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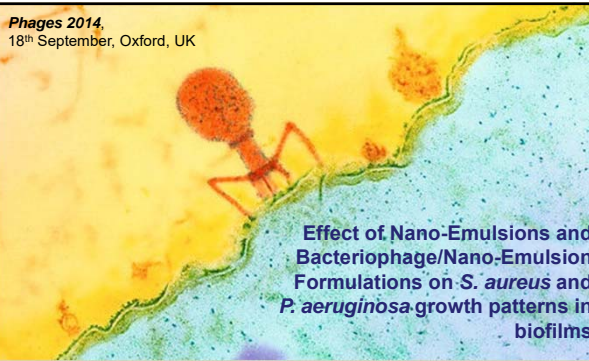
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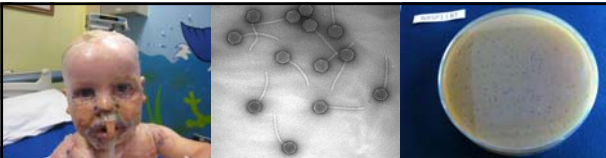


Effect of Nano-Emulsions and Bacteriophage/Nano-Emulsion Formulations on *S. aureus* and *P. aeruginosa* growth patterns in biofilms

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EPSRC Engineering and Physical Sciences Research Council




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

• Burns and burn infections¹

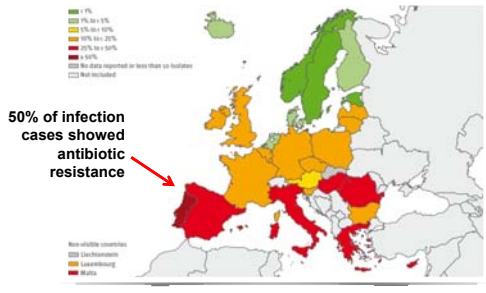
Burns are experienced by thousands of people every year in the UK with high associated costs.

- 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**.



¹UK Burn Injury Data (1986-2007 inc.), 2008. [Online]. Available: <http://www.ibidb.org/>. [Accessed: 02-Feb-2012].

• Antibiotic Resistance – Evolution in Europe²

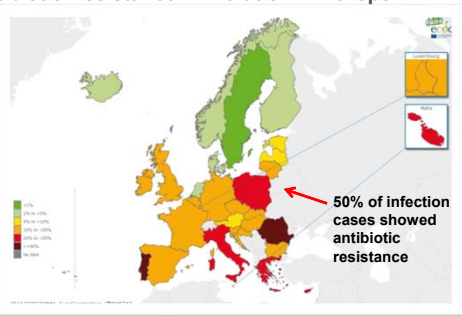


Methicillin Resistant *S. aureus* in Europe (2010)

²European Centre for Disease Prevention and Control, "Antimicrobial resistance surveillance in Europe 2010. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net).", 2011.

• Antibiotic Resistance – Evolution in Europe³

- Increasing bacterial antibiotic resistance in Europe due to misuse or abuse
- Necessity of alternatives



Methicillin Resistant *S. aureus* in Europe (2012)

³European Centre for Disease Prevention and Control, "Antimicrobial resistance surveillance in Europe 2012. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net).", 2013.

• Alternatives to antibiotics

Alternative therapies are emerging as a consequence of the widespread antibiotic resistance⁴:

- 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**

⁴P. W. Taylor, P. D. Stapleton, and J. Paul Luzio, "New ways to treat bacterial infections.," *Drug discovery today*, vol. 7, no. 21, pp. 1086-91, Nov. 2002.



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- **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* ⁷.
- 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 ⁸.
- There are challenges to implementing phage therapy *in vivo*, which may be partially addressed by modelling of population dynamics.

⁷ Ahmad SI. 2002. Treatment of post-burns bacterial infections by bacteriophages, specifically ubiquitous *Pseudomonas* spp. notoriously resistant to antibiotics. *Medical Hypotheses* 58:327–31.

⁸ Hanlon GW. 2007. Bacteriophages: an appraisal of their role in the treatment of bacterial infections. *International Journal of Antimicrobial Agents* 30:118–28.

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- **Available Emulsification Technologies**

PIT Emulsification

Homogenisation

Labels in diagram: Coarse Emulsion, Impact Ring, Fine Emulsion, Valve, Valve seat.

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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**.

