E-101 Solution (E-101) is a novel cell free myeloperoxidase-mediated microbicidal agent. This study was undertaken to evaluate the effect of E-101 on bacterial growth in wound or surgical incision sites. Hydrogen peroxide (H₂O₂) is produced in situ by the GO dehydrogenation of glucose and reduction of oxygen. The MPO-catalyzed oxidation of chloride by H₂O₂ generates hypochlorous acid (HOCl). Once generated, HOCl reacts in a diffusion-controlled reaction with a second H₂O₂ molecule to yield singlet oxygen. We evaluated the treatment effects of E-101 and its oxidative products on ultrastucture changes and microbicidal activity against methicillin-resistant Staphylococcus aureus (MRSA) and E. coli.

**Methods:** Time kill and transmission electron microscopy (TEM) studies were performed in MRSA ATCC 43300 and E. coli ATCC 25922 using enzymatic titrations of E-101 (150 and 300 µL/mL) and formulations with pMPO or GO omitted. TEM of TEM, early exponential phase growth were exposed to each formulation and sampled at 0, 30, 60, and 120 minutes. Bacteria were fixed in 3.0% glutaraldehyde, embedded and thin sectioned for TEM analysis.

**RESULTS:** E-101 demonstrated rapid (at 30 min) bactericidal activity. At 30 min morphologic changes were minimal, but at 60 and 120 min dramatic effects on S. aureus and E. coli morphology were observed as septal deformation. Both formulations of E-101 induced cytotoxic membrane inclusions (mesosomes) in S. aureus and E. coli indicating a membrane effect. Increase vacuolization of the cytoplasm and cell ghosts were also observed. E. coli appeared elongated with no visible septa, highly vacuolated cytoplasm with pleated cell walls compared to controls. When GO was omitted from the formulation, no antimicrobial activity or cellular damage was observed. When pMPO was omitted from the formulation, only increased vacuolization due to H₂O₂ was observed at the longer exposure times. Prolonged exposure to high levels of H₂O₂ generated from GO and glucose produced oxidative activity with no visible cellular damage.

E-101 is a potent myeloperoxidase enzyme system with multiple oxidative mechanisms of action. Ultrastuctural analysis following E-101 treatment showed targeted vacuolution of the cytoplasmic membrane inclusions (mesosomes) in S. aureus and E. coli by E-101 indicative of an effect on the cytoplasmic membrane.

**Conclusion:** E-101 demonstrated rapid (at 30 min) bactericidal activity. At 30 min morphologic changes were minimal, but at 60 and 120 min dramatic effects on S. aureus and E. coli morphology were observed as septal deformation. Both formulations of E-101 induced cytotoxic membrane inclusions (mesosomes) in S. aureus and E. coli indicating a membrane effect. Increase vacuolization of the cytoplasm and cell ghosts were also observed. E. coli appeared elongated with no visible septa, highly vacuolated cytoplasm with pleated cell walls compared to controls. When GO was omitted from the formulation, no antimicrobial activity or cellular damage was observed. When pMPO was omitted from the formulation, only increased vacuolization due to H₂O₂ was observed at the longer exposure times. Prolonged exposure to high levels of H₂O₂ generated from GO and glucose produced oxidative activity with no visible cellular damage.

**References:**


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**ABSTRACT**

E. coli ATCC 25922 demonstrated rapid bactericidal activity of E-101 (pMPO + GO). High levels of H₂O₂ generated from glucose and GO showed some bactericidal activity but at a much slower rate. Catalase production by S. aureus competitively destroys H₂O₂ but was ineffective in preventing the rapid killing activity of E-101. Formulation without GO (pMPO only) showed no antimicrobial activity.

**METHODS**

**INTRODUCTION AND PURPOSE**

**RESULTS**

**CONCLUSIONS**

- Time-kill and TEM studies showed that E-101 oxidative products damage cells in a time- and concentration-dependent manner if pMPO, halide and a source of hydrogen peroxide are present.
- The pMPO-H₂O₂ microbial action is several orders of magnitude more potent and than H₂O₂ alone.
- E-101 induced cytotoxic membrane inclusions (mesosomes) and septal deformation in MRSA. The presence of mesosomes is the indication of an effect on the cytoplasmic membrane.
- E-101 induced cellular elongation (septal deformation), cytoplasmic vacuoles, and pleated cell walls in E. coli. These findings are consistent with results observed with multiple mechanisms of E-101 activity.
- The lack of obvious ultrastructure change but a 6 log₁₀ kill at 30 minutes of treatment with E-101 suggest that the rapid combustive denaturation of key enzymatic components and/or destruction of membrane integrity are probable microbial events.