Microwaves: a potential new therapy for HPV infected ano-genital lesions?

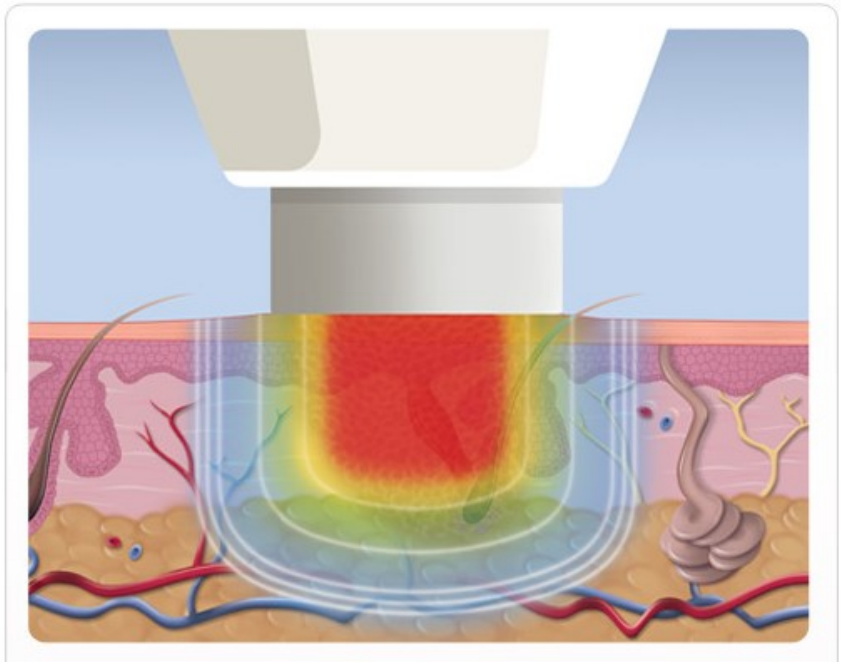
Anna Kirk
PhD Student, Graham Group

Currently there are no good clinical approaches to treating HPV-associated ano-genital lesions

- No antiviral for HPV
- Vaccine exists but…
  - Generational
- Low uptake
  - Religious reservations
  - Cost associated
  - Anti-vax propaganda
- Current therapies for ano-genital cancers/ their precursors: cryotherapy/ ablation
  - Associated pain and high recurrence rate
  - Risk of premature birth

Swift device – candidate for treatment of HPV-positive ano-genital lesions?

- Swift device delivers microwaves through a probe applicator tip
- Microwaves:
  - Linear radiation (precise targeting)
  - Heating effect: coagulation necrosis



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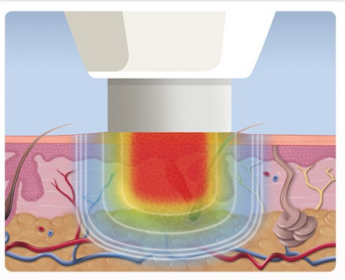
emblation | swift

Swift device – candidate for treatment of HPV-positive ano-genital lesions?



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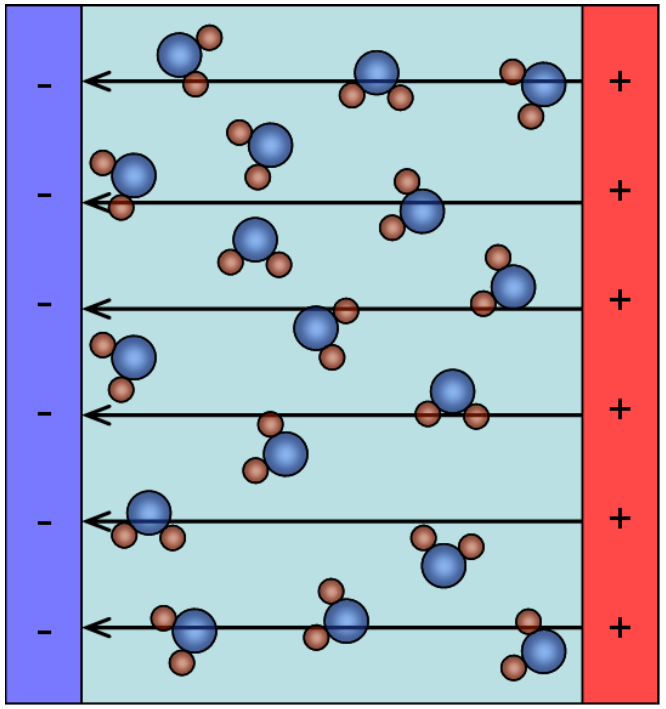
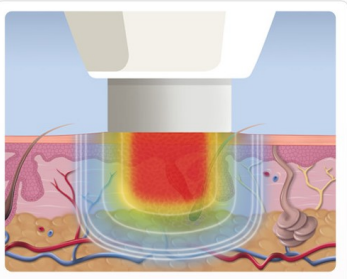


Swift device – candidate for treatment of HPV-positive ano-genital lesions?



- Used successfully for:
  - HPV-positive verrucas – 75.9% clearance (*Bristow et al., 2017*)
  - Actinic keratoses – 92% clearance (*Jackson et al., 2020*)
  - High efficiency and upregulation in immune response

OVER 100,000 SWIFT TREATMENTS CARRIED OUT ACROSS THE UK AND IRELAND

06 June 2022

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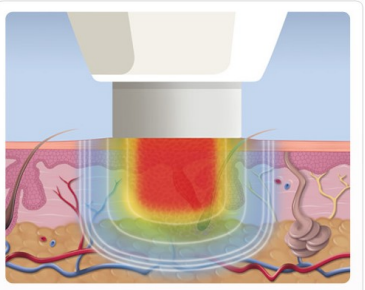
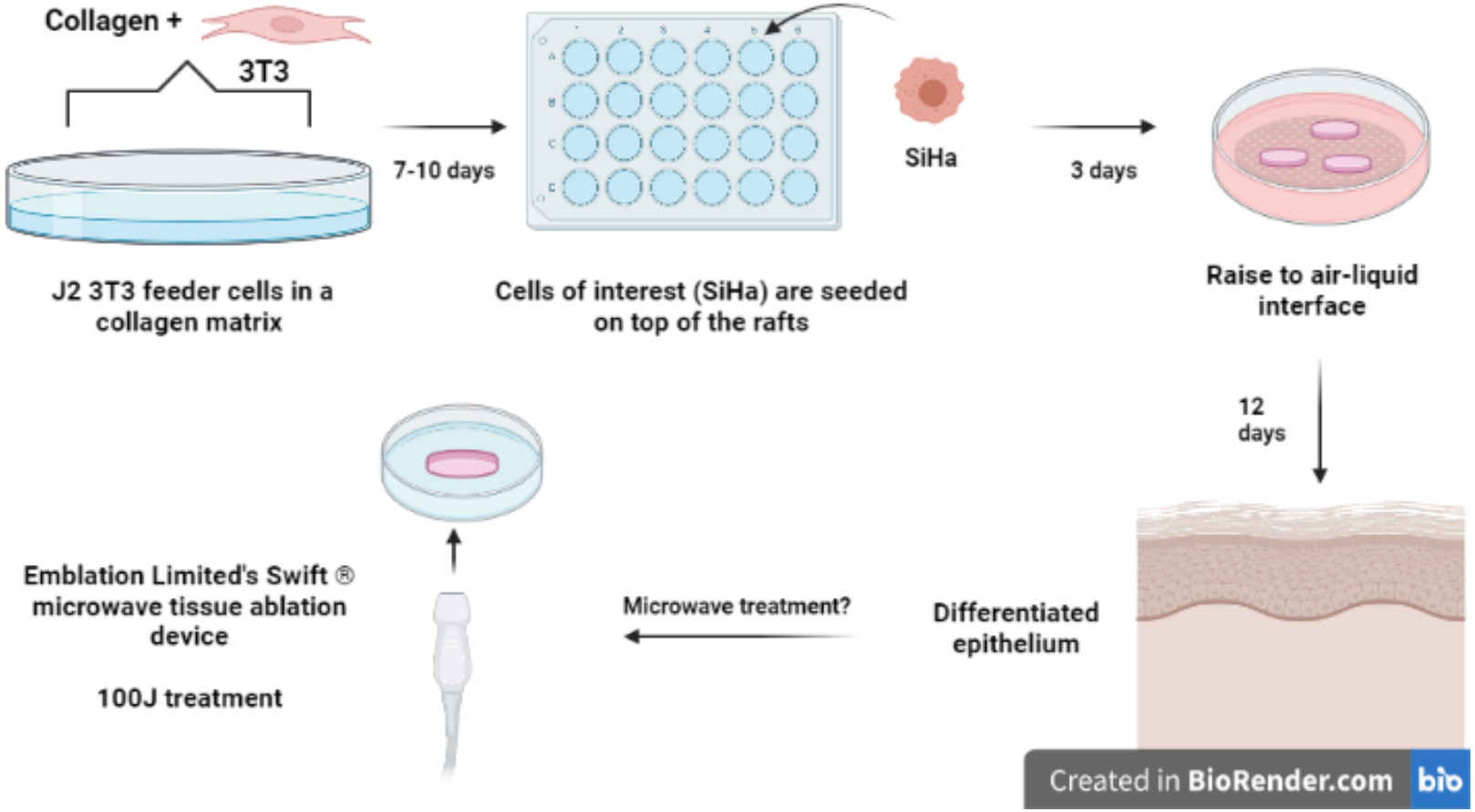
emblation swift

