



ENOGENESIS



# Disinfection in Hospital and Public Settings



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# Hospital Acquired Infections

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Pneumonia

Urinary Tract  
Infections

Wound Infections

Line Infections



# Etiology

- Prolonged supine patient positioning
- Poor patient ambulation
- Crowded hospital floors
- Poor cleaning practices
- Poor ventilation
- Poor patient hygiene
- Incapacitation or patient Intubation
- Normal flora

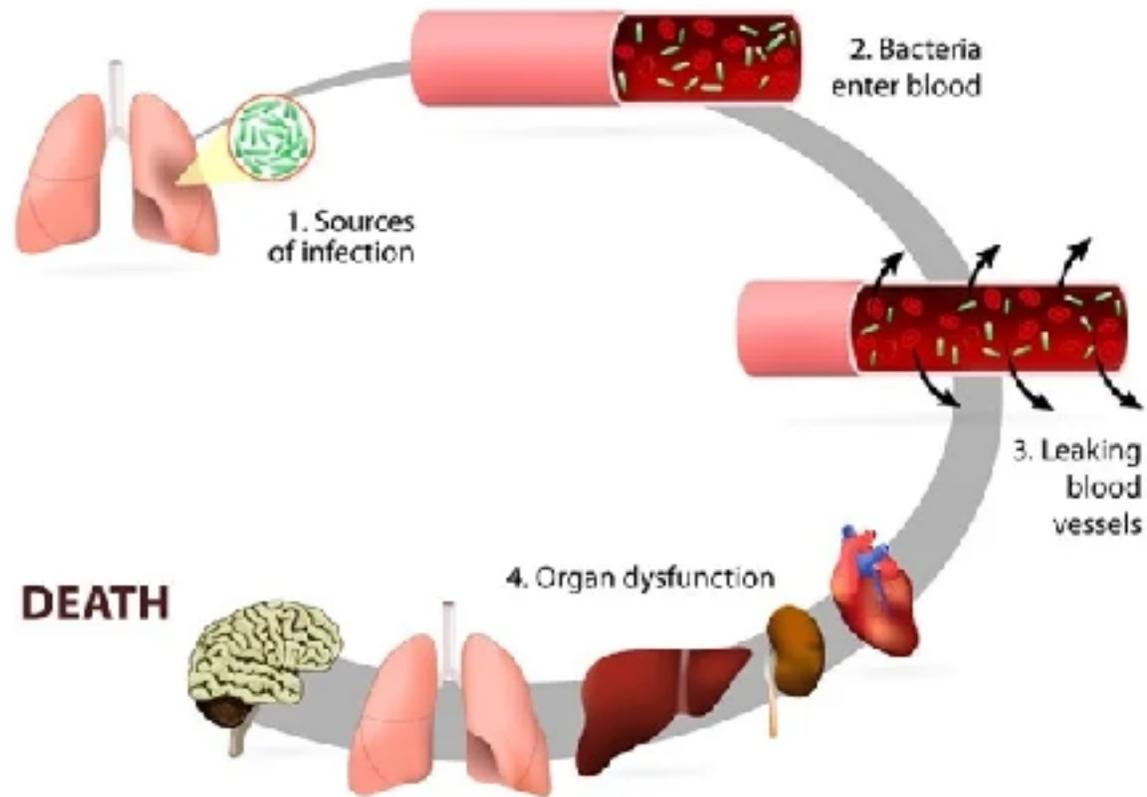


# Common Pathogens

- Bacteria like Strep Pneumo, H. Influenza, Klebsiella pneumo, Pseudomonas Aeruginosa
- Viruses like RSV, influenza, COVID
- Fungi like Candida and pneumocystis carinii

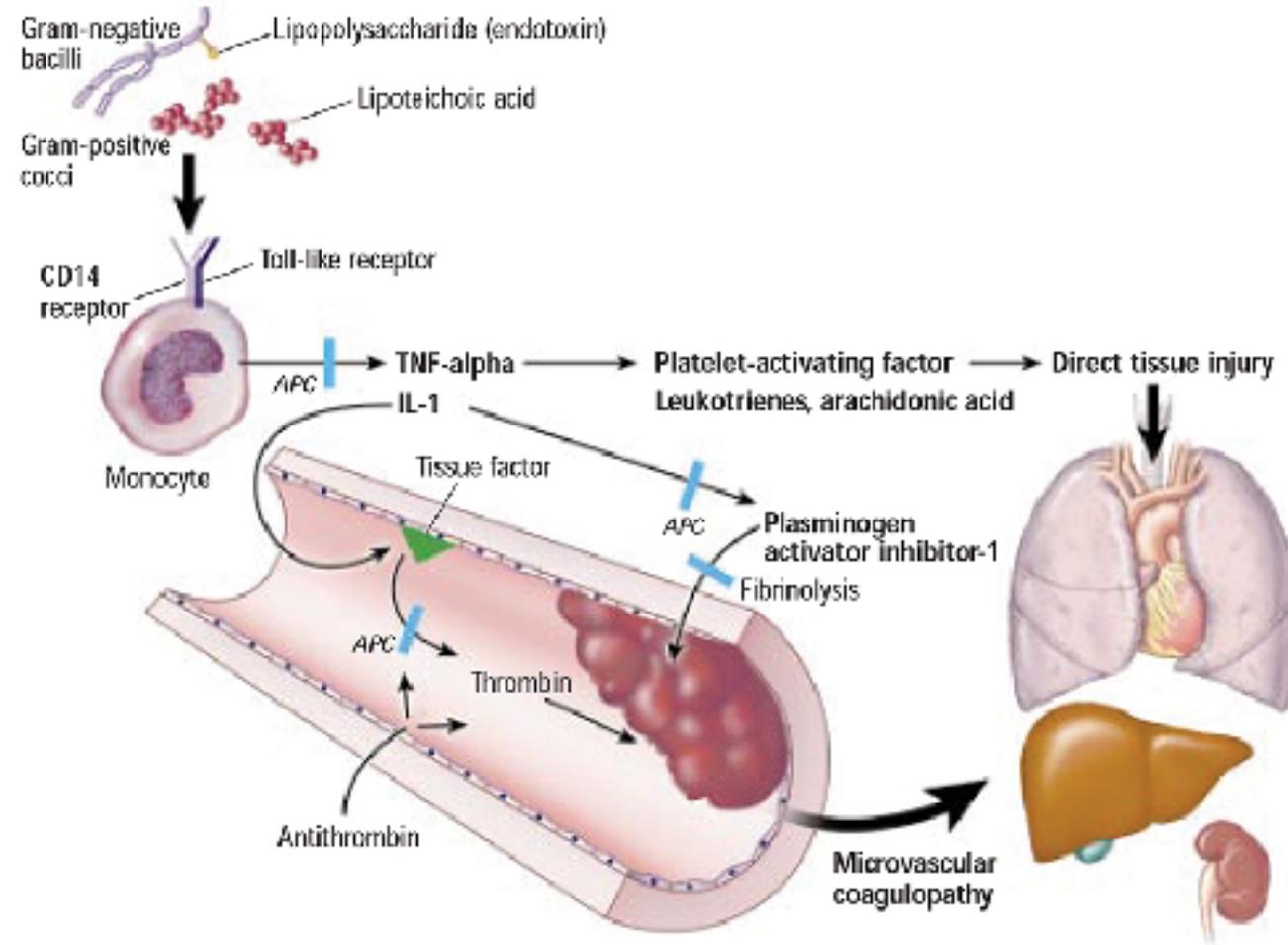
# Mechanism

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# Mechanism

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# Purpose

To disinfect spaces and surfaces to prevent transmissions to patients.  
To disinfect spaces and surfaces in a safe, easy and economical way.

## Solution



*nitric oxide*

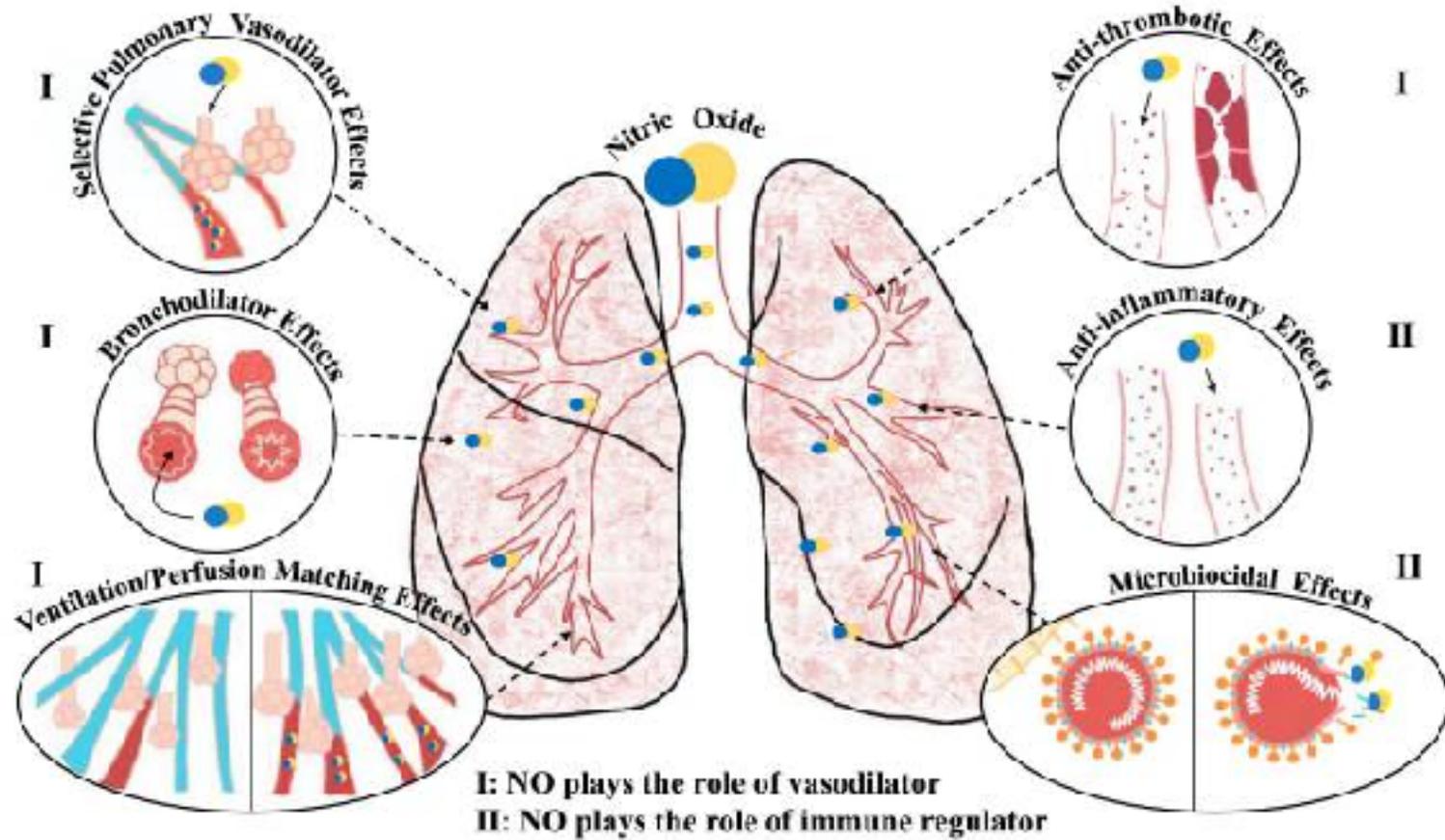




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# Nitric Oxide and COVID Prophylaxis / Sanitization / Disinfection

