Wound Healing Proof of Concept and Current Development Summary

Background

- Selenium, Ltd ("Selenium") has developed a number of technologies for coating or incorporating our proprietary Seldox™ technology into various substrates which are relevant to the field of wound treatment. Our technology has proven itself to be effective and long lasting under a variety of conditions and with a variety of materials, often showing 4 logs or greater of bacterial inhibition, pending optimization to partner specification.

- Development of the Seldox-enabled cellulose bandage was among the first projects undertaken by Selenium in this application area. The project built upon the work done by Dr. Phat Tran for his dissertation. Recent developments in this project have shown the material's stability such that it may be marketed as a reusable bandage. In 2006, Selenium partnered with ClassOne Orthodontics to develop orthodontic applications, including an antimicrobial medical adhesive that would hold the bracket in place on the tooth surface. This product line received FDA 510(k) approval and was introduced to the market in 2009. An anti-microbial cream project for use on burn wounds was undertaken in early 2009, and is currently in animal trials. Polyurethane foam, thermoplastic polyurethane, and silicone rubber substrates are all under current development, and each shows a great deal of promise for numerous applications including wound healing.

Assay Descriptions

- Selenium's CFU Assay:
  - The purpose of Selenium's CFU assay is to determine the number of colony forming units of *Staphylococcus aureus 31* (SA 31) that adhere on control and Seldox treated or enabled materials. This internally-generated data is used as a proxy for the inhibition of other gram negative or gram positive bacteria and is highly correlative with industry standard tests such as the Japanese Industrial Standard (JIS z2801) assay. When specified, other strains of bacteria may be used instead of SA31.
  - Description: Control and Seldox treated materials are placed into 2.5 mL wells containing a growth medium and approximately 1,000 cells of SA31. The wells are left in a 37^{o}C incubator overnight. The material samples are then removed from the wells and placed in 1mL tubes and vortexed to remove the bacteria. The resulting solution is then diluted by serial dilution down to 10-7 (seven tenfold dilutions). Each of the resulting solutions is then "spotted" on a nutrient rich agar plate in three separate spots and the plates are incubated overnight.

Any viable cell will then expand over the surface of the agar forming a spot, or colony. The lowest dilution in which colonies are visible is counted, and the results tabulated to provide a comparative inhibition profile between Seldox-enabled and control materials.

- Accelerated Aging Studies:
  - Selenium uses industry standard accelerated aging studies to demonstrate coating or material stability.
  - Description: Accelerated aging is achieved by storing materials in a 67 degree Celsius oven in phosphate buffered saline, pH 7.4. Under these conditions, every week of storage is considered to simulate 16 weeks of time in a wound bed.

- JIS Z2801
  - Selenium uses a Specialty Scientific (www.specialtyscientific.com) as a third party lab to perform industry standard biofilm prevention assays such as the Japanese Industrial Standard (JIS) Z2801 assay.
  - Description: Antimicrobial activity is measured by quantifying the survival of bacterial cells which have been held in intimate contact for 24 hours at 35°C with a surface that contains an antibacterial agent. The antimicrobial effect is measured by comparing the survival of bacteria on a treated material with that achieved on an untreated material.

Cellulose Bandages

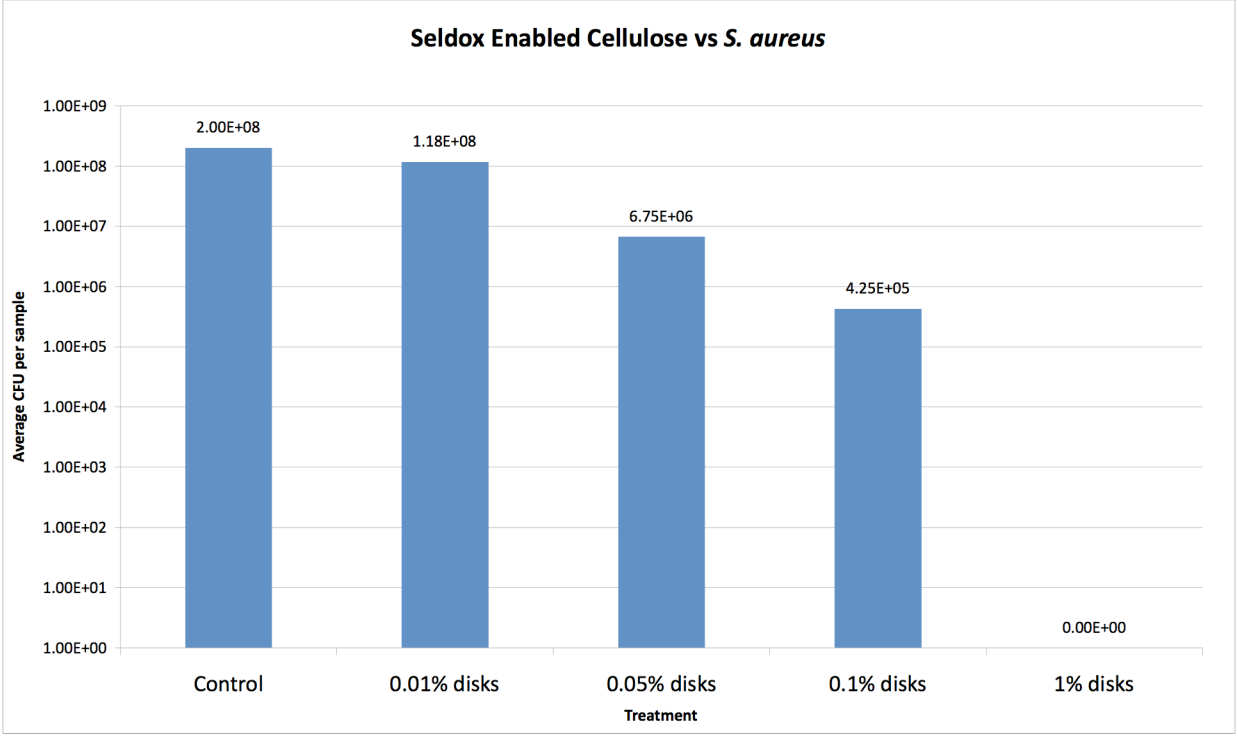
- **Development Notes**
  - Selenium has pursued development of Seldox-enabled cellulose bandages as it provides an opportunity for simplified incorporation during manufacturing via a dip coat process and protection against cuts, scrapes and shearing as the active compound is absorbed into the fiber and polymerized there.