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3. RESULTS

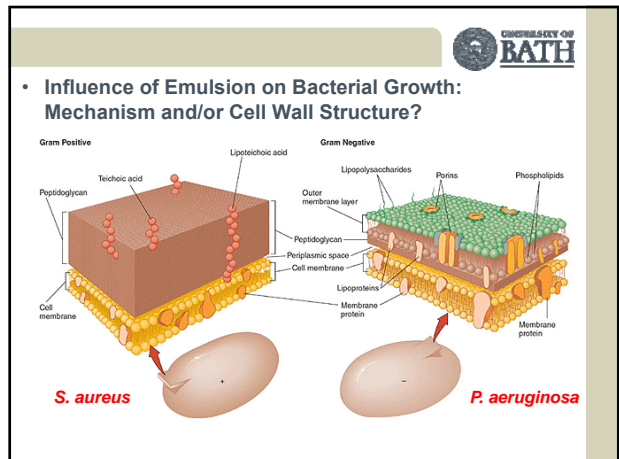
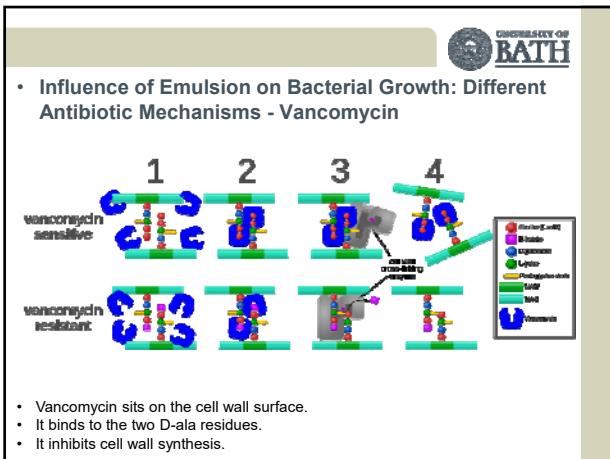
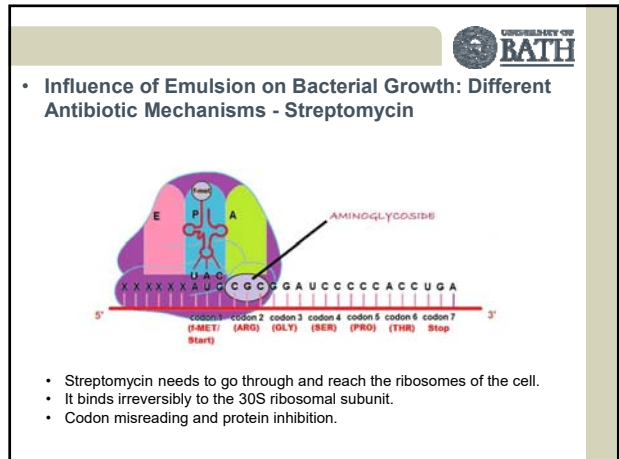
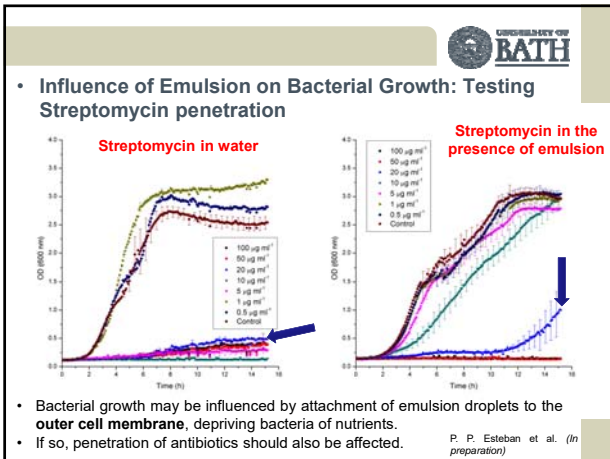
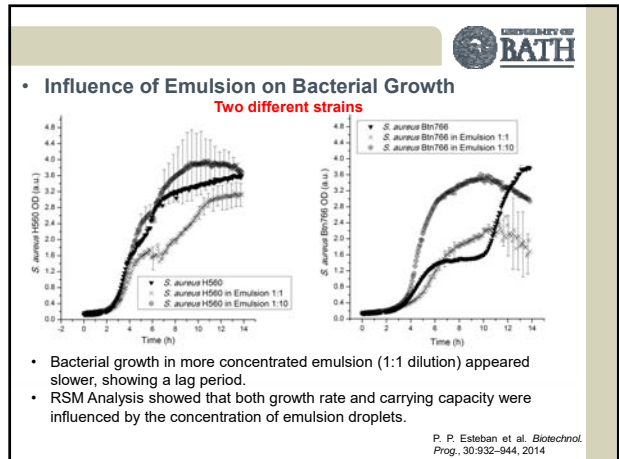
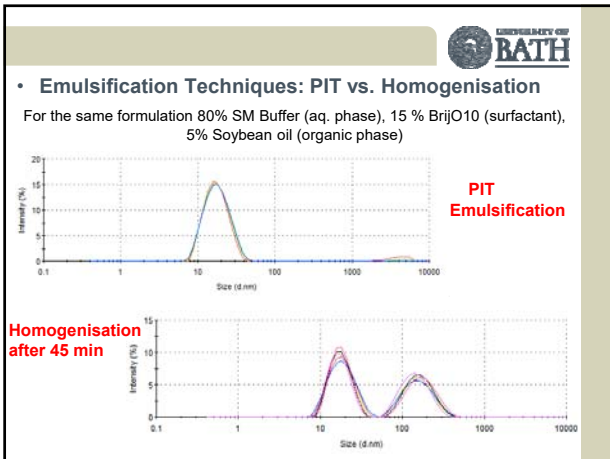
- Emulsification techniques
- Influence of oil-in-water Nanoemulsions on bacterial growth
- Influence of oil-in-water Nanoemulsions on bacteriophage lytic activity
- Product shelf-life
- Bacterial Biofilms

