

Carbon Nanotube-Based Biosensors For DNA Sequencing

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Abstract. DNA sequencing has enormous potential for biology and medicine, especially for a personalized diagnosis and treatment. Today, however, sequencing technology remains slow and expensive, elements prohibitive for most clinical applications. The development of biosensors based on carbon nanotubes will allow us to detect genetic sequences thanks to the electrical signal that is generated when two complementary DNA strands are joined (hybridization). Next, the most relevant aspects of the operation of the same will be detailed and it is necessary to emphasize that our work is purely theoretical in order that in the future it will serve for other researchers the experimentation of the same one.

Key Words: Sequencing, DNA, Biosensor, Nanotube, Hybridization , Oligonucleotides.

INTRODUCTION

Although Sumio Iijima was the first to see a nanotube; others made them before him. Unbeknownst to them, Neanderthals made small quantities of nanotubes in the fires they used to heat their caves. Separated by the heat, the carbon atoms recombine in the soot; some form amorphous globules, others spheres called “fullerenes,” and others long cylindrical capsules, called “nanotubes.”

Science has discovered three ways to make soot containing a significant proportion of nanotubes. So far, however, all three methods suffer from some significant limitations: they all produce mixtures of nanotubes with a wide range of lengths, many defects, and a variety of twists.

DNA sequencing has established itself as the cornerstone of precision medicine, personalized genomics, and advanced clinical diagnostics. Since the completion of the Human Genome Project, the demand for sequencing technologies that are rapid, cost-effective, and high-throughput has grown exponentially. Although Next-Generation Sequencing (NGS) techniques have revolutionized the field, they still face significant limitations regarding reagent costs, read lengths, and the complexity of data processing (Shendure et al., 2017). In this context, nanotechnology has emerged as a promising frontier to overcome these barriers, with carbon nanotubes (CNTs) standing out as ideal candidate materials for the next generation of genomic biosensors.

Carbon nanotubes, due to their exceptional electrical, mechanical, and thermal properties, as well as their high surface-to-volume ratio, offer a unique platform for ultrasensitive biomolecular detection. Unlike traditional optical methods that require fluorescent labeling which can alter molecular structure and increase costs sensors based on CNTs enable direct, label-free electronic detection. According to Sireesha et al. (2018), the ability of CNTs to act as channels in field-effect transistors (FETs) allows even the

hybridization of a single DNA strand to modify the device's conductance, translating biological events into measurable electrical signals with high precision.

The fundamental mechanism that makes this technology viable lies in the electrostatic interaction and π - π stacking between the nitrogenous bases of DNA and the nanotube wall. Recent investigations have demonstrated that the functionalization of CNTs whether single-walled (SWCNT) or multi-walled (MWCNT) is critical for improving sensor specificity. Liang et al. (2020) highlight that by immobilizing specific DNA probes onto the nanotube surface, the complementary sequence can be detected with detection limits in the femtomolar range, surpassing the sensitivity of many conventional techniques. Furthermore, the nanometric dimension of CNTs is comparable to the diameter of the DNA helix, facilitating intimate interaction and improving the signal-to-noise ratio.

However, the development of CNT biosensors for sequencing, particularly those based on nanopores, presents considerable technical challenges. The nanopore sequencing technique aims to read the base sequence as a DNA strand translocates through a nanometric pore, measuring fluctuations in ionic current. The use of carbon nanotubes as artificial pores has shown potential to reduce the translocation speed of DNA, a major obstacle in conventional solid-state nanopores. Kowalczyk et al. (2011) suggest that the hydrophobicity and the atomically smooth structure of CNTs can optimize ionic and molecular transport, potentially allowing for sufficient spatial resolution to distinguish individual nucleotides.

Despite these advancements, the transition from laboratory proofs-of-concept to reliable commercial devices requires addressing reproducibility issues in device fabrication and the long-term stability of chemical functionalization. Authors such as Chakraborty et al. (2023) emphasize the need to integrate these nanosensors with microfluidic circuits and real-time data processing systems (IoMT – Internet of Medical Things) to make their clinical application viable. Consequently, this research focuses on analyzing the state of the art of CNT-based biosensors, evaluating their efficacy, sensitivity, and viability as a disruptive alternative for third and fourth-generation DNA sequencing.

