

Coincident light/ultrasound therapy to treat bacterial biofilms

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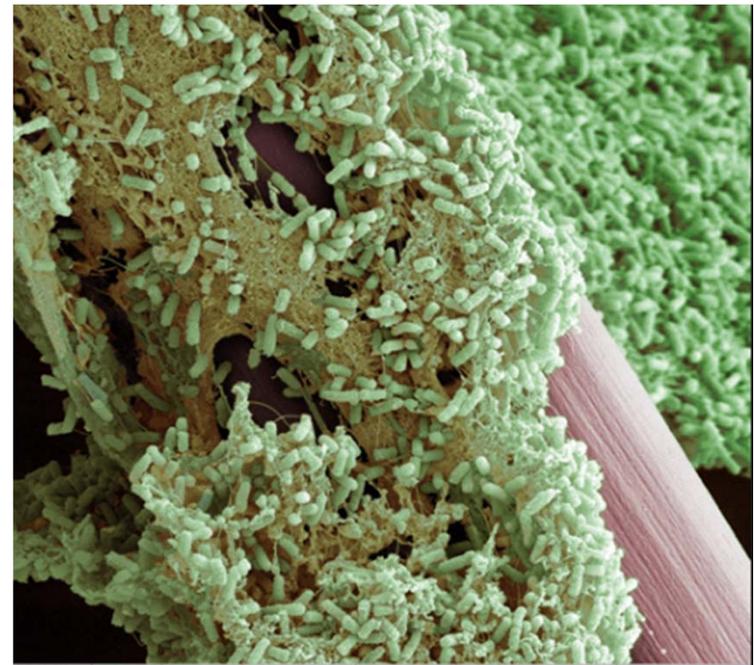
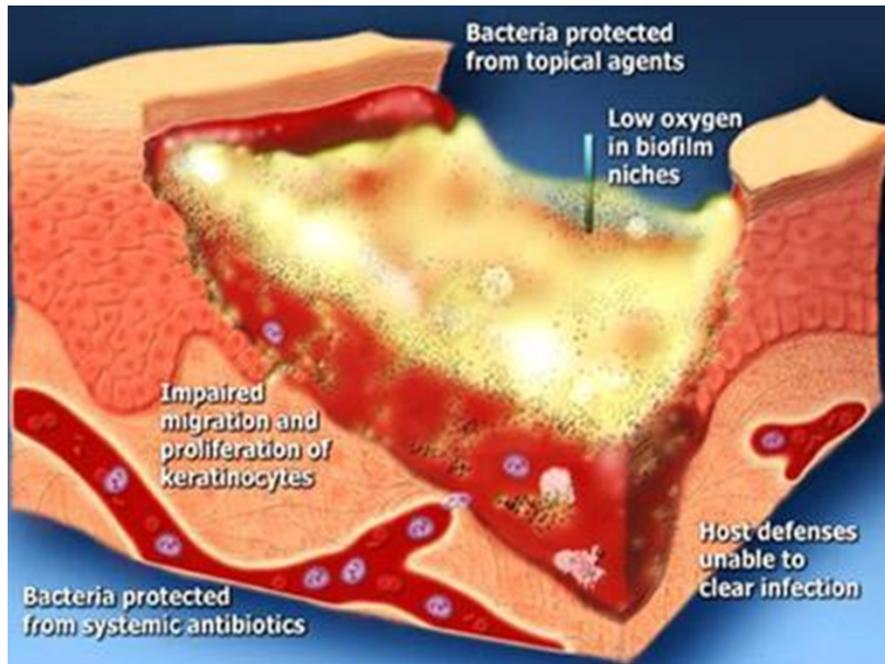
Disclosure: Both authors are employees of and have an ownership interest in Photosonix Medical