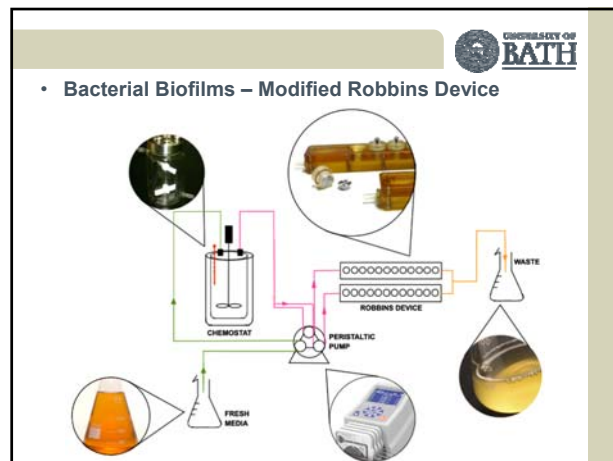
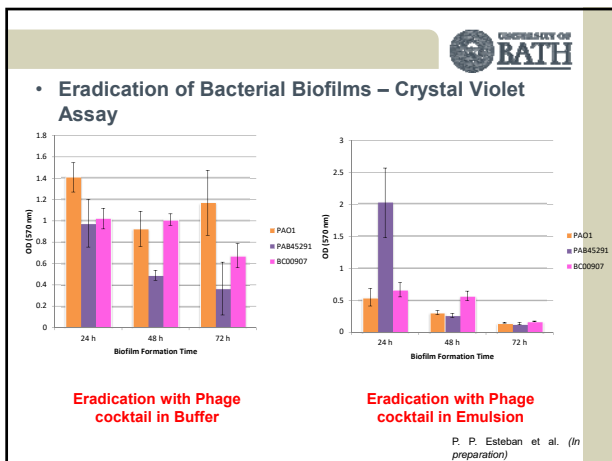
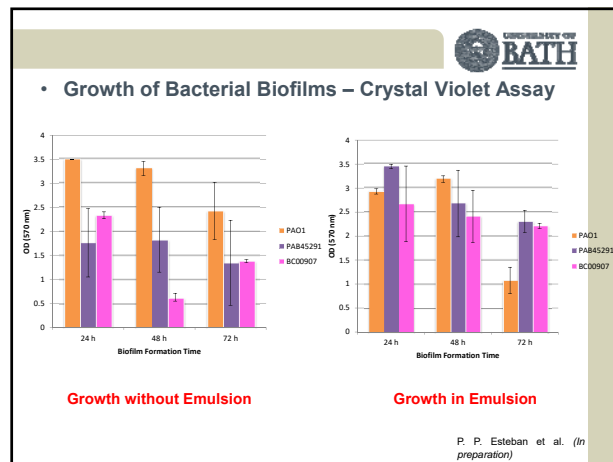
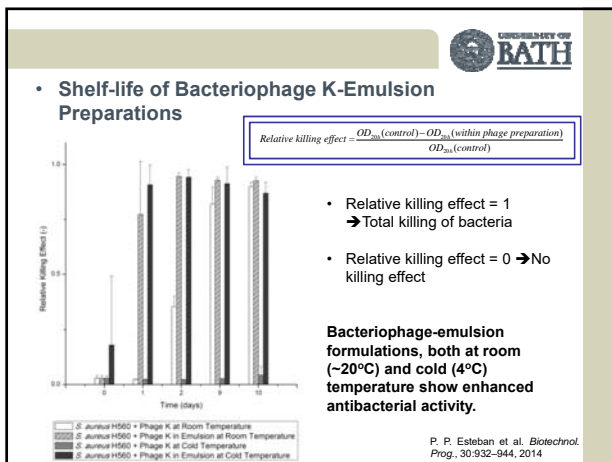
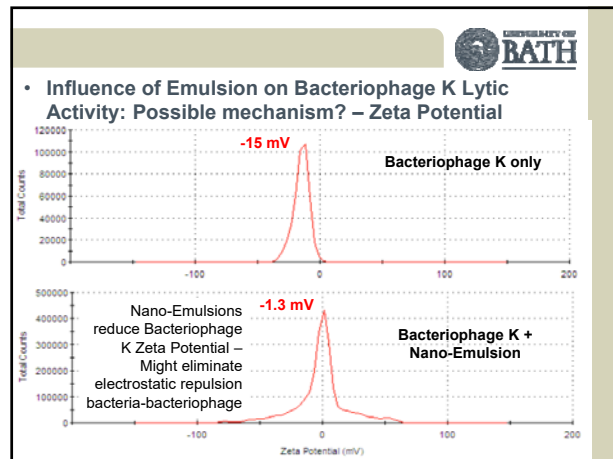
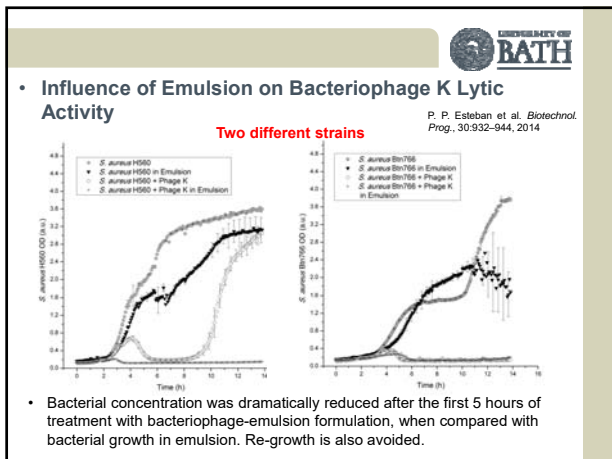
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- **Emulsification Techniques: PIT vs. Homogenisation**