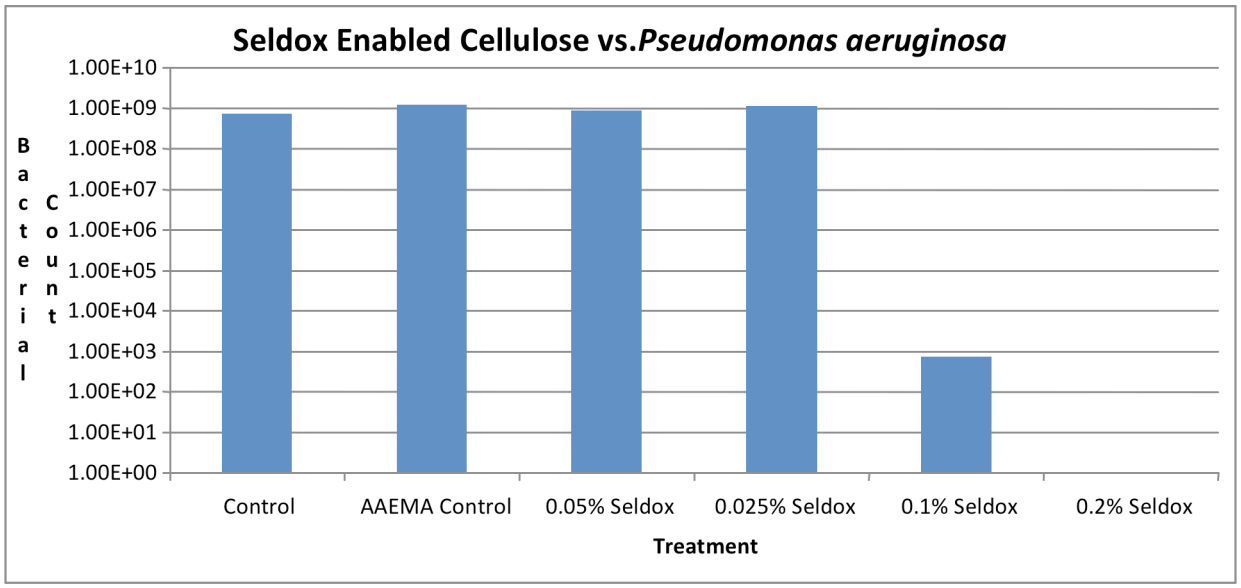
- **Material Description**

  - Seldox enabled materials are impregnated with the active material and polymerization is triggered. Non-optimized samples produced by Selenium can have a light yellow tint depending on the Seldox concentration. Lower effective doses have no apparent color. There is a slight odor associated with the completed bandage. There appears to be no change in physical characteristics aside from the color change in the Seldox-enabled samples. Optimization is pending partner specifications.

