Development of robust *in vitro* 3D models of human tumours for the identification and evaluation of anti-cancer drugs

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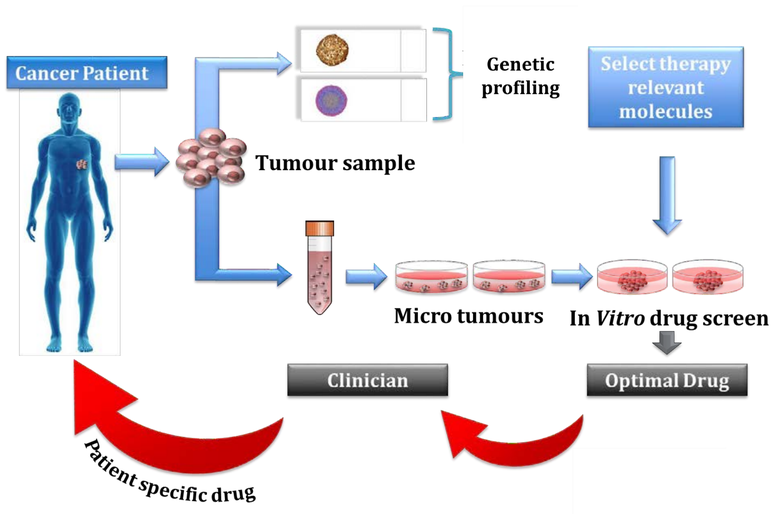
Background

Testing compounds in vivo is still the gold standard for pre-clinical drug development and evaluation of potential toxicity. However, with the rising demand for non-animal alternatives, and patient specific therapy (scheme 1) there is a pressing need for the introduction of reliable and predictive in vitro models for drug development. We are developing 3D culture models of human tumours and here we compare the functionality of two systems: Alginate tumour spheroids and OrganDot™ organotypic cultures.



Scheme 1: Patient specific treatment workflow. This represents the final output aims of this project.

Tissue-engineering has enabled the development of 3D tumour spheroids.

In this study isogenic tumour spheroids are produced within alginate beads to form both single and multi-cell tumour spheroid cultures.

Cancer cell lines can be suspended in alginate beads to enable 3D growth Within 2-3 weeks spheroid size >200 µm. Whole or bisected beads can be imaged with fluorescent microscopy.

Primary lung tumours from Asterand Bioscience and Cellaria Biosciences patient specific cell lines have enabled the growth of primary cells for this study with the prospective of achieving in vivo like responses within in vitro tumour spheroids. These results would enable patient specific treatments. (Scheme 1)

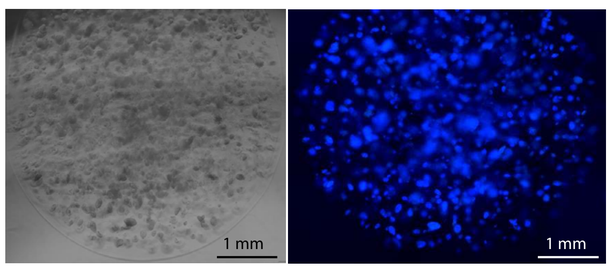


Figure 1: Spheroid bead culture (bright-field) and Hoechst 33342 co-stain showing individual spheroids within an alginate bead.

