Nanoimmunosensors for health diagnostic

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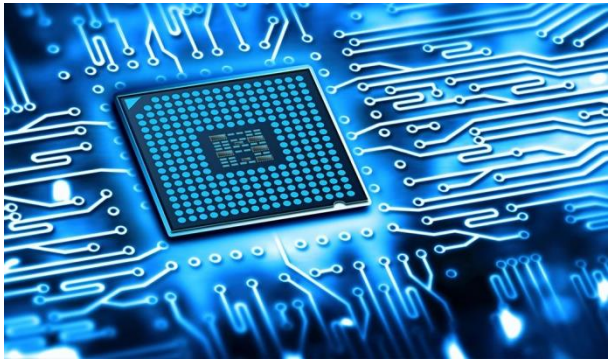
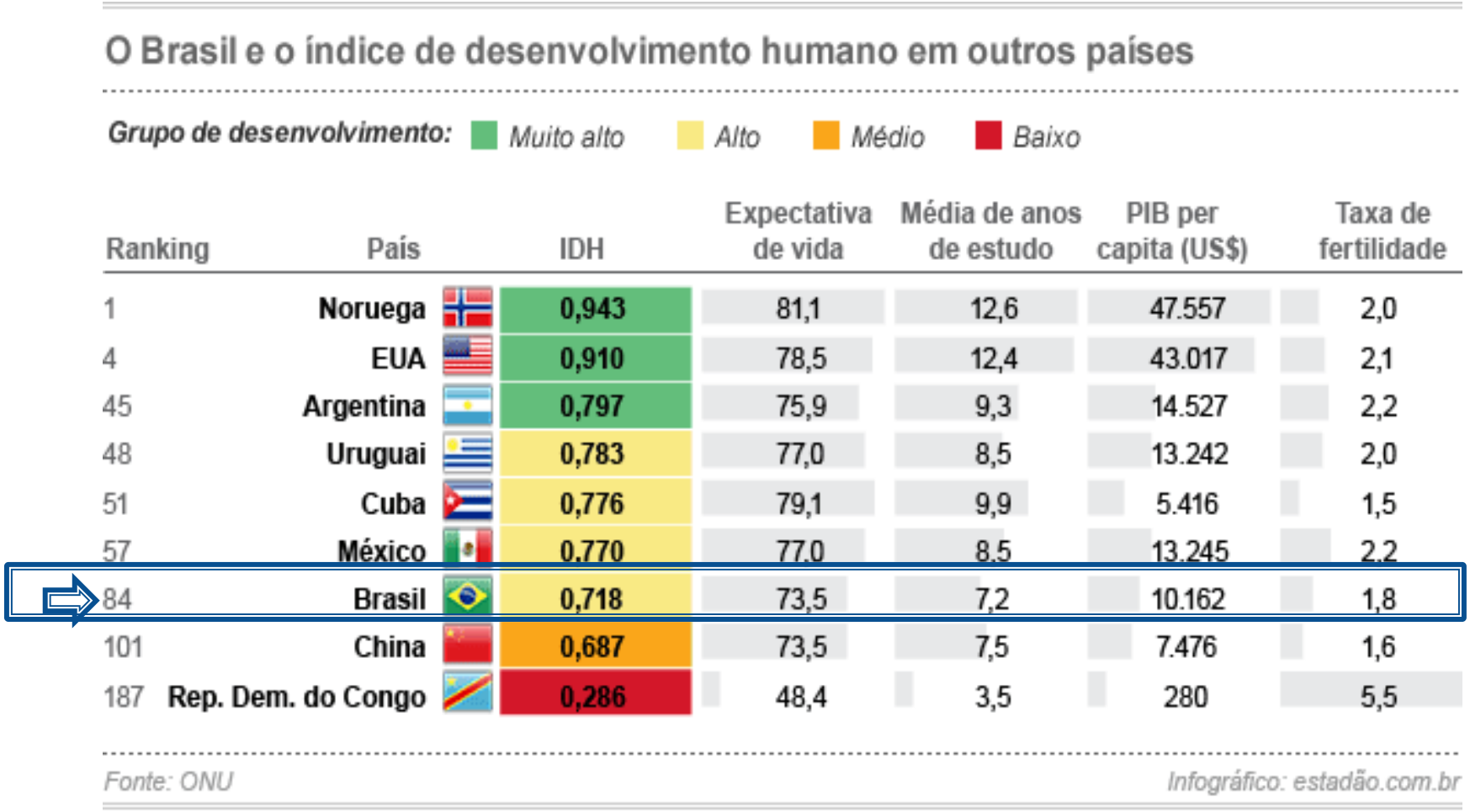
Universidade Federal de Pernambuco

Londrina, 2018

Brazil : a big country!



Health, human and social index in Brazil (ranking: 84º)



O Brasil e o índice de desenvolvimento humano em outros países

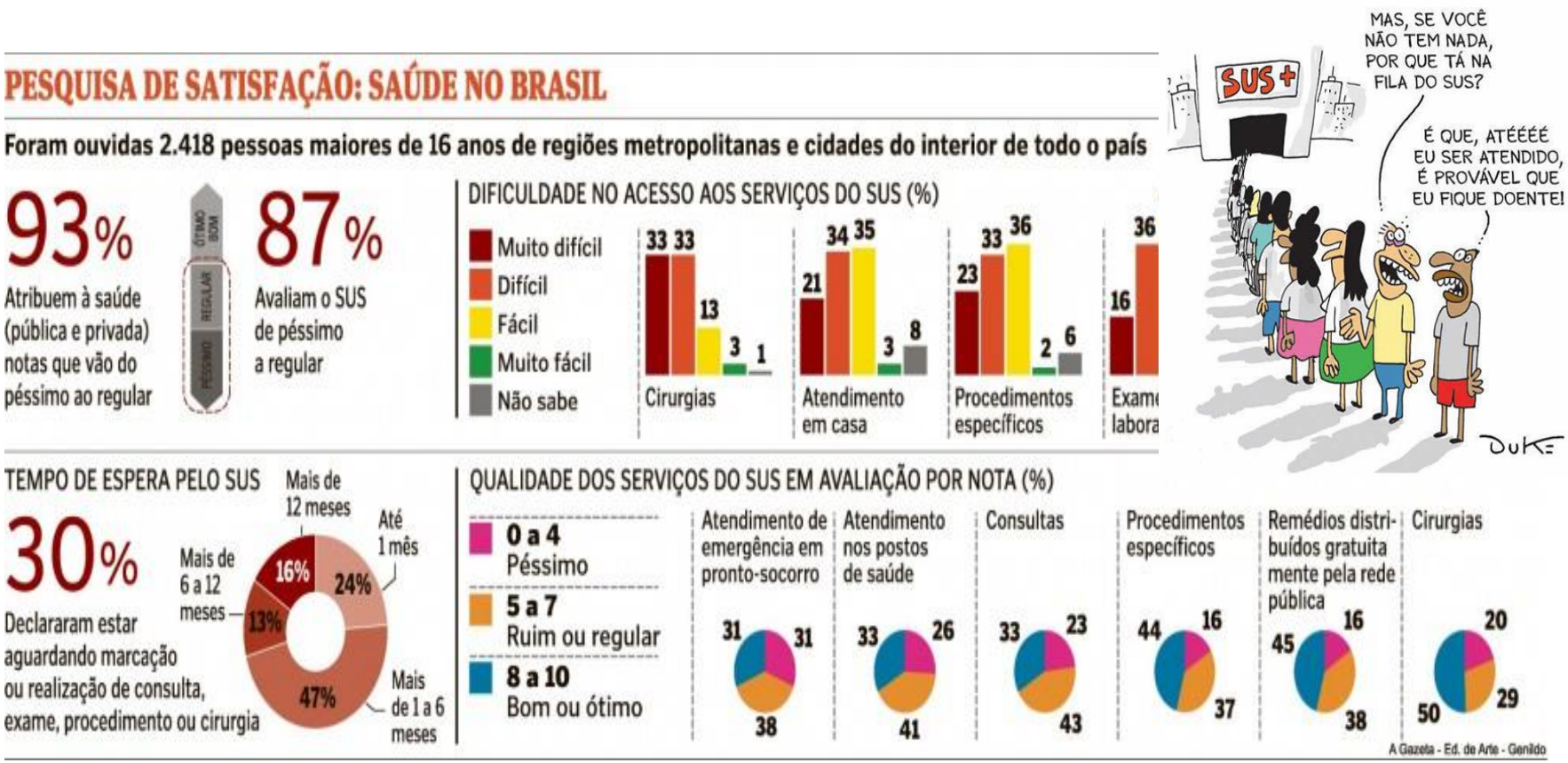
Grupo de desenvolvimento: Muito alto · Alto · Médio · Baixo

Ranking	País	IDH	Expectativa de vida	Média de anos de estudo	PIB per capita (US$)	Taxa de fertilidade
1	Noruega	0,943	81,1	12,6	47.557	2,0
4	EUA	0,910	78,5	12,4	43.017	2,1
45	Argentina	0,797	75,9	9,3	14.527	2,2
48	Uruguai	0,783	77,0	8,5	13.242	2,0
51	Cuba	0,776	79,1	9,9	5.416	1,5
57	México	0,770	77,0	8,5	13.245	2,2
84	Brasil	0,718	73,5	7,2	10.162	1,8
101	China	0,687	73,5	7,5	7.476	1,6
187	Rep. Dem. do Congo	0,286	48,4	3,5	280	5,5

Fonte: ONU

Infográfico: estadão.com.br

Public and private healthcare service in Brazil



Pan American Health Organization

World Health Organization
Regional Office for the Americas

Health in the Americas+

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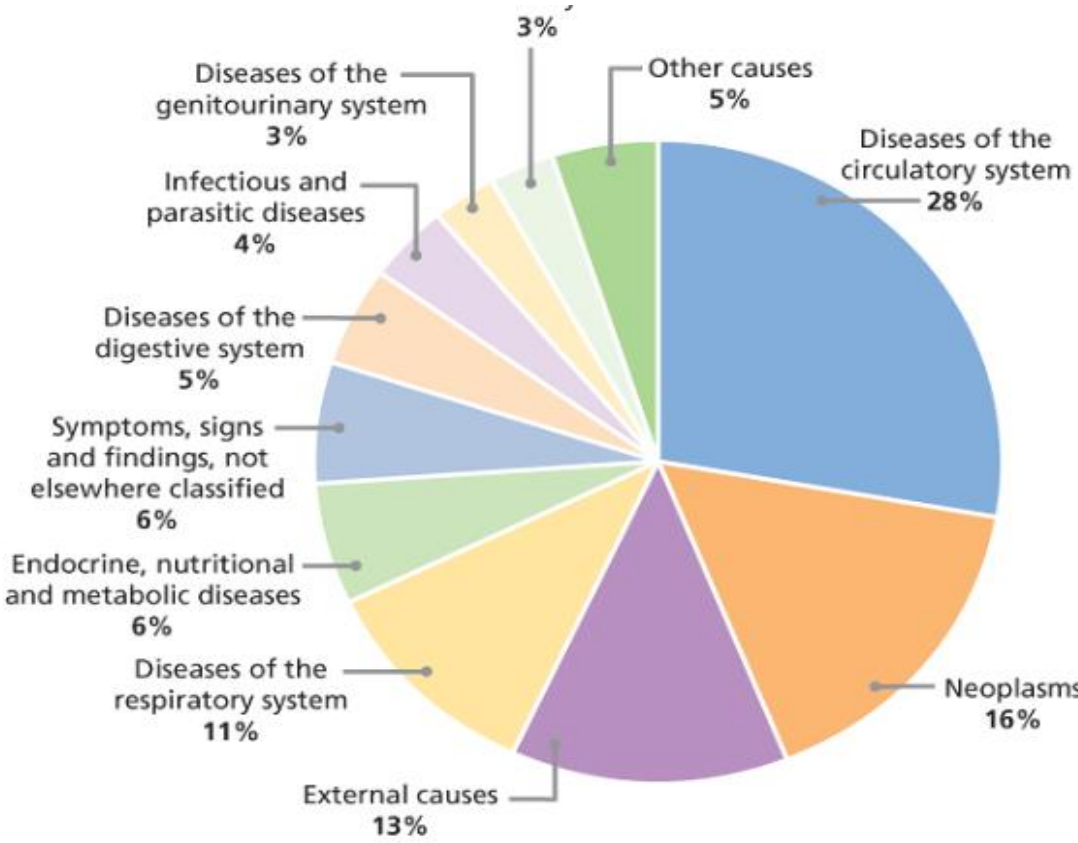
Country Report: Brazil

Mortality rate in Brazil according to WHO



Source: Pan American Health Organization. Health

The Age of Personalized Medicine

What Is Personalized Medicine?

Personalized medicine is the tailoring of medical treatment to the individual characteristics of each patient. The approach relies on scientific breakthroughs in our understanding of how a person's unique molecular and genetic profile makes them susceptible to certain diseases. This same research is increasing our ability to predict which medical treatments will be safe and effective for each patient, and which ones will not be.

Personalized medicine may be considered an extension of traditional approaches to understanding and treating disease. Equipped with tools that are more precise, physicians can select a therapy or treatment protocol based on a patient's molecular profile that may not only minimize harmful side effects and ensure a more successful outcome, but can also help contain costs compared with a "trial-and-error" approach to disease treatment.

Personalized medicine has the potential to change the way we think about, identify and manage health problems. It is already having an exciting impact on both clinical research and patient care, and this impact will grow as our understanding and technologies improve.

Personalized Medicine Is Impacting Patient Care in Many Diseases. For Example...

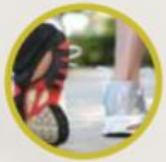
...in Breast Cancer: One of the earliest and most common examples of personalized medicine came in trastuzumab. About 30% of patients with breast cancer have a form that over-expresses a protein called HER2, which is not responsive to standard therapy. Trastuzumab was approved for patients with HER2 positive tumors in 1998 and further research in 2005 showed that it reduced recurrence by 52% in combination with chemotherapy.[1]

...in Melanoma: BRAF is the human gene responsible for the production of a protein called B-Raf, which is involved in sending signals inside cells to direct cell growth, and shown to be mutated in cancers. In 2011, a drug called vemurafenib, a B-Raf protein inhibitor, and the companion BRAF V600E Mutation Test were approved for the treatment of late stage melanoma. Vemurafenib only works in the treatment of patients

Personalized Medicine Is...

Personalized medicine is a multi-faceted approach to patient care that not only improves our ability to diagnose and treat disease, but offers the potential to detect disease at an earlier stage, when it is easier to treat effectively. The full implementation of personalized medicine encompasses:

Risk Assessment: Genetic testing to reveal predisposition to disease



Prevention: Behavior/Lifestyle/ Treatment intervention to prevent disease

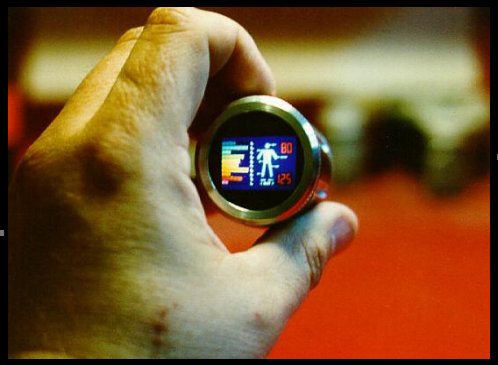
Detection: Early detection of disease at the molecular level

Diagnosis: Accurate disease diagnosis enabling individualized treatment strategy

Treatment: Improved outcomes through targeted

Dr. McCoy's scanner

(In "Star Trek VI: The Undiscovered Country")



How to minimize negative impacts in health?

- Early diagnosis;
- Decentralization of healthcare services;
- More effectiveness in prevention and control of diseases;
- More practical and faster diagnostic ;
- Low cost diagnostic analysis;
- Humanization in health care with ambulatorial attendance...

Portable analytical methods for diagnosis, control and vigilance

POCT based-biosensor

Point of Care Testing

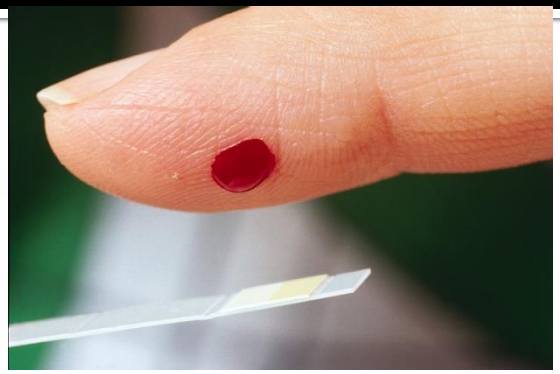
POCT has come a long way from a handful of simple waived tests to what is today a multibillion dollar global market that holds great promise for the future

Not so long ago, laboratory data would often arrive at the bedside too late to be of significant use in the active, continuing care of critically-ill patients. Now, most clinicians acknowledge that point-of-care testing (POCT) is a prerequisite for early recognition of life-threatening conditions as they require that laboratory results are made available in real-time and, if possible, at the critically ill patient's point of care.

The College of American Pathologists defines POCT as tests designed to be used at or near the site where the patient is located, that do not require permanent dedicated space, and that are performed outside the physical facilities of the clinical laboratories.

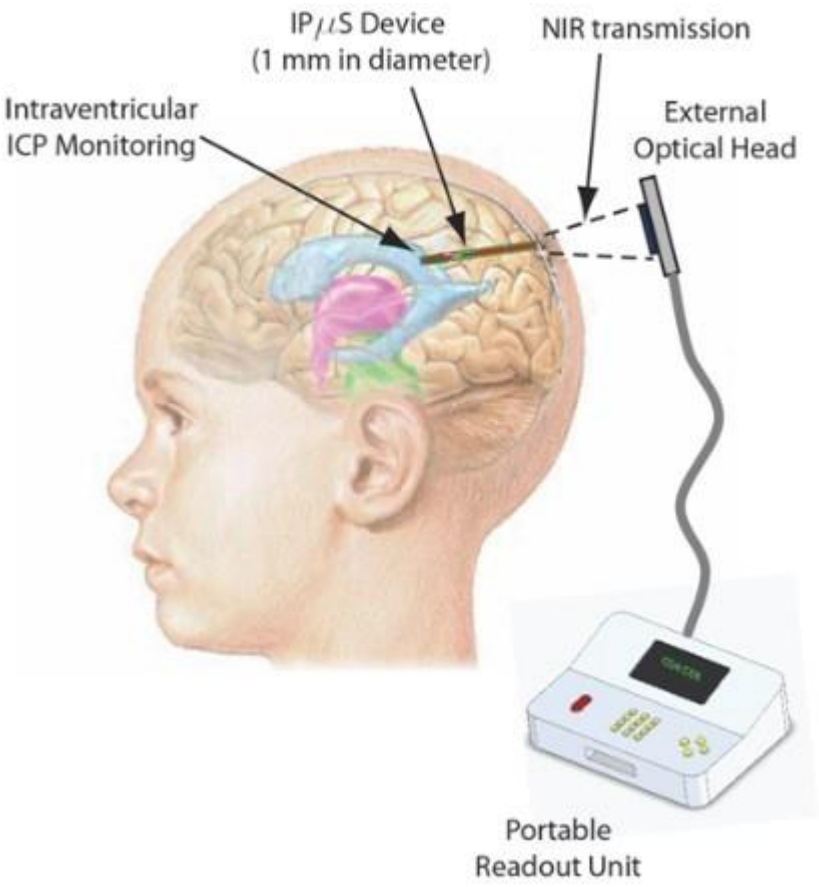
Examples include kits and instruments that are hand-carried or otherwise transported to the vicinity of the patient for immediate testing at that site (eg., capillary blood glucose) or analytical instruments that are temporarily brought to a patient care location (like operating room, intensive care unit).

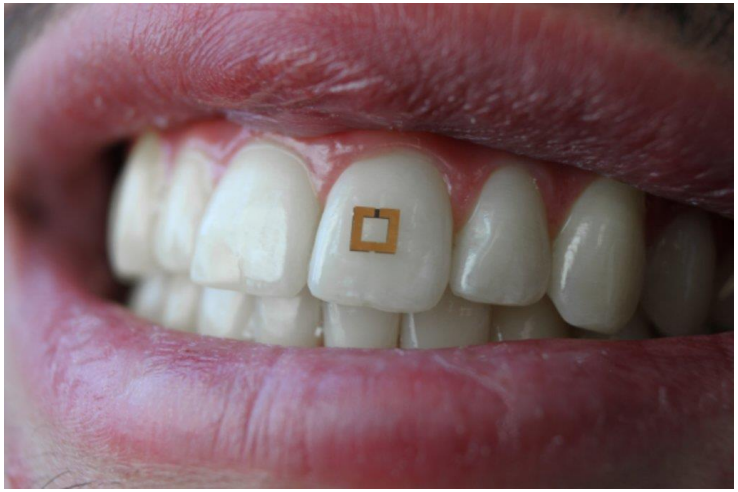
Timely, accurate diagnosis can mean the difference between life and death for a patient. The driving notion behind POCT is to bring the test conveniently and immediately to the patient. This increases the likelihood that the patient will receive the results in a timely manner.



Source: Health Express

Implantable biosensors

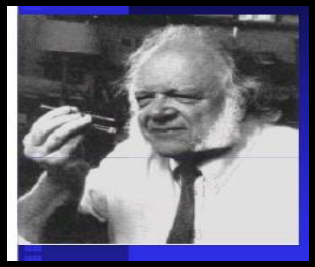




Wireless / bluetooth biosensors

A rapid point of care (POC) diagnosis of flu and other respiratory conditions

The state -of -art" … Evolution of POC



ENZYMATIC POCT

Glucose Sensors

Cholesterol, uric acid, etc

POCT FOR IMMUNOASSAY AND MOLECULAR BIOLOGY

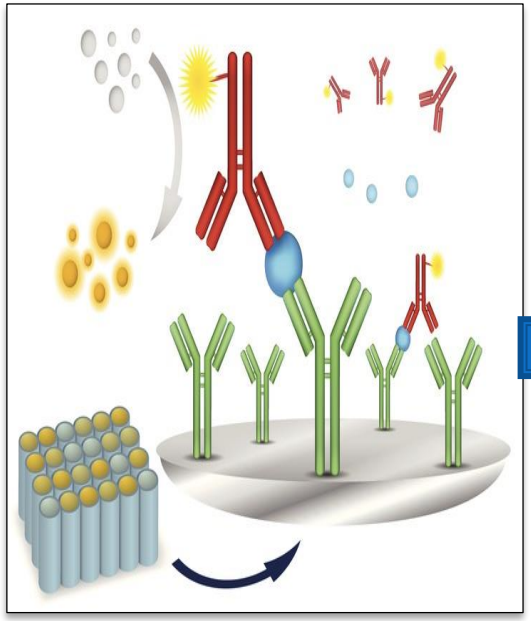
Disease Diagnostic

Terapeutic Monitoring

Immunoassay
Biosensor and Chemical analysis

ELISA METHOD

ECLIA METHOD



BIOSENSOR METHOD

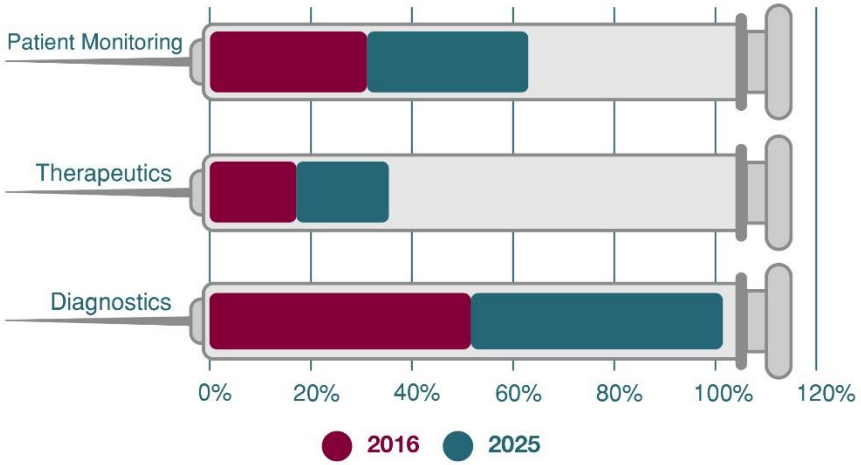
BIACore 2000- Optical SPR Technology (Biosensor)

GLOBAL DISPOSABLE MEDICAL DEVICE SENSOR MARKET (2017-2025)

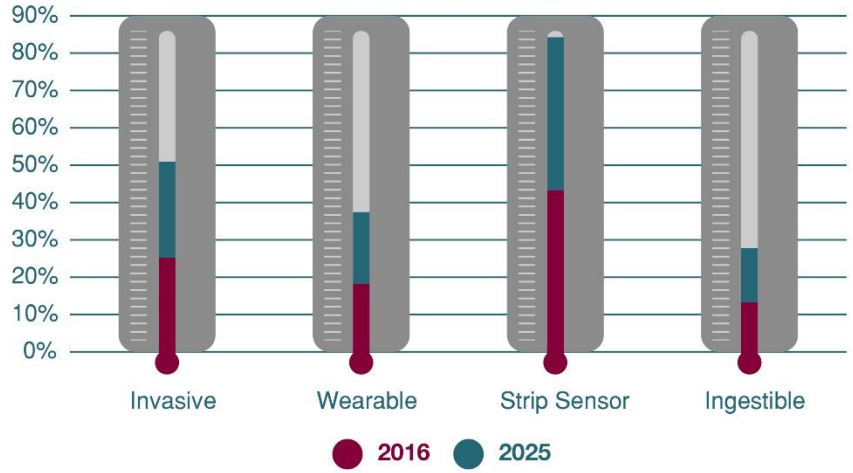
TOP COMPANIES

- ANALOG DEVICES
- COVIDIEN PLC
- GE HEALTHCARE
- HMICRO

MARKET BY APPLICATION



MARKET BY PLACEMENT



www.inkwoodresearch.com

Point-of-care Immunosensors : Challenges…

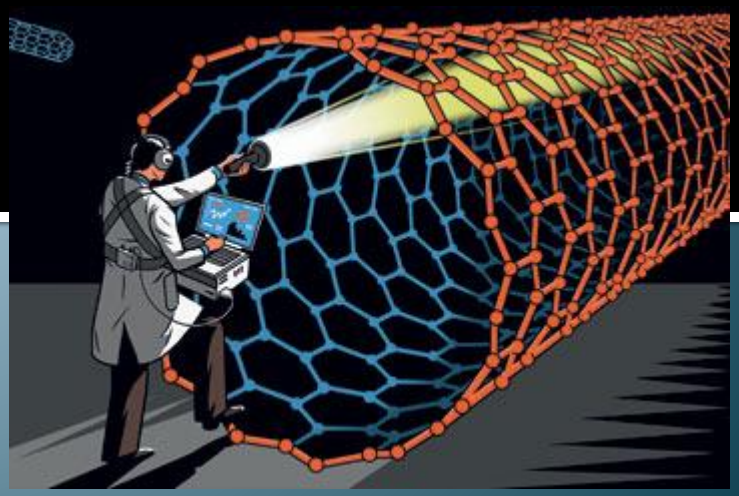
- Free sample preparation"mix and measure";
- User friendly processing (practical analyses)
- Lower limit of detection;
- Lower cost;
- More reliability;
- More reproducibility;
- Compatible with established readout systems like the glucose

- Adaptable to run or read in smartphone, tablet,
- Lower power consum, etc…

LAPED

Laboratório de Pesquisa em Diagnóstico

Our attempting to solve some problems in health



Strategies:

- Nanomaterial synthetically dedicated;
- Bioreceptors with high specificity (or non);
- Transducers with simple electronics;
- Friendly use readout system;
- Non redox-probe (probeless), and label-free detection.

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NATURE MATERIALS | COMMENTARY

The era of carbon allotropes

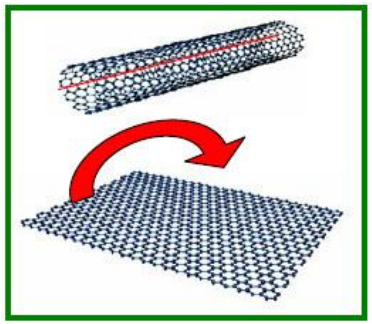
Andreas Hirsch

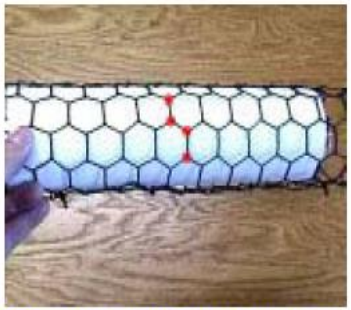
Nature Materials 9, 868–871 (2010) | doi:10.1038/nmat2885
Published online 22 October 2010

Twenty-five years on from the discovery of C_{60}, the outstanding properties and potential applications of the synthetic carbon allotropes — fullerenes, nanotubes and graphene — overwhelmingly illustrate their unique scientific and technological importance.

Chemical structures of carbon nanotubes

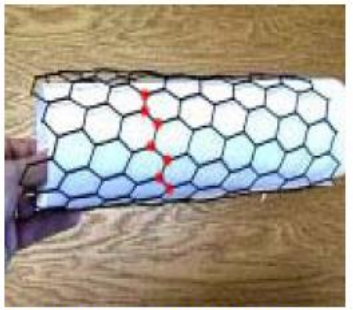
- The way how the graphene sheet is rolled up into a cylinder can form different types of CNTs



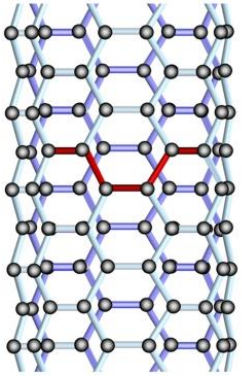


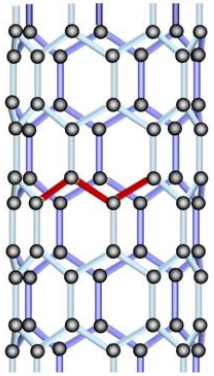
Armchair arrangement of carbon atoms

Zig-zag arrangement of carbon atoms



Chiral arrangement of carbon atoms





Metallic
Airmchair

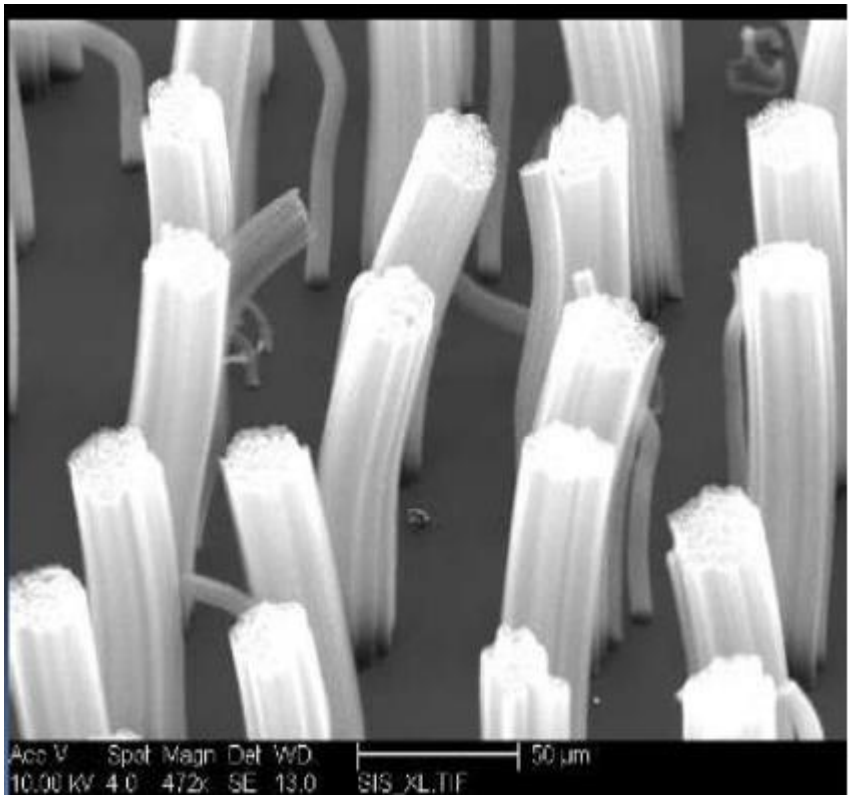
Semiconductor
Zig-Zag

- The structure of carbon nanotubes also determines their physical and chemical properties, where armchair nanotubes are always metallic in terms of electrical conductivity, zig-zag and chiral forms are semiconducting.

