of the assembly of supramolecular complexes of SDBS, triggered by detecting target RNA or DNA based on an illustrative embodiment of the invention[63].

independently. The ligand targets B , T , dendritic, macrophages, and/or cancer cells. The adjuvant based on the invention was a composition including (a) an RNA-oligonucleotide, in which the RNA-oligonucleotide comprised single-stranded or double-stranded oligonucleotide, while RNA-oligonucleotide was about 8-50 bases in length, and (b) an immunostimulatory motif in which the immunostimulatory motif was conjugated to the RNA-oligonucleotide. Figure 17 shows the details of the structural features of the pRNA 3WJ motif [51].

DNA-nanostructures including at least one targeting moiety connected to the nanostructure as the nicotine analog or nicotine was one of the embodiments of DNA nanovaccines. These compositions extract an immunogenic response in individuals, appropriate as vaccines to ameliorate nicotine dependency. The invention applies DNA nanotechnology (building DNA nanomaterial to construct nanodevices with precise control over 3-D configurations) to rationally design and assembles DNA-assembled nicotine-vaccines (DNA-Nic). The data indicated that DNA-Nic vaccine complexes induce anti-Nic antibody production in mice after primary immunization, which helps treat nicotine addiction and/or prevention in mammals, especially humans. This technology utilized DNA nanostructures as a synthetic platform for vaccine construction. Specifically, the DNA-nanostructures could be scaffolded to assemble nicotine or nicotine analog as an antigenic component [52]. Figure 18 shows a schematic plan of a 12-arm DNA-nicotine nanostructure nano-vaccine.

Preparation of the isolated polynucleotides which encoded a chimeric polypeptide where the chimeric polypeptide consisted of (a) peptide domains in which each second domain of peptide was a CD4+ epitope

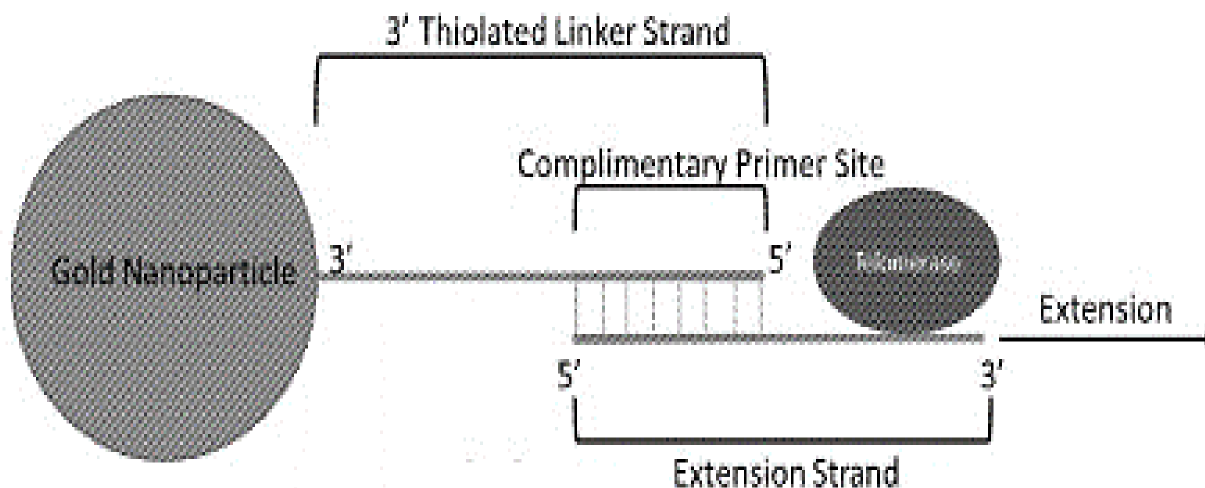


Figure 24. Diagram of the linker functionalized AuNP telomerase activity assay (A gold nanoparticle was coupled to a 3' thiolated linker nucleic acid strand which contained a complementary sequence for an extension nucleic acid strand. The extension nucleic acid strand contained a binding site for telomerase and was elongated by telomerase)[64].