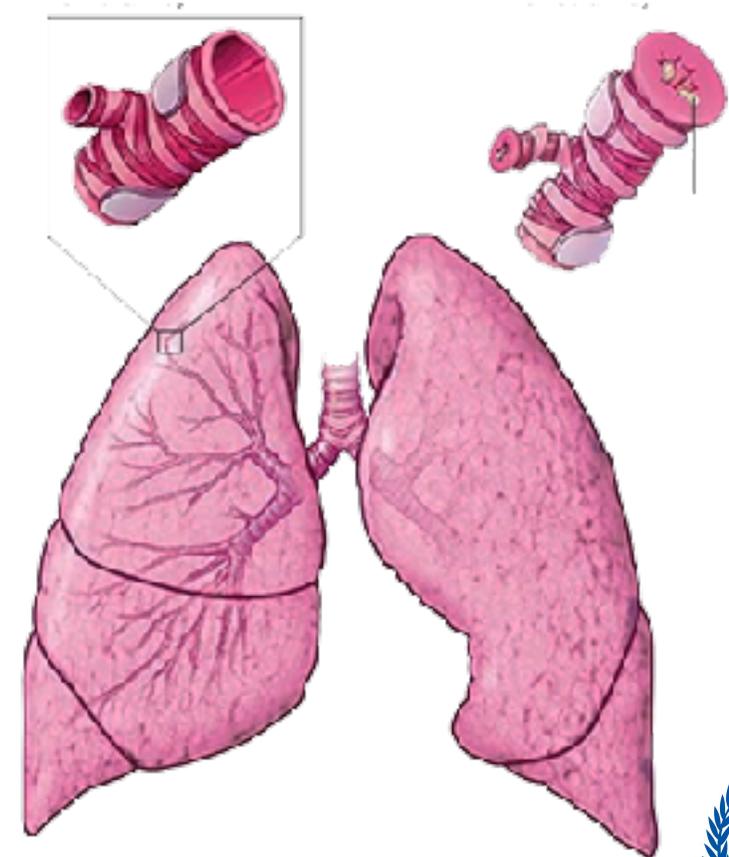
# Nitric Oxide



# Nitric Oxide

## FDA Approved Nitric Oxide Gas for Neonatal Pulmonary Hypertension in 1999

- Medical dosing of inhaled NO at 1-20 ppm
- NO – a selective pulmonary vasodilator
  - Increases blood flow to well ventilated regions of lungs
  - Improve ventilation-perfusion relationships and oxygenation
- Pulmonary Conditions Approved for NO Therapy
  - Pulmonary hypertension
  - Acute Respiratory Distress Syndrome (ARDS)
  - Chronic Obstructive Pulmonary Disease (COPD)
  - Cystic fibrosis
  - Pediatric bronchiolitis
- Primary Risks of NO Gas Therapy
  - Hypoxia from Methemoglobinemia
  - Airway Injury from Nitrogen Dioxide



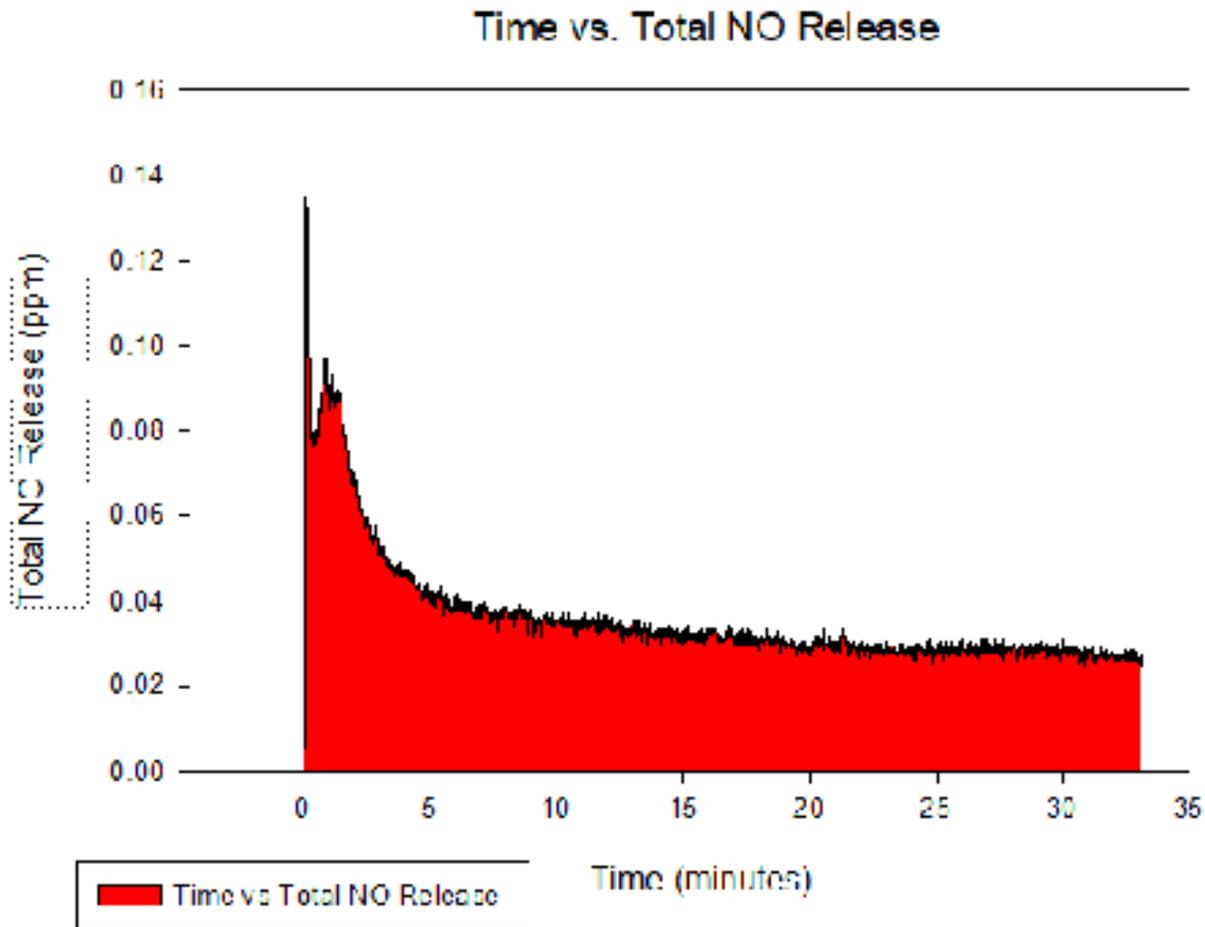
# Nitric Oxide

Nitric Oxide has two notable uses:

- 1 Non Prescription: 15 ppm to 50 ppm for personal, facility or community use, to kill bacteria, viruses or fungi
- 2 Prescription: 20 ppm and up for inpatient use

# Nitric oxide release and biological data

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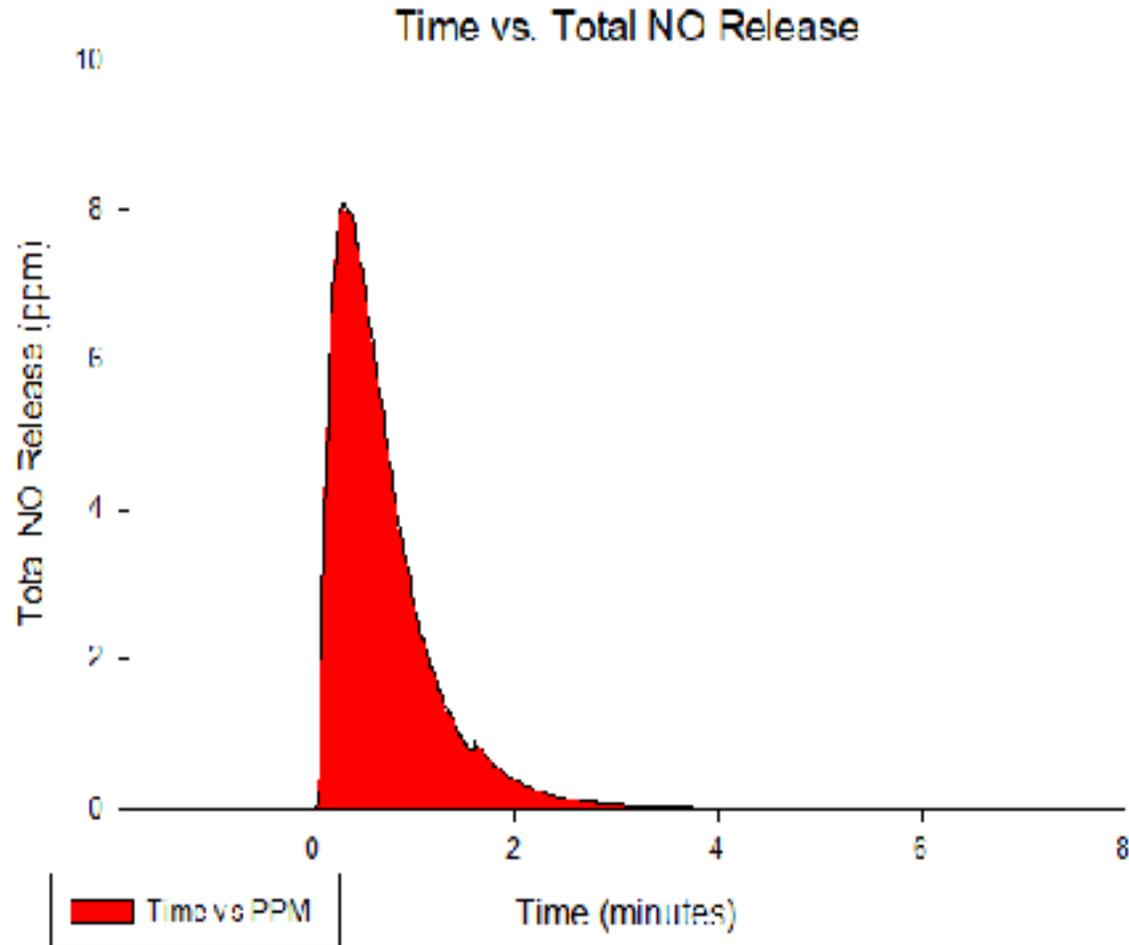
- Expected Value: 1.2225 ppm
- Actual Value: 1.2015 ppm

