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.


- Antibiotic Resistance – Evolution in Europe

50% of infection cases showed antibiotic resistance

Methicillin Resistant S. aureus in Europe (2010)


- 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

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.

References:

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 % Brij010 (surfactant), 5% Soybean oil (organic phase)
**Emulsification Techniques: PIT vs. Homogenisation**

For the same formulation: 80% SM Buffer (aq. phase), 15% Brij35 or Brij30 (surfactant), 5% Soybean oil (organic phase)

**Homogenisation after 45 min**

**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-ala 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

-15 mV Bacteriophage K only
-1.3 mV Bacteriophage K + Nano-Emulsion

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

Shelf-life of Bacteriophage K-Emulsion Preparations

- Relative killing effect = 1
  - Total killing of bacteria
- Relative killing effect = 0
  - 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

Growth without Emulsion
Growth in Emulsion

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>
</tr>
</thead>
<tbody>
<tr>
<td>Logistic</td>
<td>( \frac{dS}{dt} = aS \left( 1 - \frac{S}{K} \right) )</td>
<td>( a ) = Growth rate (time(^{-1})) ( K ) = Carrying capacity (concentration)</td>
</tr>
<tr>
<td>Gompertz</td>
<td>( \frac{dS}{dt} = aS \left( K - S \right) )</td>
<td>( a ) = Growth rate (time(^{-1})) ( K ) = Carrying capacity (concentration)</td>
</tr>
<tr>
<td>Richards</td>
<td>( \frac{dS}{dt} = aS \left( 1 - \frac{S}{K} \right)^\mu )</td>
<td>( a ) = Growth rate (time(^{-1})) ( K ) = Carrying capacity (concentration) ( \mu ) = Parameter</td>
</tr>
<tr>
<td>Hyperbolic</td>
<td>( \frac{dS}{dt} = \frac{aS}{K + \theta} \left( 1 - \frac{S}{K} \right) )</td>
<td>( a ) = Intrinsic growth rate (time(^{-1}) concentration(^{-1})) ( K ) = Carrying capacity (concentration) ( \theta ) = Parameter</td>
</tr>
</tbody>
</table>

- 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.

Alternatives to antibiotics: Bacteriophages’ mode of action

General system of ODEs:
\[
\begin{align*}
\frac{dS(t)}{dt} &= \text{Rate of appearance of bacteria by growth} - \text{Rate of disappearance of bacteria by infection} \\
\frac{dI(t)}{dt} &= \text{Rate of appearance of bacteria} + \text{Bacterial Infection Rate} - \text{Bacterial Inactivation Rate} - \text{Bacterial Lysis Rate} \\
\frac{dP(t)}{dt} &= \text{Phage Inflow Rate} - \text{Phage Inactivation Rate} - \text{Phage Adsorption Rate} + \text{Phage Release of Progenie Rate}
\end{align*}
\]

Infectivity models - Example

\[
\begin{align*}
\frac{dS(t)}{dt} &= aS(t)\left(1 - \frac{S(t)}{C}\right) - bS(t)P(t) \\
\frac{dI(t)}{dt} &= aI(t)\left(1 - \frac{I(t)}{C}\right) + bS(t)P(t) - kI(t) \\
\frac{dP(t)}{dt} &= kL(t)I(t) - bS(t)P(t) - mP(t)
\end{align*}
\]

5. CONCLUSIONS

We present a novel approach for the efficient storage and delivery of Bacteriophage K for the treatment of Staphylococcus aureus infections.

More concentrated oil-in-water nano-emulsions had a bigger effect on bacterial growth.

The nano-emulsion-bacteriophage preparations show enhanced and stable antimicrobial activity, with reduced fluctuations of infectivity over time, when compared to a simple phage suspension.

This work demonstrates the potential for a responsive wound dressing preparation.
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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