International Advance Journal of Engineering, Science and Management (IAJESM)
Multidisciplinary, Indexed, Double Blind, Open Access, Peer-Reviewed, Refereed-International Journal.
SJIF Impact Factor =7.938, January-June 2024, Submitted in June 2024, ISSN -2393-8048

DRUG- DNA DELIVERY

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ABSTRACT

One of the most cutting-edge areas of study is the delivery of drugs to cancer cells during chemotherapy. Current cancer therapy medications are ineffective because they can't differentiate between cancer cells and normal cells; as a result, they destroy both types of cells. To get around this problem and take advantage of the fact that cancer cells differ from normal cells in terms of their physical and chemical properties, drug-delivering nanoparticles (NPs) are engineered in a particular way that allows them to identify cancer cells and target them specifically. There are a number of drug delivery methods available today, each with its own set of benefits and drawbacks. Some of them include poor stability, limited encapsulation capacity, and trouble in controlling the size. Thanks to the advancements in DNA nanotechnology, NPs containing drugs can now be made using DNA strands. These NPs have a perfectly regulated size and structure, are safe to use, and are highly stable. In this paper, we detail our research into DNA nanostructure drug encapsulation, specifically looking at the loading of docetaxel and curcumin, with the goal of developing a novel, highly compatible drug delivery method.

Keywords: nano DNA, drug delivery, origami, DNA-based nanomaterials.

INTRODUCTION

There are a number of issues that are associated with the majority of traditional cancer therapies, including toxicity, a lack of selectivity, and the accumulation of tumors. It has been shown via studies conducted in both the laboratory and in clinical settings that a number of these drugs enable cancer cells to develop resistance to several therapies. Micelles, nanoparticles (NPs), stealth liposomes, polymer-drug conjugates, and other nano-sized drug carriers have been the subject of intensive research in recent years. The investigation has been conducted with the objective of minimizing the adverse effects of anticancer medications while simultaneously enhancing the efficacy of antitumor drugs in the treatment of cancer. According to the first clinical evidence, the processes by which nanoparticles (NPs) increase delivery efficiency and minimize undesirable effects seem to be more accurate tumor localization and more active cellular absorption. Doxorubicin, for instance, has been supplied to patients in a pegylated liposomal formulation for more than twenty years, indicating the extensive employment of the first generation of NP drug delivery systems. A surprisingly little increase in anticancer activity is seen as a consequence of the progressive liberation of doxorubicin from its liposomal sheaths. Additionally, prospective drug delivery vehicles based on metal nanoparticles have been looked for. For instance, the controlled release of chemotherapeutic drugs has been accomplished by the use of gold nanoparticles. Metal nanoparticles have the ability to accumulate in the body and induce toxicity, even after the drug has been provided, despite the fact that they are biocompatible and contain no chemically reactive substances. Consequently, the development of nanocarriers for the delivery of medicine that are not only safe but also biocompatible and effective remains a source of concern.

Self-assembled DNA nanostructures, which have good structural programmability and unambiguous biocompatibility, are one of the most exciting options for drug delivery nanocarriers. They are also one of the most fascinating possibilities. Building complex DNA nanostructures with well-defined shapes and dimensions is a relatively simple process that may be accomplished by using the principles of logical design. To be more specific, DNA origami is capable of producing very high yields of structures that are totally programmable. To fold a lengthy single strand of DNA, known as the scaffold strand, which is often viral genomic DNA, into any shape that the scientist chooses, this process includes the use of hundreds of tiny strands of DNA, such as staples or synthetic oligonucleotides. Not only do staples assist in maintaining the scaffold in its position, but they also perform the role of

addressable units that are capable of being functionalized by a variety of biomolecules and nanoparticles. It is possible that these modifications will make imaging, targeted delivery, and controlled release of therapeutic compounds more straightforward. There is a lot of excitement about DNA origami structures and the potential use of these structures in nanomedicine. Recent studies have shown that DNA macromolecules do not possess the characteristics that are characteristic of effective drug delivery vehicles, namely cytotoxicity and immunogenicity. The fact that DNA origami constructs were relatively stable in cell lysate was another significant discovery. This stability is essential for the controlled release of medications to subcellular destinations, which is why it was discovered. The successful elimination of cancer cells was made possible by Chang et al.'s discovery of doxorubicin aptamer conjugated DNA icosahedra. These researchers reported one of the earliest instances of a drug delivery platform that was based on a DNA nanostructure. scaffolded DNA origami is a more promising drug delivery technology than polyhedral wireframe structures. This is due to the fact that scaffolded DNA origami has a greater number of layers of tightly packed double-helices, which results in a greater number of docking sites for intercalation. Because of the high drug density, there is a possibility that enzymatic degradation and accidental drug release will be reduced.

