CAEN Award Report

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The CAEN Award was awarded me on February 11, 2014 under the category 1A_ Visit to the laboratory of Dr Stephen Price at the University College London (UCL) Research Department of Cell and Developmental Biology.

The award was utilized within a period of three months (between June23 and September 11, 2014).

I was given a very warm reception on my first visit in the lab and was introduced to other members of the lab. I was taken through a routine brief security orientation within the Department and was issued an identity card so I can have access to certain facilities within the building.

The proposed research work to be carried out titled "**Effect of Vanadium Exposure to Mouse Oligosphere on the Long Term Myelination Infrastrucure**" could not be done due to inability to secure mouse fetuses. However, alternatives were designed, which were (1) the use of chicken embryo at embryonic day 6 which is equivalent to embryonic day 14.5, 65 somites in mouse and (2) the use P19 cell line which is known to be *olig2* positive.

Benefits from my visit to the lab include:

1. Experience in dissection of chicken embryo brain
2. Training in the basics of cell culturing
    a. Primary culturing with chicken embryo brainstem
    b. Secondary culturing with P19 cell line
3. Training in immunocytochemistry

Prior to the commencement of my research work plan, I was taken round the lab to familiarize with the various sections (the store, dissection room, cell culture room, study room, dry ice room and the photography room) and the various equipment.

Among the basics of cell culturing is safety lab practices which include

1. wearing appropriate personal protective equipment (PPE) such as gloves, laboratory coats and gowns, safety glasses or goggles,
2. washing of hands before leaving the lab
3. decontamination of all work surfaces before and after experiments
4. reporting any incidents resulting in exposure to infectious materials to appropriate personnel

Some basic equipment of cell culturing include cell culture hood (laminar-flow), incubator, water bath, refrigerator, liquid nitrogen freezer, sterilizer, aspiration pump, confocal microscope and additional supplies such as cell culture vessels, multi-well plates, petri dishes, pipettes and pipettors, waste containers, media, sera and reagents. The cell culture hood is regarded as the simplest and most

economical way to provide aseptic work area while allowing containment of infectious splashes or aerosols generated by many microbiological procedures.

Although the dissection of the chicken embryo was done outside the cell culture room, aseptic procedures such as sterilizing the work area with 70% ethanol, wiping of the egg shell with 70% ethanol and the use of sterile (in 70% ethanol) micro scissors. The egg shell was then drilled with blunt forceps and chipped away to expose the embryo. The embryo was then carefully lifted with its membranes and the attachments were trimmed off to release the embryo. The embryo was then transferred to petri dish with phosphate buffered saline (PBS) to trim off unwanted parts. The head was dissected out, washed in PBS and then transferred to the dissecting microscope. The brainstem was then dissected off the meninges with sharp forceps and fine scissors.

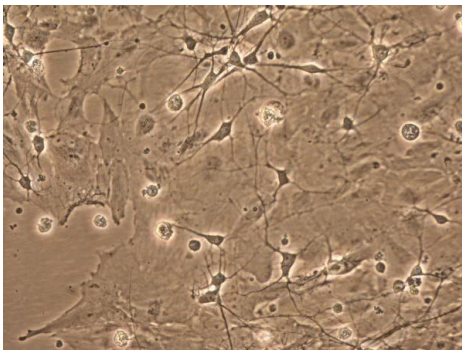
The brainstem was then transferred immediately into a 15ml falcon tube with PBS and kept in ice while more brainstems were dissected out.

Myself in the lab dissecting out chicken embryo

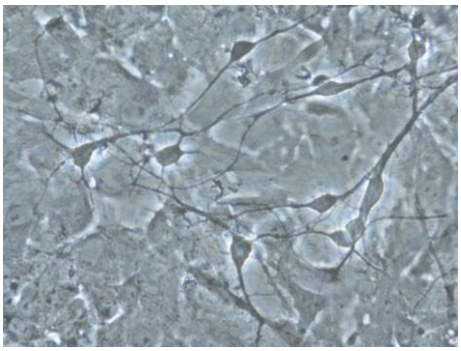
Chicken embryo at day7

Brainstem dissected out

Subcultures at different times/stages were developed according to standard procedure.



