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Antibody Immobilization in ZnO-Thin film transistors for low-cost biosensors applications

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Abstract: The antibody immobilization with low-cost materials and label-free methods are a challenge for the fabrication of biosensor devices. In this work, it was developed a strategy for antibody im-mobilization on ZnO TFTs over polyethylene terephthalate (PET) as a recyclable plastic substrate. Antibodies were biofunctionalized using a label-free strategy for enteropathogenic E. coli detec-tion. The use of a recyclable plastic substrate PET enables the compatibility with flexible electronics that could contribute for a low-cost biosensor useful in rural communities that do not have the necessary infrastructure and trained personnel for pathogenic bacterial detection in food or water. The development of this technology has the versatility to be extrapolated to different testing models, allowing the early detection of emerging diseases (bacterial or viral), and provides the opportunity to end-users for self-testing.

Keywords: Antibodies; ZnO TFTs; Biosensors

Introduction

The foodborne disease sick to nearly 600 millions of people every year resulting in 420.000 deaths. Diseases have been associated to mainly 31 risk agents (virus, toxins, parasites, chemical products and bacteria).

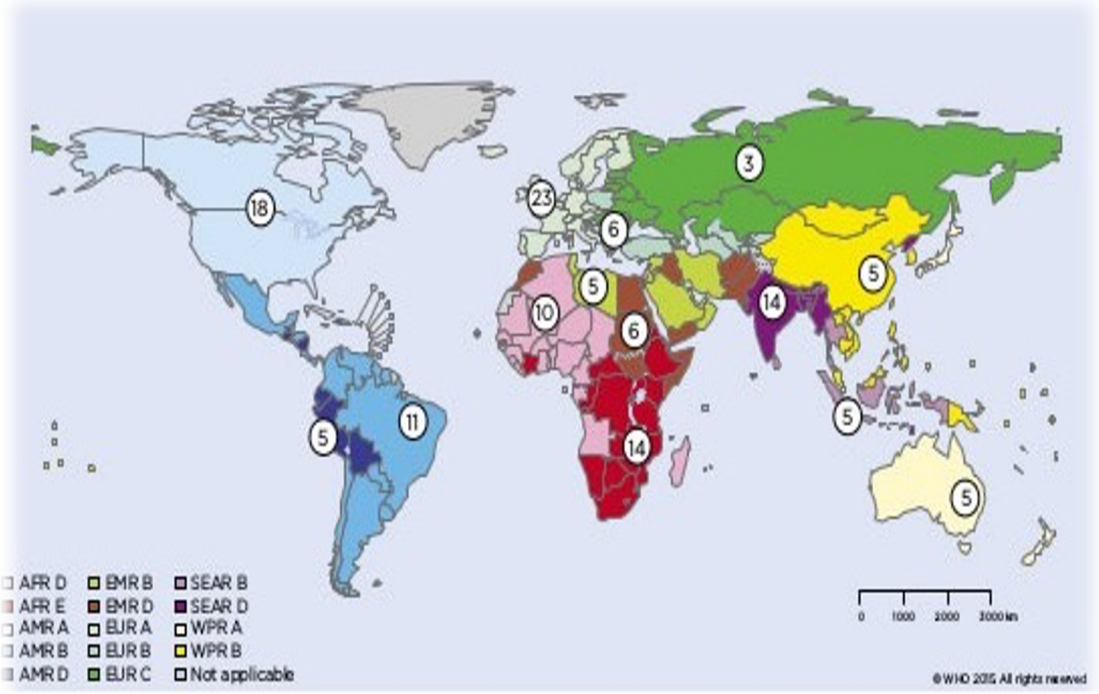


Figure. 1 Geographic distribution of countries by regions and sub-regions.

- ***Salmonella sp***
- ***Campylobacter sp***
- ***Escherichia coli sp***
- ***Listeria sp***
- ***Vibrio cholerae sp***

[WHO, 2010-2015]

