Use of Bacteriophages to Treat Bone and Prosthetic Infections in War Fighters

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The increasing problem of treating infections caused by antibiotic resistant bacteria is pushing the medical and research community to look at alternative antimicrobial agents. Bacteriophages (viruses that kill target bacteria) can be harnessed to aid the fight against pathogens and yet leave our microbial flora untouched. Poly-trauma and blast injuries that are incurred in today’s battlefields, although treated sooner and result in lower mortality rates have still devastating effects on the amputees who experience them. Osseointegration technology may be a new approach to improving the quality of life for short residual limb amputees and provide them with a functional outcome that can allow them to return-to-duty and or/community reintegration. However, a significant rate of these amputees suffers from infections that can lead to devastating consequences including further limb amputation. Our research is aimed at reducing that rate of infection.

Historical Overview

Bacteriophages (bacterial viruses - also known as phages) were discovered in the early 1900’s. Due to the lack of remedies available to treat infections at the time, phages were employed as bactericidal agents against human bacterial infections. Early clinical work by Felix d’Herelle (who discovered the lytic property of phages) showed great promise. He used oral administration of phage therapy to successfully treat several children suffering from severe dysentery. After the effective treatment of staphylococcal bacteremia through intravenous administration, d’Herelle established his own company, which produced the first commercially available phage cocktails (Abedon et al. 2011). This action was followed by many of the major pharmaceutical firms selling phage preparations (e.g. Parke-Davis and Lilly). Unfortunately, due to the lack of understanding phage biology, phage therapy trials were also plagued by a number of conflicting reports on their efficacy (Summers 2001).

By the early 1940’s, phage therapy research was discontinued in the West due to the discovery of antibiotics. Their broad spectrum, ease of large-scale manufacture and prescription, made them much more convenient than phages.

“Every convenience brings it’s own inconvenience” – Roman proverb. The widespread use of antibiotics for over half a century has contributed to the emergence of antibiotic resistant bacteria. The static property of antibiotics, are no match for the rapid evolutionary characteristics of bacteria. Thus, alternative means are currently being sought to take on the superinfection problems of today.

Current State of Microbial Research

Over the past 60 years, we have gained considerable understanding of phage biology and more recently the world of microbiomes present in humans. Bacteria inhabit the human body as symbiotes, contributing to the structure and function of tissues around them and playing an important part in the equilibrium between health and disease by promoting the development and function of our adaptive immunity (Lee & Mazmanian 2010).

One could therefore postulate that it would be paramount to minimize the disturbance of commensals in the human body when treating infections. The ability of phages to attack targeted bacteria may prove to be the natural solution.
Re-Evaluating Phage Therapy

From the mounting research on phage therapy and phage biology, the pros and cons of phage therapy has become more apparent. Early clinical trials failed to differentiate between using temperate (bad) and obligately lytic (good) phages. Fundamental phage properties such as stability under typical storage conditions and temperatures, testing of appropriate efficacy and safety studies, and confirmation of the absence of undesirable genes such as toxins (through sequencing) were either infrequently contemplated or were not technically possible at the time. Phage properties that can contribute to the utilization of phage therapy include: their ability to maintain the required concentrations in the presence of high bacterial densities; they are inherently non-toxic; they cause minimal disruption to the normal flora; they have a narrower potential to induce resistance within the residing bacterial population; their preparations may be formulated to be used with antibiotics or as phage cocktails and applied in a variety of forms such as liquids, creams or impregnated in solids (Loc-Carrillo & Abedon 2011).

Prevalence of Bacterial Infections in Amputated War Fighters

In recent wars, the rate of survival from injuries has dramatically increased in comparison to that of World War II, from 69.7% to 90.4%. Improvements in body armor and advances in medical care provide a better chance of survival. Approximately 70% of combat injuries involve the musculoskeletal system. In 2009, the United States Military Casualty Statistics report, published by the Congressional Research Service, stated that the population of amputees occurring from the Operation Iraq Freedom (OIF) consisted of 1,091 service members.

In the civilian population there is a 15% infection rate in amputations, although this is speculated to be higher for wounded war fighters who have had their injuries exposed to dirt resulting from detonated improvised explosive devices (IEDs).

With the emergence of antibiotic resistant bacteria, treatment of infections has become more challenging.

Treating Osteomyelitis and Prosthetic Infections with Phages

Although there are a few publications noting the use of phages to treat osteomyelitis in humans, both in Europe and U.S. back in the 30’s and 70’s (Albee 1932; Abedon et al. 2011), there are no published studies that have observed the efficacy of phage therapy on pre-clinical trials.

By using experimentally infected rat models and administering well-characterized phages through a parental route, we are able to investigate their pharmacokinetics - rate of decay and efficacy of the studied phages, against a bioluminescent Staphylococcus aureus strain. This bioluminescent bacterial strain has been previously used to establish a ‘real-time’ quantitative rodent model of Staphylococcal osteomyelitis (Funao et al. 2010), allowing the infection progression to be continuously recorded within each animal subject over a period of weeks. This new technology can be paired with established histological and microbiological analysis (at study end-point) to determine the efficacy of phage therapy to control the infection.

Fig. 1 Diagram of animal groups involved in pre-clinical studies. Group 1 will be used to compare histological results between control and test groups; group 2 will determine phage decay rate, group 4 will determine if ‘inactive’ phages induce a placebo effect and groups 3 and 5 will be used to investigate if the presence of active phage has an effect at reducing the problematic bacterial load on the infected site. The red-cross indicates groups which are expected to not recover from the infection.
A methodical scientific approach is currently being followed in order to determine: the best route of administration, and the optimum dosage and timing a single phage or phage cocktail be required to treat osteomyelitis. Pre-clinical studies will include all required control sets (Fig. 1).

**Percutaneous Osseointegrated Prosthesis Attachment for Military Amputees**

Osseointegration is the direct structural and functional connection between living bone and the surface of an implant (Branemark et al. 2001). This technology has been clinically utilized for dental implants since 1965, with much success.

The use of OI technology was extended to orthopaedic applications by the 1990’s and although there are still issues with infection rates (up to 18%) (Tillander et al. 2010), many patients (29/32) who have undergone the procedure would still opt-in and participate again (Personal communications with H-H Aschoff).

The advantages of skeletally attaching an artificial limb include avoiding ‘custom-made’ sockets to complement the constantly morphing stump; and the occurrence of osseoperception, which provides the subject with an ability to identify vibrations around the implant (Branemark et al. 2001).

Osseointegrated prosthesis attachment can bring freedom and improved quality of life to military amputees. This technology could also add to the number of major limb amputees returning to active duty, which has seen a significant increase from 2.3% (during the 1980’s) to 16.5% (from recent conflicts in Afghanistan and Iran) (Stinner et al. 2010).

**References**


**BJRL & Micro-Phage Lab**

The Bone and Joint Research Laboratory (BJRL) began working on OI technology in 2006 with specific interest in developing a new device only requiring a single stage implant surgery. The Micro-Phage Lab was established in 2010 within the Department of Orthopaedics, University of Utah, to work on ways to aid the diagnosis, prevention and treatment of bone and orthopaedic implant infections.

Further information on the Micro-Phage Lab can be found on their website: www.golden-ratio-microbes.org