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Synergy of Nitric Oxide and TGF-B1 in an Antimicrobial Osteoinductive Hydrogel for Field and Hospital Care of Open Bone Wounds

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Non-Disclosure

- Juan Taboas, PhD, has no conflicts to report.

Clinical Need

The prognoses for open bone wounds (**OBW**) are not ideal due to contamination, tissue trauma, and delayed treatment.

Incidence of OBWs

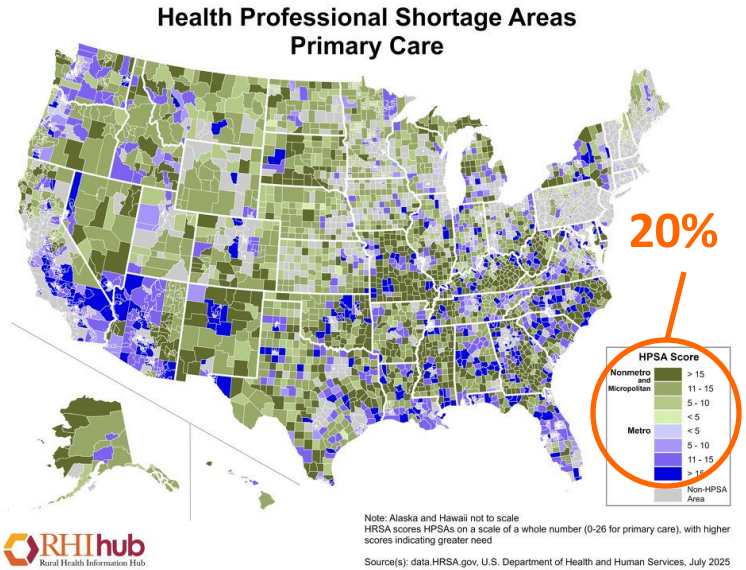
- Military: 4,200/year Iraq War (1/6 combat injuries)
- Civilian: >105,000/year (31/100,000 persons)

Incidence of Complications

- Military: 20% acute & 30% chronic infection
- HPSA: 33%-58% 2-year post-op infection
  42% rate of chronic non-union
- Low-income countries: Similar infection to HPSA
  Greater non-union

Antibiotic Resistant Infections in OBWs

- Military: ≈ 15% incidence with various species
- Civilian: ≈ 40% with MRSA



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Clinical Need

Critically sized OBW bone wounds have poor repair potential.

- Infection
- Ischemia
- Inflammation
- Soft tissue damage
- Few progenitor cells
- Often large volume

Implants

Biologics: BMP-2 in ACS

PDGF in β-TCP

Antimicrobial fillers:

$CaSO_4$, $CaCO_3$, HA, bioglass + antibiotics

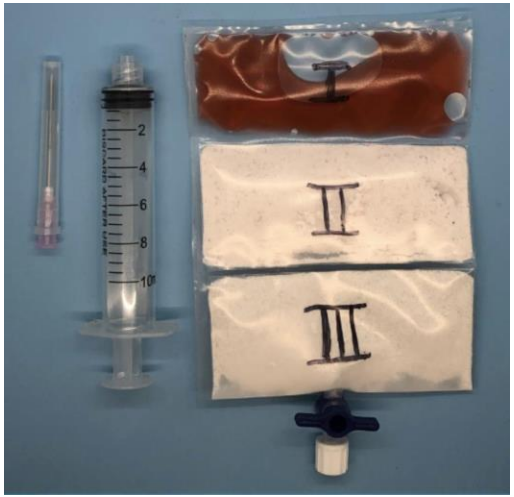
Early treatment is crucial to stop infection and promote healing.

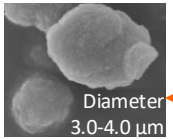
- Critically sized OBWs take about 7-8 months to heal.
- Advanced care is often unavailable at field hospitals and at first care facilities in HPSAs.
- The delay in wound-specific treatment complicates OBW healing and increases disability.

Solution

Antimicrobial & regenerative hydrogel that is easy to deliver in the continuum of care.

