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DNA ORIGAMI AND BIONANOTECHNOLOGY: AN EFFICACIOUS TOOL FOR MODERN THERAPEUTICS AND DRUG DELIVERY

***[1,2]Mirza Sabuktgeen Baig, [3,4]Usman Babar, [3,4]Usama Arshad and [2,3]Muhammad Asad Ullah**

[1]Institute of Molecular Biology and Biotechnology (IMBB), University of Lahore, Pakistan
[2]Centre for Research in Molecular Medicine (CRiMM), University of Lahore, Pakistan
[3]Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad
[4]Centre of Advanced Studies in Agriculture and Food Security (CAS-AFS)

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ABSTRACT

DNA has long been studied because of its irrefutable significance and interaction in all the essential biomechanisms. With the help of Bionanotechnology bottom-up approach, complex 2D and 3D DNA structures can be constructed as DNA origami by using short staple strands. DNA is biocompatible to different materials or particles such as drugs, antibiotics, inorganic molecules, metal particles and small molecules that can be attached precisely at nanometer scale with the DNA origami structures to generate nanodevices and nanomedicine. DNA origami devices or structures exhibit remarkable potential regarding biomedical applications, targeted drug delivery, biosensing, diagnostics, therapeutics, nanomedicine, nanoelectronics and photonics. DNA origami is still in its initial phase and is being focused to overcome the challenges and meet up the need of timeby exploring nascent opportunities for its rapid expansion.

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INTRODUCTION

DNA, the genetic material that can stably carry genetic instructions for thousands of years because of its non-reactive chemical nature and stable structure.[2] Because of this inspiring property of nature, nucleic acids became a potential building materials for designing peculiar nanostructures for past few decades.[3] DNA exhibit exclusive properties to act as a template and building block for nanomaterials. As DNA obeys the Watson and crick base pairing due to which it can be easily programed for the designing of desired DNA constructs.[4, 5]Bionanotechnology involves the construction and designing of different and unique DNA motifs in order to exploit basic DNA structure for building 2D and 3D Nanodevices.[6] Chemically specific nucleotide sequences retain an organized pairing characteristic that are likely to be simply and accurately designed at nanoscale.

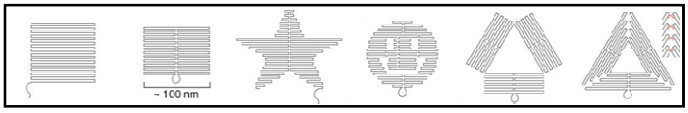
Corresponding Author: **Mirza Sabuktgeen Baig,**

Such synthesized or preordained sequences can be readily obtained from genomic DNA. This designing and binding interaction of DNA has opened a novel approach towards DNA origami.[7] The word 'Origami' originated from Japanese craft by folding paper to create special 3D shapes. Therefore, DNA origami method involves the folding of a long DNA strand with the attachment of smaller pieces of DNA usually called as staple strands to design desired nanostructures which was first introduced by Rothemund in 2006.[8] DNA nanotechnology or Bionanotechnology was pioneered by Ned Seeman and colleagues while working on DNA lattices and holiday junctions and since then this field has developed to construct and design multiple DNA structures and shapes[9] that swiftly made advancements to create DNA origami[7] or scaffolding of DNA to make modified constructs and shape the DNA structures to create 2D and 3D lattices.[10, 11] Not only DNA can maintain and store genetic information but it also has structural role in maintaining linear genome and in the regulation of gene expression in the cell to create G-quadruplex structures.[12] The progress in nucleic acid