Statistics of worldwide burden of foodborne diseases

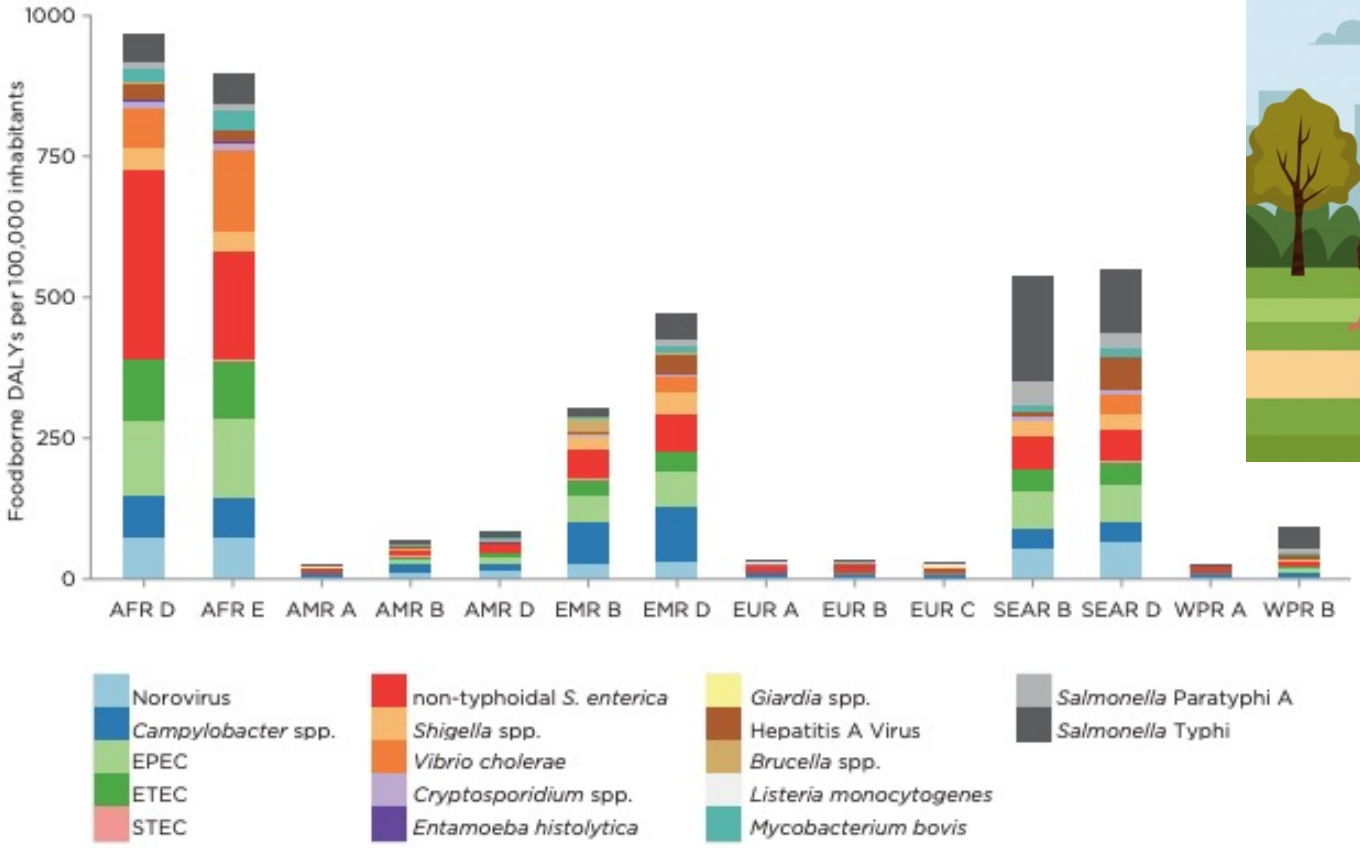


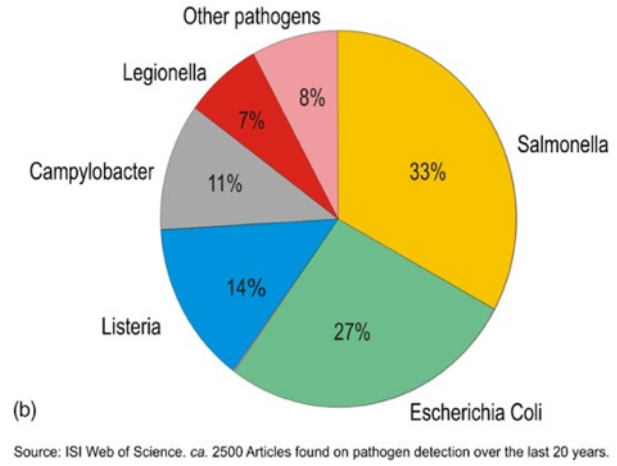
Figure. 2 Worldwide burden of foodborne diseases caused by enteric pathogens.

