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Surface properties of 1069C85 and interaction with phospholipid in mixed monolayers and bilayers

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1069C85 is a potent microtubule inhibitor effective against a range of p388 tumours resistant to other drugs. It has poor water solubility, which poses formulation difficulties. The aqueous solubility of hydrophobic drugs can be improved by incorporation in bilayer regions of liposomes, Lian & Ho (2001). Drug–lipid interactions are likely to be important in understanding this process and it may be possible to predict vesicle stability and solubilisation from parameters measured in a monolayer model. Surface properties of 1069C85 were examined and mixed monolayer technique used to investigate interaction with dipalmitoyl-phosphatidylcholine (DPPC). Solubilisation, interaction with DPPC bilayers and vesicle stability were examined in liposomes.

Monolayers were spread at the air–water interface from chloroform onto the Langmuir trough. In mixed monolayers, total number of molecules and volume of spreading solution were kept constant while mole fractions of drug and phospholipid varied. Surface pressure was measured by Wilhelmy plate and pressure versus area per molecule isotherms (25°C) were generated. In mixed systems, interactions were determined through changes in surface free energy (ΔG) and quantified by an interaction parameter (α), Joos & Demel (1969). Liposomes were prepared by hydrating DPPC films containing 2 mol% 1069C85 with water at 50°C. Vesicle size was reduced by extrusion (20 passes) through polycarbonate membranes, pore size 100 nm, under pressure of nitrogen and z-average hydrodynamic diameters were determined using photon correlation spectroscopy. Unentrapped drug was removed by gel filtration chromatography. Enzymatic colorimetric assay and HPLC determined phospholipid and drug content, respectively. The effect of 1069C85 on the DPPC main gel–liquid crystalline phase transition was examined using differential scanning calorimetry. Liposomes were heated from 10 to 55°C at a rate of 5°C min^{-1}, using water as a reference. The time taken for the vesicle diameter to increase by 25% (t_{25}) indicated relative stability.

1069C85 is surface active, forms Langmuir monolayers and interacts with DPPC in mixed systems. For all compositions, the interaction was attractive (negative α in range 0.5–2.5), induced condensation and improved stability (negative ΔG in range 0.8–2.0kJmol^{-1}). Liposome results (mean ± s.d., n = 5) are summarised in Table 1.

Table 1 Liposome characteristics

	Control	1069C85
Drug content (mol%)	—	0.035
T_{onset} (°C)	40.7 ± 0.1	40.3 ± 0.03
T_{peak} (°C)	42.6 ± 0.8	41.7 ± 0.04
Size (nm)	84 ± 1	100 ± 3
t_{25} (days)	1	5

1069C85 lowered onset and peak temperatures of the DPPC phase transition by a small but significant ($P < 0.05$) amount and improved stability. As predicted by monolayer studies, 1069C85 was solubilised as part of a (comparatively) stable bilayer at low concentrations. Further work is warranted with a range of compounds and liposome components to evaluate whether mixed monolayer stability may correlate with bilayer stability and solubilisation.

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The formulation and thermomechanical properties of soft gelatin capsule shells

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Gelatin is the principal component in mass-produced capsule shells and in the case of softgels, its properties are modulated by the inclusion water and of plasticisers such as glycerol. Such mixtures can be easily formed into ribbons having suitable thermomechanical properties, being mechanically robust enough for use in high-speed softgel capsule filling equipment, suitably elastic for filling and having softening characteristics that allow the capsule to rapidly seal after filling. However, the manufacture of softgel capsules has evolved empirically and there is still a relatively poor understanding of the relationship between a ribbon's composition and its thermomechanically important properties, such as tensile strength, elasticity and softening behaviour. Understanding this relationship would allow the rational optimisation of ribbon composition.