nanotechnology has opened ways in the static and dynamic functional nanodevices that can be built by natural or synthetic materials for novel application in different areas of study.[13] The advancement in bionanotechnology has revolutionized the DNA origami method to make complex Nano-architectures with simplicity and to functionally form the single molecule analysis systems. By utilizing ssDNA of M13 bacteriophage[14] we can create desired 2D or 3D shapes involving cubic and octahedron nanostructures using small synthetic oligonucleotides to bind the folds of longer ssDNA in specific compact shape with nanometer precision but these nanostructures are sensitive to stoichiometry.[15, 16]Different computer aided techniques and software programs like caDNAno[17] and GIDEON are used to design the 2D or 3D DNA origami structures in a virtual interface for better understanding of DNA construct and to minimize errors.[18] Due to defined structure and molecular system DNA origami can be used as a tool that is highly specific in function for number of applications like to build Nanobots, to direct the particle to specific position, in molecular pattering, drug carries and particles delivery systems, molecular sensors and as Nanocarrier.[19] DNA scaffolds structure is changed with the binding of specific molecule like drug molecule. DNA origami is configured to target cells for drug delivery. The attachment of desired molecules to DNA scaffold was detected by using fluorescence techniques and this conformation put forward the different uses of DNA origami.[20, 21] The drug delivery to cancerous cells by creating DNA Nano-architectures with Nanofilms is a novel technique because of the versatile properties of DNA to build multilayered nanostructure. The approach is to release the anticancer drug using DNA origami in controlled fashion.[22] This precise and controlled fashion delivery of DNA based nanostructures have opened up a way to novel techniques that can be used in therapeutics and a range of medical tasks.[23]Therefore, DNA origami based on DNA nanostructures has been promising to deliver different materials in the living cells and has been promising for therapeutics and health applications.[24]In this review a novel technique of structural bionanotechnology known as DNA origami has been discussed and how it will impact the modern therapeutics and research in different field of study. Although, this technique is expensive but because of modern technologies and research the rate of development has been increasing rapidly.

Applications of Bionanotechnology

The outstanding internalization of nanostructures (Figure 1) based on DNA into the cell has opened new possibilities for overcoming different clinical and medical challenges.[23]



Some of them are as follows;

Figure 1. DNA Origami Structures[7] consisting DNA scaffolds and staple strands, presented as model for designing

Nanomedicine

Nanomedicine or localized drug delivery is currently one of the major objectives of bionanotechnology. Such drug delivery which could only be released onto infected cells, lead to decreased dosage of drug intake and lower harm to the surrounding unaffected cells.[25] The release of the materials can naturally be controlled similar to release of genome out of the cell from a viral capsid[26]by adding functional strand that are susceptible to natural stimuli in the environment of the cells. Such strands include strands of DNA that have undergone structural deformity due to pH or an innate cellular material or deoxyribozymes that are sensitive to potassium.[27] Apart from targeted drug delivery, these DNA based nanostructures can be used for regulated silencing of the genes through a transfection of intrusive siRNA molecules. Moreover, these nanostructures can be utilized for reticence of protein expression through degradation of in vitro mRNA and they can also be programmed for in vivo imaging.[28, 29] A study tried to perform targeted gene silencing by integrating a minute DNA nanotube customized with PEGylated folate with molecules of siRNA, resulting in a failure.[30] A similar study succeeded in localizing the effect on the genes within tumor cells by using peptide or folate-modified DNA nanoparticle with a tetrahedral structure that were fitted with the siRNA. This result showed that the geometrical mapping and the density of the tumor-targeting ligands within the DNA based nanostructure played a critical role in the delivery of the payload. It was also observed that signals from the receptor on the membrane of tumor cells can be controlled with the aid of "nanocalipers" based on DNA origami by changing the geometric mapping of the ligands that bind the membrane.[31] Nucleosome structural changes were investigated by using DNA origami nanocaliper. This allowed the dynamic DNA origami to probe and regulate biomolecules.[32] These results clearly show the potential of the nanovehicles based on DNA origami since they are programmable and can be customized at will.