Culturing 3D organotypic cultures to model the cervical epithelium *in vitro*



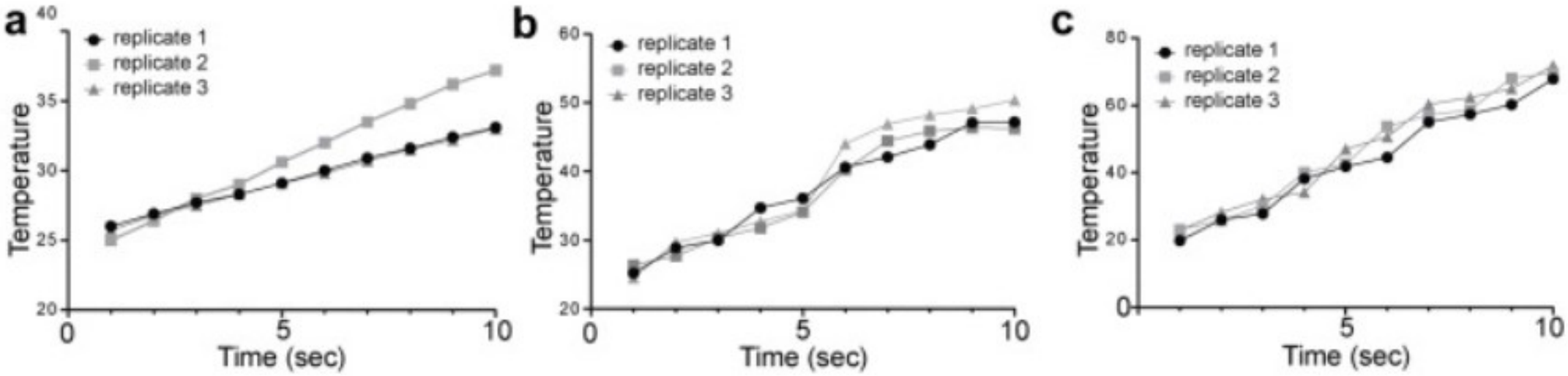


Temperature increase following microwave treatment

5W, 10 seconds

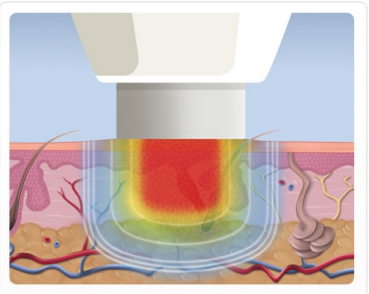
10W, 10 seconds

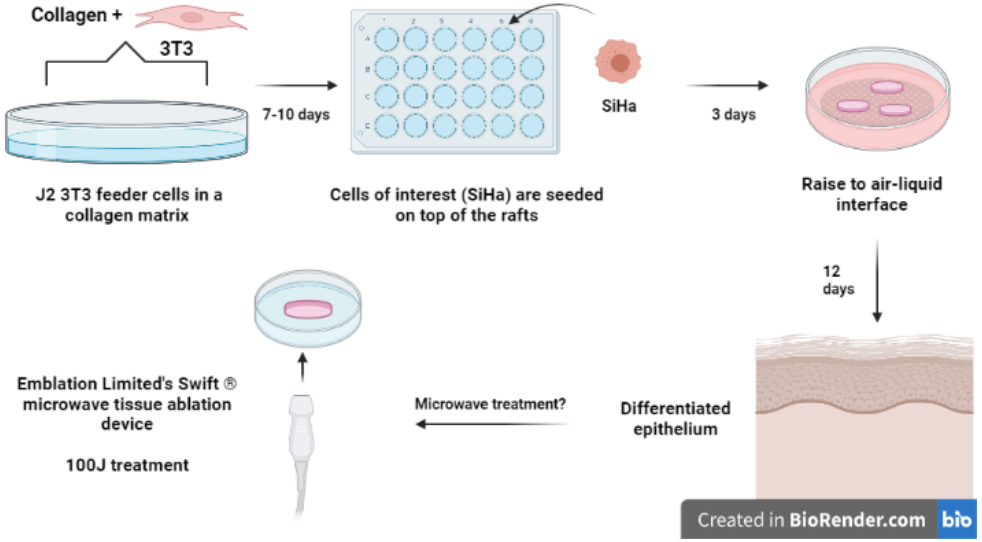
15W, 10 seconds



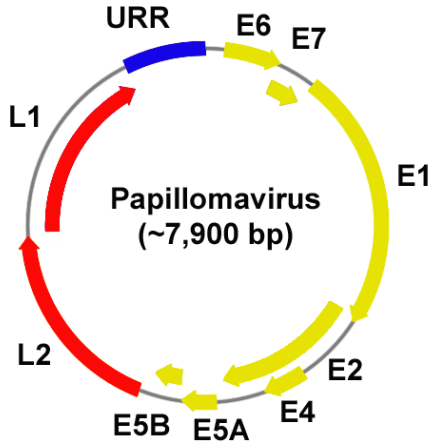
Treatment condition of 10W, 10 seconds chosen for proceeding work

Culturing 3D organotypic cultures to model the cervical epithelium *in vitro*





- Model of cervical cancer
- 2 integrated HPV16 copies
- Cannot support productive viral cycle



Integration disrupts expression of the late viral proteins

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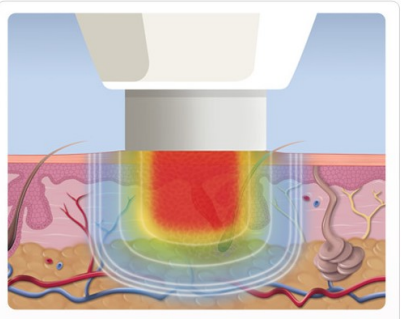
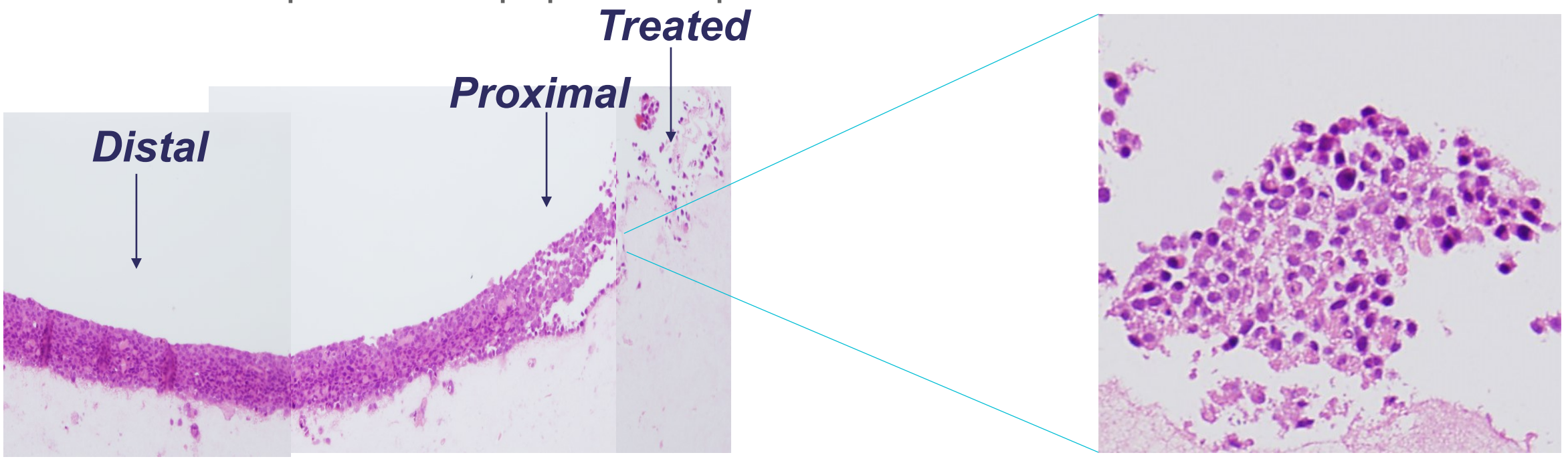
Swift device – candidate for treatment of HPV-positive ano-genital lesions?