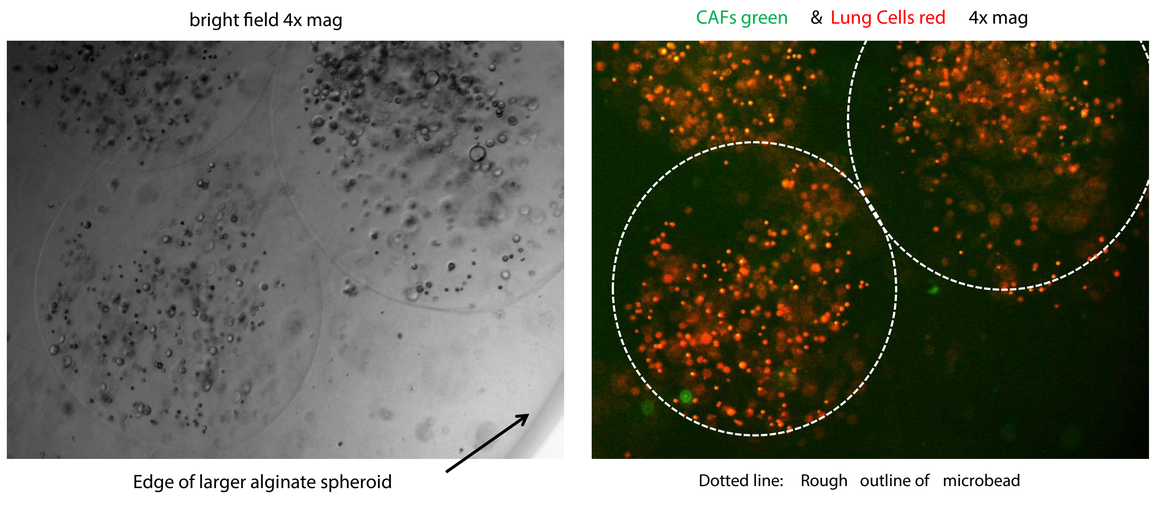


Figure 2: Co-culture with PKH26/67 labelling within microbead alginate spheroids. H1650 Tumour cells (red) PKH26 suspended in polymerised alginate results in isogenic cell clones, each producing individual spheroids in the alginate matrix; when these spheroids are suspended in further alginate containing PKH67 CAFs (green). Thus allowing the cellular growth of individual colonies in the presence of CAFs to be observed.

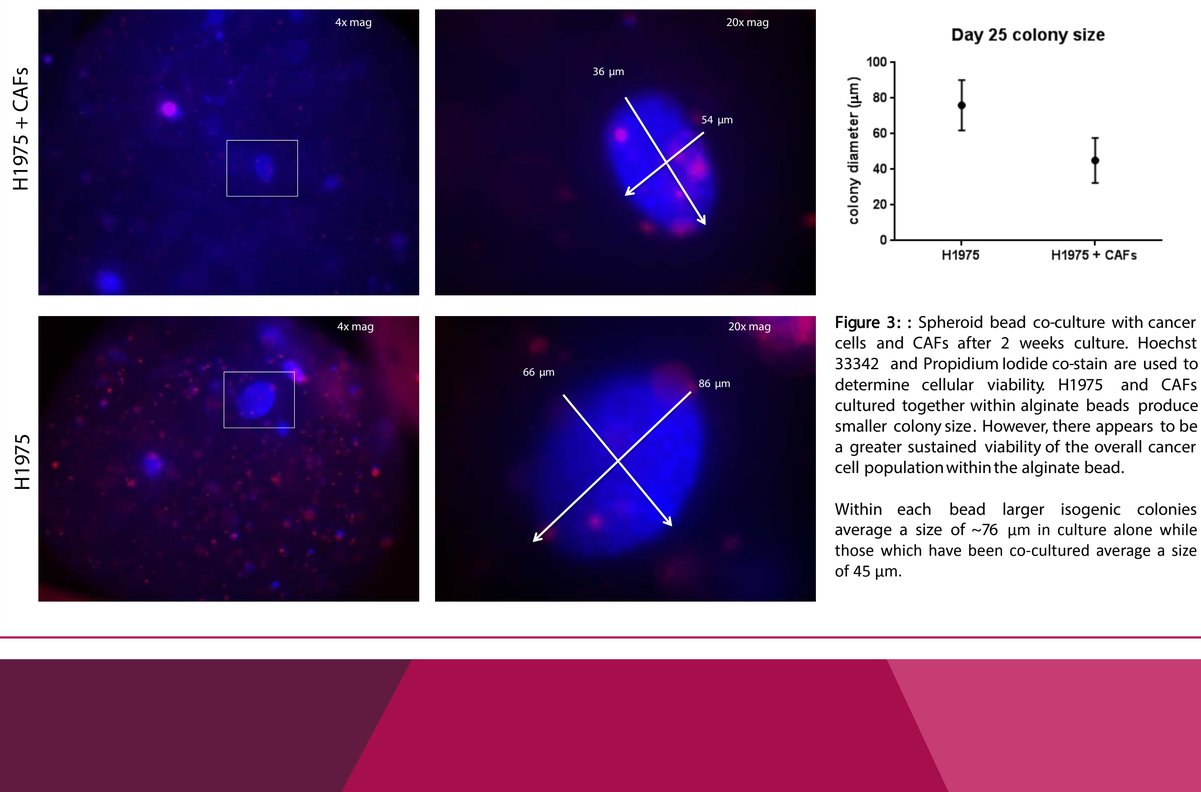


Figure 3: : Spheroid bead co-culture with cancer cells and CAFs after 2 weeks culture. Hoechst 33342 and Propidium Iodide co-stain are used to determine cellular viability. H1975 and CAFs cultured together within alginate beads produce smaller colony size. However, there appears to be a greater sustained viability of the overall cancer cell population within the alginate bead.

