Epithelial exosomes: Key modulators of neural function in human trigeminal nerves?

Background:
Pain is a natural, protective response that helps us avoid injury. Sometimes this protective response becomes deregulated and this often occurs when pain is associated with chronic inflammatory conditions. It is well recognised that during inflammation, different cell types communicate with nerves, and this communication contributes to changes within the nerves which are typical of chronic pain syndromes. Communication between different cell-types in chronic inflammatory pain is not fully understood but recent work points to a new mechanism involving small vesicles, called exosomes, which carry genetic and protein information from cells, in the surrounding tissue environment, to the nerves to influence their function.

Epithelial cells have particularly important roles in protecting us from injury and infection because the line the majority of our body surfaces. In this study we are determining the role of small vesicles, known as exosomes which are produced by many cell types, including epithelial cells. We are interested in determining whether epithelial exosomes alter the expression and function of a certain class of pain receptor on human nerves. In pain research it is recognised that animal studies may not always faithfully reproduce human biological responses. Thus, in this study we use a human epithelial cell lines and a unique model of human nerves (nerves which are differentiated from human dental pulp stem cells) so that our work has direct relevance to human rather than animal physiology. We investigate how these recently discovered exosomes influence human nerves and provide entirely new data to improve our understanding of their role during inflammation. We firmly believe that preclinical studies investigating new targets for chronic pain therapy will benefit from models which use human cells and in this way enhance the translation of research studies into new medications that can benefit patients who are coping with the daily burden of pain.

Progress to date:

The effects of epithelial exosomes on cultured neuronal cells and the influence of the epithelial microenvironment

Our research group has expertise in differentiating adult dental pulp stem cells (DPSCs) towards a functional neuronal lineage [1] and in the culture of oral epithelial cells [2] relevant to the project. Both epithelial and neuronal cultures are ideal for studying exosomes as neither cell type requires serum as a media component (which can confound exosome experiments unless exosome-depleted serum is prepared by a lengthy protocol, or purchased at great expense).

Preparation of epithelial exosomes

Exosomes are only just beginning to be studied in detail by numerous research groups around the world and techniques are constantly evolving to allow better classification of these exciting vesicles. In order to prepare exosomes from epithelial cells it is necessary to grow the epithelial cells in culture and then remove the cell supernatant for exosome preparation (Figure 1).

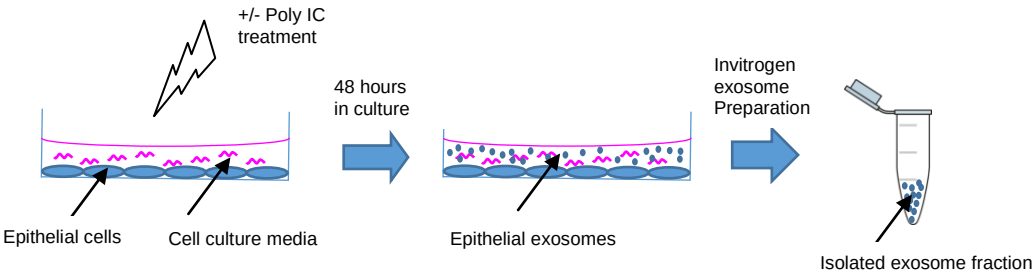


Figure 1: Preparation of epithelial exosomes from cultured human epithelial cells

We isolated exosomes from epithelial monolayers using a validated exosome isolation protocol for cultured cells (Invitrogen Total Exosome Isolation Reagent. The technique uses a protocol that facilitates isolation of intact exosomes from cell culture media and the isolated exosomes are considered suitable for further downstream analysis.

Cells can produce vesicles of all sizes and, in order to be fully classified as an exosome, the vesicle size should be in the region of 300 nm or below. We had the opportunity to use cutting-edge facilities for particle measurement in the School of Chemistry at Queen's University and have indeed have been able to show that the exosomes prepared by us from human epithelial cells are of the correct size to be defined as true exosomes (Figure 2).

