Validating Cellesce's bioreactor technology for the expansion of patient-derived breast cancer organoids

Cellesce develops bioprocessing systems for the expansion of organoids in significant volumes in a way that ***minimises*** handling time and ***maximises*** reproducibility, with the long-term goal of positioning organoid technology as a cost-effective model for early-stage drug discovery.

Following the successful expansion and commercialisation of 10 human colorectal cancer organoid lines using proprietary bioreactor technology, Cellesce is now broadening its product range to include other tissue types. Through an ***InnovateUK*** funded project, and in collaboration with ***Cardiff University***, Cellesce has been working to optimise it's patented expansion process to allow the large scale expansion of ***patient-derived breast cancer organoids***.

Here, in a proof of principle study, Cellesce has demonstrated the stability of breast cancer organoid phenotype, genotype and drug response following bioreactor expansion. Moreover, bioreactor-expanded organoids demonstrate reduced well-to-well variability and greater batch-to-batch reproducibility than their manually grown counterparts in drug response assays, thus reinforcing their utility in high throughput drug screening.

Cellesce will use the results of this study to direct the expansion of further breast cancer organoid lines in sufficient quantities to produce frozen batches that can be provided for sale for use 'off the shelf'.

Experimental set up

An ER-/PR+/Her2- patient-derived breast cancer organoid line (BrC-001) derived externally was used for this study. Two further PDX organoid lines (BrC-002 and BrC-003) were also derived in-house from supplied PDX tumour material, and used as controls where indicated.

All organoids were initially cultured manually according to previously described protocols (Sachs et al., 2018).

To compare outputs from the two culture methods, organoids were divided between **(a)** manually expanded culture conditions and **(b)** Cellesce's patented bioreactor expansion process **(Figure 1)**.

Organoids were cultured for the same duration in each process before harvesting from their matrices by the same protocols.

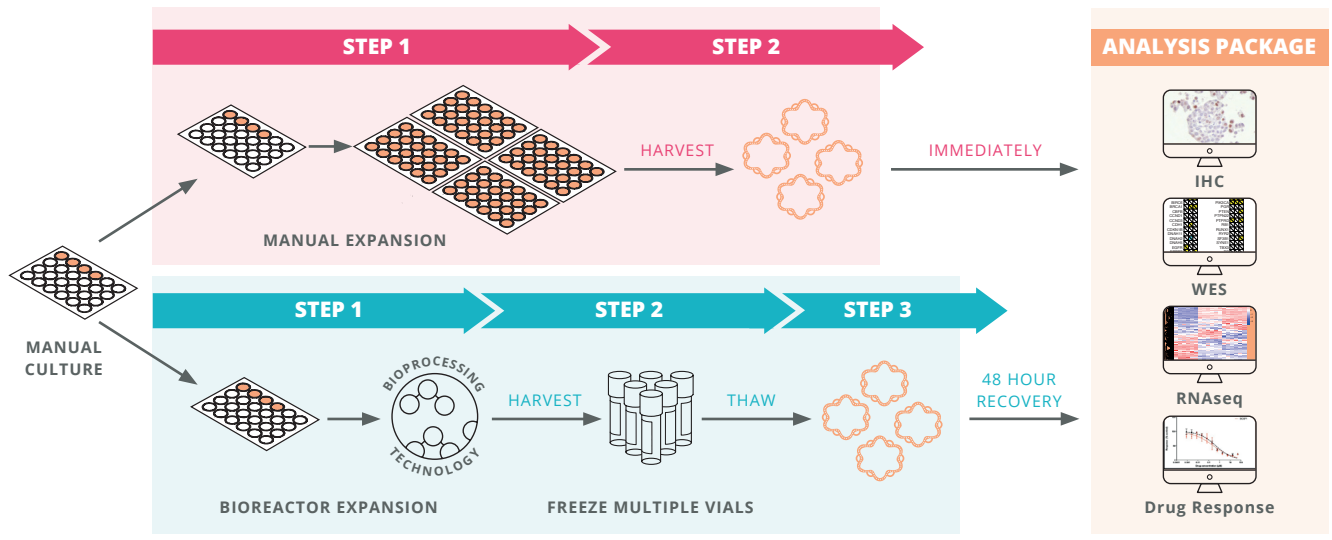

Figure 1. Experimental workflow used for comparison of organoids grown under manual or semi-automated culture methods.

