Chemical Engineering Journal xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Neuronal membrane bioreactor as a tool for testing crocin neuroprotective effect in Alzheimer's disease

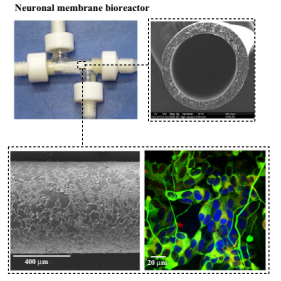
Sabrina Morelli [a], Simona Salerno [a], Antonella Piscioneri [a], Franco Tasselli [a], Enrico Drioli [a,b], Loredana De Bartolo [a,*]

[a] Institute on Membrane Technology, National Research Council of Italy, ITM-CNR, c/o University of Calabria, via P. Bucci cubo 17/C, I-87030 Rende (CS), Italy
[b] WCU Energy Engineering Department, Hanyang University, Seoul, Republic of Korea

HIGHLIGHTS

- PAN HF membrane bioreactor was developed for neuronal cell culture.
- A homogeneous and controlled microenvironment was realized into the bioreactor.
- Neuronal membrane bioreactor created a functionally active neuronal network.
- The neuronal bioreactor acts as a tool to test neuroprotective molecules.
- Crocin inhibits Aβ neurotoxicity associated to Alzheimer's disease.

GRAPHICAL ABSTRACT



Neuronal cells adhered around fibers in the membrane bioreactor

ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Membrane bioreactor
Hollow fibers
Neuronal cells
Crocin
Anti-amyloidogenic activity
Alzheimer's disease

ABSTRACT

In this paper a neuronal membrane bioreactor was developed as platform for the in vitro reconstruction of a neuronal network with defined functional, geometric and neuroanatomical features. The bioreactor consists of modified polyacrylonitrile hollow fiber membranes that were assembled in parallel in order to establish two separate compartments: an intraluminal compartment within the fibers, in which the medium flowed, and an extraluminal compartment or shell outside of the fibers where cells were cultured. We explored the ability of the membrane bioreactor to promote the growth and functional differentiation of neuronal cells up to 2 weeks.

Neuronal cells in the bioreactor covered completely the fiber surface and exhibited a high density of the axonal network reaching a very complex 3D structural organization characterized by the expression of presynaptic vesicle protein synaptophysin. Cells were also functionally active as demonstrated by the oxygen uptake rate and glucose consumption that increased with culture time achieving values of 17.7 nmol/min and 879 ± 113 nmol/min at day 15, respectively.

The neuronal membrane bioreactor was used as in vitro model of Aβ-induced toxicity associated to Alzheimer's disease to test for the first time in cells the neuroprotective effect of crocin. The administration of the Aβ produced a dramatic decrease of cell viability and induced the reactive oxidative species (ROS) generation and apoptosis. When Aβ-peptide was administered together with crocin a significant dose-dependent inhibition of apoptosis and ROS production was observed pointing out the capability of crocin to prevent the aggregation of Aβ peptide and subsequent neurotoxicity associated to Alzheimer's disease.

* Corresponding author. Tel.: +39 0984 492036; fax: +39 0984 402103.
E-mail addresses: l.debartolo@itm.cnr.it, loredana.debartolo@cnr.it (L. De Bartolo).

http://dx.doi.org/10.1016/j.cej.2016.01.035
1385-8947/

1. Introduction

Recent advances in nanotechnology, biomaterials and tissue engineering offer new strategies to mitigate or reverse the effects of disease, aging, or injury in the nervous system. Many individual and combined approaches including the development of biomaterial scaffolds, biological grafts and stem cell therapy have been proposed for numerous nervous system applications [1]. The development and implementation of living, 3D neural cellular based constructs consisting of neural cells in 3D matrices is crucial to directly replace lost function and/or to facilitate and augment the capacity for host nervous system regeneration. Biomaterial-based approaches with micro-and nanoscale features have been proposed to promote and guide neural outgrowth [2–7]. Similarly tubular guidance conduits, nano- and/or micro-fibers and microchannels have been engineered to elicit axonal outgrowth [8–12]. The creation of tissue engineered neural constructs involves neuronal/stem cell expansion and differentiation in a well-controlled microenvironment at molecular level. This requires an advanced and robust technology that overcomes the diffusion limitations of traditional static culture systems through a continuous supply of nutrients and metabolites. During the last years several devices as stirred suspension systems, rotating wall bioreactor, rotary cell culture system and miniaturized cell chamber bioreactor have been developed for culturing neuronal cells and/or neural progenitor cells [13–19]. Within this context membrane bioreactors compared to the devices based on direct perfusion have the advantage to ensure uniform nutrient and metabolite exchange in the cell compartment protecting cells from shear stress. Our overall strategy was to develop a neuronal hollow fiber (HF) membrane bioreactor as platform for the in vitro reconstruction of a neuronal network with defined functional, geometric and neuroanatomical features. The bioreactor consisted of polyacrylonitrile (PAN) HF membranes that established two separate compartments: an intraluminal compartment within the fibers, in which the medium flowed, and an extraluminal compartment or shell outside of the fibers where cells were cultured. The two compartments communicated through the pores in the fiber wall governing the transport of molecules. Cells were grown around the fibers that offered a wide surface area for their adhesion reaching high densities in the extracapillary space. Neural cells are anchorage-dependent cells that require an extracellular matrix for their adhesion, growth and differentiation. A key design consideration of the bioreactor was the use of PAN HF membranes that were modified with surface coating of poly-L-lysine (PLL) in order to promote the adhesion and growth of hippocampal neurons and to enhance neuronal differentiation and neurite alignment. Previous studies highlighted that such kind of fibers thanks to their structural, physico-chemical and permeable properties could facilitate the neural regeneration recapitulating the in vivo neural architecture [8,20].