[WHO, 2010-2017]

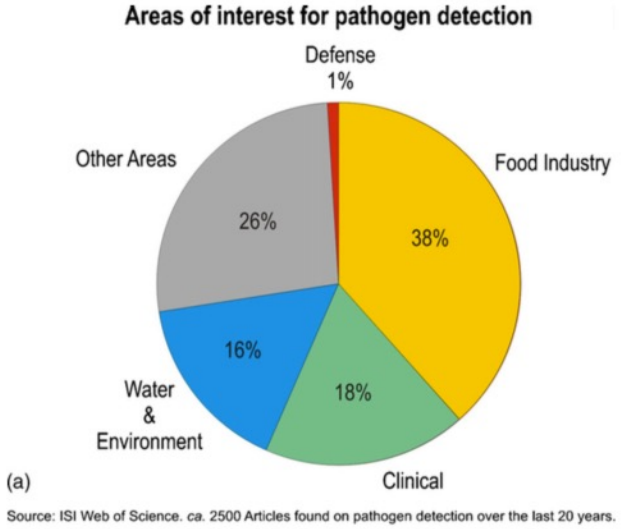
Pathogen Detection

Table 1. Pathogenic detection techniques over the last 20 years.

Detection technique	Sample type	Time of analysis	Working range	Detection limit CFU/mL
ELISA	Ground beef	Next day	10^3 - 10^4	1.2 x 10^3
PCR-ELISA	Milk	5 h	10^0 - 10^4	100
PCR-electrophoresis		2 h	10^1 - 10^4	1000
Real-time PCR	Culture medium, ground beef	5 h 20min 3 h 20min	5 – 5 x 10^4	5
RT-PCR coupled to fluorescence	Drinking water	30 min	1 - 10^6	1.6 x 10^3 CFU/mL
Fiber optic immunosensor	Culture	10 h	6.5 x 10^4	10^2
SPR biosensor	Culture	Not quoted	10^2 - 10^9	10^3
QMC immunosensor	Culture/water	170 min	10^3 - 10^8	
Amperomety	Culture	30 min	100-600	79
Conductimetric biosensor	Mixed culture, wáter, vegetable wash	10 min	10 - 10^6	81
Impedimetric immunosensors	Culture/water	10 min	10^4 - 10^7	10^4 in culture 10^7 in water



Source: ISI Web of Science. *ca.* 2500 Articles found on pathogen detection over the last 20 years.



Source: ISI Web of Science. *ca.* 2500 Articles found on pathogen detection over the last 20 years.

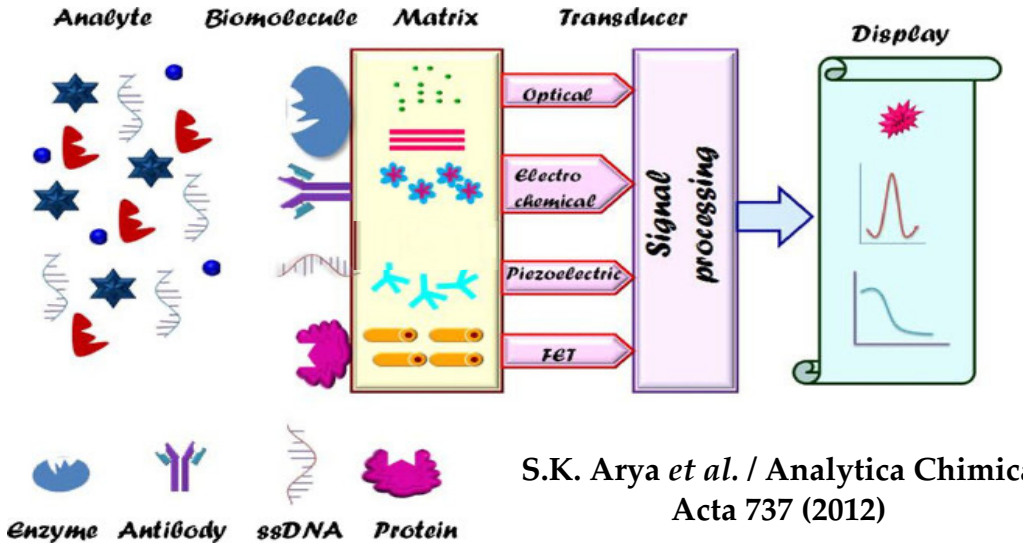
A new field of study has been developed consisting on biosensor devices

O. Lazcka et al. / Biosensors and Bioelectronics 22 (2007) 1205–1217

Biosensor

A biosensor is an integrated receptor-transducer device that uses a biological recognition element to provide selective, quantitative and semi-quantitative information.

