Development and adaptation of an in vitro rainbow trout gill model for use as an alternative to live fish studies

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In line the 3Rs, our work involves the development and validation of novel, reliable in vitro fish methods as an alternative to in vivo studies. The rainbow trout (Oncorhynchus mykiss) gill has a long history of cell and tissue models, which have developed over time from a single to a double seeding of cells in flasks and then micropatterne inserts 1,2. One issue with the existing gill model for toxicological testing is the relatively short (~2 days) duration of viability after apical exposure to water. If this time could be extended, potential applications for the model would be increased, e.g. to include longer term chronic testing to better represent current in vivo ecotoxicological tests. Such a model would reduce the number of fish used in existing tests and refine the method of exposure to test chemicals.

Materials & Methods
We have investigated various ideas to improve the time the model remains viable after addition of apical water. The methods reported here include: 1 Culture serum type. 2 Pre-perfusion of gills, and 3 Use of different membrane materials.

Blood collection from a fish before perfusion or harvesting gill cells. The serum can be used instead of FBS in later culture of cells.

Results & Discussion

The use of TS appears to show some improvements to TEER compared to FBS cultures. TS cultures reach 5000 Ω cm² (a standard threshold) a day sooner than FBS under symmetrical conditions and remain above 5000 cm² for several days more after water addition. FBS cultures appear to reach a higher maximal TER immediately after water addition. Both of the culture types are extending far beyond the ~48 hours previously reported.

SEM analysis of surface ultrastructure of a typical culture for FBS or TS at Day 5 post water addition (PWA); top reveals microvilli, tight junctions and varying cell types. After ten days PWA only TS cultures remain viable. Interestingly, TS cultures (e.g. bottom; Day 18) appear to exhibit depressed, or coated microvilli with development of a larger scale ridge structure, similar in scale to in vivo apical crypts. Further work on characterisation of this version of the model and explore its use in longer term exposures is currently being explored.

We tested primary and/or perfused cell preparations for the two cell seeds. NSD was observed between any of the 4 combinations of cell seeding were found. Although no apparent benefit for TEER, the perfusion method does reduce the protocol time for culture preparation by limiting the wash stages. Other methods are thus required.

The Trans-Epithelial Electrical Resistance (TEER) across a cell culture can be measured by a WPI EVOM2 ‘chopstick’ electrode. It is a standard measure of the cell membrane ‘tightness’ and can be interpreted as a measure of culture quality.

The alternative insert membrane types generally displayed low cell adhesion and low TEER. The identical PET membrane with a higher pore density did not attain TEER values above background. The Alv vetex membrane was not fully compatible with the chopstick electrode, so although the TEER appeared to be low, it could not be accurately measured. Only the Transwell (polycarbonate) membrane produced similar growth to the control (data not shown). It is unclear why the cells did not attach, but the seeding densities might require optimisation for specific insert types, particularly the 3D scaffold-like Alv vetex. We are also yet to try collagen-coated inserts from the major suppliers which are successfully used in mammalian in vitro cell culture.

References