The purpose of this evaluation was to determine the ability of selected antimicrobial agents and products as well as PluroGen Therapeutics, Inc novel surfactant compounds to prevent biofilm formation and to disperse and/or destroy preformed biofilms associated with wounds.

In a biofilm state, bacteria are typically less susceptible to antibiotics, antimicrobials and biocides. In some cases bacteria can be up to 4,000 times more resistant than the same organism in a free-floating state. Comparisons of MIC (minimum inhibitory concentration) tests with MBEC (minimum biofilm eradication concentration) tests vividly display the differences in susceptibility from the planktonic (free floating) to the biofilm state. In fact, using an MIC concentration of antibiotic in a biofilm infection can inadvertently expose the biofilm to a sub-lethal concentration of antibiotics thereby increasing the chances of development of resistance.

The evaluation utilized the MBEC Assay developed by Innovotech, Inc, Edmonton, Alberta based on the Calgary Biofilm Device developed in 1996 by microbiologists working at the University of Calgary. This is a simple assay that reliably cultures 96 identical biofilms at a time. The assay allows microorganisms to grow on 96 identical pins protruding down from a plate lid. By placing the biofilms on the pins into the wells of a micro titer plate, a matrix of compounds, concentrations and synergistic effects can easily be assessed. This allows rapid testing of compounds for anti-biofilm activity. The Assay produces both MBC (minimum bacterial concentration) values and MBEC (minimum biofilm eradication concentration) values from the biofilm growing on the 96-peg lid. The MBC (minimum bacterial concentration) results are derived from the organisms suspended in the wells of the microtitre plate as they are in a planktonic state. The PluroGen novel compounds were tested against the most prevalent organisms in biofilm infections namely Pseudomonas aeruginosa (5 x 10^6 CFU/mL), Staphylococcus aureus (15 x 10^6 CFU/mL), and Staphylococcus epidermidis (11 x 10^6 CFU/mL).

The PluroGen Therapeutics novel compounds tested included:
- PluroGel™ – with the Poloxamer 188 portion at concentrations of 45%, 50% and 55%.
- PluroGel™ Silver Sulfadiazine (PSSD) - silver sulfadiazine 1% with the Poloxamer 188 portion at 50% at 100%, 90%, 80%, 70%, 60% and 50% concentrations. The compounds were tested against Silvadene cream (silver sulfadiazine 1%) at concentrations 100%, 90%, 80%, 70%, 60% and 50%.
- PluroGel™ TAG (Polymyxin B [10,000 U/g], nitrofurantoin [0.3%], nystatin [4,000 U/g]) and the Poloxamer 188 portion at 50% at concentrations of 100%, 90%, 80%, 70%, 60% and 50%.

The data is presented as the breakpoint for MBC (minimum bacterial concentration) from organisms suspended in well and MBEC (minimum biofilm eradication concentration) values from the biofilm growing on the 96-peg lid.

The results of MBEC assay as performed by Innovotech, Inc demonstrated:
- All of PluroGen’s compounds tested had some degree of antimicrobial biofilm activity against the organisms tested under the conditions of this experiment.
- All of PluroGen’s compounds were able to both prevent and have efficacy against biofilms under the conditions of this experiment.
Specifically:

**Against Biofilm Prevention**

1. PluroGel™ at >50% concentration was effective against *S. epidermidis* in biofilm prevention.
2. PluroGel™ at >45% concentration was effective against *P. aeruginosa* in biofilm prevention.
3. PluroGel™ Silver Sulfadiazine (PSSD) at >80% concentration was effective against *S. aureus, S. epidermidis* and *P. aeruginosa* in biofilm prevention.
4. PluroGel™ TAG at <50% concentration was effective against *S. aureus, S. epidermidis* and *P. aeruginosa* in biofilm prevention.
5. Silvadene was not effective against *S. aureus, S. epidermidis*, or *P. aeruginosa* in biofilm prevention.

**Against Pre-formed Biofilm**

1. PluroGel™ at >50% concentration was effective against *P. aeruginosa* against pre-formed biofilms.
2. PluroGel™ at >45% concentration was effective against *S. epidermidis* against pre-formed biofilms.
3. PluroGel™ Silver Sulfadiazine (PSSD) at >80% concentration was effective against *P. aeruginosa* against pre-formed biofilm.
4. Silvadene was not effective against pre-formed biofilms.
5. PluroGel™ TAG at >50% concentration was effective against *S. aureus* against pre-formed biofilms.
6. PluroGel™ TAG at >60% concentration was effective against *S. epidermidis* against pre-formed biofilms.

In summary these data support the claim of a method for treating and/or preventing microbial biofilm topically administered as a therapeutically effective amount of surfactant composition and comprising antimicrobials in a sub-therapeutic amount.

Taking into account the published scientific literature which reports that bacteria can be up to 4,000 times more resistant than the same organism in a free-floating state\(^1,2,5,6,7,8,9\), it was postulated for these experiments, that the standard therapeutic amounts of antimicrobial drugs, which have been established based on their effectiveness against bacteria in their free-floating state, would have no or limited effect on biofilms. This is supported in the Innovotech data with the results for Silvadene®, which delivers the standard therapeutic dose of silver sulfadiazine (i.e. 1% silver sulfadiazine) in a non-surfactant delivery material. Innovotech found that Silvadene® had no effect against pre-formed biofilms, whereas PluroGel™ without any antimicrobial did have effect against pre-formed biofilms, and PluroGel™ with Silver Sulfadiazine at a lesser concentration than Silvadene® also had an effect against pre-formed biofilms. The Innovotech data further show that PluroGel™ alone, as well as PluroGel™ Silver Sulfadiazine and PluroGel™ TAG had considerably superior effect versus Silvadene® in preventing biofilm. These results support the claims around a surfactant alone and with antimicrobials in a sub-therapeutic amount having an effect on biofilms. These results also show a new finding that the normal therapeutic level of an antimicrobial when not in the presence of a surfactant has no effect on pre-formed biofilms.

References