Microwave treatment of 3D organotypic rafts causes:

- Cell death in the treated area
- Tissue disruption and apoptosis in proximal area



Pyknosis, a marker of apoptosis evident in the proximal area

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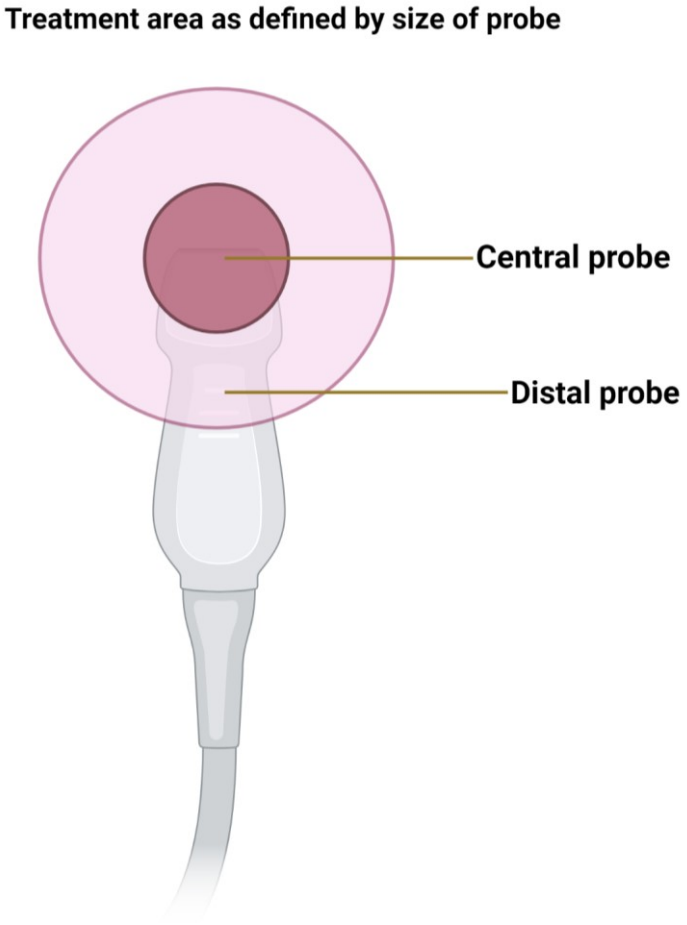
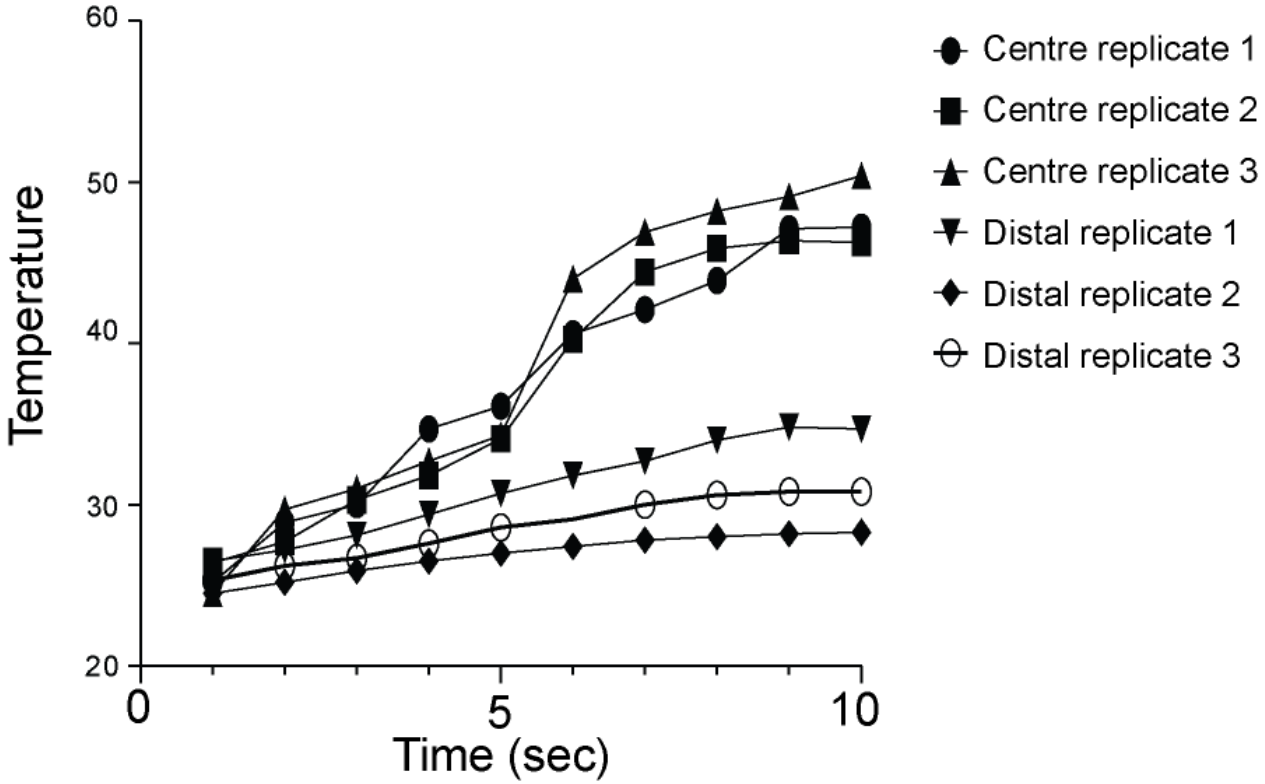
Temperature increase following microwave treatment



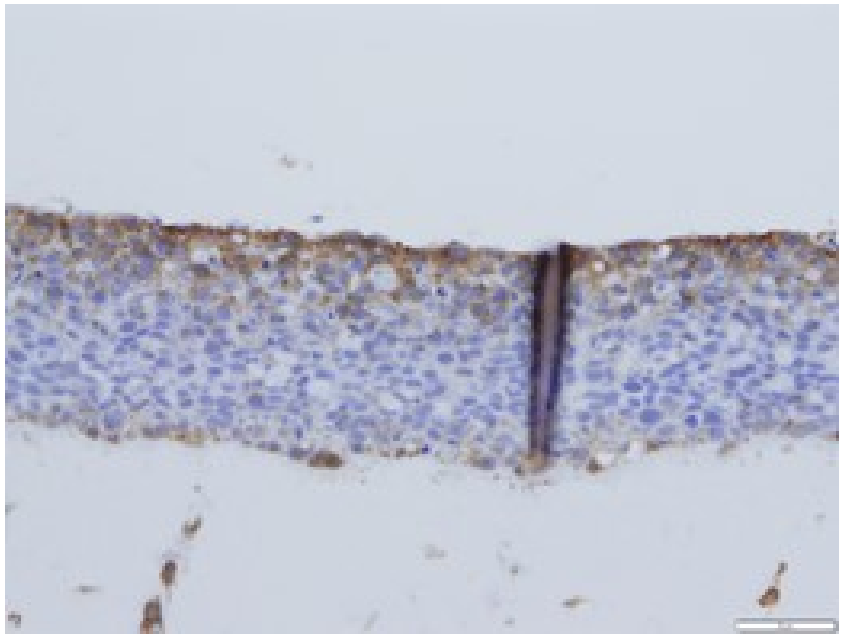


Large temperature increases in the raft following treatment are limited to areas below the probe contact site

Microwave treatment upregulates a stress response

Heat shock response

Untreated, 16 hours



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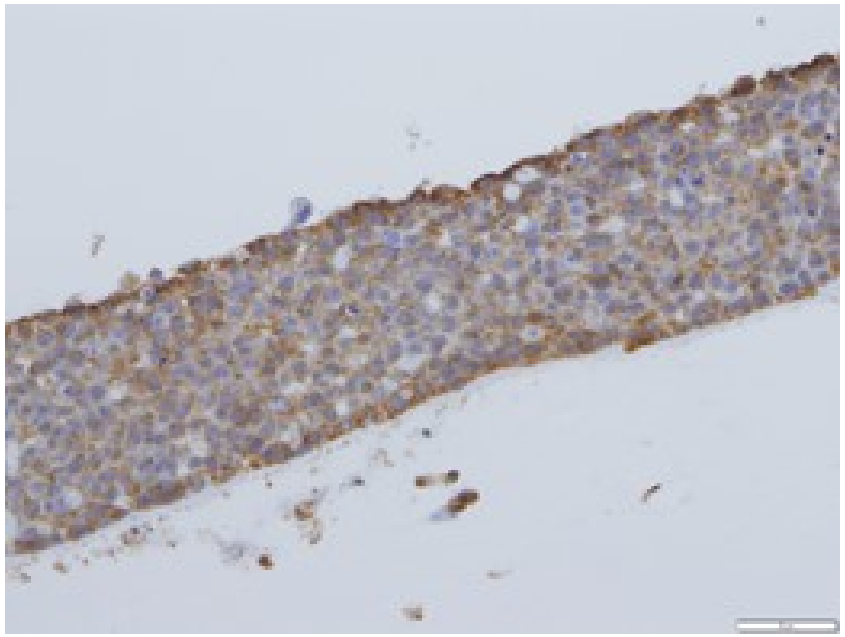
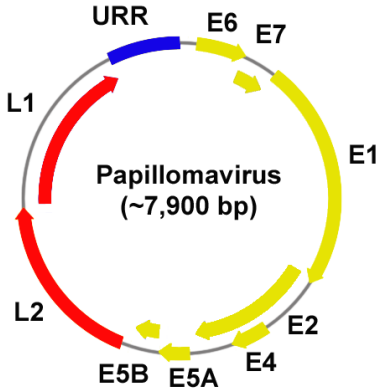
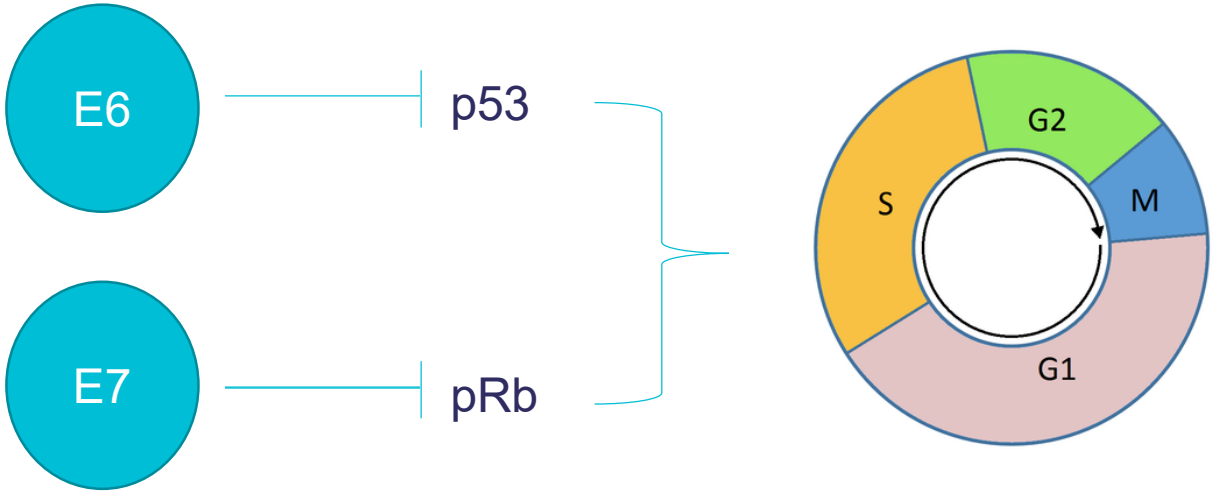