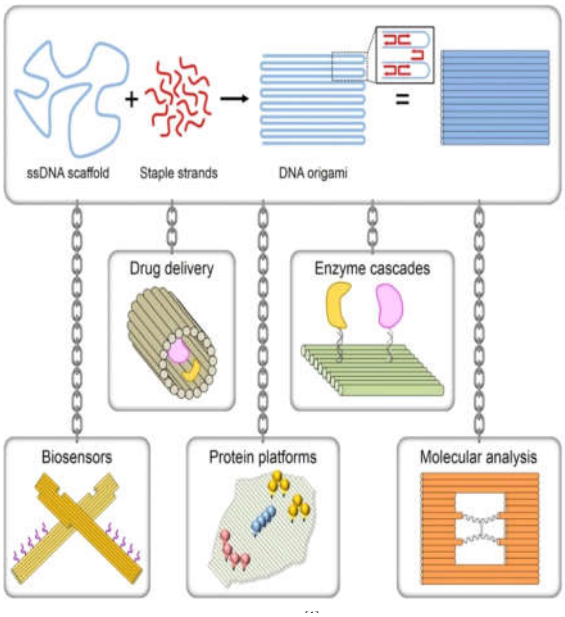


Figure 2. DNA Origami and its Biological Applications[1] The synthetic DNA Origami could be used by diverse means which involve drug delivery, enzyme cascades, bionsensors, protein platforms and molecular analysis

Nanodevices

Computational devices made of nucleic acid that have the ability to copy ncRNAs are being assembled to realize this objective of a more controlled delivery. These structures can be made by utilizing self-cutting deoxyribozyme hairpins that have a short strand of single stranded DNA that is reciprocal to

the section of mRNA that must be suppressed, inside them. These are inactive unless a disease indicator in the cellular space activates them.[33-35] DNA origami provides great potential for custom made bio-machinery and for regulating reaction at the level of individual molecules due to its highly designable geometry. Abundant studies support this notion. One study used a rectangular platform made of DNA origami to regulate and produce consecutive chemical reaction.[36] Recently, a study shows the use of DNA origami tube structures to be captured by lysosomes and degraded with time in NIH-3T3 cells by fluorescence localization method and can help us to study how DNA origami can affect the mechanism of living cells.[37]DNA nanomachines have been used to control and start enzymatic reactions in a complex style.[38]Tweezer resembling DNA structures with have also been made that can pick enzymes and their cofactors granting the ability to regulate the activity of the enzyme by opening and closing the arms utilizing oligonucleotides DNA as fuel for functionality.[39] Collection of multiple enzyme have also been constructed on templates of DNA, where by using a strand of DNA as an arm that could stretch and swing between impaired enzymes, substrate transport was obtained.[40] Another useful creation that could be used as a transport vehicle for the enzymes in replacement therapy or as a biological sensor, is a tube like enzyme cascade nanoreactor. Such a device was formed by combining interchangeable building-blocks made of DNA origami and could detect glucose.[41]Artificial multiple enzyme pathways involving DNA origami for substrate channeling has been used to design smart enzyme networks and to make catalytic nanodevices to build biological circuits.[42]A molecular shutter was built in a DNA origami nanochannel that could be opened or closed when required by DNA hybridization and to be used to study transportation of materials at nanoscale.[43] Since the DNA can release the payload before even activated to do so, because of nucleases, this drawback of the DNA can be overcome by integrating modified nucleic acids.[44] However, the introduction of an external material may lead to cytotoxicity or other unintended side effect. Therefore, such an approach must be heavily tested before implementation. Moreover the inclusion of failsafe features that would make the device unstable before reaching the target area will also be helpful in prevention of unwanted side effects.[45]

Gold nanorods

Molecular structures made using nobel metals like gold nanorods (GNRs), gold nanocages and nanoshells, hollow dendrites made of silver or gold and aggregated gold nano spheres displayed photothermal and optical conversion attributes because of localized surface plasmon resonances (LSRPs) thus offering great susceptibility for molecular imaging and photothermal therapy of cancer at the same time.[46] Particularly, such GNRs that have the ability to absorb and distribute effectively in the NIR region are exceptional successor for florescent visualization and plasmonic photothermal therapy.[47,48] Under such developments, photothermal effects of the GNRs and the exceptional carrier effect of the DNA origami approach can be combined together to achieve improved therapeutic affinity.[49]

Malaria and Bionanotechnology

DNA origami has also been used in the detection of malaria. For that signaling molecules were packed into the DNA origami construct and can only be discharged from the origami capsule in the presence of malaria biomarker *Pf*LDH.[50, 51] Another study shows that 12 modified DNA aptamers acting as a signaling molecules to detect the malarial biomarker *Plasmodium falciparum* lactate dehydrogenase (*Pf*LDH) were combined with rectangular DNA origami structure and were proven to be stably bind to *Pf*LDH detected with the help of AFM. This opens up new possibilities towards making smart devices to diagnose malaria[52]as different reports shows the resistance of malarial parasite to existing drugs and treatments.[53, 54]

