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Using FBS-free media in *in vitro* cell cultures: A case study in transitioning and characterizing A549 cells

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Introduction

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 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 more 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 medium containing FBS to four commercially available chemically defined media or media containing serum substitutes. To determine the success of the transitions, we evaluated and compared cell morphology and function. Results suggest that two media, X-VIVO™ and CnT-Prime Airway, 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.

Transition to FBS-Free media

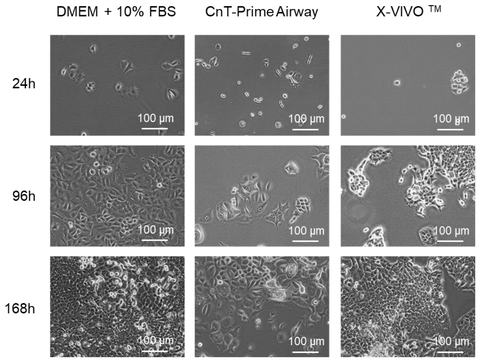
Medium	CnT-Prime Airway	X-VIVO™	HL-1 ™	XerumFree™ XF212
Supplier	CELLnTEC	Lonza	Lonza	TNC Bio
Composition	Complete medium Chemically defined Animal-component free	Complete medium Xeno-free Human serum albumin & human Transferrin	1% in DMEM Serum-free Bovine catalase	10% in DMEM Chemically defined, Animal-component free

Transitioning the cells directly to the new media was not successful; however, cells successfully transitioned using a weaning process to CnT-Prime Airway and X-VIVO™ media.

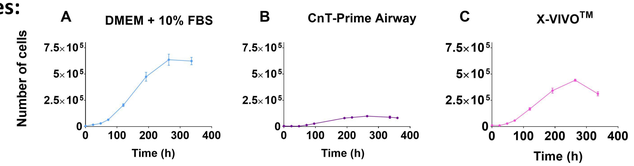
Characterization of Transitioned Cells

Morphology:

A549 cells grown in either DMEM + 10% FBS or XVIVO™ media showed a similar morphology of smaller, cuboidal cells. CnT-Prime Airway cultured cells were a more heterogeneous mixture of large, medium, and smaller, cuboidal cells.



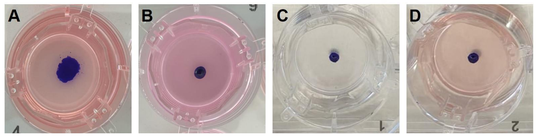
Growth curves:



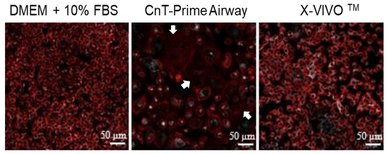
Doubling Times: 21h | 51h | 33h

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 – Cellmask (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-Prime Airway medium were negative for MLBs (see arrows) .

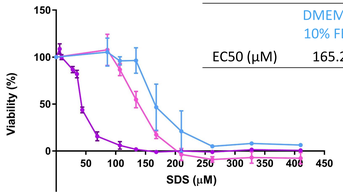
Gene expression:

		DMEM +10% FBS		CnT-Prime Airway		X-VIVO™	
		ALI	Submerged	ALI	Submerged	ALI	Submerged
Alveolar type 1	AQP5	1.00 ± 0.18	1.00 ± 0.12	0.48 ± 0.15*	0.71 ± 0.23*	0.65 ± 0.16	0.72 ± 0.13
	PDPN	1.00 ± 0.09*	1.00 ± 0.18	**150.19 ± 66.25**	**161.46 ± 60.74**	0.96 ± 0.27*	0.69 ± 0.18*
Alveolar type 2	SFTPB	1.00 ± 0.15	1.00 ± 0.25	**52.88 ± 15.10**	**45.46 ± 22.14**	0.93 ± 0.24*	0.76 ± 0.20*
	SFTPD	1.00 ± 0.47	1.00 ± 0.18	**183.24 ± 52.55**	27.60 ± 5.33	1.61 ± 0.71	0.26 ± 0.14
Secretory	MUC5AC	1.00 ± 0.46	1.00 ± 0.49	0.01 ± 0.00*	0.00 ± 0.00*	**28.06 ± 6.17**	1.77 ± 0.96
	MUC5B	1.00 ± 0.48	1.00 ± 0.33	0.23 ± 0.13	0.04 ± 0.02	**18.03 ± 4.95**	**1.84 ± 0.86**
	CFTR	1.00 ± 0.66	1.00 ± 0.36	0.08 ± 0.03*	0.02 ± 0.00*	1.34 ± 0.09	0.24 ± 0.03
Lung progenitor	TTF-1	1.00 ± 0.36	1.00 ± 0.41	0.74 ± 0.33	0.69 ± 0.08	0.79 ± 0.20	0.66 ± 0.23

At ALI and submerged conditions, genes expressed by alveolar type 1 and 2 cells were upregulated in CnT-Prime Airway cultured cells and the expression of secretory cell genes were upregulated in X-VIVO™-cultured cells compared to DMEM +10% FBS.