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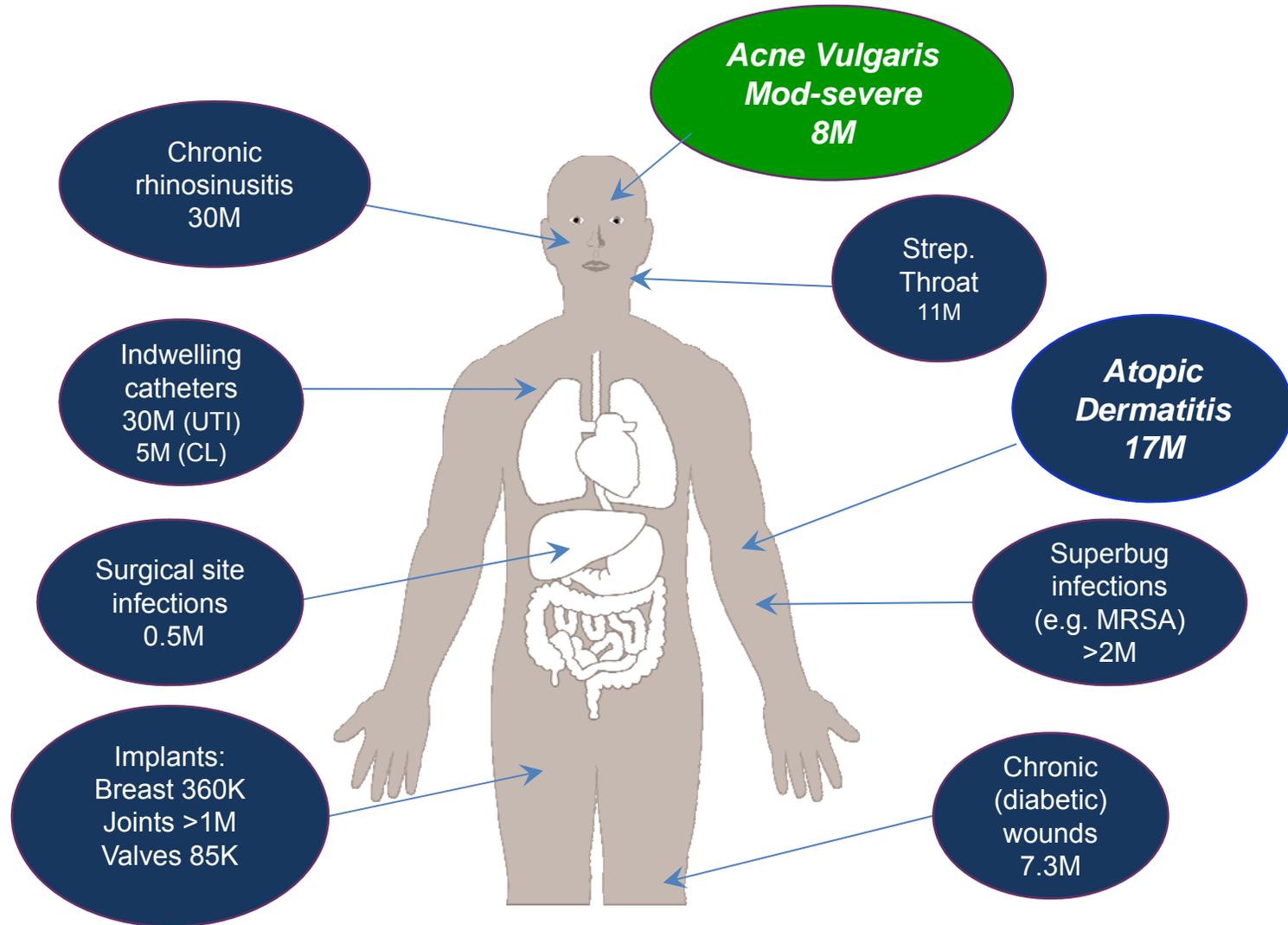
BIOFILMS: BACTERIAL BODY ARMOR

Persistent communities of bacteria



- Form polysaccharide matrix; reduce metabolism
- Physical & metabolic barrier
- Treatment requires physical stress + antibacterial

BIOFILMS: EXTENSIVE CLINICAL IMPACT



>80% of all persistent infections thought to be biofilm mediated

Numbers = pt populations



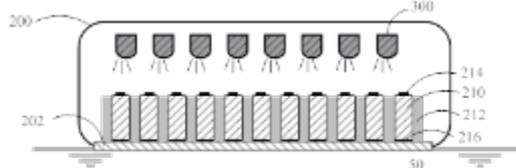
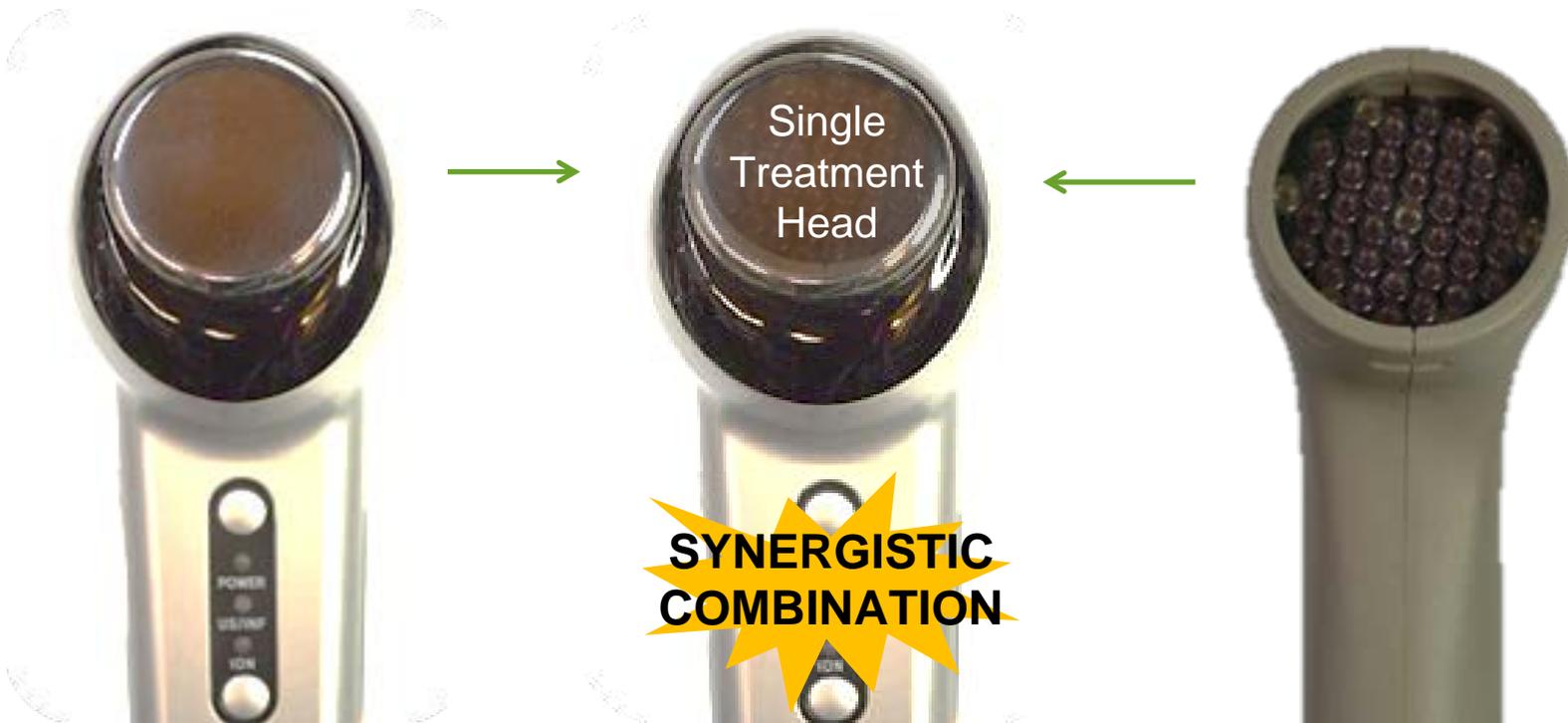
PhotoSonix Medical, Inc