Carbon nanotube synthesis

- Techniques have been developed to produce CNTs in sizeable quantities, some of them are:

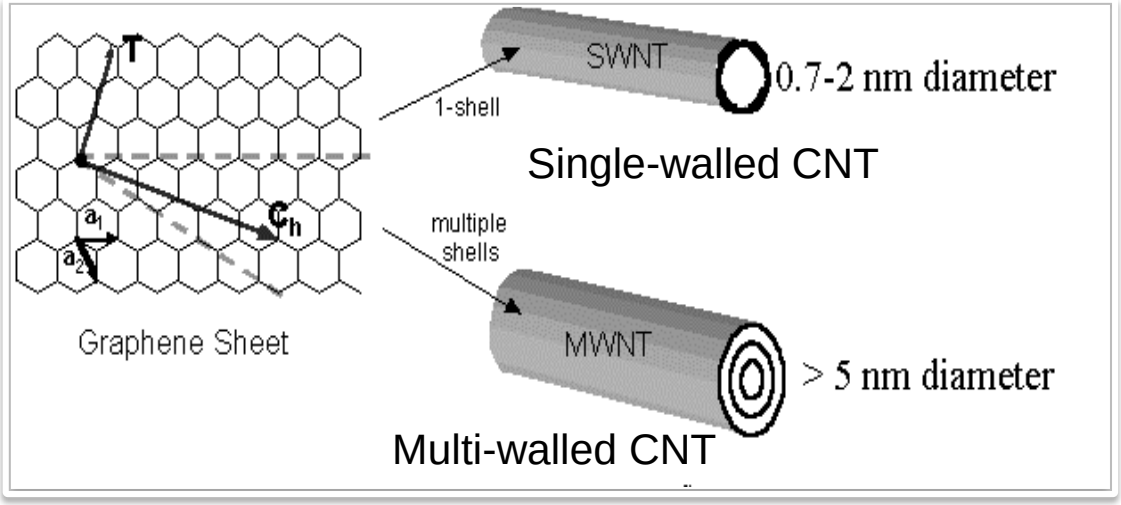
  - Arc discharge
  - Laser ablation
  - Chemical vapor deposition (CVD).

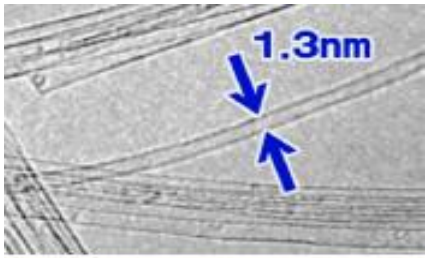


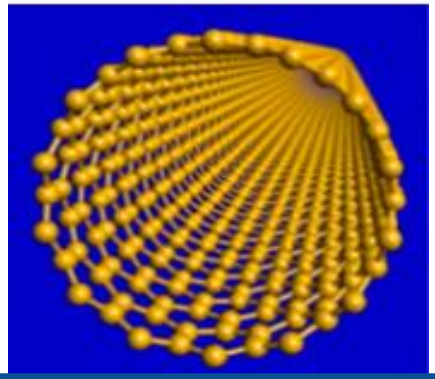
CNTs were firstly discovered by Sumio Iigima (1991).

Single and Multiple walled CNTs

- Carbon nanotubes are extremely small! They have diameters on the order of a few nanometers and lengths that can be as much as several millimeters.



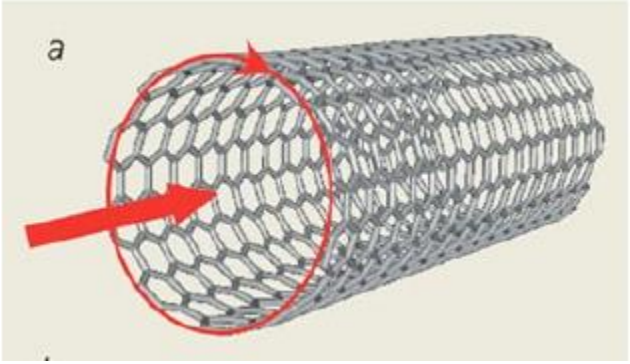




Single walled carbon nanotube -SWNT

Interesting properties of carbon nanotubes!

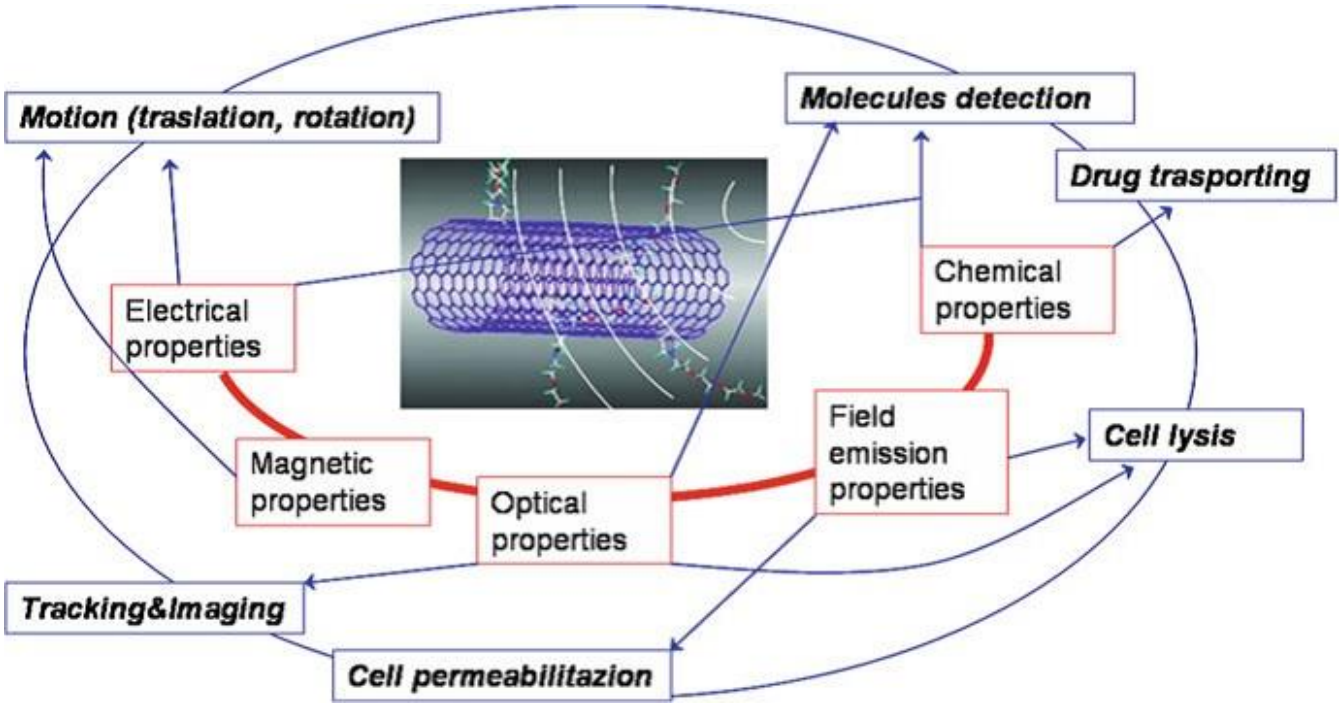
- High electrical conductivity (10^{-6} ohm typically)
- High thermal conductivity (1750-58 00 W/mK)
- High mechanical strength (tensile strength 60GPa) and modulus (Young's modulus 1TPa).
- High aspect ratio structures with diameters in nanometers, lengths in microns. Collectively, nanotubes can exhibit extremely high surface area

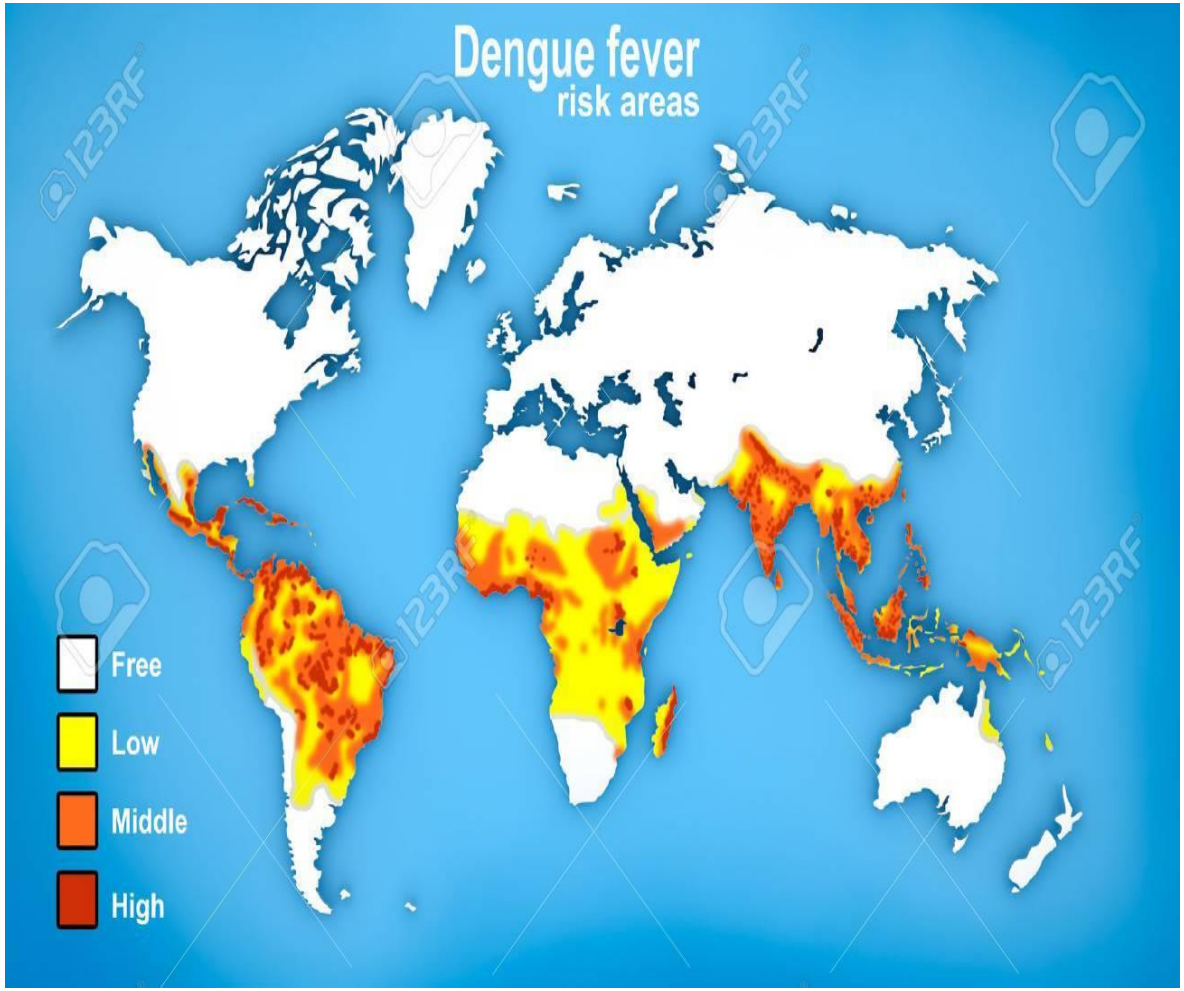


Magnetic field introduces a phase factor to the electron wavefunction in the circumferential direction. As a result, the electronic properties of a nanotube can be modulated by a magnetic field.

Discovered about new properties of nanomaterials !

- Currently, the physical properties of carbon nanotubes are still being discovered and described!!





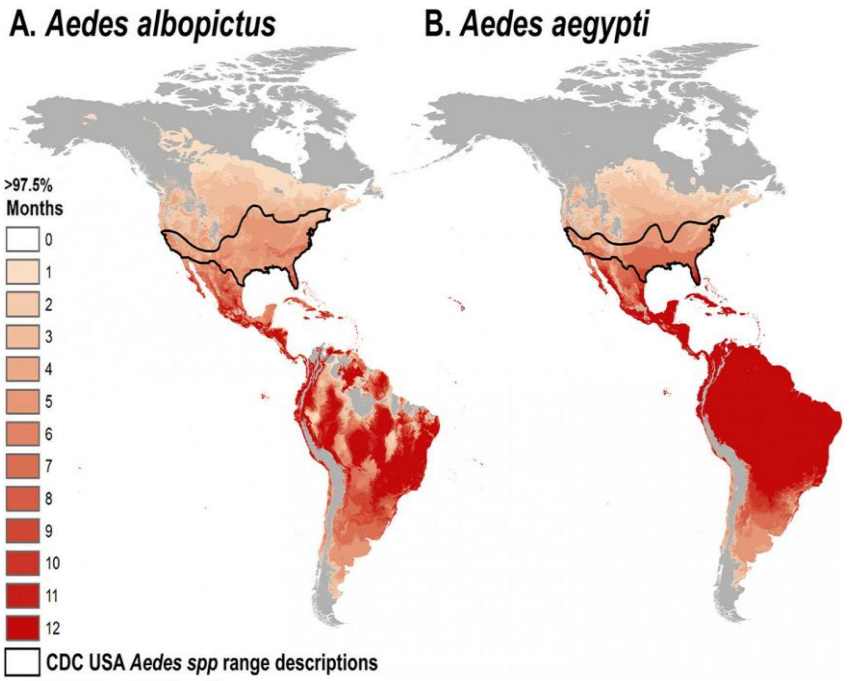
More than 40 percent of the **world's** population, in more than 100 countries are at risk of **dengue** infection.

The most significant **dengue** epid emics in recent years have occurred in Southeast Asia, the Americas and the Western Pacific. Each year, an estimated 390 million **dengue** infection s occur around the **world**.

VACCINE

Chemeri Vax Dengue

- A tetravalent vaccine
- Uses Yellow Fever vaccine as base
- 20% sero conversion
- Still under research



First , what is the criteria's of dengue hemorrhagic fever ???

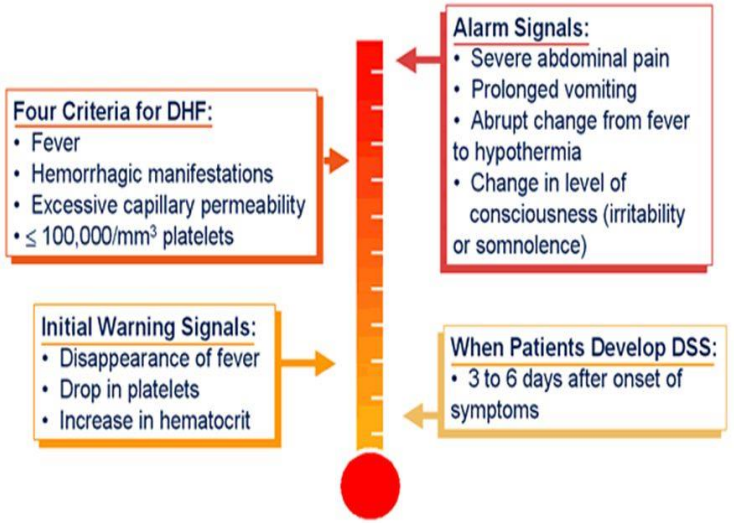
The 4 WHO Criteria for DHF

- ✓ **Fever**
- ✓ **Hemorrhagic manifestations(Symptoms)**
- ✓ **Low platelet count (100,000/mm ^{3} or less**
- ✓ **Elevated hematocrit (>20% then normal) or (> 50% THEN BASELINE)**

World Health Organization

2014 GUIDELINESS

Danger Signs in Dengue Hemorrhagic Fever



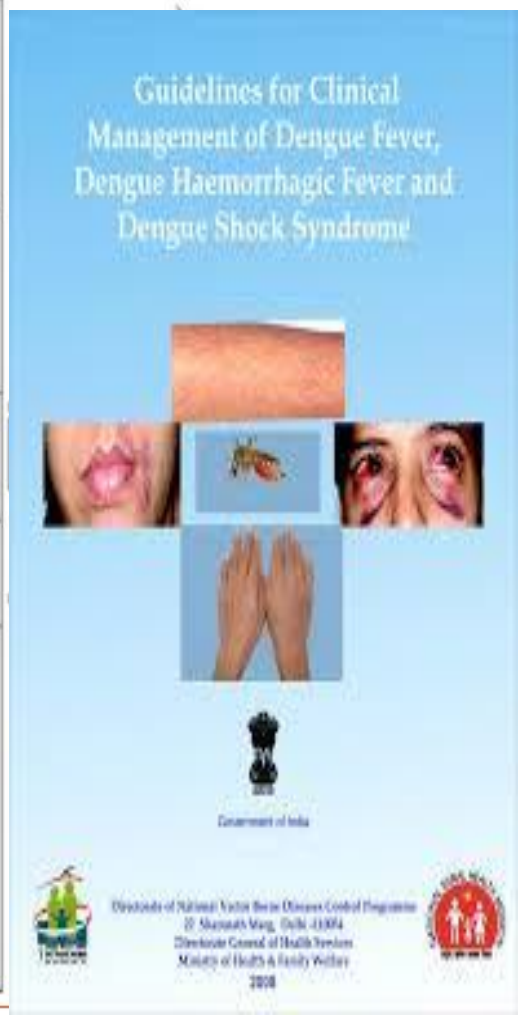
Clinical Syndrome

Dengue

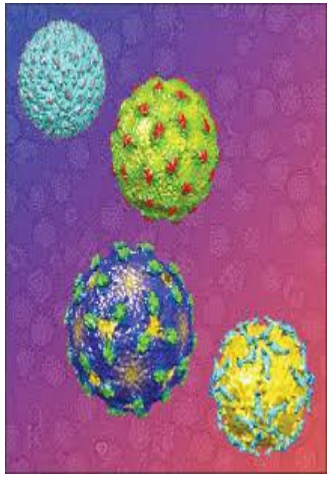
- **Most infections are benign & self-limiting**
  - 50-80% are asymptomatic or have undifferentiated fever
  - 4-7 day incubation period
- **Classic Dengue Fever**
  - **"Breakbone Fever"**
  - Severe back and bone pain
  - Morbiliform maculopapular rash sparing palms and soles
- **Dengue Hemorrhagic Fever**
  - Bruises, epistaxis, gum and GI bleed
- **Dengue Shock Syndrome**
  - Hypotension

2-3 days

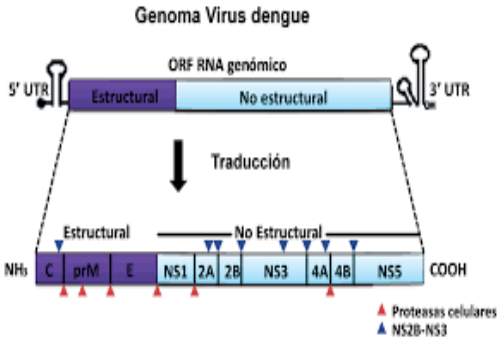
3-7 days



04 serotypes

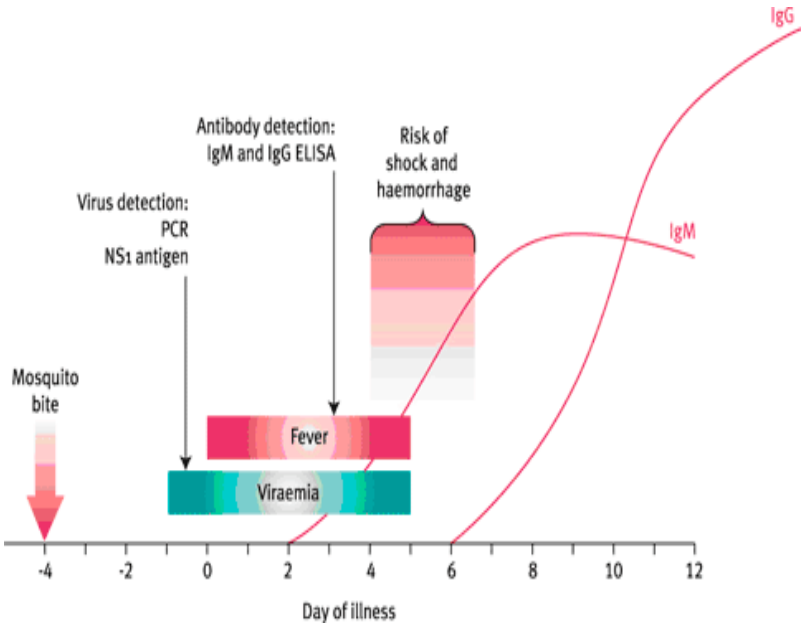


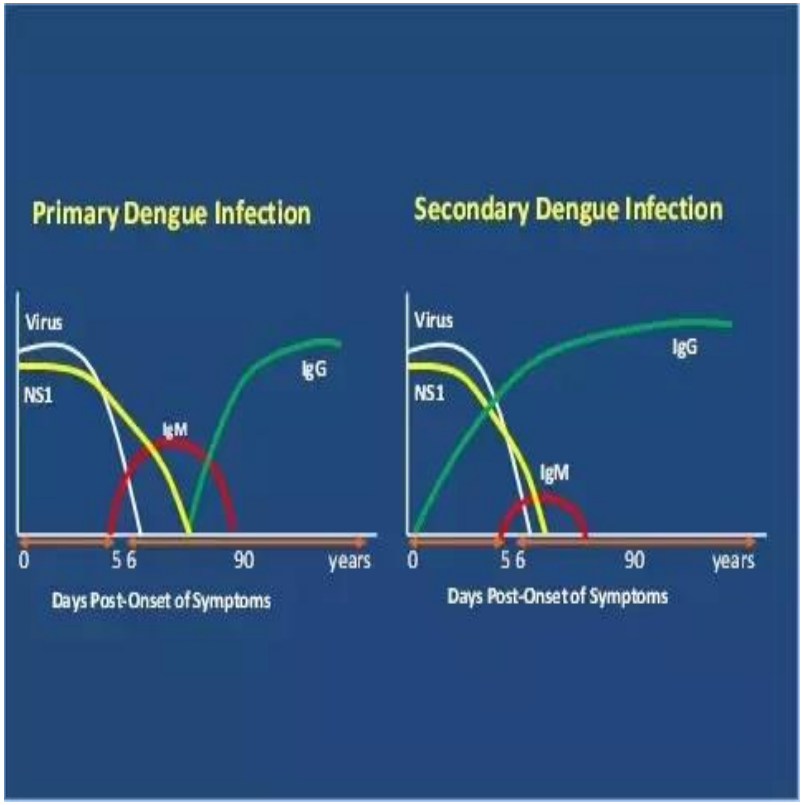
DENV1,DENV2, DENV3, DENV4



NS1 Primary and Secondary DENV

Why NS1 for Dengue acute

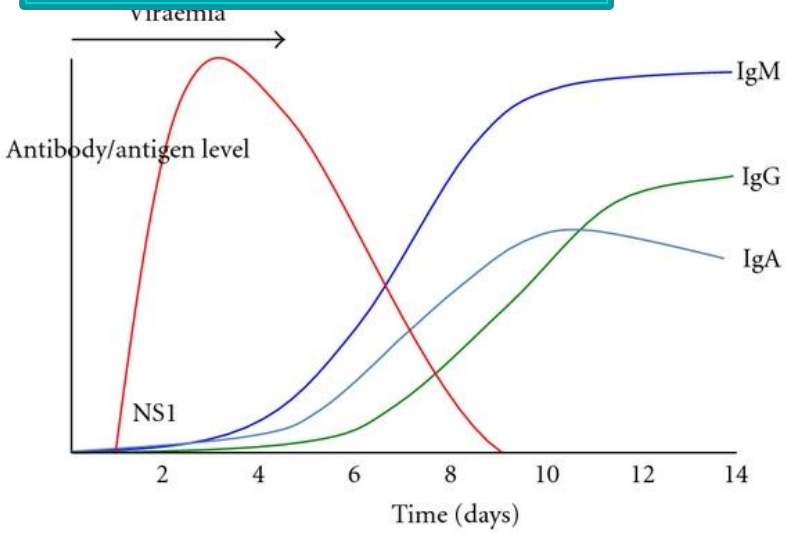




Dengue virus diagnostic

Dengue fever is considered one of the most emerging diseases causing successive epidemics practically over all tropical and subtropical regions of the world . It is estimated that 50 to100 million of dengue cases in worldwide.

Biomarkers for dengue virus : NS1



Hangzhou Clongene Biotech Co., Ltd

Read out Time: 15-20minutes

Research Article

Received: 29 September 2013 Revised: 15 December 2013 Accepted article published: 6 January 2014 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jctb.4305

Electrochemical detection of dengue virus NS1 protein with a poly(allylamine)/carbon nanotube layered immunoelectrode

Mízia M. S. Silva,[a] Ana C. M. S. Dias,[a] Bárbara V. M. Silva,[a] Sérgio L. R. Gomes-Filho,[a] Lauro T. Kubota,[b,c] Marília O. F. Goulart[c,d] and Rosa F. Dutra[a]*

Abstract

BACKGROUND: A sensitive nanostructured immunoelectrode based on poly(allylamine) (PAH) sandwich is developed for non-

A nano-hybrid (CNT and polymer) immunosensor

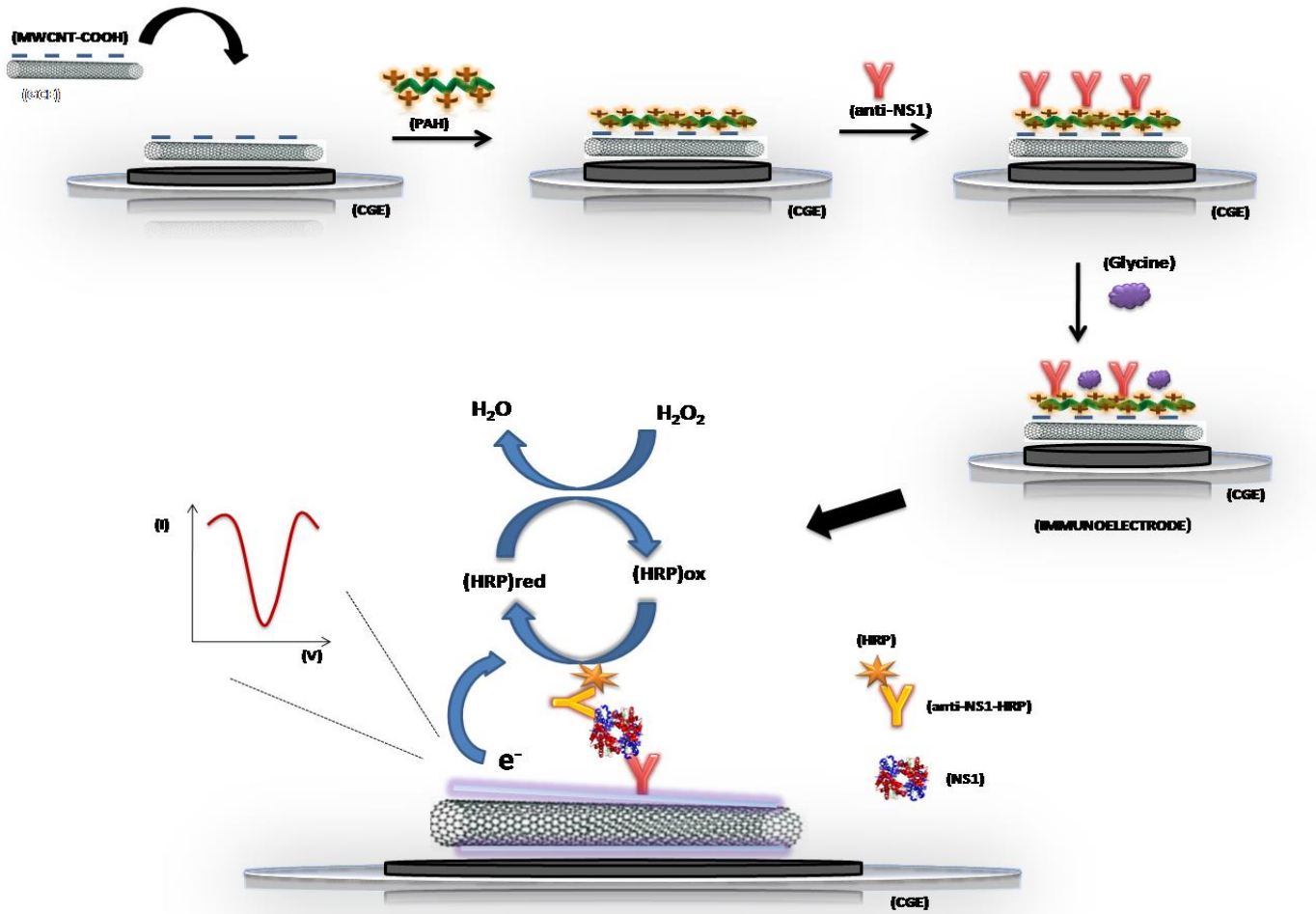
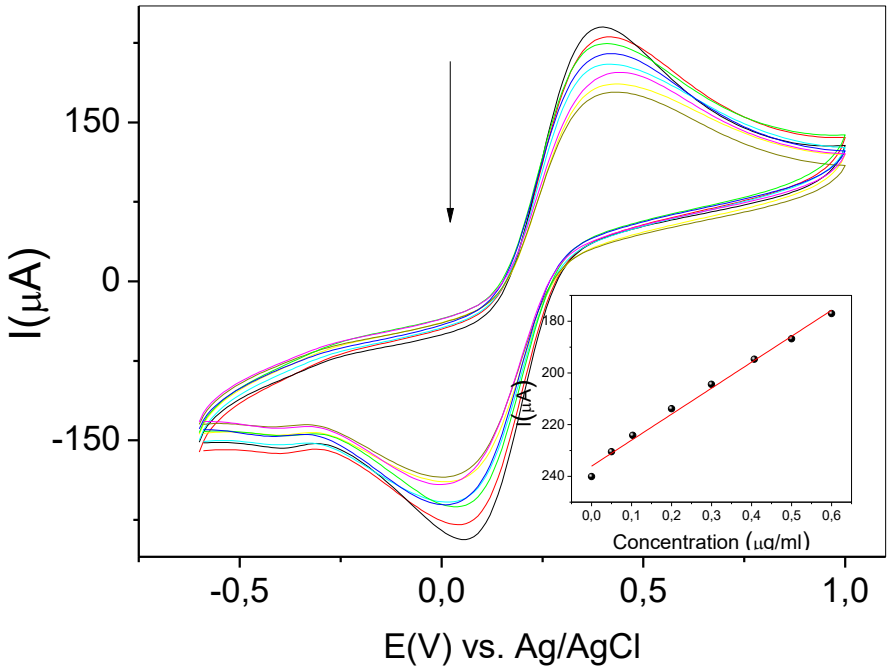


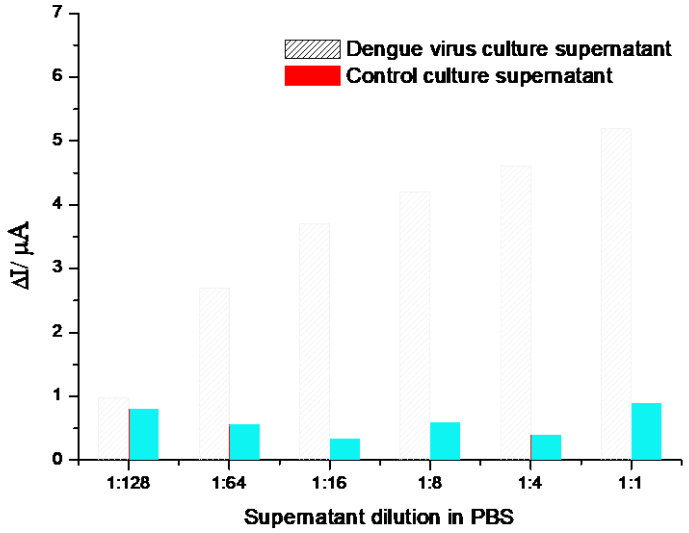
Figure 4. (A) Principle of the amperometric immunosensor showing the stepwise construction of immunoelectrode and principle of immunoassay

Response to NS1

(a)



(b)



Analytical responses of immunosensor (a) at different concentrations of NS1 (b) NS1 native glycoprotein from virus culture and control (CD4 cells) in serial dilutions. The amperometric signals obtained by DDP in 5mmol L^{-1} of $K_3Fe(CN)_6/K_4Fe(CN)_6$.

