MACQUARIE University

Superior Nanofiber Substrates for Long-Term Culturing of Primary Neurons

BACKGROUND

Neuronal cultures are a powerful and highly versatile tool for dissecting molecular and cellular mechanisms in neuroscience. Primary neuronal cultures are used to mimic the brain more realisticly *in vitro* than cell lines. As primary neurons are notoriously sensitive and do not proliferate, initial adhesion of neurons to the culture substrate is of paramount importance. The most common technique for growing primary neurons is seeding on a thin film of poly-D-lysine. This and other materials (including peptides) have numerous shortcomings or limited uses, as described in the attached publication.

OUR SOLUTION

We have developed two novel tetrapeptides, which are positively charged, bear two lysine groups and self-assemble into fibers when dissolved in water: (1) Fmoc-FFkk → Fmoc-Phe-Phe-D-lys-D-lys and (2) Fmoc-FkFk → Fmoc-Phe- D-lys-Phe-D-lys. These tetrapeptides offer, amongst other benefits, significant time savings and reproducibility over the widely used poly-D-lysine.

KEY DATA:

- Permissive for neuronal growth (do not invoke neuronal cell death or promote growth of non-neuronal cell types)
- Support normal neuronal maturation: formation of synapses and dendritic spines
- Development of electrically active neuronal networks (quantified synchronous firing)
- Fiber diameters for Fmoc-FFkk and Fmoc-FkFk are 7.7±0.3 and 5.1±0.8 nm respectively.
- Neural pH upon addition of culture media

APPLICATIONS

- Long-term neuronal cultures *in vitro*
- Suitable for AAV-mediated transduction
- Suitable for liposome-mediated transfection
- 3D neuronal cultures relevant to *in vivo* environment for:
  - Drug delivery/treatment
  - Neurogenesis
  - Disease models

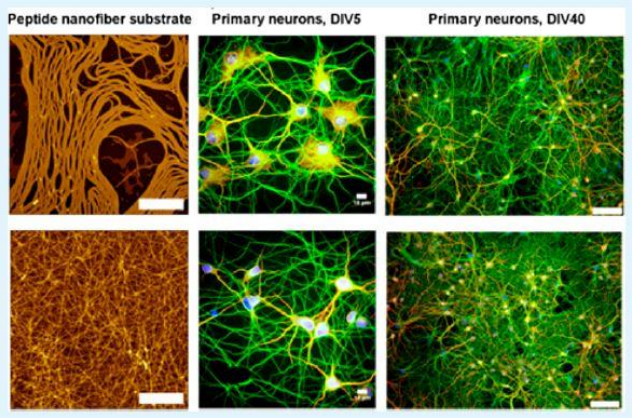

Figure 1. (left panels) Peptide nanofiber substrate self-assembly; atomic force microscopy images; (middle and right panels) development of primary neurons seeded atop peptide nanofibers at 5 days and 40 days *in vitro* (DIV) respectively;

KEY FEATURES AND ADVANTAGES
Well-defined, chemically uniform, reproducible short peptide nanofiber substrate (no batch-to-batch variation)
Facilitates adhesion and development of primary neurons
Supports long-term survival (>40 days)
Supports 2D and 3D cultures
Water solubility significantly simplifies current procedures for preparing neuronal cultures
Eliminates washing procedures required for poly-D-lysine prior to seeding cells

PUBLICATIONS

Martin, A.D. *et al* (2018) ACS Appl. Mater. Interfaces 10, 25127.

INTELLECTUAL PROPERTY POSITION

PCT patent application filed

PARTNERING OPPORTUNITY

We are seeking an industry partner for further development and commercialisation of this technology through a research collaboration or technology licence.

WOULD YOU LIKE TO KNOW MORE?

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