CLENS™

COINCIDENT LIGHT ENERGY AND NON-FOCUSED ULTRASOUND

Ultrasound

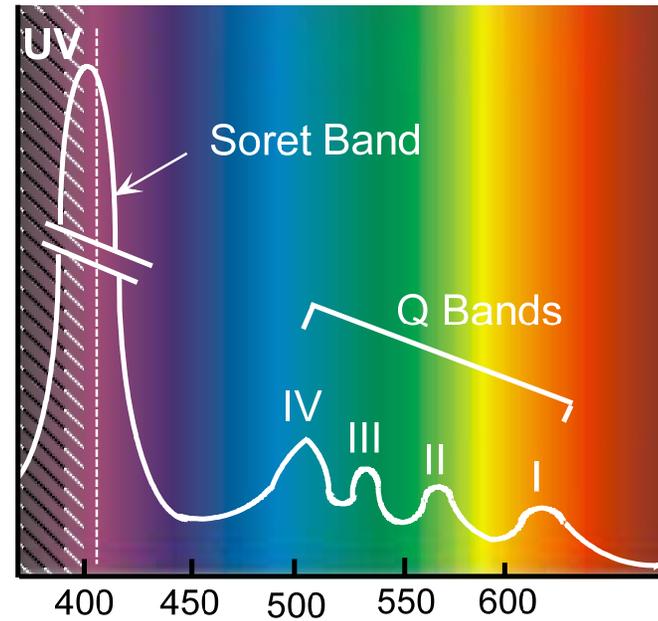
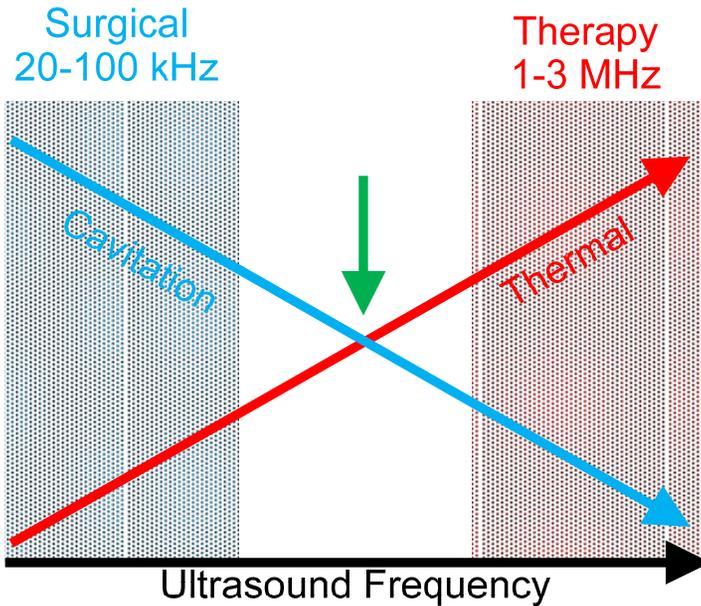
Light



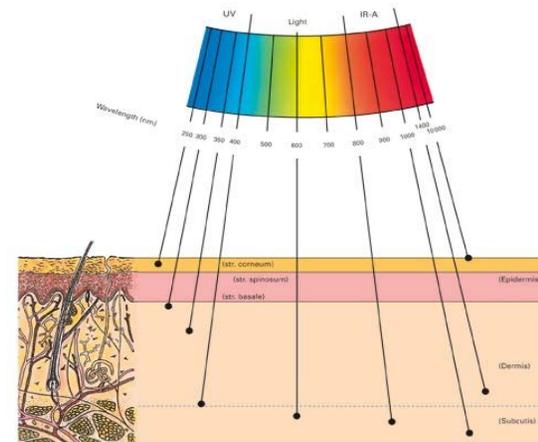
- *Sound activates, light kills*
- *Must happen coincidentally*
- *3 issued patents; 3 pending*



