UKICRS Symposium 2014

United Kingdom & Ireland Controlled Release Society

Thursday 10 April 2014
Workshop & Dinner

Friday 11 April 2014
Symposium

University College Cork
Ireland

WELCOME TO CORK!

A very warm welcome to the 2014 UKICRS annual symposium which is being held in Cork for the first time. On behalf of the committee, I am delighted to welcome you to the School of Pharmacy, UCC which celebrated its 10th anniversary in 2013. The symposium will take place in the purpose built Cavanagh School of Pharmacy and is again geared towards ensuring that postgraduate students and early-career postdoctoral researchers have ample opportunity to network and present their research in a friendly and supportive environment.

We have a very engaging programme beginning with the workshop session on the afternoon of Thursday April 10th featuring short-presentations and hands-on demonstrations from a number of industrial exhibitors including Anton Paar, BARDS, Biopharma Process Systems (BPS), ISAC, Pfizer, Stable Micro Systems and Surface Measurement Systems who will showcase current technologies available for analysis and characterisation of drug delivery systems.

In line with the title of this year's symposium, 'Drug Delivery UnCorked', the scientific programme on Friday 11 April aims to highlight the diverse array of current research in the field of pharmaceutical sciences. Two distinguished industrialists, Dr. Marcus Brewster (Janssen R&D, Belgium) and Dr. Andy Lewis (Ipsen, France) will bring a wealth of experience in drug delivery and controlled release as they deliver the keynote lectures. Complemented by an excellent series of talks and poster presentations by postgraduate and postdoctoral participants from across Ireland and the UK, the symposium promises to be an exciting and stimulating meeting.

We do hope you enjoy the meeting and our hospitality here in University College Cork!

Katie Ryan

Dr. Katie Ryan
UKICRS Symposium 2014 Organiser

WORKSHOP & SPONSORS

The UKCIRS is hosting a free workshop (2–5 pm 10 April) in University College Cork for all attendees at this year's symposium. The workshop will feature short presentations and hands-on demonstrations of equipment and technology from a number of industrial exhibitors.

The UKICRS is grateful to the sponsors of the workshop: Biopharma Process Systems (BPS), Broadband Acoustics Resonance Dissolution Spectroscopy (BARDS), Interface and Surface Analysis Centre (ISAC), Pfizer, Stable Micro Systems, Surface Measurement Systems (SMS)

DINNER

The Symposium Dinner is at 7.30pm in 'Amicus Restaurant', Paul St, Cork (www.amicusrestaurant.ie).

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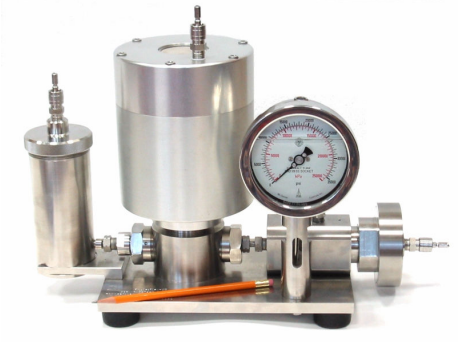

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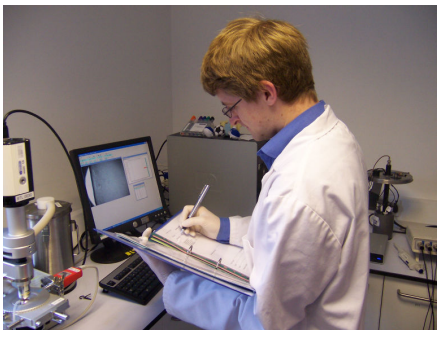

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PROGRAMME

8.30 am	Registration / Poster Setup
9.30 am	Welcome & Opening Remarks / Dr Katie Ryan (University College Cork)
9.40 am	**Dr. Marcus Brewster** (Janssen Pharmaceuticals R&D, Belgium) Designing Amorphous Solid Dispersions: In Silico Tools and Down-Scaled Automation
10.20 am	**Miss Hiteshri Makwana** (University of Nottingham) / Location! Location! Location! Site-specific conjugation of recombinant transferrin to polymers for applications in targeted drug delivery
10.35 am	**Dr. Waleed Faisal** (Minia University, Egypt) / A novel lipid-based solid dispersion for enhancing oral bioavailability of fenofibrate -in vivo evaluation using a pig model
10.50 am	Tea / Coffee
11.10 am	**Mr. Éanna Forde** (Royal College of Surgeons in Ireland) / Evaluating host defense peptide prodrugs targeted for use in the cystic fibrosis lung
11.25 am	**Miss Elisabeth Kastner** (Aston University) / Manufacturing of liposomes with desired particle characteristics by factorial design
11.40 am	**Dr. Yiwei Tian** (Queen's University Belfast) / The understanding of phase separation of amorphous drug polymer solid dispersions based on Flory-Huggins theory
11.55 am	Poster Session 1
12.40 pm	Lunch
1.25 pm	UKICRS Annual General Meeting
1.40 pm	**Dr. Andy Lewis** (Ipsen, France) / Patient focused peptide delivery
2.20 pm	**Miss Rachel Evans-Hurson** (University College Cork) / Application of Broadband Acoustic Resonance Dissolution Spectroscopy in pharmaceutical formulations
2.35 pm	**Miss Vanessa Loczenski Rose** (University of Nottingham) / Novel CRP routes to phosphonium polymethacrylates for siRNA delivery
2.50 pm	**Dr. Joanne Ramsey** (Royal College of Surgeons in Ireland) / Nebulised siRNA nanoparticles can induce knockdown in healthy and inflamed pulmonary epithelial models
3.05 pm	Tea / Coffee / Poster Session 2
3.50 pm	**Mr. Edward Mansfield** (University of Reading) / Probing diffusion of functionalised nanoparticles in gastric mucin using nanoparticle tracking analysis.
4.05 pm	**Miss Rosanne Raftery** (Royal College of Surgeons in Ireland) / Gene-activated scaffolds facilitate controlled release of growth factor for bone regeneration
4.20 pm	**Dr. Meenakshi Malhotra** (University College Cork) / RVG tagged modified cyclodextrins for receptor-mediated delivery of siRNA in glioblastoma cells
4.35 pm	Closing Remarks / Poster Prizes / Close of Meeting / Dr Gavin Andrews (QUB)