Thus, the thermal and mechanical properties of a range of softgel ribbons, comprising of gelatin, glycerol and water were evaluated using modulated differential scanning calorimetry, thermomechanical analysis and tensiometry. The standard mixture comprised gelatin, water and glycerol in an approximate weight ratio of 40:40:20 and variants containing 20–50% gelatin, 30–50% water (nominal) and 5–40% glycerol were tested. Data were obtained that indicated that increasing the gelatin concentration (fixed water:glycerol ratio) increased the softening and glass-transition points and decreased elasticity (Table 1). Conversely, increasing either the glycerol content or the water content (at fixed ratio of other components) decreased both the softening and glass-transition points and increased the elasticity, with there being no apparent distinction seen between the effect of water or of glycerol.

Table 1 Effect of increasing gelatin content

Gelatin content (%w/w)	Softening point (Tm, °C)	Glass transition point (Tg, °C)	Elasticity (Young's modulus; N mm^{-2})
20	31.0	n.a	n.a
30	35.3	35.5	0.227
45	37.8	37.7	0.270
50	41.2	40.4	0.293

Gelatin imparts a rigid structure to the ribbon, presumably due to the interactions between the polymer chains and increasing gelatin content increases these interactions, stabilising the three-dimensional structure and leading to higher temperatures being required to induce a helix-coil transition within the material. Such a crystalline system displays increased thermal stability with decreased flexibility. Water and glycerol interact with hydrophilic sites on the gelatin chains, reducing the number of molecular interactions among the crystalline side chains and reducing the density of the crystalline three-dimensional network. This allows helix-coil transitions to take place at lower temperatures (= lower softening point) and increasing mobility to the three-dimensional gelatin network due to an increase in the free volume of the system (= increased flexibility). It was found that mixtures containing gelatin outside the range 20–50% w/w were either too rigid to be poured or yielded ribbons that were too fragile to be handled.

The simple relationship observed and the analytical techniques developed herein can potentially be used to rapidly screen novel ribbon formulations proposed as candidates for use in the manufacture of softgel capsules.

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Lower Critical Solution Temperature (LCST) as a criterion to anticipate stability conditions of cellulosic solution formulations

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Pharmaceutical polymers give colloidal systems with different affinity to solvents. The opaque appearance is the main objective that could be quantified. To describe this affinity and thereby anticipate their stability, lower critical solution temperature (LCST) is defined. This temperature is the inclination point of plotting UV absorbency against temperature below which anticipates best storage conditions.

In this study two soluble cellulosic derivatives, hydroxypropylmethyl cellulose (HPMC) with different molecular weights and percentage of substitutions (E5, E15, E50, K100M, E4M, F4M and K4M) and methyl cellulose(MC) were chosen, made aqueous solutions and tested for two degrees intervals between 15°C and 90°C temperatures and different concentrations (0.25% for higher molecular weights, 2% and 10% for the first three lower molecular weights) of the polymers and with 0.1 mole of sodium chloride or sodium benzoate as common additives, using spectrophotometry at 550 nm.

The main results are shown in Tables 1 and 2. As the results showed, all materials studied had LCST of about the same for lower molecular weights whereas the percentage of the substitutions, K4M, K100M and F4M, raised LCST. The results also indicated no effect of polymer concentrations whereas the salts highly affected LCST, with the more effect of the higher dissociation NaCl. The more polar polymer, HPMC, the more effect on LCST.

Table 1 LCST for different molecular weights and substitution of HPMC

HPMC grade	E5	E15	E50	E4M	K100M	K4M	F4M
LCST	56	57	57	58	68	80	61.5

Table 2 LCST for different formulations

Formulation	LCST
HPMC E15 2%	57
HPMC E15 10%	57
HPMC E15 2% + NaCl	37
HPMC E15 2% + Na.Benzoate	41.5
HPC 2%	41
HPC 2% + NaCl	28
HPC 2% + Na Benzoate	33
MC 2%	68
MC 2% + NaCl	57
MC 2% + Na Benzoate	63

It was concluded that, beside other properties such as viscosity, LCST could be used as a suitable simple criterion to anticipate best formulation for specific conditions and could be controlled with appropriate salts.

