Improving the longevity of neural recordings of long-term implanted carbon nanotube electrodes in insects.

John O'Toole[1], Carly Kelley[1], Noe Alvarez[2], Elke Buschbeck[1]

1 Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio, 2 Department of Chemistry, University of Cincinnati, Cincinnati, Ohio

Introduction

The implantation of electrodes to measure changes in voltage has been used clinically and in research to further develop knowledge of neural activity. These electrodes typically consist of rigid, metallic fibers. Over time, it has been shown that such electrodes tend to become less efficient, due to micro-lesions and scar-tissue formation in response to rigid materials interacting with the soft and moveable tissue of the nervous system. In this project we evaluate the use of carbon nanotube (CNT) electrodes, as a soft, and chemically inert material and test how durable such electrodes are for long-term neural recordings. Specifically, we monitor neural responses from-long term implanted electrodes in insects using electroretinography (ERGs), an extracellular measurement of the photoreceptor response to light stimuli. In addition to neural recordings, we use histological examination of the tissue-fiber interface of the insect subjects following their implantation and recording period.

Preliminary findings suggest that CNT wires integrate particularly well with surrounding tissue. Our project focuses on testing fibers that have been further modified by functionalization with carboxyl groups, or with molecules that naturally occur in the nervous system, such as the neurotransmitter glutamate. Such treatments will enhance material properties including their hydrophobicity, and our studies on insects represent an important screening tool for further improvements of electrodes that maintain proper electron transfer with enhanced bio-cooperability.

Approach

In order to test the stability of neural recordings achieved from CNT electrode implants functionalized with carboxyl groups, Madagascar hissing cockroaches (*Gromphadorhina portentosa)* and grey flesh flies (*Sarcophaga bullata)* were used as subjects. For the roaches, each subject had a connector attached to the middle of the dorsal thorax. Wrapped around each node of the connector with silver conductive paint as adhesive were 3 CNT-COOH electrodes, 2 implanted into each eye, and 1 implanted into the upper dorsal thorax as a ground wire. The wires were held in place near their respective sites of implantation by wax. As for the flesh flies, a similar method of implantation was used. Furthermore, silver and gold were used as inert and noninert metallic controls when testing the longevity of CNT-COOH implants in the flies. Implanted subjects were exposed to an LED light stimulus and their response to this stimulus was measured using ERGs. The recording setup consisted of a faraday cage, amplifier, digital-analog converter, and a computer running LabScribe software.