Tuberculosis and Bionanotechnology

DNA origami drug delivery system for tuberculosis treatment has been constructed to improve the effectiveness of chemotherapy as the patient suffering from tuberculosis have to take a six month chemotherapy course and have poor curing results. Therefore a DNA aptamer was constructed based on the lock mechanism when the ligand key sequence is detected the DNA origami box opens up allows the loaded drug to bind with the bacteria to achieve its function.[55, 56]

DNA Origami as Tool for Targeted Drug Delivery

Drug delivery to specific locations in the body requires precision and research which has been done and improved on for the last decade. It has been observed that the nanoscale particles show exceptional potential to be used for drug delivery and release due to their small size and specific geometrical and chemical attributes.[57] The process of DNA origami has given rise to newer possibilities related to localized drug delivery since a large variation of nanoscale materials can be constructed to control the release of drugs by encapsulation and coordinated delivery. Structures based on DNA-origami techniques have shown diverse compatibility with other biological materials and have displayed remarkable stability in mixtures of lysate[58], staying unaltered even after being placed in temperature of 37°C for up to 24 hours.[59] This has enabled them to be used in a wide range of in vivo and in vitro applications.[19] Since the naked DNA based molecular structures cannot provide therapy and imaging functionality itself, this is usually overcome by binding some type of nano payload such as antibody protein[19], immunostimulatoryoligo DNA[60], drug molecules[23, 61] or fluorescent probes on to the nanostructure.[62]

Cancer Therapy and Bionanotechnology

The targeted delivery of molecular drugs to particular localized sites with minimal side effects, is a need for an effective drug delivery system. Due to absence of an efficient drug delivery mechanism for an individual or a joint chemotherapy, such need is still a clinical challenge. By using a diverse variety of organic and inorganic molecules, Nanomachines that can aid in the drug delivery have been constructed over the last two decades. Specifically, drug delivery systems based on Nanomachines have displayed enhanced permeability and retention effects (EPR), resulting in passive aggregation of tumor drugs.[63] Against liposomes and polymeric Nanomachine based drug systems which have been accepted for clinical use[64, 65], the diverse compatibility with other biological materials and biodegradability for easier elimination, usable modification sites and easier techniques for encapsulating drugs, make the highly programmable