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The use of ethanol to manipulate the density of HFA-227 for the delivery of porous polymer particles to the respiratory tract

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Extensive work has been performed on the viability of the inhaled route for the delivery of proteins and peptides, both locally and systemically (Patton et al 1999). This work has included the use of porous polymeric particles (Edwards et al 1998; Dellamary et al 2000) that have a relatively small aerodynamic diameter and therefore can reach the lower regions of the lungs (Edwards et al 1997). In this instance, pressurised metered dose inhaler (pMDI) suspension formulations have been prepared containing porous poly(DL-lactide-co-glycolide) (PLGA) particles containing bovine serum albumin with a particle size of approximately 25 μm and density of 1.35 g cm^{-3}. This investigation concentrated on the formulation of pMDI suspensions by matching the density of the propellant, 1,1,1,2,3,3,3-heptaflouropropane (HFA 227) with a density of 1.41 g cm^{-3} to that of the particles using ethanol as a co-solvent. MDI suspension formulations were prepared with a concentration range of ethanol, 0–15% w/w, in HFA 227. Stokes law indicates that if the density of HFA 227–ethanol propellant mix matches that of the porous particles then the creaming rate will be zero.

Microspheres were prepared using a double emulsion technique, following which they were formulated into pMDIs in polyethylene terephthalate (PET) vials or standard aluminium canisters using a transfer fill technique. The results show that the suspension containing 5% w/w ethanol exhibited the greatest physical stability over 5 min when subjected to visual and optical suspension characterisation (OSCAR). This is in accordance with Stokes law as the density of the HFA 227–ethanol mix was 1.38 g cm^{-3}. At 15% w/w ethanol, sedimentation of the suspension was exhibited. This would be expected as the density of HFA 227–ethanol mix (1.27 g cm^{-3}) is less than that of the porous particles and so the particles would sink to the bottom. In addition, the studies to determine the content of micro-spheres delivered by actuation of the valve were performed in accordance with the British Pharmacopoeia 2002 following the actuation of n = 25 shots, for suspensions that exhibited the greatest stability. Using this method, 84.84 (± 3.17)% of BSA was delivered by actuation of the valve of a formulation employing HFA 227–5% w/w ethanol compared with that of HFA 227 alone, which showed 53.11 (± 4.80)% of BSA delivered.

We can conclude that ethanol can be successfully employed for the stabilisation of pMDI suspensions containing porous particles by density matching. There is a significant increase in the content of microspheres delivered by actuation of the valve with the inclusion of 5% ethanol, which can be attributed to the formation of a stable suspension and hence consistent metering of the valve. OSCAR assessment correlates with visual assessment and is a useful tool for the rapid screening of pMDI suspension formulations.

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Microscopic characterisation of polymeric gene delivery complexes

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For many years, the concept of DNA as a therapeutic agent has been a focus of biomedical sciences. The development of cationic polymer delivery systems for gene therapy applications has attracted much interest as vectors in non-viral delivery systems. The increased recent interest in non-viral gene therapy can be attributed to promising results in-vitro and in-vivo (Bloomfield 1998). Using atomic force microscopy (AFM) both in air and in liquid environment we assess the structural characteristics of DNA–polymer complexes. This work focuses on the physicochemical characteristics of methacrylate-based polymer interaction with DNA (Rungsardthong et al 2001). Key variables that will be studied include different monomer nucleotide ratios and the effect of other environmental stresses. Supportive transmission electron microscopic (TEM) investigations have also been employed. To appreciate the complex architecture (Benjamin 2001) naked plasmid

DNA has been imaged for a comparison. AFM and TEM images of the relaxed plasmid DNA show that it is an open-loop structure with little twisting. AFM images of the complex at ratio 2.5:1 show that plectonomic supercoiled DNA originates from an aggregated condensate structure, while TEM images at 2:1 show ring-like structures with linear morphology consistent with plectonomic supercoiled DNA. However, ratio 1:1 shows many aggregates connected with each other by filament-like structures. We propose that the thin filaments are uncondensed DNA.