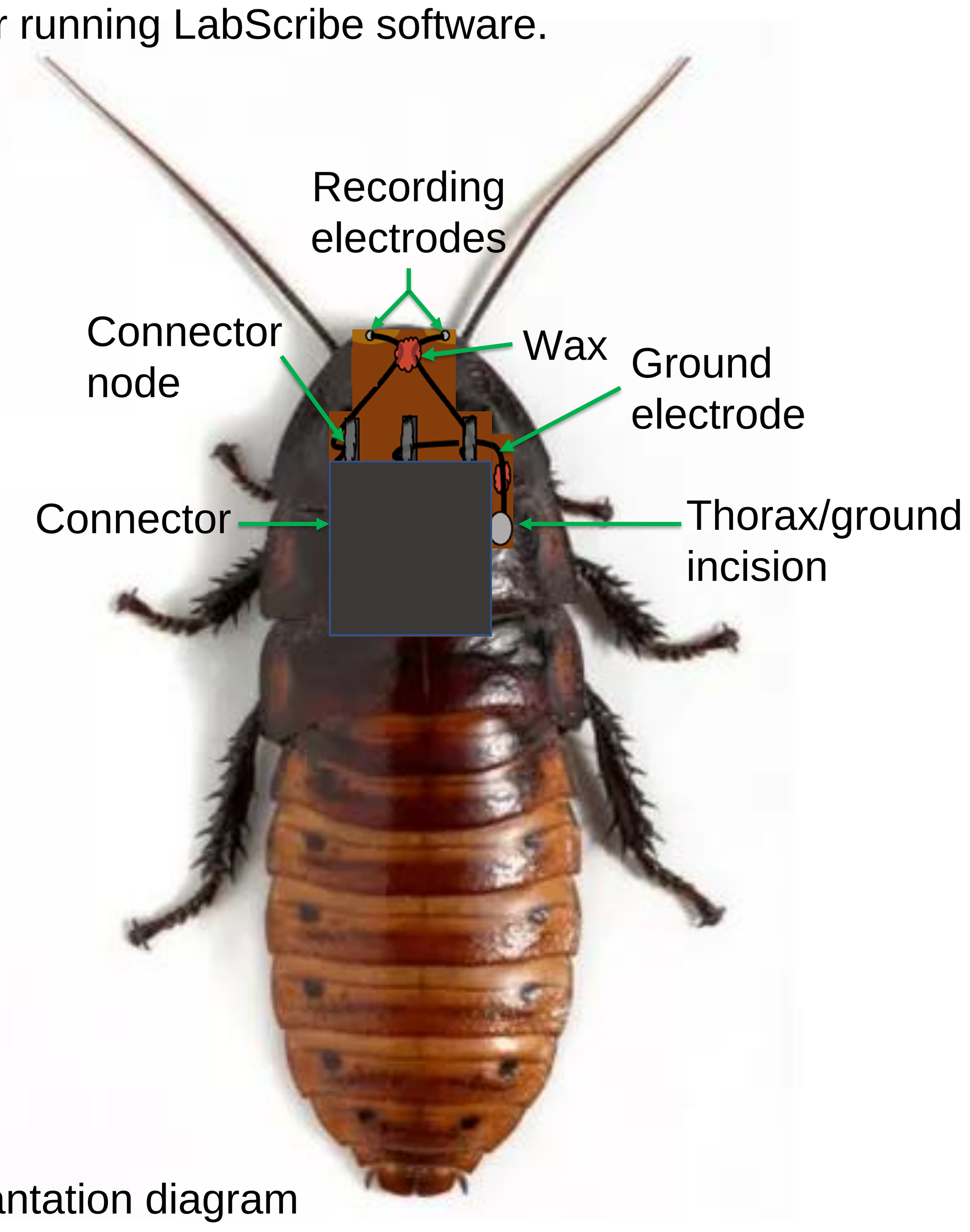

Figure 1: Implantation diagram

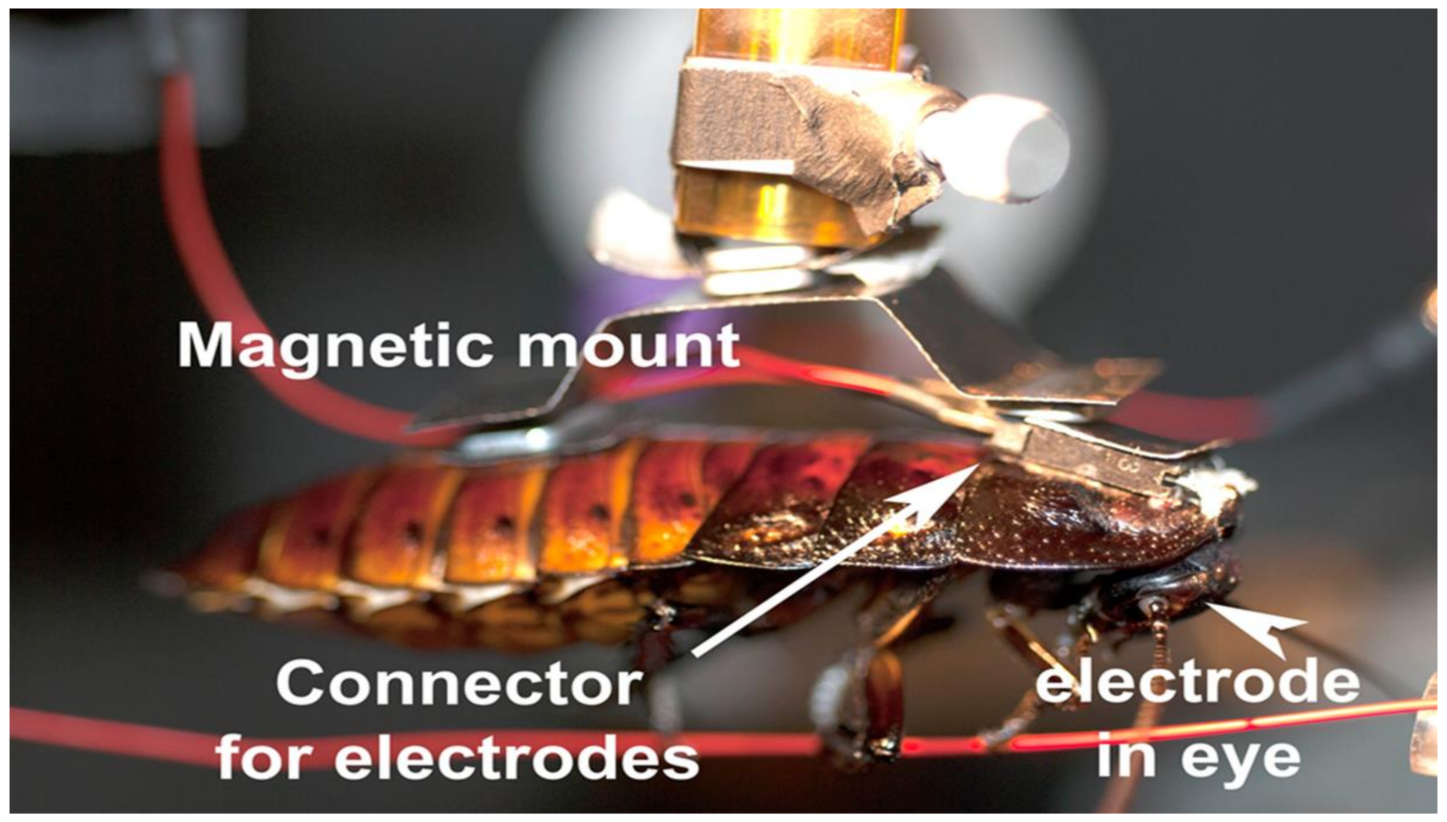

Figure 2: Electrode connector allows for easy weekly plug-in to amplifier

After a recording period of 6-10 weeks post implantation for the cockroaches, and 3 days post implantation for the flies, the subject's eyes and/or head were encased in polymer to be cross-sectioned using a microtome. These cross-sections were then used to examine the effect of the CNT-COOH, gold, and silver electrode implants on the neural tissue of the subjects.

