

Ablation II

Electroporation

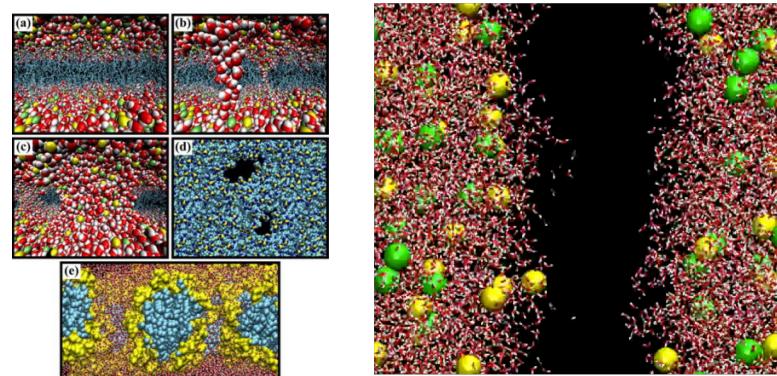
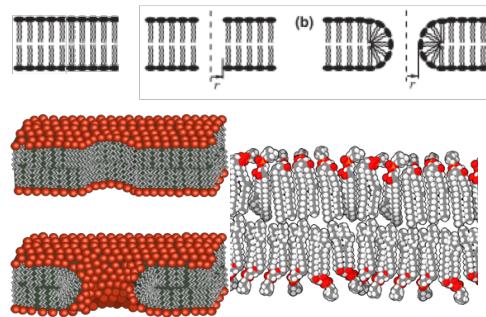
2019/03/05

What you can expect to learn today

- What is electroporation
- General theory of electroporation
- Applications of Electroporation
 - Electroporation for ablation
 - Electroporation for delivery of different agents into cells
- Review

What is electroporation?

- High strength electric field that increases the permeability of the cell membrane by the formation of small pores (also called electropermeabilization)
 - Pores can be hydrophilic or hydrophobic
 - Pore formation is initiated by the penetration of water molecules into the membrane
 - This leads to the formation of hydrophilic pores
- The pores can be temporary (reversible electroporation) or permanent (irreversible electroporation)
- It is generally thought to proceed in three stages:
 - Membrane charging phase
 - Pore creation
 - Pore evolution
- And then can reverse
 - Pore shrinkage
 - Pore resealing
- Dielectric breakdown is an irreversible process that results in destruction of the insulating material, so pore formation is a distinct process



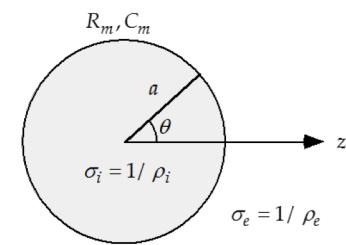
A simple model: Pore energy

- Pore energy is the energy required to introduce a single pore of radius, r .
- Only hydrophilic pores allow the passage of water-soluble substances like ions.
 - They conduct current
- The energy for creating the two types of pores are different.
 - $U(r)$ =hydrophobic energy
 - $E(r)$ =hydrophilic energy

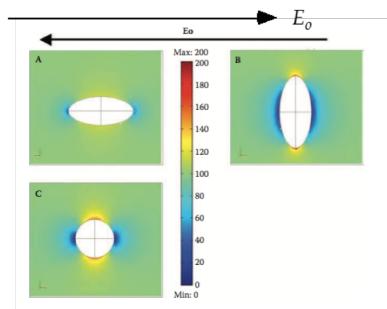
Energy to create a cylindrical gap Effect of transmembrane potential

$$U(r) \approx E_* \left(\frac{r}{r_*} \right)^2 - \frac{1}{2h} (\varepsilon_w - \varepsilon_m) V_m^2 \pi r^2$$

Recall:



$$\begin{aligned} v_m &= \frac{3\sigma_i \sigma_e R_m E_o a \cos \theta}{a\sigma_i + 2a\sigma_e + 2\sigma_i \sigma_e R_m} (1 - e^{-t/\tau'}) \\ &= \frac{R_m}{R_m + R_a} \left(\frac{3}{2} E_o a \cos \theta \right) (1 - e^{-t/\tau'}) \end{aligned}$$



$$\begin{aligned} \frac{1}{\tau'} &= \frac{1}{R_m C_m} + \frac{2\sigma_i \sigma_e}{a C_m (\sigma_i + 2\sigma_e)} \\ &= \frac{1}{R_m C_m} + \frac{1}{R_a C_m} \end{aligned}$$

$$R_a = a(\rho_i + 0.5\rho_e)$$

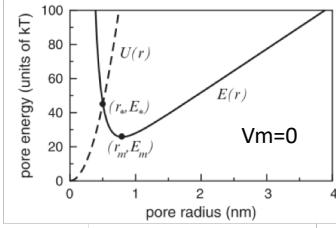
A simple model: Pore energy

- The hydrophilic pores are a bit more complicated
 - Why?
 - Need to represent them with terms that aren't dielectric since they're conductive openings

