Author’s response to reviews

Title: A novel microfluidic device capable of maintaining functional thyroid carcinoma specimens ex vivo provides a new drug screening platform

Authors:

Andrew Riley (a.s.riley@2016.hull.ac.uk)
Victoria Green (V.L.Green@hull.ac.uk)
Ramsah Cheah (R.Cheah89@gmail.com)
Gordon McKenzie (Gordon.Mckenzie@hyms.ac.uk)
Laszlo Karsai (Laszlo.Karsai@hey.nhs.uk)
James England (James.England@hey.nhs.uk)
John Greenman (j.greenman@hull.ac.uk)

Version: 1 Date: 13 Feb 2019

Author’s response to reviews:

Dear Dr Si,

Please find below a detailed list of alterations made to the manuscript made in light of the reviewers’ comments. In a small number of cases, we feel that suggestions made are beyond the scope of the research, and in some cases alter the work’s direction, and in these cases we have endeavoured to fully explain our point of view.

Aaron Diaz (Reviewer 1):

1. While the authors do a reasonable job of demonstrating that their culture system enables slice culture of thyroid carcinomas for a few days with decent fidelity, it is not clear that the system described is superior to other systems for organotypic slice culture. It is incumbent on the authors to demonstrate a significant improvement over status quo approaches via a quantitative comparison.
To the best of the authors’ knowledge, there is no other published research where thyroid tissue explants have been maintained in this manner. In fact, there is scant research detailing the ex-vivo culture of human thyroid tissue per se. Two other articles (now cited within the manuscript) describe static culture of human thyroid tissue explants, however we believe a strength of the system is in its more faithful reproduction of the in-vivo physiology, i.e. in terms of nutrient (or drug) delivery, waste removal and ability to maintain the spatial orientation of tissue. To this end we are always trying to demonstrate the similarities of the tissue on the device to the clinical situation, e.g. lower production of thyroxine by cancerous tissue when compared with benign biopsies (Figure 7). In short, the system is occupying a unique niche in terms of ex-vivo thyroid tissue culture. Furthermore, even if there were alternative models to which our system could be prepared it would not be possible to recruit sufficient patients in the 28 days given to revise the work as the current study has taken 18 months to complete.

2. If the platform is to be used for screening then it is necessary to assess inter-culture heterogeneity, under treatment with positive and negative controls, i.e. demonstrate that technical variability across cultures is low compared to treatment effects. How many replicate cultures would be required to observe a treatment effect of a given magnitude?

Since the submission of this manuscript, the authors have tested the system for its drug delivery capabilities and can confirm that versus controls, thyroid tissue treated with two drugs (etoposide and SP600125) possessed a significantly higher number of apoptotic cells and this was determined in 5 patients. The data are shown in Figure 4; standard error of the mean depicts the relatively small inter-experimental heterogeneity.

3. I may have missed it, but I don’t find a statement of informed consent and IRB approval for tissue use.

We can confirm that the study had all necessary UK ethics approvals (Local Research Ethics Committee and Hull & East Yorkshire NHS Trust - the relevant information is given in the ‘Materials and methods’ as well as under the ‘Ethics approval and consent to participate’ section of the manuscript.
Dongxia Ye, Ph.D. (Reviewer 2):

Questions for experimental design:

1. The main point for this device is to keep thyroid explant alive and functionally.
   
   Since LDH/DCP, Propidium Iodine staining and Trypan Blue Exclusion assay only illustrate cell membrane integrity, it cannot suggest the cells are viable. Probably they are in status of apoptosis. And TUNEL data demonstrated increasing apoptotic cells in post-culture samples.

   Although it is true that those assays mentioned do indeed examine cellular membrane integrity, we disagree with the statement that the cells ‘are in status of apoptosis’ as the assays are not designed to assess the induction of apoptosis. In addition, labelling of apoptotic cells by TUNEL assay demonstrated that samples maintained in the device for 96hr showed no significant increase (2.81%) in apoptosis.

   2. Since Ki-67 can also be detected in fixed tissue, Ki-67 positivity data, even no significant difference, is not convincing of tissue viability and proliferation.

      We are not exactly clear what was meant by this point. It is true that the Ki-67 data was from fixed thyroid tissue; those cells expressing the Ki67 antigen are proliferating at the point of fixation and the expression is not altered once fixed. The assay is commonly used in this way.

   3. The author did not give enough explanation for using benign tissue as control. In each experiment, the sample numbers were different, for example, LDH release at 2h, n=8 or 7, at 24h, n=14 or 10.

      We apologise for not making this clearer, the differing samples sizes at each time-point are due to difficulties in collecting effluent at certain timepoints. This is now detailed in the figure legend.
Questions for Figures:

Figure 1, no Schematic diagram of the device

Please find a schematic drawing of the culture device in a newly updated figure 1, thanks for this suggestion.

Figure 4, no methods introduction for FITC pictures

Apologies for not explaining the nature of the TUNEL assay used, this has been rectified.

Figure 5, the sample size (n=2) for BrdU staining was too small.

Since initial submission, we have been able to study one further patient, this is now incorporated into figure 5 (n=3). All three samples show very similar results.

Typos:

1, Page 9 and 14, Line 157 and 312, Error! Reference source not found

Apologies for these slips these errors should have been removed when the paper was proof-read; these are due to the removal of cross-referenced figures within the main text document in order to prepare a separate figures document.

2, Page 16, Line 362, easy to use should be easy-to-use.

This has been rectified.
The insertion of LZ’s initials here was to reference his position as a Consultant Histopathologist, however this has been removed as requested.

Questions for References:
Ref 2, 9, 25, 32, 36,

The references referred to here have been checked for their relevance and they are appropriate to the sentence or paragraph and have been retained. Formatting has been checked.

Xueying Sun, Ph.D., M.D. (Reviewer 3):

1. According to the information described in the article, the device invented by the authors may not be restricted to the application in thyroid tissues. Can this device be used for maintaining other types of tissues? Have they applied for a patent for the invention?

The device could theoretically be used to house any solid tumour tissue; another manuscript produced by the research group describing the maintenance of head and neck squamous tumour tissue is currently being finalized for submission. No patent has been sought for the system as the authors are keen for it to be adopted widely.

1. What is the currently used methods for maintaining fresh thyroid tissue slices in vitro? Can the authors perform a comparative study by comparing the novel device with the conventional one or the conventional methods for maintaining thyroid tissue slices?

We refer this Reviewer to our first response to Reviewer 1.
2. The authors should make the manuscript shorter and convert the current form as a full article to a communications or letter. Particularly the introduction and discussion sections should be made more concise.

The manuscript describes a new technology and thus requires a panel of different techniques to demonstrate its novel contribution to the field. We do not feel we can reduce the paper and still retain the depth of work required to validate the approach, and other reviewers have asked for additional data and further explanation of topics.

3. There are some mistakes of grammar, spelling or typos. It appears this version has not been finalized since the comments by co-authors are still marked in the manuscript.

We apologise for this and have meticulously checked the revised manuscript for faults and have altered these as required.