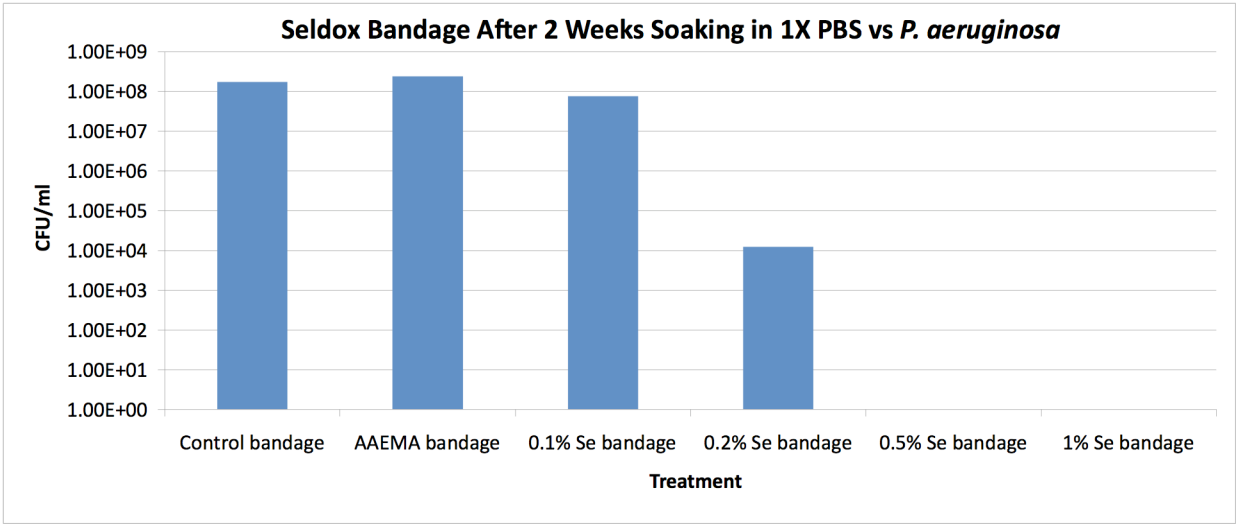
- **Dose Response/Efficacy**

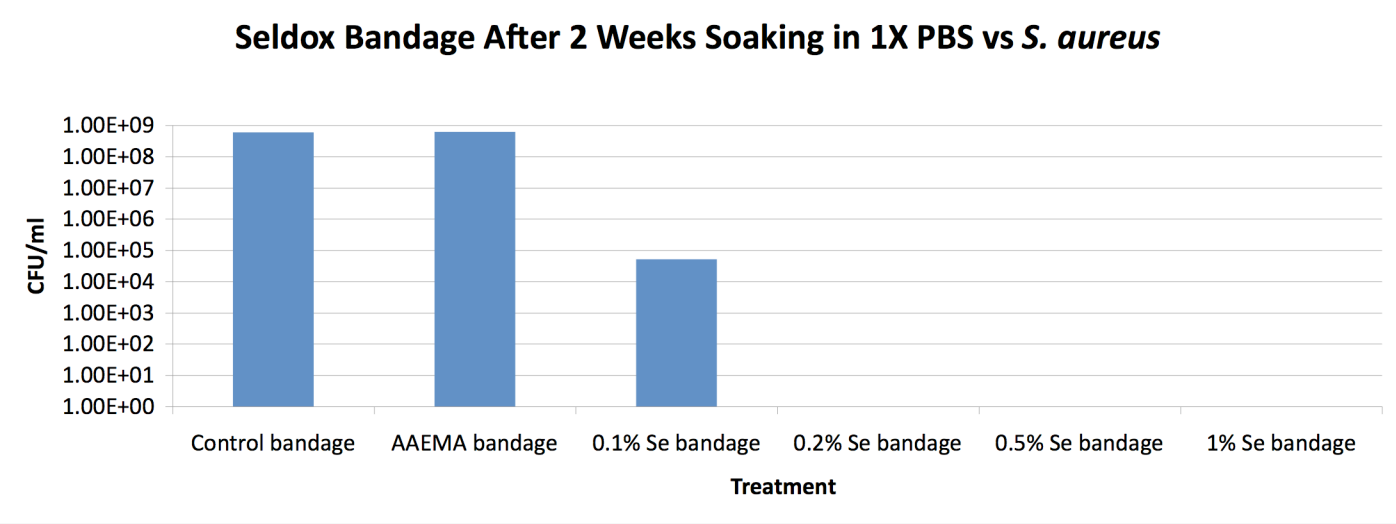
  - Selenium CFU Assay Results: A total inhibition of 8.86 logs of killing was observed at 0.2% Seldox vs *Pseudomonas*. AAEMA controls are used as a process control, using a different linker without Seldox to ensure that there is no antimicrobial activity stemming from sources other than the Seldox.



