A Synergistic DispersinB™ and Bacteriophage Combo for Wound Healing

Background

The healing of chronic wounds is a huge unmet clinical need that costs the US health care system $20 billion per year. In the United Kingdom, management of decubitus ulcers (bedsores) costs approximately 3-4 billion dollars annually, which is over 4% of the total National Health Service expenditure. The incidence of wound etiologies including diabetic foot ulcers, venous leg ulcers, decubitus ulcers, and surgical site infections are increasing at alarming rates. The current use of systemic and topical antibiotics and of topical antiseptics in the treatment of chronic wounds is no longer effective due to infections involving biofilms. There is increasing evidence to believe that biofilm formation in wounds is the best unifying explanation for the failure of chronic wounds to heal. Anecdotal clinical evidence indicates improved healing when chronic wounds are treated with the assumption that biofilm is the cause of the failure to heal. This treatment can comprise a number of approaches: use of antibacterial biofilm agents, debridement, anti-biofilm dressings, phages and biocides.

One attractive strategy for biofilm control is to target the gelatinous matrix of the biofilm rather than the cells themselves. If the extracellular polymers that hold the biofilm together could be disrupted or degraded, the biofilm would disperse and its innate defences would be subverted. Enzymes are one way this might be realized. For example, DispersinB™ enzyme that specifically cleaves the primary extracellular polymer of many staphylococcal biofilms including the major wound-associated pathogen *Staphylococcus aureus* biofilm has been recently described. This anti-biofilm enzyme has been shown to have a remarkable efficacy in disrupting and removing *Staphylococcus epidermidis* biofilms by several researchers including those at the Center for Biofilm Engineering (CBE), Bozeman, MT. Thus, DispersinB™ enzyme in combination with a broad-spectrum antibiotic/ antimicrobial could be used for effective topical wound treatment as a way of loosening and removing biofilm.

DispersinB™ Technology

Kaplan, et al.[1], discovered a novel DispersinB™ enzyme that inhibits and disperses biofilms of important wound-associated pathogens such as *Staphylococcus aureus*, *Escherichia coli* and *S. epidermidis*. The unique ability of enzyme to specifically inhibit biofilm formation and disperse preformed biofilms makes it a first of its kind antibiofilm enzyme. Kane Biotech Inc. has an exclusive worldwide licence agreement with the University of Medicine and Dentistry of New Jersey to use this technology, and is presently applying the technology to develop a wound care product.

Bacteriophage

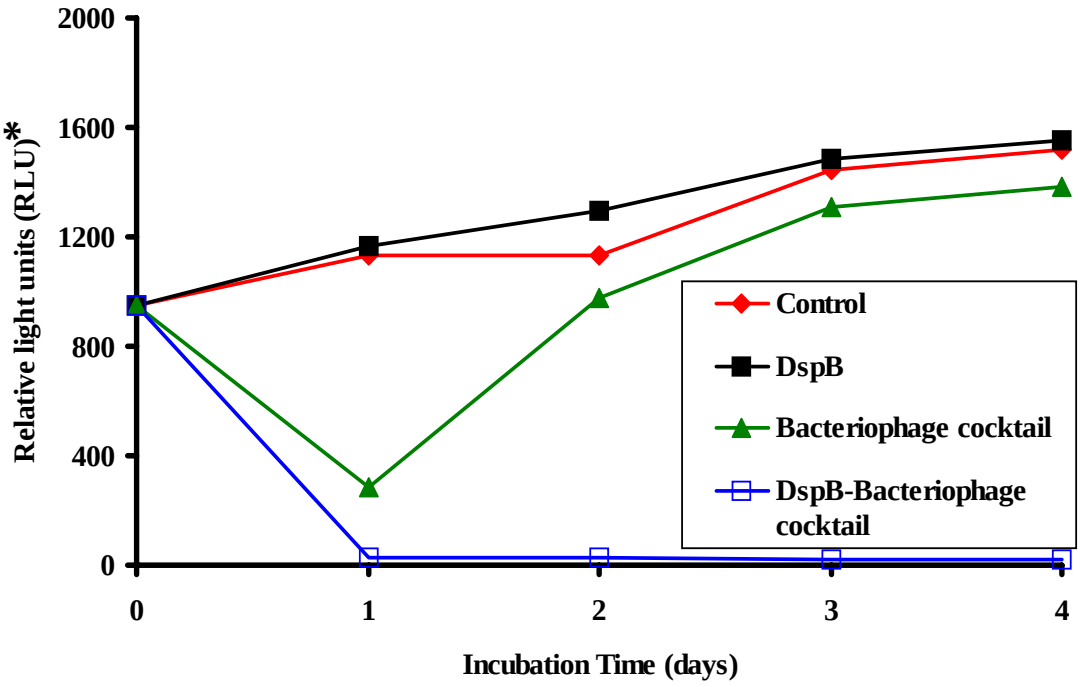
Bacteriophages are viruses that infect bacteria. Many phages have the ability to lyse bacteria (lytic bacteriophages); usually occurring after viral assembly is complete so that fully assembled virus can exit the host cells and infect new bacterial cells.