SETTING ENERGY PARAMETERS

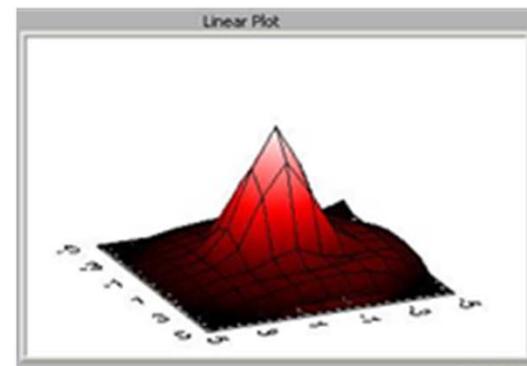
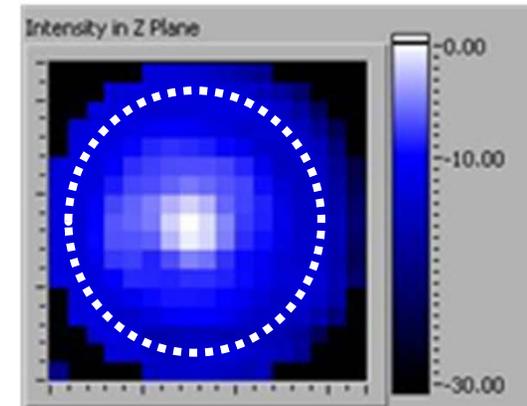
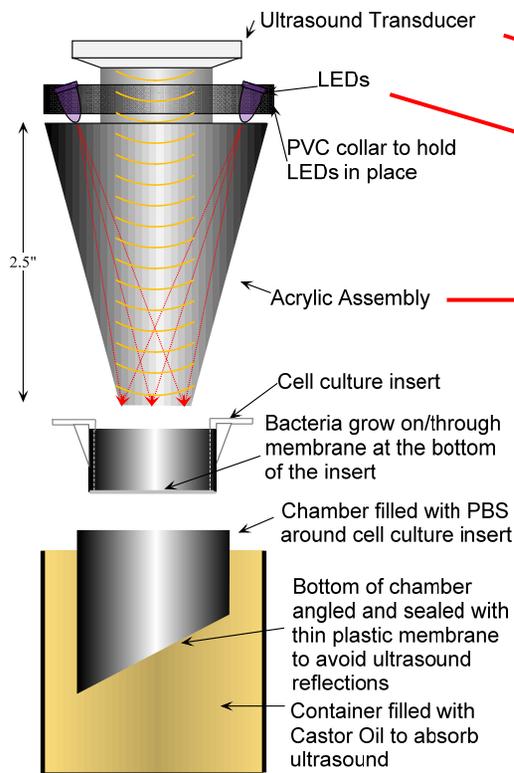


- Ultrasound
 - Creates mechanical stress
 - Minimize thermal or cavitation effects
- Light
 - W/in Soret absorption band for Protoporphyrin IX, above UV
 - Penetrates through dermis