Therapeutic





Antimicrobial = Nitric Oxide

Purpose

- No known bacterial resistance
  - Innate and adaptive immunity
- Potentiates GPCR signaling
- Promotes fracture healing

Composition

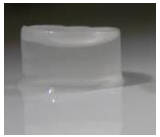
- Nitrosylated silica microparticles

NO* Donor	Half-life (37C)	Deep Tissue
GSNO	Hours – 1 day	✓
Nitrite salt	Sec - min	✗
SNP	2 minutes	✗
SNAP	6 hours	✓
NONOates	1.8s - 20 hours	?
NOC-18	20 hours	?
MSNO	18 hours	?

Hydrogel = RTC

Purpose

- Carrier for biologics (hold in defect)
- Progenitor infiltration
- Control differentiation



Composition

- RTC = Collagen
- Prior: light curable
- Testing: self-gelling shelf-stable

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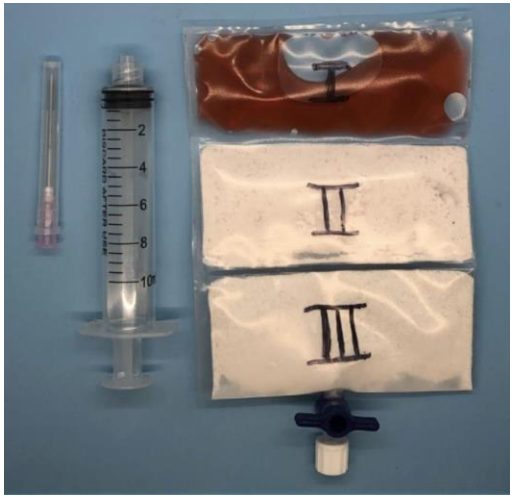
Solution

Antimicrobial & regenerative hydrogel that is easy to deliver in the continuum of care.

Therapeutic



Immune Modulator = TGF-β1

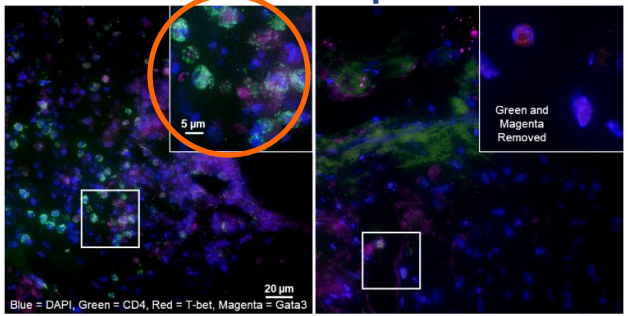
Purpose

- Highly sequestered in bone
- Immunomodulatory
- Promotes osteogenesis
- Stabilizes blood vessels

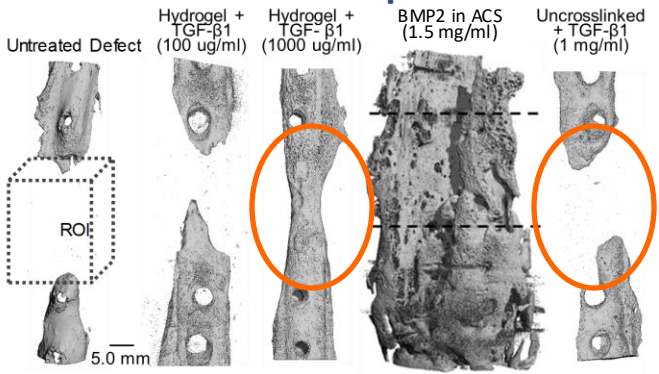
Prior testing in minipig tibial defect

- Hydrogel is essential to delivery.
- Supraphysiologic dose was required.

