Nano-Pulse Stimulation Technology is a Promising New Energy Modality for Barrett's Esophagus

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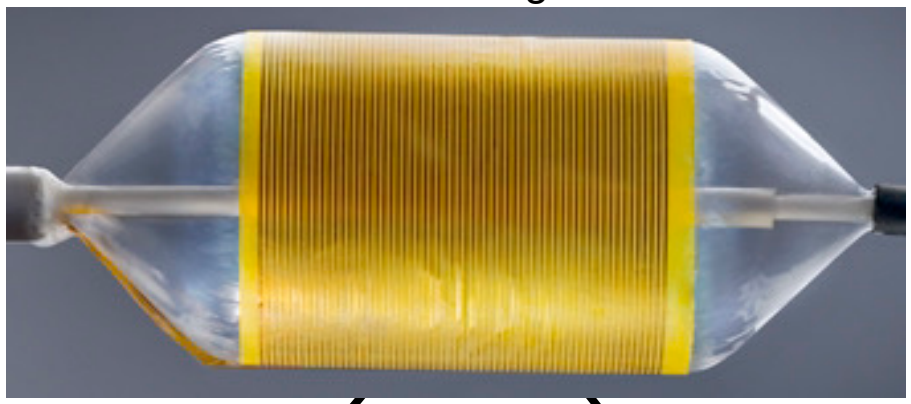
Disclosures

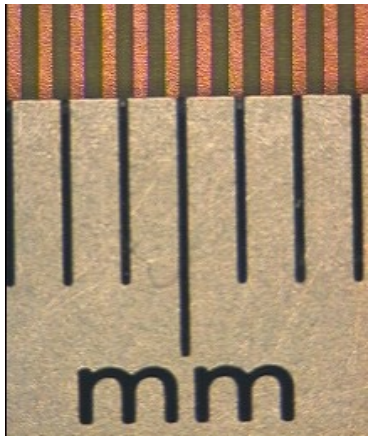
- Robert A. Ganz, MD, MASGE, S.A. – Consultant to Pulse Biosciences
- Pulse Biosciences – Study sponsor
  - Co-Authors David Danitz, Kevin Moss, Holly Hartman, Mitchell Levinson, Richard J. Connolly are all employees of Pulse Biosciences

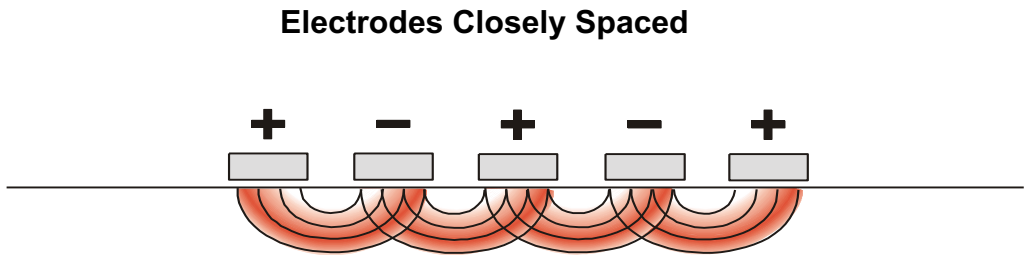
RFA - Balloon-based Electrode

3 cm length



Magnified Electrode





- **Energy delivery in < 1 sec**
- **3cm circumferentially**
- **Standardized energy density**

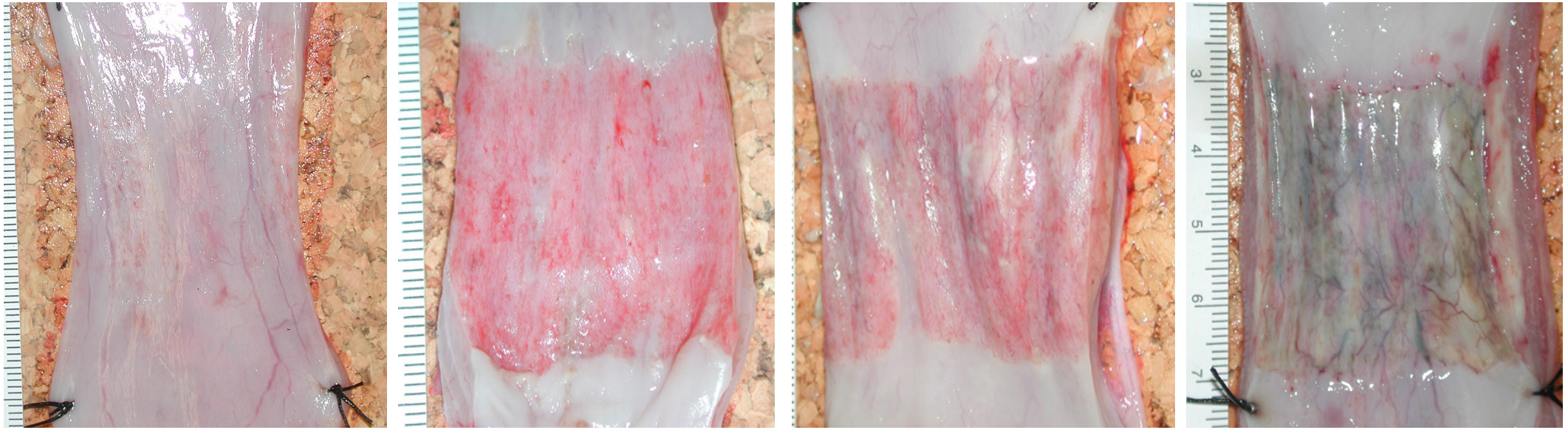
Results

- **Controls depth of ablation**
- **Enables uniform ablation**
- **Helps prevent strictures, buried glands, and perforations**

Ganz R, Zelickson B, Stern R: System and Method for Treating Abnormal Tissue in the Human Esophagus. Patent #6,551,310, issued 4/22/03