The interesting morphology of the condensed polymer–DNA complexes has been shown by both AFM and TEM. Classical condensate structure has shown in ratio 2.5:1 by AFM and 2:1 by TEM, while ratio 1:1 tends to form a loose aggregated structure.

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Preformulation salt selection studies on AR-C89855, a compound for inhalation administration

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AR-C89855 was to be formulated as a pressurised metered dose inhaler (pMDI) or dry powder inhaler (DPI) for inhalation administration. AR-C89855 is a weak base. However, attempts to isolate the free base in a crystalline form failed. A salt selection study was performed in order to identify the most suitable salt form.

AR-C89855 was prepared as the acetate, benzenesulphonate (besilate), hemifumarate, hemisuccinate, hydrochloride, 1-hydroxy-2-naphthoate (xinafoate), nitrate and *p*-toluenesulphonate (tosilate) salts. The salts were evaluated by scanning electron microscopy (SEM), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and X-ray powder diffractometry (XRPD). Hygroscopicity profiles were assessed by gravimetric vapour sorption (GVS) analysis and the solubility of the salts in water, saline and simulated lung fluid (SLF; Kanapilly et al 1973) at 37°C determined by HPLC. Following micronisation, the salts were suspended in a standard hydrofluoroalkane (HFA) formulation vehicle and cold filled into PET vials in order to assess suspension stability.

XRPD analysis demonstrated that the acetate, besilate, xinafoate and tosilate salts were highly crystalline; crystallinity of the remaining salts was poor. The besilate was found to form a solvate when recrystallised from propan-2-ol. By SEM, only the besilate, xinafoate and tosilate salts possessed well-defined crystal habits. From the results of GVS analysis, the nitrate and hemisuccinate salts were classified as hygroscopic (Ph. Eur. 1999), the remaining salts were slightly hygroscopic. Table 1 records the solubility of the AR-C89855 salts.

Table 1 Solubility of AR-C89855 salts

Salt	Solubility at 37°C (mg AR-C89855 free base equiv. ML^{-1})		
	Water	Saline	SLF
Acetate	6.80	0.80	0.11
Besilate	1.70	0.60	0.15
Hemisuccinate	0.53	0.58	0.10
Hydrochloride	8.50	0.70	0.17
Xinafoate	0.06	0.08	0.07
Tosilate	1.00	0.90	0.12

No significant phase changes were induced by micronisation. The hydrochloride and nitrate salts formed poor suspensions in the pMDI vehicle and flocculated rapidly. Organic acid salt forms dispersed readily in the propellant and yielded suspensions of suitable stability.

From an evaluation of the data collected, the besilate and tosilate salts were considered to have superior solid state and pharmaceutical properties. Crystallisation of the besilate salt proved to be less robust than the tosilate. Consequently, the tosilate salt of AR-C89855 was recommended as the most suitable salt form.

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Effect of freeze drying on vesicle size, aggregation and entrapment for liposomes formulated with polyvinyl alcohol

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Recently, alternative materials have been implicated in stabilisation of vesicles during processing to enhance size, entrapment efficiency and long-term storage potential (Takeuchi et al 1998). The aim of this study was to evaluate the effects of concentration and molecular mass of the synthetic polymer poly(vinyl alcohol) (PVA) on liposome stability, in terms of maintenance of vesicle size following freeze drying, aggregation and entrapment. PVA is used extensively in the pharmaceutical and food industries as an emulsion stabilizer and viscosity-increasing agent. Liposomes composed of phosphatidylcholine (PC; Lipoid) and cholesterol (CH; Sigma Chemical Co.) 2:1 and PC, CH and stearylamine (SA; Sigma Chemical Co.) 16:8:4 were prepared by the reverse-phase evaporation vesicle (REV) method. The required concentration (1.0 % w/v and 0.1 % w/v) of various PVA molecular weights (13–23 K, 31–50 K and 85–146 K) (Sigma Chemical Co.) was added as the aqueous phase during REV formation. Entrapment efficiency (EE %) was assessed using riboflavin (Sigma Chemical Co.). Liposomes were disrupted with isopropanol (Fisher Scientific) to determine entrapment. Results were assessed using laser diffraction analysis (Malvern Instruments Ltd) and a Multilabel Fluorescence Counter (Wallac). Table 1 shows sample size data before and after freeze drying, with EE % for the former in brackets for liposomes formulated with PVA 31-50 K.