KEYNOTE SPEAKERS

ANDY LEWIS - IPSEN

Andy Lewis, BPharm (Hons) MAPS PhD is currently Director of Novel Drug Delivery Technologies at Ipsen where he has global responsibility for the development of new products utilising innovative delivery technologies for their peptides portfolio encompassing novel formulation technologies and drug delivery devices. Prior to joining Ipsen he helped set up and grow two venture capital funded start-ups RegenTec and Critical Pharmaceuticals, where he lead the development and commercialization of novel technologies in the fields of tissue engineering and drug delivery, taking them from concept into clinical development. His work has focused on the delivery of macromolecules, particularly the sustained release and transmucosal delivery of proteins and peptides, and he has filed a number of patents in the field. He is a member of the Academy of Pharmaceutical Scientists of Great Britain, and has served on the Membership Committee, Board of Scientific Advisors and is currently Director-at-Large of the Controlled Release Society (CRS) the premier international scientific society for delivery science.

MARCUS BREWSTER - JANSSEN PHARMACEUTICALS

Marcus E. Brewster is a Vice President and Scientific Fellow at Janssen Research and Development (The Pharmaceutical Companies of Johnson & Johnson) based in Beerse, Belgium. Dr. Brewster has focused his research interests on solubilizing delivery technologies for the development of bioavailable formulations for poorly water-soluble drugs and drug candidates including the use of nanotechnology, solid dispersion approaches, novel surfactant and polymeric micelles, polyester devices and complexing agents including chemically modified cyclodextrins. He is a Fellow of the American Association of Pharmaceutical Scientists and the Treasurer and a member of the Board or Directors of the Controlled Release Society as well as a former co-chair of the Board of Scientific Advisors and a former co-chair of the workshop committee for that organization. Dr. Brewster is an Editor of the Journal of Pharmaceutical Sciences (drug delivery and biopharmaceutics), a member of the Editorial Board of Die Pharmazie, a co-organizer of the European Drug Absorption Network (EDAN) and a co-work package lead on the IMI program, Orbito. Dr. Brewster has been honored with a number of J&J distinctions as well as prizes from PARC, FACCS and the European Journal of Pharmaceutical and Biopharmaceutics. Dr. Brewster has published over 265 peer-reviewed journal articles, book chapters and proceeding, has co-edited a monograph on solvents systems and their use for AAPS Press/Springer, presented over 370 meeting abstracts and was named as inventor or co-inventor on approximately 75 patents. He received his BA from Mercer University and his Ph.D. from the University of Florida.

ORAL ABSTRACTS

1 DESIGNING AMORPHOUS SOLID DISPERSIONS: USE OF IN SILICON TOOLS AND DOWN SCALED AUTOMATION

Dr Marcus Brewster
Vice President and Scientific Fellow at Janssen Research and Development, Janssen Pharmaceuticals