Xin Du-2018



S.K. Arya *et al.* / Analytica Chimica Acta 737 (2012)

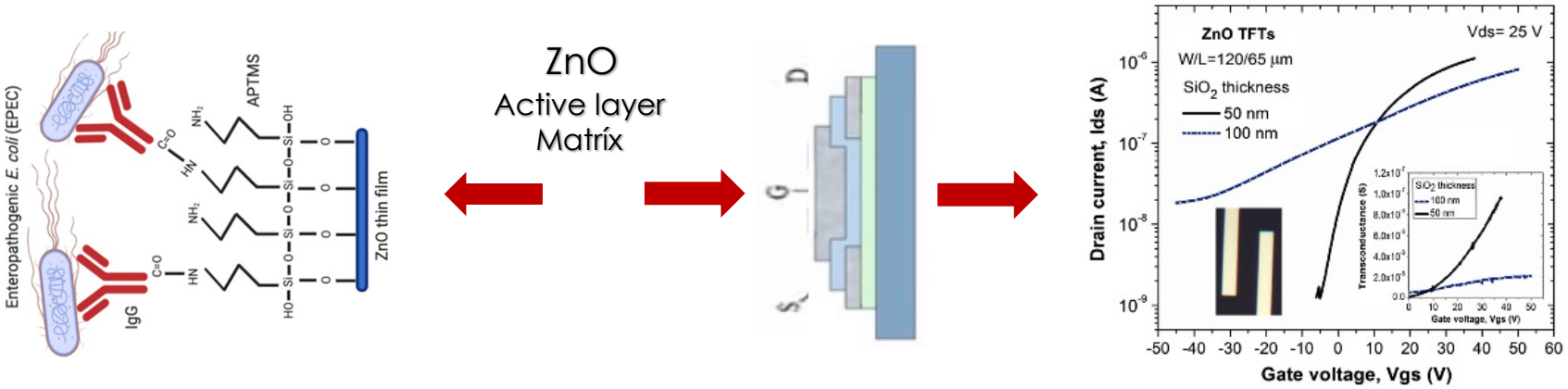


Figure. 3 Schematic view of the general components of a biosensor.

M.A. Dominguez *et al.* / Solid-State Electronics 109 (2015) 33–36

ACS Omega 2020, 5, 20473−20480

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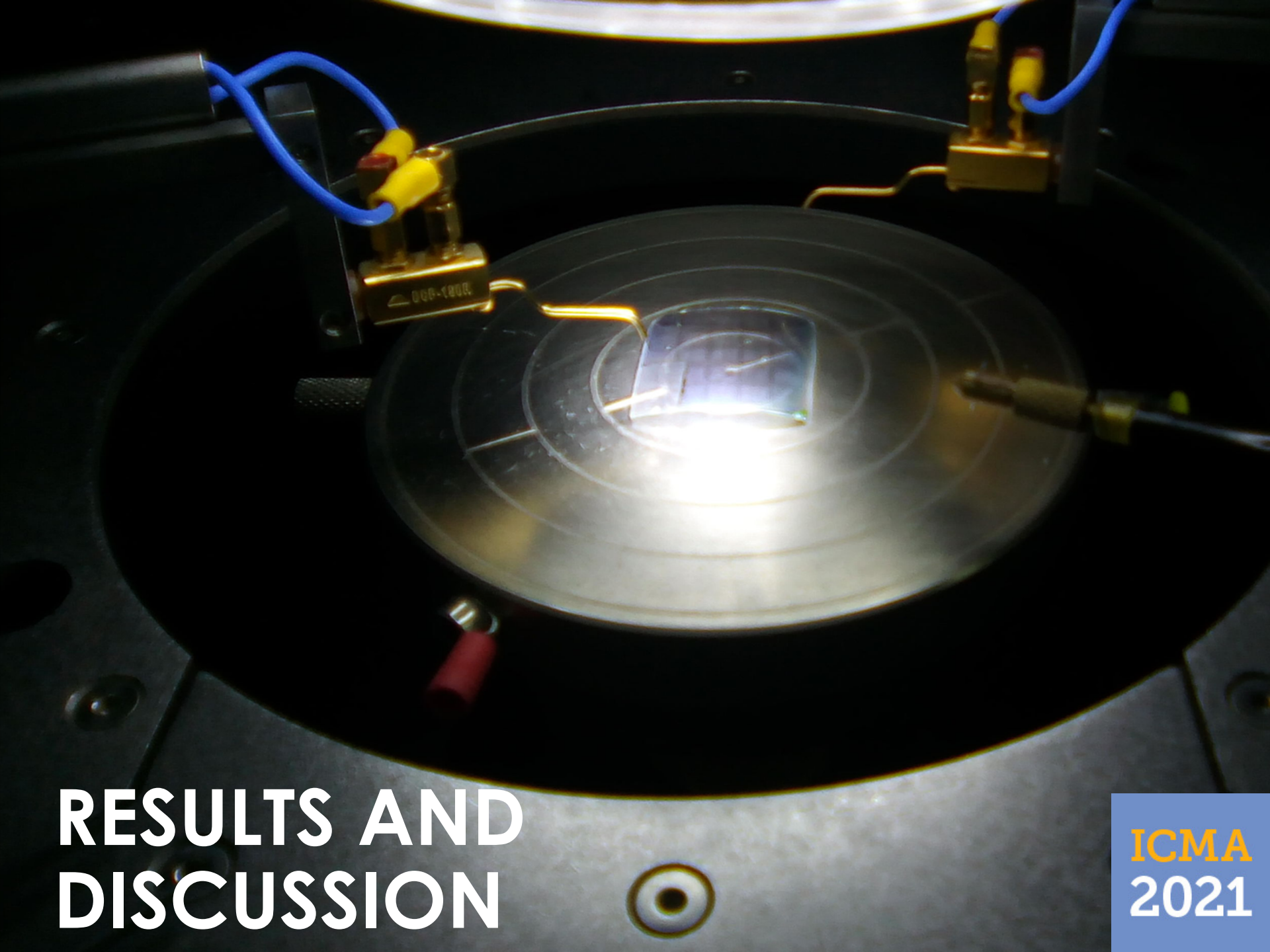