2-Weeks Post Implantation



5-Months Post Implantation



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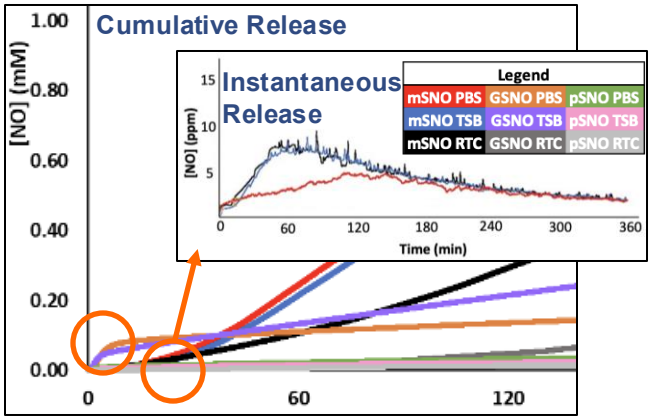
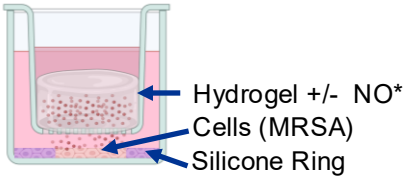
Therapeutic Characterization

A 43 mM concentration of MSNO is powerfully antibacterial and biocompatible.

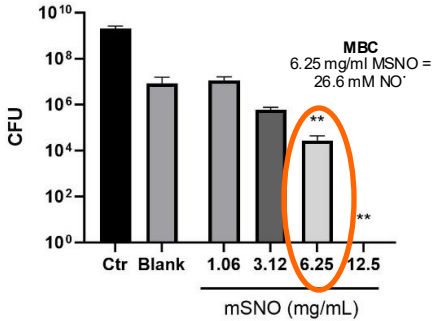
NO* Delivery at 37C



MRSA Cytotoxicity

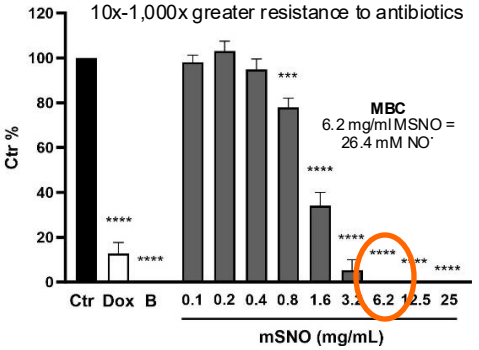


Planktonic



Biofilm

10x-1,000x greater resistance to antibiotics



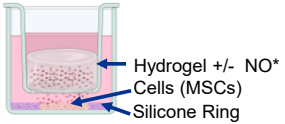
Summary

- 4253 nmols/mg (NO*/particle)
- Burst: << GSNO and tempered in RTC
- Half-life: 12 hours in PBS, 18 hours in RTC
- MBC (4-log) = 26.6 mM (6.25 mg/ml NO*/RTC)
- 43 mM = complete eradication

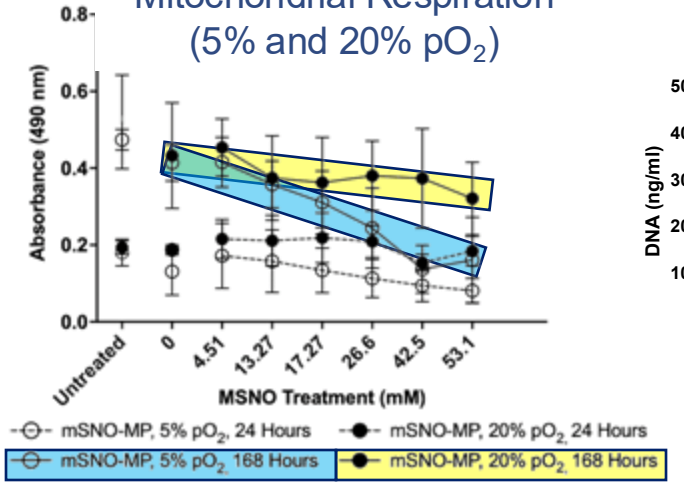
Therapeutic Characterization

A 43 mM concentration of MSNO is powerfully antibacterial and biocompatible.

