Biomaterials 105 (2016) 117–126

Contents lists available at ScienceDirect

Biomaterials

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

Human astrocytic grid networks patterned in parylene-C inlayed SiO_2 trenches

M.D. Jordan [a], B.J. Raos [a], A.S. Bunting [c], A.F. Murray [c], E.S. Graham [b], C.P. Unsworth [a, *]

[a] Department of Engineering Science, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand
[b] Department of Pharmacology & Centre for Brain Research, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand
[c] Institute for Integrated Micro & Nano Systems and The Scottish Microelectronics Centre, School of Engineering & Electronics, The University of Edinburgh, EH9 3JL, UK

ARTICLE INFO

Article history:
Received 2 May 2016
Received in revised form
9 July 2016
Accepted 2 August 2016
Available online 4 August 2016

Keywords:
Cell patterning
Human astrocyte
Astrocytic network
Parylene-C
Glia
hNT

ABSTRACT

Recent literature suggests that glia, and in particular astrocytes, should be studied as organised networks which communicate through gap junctions. Astrocytes, however, adhere to most surfaces and are highly mobile cells. In order to study, such organised networks effectively *in vitro* it is necessary to influence them to pattern to certain substrates whilst being repelled from others and to immobilise the astrocytes sufficiently such that they do not continue to migrate further whilst under study.

In this article, we demonstrate for the first time how it is possible to facilitate the study of organised patterned human astrocytic networks using hNT astrocytes in a SiO_2 trench grid network that is inlayed with the biocompatible material, parylene-C. We demonstrate how the immobilisation of astrocytes lies in the depth of the SiO_2 trench, determining an optimum trench depth and that the optimum patterning of astrocytes is a consequence of the parylene-C inlay and the grid node spacing. We demonstrate high fidelity of the astrocytic networks and demonstrate that functionality of the hNT astrocytes through ATP evoked calcium signalling is also dependent on the grid node spacing. Finally, we demonstrate that the location of the nuclei on the grid nodes is also a function of the grid node spacing.

The significance of this work, is to describe a suitable platform to facilitate the study of hNT astrocytes from the single cell level to the network level to improve knowledge and understanding of how communication links to spatial organisation at these higher order scales and trigger *in vitro* research further in this area with clinical applications in the area of epilepsy, stroke and focal cerebral ischemia.

1. Introduction

1.1. The astrocyte & astrocytic networks

The human brain contains approximately 86 billion neurons [1] with traditional network models of the brain being based around the neuron and neural network behaviour [2]. However, the brain also consists of a similar number of non-neuronal cells [1] and in the last 10 years, research has revealed that the glial cell, in particular the astrocyte [3], has more functionality than the simple perfunctory support role to the neuron than was traditionally believed [4–6]. This functionality has spanned immunological signalling [7–9], endocannabinoid synthesis [10], a source of cytokines and chemokines [11] with the most unanticipated functionality being discovered in the direct modulation of the synapses of neurons through gap junctions of astrocytes [12–14] which has attracted wide attention and study. Furthermore, it has been reported how the dysfunction of astrocytes and their intimate relationship with neurons can lead to neurological disorders [15–17].

In this article, we were motivated to employ the human hNT astrocyte derived from the human teratocarcinoma cell line (NTera2/D1) (ATCC® CRL-1973™) [18]. hNTs have been used for cell transplantation in stroke therapy [19], express ubiquitous neuronal/astrocytic markers [20] and have been directly compared to the properties of primary human cells and demonstrated to be a valid alternative to human primary astrocytes [21]. In addition, hNTs raise no ethical concerns as the derived astrocytes and neurons come from an immortalised stem cell line [20]. Thus, hNTs

* Corresponding author.
E-mail address: c.unsworth@auckland.ac.nz (C.P. Unsworth).

http://dx.doi.org/10.1016/j.biomaterials.2016.08.006
0142-9612/

provide a simple and convenient human model for pursuing the aim of this work.

Recent contemporary research has posed a new challenge for scientists to now investigate the 'Astrocytic Network', as a parallel to neural networks, in order to understand how this form of network communicates through gap junctions and interacts with neurons at larger network scales [22]. Common existing *in vitro* experimental platforms that have been used to study network function of astrocytes cells are: cell assays under fluorescence [21]; the patch clamp [23] and more recently multi-electrode arrays (MEAs) [24]. However, because astrocytes and neural cells in general, grow in a complex interwoven fashion (Fig. 1B) it becomes increasingly difficult to extrapolate the connective links that exist between them and how their calcium communication behaves at the network scale. Thus, the experimental platform proposed here will benefit the neurosciences by allowing scientists to construct organised networks of astrocytes in order to understand how their calcium communication maps from the single cell level through to larger network scales in a predictable manner, important for experimental repeatability and comparisons to be made. Hence, *in vitro* research into this type of network could hold clues into the development of novel strategies to target subsets of astrocytes for the treatment of neurological conditions such as epilepsy, stroke and focal cerebral ischemia [15–17].