RESULTS AND DISCUSSION

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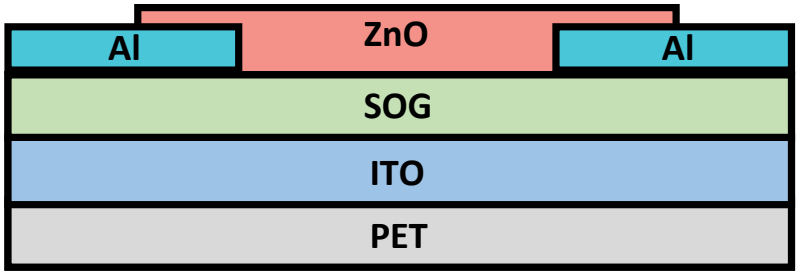
Schematic View

Platform
(Polyethylene terephthalate – PET / Indium tin oxide - ITO)

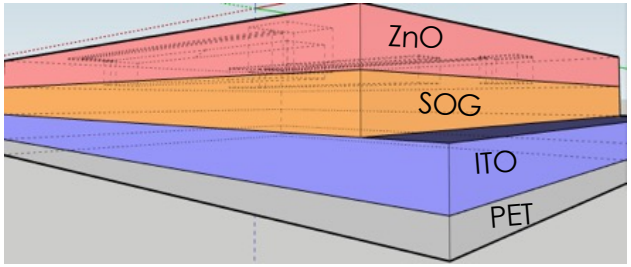
SOG – SiO_2
(Silicon oxide)

Photolitography (contacts)

Active layer (ZnO)



Transversal section



Temperature = 200˚ C was used for fabrication

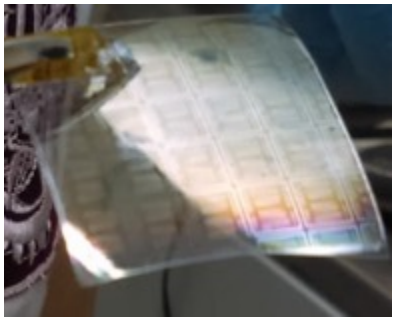
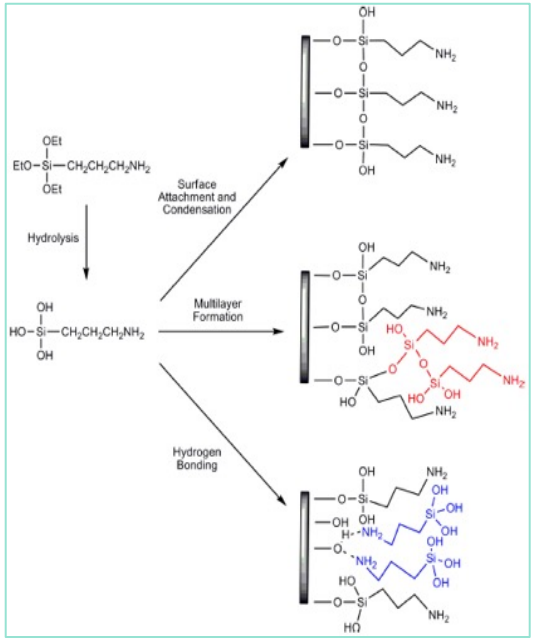


Figure. 4 Schematic view of the ZnO-TFTs (Sketchup 2020)

Biofunctionalization

1 **Surface hydroxylation. Silanization: APTMS**



Modified from S. Kumar et al. "Immobilization of Antibodies and Enzymes on 3-APTES" (2014)

