Moving up a dimension: 3D *in vitro* models as effective alternatives to live fish studies

A BBSRC, NERC & AstraZeneca funded research project at Plymouth University

Background

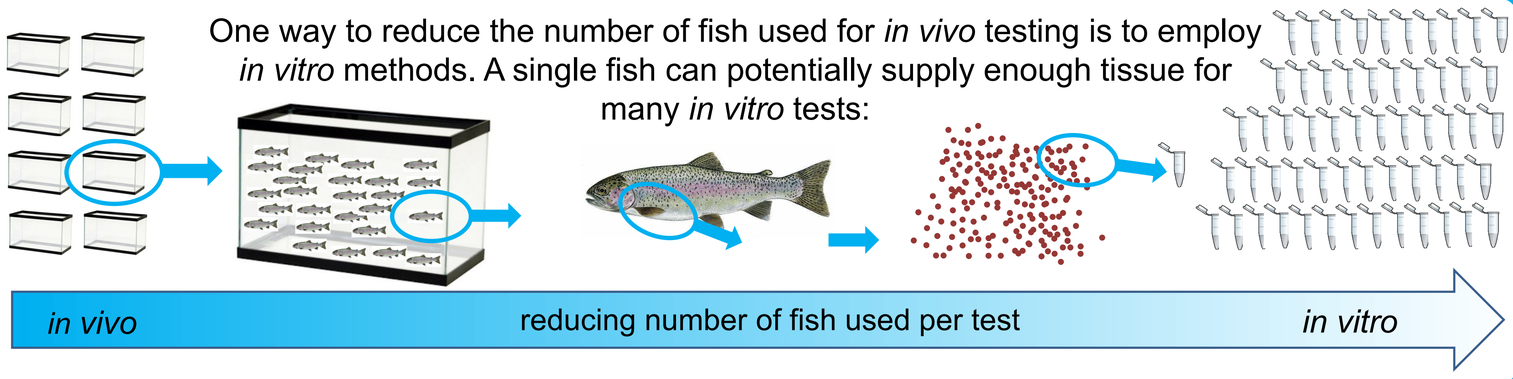
After use, a variety of chemicals get washed directly into streams and rivers. Others are treated in sewage treatment works, but still ultimately end up in the aquatic environment

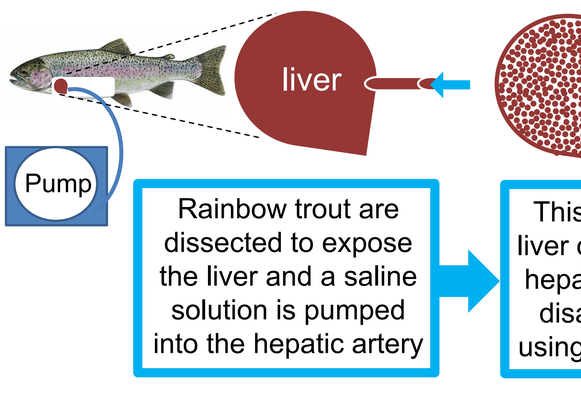
The potential impact of these substances on the environment needs to be addressed

- Chemicals in the environment are regulated by several guidelines (e.g. REACH[1] and PPPD[2]).
- Large numbers of compounds (>140,000 in Europe) face regulatory testing, involving standardised toxicity tests to algae, invertebrates and fish.
- Single tests can use hundreds of fish (e.g. OECD 305)[3].
- Biomedical tests also use many fish developing and testing drugs prior to human use.
- For ethical, scientific, economic and regulatory reasons there is a growing demand to reduce the numbers animals used in scientific studies.

Approach

Our work involves the development and validation of novel, reliable *in vitro* fish methods as an alternative to *in vivo* studies. We have already developed a method of creation and testing of liver 'spheroids.'[4] The spheroid method is more comparable to *in vivo* testing than existing methods based on increased functionality and a ten-fold increase in their duration of viability.



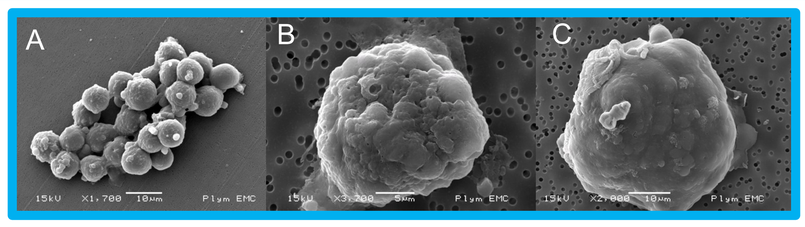


Rainbow trout are dissected to expose the liver and a saline solution is pumped into the hepatic artery

This clears the liver of blood and hepatocytes are disassociated using an enzyme

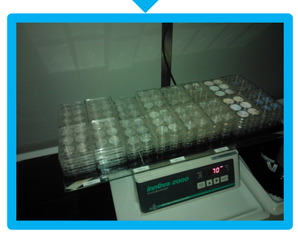
Hepatocytes are cleaned, added to microplate wells, incubated at 15ºC and oscillated

Over time the cells collide and form together into spheroids, of ~500 cells

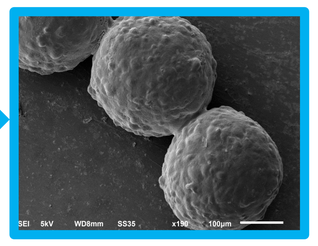


Scanning electron microscopy (SEM) during cell aggregation and spheroid formation from liver cells. (A) small aggregates 24h after plating; (B) immature spheroids, 4 days after plating; (C) mature spheroids, 8 days after plating.

The spheroids can be used in a similar manner to existing in vitro tests but have a higher functionally when compared to the intact organ



We are also developing techniques to produce spheroids and 3D layers of gut cells.
Image: SEM of 14 day old gut spheroids



Current / Future work

As well as the liver and gut, the gills are largely involved with absorption, distribution, metabolism and excretion (ADME) of environmental contaminants.

We aim to develop and validate 3D models for gill and gut tissues and then combine the cultures with liver spheroids in microplate wells to produce an *in vitro* 'virtual fish'

A similar model could incorporate gut cells, and be able to measure ADME within a simulated dietary exposure scenario

This project has the potential to develop novel, 3D *in vitro* techniques that are functionally superior to the existing methods. These techniques would facilitate a significant reduction in the numbers of fish used in environmental regulation, and remove the potential suffering of fish from direct exposures to chemicals.

References

[1] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency.
[2] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.
[3] Organisation for Economic Co-operation and Development Test Guideline No. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure.
[4] Baron et al (2012). *Ecotoxicology*, ***21*** (8), 2419-2429.

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