DispersinB™and Bacteriophage Combo

Using our DispersinB™ technology, Dr. Randy Wolcott's team at the Southwest Regional Wound Care Center in Lubbock, Texas formulated a composition comprising

DispersinB™ enzyme and a lytic phage mixture, which disperses and kills biofilm embedded *E. coli* cells. The combination of DispersinB™ and phage mixture showed almost 99% inhibition of *E. coli* biofilm as compared to only 9% inhibition by the phage mixture alone over the four day period of treatment (**Figure 1**). This combination therapy could provide a new and highly effective method of treating chronic wounds such as diabetic foot ulcers.

Figure 1: Effect of DispersinB™ (DspB) and bacteriophage mixture on biofilm-embedded *E. coli.**

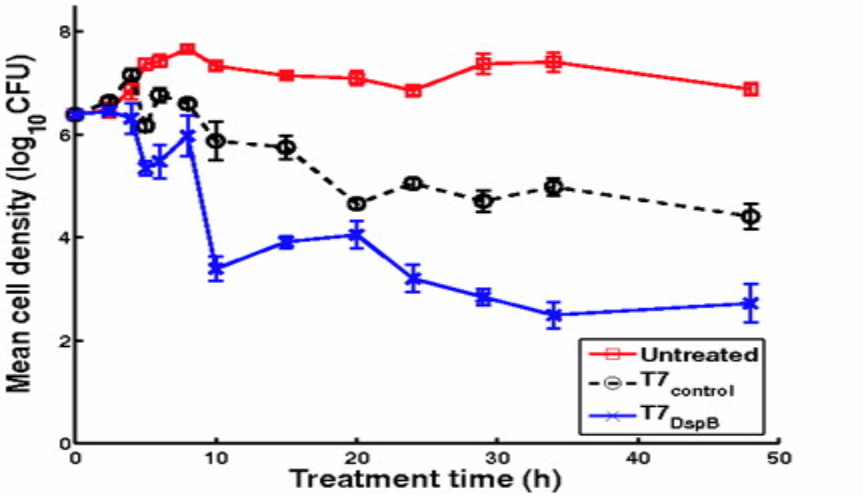


***Biofilm embedded cells were measured in terms of bioluminescence (Relative light units, RLU)

Recently, Lu and Collins[2] (2007) from Harvard-MIT, MA, USA engineered *E. coli* T7 bacteriophage to express DispersinB™, in order to simultaneously attack the bacterial cells in the biofilm and the biofilm extracellular polymeric substances. This two-pronged DispersinB™-expressing bacteriophage strategy removed 99.99% of bacterial biofilm, which were two orders of magnitude better than that of bacteriophage alone (**Figure 2).**

Thus, this study on DispersinB™-expressing bacteriophage and Dr. Wolcott's research on DispersinB™ and bacteriophage mixture combinations provide a novel strategy for treating chronic wounds more effectively than the current antibiotic/antimicrobial therapies.

Figure 2: Effect of engineered DispersinB™–expressing phage treatment on *E. coli* biofilm.



Applications

- Treatment of acute and chronic wounds in humans and animals: (i) acute wounds include surgical wounds, bites, burns, minor cuts and abrasions, and more severe traumatic wounds such as lacerations and those caused by crush or gun shot injuries, and (ii) chronic wounds include venous leg ulcers, diabetic foot ulcers, pressure sores or impaired venous drainage, and decubitus ulcers
- Food and feed additives, cosmetics, disinfectants and device coatings
- Other industrial, environmental, agricultural and medical uses

Desirable Features

- DispersinB™ increases the sensitivity of bacteria to bacteriophage either by inhibiting biofilm formation or by dispersing preformed biofilms
- As each bacteriophage is capable of infecting only a specific host bacterium, it doesn't affect useful bacteria
- Use of bacteriophage specific to *Listeria* monocytogenes as a food additive has already been approved by FDA
- DispersinB™ being a naturally occurring enzyme and further in combination with bacteriophages is unlikely to pose any safety or bacterial resistance concerns

Reference

1. Kaplan, et al., Enzymatic detachment of *Staphylococcus epidermidis* biofilms, Antimicrob. Agents. Chemother. 48: 2633-2636, 2004.
2. Lu and Collins, Dispersing biofilms with engineered enzymatic bacteriophage, PNAS. 104: 11197-11202, 2007.

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