95 GHz Radiofrequency Energy Elicits Unique Functional and Molecular Changes in Immune and Vascular Endothelial Cells

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- There are no conflicts of interest to declare.
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NMR&D

- Naval Medical Research Unit (NAMRU) San Antonio plays a vital part within Navy Medicine Research & Development (NMR&D), supporting medical readiness to ensure warfighter lethality.

- In addition to Navy platforms, NMR&D is heavily engaged in research addressing health and readiness threats in a range of operational environments, including the undersea domain, remote areas, and environments where extreme cold, heat, and altitude are factors.

- Understanding and addressing the evolving health concerns in these environments is critical to service member readiness, fitness, and lethality.

- NMR&D, with its insight into challenges faced by Sailors and Marines, is uniquely qualified to lead U.S. Navy research efforts to ensure healthy, fit, and lethal service members.

DIRECTED ENERGY INJURIES

- Directed-energy related injuries remain a high concern for the warfighter in both occupational and combat scenarios.
- DE injuries often are treated as contact burn wounds; characterization of epidermal and dermal injuries may lead to optimized mitigation strategies in combat casualty care.
- We previously identified unique molecular alterations of dermal fibroblasts in response to 95 GHz radiofrequency (RF) energy.

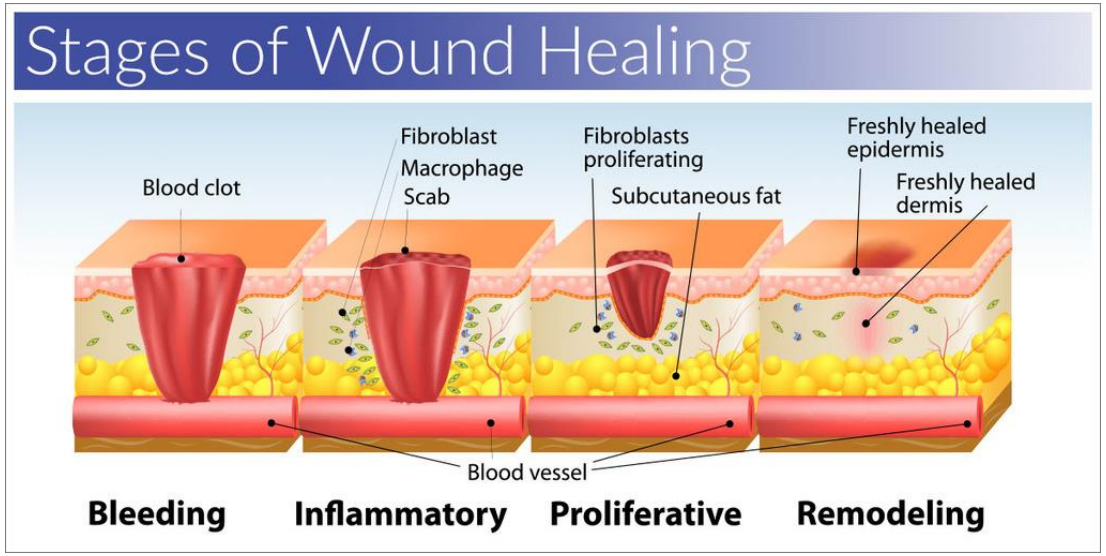


Image credits: quizlet.com, wound healing stages

95 GHz EXPOSURE TO SKIN

- Frequency: 95 GHz
- Power Density: 590 $\mathrm{mW/cm^2}$
- Penetration Depth: ~0.3 mm

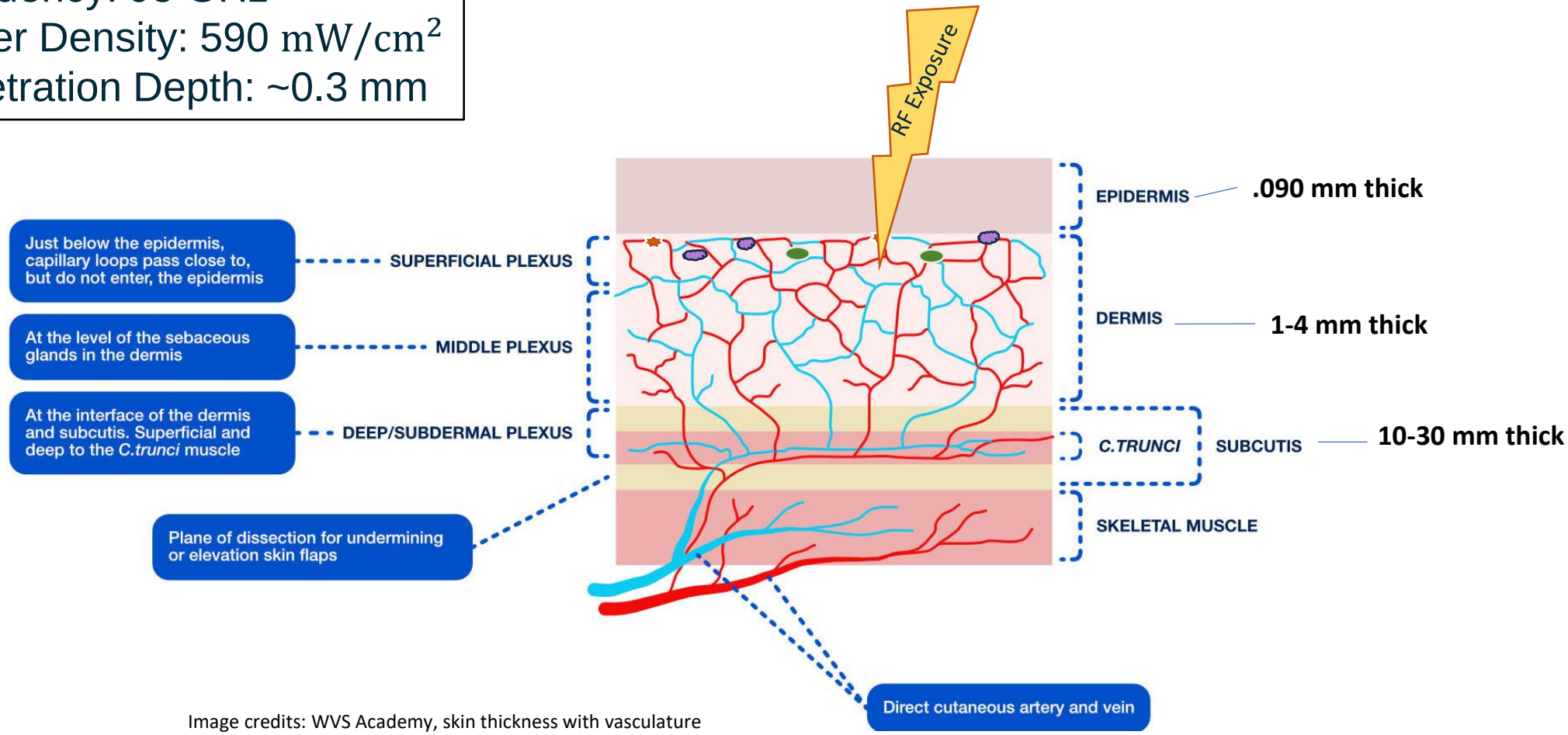


Image credits: WVS Academy, skin thickness with vasculature

OBJECTIVE

Characterize the acute molecular and functional effects of 95 GHz RF energy on vascular endothelial and immune cells that are present within the dermis

HYPOTHESES

1. RF exposure will induce a pro-inflammatory state in monocytic cells, and the activation of vascular endothelial cells.
2. RF exposed monocytic cells will induce greater vascular endothelial barrier permeability compared to non-exposed monocytic cells.