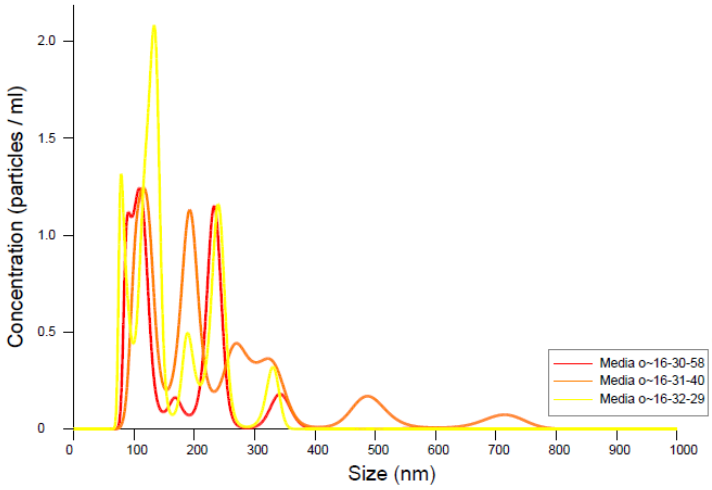


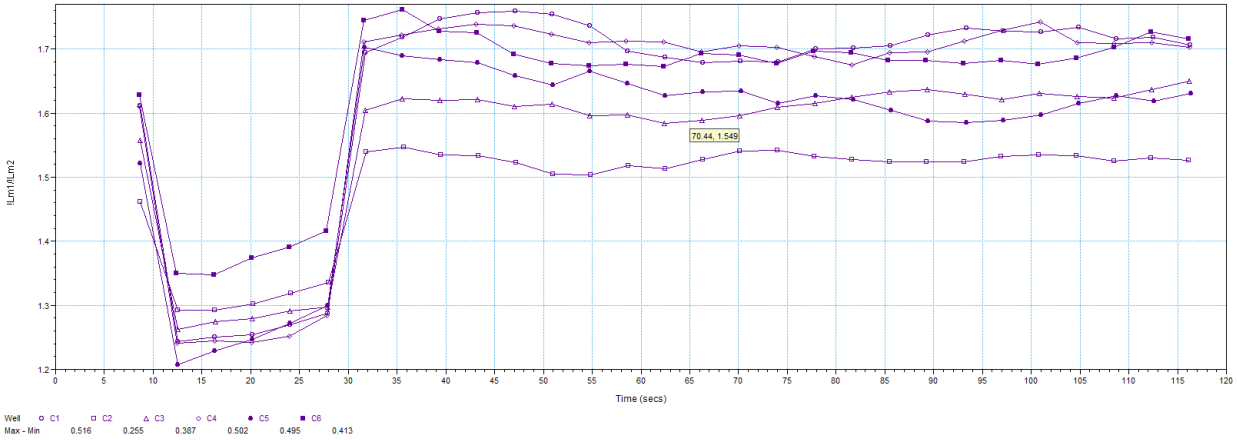
Figure 2: **NanoSight analysis of exosomes recovered from human epithelial cells with Total Exosome Isolation Reagent.** Three analyses are shown (in yellow, orange & red lines), the vast majority of particles are less than 300 nm in size.

Do epithelial exosomes communicate with neuronal cells?

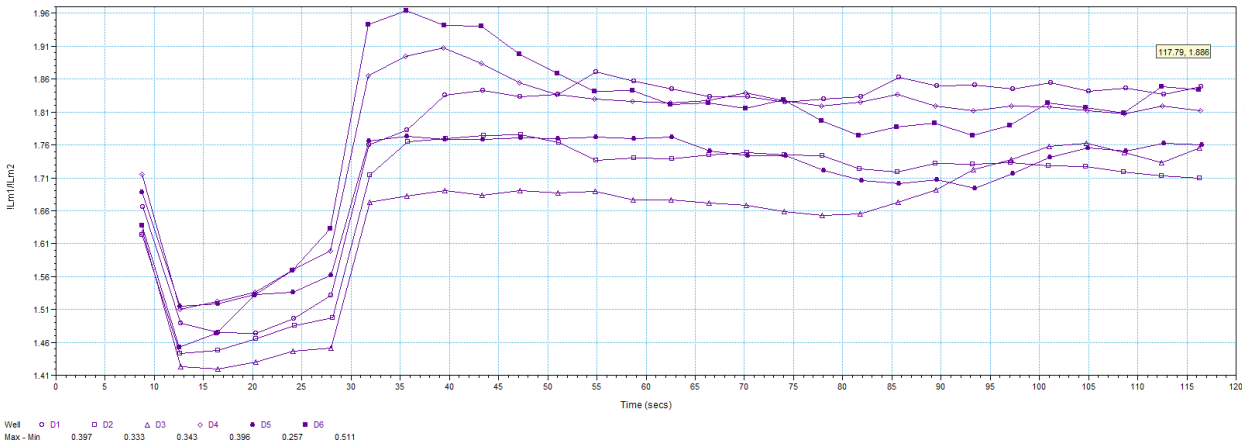
Having validated the authenticity of our exosomal preparation from human epithelial cells we sought to determine whether the contents of the exosomes were able to influence the function of human nerves. It has previously been shown that exosomes contain proteins [3] that could have the potential to alter the responsiveness of certain ion channels, known as transient receptor potential (TRP) channels on recipient (neuronal) cells. In this part of the project exosomes isolated from epithelial cells (as oultined above) were used to treat neuronal cells for 20 minutes. Subsequently we monitored changes in intracellular calcium level (indiciative of ion channel activity) using a Flexstation 3 calcium imaging approach.

