

Quality of Life and Management of Living Resources

Key Action 4 – Environment and Health

THz-BRIDGE

*Tera-Hertz radiation in Biological Research, Investigations on Diagnostics
and study on potential Genotoxic Effects*

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Database and report damage conditions for epithelial cultures

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INTRODUCTION

As part of Workpackage 2 and under the section 2.6 which relates to the report on damage conditions for epithelial cells the present database uses three THz sources, one being a portable THz source made available to the group at Nottingham for 10 days to date. Therefore, this database relates to those experiments that include transporting the cells to and from the available THz sources.

The mid-term reviewers suggested that the relevance of the corneal models was questionable and in the light of the minimal effects noted on the human epidermal stratum basale equivalent layer *in vitro*, the evaluation of complex 3D skin models was also considered unproductive. This was since the THz radiation would have to penetrate through the hydrated upper epidermal equivalent layers, or through the dermal equivalents hence attenuating the intensity that reaches the stratum basale equivalent. As there would be even less THz radiation reaching the most sensitive dividing cells it is considered pointless to pursue the research into the complex 3D skin model at present.

Therefore, the innervated epithelial model systems, that we have been developing via other grant funding, is being pursued. There is evidence that the initial formation in the embryo and also recovery from damage of epithelia, particularly the skin and eye, are in part dependent on the presence of peripheral sensory innervation. The damage to epithelial cell membranes will have effects on membrane transportation, intracellular redox potential and also cell to cell communication.

For this reason, the resazurin assay (commercially available as the Alamar Blue assay) reflecting both membrane transportation and intracellular redox potential is employed. This dye is transported into cells is then reduced to resorufin and finally exported from the cells as resorufin. To obtain differentiation of the keratinocytes it is necessary to signal to the cells and drive them to switch on the transglutaminase I enzyme and form crosslinked envelopes. This can be readily monitored using the incorporation of fluorescein cadaverine into the cells permanently as the cornified envelopes are formed.

The culturing of the neural cell line ND7/23 in Clonetics KGM medium does cause them to differentiated produce neurites and stop dividing.

RESULTS

Cells:- Primary human keratinocytes Passage 2

THz:-1-3 Energy exposure 0, 15, 30, 45, 150, 300, 450 mJ/cm²

Assays:- Resazurin and fluorescein cadaverine uptake

Culture Conditions

Grown in Clonectic KGM medium for 3 days after exposure to primary passage 2 keratinocytes that were confluent, i.e. with low cell division rates.

On day 3 the medium was changed to drive them to differentiate. They were assayed at day 6 and 8 post exposure (3 + 5 days in differentiation medium).

Results for cell membrane and redox potential as measured by resazurin to resorufin production.

Days of exposure	Medium Conditions	Exposure dose mJ/cm ²						
		0	15	30	45	150	300	450
0	Growth D	100*	87±18	88±4	96±9	93±12	109±25	104±11
3	Growth	100	79±13	88±13	92±8	76±27	74±29	73±4
6	Differentiation	100	82±22	86±16	90±36	89±19	81±22	100±22
8	Differentiation	100	92±80	70±20	70±20	76±35	90±9	95±19

· The figure represents the average percentage of resorufin production of exposed cells compared with non-exposed controls set at 100% (n=3 mean±SD).

D There were two media used. The growth medium is low in calcium (Clonetic KGM) and the differentiation medium is high in calcium and contains 20mM Fluorescein Cadaverine (Greens + FC).

Results for differentiation as measured by cornified envelop formation.

Days after exposure	Medium Condition	Exposure dose mJ/cm ²						
		0	15	30	45	150	300	450
6	3 days in differentiation medium	100*	76	141	80	68±17	78±18	73±12
8	5 days in differentiation medium	100	140	67	121	76±23	66±18	77±21

The figure represents the mean percentage cadaverine incorporation by exposed cells compared with non-exposed controls set at 100%. (mean±SD, n=3 or mean only when n=2).

Cells:- Primary human keratinocytes Passage 2
THz:-1-3 Energy exposure 0, 54, 108 mJ or 135mJ/cm²

Assays:- Resazurin and flourescein cadaverine uptake

Culture Conditions

Grown in Clonectic KGM medium for 3 days after exposure to primary passage 2 pooled donor keratinocytes that were subconfluent, i.e. in cell division.

On day 3 the medium was changed to drive them to differentiate. They were assayed at day 6 and 9 post exposure (3 + 6 days in differentiation medium).

Results for cell activity and differentiation following THz exposure to dividing human keratinocytes.

NHK cells exposed to THz then returned to culture medium directly, no travel too or from THz source involved.

Days after exposure	Media Conditions	Exposure mJ/cm ²			
		Resazurin 0mJ/cm ² 108mJ/cm ²	54mJ/cm ²	Fluorescein Cadaverin 0mJ/cm ² 54mJ/cm ² 108mJ/cm ²	
Pre-exposure	Growth	100 107±10	108±11		
Post exposure	HBSS	67±19 63±17	89±9		
3 days	Growth	229±21 231±5	269±3		
6 days	Differentiation	248±57 232±5	289±60		
8days	Differentiation			100 85±46	84±19
9 days	Differentiation	269±34 242±155	201±61	115±96 82±62	69
Values are expressed as percentage of the non-exposed pre-treated control.					

NHK cells exposed to THz then transported to Nottingham for assay.