nanoparticles made of DNA origami a favorable candidate to be used in drug delivery systems. The ability of the DNA nanoparticles to easily enter a cell without any help of a transfection agent could prove to be an efficient drug delivery system when densely packaged.[66, 67] As an alternative to using florescent assays to detect DNA origami within the targeted cell, a technique which uses florescent labels, a quantitative real time polymerase chain reaction (qPCR) M13 can be used to report the rate of cellular absorption of the materials based on DNA origami.[68] By incorporating capsid proteins of viruses with the DNA origami, the absorption rate can be improved and increased. These capsid proteins have the ability to assemble themselves and bind on the DNA origami surface through bonds that are electrostatic in nature and bind the DNA structures within themselves.[69] *Cisplatin*, *oxaliplatin* and *carboplatin* are all platinum drugs that can cause cytotoxicity because they bind with two guanine (G) DNA bases at the nitrogen positons (N).[70] Recent studies have designed cationic nanomaterials with core-shells that have a hydrophobic center and a shell that is cationic, through a biodegradable copolymer by the technique of self-construction. Drugs that are hydrophobic in nature can be combined within the core during the self-construction and the cationic shell of the nanomaterial that is fitted with the cargo, binds with the DNA through electrostatic bonds between positive polarity of the nanomaterial and the negative polarity of the DNA.[71] Paclitaxel is a hydrophobic drug that can be loaded into these structures. While the diagnosis and treatment of cancer are one of the most challenging feats in modern medicine, we have come to enhance our knowledge through understanding of the tumor cell. New innovations in designs for cancer therapy have been made using information such as the permeability of the tumor vessels to molecules that are larger compared to normal tissue vessels and the retain-ability of these molecules due to lesser clearance in the pathway at the tumor area.[72, 73] Different sensors to diagnose cancer and more effective therapy against tumors have been designed by using the selectivity of the tumor cells.[74] Since DNA origami enables accurate construction of functional groups at particular locations within the living system, these molecular materials are therefore very helpful in providing structural control over such moieties that are aligned for example ligands and receptors bound on membranes. This can be used in tumor cells that have an excessive and uncontrolled cellular signaling process. By using specialized assembly, DNA "Nanocalipers" were used to maneuver ephrin-A5 ligands at periodic intervals, so as to bind with their compatible EphA2 receptors in order to bind and activate them.[31] This molecular delivery of such ligands increases the activation of receptors and more receptors are collected on the cell surface thus enhancing the effect of receptor activation. Therefore, a novel approach to the drug delivery system is required. One that would display a diverse biocompatibility, constructability for both passive or negative cancer cell, the ability to regulate the drug release within the tumor area, increased uptake and capacity for loading drugs for an efficient inhibition of the tumor cell and would offer safety and minimal side effects.[75, 76] Doxorubicin, an anticancer drug was released using triangular and tube like origami structures. This enhanced the cytotoxicity against such tumor cells that had developed drug-resistance. Such effectiveness of the origami based carrier has been associated with the cellular internalization of the anti-cancer drug with the help of the DNA origami walkers.[23]By regulating the design of the DNA origami vehicle, the encapsulation of the doxorubicin and the rate at which it is released on the site can also be adjusted at will. This resulted in higher cytotoxicity and reduced rate of intracellular elimination in comparison to the free drug system.[61] By using DNA origami, we can assemble nanobots that have the capacity to interact with each other within a living organism which, in this case were living cockroaches model.[77] DNA origami based structures have shown successful delivery of the drug to the targeted tumor cell in the cancer affected mice model.[62] This *in vivo* delivery is a confirmation that the DNA origami approach has made the effects of the improved passive targeting and long lasting build-up at the infected regions into a possibility. Not only that, DNA origami based drug delivery also showed flawless antitumor affinity in vivo without resulting in any reportable toxicity in the system. Chemotherapeutic doxorubicin is the only drug that has been used in all every documented DNA-based drug delivery system.[78, 79]

Advancements in Cancer Therapy

Given the fact that Bionanotechnology is fairly new and still in research, it is nearly impossible for a healthy comparison with an alternate drug delivery system. One of rare candidate for such a task is the clinically successful Liposomal formation of the anticancer drug doxorubicin.[80, 81] Based on the practical application of small molecules (such as Gifitinib which is an anti-EGFR) or monoclonal antibodies such as Herceptin, an anti-HER2, localized therapy is now the main objective of cancer therapy. In comparison to doxorubicin, these particles do not possess the ability to intercalate, therefore they must be integrated on the exterior of the DNA origami nanoparticle using linking agents which are covalent and non-covalent or they should be encapsulated within a DNA origami cage-like structure, in which scenario logic gates of diverse shapes and measurements could be designs to keep the payload within the cage.[19]DNA origami cages with metal complexes were observed to enhance the anticancer drug efficiency in HepG2 cells in mice model moreover to show the biocompatibility and reduced toxicity for cancer therapy.[82] Compared to non-origami based structures, DNA origami structures display increased stability of more than 24 hours, both in serums and in physiological buffers. This stability could be because of the greater density of the double helices in the nanoparticles made of DNA origami.[83] While such results are documented in abundance, the data regarding stability of the DNA origami within in vivo is still not enough. Recent studies have shown that duration of circulation with in the blood can be enhanced from 6 to 24 minutes due to the half-life of tetrahedron DNA combined with siRNAs.[84] These reports display that the stability of the DNA origami nanostructures is still one of the main reasons why they are undermined for therapeutic applications. Factors relevant to the RNA interference such as permeability of the cells and their degradation can be eliminated by encapsulating siRNA within the lipid nanoparticles (LNP).[85] Alternatively, affinity of treatment can also be decreased by encapsulation of LNP-siRNA within the endosomes[86], a problem which can be taken care of by unification of polymers with the siRNA.[87] Nanostructures made of DNA can also be used to regulate the tumor growth and to minimize pathways for the tumor to spread through by acting as scaffolds since the cell signaling, growth and differentiation are all controlled by a similar geometric relationship of ligands and receptors. Dox (Doxorubicin), an anticancer drug, can also be administered by using specialized carriers made up of DNA origami. A two dimensional DNA origami triangle and a three dimensional DNA origami

