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DNA Origami and its Applications in Therapeutics

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Abstract: **DNA is a stable and biocompatible molecule due to its chemical and physical properties. Recent advancements in DNA nanotechnology allow the application of DNA origami nanostructures in a wide range of biomedical settings. The methods for the design, synthesis and analysis of DNA origami nanostructures are discussed in this study. One of the major applications of DNA nanotechnology is in therapeutics, as a delivery vehicle for chemotherapeutic drugs (such as doxorubicin and daunorubicin), as well as applications in photothermal and photodynamic therapy, all of which are discussed in this study. The study also discusses design parameters that need to be taken into consideration for the suitability and efficacy of the DNA origami nanostructures for different therapeutic functions.**

Keywords: **DNA origami, Nanostructures, Drug delivery, Photothermal, Photodynamic.**

I. INTRODUCTION

DNA (deoxyribonucleic acid) molecules consist of two flexible single strands that turn around each other to form a double helix structure (Watson and Crick, 1953; Franklin and Gosling, 1953; Wilkins et al., 1953), which is approximately 2nm across (Berg et al., 2010). Berg et al. (2010) also described the two polynucleotide strands as antiparallel as the 3' end of one strand corresponds to the 5' end of the other. DNA is a biological polymer and its monomers, nucleotides, are each attached to a phosphate group covalently, forming the sugar-phosphate backbone that holds the polynucleotide structure together. Each nucleotide is also attached to a nitrogenous base and there are four types of such, being adenine (A), guanine (G), cytosine (C), and thymine (T) (Alberts et al., 2002). A and G are purines, while C and T are pyrimidines (Voet and Voet, 2004). Adenine forms two hydrogen bonds with thymine and cytosine forms three hydrogen bonds with guanine, and it is these hydrogen bonds that cause the attraction between the two DNA polynucleotide strands (Phillips et al., 2012).

DNA is a relatively stable biomolecule, both thermally (Blake and Delcourt, 1998) and mechanically (Clausen-Schaumann et al., 2000). The nitrogenous bases in DNA are hydrophobic and have less exposure to water due to being positioned in the middle of the double helix, which maximises the entropy of the system as it reduces the disruption to the hydrogen bonds between these bases (Berg et al., 2010). Berg et al. (2010) also found that the phosphate groups attached to nucleotides are negatively charged, reducing the likelihood of the phosphodiester bonds being hydrolysed, also contributing to the molecule's stability. This stability is further enhanced by the bonds between the complementary bases of the two polynucleotide strands, which are strong and tolerant to high temperatures (SantaLucia and Hicks, 2004).

Seeman (1982) proposed the idea of DNA Nanotechnology, aiming to simplify crystallography-based structure determination using a DNA scaffold. He used the specificity of nitrogenous base pairing in double stranded DNA (dsDNA) to design Holliday junctions (Holliday, 1964). These junctions became the basis of designing 3D shapes like cubes (Chen and Seeman, 1991) as well as other 2D structures.

II. DNA ORIGAMI

A. Design

The concept of DNA origami introduced by Rothemund (2006) allows the formation of DNA nanostructures using a scaffold, which is a long single stranded DNA (ssDNA), typically a few thousand nucleotides long. Rothemund used bacteriophage genome M13mp18 as a DNA scaffold, together with about 200 oligonucleotides to create a variety of nanostructures. The scaffold has seams, which are lines that are not crossed by the scaffold. The shape of the scaffold strand is manipulated using staple strands, typically about 40 nucleotides long, which bind to specific sections on the scaffold due to specific base pairing, altering it into the desired shape by forming crossovers. These crossovers are often considered to have negligible lengths and their positions largely affect the nanostructure's stability. The double helical structure and twist of the strands should be carefully considered when choosing the positions for crossovers. Otherwise, there will be a strain on the structure, causing it to twist or bend in an undesired manner. However, in some cases, this additional twist could be used to form molecules with more complex curvatures (Dietz et al., 2009), such as spheres, nanoflasks (Han et al., 2011), and Möbius strips (Han et al., 2010).

Several software have been developed to allow the computational design of DNA origami. One of the most commonly used ones is caDNAno, an open-source software (Douglas et al., 2009). DNA origami designs using caDNAno can be inputted into another software called CanDo (Kim et al., 2012; Castro et al., 2011), which can be used to conduct Finite Element Analysis (FEA) (Szabi et al., 1991), testing the stability of the nanostructures. Other software are also used for the design of DNA origami, such as scaDNAno (scriptable caDNAno) (Doty et al., 2020), oxDNA, TALOS etc. (Dey et al., 2021).