In this work, we used six different oligonucleotides to construct two different drug-loaded DNA NP complexes in three dimensions. One of these complexes included docetaxel, while the other contained curcumin. The complexes were administered to A549, which is a kind of lung cancer cell seen in humans. Using a lung cancer cell line, the researchers investigated the extent to which drug-DNA nanoparticles (NPs) and the medicine itself posed a threat to the cells.

OBJECTIVE

1. Target medications or DNA to sick cells or tissues, limiting harm to healthy cells.
2. Limit off-target interactions and systemic exposure to reduce unwanted effects and toxicity.

MATERIALS AND METHODS

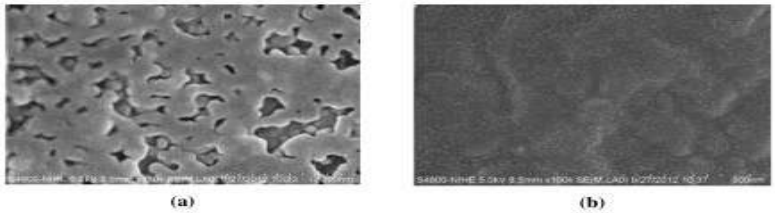
Marcrogen was responsible for the creation and production of each and every oligonucleotide that had the sequence that is listed below:

MicelT1: 5 0 -CTCAGTGGACAGCCGTTCTGGAGCGTTG GACGAAACT-30 ;

MicelT2: 5 0 -CCAGAACGGCTGTGGCTAAACAGTAACC GAAGCACCAA-CGCT-30 ;

Table 1. Loading efficiency of curcumin into DNA NP

Sample	Initial curcumin Concentration (mM)	$OD_{430\ nm}$	Unloading curcumin(mM)	Loading efficiency of curcumin into DNA NP(%)
	0.4	0.143	0.0115	97.13
	0.8	0.289	0.0233	97.08
	1.6	1.421	0.1156	92.56
	3.2	2.945	0.2375	92.75



(a) (b)

Figure 1. SEM images of DNA nanostructure: (a) in 300 nm and (b) in 500 nm.

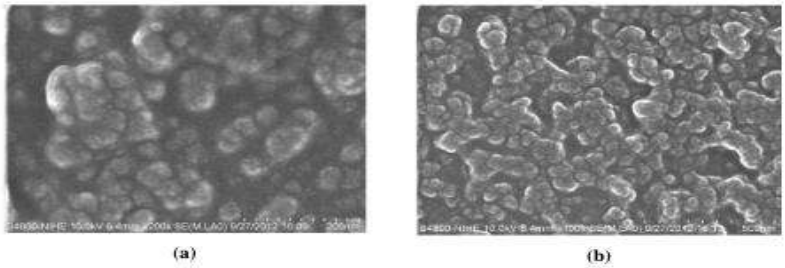


Figure 2. SEM images of DNA–curcumin complexes: (a) in 200 nm and (b) in 500 nm.

MicelT3: 5 0 -AGTTTCGTGGTCATCGTTTGGTGGTGGT GGTT-GTGGTGGTGGTGG-30;
MicelD1: 5 0 -CGATGACCTGCTTCGGTTACTGT-TTAGC CTGCTCTAC-30;
MicelD2: 5 0 -AATGCGTAGAGCACCACTG-AGGCATT-30 ;
MicelD3: 5 0 -TTTGGTGGTGGTGGTTGTGGTGGTGGTG G-30.

Constructing the model of DNA NPs

Every single one of the six oligonucleotides that were discussed previously were diluted in a single eppendoft with the same molar ratio in a 1 × TAE/Mg2 + buffer. This buffer included 40 mM Tris, 20 mM acetic acid, 2 mM ethylen diamin tetraacetic acid (EDTA), and 12.5 mM magnesium acetate. At a pH of 8.0, the solution was measured and adjusted. After being heated to 90 degrees Celsius for a duration of five minutes, the samples were allowed to drop down to ambient temperature before being self-assembled into a nanoparticle form.

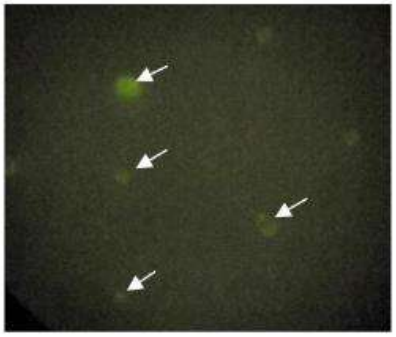


Figure 3. Uptake of DNA–curcumin complexes by lung cancer A549 under fluorescent microscope.