Table 1 Vesicles formulated with PVA 31–50 K: size (μm) before and after freeze drying (EE %)

	Alone	+ SUC	0.1%	1.0%	0.1%+SUC	1.0%+SUC
B[a]	13.94 (1.71)	23.75 (14.83)	9.94 (18.41)	6.45 (17.17)	7.51 (23.48)	14.28 (16.64)
A[a]	20.70	23.61	5.44	4.02	5.40	5.13
B[b]	17.98 (6.34)	16.76 (5.16)	6.18 (23.66)	6.49 (22.97)	4.94 (20.04)	3.64 (8.65)
A[b]	29.69	19.01	8.13	93.4	29.95	11.34

[a]PC–CH formulations, [b]PC–CH–SA formulations; Suc, sucrose; B, before freeze drying; A, after freeze drying

Although vesicle diameters are relatively constant initially, after freeze drying the positively charged formulations show consistent aggregation. Liposomes formulated with PVA 31–50 K showed the smallest size increase after freeze drying. Entrapment was variable, but the addition of sucrose conferred greater entrapment overall.

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Dielectric properties of hydrated pharmaceutical-grade microcrystalline cellulose

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Pharmaceutical granules are normally produced by a process known as wet granulation, in which an aqueous binder solution is used to aggregate the powders

in a blend. Dielectric spectroscopy is particularly suited to the investigation of hydrated powders because the low frequency dielectric spectrum is sensitive to proton currents in the hydration surface of the granules. This study examines the dielectric properties of a model pharmaceutical powder (microcrystalline cellulose) as a function of water content.

Hydrated microcrystalline cellulose (MCC; grade, Avicel PH101) was prepared from samples that were first dried to a moisture content of < 0.1% w/w. Routinely, samples of this dry MCC (50 g) were transferred to a small rotary blender, where concentrations of water (up to 40% w/w) were added drop-wise. Following each addition, the mixture was blended for two min. Samples were then stored in sealed containers for 24 h to allow the moisture to equilibrate. Low frequency dielectric measurements were carried out at 298 K using a dielectric interface (Solartron 1296 Dielectric Interface) connected to a frequency response analyser (Solartron 1255 FRA). The dielectric response of each hydrated MCC was measured between two conventional brass electrodes, with and without the inclusion of polyethylene spacers as blocking-electrodes (0.1 mm thickness).

The shape of the dielectric spectra (measured without spacers) changed significantly with increasing hydration. At low hydration (< 5%) the spectra were characterized well by the linear combination of an LFD and a Davidson-Cole function. The independence of these processes on the value of the applied potential and the thickness of sample, indicated that these were characteristics of the bulk sample. At higher hydration (> 5%) it was difficult to observe the Davidson-Cole type dispersion. The LFD process was still detected but the overall response was increasingly affected by electrode polarization.

The inclusion of blocking electrodes transformed the dielectric response into a peak, which was well characterized by a single Davidson-Cole function. Modeling this response, using a capacitance element in series with an appropriate model for the sample impedance, confirmed that this peak was associated with the composite dielectric properties of the system under test. This process could therefore be described in terms of a Maxwell-Wagner-type (MW) dispersion. Although a result of the composite nature of the sample, nevertheless, it can be taken that the sensitivity to water content of this MW response is indeed a direct reflection of the bulk sample properties, in particular the diffuse polarization of protons in the hydrated cellulose matrix.

The relaxation time of the MW process increased by 4–5 orders of magnitude, across the hydration range studied. Breaks in the plot of relaxation time with water content were interpreted in terms of the changing state of association of water with the underlying matrix. From this observation, it was possible to suggest that water molecules bind to MCC monomers at molar ratios of 1:1 and 3:2, and thereby infer that water associates intimately with this material at the molecular level of the materials substructure.