50 micromolar - Graph & Area Under the Curve



# Nitric oxide release and biological data

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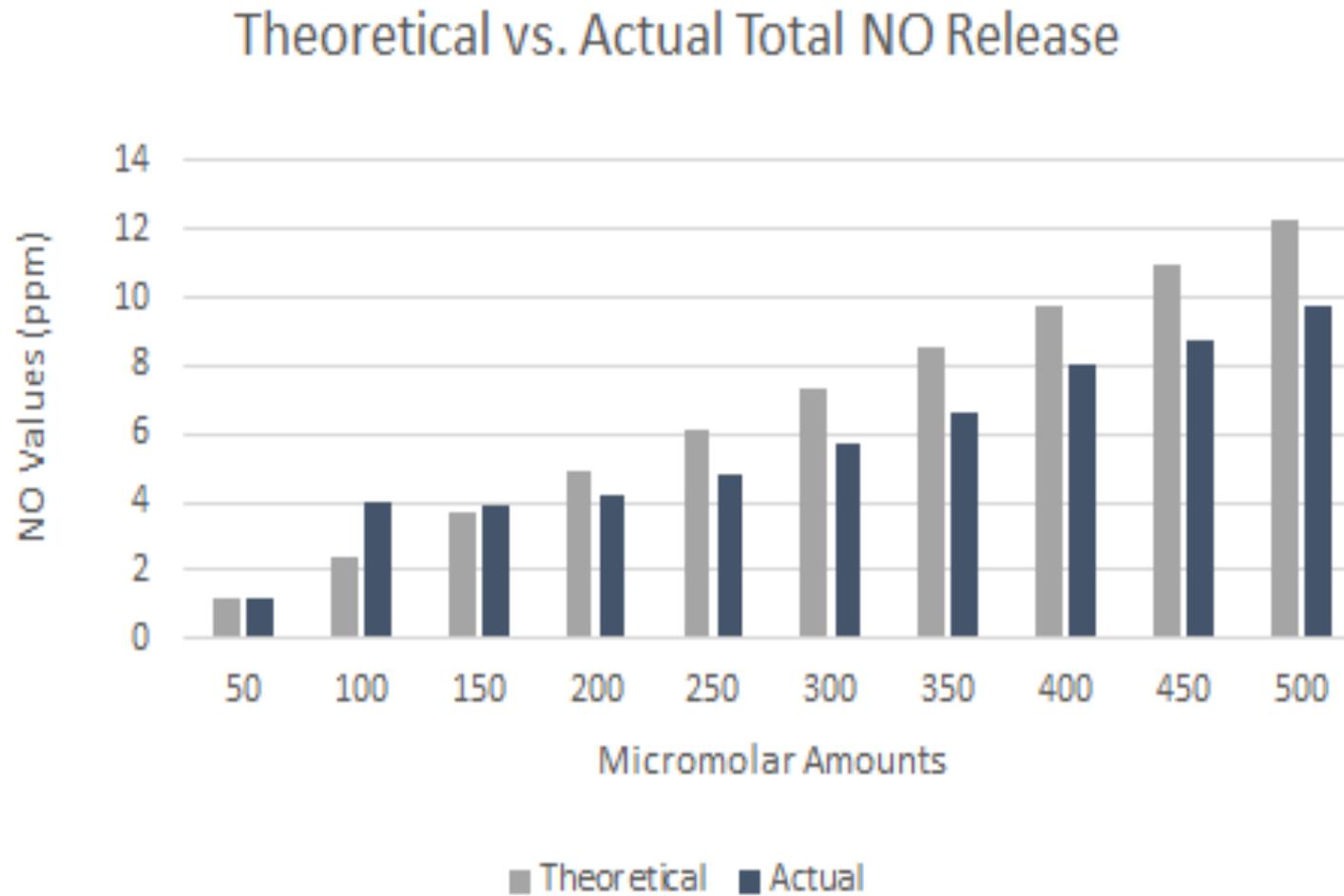
- Expected Value: 8.5575 ppm
- Actual Value: 6.634285 ppm

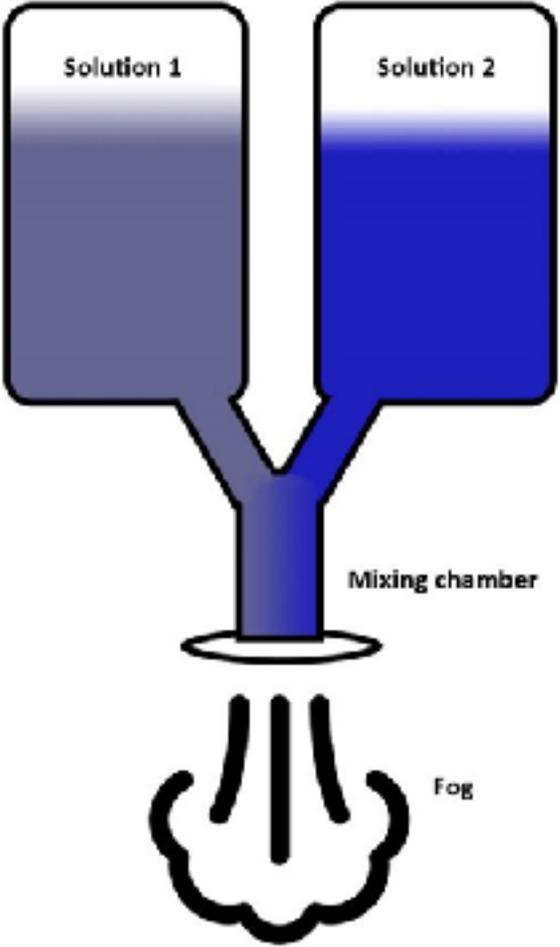
350 micromolar - Graph & Area Under the Curve



# Nitric oxide release and biological data

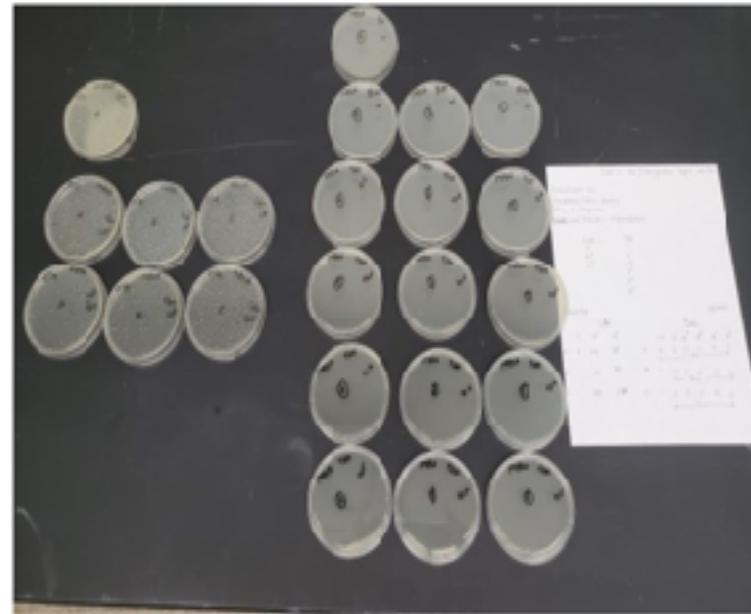
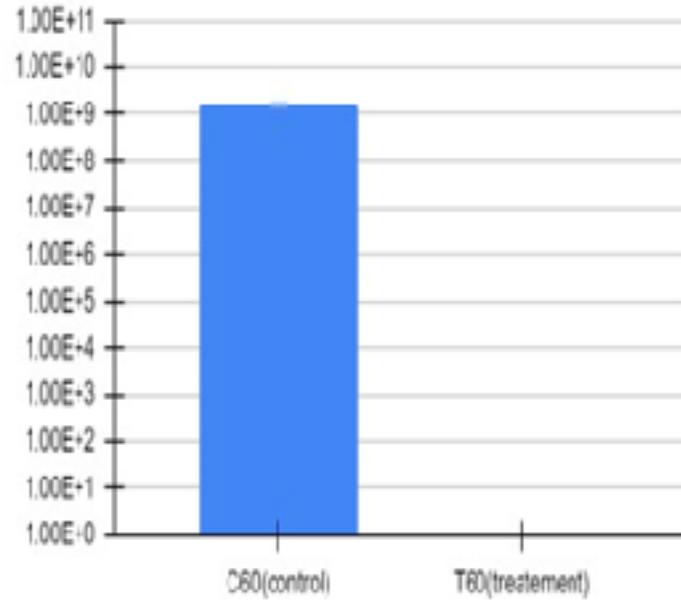
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# Disinfection Project Data

CFU Assay on MRSA Bacterial strain :