Biosensors and Bioelectronics 44 (2013) 216–221

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A sensor tip based on carbon nanotube-ink printed electrode for the dengue virus NS1 protein

Ana Carolina M.S. Dias, Sérgio L.R. Gomes-Filho, Mízia M.S. Silva, Rosa F. Dutra*

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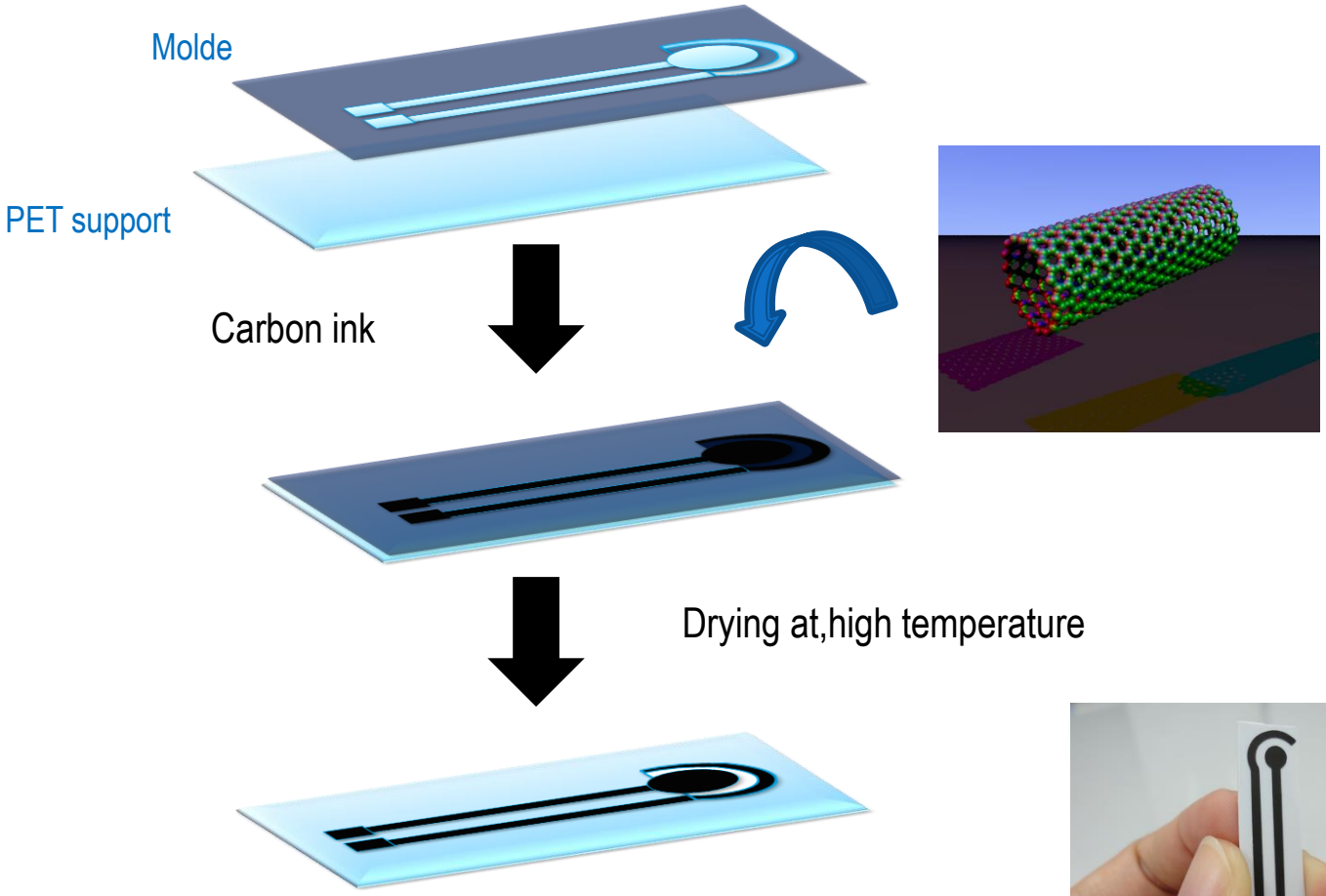
ABSTRACT

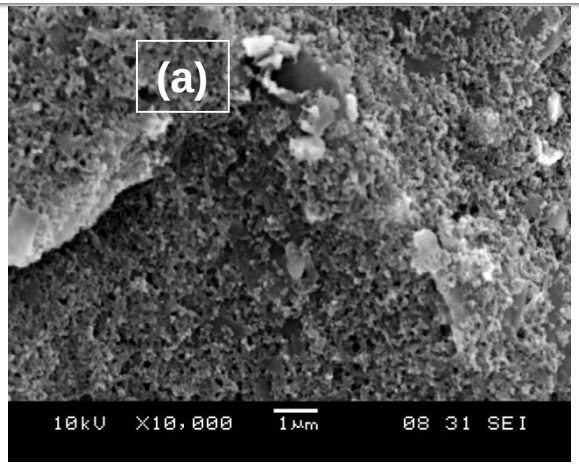
An immunosensor for the non-structural protein 1 (NS1) of the dengue virus based on carbon nanotube-screen printed electrodes (CNT-SPE) was successfully developed. A homogeneous mixture containing carboxylated carbon nanotubes was dispersed in carbon ink to prepare a screen printed working electrode. Anti-NS1 antibodies were covalently linked to CNT-SPE by an ethylenediamine film strategy. Amperometrical responses were generated at −0.5 V vs. Ag/AgCl by hydrogen peroxide reaction with peroxidase (HRP) conjugated to the anti-NS1. An excellent detection limit (in the order of 12 ng mL^{-1}) and a sensitivity of 85.59 μA mM^{-1} cm^{-2} were achieved permitting dengue diagnostic according to the clinical range required. The matrix effect, as well as the performance of the assays, was successfully evaluated using spiked blood serum sample obtaining excellent recovery values in the

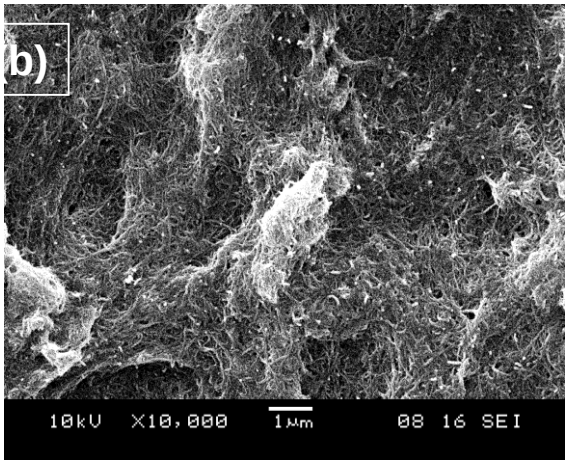
A screen printed electrode based on carbon nanotube for dengue virus NS1 protein



Dutra et al., Biosensors & Bioelectronics, 2012

Carbon nanotube – Modified screen printed electrode





Micrography of **(a)** EI without NTC e **(b) EI with** MWCNT-NH_2 (1 mg/mL).

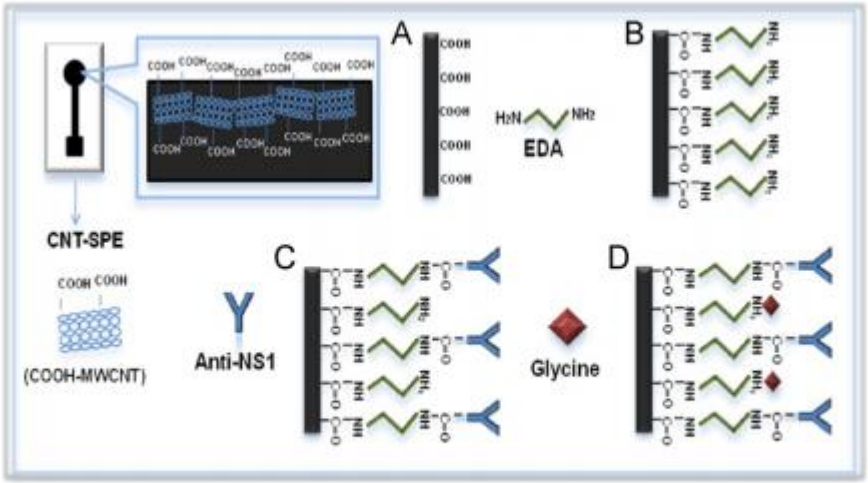
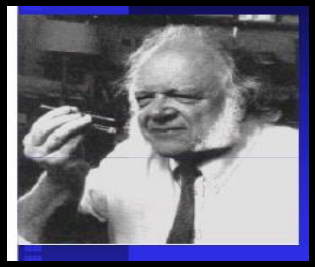


Fig. 2. Schematic illustration of the stepwise preparation of the immunosensor: (a) bare CNT-SPE, (b) EDA film formation, (c) anti-NS1 immobilization, and (d) blocking with glycine.

The state -of -art" … Evolution of POC



ENZYMATIC POCT

Glucose Sensors

Cholesterol, uric acid, etc

POCT FOR IMMUNOASSAY AND MOLECULAR BIOLOGY

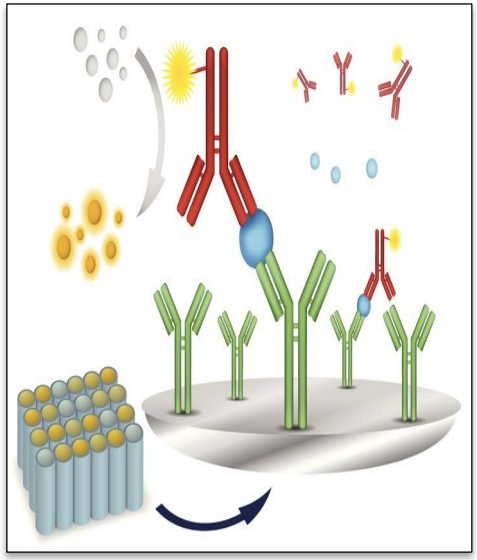
Disease Diagnostic

Terapeutic Monitoring

Immunoassay
Biosensor and Chemical analysis

ELISA METHOD

ECLIA METHOD



BIOSENSOR METHOD

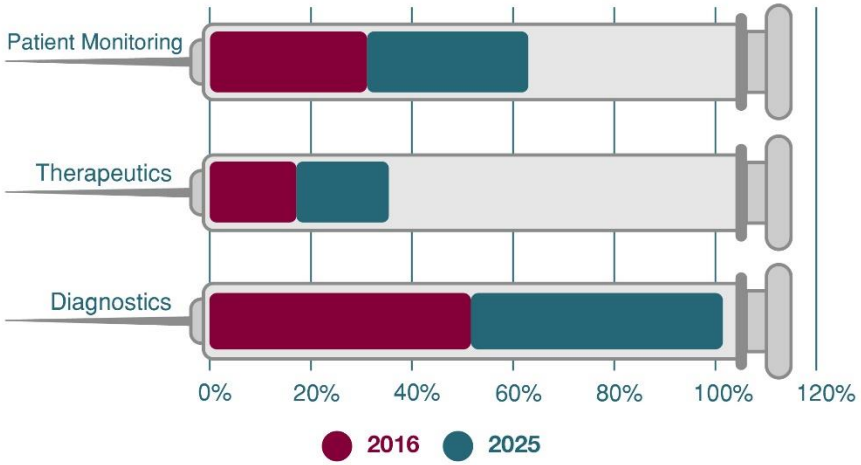
BIACore 2000- Optical SPR Technology (Biosensor)

GLOBAL DISPOSABLE MEDICAL DEVICE SENSOR MARKET (2017-2025)

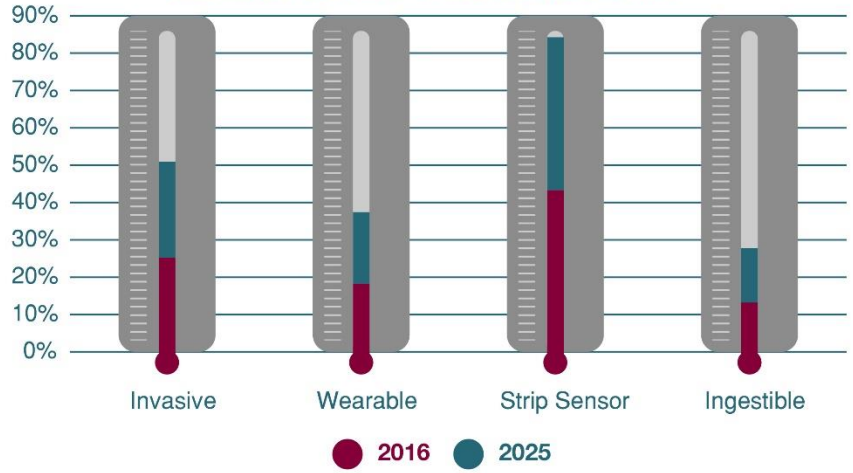
TOP COMPANIES

- ANALOG DEVICES
- COVIDIEN PLC
- GE HEALTHCARE
- HMICRO

MARKET BY APPLICATION



MARKET BY PLACEMENT



www.inkwoodresearch.com

Point-of-care Immunosensors : Challenges…

- Free sample preparation"mix and measure";
- User friendly processing (practical analyses)
- Lower limit of detection;
- Lower cost;
- More reliability;
- More reproducibility;
- Compatible with established readout systems like the glucose

- Adaptable to run or read in smartphone, tablet,
- Lower power consum, etc…

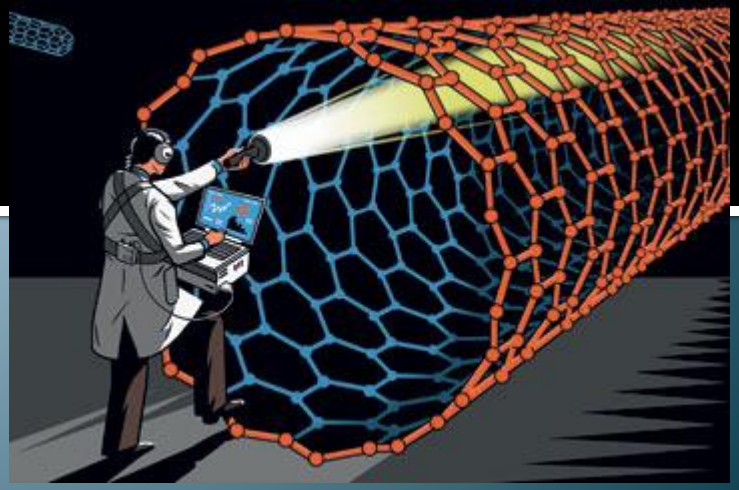
LAPED

Laboratório de Pesquisa em Diagnóstico

Our attemptings to solve some problems in health

LEB

Laboratório de Engenharia Biomédica



Strategies:

- Nanomaterial synthetically dedicated;
- Bioreceptors with high specificity (or non);
- Transducers with simple electronics;
- Friendly use readout system;
- Non redox-probe (probeless), and label-free detection.

ARTICLE PREVIEW

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NATURE MATERIALS | COMMENTARY

The era of carbon allotropes

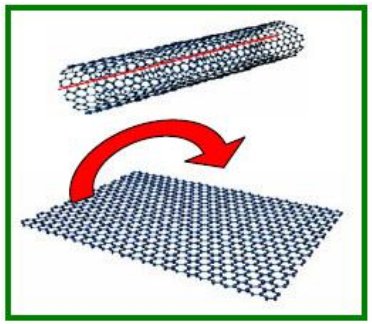
Andreas Hirsch

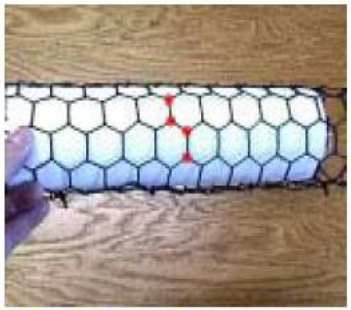
Nature Materials 9, 868–871 (2010) | doi:10.1038/nmat2885
Published online 22 October 2010

Twenty-five years on from the discovery of C_{60}, the outstanding properties and potential applications of the synthetic carbon allotropes — fullerenes, nanotubes and graphene — overwhelmingly illustrate their unique scientific and technological importance.

Chemical structures of carbon nanotubes

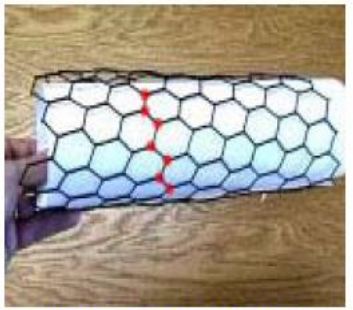
- The way how the graphene sheet is rolled up into a cylinder can form different types of CNTs



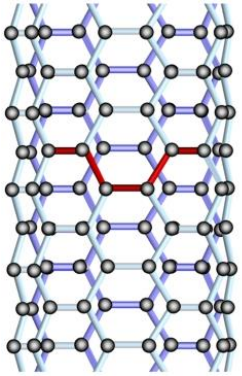


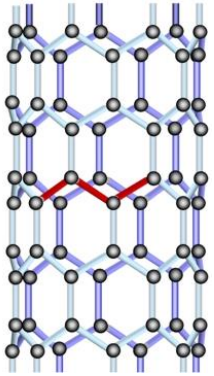
Armchair arrangement of carbon atoms

Zig-zag arrangement of carbon atoms



Chiral arrangement of carbon atoms





Metallic
Airmchair

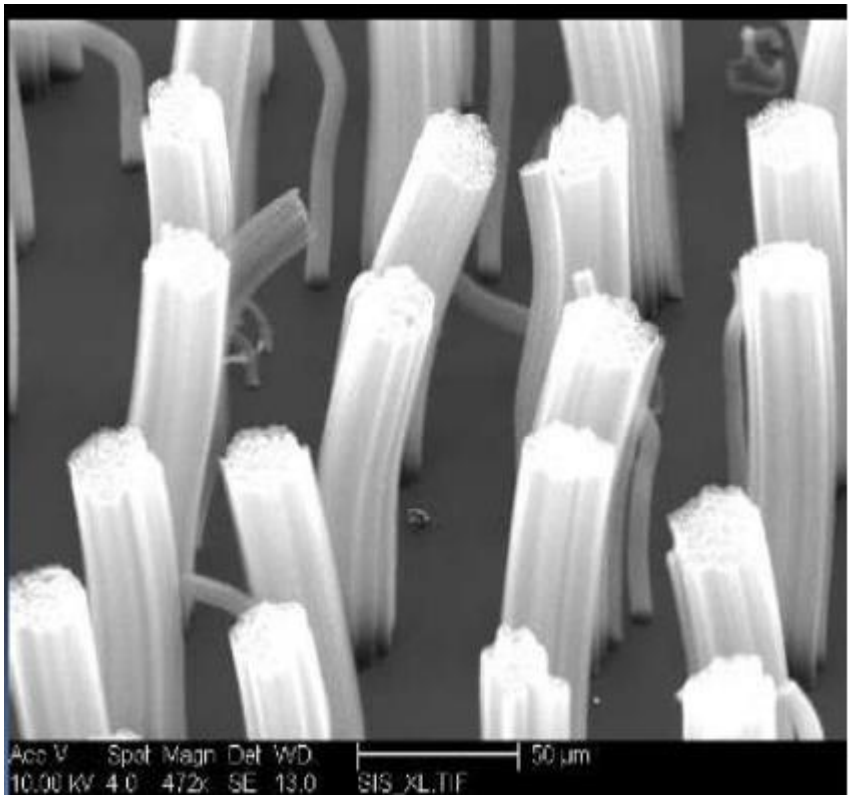
Semiconductor
Zig-Zag

- The structure of carbon nanotubes also determines their physical and chemical properties, where armchair nanotubes are always metallic in terms of electrical conductivity, zig-zag and chiral forms are semiconducting.

Carbon nanotube synthesis

- Techniques have been developed to produce CNTs in sizeable quantities, some of them are:

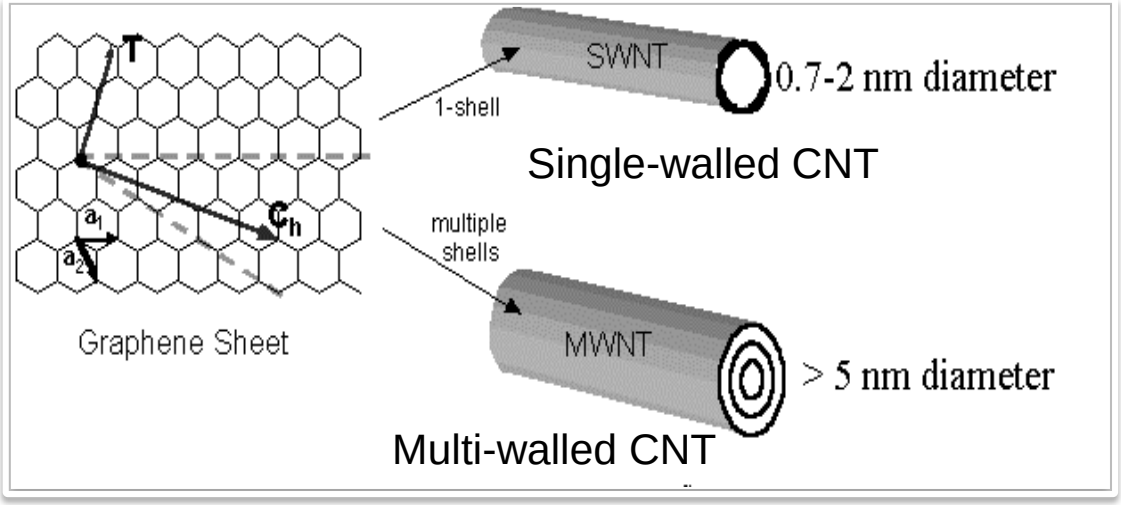
  - Arc discharge
  - Laser ablation
  - Chemical vapor deposition (CVD).

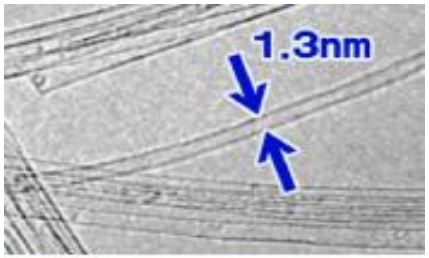


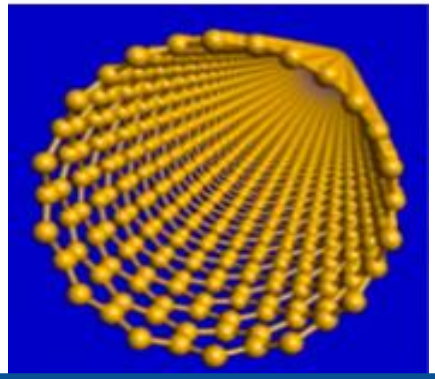
CNTs were firstly discovered by Sumio Iigima (1991).

Single and Multiple walled CNTs

- Carbon nanotubes are extremely small! They have diameters on the order of a few nanometers and lengths that can be as much as several millimeters.



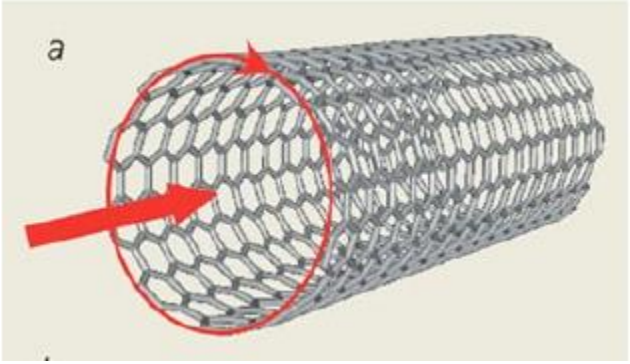




Single walled carbon nanotube -SWNT

Interesting properties of carbon nanotubes!

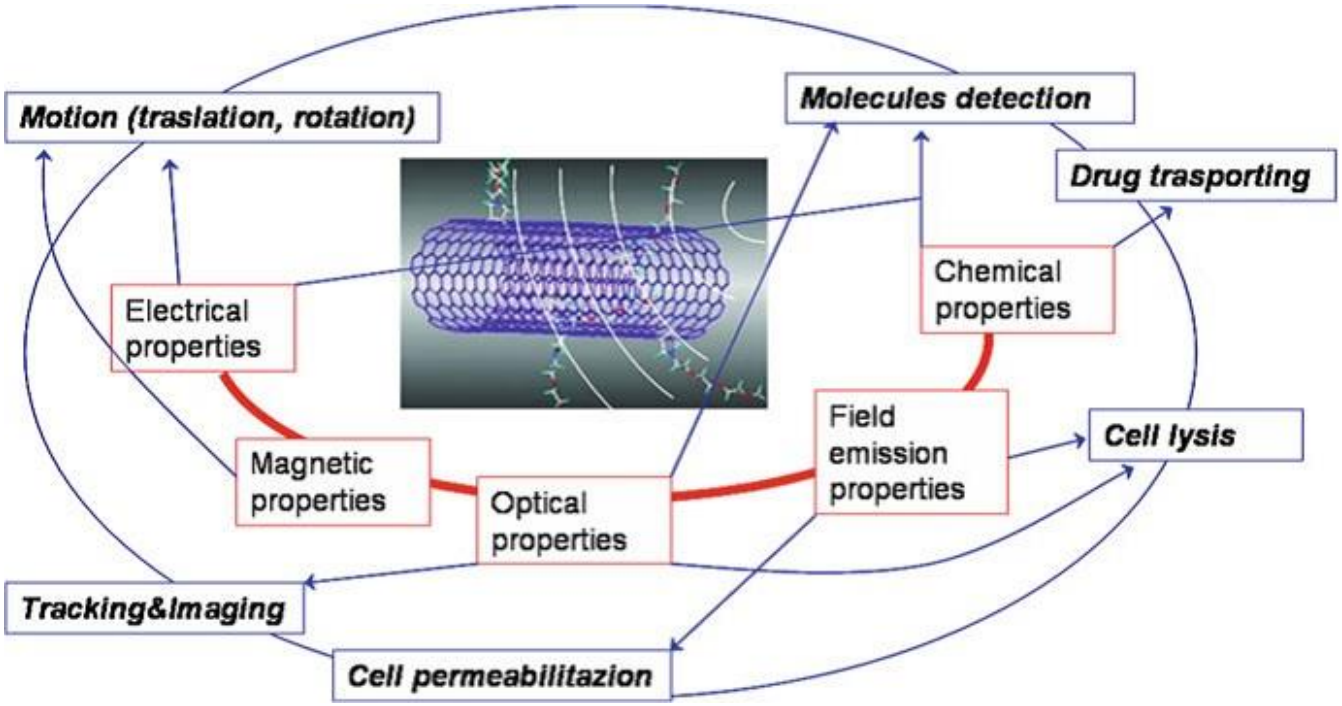
- High electrical conductivity (10^{-6} ohm typically)
- High thermal conductivity (1750-58 00 W/mK)
- High mechanical strength (tensile strength 60GPa) and modulus (Young's modulus 1TPa).
- High aspect ratio structures with diameters in nanometers, lengths in microns. Collectively, nanotubes can exhibit extremely high surface area



Magnetic field introduces a phase factor to the electron wavefunction in the circumferential direction. As a result, the electronic properties of a nanotube can be modulated by a magnetic field.

Discovered about new properties of nanomaterials !

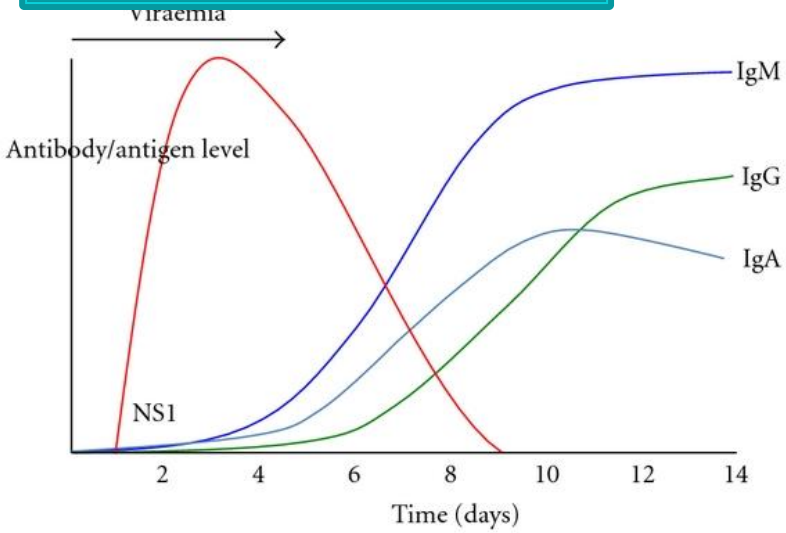
- Currently, the physical properties of carbon nanotubes are still being discovered and described!!



Dengue virus diagnostic

Dengue fever is considered one of the most emerging diseases causing successive epidemics practically over all tropical and subtropical regions of the world . It is estimated that 50 to100 million of dengue cases in worldwide.

Biomarkers for dengue virus : NS1



Hangzhou Clongene Biotech Co., Ltd

Read out Time: 15-20minutes

Research Article

Received: 29 September 2013 Revised: 15 December 2013 Accepted article published: 6 January 2014 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jctb.4305

Electrochemical detection of dengue virus NS1 protein with a poly(allylamine)/carbon nanotube layered immunoelectrode

Mízia M. S. Silva,[a] Ana C. M. S. Dias,[a] Bárbara V. M. Silva,[a] Sérgio L. R. Gomes-Filho,[a] Lauro T. Kubota,[b,c] Marília O. F. Goulart[c,d] and Rosa F. Dutra[a]*

Abstract

BACKGROUND: A sensitive nanostructured immunoelectrode based on poly(allylamine) (PAH) sandwich is developed for non-

A nano-hybrid (CNT and polymer) immunosensor

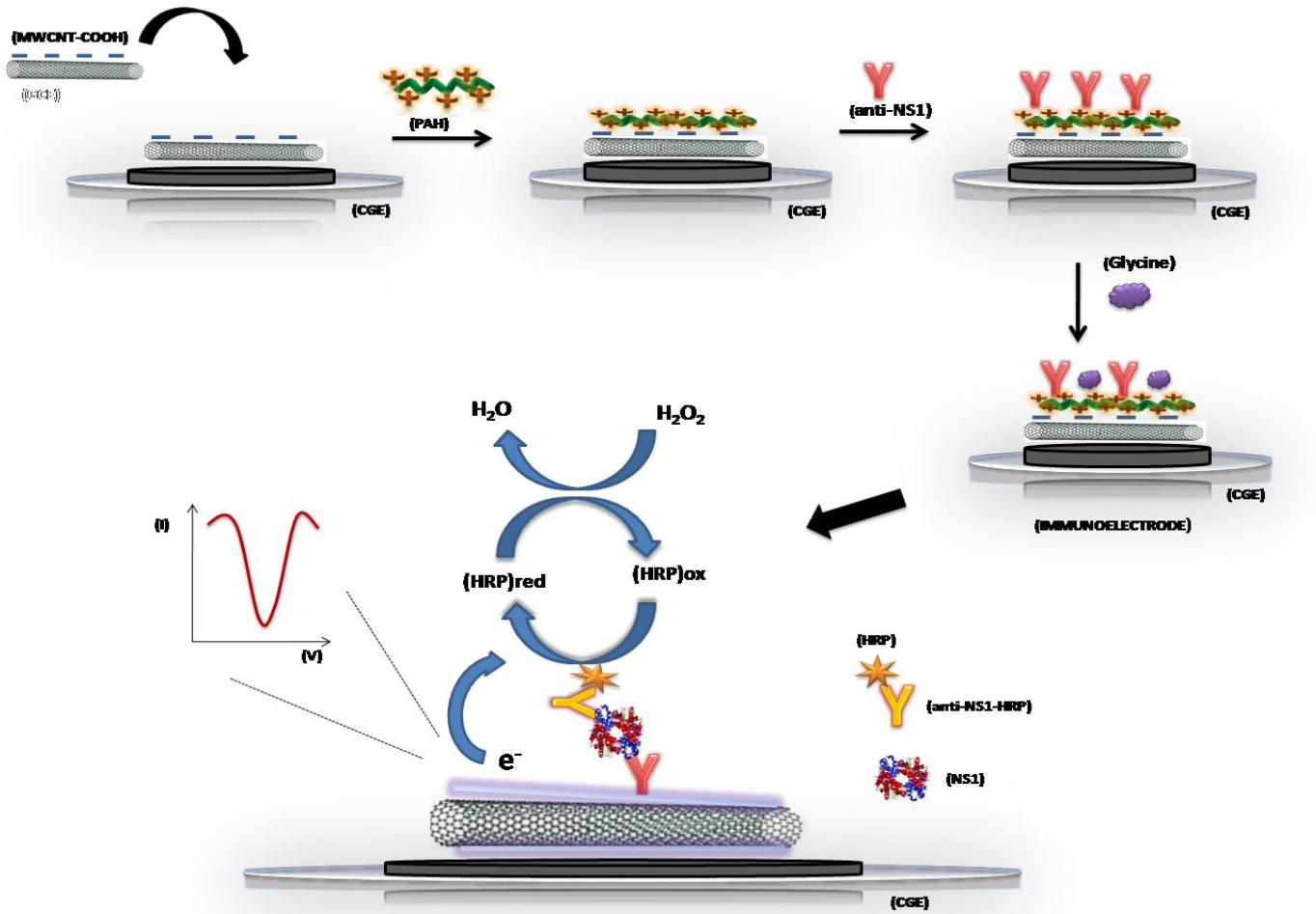
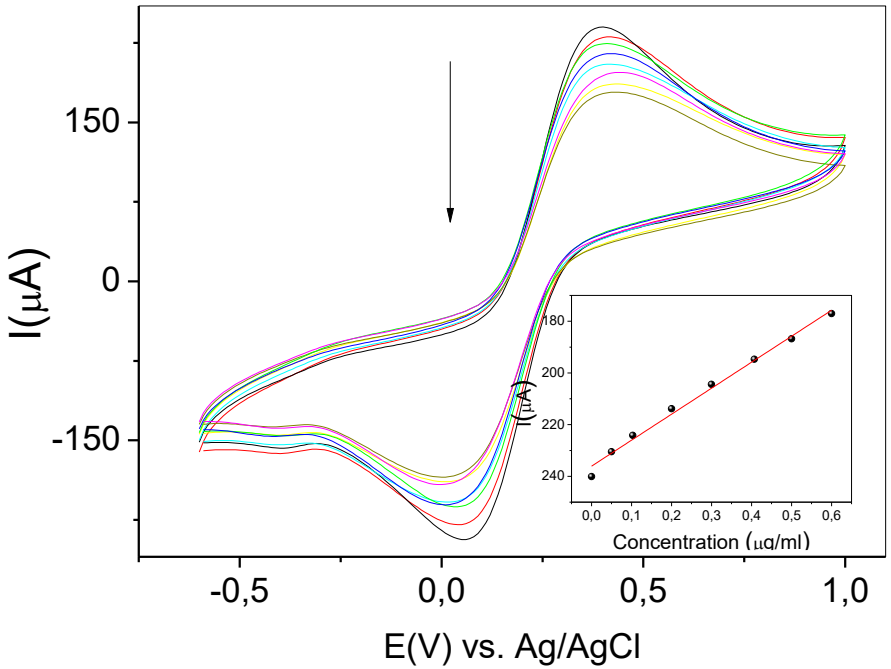