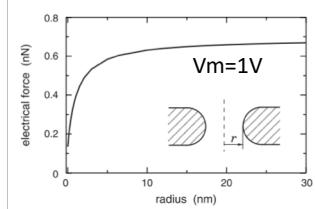
Energy to create a cylindrical gap
Effect of trans-membrane potential

$$U(r) \approx E_* \left(\frac{r}{r_*} \right)^2 - \frac{1}{2h} (\varepsilon_w - \varepsilon_m) V_m^2 \pi r^2$$

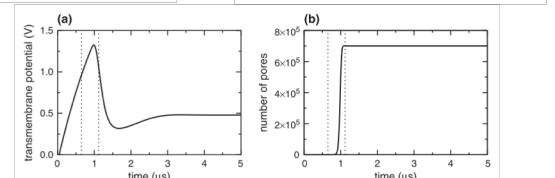
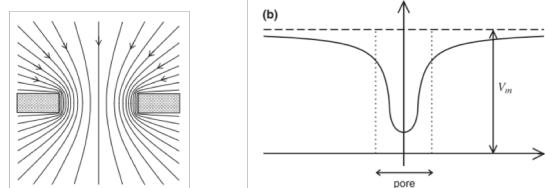
$$E(r) = \beta \left(\frac{r_*}{r} \right)^4 + 2\pi\gamma r - \sigma\pi r^2 - \int_r^\infty F(r', V_m) dr'$$



$$F(r, V_m) = \frac{F_{\max}}{1 + r_h/(r + r_t)} V_m^2$$



I-V Relation for Pores

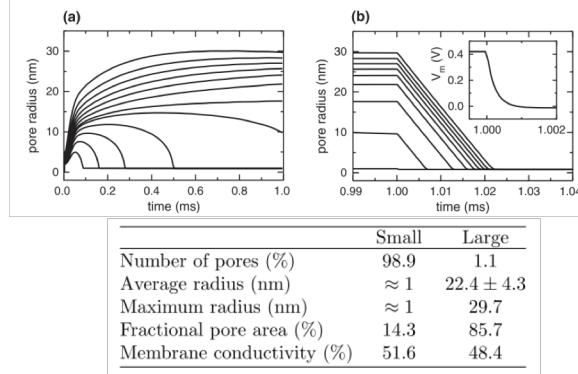


Time	1.119 μs	1 ms	1.05 ms	10 s
Number of all pores	700,311	700,314	700,291	1,719
Number of large pores	700,311	7,582	0	0
Radius of large pores ^a (nm)	1.50 ± 0.28	22.4 ± 4.3	—	—
Maximum radius (nm)	3.83	29.7	0.804	0.804
Fractional pore area ^b (×10 ⁻⁶)	162.2	460.4	45.2	0.11
Membrane conductivity ^b (S m ⁻²)	4.35 × 10 ⁴	3.91 × 10 ⁴	1.44 × 10 ⁴	35.5
Transmembrane potential (V)	1.056	0.420	-0.024	-0.0796

^aMean ± standard deviation

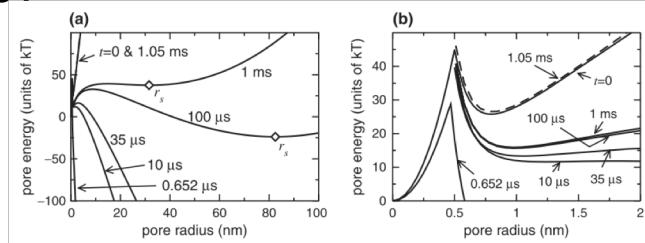
^bIncludes conductivity of membrane without pores, 20 S m⁻²

Pore Evolution and Shrinkage

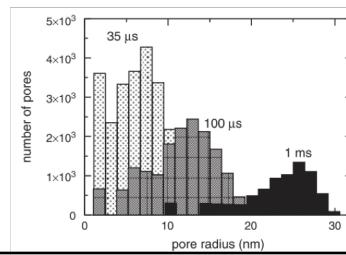


- Some pores approach ~ 1 nm because the formation of pores decreases V_m and causes them to approach r_m
- Others are larger and are limited by changes in membrane tension due to pore formation

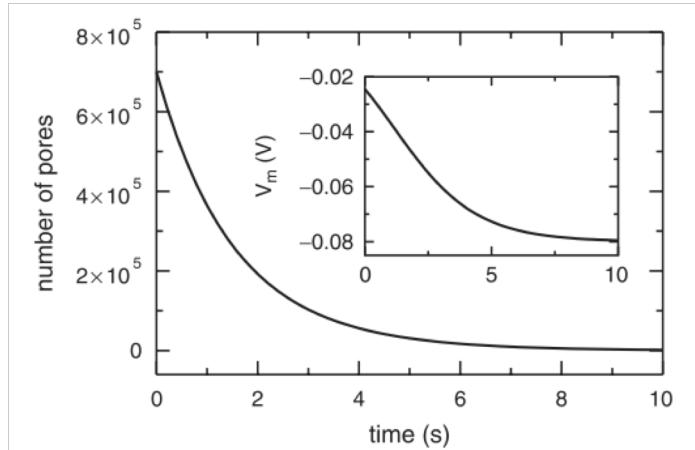
Why are there two groups of pore sizes?