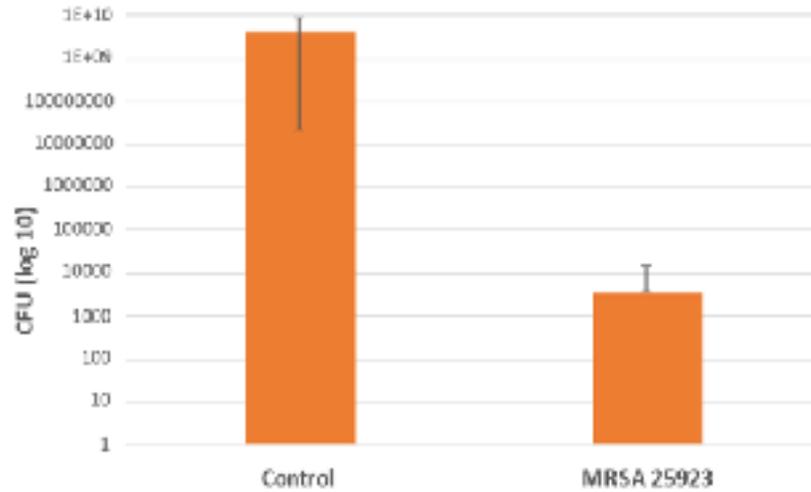


Control vs Treated MRSA bacterial plates

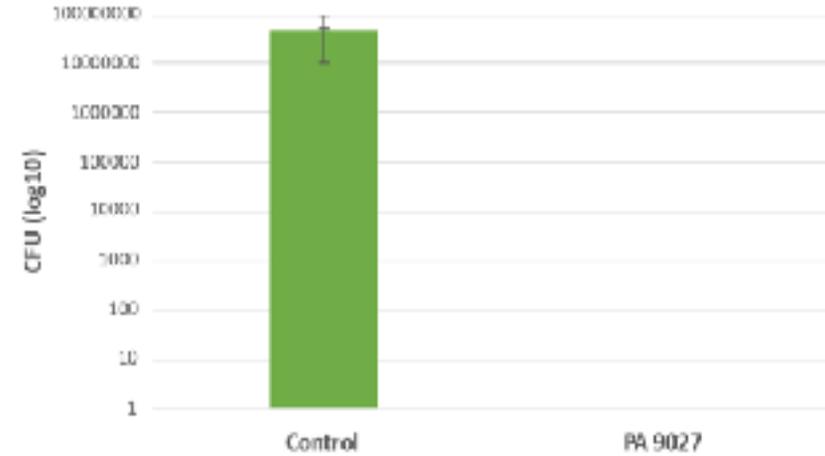
# Disinfection Project

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Antimicrobial Efficacy of NO on MRSA



Antimicrobial Activity Of NO On PA9027



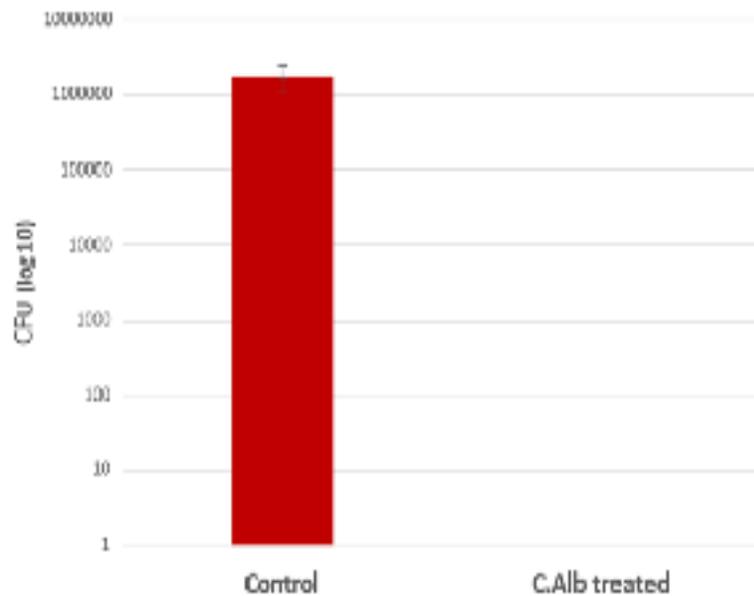
MRSA : Control v/s Treated plates



PA: Control v/s Treated plates

# Antimicrobial efficacy of NO on C.albicans 2876

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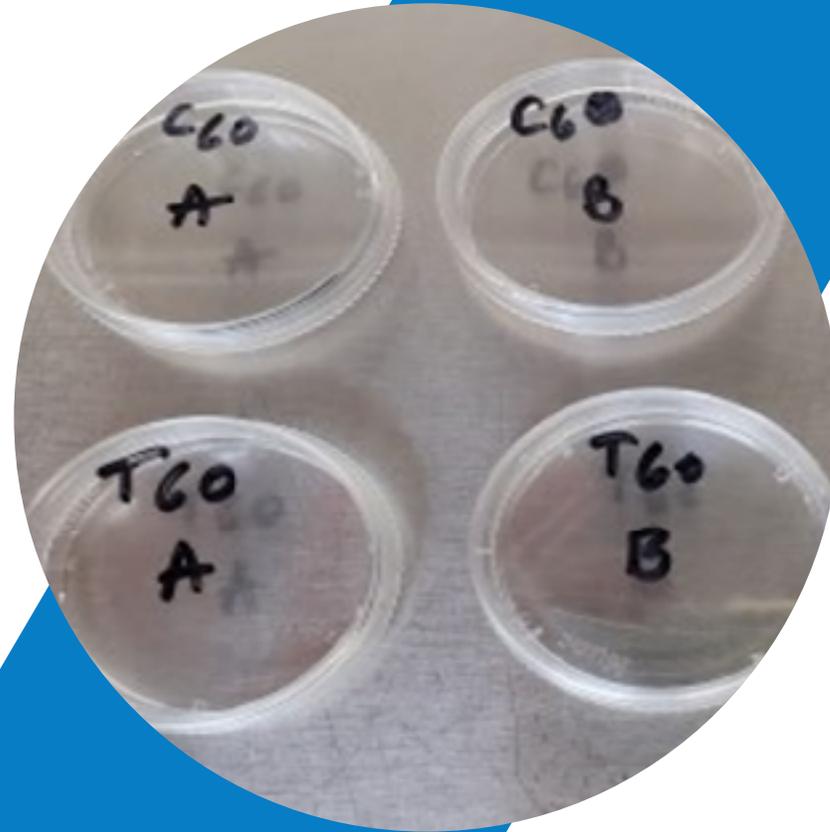


C. Albicans 2876 : Control plates

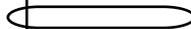
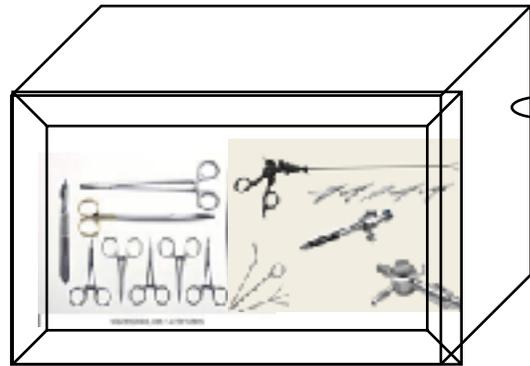


C. Albicans 2876 : Treatment plates

# Strlization Treatment



# Medical and Surgical instrument disinfection



Nitric Oxide fog / mist

# Nitric Oxide Inhibits the Replication Cycle of Severe Acute Respiratory Syndrome Coronavirus

Nitric oxide (NO) is an important signaling molecule between cells which has been shown to have an inhibitory effect on some virus infection

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Nitric oxide mist/fog

Mist/fog produced here with Nitric oxide  
~15-20ppm (OTC)



# Nitric Oxide is a powerful virucidal agent that acts within hours

- Nitric oxide (NO) is a small endogenous messenger molecule
- Inhibit replication of severe acute respiratory syndrome coronavirus (SARS-CoV-1) in vitro,
- exogenous gaseous NO (gNO) at concentrations = 150ppm has been shown to act as a potent antimicrobial agent.
- Multiple clinical trials, administration of high dose (150 to 250ppm) intermittent
- Gas NO (30-40 min cycles, 2-5 cycles a day) was safe and well-tolerated
- OC43 human coronavirus was exposed to 150-250ppm gas NO, for up to 8 hours intermittently
- (1-hour alternating) both before and after infection of human HCT-8 cells.
- Upon exposure of coronavirus infected cells to 150ppm NO. While coronavirus lost 50% of its infectivity after 4 hours of treatment with 150ppm NO, complete inhibition of infectivity was achieved after 8 hours of treatment.