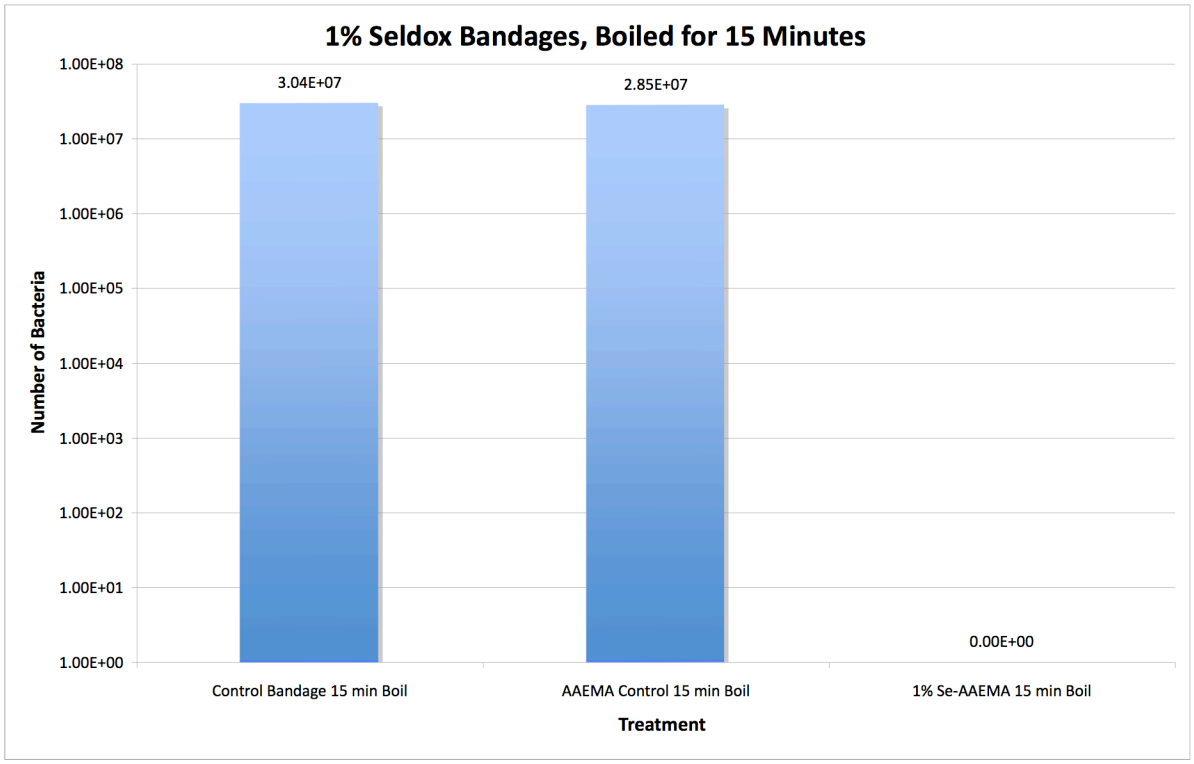


- **Stability**
  - This study was carried out by submerging Seldox enabled bandages in phosphate buffered saline for a period of two weeks at 37°C, which is approximately equal to four weeks in a wound bed.
  - Selenium CFU Assay Results: Internal testing shows a strong antimicrobial response against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Stability studies showed continuing absolute inhibition at 0.5% Seldox and above for *Pseudomonas*, and 0.2% and above for *Staphylococcus*.





- **Boil Study**
  - This test was carried out in order to determine if Seldox bandages would be suitable for reuse, especially in third world applications. The preliminary results are encouraging.



- **External Testing**
  - JIS Z2801

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TEST FOR ANTIMICROBIAL ACTIVITY AND EFFICACY
JIS Z 2801

Test Article:	Selenium Ltd Cloth
ID No.	Selenium LTD 122009001
Date of Receipt:	December 12, 2009

Test Organisms:	Staphylococcus aureus Serratia marcescens Pseudomonas aeruginosa	
Sample Size:	2cm x 2.5 cm	
Number of Layers:	One	
Neutralizer:	Letheen Broth	
Target Inoculum Level:	(1-2) x 10^5(CFU) / 1mL	
Inoculum concentration:	S. aureus S. marcescens P. aeruginosa	1-2 x 10^5 (CFU) / 1mL 1-2 x 10^5 (CFU) / 1mL 1-2 x 10^5 (CFU) / 1mL

Test Organisms	*Zero Contact Time*	*Percent Reduction*
S. aureus	1-2 x 10^5 (CFU)	> 99.990
S. marcescens	1-2 x 10^5 (CFU)	> 99.900
P. aeruginosa	1-2 x 10^5 (CFU)	> 99.900

Date completed December 17, 2009 Approved By
Ye-Sun Lee, Ph.D

Performed By
Lynn Allison

All raw data pertaining to this study and a copy of the final report will be retained in hard copy and electronic form by Specialty Scientific.
Results and conclusions apply only to the test sample submitted. No further evaluation of these results is made by Specialty Scientific. Any extrapolation or interpretation of this data to other sample is the responsibility of the sample submitter. All procedures were conducted in conformance with good laboratory practice and EN45001 Quality Standards.

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- Toxicity Data—Toxicity testing by Toxicon, a third party lab specializing in toxicology, showed that water that was in contact with the bandage contained no toxic substances, even when exposed for a month.

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TEST RESULT CERTIFICATE

Sponsor	Selenium Ltd.	**Technical Initiation**	8/29/2007
Address	3601 4th Street Lubbock, Texas 79430	**Technical Completion**	8/31/2007
Contact	Tracy Gray	**Report Date**	9/4/2007
P.O. Number	SEL-LS-10136	**Project Number**	07-3725-N1

Test Article	Wash Solution of 0.2% DiSe 1week
Lot/Batch #	081607
Study	Agar Diffusion Test – USP
Comments	None

REFERENCES: The study was conducted based upon the following references: USP 30, NF 25, 2007. <87> Biological Reactivity Tests, *in vitro*.

ISO/IEC 17025, 2005, General Requirements for the Competence of Testing and Calibration Laboratories.

GENERAL PROCEDURE: The biological reactivity of a mammalian monolayer, L929 mouse fibroblast cell culture, in response to the test article was determined. The monolayer was protected from mechanical damage, while allowing diffusion of leachable chemicals from the test article, with a layer of agar stained with a vital dye (neutral red). The test article (100 µL) was placed in a sterile filter disc with a surface area ≥ 100 mm^2 at 100% concentration. This disc was applied directly to the surface of the agar, in duplicate. Positive (Buna–N–Rubber) and negative (Negative Control Plastic) control articles were prepared to verify the proper functioning of the test system. The cultures were incubated at 37 ± 1 °C, in a humidified atmosphere containing 5 ± 1% carbon dioxide, for 48 hours. Biological reactivity (cellular degeneration and malformation) was rated on a scale from Grade 0 (No reactivity) to Grade 4 (Severe reactivity). The test article met the requirements of the test if none of the cultures exposed to the test article showed greater than a Mild Reactivity, Grade 2.

