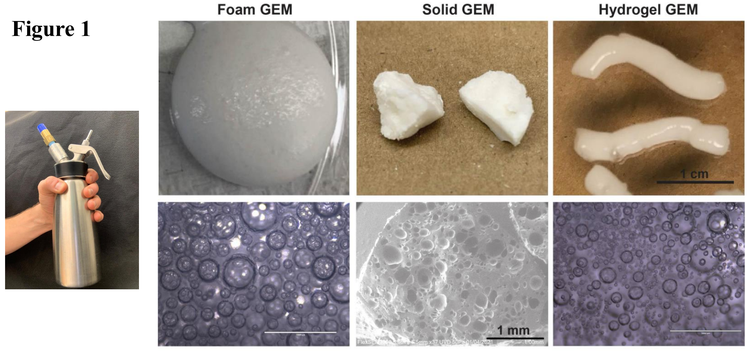
Carbon Monoxide Gas Entrapping Material to Promote Wound Healing and Reduce Bacterial Infection After Trauma

Leo E. Otterbein[1], Anupamaa Seshadri[1], Hyoin Kim[1], Paula Alves[1], Bruna Santos[1], Golda Gershanok[2], Giovanni Traverso[2], James Byrne[3]
[1]Beth Israel Deaconess Medical Center/Harvard Medical School [2]Massachusetts Institute of Technology, [3]University of Iowa

Background

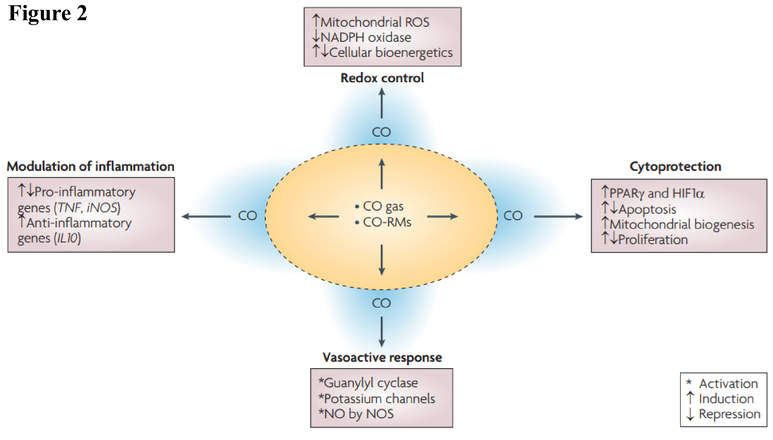
Carbon monoxide (CO) is a colorless, odorless and tasteless gas that is highly toxic at industrial doses released during combustion. CO is synthesized endogenously in all cells by the enzyme Heme Oxygenase-1 (HO-1)[1] which is vital for the host's ability to appropriately respond to stress[2]. CO is now accepted as possessing critical physiological and therapeutic properties at low concentrations yet the ability to deliver it has been met with challenges. Recently we developed **G**as **E**ntrapping **M**aterals (GEM) to safely deliver CO (and other gases) safely and effectively (**Figure 1**).

Figure 1



The exact mechanism of CO's protection is poorly understood; however, studies indicate that CO modulates inflammation and regulates innate immune responses in pathogen invasion and sterile inflammation by targeting different cell types and cellular and molecular signaling pathways. (**Figure 2**)[7].

Figure 2



Hypotheses

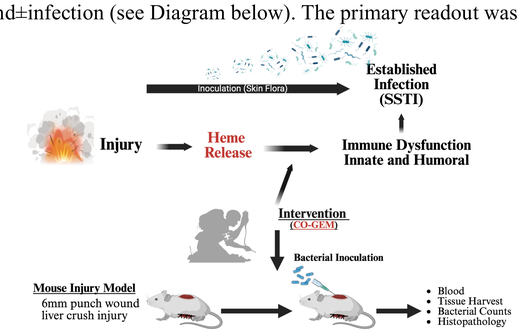
CO treatment enhances bacterial killing, including sepsis in part by modulating the innate immune response[5]. Whether CO treatment would augment the host's ability to clear bacteria in a wound has not been explored primarily because the technology for topical delivery of CO gas has not been established. Bacterial infection as well as additional trauma would slow wound healing. We therefore developed a three-hit military-relevant model in mice where a liver crush injury was accompanied by a punch wound that was subsequently inoculated with *Staphylococcus aureus*. We hypothesized the following :

(1) Effective amounts of CO can be delivered to wounds using Gas Entrapping Materials (GEM).
(2) A CO-GEM will enhance wound healing in mice and pigs
(3) Infected wound and wound in the setting of liver trauma will heal slower.
(4) A CO-GEM will enhance clearance of a bacterial wound infection
(5) A clinically and military-relevant trauma+wound infection model can be developed in mice to assess the effectiveness of CO-GEM and other interventions.