CELL LINES

Primary Human Umbilical Vein Endothelial Cells (HUVEC)

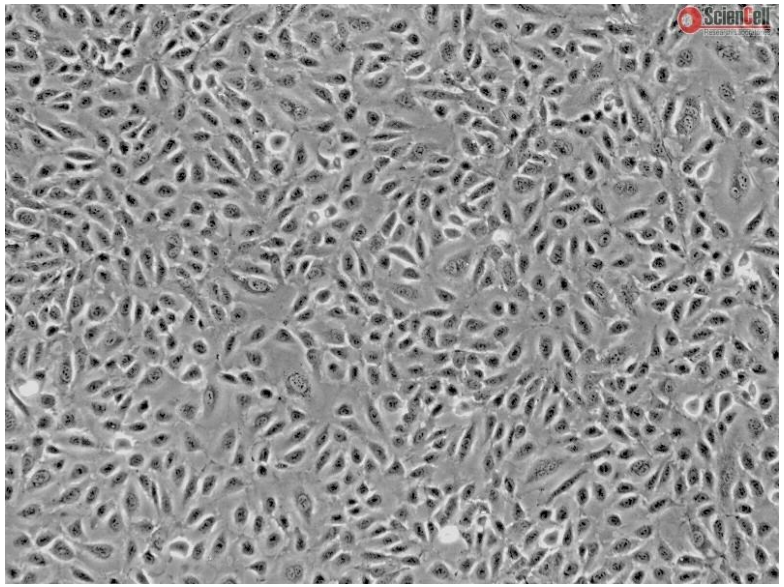
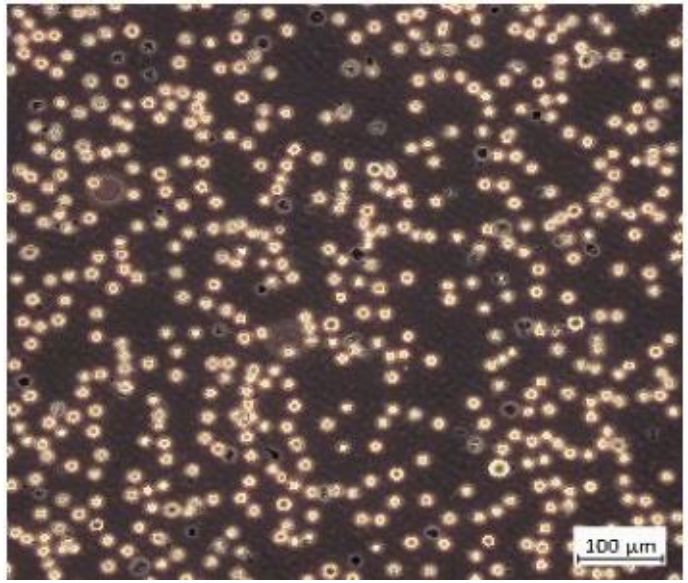


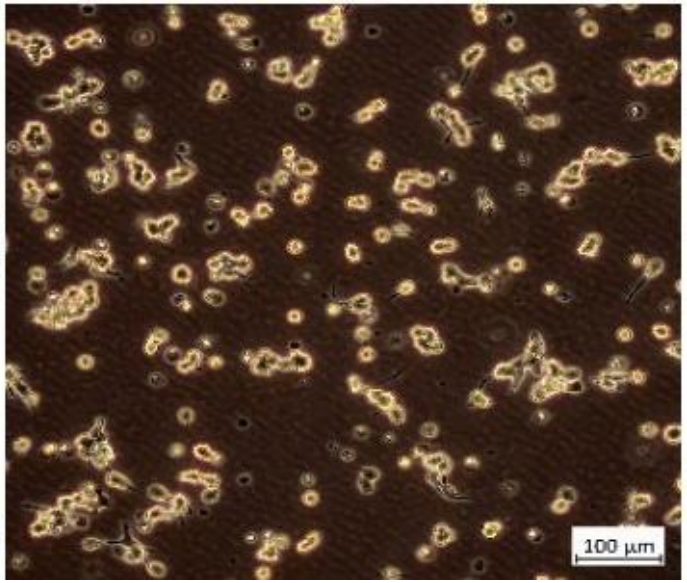
Image credits: ScienCell Research Laboratories

Immortalized Human Monocytic Cells (THP-1)

Differentiation of THP-1 with phorbol 12-myristate 13-acetate (PMA)



