BASIC SCIENCE

Nanomedicine: Nanotechnology, Biology, and Medicine
11 (2015) 77–87

nanomedicine
Nanotechnology, Biology, and Medicine

Original Article

nanomedjournal.com

Development of a nanomaterial bio-screening platform for neurological applications

Stuart Iain Jenkins, PhD[a], Paul Roach, PhD[b,1], Divya Maitreyi Chari, DPhil[a,*,1]

[a]*Cellular and Neural Engineering Group, Institute for Science and Technology in Medicine, Keele University, UK*
[b]*Institute for Science and Technology in Medicine, Keele University, UK*

Received 22 January 2014; accepted 22 July 2014

Abstract

Nanoparticle platforms are being intensively investigated for neurological applications. Current biological models used to identify clinically relevant materials have major limitations, *e.g.* technical/ethical issues with live animal experimentation, failure to replicate neural cell diversity, limited control over cellular stoichiometries and poor reproducibility. High-throughput neuro-mimetic screening systems are required to address these challenges. We describe an advanced multicellular neural model comprising the major non-neuronal/glial cells of the central nervous system (CNS), shown to account for ~99.5% of CNS nanoparticle uptake. This model offers critical advantages for neuro-nanomaterials testing while reducing animal use: one primary source and culture medium for all cell types, standardized biomolecular corona formation and defined/reproducible cellular stoichiometry. Using dynamic time-lapse imaging, we demonstrate in real-time that microglia (neural immune cells) dramatically limit particle uptake in other neural subtypes (paralleling post-mortem observations after nanoparticle injection *in vivo*), highlighting the utility of the system in predicting neural handling of biomaterials.

From the Clinical Editor: The authors describe an advanced multicellular neural model comprising the major non-neuronal/glial cells of the central nervous system, shown to account for approximately 99.5% of CNS nanoparticle uptake. They demonstrate that this novel model offers critical advantages for neuro-nanomaterials testing, while reducing the need for experimental animals.

Key words: Biomaterials screening; Multicellular models; Neural cells; Glia; Protein corona

Advanced functional material design has led to a global increase in clinical nanomaterial use for regenerative medicine, particularly platforms such as magnetic particles (MPs), in applications including imaging and biomolecule delivery, with several therapeutic nanoparticles under clinical trials or in pre-clinical development.[1,2] Identification and optimization of such medical biomaterials require dedicated design and realization of surface functionalization with appropriate materials characterization tools, and parallel biomedical testing using relevant biomimetic screening models. Neurological applications represent a unique challenge in this regard, given the complex, multicellular composition of the brain and spinal cord (termed the central nervous system or CNS).[3] Neural cells are classed into neurons (transmitters of electrical information) or glia (the supporting cells). Glia outnumber neurons by about 10-fold[4-7] and comprise several subtypes that regulate the neural environment including, critically, clearance of nanomaterials.[8] One study recently proved that glial uptake of nanoparticles accounts for *ca.* 99.5% of nanoparticle clearance in the CNS, with neurons accounting for the small balance[9] — identifying the former as the overwhelmingly dominant population governing CNS nanoparticle uptake. Consequently, the overall response of the glial population to introduced nanomaterials is the most critical predictor of the CNS characteristic response as a whole.

We recently reported major differences in MP uptake/handling between glia.[10] The immune components (microglia) showed rapid and avid particle uptake with extensive degradation. In contrast, other glial subtypes (the astrocytes, oligodendrocytes and their precursors) showed significantly lower but stable particle accumulation. Based on these observations, we predicted that the rapid and high particle accumulation by a dominant cell population, such as microglia, would constitute a critical

This work was funded by a Biotechnology and Biological Sciences Research Council (BBSRC; UK) grant (DMC) and an Engineering and Physical Sciences Research Council (EPSRC; UK) Engineering Tissue Engineering and Regenerative Medicine (E-TERM) Landscape Fellowship (SIJ).

A similar abstract and poster were presented at the Mercia Stem Cell Alliance meeting, Keele University, Dec 2013.

*Corresponding author.
E-mail address: d.chari@keele.ac.uk (D.M. Chari).