Control of Ablation Depth

Dosimetry Study to Validate Depth of Penetration



5 J/cm^2 10 J/cm^2 12 J/cm^2 20 J/cm^2

Linear Response to Varying Energy Density

Ganz, et al GIE - 2004

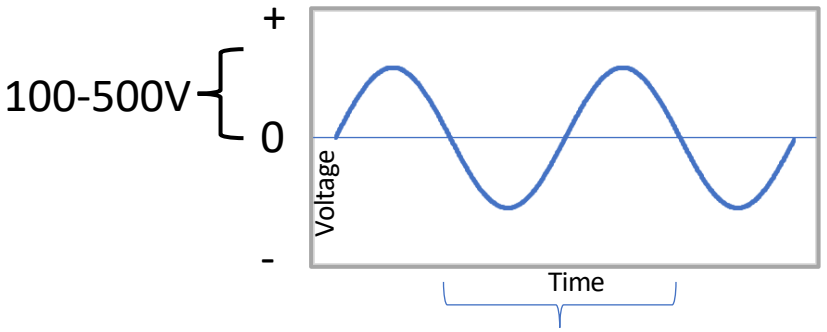
RFA Issues:

- Incomplete ablation; takes 2-3 sessions for complete treatment
- 5-10% strictures
- Post-ablation pain
- Target depth…deep muscularis mucosa/superficial submucosa; incomplete ablation of submucosal glands

Ganz R, Overholt BF, Sharma VK, et al, Circumferential Ablation is Safe and Effective for the Treatment of Barrett's Esophagus with High Grade Dysplasia: A U.S. Multi-Center Registry. Gastrointestinal Endoscopy 68: 35-40, 2008.

Therapeutic Electrical Energy Modalities

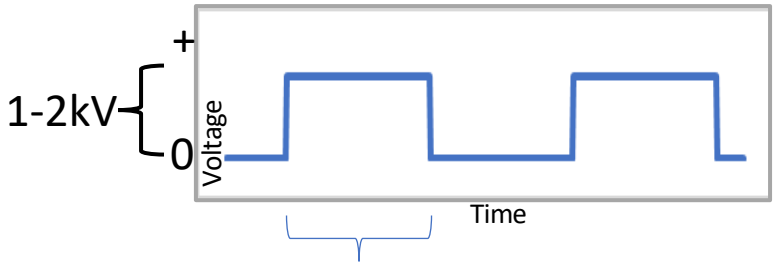
Radiofrequency
(Radiowave spectrum)



Microseconds (millionths)

- Alternating current (AC)
- Heats tissue by electrical resistance.
- Damage is thermal and non-selective.
- Dominant manner of injury: thermal, immediate necrosis

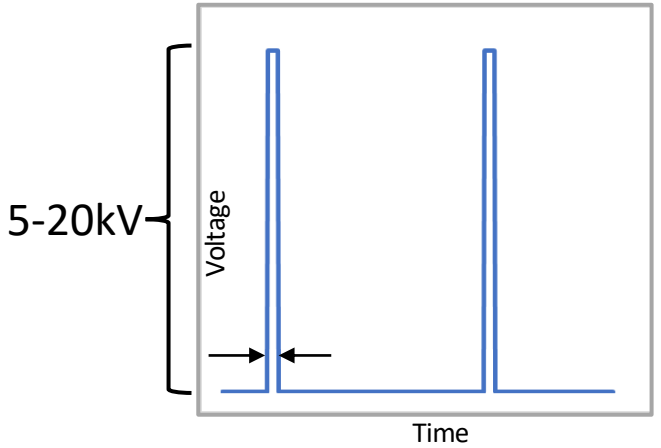
Irreversible Electroporation
(Microsecond pulsing)



Microseconds (millionths)

- Direct current (DC)
- Electrical field effect
- Destroys cell membranes.
- Damage is selective for cellular structures.
- Dominant manner of injury: traumatic, immediate necrosis

Nano-Pulse Stimulation, NPS™
(Nanosecond Pulsing)

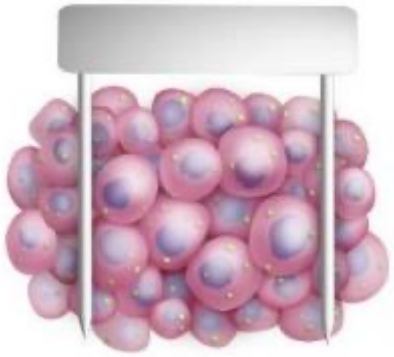


Nanoseconds (billionths)

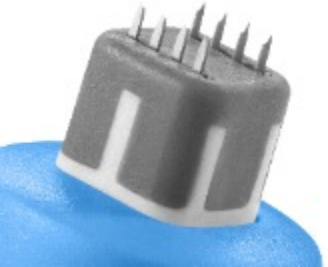
- Direct current (DC)
- Electrical field effect
- Damages cell organelles.
- Damage is selective for cellular structures.
- Dominant manner of injury: atraumatic, regulated cell death (like apoptosis)