Within each bead larger isogenic colonies average a size of ~76 µm in culture alone while those which have been co-cultured average a size of 45 µm.

Methods

3D tumour spheroids were generated from lung cancer cell lines (NCI-H1650 , NCI-H2170 , NCI-H1975 and HCC-827) with known EGFR pathway mutations, and also primary isolated lung cancer epithelial cells, by encapsulation within an Alginate matrix to produced isogenic cell populations. It was also possible to produce multi cellular tumour spheroids (MCTS) in the same manner. Additionally, spheroids were co-cultured alongside primary Cancer-Associated Fibroblasts (CAFs). Following generation (7 days), spheroids (up to 500 µm diameter) were treated with cytotoxic agents.

The OrganDot™ culture system (Asterand Biosciences) was used to generate 3D cultures of the same lung cell lines and primary lung cells.

Results

Consistent responses were observed between the OrganDot™ cultures and 3D Alginate spheroids. Both systems allowed long-term viability of cultures enabling sequential or chronic compound testing. These 3D culture systems produced responses to treatments consistent with known genotypes for those cell lines.

Ongoing studies using these models are focussed on assessing whether anti-EGFR responses may predict the presence or absence of EGFR-pathway mutations in freshly isolated primary lung tumour cultures.

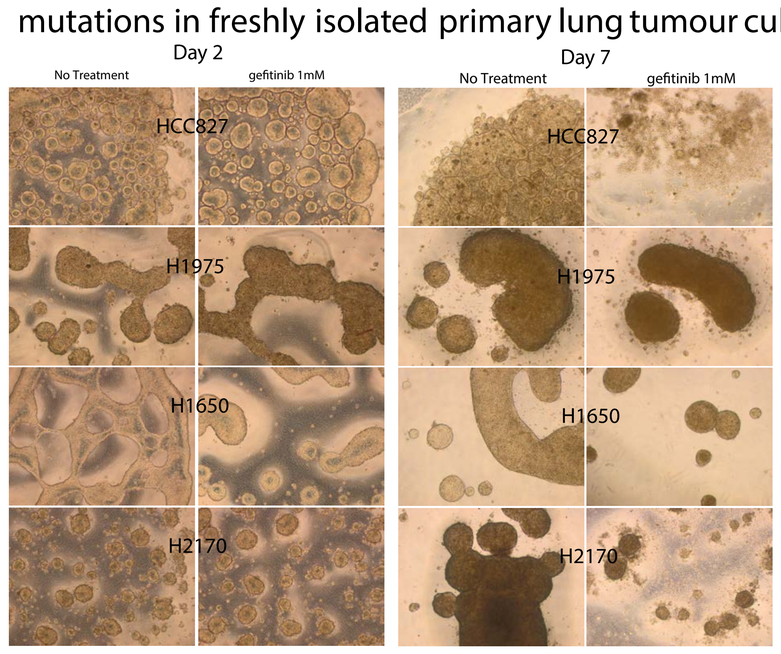


Figure 4: Lung cancer OrganDot™ spheroid growth over 7 days for 4 difference cell lines treated with 1mM gifitinib. Reduction to spheroid growth is observed in HCC827 , H1650 and H2170 cell lines with no effect visualised with H1975 . Preliminary results suggest the OrganDot™ platform responds similarly to alginate spheroid culture for the examination of cell behaviour.