Large pore distributions:



Pore resealing



Experimental observations

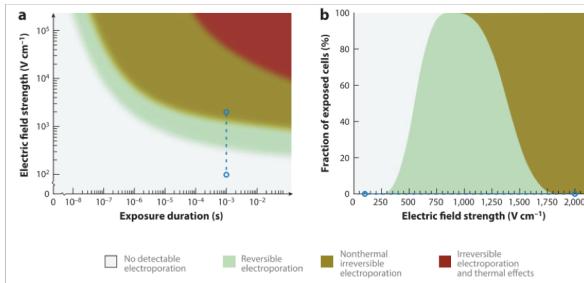
Membrane system	Threshold voltage (mV)	Reference
Squid giant axon	~1V	Benz and Conti [2]
Frog skeletal muscle	250-350mV	Chen and Lee [7]
Valonia utricularis	~-0.85V	Coster and Zimmermann [10]
Lipid vesicles	~210mV	El-Mashak and Tsong [12]
Frog cardiac muscle	0.6-1.1V	O'Neill and Tung [32]
Human erythrocytes	~1V	Kinosita and Tsong [22]

Membrane system	Resealing time	Reference
Squid giant axon	<5μs	Benz and Conti [2]
Artificial lipid bilayers	~100μs	Chernomordik et al. [8]
Artificial lipid bilayers	several seconds	Glaser et al. [15]
Human erythrocytes	~2hrs (37°C), ~20hrs (3°C)	Kinosita and Tsong [23]
Frog (Rana pipiens) skin	2-3 min	Powell et al. [34]
Sea urchin eggs	~20s	Rossignol et al. [37]
Erythrocyte ghosts	~200ms	Sowers and Lieber [42]

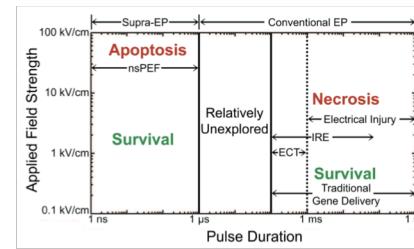
Membrane system	Pore size	Reference
Artificial lipid bilayers	40Å	Benz and Zimmermann [3]
Human erythrocytes	20-120nm	Chang and Reese [6]
Artificial lipid bilayers	4.3-30Å	Chernomordik et al. [8]
Artificial lipid bilayers	6-10Å	Glaser et al. [15]
Human erythrocytes	3.5-4.2Å	Kinosita and Tsong [23]
Erythrocytes ghosts	~8.4nm	Sowers and Lieber [42]

Applications of Electroporation

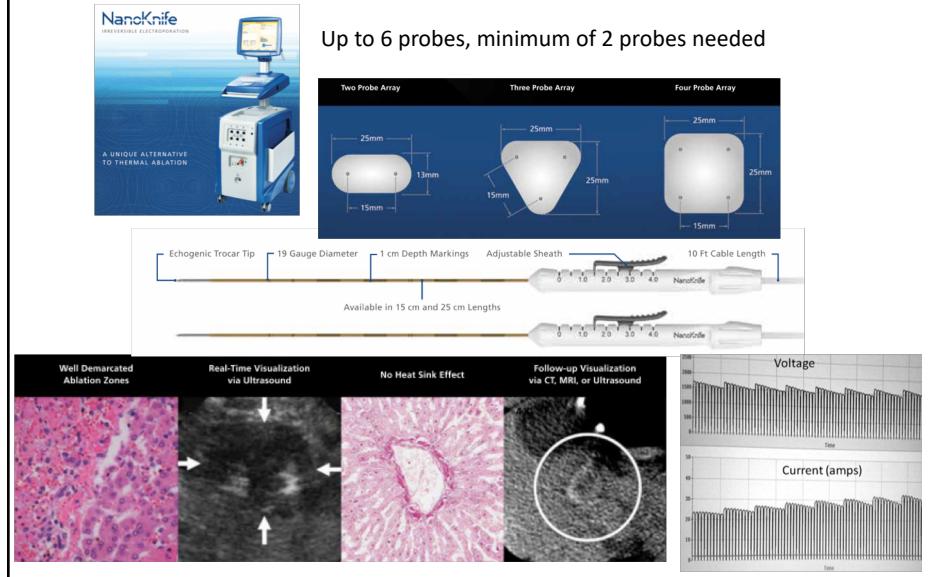
- Medical
 - Electrochemotherapy
 - Nonthermal ablation
 - Gene transfer (electrotransfer)
 - Transdermal drug delivery
- Industrial/energy
 - Decontamination of waste water
 - Biorefineries
- Food processing
 - Project FieldFOOD



- *No detectable electroporation*: No molecular transport observed (e.g. with propidium iodide uptake).
- *Reversible electroporation*: Temporary pores for molecular transport are formed, but they reseal, transport ceases, and the cells are viable after shock termination.
- *Nonthermal irreversible electroporation*: Membrane transport and intracellular leakage is extensive and/or the resealing is too slow for the cells to recover. They die, but there is generally no thermal damage to the cell.
- *Thermal Irreversible electroporation*: Strong fields lead to high electric currents and a temperature increase high enough for thermal damage to the cell (e.g. protein denaturing).