and (b) a multiplicity of first peptide domains where each first peptide domain was an eliciting epitope of CD8+ T cell. In one visualization, the multiplicity of the first peptide domains at least consisted of five peptide domains. In another perspective, the chimeric polypeptide included the amino acid subsequence. The invention prepared recombinant expression vectors consisting of the isolated polynucleotide of any combination or embodiment of the invention connected to a control subsequence, operatively. In a more embodiment, the invention prepared chimeric polypeptides, including the chimeric polypeptide-encoding with the expression vector or the polynucleotide of any combination or embodiment of an embodiment of the assertions. Further, the invention provided assemblies consisting of a pluralism of the RNAs and polypeptides, which could prepare the pharmaceutical composition (a) in the case of one assurance in which the composition includes an assistant like a glucopyranosyl lipid or TLR4 ligand assistant in a stable emulsion (GLA-SE) admissible carrier pharmaceutically, and (b) in expression vector where there are chimeric polynucleotide, chimeric RNA, chimeric polypeptide, as well as the production of any combination or embodiment. The invention presented approaches to limit or treat *Toxoplasma gondii* infection such as processing a topic at danger of *Toxoplasma gondii* infection as a value affective for limiting or treating the decadence of the chimeric polynucleotide, chimeric polypeptide, expression vector, and assembly of an embodiment, chimeric RNA and composition of embodiments in invention[53]. Figure 19 shows a schematic diagram of the DNA vaccine construct.

Preparation of DNA nanovaccine consisted of DNA with nanostructure morphology, the compound of tumor-antigen peptide-DNA, and immunologic adjuvant including CpG or double-stranded RNA was the other embodiment. The invention fabricated a type of nanostructure assembled as profiling DNA, folding assisted chain of DNA, capturing a chain of DNA, finding out immunologic adjuvant molecule appropriately, and tumor antigen molecule in the precise positioning onto self-assembled nanostructure surface of DNA by using the capture of DNA chain. Controllable DNA molecular (switch) was fabricated in the tubular nanostructure DNA side, the endosome acid environment was responded after using antigen-presenting cell for releasing immunologic adjuvant and tumor antigen molecule, which is represented as a type of novel efficacious and safe tumor vaccine

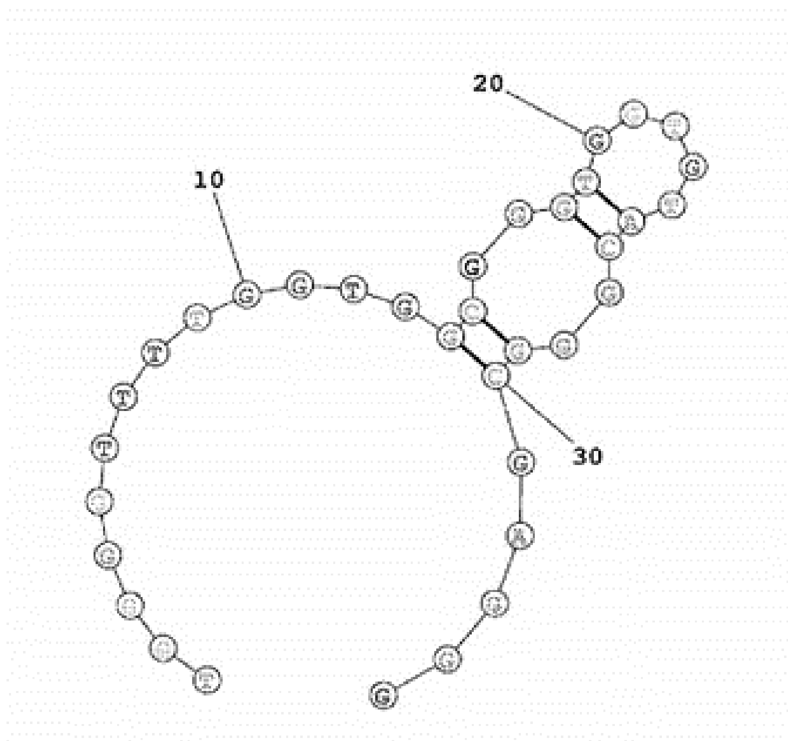


Figure 25. Modeling of the secondary structure of oligomer against AFB1 in web software of RNA Structure[65].