# Sanitizing mask, PPE kits for regular disinfection

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**Nitric oxide spray**



**Nitric oxide spray**



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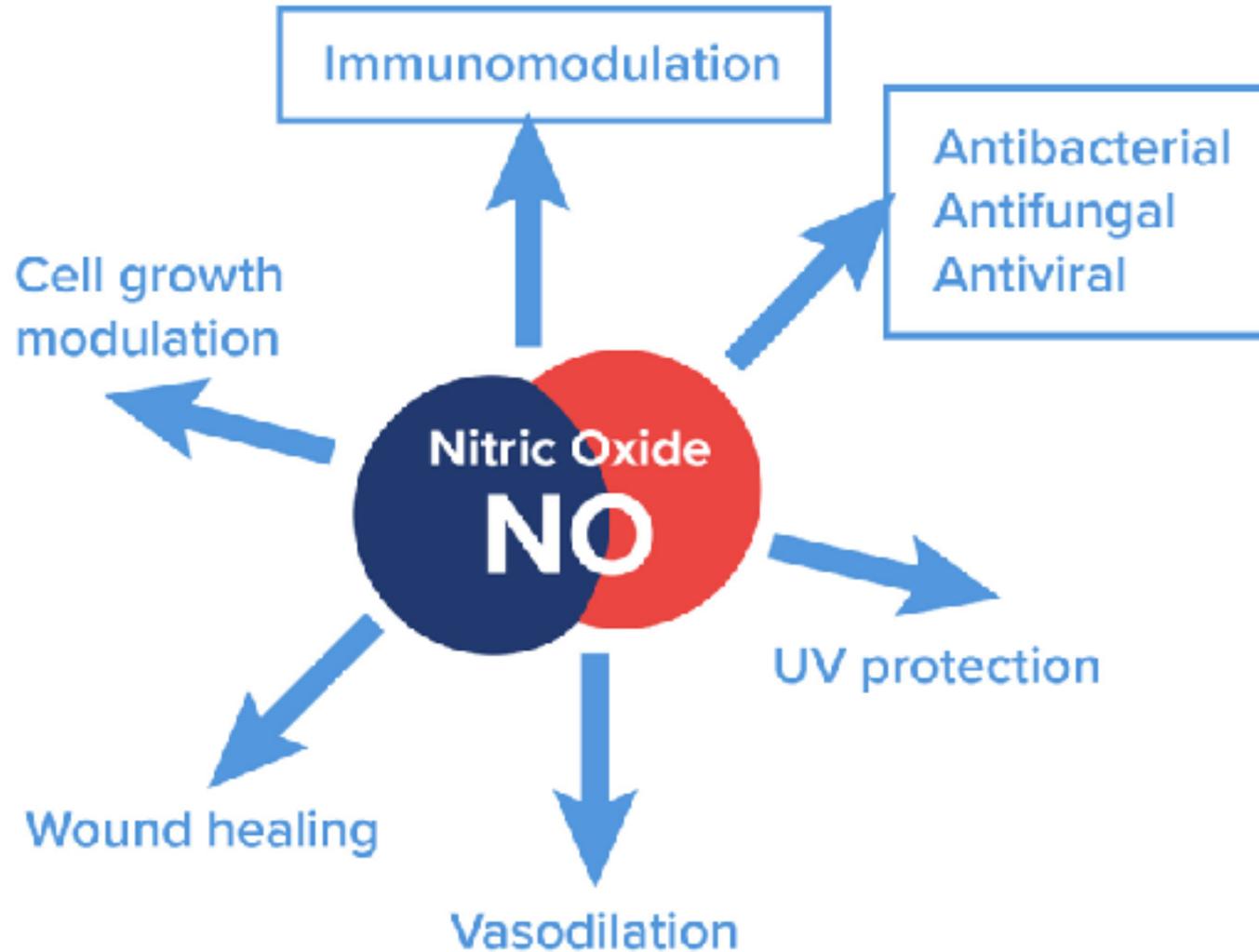
# Wound healing



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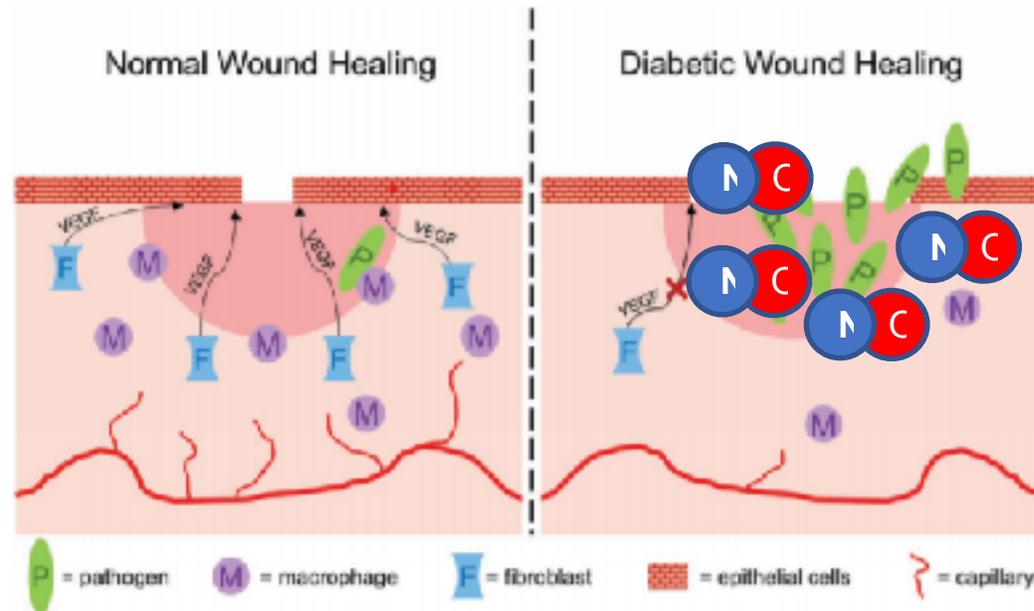
# Wound healing

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# Accelerated wound healing in presence of Nitric Oxide

Nitric oxide (NO) is a therapeutic agent due to its ability to regulate inflammation and eradicate bacterial/fungal/viral infections.



# Steps involved in wound healing process using NO which is disrupted in the event of an injury.

1. Vascular homeostasis
2. Inflammation
3. Antimicrobial action

WOUND HEALING PRODUCTS band aid with nitric oxide + antibiotic ointment for accelerated wound healing:

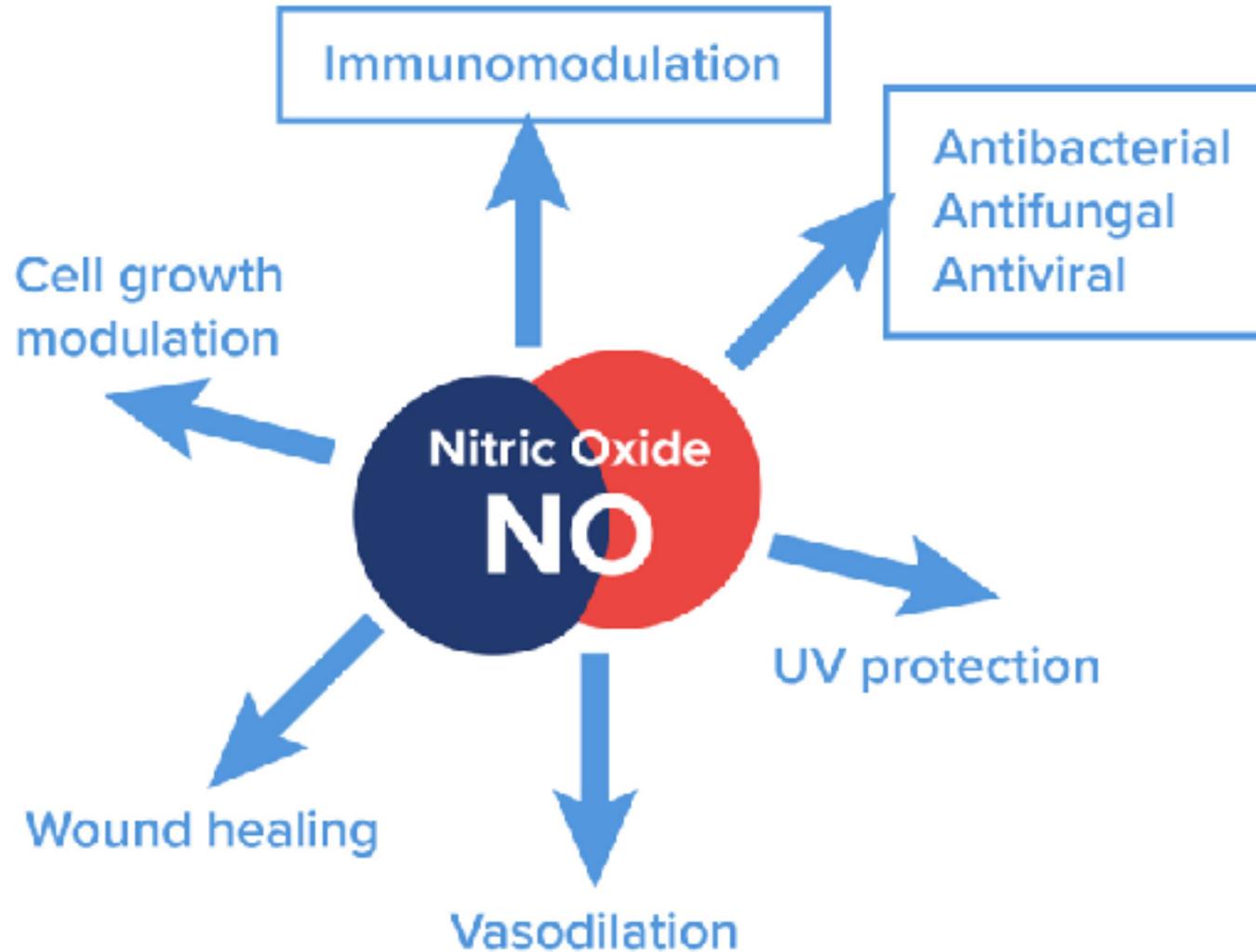


# Pain management

- Capsicum Plaster consists of a suitable cloth of cotton or rayon or both mixed, evenly spread with self adhesive mass containing Capsicum Oleoresin. The plaster is performed evenly & the adhesive surface is protected with silicon based
- Capsicum Oleoresin + curcumin and Nitric Oxide powder for osteoarthritis in the form of plaster consists of a suitable cloth of cotton or rayon or both mixed, evenly spread with self adhesive.
- Belladonna Plaster consists of a suitable cloth of cotton or rayon or both mixed, evenly coated with adhesive mass containing Belladonna Extract. The plaster is perforated & the adhesive surface is protected with printed /plain silicon paper.

