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HON 301
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The Application of DNA Nanotechnology for Guided Drug Delivery

Introduction:

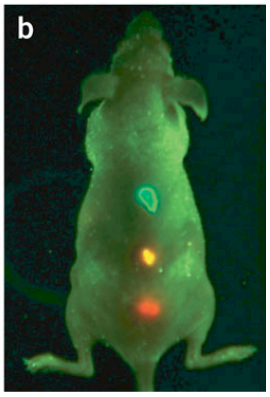
A major problem that exists in drug-based disease treatments is the lack of specificity in drug delivery. This is none the more prevalent than in the current state of cancer treatment. Chemotherapy, one of the most commonly used forms of cancer treatment, consists of using very powerful drugs to stop or slow the growth of cells. While chemotherapy is very effective at killing cancer cells, a major side effect of this form of treatment is that it is indiscriminate in the types of cells it kills. As such, regularly dividing cells found in the blood, mouth, intestines and hair are killed as well, leading to the bald look found on chemotherapy patients. These patients will also experience several side effects that include pain, fatigue, blood disorders and nervous system effects among others [1]. For example, anywhere from 70% to 90% of cancer patients being treated with chemotherapy or radiation therapy exhibit anemia [2]. Consequently, there is a need in medicine for the delivery of drugs to specific locations of the body. Since the cells in our bodies operate on the nanoscale, developments in nanotechnology have opened up new possibilities in the field of medicine. More specifically, the exploitation of the properties of DNA have allowed for the development of drug delivery vectors that can target specific locations of the body.

Definition and History of Nanotechnology

The field of nanotechnology can be described as the application of technology and engineering to interactions on the nanoscale, which encompasses a range between 1-100 nanometers. Utilization of technology at this scale allows for a more direct manipulation of the atoms and molecules that make up our world. This allows for the production of materials that exhibit greater strength, reactivity and versatility [3]. While the term "nanotechnology" and its field of study was not truly defined until the mid-1900s, the use of nanotechnology has existed in the pre-modern era. As early as the 4th century, talented craftsmen were found to have used colloidal gold and silver to create dichroic glass, or glass that changes color based upon conditional lighting. The vivid colors found in stained-glassed windows can also be attributed to the properties of gold nanoparticles [4]. The advent of modern nanotechnology can be traced to Richard Feynman's lecture "There's Plenty of Room at the Bottom" in 1959, in which he proclaims the possibilities of rearranging molecules at the atomic scale. One "wild idea" that he proposes is the possibility to "swallow the surgeon", or create a tiny robot that could enter one's body and complete surgical operations within the body [5]. While Feynman provided the conceptual basis for the field of nanotechnology, research in the field did not truly begin until the 1980s, when Binnig and Rohrer developed the scanning tunneling microscope, which allowed researchers to view the structures of individual atoms [4]. Subsequently, several discoveries were made, including that of carbon nanotubes, fullerenes and quantum dots, leading to the conception of the modern nanotechnology field.

The Lycurgus Cup, an early example of dichroic glass [4]

Applications of Nanotechnology in Medicine



Use of quantum dots for multi-color imaging. Figure adapted from [6]

With the natural progression of research in nanotechnology, potential applications in the field of medicine became apparent. One such application is the use of quantum dots for *in vivo* tumor imaging. Quantum dots are semiconducting nanocrystals that exhibit fluorescent properties when exposed to ultraviolet light. For the purpose of tumor imaging, these nanocrystals are encapsulated by a polymer coating that contains biomolecules which can recognize tumor-specific peptides and antigens. This results in the accumulation of quantum dots at the location of tumor, allowing for the ability to take higher contrast images of the tumor itself. Unique optical properties of quantum dots also allow for multi-colored fluorescent imaging, further highlighting the versatility of the technology [6]. However, while *in vivo* imaging has already been accomplished in mice, the toxicity of the nanocrystals used in current quantum dots have prevented there use in humans. Lipid nanotechnology is also a field that has great implications in medicine. Lipids possess several desirable properties for medical nanotechnology, including that of self assembly and biocompatibility. For example, lipids can form liposomes, or little capsules of lipids, that can contain substances such as drugs or DNA for delivery to the body. The surfaces of these liposomal vectors can be modified to target specific proteins, allowing for specificity in drug delivery, and/or to allow for protection from the human immune system. Liposomal vectors are also desirable because they can readily fuse with the lipid bilayer of a normal cell [7]. While lipid nanotechnology does possess a level of versatility and provides for many applications in medicine, an even more versatile field in nanotechnology for medical use can be found in DNA.