RESULTS: No signs of reactivity (Grade 0) were exhibited by the cell cultures exposed to the test article or the negative control article at the 48 hour observations. Moderate signs of reactivity (Grade 3) were observed for the positive control article at the 48 hour observation.

CONCLUSION: The test article is considered non–cytotoxic and meets the requirements of the Agar Diffusion Test, USP guidelines.

AUTHORIZED PERSONNEL:

Franck Grall, Pharm. D., Ph.D.
Study Director

Morgan Sessoms, B.A.
Quality Assurance

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TEST RESULT CERTIFICATE

Sponsor	TTUHSC/Selenium Limited	**Technical Initiation**	10/5/2007
Address	3601 4th street Lubbock, Texas 79430	**Technical Completion**	10/7/2007
Contact	Tracy Gray	**Report Date**	10/12/2007
P.O. Number	SEL-LS-10145	**Project Number**	07-4363-N1

Test Article	0.2% DiSe 1 month solution
Lot/Batch #	Not Supplied by Sponsor
Study	Agar Diffusion Test – USP
Comments	None

REFERENCES: The study was conducted based upon the following references: USP 30, NF 25, 2007. <87> Biological Reactivity Tests, *in vitro*.

ISO/IEC 17025, 2005, General Requirements for the Competence of Testing and Calibration Laboratories.

GENERAL PROCEDURE: The biological reactivity of a mammalian monolayer, L929 mouse fibroblast cell culture, in response to the test article was determined. The monolayer was protected from mechanical damage, while allowing diffusion of leachable chemicals from the test article, with a layer of agar stained with a vital dye (neutral red). The test article (100 µL) was placed in a sterile filter disc with a surface area ≥ 100 mm2 at 100% concentration. This disc was applied directly to the surface of the agar, in triplicate. Positive (Buna–N–Rubber) and negative (Negative Control Plastic) control articles were prepared to verify the proper functioning of the test system. The cultures were incubated at 37 ± 1 °C, in a humidified atmosphere containing 5 ± 1% carbon dioxide, for 48 hours. Biological reactivity (cellular degeneration and malformation) was rated on a scale from Grade 0 (No reactivity) to Grade 4 (Severe reactivity). The test article met the requirements of the test if none of the cultures exposed to the test article showed greater than a Mild Reactivity (Grade 2).

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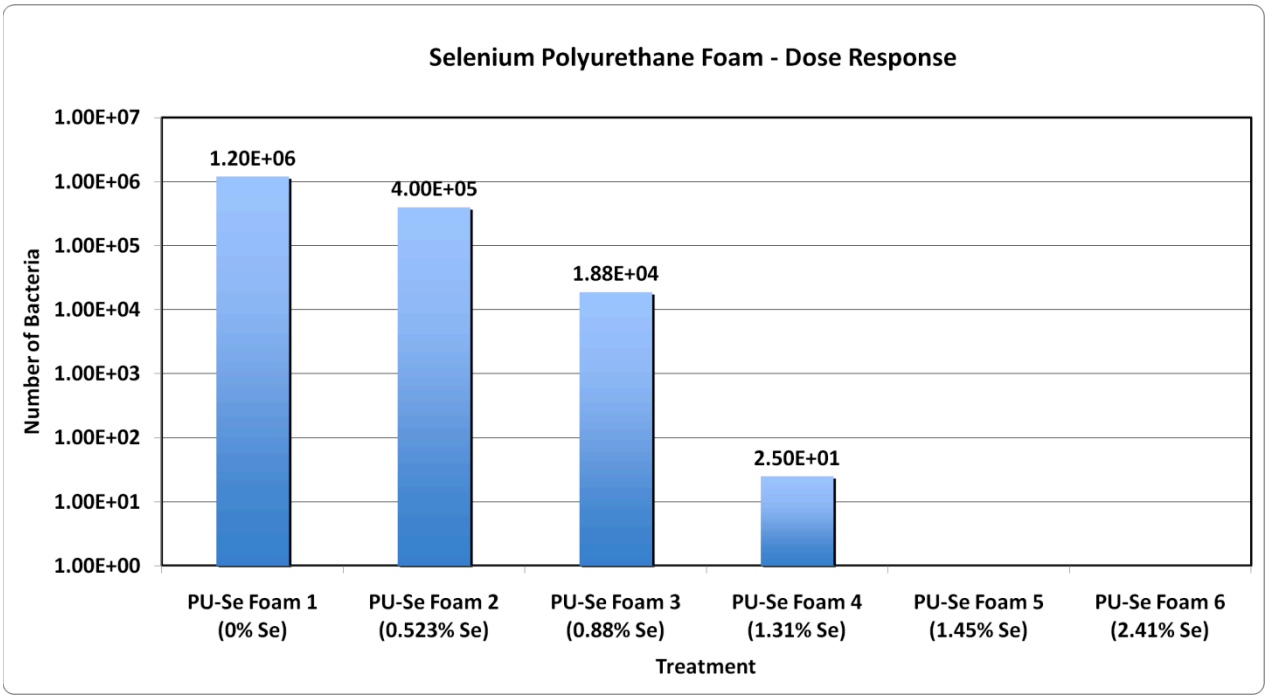
- **Conclusion**
  - Internal testing has shown that Seldox enabled cellulose bandages exhibit a high degree of antimicrobial efficacy, and remains stabile for at least two weeks in fluid at body temperature. External testing showed high inhibition of *S. aureus,*

