Abstract # 1018

Transitioning A549 Cells to FBS-Free Media: Process and Determining Success

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Background

Conventional cell culture practices include supplementation with fetal bovine serum (FBS) for growth and maintenance. The undefined nature and batch-to-batch variation of FBS introduces substantial uncertainty and variability in composition of cell culture media and cell fate. Conversely, the nutrients required for the growth and maintenance of cells can be controlled, systematically evaluated, and reliably reproduced in serum-free media, providing increased control of cellular fate. In this study, we evaluated the effects of transitioning A549 cells, a human lung alveolar-like cell line commonly used in respiratory research, from FBS-containing medium to four commercially available chemically defined media or media containing animal-free serum substitutes. To determine the success of the transitions, we evaluated and compared cell morphology and function. Results suggest that two media, XVIVO and CnT-PRA, are viable alternatives to FBS-supplemented media to culture A549 cells, and the choice of medium will be dependent on the objective of the study.

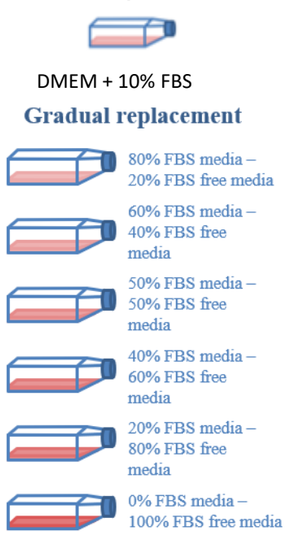
Media Transition

Serum-free media:

Medium	CNT-PRA™	XVIVO-10™	HL-1 ™	XerumFree™ XF212
Supplier	CELLnTEC	Lonza	Lonza	TNCBIO
Composition	Chemically defined complete medium, animal-component-free	Xeno-free complete medium with human serum albumin and human transferrin	1% supplement in DMEM, serum-free medium with bovine catalase from bovine liver	10% supplement in DMEM, chemically defined, animal-component-free

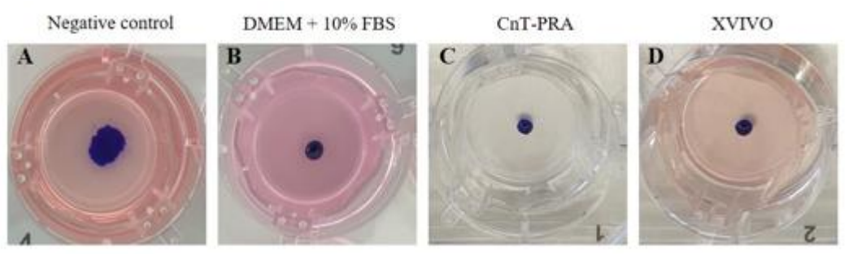
Transitioning the cells directly to the new media failed; however, cells successfully transitioned using a weaning process to CnT-PRA and X-VIVO media.

Transition process:



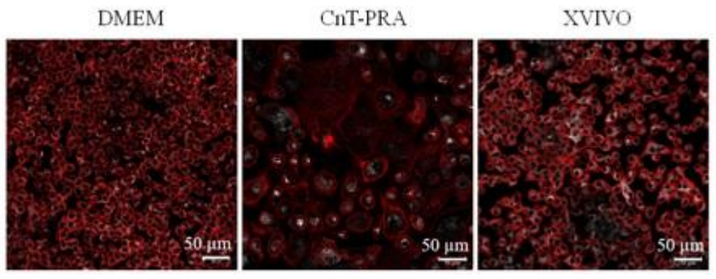
Functionality Assessment

Surfactant production at the air-liquid interface (ALI)



An important function of alveolar type 2 cells is to secrete lung surfactant. In the surfactant droplet test, a small diameter of the droplet indicates a lower surface tension and the presence of surfactant. All media types resulted in cells with similar surface tension, thus some surfactant was secreted.