Undifferentiated



Differentiated

Image credits: ATCC

95 GHz RF EMITTER

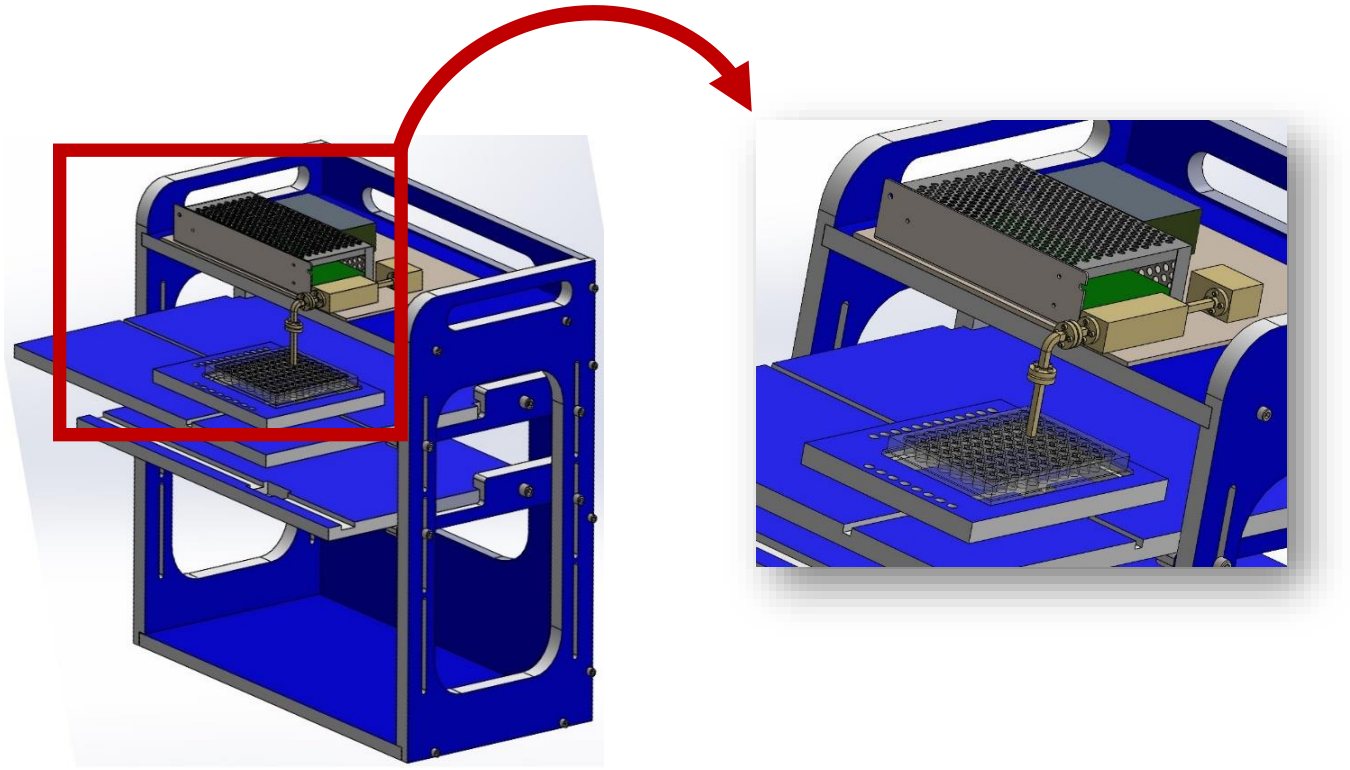
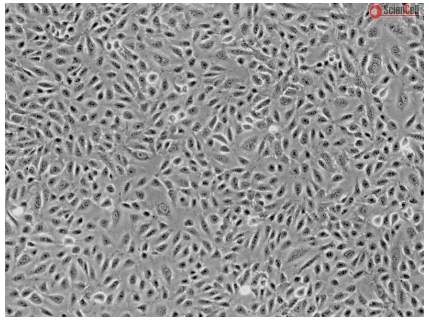
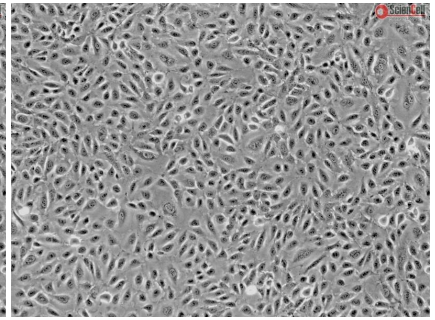
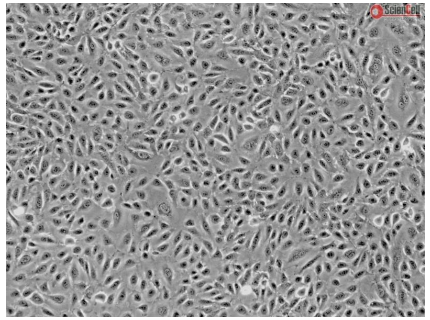
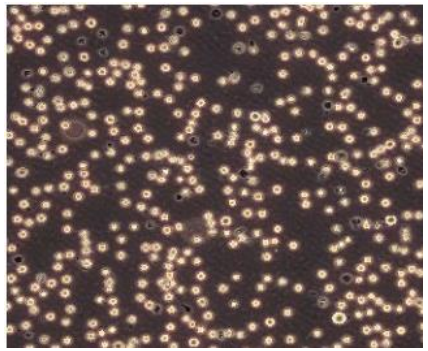
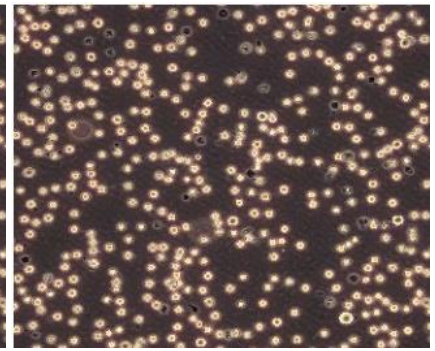
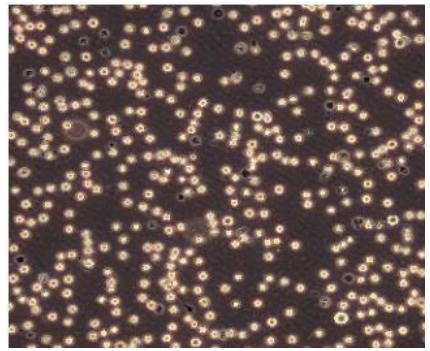