Contemporary drug pipelines are increasingly populated with difficult-to-formulate drug substances. The genesis of this heightened complexity is rooted in three confluent trends including (1) the reliance of the drug discovery process on high throughput screening, (2) growing issues with drug form and (3) the nature of current drug targets which are often associated with structure-activity relationships (SAR) which deviate or disconnect from the chemical space associated with good oral bioavailability and acceptable biopharmaceutical fitness. Taken in aggregate, this evolution in dosage form development challenges has forced formulators to innovate with a variety of novel approaches promulgated in the last few years. Generally these approaches are associated with either increasing the chemical potential of the drug substance in the solid state through a change in the drug form or reducing the chemical potential of the drug in its dissolved state. Altering the drug form can be executed through the use of amorphous, smectic or nematic mesophases of the drug or drug candidate. The selection of an appropriate candidate formulation is generally tied to assessments of the active pharmaceutical ingredient (API), aspects of its formulation as well as biopharmaceutical factors. This entails assessing design space elements and drug-ability information associated with preformulation, the dosage form and screening evaluations in animals. Generally the decision tree includes deciding whether a conventional or enabling formulation strategy is needed, if enablement is needed, then what is the best strategy and finally what process or excipient design space features should be considered for the selected trajectory. A biopharmaceutical dimension is also added usually by an indirect assessment of the in vivo dissolution rate in the rat or dog. Additional in silico or down-scaled (96-well plate) endpoints might then be applied to generate data-driven decisions related to which technology is best aligned with the specific task indicated. If a non-crystalline concept is suggested, the pure amorphous phase is only possible in a limited number of cases since this physical form is thermodynamically unstable requiring manipulation to render it pharmaceutically useful. These pharmaceutical interventions usually include stabilizing the non-crystalline phase by vitrifying/dispersing it in a glassy carrier, by increasing the aggregate/mixing glass transition temperature (Tg) of the composite though the use of a high Tg carrier and by encouraging specific drug-carrier interactions through selecting matrices with appropriate functionality and chemistry. Examples of marketed product and drug candidate development show that these approaches can increase formulation finding efficiency and robustness and contribute to the ability to get important products to patients.

2 LOCATION! LOCATION! LOCATION! SITE-SPECIFIC CONJUGATION OF RECOMBINANT TRANSFERRIN TO POLYMERS FOR APPLICATIONS IN TARGETED DRUG DELIVERY

Hiteshri B Makwana[1], Cameron Alexander[1], Karl Nicholls[2], Tara Sharpe[2], Dave Mead[2], Darrell Sleep[2] & Stephanie Allen[1]. [1] Laboratory of Biophysics and Surface Analysis and Drug Delivery and Tissue Engineering, School of Pharmacy, University of Nottingham, Nottingham, UK; [2] Novozymes Biopharma Ltd UK, Castle Court, Nottingham, NG7 1FD, UK

Background: The up-regulation of the transferrin (Tf) receptor CD71 in malignant disease has been suggested as a potential therapeutic target for drug carrying protein-polymer conjugates (PPCs). Traditionally, PPCs are synthesized via a non-selective conjugation route that can lead to a loss in biological activity and lack of reproducibility. Recombinant protein technologies can serve as a platform to circumvent such limitations. In this abstract we describe how variants of Tf can be expressed with a single free cysteine reside for the site-specific conjugation to a synthetic polymer. Using sophisticated controlled polymerisation chemistries, we are able to generate Tf-PPCs for evaluation as suitable drug delivery systems.

Methods: Tf variants were expressed and characterised (Urea gel and Ellman's assay). Polymers were synthesized using atom-transfer radical polymerization (ATRP). Tf-polymer conjugates and their assemblies were characterised using high-performance liquid chromatography (HPLC), matrix-assisted laser desorption time-of-flight (MALDI-TOF), atomic force microscopy (AFM), transmission electron microscopy (TEM), ELISA and gel electrophoresis (SDS-PAGE). The drug loading potential of Tf-PPCs were evaluated and release profiles were studied. In vitro cytotoxicity assays were performed on the colon cancer cell line HCT116. Tf-mediated uptake was antagonised using an anti-CD71 antibody.

Results: Non-selective conjugation of Tf can lead to a loss in biological activity and thus targeting ability. To overcome this, we employed recombinant protein technologies to engineer a free cysteine residue on Tf. A number of Tf variants were expressed, each with the cysteine residue mutation at different locations of the protein structure. Using ATRP synthesis, polymer chains (9-10 kDa, PDI 1.16) were grown from each Tf variant. Tf-polymer conjugates have the ability to self-assemble into nano-scaled structures of 20-30 nm in diameter and have high paclitaxel (PTX) encapsulation efficiencies (64.1% (± 2.7 SD)). Tf-PPCs demonstrated significant retardation of PTX release in vitro. The drug loaded systems exhibited cytotoxicity against a CD71-high expressing cell line. Inhibition of Tf-mediated uptake demonstrated that the cytotoxicity of Tf-PPCs was Tf-mediated. When evaluating cytotoxic performance, differences in potency were noted between the different Tf- PPCs and were correlated to the location of the cysteine mutation on the protein. As a result the best and worst sites for modification on the Tf structure have been identified.

Conclusions: Herein, we describe how the location of polymer engraftment on a protein can impact biological activity. Using recombinant protein technologies we have demonstrated that Tf can be utilized to develop PPCs that can self-assemble, can carry cytotoxic agents and retain biological affinity for its target. It is hoped that employing strategies as described above can yield effective drug delivery systems that can target an array of diseases.

3 A NOVEL LIPID-BASED SOLID DISPERSION FOR ENHANCING ORAL BIOAVAILABILITY OF FENOFIBRATE – IN VIVO EVALUATION USING A PIG MODEL

Waleed Faisal[1], Therese Ruane O'Hora[2], Caitriona M. O'Driscoll[3] & Brendan T. Griffin[3], [1] Department of Pharmaceutics, Minia University, Egypt; [2] Department of Physiology, University College Cork, Ireland; [3] Pharmacodelivery Research group, School of Pharmacy, University College Cork, Ireland.