HSP70 is upregulated 16 hours post treatment

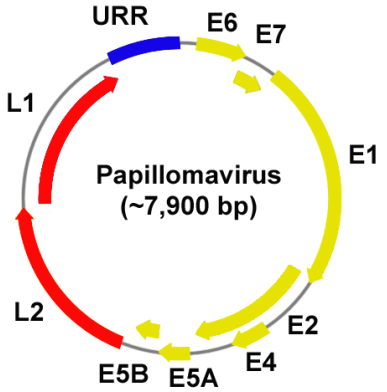
HPV E6 and E7 proteins

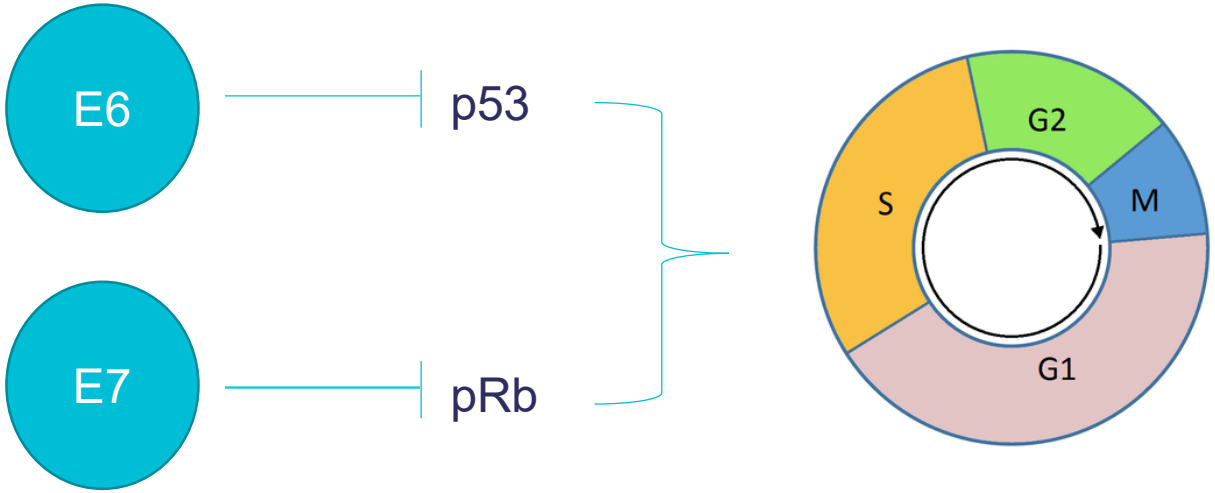




E6 and E7 mediated inhibition of p53 and pRb results in continued cell proliferation with differentiation

HPV E6 and E7 proteins

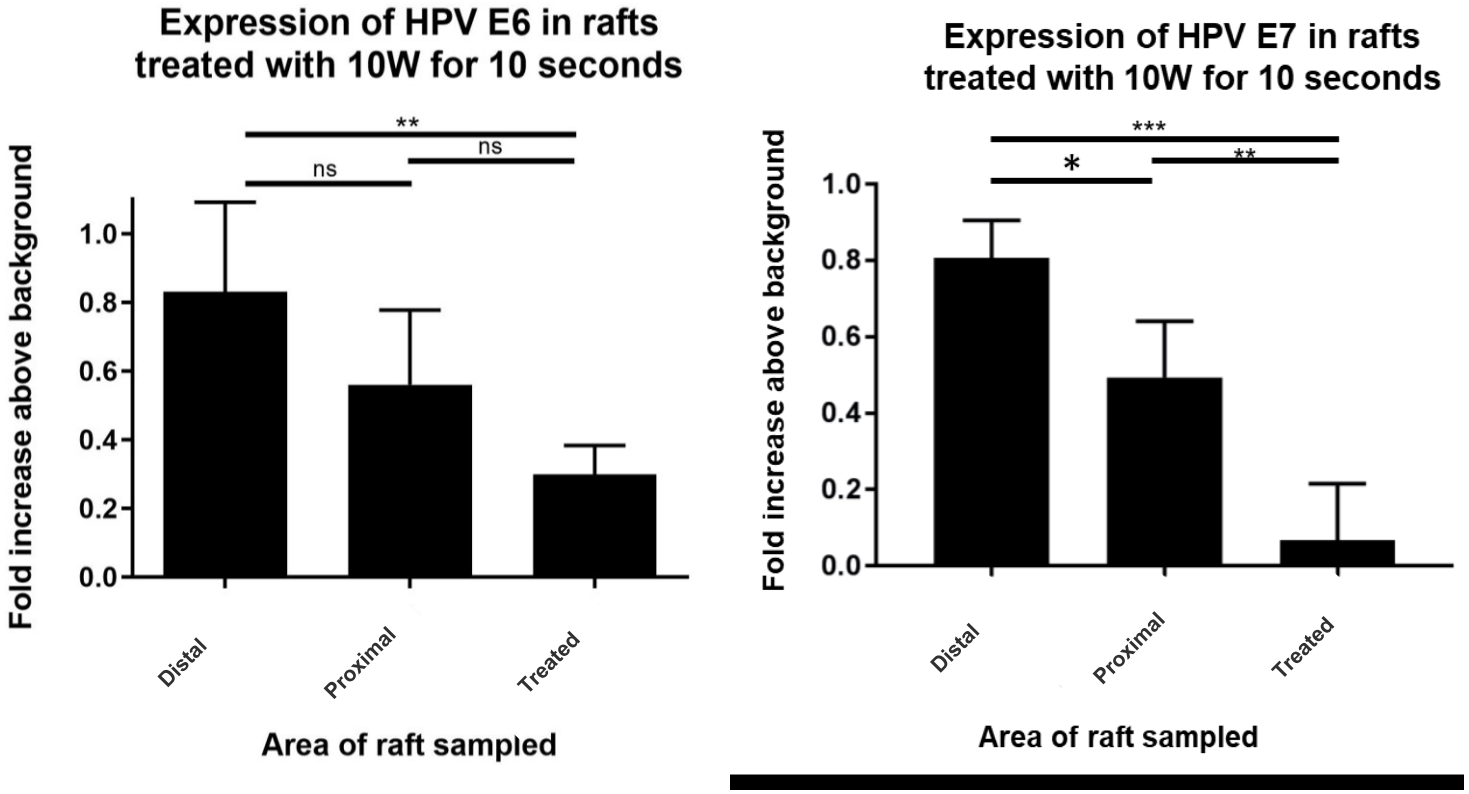




E6 and E7 mediated inhibition of p53 and pRb results in continued cell proliferation with differentiation

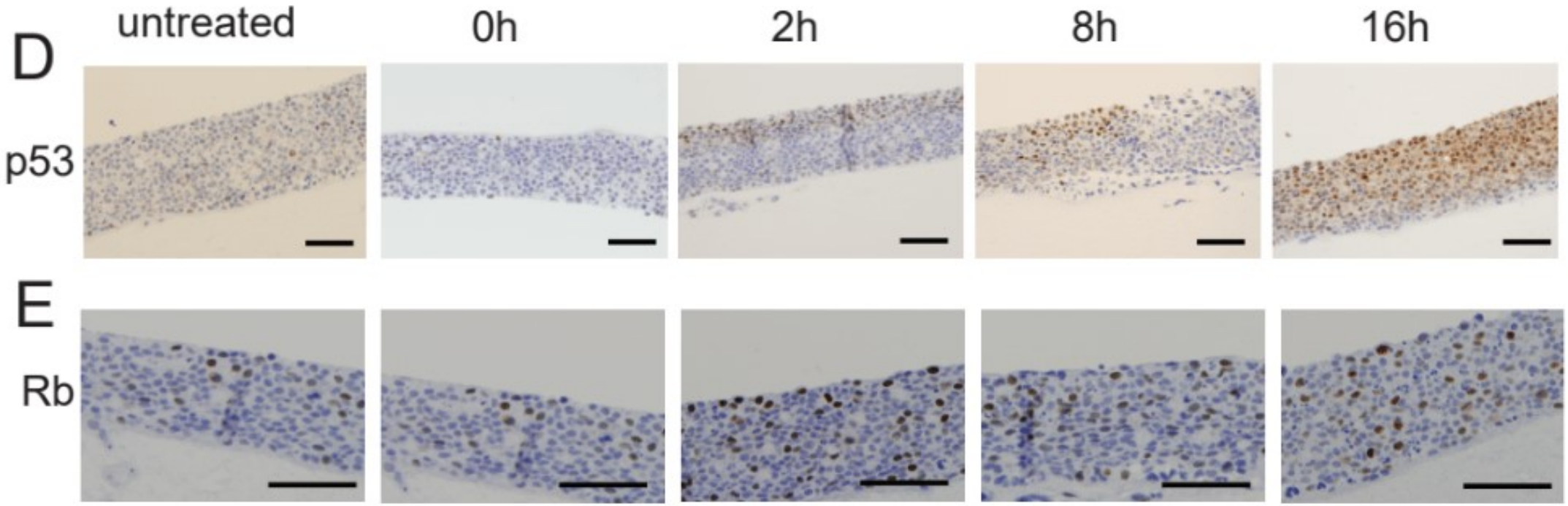
Unregulated expression of E6 & E7 → cancer