Presence of multilamellar bodies (MLBs) in submerged conditions

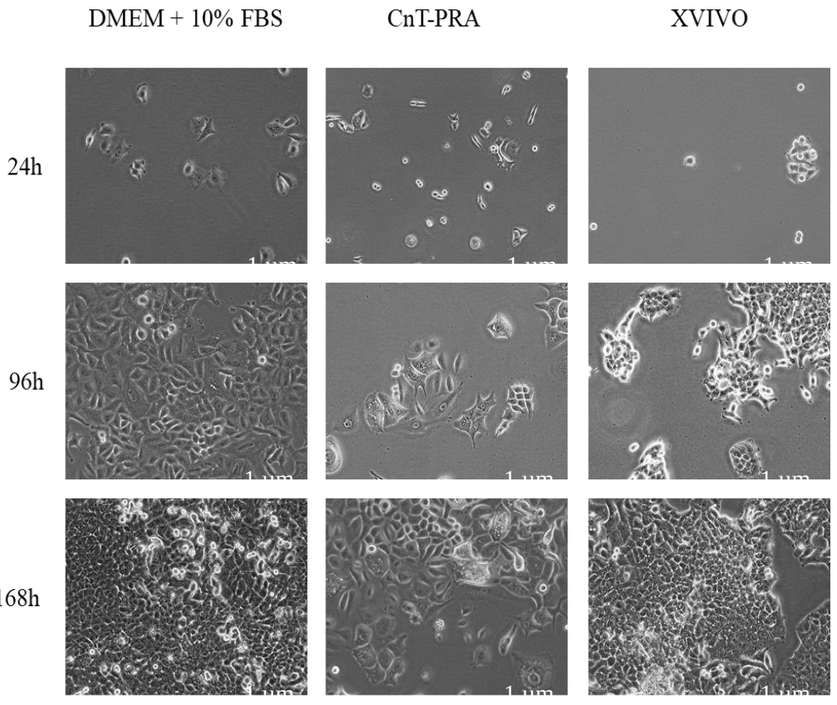


Gray – Lysotracker (MLBs)
Red – Cellmaks (cell membranes)

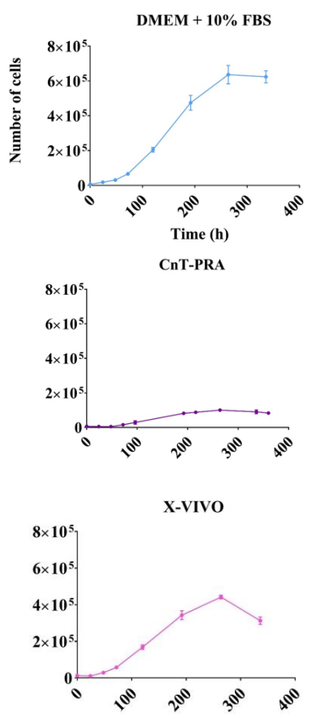
LysoTracker is used as a marker for MLBs that are typical for type 2 alveolar cells. MLBs were stained in A549 cells in all media. The very large cells found only in A549 cells cultured in CnT-PRA medium were negative for MLBs .

Characterization of Transitioned Cells

Morphology:



Growth Curves:



Doubling:

21 hours

51 hours

33 hours

A549 cells grown in either DMEM + 10% FBS or XVIVO media showed a similar morphology of smaller, cuboidal cells. CnT-PRA-cultured cells were a more heterogeneous mixture of few very large cells, a majority of medium sized cells and smaller, cuboidal cells. Growth rate was highest for DMEM +10% FBS followed by X-VIVO and CnT-PRA, with the doubling time for CnT-PRA significantly greater at more than twice that of DMEM+10% FBS

Gene expression:

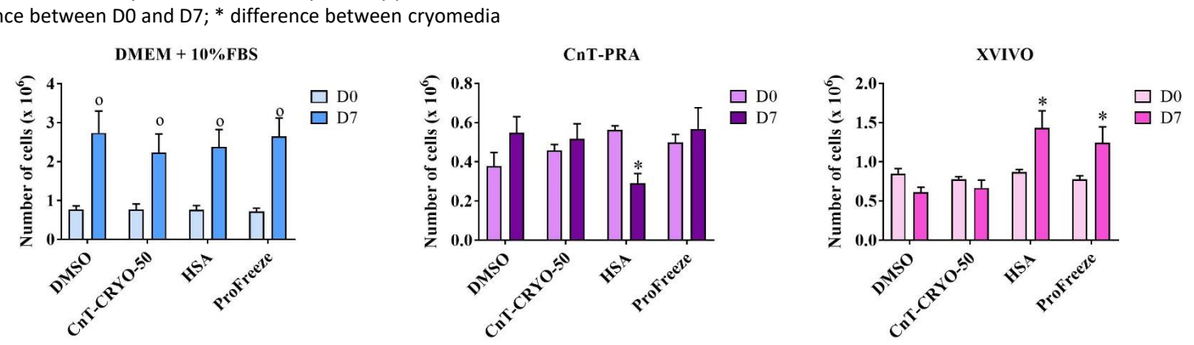
Cell type	Gene marker	DMEM + 10% FBS ALI	DMEM + 10% FBS Submerged	CnT-PRA ALI	CnT-PRA Submerged	XVIVO ALI	XVIVO Submerged
Alveolar type 1	**AQP5**	0.22 ±0.02	1.01 ±0.13	ND	ND	0.11 ±0.03	0.99 ±0.28
	T1a	ND	1.13 ±0.68	**101.6 ±37.7**	**141.33 ±36.67**	ND	ND
Alveolar type 2	**CFTR**	1.2 ±0.8	1.31 ±0.91	ND	ND	1.61 ±0.11	0.43 ±0.06
	SPB	1.08 ±0.26	1.07 ±0.51	**43.71 ±12.49**	**36.25 ±17.65**	ND	ND
	SPD	0.97 ±0.46	1.28 ±0.78	**177.7 ±50.96**	**48.56 ±9.38**	1.56 ±0.69	0.45 ±0.25
Goblet	**MUC5AC**	0.82 ±0.38	1.79 ±1.55	ND	ND	**24.21 ±6.91**	4.55 ±2.48
	MUC5B	0.82 ±0.4	1.59 ±1.19	0.19 ±0.11	0.09 ±0.05	**15.29 ±3.29**	**4.16 ±1.95**
Lung progenitor	**TTF-1**	0.66 ±0.28	1.11 ±0.5	0.92 ±0.34	0.62 ±0.07	0.73 ±0.19	0.59 ±0.23