Observations

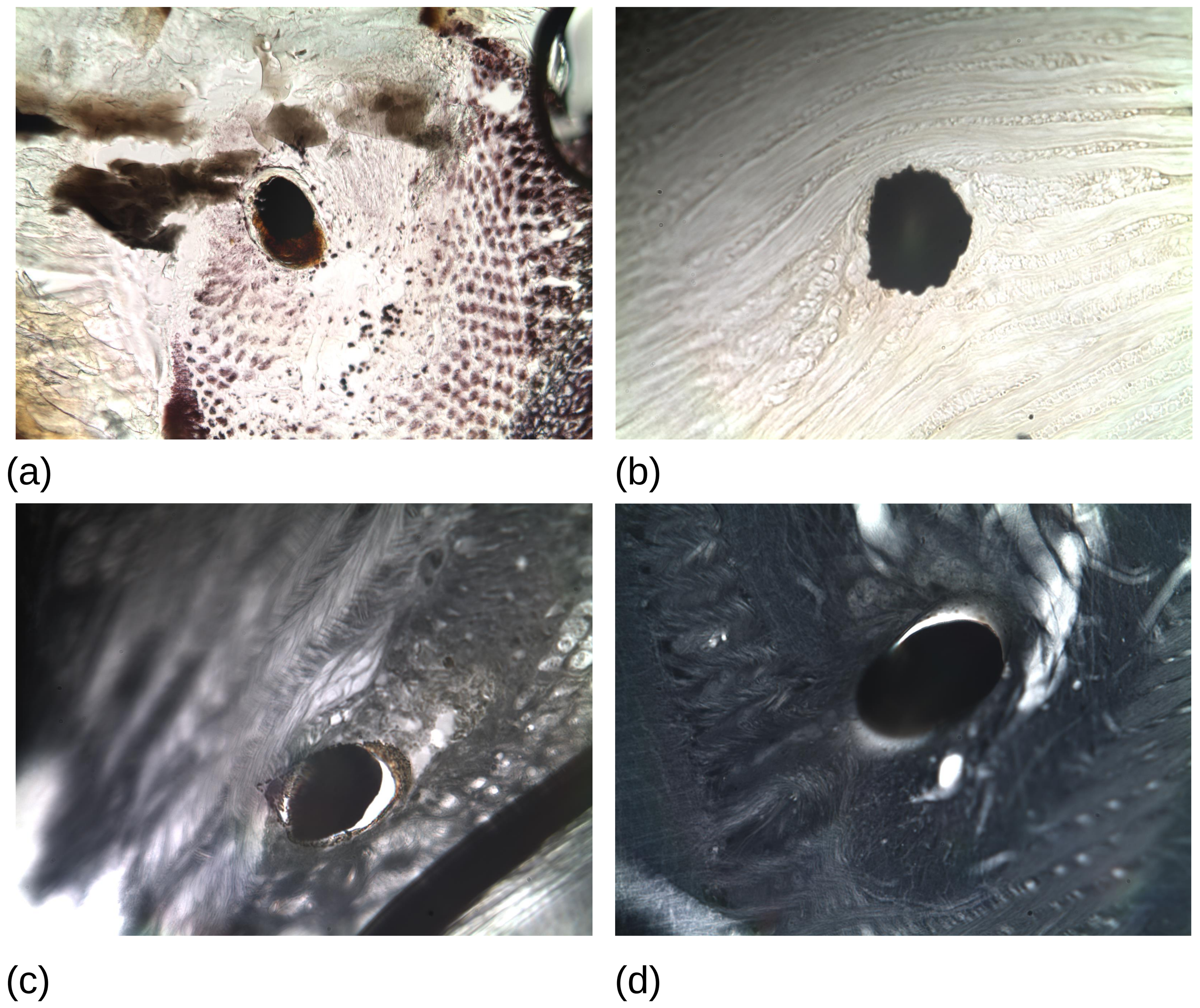

Figure 3: electrode integrated in neural tissue
(a) Roach – CNT-COOH
(b) Fly – CNT-COOH
(c) Fly – Silver (inert control)
(d) Fly – Gold (noninert control)

Results

After initial analysis of the data acquired from ERG recordings of roaches with CNT-COOH implants (Fig. 4), it is shown that these electrode fibers are an excellent material for long-term use. However, since the preps generally did not last much past 8 weeks, it is necessary to examine the post implant histology. Upon observation of the two controls used in the fly implants, especially silver, the toxicity of the metallic fiber gave rise to degradation of tissue, made clear from the vesicles present surrounding the electrode (Fig. 3c & 3d). In comparison, the CNT-COOH electrode in both the roaches and flies showed a much better integration with the surrounding tissue. However, further examination of the tissue of the roach shows the formation of a callus around the electrode (Fig. 3a).