2 **Immobilization.**

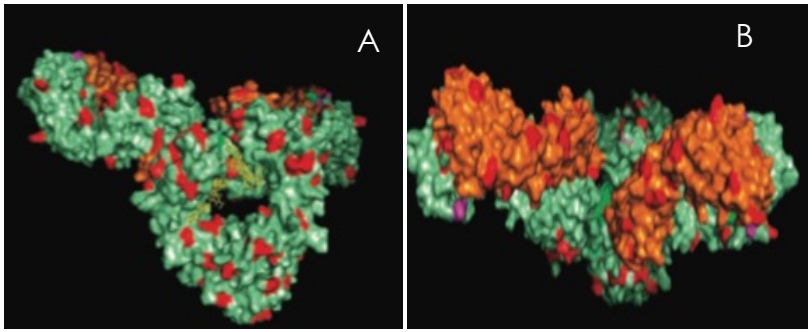
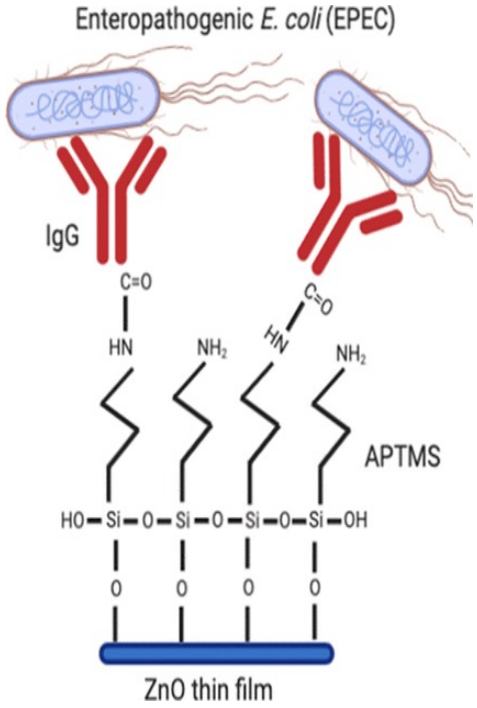


Figure. 5 3D structure of Ig G from rabbit (Anti-HRP). Protein Data Bank (PDB). The heavy chains are represented in green, the light ones in brown. In red are represented the carboxyl groups and in purple the amino terminal groups.



Biomacromolecules 2008, 9, 2230–2236

ACS Omega 2020, 5, 20473–20480

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Antibody Immobilization

Bradford reaction

Specific bond between blue Coomassie dye G250 and aminoacid (Arg, Trp, Tyr, His and Phe) from proteins.

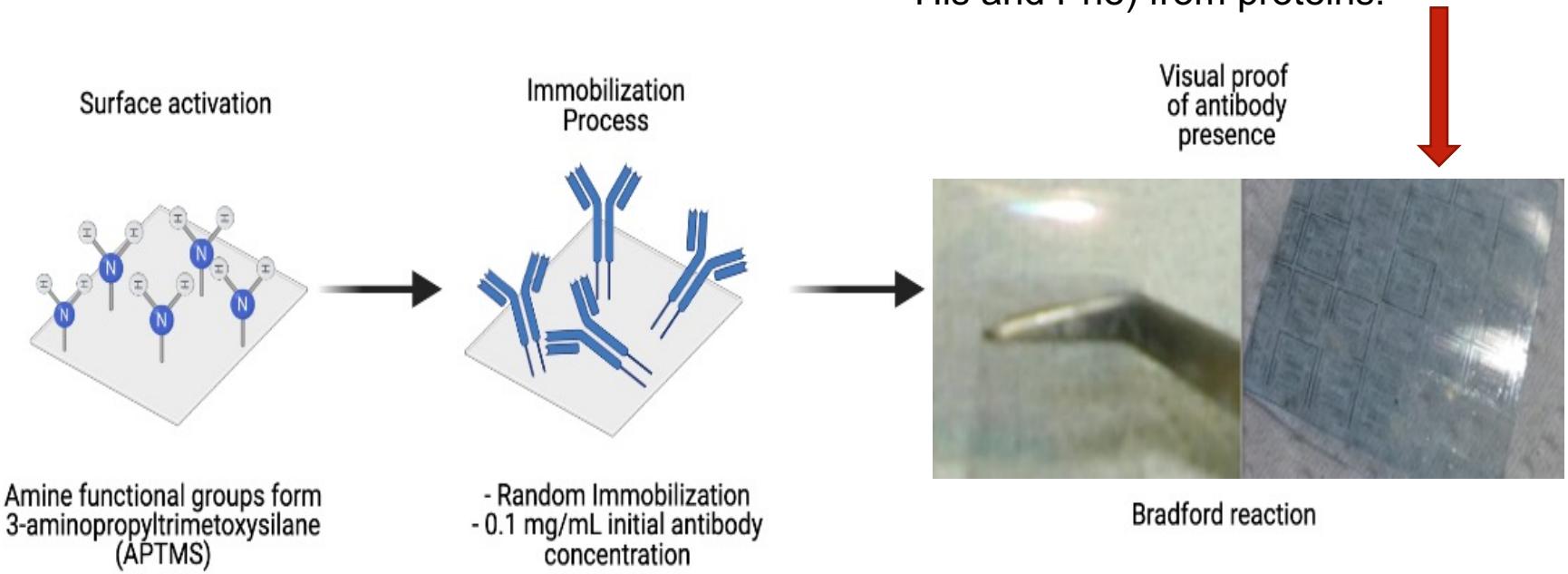
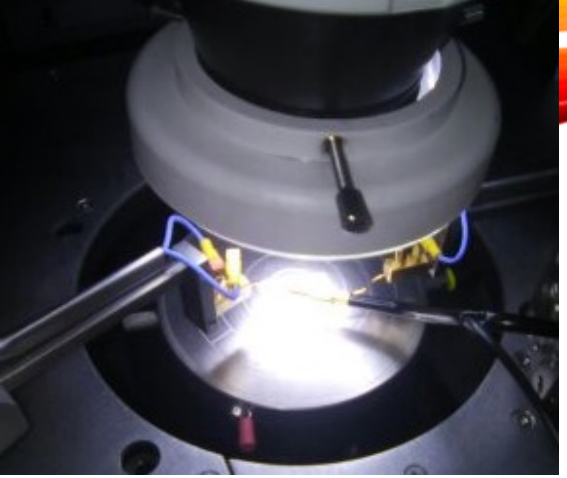