Commercial device



Project FieldFOOD



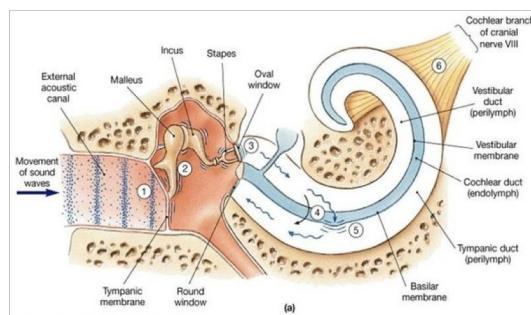
- “Use of Pulsed Electric Field (PEF) technology in producing fruit juice, tomato products, wine, cider and olive oil will be analyzed and optimized. To these ends flexible and portable low-cost pulse generators will be designed. The results of the project will also be of interest for the food sector as a whole and for the pharmaceutical and biotechnological sector.”
- Used to modify food textures by breaking down cell membranes in plant/animal tissue
- Kills microbes to increase shelf life without damaging other proteins and impacting taste
- Increasing bioavailability
- “The lack of industrial-scale equipment and the high costs involved have limited the commercial use of PEF technology in the food industry... To eliminate these bottlenecks the European FieldFOOD project has been established.”

Biorefineries

- Extracting biomolecules and biomass
- Uses the electrolysis and associated shock waves to fragment particles
- Antioxidant activity of grape seeds could be increased after exposure to 80 kJ/kg

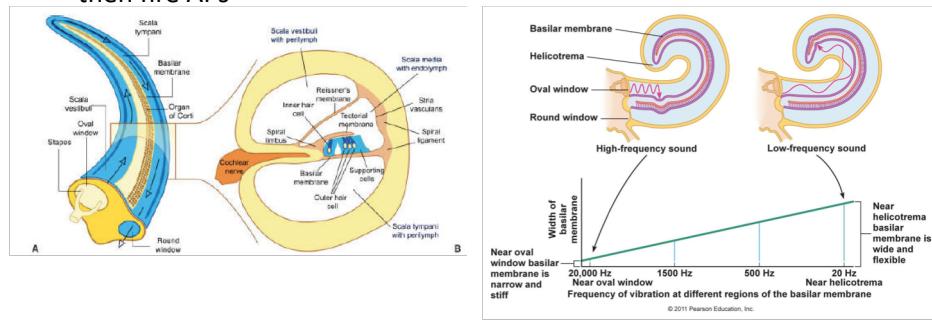
Hearing overview:

- The outer ear focuses sound waves toward the tympanic membrane.
- The pinna (visible portion) and ear canal act as an acoustic filter that has different properties for sound coming from different directions
- The tympanic membrane vibrates when acoustic waves hit it and it causes the middle ear ossicles to move
- The middle ear acts to overcome the mechanical impedance mismatch of air and water by “impedance matching” with the malleus, incus, and stapes.
- The stapes causes another membrane at the oval window to vibrate and transmits the acoustic wave to the fluid-filled inner ear.
- The round window is also flexible to allow for fluid displacement



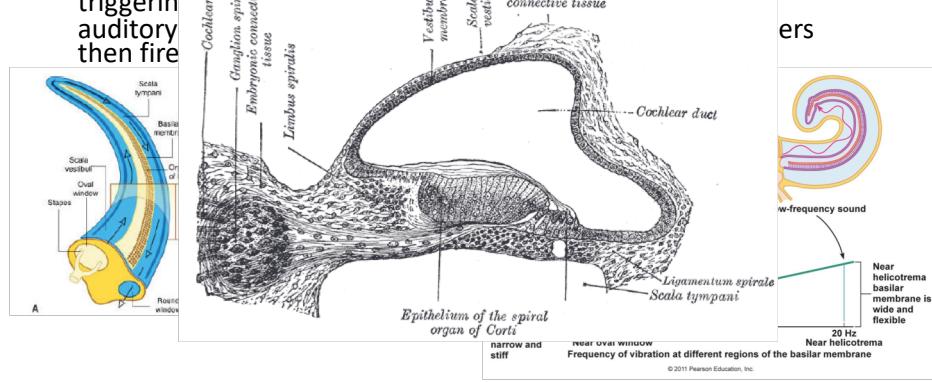
Cochlea structure and function

- The cochlea is a spirally shaped bony path (makes 2.5 turns) that contains the Organ of Corti
- The Organ of Corti transduces vibration to APs. It is distributed along the length of the cochlea between fluid-filled chambers
- Basilar membrane displacement causes depolarization of hair cells (via K^+ influx), which in turn causes calcium influx, triggering exocytosis of neurotransmitters at synapses with auditory nerve fibers of the spiral ganglion. These nerve fibers then fire APs