Mixed population of cells at day6 of culturing x20

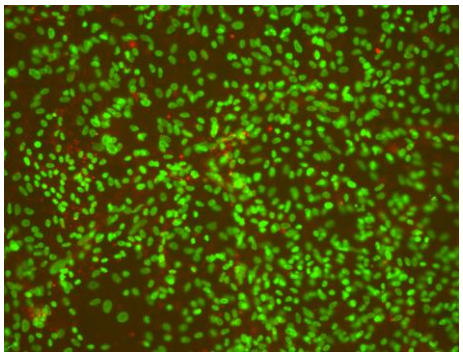


Mixed population of cells at day7 of culturing x40

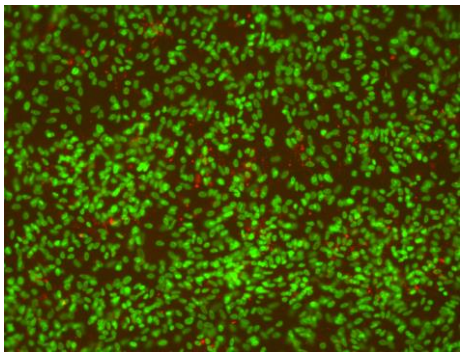
Immunocytochemistry (ICC)

ICC was carried out on the mixed cell populations of the chicken embryo cultures using marker for neuron (TAG1, NCAM, Neurofil and MAP2) and oligodendrocyte (O4). The protocol used is as stated briefly below:

1. Preparation of fixing mixture (4% paraformaldehyde)
2. Addition of 0.5ml of cell fix to each well containing coverslip (on which cell were growing)
3. Washing with 0.5ml PBS
4. Addition of blocking solution (PBS + 1% FCS + 0.1% Triton)
5. Incubation with appropriate primary antibodies and keeping in fridge overnight
6. Incubation with respective secondary antibodies for about 30mins
7. Application of mounting medium (DAPI) on glass slides
8. Mounting of coverslips on glass slides



Chicken embryo at day6 (O4)

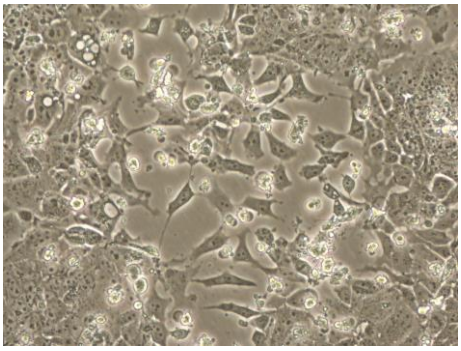


Chicken embryo at day7 (O4)

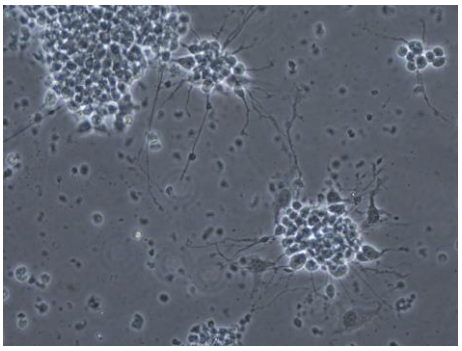
P19 Cell Line

They are embryonic carcinoma cell lines from embryo-derived teratocarcinoma in mice. Just as embryonal carcinoma can differentiate into cells of all 3 germ layers, so also P19, by aggregating the cells into embryonic body. The cell line can be induced with retinoic acid to differentiate into glia cells.

The frozen cell line was immediately thawed upon acquisition using a standard procedure. When needed, the P19 cell culture was splitted into 10cm well plates after 3days and confluency was reached after another 3days.



P19 cell line morphologic appearance after 24hours of culture



Morphologic differentiation of P19 cell line with development of cellular processes

Immunocytochemistry was carried out to establish any expression of oilgodendrocyte marker using O4 but it was negative. This may be due to early differentiation in which O4 was yet to be expressed.

In conclusion, my visit to an established laboratory as Dr Stephen Price's has facilitated my interest in exploring cell culture as a tool in neuroscience research. I also enjoyed the conducive working environment provided by the entire team in the laboratory. Furthermore, my hands-on experience in the techniques learnt under the tutelage of Dr Stephen is worthwhile and has added more value to curriculum vitae (CV).

In this regards I will like to appreciate Dr Stephen Price (with his entire team) of the Research Department of Cell and Developmental Biology, University College London (UCL) for such a wonderful and resourceful opportunity to work in his laboratory.

I also acknowledge the Committee for Aid and Education in Neurochemistry (CAEN) / International Society for Neurochemistry (ISN) for the grant awarded me for this work.