1. INTRODUCTION

- Description of the problem of burns and burn site infections
- Alternatives to antibiotics: Bacteriophages
- Available technologies and implementation of the emulsification technique as an effective storage/delivery medium

1. INTRODUCTION

- Burns and burn infections
  Burns are experienced by thousands of people every year in the UK with high associated costs.
  - Children under two years old are the most vulnerable to burn wound infections.
  - Minor and moderate burns caused by scalds or spillages are the most common.
  - The majority of cases are domestic injuries (79%).
  - Complications caused by Toxic Shock Syndrome.

- Antimicrobial resistance in Europe
  - 50% of infection cases showed antibiotic resistance

- Alternatives to antibiotics
  Alternative therapies are emerging as a consequence of the widespread antibiotic resistance:
  - Improved antibiotics – difficult to make
  - Example: Only Linezolid has been approved for the treatment of acute skin infections since 2000, although Tedizolid is currently being developed
  - Molecular Biology techniques to make bacteria more susceptible to antibiotics
  - Activated antibacterial agents

Bacteriophages

Methicillin Resistant S. aureus in Europe (2010)


Methicillin Resistant S. aureus in Europe (2012)


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Bacteriophages
Phage therapy as an alternative to antibiotics

- Bacteriophages have been used against skin and wound infections, with reported success rates of up to 90% against S. aureus.
- The advantages of bacteriophage therapy include their abundance and ecological ‘friendliness’; they can be used as a ‘phage-cocktail’, they multiply exponentially, and they do not generate unwanted side-effects.
- There are challenges to implementing phage therapy in vivo, which may be partially addressed by modelling of population dynamics.


2. PROJECT AIMS

- Delivery of phage or ‘phage-cocktail’ to the point of infection without losing efficacy, either during delivery, or prior storage.
- Use of oil-in-water nano-emulsions as a stabilising / delivery vehicle, due to their capacity to prevent virus precipitation, and to enhance transdermal penetration.
- Understanding the mechanisms of interaction in a mixture containing emulsion droplets, bacteriophage, and bacteria, and the relative effects of emulsion and phage on bacterial growth.

3. RESULTS

- Emulsification techniques
- Influence of oil-in-water Nanoemulsions on bacterial growth
- Influence of oil-in-water Nanoemulsions on bacteriophage lytic activity
- Product shelf-life
- Bacterial Biofilms
**Emulsification Techniques: PIT vs. Homogenisation**

- For the same formulation: 80% SM Buffer (aq. phase), 15% BrijO10 (surfactant), 5% Soybean oil (organic phase).

**Homogenisation after 45 min**

![Graph showing emulsification techniques comparison]

**Influence of Emulsion on Bacterial Growth**

- Bacterial growth in more concentrated emulsion (1:1 dilution) appeared slower, showing a lag period.
- RSM Analysis showed that both growth rate and carrying capacity were influenced by the concentration of emulsion droplets.

**Influence of Emulsion on Bacterial Growth: Testing Streptomycin penetration**

- Bacterial growth may be influenced by attachment of emulsion droplets to the outer cell membrane, depriving bacteria of nutrients.
- If so, penetration of antibiotics should also be affected.

**Influence of Emulsion on Bacterial Growth: Different Antibiotic Mechanisms - Streptomycin**

- Streptomycin needs to go through and reach the ribosomes of the cell.
- It binds irreversibly to the 30S ribosomal subunit.
- Codon misreading and protein inhibition.

**Influence of Emulsion on Bacterial Growth: Different Antibiotic Mechanisms - Vancomycin**

- Vancomycin sits on the cell wall surface.
- It binds to the two D-alu residues.
- It inhibits cell wall synthesis.

**Influence of Emulsion on Bacterial Growth: Mechanism and/or Cell Wall Structure?**

- S. aureus
- P. aeruginosa
Influence of Emulsion on Bacteriophage K Lytic Activity

Bacterial concentration was dramatically reduced after the first 5 hours of treatment with bacteriophage-emulsion formulation, when compared with bacterial growth in emulsion. Re-growth is also avoided.

