Coincident light/ultrasound therapy to treat bacterial biofilms

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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
CLENS™  
COINCIDENT LIGHT ENERGY AND NON-FOCUSED ULTRASOUND

Ultrasound ➢ Sound activates, light kills
➢ Must happen coincidently
➢ 3 issued patents; 3 pending

Light ➢ Synergistic combination
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 (∼ 400kPa)
- Fully calibrated dosimetry
- Test wells (inserts) suspended in angled chamber coupled to castor oil to eliminate acoustic reflections
**BIOFILM METHODOLOGY**

Inoculate chamber with bacteria

- 30mm
- 2 mL medium
- 4 mL growth medium

Biofilm grows on membrane

- 24-96hr
- 37°C

Wash away free swimming bacteria, leaving biofilm on membrane

Permeable PET membrane in Millicell Hanging insert, 0.4 μ pores

Biofilm grows on membrane

Replace growth medium with saline, Place insert in treatment chamber

Treatment chamber absorbs the ultrasound energy after it passes through the PET membrane, mimicking the in-vivo exposure condition

Treat with CLENS™ device 5 - 60 min, ambient temp

Analyze CFU

Stain biofilm, or

Disrupt biofilm followed by serial dilution and plating on agar for colony counts

Stain with Crystal Violet or Live/Dead stain and evaluate status of biofilm

6 well plates

inserts
FIRST RESULTS

30 minute treatment results

24Hr RPMI Control

24Hr RPMI Treated

72Hr TSB Control

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
- Effects are visible on both microscopic and macroscopic levels
- Only central region exposed to both types of energy
- Live (green) / dead (red) stain

![Images of lower and high magnification views of cell structures exposed to light and ultrasound energy.](image)
QUALITATIVE TO QUANTITATIVE

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

![Graph showing efficacy vs. length of exposure and 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

*P. acnes* NTCT737 ATCC 6919
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

- No measurable effect

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