content technique allowed the visual simplification of the re-crystallization process. By plotting the percentage solid against the temperature for each individual cooling rate it is possible to identify the amount of Gelucire 44/14 present in the solid state at any temperature point during the re-crystallization process. The quasi-isothermal modulated-temperature DSC method allowed the isolation of the temperature at which Gelucire 44/14 re-crystallization occurred by holding the molten sample at each temperature increment for an extended period. This was detected by the use of Lissajous figures, whereby the modulated heat flow is plotted against modulated temperature. This in turn allows observation of the reproducibility of the sine-wave heat-flow modulations within a single isothermal period. The re-crystallization could be observed in real time by noting the deviation of the sine-wave curves from the steady state through the course of the crystallization process, thereby providing a novel means of deconvoluting the heat-flow processes associated with the thermal event as a function of time. It was noted that the re-crystallization temperature of Gelucire 44/14 was 32–31°C with an isotherm of 40 minutes, and 30°C with an isotherm of 10 minutes.

Conclusions Quasi-isothermal modulated-temperature DSC appears to be a very promising new tool in the investigation of the Gelucire 44/14 re-crystallization process. By holding the sample at each temperature increment for an extended period, it is possible to isolate the re-crystallization process. This in turn leads to the possibility of mathematically modelling the associated kinetics; work is ongoing to this effect.

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Rheological investigation of some common natural polymer gels and their synergistic combinations

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Objectives To investigate rheological synergies evident from simple combinations of natural polymers used to produce lyophilized wafers. Sodium alginate (SA) and a selection of natural gums, including xanthan gum (XG), locust bean gum (LBG), guar gum (GG), ghatti gum (GhG) and karaya gum (KaG), alone or in combination, are useful for the production of lyophilized wafers. These wafers have potential use as stable vehicles for the topical delivery of anti-microbial compounds to suppurating wounds and control of their flow properties is essential for optimizing their performance in a variety of wound environments.

Methods All gels were prepared in distilled water at concentrations ranging from 0.5 to 3.5% w/v. Individual gels and simple combinations of two gels (50:50) were prepared and characterized by continuous flow rheometry at 25°C. A cone-and-plate geometry was used (40 mm/2° steel) and flow measurements were conducted between 0 and 600 s^{-1} using an AR1000 dynamic rheometer (TA Instruments). Analysis of flow curves was undertaken with the system software using Newtonian, Power Law and Herschel–Bulkley models, *viz* $\sigma = \eta'\gamma$ (Newton), $\sigma = \eta'\gamma^n$ (Power Law) and $\sigma = \eta'\gamma^n + \sigma_o$ (Herschel–Bulkley), where σ is shear stress (Pa), η' is viscosity coefficient or 'consistency' (Pa·s), γ is shear rate (s^{-1}), n is rate index of pseudoplasticity and σ_o is yield stress (Pa).

Results Results are shown in Table 1.

Conclusions All mixtures showed increased consistencies compared with the respective homopolymers. This is indicative of synergistic interactions between constituent polymers. XG/LBG and KaG/SA exhibited yield stresses of 100.60 and 193.26 Pa respectively. This is in agreement with Higiro et al (2006), who reported strong intermolecular interactions between the trisaccharide side chains of XG and the 1–4-linked β-D-mannan backbone of LBG. The greatest consistency of 53.69 Pa·s was displayed by GG/GhG (1:8) compared with 0.16 and 0.38 Pa·s for the individual gels. Control of the rheological properties of gels formed from these natural polymers and their combinations are critical when producing lyophilized wafers for the topical delivery of therapeutic agents to suppurating surfaces such as chronic wounds.

Higiro, J. et al (2006) *Food Res. Int.* **39**: 165–175

Pharmaceutical Microbiology

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Application of a novel drug-delivery device with photodynamic anti-microbial chemotherapy: potential treatment of methicillin-resistant *Staphylococcus aureus* wound infection

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Objectives Photodynamic anti-microbial chemotherapy (PACT) is one alternative approach to the selective killing of methicillin-resistant *Staphylococcus aureus* (MRSA), whereby a combination of a sensitizing drug and visible light causes the selective destruction of microbial cells via singlet oxygen production. The objectives of this study were to (1) determine the sensitivity of MRSA to methylene blue (MB)- and meso-tetra(*N*-methyl-4-pyridyl)porphine tetratosylate (TMP)-mediated lethal photosensitization and (2) develop a dosage form for delivery of adequate photosensitizer doses to infected wounds.

