RESEARCH ARTICLE

# DNA Nanotubes Coupled with Magnetic Nanoparticles as a Platform for Colorimetric Biosensors

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**ABSTRACT**

This study describes the fabrication techniques for two forms of magnetic DNA nanotubes (MDNTs) and their applications as platforms for developing colorimetric assays. The first form of MDNTs was DNTs filled-up with magnetic nanoparticles (MNPs) and the second one was DNTs arayed with MNPs on their extrior surfaces. Then the both forms of MDNTs were employed as platforms for attaching a specific insulin aptamer. The sensitivity and accuracy of the insulin measurement using both platforms were studied and compared with enzyme-linked immunosorbent assay (ELISA) as the standard method for the measurement of insulin in cliniccal laboratories. Applying the magnetic field to MDNTs led to enhance the ability of insulin capturing by the aptamer array in serum and subsequently by removing unspecified contents led to precised detection. For specific detection of insulin and its measurement in this study, a G-rich DNA aptamer with HRP-mimicking activity was used that simulated the peroxidase performance when the insulin was trapped with the aptamer. The presence of MDNTs made a high flexibility, a greater convergence of the connectivity to superficial aptamers, and hence the availability of these aptamers to increase the target molecules and subsequently increased the sensitivity of the measurements. This colorimetric method can be used as a novel biosensores for detection of any target molecules.

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## INTRODUCTION

Nanomaterials that can be applied in a variety of fields such as the field of medical treatment, medical diagnosis and imaging, is one of the greatest needs of medical sciences today due to the considerable characteristics of nanoscale materials. The remarkable development of nanotechnology has provided many opportunities for designing and manipulating nanomaterials with multiple new functions [1,2].

Nanoparticles have found significant importance in improving the performance and efficiency of nanotechnology in modern biomedical sciences and other biotechnology areas. Compared to their massive scale, nanoparticles in both organic and inorganic categories exhibit significant characteristics, including: 1) a very small size below 100 nm, 2) high aspect ratio and 3) Improvement of the physical and chemical properties in nanoscale [3, 4].

All of nanoparticles can be functionalized by organic and inorganic components through various synthesis methods, with the aim of acquiring to new nanomaterials suitable for biomedical applications. For example, the effective materials of diagnostic and medical imaging can be attached to the surface of inorganic nanoparticles to create nanoparticles with new characteristics. Also, these nanomaterials can be functionalized with target functional group such as cell receptors, antibodies, peptides, aptamer, and nucleic acids to create specific nanostructures for detection of pharmaceutical and chemical compounds for various purposes. This category of

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new materials is called hybrid nanomaterials [5, 6.]

In fact, hybrid nanomaterials consist of two components of inorganic/organic nanoparticles and organic functional groups that not only maintain both good and proper properties but also provide new properties for therapeutic, diagnostic and imaging applications. The goal is to acquire new nanomaterials with multiple function that must certainly take precedence over the characteristics of each component. Obviously, the hybrid nanomaterial resulting from the attachment of molecular probes as the detector agent to nanoparticles is very desirable in the design of novel nanobiosensors [7].

Meanwhile, the use of probe-magnetic nanoparticles in designing nanobiosensor has become increasingly important because of these nanoparticles accumulate with the use of external magnetic field and allow the elimination of unwanted agents, lead to exact measurement of target. Subsequently, the need for multi-step purification and preparation of biological sample was eliminated and the process for identifying the target molecule takes place in the shortest possible time [8-10].

In the meantime, a suitable platform with a high aspect ratio to carry functional subunits will have a significant role in increasing the final sensitivity of the nano-biosensores. Although the use of nanoparticles has always been a good option for increasing the transport capacity of detectable molecules, such as aptamer, due to the high surface-to-volume ratio, but with recent advances in the field of nanotechnology, there are good alternatives to carry the functional subunits of aptamer or even enzymes, all of which aims to increase the final sensitivity of the biomedical sensor [11-14].

Previous studies on the use of tubular nanostructures to carry enzyme markers such as peroxidase enzymes have led to the development of high-sensitivity ELISA-based diagnostic kits. Because in these kits, by attaching of enzyme molecules at the tubular nanostructure surface at the end of the antibody, signal amplification was occurring which itself causes the LOD decreases [15-16].

With the advent of DNA nanotechnology, new strategies for designing sensors based on aptamer have been developed to increase the accuracy and sensitivity of target molecules based on increasing the loading factor of the probe such as aptamer [17-20]. Among the wide range of DNA nanostructures, DNA nanotubes (DNTs) have attracted the attention of many enthusiasts in the field of making DNA nanostructures due to the surface-to-volume ratio, and the ability to carry materials on the surface and to enclose materials in the central channel. Also, the versatility and flexibility of this nanotube can provide a suitable platform for placement and orientation of the various types of hybrid nanomaterials that were previously discussed [21-23].