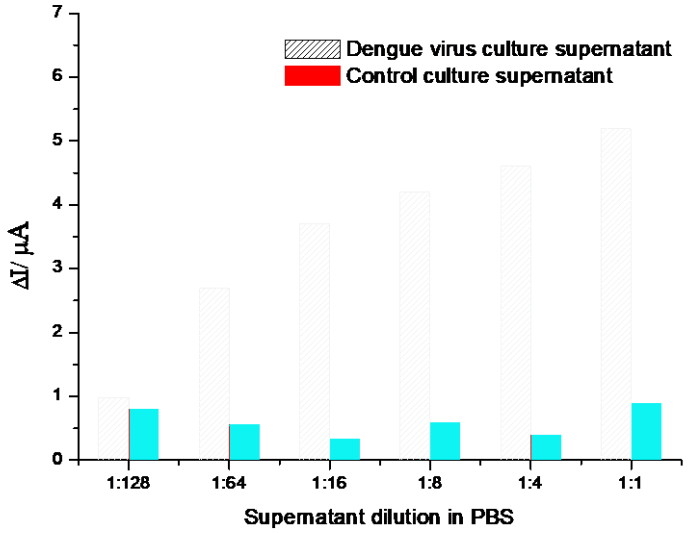
Figure 4. (A) Principle of the amperometric immunosensor showing the stepwise construction of immunoelectrode and principle of immunoassay

Response to NS1

(a)

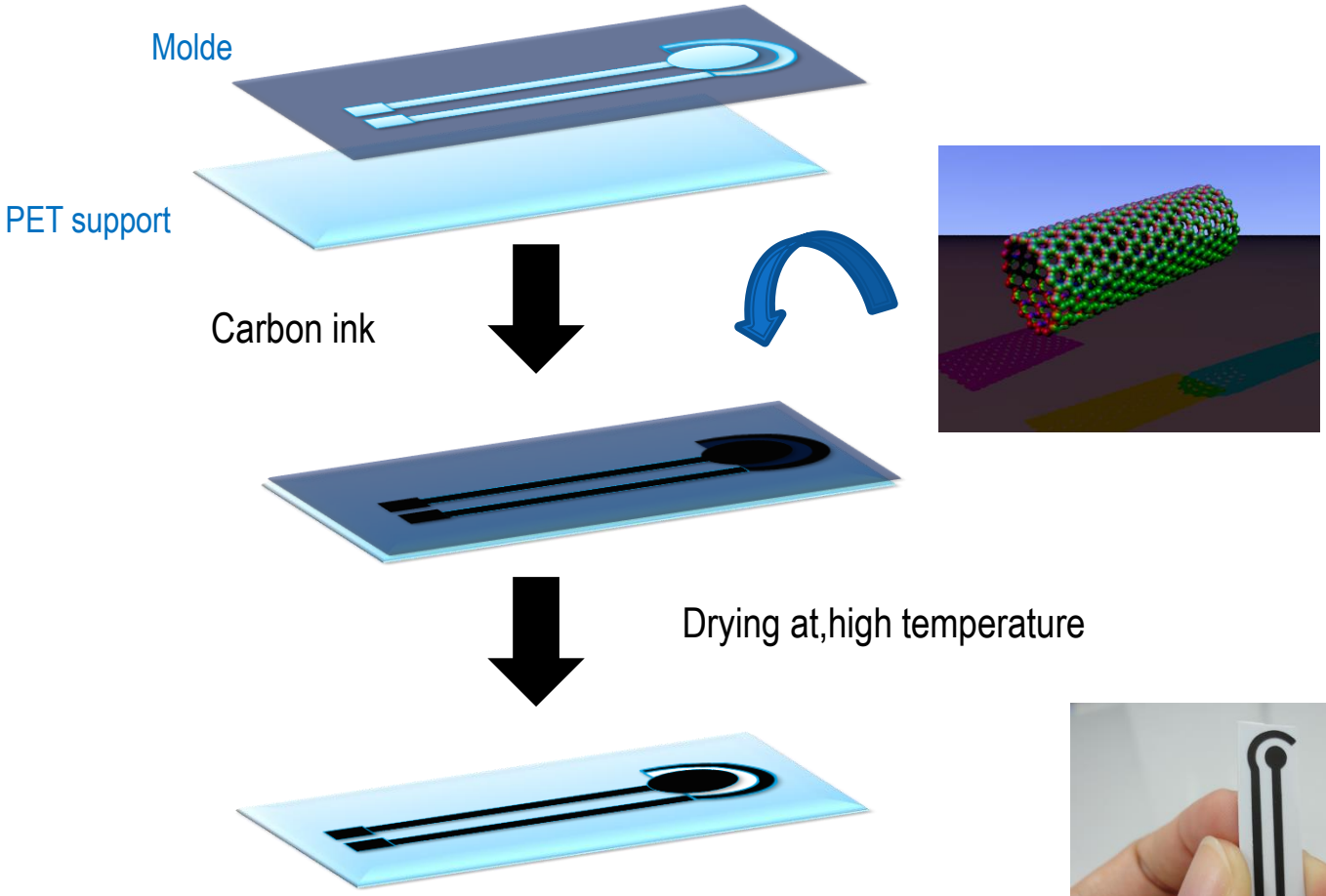


(b)



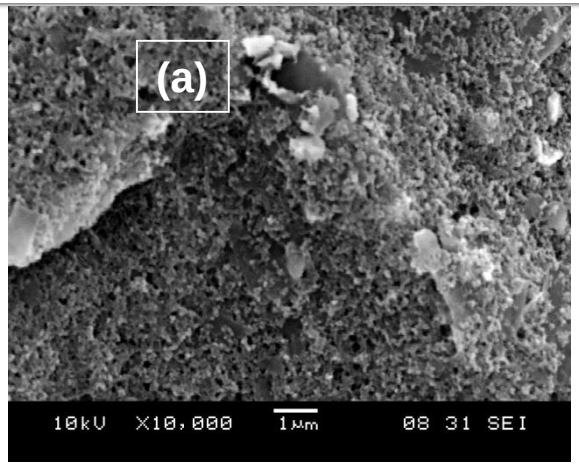
Analytical responses of immunosensor (a) at different concentrations of NS1 (b) NS1 native glycoprotein from virus culture and control (CD4 cells) in serial dilutions. The amperometric signals obtained by DDP in 5mmol L^{-1} of $K_3Fe(CN)_6/K_4Fe(CN)_6$.

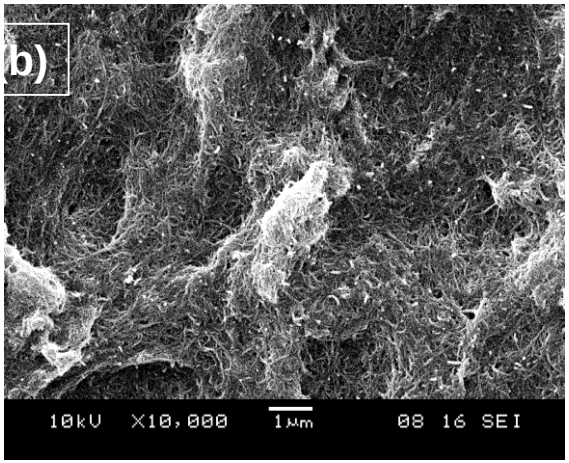
A screen printed electrode based on carbon nanotube for dengue virus NS1 protein



Dutra et al., Biosensors & Bioelectronics, 2012

Carbon nanotube – Modified screen printed electrode





Micrography of **(a)** EI without NTC e **(b) EI with** MWCNT-NH_2 (1 mg/mL).

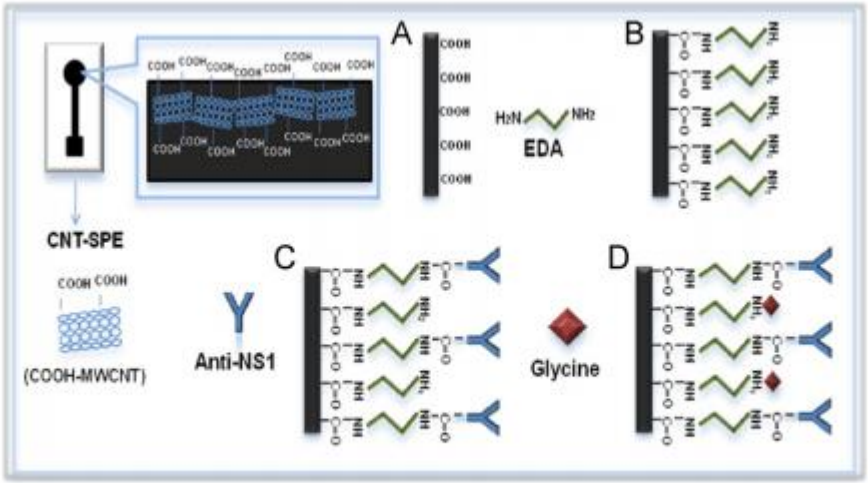
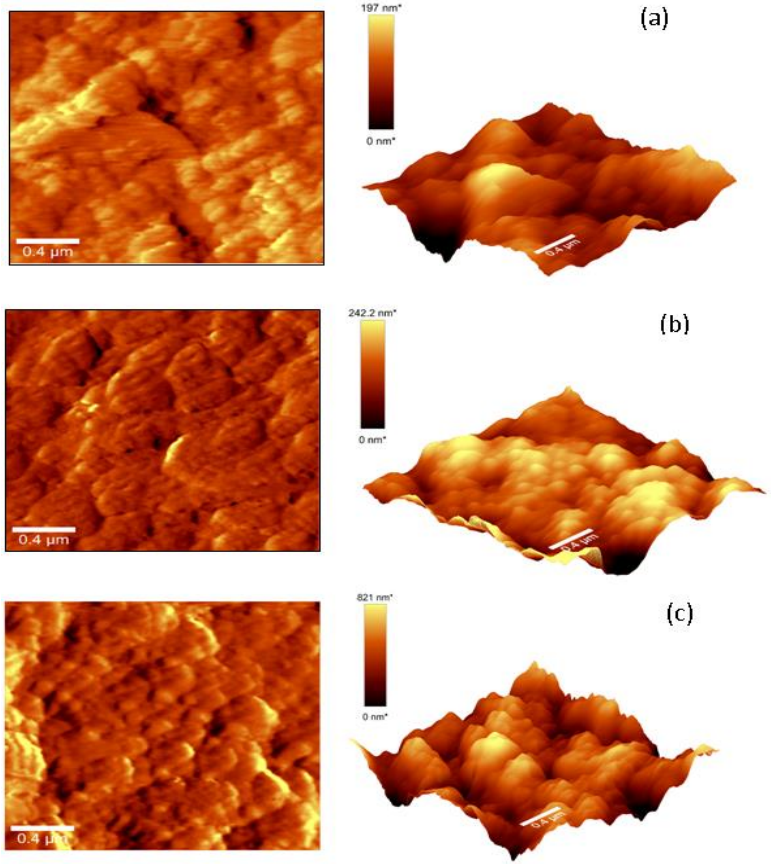
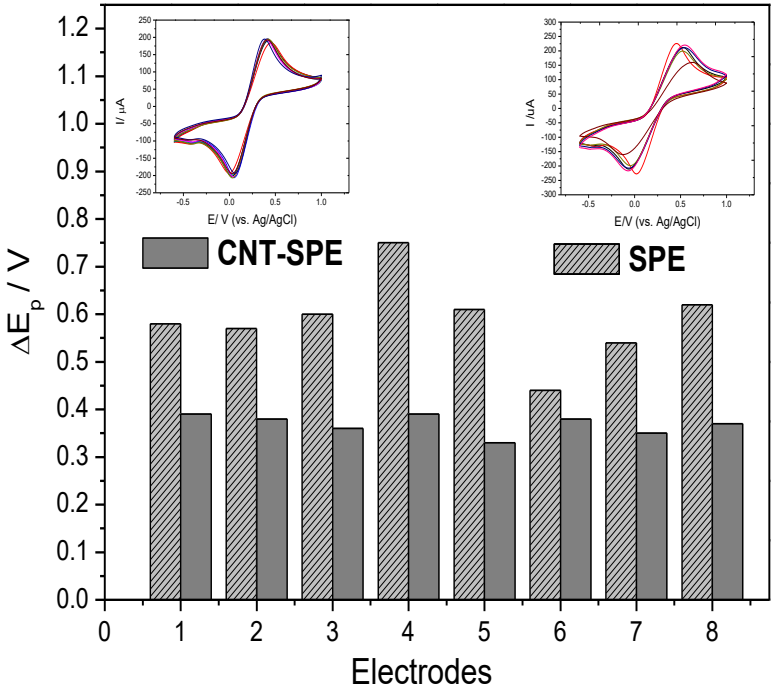


Fig. 2. Schematic illustration of the stepwise preparation of the immunosensor: (a) bare CNT-SPE, (b) EDA film formation, (c) anti-NS1 immobilization, and (d) blocking with glycine.

Characterization of SPE-NS1



AFM images: (a) bare CNT-SPE; (b) CNT-SPE modified by EDA and (c) CNT-SPE with immobilized anti-NS1.



Effect of CNT on the reproducibility of SPEs. CV = 3.1% against 12,9% (without CNTs)

Analytical response to NS1

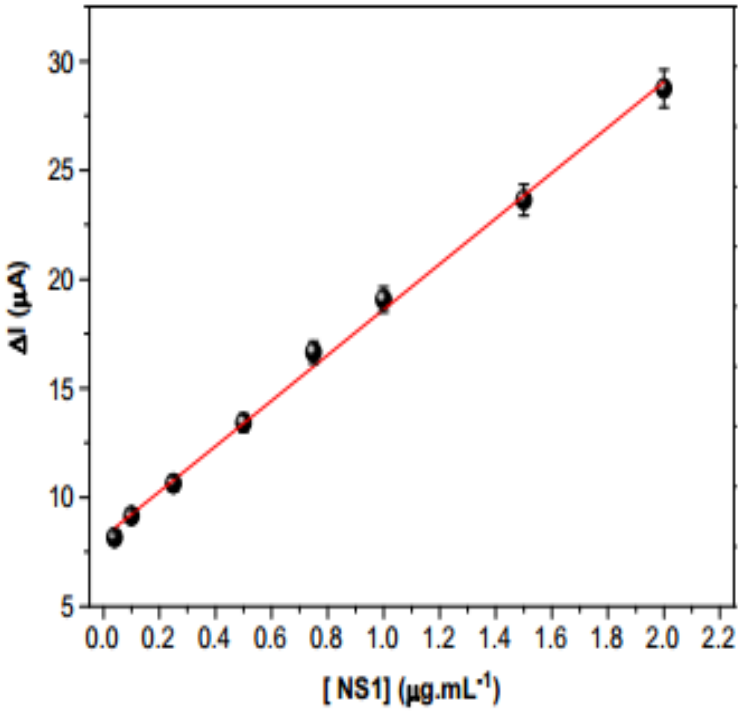


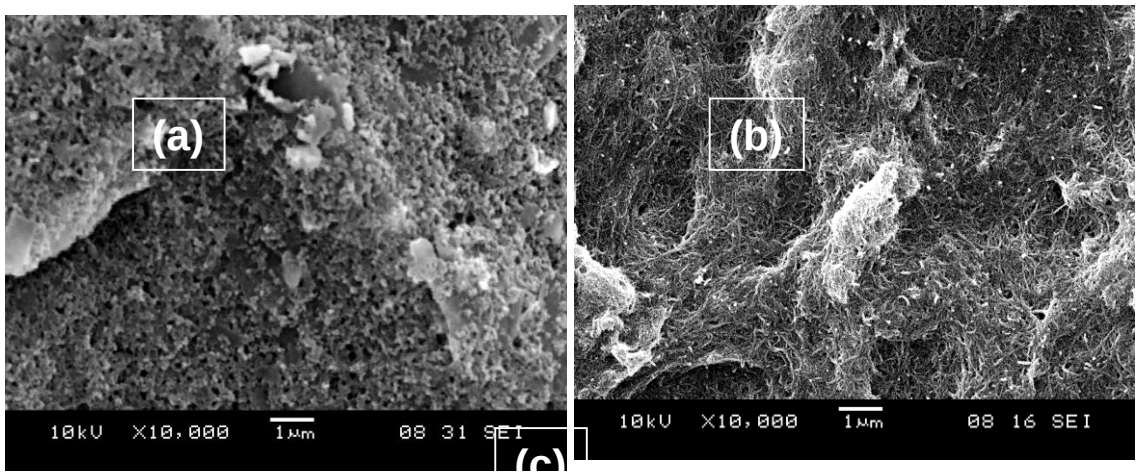
Fig. 5. Calibration curve of the immunosensor for NS1 protein of dengue virus.

Table 1
Recovery values in spiked PBS and serum samples based on the immunosensor. In all cases, $n=3$.

Concentration (ng mL^{-1})	PBS		Serum	
	Found concentration (ng mL^{-1})	Recovery (%)	Found concentration (ng mL^{-1})	Recovery (%)
0.10	0.104	104.0	0.116	116.0
0.50	0.510	102.0	0.512	102.4
1.00	1.030	103.0	1.010	101.0
1.50	1.480	98.7	1.470	98.0

An excellent reproducibility was obtained (coefficient of variation CV = 3.4%). The proposed immunosensor also showed good repeatability by measuring 10.
The proposed immunosensor also showed good repeatability by measuring 10, CV = 3,2%

Carbon nanotube – Modified screen printed electrode for troponinT : detection of myocardial infartion – Label free detection



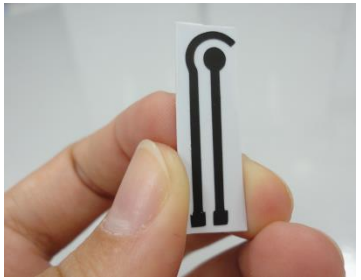
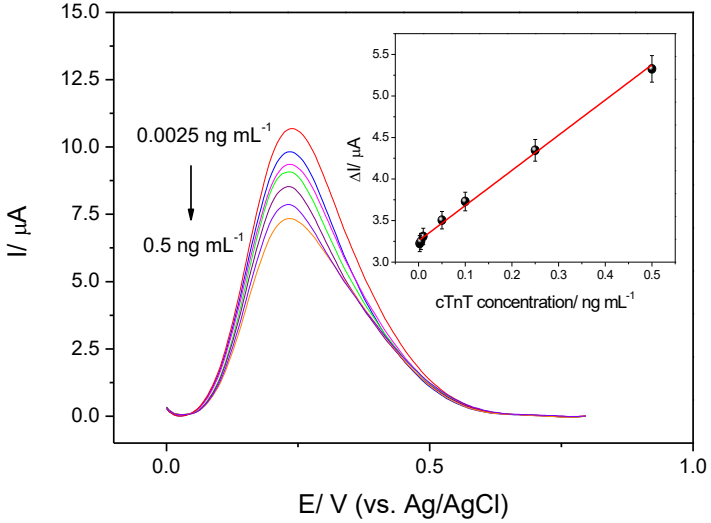
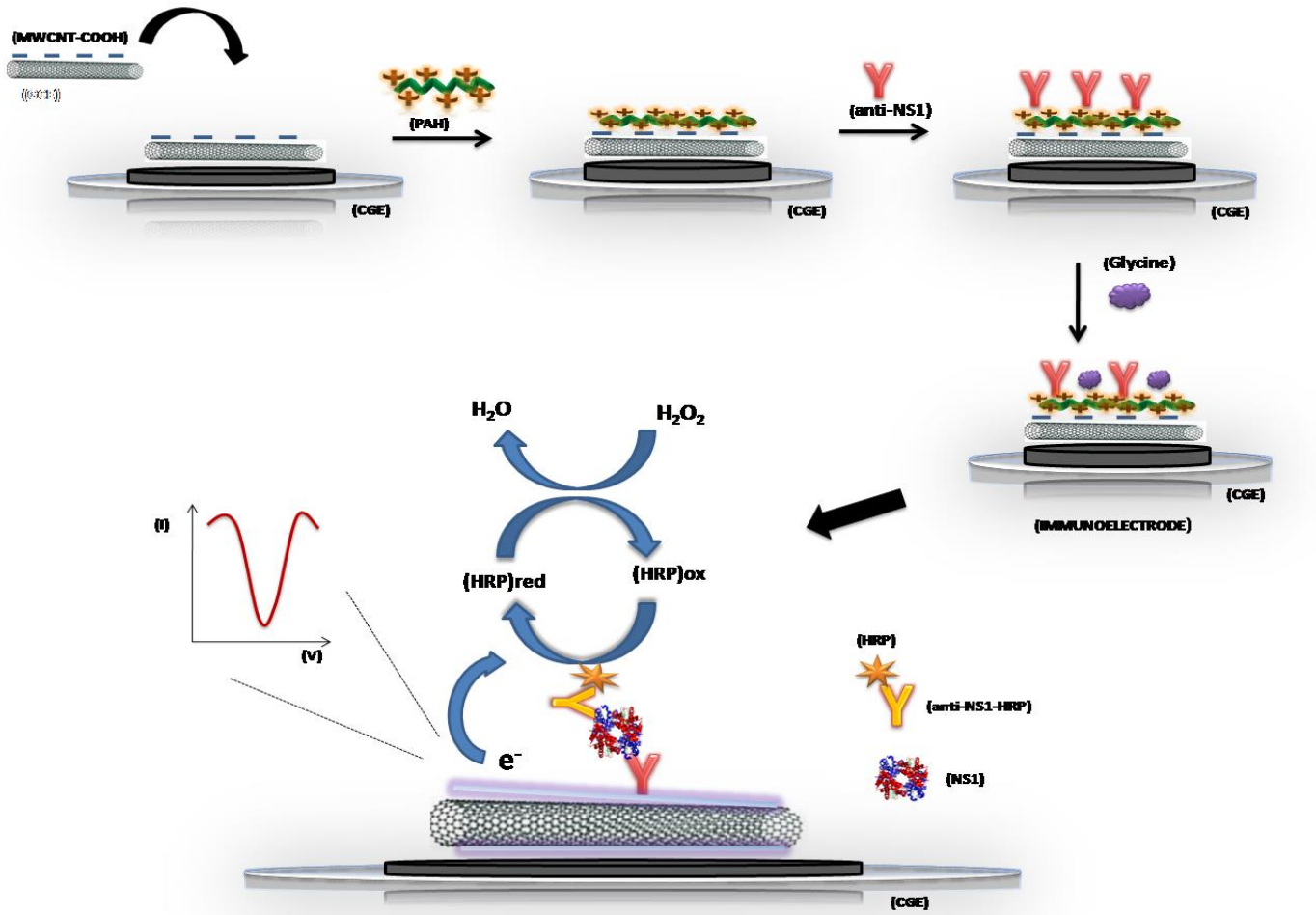


Fig. 16: MEV of **(a)** EI without NTC e **(b) EI with** MWCNT-NH_2 (1 mg/mL).

Silva et al., Talanta, 2013



A nano-hybrid (CNT and polymer) immunosensor



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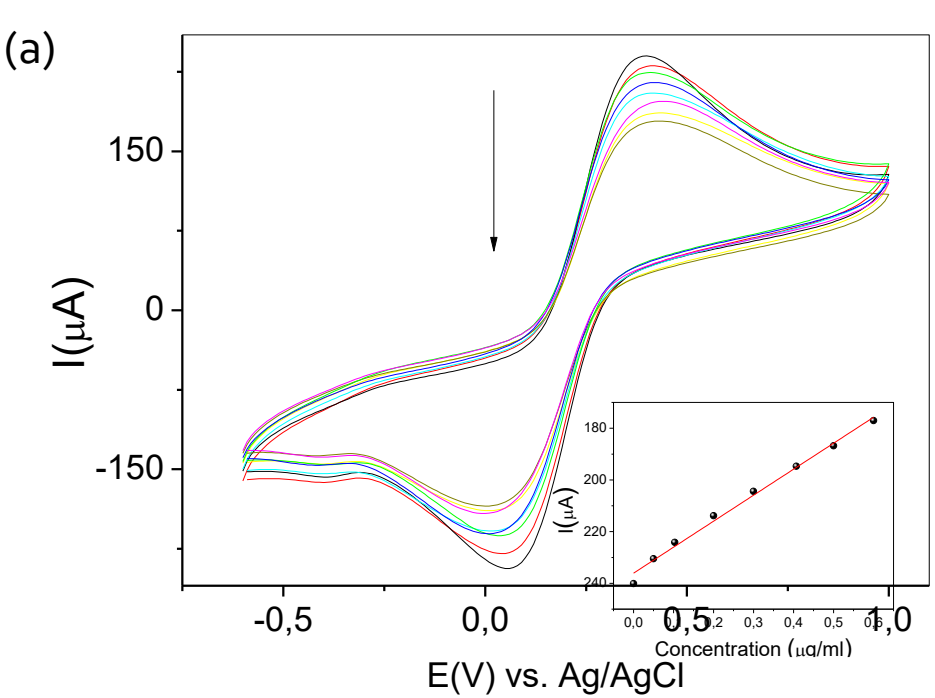

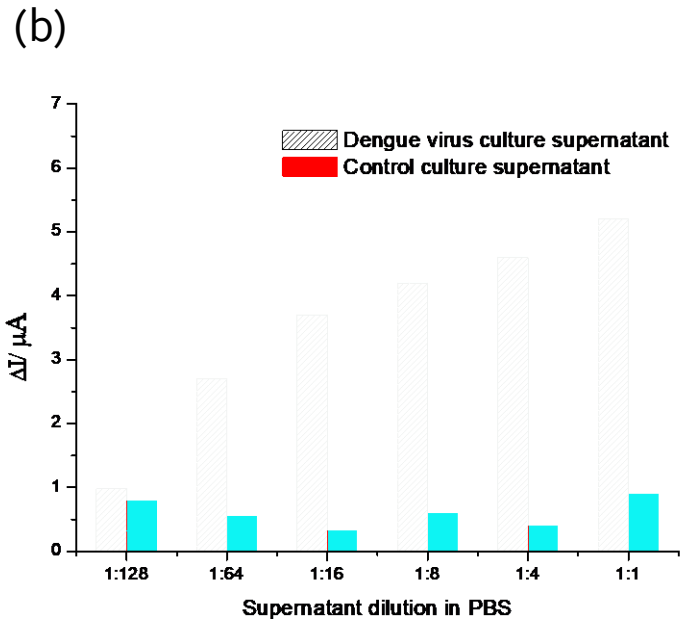

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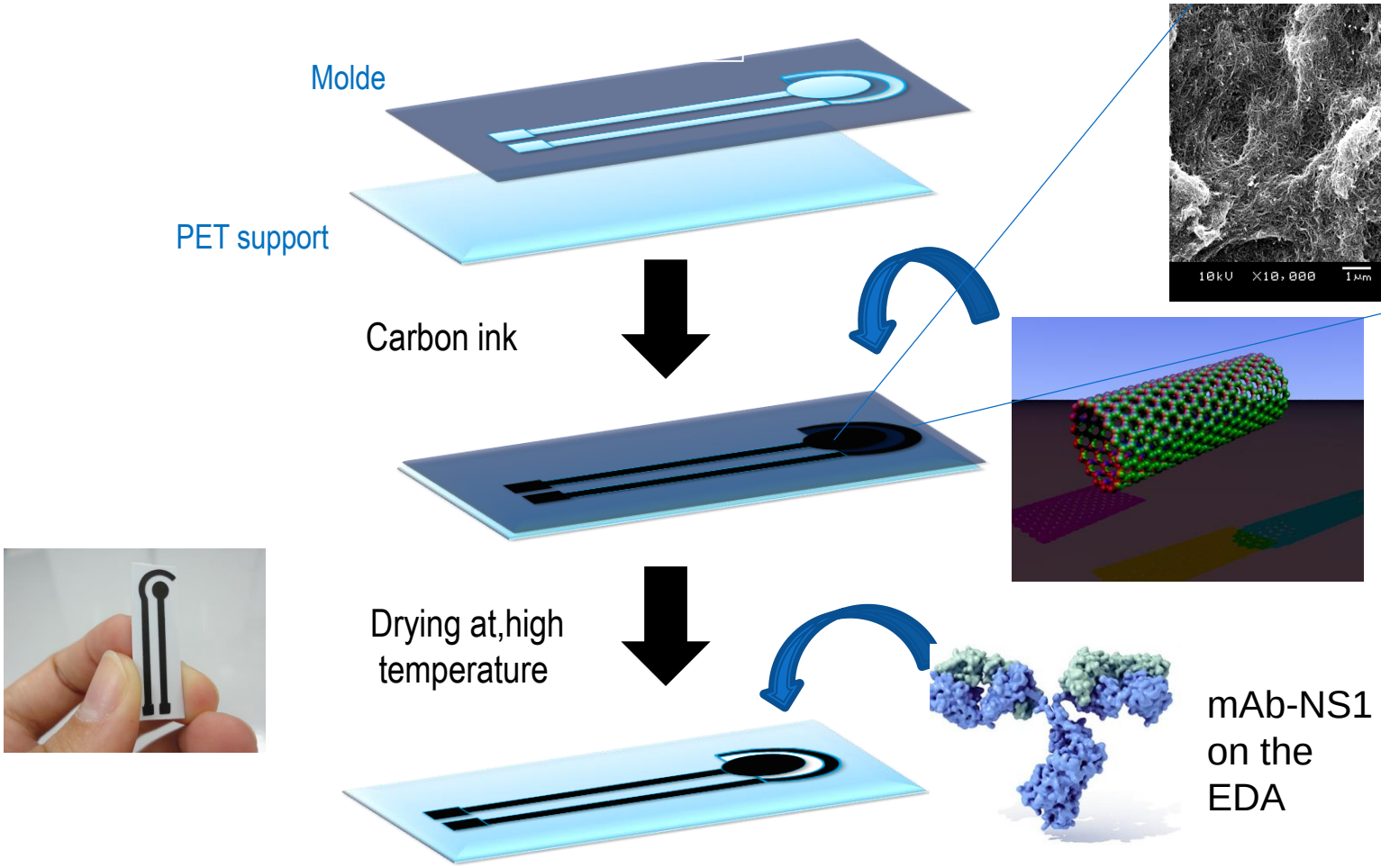


Dias et al., Biosensors & Bioelectronics, 2013

Homemade 3-carbon electrode system for electrochemical sensing: Application to microRNA detection

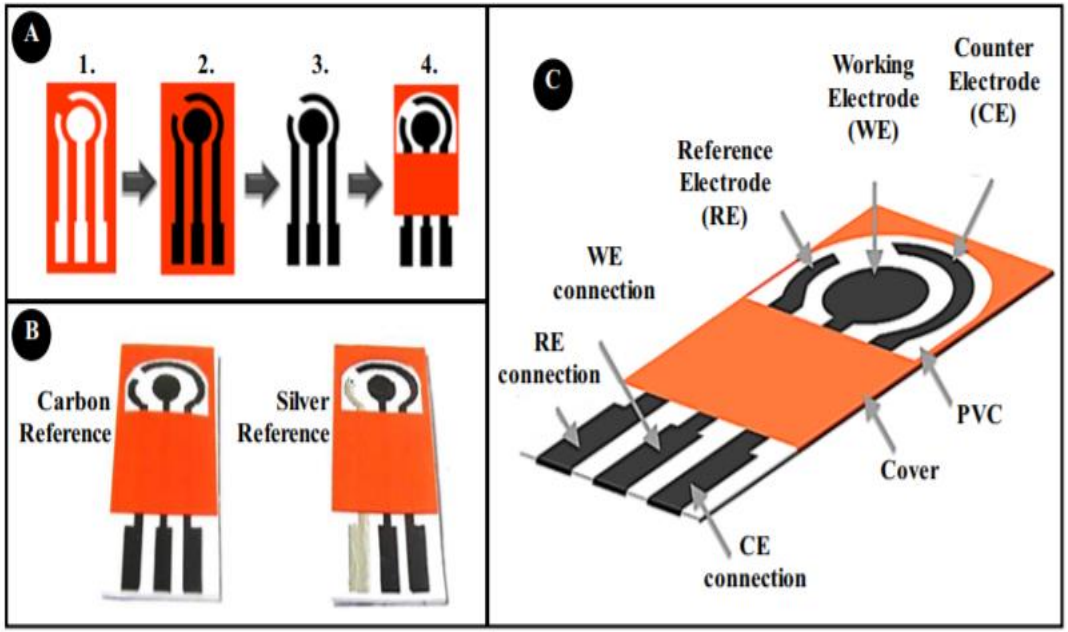


Fig. 1. Schematic representation of the development steps of the C-SPEs production (A), a picture with the real electrodes having different REs (B); and the identification of all components (C).