Methods For TMP and MB release from a novel poly(vinyl alcohol) (PVA; 8% w/w)/borate (2% w/w) hydrogel, gel-loaded inserts (25 mm diameter × 10 mm height) containing 4 g gel (1.0 mg mL^{-1} drug) were placed in 100 mL phosphate buffer. At defined times, 5.0 mL of receiver phase was removed. Photosensitizer concentration was determined spectrophotometrically at their λ_{max}. Light diffusion studies through photosensitizer solutions and newborn calf serum were carried out at 635 nm. Planktonic cultures of a clinical MRSA isolate were prepared by overnight incubation at 37°C. Biofilm samples were prepared by incubation of PVC discs with inocula equivalent to 10^7 organisms for 24 hours before use. Test samples were incubated for 30 minutes with solutions of photosensitizers of concentrations 2, 5, 10, 50 and 250 μg mL^{-1} and irradiated (635 nm, 100 mW cm^{-2}, irradiation time 5 minutes, distance from sample 1.8 cm, total light dose 100 J cm^2). The number of surviving bacteria was determined using the Miles and Misra technique. The experiment was repeated using photosensitizer solutions in newborn calf serum to simulate conditions in wounds. Where appropriate, the Mann–Whitney U test was used for statistical analysis.

Results Photosensitizer release from PVA/borate hydrogels showed receiver compartment concentrations of MB and TMP of more than 10 μg mL^{-1} after a 6-hour release period. Serum depths up to 3.5 mm did not affect light diffusion. The presence of increasing concentrations of photosensitizers caused significant reductions in light transmission. For example, increasing MB concentration from 50 to 500 μg mL^{-1} reduced measured fluence from approximately 1.5 to 0.3 mW cm^{-2} ($P < 0.0001$). In PACT, the kill rate was dependent on photosensitizer concentration, even in the absence of irradiation. For example, in the case of a planktonic culture incubated with 2 μg mL^{-1} MB in phosphate-buffered saline, pH 7.4, the percentage kill was 29.73%, while that for 250 μg mL^{-1} MB was 99.46% ($P = 0.0339$). The growth of bacteria in a biofilm and the presence of serum decreased the kill by MB-PACT. For example, irradiated planktonic MRSA incubated with 10 μg mL^{-1} MB reduced survivors to below detection levels, while the equivalent biofilm culture had a reduction of 88.19% ($P = 0.0209$). TMP-PACT was unaffected. For example, irradiated planktonic culture exposed to 10 μg mL^{-1} TMP reduced survivors to below detection levels while that of the equivalent biofilm culture was 99.71% ($P = 0.0833$).

Conclusions MB- and TMP-mediated photosensitization significantly reduced the bacterial load of both planktonic and biofilm cultures. Factors such as light

Table 1 Viscosity coefficient or 'consistency', η' (Pa·s), and yield stress, σ_o (Pa), for six natural polymers and their binary mixtures (50:50). Note the particularly large yield stresses for the synergistic mixtures of KaG/SA and LBG/XG

Polymer (% w/v)	SA η' (σ_o)	XG η' (σ_o)	LBG η' (σ_o)	GG η' (σ_o)	KaG η' (σ_o)	GhG η' (σ_o)
SA (2.5%)	1.17 (0.00)	–	–	–	–	–
XG (0.5%)	2.00 (0.00)	0.17 (7.71)	–	–	–	–
LBG (0.5%)	9.66 (0.00)	2.08 (100.60)	0.04 (4.87)	–	–	–
GG (0.5%)	6.89 (0.00)	2.35 (23.04)	10.50 (34.43)	0.16 (6.19)	–	–
KaG (1.5%)	22.57 (193.26)	0.94 (22.78)	9.49 (34.01)	3.15 (24.31)	0.03 (5.27)	–
GhG (3.5%)	9.35 (0.00)	3.54 (35.76)	20.58 (70.62)	53.69 (52.49)	4.54 (24.30)	0.38 (16.09)

penetration through photosensitzer solutions may contribute to incomplete killing, especially for MB, which prevented light diffusion to a greater extent than TMP, and showed reduced rates of killing. The presence of serum also reduced kill rates. Release of a photosensitizer from a PVA/borate hydrogel and subsequent PACT could potentially be used in the treatment of infected wounds.

