

Neuroblastology is a success!!

Dr Hannah Davies

BACKGROUND

This project arose from a visit to Liverpool Life sciences UTC (LLS-UTC) (a specialist free school) where my colleagues and I spoke to the students about their research projects during their enrichment sessions. Many of the students expressed a wish to design projects investigating the physiological effects of compounds on cells, e.g. the effect of common pharmaceutical agents such as paracetamol. At the time, they had equipment suitable for cell culture (CO₂ incubators and laminar flow hoods) but they they did not have the expertise or reagents to enable experiments with live cells despite interest from many of their students. We aimed to assist the school in setting up their cell culture facilities and teach the staff and students how to culture mammalian cells. This would be carried out in the context of investigating the brain in ageing and disease.

INTRODUCTION TO CELLS

The first stage of this project was to facilitate the introduction of cell culture into LLS-UTC through provision of a starter culture of SH-SY5Y neuronal cell line, support and expertise. Initially, University of Liverpool staff trained Dr John Dyer, a teacher, and a small number of students to culture the cells (Fig. 1).

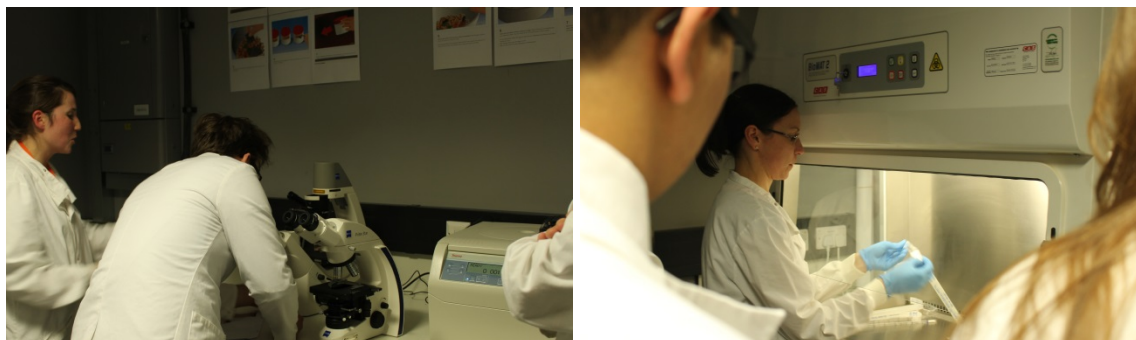


Figure 1. Introduction to the skills and techniques required to culture SHSY-5Y neuroblastoma cells

CONTINUING IN OUR ABSENCE

We then left the school with detailed protocols and the cells and the students continued to culture the cells with the support of their teacher until they had sufficient numbers for their planned experiments (Fig. 2).

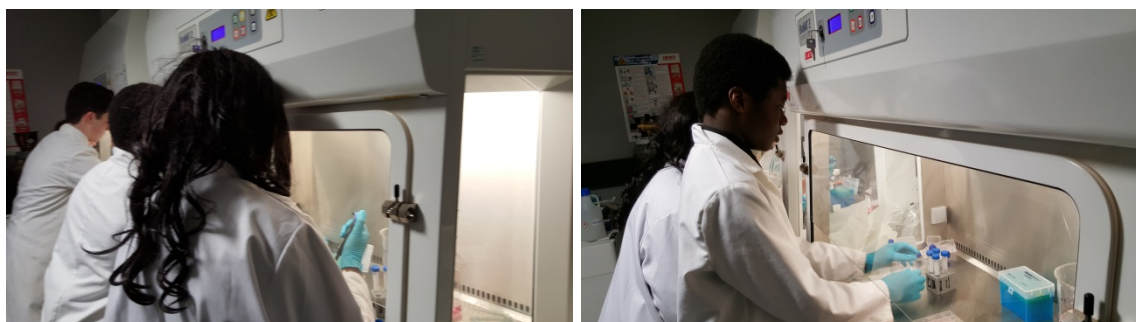


Figure 2. The students culturing the cells in our absence to generate sufficient numbers for experimentation.