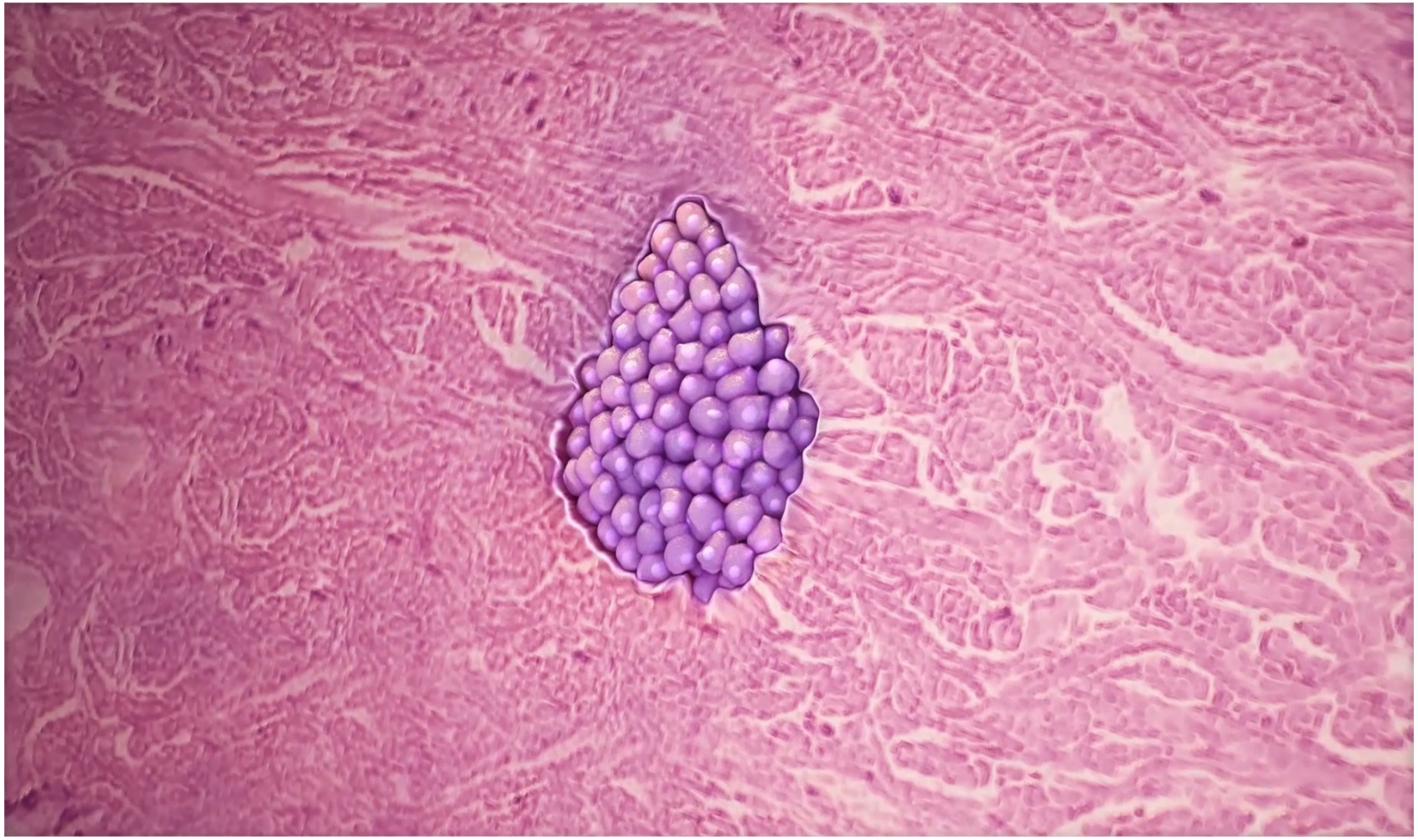
Nano-Pulse Stimulation™ (NPS) is a new, non-thermal energy modality that stimulates regulated cell death (RCD) while sparing non-cellular tissue

NPS Treatment of Tissue



Ultra-short (billionths of a second) electrical energy pulses cause internal organelle disruption leading to Regulated Cell Death

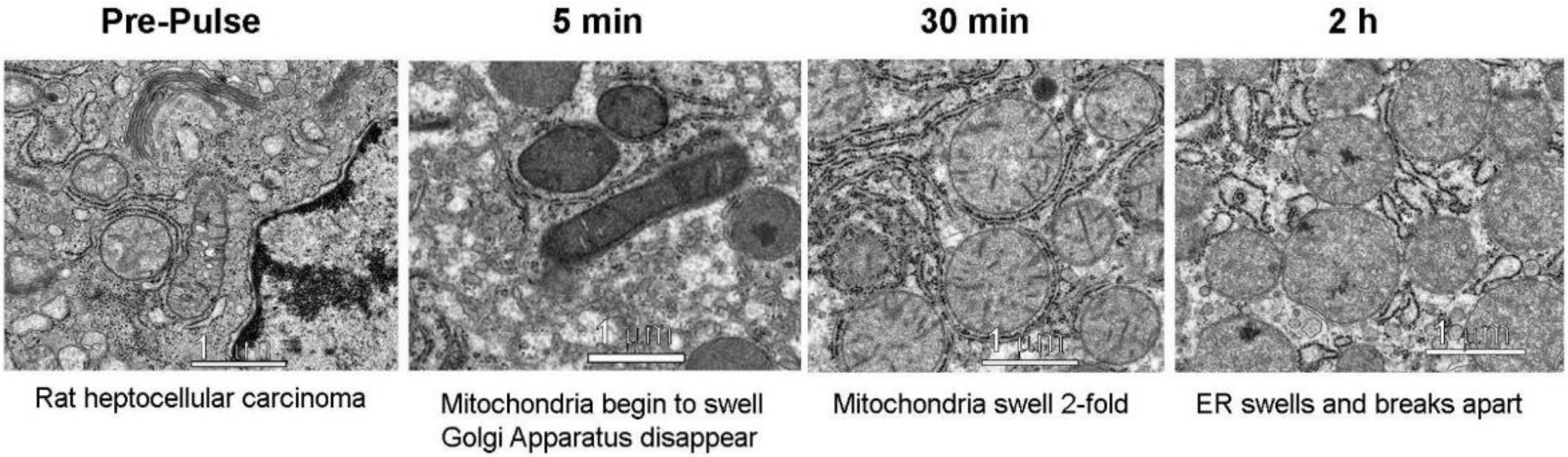




Nano-Pulse Stimulation and NPS are trademarks of Pulse Biosciences, Inc.