Given the recent applications of DNTs as carriers for the transport of various molecules and metal nanoparticles, the use of these nanostructure can provide a suitable platform for homogeneous loading of a significant number of aptamer nanostructures [24-29]. In addition, the presence of a central channel in this nanostructure provides the ability to encapsulation of magnetic metal nanoparticles, which is the basis for the high cluster of the target molecule [30]. Therefore, the design and construction of magnetic DNA nanotubes (MDNTs) can be a new step in the field of intelligent nanostructures to make nanoscale-based diagnostic kits for detecting high-sensitivity target molecules [29-31].

The fabrication of aptamer array on MDNTs can provide the basis for designing and developing new hybrid nanomaterial for identifying the desired biomarkers, such as insulin as a target molecule in this study (Fig. 1)

The purpose of this study was to acquire the technical knowledge of designing the new colorimetric sensing platform using magnetic DNA nanotubes as hybrid nanomaterials with the array of specific oligo-aptamers to identify insulin. In addition to the identification function, the selected oligo-aptamer also has the ability to mimic peroxidase activity, which is due to the presence of a rich region of guanine that can form the G-quadruplex structure in the presence of the target molecule. This means that a nucleotide sequence can simultaneously show the role of aptamer for insulin detection and then the role of DNAzyme as a result of insulin binding.

## MATERIALS AND METHODS

*Chemicals*

BIO-RP-purified DNA oligonucleotides (such as biotin-5′ TGGTGGGGGGGGTTGGTAGGGTGT-CTTC3′, as a biotin-labelled G-quadruplex insulin aptamer[29], and 24 DNA oligonucleotides as staples in DNA nanotube) were synthesized by Bioneer, Korea. In addition, to rule out non-specific insulin binding, a control 30 mer DNA oligonucleotide was synthesized by Bioneer with a random sequence. M13mp18 phage genome and T4 DNA ligase were purchased from New England Biolabs

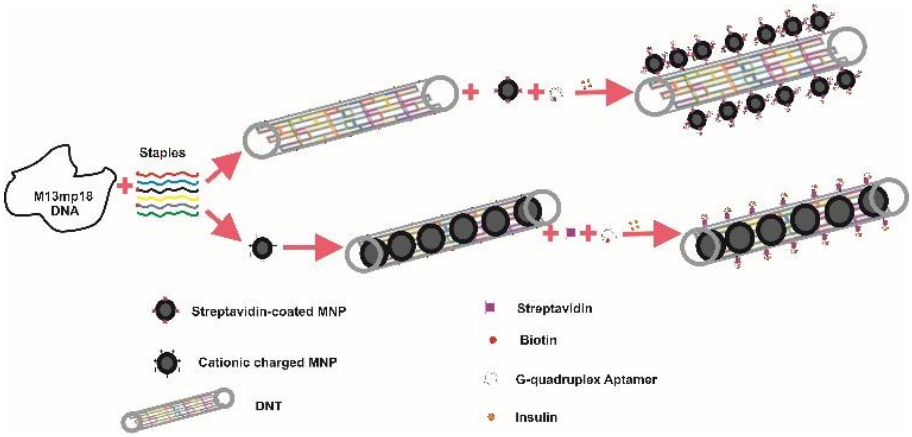


Fig. 1. Graphical abstract of MDNTs fabrication with DNA origami as a colorimetric sensing platform

(Massachusetts, USA). Quantum Prep Freeze 'N Squeeze DNA gel-extraction spin columns were obtained from Bio-Rad. Streptavidin (fluidMAG-Streptavidin) and cationic charged magnetic nanoparticles (fluidMAG-UC/C) with 100 , 50 nm in diameter size respectively and a Magneto PURE-Micro separator were purchased from Chemicell (Germany). 3, 3′, 5, 5′ tetramethylbenzidine (TMB), hemin (Bioextra, from porcine), insulin and stop reagent for the TMB substrate were provided from Sigma, USA. Hemin stock solution (5 mM) was prepared in dimethyl sulfoxide (DMSO) (purchased from Bio Idea Company, Tehran, Iran), stored in the dark at – 20°C and diluted to the required concentration with buffer solution (20 mM Tris-HCl, 40 mM KCl, 200 mM NaCl, 0.06%(v/v) Triton X-100, pH 7.4). The desired concentration of insulin was dissolved in binding buffer (20 mM Tris-HCl, 140 mM NaCl, 5 mM KCl, 1 mM CaCl2 and 1 mM MgCl2, pH 7.4). The insulin ELISA kit was purchased from Demeditec, Germany.

*Instruments*

The thermal condition for self-assembly of the DNA nanotube in origami reaction was set using the Rotor Gene Q machine (QIAGEN, Germany). All the assays were performed using a 96-well immunoplate from SPL Life Science (Gyeonggi-do, Korea). For any level of heating interference, a Master Cycler Personal (Eppendorf, Germany) was used. For shaking of the ELISA plates a rotator 430 from the Pole Idea Pars Company (Tehran, Iran) was used. Ultraviolet (UV)–visible absorption spectra were measured using a spectrophotometer (JenWay 6505, UK). Topographic characteristics of the nanotubes were studied by atomic force microscope (JPK-AFM, Germany). Transmission electron microscope (TEM) was used to assess the size and morphology of magnetic DNA nanotubes with TEM EM900 (Zeiss, Germany).