utilized for immunizing therapy and limiting many types of malignancy by considering specific tumor antigen immunostimulating influence. The tumor-antigen peptide is reacted by "click" connect with DNA chain which forms tumor-antigen peptide-DNA multiple close objects. Double-stranded RNA immunologic adjuvant used DNA as a template, and in-vitro transcription formed single-stranded RNA and two single-stranded RNAs according to 1:1's molar ratio mixing after annealing double-stranded RNA. The synergistic effect was played using tumor-antigen peptide and two kinds of immunologic adjuvants by being precisely controlled three relative positions, while two signal paths of TLR3 and TLR9 were activated better than the structure for only including a kind of immunologic adjuvant. The DNA nanostructure was assembled and formed by creating DNA template chain, folding assisted DNA chain, and capturing DNA chain. The DNA template chain included M13mp18 phage genome DNA and/or λ bacteriophage genomic DNA, preferably M13mp18 phage genome DNA. Single-stranded as the main chain used the cyclic DNA of M13mp18 bacteriophage, excessive short-chain DNA was as auxiliary chain, which was complementary in the hybridization of specific position with programmable auxiliary chain by the main chain, and folding formed a kind of two-dimensional rectangular lamella DNA and received Rice structure. The M13 phage genome DNA or asymmetric PCR product by genetic modification was made for DNA template chain to construct DNA nanostructure[54]. Figure 20 displays the atomic force microscope morphology of DNA nanostructures.

2.4. Diagnostic applications

Recently, molecular methods have been utilized appropriately for quantitative and qualitative analyses. The enhanced knowledge in computer science in computational biology and bioinformatics results in designing the probes of DNA/RNA faster than before. The probes of DNA were used in many fields like veterinary, food, environment, security, and medicine for limitation, treatment, and diagnosis [55]. Hence, it is recognized as a great amount for providing a rapid and

easy screening probability that can permit testing many samples quickly by having a high degree of reliability simultaneously, which is beneficial for preparing kits to include the reagents standardized and enhanced for sensitivity and precision for commercial applications[56].

A new type of magnetic nano-treatment diagnostic reagent was based on DNA-based materials such as DMSA Zn0.4Fe_2.6O_4. The synthesis of nanoparticles and tetrahedral provided DNA and treatment diagnostic reagent TDN Zn0.4Fe_2.6O_4. In synthesizing RGD nanoparticles, the approach control reaction condition provided the DMSA Zn together with a high magnetic saturation amount of 0.4Fe2.6O4NPs. This NP could be used as a substrate, targeted molecule (RGD), and pharmaceutical carrier DNA tetrahedrons linked with simultaneously to achieve the reagent by magnetic resonance image-forming recognize and therapy by the drug. Treatment distinction indicated good biocompatibility, stable performance, and target managing the attributes and magnetic resonance signal, which was strong relatively. The product could satisfy the practical demand. The magnetic resonance image-forming diagnosis part of the described treatment diagnostic reagent was Zn0.4Fe2.6O4NPs, the drug therapy carrier part of the described treatment diagnostic reagent is DNA tetrahedrons, and the targeted target molecule of described treatment diagnostic reagent was amido modified RGD. DNA tetrahedrons（TDN） as the amido modified TDN-4 by TDN-1, TDN-2, TDN-3 was modified and designated at 5 ' ends NH3-TDN-4, four single-stranded to be assembled[57]. The advantage of this invention was that the DMSA-Zn of high magnetic saturation value Zn0.4Fe2.6O4NPs was substrate by using the method coupled with targeted molecule (RGD) and DNA tetrahedrons connected to its surface was obtained as the treatment diagnostic reagent with targeting. Diagnosis and treatment reagent prepared by the invention had good physical and chemical stability, targeting nuclear magnetic resonance and medicine. The performance of a conveying drug conveying carrier DNA tetrahedrons used by this technology had good cell-permeable and bio-compatible properties. The present invention's preparation method was simple, workable, and could meet production and apply more.

The amido modified gold nanocarrier for sensing cell C/EBP α was the other approach for applying DNA nanostructures. The kit included amido enhanced a gold nanocarrier, double-stranded film reagent, and DNA penetrated albumen (DNA reagent included C/EBP α binding surface and fluorescent marker). Since charged amidized AuNPs (positive charge) adsorbed the electronegative double-stranded DNA including C/EBP α specific binding surface, entrained using fluorescent molecule was quenched due to the impressive luminous influence of AuNPs. C/EBP α could particularly bind double-stranded DNA at the existence of C/EBP α. Hence, AuNPs were hindered for causing fluorescence intensity restored the dsDNA suction-operated. The kit of a kind of amido modified gold nanocarrier sense C/EBP α of the present invention includes amido, modified gold nanocarrier, film penetrate albumen, and double-stranded DNA reagent. In addition, it could include the necessary PBS buffer system. The double-stranded DNA contained a C/EBP α binding site, 3' as the end fluorochrome labels containing C/EBP α chain in the double-stranded DNA[58].