Image credits: Mr. Justin Bequette

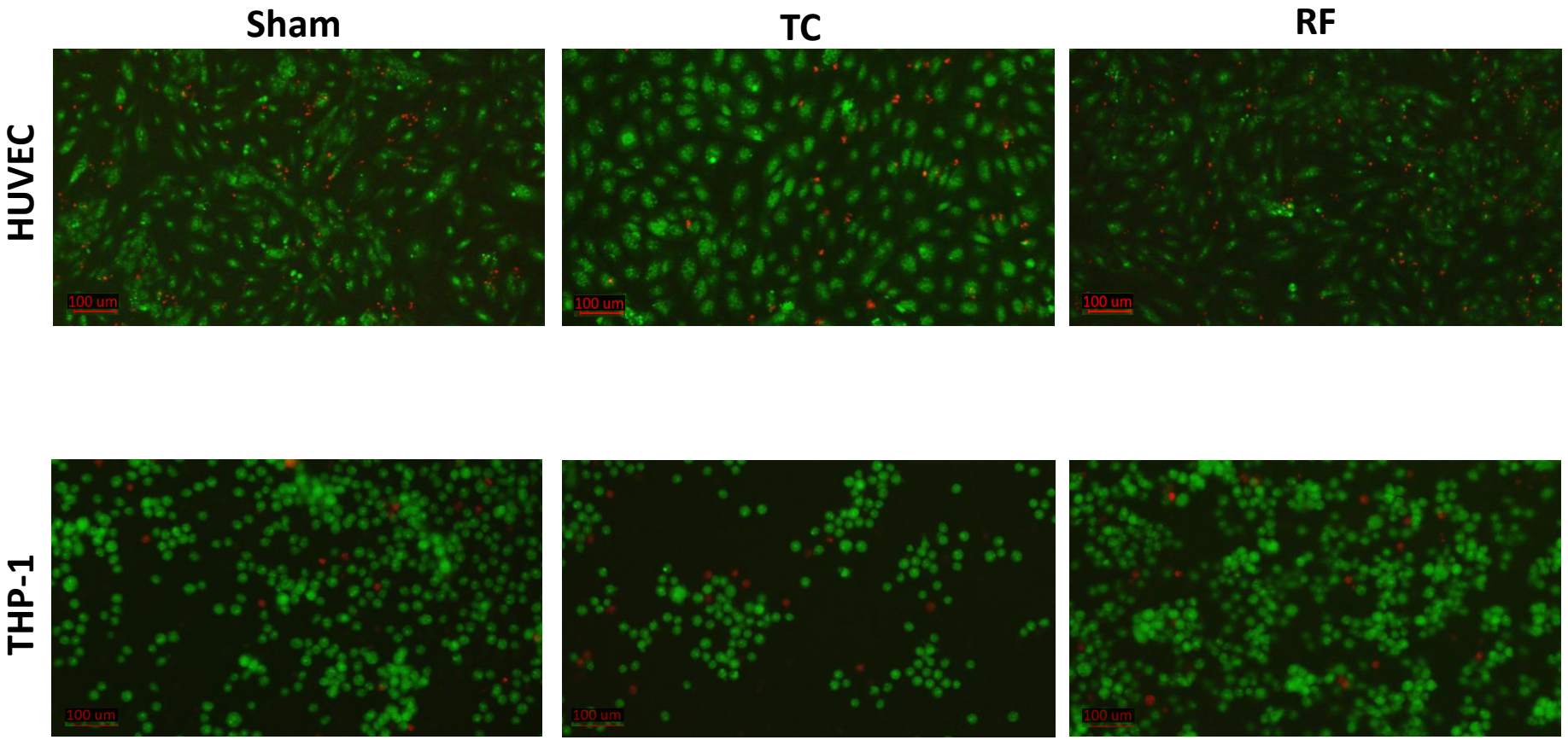
HYPOTHESIS 1
EXPERIMENTAL DESIGN

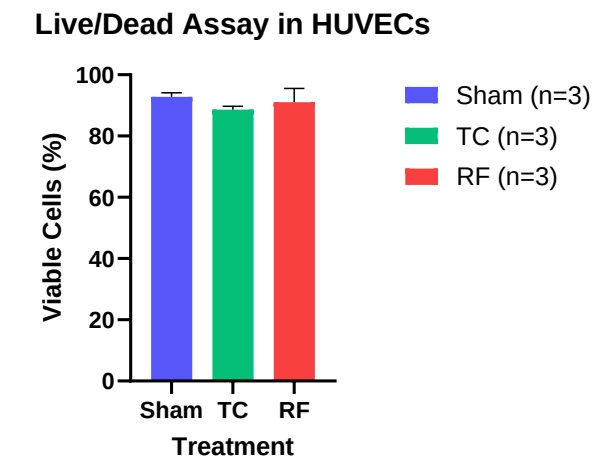
	Sham Control (Underwent all procedures except RF or temp exposure)	**Temperature Control (TC)** (Exposure to RF-equivalent temperature for 6 min)	**RF Exposure (RF)** (Exposure to 95 GHz, 590 mW/cm^2 for 6 min)
HUVEC			
THP-1			

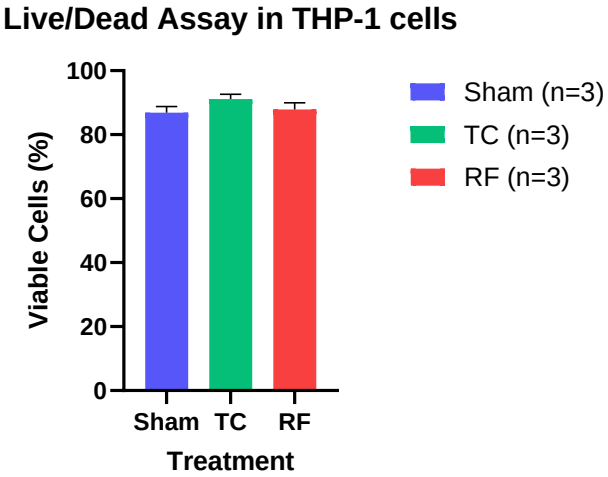
Outcomes (assessment within 1 hr post-exposure)

	Cell Viability	Gene Expression
HUVEC	• Acridine orange (AO) and propidium iodide (PI), nuclear staining	• RT^2 Profiler™ PCR Array Human **Endothelial Cell Biology** (84 different genes involved in endothelial cell activation)
THP-1	• Acridine orange (AO) and propidium iodide (PI), nuclear staining	• RT^2 Profiler™ PCR Array Human **Cytokines & Chemokines** (84 different genes involved in monocyte immune response)

RESULTS: CELL VIABILITY



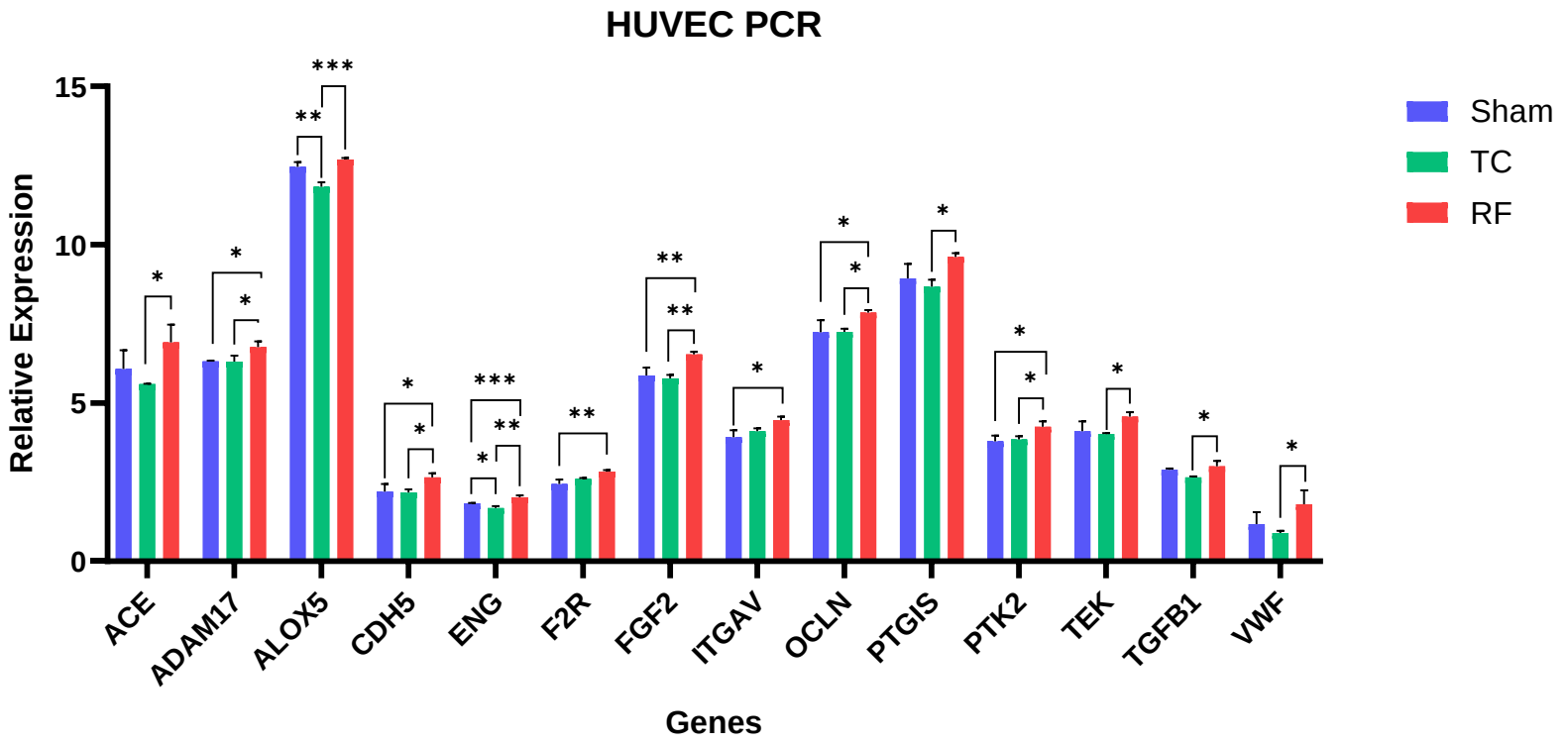




One-way ANOVA w/ Tukey post hoc; n=3 for each group

RESULTS:

HUVEC GENE EXPRESSION



One-way ANOVA w/ Tukey post hoc
n=3 for each group; *p<0.05, **p<0.01, ***p<0.001

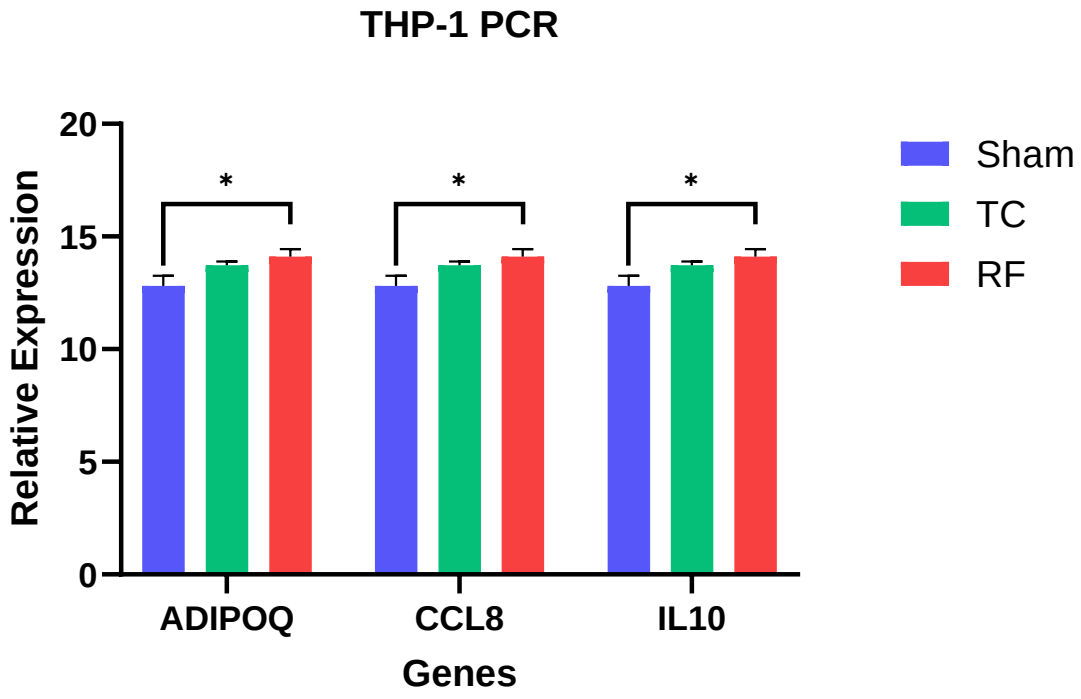
Gene List

ANGPT1	MMP2	ICAM1	PECAM1
CCL2	MMP9	PTGIS	PLG
CCL5	NOS3	SOD1	SELL
CX3CL1	NPPB	SELE	SELPLG
EDN1	NPR1	TGFB1	VWF
EDNRA	PF4	TNF	MMP1
ENG	PGF	VCAM1	PLAT
F3	PLAU	ANXA5	PROCR
FASLG	PTGS2	BAX	TFPI
FGF1	SERPINE1	BCL2	THBD
FGF2	SPHK1	BCL2L1	TIMP1
FLT1	TEK	CASP1	IL11
FN1	THBS1	CASP3	ACTB
HIF1A	TYMP	CFLAR	B2M
HMOX1	VEGFA	FAS	GAPDH
IL1B	ACE	IL3	HPRT1
IL6	AGT	IL7	RPLP0
ITGA5	AGTR1	OCLN	
ITGAV	ALOX5	PTK2	
ITGB1	APOE	TNFSF10	
ITGB3	CALCA	ADAM17	
KDR	CAV1	CDH5	
KIT	EDN2	COL18A1	
KLK3	F2R	PDGFRA	

Differentially expressed genes
Housekeeping genes

RESULTS:

THP-1 GENE EXPRESSION



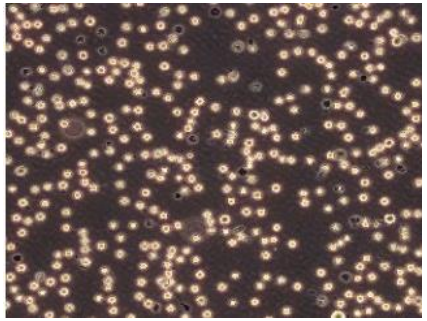
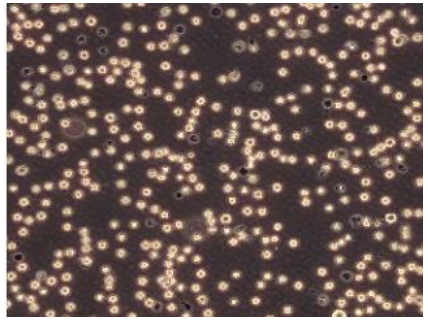
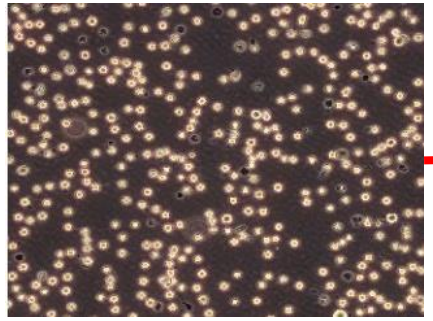
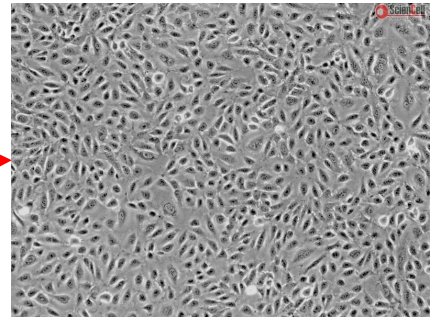
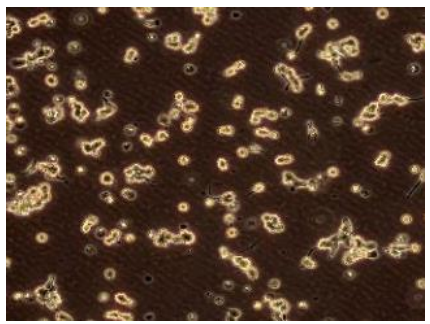
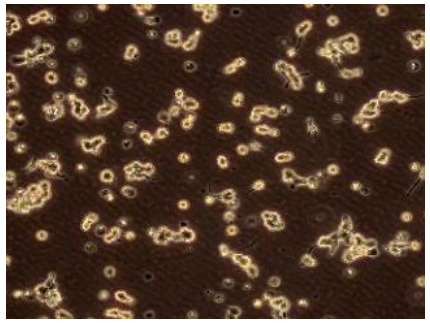
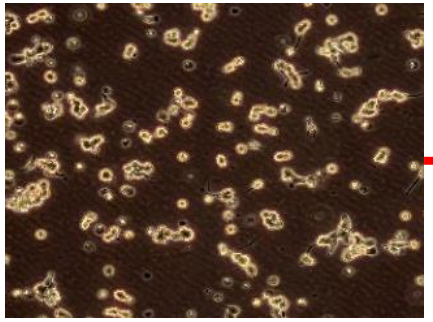
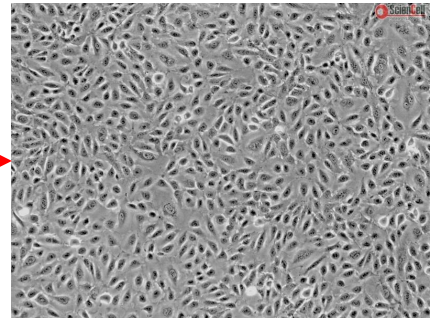
One-way ANOVA w/ Tukey post hoc
n=3 for each group; *p<0.05