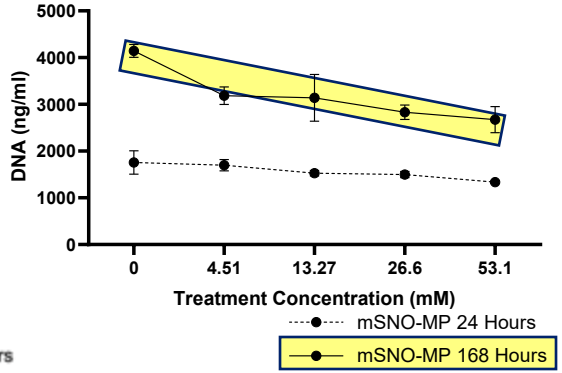
Cytocompatibility with Human MSCs



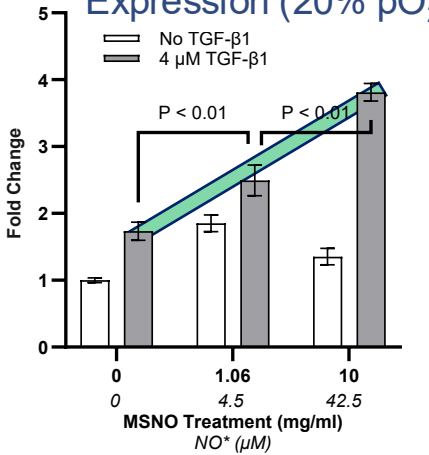
Mitochondrial Respiration (5% and 20% pO_2)



Proliferation (20% pO_2)



Osteocalcin Gene Expression (20% pO_2)



Summary

- NO* effect depends on oxygen tension
- NO* potentiates osteogenesis by TGF-β1

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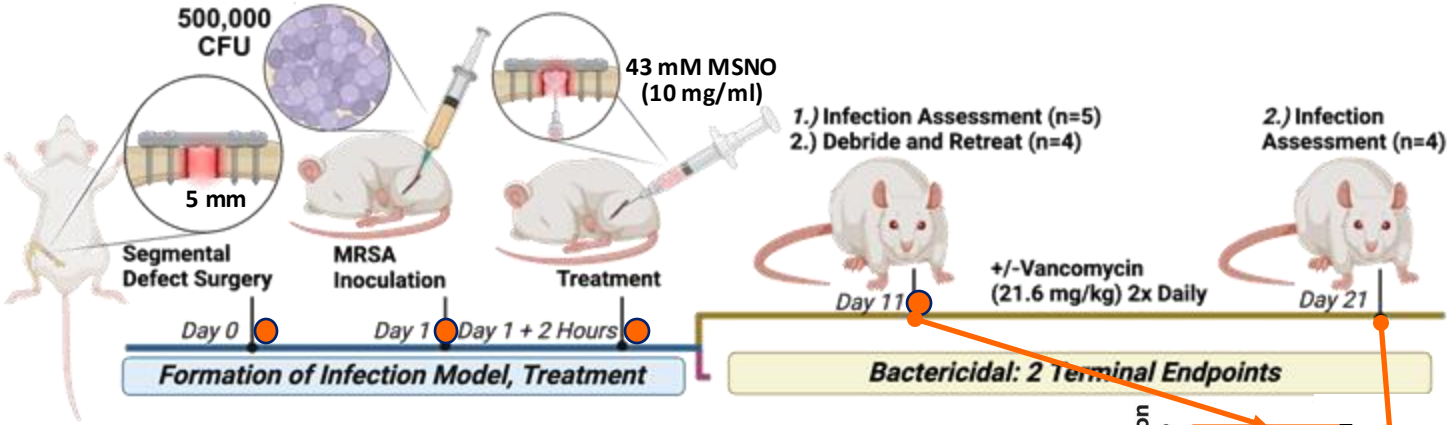
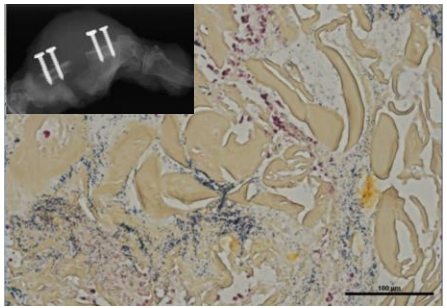
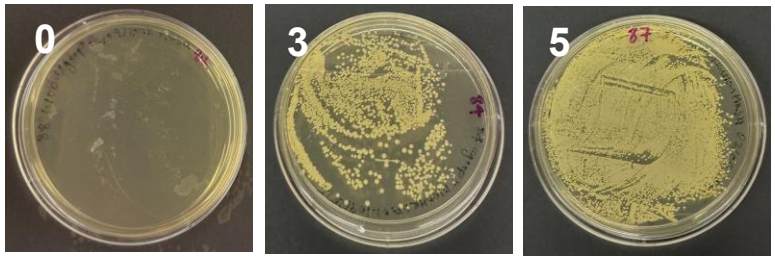
Efficacy: Antimicrobial (*in vivo*)