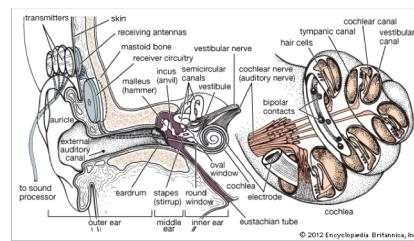
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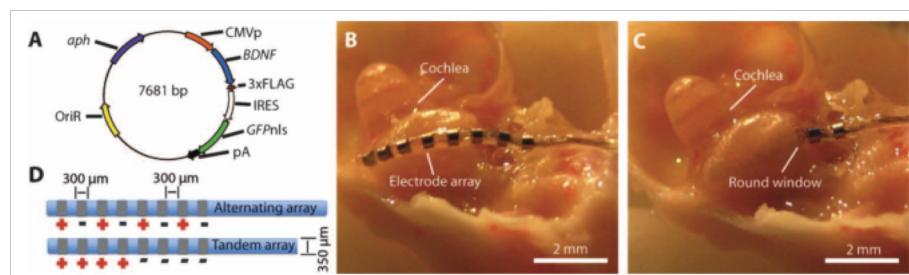
Cochlear implant

- An electrode array is implanted through the round window to be in close proximity to the damaged hair cells and spiral ganglion neurons (SGNs).
- SGNs are directly stimulated by the implant, typically in a monopolar configuration
 - Each platinum electrode is ~500 µm in diameter
- In profound hearing loss SGNs atrophy and retreat back from the Organ of Corti
 - This is thought to be caused by loss of Brain-derived neurotrophic factor (BDNF) in the Organ of Corti
- This results in a large gap between the electrode and the SGNs
 - Why is this a problem? Can't we just increase the amplitude?

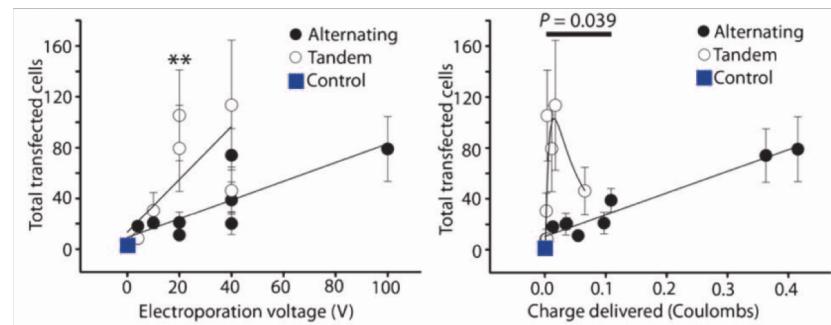


Gene therapy: a unique approach

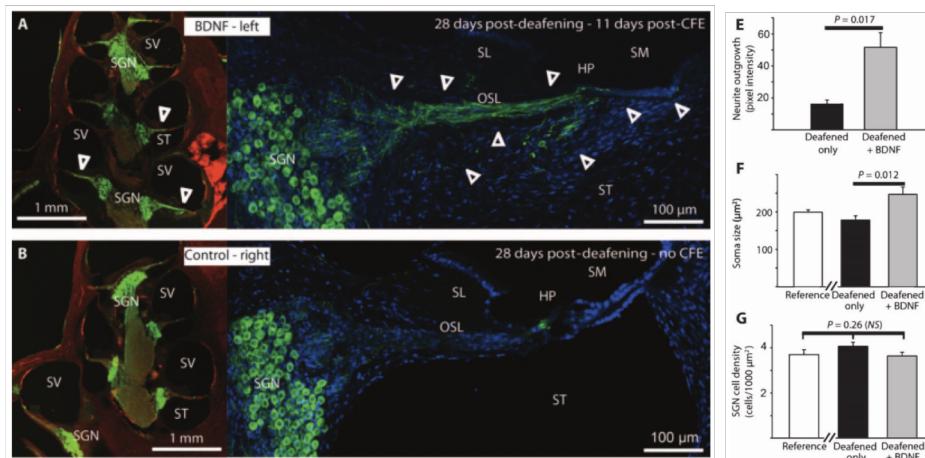
- Plasmid was injected into the perilymph of the scala tympani and scala vestibule
- Promoter to drive expression of BDNF and a GFP reporter
- Cells were transfected with the BDNF gene cassette via electroporation with voltages from 4 V - 100 V (5 - 50 pulses, 50 ms duration).



Electroporation efficiency



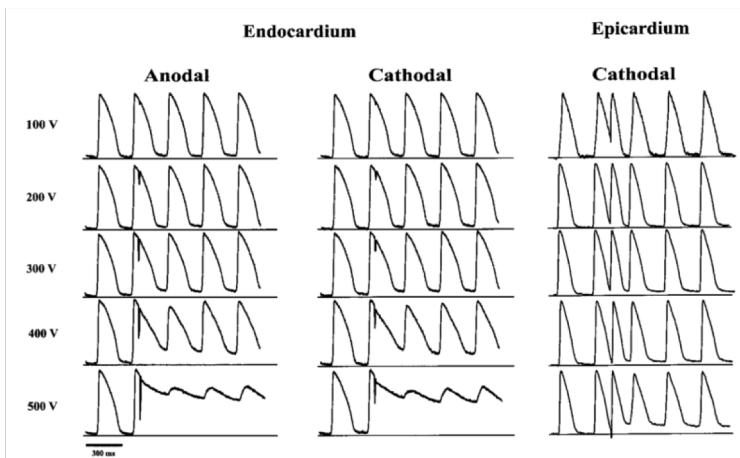
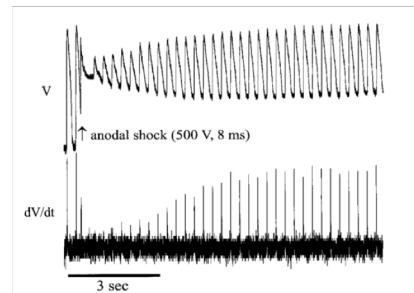
SGN growth



