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Electro-addressable conductive alginate hydrogel for bacterial trapping and general toxicity determination

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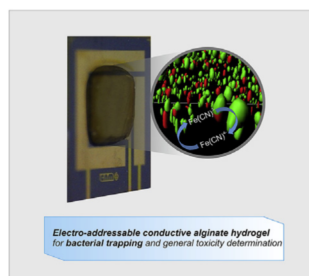
HIGHLIGHTS

- Electro-addressable conductive alginate hydrogels.
- Live bacteria electrotrapping for biosensing.
- Enhanced general toxicity assessment through ferricyanide respirometry.

GRAPHICAL ABSTRACT



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ABSTRACT

In biosensors development, alginate hydrogels are a first choice for enabling stable biomolecules entrapment in biocompatible membranes obtained under soft physiological conditions. Although widely exploited, most alginate membranes are isolating and poorly repetitive, which limit their application in biosensing. Significant steps forward on improving repeatability and conductivity have been performed, but to date there is no single protocol for controlled deposition of live cells in replicable conductive alginate layers. Here, cell electrotrapping in conductive alginate hydrogels is examined in order to overcome these limitations. Conductive alginate-coated electrodes are obtained after potentiostatic electrodeposition of graphite-doped alginate samples (up to 4% graphite). The presence of graphite reduces electrode passivation and improves the electrochemical response of the sensor, although still significantly lower than that recorded with the naked electrode. Bacterial electrotrapping in the conductive matrix is highly efficient (4.4×10^7 cells per gel) and repetitive (CV < 0.5%), and does not compromise bacterial integrity or activity (cell viability = 56%). Biosensing based on ferricyanide respirometry yielded a four times increase in biosensor response with respect to non-conductive alginate membrane, providing toxicity values completely comparable to those reported. Cell electrotrapping in conductive hydrogels represents a step forward towards in high-sensitive cell-based biosensors development with important influence in environmental analysis, food and beverage industry as well as clinical diagnosis.

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1. Introduction

Biosensors development requires stable immobilization of biomolecules, including enzymes, antibodies, aptamers and cells,

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among others, on the transducer surface. A good immobilization method may allow to control and maintain the amount of biomolecules without compromising the integrity and activity of the biomolecule or the biosensor performance. Cell immobilization is particular because, apart from previous stability and reproducibility requirements, the immobilization methods should ensure cell viability and metabolic activity [1] The methods for cell immobilization include adsorption [2–4], covalent binding [5], encapsulation [6,7] and entrapment. Although this variety, cell entrapment in polymeric matrices is advantageous for preserving cell integrity and viability, at the time that ensures stability in a physiological environment [8,9].

A number of polymeric matrices have been used for entrapping cells, such as gellan/xanthan matrix [10], SiO_2 sol-gel [11], PVA/alginate [12], alginate [13] and polypyrrole [14,15], among others. From all of them, alginate is one of the preferred for enabling gel formation at very soft experimental conditions, i.e. room temperature, aqueous medium and pH 7, through a cation-mediated cross-linking process [13,16]. Additional advantages are in the low toxicity, low antigenicity and permeability of these membranes, allowing the diffusion of small molecules [16,17]. Main limitations of alginate hydrogels for biosensors are the lack of standardization and repeatability of the gelling protocols, and the electrical insulation of alginate membranes, particularly relevant in the development of electrochemical biosensors. It is worth mentioning that electrochemical transduction, for simplicity, cost-efficiency and miniaturization/integration capacities is still the first choice in biosensors development.