nanotube has acted as a carrier to encapsulate and deliver drugs for this purpose. These nanostructures have proven to be more effective in tumor selectivity, increased cellular absorption in cancer infected breast cells, and much longer duration of therapeutic effects.[23, 62] Another report using DNA origami nanotubes shaped like rods, showed similar effectiveness of the drug delivery by inserting daunorubicin.[88] These studies have shed a light on the shortcomings of the typical drug delivery to tumors that can and have been overcome by utilizing nanostructures assembled by DNA origami as agents for localized drug delivery. Alternate drug transports such as macromolecule and nanoparticles (NPs) based on metal are also very good candidates in Nanomedicine.[89] It has been reported that nanoparticles that have encapsulated drugs display improved efficiency in drug delivery and reduced side effects because of their flawless absorption at cellular level.[90, 91] The first generation of the molecular drug delivery system such as Doxil (Ortho bio-tech), a constructed nanoparticle of PEGylated liposome for doxorubicin has already been used to treat breast and ovarian cancer, Kaposi's sarcoma which is related to AIDS and other cancerous cells since the last two decades.[92] Another nanoparticle stabilized from paclitaxel albumin, called the Abraxane was also accepted to be used in the treatment of similar tumor formations in the breast, ovaries and even for non-small-cell lung cancer (NSLC).[93] However, these macromolecular constructs only provided a minor enhancement in therapeutic adequacy because of the gradual and free release of the specific drug from the sheath of the Nano material.[94] Although metal particles provided good compatibility with other biological materials, were unaffected by chemical change and were even used for regulated release of doxorubicin within a laboratory experiment, they still posed a side effect by staying within the body even after the release of the drug and resulting in increased toxicity.[95, 96] Some problems are still there such as the molecules that can be integrated within the nanostructure do not always resemble the documented reports therefore leading to an overload of cargo within the DNA nanoparticle. Deformation of the DNA nanoparticle can also occur due to an oversaturation condition during an in vivo experiment.[97]

Immune Stimulation

It has been discovered in recent researches that the nanocages made out of DNA polyhedral wireframe can be used as transport for the enhanced delivery of cargo such as molecular anti-cancer drugs and oligo DNA (CpG sequences) for stimulation of the immune system.[98] These DNA nanostructures were also tested along with other substances such as Cytosine-phosphate-guanine oligodeoxynucleotides (CpG ODNs) resulting a response of the internal immune system. The DNA nanotubes were decorated with the CpG ODNs and were incorporated in tissues. The results showed that the nanotubes lined with the CpG ODNs showed higher absorption of leukocytes into the neighboring tissues in comparison to the regular DNA nanotubes, resulting in the activation of successive immunostimulation.[60, 99] These results showed that due to the compatibility and stable nature of these nanostructures based on DNA origami, they prove to show great potential for delivery vehicles for the activation of the internal immune system. In search of a simpler answer to this approach, RCA (rolling circle amplification) was introduced to construct DNA origami. RCA enabled the development of strands that made DNA origami by having short periodic sequences that bonded with lesser staple strands. By using this technique, nanoribbons of DNA were made with varied lengths and thickness which formed multi-layered 3-D structures, when assembled. These three dimensional structures also showed potential for drug delivery.[100] Due to their enhanced size and more structural control for the assembly of multipurpose transport for delivery and release of localized drugs, DNA origami show more favorable potential for being a candidate in carrier assembly. Multilayered nanostructures made of DNA origami have been considered more due to their higher affinity with the targeted cells and lesser sensitivity with the untargeted cells. One specific design, the barrel shaped hexagonal DNA origami has been used for the delivery of payload. It works by activation by reconfiguration of its compatible DNA aptamers sites.[19] Tubular DNA origami objects have proven to be an effective transport and have shown to cause activation of strong immune responses of splenocytes.[60] The earliest and the most significant drug delivery system utilized a DNA "Nanopill" based on the DNA-origami approach.[19] This 'Nanopill" was a hexagonal barrel that consisted of two halves which were joined from the back using hinges made of scaffold strands. The front of the cylindrical structure contained "locks", certain DNA aptamers that could be opened under the influence of the compatible aptamer antigen called the "key", there by releasing different moieties, which were stored in 12 cargo-binding-sites within the nanopill, at the same time. Further control of the process was also achieved by comparable nanorobots which were designed and programmed to form logical results in isolated cells and in the living body of a cockroach model *(Blaberus discoidalis)*. This enabled the possibility of altering the delivery of the nanoscale cargo in the living system to an on or off state providing more control over the process.[77]