Dutra et al., Microchemical Journal 138 (2018) 35–44

The homemade production of carbon screen-printed electrodes (C-SPEs) with a three carbon electrodes and application

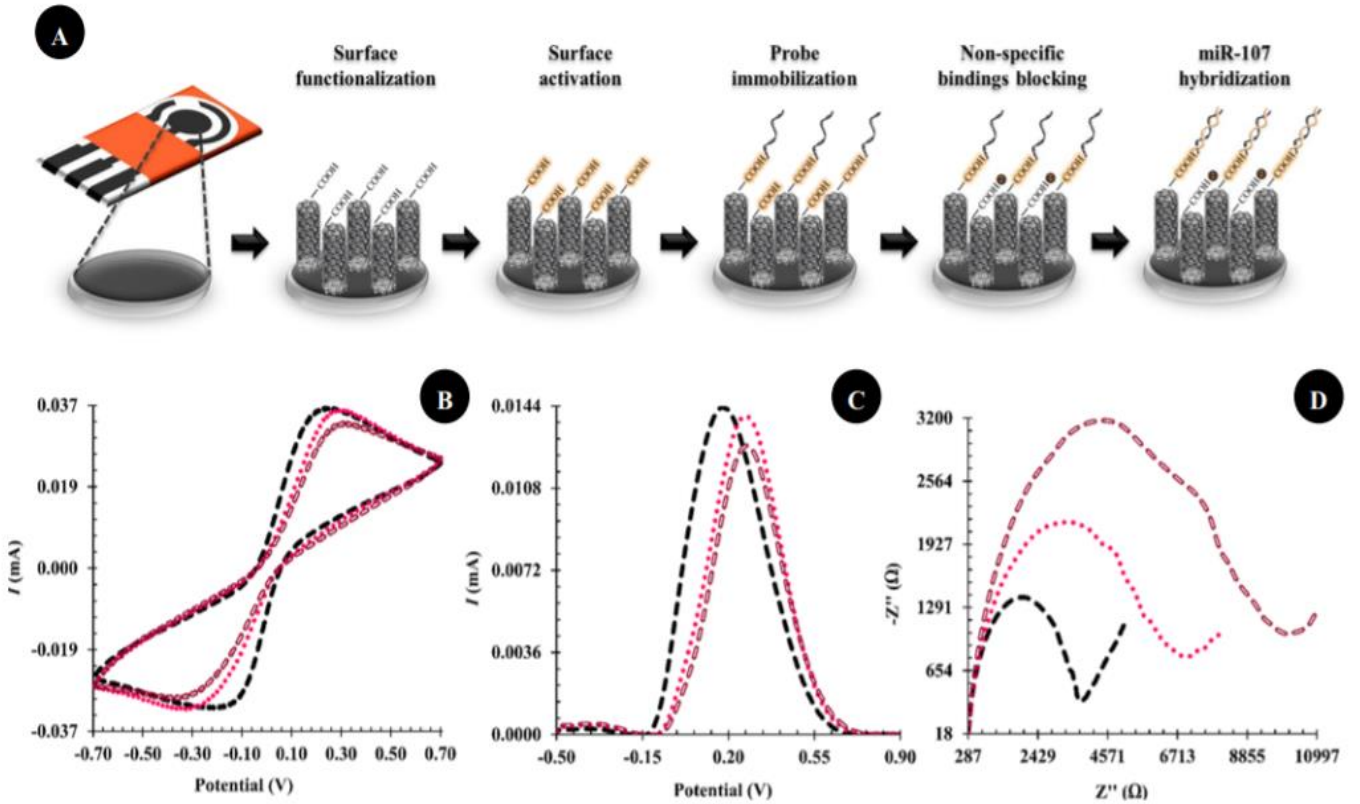


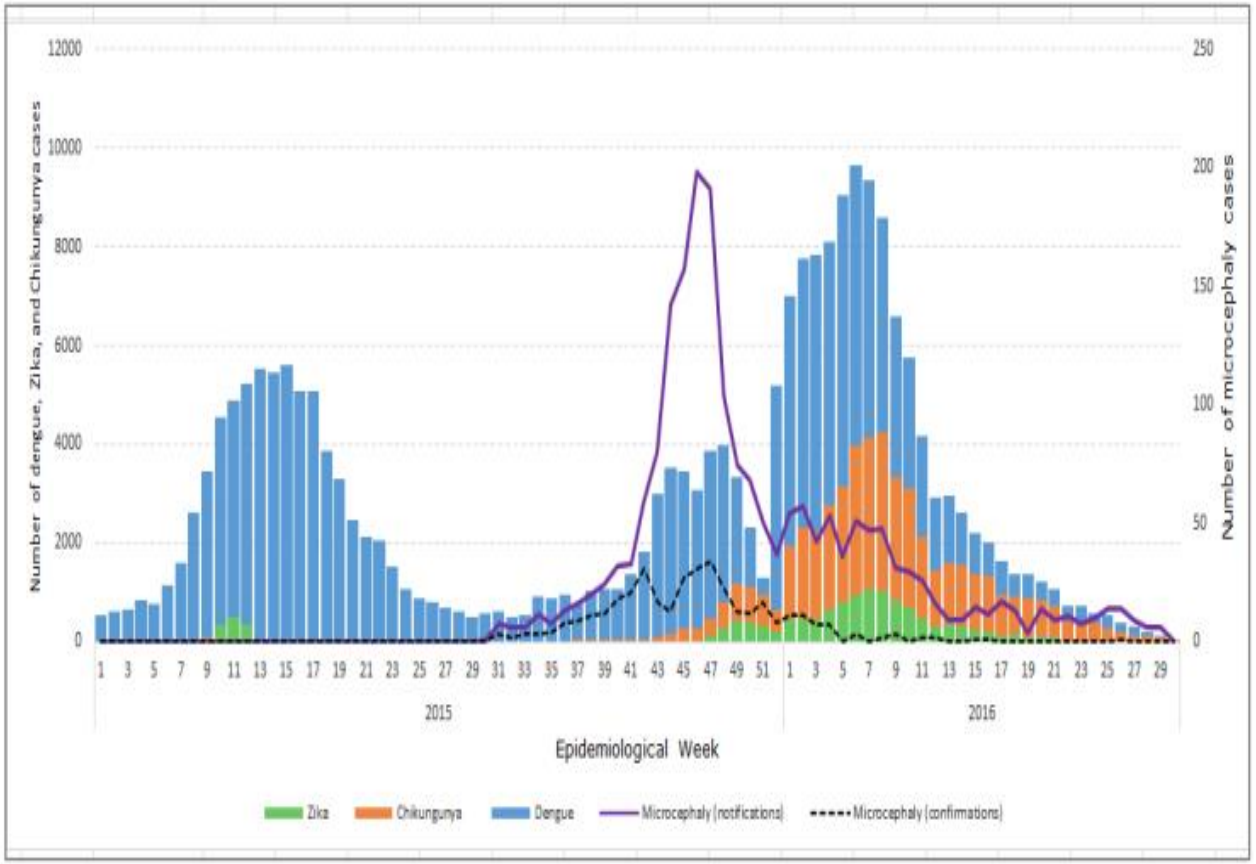
Fig. 5. Schematic representation of the assembly of the biosensor (A) and its electrochemically generated signals by CV (B), SWV (C) and EIS (D) after MWCNTs-COOH deposition (---), anti-miRNA binding (•••), and L-asparagine binding (–·–).

Dutra et al., Microchemical Journal 138 (2018) 35–44

Sensor para Detecção do Zika vírus

- Epidemia da Zika em 2016 (OMS: ZIKA como emergência global);
- Transmissão vertical,
- Vigilância no período pré-natal : MS apontou a obrigatoriedade de três testes no período pré-natal;
- Necessidade de desenvolver alternativas point-of-care devido à urgência na intervenção e inquéritos epidemiológicos;
- Os testes atuais são realizados em laboratório (PCR, ELISA, Mac Elisa);
- Surtos e semi-surtos em regiões tropicais, e outras regiões;

Figure 9. Chikungunya, dengue, Zika and microcephaly cases reported in the state of Pernambuco, by EW. Brazil. 2015 to EW 29 of 2016.



Source: Data provided by the Pernambuco Secretary of Health and reproduced by PAHO/WHO[13]

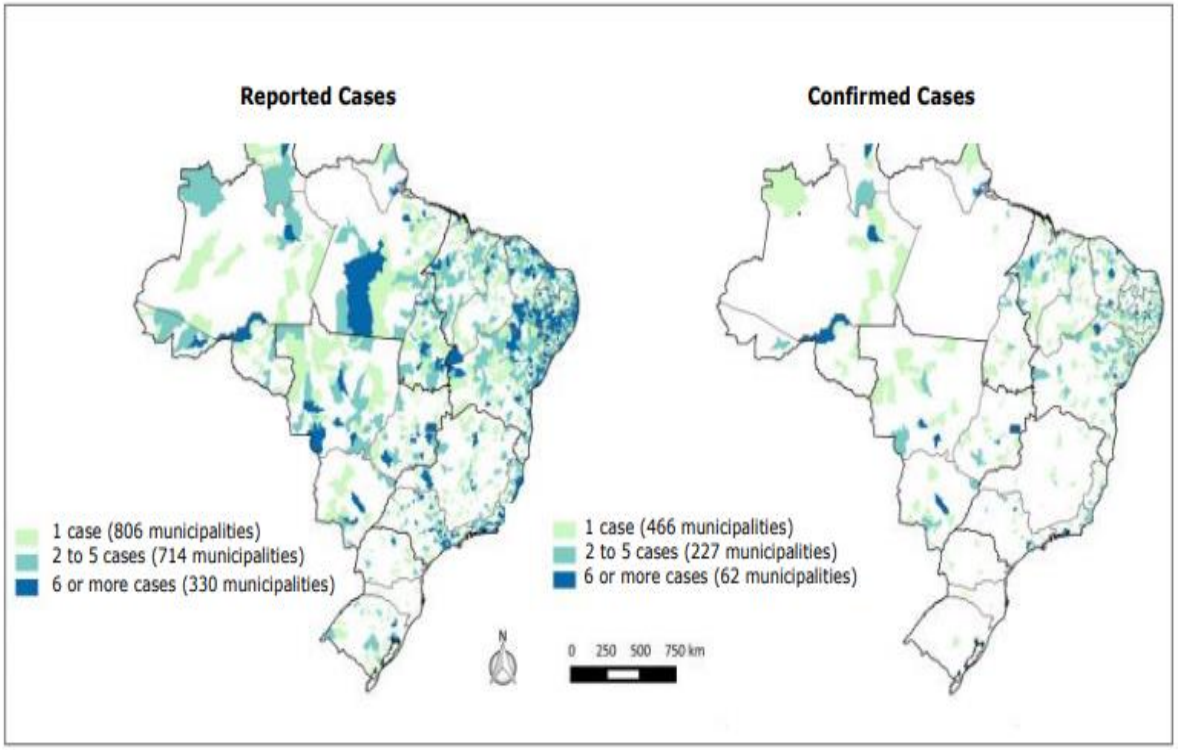
→ O aumento no número de casos de Dengue pode ser reflexo de um monitoramento inadequado, uma vez que os flavivírus possuem 60% de homologia geneticamente.

Pan American Health Organization

World Health Organization
Regional Office for the Americas

Brazil: Areas with Risk of Zika

Figure 8. Number of municipalities in each state with reported and confirmed cases related to Zika virus infections and other etiological infections. Brazil. EW 45 of 2015 to EW 52 of 2016



Source: Data published by the Brazil Ministry of Health and reproduced by PAHO/WHO[12]

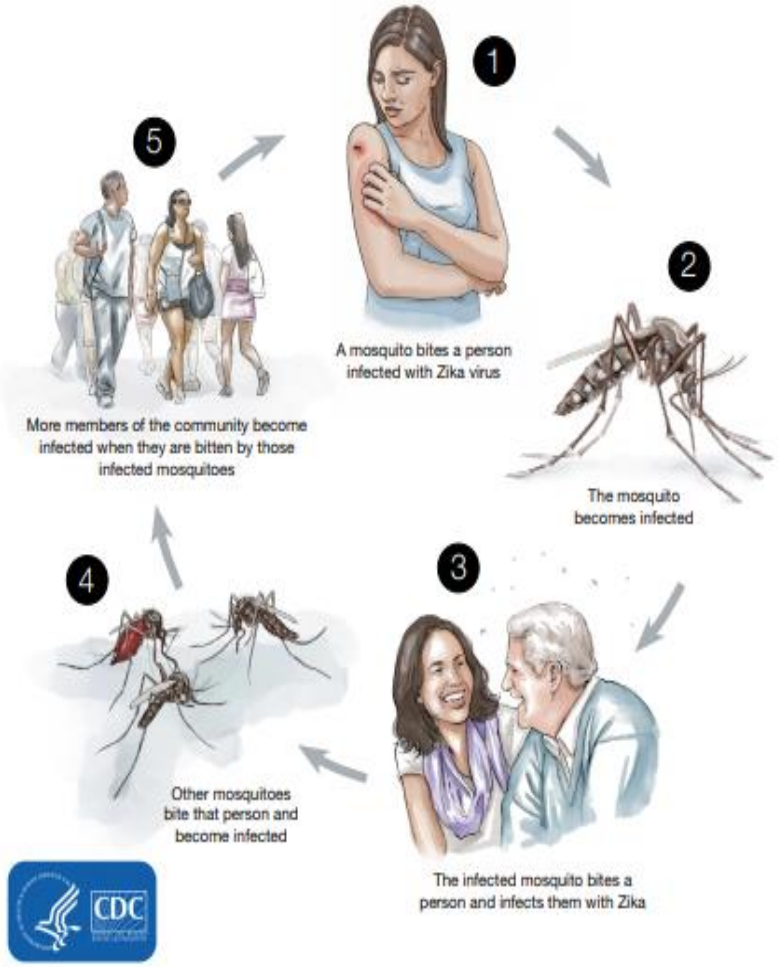
* Few confirmed cases: Sub-notification and non-mandatory declaration, and non accurated methods!

PROTECT YOUR FAMILY AND COMMUNITY

HOW ZIKA SPREADS

Accessible Version: https://www.cdc.gov/zika/transmission/index.html

Most people get Zika from a mosquito bite



Other ways people get Zika

During pregnancy
A pregnant woman can pass Zika virus to her fetus during pregnancy. Zika infection during pregnancy can cause serious birth defects and is associated with other pregnancy problems.

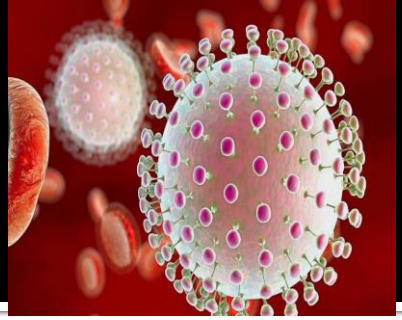
Through sex
Zika virus can be passed through sex from a person who has Zika to his or her sex partners.

Through blood transfusion
Zika virus may be spread through blood transfusion.

CDC

284550

ZIKV biomarkers

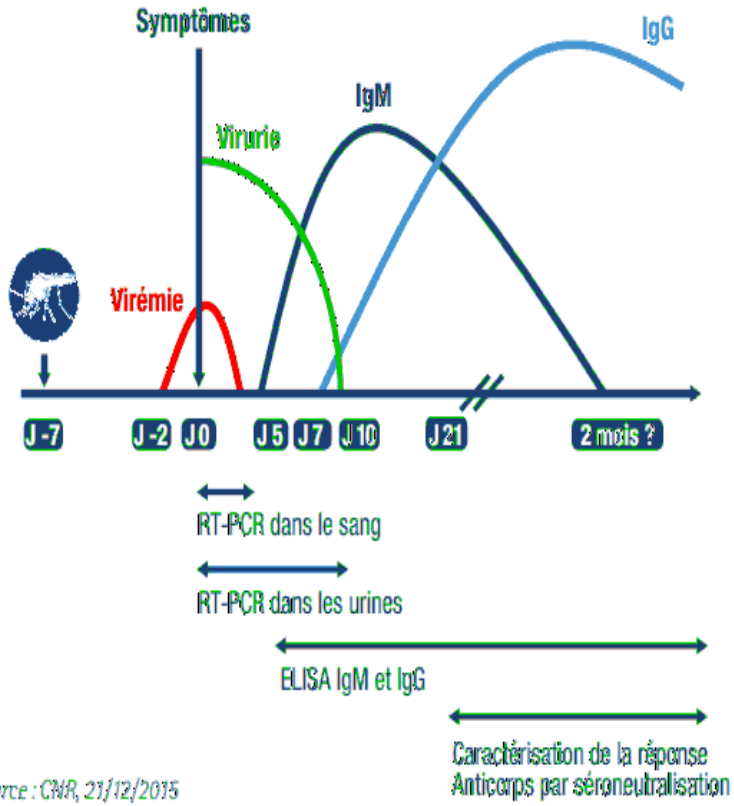


La stratégie diagnostique des infections à virus Zika dépend du moment où le prélèvement est réalisé par rapport à la date de début des signes :

- de J0 à J3/J5 : RT-PCR sur prélèvement de sang et d'urine ;
- de J0 à J10 : RT-PCR sur prélèvement d'urine.

http://www.inpes.sante.fr/CFESBases/catalogue/pdf/1708.pdf

LA CINÉTIQUE DE L'INFECTION PAR LE VIRUS ZIKA
(État actuel des connaissances) 28 décembre 2015.



Source : CNR, 21/12/2015

Definição de marcadores sorológicos e alvos moleculares

Ernesto Marques (FIOCRUZ-PE/Pitts-USA)

RESULTADOS

Construção de Painel Sorológico

	ZIKA	DENGUE
Painel A	+	-
Painel B	+	+
Painel C	-	+
Painel D	-	-

! Coorte ~400 pacientes ZIK, porém **19 ZIK+ DENV-**

Estudo de reatividade das Proteínas do Capsídeo, M, Envelope,NS1e NS2B;

NS2B

NS2B *é uma proteína de membrana integral de 14 kDa*

Estudo de reatividade (andamento): NS3, NS4B e NS5

RESULTADOS

- ✓ **NS2B** é uma proteína de membrana integral de 14 kDa que contém três d
  transmembranas locali
  e um domínio central

Science News

from research organizations

New test extends window for accurate detection of Zika

The test improves on existing o
means to determine whether a
exposure

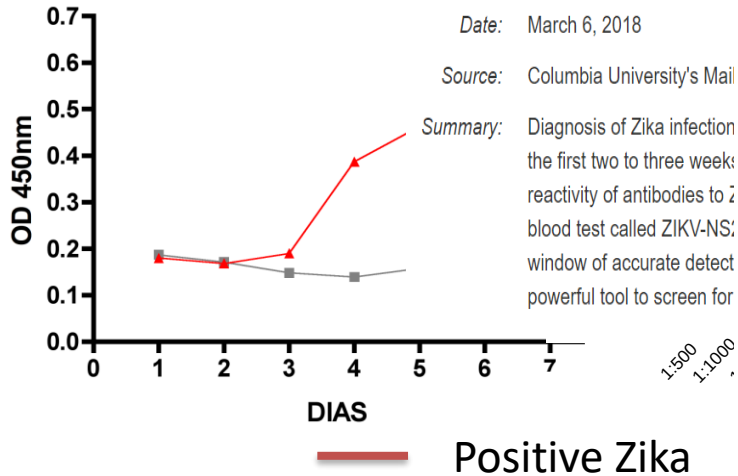
Date: March 6, 2018

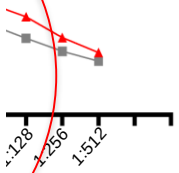
Source: Columbia University's Mail

Summary: Diagnosis of Zika infection the first two to three week reactivity of antibodies to blood test called ZIKV-NS window of accurate detect powerful tool to screen for

tests for exposure are only reliable in the first two to three weeks after infection while the virus is circulating in the bloodstream. Antibody tests are confounded by cross-reactivity of antibodies to Zika with dengue, yellow fever, and Japanese encephalitis viruses following infection or vaccination. A new blood test called ZIKV-NS2B-concat ELISA is faster, less expensive, and extends the window of accurate detection from weeks to months after the onset of infection, giving clinicians a powerful new tool to screen for Zika throughout pregnancy.

The new Zika test is detailed in the scientific journal *mBio* and was developed by scientists at the Center for Infection and Immunity (CII) at Columbia University's Mailman School of Public Health and their colleagues at the University of California, Berkeley; Ministry of Health of Nicaragua; Walter Reed Army Institute of Research; Erasmus University Medical Centre; New York City Department of Health and Mental Hygiene; New York State Department of Health;





- ✓ **Anticorpos antiIgG → Partícula vi**

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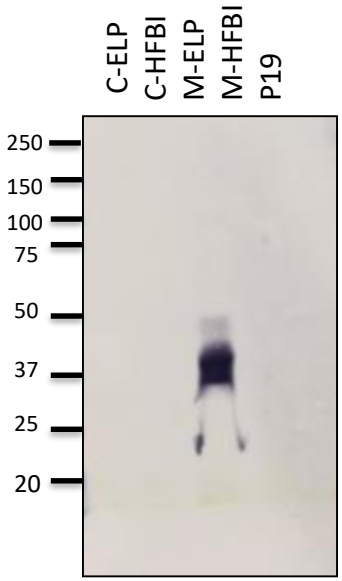
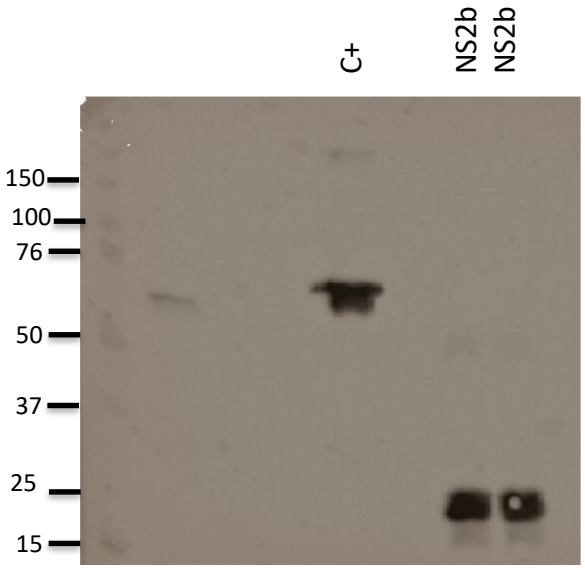
Produção dos Biomarcadores (Proteínas e anticorpos)

Maria Izabel F. Guedes (UECE)

Sistema vegetal : *Nicotiana benthamiana*

Proteínas **NS2B, M ,** NS1, Capsideo, NS3, NS4B.

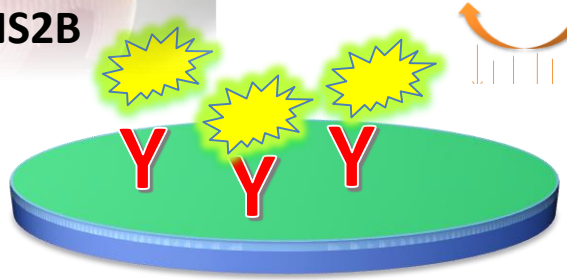




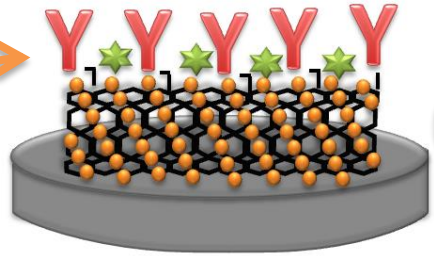
Yield	M-ELP	NS2B-ELP
% TSP	1,27	1,10
Fresh Weight ($\mu g/g$)	170	155

Desenvolvimento dos Sistemas Sensores (Detecção de NS2)

Rosa Fireman Dutra (UFPE)



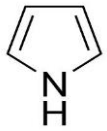
$[Fe(CN)_6]^{3-}$ $[Fe(CN)_6]^{4}$ e^-



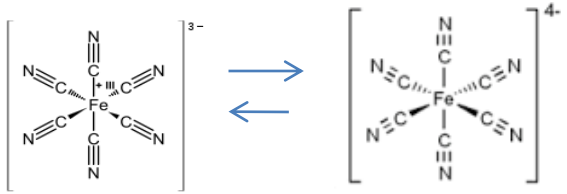
$[Fe(CN)_6]^{4-}$

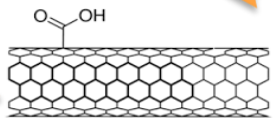
$[Fe(CN)_6]^{3-}$

Pyrrole



Chemical mediator





Carbon nanotube

Vantagens

- Detecção direta em tempo real, amostra de sangue ou saliva.
-Resultados em menor tempo.

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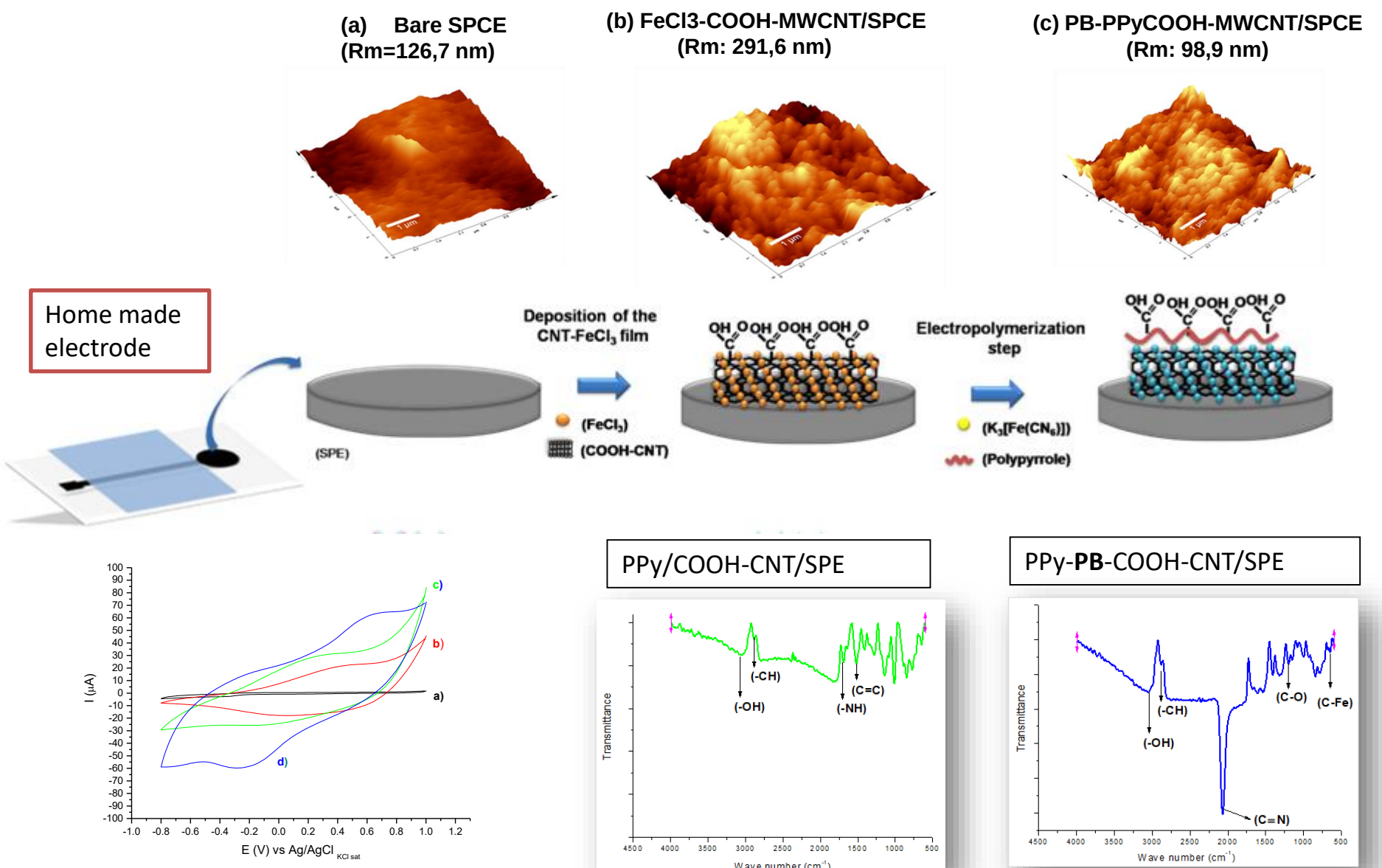
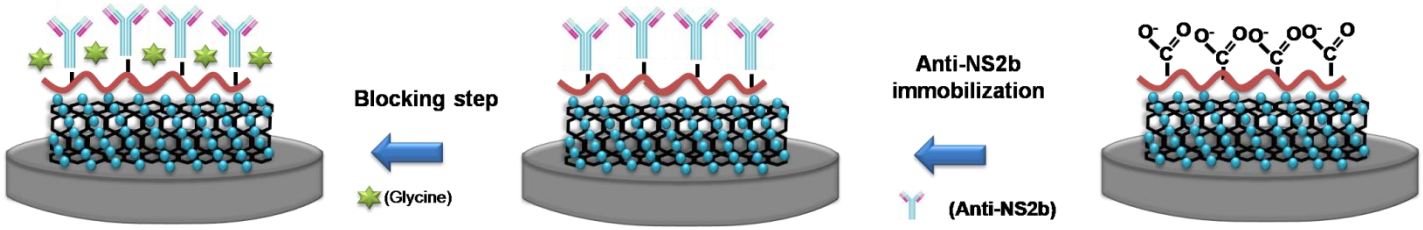


Figure 1. Electrochemical profile of the modifications of the SPE: (a) bare SPE; (b) only PPy; (c) PPY/COOH-CNT and (d) PB/PPy/COOH-CNT. Measure performed in KCl (0.1 M) solution at 0.05 V/s.



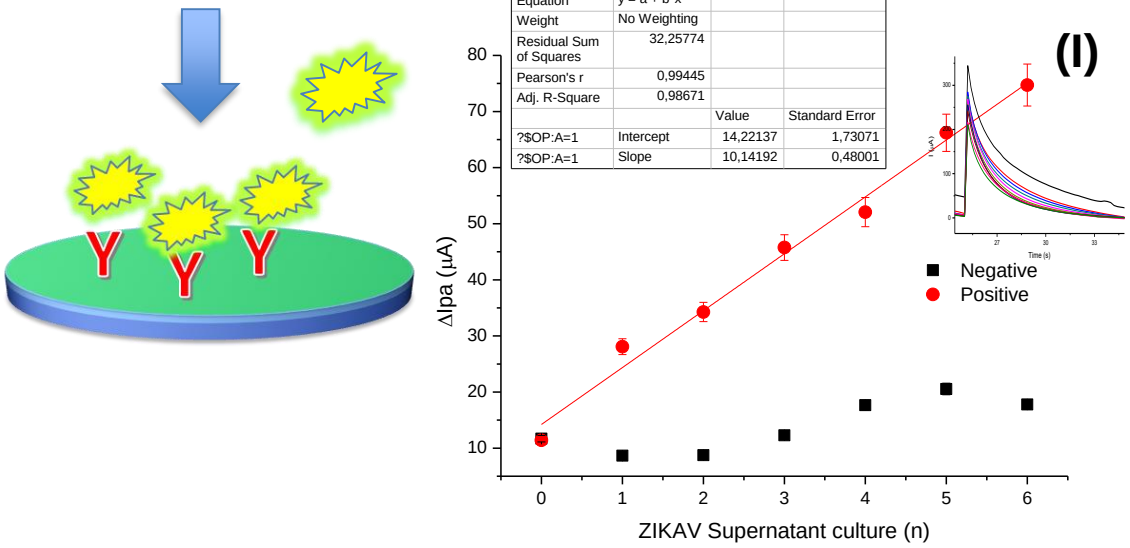


Figure 2. I) Analytical response of the immunosensor to supernatant culture with and without ZIKV isolated (positive and negative); **(II)** Cyclic voltammograms of the immunosensor to successive injection of the supernantant culture with ZIKV. Measure performed in KCl (0.1 M) solution at scan rate of 0.05 V/s;

Próximas Etapas: Resposta em Saliva e Sangue total. Uso do sistema embarcado. Validação

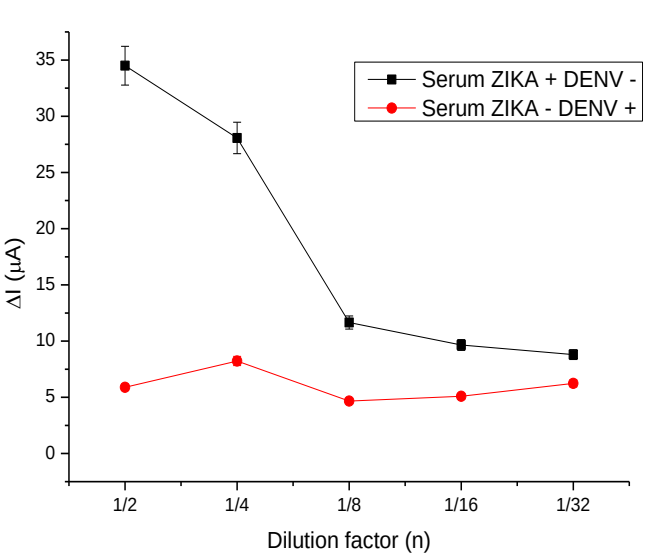
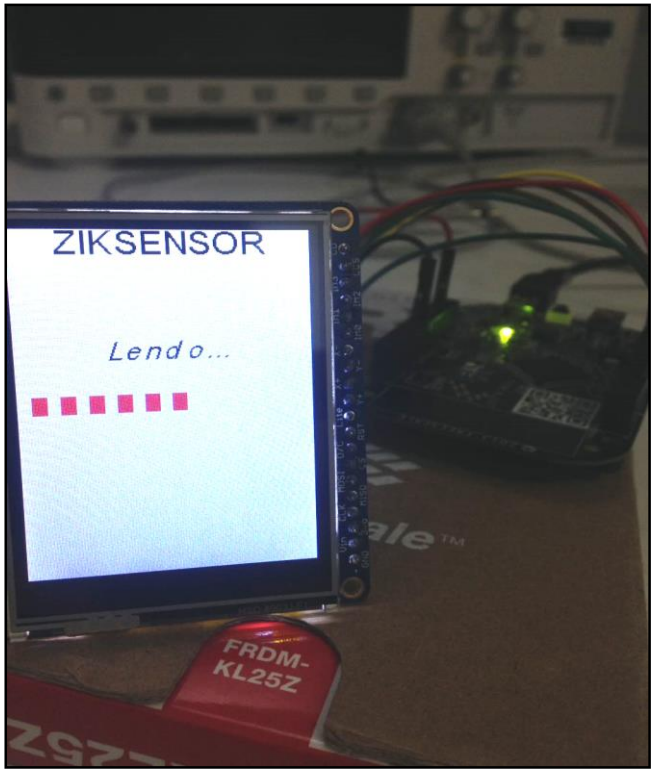


Figure 3. I) Analytical response of the immunosensor to successive injections of the pool serum. Measure performed in KCl (0.1 M) solution at scan rate of 0.05 V/s;

Obtenção do Sinal Método Similar aos Glicosímetros

RESULTADOS

Tela gráfica para a interface Homem-máquina



Protótipo Final

- ✓ Imunossensor Point-of-care (Leitura em Sangue e Saliva)

Sistema de Geo-referenciamento para ZIKV.