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Investigation into the potential of sub-lethal photodynamic anti-microbial chemotherapy to increase resistance to antibiotics

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Objectives Photodynamic anti-microbial chemotherapy (PACT) combines a sensitizing drug and visible light to cause the destruction of bacteria via singlet oxygen production. This is a non-specific oxidizing agent present only during illumination, and therefore development of resistance is considered unlikely. This study aimed to determine (1) whether treatment of *Pseudomonas aeruginosa* PAO1 and *Staphylococcus aureus* AH7 with sub-lethal doses of methylene blue (MB) and meso-tetra(*N*-methyl-4-pyridyl)porphine tetratosylate (TMP)-PACT result in increased antibiotic resistance, and (2) whether sub-lethal PACT increases resistance to subsequent PACT.

Methods Initial studies determined lethal and sub-lethal concentrations of photosensitizers for each strain in combination with a light dose of 100 mW cm^{-2} (5 minutes' exposure time, 1.8 cm from sample, total light dose 100 J cm^{-2}). Planktonic cultures were prepared by incubation at 37°C overnight and 10^7 organisms were incubated with solutions of photosensitizers at concentrations of 2.5, 25 and 250 μg mL^{-1} and then irradiated (635 nm). Surviving bacteria were enumerated following serial dilution using the Miles and Misra technique. Planktonic cultures of both organisms were incubated for 72 hours with sub-lethal photosensitizer concentrations. At 24 hour intervals, samples were irradiated, washed and inoculated into fresh broth/photosensitizer. After 72 hours sensitivity of surviving organisms to a range of antibiotics was determined using E-test® strips (AB Biodisk, Sweden) and compared with the sensitivity of an untreated control, in line with BSAC and manufacturer guidelines. E-tests® contain a gradient of 15 reference minimum inhibitory concentration (MIC) dilutions of an antibiotic, and the zone of inhibition intersects the scale on the strip at the MIC. Cultures were spread on nutrient agar plates, and E-test® strips positioned in the centre. MICs were determined as the lowest concentration that inhibited visible growth. Surviving bacteria were treated with previously lethal photosensitizer light combinations to determine whether susceptibility to PACT was affected by exposure to sub-lethal levels. Where appropriate, the Mann–Whitney U test was used for statistical analysis.

Results Treatment of both strains with sub-lethal concentrations of photosensitizer resulted in significant decreases in susceptibility to previously lethal photosensitization. For example, for PACT of *P. aeruginosa* PAO1 using 250 μg mL^{-1} TMP, percentage kill dropped from 99.96 to 91.15% after exposure to sub-lethal TMP concentrations ($P = 0.0209$). E-test® results for strain PAO1 revealed that exposure to sub-lethal photosensitizer concentrations caused a decreased susceptibility to meropenem (MIC increased from 0.125 to 0.19 μg mL^{-1} after exposure to MB-PACT and 0.47 μg mL^{-1}after exposure to TMP-PACT). Exposure to TMP-mediated sub-lethal photosensitization caused an increase in resistance to ceftazidine, with MIC increasing from 0.38 to 4 μg mL^{-1} on exposure. Susceptibility to tobramycin and piperacillin was unaltered. E-test® results for *S. aureus* AH7 demonstrated that exposure to sub-lethal concentrations of both photosensitizers caused a decreased susceptibility to mupirocin, with the MIC increasing from 0.125 to 0.94 μg mL^{-1} after MB- and TMP-PACT. Exposure to TMP-mediated sub-lethal photosensitization only caused a decrease in susceptibility to vancomycin (MIC increased from 1.0 μg mL^{-1} for the untreated control to 1.5 μg mL^{-1} after TMP-PACT). Sensitivity to fusidic acid and linezolid remained unchanged.

Conclusions Sub-lethal PACT can decrease susceptibility of *P. aeruginosa* and *S. aureus* to antibiotics. In all cases, sub-lethal PACT increased the resistance of the test organism to previously lethal PACT. It is notable, however, that, despite reductions in sensitivity to PACT, the percentage kill of each organism remained high.

