Chitosan biopolymer: Alternative adhesion factor and scaffold matrix for 2D and 3D neuronal cultures

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Abstract

The increase of different types of cell cultures, which can be used for the *in vitro* studies of physiological and/or pathological processes, has introduced the need to improve culture techniques through the use of materials and culture media that promote growth, recreating a cellular micro-environment that can be asserted in *in vivo* condition. The standard methods for the functionalization of supports used for cell cultures are based on the use of synthetic or natural biopolymers, which generally have high costs, such as poly-lysine and poly-ornithine. The aim of this work is to demonstrate the alternative use of the polysaccharide chitosan as adhesion factor and structural component for 2D/3D neuronal cultures. Thanks to its versatility, it could be easily functionalized for the fabrication of personalized of *in vitro* models.

Introduction

Cell cultures are fundamental for a wide of applications involving both research and industries. The increase of different types of cell cultures, which can be used for the *in vitro* studies of physiological and/or pathological processes, has introduced the need to improve culture techniques through the use of materials and culture media that promote growth, recreating a cellular micro-environment that can be asserted in *in vivo* condition. Therefore, it is important to design and develop new biologically sustainable methods, such as to contribute to the "closer-to-*in vivo*" condition.[1]

Related to that, in this work, we present the biopolymer Chitosan (CHI) as support for 2D and 3D neuronal cell cultures. Chitosan is a copolymer of glucosamine and N-acetyl-glucosamine, obtained by the deacetylation of chitin; it is well known for its low-cost, biocompatibility, biodegradability, muco-adhesiveness, antibacterial activity as well as its bioaffinity.[2]

Materials and Methods

CHI was dissolved in 0.1 M acetic acid at different concentrations (0.01% - 2% w/v); 2% sodium hydroxide solution. For 2D cultures only, Poly- ornithine (PORN) solution 0.15 mg/mL in water, as control.

2D: Chitosan nanometric films were obtained by dip coating.

3D: Chitosan microspheres were fabricated by a phase-inversion process using an aerodynamic encapsulator.

Chitosan films and microspheres were then used as support for the *in vitro* growth of primary neuronal cells. To validate the ability of chitosan to support neuronal adhesion, networks development and the differentiation capacity, morphological and functional characterization were carried out by confocal, transmission electronic and atomic force microscopies. A preliminary electrophysiological characterization of spontaneous activity was conducted by Micro-Electrode Arrays (Figure 1).

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Results

Chitosan films showed the ability to support the adhesion and differentiation of neuronal culture. The growth of neurons plated on chitosan films is comparable with

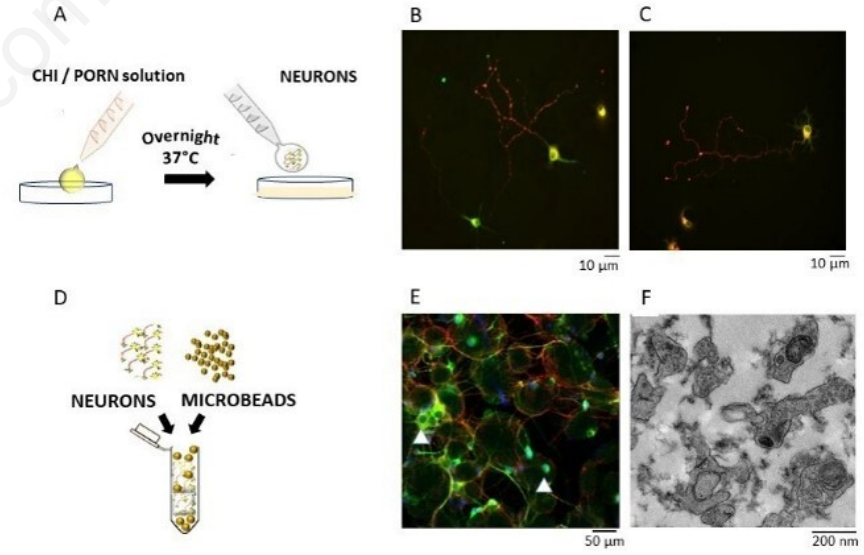

Figure 1. A) Scheme of adhesion factor deposition for 2D cultures. B-C) Hippocampal culture development on dip-coating chitosan and on poly-ornithine staining staining for MAP 2 (green) and TAU (red) at 7 *days in vitro*; D) Scheme of 3D cell cultures assembly. E) Confocal microscope images of *3D neural network* at DIV 25 on 2% CHI microbeads labeled for MAP-2 (green), Tubulin βIII (red) and DAPI (blu). F) Low-mag TEM micrograph of a portion of chitosan scaffold with the neuronal network: neuritic processes inside microbeads.

ones on standard adhesion factors (poly-ornithine). Furthermore, it is noted that 3D cultured neurons, show distinct morphologies that are more representative of the *in vivo* environment. In particular, these results have been confirmed by a preliminary electrophysiological characterization.[3]

Conclusions

We successfully demonstrate the alternative use of the polysaccharide chitosan as adhesion factor and structural component for 2D/3D neuronal cultures. Thanks to its low cost and versatility, it could be easily functionalized for the fabrication of personalized of *in vitro* models.

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