We determined whether the exosomes themselves could interact with nerves and also whether the exosomes could modulate the activity of neuronal TRP channels that were incubated with standard TRP agonists. Furthermore, to determine whether the microenvironment of the cells influenced the function of exosomes we 'stressed' the cultured epithelial cells by exposing them to a molecule known as Poly I:C (see Figure 1 for Poly I:C treatment) which is used in research studies to mimic the effects of a virus. Thus we could determine whether exosomes produced by the epithelial cells under stress (similar to that encounted by a viral infection), could alter neuronal function compared with exosomes produced under control (normal) conditions (Figures 3,4).

(A)



(B)



(C)

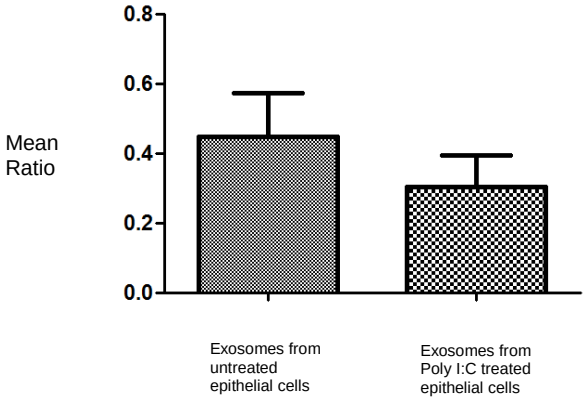


Figure 3: Flexstation 3 calcium imaging traces (A & B) and bar chart summary (C) of direct effects of epithelial exosomal on human neuronal cells.

Effects of exosomes from untreated epithelial cells on human neurones (A). Effects of exosomes from Poly I:C treated epithelial cells on human neurones (B). Bar chart summary of responses (C).

Our results show that epithelial exosomes are capable of communicating directly with neuronal cells and in so doing modify their responsivness. The data above suggest that esoxomes produced following epithelial cell treatment with Poly I:C produce slighlty lower calcium responses in neuronal cells.

We also studied the indrect effects of epithelial exosomes on neuronal cells, by determining their ability to alter TRP channel responses. As outlined above for the direct effects of epitheial exosomes, Poly I:C-treated epithelial cells produced exosomes that indirectly appear to slightly lower intracellular calcium levels following TRPA1 activition with its agonist cinnamaldehyde.

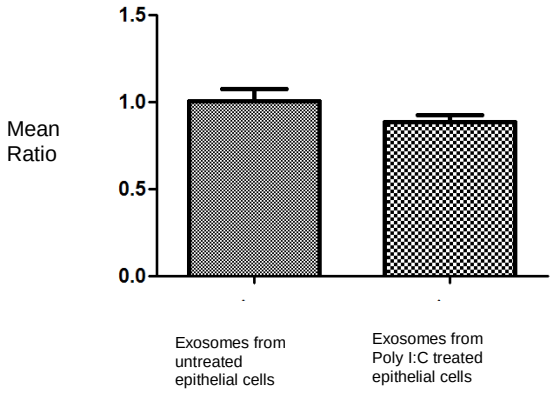


Figure 4. Bar chart summary of indirect effects of exosomes on human neuronal cells

Further work will be required over the reaming six months of the project to determine the robustness of these initial data on the role of exosomes in modulating neuronal function. We are also now progressing to Aim 2 of the original project to begin to study the effect of miRNA within the exosomes on neuronal TRP expression and function.

Summary

Using this model to study the effects of epithelial exosomes on neuronal function, we have now begun to unravel how epithelial cells communicate with neuronal cells using small vesicles known as exosomes. It is hoped that knowledge gained in this work will be important in guiding future research studies, clinical trials and treatments to modulate and alleviate human pain

References

[1] Clarke..**Curtis..McGarvey..Lundy** (2014) *J Dent Res* 93: Sp Issue B.
[2] McKeown..**Lundy** (2006) *Oral Oncol* 42, 685-90.
[3] Biasutto (2013) *Exp Cell Res* 319, 2113-23.