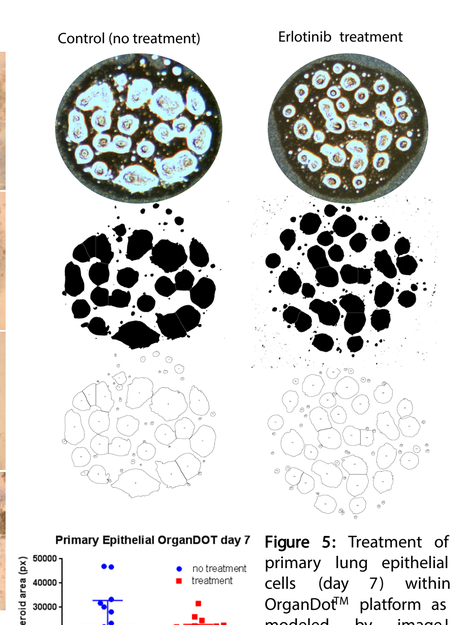
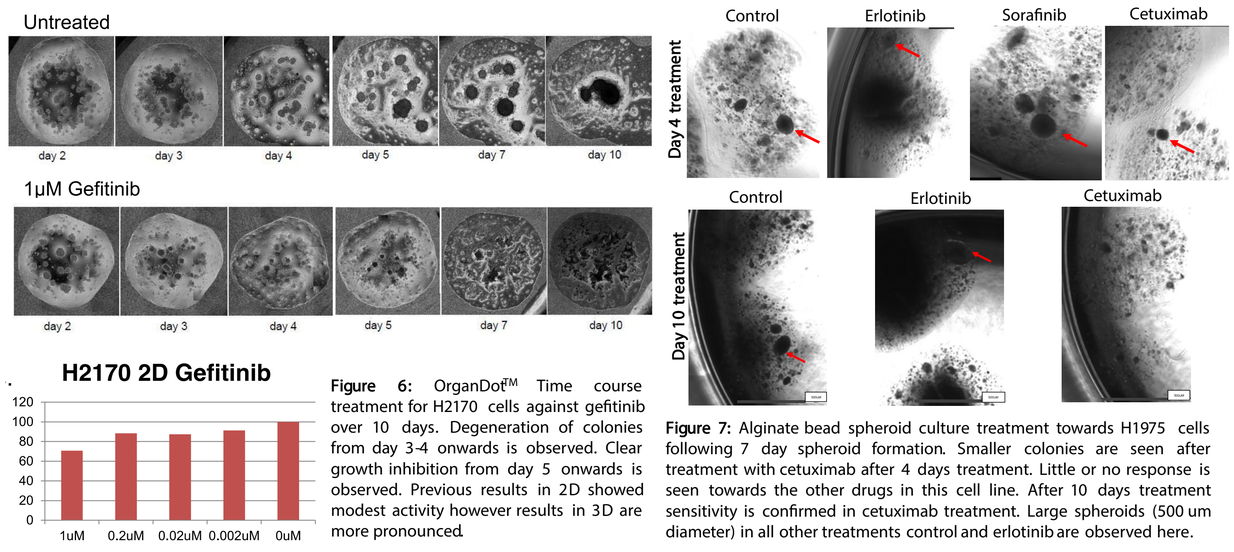


Figure 5: Treatment of primary lung epithelial cells (day 7) within OrganDot™ platform as modeled by imageJ analysis. Each spheroid pixel size was measured and the relative tumour area was calculated.



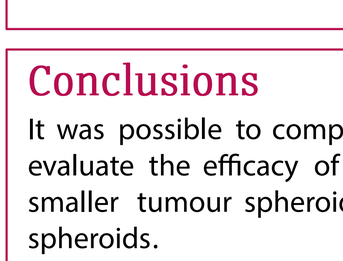


Figure 6: OrganDot™ Time course treatment for H2170 cells against gefitinib over 10 days. Degeneration of colonies from day 3-4 onwards is observed. Clear growth inhibition from day 5 onwards is observed. Previous results in 2D showed modest activity however results in 3D are more pronounced.

Figure 7: Alginate bead spheroid culture treatment towards H1975 cells following 7 day spheroid formation. Smaller colonies are seen after treatment with cetuximab after 4 days treatment. Little or no response is seen towards the other drugs in this cell line. After 10 days treatment sensitivity is confirmed in cetuximab treatment. Large spheroids (500 um diameter) in all other treatments control and erlotinib are observed here.

Conclusions

It was possible to compare the two 3D in vitro models as valuable systems to identify and evaluate the efficacy of anti-cancer drugs. The presence of CAFs attenuated the growth of smaller tumour spheroids although drug responses were not markedly changed in larger spheroids.

These systems hold the potential to better understand and enable patient specific therapies while fast-tracking drug development.

Acknowledgements

We would like to thank Innovate UK for funding this project.

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