In recent years, significant efforts to improve hydrogels repeatability and conductivity have been performed in separate. Regarding repeatability and homogeneity of the hydrogels, electrodeposition techniques are preferred for providing strict control of the deposition region and the architecture of the hydrogel, i.e. size, shape, thickness and porosity [18–21], properties that can be modulated by adjusting the potentiostatic conditions [21]. Mammalian and bacterial cells have been successfully immobilized by alginate electrodeposition [22–25]. On the other hand, a growing trend to improve hydrogel conductivity is the inclusion of conductive nanomaterials within the polymer matrix [26]. Conductive hydrogels based on graphite [27], graphene [28,29] and carbon nanotubes [30], have attracted a great interest [31]. These conductive matrices combine advantages of hydrogels and conductive composites in a hybrid material, the properties of which can be modulated by shifting the ratio between the alginate and the doping conductive nano-material [26]. These conductive hydrogels have been used to immobilize bacteria and to demonstrate their electroactivity for energy production [32]. However, to the best of our knowledge, both improvements have been never combined in a single electrodepositable and conductive alginate hydrogel for cell trapping.

In this work, bacterial electrotrapping in conductive alginate hydrogel-coated electrodes is examined and applied to biosensing. Graphite microparticles are used as conductive doping material. The analytical performance of the conductive hydrogel-coated electrodes is characterized and hydrogel thickness is measured by profilometry. The biocompatibility and reliability of the electrotrapping protocol is assessed by confocal microscopy and plate counting. Biosensing is performed based on ferricyanide respirometry [33–36] and applied to toxicity assessment.

2. Materials and methods

2.1. Chemicals

Alginic acid, calcium carbonate, calcium chloride, potassium ferricyanide, potassium ferrocyanide, glucose, graphite, 3,5-dichlorophenol, potassium di-hydrogen phosphate and di-potassium hydrogen phosphate 3-hydrate were purchased from Panreac (Spain) and were of analytical grade and all the solutions were prepared with distilled water.

2.2. Alginate electrodeposition and electrochemical analysis

Gold screen printed electrodes (SPE; Dropsens 220BT) integrating a round gold working-electrode (4 mm of diameter), a gold counter-electrode and a Ag pseudo-reference electrode were used in this work. Alginate electrodeposition and electrochemical measurements were performed with a Dropsens μSTAT8000 potentiostat controlled by Dropview 8400 software.

Briefly, precursor solution containing 1% (w/v) sodium alginate, 0.125% (w/v) $CaCO_3$, graphite (between 0 and 4% w/v) and a bacterial concentration up to 1×10^9 colony forming units (cfu) per mL were prepared in distilled water. Graphite was pre-treated and introduced in the solution following the protocol described elsewhere [37].

A volume of 100 μL of the precursor solution was dropped on the SPE surface and electrodeposited at 1.5 V (vs. Ag pseudo-reference) for 90 s. Thick and stable conductive alginate hydrogels were obtained.

2.3. Microorganisms

Escherichia coli ATCC 10536 was grown aerobically in LB broth for 18 h at 37 °C in a shaker bath (110 rpm). Grown cultures were centrifuged at 10100 g for 10 min and re-suspended in distilled water. For cell quantification, optical density of re-suspended bacteria was measured at 550 nm in a SmartspecTM Plus spectrophotometer (BioRad). Agar plate counting was carried out for viable counting.

2.4. Confocal microscopy

Three-dimensional reconstructions of bacteria-containing conductive alginate hydrogels images were used to evaluate the biocompatibility of the electrodeposition protocol (through the number of live and dead bacteria) and the distribution of bacteria in the gel.

Electrotrapped bacteria were stained with the Live/Dead Invitrogen Kit BacLight (Invitrogen) following the protocol detailed by the supplier. The conductive alginate hydrogel was incubated in 100 μL of Live/Dead staining solution for 15 min, washed with water (three times) and imaged by confocal microscopy (Leica TCS SP5) at excitation wavelength of 470 nm. Z-stacks were acquired every 1 μm for a total thickness of 300 μm. Reconstruction of individual stacks was performed with the IMARIS software. In the three-dimensional reconstruction, live bacteria, stained with SYTO9, appeared in green (emission wavelength = 630 nm), while dead bacteria, stained with propidium iodide, emitted in the red region of the visible spectra (emission wavelength = 530 nm).

2.5. Profilometer

Hydrogel thickness was measured with an optical profilometer PLμ2300 from Sensofar controlled with PLμ Confocal Imaging Profiler.

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