As per conventional manual culture protocols, manually expanded organoids harvested from their matrix were immediately used for ongoing analysis (RNAseq, immunohistochemistry or Whole Exome Sequencing) or plating in drug response assays.

In contrast, organoids obtained from the bioprocess were frozen in cryovials following harvest, at a fixed density. Cryovials were then thawed, plated and given 48 hours recovery time prior to analysis or use in ongoing assays, to limit effects of freeze-thaw stress on analysis results.

Comparative analysis performed on organoids included Whole Exome Sequencing (WES) for comparison of key driver gene mutations, immunohistochemistry, RNAseq and assessment of organoid drug responses.

Results

Immunohistochemistry confirmed that BrC-001 breast cancer organoids retained their original ER, PR and HER2 subtype status following expansion in the bioprocess, with similar results shown for additional markers p53 and the cytokeratin proteins CK14 and CK18 **(Table 1, Figure 2)**.

From WES datasets, specific driver genes were chosen for investigation, based on a list previously described by Sachs et al. (2018). Analysis indicated no change following BrC-001 organoid expansion in the bioreactor **(Figure 3)**.

RNA was harvested from BrC-001 organoids and two additional organoid lines of ER+/PR-/HER2+ subtype (BrC-002) or triple negative (ER-/PR-/HER2) (BrC-003) subtype, the latter as controls where gene expression would be expected to be altered compared to our test conditions.

Differential expression analysis indicated that no genes were significantly differently expressed between the two expansion methods in the BrC-001 line. In contrast, comparison of two organoid lines both expanded in the bioreactor indicated large variations in gene expression levels. Hierarchical clustering analysis detected distinct gene expression characteristics of the three different organoid lines, but no discernible differences were found in gene expression patterns between culture methods, with analysis unable to clearly group biological replicates from manual expansion as separate to biological replicates from bioreactor expansion **(Figure 4)**.

Overall, all RNAseq evidence verified that the Cellesce bioprocess does not significantly alter organoid gene expression.

Marker	Manual	Bioprocess
ER	✗	✗
PR	✓	✓
Her2	✗	✗
p53	✗	✗
CK14	✓	✓
CK18	✓	✓

Table 1. Summary of immunohistochemical analysis of BrC-001 organoid marker expression.

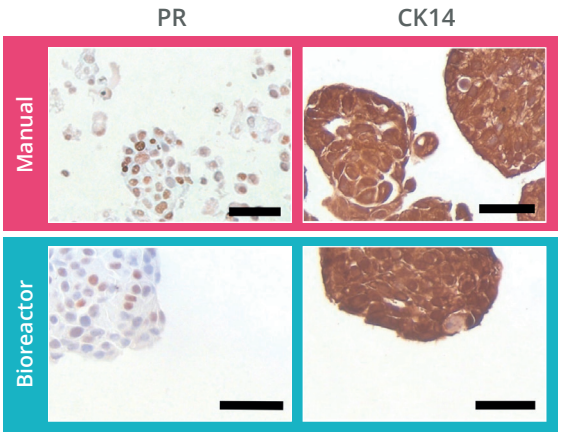

Figure 2. Immunohistochemical analysis of BrC-001 organoid marker expression. *Slides were imaged using a Zeiss Axioscan Z1 Slide Scanner. Scale bars 50µM.*

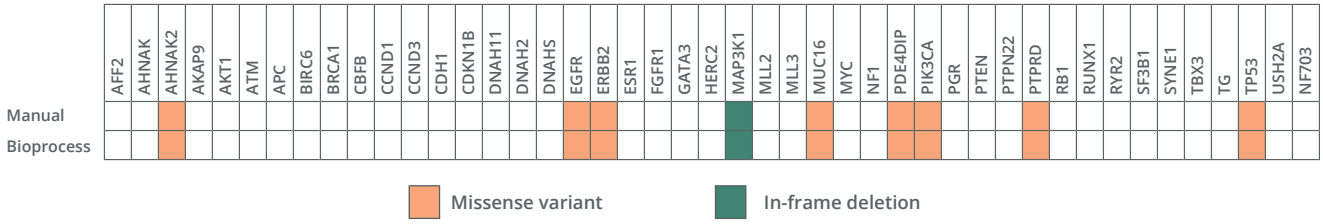

Figure 3. Comparison of driver gene mutations between manually expanded and bioreactor expanded BrC-001 organoids.