Background: Fenofibrate represents a class of drug that may benefit from formulation in a lipid-based drug delivery system. The bioavailability of fenofibrate in conventional dosage forms is low, variable and increased when administered after food. The aim of this study was to develop an optimised formulation, which allows for efficient absorption following oral administration.

Methods: A self-emulsifying drug delivery system (SEDDS) and solid dispersion of fenofibrate were developed initially. Subsequently, a novel lipid based solid dispersion (LBSD) was designed. Characterization of drug in various formulations was performed by differential scanning calorimetry (DSC) and X-ray diffraction (XRD).

Results: The bioavailability of fenofibrate was significantly increased after oral administration of LBSD to fasted pigs. A clear distinction in terms of Cmax and AUC was observed between LBSD and the commercial product Lipanthyl.

Conclusions: In conclusion, a novel LBSD formulation was developed to enhance the oral bioavailability of the model lipophilic compound, fenofibrate, by enhancing dissolution in the gastrointestinal tract and promoting intestinal lymphatic uptake utilising digestible lipid excipients.

4 EVALUATING HOST DEFENSE PEPTIDE PRODRUGS TARGETED FOR USE IN THE CYSTIC FIBROSIS LUNG

Éanna Forde[1,2], Hilary Humphreys[2,3], Catherine Greene[4], Deirdre Fitzgerald-Hughes[1] & Marc Devocelle[2], [1]Department of Clinical Microbiology, Royal College of Surgeons in Ireland, Dublin 9, Ireland. [2] Centre for Synthesis and Chemical Biology, Department of Pharmaceutical and Medicinal Chemistry, Royal College of Surgeons in Ireland, 123, St. Stephen's Green, Dublin 2, Ireland. [3] Department of Microbiology, Beaumont Hospital, Dublin 9, Ireland. [4] Pulmonary Research Division, Department of Medicine, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin 9, Ireland.

Background: Host defence peptides (HDPs) are short antimicrobial peptides forming part of the innate immune system. Deficiencies in HDPs can lead to enhanced susceptibility to infections, e.g. in the cystic fibrosis (CF) lung, rationalising their exogenous application. However, their development as antimicrobial therapeutics is limited by cytotoxicity. Previously, three HDP-based peptide prodrugs were designed so that their positive net charge, which contributes to their antimicrobial activity, was masked by a promoiety containing a substrate for neutrophil elastase (NE), allowing targeted release of the active HDP. The promoiety was successful in reversibly-reducing antimicrobial activity and reducing cytotoxicity, potentially limiting host toxicity (1). However, remaining issues with selectivity and cytotoxicity required the optimisation of the promoiety and peptide sequence. The aim of this study was to further refine these prodrugs and elucidate their potential effects on CF airway cells.

Methods: HDP-prodrugs were synthesised using L- and D-amino acids. Enzyme-lability of the promoiety, required for prodrug activation was investigated using HPLC and MALDI-TOF MS with purified NE. New HDPs such as WMR (2) were incorporated into the prodrug model. The susceptibility of Pseudomonas aeruginosa to the parent and prodrug peptides was compared in the presence and absence of NE-rich CF bronchoalveolar lavage (BAL) fluid and in different concentrations of NaCl. The effect of the promoiety on HDP cytotoxicity was determined using the MTT assay with CF bronchial epithelial (CFBE41o-) cells.

Results: Modification of the promoiety, while still leading to reduced cytotoxicity, did not improve selectivity. However, the use of new peptide sequences yielded the required improvements. The new pro-HDP, pro-WMR, maintained low antimicrobial activity in the absence of NE, performing better than the previous pro-HDPs, with a bactericidal activity at 25μg/ml that increased from 8.4% to 91.5% with the addition of 25% v/v BAL fluid ($p=0.0008$). Selectivity was also greatly improved (IC50 against CFBE cells ≥300 μM).

Conclusions: Selective activation by a host disease-associated enzyme at concentrations within the ranges of CF lung was demonstrated here. The promoiety was successful in reversibly-reducing antimicrobial activity and reducing cytotoxicity. The further refinement of the approach has produced pro-HDPs, with greatly reduced host toxicity, that function in the challenging conditions of the CF lung.

5 MANUFACTURING OF LIPOSOMES WITH DESIRED PARTICLE CHARACTERISTICS BY FACTORIAL DESIGN

Elisabeth Kastner, Andrew J. Ingham & Yvonne Perrie, Aston Pharmacy School, Aston University, Aston Triangle, Birmingham B4 7ET, UK

Background: The increased application of liposomes as carrier vesicles and immunological adjuvants necessitates an improved understanding in process control and product characteristics. Characterisation of liposomes is not only crucial in manufacturing but may also dictate the immunological activity of the adjuvant. Design of Experiments (DoE) is a method frequently used for describing a manufacturing process, which increases the process reproducibility by generation of a design space. Therefore, the aim of this study was to develop such a mathematical model that would allow for predictions of key liposome characteristics.