*Simulation and modelling of DNA nanotubes via caDNAno*

The caDNAno software was used for designing desired DNA nanotubes in this study https://cadnano.org[30,31] based on DNA Origami, the method of construction is as mentioned in previous authors' articles that is briefly stated here. DNA sequence of M13mp18 phage genome was applied in the software as the scaffolded strand to achievement of oligonucleotides sequences of DNA staple as a helper for self-assembly of scaffolded strand into desired DNA nanotubes. The staple strands sequence was determined based on their complementary with the special site of scaffold that bind to form desired structure. The honeycomb style was introduced in the caDNAno for properly folding of DNA nanotubes within a DNA origami reaction [21, 34]. To achievement of the biotin-functionalized DNA nanotubes, after the design of a nanotube and the placement of each of the staple strands in the best position, staple strands that one of the two end were placed at the surface of the nanotube after the folding of this, were synthesized by biotin modification at the 5` end. Assuming that after self-assembly of these nanotubes, the biotin groups appear at the surface of DNA nanotube [29].

*Fabrication of DNA nanotube*

A biotinylated DNT was fabricated via an origami reaction that is stated in previous authors articles, prepared by combining 20 nM DNA

Table 1. Staple oligonucleotide sequences for self-assembly of DNTs

| No. | Sequence (5′ to 3′) |
|---|---|
| 1 | Bio-CCAACGTGCAGGTCATTCGTA |
| 2 | Bio- CACTATTCCGGTTCATGGTCG |
| 3 | Bio- TTCCAGTTCCCTTAAGCAGGC |
| 4 | Bio- GAGATAGGGTTGACGCGCGGGGAGAGGCGGT |
| 5 | Bio- ACGGCCAGTGCCTGTTTCCTG |
| 6 | CATGCCTCAAAGGGGCGCTCA |
| 7 | GAGGATCAAAGAACGTCGGGA |
| 8 | GGCAAAATTGGAACGCTGCAT |
| 9 | ATCATGGGCTCACAAATGAGTGAGCTAACTCAC |
| 10 | GGTACCGACGAGCCAGTGTAA |
| 11 | GAAAATCTTGCCCTCACCAGT |
| 12 | Bio- CATGCCTCAAAGGGGCGCTCA |
| 13 | Bio- TGTGAAATTGTTATCCTCATAGCAAGCTTG |
| 14 | ACAACATAGCTCGAGACTCTA |
| 15 | CAGCTGACTGTTTGCGAAATC |
| 16 | CTGGCCCTTGCCCCTAAATCAAAAGAATAGCCC |
| 17 | AGCCTGGCTTTCCAGTGGACT |
| 18 | GAGACGGCGTGCCAAAGAGTC |
| 19 | GTGGTTTTCGGCCAAGTGTTG |
| 20 | Bio- TTGCGTATTGGGGTTGCAGCA |
| 21 | Bio- ATTAATTGCGTTCGAAAAACCGTCTATCACG |
| 22 | Bio- CTGCCCGGGTGCCTATTCCAC |
| 23 | Bio- AACCTGTGCCATAAGGAAGAA |
| 24 | Bio- TAATGAATTCTTTTTCACCGC |

scaffold M13mp18 single-stranded DNA and 100 nM of each staple oligonucleotide (Table 1) that were diluted in 1× Tris base, acetic acid and EDTA buffer (40 mM Tris–acetic acid buffer (pH 8.0) and 12.5 mM magnesium acetate). The mixtures were kept at 95°C for 5 min and then annealed from 95°C to 20°C at a constant rate of –1°C $min^{-1}$ in the thermocycler. For the fabrication of sturdier DNTs, the origami products were treated by a ligation process with T4 DNA ligase. The ligation–reaction mix was prepared containing 2 µl of 10× T4 DNA ligase reaction buffer, 10 µl of self-assembled DNTs, 2 µl of 50% polyethylene glycol and 1 µl of T4 DNA ligase enzyme. Finally, the ligation mix was incubated at 37°C for 1 h [21,34].

*Fabrication of DNA nanotube with carrying of MNP on the surface*

Similar to what was stated in the previous article by the authors, The biotinylated DNTs were conjugated with $2.5 \times 10^{-3}$ mg $ml^{-1}$ streptavidin-coated MNPs and then incubated at 37°C for 5 min. Then the nanotubes were separated from the reaction materials via electrophoresis in 1% agarose gel. The attended bond on the gel for MNP-coupled DNTs was extracted by the use of quantum prep freeze N squeeze DNA gel extraction spin columns according to the manual instruction [29].

*Fabrication of DNA nanotube with encapsulated MNP inside the channel*

For the firs time, fabrication of DNA nanotubes including magnetic nanoparticles inside the channel, was done. Cationic-charged MNPs were used with the hypothesis that these MNPs have tendency to physical adsorption to DNA strands and was stabilized by cross-linking with the internal negative charge of DNA nanotube resulting from anionic phosphate group. By considering it, fabrication of DNA nanotube by top-down method was accompanied and joined by including process of cationic charged MNP (C-MNP). For this purpose, $2.5 \times 10^{-3}$ mg/ml C-MNP was combined with the origami reaction mix of DNA nanotube. The mixture was kept at 95°C for 5 min and then annealed from 95°C to 20°C with a constant rate of –1 °C/min in the

thermocycler. For the fabrication of long and sturdier DNA nanotubes, the origami products were treated by ligation process with T4 DNA ligase treatment. The fabricated magnetic DNA nanotubes were separated from simple DNA nanotubes and others by Magneto PURE-Micro separator.