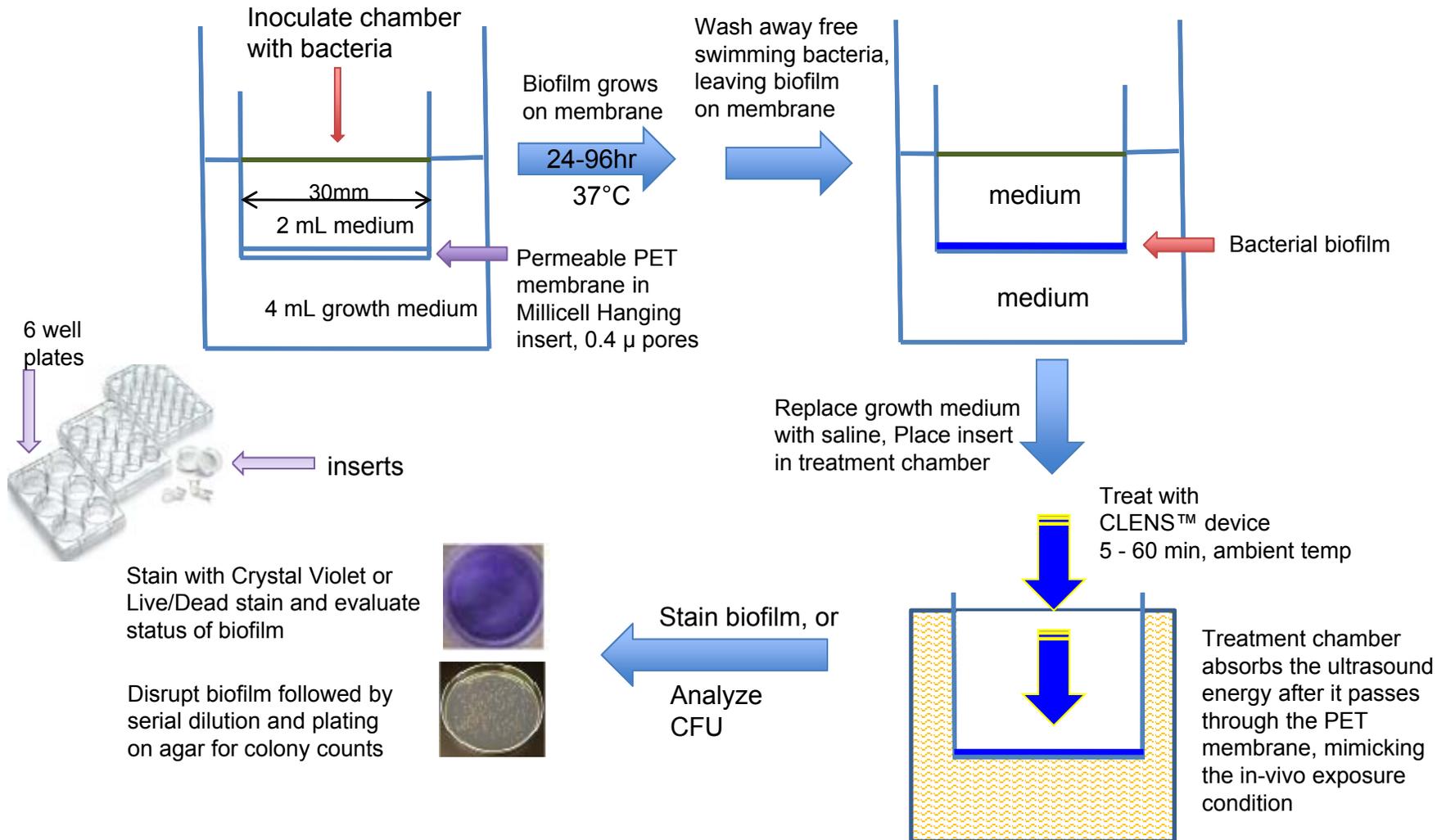


ULTRASOUND EXPOSURE METHODOLOGY

- Conical Acrylic assembly combines ultrasound and light energy
- Range of frequencies (150kHz – 2MHz), pressures ($\approx 400\text{kPa}$)
- Fully calibrated dosimetry
- Test wells (inserts) suspended in angled chamber coupled to castor oil to eliminate acoustic reflections



BIOFILM METHODOLOGY



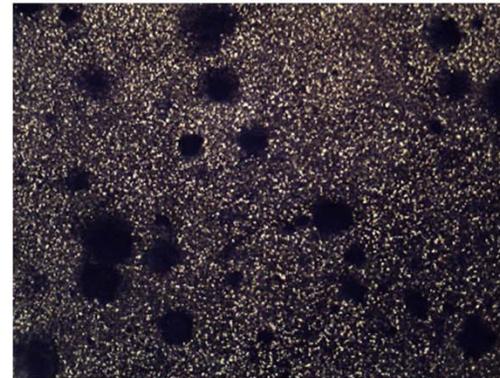
FIRST RESULTS

30 minute treatment results

24Hr RPMI Control



72Hr TSB Control



24Hr RPMI Treated



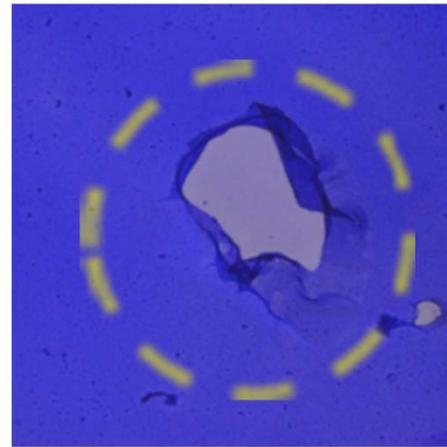
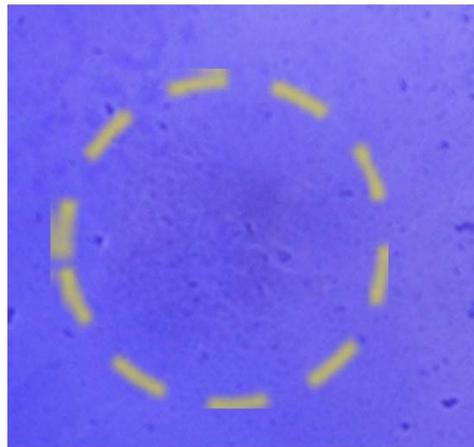
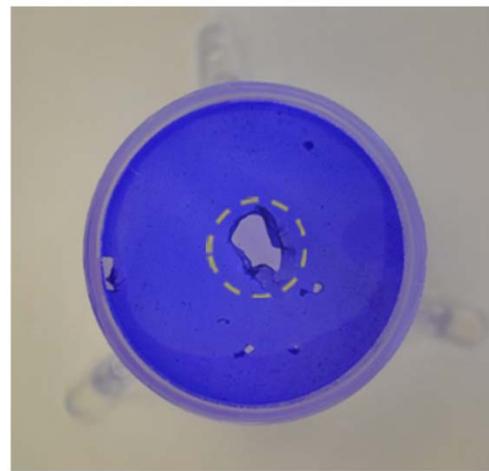
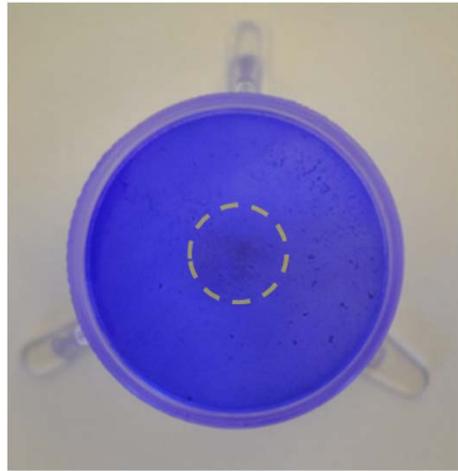
72Hr TSB Treated