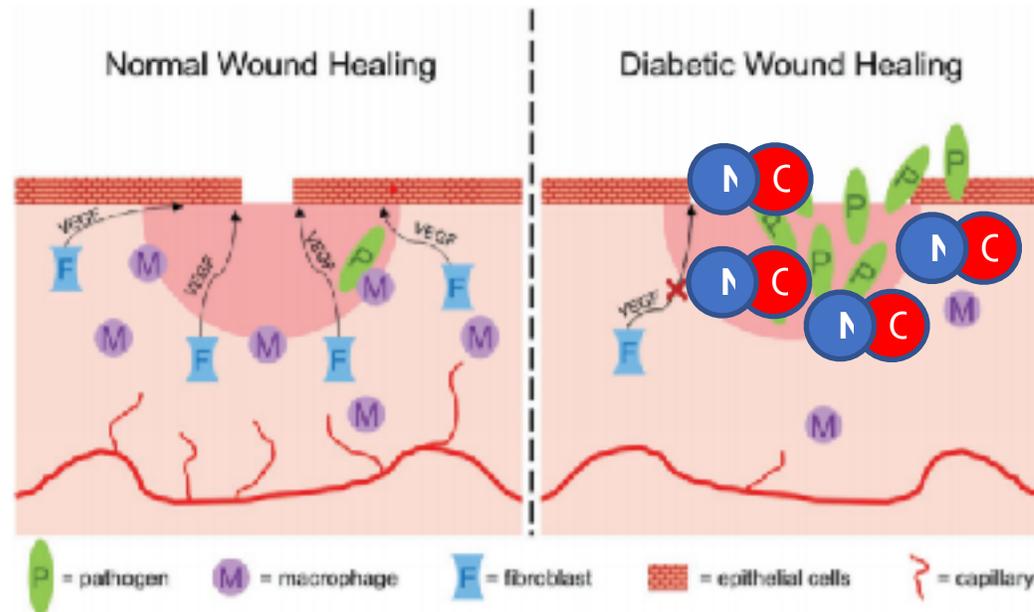


# Pain management



# Accelerated wound healing in presence of Nitric Oxide

Nitric oxide (NO) is a therapeutic agent due to its ability to regulate inflammation and eradicate bacterial/fungal/viral infections.



# Surgical tapes



Zinc Oxide Plaster: Adhesive Tape consists of a suitable cloth of cotton or rayon or both mixed, evenly spread with self adhesive mass containing zinc oxide. The cloth is finished to good white or dyed flesh(skin) color with nontoxic dye.

## Plastic Spool and Metal Sleeve Pack

Product Size	No. of Spools per inner	No. of Inners per case	No. of spools per shipper	Shipper size (mm)	Weight per shipper (kg)
1.25cm x 1m	60	18	1080	405 x 335 x 255	8.5
2.50cm x 1m	40	18	720	405 x 335 x 255	9
1.25cm x 5m	50	6	300	405 x 335 x 255	9.5
2.50cm x 5m	30	6	180	345 x 295 x 255	8
5.00cm x 5m	20	6	120	345 x 295 x 265	10.2
7.50cm x 5m	10	8	80	295 x 235 x 390	10
10.0cm x 5m	10	9	90	345 x 295 x	14.3

Product Size	No. of Rolls per tube	No. of tubes per Shipper	No. of spools per Shipper	Shipper Size (mm)	Weight per shipper (kg)
2.50cm x 8m	12	25	300	445 x 300 x 355	15
5.00cm x 8m	6	25	150	430 x 290 x 340	16.7
7.50cm x 8m	4	25	100	445 x 300 x 355	18
10.0cm x 8m	3	25	75	430 x 290 x 340	18
2.50cm x 9m	12	25	300	445 x 300 x 355	22
5.00cm x 9m	6	25	150	430 x 290 x 340	22
7.50cm x 9m	4	25	100	445 x 300 x 355	22
10.0cm x 9m	3	25	75	430 x 290 x 340	22
2.50cm x 10m	12	25	300	430 x 430 x 345	25
5.00cm x 10m	6	25	150	430 x 430 x 345	25
7.50cm x 10m	4	25	100	430 x 430 x 345	25



# Topical or transdermal drug delivery

## ways to administration

- Powder
- Paste
- Ointment or gel



Make 2 slides



Nitric oxide gel for radiation dermatitis

Nitric oxide + Melanin gel for radiation dermatitis

Radiation dermatitis



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# Nanoparticles Used to Prevent Inflammatory Acne Through Slow-Released Nitric Oxide

- NO-inhibit the bacteria-stimulated inflammatory cascade associated with acne with minimal or no toxicity to keratinocytes.
- prevent *Propionibacterium* acnes induced inflammation by both clearing the organism and inhibiting microbial stimulation of the intrinsic immune response,



**NO**  
→



<http://www.nature.com/jid/journal/vaop/naam/pdf/jid2015277a.pdf>.



# Title: Nitric Oxide-based Contact Lens Care Systems

*Jimmy K. Lee, MD<sup>2</sup>, Jyothishree Tholalu Jayadeva, BTech<sup>1</sup>, Shivaleela Keerthy, MS<sup>1</sup>, Jee Young Moon, PhD<sup>2</sup>, Joseph Yi, BA<sup>2</sup>, J. Christian Hein, BS<sup>2</sup>, Robert Kremer<sup>1</sup>, and Mahantesh S. Navati, PhD<sup>1</sup>*

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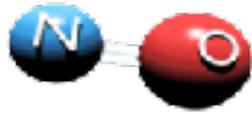
## Purpose:

The widespread application of therapeutic and cosmetic contact lenses has significantly increased the incidence of infectious keratitis among millions of people yearly. Despite adopting various standard cleaning procedures, microbes can still establish biofilms on the contact lenses and their cases. In addition to resistance to lens disinfectant systems, biofilms formed by pathogenic organisms are of increasing clinical importance due to their antimicrobial resistance. Nitric oxide (NO), a small lipophilic molecule has been well studied as an antimicrobial with no known reports of antimicrobial resistance (Privett BJ, Broadnax AD, Bauman SJ, Riccio DA, Schoenfisch MH. Examination of bacterial resistance to exogenous nitric oxide. Nitric Oxide. 2012;26(3):169-73). We exploited this property of NO to create biodegradable and water-soluble NO releasing powder- ENO-Vision™ capable of killing both planktonic and biofilm-associated microbes. We envision to use this NO powder as a novel lens sterilization system.



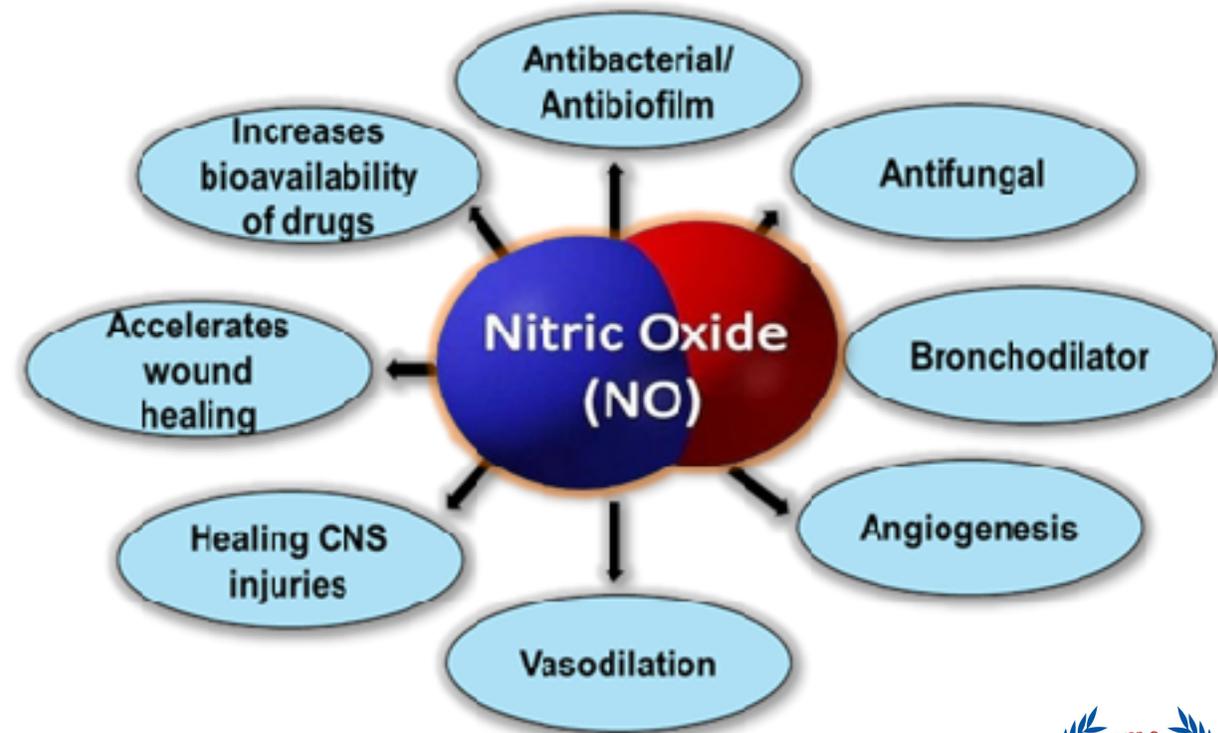
# ENO-vision based on Nitric Oxide (NO)

What is Nitric Oxide?



- Unstable free radical gas
- Lipid Soluble, very small
- Highly reactive

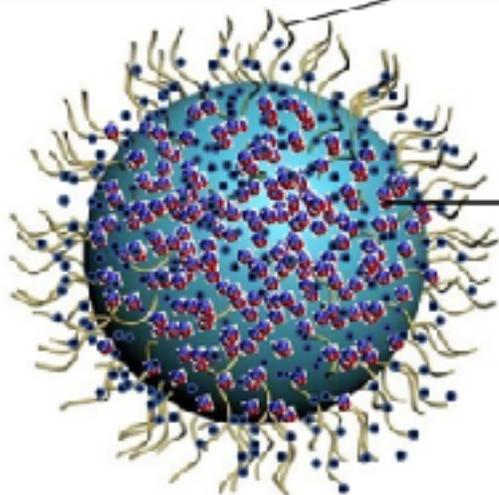
## Biological properties of NO



Nano-NitricOxide

Drug molecule  
(optional)

Nitric oxide



# Role of Nitric Oxide in Ophthalmological Treatments

- Antimicrobial properties of nitric oxide
  - NO is an excellent broad-spectrum antimicrobial and anti-biofilm agent with NO known antimicrobial resistance
  - NO induces nitrosative and oxidative stress – targets cell membranes, structural proteins, metabolic enzymes
  - NO interferes with adhesion, colonization and dissemination of fungal cells
  - NO inhibits viral replication
- Hypotensive properties of NO
  - Reduces intraocular pressure
  - Improvement of ocular blood flow