MATERIAL AND METHOD

Among the most effective methods that have been used, especially in the biomedical field, are:

- Synthesis by the arc method (Daboussi, 1997).
- Synthesis by laser vaporization method.
- Synthesis by the Vapor Growth Method (CVD) (Jasti et al., 2008).
- Pyrolysis of graphite electrodes in a controlled helium atmosphere.
- Catalytic synthesis: using transition metals on silica supports alumina and also on zeolites (Collins & Avouris, 2000).

Synthesis of Carbon Nanotubes (SWCNTs)

Instead of listing obsolete methods, this study selects Chemical Vapor Deposition (CVD) as the standard for fabricating electronic devices.

"The synthesis of single-walled carbon nanotubes (SWCNTs) will be carried out using catalytic Chemical Vapor Deposition (CVD) on silicon/silicon dioxide (Si/SiO₂) substrates. This method is preferred over arc discharge or laser ablation due to its ability to control the diameter and chirality of the nanotubes, critical factors for ensuring the semiconductor properties required in a field-effect transistor (FET) biosensor (Yang et al., 2014)."

Purification and Biochemical Functionalization

The raw nanotubes are hydrophobic and do not interact with DNA on their own. This is the missing piece in your methodology: explaining how they become "biocompatible."
 "To give the nanotubes biological specificity, a non-covalent functionalization will be performed using a molecular linker, such as 1-pyrene-butyric acid succinimidyl ester (PBASE). The pyrene group attaches to the nanotube wall via π - π stacking, preserving the electronic properties of the SWCNT, while the succinimidyl group anchors the complementary single-stranded DNA (ssDNA) probes to the target sequence (Layqah y Eissa, 2019)"

Biosensor Device Fabrication (FET)

"The device will be configured as a field-effect transistor (CNT-FET). Using standard photolithography, titanium/gold (Ti/Au) source and drain electrodes will be deposited at the ends of the nanotube network. This configuration allows for real-time measurement of channel conductance. Immobilization of the DNA probe on the nanotube channel will serve as the biological 'gate' that modulates the current upon hybridization with the sample (Sireesha et al., 2018)"

Detection and Data Analysis Protocol

"Detection will be based on measuring changes in the electrical resistance (or conductance) of the device upon introduction of the DNA sample. Hybridization between the probe and the target DNA induces charge doping or carrier dispersion in the nanotube, generating a measurable electrical signal without the need for PCR amplification or fluorescent labeling (Mishra et al., 2022)"

Synthesis by the steam growth method (BIOMEDICAL EFFICIENCY)

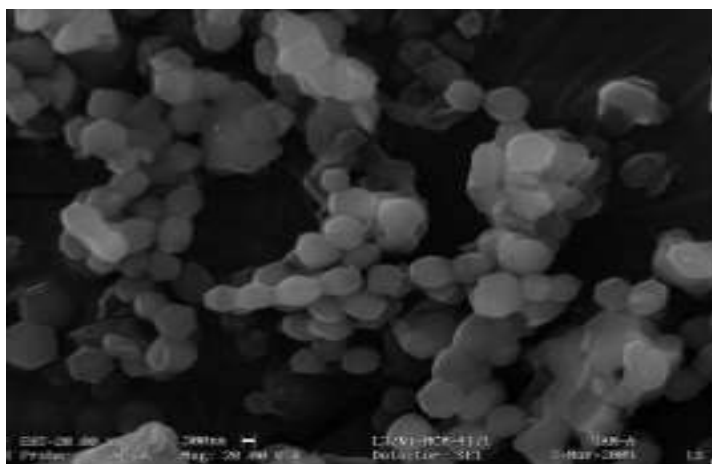


Fig. 1. Transmission Electron Microscopy (Morphology of crystals synthesized by the vapor growth method). Crystals synthesized by the vapor growth method are shown, displaying a well-defined hexagonal morphology and compact distribution. Variations in size suggest hierarchical growth influenced by synthesis conditions, such as temperature and pressure, key factors influencing their physicochemical properties.

A substrate that acts as a catalyst (Fe, Co, Ni) forming a thin film of 1 to 50 nanometers (nm) thick is placed in a low-pressure helium inert atmosphere oven, heated to 600 degrees Celsius and methane, acetylene or benzene gas is slowly added, releasing carbon atoms, which can recombine in the form of nanotubes ⁶ (Figure 1).

Due to the high temperatures, the metal (catalyst) clumps into separate nanoparticles that serve as growth centers (Figure 2) that form the basis of the nanotubes; therefore, the particle size determines the diameter of the resulting nanotube.

Normal performance: 20 to almost 100 percent.