Methods: A 2 level CCF response surface design was used to compose an orthogonal construct of experiments, which investigated the effect of lipids to liposome size, zeta potential (ZP) and polydispersity (PDI). The two factors used were the lipids DDA (50-250 ug/mL) and DSPC (50-250 ug/mL). Responses selected were the liposome size, determined as z-average by DLS measurements, ZP and PDI measurements, which were regarded as critical quality attributes in liposomal adjuvants.

Results: A statistically valid model was generated for all three responses; liposome size, ZP and PDI. The significance of the net charge of the lipids was linked to the overall zeta potential, revealing the model terms DSPC and DDA*DSPC as the most important factors in the process. The response surface plots revealed a predicted PDI from 0.3 to 0.8, a ZP from +70 to -10 mV and a size from 500 to 4800 nm. We could see that a high amount of DDA in the formulation is related to a low PDI, a high ZP as well as low liposome size. Increasing the amount of DSPC into the formulation leads to an increase in PDI and size; as well as a decrease in zeta potential. We performed a sweet spot analysis that exemplified the application of a response surface modeling for controlling the manufacturing process of liposomes with desired particle characteristics.

Conclusions: A model could be generated which allows for predictions of the main particle characteristics in a liposome formulation, size, ZP and PDI, as a function of lipids used. Results emphasize on the potential and significance of applying statistical based process control for generating a design space and allowing for reproducible manufacturing of pharmaceutical and biopharmaceutical products.

6 THE UNDERSTANDING OF PHASE SEPARATION OF AMORPHOUS DRUG POLYMER SOLID DISPERSIONS BASED ON FLORY-HUGGINS THEORY

Yiwei Tian, David S. Jones & Gavin P. Andrews, School of Pharmacy, Queen's University Belfast, BT9 7BL, UK

Background: The thermodynamic status of amorphous felodipine within different polymeric carriers is defined using Flory-Huggins phase diagrams. It in principle reveals the variations in the solubility and miscibility of felodipine in different polymers, which allows ranking and selection of the most suitable polymer for stabilization of amorphous BCS II drugs. Here, the effect of drug loading and polymer type at defined thermodynamic conditions were investigated. The phase separation and crystallization of these amorphous solid dispersions were studied and an in-depth treatise of the outcomes in relation to F-H theory is discussed.

Methods: To gather a detailed understanding of the theoretical F-H model constructed for amorphous drug polymer solid dispersion systems, quench-cooled melts of homogenized ball milled samples were used in this study. Sample containing felodipine and polymer HPMCAS/ Soluplus/PVPK15 at specified compositions were prepared using a ball mill (Restch MM200). The mixture was placed on a clean glass slip at 150 °C until completely liquified, then covered with another pre-heated glass slip to form a thin amorphous disc. A pre-cut aluminium ring (50µm thick) was sandwiched between to unify the thickness of the solid dispersion. The top slip was subsequently removed after cooling. Immediately prepared samples were confirmed to be crystal free by observation in the dark field of a polarized light microscope (PLM Olympus BX50). The resultant amorphous solid dispersion discs were stored at 80°C, 0% RH. During the study, PLM and Raman microscopy were both employed and the crystallization rate and surface behavior were studied.

Results: In order to better understand the solubility/miscibility of a model drug, felodipine, with selective polymers namely PVPK15, Soluplus and HPMCAS, phase separation and crystal growth rate of the amorphous felodipine from homogenous amorphous solid dispersions was studied. The phase separation and crystallisation were monitored using Raman chemical imaging and PLM. No correlation was found between the T_g of polymer or T_g of felodipine-polymer solid dispersion and the extend of crystal growth rate. PVPK15 and Soluplus® was highly effective in stabilising amorphous felodipine relative to HPMCAS-HF; as predicted by constructed phase diagrams. The differences in terms of nucleation and crystal growth for amorphous solid dispersion systems were also discussed. FD with PVPK15 and Soluplus® appeared to be an upward surface crystal growth mechanism and FD/HPMCAS-HF showed a surface enhanced and surface to bulk penetration type crystal growth. Due to the high immiscibility between FD and HPMCAS, the surface to bulk type crystal growth was not retarded by coating with a thin layer of pure polymer. Conversely, a thin layer of polymer coating on FD/PVPK15 and FD/Soluplus significantly slowed crystal growth. Most interestingly, an amorphous-amorphous phase separation was observed in both 50% and 60% FD/ PVPK15 solid dispersions whereas the 50% FD/PVPK15 exhibited a classic nucleation and growth mechanism.

Conclusions: Classic Flory-Huggins theory has provided sufficient information for the understanding of phase separation and crystallisation of amorphous drug polymer solid dispersion systems.