The other application was prepared for detecting schizoid peripheral blood indication plasma DNA. Particularly, the invention prepared a nucleic acid type or the targets of its determination reagent, as well as the nucleic acid, and its determination reagent was selected for dissociating dsDNA within the blood, segments of ALU 115 bp as well as its amplimer, segments of ALU 247 bp and its amplimer; nucleic acid, and its determination reagent was utilized for preparing diagnosis schizophrenia, and the determination reagent and kit in the cases of its prognosis. Further, the invention presented the determination kit for detecting schizophrenia and its nucleic acid array or chip, prognosis, and application from there, the aims and schizophrenia of dsDNA reagent box to determine content, as well as the diagnostic approaches of its foresight. When the concentration for the dsDNA always dissociated in the sample was higher than 16.65 ng/mL, the patient suffered from schizophrenia disease risk was higher than the normal population. On the other hand, when the relative concentration of ALU-115 bp segments in the sample was higher than 7.15 × 10-8, the patient

suffered from essence refreshing split disease risk was higher than the normal population[59].

Using Sequenom Mass-ARRAY for investigating methylated DNA was based on tech cell gene methylation level of VDR, and the pathogenetic assembly of glycosuria, which happened by diabetes as preliminary screening marker. It was determined that multiple methylation positions of DNA in people's blood in gene VDR transcription started point context point, which happened considerably related to diabetes. Hence, the invention prepared the DNA methylation marks for diabetes screening, detection, and danger profile object, and the markers were determined to be seven sites of CpG gene VDR methylate. Second, the invention prepared the primers to determine DNA methylation markers. Finally, the invention prepared a type of DNA methylation marker determination kit consisting of this pair introduced in the second invention. The inventors discovered that in gene VDR in the CpG groups related to diabetes generation, these methylation sites of little sequences could be used as the prediction pathogenetic DNA methylation marker of glycosuria for diagnosis and screening and risk profile of diabetes[60].

A chip-based kit for determining a DNA sample was the other embodiment via a signal amplification approach. The disclosure consisted of incorporating the compounds to be investigated by using a biotransformation approach conducted for the test system to determine the fragments of DNA. Hence, the chip substrate is just related to a united standard. Based on the results, a unified chip together with the substrate was utilized for detecting the pluralism of objects, while the provision of the chip was simplified, and cross-contamination made by many substrates was eluded. Further, the kit included a signal amplification approach leading to higher determination sensitivity as the same was utilized for detecting DNA. The invention related to a kit for detecting a DNA sample, which comprised a capture probe, a mediator and a terminal signal, an unmodified solid-phase carrier, and magnetic beads modified with an affinity substance (i.e. M5), while the capture probe was a DNA fragment containing a DNA fragment complementary to and paired with a partial nucleotide sequence (i.e. B1) of a DNA fragment to be tested in the DNA sample, and the partial nucleotide sequence B1 contained a site to be tested. The mediator and terminal signal were both DNA fragments in which the nucleotide sequence (i.e. L1) at one end of the mediator was complementary to and paired with a partial nucleotide sequence B2 of the DNA fragment to be tested in the DNA sample and the nucleotide sequence L2 at the other end of the mediator was complementary to and paired with a partial nucleotide sequence C of the terminal signal. In addition, the kit further included a chip with a substrate which was a DNA fragment, and part or all of the fragment of the substrate was complementary to and paired with a partial nucleotide sequence C2 of the terminal signal. Further, the kit further comprised a signal conversion fragment, a DNA fragment, and part or all of the fragment of the signal conversion fragment was complementary to and paired with a partial nucleotide sequence C3 of the terminal signal. However, the terminal signal or signal conversion fragment was labeled with an affinity substance (i.e., M6) with an affinity with an affinity substance M5[61]. Figure 21 shows a schematic diagram of a method for detecting a DNA sample.