EXPERIMENTING WITH CELLS

The students then designed different experiments to challenge the cells with a variety of compounds. They assessed the effect of the compounds on their cells using existing technologies at LLS-UTC, such as microscopy to investigate cell morphology and determine dead Vs alive populations. The majority of students chose to test compounds known to have an effect on brain health such as green tea and spices such as cumin and turmeric.

RESULTS AND POSTER SESSIONS

The students presented the results of their work at a poster session at a LLS-UTC partner's evening, discussion their work with a variety of local representatives from clinical and industrial settings (Fig.3). Furthermore, the students were also invited to present their work at the University of Liverpool's Genomes to systems away day. Here, the students discussed their work and their future plans with students and members of staff from the University of Liverpool.

Neuroblastology - Brain in a Bottle

Shane Conlon, Tom Smith, Martyna Skorek, Megan Coughlin & Ellen Cook

Introduction
Neuroblastoma cells (SHSY-5Y) were used to introduce cell culture to students at Liverpool Life Sciences UTC and to investigate the potential protective effects of natural substances against neurodegenerative diseases such as Alzheimer's. Natural substances are receiving increased attention from researchers due to their potential neuro-protective effects. In this study we investigated the effect of green tea on the growth of SHSY-5Y cells.

Methods
The cell media was changed every other day to ensure the cells have access to nutrients. When the cells reach >80% confluence they were split into 4 new flasks using trypsin to release them from the bottom of the original flask.

The cells were loaded onto the plate and incubated for 24 hours at 37°C and 5% CO₂. Confluence was then checked and recorded as initial confluence. 0.5ml of each different solution was added to each well mixed in with 0.5ml media (see below). The plate was incubated for a further 24 hours. After 48 hours % confluence was estimated visually.

24 well plate used to set up and carry out experiments

Row A was used as a control

Results

Treatment	% Confluence	% Live Cells
Green Tea	~65	~75
Green Tea (decaffeinated)	~65	~15
Caffeine Solution (30mg/ml)	~85	~45
Control (media only)	~85	~45

A - Green tea
B - Green tea (decaffeinated)
C - Caffeine Solution (30mg/ml)
D - Control (media only)

Future Work
We would like to test a range of concentrations of green tea. We would also like to investigate curry spices.

Acknowledgements
We would like to thank Dr Hannah Davies and Dr Jill Madine for making this work possible. Thank you to the Biochemical Society for funding the project.

Liverpool Life Sciences UTC UNIVERSITY OF LIVERPOOL

LAVENDER ON THE BRAIN - Tera Birchall

OVERVIEW
Preliminary investigation into the effects of lavender extract of *Lavandula angustifolia* on SHSY-5Y cells.

BACKGROUND INFORMATION
Lavandula angustifolia is a species of lavender, a genus of plants in the family Lamiaceae. It is a perennial herbaceous plant with a woody stem and is native to the Mediterranean region. It is widely used in perfumery and as a natural remedy for various ailments. The study was conducted to investigate the effects of lavender extract on SHSY-5Y cells. It is hypothesized that lavender extract will lead to an increase in cell growth compared to control.

CELL CULTURE AND PLATING
Cells were cultured in various T25 flasks at 37°C in 5% CO₂ and 95% air. 12 hours media was changed every 24 hours, and after 48 hours, the cells were counted in a vertical counter flow rack.

EXTRACT METHOD
Dried lavender angustifolia flowers were purchased from Doreen Ltd. The flowers were washed free of surface growers in Provence, France. The flowers were placed in a blender and 200ml water was added to 200g of water in a blender. The mixture was blended for 30 seconds and filtered through a coffee filter and evaporated to a thick, yellowish residue. The extract was added to 100 and 150 volumes of 95% cell media. One half of each volume was treated to 48 hours at 37°C and the other half was used as a control.