They are now developing an electrode array with a fluid-filled lumen to inject the plasmid near the electrodes

Electroporation of Cardiac Tissue – Defibrillation

- Electrophysiological definition of electroporation:
 - A 10% reduction of any one of:
 - MDP
 - APA
 - dV/dt_{max}
 - Comparing the beats immediately before and after the shock

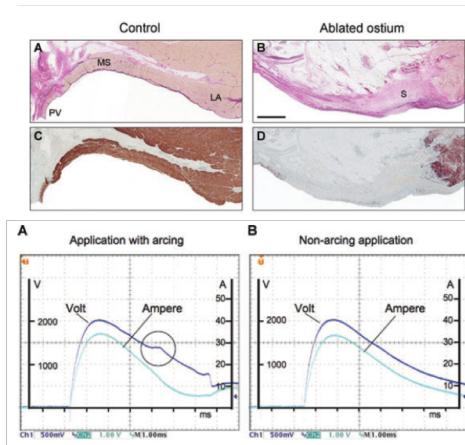


Non-thermal ablation

- Much like your homework we know that a conductive fluid will heat up when an electric field is applied:
 - $\Delta T = (V/L)^2(\sigma/\rho c)\Delta t$
 - L is chamber length
 - σ is conductivity
 - ρ is fluid density
 - c is specific heat
 - Δt is pulse duration

PVI with electroporation

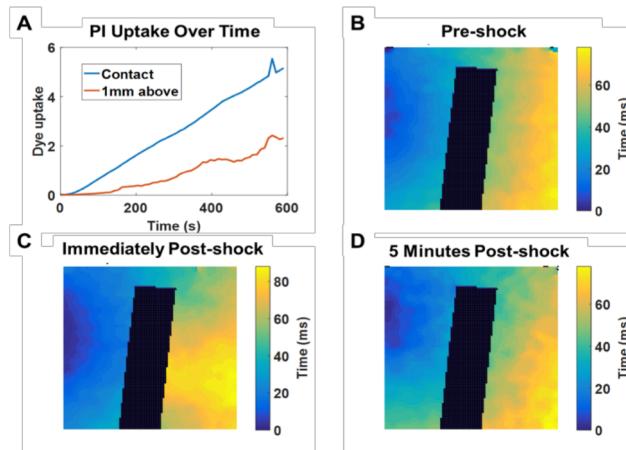
- Modified pacing catheter with 10 electrodes
- Attempt to get one location like the cryoballoon
- 200 J defib shock
- Pulmonary vein stenosis was less than for RF
- No phrenic nerve damage
- What will the issues be?



PVI with electroporation vs other modalities

- Cryoablation has little impact on the extracellular matrix (ECM)
- RF severely damages ECM
 - Since atrial tissue is thin and relatively fragile this limits the number of RF applications
- Nanosecond electroporation (NEP) is thought to preserve ECM structure
- Cryoablation, unlike RF, yields relatively little control over the size and shape of the lesion
- NEP confers similar control as RF
- NEP has good depth penetration and only requires seconds (<10) to achieve it.
 - Cryoablation needs significant application time (2-5 min), and RF requires multiple (30 s-2 min) applications

The issue of tissue contact and reversibility



Clinical Trial for AF

Ablation of Atrial Fibrillation With Pulsed Electric Fields

An Ultra-Rapid, Tissue-Selective Modality for Cardiac Ablation

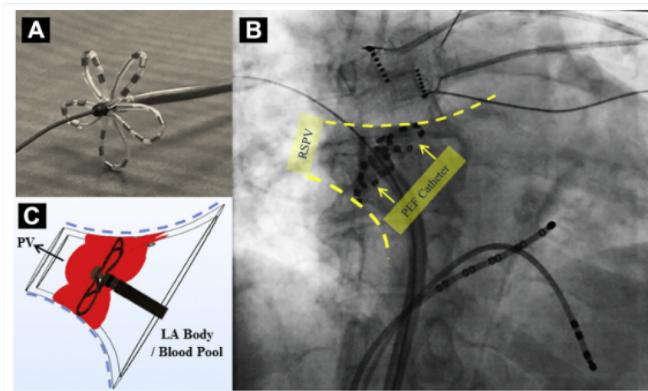
Vivek Y. Reddy, MD,^{a,b} Jacob Koruth, MD,^c Pierre Jais, MD,^d Jan Petru, MD,^d Ferdinand Timko, MD,^d Ivo Skalsky, MD,^d Robert Hebler, MD,^e Louis Labrousse, MD,^f Laurent Barandon, MD,^f Stepan Kralovec,^b Moritoshi Funosako, MD,^b Boochi Babu Mannava, MD,^b Lucie Sediva, MD,^b Petr Neuzil, MD, PhD^b

OBJECTIVES The authors report the first acute clinical experience of atrial fibrillation ablation with PEF—both epicardial box lesions during cardiac surgery, and catheter-based PV isolation.