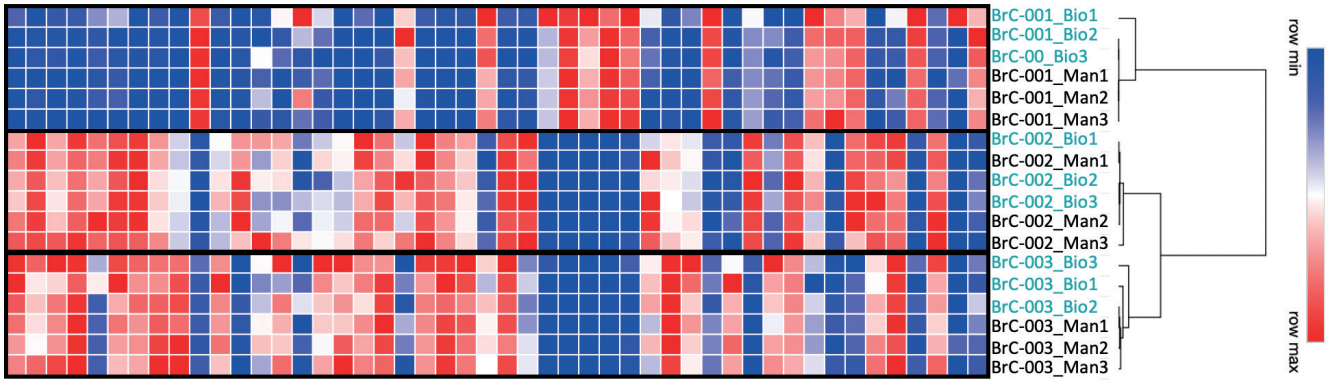

Figure 4. Gene expression analysis of organoids using RNAseq.

Heatmap of FPKM reads of PAM50 genes in all samples. The colour scale is set by row (gene) minimum and maximum FPKM reads, where red is a higher number and blue is lower. Bioreactor samples are labelled in green for distinction.

Drug treatment assays performed on BrC-001 organoids expanded manually and using bioreactor technology, according to the workflow shown in **Figure 5**, broadly indicated that bioreactor expansion does not alter drug response; in fact in the majority of cases, analysis indicated that individual drug EC50s were not statistically different between the two organoid sets **(Figure 6A, indicated by *)**.

Moreover, dose response data indicated that bioprocessed organoids generated tighter error bars (n=3), and thus more reproducible data, when compared to those grown manually **(Figure 6B)**.