Gene List

CCL1	CXCL9	IL5	LTA
CCL11	PPBP	IL6	LTB
CCL13	XCL1	IL7	TNF
CCL17	IL10	CXCL8	TNFRSF11B
CCL18	IL11	IL9	TNFSF10
CCL19	IL12A	IFNA2	TNFSF11
CCL2	IL12B	IFNG	TNFSF13B
CCL20	IL13	BMP2	ADIPOQ
CCL21	IL15	BMP4	C5
CCL22	IL16	BMP6	MIF
CCL24	IL17A	BMP7	SPP1
CCL3	IL17F	CNTF	TGFB2
CCL5	IL18	CSF1	ACTB
CCL7	IL1A	CSF2	B2M
CCL8	IL1B	CSF3	GAPDH
CX3CL1	IL1RN	GPI	HPRT1
CXCL1	IL2	LIF	RPLP0
CXCL10	IL21	MSTN	
CXCL11	IL22	NODAL	
CXCL12	IL23A	OSM	
CXCL13	IL24	THPO	
CXCL16	IL27	VEGFA	
CXCL2	IL3	CD40LG	
CXCL5	IL4	FASLG	

Differentially expressed genes
Housekeeping genes

HYPOTHESIS 2
EXPERIMENTAL DESIGN

Sham Control (Underwent all procedures except RF or temp exposure)	Temperature Control (Exposure to RF-equivalent temperature for 6 min)	RF Exposure (Exposure to 95 GHz, 590 mW/cm^2 for 6 min)	HUVEC (Mature Monolayer)
THP-1				
THP-1 + PMA				

Outcomes

- Endothelial Barrier Function
- Immunocytochemistry (ICC)

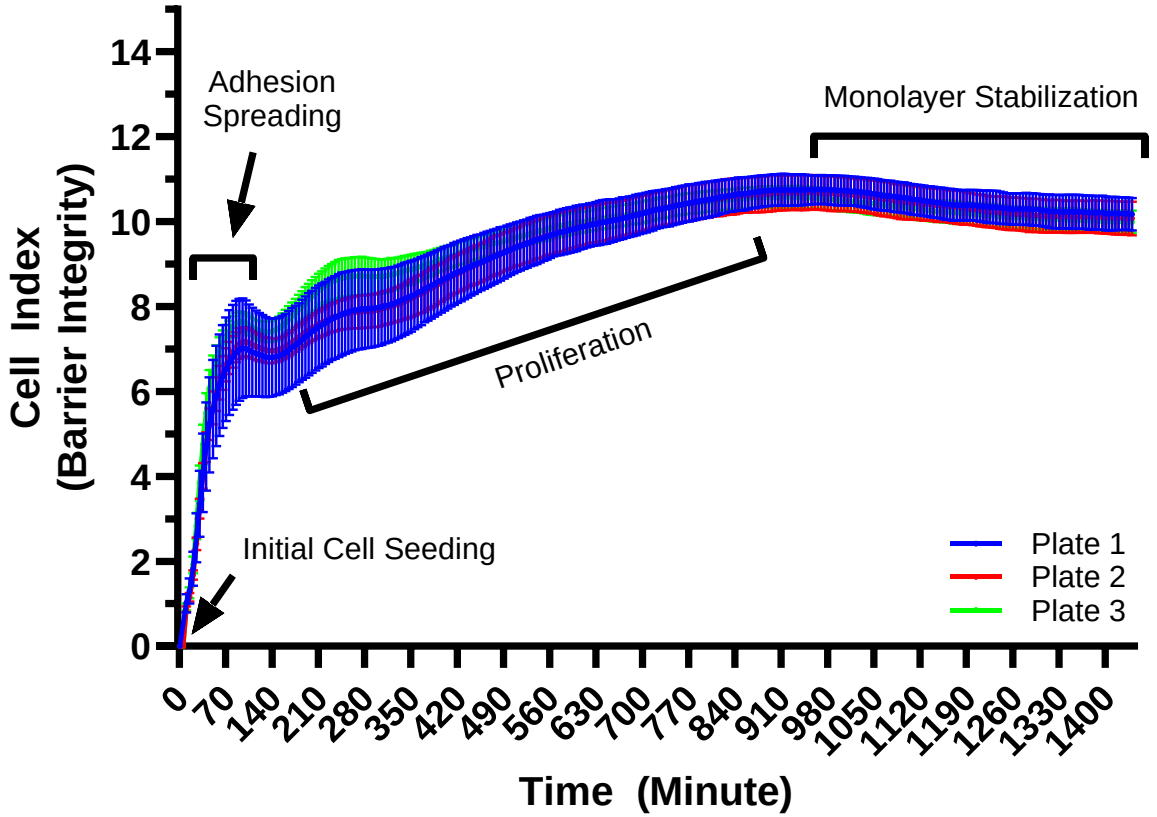
- Endothelial Barrier Function
- Immunocytochemistry (ICC)