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

PIT Emulsification **Homogenisation after 45 min**





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.

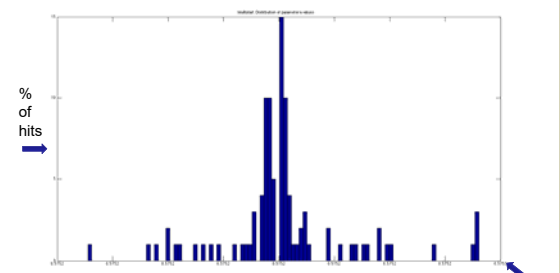
• Modelling bacterial growth

Model	Formulation	Parameters
Logistic	$\frac{dS(t)}{dt} = aS(t)\left(1 - \frac{S(t)}{K}\right)$	a=Growth rate (time ⁻¹) K=Carrying capacity (concentration)
Gompertz	$\frac{dS(t)}{dt} = aS(t)\log\left(\frac{K}{S(t)}\right)$	a=Growth rate (time ⁻¹) K=Carrying capacity (concentration)
Richards	$\frac{dS(t)}{dt} = aS(t)\left(1 - \frac{S(t)}{K}\right)^\eta$	a=Growth rate (time ⁻¹) K=Carrying capacity (concentration) η =Parameter
Hyperbolic H1	$\frac{dS(t)}{dt} = \frac{1}{K}S(t)(K - S(t))\left(Ka + \frac{\theta}{\sqrt{1+t^2}}\right)$ If $\theta=0$ - equivalent to the Logistic model.	a=Intrinsic growth rate (time ⁻¹ concentration ⁻¹) K=Carrying capacity (concentration) θ =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.