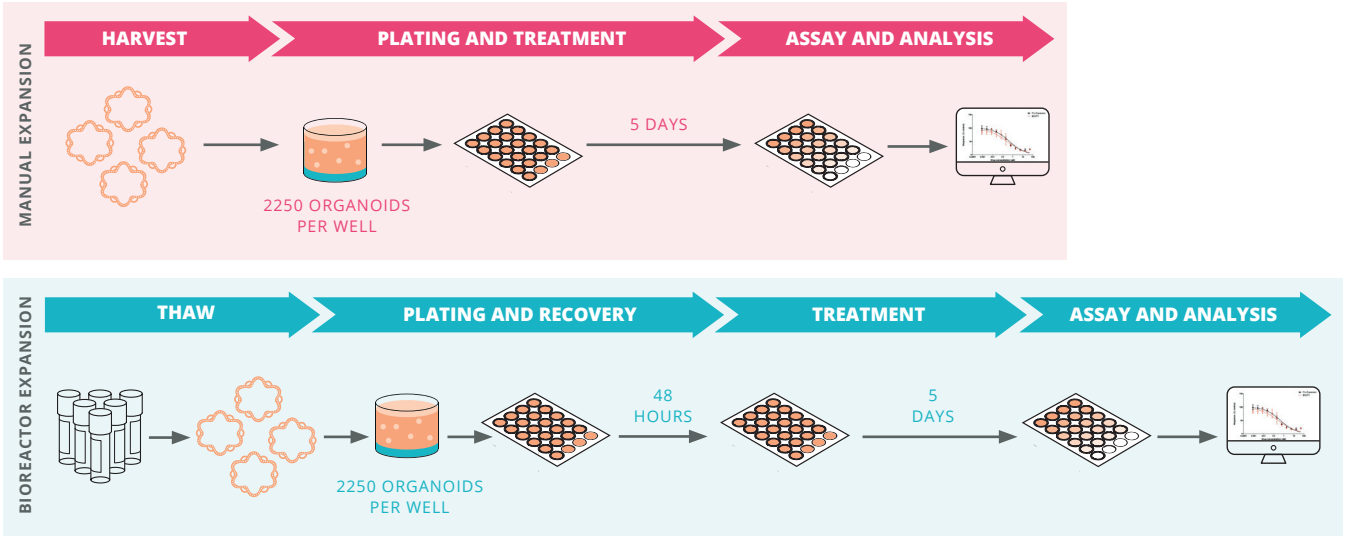

Figure 5. Drug response assay workflow.

Organoids from each source were resuspended at 90 organoids per µl in full growth medium supplemented with 5% hydrogel matrix. 25µl of this organoid suspension was dispensed into each well of a white, clear-bottomed 384 well plate (Greiner) pre-coated with 10µl matrix. Organoids from frozen stocks of bioreactor expanded organoids were given a 48 hour recovery period before treatment with compounds, while organoids harvested directly from manual culture were treated immediately. 11 point compound titrations (2 or 3 fold) were applied in quadruplicate alongside DMSO controls. After 5 days, cell viability assessments were made using an end-point Cell Titer Glo 3D assay. Experiments were performed in triplicate, from three independent bioreactor or manual expansions.

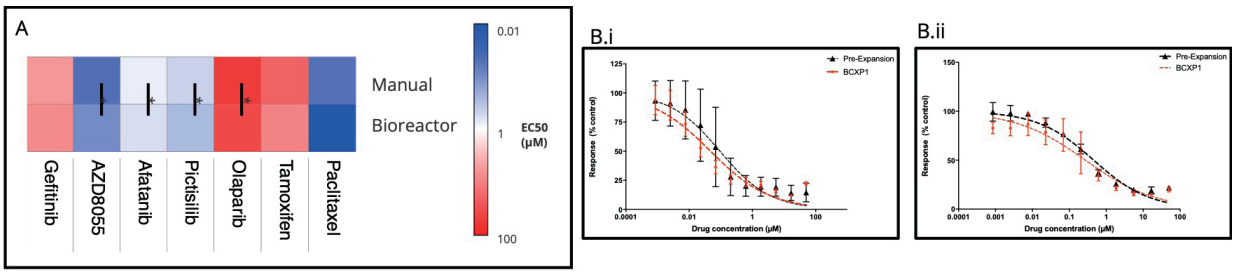

Figure 6. Drug response data.

BrC-001 organoid response to a variety of compounds was measured using Cell Titer Glo 3D on day 5 of treatment. Data was normalized to DMSO control conditions (100%) and analysed by non-linear regression analysis. Resulting dose-response curves were used to calculate EC50 values for each drug, for each organoid expansion condition (n=3). **(A)** *Heatmap of calculated EC50 values for all drugs and organoid expansion conditions. Lower values are depicted by blue colour, while higher values are depicted by red colour. Further analysis of curve fitting was performed, with * indicating pairs of responses that fit to the same curve, and are therefore not statistically different from one another.* **(B)** *Dose response curves following treatment with (i) AZD8055 or (ii) Paclitaxel, expressed as % response compared to control DMSO wells (mean ±SD, n=3 for each condition)*

Conclusion

When taken together, the results of this study confirm that Cellesce's bioprocess is compatible with the large scale expansion of breast cancer organoid lines. Moreover, batches of organoids generated in this way produce more reproducible data than those produced by more labour intensive and costly manual methods.

Following the success of this study, Cellesce is currently working alongside Cardiff University to generate large scale batches of organoid lines from a variety of molecular subtypes of breast cancer, with the first lines available in late 2020.

Cellesce Ltd • Cardiff Medicentre • Heath Park • Cardiff • CF14 4UJ
T +44 (0)292 280 2820 **W** cellesce.com **E** info@cellesce.com