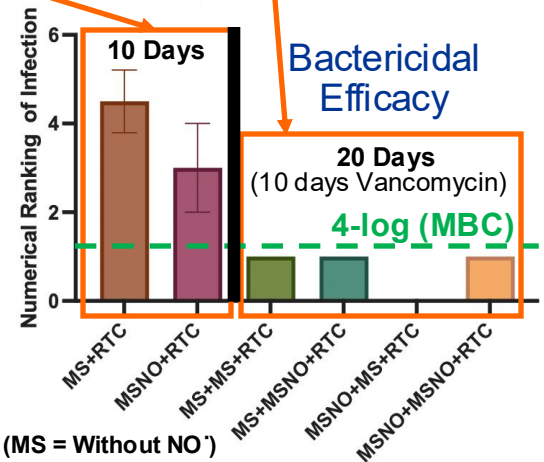
Brown & Brenn Staining



Ranking of Infection (1:100 plated dilution)



Bactericidal Efficacy



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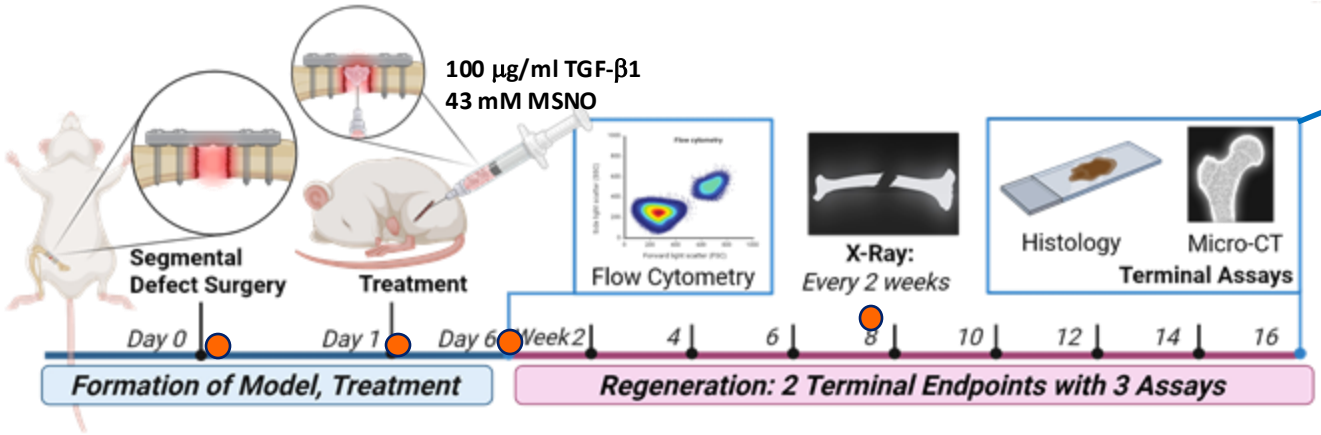
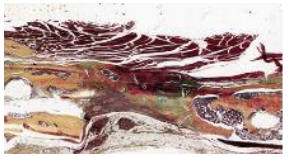
Efficacy: Regenerative (*in vivo*)