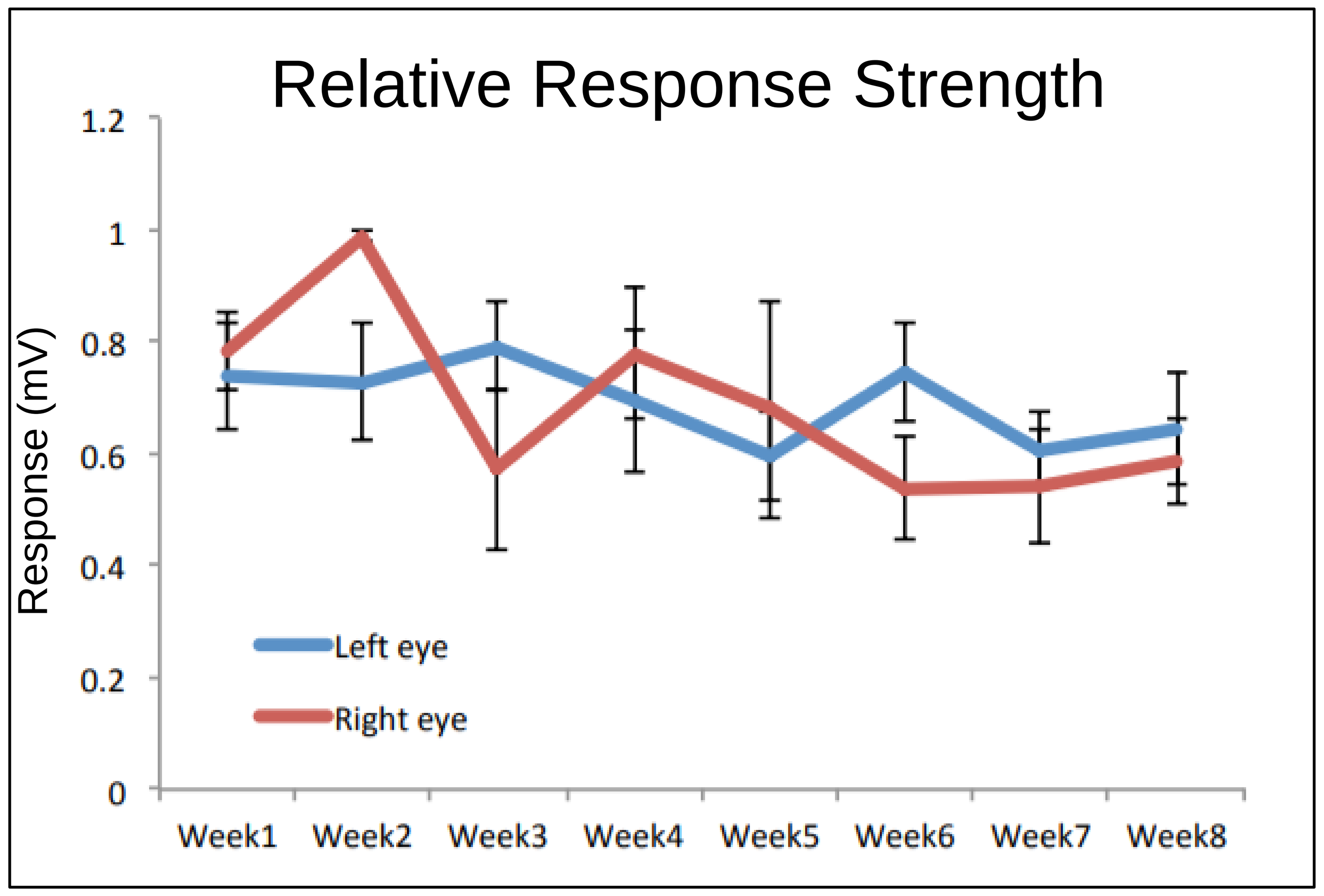

Figure 4: Relative response strength of roaches implanted with CNT-COOH electrodes over the course of 8 weeks

Future Studies

The results suggest that there exists room to improve in terms of hydrophilicity, electron transfer, & bio-cooperability. With this, the next step of this project would be to test CNT fibers functionalized with glutamate, a molecule naturally occurring in the nervous system. However, before continuing, it is beneficial to observe the results of similar experiments.

Murugan Keerthi et al compare the characteristics of pristine screen-printed carbon electrodes to those of (1) CNT functionalized with sulfuric and nitric acid, and to (2) CNT functionalized with Molybdenum nanoparticles in addition to the two latter. The chemical oxidation of (1) introduces more oxygen functional groups on their surface, which improve their hydrophilicity and chemical reactivity. The acid mixture can break the tubes of CNTs resulting in an increase in their electrical conductivity and corrosion resistance. Electrochemical impedance spectroscopy (EIS) showed that (2) has superior electron transfer ability and cyclic voltammetry showed that (2) also exhibited fast electron transfer in the system[1]. C.I. Santana et al focus their study on the cardiovascular system but observe the effects of CNT functionalized with the organic molecule 2-(methacryloyloxy) ethyl phosphorylcholine. When compared to pristine CNT, these fibers showed a very high contact angle with, indicating the repellence of, highly oxidized low-density lipoproteins (oxLDL). OxLDL is known to enhance the expression of pro-inflammatory genes causing dysfunction of vascular endothelial cells present in endothelium[2].

Acknowledgements

This research was supported by a grant from the UCGNI-Neurobiology Research Center Pilot Research Program to NTA and EKB and the National Science Foundation under IOS-1456757 and IOS-1856341 to EKB.

References

1. Keerthi, M., Boopathy, G., Chen, S.-M., Chen, T.-W., & Lou, B.-S. (2019). A core-shell molybdenum nanoparticles entrapped f-MWCNTs hybrid nanostructured material based non-enzymatic biosensor for electrochemical detection of dopamine neurotransmitter in biological samples. *Scientific Reports*, *9*(1), N.PAG.
2. Santana, C., Hoyos, L., Pérez, J., Bustamante, J., & García, A. (2017). A novel functionalization method for carbon nanotubes to repel ox-LDL in treatments after stent placement. *Materials Science & Engineering* C, 79, 30-36.