FIRST RESULTS

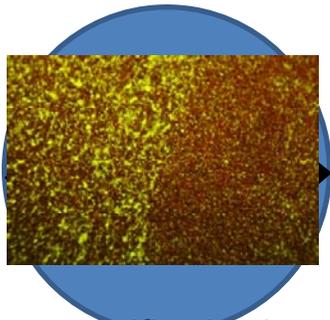
30 min Tx

60 min Tx



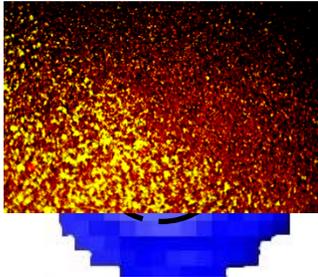
LIGHT & ULTRASOUND SYNERGY

Extent of blue light illumination

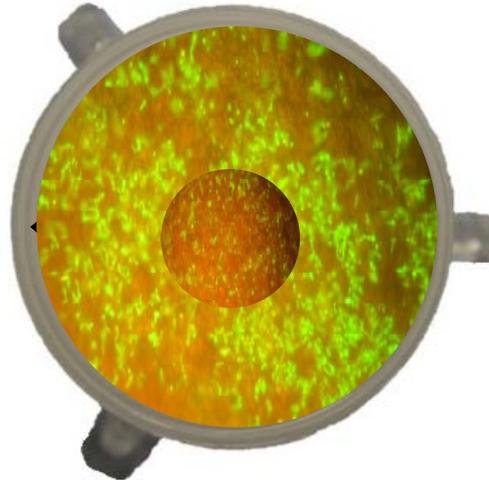


Lower magnification images

Extent of ultrasound energy

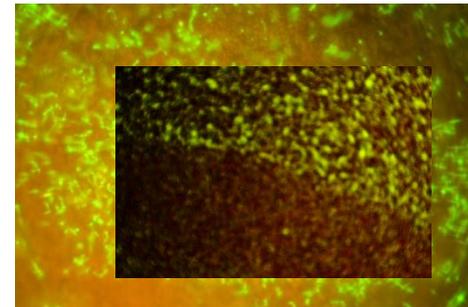


Only central diameter exposed to both types of energy

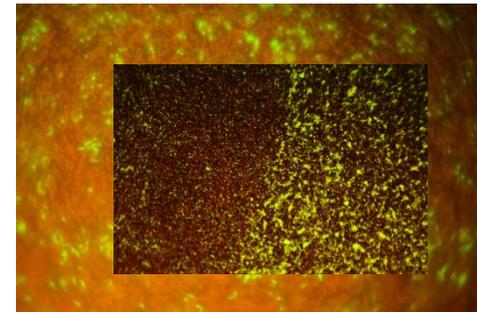


Effects are visible on both microscopic and macroscopic levels

High magnification - outer zone



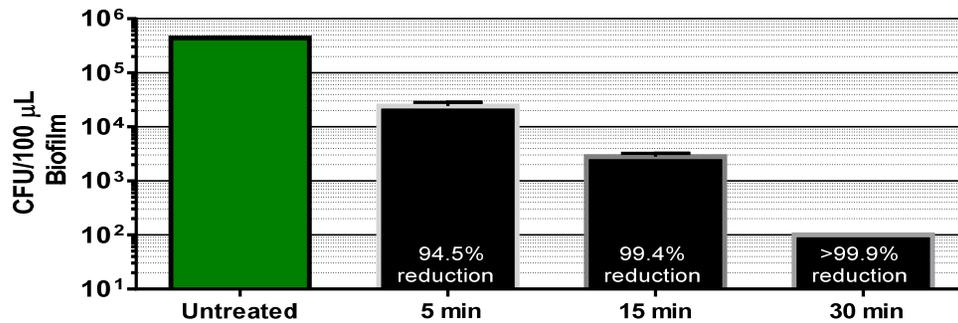
Lower magnification images
High magnification - overlap zone



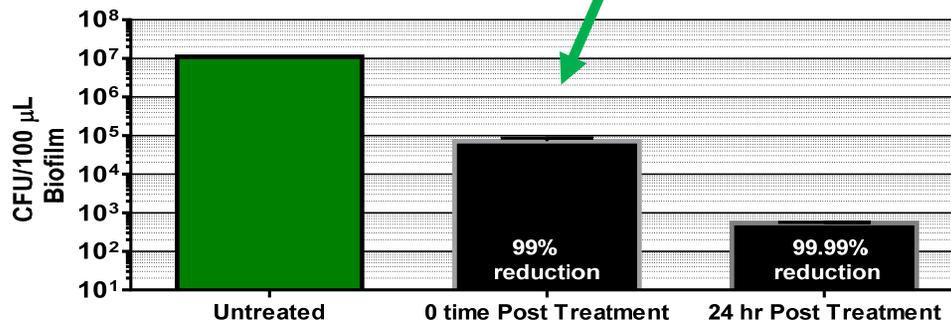
Live (green) / dead (red) stain

QUALITATIVE TO QUANTITATIVE

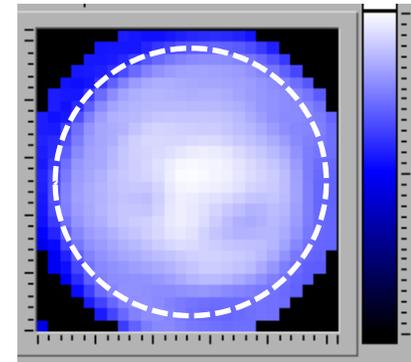
- Redesigned treatment head
 - Allows more LEDs, higher light output
 - Insonifies entire surface of insert
 - Quantification of bacteria via CFUs



