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## Poster Session 3 – Pharmaceutical Microbiology

### 242
### Disinfection of biofilms using Toluidine Blue O and Red Light

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As current antimicrobial agents are gradually being rendered ineffective by resistance developing in target organisms, there is an urgent need for alternative antimicrobial approaches. Toluidine Blue O (TBO) is a light-activated antimicrobial agent that has been shown to be effective against a wide range of bacteria. The aim of this investigation was to determine the efficacy of TBO against biofilms grown on silicone surfaces when impregnated into the substrate and when applied externally. Silicone discs were impregnated by swelling the discs with chloroform for 2 h and then applying 1 mg mL$^{-1}$ TBO solution (or water for controls) for 16 h. Discs were then rinsed of excess TBO. Biofilms of *Proteus mirabilis* and *Staphylococcus epidermidis* were grown by seeding for 4 h with the appropriate culture in TSA at 37°C ($1 \times 10^5$ CFU mL$^{-1}$). The culture was then removed from each disc and replaced with fresh medium. Discs were incubated for 48 h. Photo-activated disinfection (PAD) was initiated by activating the biofilms for 15 min with red light from a diode laser (wavelength: $633 \pm 2$ nm) to apply a total energy dose of 59 Joules. In an alternative treatment protocol, discs were washed with a TBO solution (25 $\mu$g mL$^{-1}$) followed by light activation as before. Following treatment, biofilm viability was assessed by removal of adherent bacteria and enumeration by viable counts. *S. epidermidis*: washing with TBO led to a 3.2 log reduction in cell numbers. Impregnated discs not exposed to red light resulted in a 1.1 log reduction and when exposed to red light a 1.2 log reduction was observed. Washing of biofilms on impregnated discs gave 1 log reduction in the non-light-activated control and 2.4 log reduction when exposed to red light. *P. mirabilis*: washing led to a 1.1 log reduction in viability. Impregnated discs showed no significant reduction. Washing *P. mirabilis* biofilms on impregnated discs gave no reduction in the control and a 1 log reduction when exposed to red light. Successful disinfection according to the British Standard (BS EN 1276, 1997) occurs when there is a five log reduction in cell number. This did not occur in any of these data. When you compare the two organisms, it can be clearly demonstrated that *S. epidermidis* is more susceptible to disinfection using PAD than *P. mirabilis*. Presently, washing the biofilms with TBO is the most successful method of applying the photoactivated dye to use PAD technology for the disinfection of silicone biofilms.

British Standard (1997) *Chemical disinfectants and antiseptics – quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas – Test method and requirements (phase 2/step 1)*. BS EN 1276

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### Conformable semisolids for photodynamic inactivation of MRSA