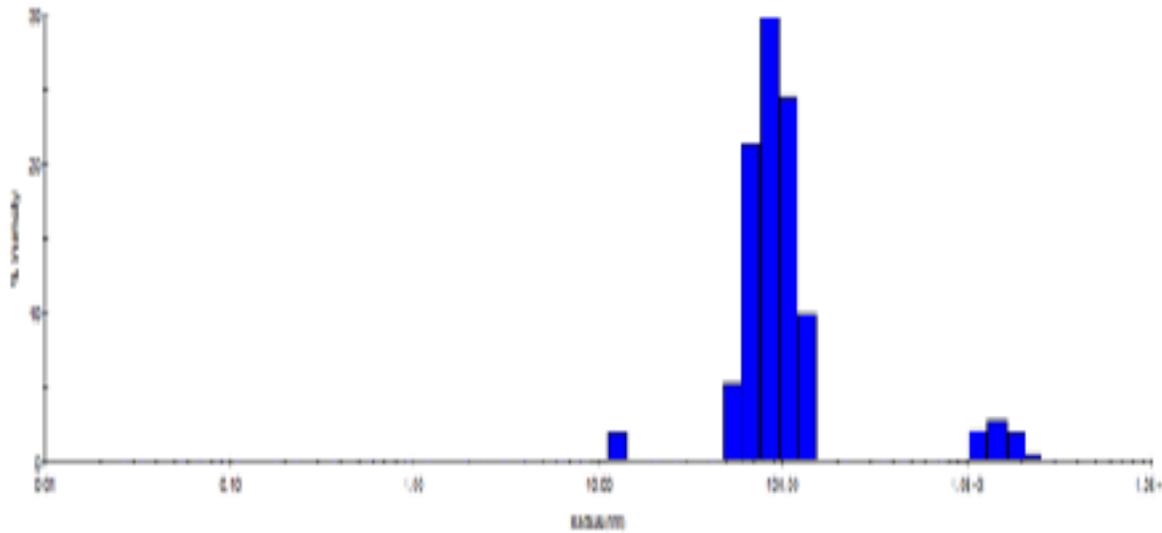
1. Virulence 3:3, 271–279; May/June 2012; G 2012 Landes Bioscience.  
The potential of nitric oxide-releasing therapies as antimicrobial agents  
David O. Schairer, Jason S. Chouake, Joshua D. Nosanchuk, and Adam J. Friedman

2. J Clin Invest. 1997;99(12):2818-2825  
Perspectives series: host/pathogen interactions. Mechanisms of nitric oxide-related antimicrobial activity by [F.C.Fang](#)

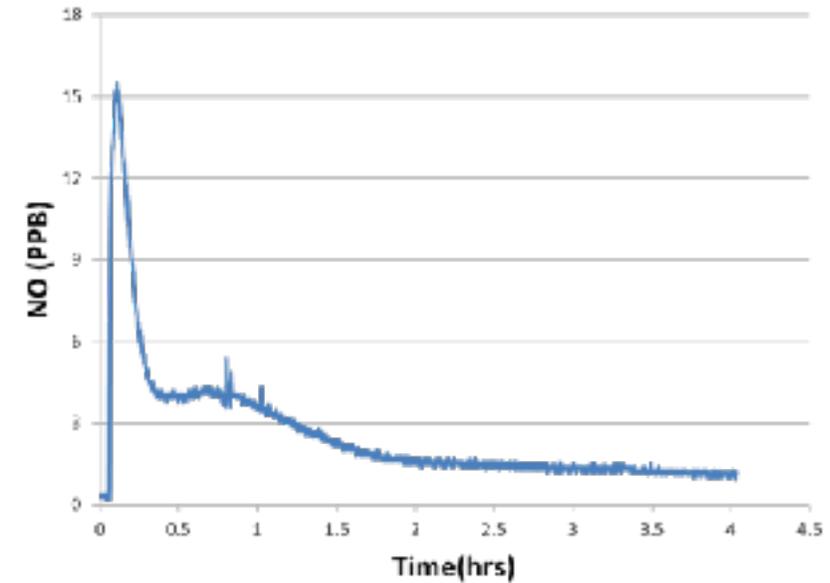


# Physico-chemical characterization of ENO-vision

## A. Size-distribution



## B. NO release



- Size distribution from 90 – 100 nm through dynamic light scattering
- NO release rate determined as 0.025 nmoles/mg/min
  - Sustained NO release
  - Tunable NO release

# Most Common Pathogens for Contact Lens-Related Keratitis

- Bacteria (71%-88%)
  - Gram Positive
    - Staphylococcus spp. (20%)
    - Coagulase-negative Staphylococci (16.8% - 49%)
    - S. aureus (5.9%)
    - S. epidermidis (4.8%)
  - Gram Negative
    - **Pseudomonas (19% - 73.5%)**
    - Serratia (4.8% - 23%)
- Fungal (2.6% - 5%)
  - Filamentous
    - Fusarium (51% - 68%)
    - Aspergillus (9.2% - 11%)
  - Yeast
    - Candida (2% - 12%)
- Acanthamoeba (0.9% - 4%)
  - Acanthamoeba castellanii (38% - 66%)
  - Acanthamoeba polyphaga (30%)



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# Methods

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Eno-Vision was developed using ENOGENESIS Therapeutics proprietary nanotechnology and the NO release rates were measured using a NO analyzer (Sievers 280i, Boulder, CO). The antimicrobial properties of ENO-Vision™ with contact lenses infected with microbial strains *Pseudomonas aeruginosa* (ATCC® 9027™) and *Candida albicans* (ATCC® MYA-2876™) purchased from American type culture collection (ATCC, Manassas, VA) was studied through a colony forming unit (CFU) count assay. Briefly, an overnight grown culture of *P. aeruginosa* in tryptic soy broth (TSB; MP Biomedicals, Santa Ana, CA) and *C. albicans* in Sabouraud Dextrose broth (SDB; BD Biosciences, San Jose, CA) at 37°C was diluted to 10<sup>8</sup> cells ml<sup>-1</sup> in their respective media based on McFarland equivalence turbidity standard 0.5 (Thermo Fisher Scientific, Waltham, MA). Soft contact lenses (Acuvue® Moist) from Johnson & Johnson were co-cultured with microbial strains for 24h in a 24-well polystyrene plates (Thermo Fisher Scientific, Waltham, MA) at 37°C. Post this, the infected lenses were removed and gently washed twice with sterile saline. They were then transferred to a new 24-well plate and treated with 20mg/ml of ENO-Vision™ in either TSB/SDB media for 8 and 12h at 37°C. Infected lenses in media alone served as the control in this experiment. After incubation, both the media and lens (control and treated) were separated, the microbial cells in the lens were isolated in 1ml of fresh media using gentle vortexing for 1 min. Various dilutions of these suspensions in 1X phosphate buffered saline were plated on either tryptic soy agar (MP Biomedicals, Santa Ana, CA) or Sabouraud dextrose agar (BD Biosciences, San Jose, CA) plates. Microbial viability was assessed by counting the number of colonies established on the agar plates after overnight incubation at 37°C.



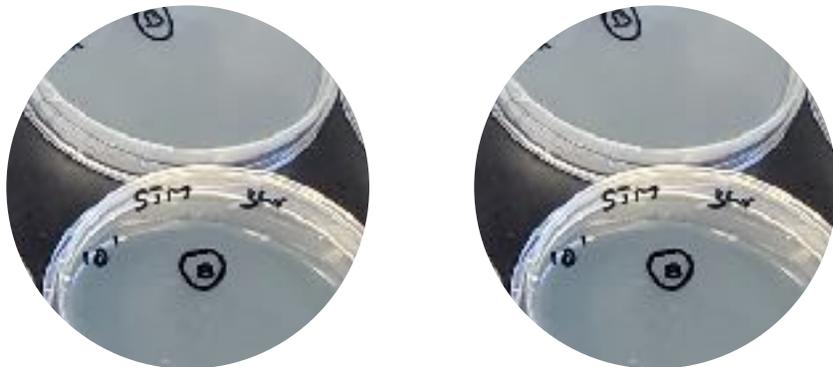
# Plating of Pseudomonos Aeruginosa microbial cells after Treatment