In this study the ability of a small-scale HF membrane bioreactor to promote the differentiation and the functionality of neuronal cells was explored. To this purpose human neuroblastoma derived SH-SY5Y cells were used as a model cell system [21], as these cells may differentiate into adrenergic or cholinergic neurons whose proliferation, alignment and direction/length depend on the substrate surface characteristics [22] and in addition their human origin makes them an appealing model for studying neurodegeneration and neurorepair mechanisms. Thus, we tested the ability of the bioreactor to elicit the differentiation of neuronal cells and to maintain their functionality up to 2 weeks. Finally, the neuronal membrane bioreactor was used to test the antioxidant properties and the inhibitory activity of a carotenoid crocin on amyloid-β (Aβ) aggregation. Deposition of Aβ in the brain is a neuropathological hallmark of Alzheimer's disease that leads to synaptic function impairment, ultimately producing massive local neurodegeneration. Crocins are bioactive constituents found in the fruit of gardenia and in the stigmas of saffron, that have various health-promoting properties, including antitumoral, anti-inflammatory, anti-hyperlipidemic, antidepressant, anxiolytic, anti-atherosclerotic properties [23] and the neuroprotective potentials of crocin have been reported largely in association with their antioxidant capacities [24–26]. Recently, Karakani et al., showed that crocin can inhibit the aggregation of human tau protein [27]. Moreover, in vitro studies that were carried out in solutions containing Aβ peptide and the carotenoid, demonstrated the anti-aggregation activity of crocin on $A\beta_{1-40/1-42}$ peptides [28,29]. To the best of our knowledge, there is no study on the anti-amyloidogenic effect of crocin in a neuronal cellular system in vitro. To this purpose the neuronal membrane bioreactor was used as in vitro system in which Aβ-mediated toxicity associated to Alzheimer's disease was induced to assess for the first time in literature the capability of the crocin to interfere with Aβ aggregation and to protect against Aβ-induced cytotoxicity.

2. Materials and methods

2.1. Hollow fiber membrane bioreactor

The hollow fiber membrane bioreactor consisted of PLL modified PAN HF membranes assembled in parallel within a tubular glass housing (Fig. 1). The fibers were potted with polyurethane adhesive (Polaris Polymers, OH, USA) at each end in order to estab-

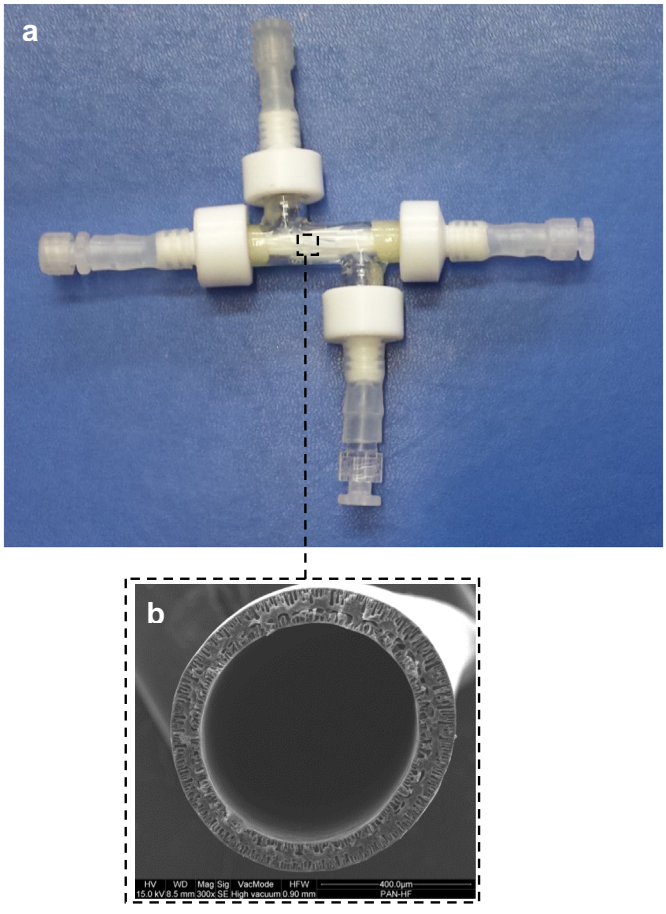


Fig. 1. PAN HF membrane bioreactor (a) and SEM micrograph of cross-section of PAN-HF membranes (b).

Please cite this article in press as: S. Morelli et al., Neuronal membrane bioreactor as a tool for testing crocin neuroprotective effect in Alzheimer's disease, Chem. Eng. J. (2016), http://dx.doi.org/10.1016/j.cej.2016.01.035