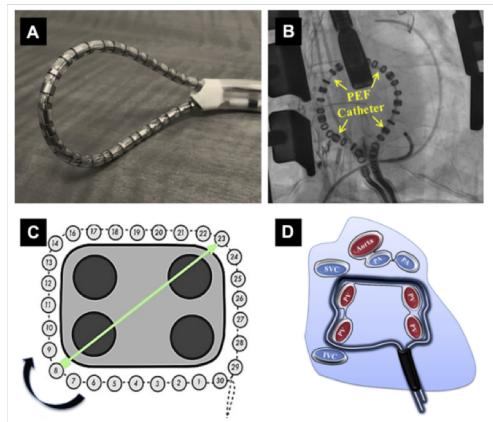
BACKGROUND Standard energy sources rely on time-dependent conductive heating/cooling and ablate all tissue types indiscriminately. Pulsed electric field (PEF) energy ablates nonthermally by creating nanoscale pores in cell membranes. Potential advantages for atrial fibrillation ablation include: 1) cardiomyocytes have among the lowest sensitivity of any tissue to PEF—allowing tissue selectivity, thereby minimizing ablation of nontarget collateral tissue; 2) PEF is delivered rapidly over a few seconds; and 3) the absence of coagulative necrosis obviates the risk of pulmonary vein (PV) stenosis.

METHODS PEF ablation was performed using a custom over-the-wire endocardial catheter for percutaneous transseptal PV isolation, and a linear catheter for encircling the PVs and posterior left atrium during concomitant cardiac surgery. Endocardial voltage maps were created pre- and post-ablation. Continuous and categorical data are summarized and presented as mean \pm SD and frequencies.

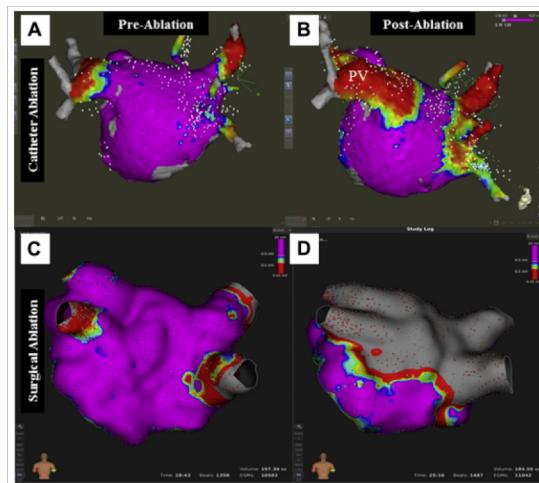
The electrode and location (endocardial)



The electrode and location (epicardial)

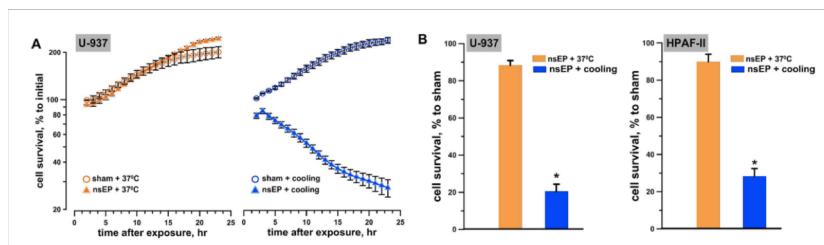


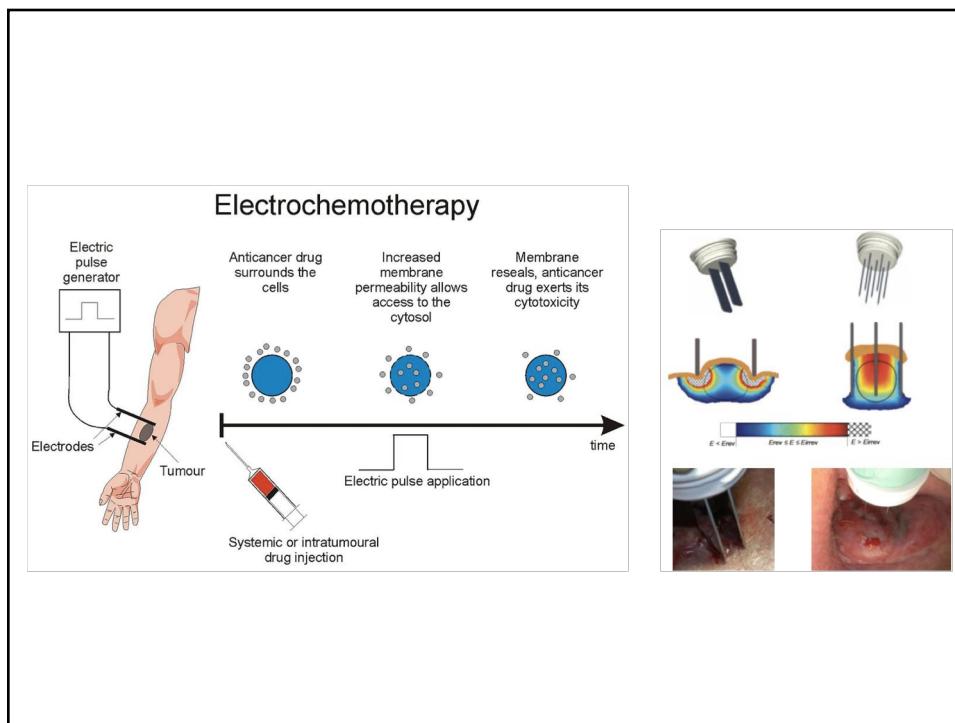
Baseline and Post-Ablation Electroanatomical Voltage Mapping