Days after exposure	Media Conditions	Exposure to 135 mJ/cm ²	
		Resazurin	Fluorescein Cadaverin
Pre-exposure	Growth	100	
2 hours post exposure	HBSS	117/38	
3 days	Differentiation	106±15	129±22
6 days	Differentiation	109±22	99±35
8 days	Differentiation	91±40	99±13
Values are expressed as percentage of the non-exposed controls for the relevant day.			
NB. Here is no data for the NHK cells since these died after the transportation. It is thought that the transportation conditions were to blame since the controls (non-exposed) died also.			

Cells:- ND7/23 neural cell lines.,

THz – 1-3 Energy exposure 0, 54 or 108mJ or 135mJ/cm².

Assays:- Resazurin and flourescein cadaverine uptake

Culture Conditions

Grown in DMEM medium for 3 days after exposure and were subconfluent, i.e. in cell division.

On day 3 the medium was changed to drive them to differentiate. They were assayed at day 6 and 9 post exposure (3 + 6 days in differentiation medium).

Results for cell activity and differentiation following THz exposure to ND/7/23 cells.

ND7/23 cells exposed to THz then returned to culture medium directly, no travel too or from THz source involved.

Days after exposure	Media Conditions	Exposure mJ/cm ²			
		Resazurin 0mJ/cm ² 108mJ/cm ²	54mJ/cm ²	Fluorescein Cadaverin 0mJ/cm ² 54mJ/cm ² 108mJ/cm ²	
Pre-exposure	DMEM	100 105±7	115±4		
Post exposure	HBSS	125±43 109±42	142±37		
3 days	DMEM	151±35 142±25	174±21		
6 days	Differentiation	267±81 194±1	283±31		
8days	Differentiation			100 96±34	102±48
9 days	Differentiation	282±78 256±31	273±17	49±18 50±13	51±10
Values are expressed as percentage of the non-exposed pre-exposed control.					

ND7/23 cells exposed to THz then transported to Nottingham for assay.

Days after exposure	Media Conditions	Exposure to 135 mJ/cm ²	
		Resazurin	Fluorescein Cadaverin
Pre-exposure	DMEM	100	
2 hours post exposure	HBSS	117/38	
3 days	Differentiation	106±15	129±22
6 days	Differentiation	109±22	99±35
8 days	Differentiation	91±40	99±13
Values are expressed as percentage of the non-exposed controls for the relevant day.			
NB. Here is no data for the NHK cells since these died after the transportation. It is thought that the transportation conditions were to blame since the controls (non-exposed) died also.			

Cells:- Human primary keratinocytes co-cultured with ND7/23 neural cell.,
THz – 1-3 Energy exposure 0, 22.5 mJ/cm².

Assays:- Resazurin and flourescein cadaverine uptake

Culture Conditions

The ND7/23 cells were seeded into three wells of a 4 well cluster on a 96 well plate in DMEM. After attachment NHK cells were also added to three wells, two with ND7/23 cells and the empty one. Two days later keratinocytes were seeded into the insert and the medium was changed to KGM. Four days later the inserts were exposed to THz either ND7/23 or NHK cell side. They were grown in Clonectic KGM medium for 1 day after exposure. They were then changed to drive them to differentiate. They were assayed at 2 days later.

Results.

Effects of THz on ND7/23 cells, Normal human keratinocytes, and co cultures.

Day after Exposure	Medium conditions	Cell type	Exposure dose 22.5 mJ/cm ²	
			Resazurin uptake	Cadaverine
3 days	In differentiation medium	Co cultures	99	73
		NHK alone	96	117
		ND7/23 alone	124	315
9 days	In differentiation medium	Co-cultures	100	72
		NHK alone	139	80
		ND7/23 alone	110	99
12days	In differentiation medium	Co-cultures	100	80
		NHK alone	113	80
		ND7/23 alone	174	112

Values are expressed as percentage of the non exposed controls upon the same day post exposure and in differentiation medium. N=1 (to date).

Culture Conditions

The ND7/23 cells were seeded onto the underside of a 0.4µM polycarbonate insert in DMEM. Two days later keratinocytes were seeded into the insert and the medium was changed to KGM. Four days later the inserts were exposed to THz either ND7/23 or NHK cell side. They were grown in Clonectic KGM medium for 1 day after exposure. The barrier function was checked with a fluorescein leakage assay. They were then changed to drive them to differentiate. They were assayed at 2 days later. *Sodium fluorescein passage across a culture is dependent on the barrier capacity of the cells. Thus if the barrier is damaged leakage will occur. The normal maximal leakage for an undamage less is <6% of the total amount added.*

Effects of THz on ND7/23 cells, Normal human keratinocytes, and co cultures on inserts.

Day after Exposure	Medium conditions	Cell type	Exposure dose 22.5 mJ/cm ²		
			Resazurin Fluorescein leakage		Cadaverine uptake
1 day	Growth	Exposed ND7/23 side	96		0.45
		Exposed NHK side	104		0.49
		NHK alone	113		0.55
		ND7/23 alone	87		no barrier
1 day Not exposed	Growth	Exposed ND7/23 side	103		0.88
		Exposed NHK side	112		0.46
		NHK alone	125		0.58
		ND7/23 alone	75		no barrier
3days	In differentiation medium	Exposed ND7/23 side	98	51	
		Exposed NHK side	93	32	
		NHK alone	97	58	
		ND7/23 alone	103	142	
3days	In differentiation medium	Exposed ND7/23 side	97	152	
		Exposed NHK side	116	88	
		NHK alone	73	105	
		ND7/23 alone	127	95	

Values are expressed as percentage of the mean of the NHK plus the ND7/23 cells alone values for the resazurin and the FC. The fluorescein leakage is expressed as a percentage of the maximal amount added. N=1 (to date).