*Electrophoresis behaviour of three types DNA nanotubes*

For assessing the electrophoretic behaviours of these nanotubes (simple, carrying MNP at the surface and filled-up with MNP) 5 µL of the nanotubes were loaded in %1 agarose gel and the assay was run within an electrophoresis experiment at 0.5X TBE buffer pH 8.2. The experiment was performed using an electrophoresis mini set. Then the electrophoresis behaviour of three types of DNA nanotubes were compared to each other's. The attended bond on the gel for DNTs was extracted by the use of quantum prep freeze N squeeze DNA gel extraction spin columns according to the manual instruction. Then, all of the extracted DNTs were characterized by microscopy study [21.29.34].

*Microscopy Characterization of three types of DNA nanotubes with Atomic Force*

Topographic characteristics of the nanotubes were studied by atomic force microscope. For this purpose, 5 µL of the nanotubes was immobilized on a mica surface for 4 hours at 25°C. The AFM was performed in contact mode with JPK-AFM (with 150 Hz IGain, 0.0048 PGain, and 1.0 V set point). The cantilever was ACTA-10 probe model. The rough data were analysed using the JPK Nano analyser software.

*Characterization of three types of DNA nanotubes with Transmission Electron Microscopy*

Transmission electron microscope (TEM) was used to assess the size and morphology of magnetic DNA nanotubes and also the simple DNA nanotubes. For this purpose, the DNA nanotubes were immobilized by syringe spraying on Agar Scientific (Stansted, Essex CM24 8GF, United Kingdom) from carbon layer with 300-mesh $Cu_{(50)}$. The micrographs were obtained by TEM EM900 [29].

*Fabrication of insulin G-quadruplex aptamer arrays on magnetic DNA nanotubes*

Two µM biotin-labelled G-rich aptamers were added to DNTs with streptavidin coated MNP at the surface and incubated at 37°C for 10 min. The mixture was placed in a Magneto PURE-Micro separator and washed twice with 5 mM Tris buffer to remove additional aptamer molecules [29].

Also, for fabrication of aptamers array on the surface of simple DNTs and MNP-encapsulated DNT, 5 µM of streptavidin were added to mix and incubated in 37 °C at 10 minutes. Then, 2 µM biotin-labelled G-rich aptamers were added to DNTs with streptavidin at the surface and incubated at 37°C for 10 min. The mixture was placed in a Magneto PURE-Micro separator and washed twice with 5 mM Tris buffer to remove additional aptamer molecules.

*Colorimetric and quantitative measurement of insulin by G-rich aptamer arrays on magnetic DNA nanotubes*

100 nM insulin in binding buffer was added and the solution was kept for 40 min at room temperature to allow formation of appropriate folding of the aptamer–insulin complexes. Up to 150 µl of 20 µM hemin solution in DMSO was added to the mixture, which was then kept at room temperature for 1 h to form the insulin–aptamer–hemin complexes. A 75 µl aliquot of TMB–$H_2O_2$ was added and incubated for 30 min at room temperature. Then, 75 µl of stop reagent ($H_2SO_4$) was added to the mixture and the colorimetric changes were recorded.

*Comparison of enzymatic activity of selective G-Quadruplex aptamer against insulin in conjugated and non-conjugated mode*

In order to measurement and compare the selective enzyme activity of oligo-aptamer in a non-conjugated and conjugated to MNP, conjugate to magnetic DNA nanotubes with MNP at the surface and conjugated to the magnetic DNA nanotubes with encapsulated MNP, after providing a reaction mix, containing one of the desired conjugated nanostructures, hemin and insulin (as the target molecule), was incubated at room temperature for 45 minutes, the colouring agent called TMB was added to the reaction mix and immediately placed in a spectrophotometer and absorbance changes of all relevant mixtures at 650 wavelengths in 5 minutes was studied and analysed as kinetic of the oligo-aptamer enzymatic activity. Also, the mixture with no G-rich aptamer oligonucleotide was measured as the background using UV–Vis spectroscopy. At the end of assays, 75 µl of stop reagent ($H_2SO_4$) was used to stop the enzymatic reaction and the absorbance was recorded at 450 nm.