RESULTS
There was no significant change in growth. There was an increase in cell count.

DISCUSSION
Further investigations could test a range of cell concentrations. More cell counts may not reflect change in confluence. Cell confluence should be recorded. More results could give a more accurate view. More investigations should be done to see if the effects are the same. Effects may be due to the presence of other natural compounds of the extract itself.

ACKNOWLEDGEMENTS
Thank you to Dr Jill Madine and Dr Jill Madine (UTC) for making this project possible and for funding the project.

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The Potential Miracle Spice for Alzheimer's Disease

By Laura Hurst

Overview: To investigate the claims in recent studies that the spice Turmeric could help prevent the development of neurodegenerative diseases such as Alzheimer's disease.

AIM: Using neuroblastoma cells from a cell line (SHSY-5Y) set up a laboratory to culture the cells, which can then be used in experiments to test the effects of natural compounds such as turmeric.

Introduction: Alzheimer's disease is a progressive neurodegenerative disease often occurring in those over 65. It is one of the most common causes of dementia and leads to issues with memory, language and concentration. It is believed to be caused by the formation of beta amyloid plaques and neurofibrillary tangles leading to mass cell degeneration and brain atrophy. Turmeric is a popular spice in India and South Asia. It has been noted that in both rural and urban India the rates of dementia are significantly lower than the rest of the world. This has led many to believe that the phytochemical Curcumin contained in turmeric may prevent Alzheimer's disease via its anti-inflammatory, anti-oxidative and anti-cytotoxic properties. This can help to prevent neuron degeneration and death and therefore the symptoms of Alzheimer's disease.

Methodology:

1. Changing Cell Media Protocol - for a 25 cm² culture flask, the nutrient media must be replaced with 5ml of fresh media every two days. If the cells will be left for longer replace with 10ml of media.
2. Sub-Cultivation Protocol - add 2ml of this enzyme trypsin and observe under a microscope. When cells detach incubate with 2ml of media and centrifuge. Remove supernatant to leave a pellet of cells & re-suspend in fresh media, which can then be split between 4 flasks.
3. Plating Cells - follow sub-cultivation protocol to get the pellet of cells. Re-suspend with 12ml of media and add 1ml to each well of a 6-well multi well plate, repeat to fill all 24 wells. Add varying amounts of turmeric solution and media to the wells and observe cell growth.

Future Goals: Use the lab for further research into the effect of not only turmeric, but other natural compounds on neuroblastoma cells as well as beta amyloid protein as they are believed to be one of the main causes of Alzheimer's disease.

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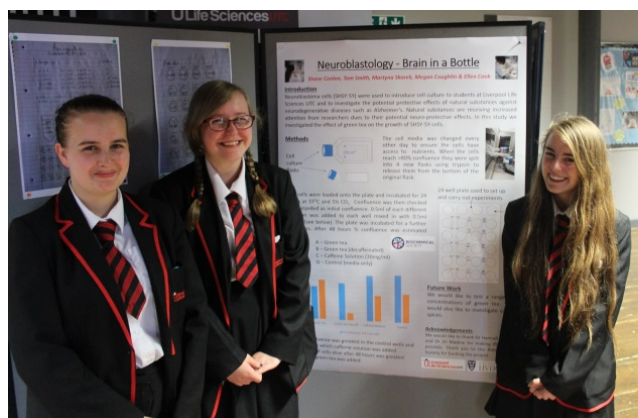


Figure 3. Example of a scientific results poster produced by some of the year 10 students (left) and year 12 students (right) participating in the project and photographs from the poster sessions.

AIMS AND OUTCOMES

The project aimed to:

1. *Provide students with hands-on experience of culturing cells and using them to test their own research hypotheses (16+).*

23 students from KS4 and 5 learnt how to culture SHSY-5Y neuroblastoma cells and tested their hypotheses in small groups.