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Synthesis and evaluation of novel N-terminal modified anti-microbial lipopeptides against bacterial biofilms

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Objectives To synthesize and evaluate a range of synthetic ultrashort lipopeptides (four amino acids) for anti-microbial and anti-biofilm activity and obtain minimum biofilm eradication concentrations (MBECs) against biofilm-producing micro-organisms. Biofilms commonly adhere to host cells or implanted medical devices such as catheters. Increasing doses of anti-microbials are required for treatment of biofilm-related infections compared with their planktonic counterparts and this often leads to an increase with the development of resistant strains of the microorganisms and/or removal of the implanted device. Cationic anti-microbial peptides, carrying a net positive charge of more than +2, with a ratio of hydrophobic to charged residues ranging from 1:1 to 2:1, attach preferentially to negatively charged lipids present on bacterial cells. The conjugation of small lipophilic moieties, including palmitic acid (C_{16}), at the terminus of the amino acid sequence leads to increases in the hydrophobicity of the molecule. There is therefore increased likelihood of the anti-microbial peptide inserting preferentially into the membrane of the microbe via the carpet, toroidal-pore, barrel-stave or aggregate models.

Methods The peptide synthesis was performed on a manual peptide synthesis apparatus utilizing standard Fmoc chemistry protocols and purified via reversed-phase high-performance liquid chromatography (RP-HPLC; >90%). The anti-microbial activities of the peptides were evaluated using a 96-well microtitre assay to evaluate minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) (starting inoculum $\approx 1 \times 10^6$ cfu/mL). The 96-well high-throughput MBEC assay system (Calgary Biofilm Device) was used to determine the MBEC (biofilm concentration $\approx 2 \times 10^5$ cfu/peg). Biofilms were grown on 96 pegs for 24 hours and placed in a challenge plate containing dilutions of the reagents for 24 hours. The pegs were rinsed in phosphate-buffered saline and transferred to a recovery plate containing 200 μL Müller–Hinton broth, sonicated and placed in a rotary incubator for 18–24 hours. Growth was analysed using a Biolise® spectrophotometer at 570 nm, and MBEC values obtained. Activity was assessed against a range of microbial pathogens including *Staphylococcus epidermidis*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* (strain PA01), *Escherichia coli* (strain PIII), *Proteus mirabilis* (strain BB2000), *Candida albicans* and *Staphylococcus aureus*.

Results The peptide sequence C_{16}-O-O-W-W had a broad anti-microbial spectrum with MBEC values as low as 125 μg/mL against *S. epidermidis* (ATCC 35984) and 31.25 μg/mL against MRSA (CI 201). Anti-microbial activity was also observed with substitution of the palmitic acid portion of the anti-microbial peptide sequence with 3-(4-hydroxyphenylpropionic) acid, malemidocaproic acid, 3,5-dimethoxy-4-hydroxycinnamic acid, lauric acid, myristic acid and *p*-anisic acid. Substitution with lauric acid to form the sequence C_{12}-O-O-W-W achieved MBEC values of 14 μg/mL against MRSA (CI 201) and *S. epidermidis* (ATCC 35984). Lower MICs, MBCs and MBECs were achieved with the addition of these hydrophobic side chains compared with the unmodified sequence (MBEC 500 μg/mL against MRSA (CI 201) and *S. epidermidis* (ATCC 35984).

Conclusions This novel class of anti-microbial peptide offers a lower-cost alternative to naturally derived AMPs and facile synthesis. The presence of the unnatural amino acid ornithine may lead to increased stability within the body as opposed to naturally occurring amino acids. These peptides may have utility in the prevention and control of device-associated infections, especially those involving *Staphylococcus* spp.

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Assessment of the activation of the *agr* quorum-sensing system on biofilm formation of *Staphylococcus epidermidis* using synthetic pheromone derivatives

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Objectives The behaviour of *Staphylococcus epidermidis* during biofilm development is greatly influenced by cell-to-cell communication, a process known as quorum sensing (QS) (Kong et al 2006). The density-dependent *agr* QS system has been assigned a key role in the regulation of several of the biofilm-associated factors of *S. epidermidis* (Kong et al 2006). The main signalling molecule of the staphylococcal *agr* system is a pheromone octapeptide containing a thiolactone ring structure (Otto 2004). As disabling the QS system reportedly enhances biofilm development of *S. epidermidis,* the aim of this study was to activate the *agr* system using synthetic pheromone peptides and assess the effect on biofilm formation of this important nosocomial pathogen.

