



## Electrode Development for BFCs and Biosensors

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### Description

In order to explore the benefits provided by the use of a CH and CB combination for wearable sensing technology, different techniques have been reviewed to create biocompatible nanomaterial-based membranes. In this sense, several examples of CH doped with carbon-based materials have been proven to have suitable conductivity properties and the ability to be shaped in the form of films, electrospun membranes, and porous structured materials such as supports for the development of bioelectrodes, wound healing, and tissue repair. In these approaches, the CH-carbon doped structured materials have presented conductivity values ranging from 0.0002 to 0.25 S·cm<sup>-1</sup>, as is the case of a structured hybrid membrane composed of CH and graphene oxide, MWCNT, or CB. Regarding CB-based conductive films, systems found in the literature include an electrospun CH-CB membrane with 62.5% w/w of CB with respect to CH with ideal mechanical properties, high conductivity, and chemical stability; the use of CH together with polypyrrole for tissue engineering applications; or the use of CB to increase conductivity in a polyurethane film. However, the former approaches involve the use of additional reagents and/or binders or solvents as is the case of PVDF or acetone and NMP for the development of the previous slurry for immunosensors and techniques more complex than knife casting such as electrospinning for the preparation of self-standing chitosan-carbon black membranes.

### Limitations of Conductive Polymers

Due to the above-mentioned limitations of conductive polymers for bioelectrode development, polymers from biological origin appear to be a very interesting alternative to overcome solubility and biocompatibility issues. This is due to their similar film and membrane forming abilities and their ideal biocompatibility. Biopolymers are usually derived from cellulose, and they include chitosan (CH), alginate, or carrageenan, among others. CH, despite being a natural insulator, presents interesting properties such as flexible film forming ability, low toxicity, biocompatibility, biodegradability, antibacterial activity, and hydrophilicity. However, its main drawback in being used for bioelectrode development is the fact that it does not present conductive properties. Nevertheless, insulating polymers with satisfactory mechanical properties has already been used to develop conductive substrates by the addition of materials such as graphite, carbon black (CB), carbon nanotubes (CNTs), or metallic particles are

such as colloidal silver or gold. Thus, electrically conductive polymer composites, including biopolymers, have attracted a great deal of interest due to the combination of simultaneous flexibility and conductivity.

### Enzyme Immobilization

For enzyme immobilization, Glucose oxidase (GOx) from *Aspergillus niger* Type VII and Laccase from *Trametes versicolor* were obtained from Sigma-Aldrich. N-(3-dimethylaminopropyl)-N-ethylcarbodiimide was purchased from Iris Biotech GmbH (Marktredwitz, Germany), and N-Hydroxysulfosuccinimide (NHS) was provided by Sigma Aldrich. 2-(N-morpholino) ethanesulfonic acid for the MES buffer and D-Glucose biotechnology grade standard was provided by VWR LIFE SCIENCE (Radnor, PA, USA). For the preparation of Phosphate Buffer solutions, 0.1 M Na<sub>2</sub>HPO<sub>4</sub> and NaH<sub>2</sub>PO<sub>4</sub> from VWR LIFE SCIENCE were used. Planetary Ball Mill Retsch PM100 as used for the preparation of the slurry for the CH membranes. An orbital shaker JP Selecta Rotaterm was used for the washing stage in the membrane preparation. A Heidenhain-METRO MT 1281 length gauge (Heidenhain GmbH, Traunreut, Germany) was used to control membrane thickness.

In this study, flexible and biocompatible conductive bioelectrodes have been developed by the combined use of CH and CB to improve electron transfer ability and minimize insulating capacity. However, there are important differences between the membranes developed in this study and previously reported membranes regarding simplicity and sustainability of the synthesis method that will not need any toxic reagents and solvents, extra energy input, or long reaction times. In addition, the CH-CB membranes developed in this study could be easily sized and shaped to suit any application, which makes them highly suitable materials for bioelectrode development, along with their ability to host specific enzymes due to their chemical and biocompatible properties.

Additionally, it has been proven that the flexible and biocompatible CH-CB membranes synthesized in this study can be used as bioelectrodes for anode and working electrode development for BFCs and biosensors, respectively. Thus, energy harvesting and glucose detection have been tested in order to test their suitability for further wearable device development.

CB displays specific properties such as high surface area, sphere diameter of nanometrical size, and high ID/IG ratio. It also exhibits excellent electrical conductivity, is dispersible in many solvents, and can be functionalized, and presents defect sites and fast electron transfer kinetics. CB has also been used as an additive in many polymers to enhance physical properties including electrostatic discharge protection, electromagnetic shielding, or thermal and mechanical resistance. Regarding CB suitability for on-body use, only a few studies regarding CB biocompatibility have been found. In this situation, the only risk associated with CB is found when it comes from CB particle inhalation and indoor exposure as opposed to skin or tissue contact because particle exposure could lead to the appearance of toxicity effects in the lung; cardiovascular disease; or malfunction, neurotoxicity, or carcinogenicity. Although biocompatibility experiments have not been carried out in this work, all the individual components used for the synthesis and that are present in the final CH-CB membrane are well reported to be non-toxic and biocompatible..

Specifically, incubation of HeLa cells with carbon nanomaterials and chitosan in a well established MTT cell viability assay where the role of chitosan to decrease the cytotoxicity of carbon nanomaterials has been proven. Furthermore, PC12 cell biocompatibility studies is a

using chitin and carbon nanomaterials exhibited no cytotoxicity in the MTT assays. The expected applicability of the developed membrane is for it to be used in contact with the skin.