Studies on Cardiac Electroporation						
Year	Author	Ablation location	Cather Design	Electroporation parameters (Energy/Pulse Number/Pulse Duration)	Outcome	
2007	Lavée	Epicardial	Hand held clamp	1000-1500V/ 8-32 / 100 μs	Lesion depth of 40-140 mm	
2009	Hong	Epicardial	Bipolar jaws + linear	10-40/ 100-400μs	Transmural except over epicardial fat	
2011	Wittkampf	PV ostia	Circular	200 J/ 4 / 6ms	Reduction in EGM amplitude	
2012	Wittkampf	Epicardial	Circular	100-200 J/ 1 / 6ms	Transmurality seen in 100% at 200J	
2013	Du Pre	Epicardial	Circular + linear	50-360 J/ 1	Transmurality seen in 32%	
2014	Neven	Epicardial	Linear	50-300J/ 1 / 6ms	Transmurality seen in 100% of the 100 and 300 J lesions.	
2014	Neven	Epicardial	Circular	50-200 J/ 1	Transmural in 20% at 200J	
2014	Van Driel	PV	Circular	200 J/ 10	No PV stenosis	
2014	Neven	Epicardial	Circular	200 J/ 1	Transmurrality seen in 4 of 13 (31%) lesions	
2014	DeSimone	PV ostia	Circular	7500 μA/ 140 μs	Ablation successful with preliminary efficacy and safety with balloon prototype	
2015	Van Driel	Phrenic Nerve	Circular	200 J/ 1	No damage. 2/19 had acute 30 min effect	
2016	Zager	Epicardial	2 needle electrode	50-500V/ 10-20 / 100 μs	High output, longer pulse duration and a larger number of pulses can be used to increase tissue damage	
2016	Madhaven	Cardiac ganglia (CG)	Deflectable multiarray + Linear "Finger"	100-1200 μA	DC of 500 μA was effective at 4/7 CG sites and 5,000 μA ablated the CG at 5/7 sites	
2016	Madhaven	Cardiac ganglia	Deflectable multiarray + Linear "Finger"	3000 μA	CG ablated at 22/23 sites	

Test your understanding: Why does this happen?

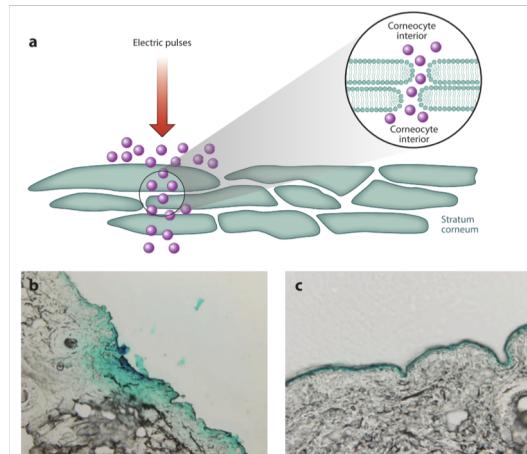




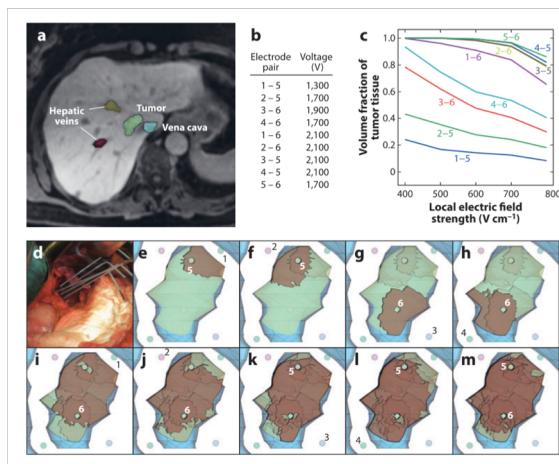
Electrochemotherapy



Transdermal drug delivery



Liver tumor



Not only cell size that matters – toward selectivity

Cell line	Organism	Type	LE50 (V/cm)	Cell size (μm)
MDA-MB-231	Human	Breast cancer	1200-1300	17.30 \pm 0.37
AsPC-1	Human	Pancreatic cancer	1100-1200	14.90 \pm 0.57
LNCaP	Human	Prostate cancer	1050-1150	19.05 \pm 0.48
HL-1	Mouse	Cardiomyocyte	1000-1100	15.03 \pm 0.31
HDF	Human	Dermal fibroblasts	950-1050	19.90 \pm 1.08
KPC	Mouse	Pancreatic cancer	900-1000	12.80 \pm 0.25
CLU-172	Mouse	Hypothalamus	700-800	19.17 \pm 1.03
TRAMP-C2	Mouse	Prostate cancer	600-700	16.92 \pm 0.40

Review