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Methicillin-resistant strains of *Staphylococcus aureus* (MRSA) are a primary aetiological factor in nosocomial infections, being difficult to treat and leading to prolonged hospital stays. Widespread resistance has been demonstrated, leaving some forms of MRSA susceptible only to glycopeptide antibiotics. Increasing resistance to vancomycin and teicoplanin has now been reported, with resistance to all antistaphyloccal agents a distinct possibility. MRSA may be present in nares and on skin of healthy people, as well as in wounds, burns and venous ulcers, and may be transmitted from patient-to-patient, doctor-to-patient or surface-to-patient. Clearly, there is an urgent need to develop alternative treatments for MRSA infections that are selective for the organism over human cells and have a low propensity for resistance development. Photodynamic antimicrobial chemotherapy (PACT) is a potentially novel approach for the selective killing of MRSA. In PACT, a combination of a sensitizing drug and visible light causes the selective destruction of microbial cells via singlet oxygen production. Importantly, as singlet oxygen is a non-specific oxidizing agent and is only present during illumination, development of resistance to this treatment is unlikely. As a result of increasing antibiotic resistance, PACT has recently come to the fore as a potential alternative antimicrobial therapy. However, due to the rapid development of the field, drug delivery research in this area is virtually non-existent (Donnelly et al 2005a). The primary objective of this study was to determine the susceptibility of a clinical MRSA isolate growing planktonically to photodynamic inactivation using a combination of methylene blue (MB) and visible light (635 nm, 100 J cm$^{-2}$). The secondary objective was to determine the susceptibility of biofilm cultures of the clinical isolates to MB-mediated lethal photosensitization. As the intended lesions for this photodynamic eradication are topical and usually venous in origin, exudation is a problem for effective drug delivery. To overcome this problem, a shear-sensitive PVA-borax gel was evaluated as a potential drug delivery system for MB. Suspension cultures of MRSA 180 were grown overnight in nutrient broth and centrifuged to pellets before resuspension ($1 \times 10^7$ cfu mL$^{-1}$) in photosensitizer solutions of various concentrations. Various incubation times (37 °C, in the dark) were investigated and irradiation was performed in 96-well plates with back well walls and clear bottoms, as described previously for *Candida albicans* (Donnelly et al 2005b). Biofilms were grown over 24 h on PVC discs in nutrient broth, which was then removed and replaced with photosensitizer solutions of various concentrations for defined time periods before irradiation. Incubation of planktonic MRSA cells with MB concentrations of 0.25 mg mL$^{-1}$ and 0.005 mg mL$^{-1}$, respectively, for 30 min before irradiation achieved greater than 6 $\log_{10}$ reductions in numbers of viable organisms in both cases. Incubation of MRSA biofilms with MB concentrations of 0.25 mg mL$^{-1}$ and 0.005 mg mL$^{-1}$, respectively, for 30 min before irradiation again achieved greater than 6 $\log_{10}$ reductions in numbers of viable organisms in both cases. Drug release studies demonstrated that PVA-borax gels could accommodate MB and release almost 60% within 15 min. In addition, they possessed tensile properties that would make them ideal for both application to, and removal from, exudative lesions.

Donnelly, R. F. et al (2005a) *Photochem. Photobiol.* In press
Donnelly, R. F. et al (2005b) *J. Control. Release* **103**: 381–392

### 244
### Photodynamic inactivation of planktonic and biofilm-grown *Pseudomonas aeruginosa* isolated from cystic fibrosis patients

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Cystic fibrosis patients accumulate thick mucus in their lungs, providing an ideal environment for development of antibiotic-resistant *Pseudomonas aeruginosa* biofilms. Persistent infection leads to pulmonary inflammation and eventually death due to respiratory failure. Recent evidence indicates that photodynamic antimicrobial chemotherapy (PACT) may be useful for infections caused by antibiotic-resistant bacteria and those in multi-species biofilms (O'Neill et al 2002). In PACT, a combination of a sensitizing drug and visible light cause the selective destruction of microbial cells via singlet oxygen production. Importantly, as singlet oxygen is a non-specific oxidizing agent and is only present during illumination, development of resistance to this treatment is unlikely (Wainwright 1998). The primary objective of this study was to determine the susceptibility of clinical *P. aeruginosa* isolates growing planktonically to photodynamic inactivation using a combination of either toluidine blue O (TBO) or meso-tetra (N-methyl-4-pyridyl) porphine tetra tosylate (TMP) and visible light (635 nm, 100 J cm$^{-2}$). The secondary objective was to determine the susceptibility of biofilm cultures of the clinical isolates to TBO-mediated lethal photosensitization. Suspension cultures of six clinical *P. aeruginosa* isolates were grown overnight in nutrient broth and centrifuged to pellets before resuspension in photosensitizer solutions of various concentrations. Various incubation times were investigated and irradiation was performed in 96-well plates with back well walls and clear bottoms, as described previously for *Candida albicans* (Donnelly et al 2005). Using a TBO concentration of 0.05 mg mL$^{-1}$ and a 30-s incubation time, the highest percentage kill achieved was 99.9% for isolate 12A and the lowest was 89.96% for isolate 6A. With TMP, the highest percentage kill achieved was 99.77% for isolate 7–6087B, and the lowest was 86.34% for isolate 6A. However, a TMP concentration of 5 mg mL$^{-1}$ was required to achieve these percentage kills, due to the poor absorptivity of TMP at 635 nm and possibly also its non-ionic nature. Biofilms were grown over 24 h on PVC discs in nutrient broth, which was