Two different strains

Influence of Emulsion on Bacteriophage K Lytic Activity: Possible mechanism? – Zeta Potential

Nano-Emulsions reduce Bacteriophage K Zeta Potential – Might eliminate electrostatic repulsion bacteria-bacteriophage

Shelf-life of Bacteriophage K-Emulsion Preparations

Relative killing effect = \( \frac{\text{OD}_{20\text{h}}(\text{control}) - \text{OD}_{20\text{h}}(\text{within phage preparation})}{\text{OD}_{20\text{h}}(\text{control})} \)

Relative killing effect = 1 \( \Rightarrow \) Total killing of bacteria

Relative killing effect = 0 \( \Rightarrow \) No killing effect

Bacteriophage-emulsion formulations, both at room (~20°C) and cold (4°C) temperature show enhanced antibacterial activity.

Growth of Bacterial Biofilms – Crystal Violet Assay

Eradication of Bacterial Biofilms – Crystal Violet Assay

Eradication with Phage cocktail in Buffer

Eradication with Phage cocktail in Emulsion

Bacterial Biofilms – Modified Robbins Device
4. MODELLING STRATEGIES

- Modelling of bacterial growth – Test of existing models.
- Influence of the ratio emulsion droplets : bacterial cells on growth parameters.
- Proposal of a modified logistic growth model in the presence of emulsion droplets.
- Infectivity models: general principles and difficulties.

<table>
<thead>
<tr>
<th>Model</th>
<th>Formulation</th>
<th>Parameters</th>
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| Logistic         | $\frac{dS}{dt} = aS(t) \left(1 - \frac{S(t)}{K}\right)$                   | $a$: Growth rate (time$^{-1}$)  
|                  |                                                                             | $K$: Carrying capacity (concentration) |
| Gompertz         | $\frac{dS}{dt} = aS(t) \log \left(\frac{K}{S(t)}\right)$                   | $a$: Growth rate (time$^{-1}$)  
|                  |                                                                             | $K$: Carrying capacity (concentration) |
| Richards         | $\frac{dS}{dt} = aS(t) \left(1 - \left(\frac{S(t)}{K}\right)^3\right)$    | $a$: Growth rate (time$^{-1}$)  
|                  |                                                                             | $K$: Carrying capacity (concentration) |
| Hyperbolic III   | $\frac{dS}{dt} = \frac{aS(t)}{K + S(t)} - \frac{bS(t)}{K^2 + S(t)^2}$      | $a$: Intrinsic growth rate (time$^{-1}$)  
|                  |                                                                             | $b$: Parameter  

- Modelling bacterial growth - METHOD

- In-built parameter estimation model of Matlab (lsqnonlin) not powerful enough for stiff systems of DEs.
- Self-made parameter estimation algorithm using Matlab.
- Multistart run for 100 random initial guesses for all growth models.
- Determination of parameters, % of hits using different initial guesses, and value of total residual after fitting.
- Preferred model – Simplest model.

- Modelling bacterial growth - RESULTS

- Logistic model yields smaller residual and all models’ parameters lead towards logistic model

- Influence of the ratio emulsion droplets : bacterial cells on growth parameters.

- First moments of growth curve can be approximated to an exponential – Fitting growth rate
- Use different initial dilution factors of emulsion (vary amount of droplets per bacteria)

- Proposal of a modified logistic growth model in the presence of emulsion droplets.

- Growth rate is dependent on the droplet : bacteria ratio.
- What about carrying capacity?
• Infectivity models
  • Steps from a microscopic point of view:
    1. Diffusion or transport from the bulk of the solution to bacterial surface.
    2. Recognition and adsorption due to specific receptors on bacterial outer membrane.
    3. Injection of bacteriophage genetic material.
  • General mass-action law.

• General system of ODEs:
  \[
  \frac{dS}{dt} = aS - S\left(1 - \frac{S}{C}\right) - bS\cdot P
  \]
  \[
  \frac{dI}{dt} = aI - I\left(1 - \frac{I}{C}\right) + bS\cdot P - kI
  \]
  \[
  \frac{dP}{dt} = kLI - bS\cdot P - mP
  \]
6. ONGOING WORK

- Investigation of the influence of outer cell wall properties on emulsion formulations performance in terms of growth and phage infectivity – Pseudomonas aeruginosa (Gram negative bacteria).
- Experimental determination of some of the infectivity parameters in order to achieve better fitting for the modelling strategies.
- Investigation of more realistic wound environments, where the presence of biofilms is determinant and critical – Use of our formulations in S. aureus and P. aeruginosa biofilms.

7. FUTURE WORK

- We are exploring the biological mechanisms within the system and evaluating more favourable formulations in terms of biocompatibility and cost.
- We are evaluating more comprehensive approaches to modelling the bacteriophage / emulsion / bacterial interactions.
- We are moving towards a more realistic wound environment.

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