- ✓ A tecnologia do biossensor para ZIK/DENV pode ser adaptável para ensaios laboratoriais automatizados

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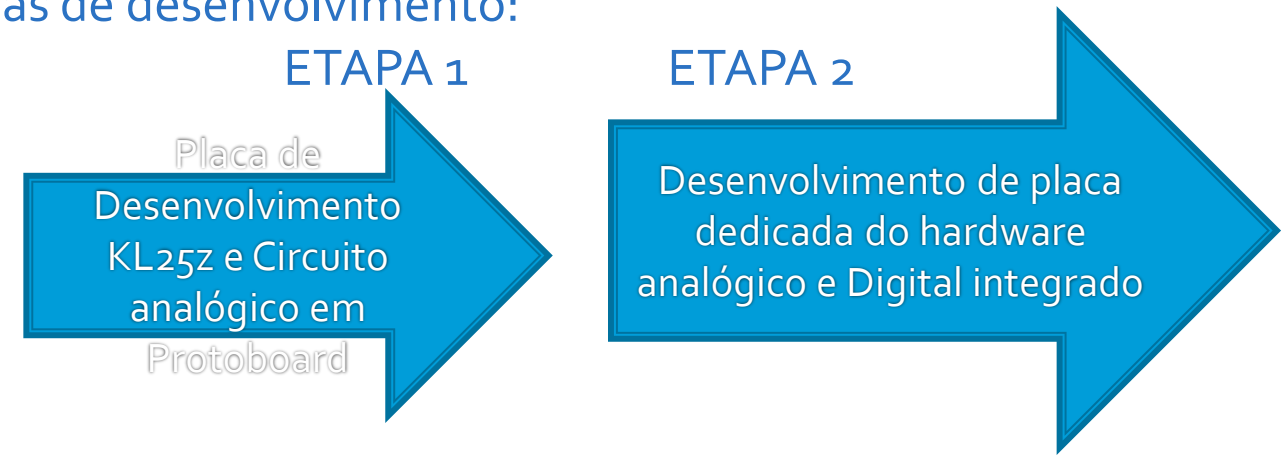
Leitor (Sistema embarcado)

Marco Aurelio Benedetti - UFPE

Escolha da tecnologia para o desenvolvimento do equipamento

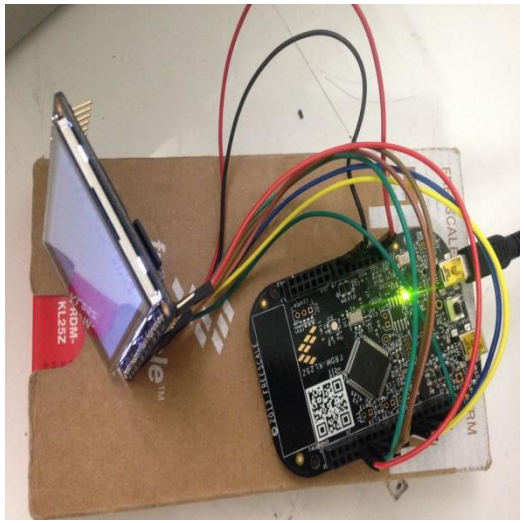
- Tecnologia Ultra Low-Power;
- Processador ARM® Cortex®-M0+, 32-Bit, 48MHz;
- Módulo de comunicação SPI, I2C e UART;
- Alimentação em 1.7 V ~ 3.0 V;
- Conversor A/D de 12 bits e 4 canais;
- Conversor D/A de 12bits;
- Circuito amplificadores de precisão.

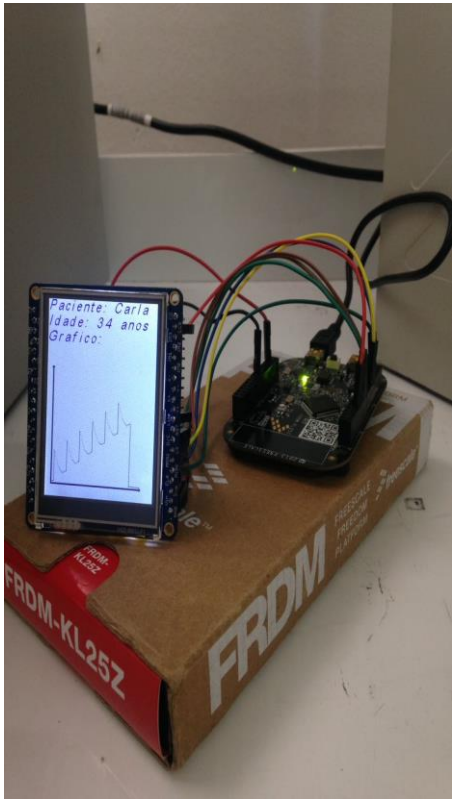
Etapas de desenvolvimento:



Sensor para leitura em tempo real

Construção do primeiro protótipo de teste



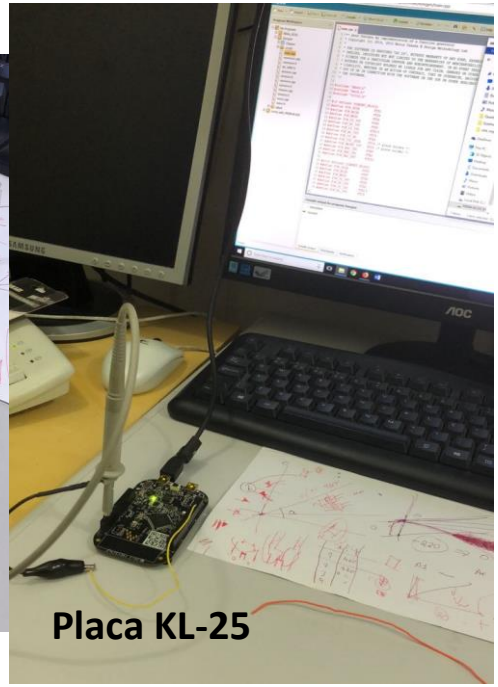


RESULTADOS

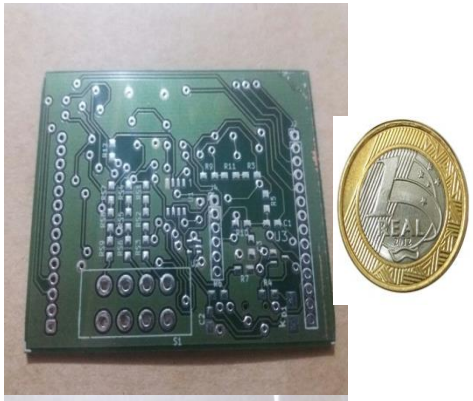
Construção de placa eletrônica (Miniaturizado)

Placa desenvolvida a partir da KL-25, compondo Hardware Digital e Analógico

Placa KL-25



Placa KL-25

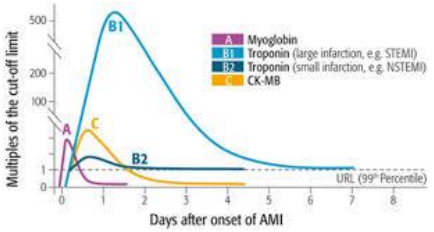


Placa desenvolvida

Check Access Export

Biosensors and Bioelectronics

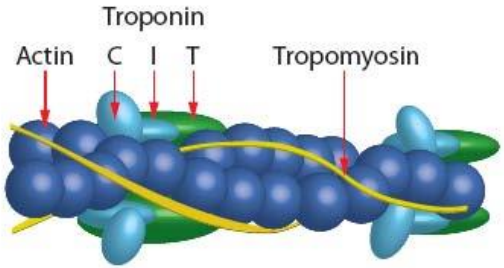
Volume 28, Issue 1, 15 October 2011, Pages 243-250



Artificial antibodies for troponin T by its imprinting on the surface of multiwalled carbon nanotubes: Its use as sensory surfaces

Felismina T.C. Moreira [a, c, d], Rosa A.F. Dutra [b], João P.C. Noronha [d], Alexandre L. Cunha [e], M. Goreti F. Sales [a]

⊞ Show more



Check Access Export

Clinica Chimica Acta

Volume 376, Issues 1–2, 1 February 2007, Pages 114-120

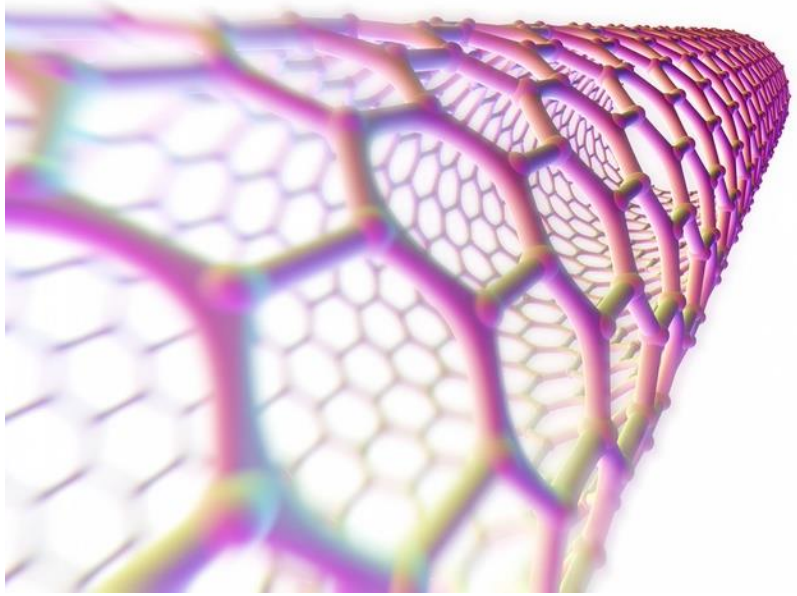
An SPR immunosensor for human cardiac troponin T using specific binding avidin to biotin at carboxymethyldextran-modified gold chip

Nanobiomimetic sensors

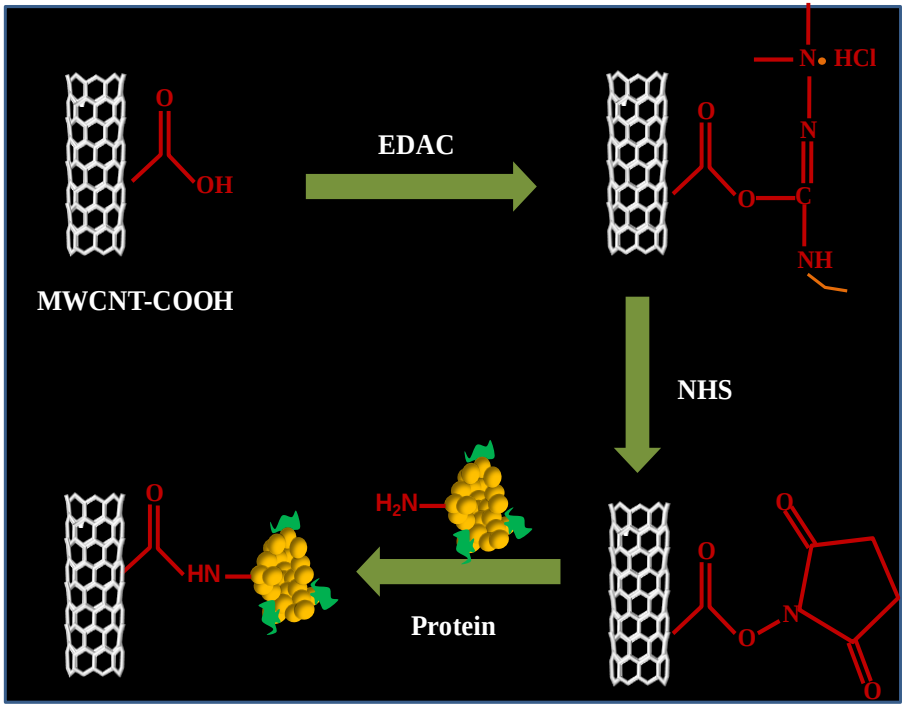
- Plastic antibodies

based on nanomaterials

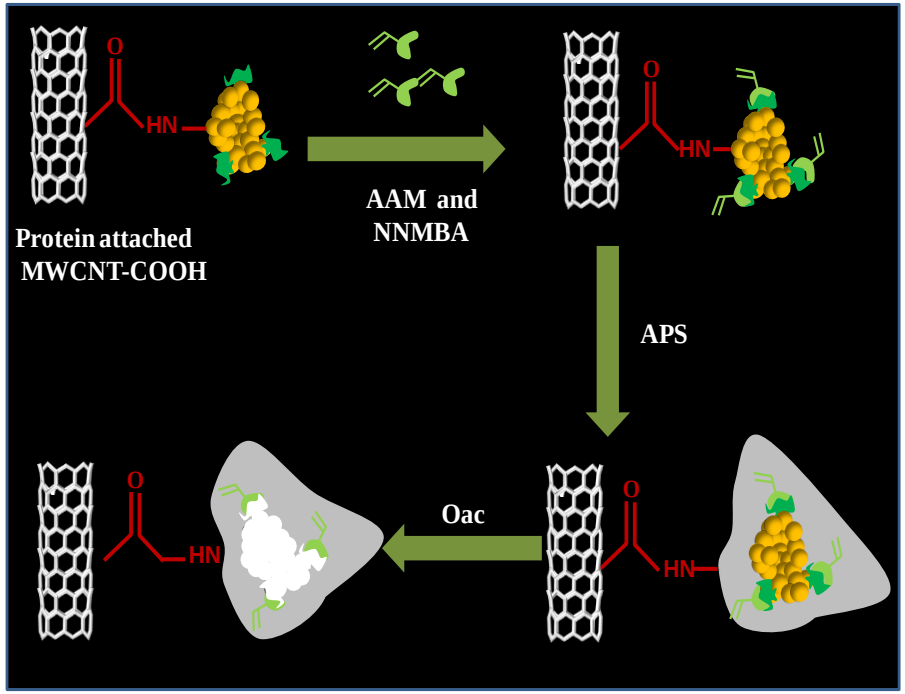
Nano-Moleculary Imprinted polymers: nanoMIPs



→ Plastic anti-cTnT obtained using by carbon nanotubes



Covalent attaching protein to carbon nanotubes via two step process of diimide-actived amidation.



Stepwise imprinting process of the troponin T on MWCNT

Moreira et al., Bionsensors and Bioelectronics 2012

Results

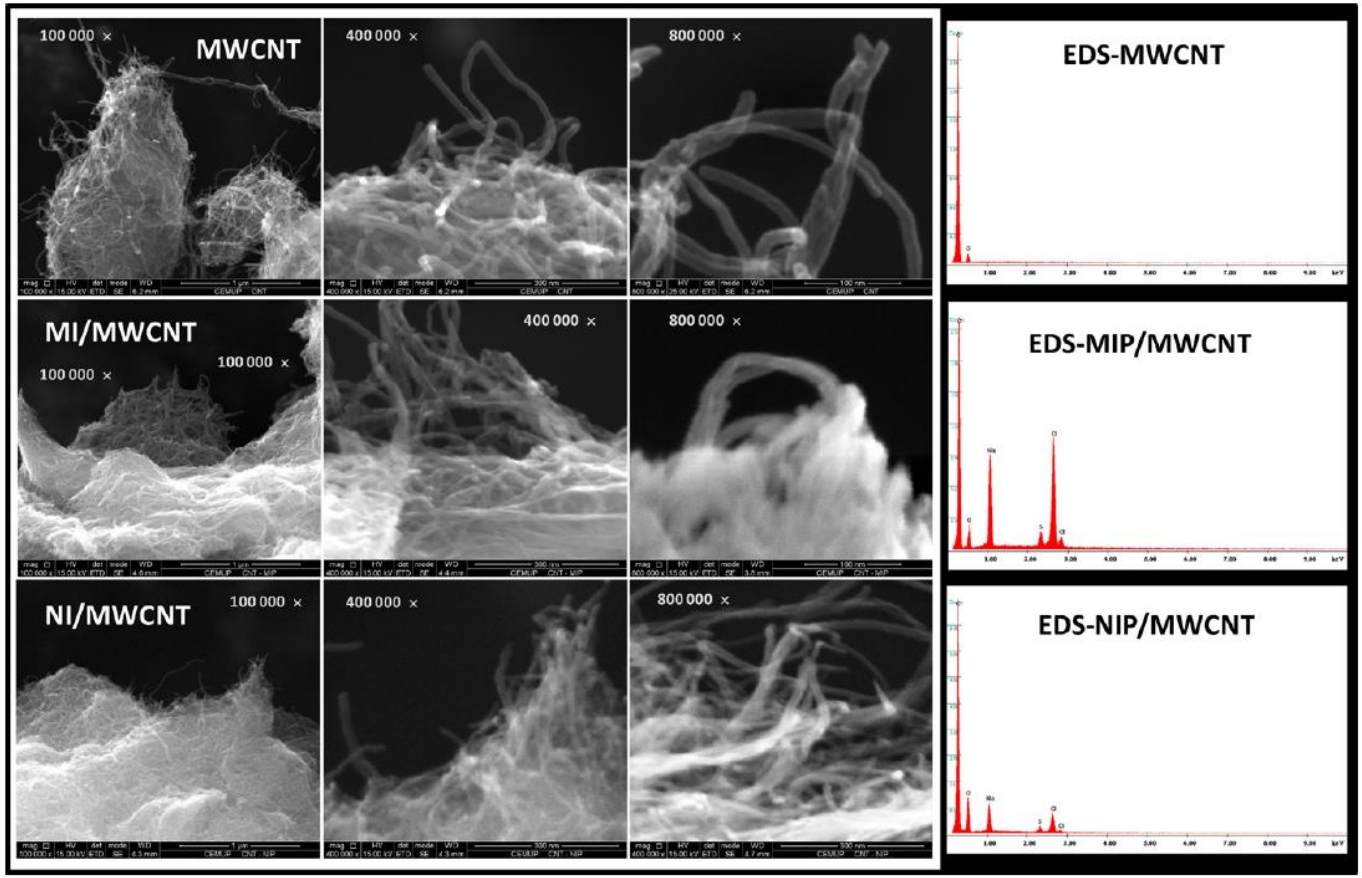


Fig. 2. SEM and EDC images of plain MWCNT (first line) and modified MWCNT by MI (second line) or by NI (third line) technologies.

Moreira et al., Bionsensors and Bioelectronics, 2012

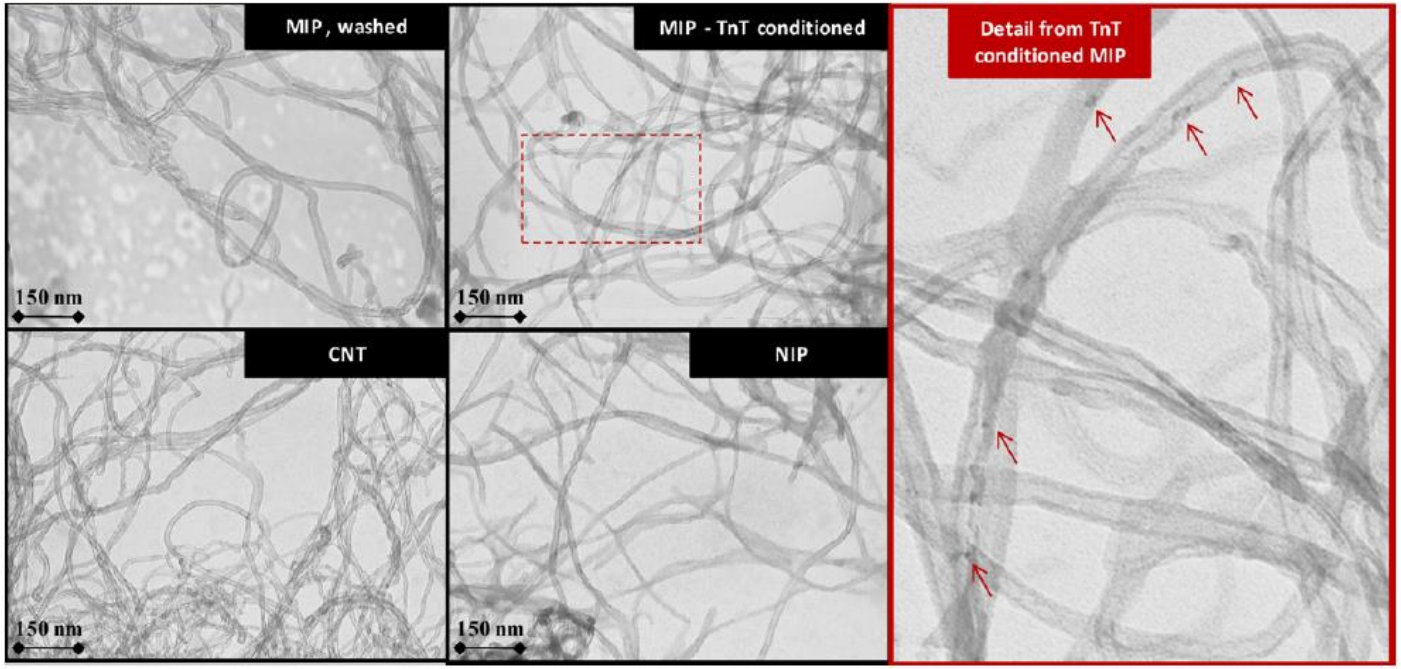
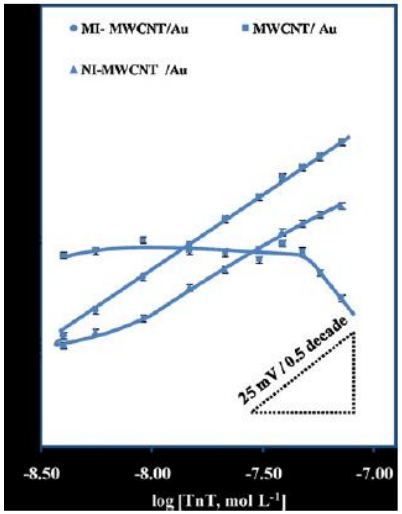


Fig. 3. TEM images of MI, NI and CNT technologies.



Potentiometric Response to cTnT

Drawbacks - LOD!!

Moreira et al., Bionsensors and Bioelectronics, 2012

Antibody-MIP produced by

Conducting and Organic Polymer + Graphene

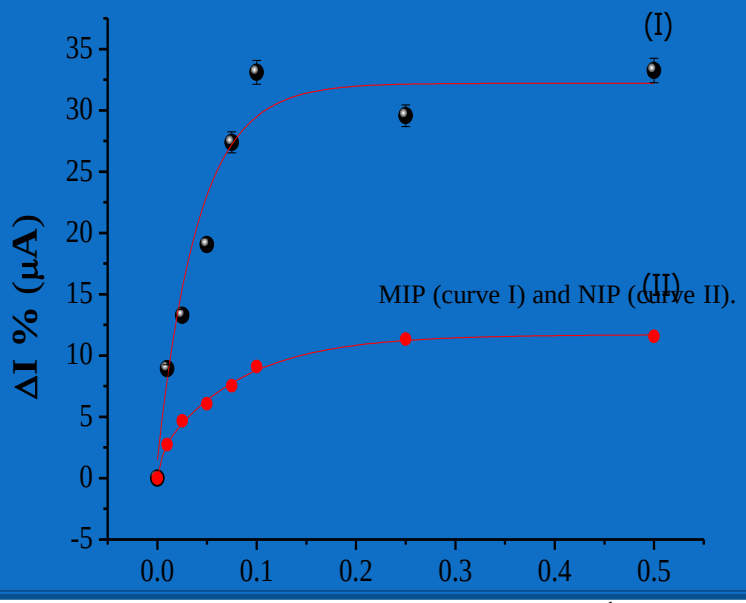
Synthetic antigen peptide

Silva et al., Bionsensors and Bioelectronics, 2015

Biosensors and Bioelectronics
Volume 77, 15 March 2016, Pages 978–985

An ultrasensitive human cardiac troponin T graphene screen-printed electrode based on electropolymerized-molecularly imprinted conducting polymer

Bárbara V.M. Silva[a], Blanca A.G. Rodríguez[a], Goreti F. Sales[b], Maria Del Pilar T. Sotomayor[c], Rosa F. Dutra[a]
Show more



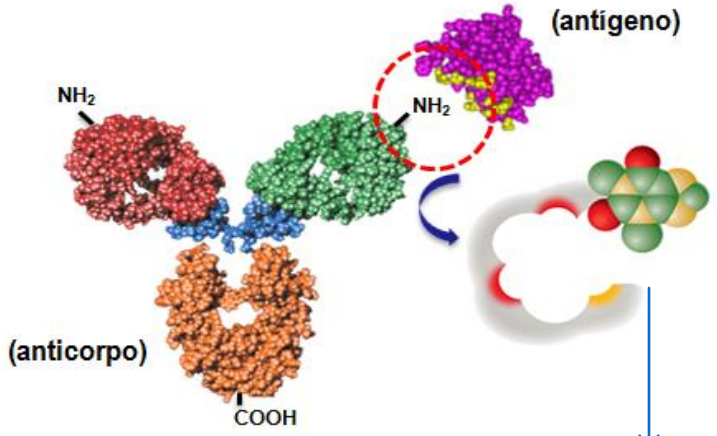
Adsorption Isotherms

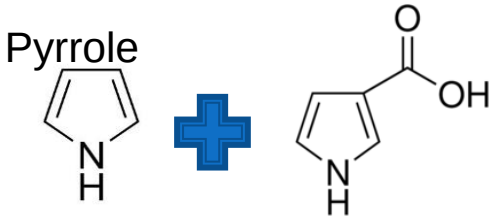
$$I_S = \frac{I_{max}}{1 + K_D/[S]}$$

K_D (MIP): 7.3 10^{-13} M
K_D (NIP): 11.6 10^{-13} M

MIP 37% NIP

Nano MIPs: organic conducting polymer + graphene

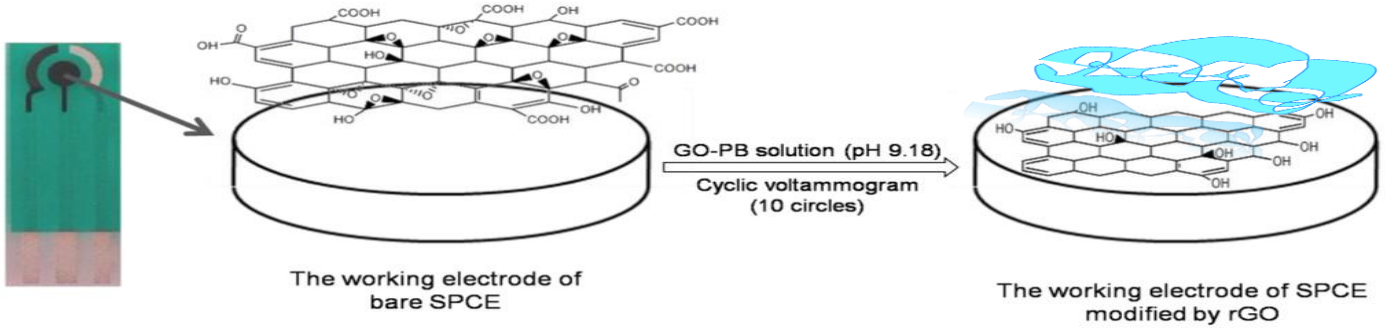


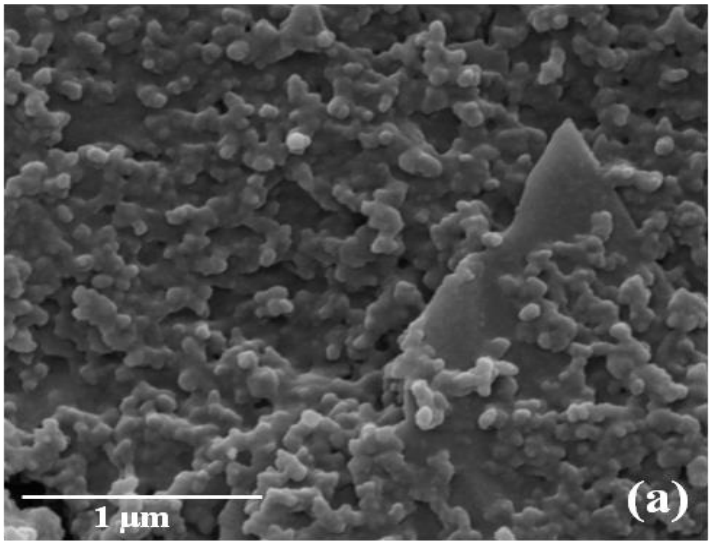


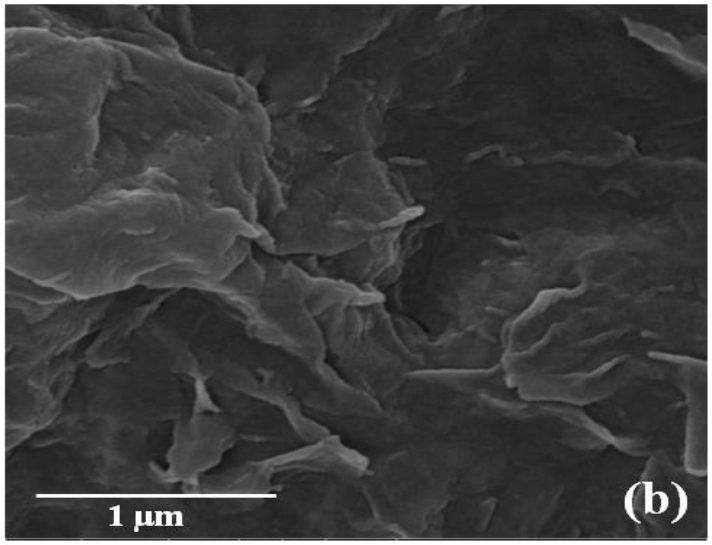
COOH-Pyrrole

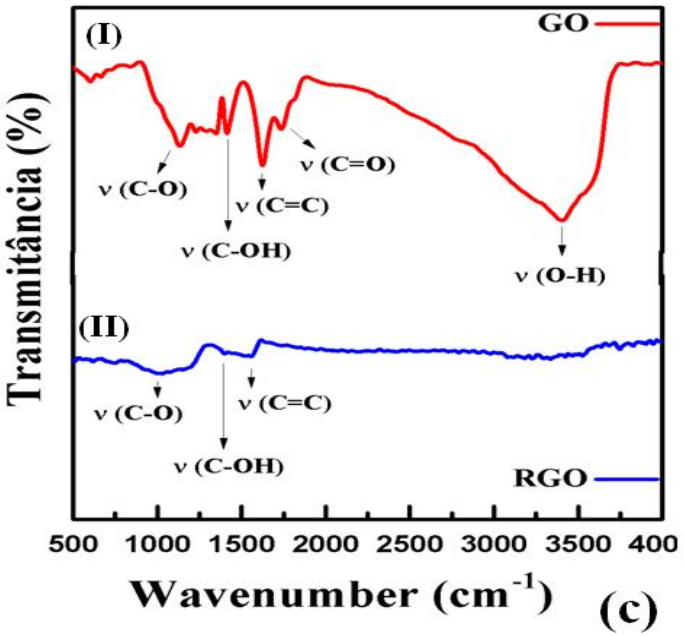
Synthetic protein

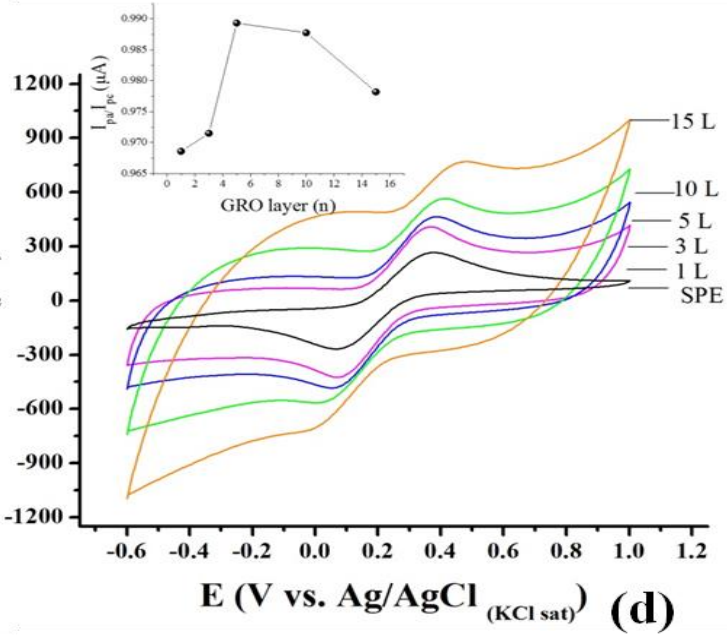
MIP grafted on the Graphene electrode



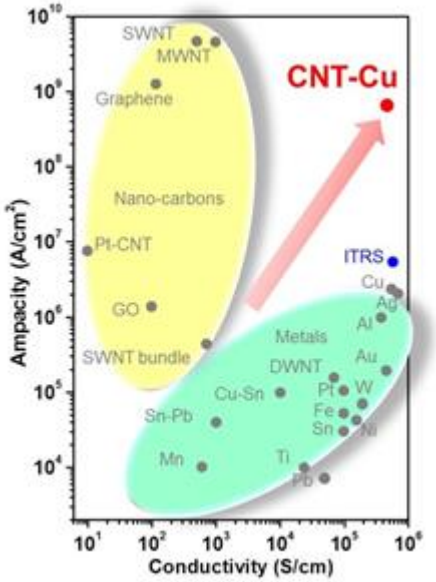

1 µm
(a)