CVD technique is the simplest of the three methods for application on an industrial, biological and medical scale since it can be used to manufacture long and symmetrical nanotubes required in the fibers used in experimental biological composite materials (Frías et al., 2015).

Limitations: The resistance portion of these nanotubes by these methods is one tenth of tensile (Carrión et al., 2010).

Synthesis by the arc method (biomedical efficiency)

It consists of connecting two graphite rods with diameters ranging from 0.5 to 40 mm to a power supply with a voltage of 20–50 V, separating them a few millimetres, and then activating a switch. When a DC spark of 50–120 A jumps between the rods and a base pressure of 400 torr of helium, the carbon evaporates into a hot plasma (Belytschko et al., 2002).

Part of it condenses again in the form of nanotubes.

Advantages: produce single and multiple wall nanotubes with few structural defects, i.e., manipulable at experimental levels and for applications, especially biological ones, to humans (Ruoff et al., 2003).

Diameter distribution: Typically, a wide range of nanotube diameters is obtained; in addition, the growth of the ~1 mm nanotube typically occurs in 0.1 s; that is, a carbon ring is added every 10-5 seconds (s): slow growth (Zoli, 2003).

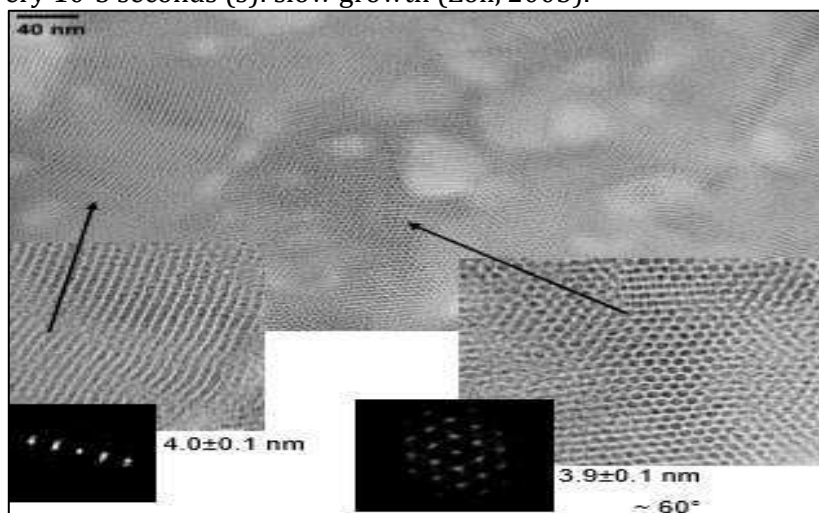


Fig. 2. High-resolution electron microscopy (mold-growth carbon nanotube synthesis, vapor growth method). The organized concentric layers are known as multi-walled carbon nanotubes. The spacing between the planes forming the structure (3.9-4 +/- 0.1 nm) and the 60° angle indicating the diffraction pattern are indicated.

Biosensors

A biosensor can be defined as a device composed of two fundamental elements: a biological receptor (e.g. proteins, DNA, cells)(Langer, 1995) prepared to specifically detect a substance taking advantage of the exquisite specificity of biomolecular interactions and a transducer or sensor, capable of interpreting the biological recognition reaction produced by the receptor and "translating" it into a quantifiable signal (Wang et al., 2024) (Figure 3)

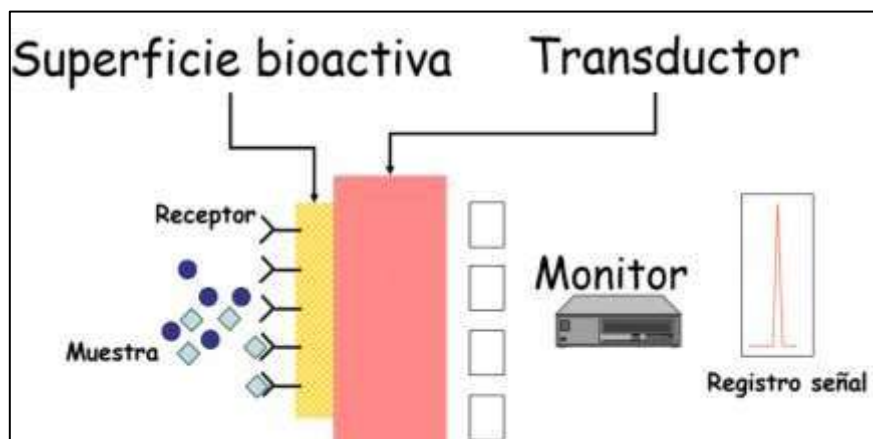


Fig. 3. Structure of a biosensor – biological receptor, transducer. It consists of a bioactive surface with specific receptors that interact with the analytes present in the sample, generating a signal that is captured by a transducer. This transducer converts the biological signal into a measurable signal, which is sent to a monitor for processing and recording, allowing the analyte of interest to be detected and quantified.