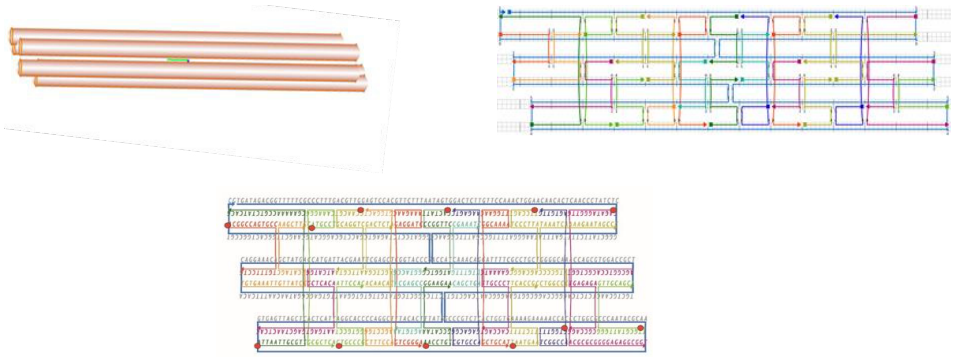

Fig. 2. Graphical view of designed DNTs with caDNAno software and the position of bioten groups

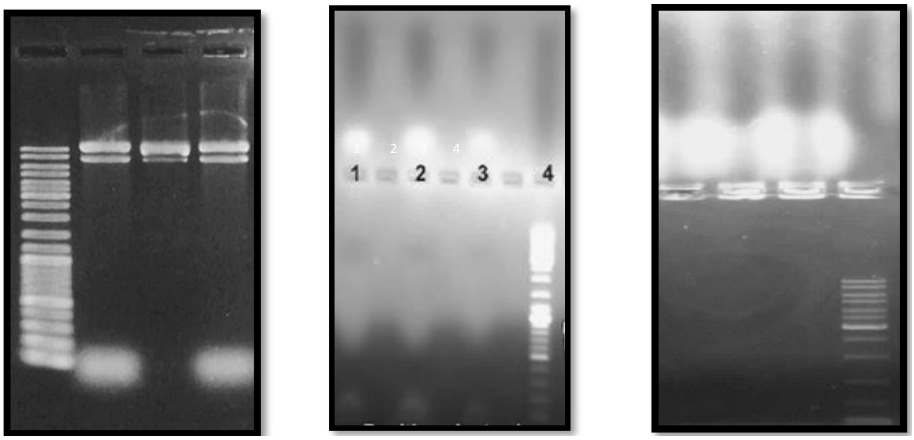


Fig. 3. Electrophoresis behaviours of DNTs on agarose gels; Left, simple DNTs; Middle, DNTs carrying MNPs on the surface and right, MNPs-encapsulated DNTs; lines 1-3, DNTs; Lines 4, DNA ladders.

## RESULT

*Staple oligonucleotide sequences as caDNAno output for self-assembly of DNA nanotube*

The computational design of the nanotube from caDNAno software was indicated in Fig. 2.

*Gel- Electrophoresis behaviour of three types DNA nanotubes*

The electrophoretic behaviour of magnetic DNA nanotubes contained C-MNPs inside at the channel or carrying at the surface were reversed in comparison to that behaviour by natural DNA (e.g. DNA ladder) and simple DNA nanotube (Fig. 3).

*Microscopy Characterization of three types of DNA nanotubes with Atomic Force microscopy (AFM)*

AFM results also demonstrated the fabrication of three typed of DNTs (Figs. 4,5.6). Alignment of MNP demonstrated the fabrication of MNP-carrying DNTs at the surface; Pea-Pod liked structure of MNP-encapsulated DNTs also demonstrate; however, the free C-MNPs among DNA nanotubes were also seen that those were not encapsulated within the nanotubes. Constructed-simultaneously encapsulation of magnetic nanoparticles into the large channel of this tubes leads to "pea-pod" particle alignment in nanotube channel which is visible in AFM topography view.

*Characterization of three types of DNA nanotubes with Transmission Electron Microscopy (TEM)*

TEM micrograph of DNA nanotubes without and included of magnetic nanoparticles were shown in Fig. 7. The nanotubes were in filamentous shapes. The TEM micrograph was demonstrated encapsulation of C-MNPs inside the DNA nanotubes, obviously. The length of these nanotubes were estimated ~1000 nm with ~100 nm diameter.

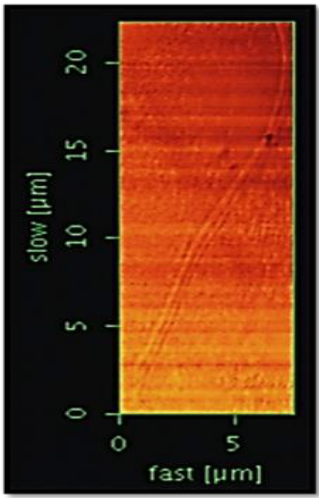


Fig. 4. AFM micrographs of DNT. The length and diameter of DNTs show confirms the fabrication of DNATs.

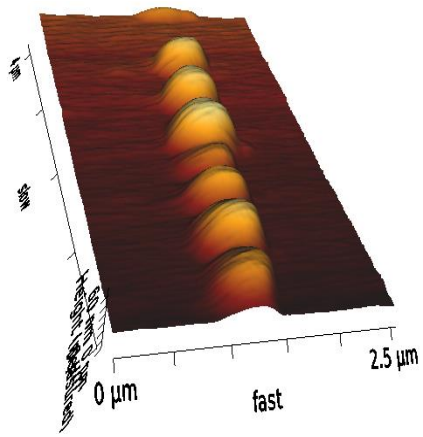


Fig. 5. AFM micrographs of magnetic DNTs whth extrier alignments of MNP in the surface of DNTs. The alignment of nanoparticles in a line indicates the occurrence of streptavidin-biotin binding between the streptoavidin coated nanoparticles and biotinylated oligo-aptamers.