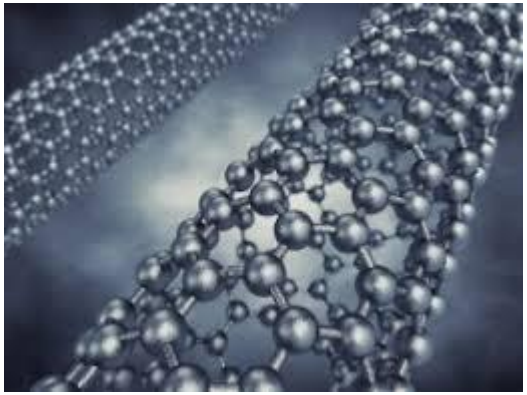

1 µm
(b)


(I)
GO
ν (C-O)
ν (C-OH)
ν (C=C)
ν (C=O)
ν (O-H)
(II)
ν (C-O)
ν (C-OH)
ν (C=C)
RGO
Transmitância (%)
500 1000 1500 2000 2500 3000 3500 4000
Wavenumber (cm-1)
(c)


15 L
10 L
5 L
3 L
1 L
SPE
I (µA)
1200
900
600
300
0
-300
-600
-900
-1200
-0.6 -0.4 -0.2 0.0 0.2 0.4 0.6 0.8 1.0 1.2
E (V vs. Ag/AgCl (KCl sat))
GRO layer (n)
0 2 4 6 8 10 12 14 16
(d)

Redox probe-free readings of a β-amyloid-42 plastic antibody sensory material assembled on copper@carbon nanotubes





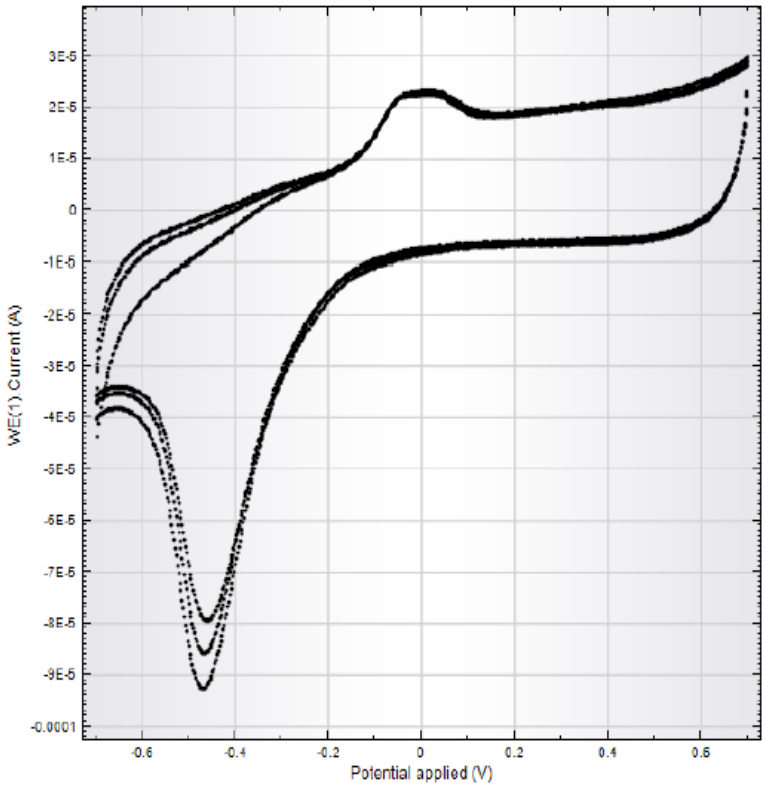
Dutra et al., 2018. Sensors and Actuators B 264 (2018) 1–9

Synthesis of electrocatalytic Cu@CNT

Leituras em KCl

CNT – Cu 2+

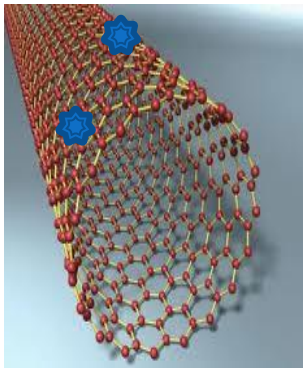
Ausência de solução de FE(II)/ FE(III)



CV em KCl (CNT- Cu 2+)

3 aplicações
3 µL de (CNT- Cu 2+)
Dispersos em DMF

30 minutos 70ºC



Cu

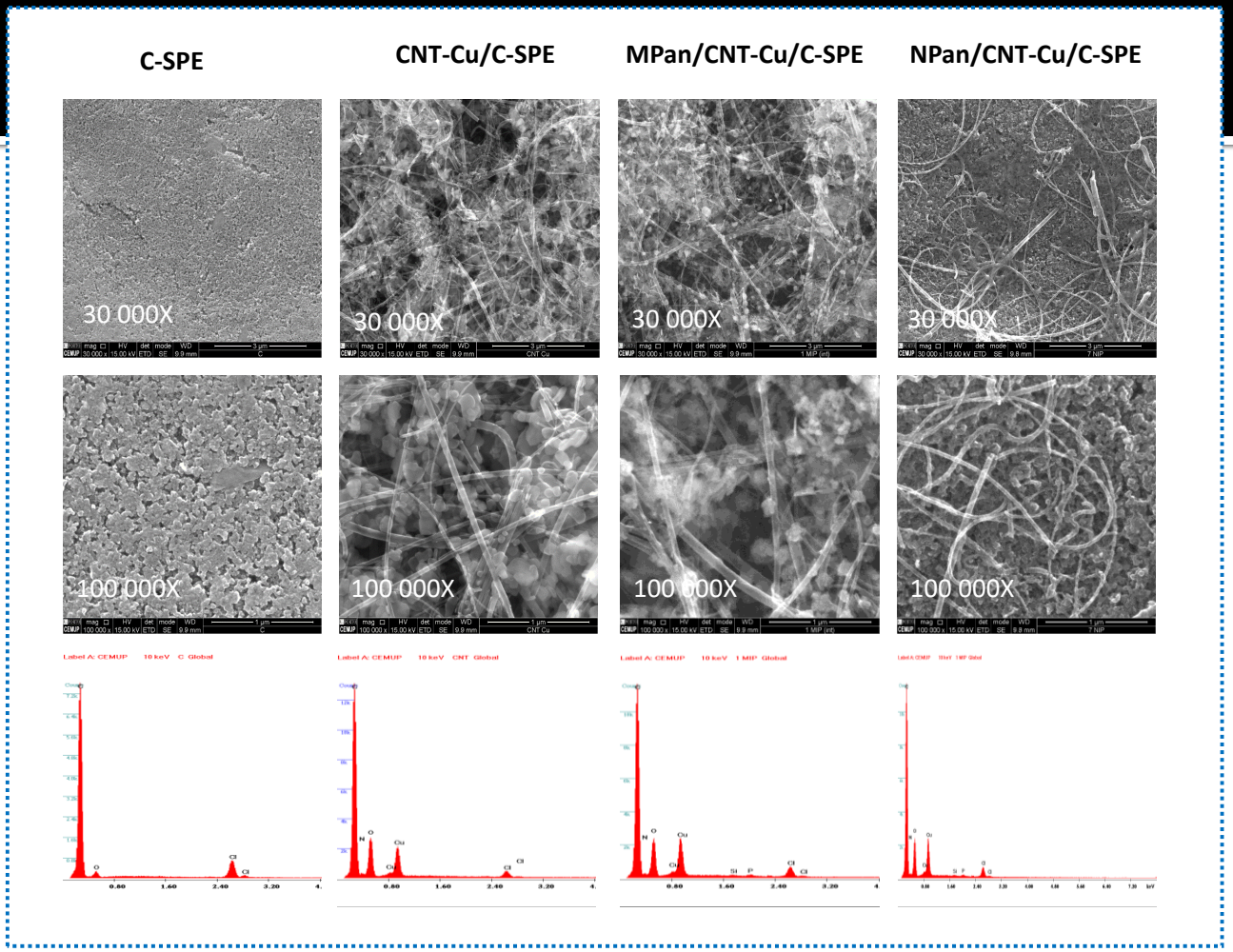


Figure 3 – SEM images of C-SPE, CNT-Cu/C-SPE, MPan/CNT-Cu/C-SPE, NPan/CNT-Cu/C-SPE **materials**.

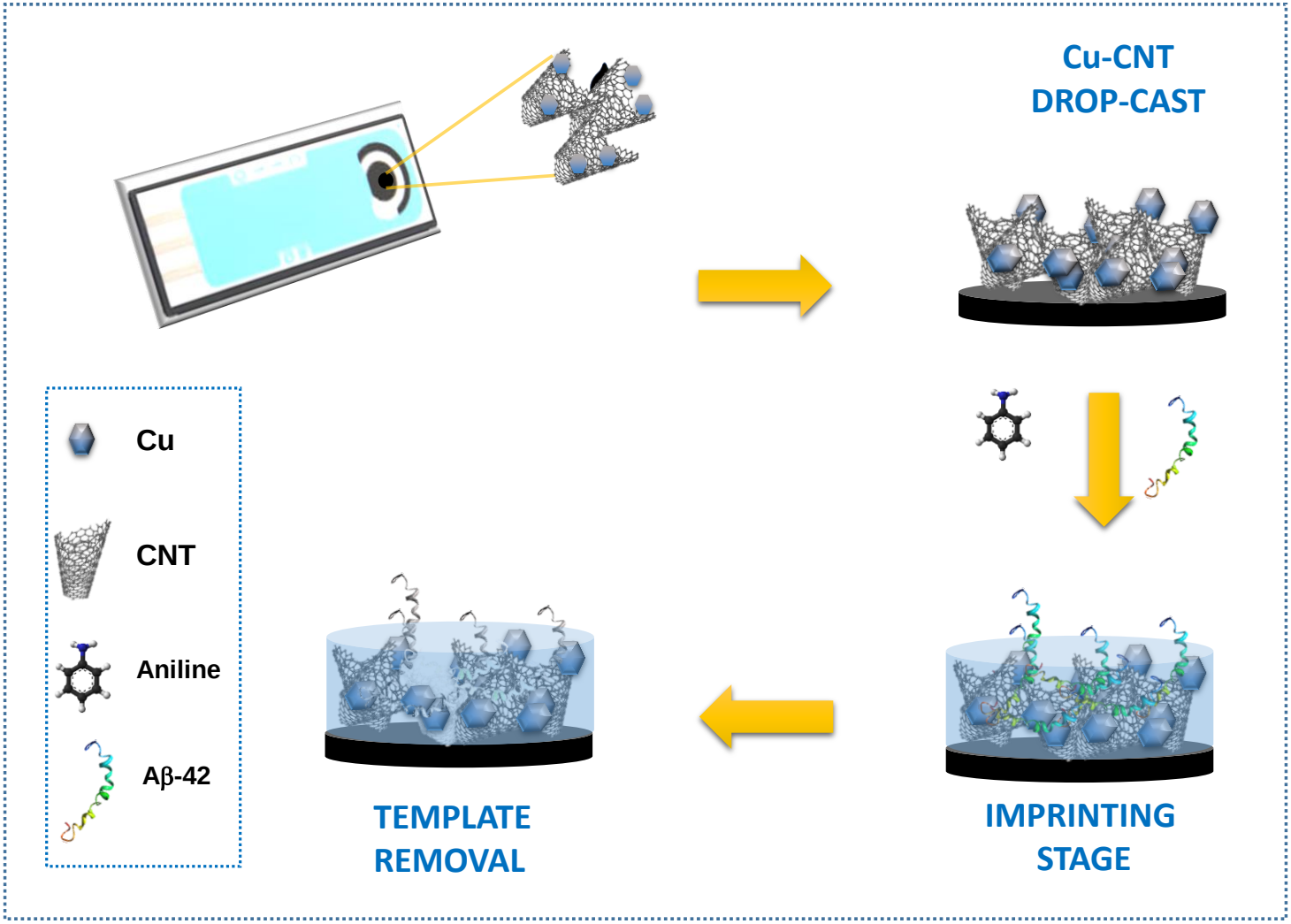


Figure1 - Schematic representation of the synthetic process of imprinting synthesis.

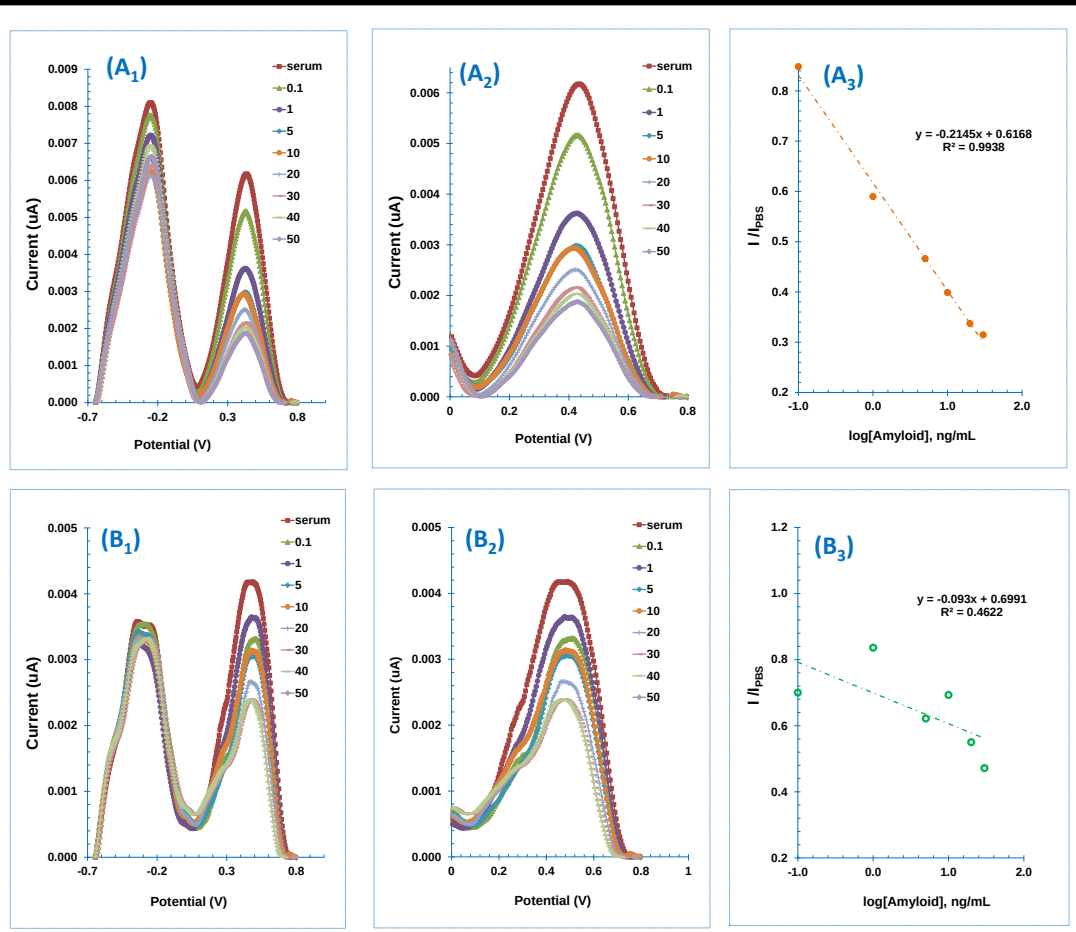
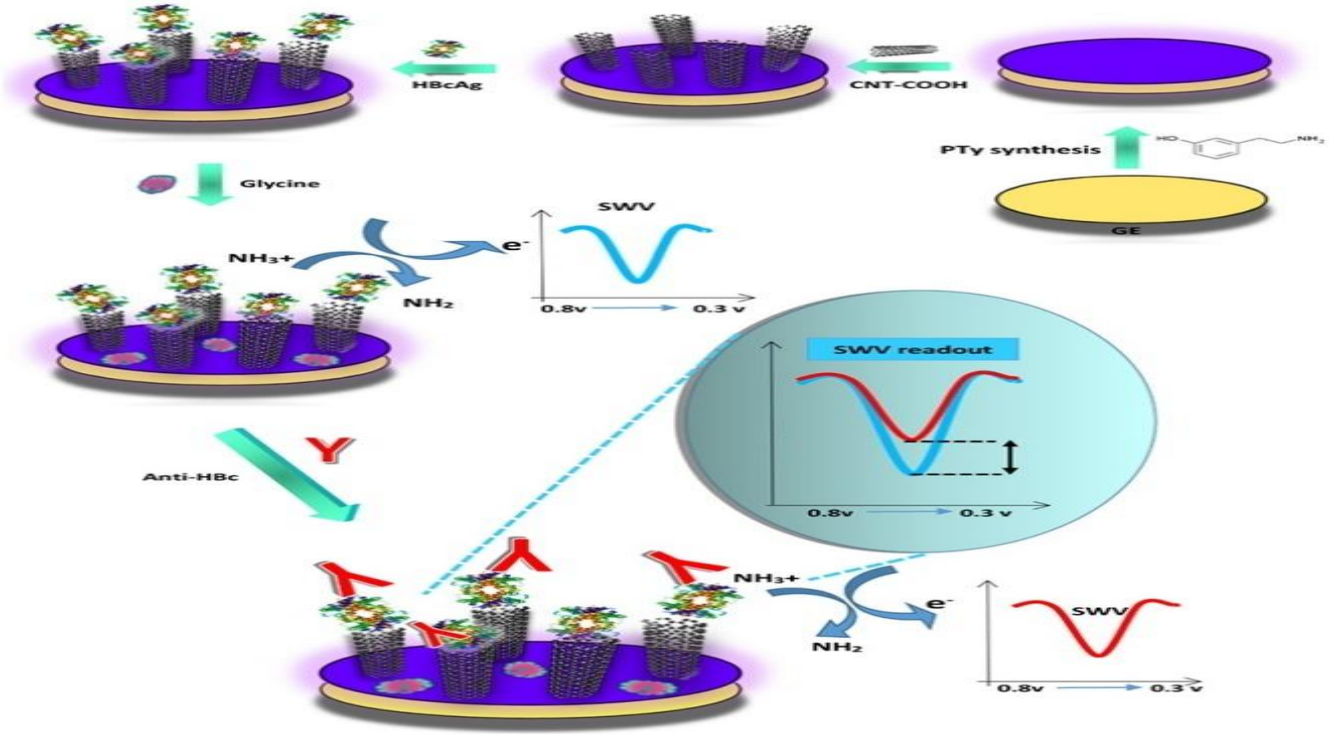


Figure 6 - SWV measurements of (A) MPan/CNT-Cu /C-SPE and (B) NPan/CNT-Cu /C-SPE based biosensor in wide potential range (1), the insite of a narrow potential range (2) and the corresponding calibration curve (3). Assays in human serum samples diluted 100 times at pH 7.4 with different concentrations of Aβ42 (ng/mL).

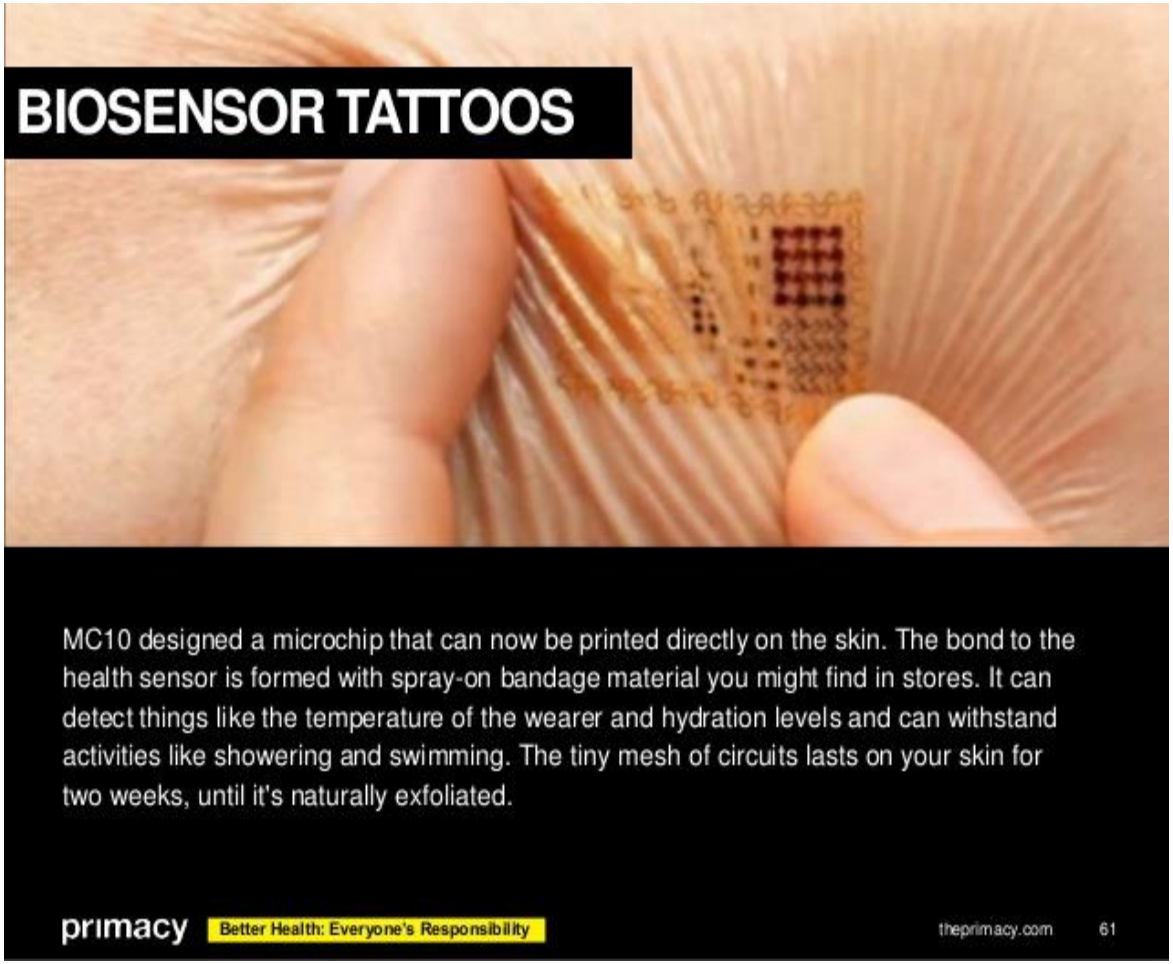
Colloids and Surfaces B: Biointerfaces

Volume 172, 1 December 2018, Pages 272-279

A label-free and reagentless immunoelectrode for antibodies against hepatitis B core antigen (anti-HBc) detection



New approaches using CNT … biomaterials to nanohybrid!



Lactate biosensor

Wang group's, 2013

Drug delivery

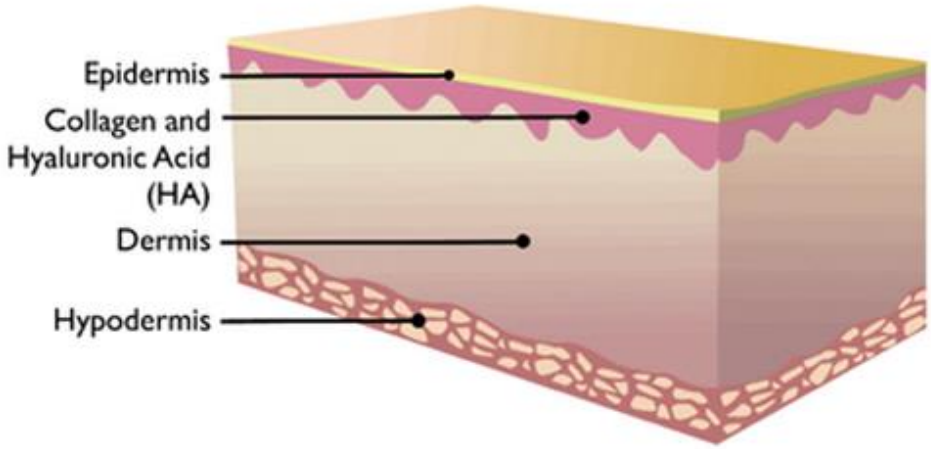
Dae-Hyeong Kim at Seoul in South Korea, Nature 2014

Stick-on skin sensor

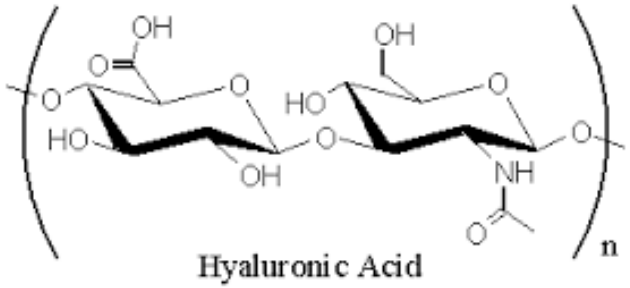
Hyalluronic acid for a nanobiosensor:

HYALURONIC ACID (HA) IS THE FOUNDATION OF OUR SERUM LINE

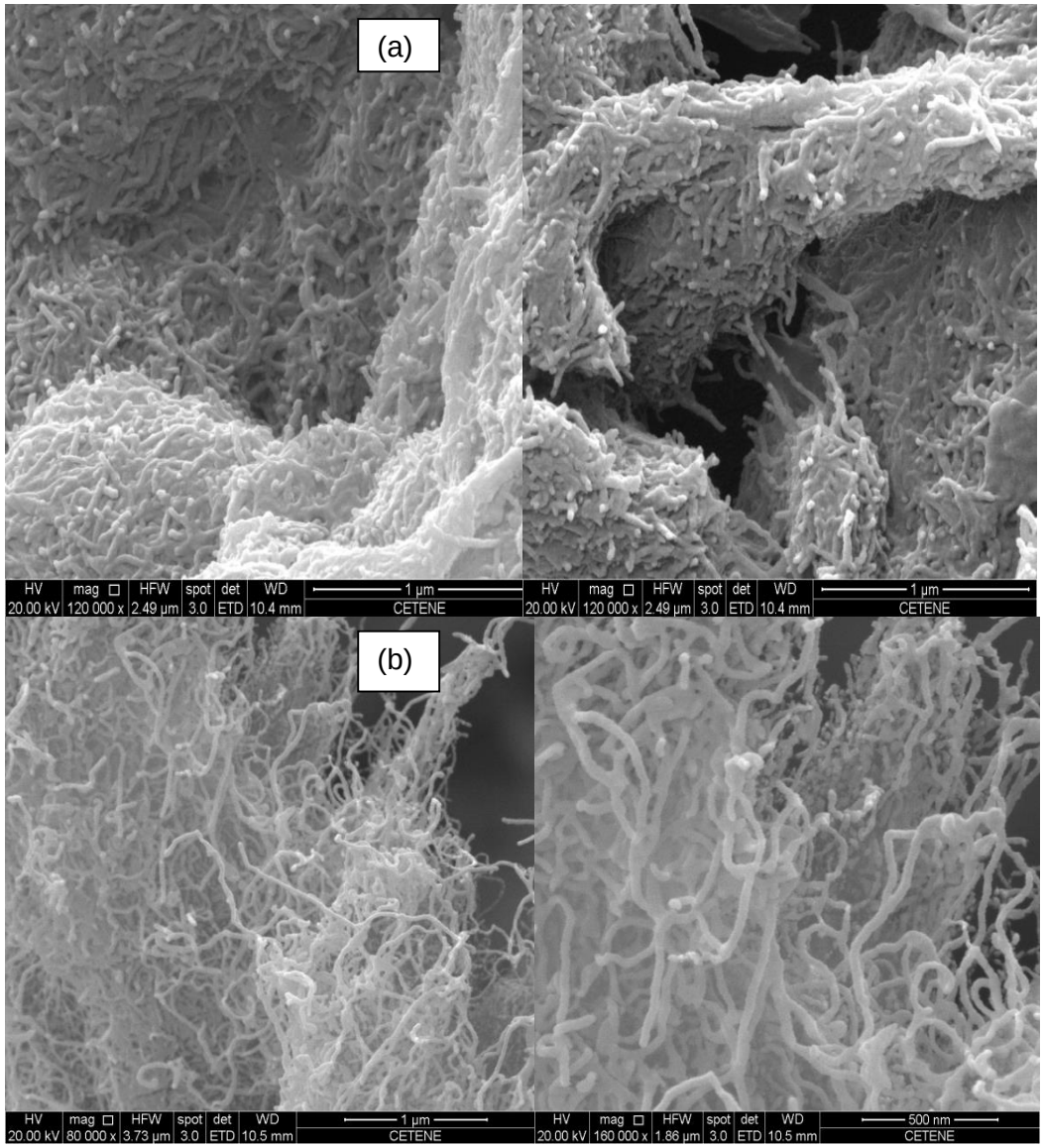
50% of the Hyaluronic Acid (HA) found in the body is found in the skin layers of the dermis and epidermis. HA keeps skin hydrated, supported and provides nourishment transport to the cells. With age, the ability of the skin fibroblasts to produce HA decreases leaving the skin unhealthy and wrinkled.

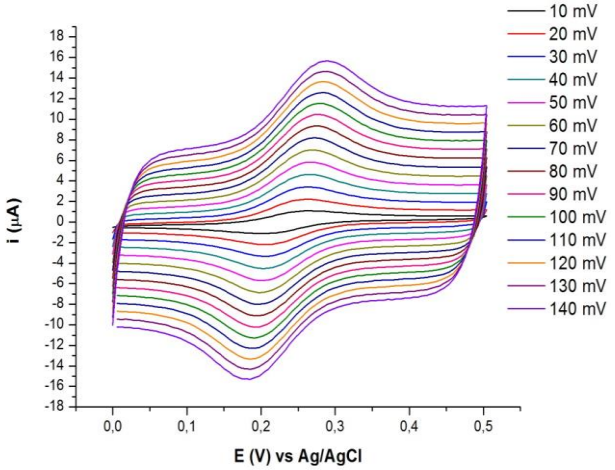


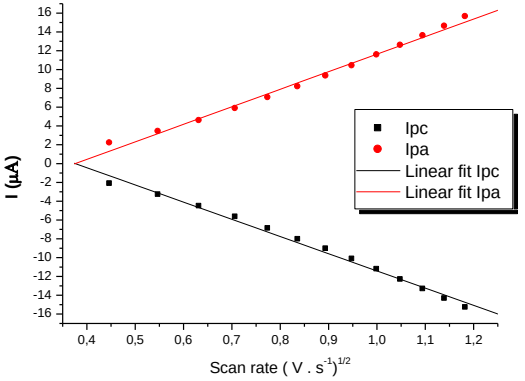
HA has been shown to be absorbed topically and keeps skin hydrated and can prevent wrinkle formation. HA is the subject of a great deal of research because of it's amazing properties to rejuvenate and moisturize the skin.