NPS induces intracellular organelle changes

Electron microscopy shows acute changes to the mitochondria, endoplasmic reticulum (ER) and Golgi apparatus in *in-vivo* rat tumor model[1]

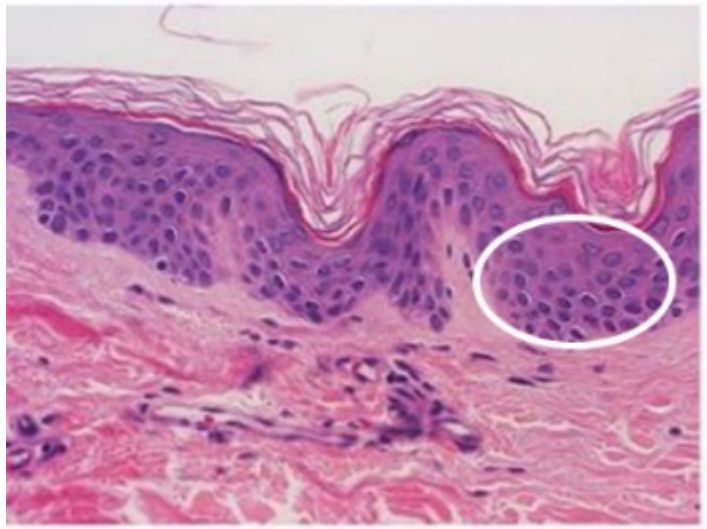


McA-RH7777 (HCC) in Buffalo rats

1. Nuccitelli R, Zelickson B, et al. Nano-Pulse Stimulation Induces Changes in the Intracellular Organelles in Rat Liver Tumors Treated In Situ. ***Lasers in Surgery and Medicine*** 2020; 52:882–889

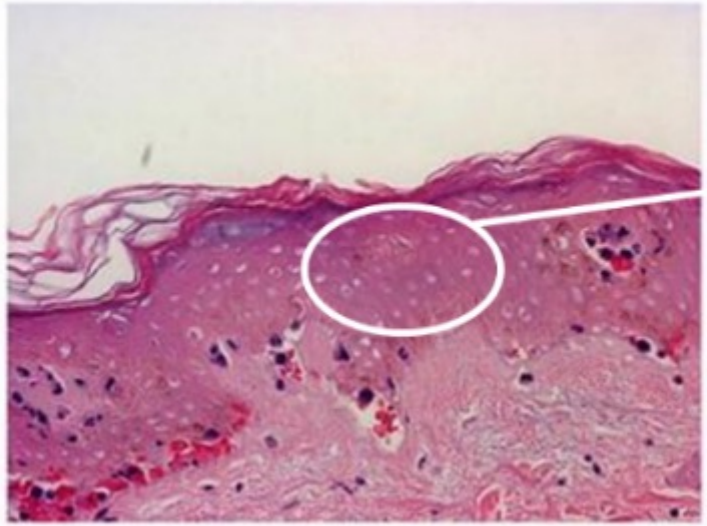
NPS stimulates RCD of the epidermis while sparing non-cellular tissue

Untreated Control



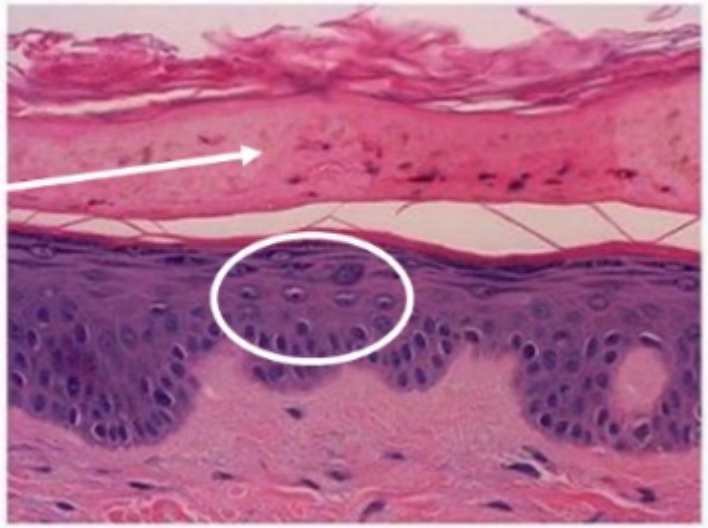
- Epidermal cells with healthy dark nuclei