Performance assessment

Dose response curves & EC50 after 24h Sodium Dodecyl Sulphate exposure in submerged conditions:

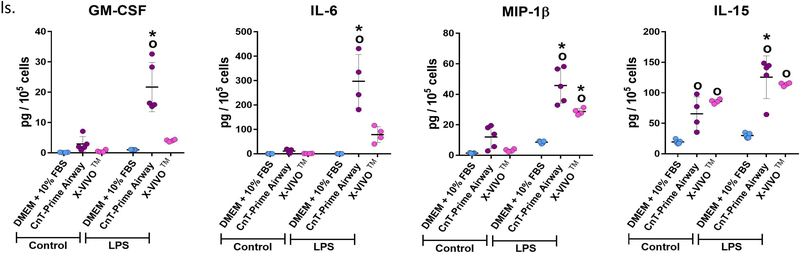


	DMEM + 10% FBS	CnT-Prime Airway	X-VIVO™
EC50 (µM)	165.2	40.9	140.8

EC50 was significantly lower in CnT-Prime Airway compared to the other media. An increased sensitivity to toxicants may indicate the presence of ATI cells.

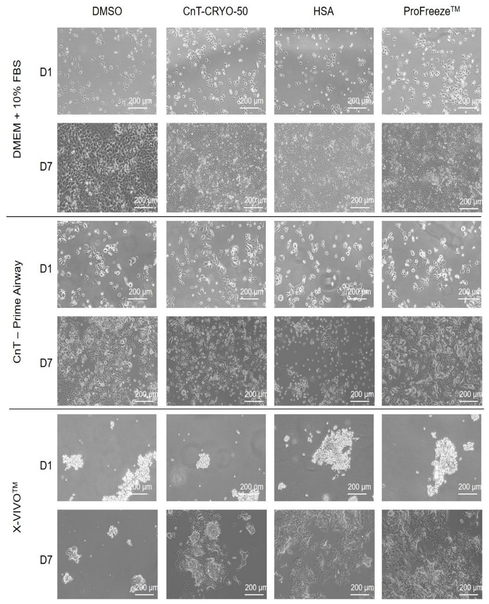
Cytokines release after 24h exposure to Lipopolysaccharide exposure in submerged conditions:

Following exposure to LPS, secretion levels significantly increased for most cytokines for CnT-Prime Airway. Most cytokine secretion levels also increased from the basal level for X-VIVO™. DMEM +10% had the lowest expression levels.



o : difference between FBS-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)

Cryopreservation in serum-free media



A comparison of microscopic images from day 1 and day 7 indicates that cells in all media were proliferating. Seven days after thawing, morphology was similar to that recorded following transition and prior to cryopreservation for all cells cultured within the same media (DMEM +10% FBS, CnT-Prime Airway, and X-VIVO™) across freezing media.

One day after thawing, cells cultured in X-VIVO™ medium were clustered. Nevertheless, they were amenable to cryopreservation without the use of FBS.

Conclusion and next steps

- A549 cells successfully transitioned to X-VIVO™ and CnT-Prime Airway media using a gradual process.
- X-VIVO™ medium retained the phenotype of cells cultured in FBS supplemented medium. Similar to DMEM +10% FBS, X-VIVO™ 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 secretory 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-Prime Airway and the other media. CnT-Prime Airway 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-Prime Airway 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-Prime Airway compared to the other media. An increased sensitivity to toxicants may indicate the presence of ATI cells and increased cytokine release. Results suggest that CnT-Prime Airway cultured cells may lose their adenocarcinomic phenotype in favor of an ATI and ATII epithelial cell phenotype.
- To improve the adhesion of the cells grown in X-VIVO™ and in CnT-Prime Airway, it may be useful to coat cell culture substrate with proteins promoting adherence.
- A549 cells are amenable to FBS free cryopreservation.
- Inter-laboratory performance will be evaluated in the next steps.