Efficacy vs. length of exposure

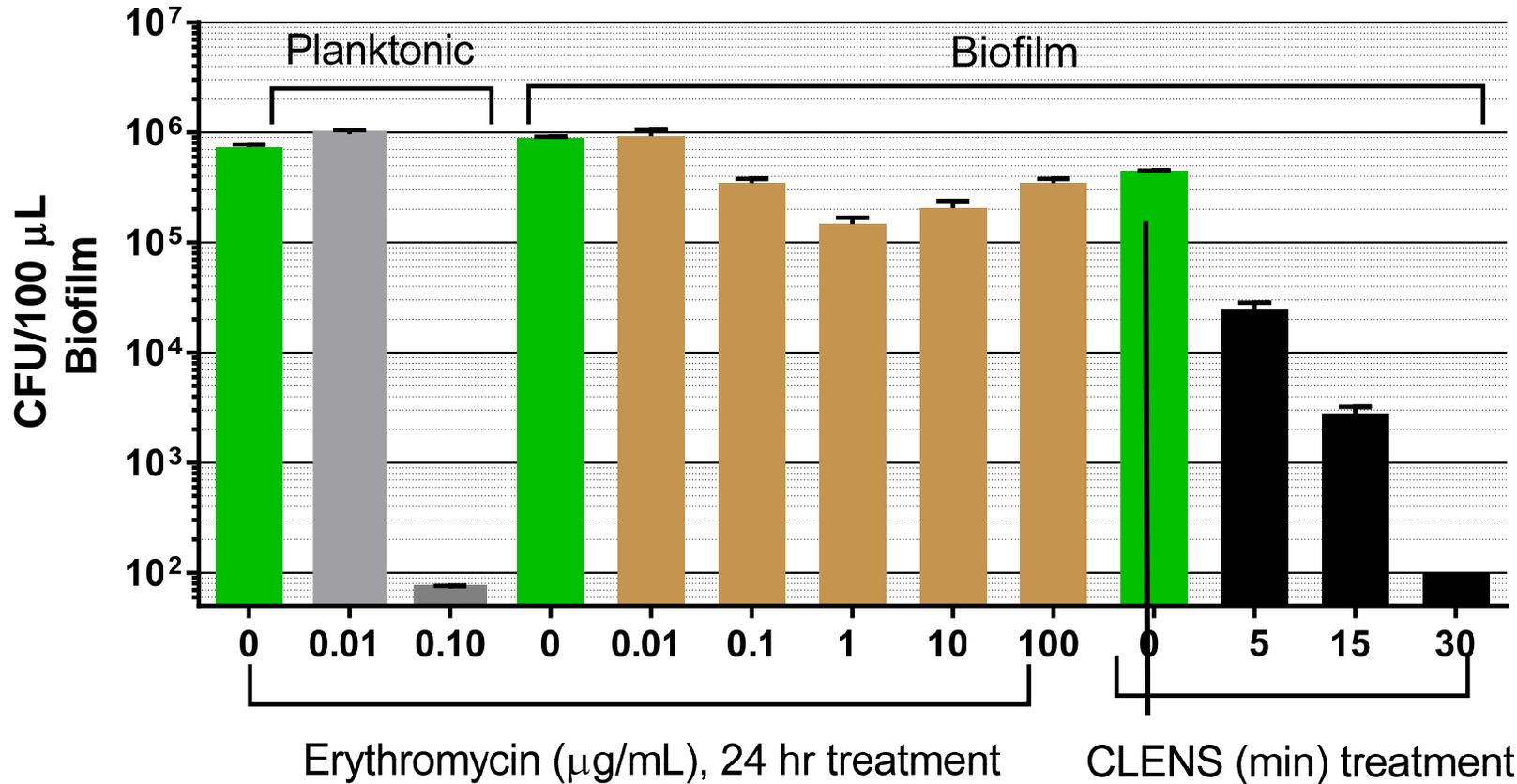


Efficacy increases with time post treatment



CLENS VS. ERYTHROMYCIN TREATING BIOFILM

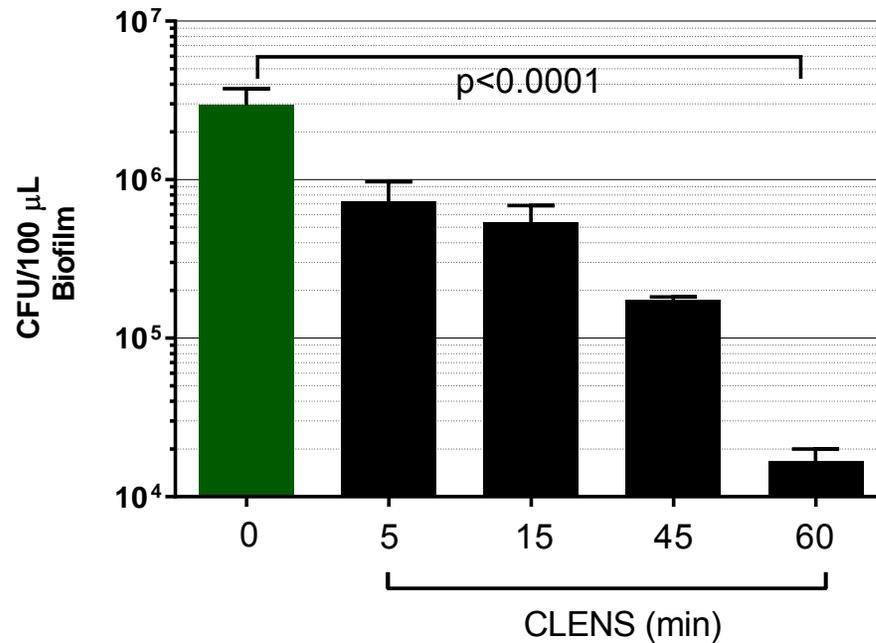
- Biofilm bacteria are >3000x more resistant than planktonic