Microwave treatment in SiHa cells results in a loss of E6 and E7 expression



Significant decrease in E6/E7 protein in treated areas 16 hours post treatment

The loss of E6 and E7 expression following microwave treatment correlates with an upregulation of p53 and Rb



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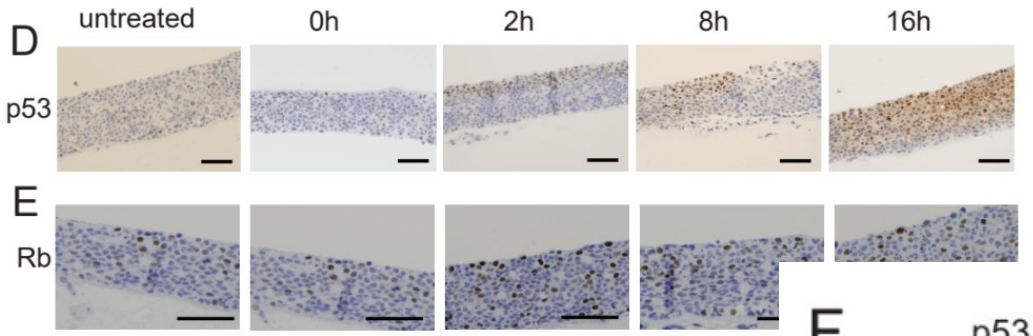
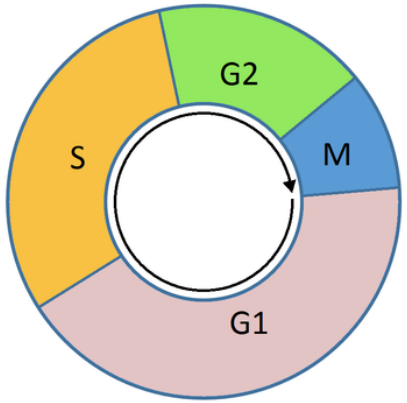
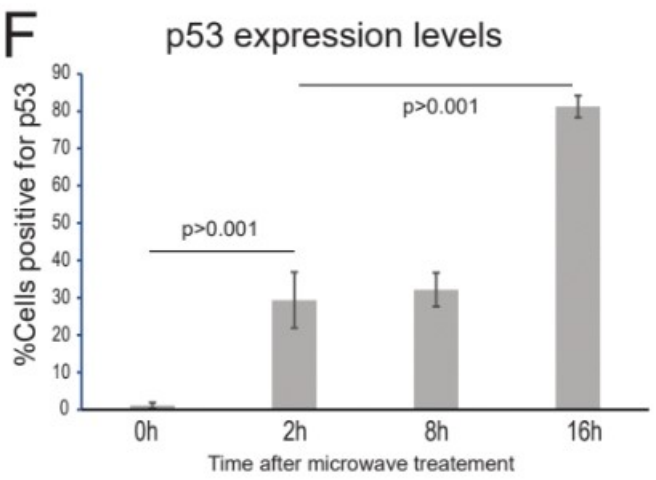
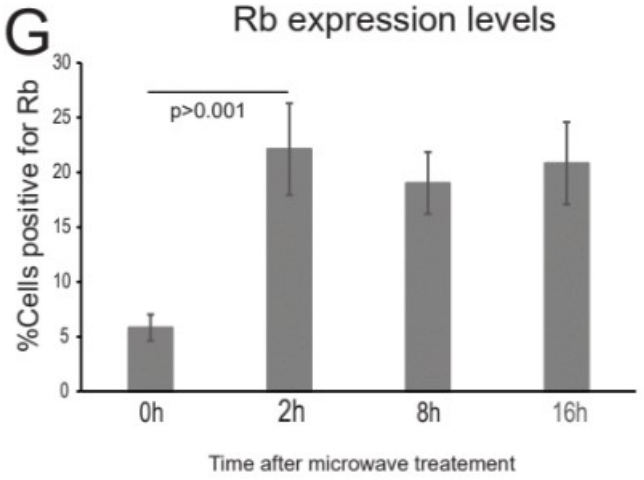




p53
Rb





Significant increase in p53 and Rb levels following microwave treatment

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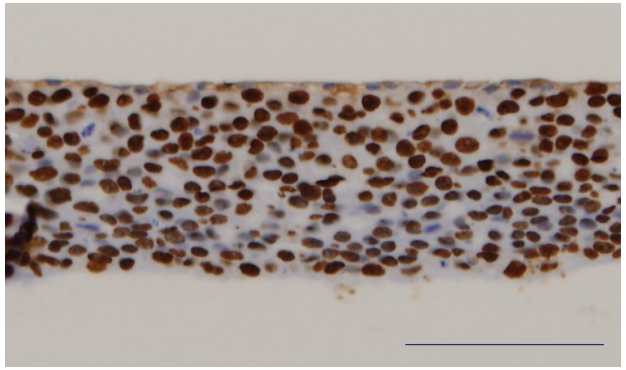
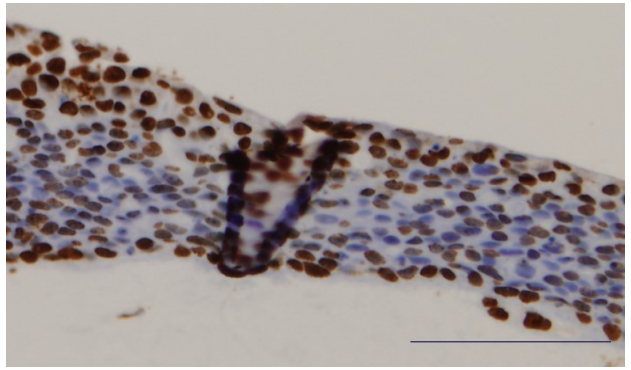
Microwave treatment reverses proliferation