At ALI and submerged conditions, genes expressed by alveolar type 1 and 2 cells were upregulated in CnT-PRA cultured cells, and the expression of goblet cell genes were upregulated in XVIVO-cultured cells compared to DMEM +10% FBS.

Cryopreservation in animal-free media

Cell growth from thawing to day 7:

Repeated Measures 2 way ANOVA followed by a Tukey post hoc test
o : difference between D0 and D7; * difference between cryomedia



Cells cultured in DMEM + 10%FBS had increased growth across freezing media compared to CnT-PRA and XVIVO cultured cells. However, cells in CnT-PRA and XVIVO media remained viable after seven days and started to demonstrate proliferation in some types of freezing media.

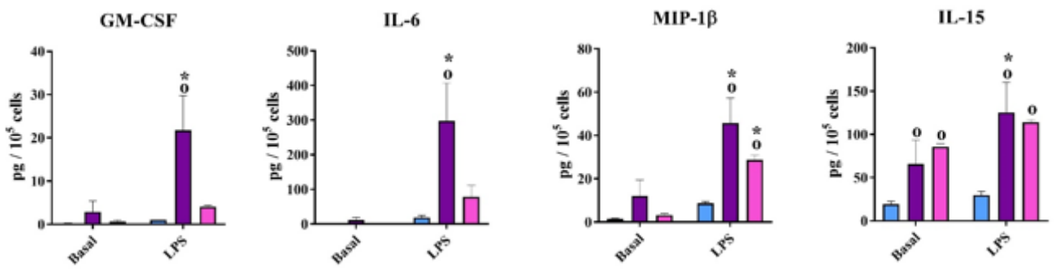
Performance Assessment

EC50 after 24h exposure to Sodium dodecyl sulphate (submerged): When exposed to SDS, a cell lysis detergent , EC50 was significantly lower in CnT-PRA compared to the other media. An increased sensitivity to toxicants may indicate the presence of ATI cells.

	DMEM + 10% FBS	CNT-PRA™	XVIVO™
EC50 (µM)	165,2	40,9	140,8

Cytokine release after 24h exposure to Lipopolysaccharide (LPS) in submerged conditions

o : difference between serum-free medium and DMEM + 10%FBS within the same treatment (medium effect)
* : difference between the basal level and LPS treated cells within the same medium (treatment effect)



Following exposure to LPS, secretion levels significantly increased for most cytokines for CnT-PRA. Most cytokine secretion levels also increased from the basal level for XVIVO. DMEM +10% had the lowest expression levels. Additional cytokine values will be made available in the upcoming publication.

Conclusions and Next Steps

- A549 cells successfully transitioned to XVIVO and CnT-PRA media using a gradual process.
- XVIVO medium retained the carcinogenic phenotype of cells cultured in FBS-supplemented medium. Similar to DMEM +10% FBS, XVIVO medium had a proliferative effect and cells cultured in this medium were of similar size and morphology, expressed similar gene markers at the ALI and in submerged conditions (with the exception of goblet cell markers), demonstrated similar lamellar body staining, secreted some surfactant, performed similarly when exposed to perturbations, and were amenable to cryopreservation without the use of FBS.
- Substantial differences existed between CnT-PRA and the other media. CnT-PRA-cultured cells showed a reduced growth rate and a heterogeneous mixture of small cells with a size similar to ATII, very large cells, and cells with sizes in-between. The very large cells did not stain for MLBs. Smaller CnT-PRA cultured cells contained MLBs and produced surfactant, hallmarks of ATII cells. There was a pronounced increase in some ATI and ATII gene expression. When exposed to SDS, EC50 was significantly lower in CnT-PRA compared to the other media. An increased sensitivity to toxicants may indicate the presence of ATI cells. Results suggest that CnT-PRA-cultured cells may lose their adenocarcinomic phenotype in favor of an ATI and ATII epithelial cell phenotype.
- A549 cells are amenable to FBS-free cryopreservation.
- Inter-laboratory performance will be evaluated in the next steps.