2. *Provide school age students (14-16) with an understanding of in vitro cell culture and its applications in research.*

Without exception all the students have now learnt the skills required to maintain mammalian cells in culture, including the preparation of media, sub-culture and preparation of the cells for experimentation.

3. *Promote discussion about cellular research models with a focus on the ageing brain and the implications for society.*

In addition to the experimental work the students have carried out comprehensive literature searches, learnt about the origins of the cell line they are studying, and the use of cell lines in research.

At the poster sessions the students were able to confidently discuss their work with a variety of audiences and how their projects fit into the wider remit of research in ageing and neurodegeneration.

Furthermore, an interactive teaching session was developed to educate students on the effect of dementia on the brain. This was delivered successfully to a large group of (30+) students on a related enrichment pathway (health and social care). These students did not participate in the cell work but were exploring the care aspects of dementia in the community in their enrichment activities. This session could be delivered in many different schools and does not require the specialist equipment available at LLS-UTC.

EVALUATION AND FEEDBACK

Facilitator perspective (Dr Hannah Davies)

Overall the project has worked really well. The initial preparation required before the students could start the experimental work was more involved than initially anticipated. It took a lot of time and work to organise gas delivery to the school, prepare the risk assessments and detailed protocols and then to deliver the teaching sessions. The students were highly motivated and had very good ideas for experiments. In some cases students asked for specialist compounds they had read about in the literature and I was able to source them from other colleagues in the University of Liverpool to enable them to conduct their first choice experiment.

LLS-UTC asked us to repeat the project next year and have now incorporated this project into part of their enrichment curriculum for the academic year 2016-2017. In order to make the project more sustainable several things will be run differently:

- The CO₂ supply and management is to be handed over to the school.
- Management of the cells over the school holidays will now be done within the school

Teacher perspective (Written by Dr John Dyer teacher at LLS-UTC)

This has been an incredibly successful project that has proved to be very popular with students from across the year groups. The students have gained valuable skills in cell culture and aseptic technique as well as a range of transferrable skills such as leadership, time management (in order to care for the cells), experimental design and evaluating and improving upon techniques and procedures. The level of enthusiasm and engagement that students have shown has been incredible and many

students have happily given up time at lunchtime breaks and after school in order to tend to the cells or collect data. This enthusiasm and engagement has certainly been increased by the fact that Hannah spent time training the students at the start as well as coming in on a number of occasions to provide technical expertise and support with experimental design. Students regularly commented that they have enjoyed these sessions and found it useful to have direct contact with active researchers. Perhaps the most striking thing for me was how disappointed the students were when some of their cells got infected. However, they responded brilliantly by developing their procedures in order to minimise the risk of further contamination. This sort of resilience, coupled with the level of care, accuracy and precision required during this project will prove invaluable to students at the start of their scientific careers. Our students have already started preparing for the next round of experiments and we intend to incorporate this project as part of our skills curriculum.

Student perspective

All the students were given feedback forms (Fig.4A) to complete at the end of the experimental work. Overall the feedback was very positive indicated by the average rating of 9.5 out of 10.