ENDOTHELIAL BARRIER FUNCTION

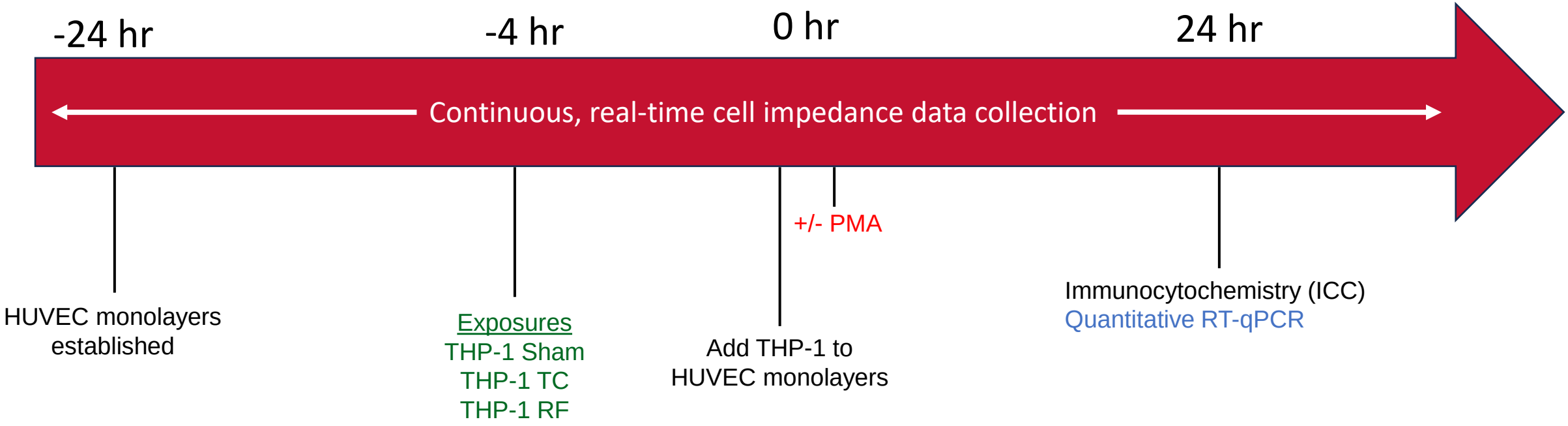
Cell Impedance System

Photo Credit: Mr. Burrell Parmer, NAMRU-SA

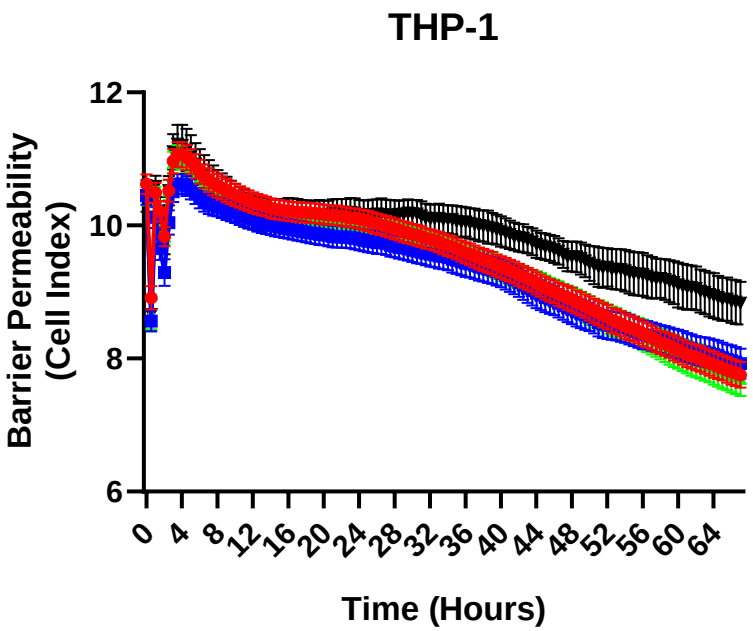
Cell Index = (impedance at time point n – impedance in the absence of cells)/nominal impedance value