- 5 min CLENS superior to 24 hr of 100 µg/mL erythromycin

CLENS EFFECTIVE AGAINST RESISTANT STRAINS

CLENS treatment of *S. aureus*, MRSA strain Mu50, biofilm

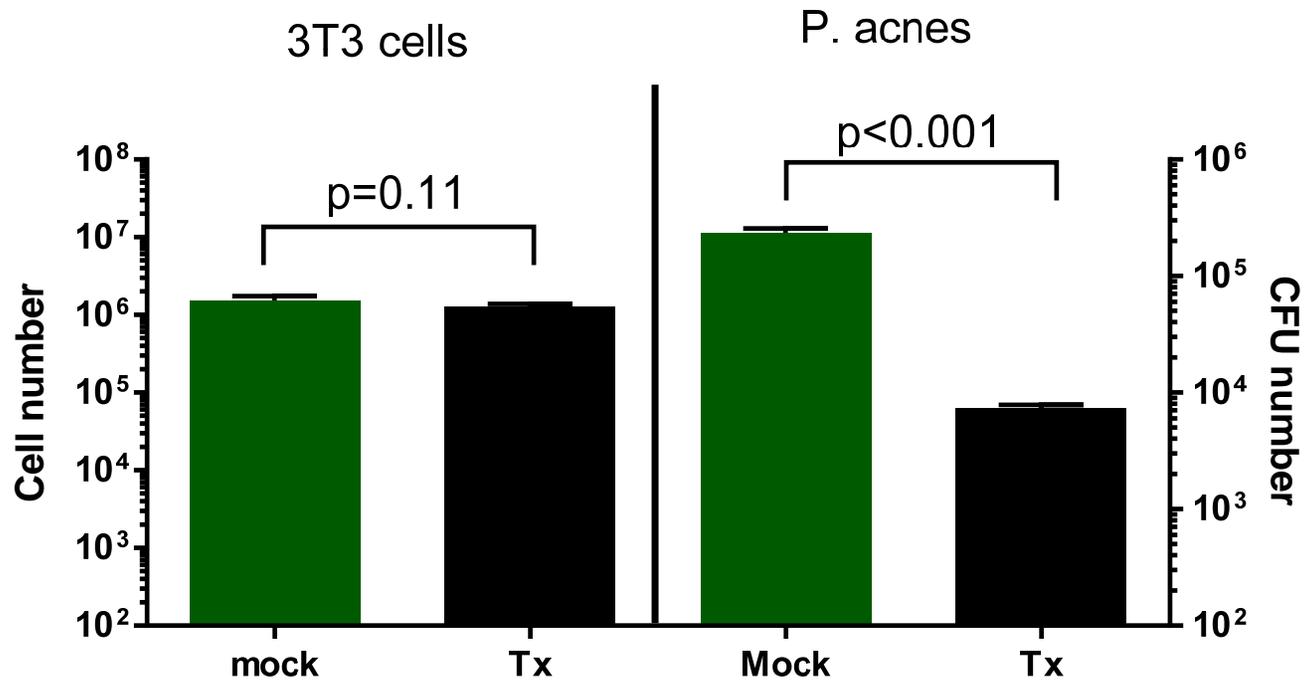


✓ Successful biofilm treatments

- *S. epidermidis*, Reference biofilm-producing bacteria
- *S. aureus*, Atopic Dermatitis, MRSA
- *P. acnes* killed within 5 mins; effect continues 24 hrs

S. epidermidis RP62A ATCC 36984

TESTING ON MAMMALIAN CELL LINES

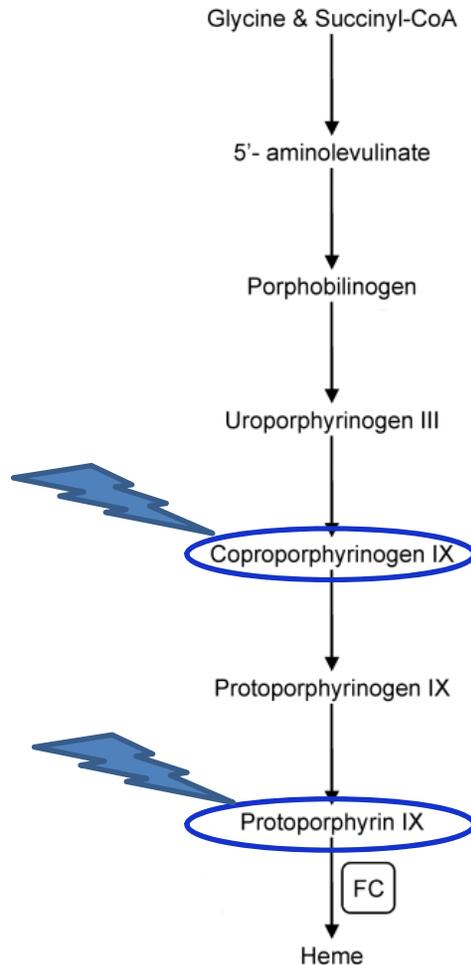


Mock or CLENS treatment 15 min
Cell or CFU numbers measured
24 hr post Tx

➤ No measurable effect



TESTING ON MAMMALIAN CELL LINES



- Porphyrin metabolic pathway common to both bacteria and mammalian cells
- In mammalian cells, pathway ends in Heme
- In bacteria, lack of Ferrochelatase stops the pathway at Protoporphyrin IX (PpIX)
- Stimulating bacteria causes a buildup of PpIX, which when subjected to blue light, produces reactive oxygen species
- *P. acnes* found to have high levels of CPp



SUMMARY

- New approach to biofilm treatment
- Proposed Mechanism of Action
 - Ultrasound stresses/stimulates bacteria
 - Light interacts with Protoporphyrin IX
 - Releases reactive oxygen species
- Next Steps
 - Optimize energies, exposure time
 - Clinical Pilot trial for bacterial reduction
- Ultimate goal
 - Treatment of dermal conditions: acne, AD