*Colorimetric and Quantitative measurement of insulin by conjugated nanostructure*

The colorimetric observations performed at this stage indicate that with improvement of detection platform for insulin, measurement of a certain concentration of insulin in the reaction mix has been done with greater accuracy. the intensity of the blue colour produced by the addition of TMB and the yellow colour produced after the addition of the stop reagent change also improved and this feature improves the sensitivity of the test that is very valuable(Fig. 8).

The sensitivity and minimum measurable

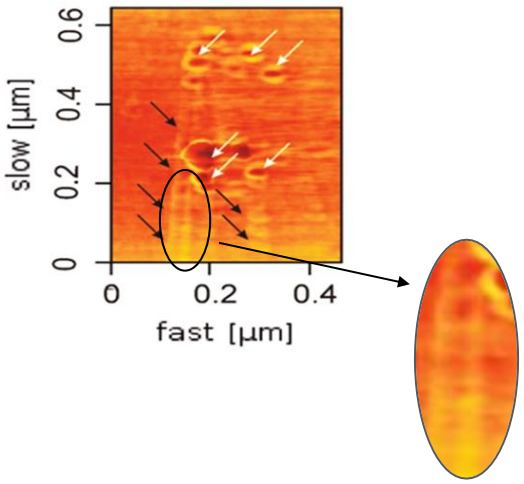


Fig. 6. AFM of DNA nanotubes filled up with an array of C-MNPs (black arrows). Also, a few magnetic nanoparticles outside of DNA nanotubes were indicated (white arrows). In AFM image, it's obvious the periodic array or pea-pod alignment of magnetic nanoparticles in DNTs central channel.

amount of insulin as a target molecule is highly dependent on the substrate used to identify the target. Some insulin that is not detectable by the free aptamer is capable of detecting and measuring high sensitivity by enhancing the diagnostic platform, which can affect the rate.

According to the result, the intensity of the yellow color changes observed in the wells from left to right shows the influence of the detection platform on the sensitivity and LoD.

*Comparison of enzymatic activity of selective G-Quadruplex aptamer against insulin in conjugated and non-conjugated mode*

The results of this study, using statistical regression or correlation statistics, clearly increase the efficacy of aptamer and the colorimetric response of aptamer in detecting and binding to insulin at the position of conjugated oligo-aptamer to DNA nanotubes containing magnetic nanoparticles inside (Figs. 9,10).

Steady-state kinetics of G-quadruplex insulin aptamer with peroxidase function in separate form and in the array on MNP-coupled DNTs were measured at the absorbance changes at 650 nm in a period of time. The results demonstrated more velocity of the aptamer array (0.0033 OD $s^{-1}$) on DNTs than the results from free aptamers separately. It seems the DNT could give a flexible area for positioning aptamers on it to perform capture

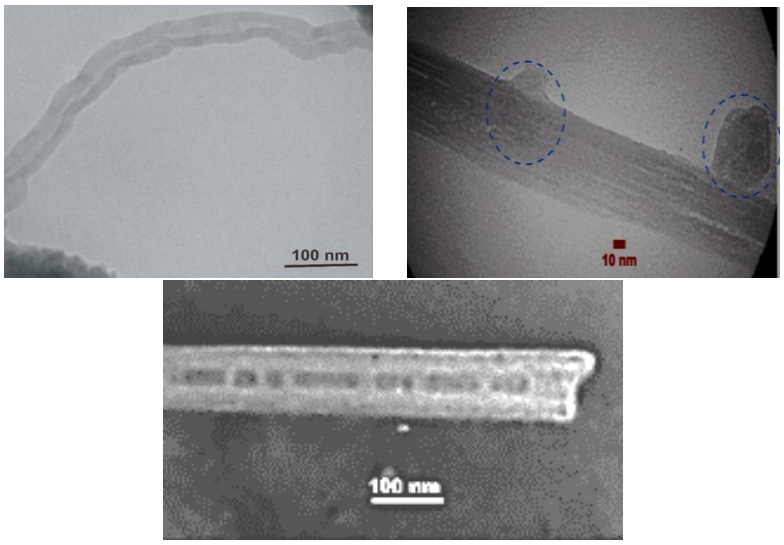


Fig. 7. TEM microscopy results of three types of DNTs; Top (left), simple DNTs; Top (right) [29], DNTs carrying MNPs on the surface [29]; Bottom, MNPs-encapsulated DNTs.