B. Synthesis and analysis

DNA origami is usually synthesised in a buffer containing 5mM trisaminomethane (Tris), 1mM Ethylenediaminetetraacetic acid (EDTA), 5mM sodium chloride, 12.5mM magnesium chloride, 20nM bacteriophage genome M13mp18, and double distilled water (ddH_2O), at pH 7.8-8.3 (Castor et al., 2011; Rothemund, 2006). Rothemund (2006) also found that the concentration of staples present in the solution must at least be 2 times more than that of the scaffold for assembly to occur properly. Further, the folding reaction can be conducted in a laboratory and the results can then be analysed using agarose gel electrophoresis and Transmission Electron Microscope (TEM) or Atomic Force Microscope (AFM) imaging (Halley, 2016).

III. USE OF DNA ORIGAMI IN THERAPEUTICS

A. Design Parameters

DNA origami nanostructures can be used in therapeutics due to their high specificity, but their suitability and efficacy in therapeutics need to be assessed before being utilized both in vitro and in vivo. The two main design parameters that are required to be taken into account for this are the geometric properties and the charge of the design (Madhanagopal et al., 2018).

The geometry and dimensions of the nanostructures affects their ability to cross biological barriers (Okholm and Kjems, 2016), their cellular uptake (Bastings et al., 2018), as well as their distribution and overall pharmacokinetics (PK) (Hoshyar et al., 2016; Caldorera-Moore et al., 2010). For instance, geometric properties such as degrees of curvature impacts the ease of control of drug release kinetics, affecting the specificity of the DNA origami and therefore its suitability in therapeutics. Besides, smaller nanostructures (typically smaller than 100nm in size) have been found to be more efficient at delivering drugs to solid tumours (Wilhelm et al., 2016), whereas larger nanostructures (bigger than 100nm) favoured a better absorption and retention by these tumours (Yu et al., 2020).

Another significant design parameter to consider is the overall charge of the DNA nanostructure. They are normally negatively charged due to the phosphate groups attached (Berg et al., 2010), but this can be altered using surface coatings. Some examples include cationic polymers such as polylysine (Ponnuswamy et al., 2017), polyethylene glycol, polyethyleneimine, and chitosan (Ahmadi et al., 2018), as well as certain virus capsid proteins (Mikkila et al., 2014). All these coatings have their advantages and disadvantages respectively, which need to be taken into account before their usage.

B. Therapeutics

One of the most popular applications of DNA origami is in targeted drug delivery, both in vitro and in vivo. 3D DNA Origami boxes have been created with programmable lids (Andersen et al., 2009). The boxes use a dual lock-key system, requiring two externally supplied key strands made of aptamers, which are ssDNA molecules and are able to form certain secondary and tertiary structures, allowing them to bind to specific target molecules. These key strands fit into the toehold binding site due to the complementary strands on the box's front face being longer than the temporary closing strands on the lid. The box is then opened irreversibly via Toehold Mediated Strand Displacement (TMSD), where the closing strands are replaced by the key strands (Yurke et al., 2000). This process was made reversible as Zadegan et al. (2012) attached a ssDNA to both the lid and box surface, leaving a loop to act as a toehold for the key strands. After the box has been opened, the key strands can be displaced by secondary input strands complementary to the key strands, rehybridizing the lock to close the lid. This reversibility allows more control over when the drug is released, reducing the severity of side effects caused by the therapeutic drugs.

DNA drug delivery systems were developed further through the design of DNA nanorobots that had a much a higher specificity as they were able to detect single cells from mixed populations (Douglas et al., 2012). These nanorobots consisted of two sections with ssDNA entropic springs (Smith et al., 1996; Kim et al., 2012) on one side and two locks on the other, similar to the 3D origami boxes. The nanorobots designed by Douglas et al. (2012) were loaded with antibodies to human CD33 and human CDw328 Fab' fragments, inhibiting growth of Natural Killer (NK) cells. Meanwhile, nanorobots loaded with antibodies to human CD3ε Fab' and flagellin Fab' were mixed with T cells and found to activate these cells, implying that the nanorobots are capable of manipulating cell behaviour. These robots also search for the targeted cells before releasing the load inside. They are able to release the drugs selectively and were tested with lymphoma and leukemia cell lines.