7

PATIENT FOCUSED PEPTIDE DELIVERY

Dr Andy Lewis
Ipsen

The high potency and specificity of peptides have made them attractive drug candidates with several blockbuster products. However, their relatively large size and hydrophilicity means that they are usually administered by injection, and early efforts in drug delivery focussed on injectable sustained release formulations to improve patient experience and compliance. The successful development of a number of such technologies has resulted in the majority of blockbuster peptide drug products exploiting some form of PLGA or PLA formulation (implants, microspheres or microparticles). Indeed parenteral sustained release formulations have become a standard of care in many indications treated by peptide drugs, and as such are now routinely integrated into a product development programme. The next generation of sustained release peptide delivery technologies are now focussed not only on achieving sustained release, but providing additional patient benefit e.g. simplifying administration, administration through narrower gauge needles, and ready to use presentations. Furthermore recent advances in peptide delivery technologies are opening up the possibility of developing oral, buccal, transdermal and inhaled routes for a wider range of peptides, with many in late stage development and close to market. Recent developments in novel peptide delivery technologies will be reviewed with an emphasis on key factors that might determine their success.

8 APPLICATION OF BROADBAND ACOUSTIC RESONANCE DISSOLUTION SPECTROSCOPY IN PHARMACEUTICAL FORMULATIONS

Dara Fitzpatrick[1], Rachel Evans-Hurson[1], Bastiaan Vos[1], Seán McSweeney[1], Jacob Krüse[2] & J.J. Keating[1,3], [1] Department of Chemistry and Analytical and Biological Chemistry Research Facility, University College Cork, Ireland. [2] Kinetox, Beilen, The Netherlands. [3] School of Pharmacy, University College Cork, Ireland.

Background: Broadband Acoustic Resonance Dissolution Spectroscopy (BARDS) is a new platform technology with key applications in Pharmaceutical Analysis including blend uniformity, profiling of drug delivery spheres, stability testing, inter-batch variation and counterfeit identification. In this study, data is present which shows the potential of BARDS in the rapid determination of the loading of API and enteric coatings on drug delivery spheres. The formulations are shown to undergo reproducible changes in the compressibility of the solvent during dissolution which is indicated by unique acoustic spectra.

Methods: A purpose built spectrometer was built and used to investigate the BARDS responses. It consists of a chamber with a glass tumbler, microphone, a magnetic stirrer and follower. There is access at the front for the dissolution vessel and at the top in order to place a sample in a weighing boat on a tipper motor for introduction of the solute. The microphone is positioned above the top of the glass within the housing. The glass, containing 25 mL of water is placed on the stirrer plate. The stirrer motor underneath is positioned so as to allow the magnetic follower to gently tap the inner glass wall. In this way, the follower acts as a source of broadband acoustic excitation, thereby inducing various acoustic resonances in the glass, the liquid and the air column above the liquid. The audio is sampled at a rate of 44.1 kHz. A fast Fourier transform is applied to the signal. The resonances of the liquid vessel are recorded in a frequency band of 0–20 kHz.

Results: Acoustic spectra are presented which show characteristic dissolution profiles of both raw materials and finished product. A steady state lag time after the addition of drug spheres until the presence of a large response for the dissolution of the core sugar sphere is indicative of the thickness of the coatings on the sphere. The method can also be used to validate the ratio of drug coated spheres to placebo spheres in a formulation.

Conclusions: BARDS analysis is proven to be a rapid new approach to characterize a range of formulations through production and post-production stages. The methodology is shown to be universally applicable to drug delivery sphere profiling due to the limited range of enteric coatings used in formulations.

9 NOVEL CRP ROUTES TO PHOSPHONIUM POLYMETHACRYLATES FOR SIRNA DELIVERY

Vanessa Loczenski Rose[1], Andrezj Gallas[1], Saif Shubber[1], Sebastian G. Spain[1], G. Sebastiaan Winkler[1], Stephanie Allen[1], Sanyogitta Puri[2] & Giuseppe Mantovani[1], [1] School of Pharmacy, Boots Science Building, University Park, University of Nottingham, NG7, UK; [2] Astrazeneca UK Ltd., Pharmaceutical Development, Alderley Park, Macclesfield SK10 2NA, UK

Background: Small interfering RNA (siRNA) therapeutics have great potential as a treatment paradigm for disease targets inaccessible by traditional small molecules drugs. siRNA via the RNAi pathway regulates gene expression by interacting with messenger RNA and can be used to selectively switch off genes. However, given its extreme instability in vivo and restricted cellular uptake, systemic delivery of siRNA requires complex formulations to prevent nuclease degradation, improve biodistribution and minimize off-target effects. Recently, the application of phosphonium containing polymers has been suggested as attractive alternatives to ammonium analogs due to their improved DNA/RNA binding and lower cytotoxicity.

Methods: We describe a novel synthetic approach to prepare ammonium and phosphonium derived polymethacrylates as potential RNA delivery system. A set of ammonium and phosphonium analogs with different macromolecular architectures was synthesized using techniques of controlled radical polymerization (RAFT and SET-LRP). Dynamic light scattering (DLS), atomic force microscopy (AFM) and zeta potential measurements have been performed to study size (nm) and charge of polyplexes at different N_+/P_- or P_+/P_- ratios. The efficiency to form polymer-siRNA complexes through ionic interactions was assessed using a gel retardation assay. A cell viability assay (human U2OS and mouse 3T3 cells) and cellular uptake studies were performed by confocal imaging and flow cytometry to further evaluate the delivery system.