Mock, 16 hours

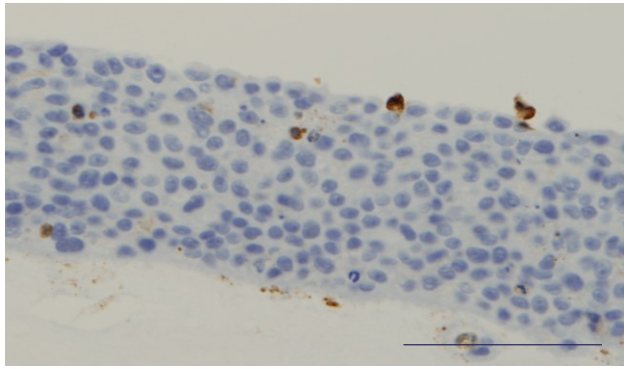
Treated, 16 hours

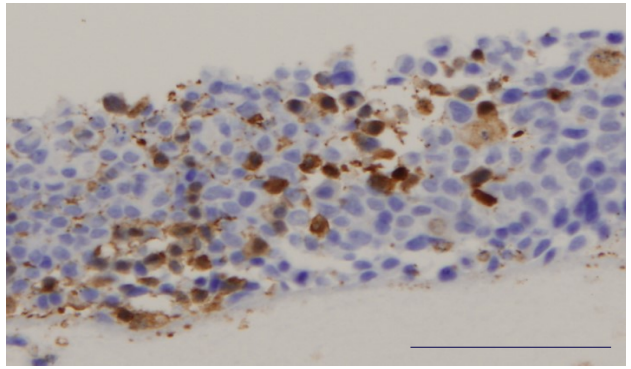
MCM2



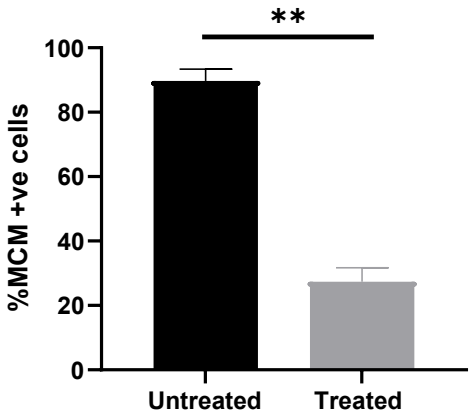


Cleaved caspase 3

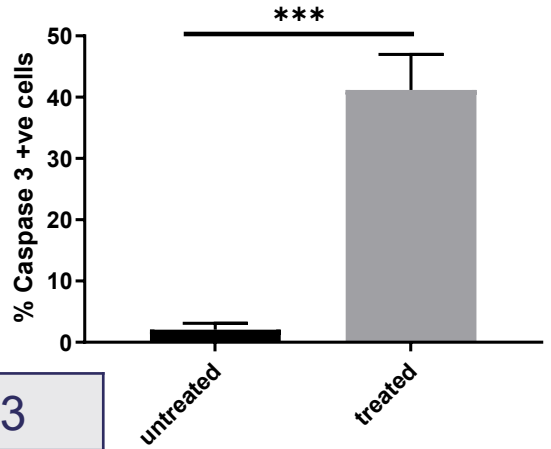




Loss of MCM expression upon microwaving



Gain of Caspase 3 expression upon microwaving



Apoptosis	Caspase 3
Proliferation	MCM2

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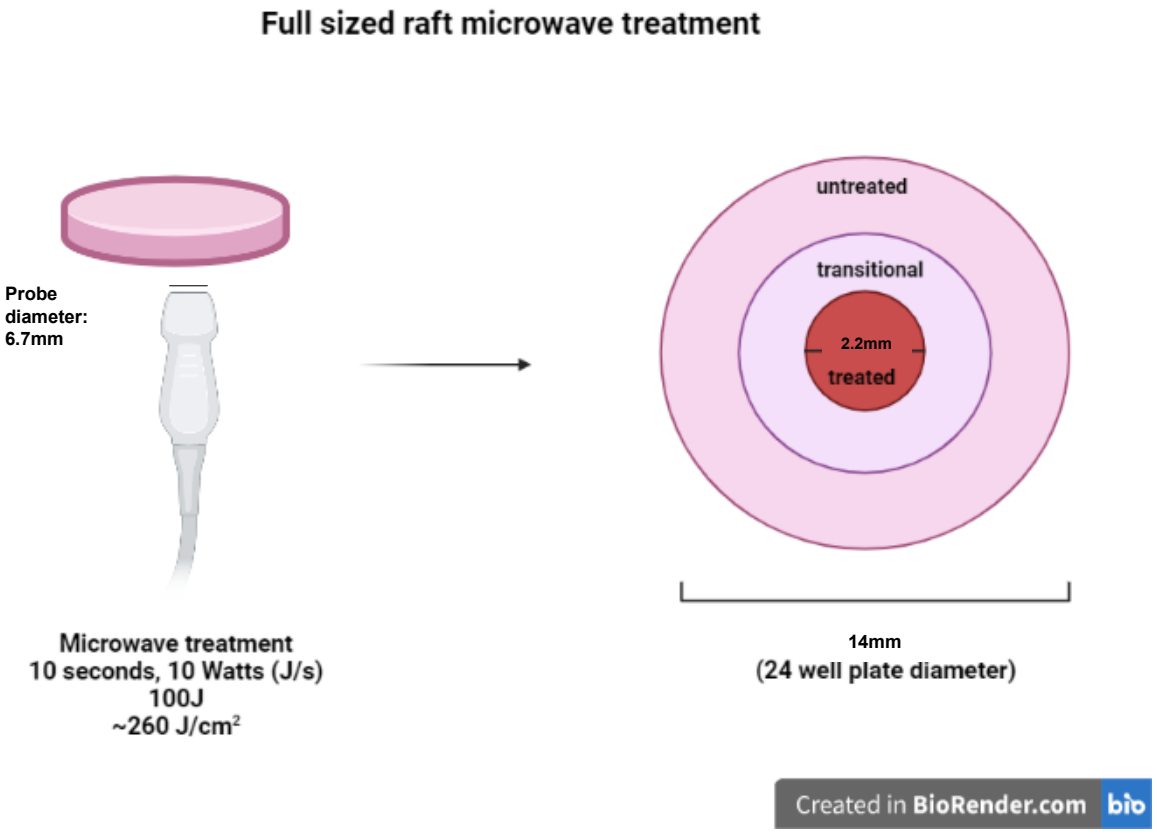
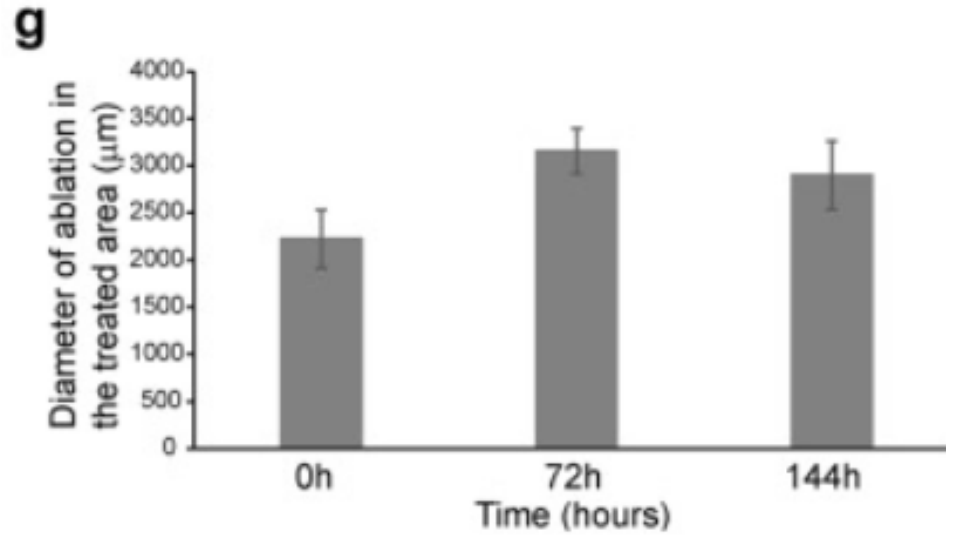
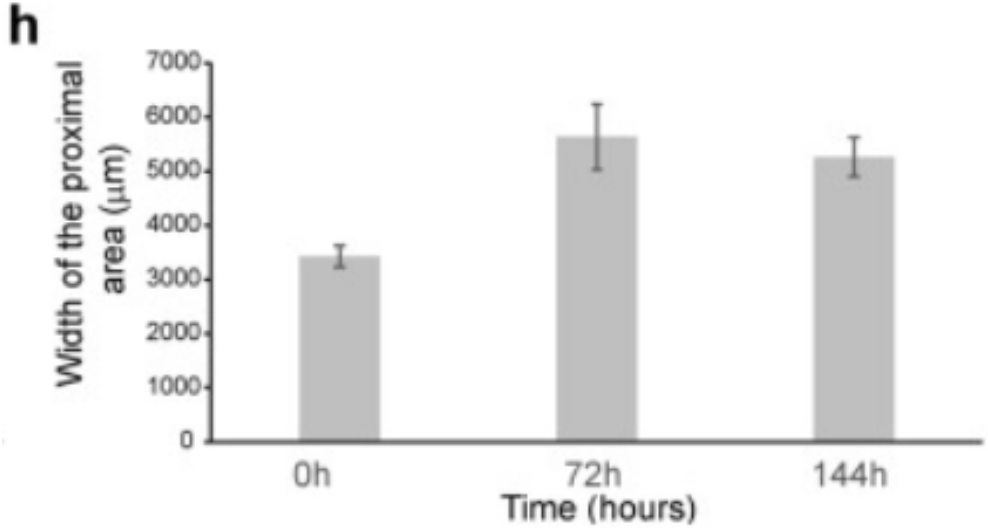
Continued cell death results in the size of the treated and proximal areas increasing over time







SiHa cell line conclusions

Microwave treatment results in:

- Cell **death** at the site of treatment
- Tissue **disruption** with markers of **apoptosis** upregulated in proximal tissue
- A **stress response** – **heat shock**
- A **reduction** in the expression of the **E6 and E7** oncoproteins
  - With a correlative increase in p53 and Rb
- A reduction in **proliferation**

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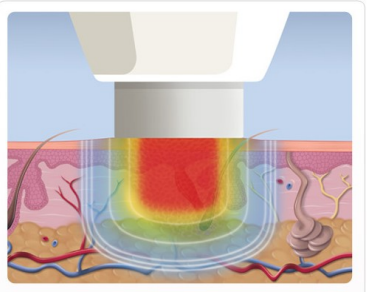
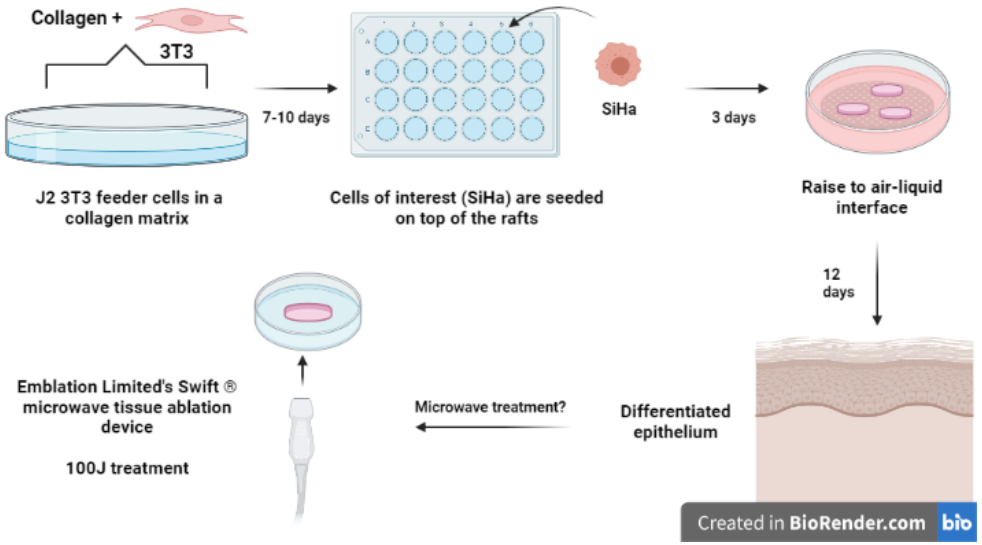
Reversal of tumour phenotype