S. marcescens, and *P. aeruginosa.* External toxicity testing showed no toxicity to bystander cells. This data is directly applicable to wound healing applications and is indicative of the platform readiness for co-development efforts to enter this market.

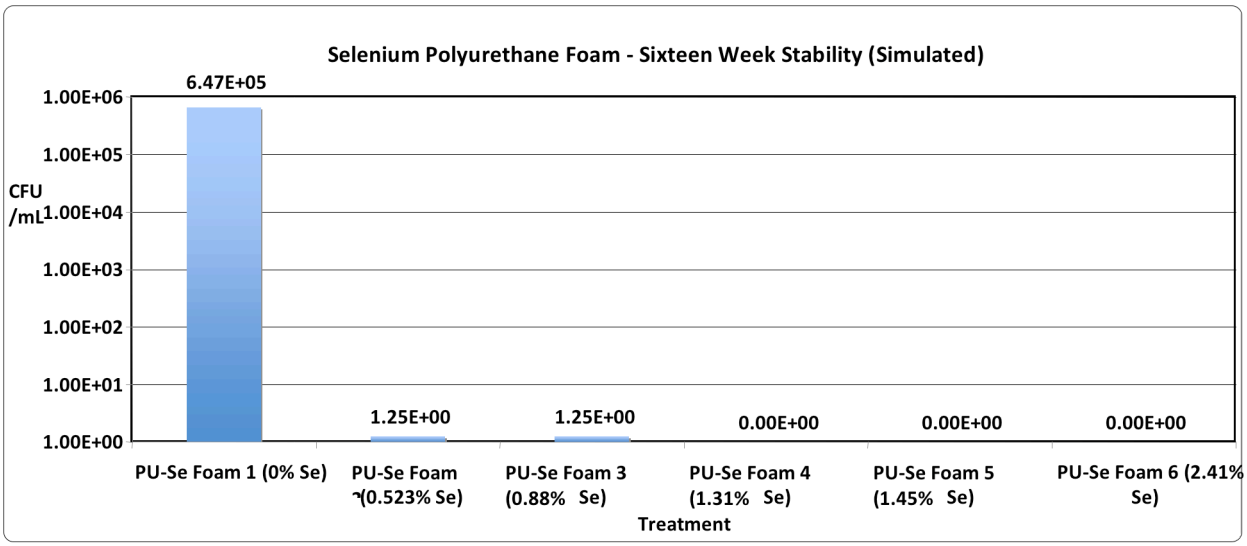
Polyurethane Foam

- **Development Notes**
  - Market research has shown that a robust and powerful anti-microbial is needed for polyurethane bandage applications. To that end, Selenium has developed a Seldox-enabled polyurethane foam as a proof of concept.
- **Material Description**
  - Seldox copolymer polyurethane foam is a light, flexible material with added color proportional to the amount of selenium added. The highest concentration has a light yellow color. There is no detectable smell or apparent change in physical characteristics associated with the samples.
- **Dose Response/Efficacy**
  - Selenium CFU Assay Results: A total inhibition of 6.0792 logs of killing on PU-Se foam formulations #5 and #6 was observed.



- **Stability**

  - Selenium CFU Assay Results: A total inhibition of 5.7138 logs of killing at 0.523% Se was observed in all treatments over 16 weeks (simulated).



- **Conclusion**

  - Internal testing has shown that Seldox-copolymerized polyurethane foam exhibits a high degree of antimicrobial efficacy, and remains stabile for at least 100 days in simulated wound bed conditions. This data represents Selenium's first efforts at copolymerized application of the Seldox technology and is indicative of the degree of flexibility in the platform.