Results: Cationic polymethacrylates are water soluble and form polymer-siRNA complexes of around (~15 nm) at different N_+/P_- or P_+/P_- ratios with positive surface charges as analyzed by DLS and zeta potential, respectively. Furthermore, all polymers efficiently bound siRNA and DNA oligonulceotides at low N_+/P_- or P_+/P_- ratios, which is advantageous to generate safer and cost-effective siRNA nanovectors. The strength of polymer binding was analyzed using a modified Hill equation and revealed slightly better binding properties for phosphonium containing analogs. The cell viability assay demonstrated the relatively non-toxic nature of polymer concentrations within the optimal range for gene knockdown. Cellular uptake studies with labelled RNA demonstrated efficient uptake as verified by flow cytometry and confocal imaging.

Conclusions: Taken together, the polymers presented here show highly desirable properties as potential RNA delivery systems, nucleic acid transfection studies are currently ongoing.

10 NEBULISED SIRNA NANOPARTICLES CAN INDUCE KNOCKDOWN IN HEALTHY AND INFLAMED PULMONARY EPITHELIAL MODELS

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Background: RNA interference (RNAi) allows specific and potent knockdown of target genes, and interest now lies in its potential as a therapeutic to mediate gene silencing in diseased cells. Inhalation offers a means of direct delivery of siRNA to the target organ, the lungs. It also offers many advantages over systemic delivery such as decreased systemic toxicity, immediate availability, and local and non-invasive delivery. In respiratory medicine siRNA is being explored for the treatment of chronic inflammatory conditions such as asthma, chronic obstructive pulmonary disease, cystic fibrosis, acute lung injury. However, clinical and commercial translation is hampered by lack of efficient delivery systems and relevant screening tools for their development.

Methods: NP Manufacture and Characterisation. Poly(ethyleneimine)-Poly(ethylene) glycol (PEI-PEG) co-polymers were combined with siRNA to form siRNA nanoparticles (NPs). The size distribution and zeta-potential of the siRNA nanoparticle dispersions were measured on a Nano-ZS (Nanoseries, Malvern Instruments). In vitro epithelial transfection. Calu-3 cells were seeded in Transwell plates at liquid-liquid and air-liquid interfaces, whilst an Aeroneb® Pro vibrating mesh nebuliser was sealed into place at the entrance of a Twin Stage Impinger. PEI/ PEI-L-PEG(10kDa) siRNA NPs were then delivered at various N/P ratios. High content analysis screening. Calu-3 cells were transfected with siRNA NPs at various N/P ratios and stained using phalloidin-TRITC and Hoechst nuclear stain. Image analysis was achieved using the InCell® 1000 High Content Analyser (GE Healthcare, UK). siRNA knockdown in LPS-stimulated rat model. Female Sprague-Dawley Rats (220-250g) were intratracheally instilled with NPs (REC743) followed by LPS/PBS 3 hr prior to 24 hr collection time points. Bronchoalveolar lavage (BAL) samples were collected. The supernatants were profiled for cytokine levels including IL8 whilst the pelleted cells were resuspended in 100μl PBS for BAL cell counts and cytology analysis.

Results: PEI grafted with linear PEG chains formed significantly ($p<0.001$) more compact NP than unmodified PEI siRNA NP. At N/P 7 and 10, PEI-L-PEG(10kDa) siRNA NP exhibited highly significant levels of cellular internalisation. For PEI siRNA NP nebulisation appeared to significantly diminish their ability to elicit GAPDH knockdown, with no knockdown observed at N/P=15 post-nebulisation. In contrast, PEI-L-PEG(10kDa) siRNA NP appeared to be much more resistant to nebulisation effects, with significant knockdown still evident post-nebulisation. Analysis of both siRNA NPs treated rat lungs, as with the PEI group, revealed no significant difference from PBS controls but PEI-L-PEG(10kDa) anti-IL-8 siRNA were found to be capable of eliciting a decrease of 65% in IL-8 mRNA expression compared to LPS treated. However, histopathology analysis revealed high levels of inflammation in both PEI-LPS and PEI-L-PEG(10kDa)-LPS treated models.

Conclusions: When combined with the Aeroneb Pro® delivery device PEI-L-PEG(10kDa) NPs demonstrated IL-8 knockdown with reduced toxicity and better stability compared PEI. Both NP systems are capable of target gene knockdown and inhibiting inflammatory cell influx in vivo in a rodent model of inflammation but their pro-inflammatory effects are dose limiting.