Methods Cyclical peptides, based on the naturally occurring *S. epidermidis* pheromone peptide structure, were synthesized using solid-phase peptide synthesis. Their effect on biofilm formation was assessed using the Calgary Biofilm Device, a platform carrying 96 pegs that fits into the wells of a standard microtitre plate. To the microtitre plate, a standardized bacterial suspension of *S. epidermidis* ATCC no. 35984 was added, pheromone peptides at final concentrations of 5, 10 and 15 μM were also included, and the peg lid placed onto the device. Biofilm development was carried out in a gyrorotary incubator at 37°C over a 24 hour period. Biofilm formation was quantified by obtaining viable counts following disruption of the biofilm by sonication.

Table 1 The effect of *S. epidermidis* pheromone peptide on biofilm formation of *S. epidermidis*

Pheromone concentration (μM)	Biofilm viable count (cfu/peg)
0 (control)	$3.77 \pm 2.12 \times 10^4$
5	$1.89 \pm 0.29 \times 10^6$
10	$3.07 \pm 1.32 \times 10^6$
15	$3.23 \pm 1.86 \times 10^6$

Results Interestingly, addition of the cyclical pheromone peptide enhanced biofilm formation of *S. epidermidis* (Table 1).

Conclusions It has previously been demonstrated that blocking the QS response results in increased biofilm formation of *S. epidermidis*, thereby promoting pathogen success during infections related to indwelling medical devices. However, this initial study on the *S. epidermidis* signalling molecule indicates that activation of the QS system, by addition of the pheromone peptide to an actively growing biofilm population, leads to enhanced biofilm formation, suggesting that *agr* is a positive, not a negative regulator of biofilm formation. Further investigation on the involvement of *agr* in biofilm formation of *S. epidermidis* is required.

Kong, K.-F. et al (2006) *Int. J. Med. Microbiol.* **296**: 133–139
Otto, M. (2004) *FEMS Microbiol. Lett.* **241**: 135–141

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Development of anti-adherent intraocular lens coatings to reduce occurrence of infectious endophthalmitis following cataract surgery

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Objectives The most serious complication of cataract surgery is infectious endophthalmitis, an inflammatory reaction of intraocular fluids and tissues. Binding of bacteria to the intraocular lens (IOL) implanted during cataract surgery represents a prominent aetiological factor in the pathogenesis of the condition, with 70% of cases caused by Gram-positive coagulase-negative staphylococci, mostly *Staphylococcus epidermidis* (Kresloff et al 1998). A novel antibiotic-impregnated IOL coating comprising 2-hydroxyethyl methacrylate (HEMA) and methacrylic acid (MAA) was proposed to prevent bacterial adherence to the IOL and hence infectious endophthalmitis.

Methods Hydrogel discs formulated from polyHEMA and 70%/30% HEMA/MAA were loaded with gentamicin, ceftazidime, and amikacin, individually or in combination. These antibiotics were selected as they provide coverage for Gram-positive and Gram-negative microorganisms and have been utilized as initial empirical treatment of suspected bacterial endophthalmitis (Benz et al 2004). The discs were applied to seeded agar plates prepared using Müller–Hinton agar and a clinical isolate of *S. epidermidis*, and incubated for 18 hours. Zones of inhibition were then measured. A range of polymers was formulated, with or without additional crosslinker, 1% ethyleneglycol dimethacrylate (EGDMA), to examine the effects of increased crosslinker and gentamicin incorporation on bacterial adherence *in vitro* over a 4 hour period, using a clinical isolate of *S. epidermidis* (1×10^7 cfu/mL bioburden). Adhered bacteria were removed from the hydrogel discs by sonication and vortexing, and viable counts were obtained (Jones et al 2005).

Results The materials formulated from 70%/30% HEMA/MAA yielded larger zones of inhibition than polyHEMA due to the higher antibiotic loading ability of this copolymer. The numbers of adhered bacteria are presented as a percentage of original inoculum (Table 1).