Characteristics of biosensors

- Possibility of performing the analysis of the substance to be determined in real time directly (without the need for a marker) unlike any biological or clinical analysis that always requires a marker (whether colorimetric, fluorescent or radioactive).
- Possibility of performing not only a qualitative (yes/no) and quantitative analysis, but also the possibility of evaluating the kinetics of the interaction (affinity constant, association, dissociation,) and, therefore, elucidating the fundamental mechanisms of said interaction (Kumar et al., 2011a) .

What are biosensors used for?

It allows the possibility of obtaining direct, continuous measurements, quickly and with high sensitivity, many biosensors also offer the advantages of maintaining compact dimensions and being highly portable, so they could be used anywhere, such as at home or in the doctor's office (Herrera, 2021). This also means that the amount of sample required for analysis is relatively low (from micro to nano-liters), which is very important if it is a blood or DNA analysis or if the sample is expensive or difficult to obtain (Ladame y Chang, 2019).

Classifications of biosensors

Catalytic biosensors, whose receptors can be enzymes, tissues, or cells. These are the best-known and most widely applied biosensors .

Affinity biosensors, where the receptors are antibodies, nucleic acids, receptors (Brumfiel, 2009) .

Biosensor translation system

Electrochemical, Optical, Piezoelectric, Thermometric, Nanomechanical (Carrillo, 2020). From all the above, the applicability of biosensors can be said in 'classical' terms as infinite, from which at the scientific-medical level the following have been obtained: biosensors as carcinogenic detectors, biosensors as food alert sources, gold biosensors to detect blood proteins, biosensors to eliminate bacteria from food, fluorescent bacterial detectors, biosensors as glucose detectors, biosensors to sequence DNA (Khan, 2012).

Biosensors made from carbon nanotubes

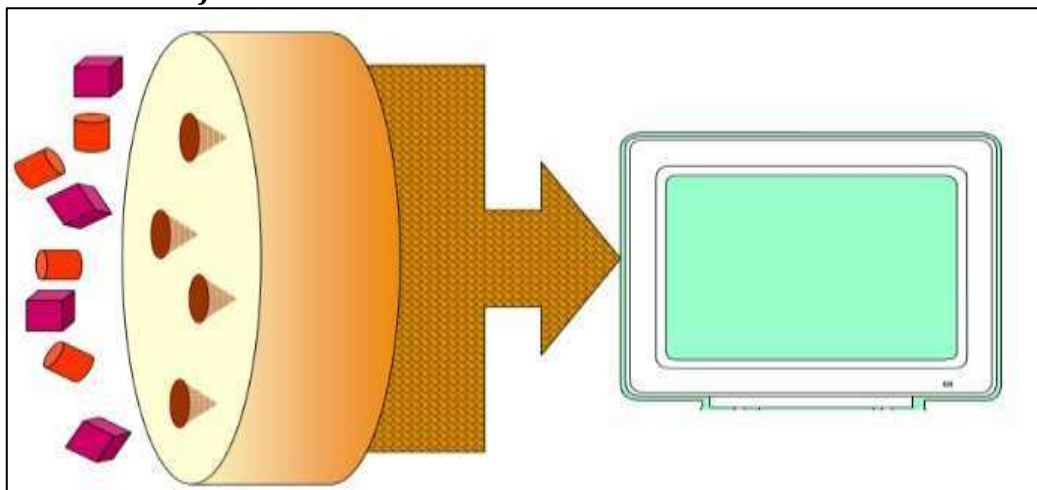


Fig. 4. Biosensor (Receptor, Transducer). Detection of protein sequences. Sample molecules are directed toward a bioactive surface with specific receptors that only recognize and capture certain proteins. This interaction generates a signal that is transmitted to a transducer, which converts the biological signal into an electronic signal displayed on the monitor, allowing the proteins present in the sample to be detected and analyzed.

To date, what has been analyzed and manufactured by humans in the synthesis of carbon nanotubes and the production of biological detector chips (biosensors) has been noted. New studies show progress in the detection of chemical structures, protein sequences, toxic samples, and pathologies. These are determined by the union of carbon nanotube-based transistors with high sensitivity and specificity. This is essential for implementing effective personalized medicine (Huhtala et al., 2002). (Figure 4).