1 Day Post-Treatment



- "Ghost cells"
- Non-viable epidermis
- Minimal inflammation

7 Days Post-Treatment

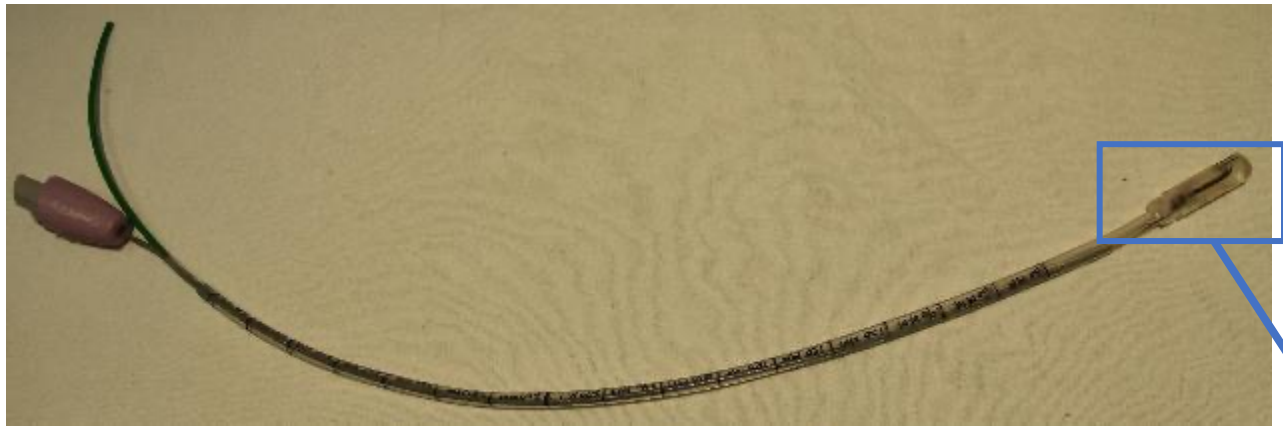


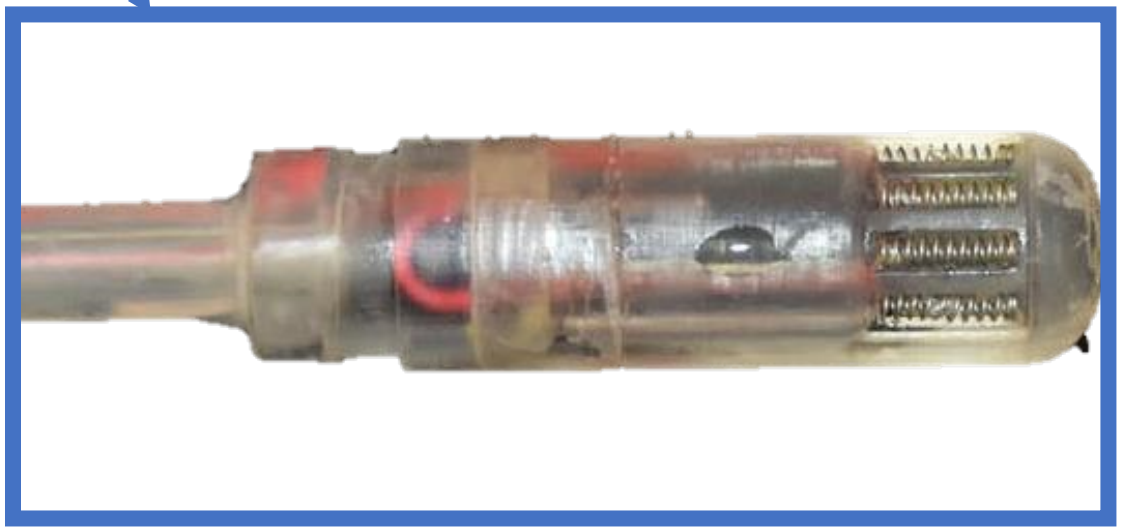
- Original necrotic epidermis peeling
- New epidermis layer, healthy nuclei
- Minimal dermal inflammation

The cell-specific effect is non-thermal, as a typical nano-pulse delivers ~0.1 Joules of energy distributed in a volume of tissue

Reference:
Kaufman, D., Mehregan, D. et al. "A Dose-Response study of a novel method of selective tissue modification of cellular structures in the skin with nanosecond pulsed electric fields," ***Lasers in Surgery and Medicine***, 2019; 52: 315-322

NPS Esophageal Catheter





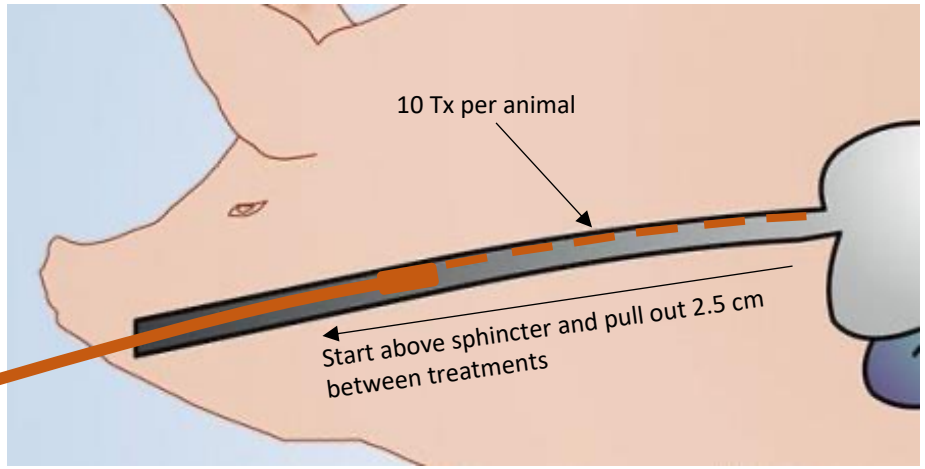
- Axial spring electrodes built into distal tip
- 1.6 cm long spring electrodes spaced at 2.5 mm
- Ablative area covers 110° of esophagus
- Vacuum assisted to enhance surface contact