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The effect of gentamicin on poly(methylmethacrylate) bone cement materials

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Self-curing polymethylmethacrylate (PMMA)-based bone cements are extensively used in orthopaedic surgery for the attachment of metal (prosthesis) to living bone. To minimise the incidence of infection, which necessitates revision surgery, it is common practice to add gentamicin to the bone cement mix. Although this may be useful in reducing the incidence of infection, the effects of gentamicin on the mechanical properties of the cement must be considered. Several reports have examined the mechanical effects of antibiotic additions on bone cement, although there is still controversy as to whether they have significant effects on the cement mechanical strength (Lautenschlager et al 1976; Nelson et al 1978; Wright et al 1984; Davies et al 1989). As the primary role of bone cement is to provide mechanical strength and support for the prosthesis, it is necessary to establish if drug additions compromise the mechanical properties of bone cement which may in turn result in joint failure.

In this study, gentamicin has been incorporated into PMMA bone cement materials formulated under controlled conditions, as opposed to other literature studies which have examined commercial cements. A novel formulation procedure involving MMA, benzoyl peroxide and dimethyl-4-toluidine was used which induces rapid polymerisation and results in a complete uniform dispersion of gentamicin throughout the PMMA film. The PMMA films incorporated gentamicin sulfate at concentrations of 1, 2 and 4% w/w. The ultimate tensile strength (UTS), strain and Young's modulus (YM) of the cements are given in Table 1. The effect of increasing gentamicin concentration on mechanical properties was statistically analysed using a one-way analysis of variance in conjunction with Tukey's HSD post-hoc test ($P < 0.05$ denotes significance).

Table 1 Mechanical properties of PMMA bone cements with gentamicin

PMMA cement (% w/w)	UTS (MPa)	Strain ($\times 10^{-3}$)	YM (MPa) ($\times 10^{-3}$)
Control	42.69 ± 4.78	45.3 ± 4.8	0.95 ± 0.20
1% Gentamicin	48.31 ± 6.42	29.7 ± 4.4	1.65 ± 0.27
2% Gentamicin	56.37 ± 18.4	39.5 ± 4.9	1.27 ± 0.42
4% Gentamicin	68.11 ± 8.68	31.7 ± 5.4	2.16 ± 0.11

The results show incorporation of gentamicin at 4% to PMMA bone cement materials causes a significant increase in both ultimate tensile strength and Young's modulus and a decreased strain with respect to PMMA. This is consistent with the effects of solid fillers on mechanical properties and it can therefore be concluded that incorporation of gentamicin at a concentration of 4% may adversely affect the properties of bone cements and their clinical performance.

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Characterisation of novel anti-adherent coatings for medical devices

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The clinical use of urinary devices (e.g. urinary catheters) is restricted due to device-related problems. The most prevalent problem is device-related infection, which occurs following bacterial adherence to, and subsequent biofilm formation on, the device. A proposed solution is the use of microbial anti-adherent coatings on the device surface thus engineering the desired surface characteristics without alteration to the mechanical properties of the device. In this study, the surface properties and the resistance to microbial adherence of a series of novel biocompatible coatings, composed of blends of lecithin and cholesterol, on polyurethane (PU) were examined as candidate coatings of urinary medical devices.

A series of lecithin (L)/cholesterol(C) solutions (100%:0% w/w, 75%:25% w/w, 50%:50% w/w, 25%:75% w/w) were prepared by dissolving the required masses of each component in dichloromethane with stirring. Samples of medical grade polyurethane were coated with these blends by a multiple dip-coating process, allowing the solvent to evaporate between each coating. The resistance of both PU and PU coated with the surfactant blends to the adherence of clinical isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* over a range of time periods (0.5–8 h), expressed as a percentage of the initial inoculum that had adhered to the medical device, was performed as previously described (Jones et al 1997). Surface hydrophobicity of the coated and uncoated PU strips was determined using a Cahn Dynamic Contact Angle Analyser employing distilled

water as the wetting medium and an immersion rate of 150 μm s^{-1}. The thermal properties of the blends were characterised using modulated DSC (TA Instruments MTDSC 2920) employing hermetically sealed pans (pinhole) and a defined heating rate (5°C min^{-1} from −10°C to 120°C. The effect of coating composition (L/C blend ratio or PU) on the resistance to microbial adherence and advancing and receding contact angles were statistically examined using a one-way analysis of variance in conjunction with Tukey's HSD post-hoc test. In all cases six replicates were examined and $P < 0.05$ denoted significance.