1.2. Cell patterning & biomaterial Parylene-C

Since astrocytes grow in a complicated Daedalean fashion, *in vitro* (shown in Fig. 1), it is difficult to map the connective architecture that links single astrocyte cells together and hence determine how this affects the downstream large scale behaviour of a network.

Thus, over the last 45 years, engineering and biology have attempted to deconstruct these component cells and re-organise them in simpler ways to allow for study. This has resulted in the development of a contemporary field of study concerned with the controlling and arrangement of cells, known as 'Cell Patterning' [25]. Cell patterning, originated from metal deposition patterning in the late 1960's by Carter [26], which broadened considerably in subsequent years to encompass photolithography, pioneered by Kleinfeld [27], and soft lithography to produce micro-contact printing introduced by Whitesides [28] and more recently inkjet printing of cells, introduced by Klebe [29] which will most likely supersede these methods when higher patterning resolutions can be obtained. Variants of these patterning methods have since been extended to encompass multiple cell types [25], co-cultures [30] and combined with Multi-Electrode Arrays (MEAs) for electrical cell recording [2].

Parylene-C [31] is a member of the parylene family which falls into the class of polymerised *para*-xylelene polymers. The chemical makeup of Parylene-C consists of polymerised *para*-xylelene where one aromatic hydrogen on each of the phenyl groups has been substituted with chlorine. Parylene-C hosts a broad span of useful properties, such as a low gas and water permeability, good electrical insulation and acid resistance. In addition, it can be fast deposited via chemical vapor deposition (CVD) [49], providing a uniform conformal surface that is pinhole free making it attractive for semi-conductor manufacture.

In addition, parylene-C is also a bio-friendly material [31] and has previously been used in stencils, 3D cages for cell guidance and hydrophobic/hydrophilic bases that could be modified electrically [32–35]. In 2009, Delivopoulos introduced a high fidelity technique for patterning rat primary neurons on parylene-C, based around Selective Molecular Assembly Patterning (SMAP) [36] demonstrating that if parylene-C was initially treated with piranha acid that it would allow for the effective absorption of equine serum [37]. Delivopoulos also demonstrated later how photo-oxidation can disrupt parylene-C patterning [38]. This was extended by Unsworth who patterned rat primaries to the single cell level on ultra-thin parylene-C [39]. Unsworth also demonstrated how parylene-C could be used to pattern the hNT neuron [40] and hNT astrocyte [41] derived from the human teratocarcinoma cell line to the single cell level and Raos demonstrated how infra-red, laser ablated parylene-C [42] could be used for rapid pattern prototyping using hNT astrocytes. This work has been further extended by Hughes [43] who demonstrated how to modulate the pattern adhesion properties for HEK293 cells on parylene-C. Most recently, Delivopoulos demonstrated how the ratio of the fibronectin and albumin on the parylene-C and SiO_2 surfaces played an important role in cell patterning [44], Trantidou [45] demonstrated parylene-C could be laser modified for the micropatterning of rat ventricular fibroblasts and neonatal myocytes and Golda-Cepa [46] demonstrated how parylene-C when functionalized *with* oxygen plasma could promote cell growth for the MG-63 human osteosarcoma cell line.

In the field of cell patterning, construction of organised networks is typically performed using simple linear links in order to easily map the flow of information from the single cell level to large network scales. Hence, grid patterns are one of the simplest linear forms commonly used in cell patterning. Whilst many groups have demonstrated grid patterning of neurons in the absence of astrocytes and co-cultures of neuronal and glial cells grid arrangements

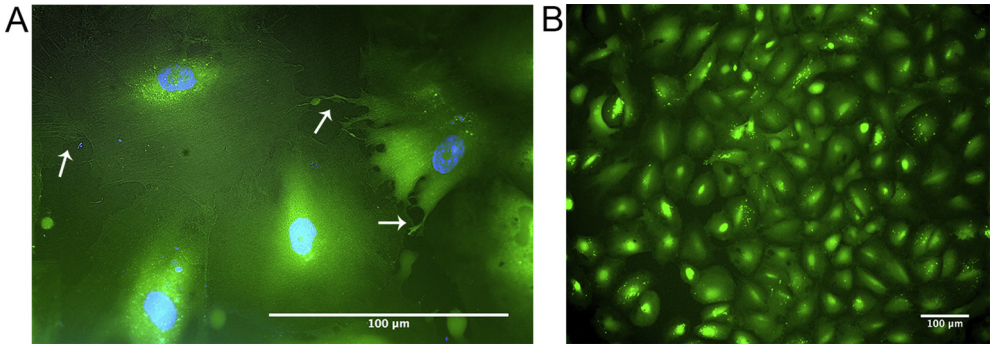

Fig. 1. Typical hNT astrocytic networks *in vitro*. (A) A three channel overlay image of multiple hNT astrocytes forming connections to each other. Channel one is bright field, channel two is fluorescence where cell cytoplasm (green) was stained live with cell tracker green CMFDA dye and channel three is fluorescence where nuclei (blue) were stained with Hoechst 33258. Arrows point to astrocytic processes. (B) A fluorescence image of a typical hNT astrocytic network *in vitro* at high confluence. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)