Materials and Methods

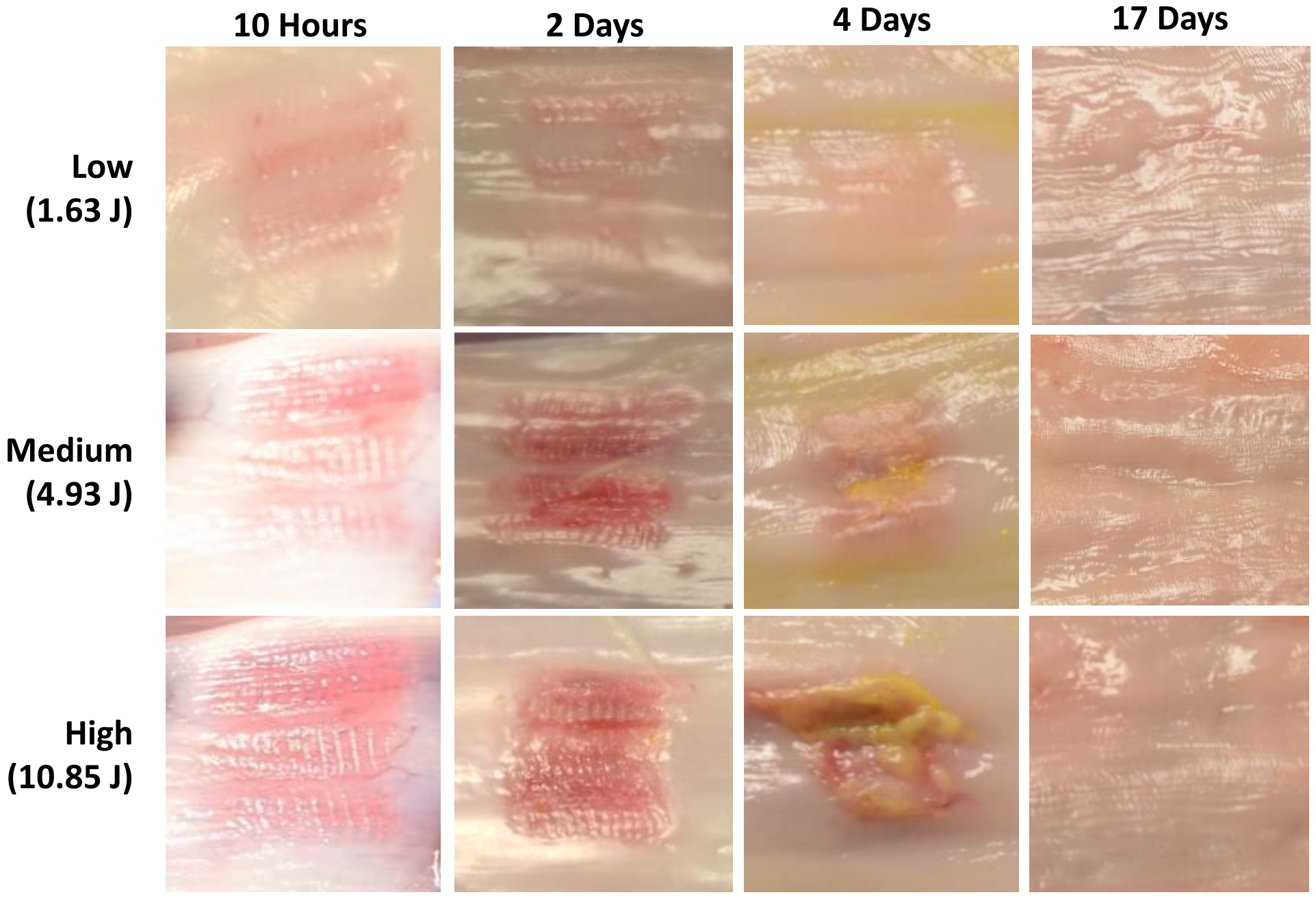
- Study performed in accordance with Sutter Institute for Medical Research Institutional Animal Care and Use Committee protocol PB.10.19.
- 10 female Yorkshire swine, 15 – 22 weeks old, 76 – 92 Kg.
- Baseline endoscopy (Olympus GIF-H180) to assess length of esophagus
- Animals treated with 3 energy levels and survived for 10 hours, 2 days, 4 days, 17 days, and 30 days.

Device Placement & Procedure

- Novel NPS device inserted to predetermined depth
  - 20 inHg vacuum applied to secure device to surface
  - 5 NPS treatments performed with each energy setting per survival timepoint; (75 total treatments)
    - Low: 1.63 ± 0.04 J ,Medium: 4.93 ± 0.12 J, High: 10.85 ± 0.29 J
    - All with average power of 0.56 ± 0.05 W.
  - Individual treatments with 1 cm spacing
- Prior to euthanasia, esophagus re-endoscoped to assess for any evidence of ulceration or stricture