Table 1 Adherence of *S. epidermidis* clinical isolate to hydrogel materials

Hydrogel system	% Adherence	
	To control polymer	To gentamicin polymer
HEMA	0.00112 ± 0.000112	0.0000998 ± 0.000110
20% MAA	0.00976 ± 0.00184	0.0000885 ± 0.000119
30% MAA	0.0126 ± 0.00344	0.0000203 ± 0.0000352
1% EGDMA/HEMA	0.00110 ± 0.000512	0.0000143 ± 0.0000221
1% EGDMA/20% MAA	0.00951 ± 0.00171	0.0000138 ± 0.0000240
1% EGDMA/30% MAA	0.0117 ± 0.00417	0.000135 ± 0.000117

Conclusions Incorporation of a combination of ceftazidime and gentamicin into 70%/30% HEMA/MAA yielded the largest zone of inhibition. Incorporation of gentamicin into the hydrogel systems significantly reduced bacterial adherence to all materials ($P \leq 0.05$, one-way analysis of variance, n = 5), and thus would be highly beneficial as an IOL coating.

Benz, M. S. et al (2004) *Am. J. Ophthalmol.* **137**: 38–42
Jones, D. S. et al (2005) *Biomaterials* **26**: 2013–2020
Kresloff, M. S. et al (1998) *Surv. Ophthalmol.* **43**: 193–220

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In vitro investigation of silicon microneedle-mediated microbial penetration across a model membrane

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Objectives Microneedles (MN) are so termed because they generally range from 100 to 1000 μm in length, and are designed to perforate the external barrier of skin, the stratum corneum, to provide a direct and controlled route of access of therapeutic molecules into the viable epidermis. MN puncture has never been associated with skin infection. However, to date, no study has investigated the ability of microorganisms to traverse MN-induced holes in the stratum corneum. In the present study, penetration of bacterial (*Pseudomonas aeruginosa* or *Staphylococcus epidermidis*) and fungal (*Candida albicans*) microorganisms across MN-treated silicone (Silescol®) membranes, used to mimic stratum corneum, was investigated. Cone-shaped MN arrays (6×5) of 280 μm in height and 250 μm base width were used.

Methods Microorganism isolates were cultured in Müller–Hinton broth at 37°C for 18 hours. Suspensions were centrifuged, drained and resuspended to 10^7 cfu/mL in sterile phosphate-buffered saline (PBS). A colorimetric absorbance of 0.1 (*P. aeruginosa* and *S. epidermidis*) and 1.0 (*C. albicans*) set at an optical density of 550 nm was carried out, to give a concentration of 10^7 cfu/mL. Penetration of microorganisms across silicone membranes was investigated using a sterile Franz cell apparatus at 37°C. Receptor compartments (12 mL) were filled with sterile PBS and silicone membranes (50 μm thick) were sandwiched between donor and receptor compartments. A 1.0 mL suspension of microorganisms was placed in the donor compartment and left for 24 hours for biofilm formation. After 24 hours, excess microorganism suspension was removed and the membrane was punctured in one of three ways: (i) MN punctured and left in place, (ii) MN punctured and removed and (iii) hypodermic needle (21G) punctured and removed. Using a long sterile needle, samples (0.1 mL) were removed from receptor compartments at defined intervals and spread over Müller–Hinton agar (MHA) plates. After incubation of plates at 37°C for 24–48 hours, colonies were counted and the cumulative numbers penetrating across the membranes determined. The Mann–Whitney U non-parametric test was used for comparison, where $P < 0.05$ was considered statistically significant.

Results Results showed the possibility of microorganisms crossing across MN-punctured membranes (Table 1). In general, the three microorganisms showed nearly the same tendency of crossing the membrane *in vitro* after 24 hours. Hypodermic needles showed significantly ($P < 0.05$) higher amounts of penetration, followed by MN-punctured and removed and MN-punctured and left in place. However, control membranes without any puncture showed no growth of microorganisms in MHA plates, which indicated the integrity of the membrane.

Conclusions In conclusion, the present study illustrated the possibility of microorganism penetration across MN-punctured silicone membranes in an *in vitro* study. The hypodermic needle-punctured membrane showed highest microorganism penetration, which was due to the holes created being of larger diameter and remaining open for more than 24 hours. On the contrary, the holes created by the MNs were only a few micrometres wide and typically contracted following

Table 1 Microorganism penetration across MN- or hypodermic needle-punctured silicone membrane after 24 hours

Conditions	Microorganisms present in Franz cell after 24 hours (cfu/Franz cell; n = 3)		
	P. aeruginosa	*C. albicans*	*S. epidermidis*
Control	0	0	0
MN punctured and left	0.20×10^4	0.21×10^4	0.09×10^4
MN punctured and removed	0.23×10^4	0.46×10^5	0.21×10^4
Hypodermic needle punctured and removed	0.96×10^5	0.39×10^6	0.31×10^5

withdrawal of the MN. Therefore, lower microorganism penetration resulted. In addition, when MNs were left in place, blockage of the holes by the MN allowed lower penetration of microorganisms than the hypodermic needle-punctured membranes.