Drug loading

After being incubated for a period of twenty-four hours in a solution that included two millimolar docetaxel and two nanometers of curcumin, the DNA nanoparticle structures were spun at a speed of ten thousand revolutions per minute at room temperature for ten minutes. Following centrifugation, the drug-loaded DNA nanoparticles and the free doxorubicin that was present in the supernatant were separated. In order to determine the amount of free doxorubicin, the absorbance of doxorubicin at 480 nm was measured using a microplate reader (TECAN, Infinite M200, Switzerland). It is possible to determine the loading content of docetaxel (or curcumin) in the DNA NP by using the formula that is shown below:

$$C_f = C_i - C_u,$$

with Cf denoting the final docetaxel/curcumin loading contents in DNA NPs and Ci denoting the initial loading contents, Cu denoting the unloaded docetaxel/curcumin, and the loading efficiency of the drug in DNA NPs being denoted by the symbol Cf.

$$\eta = \frac{C_f}{C_i} \times 100\%.$$

RESULT AND DISCUSSION

DNA NP design

After being heated to 90 degrees Celsius for five minutes, the DNA strands that comprise the structure seen in figure 1 self-assembled. This occurred after the DNA strands were progressively cooled down to room temperature.

Loading efficiency of docetaxel and curcumin in DNA NP

To begin, dissolve 0.1 milligrams per milliliter of curcumin in a solution of 0.5% DMSO. For the subsequent step, the absorbance should be measured using a wavelength range of 300-700 nm. With a wavelength of 430 nm, Figure 2 illustrates the maximal absorption of curcumin.

Due to the fact that the absorption of curcumin was shown to be connected with its concentration, the substance was diluted with concentrations of 0.01, 0.02, 0.04, 0.06, 0.08, and 0.1 mg ml−1 of curcumin. After that, the optical density was determined to be 430 nm. This method was used in order to get the OD values as well as the standard curve that are shown in table 1 and figure 3. As a result, the curcumin used in the experiment had the highest absorbance at 430 nm.

We have discovered that the variables that are linearly connected to one another are the observed absorbance (OD) and the amount of curcumin in solution (mg ml−1). This is shown by the equation Y = 33.7X + 0.151. In order to determine the effect that curcumin packing has on complicated nano-DNA, the typical curves are used. After incubating the mixture with DNA nanoparticles, the curcumin-DNA complexes were separated from the unloaded curcumin by the use of centrifugation. Quantifying the amount of unloaded curcumin in the super layer is accomplished with the help of the standard curve, which is subsequently used in the process of calculating the packing efficiency of curcumin on DNA nanoparticles. Results of the test to determine how successfully the DNA NP encapsulated curcumin are shown in table 2, which may be accessed by clicking here.

This method produced loading efficiency values for curcumin in DNA NPs that ranged from 92% to 97%, according to our findings. In addition, the findings showed that there was practically very little leftover curcumin after packing, even when the content of curcumin was considered modest. In contrast, the most beneficial dose of curcumin encapsulation was seen at a concentration of 0.8 millimolar (294.704 milligrams per milliliter).

Docetaxel, which has a maximum absorption wavelength of 234 nm, demonstrated a loading efficacy of 87% in DNA nanoparticles, which is identical to the previous example.

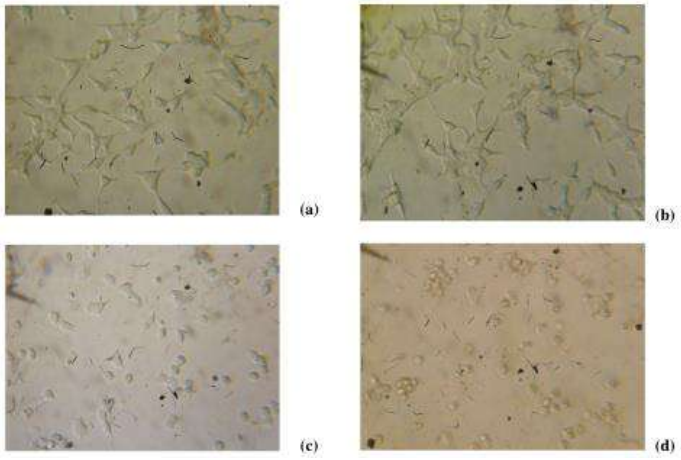


Figure 4. Cytotoxicity test of DNA NP on lung cancer cell line A549. (a) 0.5% DMSO solution addition in culture medium (for control) after 48 h; (b) DNA NP in 0.5% DMSO solution were added to culture medium; (c) DNA–docetaxel complexes in 0.5% DMSO solution were added to culture medium after 24 h; and (d) DNA–docetaxel complexes in 0.5% DMSO solution were added to culture medium after 48 h.