DNA Origami in Photodynamic therapy

Millions of people die each year across the globe because of malignancy and the number of deaths has been rising constantly. In any case, there are many treatment alternatives that are accessible clinically such as chemotherapy, surgery and radiotherapy although these treatments are restricted because of systematic symptoms, high repetition rate, and increased radiation dose that damages healthy cells.[101] Another promising therapy to kill cancerous cell is Photodynamic therapy (PDT) in which a photosensitizer is exposed to ultraviolet radiation or lasers in the presence of molecular oxygen which produces highly reactive oxygen and kills the cancerous cells.[102, 103] This technique has been reported to modify with the help of DNA origami complex where BMEPC (3,6-bis[2-(1-methylpyridinium)ethynyl] 9-pentyl carbazolediiodide) molecules were packed inside the origami structure as compared to aptamers because of denser intercalation sites and extra layers of firmly packed double helices allows to refrain DNA hydrolases to bind and to increase the density and protecting BMEPC particles from photobleaching results in increasing the effectiveness and fluorescence emission of photodynamic aspects as compared with carrier free BMEPC.[23] The use of DNA origami open up the novel methods in photosensitizer delivery applications in photodynamic cancer therapy.[104]

Current Status and Recent Advancements

DNA origami can be used to study other systems and patterns which can be visualized by using Atomic force microscopy.

DNA origami assembly was used for Nano patterning and design streptavidin Nano-arrays wells.[10] Enhanced green fluorescent protein were patterned by using DNA origami construct for biosensing.[105] By using rectangular origami with attached streptavidin assembly forms the pattern of the coat of arms of Ukraine and by utilizing DNA origami assemblies multi-protein assemblies can be created with nanometer precision.[106] DNA origami has also been used to decorate site specifically with complex patterns of random proteins.[107] Moreover, virus capsid attached to rectangular and triangular DNA origami assembly has also been reported.[108] It has been observed that thrombin molecules can be effectively attached to aptamers.[109]Furthermore, according to study on six-helix bundle DNA origami structure and rectangular DNA origami structure gold Nano particles can be linned in pattern on top of origami assembly.[110, 111] By clever placement of fluorophores as a scale on DNA origami, measuring tools can be constructed to help in super resolution optical microscopy.[112] Recently, DNA origami has been used to make hybrid nanophotonic devices by fabricating molecular emitters and photonic crystal cavities.[113]Lithographic arrangement of gold islands can also be interconnected using nanotubes made of DNA origami.[114] In addition to this DNA origami can also be metallized by using gold particles.[115]DNA origami was used as a platform for antenna array in design concepts and to organize chromophores for light harvesting applications such as artificial photosynthesis.[116] An advancement in Nano-tubes in Nano-electronics has been reported in a study which showed single walled carbon nanotubes placed on top of the rectangular DNA origami construct, demonstrating stable field effect and behavior resembling a transistor.[117] With the help of interactions between biotinylated DNA strands and streptavidin molecules that are accurately aligned on DNA origami and wrapped around nanotubes of carbon, carbon nanotubes can be placed on top of DNA origami.[118] Another advancement in DNA origami application involving the top-down and bottom up approaches of DNA nanotechnology dealing with wafer scale has been reported by adjusting and aligning origami structures on surfaces that have been patterned using lithography technique.[119] Moreover, a study showed that gold nanoparticles can be placed on top of DNA origami assembly bound lithographically at specific sites.[120] With the help of dielectrophoretic trapping, it has been shown that nanoelectrodes can contain origami structures that are placed and oriented between them. This study also showed detection of RNA without label using atomic force microscopy of hybridization of rectangular origami that had a target placed on top of it.[121] Formation of plasmonic nanostructures can also be observed by attaching gold nano particles to an origami shaped as a tube. The optical attributes of nanostructures can also be studied and predicted accurately by particular placement of nanoparticles that was possible due to the definite positioning ability specific to DNA origami method.[122] A previous study has demonstrated that single nucleotide polymorphisms (SNPs) can be distinguished by using DNA origami.[123] By arranging DNA tile based structures on a seed that has unique sticky ends at the edge of a two dimensional DNA origami structure, Algorithmic self-assembly of DNA has also been observed.[124, 125]DNA origami has further helped in research of the effects on the efficiency of DNA methyltransferase caused by dsDNA tensions.[126]Additionally, new secondary DNA binding site in the enzyme topoisomerase I has also been documented using DNA origami.[127] In a fascinating study, it has been found out that a molecular robot can be instructed to move on a pre-programmed path defined by a common enzymatic reaction on top of origami assembly.[128] A similar approach also reports a DNA molecule that has been programmed to work as a walker molecule to collect materials put on particular sites of an origami structure on predefined paths.[129]Regardless of the documented milestones of the DNA origami, a more effective technique for transfection of the DNA nanoassemblies is still required since the polar nature of the DNA origami particles make them very less transfect-able.[68] Moreover, DNA also requires alterations with lipids, cationic polymers or peptides for an accomplished delivery into the cell. This problem, however, can be overcome by enhancing the surface attributes of the DNA-origami structures using particular DNA intercalators.[130]