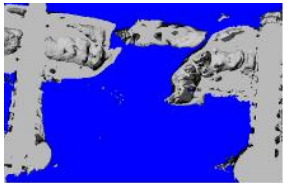
Outcomes at 16-Weeks

Movat's Pentachrome



RTC+MSNO+TGF-β1

µCT



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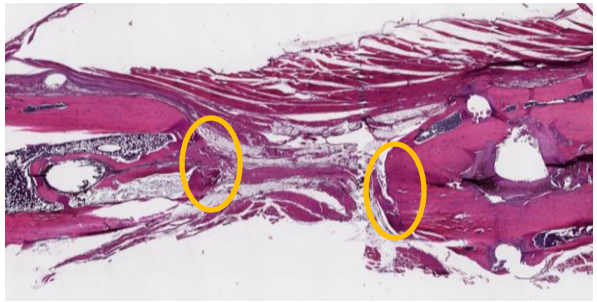
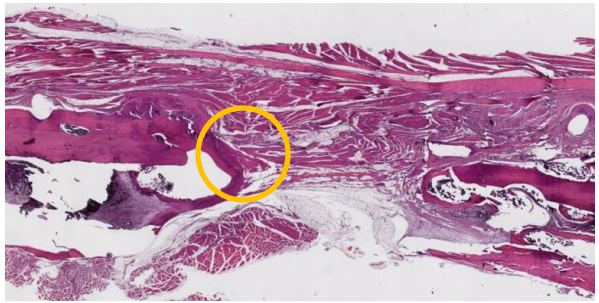
Efficacy: Regenerative (*in vivo*)

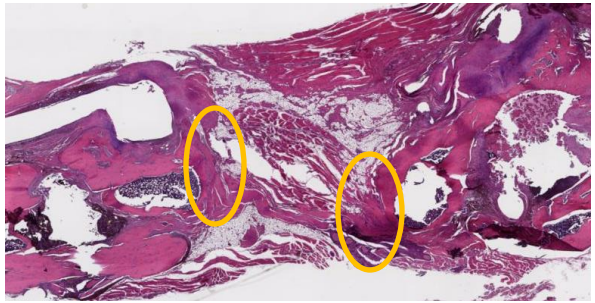
Untreated Defect



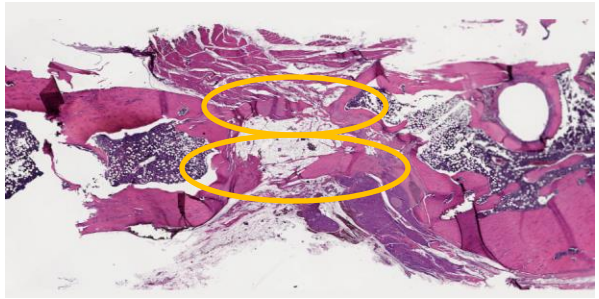
RTC+TGF-β1



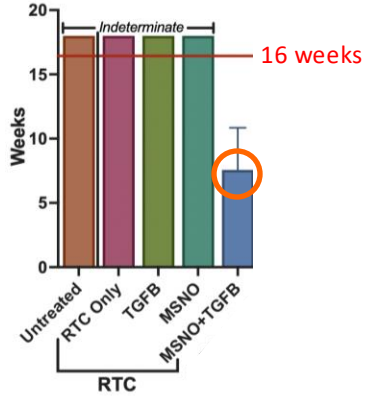
RTC+mSNO-MP



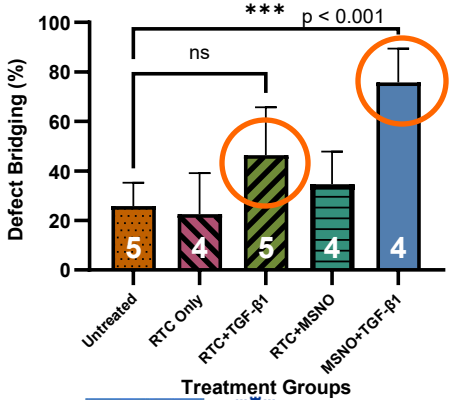
RTC+mSNO-MP+TGF-β1



Time to 50% Bridging (x-ray)