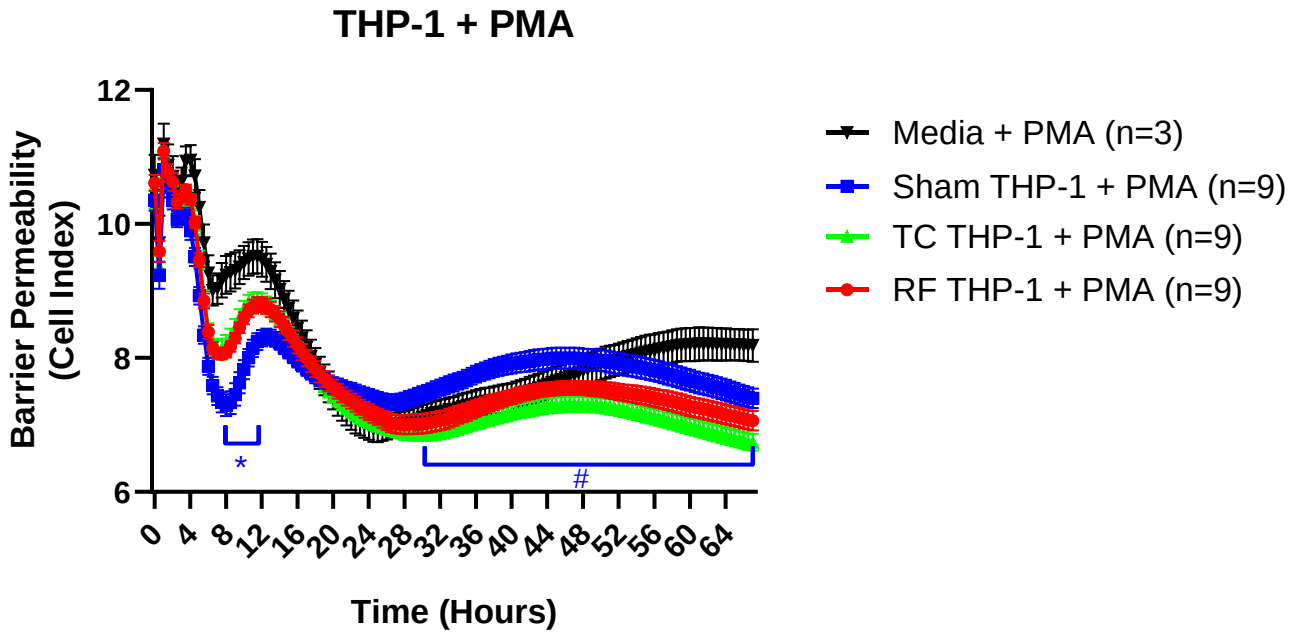


EXPERIMENTAL TIMELINE



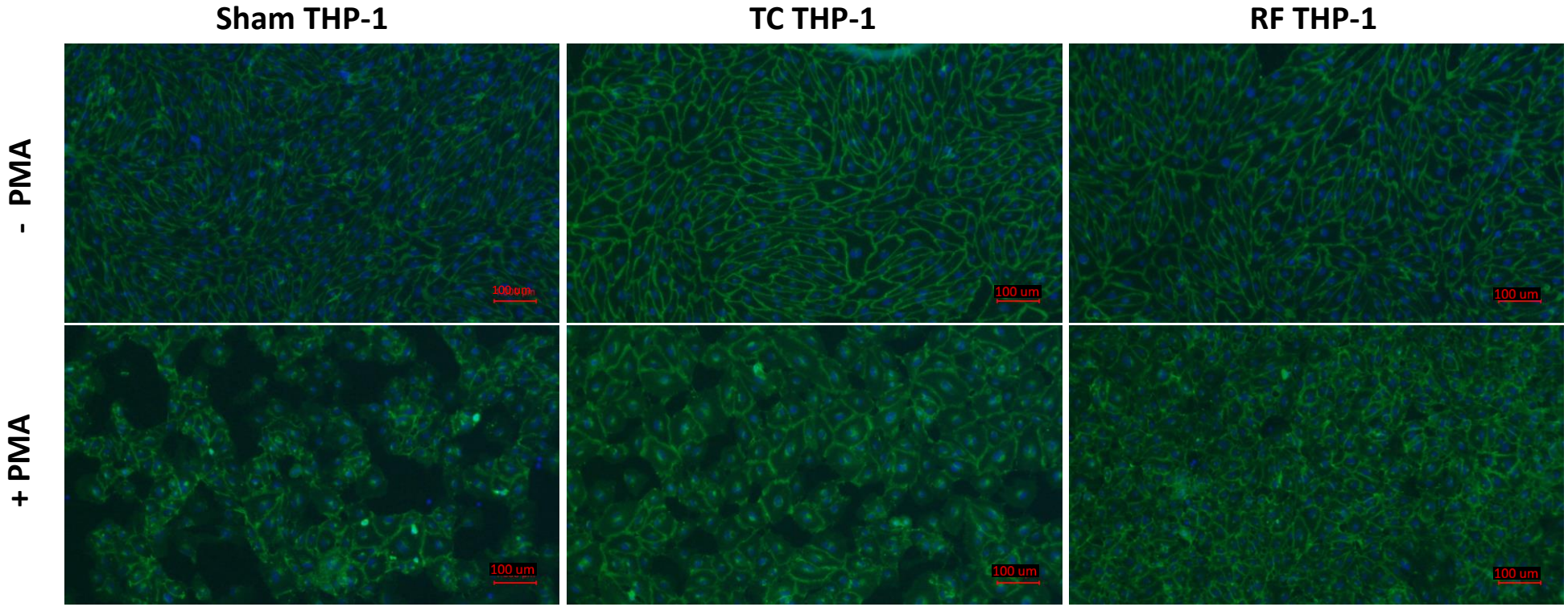
ENDOTHELIAL BARRIER FUNCTION





Two-way ANOVA w/ Tukey post hoc
*p<0.05, Sham vs TC and RF
#p<0.05, Sham vs TC

IMMUNOCYTOCHEMISTRY



Representative images of HUVEC cells; VE-cadherin (green), and nuclei (blue)

SUMMARY OF RESULTS

- **Gene Expression:**
  - **Endothelial Cells**:12 markers of endothelial activation were changed after RF exposure compared to heat block exposure
  - **Monocytic Cells:** Only RF-exposed THP-1 cells had elevated expression of ADIPOQ, CCL8, and IL10 compared to Sham Control

- **Monocytic Cell Function:**
  - **Undifferentiated THP-1**: RF or TC exposure did not affect the ability of THP-1 to disrupt the endothelial barrier
  - **Differentiated THP-1**: RF or TC exposure significantly altered the ability of THP-1 macrophages to disrupt endothelial barrier

CONCLUSION

- A novel model for in vitro analysis of superficially-penetrating RF energy was developed and used to analyze injuries to dermal fibroblasts, immune cells and endothelium, allowing for future studies of the more complex wound healing environment, both in vitro and in vivo.

- Furthermore, proof of concept was established for cellular differences in gene expression and function that suggest RF to be a unique insult to skin.

This study provides foundational data for improved treatment strategies for directed-energy related injuries

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Questions?

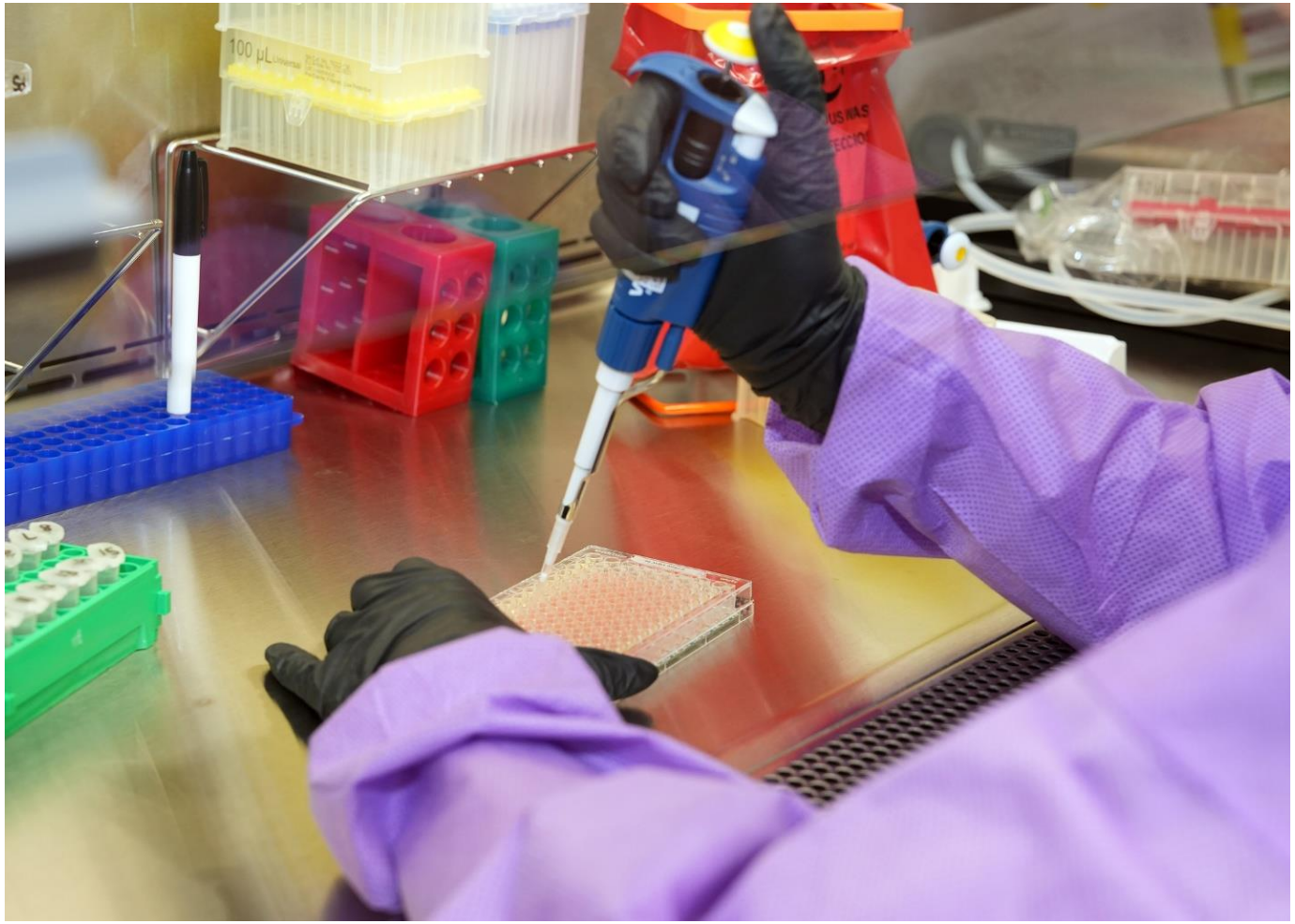

Image credits: Mr. Burrell Parmer