Table 1 Adherence and contact angle results

Coating composition	% Adherence of *S. aureus* after 0.5 h	Advancing contact angle (°)
PU (uncoated)	0.81 ± 0.05	93.2 ± 1.8
100%L:0%C	0.05 ± 0.01	61.1 ± 1.6
75%L:0%C	0.09 ± 0.04	64.4 ± 1.4
50%L:0%C	0.12 ± 0.03	71.0 ± 1.3
25%L:0%C	0.52 ± 0.01	80.3 ± 2.4

DSC traces show a broad endotherm from 0°C to 30°C which decreased in intensity with increasing cholesterol concentration. The contact angles and resistance to the adherence of both *S. aureus* and *Ps. aeruginosa* of the coated samples were significantly lower than native PU, highlighting the possible attributes of these surfactant blends as medical device coatings. Interestingly, increasing the ratio of lecithin to cholesterol significantly decreased the advancing contact angle (due to their higher HLB values), and importantly decreased the resultant microbial adherence. In conclusion, in light of the microbial anti-adherent properties, these surfactant blends offer initial promise as coatings of urinary biomaterials.

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Ionically crosslinked alginate hydrogels as scaffolds for periosteum-derived chondrogenesis

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Periosteal grafting has been explored extensively to resurface damaged articular surface (Chen et al 1999). However, the chondrogenic potential of periosteum needs to be improved and optimized to allow broad applicability and improve clinical outcome. This study involves the formulation and optimisation of ionically crosslinked alginate hydrogels as scaffolds for periosteum-derived chondrogenesis in the periosteum organ culture model (O'Driscoll et al 1994). The function of exogenous growth factors on infiltration and maturation of the cell population in these alginate gels was also studied.

A series of alginate-based gels were prepared utilizing different concentrations of alginate and $CaCl_2$ or $CaSO_4$ and their compressive strength tested using an Instron-5542 device. Periosteal explants from the tibia of skeletally mature New Zealand white rabbits were suspended in alginate gels and bathed with media supplemented with either transforming growth factor-β1 (TGF-β1), basic-fibroblast growth factor (FGF-2), a combination of the two, or in the absence of growth factors. Histological and immunohisto-chemical analysis of the explants was performed at 1, 3, 6 and 8 week time-points.

Alginate solutions (2% (w/v)) ionically crosslinked utilizing 75–150 mM $CaCl_2$ were found to produce the most homogeneous gels which exhibited nearly completely elastic behavior at low levels of deformation (< 1 mm, $R^2 = 0.99$) and compressive moduli appropriate for use in the organ culture model. Periosteum derived neo-chondrogenesis in these gels was absent in the absence of TGF-β1, with or without FGF-2. TGF-β1-induced periosteal chondrogenesis was evident at 3 weeks and increased up to 8 weeks. Further exposure of the periosteum to FGF-2 concomitantly during the first week of culture markedly enhanced periosteal chondrogenesis, as assessed by the cellular density, the intensity of safranin-O staining for glycosaminoglycans and the presence of type-II collagen.

A series of ionically crosslinked alginate hydrogels were developed for use in an organ culture model to study periosteal chondrogenesis. Utilizing this model, it was found that the addition of a mitogen, FGF-2, during the in-vitro culture of periosteum in the presence of TGF-β1, was found to enhance neo-chondrogenesis and cellularity. These results increase our understanding of the process of periosteal chondrogenesis and its regulation, so that it can be optimized and rationally controlled for articular cartilage repair and regeneration.

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