Basic model of a carbon nanotube biosensor

The method devised by the researchers at Irvine (University of California) is based on the union of a single molecule of an enzyme (lysozyme) to a tiny field-effect transistor, in which the active material is a carbon nanotube. As it moves to attack the invading bacteria, electrostatic fields are generated on the surface of the protein in contact with the nanotube, which have a "gating" effect on the device, that is, the activity of the molecule regulates one of the parts of the transistor, the gate, which acts by allowing or cutting the flow of electrons that pass through the device (Kamat, 2006).

In this way, the transistor acts as an amplifier for the protein's movements. "Our circuit is a molecular-sized microphone," says Collins, a professor of physics and astronomy in the School of Physical Sciences. "It's like a stethoscope listening to your heartbeat, but in this case we're 'listening' to a single molecule."

nanoscopic device (Conroy et al., 2009), which allows even a small local electric field to have an enormous effect and amplification," explains Federico Rosei, professor at the

Research Chair in Organic and Inorganic Nanomaterials at the University of Quebec (Canada).

The transistor does not record flows of photons, but of electrons, which are generated due to the changes in electrostatic potentials that occur on the surface of the molecule to which it is attached (James et al., 2016). Researchers take advantage of the fact that the one-dimensionality of the nanotube allows for high gate sensitivity and favors “an advantageous bandwidth” and a limited amount of interference (Hwang et al., 2016).

DNA sequencing (biosensors)

Researchers at the University of Illinois (Urbana-Champaign, USA) coated single-walled carbon nanotubes with a DNA fragment comprising a 14-mer oligonucleotide sequence. When these tubes were immersed in a solution containing complementary single-stranded DNA, a hypsochromic shift of near-infrared fluorescence of 2 meV was observed, with a detection sensitivity of 6 nm (Figure 5). This shift could be measured optically and did not require any applied voltage or current to the system (Eder, 2010).

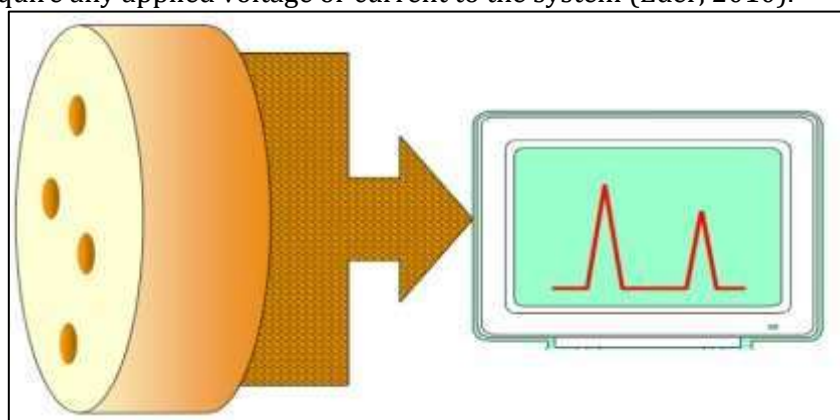


Fig. 5. Hypsochromic shift. Detection at 6 nm.

The work, published in Proceedings of the National Academy of Sciences, shows the design of a chip capable of capturing DNA (or RNA) molecules that contain a mutation, by hybridizing said molecules with a probe embedded in graphene (Terrones, 2013). Said probe consists of a double-stranded DNA sequence, complementary to the region where the mutation or SNP to be analyzed is located. In it, both strands are weakly linked to each other by the substitution, in one of them, of some guanines with iosines (Galperin y Koonin, 2010). The normal strand is firmly bound to the graphene, while the modified strand is not. Thus, when the probe is exposed to DNA (or RNA) molecules in a sample, the fragment containing the SNP displaces the complementary (but modified) sequence from the strand attached to the graphene, due to its greater complementarity with the latter (because of the absence of modifications), and binds to it, allowing the SNP to be detected (Finnegan, 1989). This union will trigger an electrical signal, which can be recorded by a computer system for subsequent analysis and processing. The system offers the possibility of being implanted in the body, facilitating real-time detection of the PNS, in addition to being able to transmit the information wirelessly to an electronic device, such as a smartphone or tablet (Biémont et al., 1999). (Figure 5).