Gross Pathology: Tissue Erythema/Mucosal Sloughing



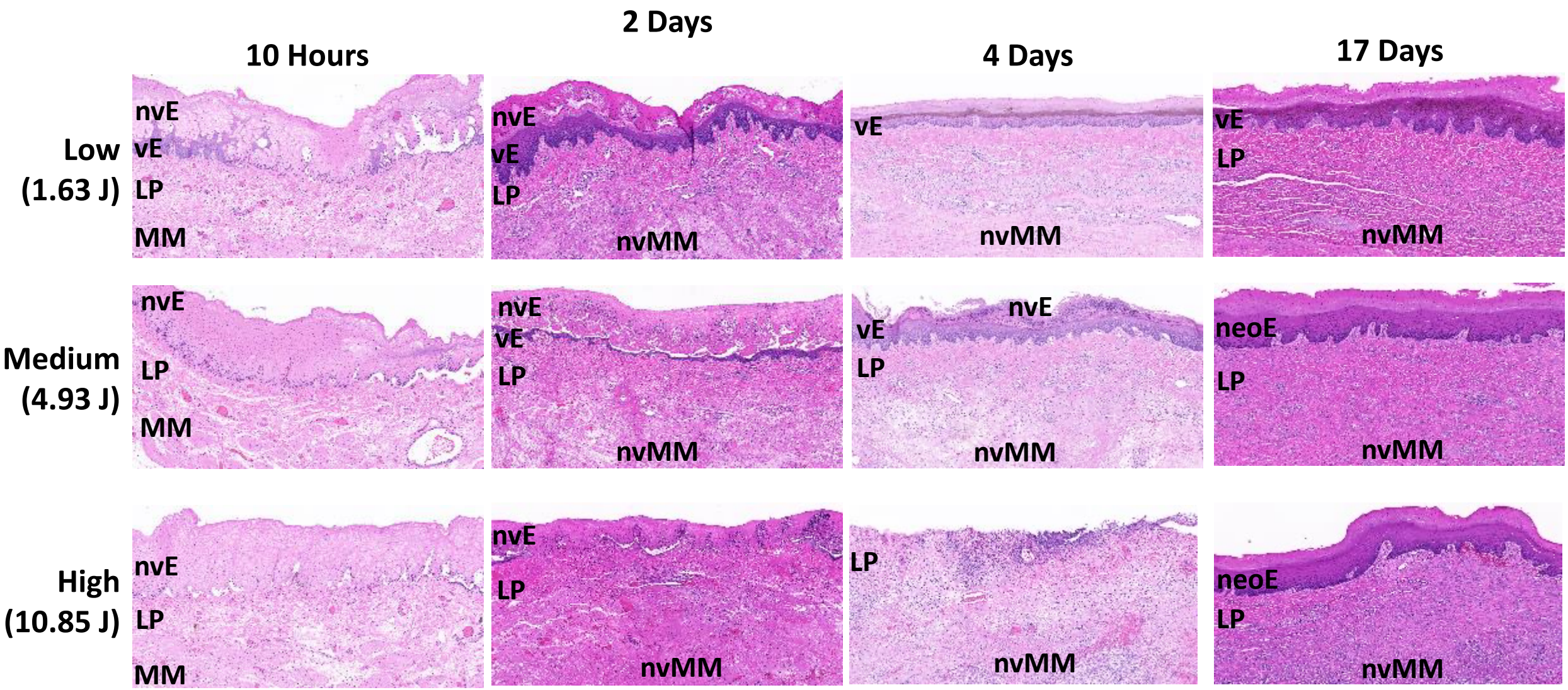
Animal Observations by Endoscopy

Time Point	
Immediately after Tx	Mild to moderate erythema at Tx sites
2 Days	Mild erythema and epithelial loss at Tx sites
4 Days	No obvious Tx-related effects for low dose; erythema, mild mucosal sloughing at medium/high dose
17/30 Days	No Tx-effects, no evidence of scarring/stricture
Throughout	No observed behavioral changes. Normal appetite.

After euthanasia, entire porcine esophagus was fixed in formalin and cross-sectioned at 3 mm increments for routine histopathology

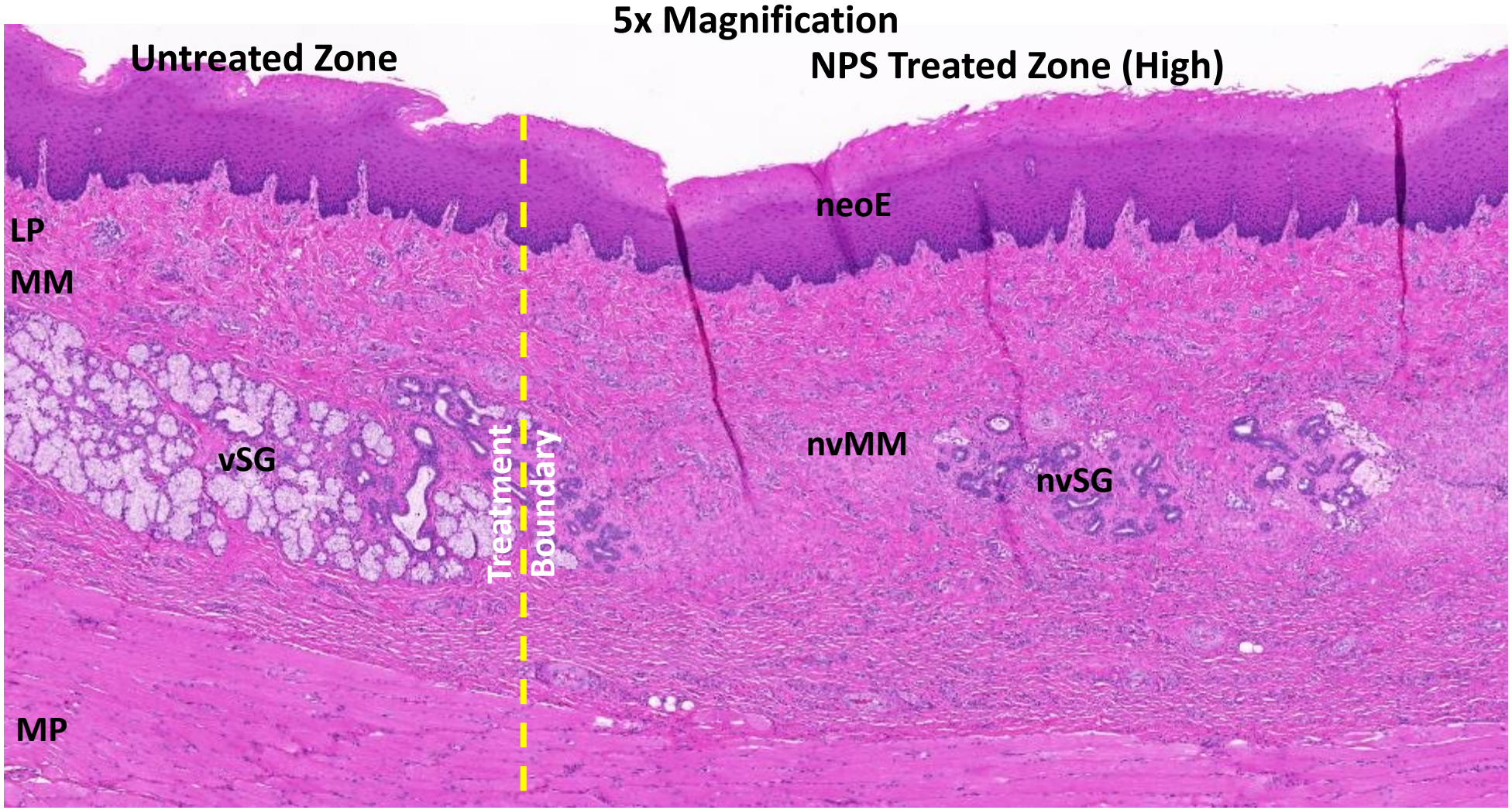
- Staining with hematoxylin and eosin, Gomori trichrome, and Movat pentachrome

Esophageal epithelium treatment



vE = Viable Epithelium; nvE = Non-Viable Epithelium; neoE = Neo-Epithelium; LP = Lamina Propria; MM = Muscularis Mucosa; nvMM = Non-viable Muscularis Mucosa