Silicone copolymer

- **Development Notes**

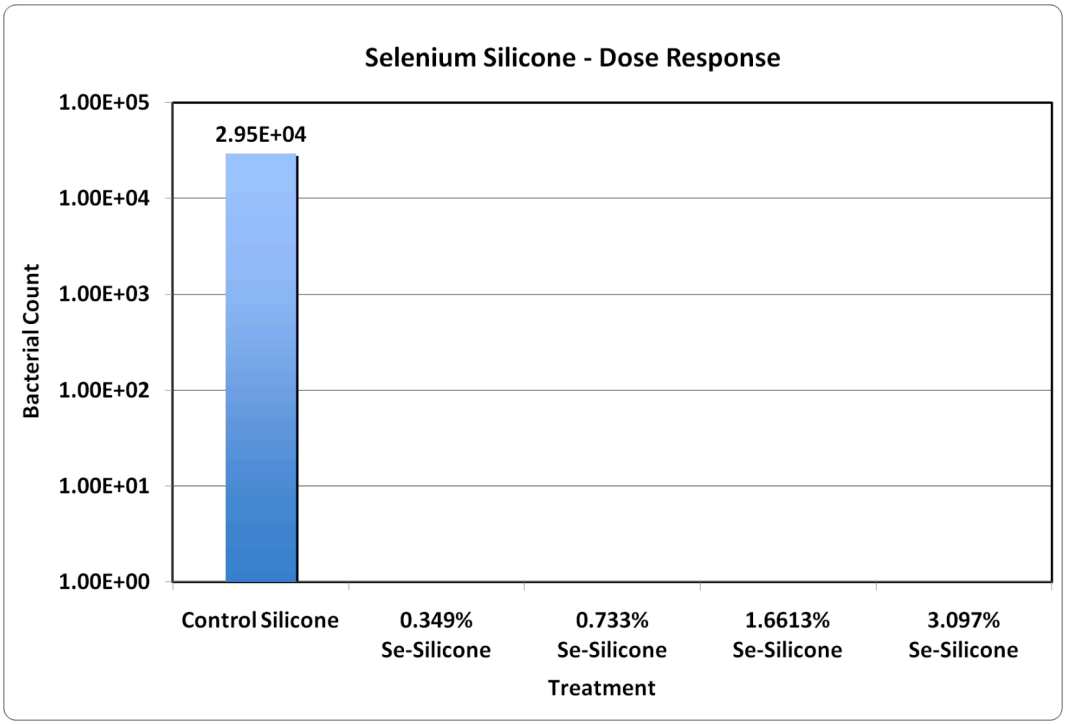
  - Selenium's work in the medical device market led it to a method to incorporate Seldox directly into the polymer chain of silicone. This is among our newest products, and has not been subjected to external testing.

- **Description of Silicone - Copolymer**

  - The incorporation of a Seldox co-monomer into silicone does not appear to have any effect on the physical properties of the final product, save for the addition of a slight yellow tint which is proportional to the amount of Seldox added, with the lowest tested effective dose having a barely visible tint.

- **Dose Response/Efficacy**

  - Selenium CFU Assay Results: A total inhibition of 4.470 logs of killing was observed in all treatments.



- **Conclusion**

  - Internal testing has shown that Seldox-enabled silicone exhibits a high degree of antimicrobial efficacy, even at low concentrations. Although internal QA/QC has not yet been completed, these results are very encouraging. The ability to block the growth of bacteria at very low concentrations of Seldox material is further evidence of the flexibility of the technology.

Topical Cream

- **Development Notes**

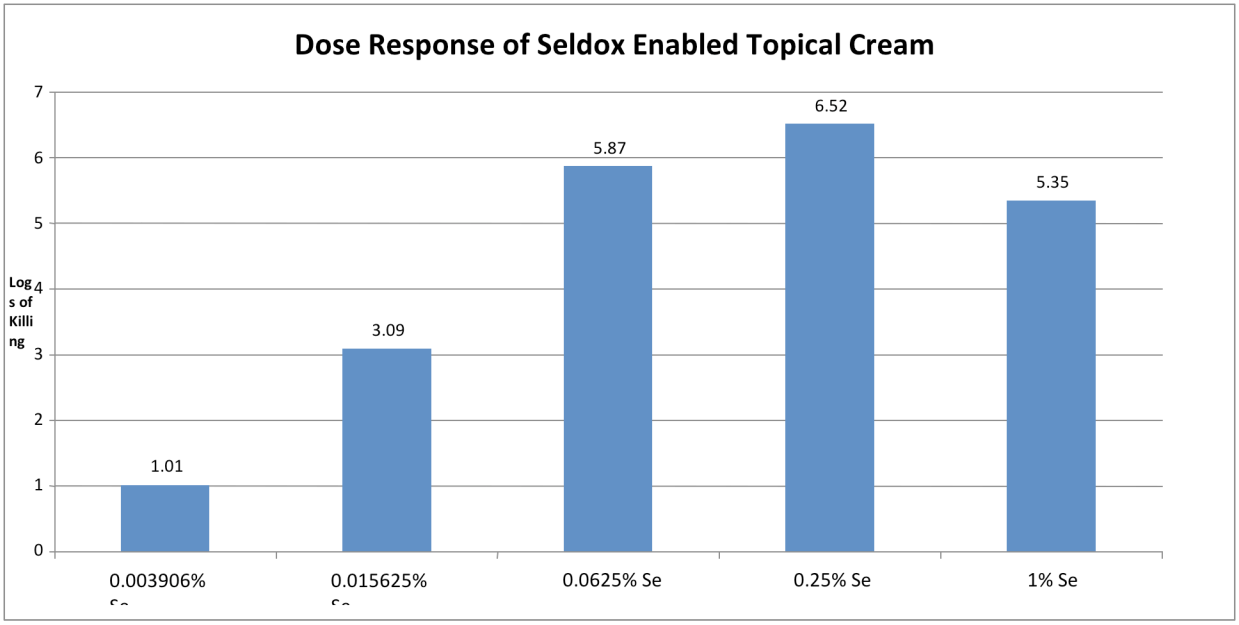
  - Clinicians have expressed a great deal of interest in a long lasting anti-microbial cream that could be used to treat burn wounds. In order to service this need, Selenium has developed a Seldox-enabled ointment.