[1] Both senior authors contributed equally to this manuscript.

http://dx.doi.org/10.1016/j.nano.2014.07.010
1549-9634/

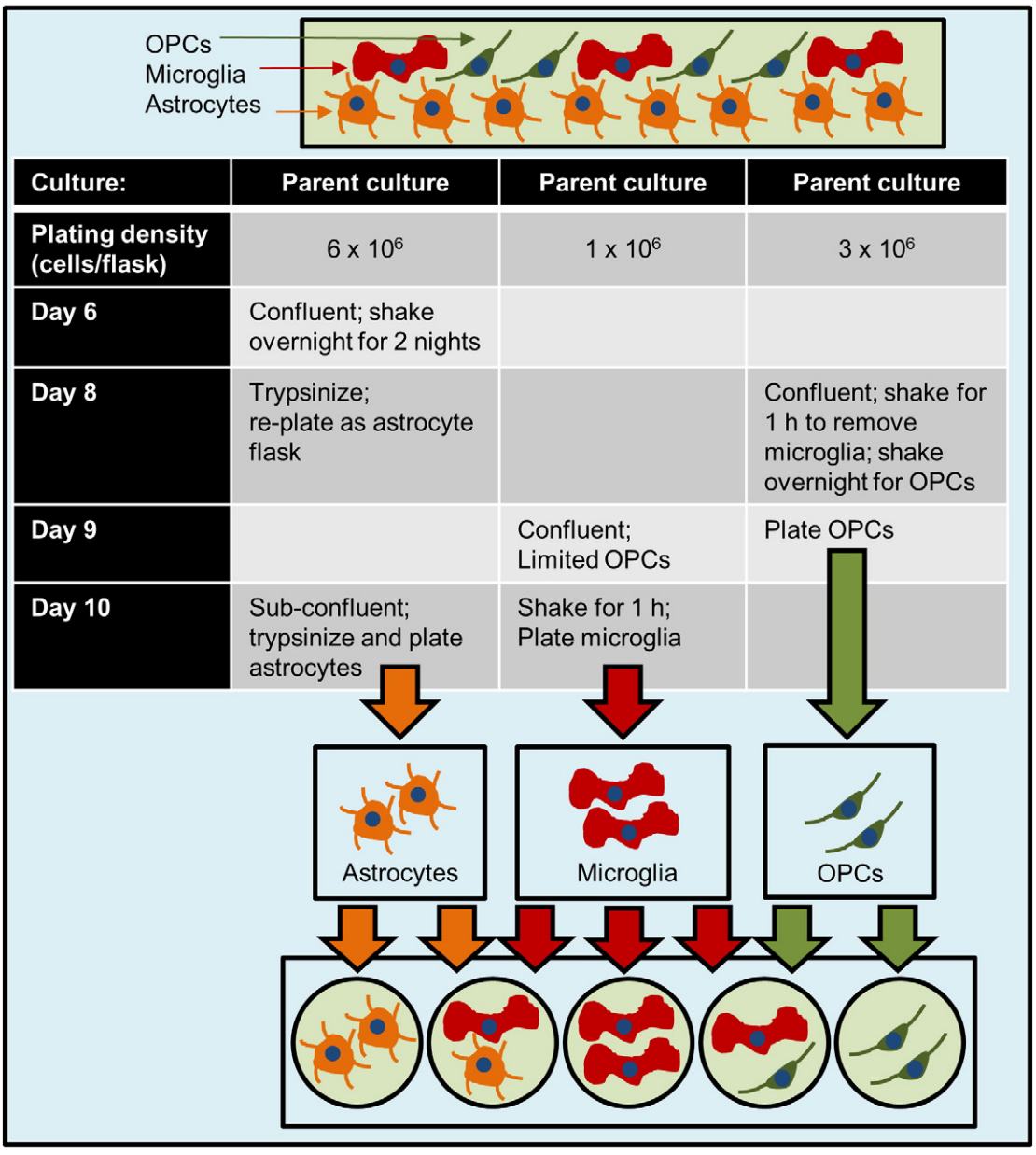

Figure 1. Schematic diagram showing 'stoichiometrically defined' co-culture method.

'extracellular barrier' to particle uptake in mixed neural cell populations, such as the intact nervous system. This is pertinent as high numbers of activated microglia are typically present in neurological pathology.[11] Accordingly, the development and testing of neuro-compatible materials for clinical use must account for both intercellular dynamics and constituent glial cell numbers, using appropriate multicellular neural models.

Despite this major need there is a substantial lack of sophisticated and accessible neural models for high-throughput screening of neuro-nanomaterials.[12] In terms of widely used current approaches, live animal models are biologically relevant but involve significant ethical issues, technical complexity and expense, while being low-throughput. 'Reductionist' models addressing the 3Rs principles (Reduction, Replacement and Refinement of animal experimentation[13,14] for which there is a current global drive) have several drawbacks, chiefly pertaining to their biological relevance. These include use of inappropriate sources/combinations of cells/tissue, *e.g.* cell lines of unknown age/provenance combined with primary cells, adult plus immature cells or peripheral nervous system (PNS) and CNS cells,[15,16] significantly limiting their neuro-mimetic capacity. Large variability is also inherent in these models, making reproducibility and robust analyses problematic. Tissue explants are technically challenging, showing uneven cellular distribution and stoichiometry, limiting robust quantification of material uptake,[16] and reducing their predictive utility.

Another major point of note is that biomolecule interactions with materials at the nanoscale – the same length scale as