Limitations and Future Considerations

Certain issues still linger that must be addressed before any practical application can be implemented. *In vivo*, the pharmacokinetic biological availability of the DNA origami nanostructures must be improved. While, the DNA origami based objects can resist nuclease digestion[131] and live in cellular milieu[58], the transmission duration of these structures must be increased which can be done by altering their spatial attributes or by assembling a variety of protective coating such as lipid or protein based coverings.[69, 132] One of the greatest discouraging factor for the adoption of DNA origami approach for both clinical and therapeutic application is the cost of designing these structures. Not only the material used in formation are costly but so is the equipment required to keep the design as accurate as possible. Interestingly however, the rate of research and development of the entire field is increasing so rapidly that it is expected to ramp-up the production quantity from a micro or a milligram unit up to a full gram. This increase in production quantity will lead to a significant price drop.[75, 133] While the current cost of synthesizing even a single gram of these nanomachines is estimated around $100,000, newer and efficient techniques for synthesizing DNA sequences, amplification of enzymatic activity and purification of the DNA origami yield could bring down this figure to measly $1,000[75], cutting back more than 100 times. This could enable the easier affordability and accessibility of larger, more complex DNA origami assembles. Such available complexes could allow the synthesis of artificial biological catalysts that could mimic and substitute enzymes. Novel sensory machines would also be a reality along with other biomimetic structures. The utilization of these structures could also enable the possibility of artificially designing a complete immune system for the body. Such a system would require a new height of spatial complexity and a quickly maturing methods of Biocomputing. Never the less, a system like that could utilize completely programmable nanobots that will be able to localize at different places within the body, fighting a wide range of reagents in vivo, thus eliminating any chances of a disease infestation.[134]

Conclusion

The field of Bionanotechnology has ramped up the development of using DNA to create 2D and complex 3D nanostructures that can be constructed easily and precisely. DNA origami has been developed in such a sophisticated manner that has paved the path in better understanding of biological phenomenon and solving diverse problems related to diagnosis, therapeutics, in targeted drug delivery, in

pharmaceutical applications and health sciences. The explosive development in DNA origami has led to explore new opportunities in technology. Despite the fact that DNA origami has potential for number of applications, there are some hurdles that need to be addressed. This technique is expensive and still under research and with the help of latest techniques and technologies researchers are working to overcome the challenges associated with DNA origami and unlocking its full potential to have great impact on various far flung disciplines.

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