Detection of caprine arthritis-encephalitis virus (CAEV) via recombinase lateral flow dipstick (LFD) and polymerase amplification (RPA) was the other embodiment [62]. The kit and approach were determined to be appropriate for the field of laboratory usage and were sensitive and particular to determine CAEV proviral DNA in cases of goats considering a fast manner. The invention was introduced as an appropriate approach due to its applications for on-site usage at farms and should be appropriate in epidemiological investigations and eradication programs. Providing a method for detecting caprine arthritis-encephalitis virus (CAEV) infection was considered as the main feature of this invention including (a) amplifying gag-segment (nt 618-803) DNA of CAEV as a target sequence in the sample by recombinase polymerase amplification (RPA) in the presence of a pair forward and reverse primers and a probe sequence which was complementary to an internal region of the target sequence, while one of the primers being labeled with a first label and the probe sequence being labeled with a second label such that amplification of the target sequence generated an

amplicon labeled with both first and second labels, (b) applying an amount of the amplification product of step (a) to a lateral flow device/strip to flow laterally towards a distal end of the lateral flow device/strip, while the lateral flow device/strip sequentially comprising (i) a sample pad, (ii) a conjugate pad, (iii) a test region, and (iv) a control region, said conjugate pad having mobile reporter labeled with a first agent which attached explicitly to the first label or the second label of the amplicon, said test region provided with a second agent which specifically attached to the second label or the first label of the amplicon to inhibit further lateral flow of the amplicon associated with the mobile reporter, and said control region being provided with a control agent, and (c) detecting any binding of the amplification product at the test and control region. Another aspect of the present invention was related to providing a kit for detection of caprine arthritis-encephalitis virus (CAEV) in a sample including a pair of forward and reverse primers targeting a gag-segment (nt 618-803) DNA of CAEV as a target sequence, a probe sequence which was complementary to an internal region of the target sequence, a reagent for conducting recombinase polymerase amplification (RPA) reaction. And a lateral flow device/strip, while one of the primers was labeled with a first label and the probe sequence being labeled with a second label such that amplification of the target sequence generated an amplicon labeled with both first and second labels; and the lateral flow device/strip sequentially comprising (i) a sample pad, (ii) a conjugate pad, (iii) a test region, and (iv) a control region. Further, the said conjugate pad having mobile reporter was labeled with a first agent which specifically attached to the first label or the second label of the amplicon, said test region provided with a second agent which specifically attached to the second label or the first label and said control region is provided with a control agent [63]. Figure 22 shows the molecular specificity of RPA-AGE and RPA-LFD by using the total DNA of animals natural infected with goat pox virus.

Optical sensors for detecting oligonucleotides were invented for point-of-care, diagnostic, theranostic, and drug screening usages. In this way, an excellent and customizable system was prepared for determining specific RNA and DNA oligonucleotides by utilizing a carbon nanotube to produce an optical signal that was useful for determining circulating oligonucleotides. This type of biomarkers possessed prognostic and diagnostic value in the cases of metabolic disease, cancer, organ rejection, infectious disease, and fetal health. Potential purposes consisted of circulating mRNA, microRNA (miRNA), and cell-free tumor DNA. Since the platform was compatible with biofluids, it was provided in various embodiments, purification-free, point-of-care diagnostics. In addition, the implants comprised the sensing platform in live organisms (e.g., humans, rodents) and methods for detecting oligonucleotides in vivo with a noninvasive method. Thus, this platform could be an implantable sensor for biomarkers, allowing for real-time, non-invasive monitoring *in vivo*. The devices were primarily a sensor comprising a single-walled carbon nanotube (SWCNT) and a polymer associated with the SWCNT. In contrast, the polymer included a first and second domain, where the first domain had a sequence complementary to a target nucleotide sequence and the second domain was a stabilizing domain[63]. Figure 23 displays a schematic plan for constructing the SWCNT sensing platform.

For determining telomerase activity, many approaches were shaped to fabricate gold NPs functionalized by a telomerase substrate that sometimes a linker nucleic acid possesses a region supplementary for a nucleic acid containing a telomerase substrate. Functionalized gold NPs had been fabricated by utilizing the prepared approach. Further, it was based on a functionalized gold NP complex, contenting gold NPs, coupled with a linker nucleic acid hybridized to a nucleic acid contenting a telomerase substrate. Figure 24 displays telomerase tests by utilizing the functionalized gold NP complexes or functionalized gold NPs of the invention and kits to detect telomerase activity of a cell, including the functionalized gold NP complexes or functionalized gold NPs of the prepared invention. Based on the invention, a functionalized gold nanoparticle complex was provided, in which the complex included (i) a gold nanoparticle coupled to a linker nucleic acid having a region of complementarity to a nucleic acid comprising a telomerase substrate, and (ii) a nucleic acid comprising a telomerase

substrate, while the nucleic acid comprising the telomerase substrate was hybridized to the linker nucleic acid to form the functionalized gold nanoparticle complex[64].