then removed and replaced with photosensitizer solutions of various concentrations. In biofilm cultures the highest percentage kill achieved was 99.99% for isolate 3-6087B, using a TBO concentration of $5\,mg\,mL^{-1}$and a 30-min incubation time, and the lowest was 90.55% for isolate PAO1. This study demonstrates that antibiotic-resistant clinical *P. aeruginosa* isolates are susceptible to photodynamic inactivation when grown in either planktonic or biofilm cultures. Unlike conventional antibiotics, the type of culture has no significantly detrimental effect on the efficacy of PACT. We are currently investigating the influence of mucus on the efficacy of PACT of *P. aeruginosa* and the efficient pulmonary delivery of both photosensitizers and light.

Donnelly, R. F. et al (2005) *J. Control. Release* **103**: 381–392
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### Photodynamic inactivation of *Candida albicans* with Toluidine Blue O released from a mucoadhesive patch

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Mucocutaneous oropharyngeal candidiasis, known informally as oral thrush, is predominantly caused by *Candida albicans* strains. The overall incidence of oral candidiasis in adults has increased dramatically with the spread of HIV/AIDS. The condition occurs in up to 84% of HIV-infected patients and is considered to be an independent predictor of immunodeficiency in patients with AIDS (Teichert 2002). Conventional treatments for oral candidiasis have been shown to assume a fungistatic effect rather than a fungicidal mechanism, a factor to which much of the inadequacy of current antifungal agents has been assigned. Resistance to such agents has also increased noticeably in recent years. Recent evidence indicates that photodynamic antimicrobial chemotherapy (PACT) may be useful for infections caused by antibiotic-resistant bacteria and those in multispecies biofilms. In addition, several studies have shown PACT to have significant antifungal action. In PACT, a combination of a sensitizing drug and visible light causes the selective destruction of microbial cells via singlet oxygen production. Importantly, as singlet oxygen is a non-specific oxidizing agent and is only present during illumination, development of resistance to this treatment is unlikely. As a result of increasing antibiotic resistance, PACT has recently come to the fore as a potential alternative antimicrobial therapy. However, due to the rapid development of the field, drug delivery research in this area is virtually non-existent (Donnelly et al 2005a). We have previously described a bioadhesive patch containing 5-aminolevulinic acid for PACT of onychomycosis pathogens, including *C. albicans* (Donnelly et al 2005b). In this study we have adapted this patch for PACT of *C. albicans* infections in the oral cavity. The primary objective of this study was to determine the susceptibility of clinical *C. albicans* isolates growing planktonically and in biofilm to photodynamic inactivation using a combination of toluidine blue O (TBO) and visible light (635 nm, $100\,J\,cm^{-2}$). The secondary objective was to determine whether the adapted patch could release sufficient amounts of TBO to allow photodynamic inactivation. Incubation of *C. albicans* ($1 \times 10^7$ $cfu\,mL^{-1}$) with either $2\,mg\,mL^{-1}$ or $5\,mg\,mL^{-1}$ for 30 min, followed by 10 min irradiation, allowed greater than 6 $\log_{10}$ reductions in numbers of viable organisms of the planktonic cells. Incubation of *C. albicans* biofilms, grown overnight on PVC discs, for 3 h with $5\,mg\,mL^{-1}$ TBO followed by 10 min irradiation again allowed greater than 6 $\log_{10}$ reductions in numbers of viable organisms. Patches containing $10\,mg\,cm^{-2}$ TBO adhered strongly (mean force of removal = 1.1 $N\,cm^{-2}$, TA-XT2 Texture Analyser, Stable Microsystems, Haslemere, UK) to excised porcine cheek tissue, obtained from a local abattoir. Concentrations of TBO achieved on the receiver compartment side of a Cuprophan dialysis membrane, used to mimic diffusion through biofilms, were an order of magnitude lower than the phototoxic concentrations after 3 h release. However, when the release surface of the patch was exposed directly to the release medium, TBO concentrations of approximately $10\,mg\,mL^{-1}$were achieved in the receiver compartment after 3 h. Consequently, with suitable modifications, the patch may be suitable for delivery of TBO to the oral cavity for PACT of planktonic and biofilm cells of *C. albicans* causing oral candidosis.