- **Material Description**

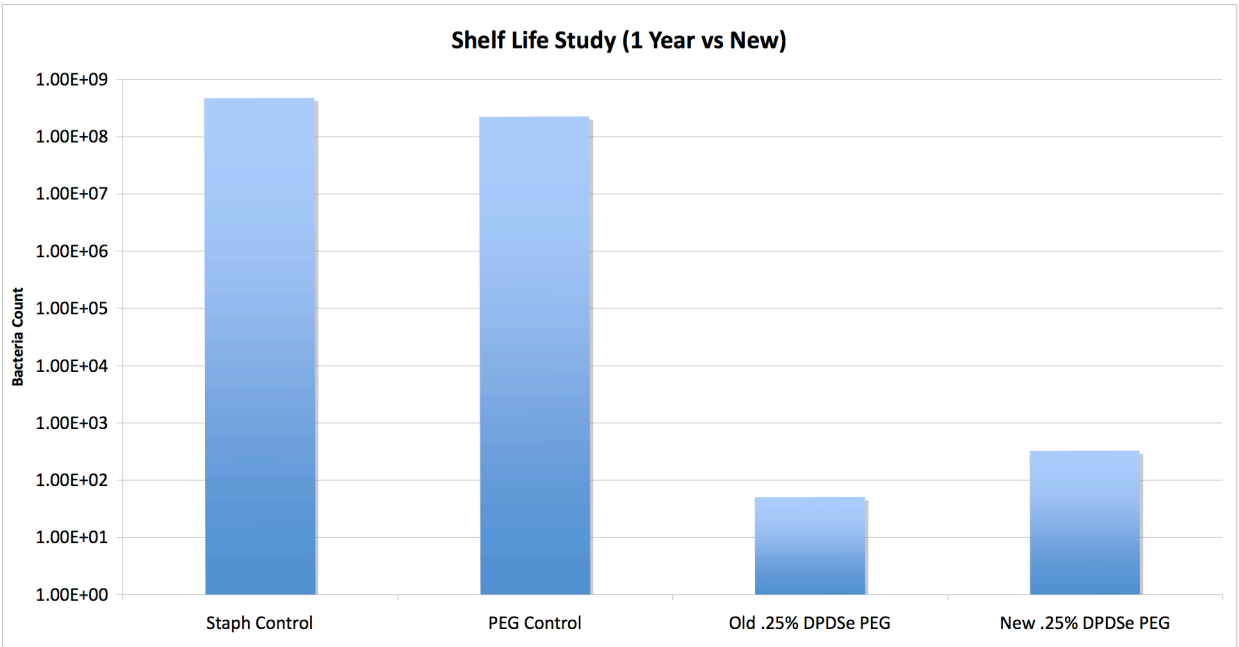
- Seldox enabled creams have a yellow color. Aside from the color, the properties of the Seldox-enabled creams are determined entirely by the choice of compounding base. We currently use a high molecular weight PEG cream as a base, which is a solid at room temperature, but a persistent, viscous gel at body temperature.

- **Efficacy/Dose Response**

  - Selenium CFU Assay Results: This material has been tested against a wide range of species, with results as follows:
    - *Staphylococcus epidermidis*—11.72 logs
    - *Enterococcus casseliflavus* (clinical isolate)—7.82 logs
    - *Staphylococcus aureus* (clinical isolate)—6.75 logs
    - *Enterococcus faecalis*—5.22 logs
    - *Klebsiella pneumoniae*—1.41 logs
    - *Pseudomonas aeruginosa*—0.47 logs
    - *Acinetobacter*—no effect
  - The data composing the above below was taken from several different studies, and as such, is reported as logs of killing rather than absolute numbers. Each is referenced to its own control.



- **Stability**
    - Shelf life of the Seldox cream has been shown to exceed a year, as year old material has approximately the same killing efficacy as newly made material.



- **Conclusion**
    - Internal testing has shown that Seldox-enabled topical cream exhibits a high degree of antimicrobial efficacy against many clinical strains of bacteria, and remains stabile for at least 1 year on the shelf. Though highly effective against gram positive strains, gram negative strains showed less susceptibility. This product remains in active development, as Selenium researchers continue to optimize the product concentration and compound form to improve efficacy against gram negative strains.

Wound Care Summary

- Selenium's Seldox materials are well suited to meet market needs in wound care applications. Internal testing has shown that non-optimized Seldox-enabled products exhibit a high degree of efficacy and stability both on the shelf and in simulated *in vivo* conditions. Results from testing by an independent third party laboratory on non-optimized materials showed greater than three logs inhibition against *Staphylococcus aureus*, and greater than 4 logs of inhibition against *P. aeruginosa* and *S. marcescens* for polyurethane products. These tests show that the Seldox-enabled polyurethane is both

highly effective and stable. Further external testing showed the material was non-hemolytic, meaning there is little chance of a bystander effect when used in wound care applications. Topical creams have also shown themselves to be highly effective against both clinical and lab strains of several relevant gram positive bacteria.