DNA origami nanostructures have also been used in chemotherapeutics, as vehicles for doxorubicin. Doxorubicin is an anthracycline used in chemotherapy (for breast cancer, leukemia, lymphoma, sarcoma etc.), which inhibits the replication and transcription of functional genes in cancer cells (Carvalho et al., 2009). This drug lacks specificity and as a result can cause a variety of side effects, highlighting the need to use DNA origami structures that are biocompatible to it as delivery vehicles (Zhao et al., 2012). Zhao et al. (2012) also found that the efficacy of doxorubicin is improved when adsorbed to a DNA origami nanostructure. This boosted the drug's ability to induce cell death in doxorubicin-resistant MCF-7 adenocarcinoma cells (Jiang et al., 2012). Jiang et al. (2012) highlighted the importance of the geometry parameter of the DNA origami nanostructure as a vehicle for doxorubicin, because rod-shaped nanostructures were shown to outperform triangular ones. Rod-shaped nanostructures in the 10nm-100nm size range have also been found to be effective in the treatment of hematologic malignancies.

Similar methods can also be employed using another chemotherapeutic drug, daunorubicin, which is commonly used for the treatment of acute lymphoblastic leukaemia as well as acute myeloid leukemia (Thakor and Gambhir, 2013; Lancet et al., 2014; Löwenberg et al., 2009; Kraft et al., 2014). Halley et al. (2016) formed daunorubicin-loaded trojan horse DNA nanostructures. These were rod-like and approximately 92.5 by 13.2 by 11 nm and were successfully taken in by HL-60 cells (Shang et al., 2014). They have also been found to efficiently circumvent the drug resistance mediated by efflux pumps in leukemia cells. This is because P-gp (permeability glycoprotein), which is overexpressed in many drug-resistant tumour cells and can act as an efflux pump, can get downregulated through the use of multifunctional DNA origamis, reducing the effect of multidrug resistance (MDR). This will reduce the need for prescribing patients with higher doses of chemotherapeutic drugs, which despite being able to mitigate the effect of MDR over a short duration, will lead to more severe MDR in the long run.

Besides, DNA origami nanostructures can also be utilised in photodynamic and photothermal therapy (Kong et al., 2016). Jiang et al. (2015) attempted to inhibit the growth of MCF cancer cells using the heat generated from near-infrared radiation (NIR) of a DNA origami gold nanorod (DO-AuNR) complex. They tested this in vivo using MCF-7 xenograft tumour bearing mice, and the results showed that triangular DO-AuNR were taken up by cells most efficiently. This technology was further developed by Du et al. (2016) who were able to develop an optoacoustic imagining agent and use DO-AuNR complexes to enhance the imaging resolution of the mouse tumour tissues. Zhuang et al. (2016) were also able to use DNA origami in photodynamic therapy. They intercalated a photosensitive agent, 3, 6-bis[2-(1-methylpyridinium) ethynyl]-9-pentyl-carbazole diiodide (BMEPC) to a DNA origami nanostructure. This was then able to trigger the apoptosis of the tumour cells through the production of free radicals.

IV. CONCLUSION AND RECOMMENDATIONS

There has been great improvements in DNA nanotechnology and its various medical applications over the years. DNA origami nanostructures have been tested on their cargo function for drug loading and release, and have been shown to be successful drug delivery vehicles, particularly for chemotherapeutic drugs doxorubicin and daunorubicin. These nanostructures specifically target cancer cells, reducing the severity of side effects, and have even been shown to circumvent drug resistance of cancer cells. The stability of DNA as a biomolecule allows these nanostructures to be able to survive for long enough arrive at the target site and complete their functions. They have also been used in cancer treatment through photothermal and photodynamic therapy, which have also been proven to be successful. However, to increase the specificity and efficiency of these nanostructures in therapeutics, the key design parameters should be carefully examined.

As for recommendations, the DNA origami technique indicated a crucial need for accelerating the advanced research field of DNA nanotechnology. Studies on the therapeutic applications of DNA origami are mainly focused on cancer treatment, and its potential use for the treatment of other diseases is often neglected. Besides, the geometry of DNA origami nanostructures has a significant impact on their functionality and efficacy, and this parameter largely depends on the computational methods used to design the origami. Currently, it is still difficult to design DNA origami with complex shapes. Therefore, more sophisticated computational methods for the design of DNA origami should also be developed to allow the design of structures that have more efficient therapeutic mechanisms.

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