Donnelly, R. F. et al (2005a) *Photochem. Photobiol.* In press
Donnelly, R. F. et al (2005b) *J. Control. Release* **103**: 381–392
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### Antimicrobial properties of surface-active agents by flow calorimetry

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Surface-active reagents (surfactants) have wide and varied applications in today's modern society; from soaps to immunosorbents they form a multi billion dollar industry with some surprisingly subtle uses. One such application of surfactants is as soaps or detergents for the removal of dirt and grease. With the ever-increasing rise of bacterial resistance and increase in public awareness, the antimicrobial aspect of cleansing products must be considered and indeed this has spawned a whole new range of antibacterial products available for the general public to buy. Biological calorimetry offers a technique to study the growth and, moreover, death of microbiological cultures in a unique fashion, distinctly different from traditional microbiological analysis. Heat energy is evolved as microorganisms grow and respire, which can be detected and converted via thermocouples, into power (Watts); the resulting data from this technique is in the form of a power-time graph. A Thermal Activity Monitor (Jarfalla, Sweden) capable of detection in the nano-watt range was employed in flow-through mode to monitor the heat evolution of microbial cultures in a simple glucose buffered medium at 37°C. Isothermal Calorimetric analysis yields real-time data from which the dynamic response of microorganisms can be observed and their thermo-kinetic response quantified. This also has the advantage that experimental time can be reduced from a minimum of 24 h using traditional microbiological techniques to ca. 3 h calorimetrically. The results (Table 1) show that calorimetry offers an excellent technique for quantitatively assessing the antimicrobial potential of surfactants to a high degree of accuracy over a short period of time. *Saccharomyces cerevisiae* has been used as a model target organism for studying the effect of surfactants. *S. cerevisiae* was chosen for its ability to easily respire on glucose media with minimal nutrient requirement and a well-defined protocol (Beezer et al 1976) for freezing/thawing with good recovery. Calorimetry also offers an opportunity to study the effects of surfactants on polymicrobial cultures with ease or for microorganisms trapped within complex matrices. Furthermore antagonistic/synergistic actions of a combination of two or more surfactants can be easily assessed, again not easily accomplished with traditional techniques. Results from this quantitative analytical approach can be used to design and optimise the antimicrobial efficiency of formulations (e.g., surgical hand washes) in which an antimicrobial cationic surfactant is usually supported by a non-ionic surfactant.

**Table 1** SDS addition after 20 min into *Saccharomyces cerevisiae* respiring on glucose buffered medium

| SDS concn (mM) | Integrated power 0–2 h ($\mu$W) | Rate of kill ($\mu W\,s^{-1}$) |
|---|---|---|
| 0 | 831992 | — |
| 0.032 | 831856 | 0.00188 |
| 0.064 | 832052 | $-4.3 \times 10^{-7}$ |
| 1 | 620369 | −0.03700 |
| 4 | 379234 | −0.11495 |
| 8 | 379854 | −0.12586 |

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## Poster Session 3 – Pharmacy Education

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### Design of a web-based tutorial for acid-base equilibrium theory

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Most drugs are either weak acids or weak bases and the ionisation state is dependent upon the pH of their environment. In solutions of these drugs, an equilibrium exists between the undissociated and the ionised form and extent of ionisation will affect solubility, partitioning, and ultimately drug absorption. The ionisation constant allows comparison of the strengths of weak acid and bases and this is an aspect of the MPharm course that students find challen-