Culturing 3D organotypic cultures using a collagen-3T3 matrix





- Model of cervical cancer
- 2 integrated HPV16 copies
- Cannot support productive viral cycle

- **Model of earlier disease state**
- **Episomal copies of HPV16**
- **Late stage of viral cycle**

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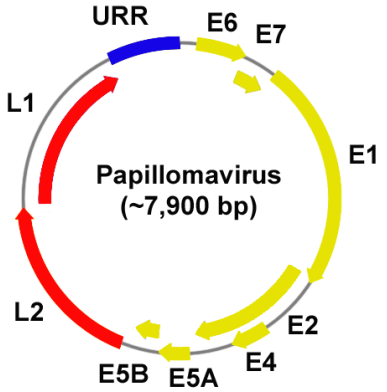
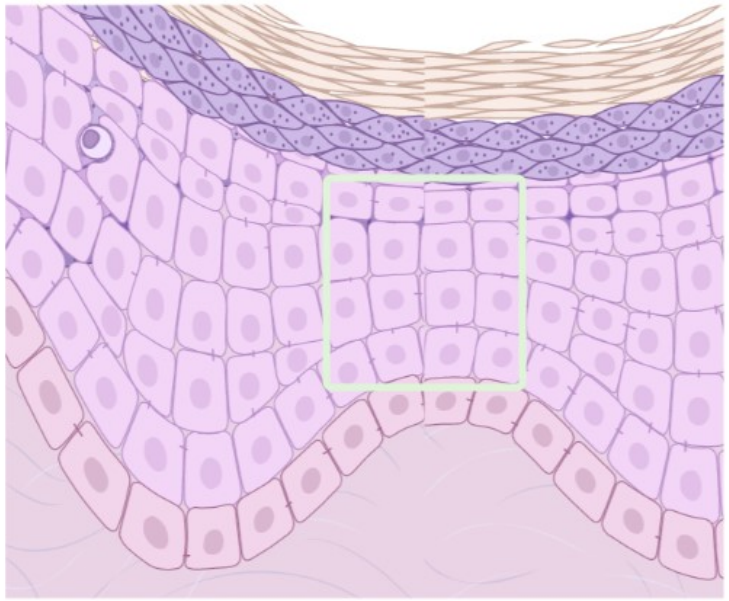
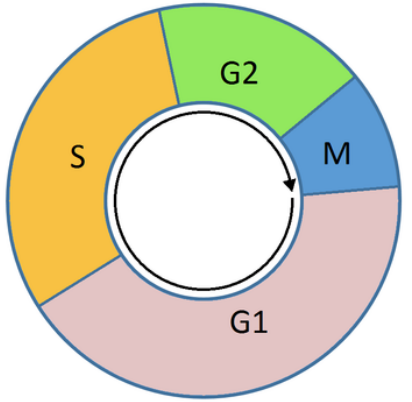
HPV E6 and E7 proteins are essential for productive replication





Suprabasal cells
Begin differentiation
Exit of cell cycle

Created in BioRender.com bio



E6 and E7 mediated inhibition of p53 and pRb results in continued cell proliferation with differentiation

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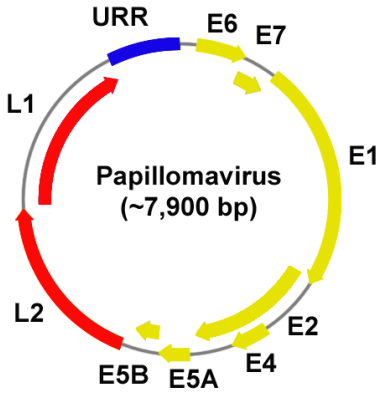
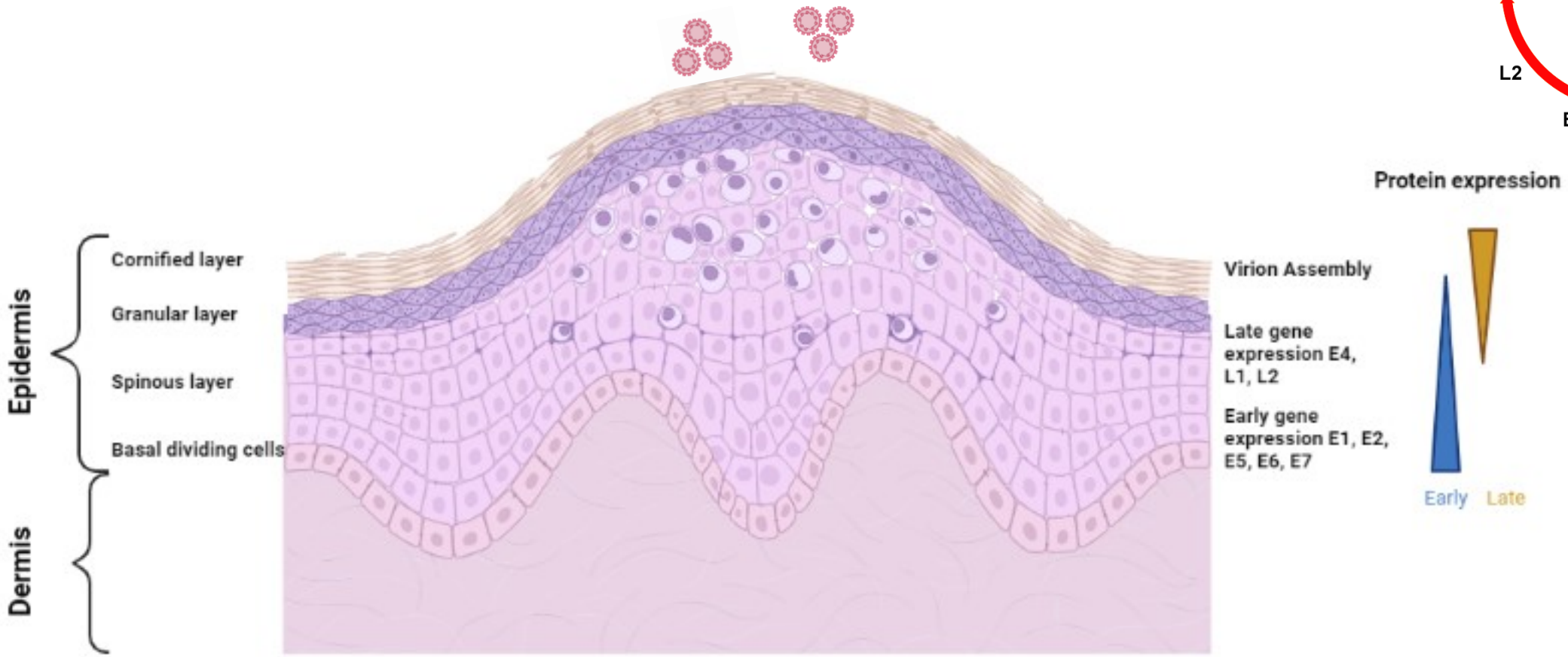
The HPV life cycle is linked to the differentiation status of the epithelium

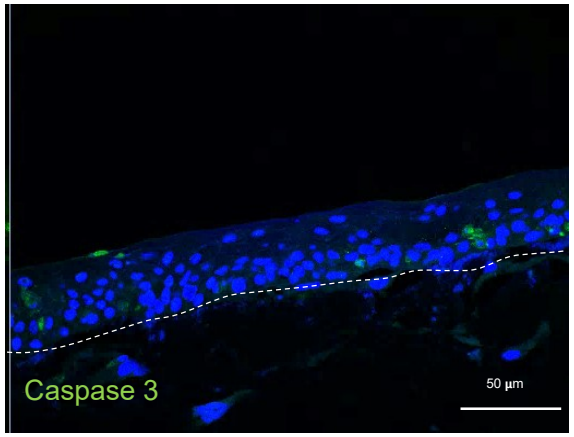


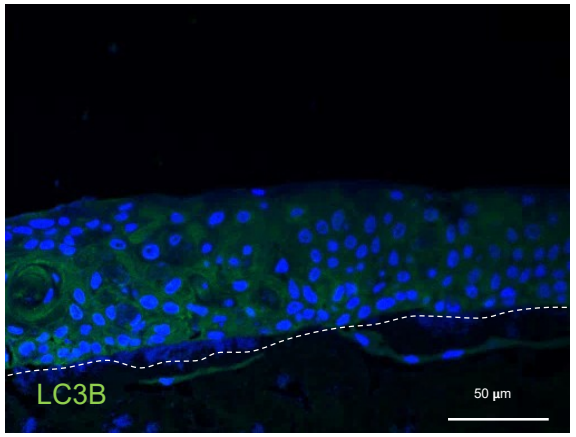


Microwave treatment results in an upregulation of apoptosis and autophagy markers