DNA sequencing

It is a set of methods and techniques whose purpose is to determine the order of nucleotides (A, C, G and T) in a DNA oligonucleotide. (Figure 6).

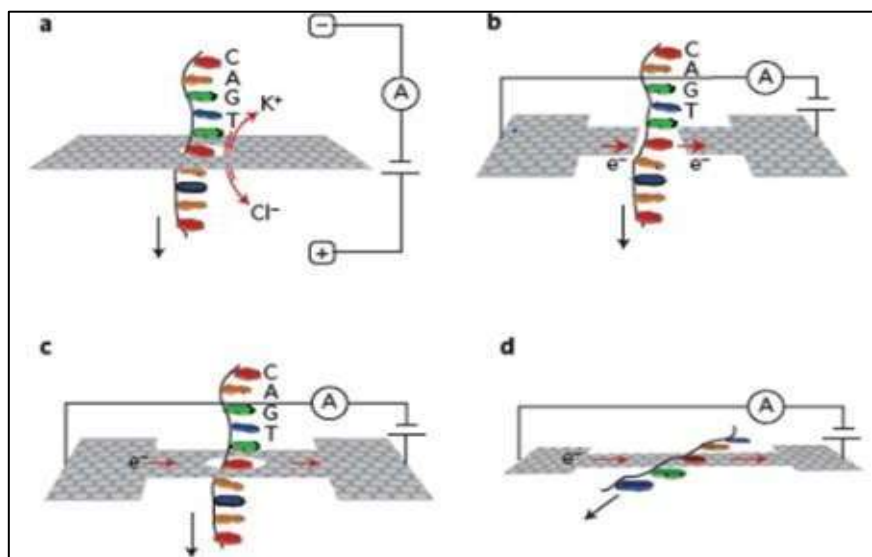


Fig. 6. Four new concepts using graphene nanostructures for DNA sequencing.

- Detection of changes in ionic current through a nanopore in a graphene membrane due to the passage of a DNA molecule.
- Modulations of a tunneling current through a nano-gap between two graphenes due to the presence of a DNA molecule.
- Variations in the in-plane current through a graphene nanoribbon due to the passage of a DNA molecule.
- Changes in a graphene current due to physisorption of DNA bases on graphene (Jorda et al., 2010).

DNA sequencing is a rapidly evolving methodology for reading the sequence of bases in a genome (Boulle et al., 2016). Given its role in human physiology and development, this sequence information is expected to have a significant impact on the diagnosis and treatment of disease, ultimately facilitating personalized medicine where the right treatment can be applied to individuals (Subramanian, 2013).

The need for cheaper and faster techniques led scientists and companies to work on new sequencing technologies (Pêgo, 2023). However, new sequencing methods, based on nanotechnology approaches, now focus on long-length single-molecule sequencing without any amplification or labeling (Duda y Tarko, 2023). (Figure 7)

But despite the various techniques that allow DNA sequencing, it is not always possible to obtain complete genome information for an organism (Mikhailovich et al., 2008). This can lead to errors in reconstructing lineages and in estimating the type of mutations and the number of mitoses generated.

Due to their unique structure and properties, carbon nanotubes offer exciting opportunities for developing new DNA sequencing technology to identify somatic mutations, such as base substitutions, generated between different organisms—in other words, a biosensor.

The development of a DNA biosensor allows the detection of genetic sequences thanks to the electrical signal generated when two complementary DNA strands join (hybridization) (Zhang et al., 2012).

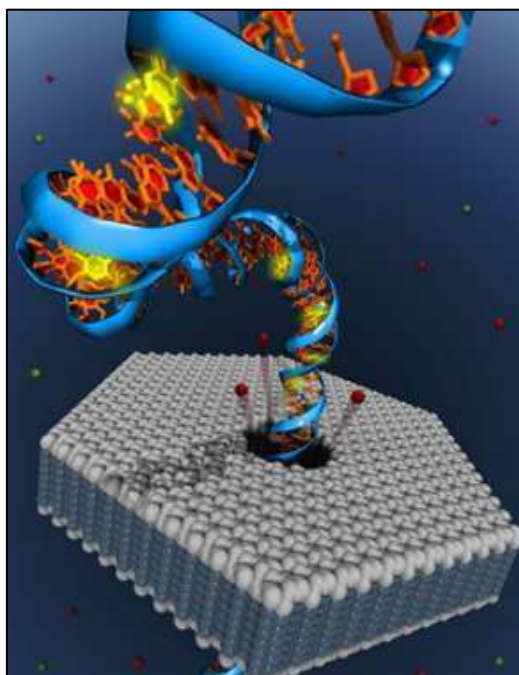


Fig. 7. DNA strands passing through the biosensor (carbon nanotubes).