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Anti-microbial activity of liposomal tea tree oil and its constituents

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Objectives The aim of these studies was to assess the anti-microbial activity of tea tree oil (TTO), liposome-encapsulated TTO emulsions and the constituents of TTO against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans* using the standard disc diffusion assay. Natural products are increasingly seen as a main source of novel therapeutic agents for infectious diseases; the monoterpene alcohols are particularly anti-microbially active because of their relatively high water solubility and the presence of the alcohol moiety (Hammer et al 2003).

Methods Emulsions of TTO with different molecular weights of poly(vinyl alcohol) (PVA) (13–23, 30–70, 70–100 kDa) at 0.1 and 1.0% w/v and subsequent liposome encapsulation were performed as previously reported (Martin et al 2007). Anti-microbial activity was assessed with a 100 μL sample of each culture suspension spread uniformly over 20 mL tryptic soy agar for *P. aeruginosa* and *S. aureus*; malt extract agar plates were used for *C. albicans*. After inoculation a 5 mm sterile filter disc was centrally placed and 50 μL of sample added: TTO, TTO emulsion, liposomal TTO emulsion and TTO components (α-terpineol, *p*-cymol, γ-terpinene, α-pinene, 1,8-cineole, α-terpinene, terpinene-4-ol, limonene and terpinolene). After disc saturation, plates were inverted and incubated at 37 ± 2°C for 24 hours and the zone of no growth (zone of growth inhibition, ZOI) around each disc was assessed (Figure 1). The ZOI radius was measured from disc centre to ZOI edge.

Results Anti-microbial activity studies reveal the efficacy of various TTO formulations and TTO components against three common microorganisms. ZOI development with free TTO, TTO-PVA emulsion and TTO-PVA liposomes was studied for 24, 48 and 78 hours; times were selected to investigate the duration of action of individual constituents. The activity of free TTO compared with TTO-PVA emulsion and TTO-PVA liposomes was much higher against *S. aureus* ($P < 0.02$) and *C. albicans* ($P < 0.025$), while *P. aeruginosa* showed less sensitivity against free TTO and after an interval of 78 hours no ZOI was shown. No activity against *P. aeruginosa* was found for TTO-PVA emulsion formulations, which may indicate resistance against PVA-formulated TTO. *C. albicans* showed the highest sensitivity to TTO for all emulsion formulations, with greatest sensitivity to $PVA_{70–100kDa}$-TTO emulsion. Results for encapsulated formulations (liposomal $PVA_{30–70kDa}$-TTO and liposomal $PVA_{13–23kDa}$-TTO) revealed anti-microbial activity against *P. aeruginosa* at less than 24 hours. The anti-microbial activity of major components present in TTO was also examined. There was little or no activity shown by the majority of TTO components against *P. aeruginosa* except terpinene-4-ol (the major TTO constituent); a 7 mm ZOI (which was the same for free TTO) was observed. Efficacy of TTO components (1,8-cineole, $P < 0.1$; terpinene-4-ol, $P < 0.005$; terpinolene, $P < 0.1$) was greatest against *C. albicans*.

Conclusions In conclusion, TTO-PVA emulsions were found most effective against *S. aureus* and *C. albicans*, with *P. aeruginosa* demonstrating resistance to most formulations. The major TTO constituent (terpinene-4-ol) displayed the greatest efficacy against all microorganisms, including *P. aeruginosa*; the other components, however, showed only limited anti-microbial efficacy with the greatest activity against *C. albicans*. Future work aims to identify potential synergistic activity of TTO and its constituents in combination with traditional anti-microbial therapies, to combat the development of antibiotic resistance.