Bridging at 16-weeks (μCT)



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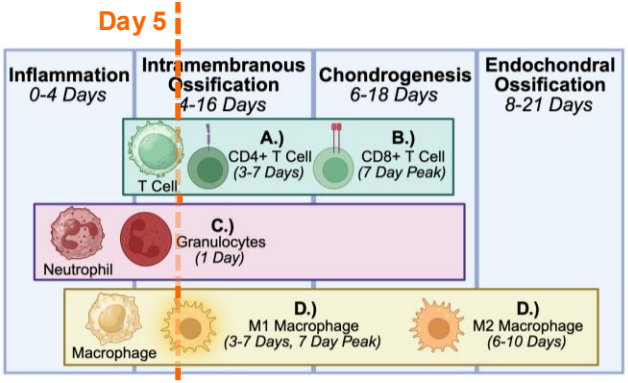
Efficacy: Osteoimmunomodulation

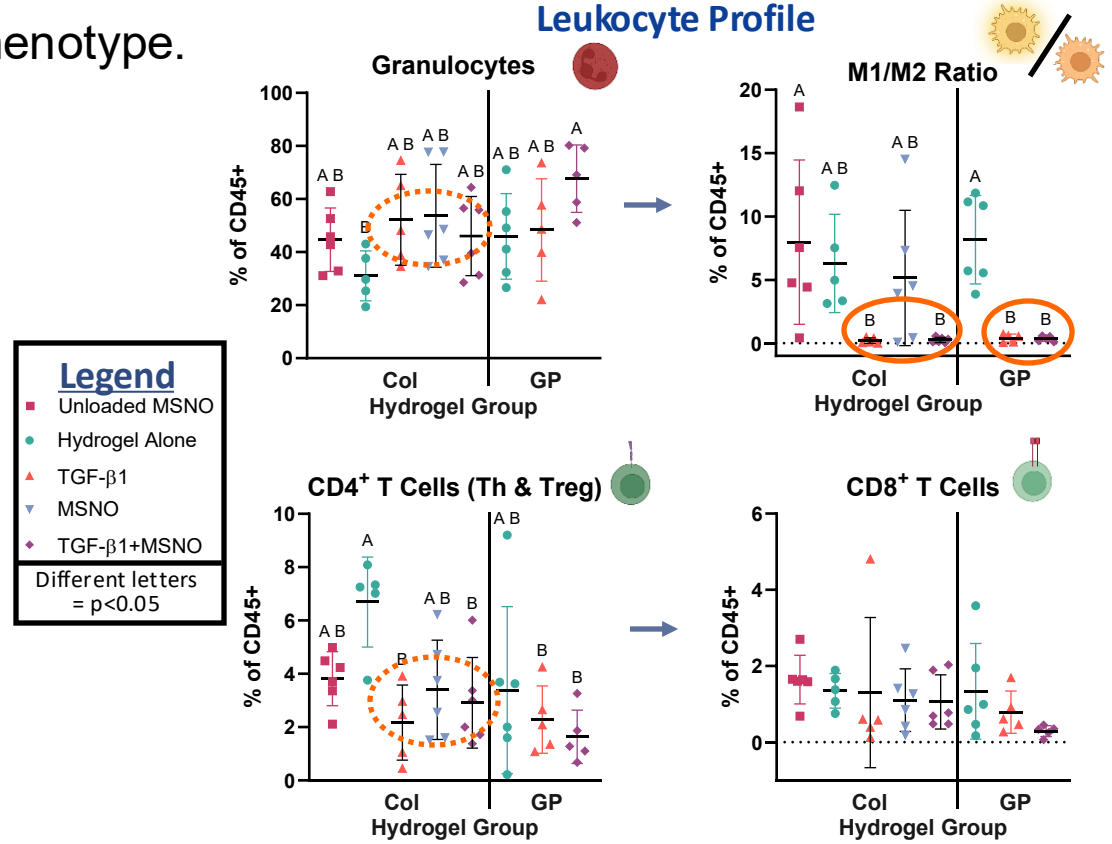
TGF-β1 suppresses the M1 phenotype.