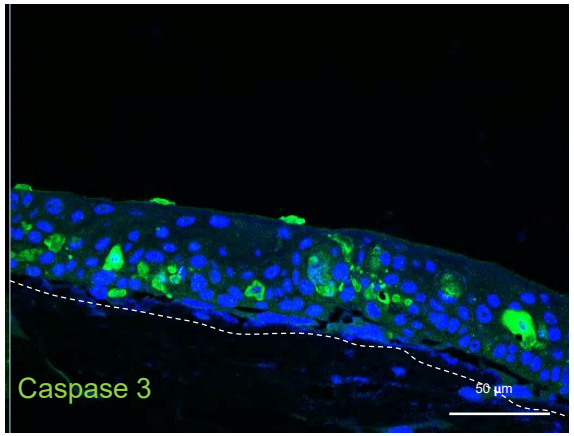
16 hours

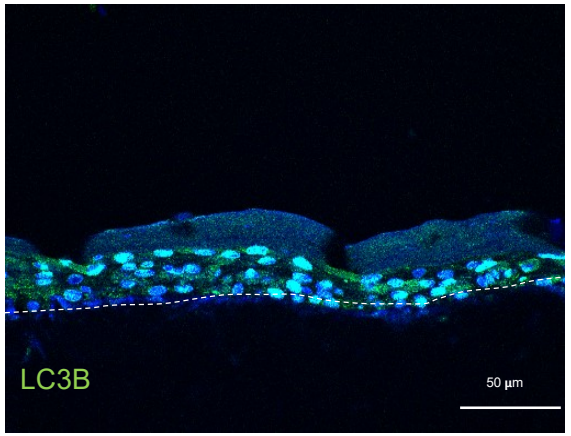
Untreated tissue

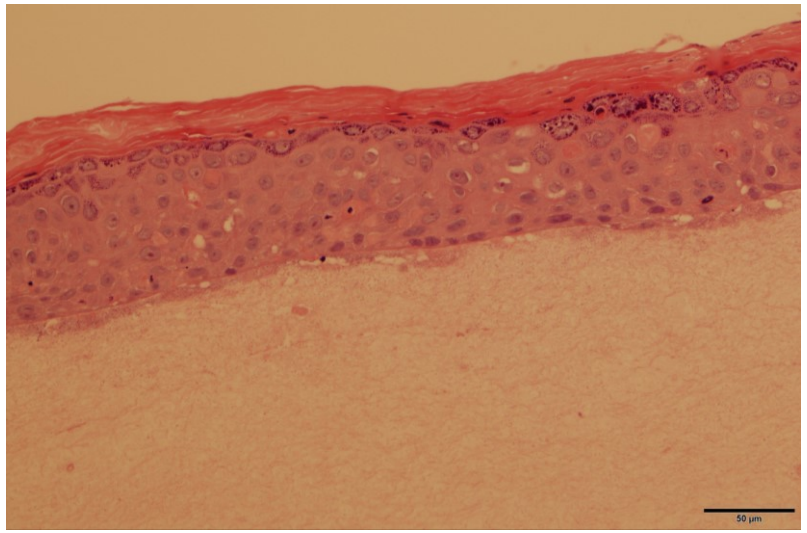




Treated tissue







Apoptosis	Caspase 3
Autophagy	LC3B

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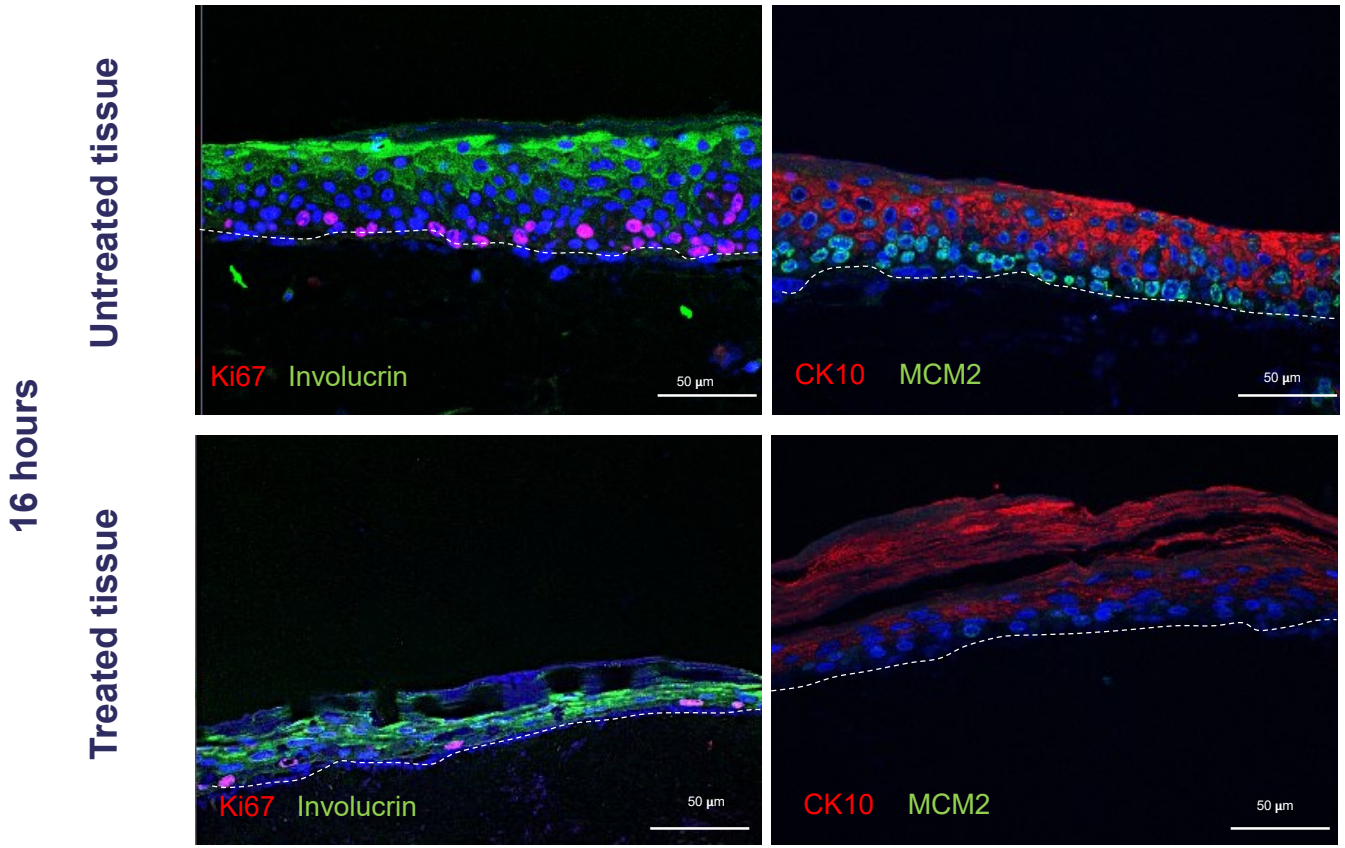
Microwave treatment causes an increase in differentiation



Proliferation	Ki67, MCM2
Differentiation	Involucrin, CK10

Conclusions

Microwave treatment results in:

- Cell **death** at the site of treatment
- Tissue **disruption** with markers of **apoptosis** upregulated in proximal tissue
- A reduction in proliferation
- A **reduction** in expression of **E6 and E7 oncoproteins**
  - **Increase** in p53 and Rb expression
- Treatment resulting in a **more differentiated phenotype**
- Stimulates the **immune** response?

Acknowledgements

Graham group:

- Sheila Graham
- Andrew Stevenson
- Ilaria Epifano
- Harry Scott
- *Michaela Conley*
- Madeleine White

- Emblation team: Matt Kidd
- John Doorbar, Nagayasu Egawa

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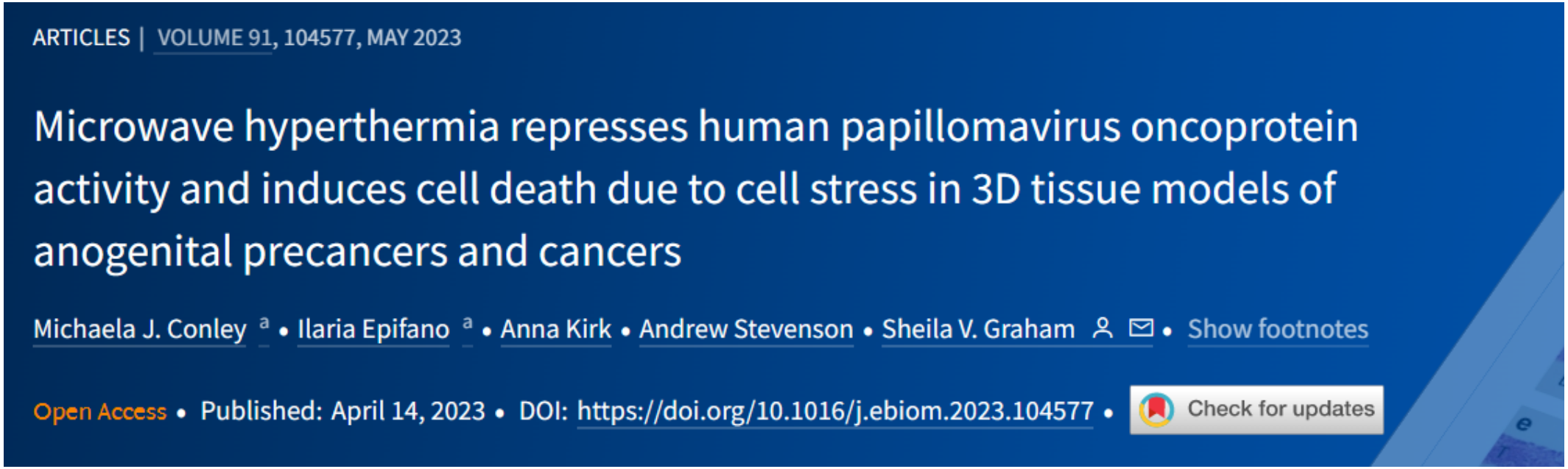
Acknowledgements



SCAN ME

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AIN study:
Katrina Knight

Thank you