Table 2: absorbance measurement of colorimetric reaction in 450 nm by spectrophotometer.

| Assay Platform | Free Aptamers | Aptamer conjugated MNP | DNTS with extrior MNP | DNTs with interior MNP |
|---|---|---|---|---|
| Absorbance in 450 nm | 0.2 | 0.3 | 0.9 | 1.1 |


Fig. 8. Comparison of colorimetric assessment of insulin detection in conjugated and non-conjugated mode. 1) aptamer in non-conjugated mode , 2) Aptamer conjugated MNP, 3) Aptamer conjugated DNTs with extrior alignment of MNP on the surface, 4) Aptamer conjugated DNTs with interior alignment of MNP in central channel of DNTs. In all wells, all the reactive factors such as insulin concentration and the recognition element are considered identical.

selectively and make peroxidase function rapidly when compared with free aptamers (Fig. 10).

## DISCUSSION

The choice of a suitable nanocarriers with a high aspect ratio to carry functional subunits will have an effective role in increasing the final sensitivity of the nanobiosensors. Previous studies on the use of tubular nanostructures for the transport of enzyme markers such as peroxidase enzymes have led to the acquisition of high-sensitivity ELISA-based diagnostic kits, because in these kits, by attaching the carbon nanotubes at the end of the antibody provide the high loading capacity for the placement of a large number of enzyme molecules, which lead to the detection of minimal concentration of target. With the advent of modern science called DNA nanotechnology, new strategies for designing aptamers-based sensors have been developed to increase the specificity and sensitivity of target molecules based on increasing the load factor of the probes, such as aptamer.

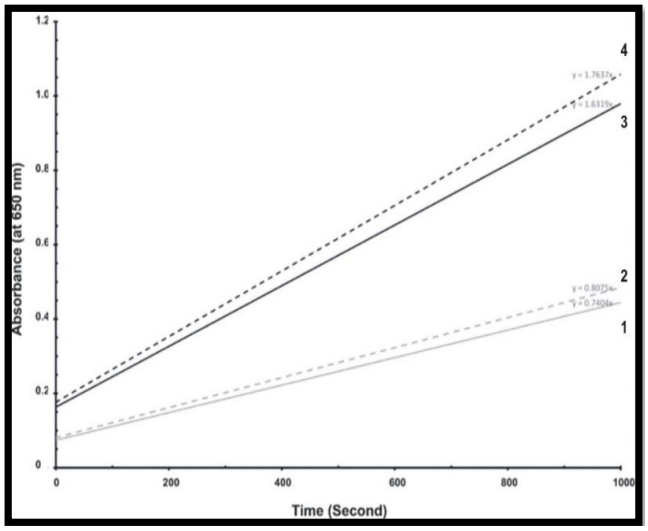


Fig. 9. Steady-state kinetic assay of G-quadruplex insulin aptamers. 1) non-conjugated aptamers. 2) conjugated aptamers on the surface of MNP, 3) DNTs with extrior MNP and 4) DNTs with interior MNP.

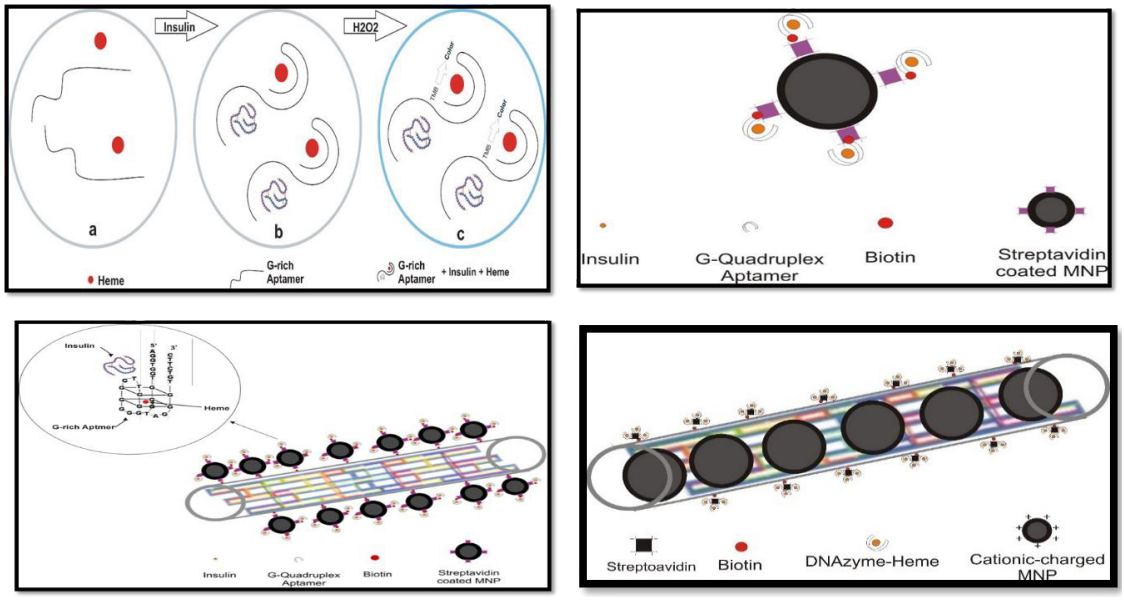


Fig. 10. Graphical abstract of conjugated and non-conjugated Insulin aptamer.