CONCLUSION

An in-depth description was provided of a method for the creation of DNA-docetaxel/curcumin oligonucleotide, which has characteristics that are unique to cancer cells

and the capacity to load medications. On the other hand, the encapsulation efficiency of curcumin was 97%, whereas the encapsulation efficiency of docetaxel in nanocomplexes was only 87%. Because of the gradual drug release mechanism that it has, it is feasible that docetaxel in the DNA-docetaxel complex might be able to kill cancer cells.

REFERENCES

1. Kopecek J, Kopeckova P, Minko T and Lu Z 2000 Eur. J. Pharm. Biopharm. 50 61
2. Konishi M, Tabata Y, Kariya M, Suzuki A, Mandai M, Nanbu K, Takakura K and Fujii S 2003 J. Control. Release 92 301
3. Gabizon A A 2001 Clin. Cancer Res. 7 223
4. Wu Y, Sefah K, Liu H, Wang R and Tan W 2010 Proc. Natl Acad. Sci. USA 107 5
5. Tong L, Wei Q, Wei A and Cheng X J 2009 Photochem. Photobiol. 85 21
6. Lee E S, Na K and Bae Y H 2005 J. Control. Release 103 405
7. Nishiyama N, Okazaki S, Cabral H, Miyamoto M, Kato Y, Sugiyama Y, Nishio K, Matsumura Y and Kataoka K 2003 Cancer Res. 63 8977
8. Chetverina H V and Chetverin A B 2008 Biochemistry 73 1361
9. Wang A Z, Langer R and Farokhzad O C 2012 Annu. Rev. Med. 63 185
10. Jiang Q, Song C,, Nangreave J, Liu X, Lin L, Qiu D, Wang Z G, Zou G, Liang X, Yan H and Ding B 2012 J. Am. Chem. Soc. 134 13396 [11] Petros R A and DeSimone J M 2010 Nature Rev. Drug Discov. 9 615
11. Pinheiro A V, Han D, Shih W M and Yan H 2011 Nature Nanotechnol. 6 763
12. Chang M, Yang C S and Huang D M 2011 ACS Nano 5 6156 [14] Manaspon N, Viravaidya-Pasuwat K and Pimpha N 2012 J. Nanomater. 2012 593878
13. Caddeo C, Nacher A, Vassallo A, Armentano MF, Pons R, Fernàndez-Busquets X, Carbone C, Valenti D, Fadda AM, Manconi M (2016) Efect of quercetin and resveratrol co-incorporated in liposomes against infammatory/oxidative response associated with skin cancer. Int J Pharm 513(1–2):153–163
14. Calixto G, Duque C, Aida K, Rodrigues dos Santos V, Massunari L, Chorilli M (2017) Development and characterization of p1025- loaded bioadhesive liquid-crystalline system for the prevention of Streptococcus mutans bioflms. Int J Nanomed 13:31–41
15. Calixto GMF, Bernegossi J, De Freitas LM, Fontana CR, Chorilli M (2016) Nanotechnology-based drug delivery systems for photodynamic therapy of cancer: a review. Molecules 21:342
16. Wang, J.; Li, Y.; Nie, G. Multifunctional biomolecule nanostructures for cancer therapy. Nat. Rev. Mater. 2021, 6, 766–783.
17. Zhang, R.R.; Schroeder, A.B.; Grudzinski, J.J.; Rosenthal, E.L.; Warram, J.M.; Pinchuk, A.N.; Eliceiri, K.; Kuo, J.; Weichert, J.P. Beyond the margins: Real-time detection of cancer using targeted fluorophores. Nat. Rev. Clin. Oncol. 2017, 14, 347–364.
18. Thakor, A.S.; Gambhir, S.S. Nanooncology: The future of cancer diagnosis and therapy. CA-Cancer J. Clin. 2013, 63, 395–418.
19. Torchilin, V. Tumor delivery of macromolecular drugs based on the EPR effect. Adv. Drug Deliv. Rev. 2011, 63, 131–135.
20. Fang, J.; Nakamura, H.; Maeda, H. The EPR effect: Unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect. Adv. Drug Deliv. Rev. 2011, 63, 136–151.