A rapid and sensitive nanomolecular detection of aflatoxin B1 (AFB1) was the other embodiment for measuring AFB1 concentrations in food samples[65]. It included (i) biotin-modified G-quadruplex aptamer against AFB1, (ii) streptavidin-coated magnetic nanoparticles, and (iii) the colorimetric measurement of aflatoxin B1 in biologically or food samples. G-quadruplex-based aptamers could enhance the hemin peroxidation property after trapping the target molecule and ultimately increase the detection's sensitivity. Among various nanomaterials, nanoparticles (NPs) based on Au/Ag, carbons (CBNs), magnetic (MNPs), quantum dots (QDs), up-conversion nanoparticles (UCNPs), and metal-organic frameworks (MOFs), magnetic nanoparticles could be used in the food industry to identify and isolate pollutants due to their superparamagnetic properties, as well as the ability to catalyze oxidation and color production reactions in the presence of chromogen substrates. Nowadays, aptamers have been suggested as suitable substitutes for antibodies due to their specific advantages over other identifiable elements, but it still needs equipment such as spectrophotometers in the laboratory to identify target biomarkers using aptamers. Hybrid nanomaterials with aptamers have been instrumental in generating and amplifying color and fluorescent signals, which can be detected and measured with smartphone surface plasmon resonance. Finally, it is possible to quickly evaluate the essential measurements required by using conjugated aptamers with magnetic nanoparticles at each location.

Meanwhile, the peroxidase property of G-quadruplex structures can dramatically increase sensitivity, accuracy, and ease of use for the amateur user of colorimetric sensors. The use of proprietary propellant conjugated with magnetic nanoparticles can provide more stability and reduce the cost of the finished product, and an increase in the surface-to-volume ratio can dramatically increase the sensitivity. On the other hand, magnetic nanoparticles can help detoxify and remove pollutants, which can be made available to anyone on a portable smartphone by using an optical sensor based on a digital camera and android application[66]. Finally, the ease of rapid, reliable, and low-cost testing can significantly contribute to food safety and human health by controlling the use of this toxin, reducing the risk of liver cancer and other disorders resulting from its unauthorized use[65]. Figure 25 shows modeling of the secondary structure of the oligomer against AFB1.

Conclusion

The emergence of DNA nanomedicine as a treatment point for several diseases has attracted much attention. Most researchers envisioned the broad potential of DNA-based nanomedicine for *in vivo* application due to their biocompatibility and biodegradability. DNA-based nanomedicine has shown excellent results for drug delivery, cancer therapy, gene therapy, nanovaccine, and several diagnostic purposes at the preclinical and clinical stages of drug development. DNA nanostructures are considered rational candidates for scheduled and smart drug/gene delivery as a molecular payload carrier or a substrate to the conjugate drug for transporting to targeted cells. The delivery of functional genes and other polynucleotides into particular target cells can be used in various contexts. For example, gene therapy can be used for immune deficiency, neurological disorders, particularly Alzheimer's disease, multidrug resistor colon cancer cell, ocular disease, and control and treatment of infected burn wounds with multi-drug resistance MDR. These unparalleled successes have led gene therapy to a special place in nanomedicine DNA research. DNA nanovaccine is another breakthrough of DNA nanotechnology in medicine. Although DNA nanovaccines are not yet available on the market, a large body of research has been conducted, which could give an excellent perspective for a new generation of the smart vaccine. The diagnosis is as important as the cure. High-sensitivity and high-speed diagnostic methods are requirements in today's modern medical world. In addition to accuracy and speed, DNA-based diagnostic methods have the advantage of reducing costs. Some of these methods allow early diagnosis of chronic diseases such as diabetes. Other diagnostic applications of the DNA-based method include its use in medical imaging, diagnosis, the prognosis of schizophrenia, and

detection of aflatoxin B1 (AFB1) concentrations in food samples. However, the safety of DNA-based nanomedicine should be considered for more effective results. DNA and DNA-based nanomaterials have provided a wide field of applications for nanotechnology approaches in nanomedicine. Hence, it seems that the DNA-based nanodevices can improve the future of miniaturized diagnostics, therapeutics, and theranostics in medical sciences, and these improvements can provide various medical goods for the health increment of humanities.

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Conflict of interest

The authors further declare that, they have no conflict of interest.

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