Methods

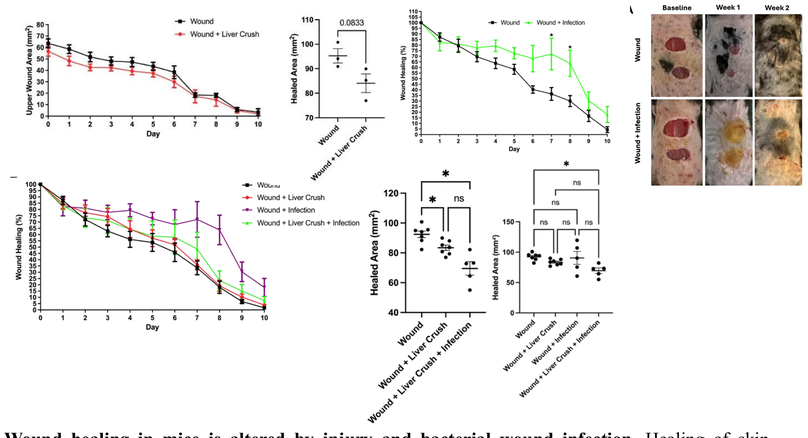
Mouse Wound, Trauma and Infection

Our goal was to simulate a blast injury in theater that resulted in release of the damage molecule heme, we combined abdominal organ damage with a skin and soft tissue injury (SSTI) with and without a bacterial infection. To accomplish this, mice were anesthetized with isoflurane and a laparotomy was performed. A Crile needle-holder (Integra Lifesciences) was modified by adding a stem and screw to the locking ratchet. This prevents the tips from closing completely and provides reproducible compression. The left liver lobe was then crushed 10 times uniformly. In After closing the laparotomy, two 6 mm punch wounds were made on the dorsal side. n To study the impact of liver crush trauma on bacterial clearance, an infection was created by adding Fluorescent *Staphylococcus aureus* (10^6-10^8 colony-forming units; 50uL) to the wound bed. Wounds were then covered in Tegaderm. Shams underwent laparotomy only ± liver crush±wound±infection (see Diagram below). The primary readout was wound healing.



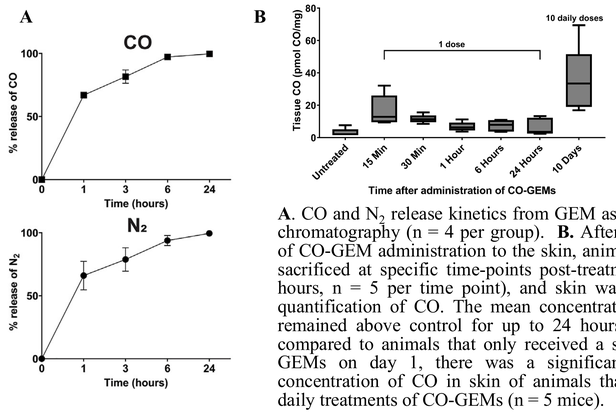
Results

Wound healing in mice in the presence and absence of trauma and infection



Wound healing in mice is altered by injury and bacterial wound infection. Healing of skin wounds was measured over time in mice that subjected to wound only, wound + *S. aureus* infection, wound + liver criush, wound + liver crush + *S. aureus* infection. N= 6-8 mice/group.

