Development of a Novel Multicellular 3-Dimensional Blood Brain Barrier In Vitro Model

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Background and aim
The blood brain barrier (BBB) is crucially important for normal brain function, many pathological diseases are characterised by BBB breakdown, such as stroke, Alzheimer’s disease, diabetes and multiple sclerosis. Despite this fact, the induction and maintenance of the BBB is still poorly understood. For example, the majority of possible candidates for central nervous system targeting drugs, which have been tested, are inefficient because of poor permeability across the BBB. At present, the majority of studies on BBB integrity and injury are performed in vivo. Current in vitro BBB models are limited, because these models do not accurately reflect the cellular interactions that occur in vivo, as they overlook the 3-dimensional structure of blood vessels and in particular the presence of pericytes that play an instrumental role in the formation of the BBB.

It is the aim of this project to establish a cell culture system that contains all three major cell types that form the BBB in vivo: endothelial cells, astrocytes and pericytes. We want to reduce and replace animal experiments with in vitro testing and refine current in vitro BBB models, by developing a new system that represents a more complete model for BBB formation and maintenance. Development of this system will reduce and partly replace the use of animals in BBB experimentation and aid identification and refinement of new therapeutic targets and protective strategies.

Method and results
Our in vitro culture should closely mimic the structure and interaction of cells comprising the BBB in vivo (Fig. 1). We have started to develop an astrocyte/endothelial 3-dimensional cell culture system. These cultures consist of cells mixed in a ratio of 1:1 and suspended in a collagen matrix that solidifies at room temperature and is then overlaid with media. After 24 hours in a 3-dimensional culture, the endothelial cells begin to rearrange and form tube-like structures, which the astrocyte end feet contact (Fig. 2). To set up the new system we will first introduce pericytes into our culture system. We then intend to:
1) Observe growth and maintenance of pericytes alone in collagen.
2) Observe growth of pericytes in the presence of either, endothelial or astrocytic cells.
3) Ascertain the optimal ratio of endothelial cell:astrocyte:pericyte to obtain the most stable multicellular 3D cultures in vitro.

Preliminary results suggest our current 2-cell model remains stable for approximately 6 days. We would like to adapt and prolong this for up to at least 2 weeks, to enable subsequent chronic experiments to be performed. We shall also try matrigel and other matrices as an alternative substrate for cell culturing and compare results. The use of matrigel would be preferable as it is more cost effective.
4) Confirm the BBB phenotype in 3D cultures. Differentiation of the cells and barrier phenotype will also be confirmed by fluorescent microscopy and/or western blotting, this will enable morphological analysis and accurate localisation of the cells within the culture. 3D reconstructions of these images will then be made.

Fig. 1: Cellular relationships at the BBB. Pericytes (P) are closely associated with capillary endothelial cells (E) and share a common basal lamina (BM). Astrocytic end feet (A) ensheath the capillary (from Abbott, 1989).
5) Characterise the in vivo model with regards to cell movement and rearrangement within the culture using live imaging and confocal microscopy combined with fluorescent tagging of cells and cell-specific molecules.

After the establishment and characterisation of the culture system, changes in gene and protein expression as a result of contact with the other cell types, can be identified and monitored, using molecular biology techniques, FACS analysis sorting on known cell-specific markers, as well as ELISA and RIA techniques to measure the release of different cytokines and growth factors such as VEGF and Epo into the culture media. These methods will lead to identification of proteins expressed in the BBB under normal physiological conditions.

Conclusions and relevance for 3R

Importantly, this model system will provide a unique opportunity to study the BBB and reduce the need for difficult, invasive animal experiments. Given the large number of diseases characterised by BBB breakdown, such a model has wide applications in the medical and pharmaceutical industries, wherein drug and chemical testing could first be carried out exhaustively on the in vitro system. Establishment of this model will therefore promote the refinement of potential therapeutic tools and strategies, prior to animal testing and ultimately reduce animal experimentation, consumables and personnel costs. Furthermore, this model system will not only provide information on specific cellular interactions and signals, that promote induction of BBB formation during development, but will be readily manipulated and subjected to different insults such as hypoxia, glucose deprivation and chemical exposure to assist in understanding the breakdown of the BBB. Thus utilisation of this model means BBB research can be more focused and directed to the specific roles of individual cell types, as well as barrier function as a whole, with a minimal use of animal experimentation.

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Adjuvanticity of Microbial-Derived Particles and Synthetic Analogs In Vitro

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Background and aim

Vaccines represent the most successful immune-based medical treatments ever discovered and provide long lasting protection against acute infections. The task of limiting adverse reactions by using highly purified antigenic formulas has provided a safer, but nevertheless poor quality of immunogenic therapeutics. Thus, future immune intervention strategies, have a crucial dependence on the identification and characterisation of molecularly defined adjuvants, as well as, on the availability of adequate applicable methods for assessing adjuvanticity.

We would like to develop a system to identify human-compatible adjuvants based on lymphocyte cultures in vitro. We have two aims. Firstly, we will assess the capacity of microbial-derived particles and synthetic analogs of enhancing antigen-specific CD4+ T cell responses in vitro. Secondly, we will monitor the capacity of compounds endowed with adjuvant