Untreated Lens @ 3hr



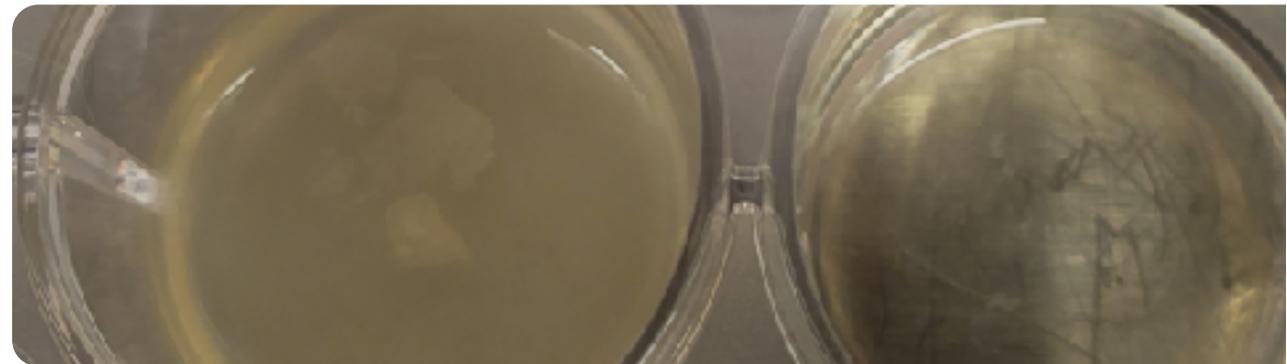
Treated Lens with Lens Solution @ 3hr



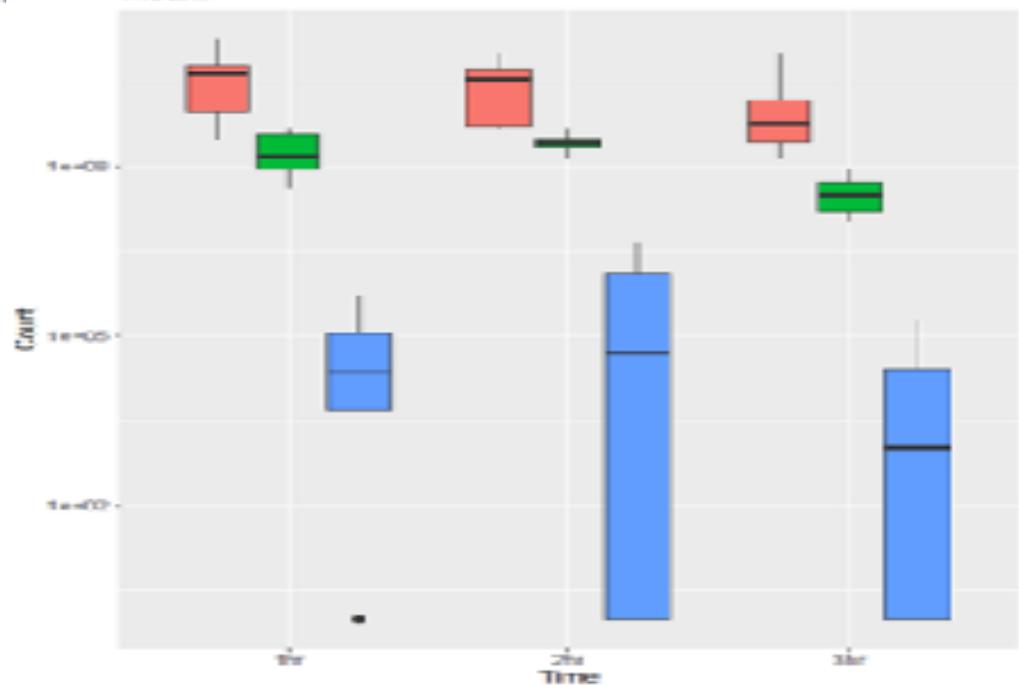
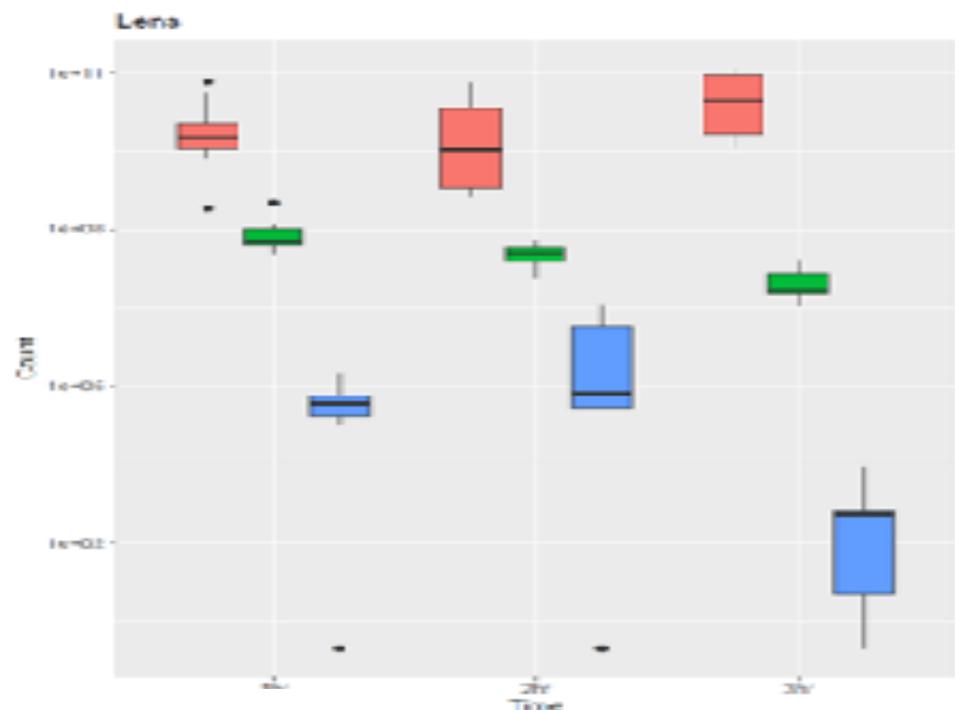
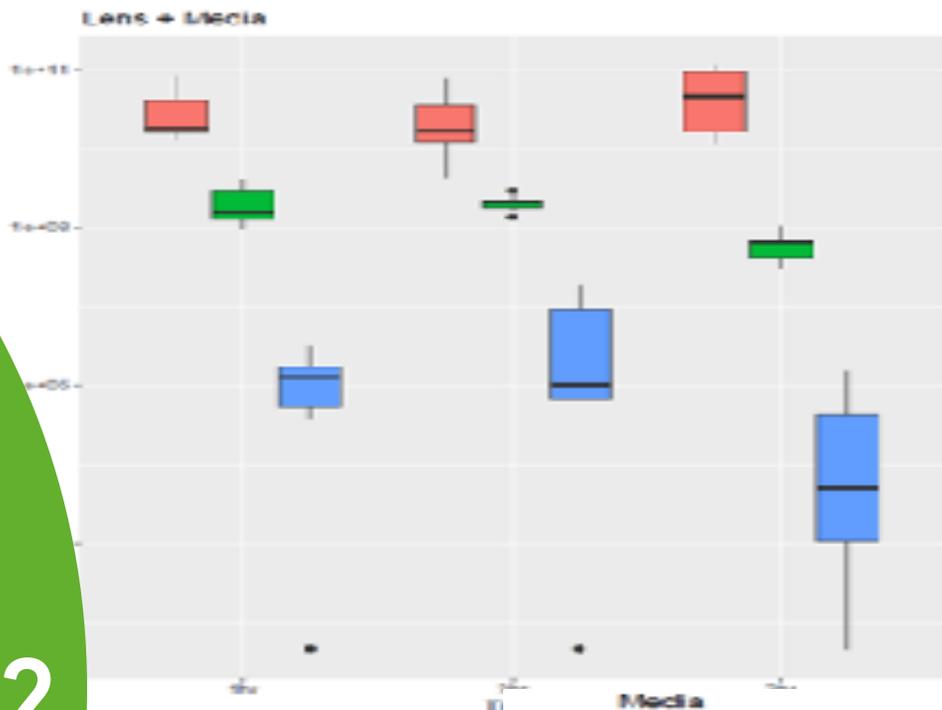
Treated Lens with NP + Lens Solution @ 3hr

Biofilm on Contact Lens

Disrupted Biofilm after treating with Nanoparticles



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# Summary

## Summary:

Bacteria on either lens, media, or combined (lens and media) in nanoparticle solution were significantly lower in their abundances than those in untreated solution and in lens solution for each time point (1hr, 2hr, 3hr). In specific, compared to the bacteria count on lens in lens solution, the nanoparticle solution showed  $2.2 \times 10^{-4}$ ,  $5.6 \times 10^{-4}$ ,  $1.1 \times 10^{-5}$  fold lower bacteria counts at 1hr, 2hr, and 3hr, respectively (Median bacteria counts were 48000, 72000, 360 at 1hr, 2hr, and 3hr in nanoparticle solution and 57000000, 35400000, 6900000 at 1hr, 2hr, and 3hr in lens solution. 95% CI on fold change=  $(1 \times 10^{-5}, 4 \times 10^{-3})$ ,  $(6 \times 10^{-6}, 5 \times 10^{-2})$ ,  $(1 \times 10^{-6}, 8 \times 10^{-5})$ ). Of a note, contact lens solution also showed significant decreases in bacteria compared to untreated solution at each time point -  $1.3 \times 10^{-2}$ ,  $7.9 \times 10^{-3}$ ,  $3.7 \times 10^{-4}$  fold lower bacteria counts on lens at 1hr, 2hr, and 3hr. Under nanoparticle solution, bacteria count at 2hr was similar with the count at 1hr, but the count at 3hr was significantly lower than the count at 2hr (0.01 fold, 95% CI=(0.00008, 0.3), P=0.015). As bacteria counts under untreated solution were quite constant over time, we assume the initial bacteria count (0 time point) to be similar with observed bacteria count under untreated solution and expect that 3.5 hrs soaking in nanoparticle solution is required to reach zero bacteria count under exponential decay over time. In summary, nanoparticle solution showed the efficacy against *Pseudomonas Aeruginosa*, more efficient than BioTrue contact lens solution. More data at more frequent time points over longer duration in addition to zero time point will better elucidate the decay patterns of bacteria under nanoparticle solution.



# Summary

## Results

The number of bacteria plated from bacteria treated with nanoparticle solution were significantly lower than those that were either untreated or treated with lens solution for every time point (1hr, 2hr, 3hr). Compared to the bacteria count from lenses treated with lens solution, the nanoparticle solution showed  $2.2 \times 10^{-4}$ ,  $5.6 \times 10^{-4}$ , and  $1.1 \times 10^{-5}$  fold lower bacteria counts at 1hr, 2hr, and 3hr, respectively (Median bacteria counts were 48000, 72000, 360 at 1hr, 2hr, and 3hr respectively in nanoparticle solution; 57000000, 35400000, 6900000 at 1hr, 2hr, and 3hr respectively in lens solution; 95% CI on fold change =  $(1 \times 10^{-5}, 4 \times 10^{-3})$ ,  $(6 \times 10^{-6}, 5 \times 10^{-2})$ ,  $(1 \times 10^{-6}, 8 \times 10^{-5})$ ). Bacteria treated with contact lens solution also showed significant decreases in bacteria compared to untreated solution at each time point ( $1.3 \times 10^{-2}$ ,  $7.9 \times 10^{-3}$ ,  $3.7 \times 10^{-4}$  fold lower bacteria counts on lens at 1hr, 2hr, and 3hr). With nanoparticle solution, the bacteria count at 2hr was similar to that at 1hr, however, the count at 3hr was significantly lower than that at 2hr (0.01 fold, 95% CI= $(0.00008, 0.3)$ ,  $P=0.015$ ).

## Conclusions

Since the number of bacteria in the untreated group did not change significantly over time, we assume that there was minimal bacterial death due to external factors and that 3.5 hrs incubation in nanoparticle solution is required to completely sterilize the lens using an exponential decay model. In summary, the nanoparticle solution showed greater efficacy against *Pseudomonas Aeruginosa* than BioTrue contact lens solution. Additional data at more frequent time points and over longer durations would better elucidate the decay patterns of bacteria treated with nanoparticle solution.





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Thank You

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