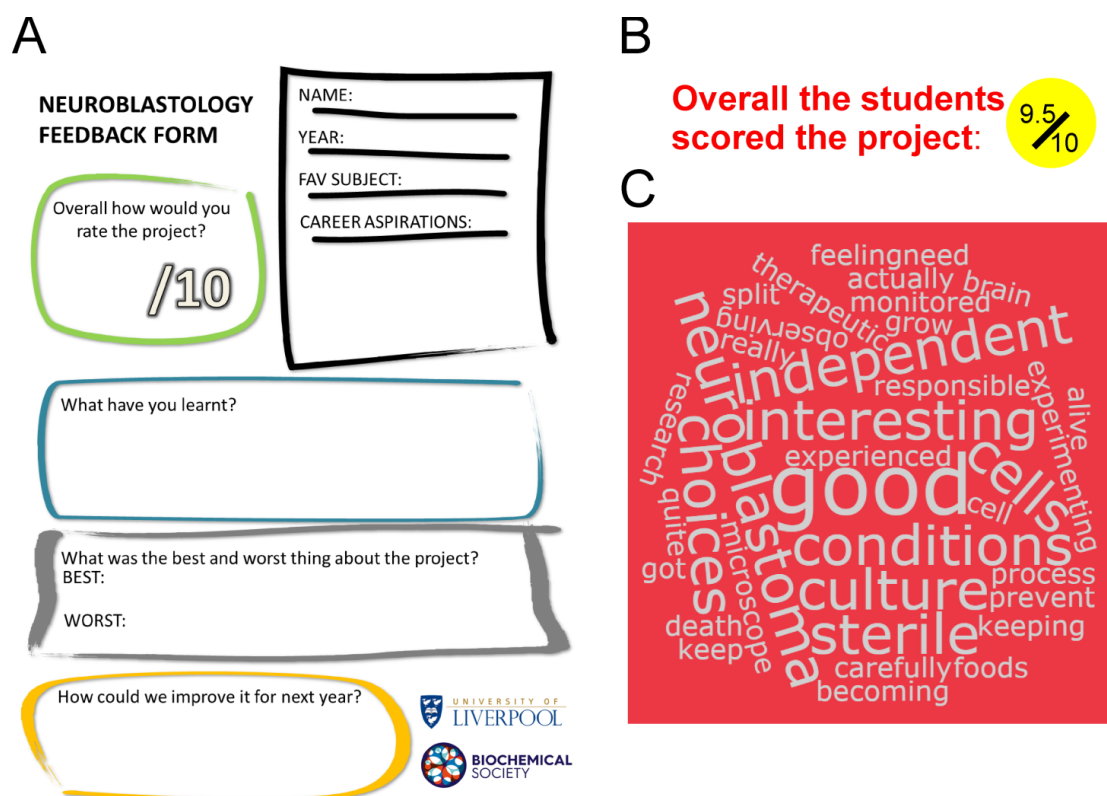


Figure 4. Feedback overview. Feedback form given to students at the end of the experimental work (A). Overall average rating given by the students (B) and a word cloud representation of the feedback (C).

FUTURE WORK

Short term

As a result of the poster session discussions some of the students have now been invited to present their work to the Dementia steering group at the Royal Liverpool University Hospital. This will take place over the coming months and will provide an excellent opportunity for the students to show off their hard work and discuss the wider impact of dementia in and ageing population.

Furthermore, some of the more senior students (KS5) are aiming to publish some of their findings in the Young Scientist's Journal (<http://ysjournal.com/about-us/>). This opportunity will provide the students with further skills and help many with their further education applications.

Long term

A key aspect of this project was to try and develop sessions and materials that could be used in different schools. To an extent this has been achieved, much of the necessary paperwork and

planning has now been carried out however many schools (if not all) lack the equipment required to recreate this project in their schools. The aim moving forward is to develop a platform that would enable more schools to participate in the project. It is hoped that we will be able to host other schools at LLS-UTC so they can experience the practical side of the project but also to develop online materials (video/discussion forums) that will other schools to access and contribute to the project remotely. In order to achieve this it will be necessary to secure further extension funding and work in collaboration with other schools and education professionals in the local area. We are currently in discussion with several partners that could assist with the creation and design of such a platform.

ACKNOWLEDGMENTS

Thanks to the Biochemical Society for the outreach grant that enables us to buy the necessary materials and reagents to allow the pupils to learn how to culture mammalian cells. Thanks to Dr John Dyer and staff at LLS-UTC for their enthusiasm and hard work. Thanks to other members of staff at University of Liverpool for helping out with teaching sessions and supplying compounds for experimentation.