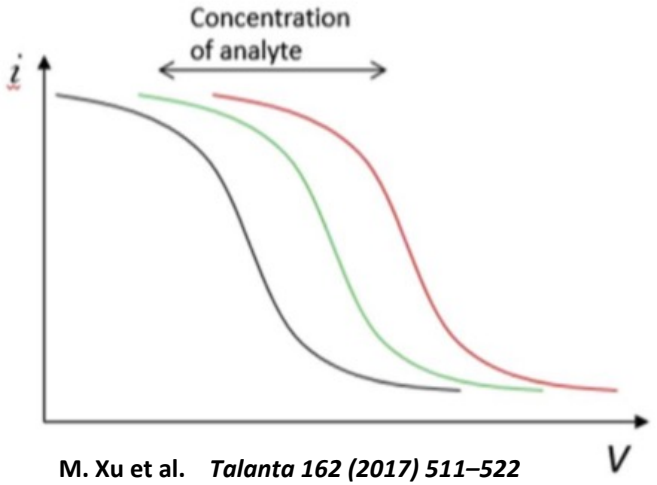
Figure 6. Methodological representation of the immobilization strategy (Biorender, 2021)

TFTs before and after biological treatment



Transfer Characteristic curves

Expected Behavior



M. Xu et al. *Talanta 162 (2017) 511–522*

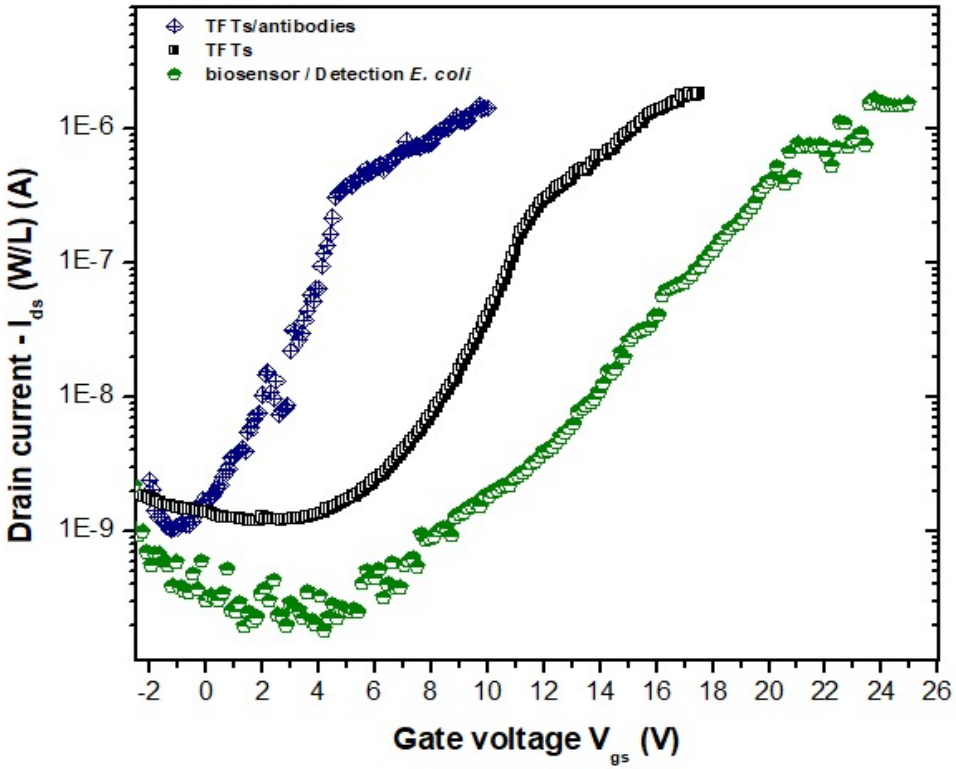


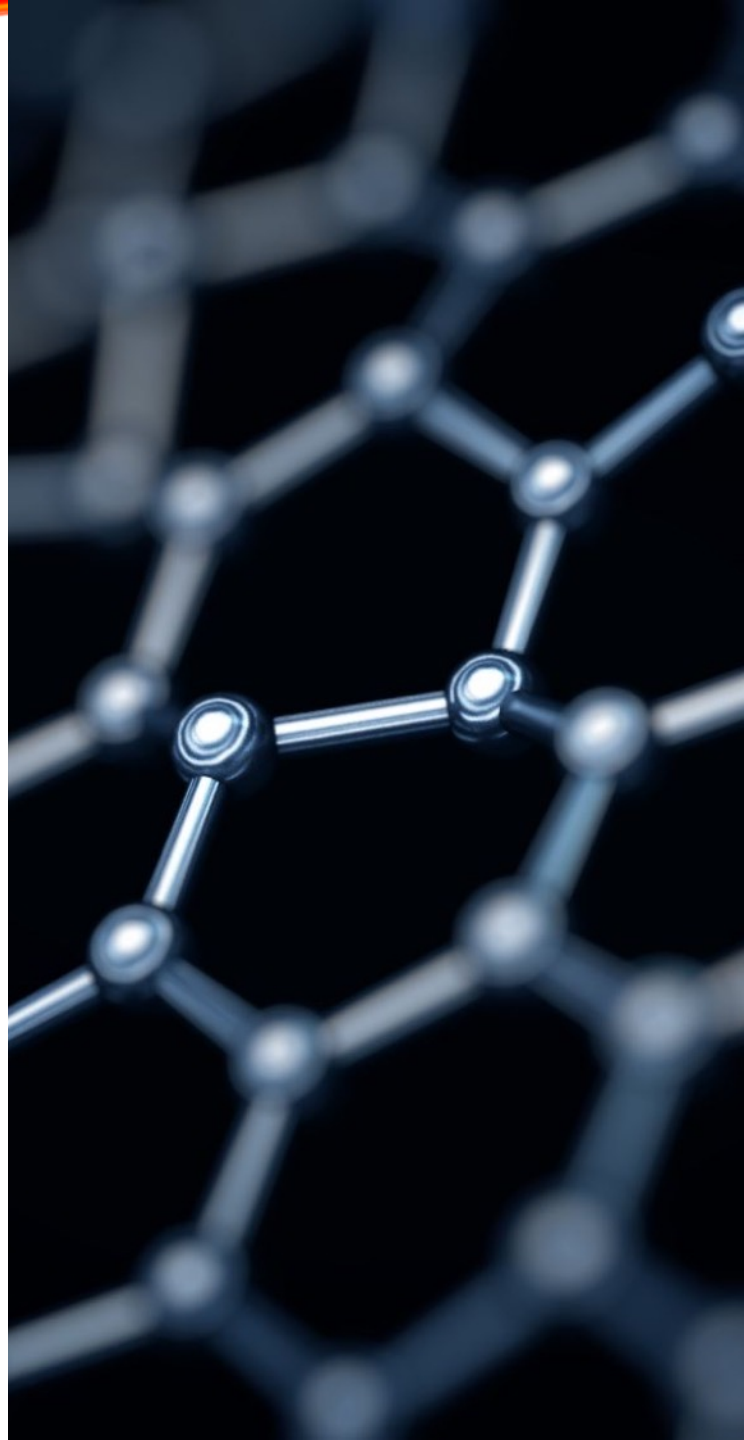
Fig. 7 Gate voltaje vs drain current in logaritmic scale from -2 volts to 26 volts.

	Threshold Voltage (V_T)
No Antibodies	10.5
Antibodies	1.45

The threshold voltage (V_T) is defined as the gate voltage at which conduction electrons begin to appear in the channel.

CONCLUSIONS

- The methodological process for immobilization of antibodies has been found to be reproducible.
- The process of chemical modification of the transistor active layer does not affect the electrical behavior or any of the components, which allows a transfer curve to be obtained, and the process conditions are reproducible.
- It is worth mentioning that the use of plastic substrates and processing at low temperature enables the development of immunosensor devices with an important projection for low cost and sustainable technologies.



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