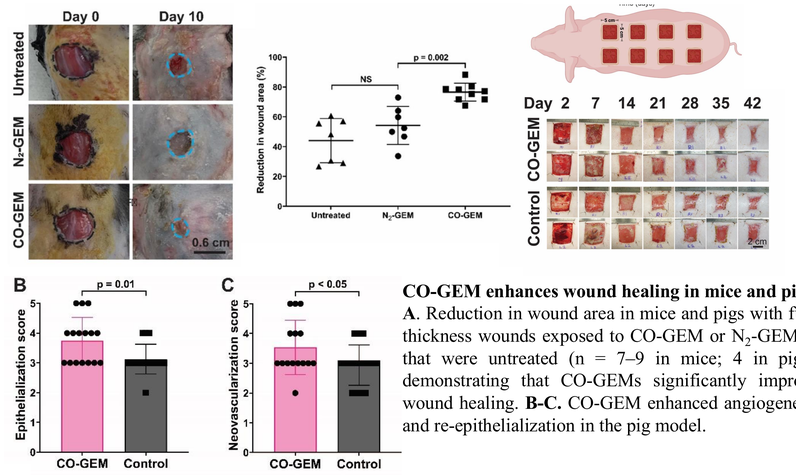
Topical CO-GEM delivery releases CO locally in the tissue



A. CO and N_2 release kinetics from GEM as determined by gas chromatography (n = 4 per group). **B.** After a single treatment of CO-GEM administration to the skin, animals were humanely sacrificed at specific time-points post-treatment (15 min – 24 hours, n = 5 per time point), and skin was collected for the quantification of CO. The mean concentration of CO in skin remained above control for up to 24 hours. Moreover, when compared to animals that only received a single dose of CO-GEMs on day 1, there was a significant increase in the concentration of CO in skin of animals that had received 10 daily treatments of CO-GEMs (n = 5 mice).

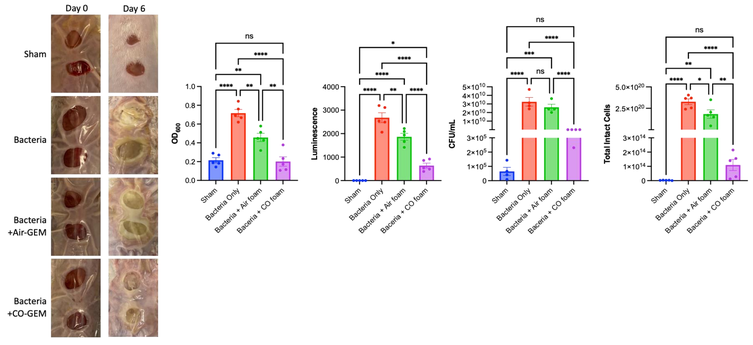
Results

CO-GEM enhances wound healing in mice and pigs



CO-GEM enhances wound healing in mice and pigs
A. Reduction in wound area in mice and pigs with full-thickness wounds exposed to CO-GEM or N_2-GEM or that were untreated (n = 7–9 in mice; 4 in pigs), demonstrating that CO-GEMs significantly improve wound healing. **B-C.** CO-GEM enhanced angiogenesis and re-epithelialization in the pig model.

CO-GEM enhances bacterial clearance of bacteria-infected wounds



Bacterial wound infection is cleared more effectively in mice treated with CO-GEM.
Mice were subjected to wounding ± infection and ± CO-GEM (50ul/wound) administered daily topically for 6 days. Bacterial counts of wounds were then done using 4 different readouts including OD, fluorescence intensity, agar plate assay, and Bactobox. In each instance, mice treated with CO-GEM showed reduced bacterial burden as compared to Air-foam control. Results are mean±SD of n=4=6/group. *P<0.05, **p<0.01, *** p<0.005, **** p<0.001.

Conclusions

1.) Gasotransmitters, modeled after molecular gastronomy with safe (GRAS) ingredients, can effectively be formulated into foams, hydrogels, and solids as a gas-entrapping material that can be safely and effectively tuned to be delivered systemically.

2.) Carbon Monoxide (CO) modulates the innate immune response to clear bacteria and promote tissue repair in models of traumatic injury. CO is in multiple clinical trials.

3.) We have created and characterized a military-relevant multiple hit injury model in mice that includes wounding, organ injury and infection for evaluation of potential therapeutic interventions.

4.) CO-GEM delivers CO effectively as a topical foam measured by systemic carboxyhemoglobin, demonstrating prototypical pharmacologic properties.

5.) CO-GEM promotes wound healing in mice and pigs and wound bacterial clearance in a mouse wound model

6.) Ongoing studies is evaluating the efficacy of CO-GEM in the wound/trauma/infection model.

Acknowledgments

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