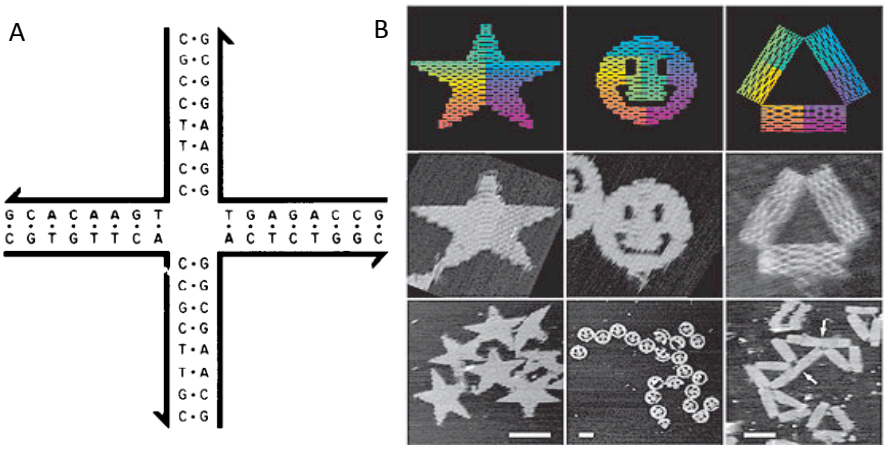
DNA Nanotechnology

Background

DNA is another naturally occurring molecule in the body that has the capability to be used for various medical purposes. Due to the fact that it is intrinsic to the human body, nanotechnology built around DNA, like lipids, possess an inherent biocompatibility with the body. This property is desirable because it lowers the chances of causing immune responses. Similar to lipid nanotechnology, another attractive property of DNA as a nanomaterial is its ability to self-assemble [8]. However, where DNA differs from lipids is the mechanism through which this self-assembly occurs. With regards to lipids, self assembly occurs simply because phospholipids have hydrophobic and hydrophilic ends. As a result, these lipids will organize themselves so that all hydrophilic portions are facing an aqueous solution while all hydrophobic portions are facing each other, generating the liposomal vector mentioned above. The limitation of this self assembly is that it effectively only allows for the formation of hollow containers. While it is true that one can alter lipids in order to give them more versatility, this process soon becomes complicated. On the other hand, DNA self assembly is predicated on base pairing. A molecule of DNA is made out of two strands of nucleotides. These nucleotides are composed of three portions: a sugar (deoxyribose), a phosphate group and a nitrogenous base. Each nucleotide is connected to an adjacent nucleotide through phosphodiester bonds formed between the sugar and the phosphate and connected to the opposing strand by hydrogen bonds formed between the nitrogenous bases. There are four nitrogenous bases: adenine, guanine, cytosine and thymine, with adenine bonding to thymine and guanine bonding to cytosine. As a result, a strand of nucleotides can only bond with another strand of nucleotides that has complementary nitrogenous bases. Exploitation of this property has allowed for the development of the field of DNA nanotechnology.

History

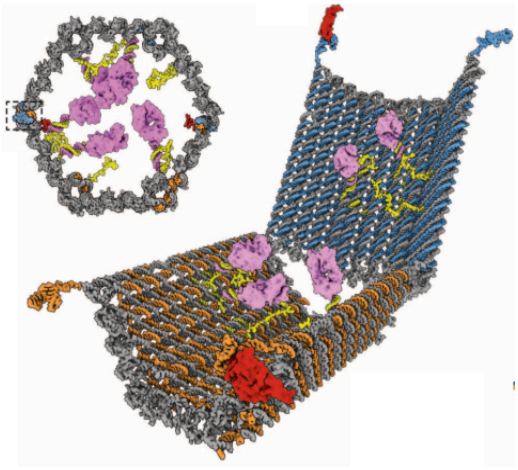
The conception of DNA nanotechnology began in the 1980s with Nadrian Seeman. In his paper "Nucleic Acid Junctions and Lattices", Seeman describes the necessary criteria for creating "immobile junctions" of DNA. Essentially, Seeman points out that if one has four single strands of DNA, and if a specific sequence of DNA on one strand is uniquely complementary to a sequence on another strand, then the DNA will bind in a cross shaped junction as shown by the figure [9]. By creating lattices out of these junctions, one could theoretically build larger nanostructures. Seeman himself sought to create nanostructures in order to assist with the crystallization of protein structures. However, Seeman soon found that his lattice proposal was incapable of supporting larger scale 3D structures. As a result, the field of DNA nanotechnology was largely considered a novelty for the early portion of the 2000s. The next major advance in the field came from Paul Rothemund, who developed a process of creating DNA nanostructures that he coined "DNA origami" in 2006. In this process, Rothemund utilized one long strand of DNA and several small strands of DNA as his base material. Upon the heating and cooling of these molecules, the smaller strands of DNA will bind to predetermined portions of the longer strand of DNA, leading to the creation of distinct structures. Utilizing this process, Rothemund was able to create a variety of structures, including that of a smiley face [10]. Another great implication of Rothemund's discovery was that his procedure was easy to carry out and required easily accessible materials. As long as one could create a algorithm for calculating the correct sizes and location of DNA binding, anyone could, in theory, create a DNA origami nanostructure. Following this discovery, it was soon demonstrated in 2009 that the concept of DNA origami could be utilized for the purpose of creating 3D nanostructures. Much like origami itself, the earliest 3D DNA nanostructure built was a simple box that was composed of six DNA origami sheets folded together [11]. With this event, it became possible to create DNA nanostructures that could hold and deliver drugs.