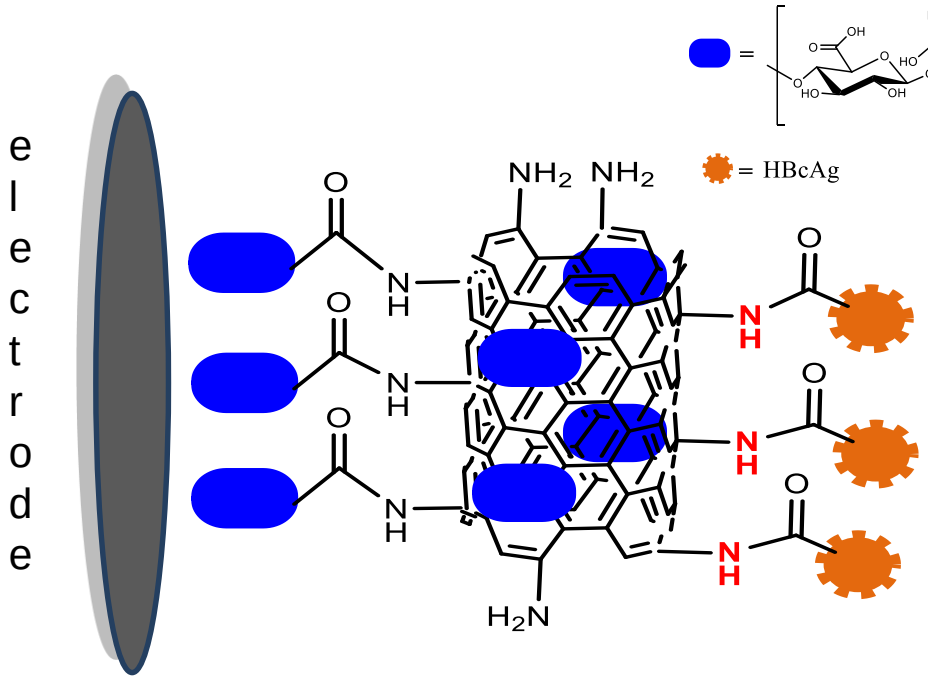
Hyaluronic Acid

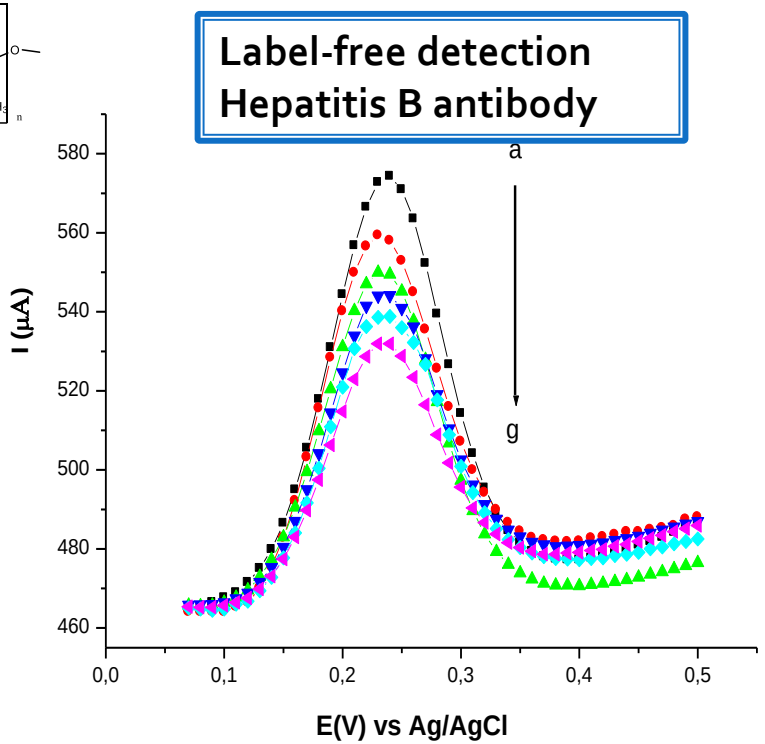




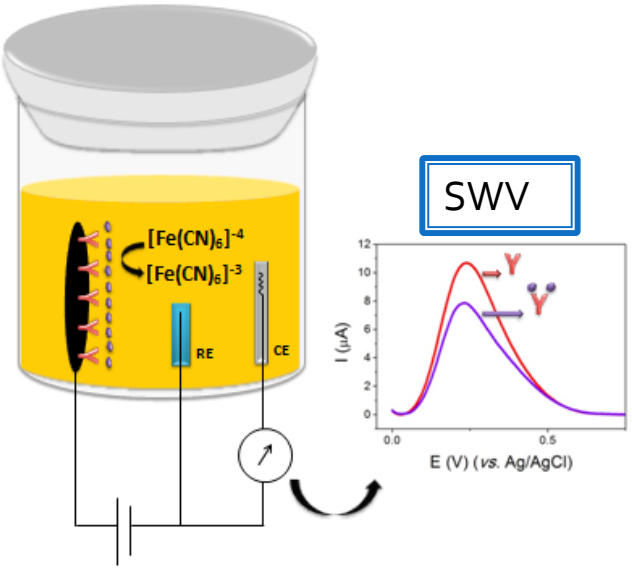


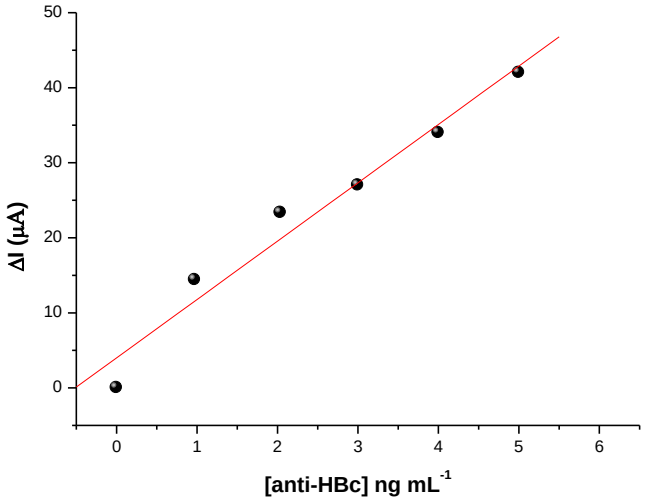
SEM micrographies of HA-CNTs (a) after EDC/NHS activation (b) control (without EDC/NHS activation).





SWV curves of analytical responses to anti-HBc





Talanta, 2015 (accepted to publication)

IMMUNOSENSOR BASED GRAPHENE-POLYSULFONE FOR DETECTION OF RHEUMATOID ARTHRITIS

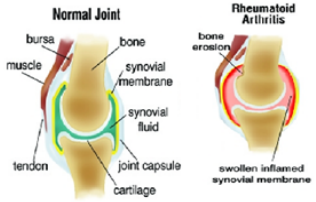
SILVA-JR, A.A.[1]; RODRÍGUEZ, B.A.G.[1], BAHAMONDE, J.P.[2], VELAZQUEZ, V.G.[2] DUTRA, R.A.F.[1]
[1] Departamento de Engenharia Biomédica, CTG – UFPE, Recife, Pernambuco, Brazil.
[2] University Carlos III de Madrid, Madrid, Spain.

Laboratório de Engenharia Biomédica

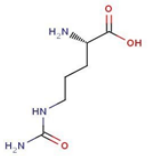
INTRODUCTION

Rheumatoid arthritis (RA) is a chronic degenerative disease manifests as specifics, auto-antibodies directed against citrullinated proteins. Cyclic citrullinated peptide antibody being (ACCP) important marker for the diagnosis and prognosis of RA. ACCP detection performed by ELISA, but has a low detection limit. Therefore, the development of high sensitivity is required, diagnostic specificity and low cost, such as immunosensors.

Rheumatoid Arthritis



ACCP



Immunological test

Immunoassays main tool for diagnosing

Immunosensors

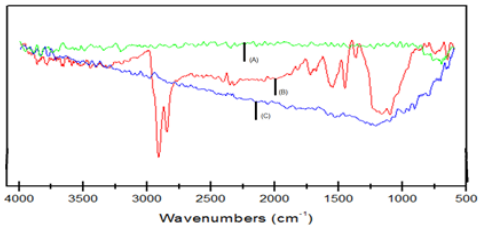
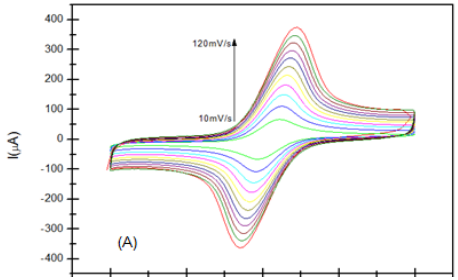
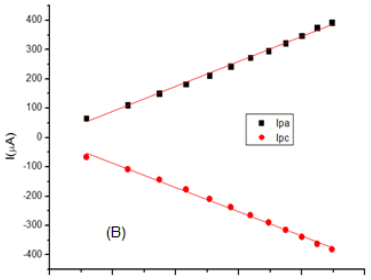
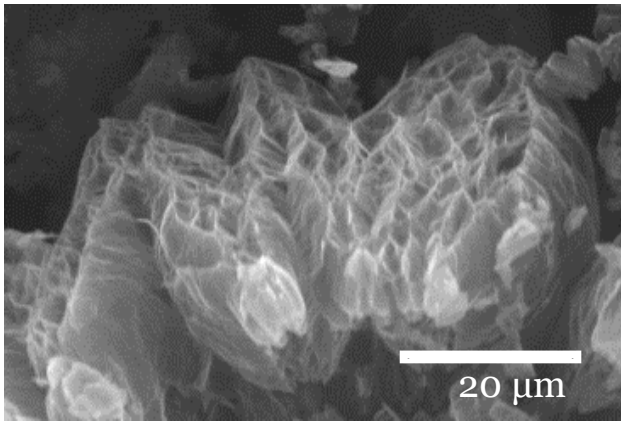
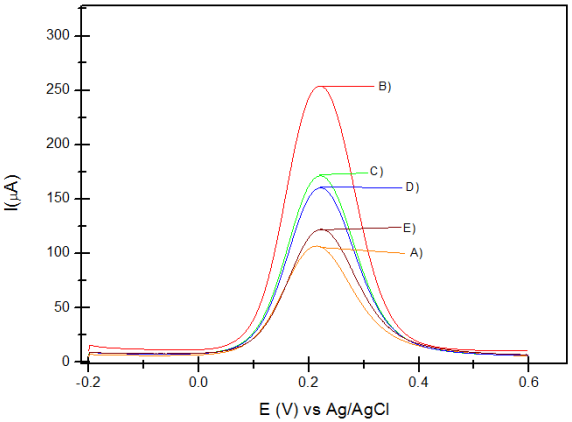


Figure 2. Figure 2. By FTIR was perceived difference in the transmittance spectra of the working electrode surface is not changed (A) of ROG-PSO (B) and ROR (C).









Biosensors and Bioelectronics 25 (2009) 870–876

Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/bios

Potential of a simplified measurement scheme and device structure for a low cost label-free point-of-care capacitive biosensor

Elder A. de Vasconcelos[a], Newton G. Peres[b], Cintya O. Pereira[b], Valdinete L. da Silva[c], Eronides F. da Silva Jr.[a], Rosa F. Dutra[b,*]

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[b] *Laboratório de Pesquisa em Diagnóstico/LAPED, PROCAPE, Universidade de Pernambuco, Rua Arnóbio Marques, Santo Amaro, 310, 50100-130, Recife-PE, Brazil*
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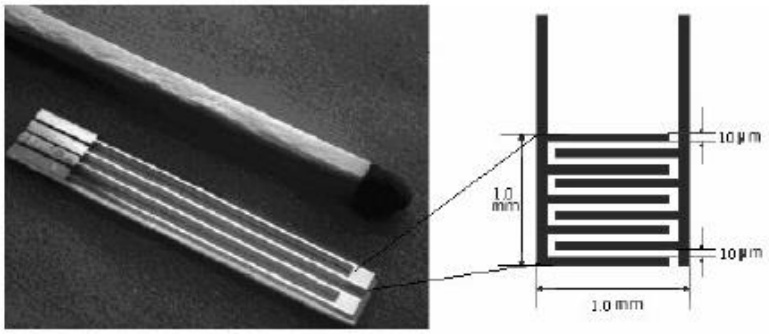
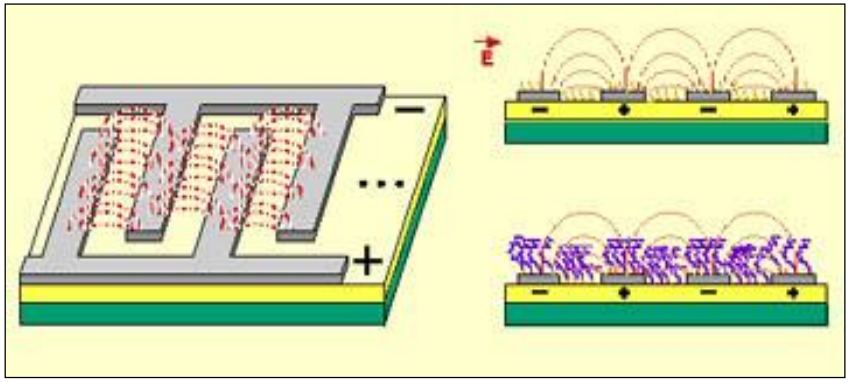
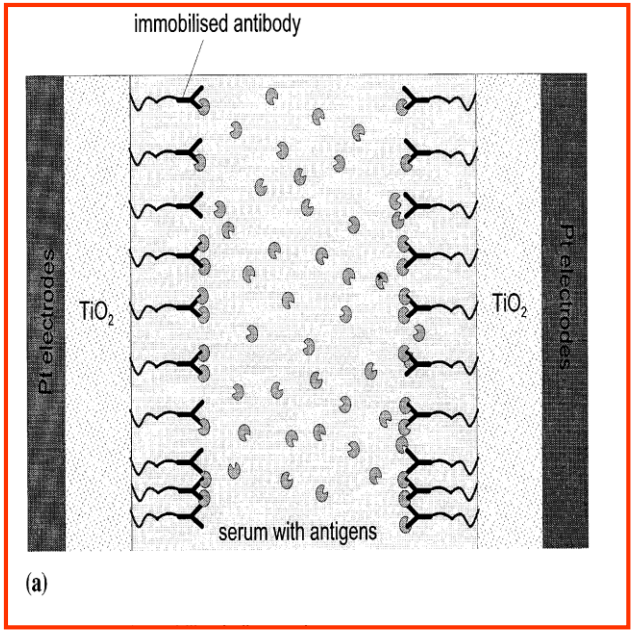
•Capacitive Transduction: a new idea

-> based on electrical conductivity changings at a constant AC voltage by antigen-antibody interactions

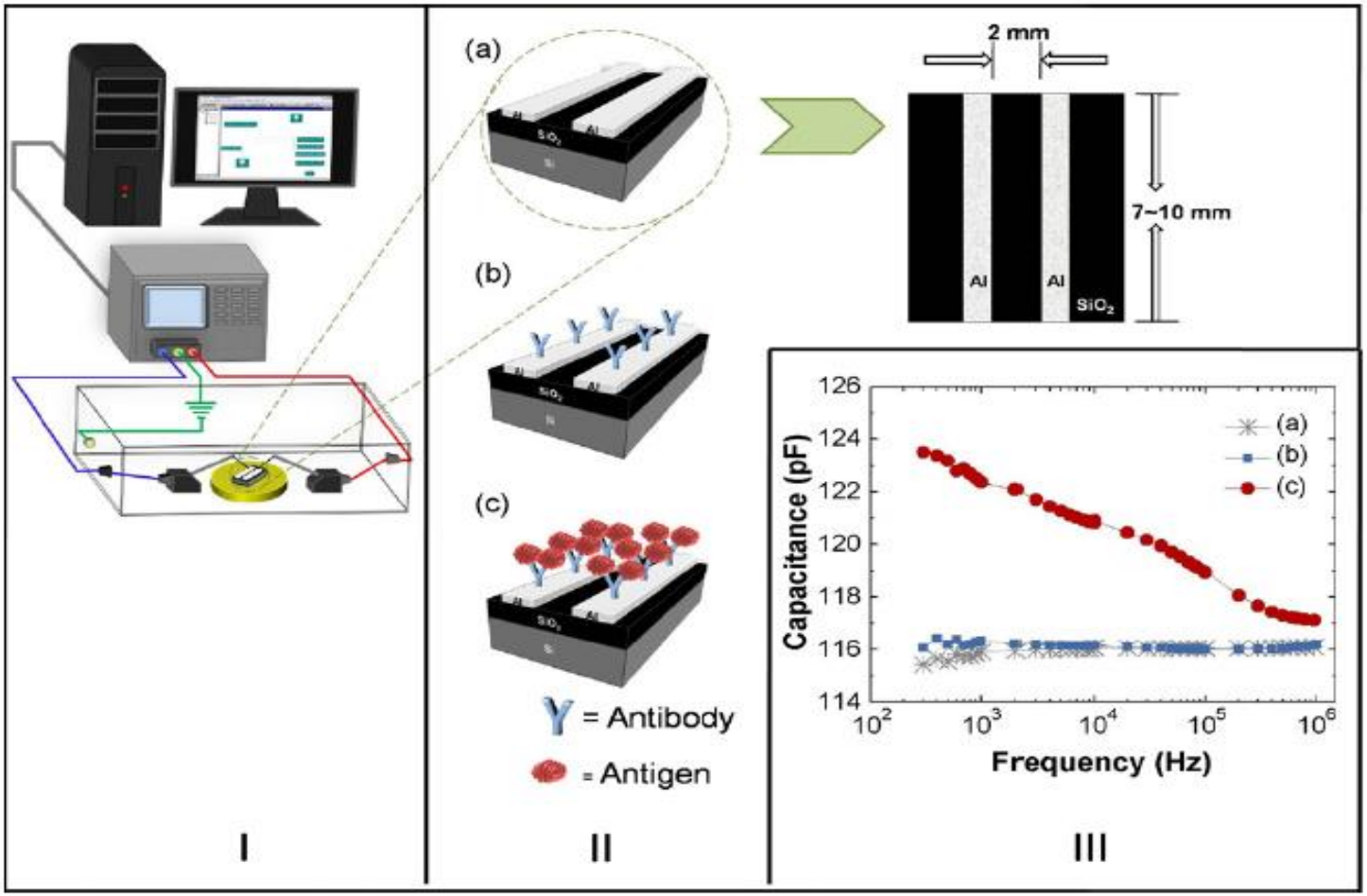






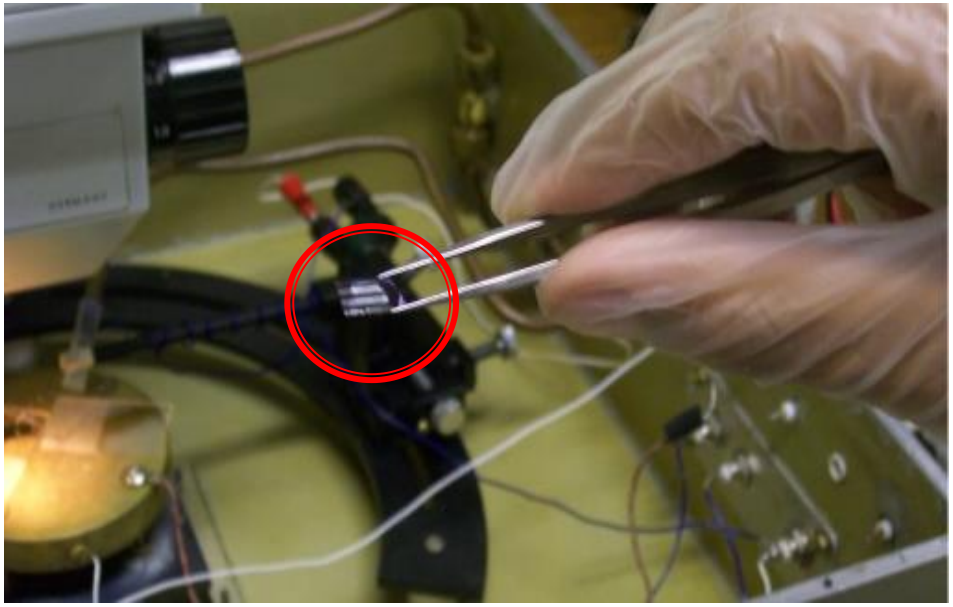
Dutra et al, 2013. Biosensors& Biolectronics

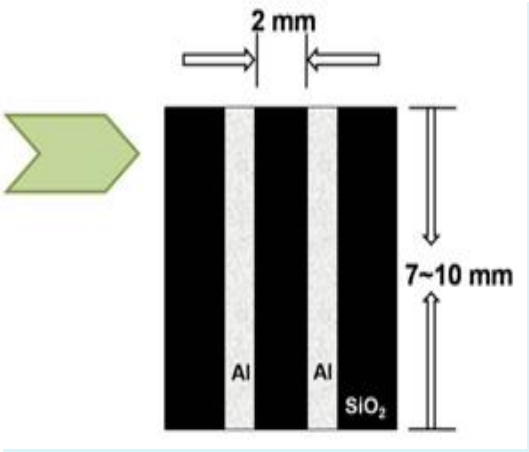
ImmunoSensing platform based a simple capacitor



Device fabrication (sensor chip)

Si <100> wafers were cleaned in ultrasonic agitation by a sequence of 5-min immersions in acetone, methanol and HF with DI water between steps. A silicon dioxide (SiO_2) layer (350 nm-thickness) was grown in a tubular furnace by wet oxidation. Two Al electrodes (80 nm thickness each) onto the SiO_2 layer were thermally evaporated from a tungstein boat followed by a 30-min anneal in forming gas at 400°C.

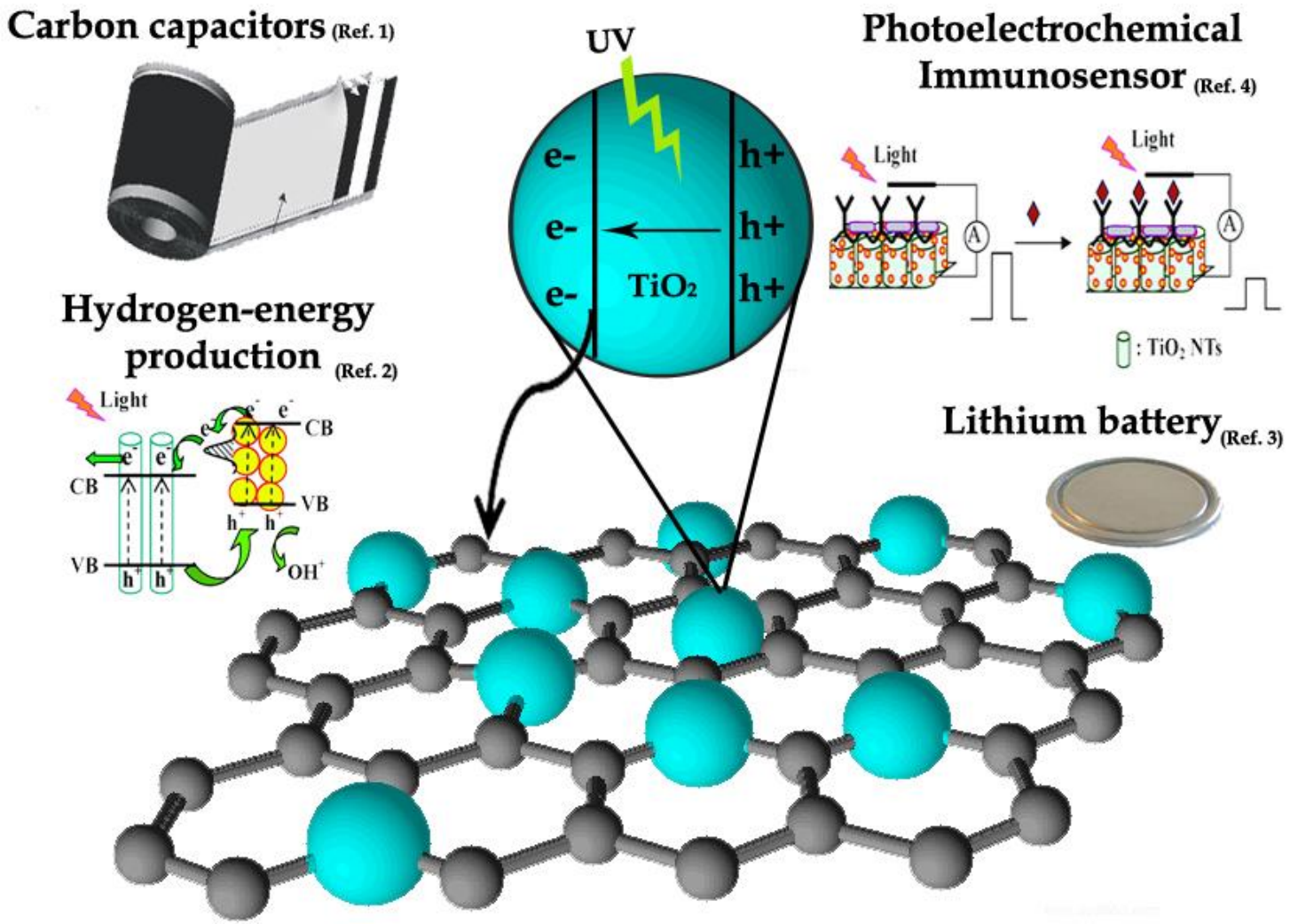


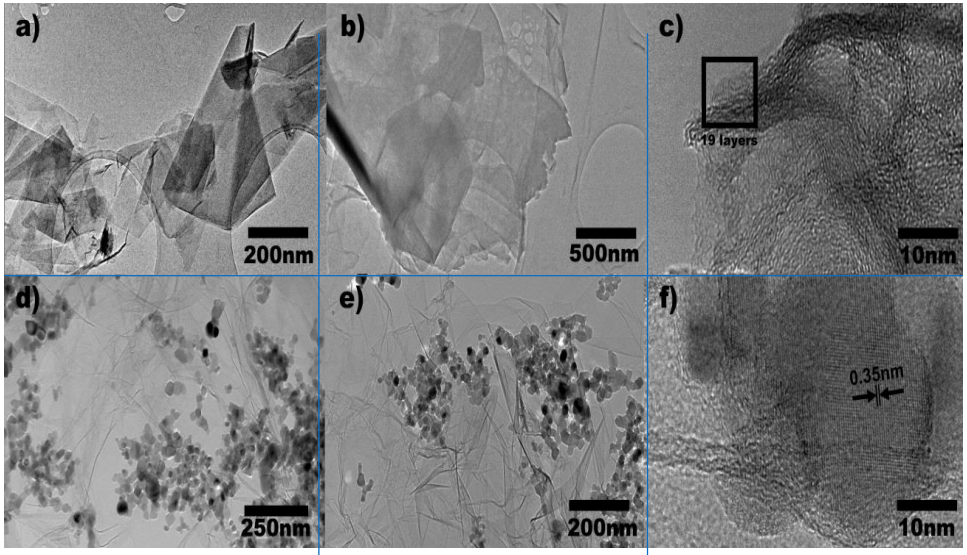


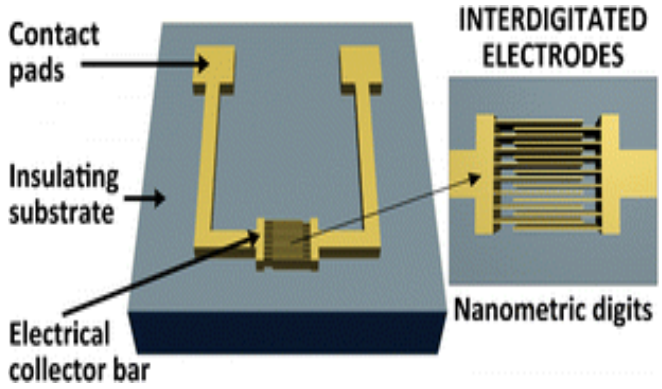
Device structure

The devices were fabricated in a Class 1000 cleanroom (Federal Standard 209D) at the Federal University of Pernambuco.

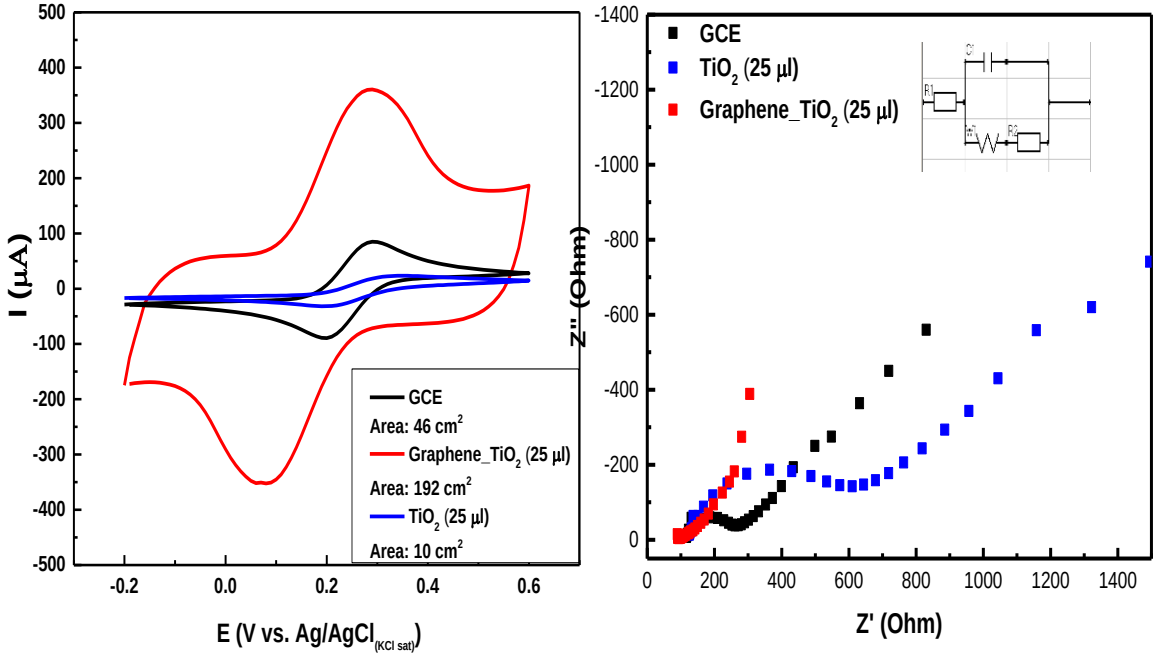
RGO_TiO_2 composite for electrochemical immunocapacitors







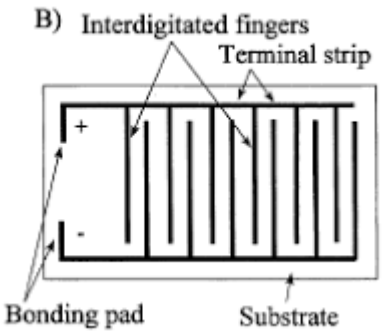
TEM and HRTEM images of GOx (a-c) and TiO_2 over GOx sheets(c-f).



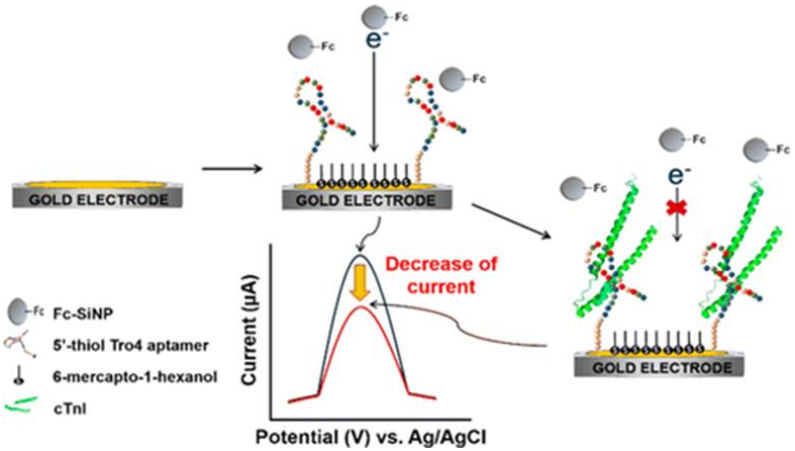
CV curves comparing TiO_2 and RGO_TiO_2, D) EIS Spectroscopy. GCE 1.17 µF, TiO_2 NPs 5.56 µF and RGO_TiO_2 30 mF.

Interdigitated planar capacitor

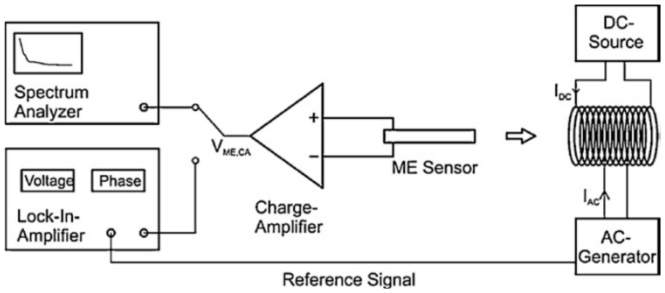
Nanocomposite “grafted’
Ppy –NTCTiO2 on IDE

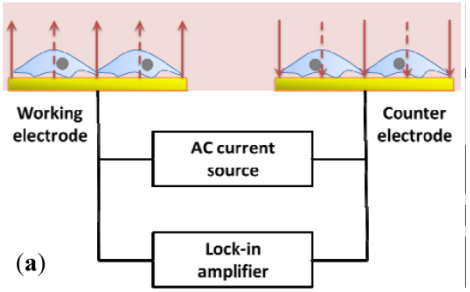


Cervical Cancer



Lockin amplifier - Capacitance





Dutra et al., 2018 submited to Analyst

Summary and outlook

- Synthesis of nanomaterial should be more controlled.
- Nanomaterials dedicated should be intended.
- Nano MIPs is a good alternative to a trial testing.
- Low cost, one step and large scale biochips and tips should be aim.

Special Thanks! Our team:

PhD Students: Cybelle Silva, Barbara Mendonça, Erika Lima, Erika Trindade, Diego Guerra, Priscila Santos, Djair, Paulo Landim, Gilvania Santana
Master Students : Diego Leite, Cybelle Silva, Priscila,
Graduating students: Carolina, Erika Trindade, Laila
PosDoctoral:.Blanca Azucena Rodrigues, Amanda Oliveira, Elaine Vaz
DTI : Heuryk Rodrigues, Huende.

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Marilia O FGoulart (UFAL)
Neuza Alcantara Neves (UFBA, FIOCRUZ)
Iolanda Vieira (UFSC)
Ernesto Marques (Pittsburgh University, USA)
Renato Araújo (DE, UFPE)
Marco Benedetti (DE, UFPE)
Elder Vasconcelos (DF, UFPE)

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Thank YOU
for your kind attention!!

Email: rosa.dutra@ufpe.br