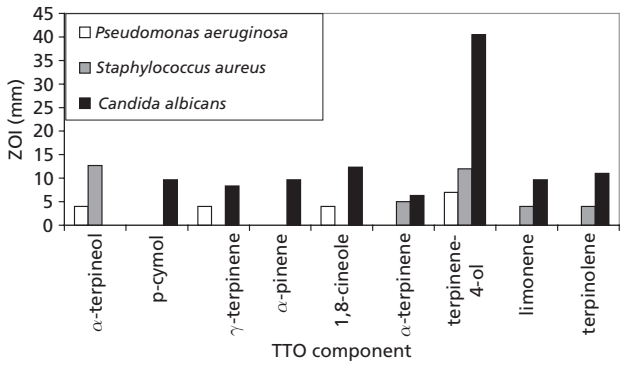

Figure 1 Zone of growth inhibition (ZOI) obtained for various tea tree oil (TTO) components against three common microorganisms.

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Martin, C. et al (2007) *J. Pharm. Pharmacol.* **59** (Suppl.): A19

Pharmaceutical Technology

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Study of *in vitro* release characteristics of etoricoxib semisolid dispersion in Gelucire-based capsules

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Objectives The aim of the present work was to prepare and characterize different dispersions of etoricoxib with Gelucire 44/14 and Gelucire 50/13 so as to improve its dissolution and stability properties.

Methods Solubility measurements were performed according to the method of Higuchi and Connors (1965) with various concentrations of aqueous solutions of Gelucire 44/14 and Gelucire 50/13 prepared in distilled water with drug. The carrier fusion method was used to prepare different dispersions of etoricoxib using Gelucire 44/14 and Gelucire 50/13. Differential scanning calorimetry (DSC) of drug dispersion into carriers was studied using a DSC-60 calorimeter (Shimadzu). The *in vitro* release of etoricoxib capsules was performed by using DisSolution test apparatus (Veego UDA-8D) to the US Pharmacopoeia (USP) standard method at a paddle rotation speed of 100 rpm, with 900 mL 0.1 M HCl or phosphate buffer, pH 6.8, as a dissolution medium at 37 ± 0.5°C. At the specified time, 10 mL samples were withdrawn and filtered through 0.45 μm Whatman filter paper and then assayed for etoricoxib content by measuring the absorbance at 233 nm using a UV-1700 Shimadzu UV-visible spectrophotometer. The optimized formulation capsules were stored in glass bottles (unpacked capsules) and subjected to accelerated stability studies as per International Conference on Harmonisation (ICH) guidelines; that is, at 40°C and 75% relative humidity, and at room temperature and normal humidity conditions.

Results The phase solubility study was carried out using different Gelucire 44/14 solutions in distilled water (1, 2, 5, 10 and 15% w/v). The 15% w/v solution showed maximum solubility. The same study was carried out using Gelucire 50/13 solutions in distilled water (1, 2, 3, 4 and 5% w/v), and the 5% w/v solution showed maximum solubility. DSC showed there was no well-defined interaction between etoricoxib and the carriers. An *in vitro* release study was carried out for prepared capsules using Gelucire 44/14 as a carrier in 0.1 M HCl or phosphate buffer, pH 6.8. 80.6% drug release was obtained within 10 minutes. The same test was done on prepared capsules with Gelucire 50/13 as a carrier in 0.1 M HCl or phosphate buffer, pH 6.8. 81.4% drug release was obtained within 60 minutes. The stability study indicated that etoricoxib was stable at room temperature and normal humidity conditions while high temperature and humidity led to crystallization of the drug.

Conclusions Etoricoxib polymer dispersions were formed at different ratios using the melting method and put into hard gelatin capsules. Solubility studies showed a solubilizing effect of this polymer on etoricoxib at different temperatures. The negative values of the Gibbs free energy and enthalpy of transfer from water to an aqueous solution of this polymer indicated the spontaneity of the transfer. DSC studies indicated a lack of interaction between carrier and etoricoxib. The dissolution rates of etoricoxib dispersions were higher than those of the pure drug; this was possibly caused by increased wettability and dispersibility of etoricoxib, the surface tension-lowering effect of polymer to the medium and the solubilizing effect of the carrier.

Higuchi, T., Connors, K. A. (1965) *Adv. Anal. Chem. Instr.* **4**: 117–212

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Dilute solution viscometry and flow properties of a new natural polymer

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Objectives A natural polysaccharide polymer was obtained by extraction from the inner-stem bark of the tropical shrub *Grewia mollis* (Family Tiliaceae) and its potential as a pharmaceutical excipient was investigated. The dried and pulverized