With the arrival of nanotubes in the nanotechnology world, the applications of this field have greatly improved, due to their structural properties, such as the surface-to-volume ratio, the ability to change in diameter, and the ability to alignment of certain materials, such as metal nanoparticles at the surface or in the central channel. Tubular nanostructures have been made up of a variety of finite materials, including carbon nanotubes, peptide nanotubes, inorganic nanotubes and DNA nanotubes. Each of these nanotubes can have diverse applications depending on gender, size and shape, but among them DNA-based nanotubes have biological origin and have therefore been able to use numerous applications in the fields of biomedicine. Also, the versatility and flexibility of this nanotube can provide a suitable platform for placement and orientation of the various types of materials such as hybrid nanomaterials that were previously discussed.

Considering the recent applications of DNA nanotubes as carriers for the transfer of various molecules and metal nanoparticles, the use of these types of nanostructures can be a suitable platform for homogeneous loading of a significant number of probes with the objective of more sensitive and specific identifying target molecules. In addition, the presence of a central channel in this nanostructure, provides the space necessary for the placement of magnetic metal nanoparticles, which provides the basis for the accumulation of high-capacity target molecules. Therefore, the design and construction of magnetic DNA nanotubes carrying target probes can be a great step in the field of intelligent nanostructures to make nanoscale-based diagnostic kits for identification with high sensitivity of target molecules. In order to improve the performance of the designed diagnostic method, iron-oxide magnetic nanoparticles are used due to having magnetic properties.

The production of aptamer arrays on magnetic DNA nanotubes can provide the basis for designing and developing new methods for identifying and measurement of desired biomarkers liked insulin. These nano-structures provide the ability to array of a high capacity for binding of insulin and detect the target molecule, based on the minimum detection of insulin in the biological sample. Also, by establishing the magnetic field, the possibility of collecting insulin in the biological sample is as high as possible. Subsequently, by accompanying the process of isolating the insulin from the patient's sample with the washing step, it is possible to remove the unwanted serum contents and cause the detection insulin is more accuracy and sensitive by using the DNAzyme structure that mimics the peroxidation activity that occurs when the analyte is trapped by aptamer.

An exclusive IGA3 aptamer, which has been investigated in this study as the best specific aptamer for identifying and trapping of the target molecule as well as the catalytic function of peroxidation imitation, has been widely studied and identified as a proprietary probe for insulin binding in order to construct specific insulin sensors are based on aptamer. In fact, IGA3 is a guanine-rich nucleic acid. Biophysical and chemical studies of this aptamer have shown that the aptamer IGA3, in the presence of its target molecules, insulin, after its identification and connection, along with its bowel structure for insulin capture, the structure of G-quadruplex in the guanine-rich sequence region. which can imitate the catalytic function of the peroxidase enzyme in the presence of the hemin molecule.

The results showed that the intensity of the absorption of the reaction mixture was significantly increased with increasing insulin concentration. In fact, with the design of this system, a G-rich aptamer plays two roles. First, it serves as the cognitive element that recognizes and identifies target molecules, and continues to create the structure of G-quadruplex and then signal transmission elements that can transform the analyte recognition and entrapment into a recognizable signal. This feature can be very important for the design of biosensors, because according to this model, two important parts of a biosensor, as mentioned above, in one part with a reduction in cost due to the replacement of antibodies by oligo-aptamer with the degree of specificity and sensitivity and accuracy more, as well as the ease of synthesis, thermal stability and non-immunity.

Of course, with regard to the comparison of the four insulin measurement methods studied in this study, it is clear that the sensitivity designed to detect insulin by magnetic DNA nanotubes that carry MNP at the surface is sensitive more than to detect insulin relative to the free state that oligo-aptamer are free in the reaction mix and even to the conjugated state to iron nanoparticles. Because oligo-aptamer in the free state have less probability of accidental collision with insulin and consequently the formation of a suitable bowl structure as the introduction to form G-quadruplex structure, they cannot detect the target molecule and the probability of correct insulin measurement is reduced. Additionally, free aptamer in the reaction medium can take any secondary structure, which leads to a lack of proper structure in dealing with the target molecule (insulin).

In the case of aptamer attached to the surface of the MNP in the reaction mix, the cause of the decrease in insulin levels compared to the other aptamer array on the surface of DNA nanotubes was the probability of regional accumulation of nanoparticles and the reduction of the availability of aptamers for binding and identification of insulin. But comparing two nanoscale aptamer arrays at the DNA nanotube surface showed when aptamer are placed at the DNA nanotube with the encapsulated MNP at the surface, more aptamers are available at the surface to detect insulin. Because for every molecule of streptavidin as a bridge for

binding of the biotinylated aptamer to the surface of DNA nanotubes, there are free third sites for the aptamer attachment. In the cases where the specific isolate of insulin aptamer is conjugate to DNA nanotubes coupled with MNP at the surface, due to the alignment of nanoparticles at the surface and proportional to the size of these nanoparticles, the spatial inhibition created by them and lead to less available aptamer and reduces insulin binding. It can thus be argued that the structure of DNA nanotubes containing magnetic iron oxide nanoparticles in their central channel can provide a suitable basis for the design and development of new substrates for targeting target molecules in a variety of biosensors including electrochemical, colorimetric and spectroscopy.

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## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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