17 Day – Submucosal Gland Clearance with High Energy

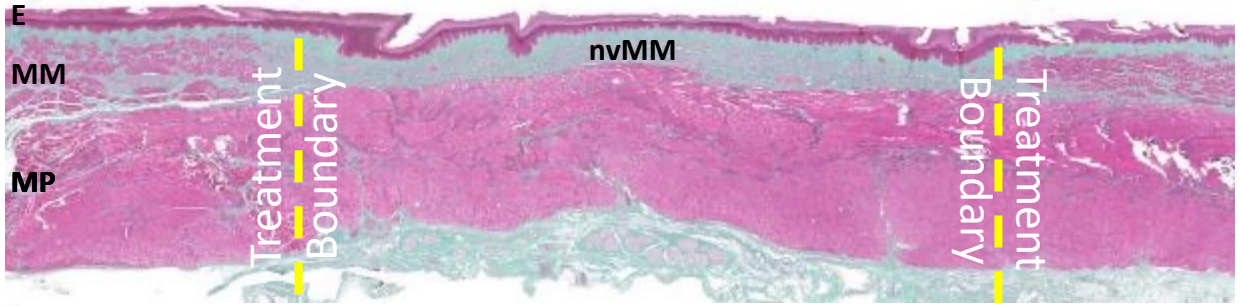


E = Epithelium ; neoE = Neo-Epithelium; LP = Lamina Propria; MM = Muscularis Mucosa; nvMM = Non-Viable Muscularis Mucosa; MP = Muscularis Propria; vSG = Viable Submucosal Glands; nvSG = Non-Viable Submucosal Glands

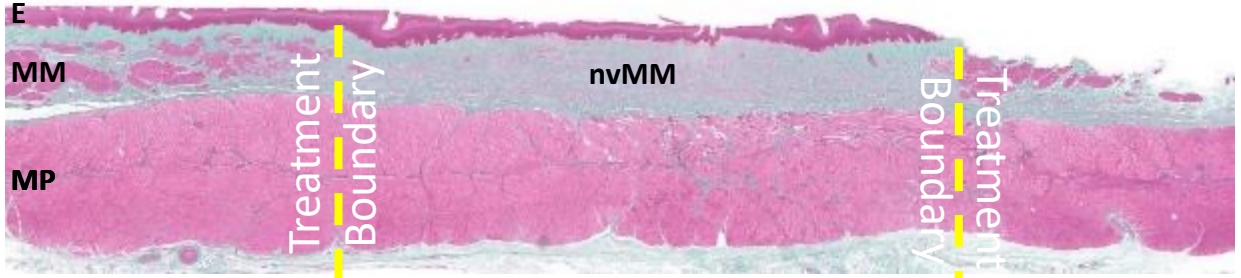
17-Day Histology – Minimal fibrosis; no evidence of scarring/stricture

1x Magnification

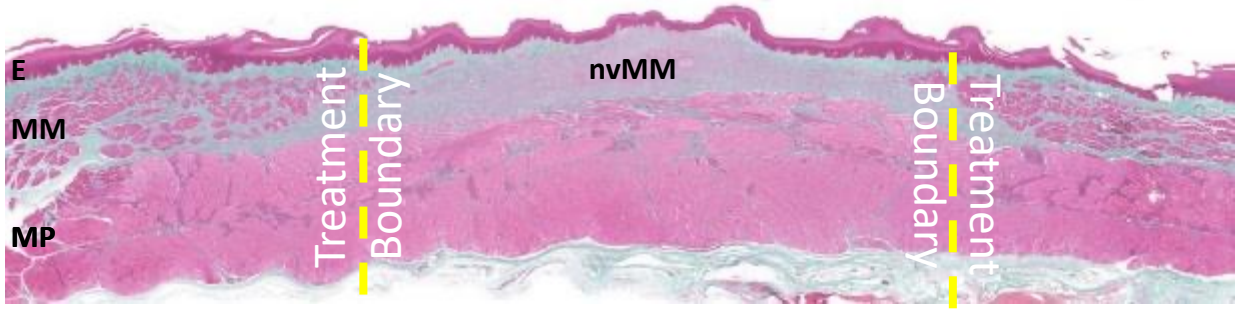
Low (1.63 J)



Medium (4.93 J)



High (10.85 J)



Control

Gomori Trichrome staining used in this histology

E = Epithelium; LP = Lamina Propria; MM = Muscularis Mucosa; MP = Muscularis Propria; nvMM = Non-Viable Muscularis Mucosa

Histology Summary

	Low Dose 1.63 ± 0.04 J	Medium Dose 4.93 ± 0.12 J	High Dose 10.85 ± 0.29 J
Removal of Epithelium	Partial	Near Complete	Complete
Re-epithelialization Observed	2 days	4 days	17 days
Elimination of Submucosal Glands	None	Partial	Complete
Treatment Depth	Top of Muscularis Propria	Top of Muscularis Propria	Top of Muscularis Propria
Inflammation	Mild	Mild	Mild

Key Findings

- Cleared esophageal epithelium and submucosal glands in a single treatment
- Mechanism of Action is regulated cell death (RCD) – histological markers include:
  - Caspase-3 up-regulation
  - Pyknosis (chromatin condensation)
  - Karyorrhexis (nuclear fragmentation)
- No evidence of thermal damage or fibrosis/stricture
- No evidence of clinically significant damage to the submucosa, muscularis propria or serosa

Conclusions:

- Nano-pulse stimulation (NPS):
  - Novel, RCD
  - Targets cellular tissue while sparing noncellular structures such as collagen, nerves, vessels.
- Ability to eliminate esophageal epithelium and submucosal glands without causing fibrosis at 17/30 days.
- Unique mechanism of action - NPS may be an appropriate technology for treating BE - targeting Barrett's epithelium and submucosal glands while sparing the submucosal stroma and nerves, potentially reducing stricture and post-procedural pain.

Thank you!