Time Course of Leukocyte Infiltrate



Flow cytometry markers: CD45, CD3, CD4, CD8, CD161, CD11b/c, CD86, CD163, and His48

Leukocyte Profile



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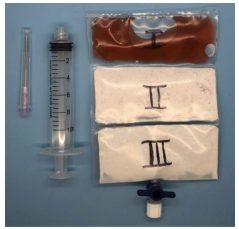
Conclusion

Validated the prototype antimicrobial and osteoinductive therapeutic for treatment of open bone wounds at the point of injury and along the continuum of care.



Determined

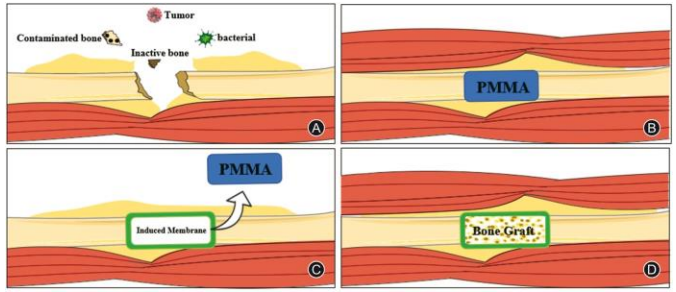
- Reduced the supraphysiologic load of MRSA in rat femurs
- Improved healing of rigidly fixed critically sized bone defects
- Potent osteoimmunomodulator

Discovered

- Novel synergy between NO* and TGF-β1
- Impactful for bone repair, bone biology, and diseases of impaired bone homeostasis

Application in Continuum of Care

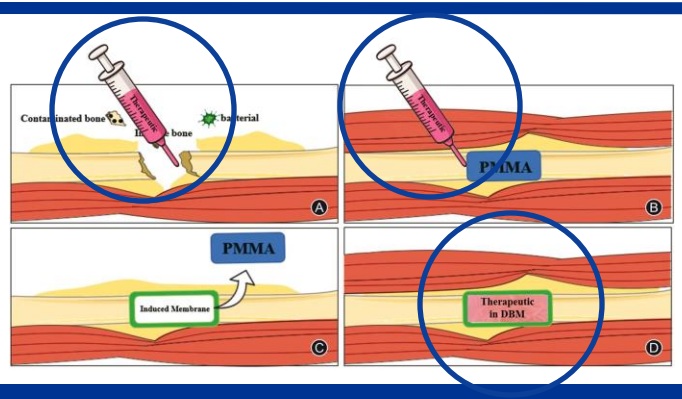
Current Treatments



Antibiotics
- Irrigated during debridement
- Eluted from spacer
- Systemic administration

Grafts
- Autologous cancellous bone
- Allogenic bone, DBM, $Ca_x(PO4)_y(Z)$ cements

Therapeutic Hydrogel



Antibiotics
- Local and/or
- Systemic administration

Grafts
- Hydrogel alone
- DBM or cements loaded with the hydrogel

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Current and Future Work

Efficacy

- Hydrogel loaded DBM in rat model
- Porcine preclinical model with physiologic initial bacterial load

MOA: Interaction Between $NO^{\cdot}$ and TGF-β1

- $NO^{\cdot}$ +/- TGF-β1 effect osteogenesis vs. chondrogenesis by hMSCs *in vitro*
- Molecular mechanisms of signaling *in vitro*

Feasibility / Utility

- Replace TGF-β1 with a small molecule agonist
- Self-gelling hydrogel formulation

IP

- US 63/755,994. TGF-B1 and Nitric Oxide Delivery Platform for Bone Wounds.

Collaborations

- Complex wounds, e.g., VML, ischemia

Team

University of Pittsburgh

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- Ingrid McNamara, MS
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Zylö Therapeutics

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