(A) Ned Seeman's DNA junction and (B) some examples of Paul Rothemund's DNA orgami. Figures adapted from [9,10]

Current State of DNA Nanotechnology in Drug Delivery

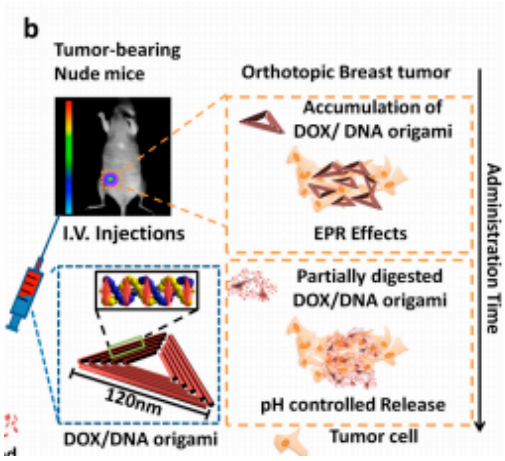
Utilizing a combination of Seeman and Rothemund's approaches, there are currently many DNA nanostructures under research that have the capabilities to deliver drugs. One simple example of such a system is found in the use of oligonucleotide nanoparticles (ONPs) for *in vivo* siRNA delivery in mice. In this research experiment, several strands of DNA were assembled into a tetrahedral structure along with a piece of siRNA (RNA that can mediate gene silencing). What's interesting in this schematic is that the "drug", which is the siRNA, is actually a part of the nanostructure itself. In this experiment, the siRNA only becomes active when specific ligands are available in the environment, which can be programmed into the nanostructure by altering the shape of the DNA sequences[12]. This study demonstrated that DNA was much easier to control than lipids because of the sequence specificity necessary for creating DNA nanostructures.



Logic gated 3D DNA nanostructure. In this figure, the "molecular locks" are the little domains of DNA sticking out of the ends of the hexagonal tube. The figure is adapted from [13]

Another example of the capability to create guided drug delivery vehicles out of DNA nanostructures is found in the 3D DNA origami technology mentioned earlier. Essentially, a hexagonal DNA tube was designed to carry specific molecules within itself, whether they be fluorescent molecules or potential drugs. What makes this vehicle extremely versatile is that it was designed with the presence of two "molecular locks", which are simply DNA structures that recognize and bind to specific molecules. For this experiment, the tube was designed so that the molecule it carried within would only be released when both "molecular locks" were open. The implication of this research is that it creates an "and" logic system that greatly increases the specificity of drug delivery. For example, if a specific tumor releases two antigens as a result of its growth, the DNA nanostructure would only open when both antigens are present. This greatly limits the chance of the drug being delivered to normally growing cells [13].

Outside of spatial considerations for DNA nanostructure based drug delivery, research is also being conducted which addresses the temporal release of drugs. One major problem that currently exists in drug delivery is the high level of toxicity that accompanies extremely potent drugs. In order to combat this, a DNA origami structure was created that had doxorubicin, a powerful cancer drug, intertwined in its structure. Because the doxorubicin was embedded within the nanostructure, the entire dose of doxorubicin was not active at once. This complex was then injected into the bloodstream of a mouse near a tumor growth. Due to the structure of the drug-DNA complex, accumulation of the nanostructure was found in the tumor region of the mouse. In order to time the release of doxorubicin, the DNA nanostructure was designed to degrade at a predetermined pH. As a result, the doxorubicin was only released and accumulated in the tumor-specific region [14]. While these examples of research have greatly increased the optimism of utilizing DNA nanostructures as drug delivery vehicles, the field is still in its infancy so there is a lack of human clinical trials.



Summary of research into time controlled drug release. Figure adapted from [14]

Summary and Future Studies

Given that the use of DNA nanotechnology has only become considered a valuable field of study over the last decade, there is currently a lack of understanding in the totality of how DNA nanostructures affect and are affected by the environment within the human body. For example, our bodies contain natural endo and exonucleases that can degrade DNA and RNA. As such, the stability of DNA nanostructures must be considered when designing them for use in the human body. Other factors that must be considered include the accidental integration of foreign DNA into a person's genome as well as

interactions between these nanostructures and the human immune system. As a result, there are currently very few, if any, clinical studies that utilize DNA nanostructures. However, *in vivo* experiments performed in mice have demonstrated several positive mechanisms that could revolutionize drug delivery, showing the value of continuing the study of creating DNA nanostructures.

Future studies in this field should seek to fully clarify the factors that can influence the stability of DNA nanostructures in order to improve their efficacy as drug delivery vehicles. Outside of drug delivery, Rothemund also mentions several other potential developments in DNA nanotechnology that could revolutionize medicine. For example, recent research done in generating tiles of DNA, or "DNA legoes" have the potential to further simplify the process of creating 3D DNA nanostructures while also increasing their versatility. Rothemund himself is currently conducting research into utilizing DNA tiles to create large-scale DNA nanostructures by programming DNA to self assemble these DNA tiles [10]. Larger scale structures could potentially act as biosensors that could alert the body of health risks before they even develop into diseases. Overall, the field of DNA nanotechnology seems to show a lot of promise in being able to alter the landscape of medicine.

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