Hybridization

Nucleic acid hybridization (DNA or RNA) is a process by which two anti-parallel nucleic acid strands with complementary base sequences are combined into a single double-stranded molecule, which takes the structure of a double helix, with the nitrogenous bases hidden inside. Nucleotides will bond with their complements under normal conditions: A=T; A=U; C≡G; G≡C ; T=A or U= A (Chen et al., 2009).

So two perfectly complementary strands will join together quickly (Martín et al., 2024). On the contrary, due to the different geometries of the nucleotides, a simple variation of the above pairs leads to inconsistencies between the strands and will make the joining difficult (Martín et al., 2024).

The relevance of the work is that it has allowed us to demonstrate two things: the electronic detection of these biological events and, secondly, that electrons circulate through DNA and that its electrical conductivity varies depending on the amino acids that make up the genetic sequence. (Figure 8) "We have been wondering for a long time about this, whether electrons could pass through DNA and whether this conductivity could be used as a method to detect different genetic strands" (Jurka, 2007).

The biosensor consists of a 1-square-centimeter chip integrating 896 field-effect transistors made from single-layer carbon nanotubes (Liu et al., 2009). The oligonucleotide sequence, developed by Ramon Eritja 's team at the CSIC's Institute of Advanced Chemistry of Catalonia, is linked using a polymer designed and developed by Cidetec, which is used to functionalize the nanotube in situ in the transistor (Khalaj et al., 2017). When the oligonucleotides, acting as a probe, bind to the complementary DNA sequence being sought, an electrical response is generated that can be detected and measured by the device. Such a system could be used in the future for medical diagnostics, for the detection of genetic mutations, or for other applications where immediate DNA detection is required (Ashraf, 2016).

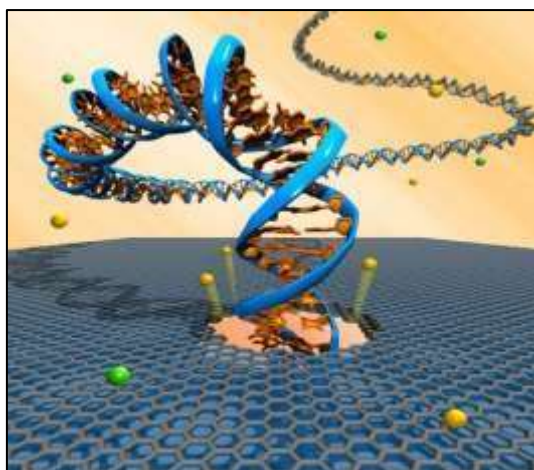


Fig. 8. DNA double helix through carbon nanotubes.

Properties of carbon nanotubes for DNA sequencing

- Depending on their diameter and helicity (Spitalsky et al., 2010), they can be semiconductors or metallic with a conductivity, the latter, 6 orders of magnitude greater than that of copper (something like a million times more) (Spitalsky et al., 2010).
- They have excellent thermal conductivity, greater than that of diamond, and have great chemical and thermal stability. (Figure 9) (Mora Bolaños & Chaves Villareal, 2021).
- As electron emitters, they emit in a very narrow energy range, 0.1 eV (electron volts), whereas the best electron emitters currently used emit in a range between 0.6 and 0.3 eV (Camacho et al., 2023).
- Very light, but with a resistance far superior to that of steel and enormous elasticity (Li et al., 2003).
- The density of nanotubes is 1.4 grams per cubic centimeter, and they have been calculated to be between 10 and 100 times stronger than steel (Kumar et al., 2011).
- The appearance of carbon nanotubes is like a black powder, "like soot" but at the nanometric scale their structure shows a high symmetry (Jastrzębska et al., 2012).

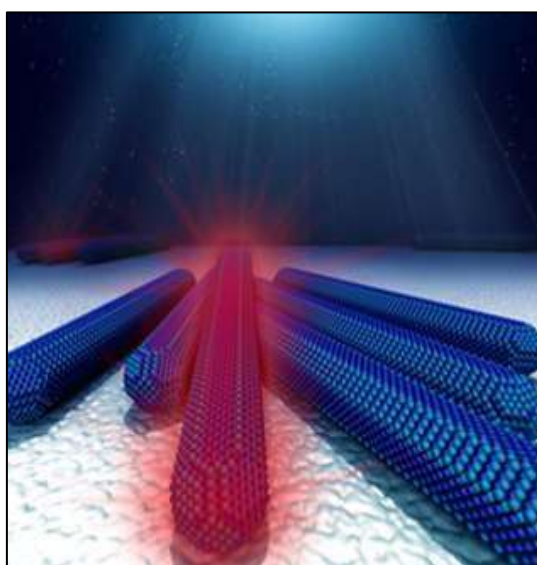


Fig. 9. Thermal conductivity of carbon nanotubes.