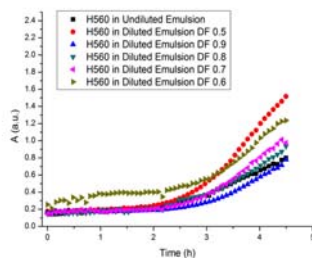
• Modelling bacterial growth - RESULTS



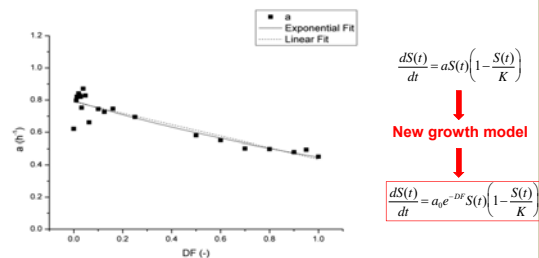
- Logistic model yields smaller residual and all models' parameters lead towards logistic model

• 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.



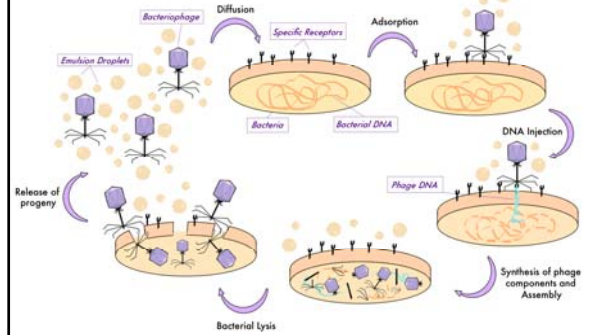
- Growth rate is dependent on the droplet : bacteria ratio.
- What about carrying capacity?

• 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.
 4. Bacteriophage self-replication.
- General mass-action law.

• Alternatives to antibiotics: Bacteriophages' mode of action



• Infectivity models

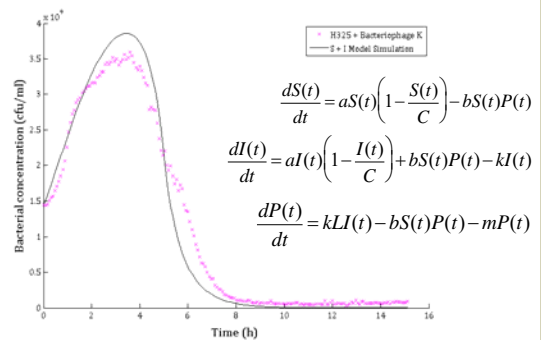
• General system of ODEs:

$$\frac{dS}{dt} = \{ \text{Rate of appearance of bacteria by growth} \} - \{ \text{Rate of disappearance of bacteria by infection} \}$$

$$\frac{dI}{dt} = \{ \text{Rate of appearance of bacteria} \} + \{ \text{Bacterial Infection Rate} \} - \{ \text{Bacterial Inactivation Rate} \} - \{ \text{Bacterial Lysis Rate} \}$$

$$\frac{dP}{dt} = \{ \text{Phage Inflow Rate} \} - \{ \text{Phage Inactivation Rate} \} - \{ \text{Phage Adsorption Rate} \} + \{ \text{Phage Release of Progenie Rate} \}$$

• Infectivity models - Example



• Infectivity models - Difficulties

- System of ODEs very non-linear.
- Parameter estimation is not trivial.
- Outcome highly dependent on initial guesses of parameters.
- Having a working and reliable parameter estimation method would help elucidating the mechanisms.
- Possible improvement: experimental determination of some parameters and use them as initial guesses/fixed parameters.

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 aureuginosa* (**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. aureuginosa* 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**.



8. ACKNOWLEDGEMENTS

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- I thank my supervisors Dr Tom Arnot and Dr Toby Jenkins for their support and advice.
- We thank North Bristol NHS Frenchay Hospital for their support.



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