Detection in minutes

This review provides an overview of the theoretical proposal of the biosensor for DNA sequencing based on carbon nanotubes (Pop et al., 2006).

With the biosensor, detection would be performed directly and in a matter of minutes. This is a record time compared to current methods, which require a couple of days, since DNA molecules must be labeled and amplified to increase sensitivity (Star et al., 2006).

DISCUSSION

Based on the characterizations we have highlighted in this work as ideal for carbon nanotubes, both in theoretical and applied fields, we determined that the vapor-phase growth method is the most suitable for their synthesis. This is due to its high efficiency, ease of production, and ability to generate long, symmetrical nanotubes—essential characteristics for biomedical applications and for the fabrication of fibers in composite biological-experimental materials. Furthermore, our findings confirm that carbon nanotubes, whether single- or multi-walled, exhibit few structural defects and are compatible with biological-scale manipulations, making them ideal for use in biosensors. In this context, we propose a biosensor composed of a biological receptor, in this case, DNA, and a transducer capable of detecting and converting the biological interaction into a quantifiable signal (sequencing). This configuration allows for the elucidation of fundamental mechanisms of biological interaction, particularly in affinity biosensors, where the receptor is compatible with the biological material of interest. Carbon nanotubes are ideal components for these biosensors, not only because of their ability to improve sensitivity and specificity, but also because of their unique physical and chemical properties, which facilitate their manipulation and optimize their performance, such as:

- They can be semiconductors or metallic with conductivity.
 - They have excellent thermal conductivity, greater than that of diamond.
 - As electron emitters, they emit in a very narrow energy range, 0.1 eV.
 - Very light, but with a resistance far superior to that of steel and enormous elasticity.
- Now in this way, with these characteristics known and widely studied, it was possible to base these biosensors on carbon nanotubes for the simple fact of the feasibility at the biomedical level for a living organism to sequence its DNA and see if it presents irregularities or mutations, prioritizing physical aspects in said receptor-transducer interaction such as the electronic detection of these biological events and, secondly, that electrons circulate through DNA and that its electrical conductivity varies depending on the amino acids that make up the genetic sequence.

It would be very helpful to be able to know when an organism's DNA presents irregularities or mutations, in a faster and more efficient way than the techniques already known (Genereux y Barton, 2010). Our proposal is purely theoretical, but we hope that in the future these new techniques will be used in genetics and biomedicine. These biosensors based on carbon nanotubes have spectacular properties that make them highly functional, a scientific advance that should be used now.

CONCLUSION

The extraordinary properties of carbon nanotubes make them excellent candidates for a large number of applications, but the field that most interested us and that we consider important is their use as sensors of genetic material.

Such a system could be applied in the future for medical diagnostics, for the detection of genetic mutations, or other applications where immediate DNA detection is required.

Another challenge we propose in this article is to perfect the synthesis processes for carbon nanotubes, since by improving their structure we make their intended use, in this case genetic sequencing, more efficient.

Financing

None.

Conflict of interest

The authors declare that there is no conflict of interest.

GRATITUDE

This research was conducted under the supervision of Dr. Hugo Cerda and TSU A. Carolina Ledezma-Carrizales, to whom we would like to express our deepest gratitude for making the completion of this work possible and for their help in successfully making its publication possible.

To our image collaborators for allowing us to use their very well prepared material: Marisela Maubert F, Laura Soto S, Ana Ma. León C. and Jorge Flores M. researchers in the area of Materials Chemistry at the Autonomous Metropolitan University in Reynosa Tamaulipas, Azcapotzalco, to researcher Ángel Maquieira from the Polytechnic University of Valencia from the Institute of Molecular Recognition and the Department of Chemistry in Valencia, Spain, to Robert Johnson from the Department of Physics and Astronomy at the University of Pennsylvania, USA, to Stephanie J. Heerema and Cees Dekker Kavli of the Kavli Delft Institute for Nanoscience at Delft University of Technology in the Netherlands.

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