11 PROBING DIFFUSION OF FUNCTIONALISED NANOPARTICLES IN GASTRIC MUCIN USING NANOPARTICLE TRACKING ANALYSIS

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Background: With the increased use of nanoparticles in the pharmaceutical, cosmetic and food industries, there is a developing need to understand how these particles behave in biological milieu in order to predict their in vivo activities. Mucus, for example, coats tissue surfaces throughout the gastrointestinal tract, and acts as a barrier to drug delivery. Here we present research focussed on the diffusion of organosilica nanoparticles functionalised with various compounds through porcine gastric mucus using Nanoparticle Tracking Analysis (NTA) developed by NanoSight Ltd. Much of the current research on diffusion through mucus looks at particles functionalised with PEG, due to its low toxicity and resistance to enzymatic breakdown1. It has also been demonstrated that coating the surface of nanoparticles with PEG will enhance their diffusion through mucus gels.

Methods: By using thiolated silica as a model, we have developed a series of nanoparticles functionalised with poly(ethylene glycol) (PEG), poly(2-ethyl-2-oxazoline) (POz) and poly(N-isopropylacrylamide) (PNIPAM). The nanoparticles were characterised using dynamic light scattering, transmission electron microscopy and NTA. The functionalised nanoparticles were fluorescently labelled by reacting with maleimide terminated BODIPY. By fluorescently labelling the particles it is possible to monitor their diffusion without interference from mucin.

Results and Conclusions: The diffusion of functionalised nanoparticles suspended in mucin was studied using NTA, and the results were compared with the theoretical estimation of diffusion coefficients using the Stokes-Einstein equation. Preliminary experimental work suggests that PEGylated particles diffuse in mucin more readily than thiolated ones, which is in agreement with the literature.{Formatting Citation} This is due to the hydrophilic nature of PEG that reduces interactions with mucin. Based on this data POz and PNIPAM should also increase the rate of diffusion, due to the hydrophilic nature of the molecules.

12 GENE-ACTIVATED SCAFFOLDS FACILITATE CONTROLLED RELEASE OF GROWTH FACTOR FOR BONE REGENERATION

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Background: Gene-activated scaffolds can facilitate localized, controlled release of growth factor at defect sites which can induce tissue regeneration. A gene delivery vector is required to 'activate' a scaffold. Chitosan is a natural cationic polymer with potential for intracellular delivery of plasmid DNA. The aim of this study is to develop a chitosan-based gene delivery vector capable of transporting bone growth factor genes into stem cells seeded onto a collagen scaffold – thereby forming a gene-activated scaffold suitable for bone regeneration.

Methods: Polychitosan (Mw 160 kDa) and oligochitosan (Mw 7.3 kDa) were assessed for stem cell transfection efficiency. Bone marrow-derived rat MSCs (rMSC) were used at passage 5. Reporter genes Gaussia Luciferase (pGLuc) and Green fluorescent protein (pGFP) were used to assess transfection efficiency. MTT assay determined vector toxicity. The optimized chitosan nanoparticles were incorporated into a collagen scaffold and growth factor expression was quantified using ELISA and calcium deposition was also determined.

Results: Following a rigorous physicochemical characterization and transfection efficiency screening process, a lead oligochitosan-pDNA nanoparticle formulation was chosen. This formulation was capable of transfection efficiency of >45% as determined by flow cytometry. Chitosan treatment did not negatively affected cell viability. The optimized transfection protocol was then applied to a collagen-based scaffold and sustained expression of bone morphogenetic protein was seen up to day 28.

Conclusions: A novel gene-delivery vector was optimized for transfection of stem cells. These oligochitosan nanoparticles were then used to deliver osteogenic genes to MSCs seeded on a collagen scaffold, thus forming a gene-activated scaffold. This resulted in enhanced mineral deposition by the MSCs indicating that this construct is highly suitable for use in bone defect regeneration.

Acknowledgements: SFI Research Frontiers Programme (Grant No. 11/RFP/ENM/3053)

13 RVG TAGGED MODIFIED CYCLODEXTRINS FOR RECEPTOR-MEDIATED DELIVERY OF SIRNA IN GLIOBLASTOMA CELLS

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Background: Glioblastoma is a malignant primary brain tumor and is most commonly found in males. The conventional therapies include surgery followed by chemotherapy, radiotherapy and immunotherapy. Most therapies have limitations due to post-surgical complications and the challenge of crossing the blood-brain barrier (BBB). Thus, continual advancements are made to develop a therapeutic modality that is non-invasive, overcomes the BBB and delivers the therapeutic at the targeted site. The current project, develops a modified RVG-tagged cyclodextrin-siRNA nanocarrier. The presence of RVG enables receptor-mediated endocytosis of the nanocarrier by the acetylcholinesterase receptors expressed on the surface of brain cancer cells.

Methods: Modified amphiphilic cyclodextrins SC12CD-propylamine, SC12CD-PEG500 and SC12CD-PEG500-RVG were chemoselectively synthesized and characterized by NMR at University College Dublin. The modified cyclodextrins were premixed at various molar ratios to form a co-formulation, followed by addition of siRNA (mass ratio-10). The prepared formulations were characterized for their size, charge, stability and in-vitro studies (uptake and cytotoxicity), performed on U87, human glioblastoma cells**.**

Results: PEI grafted with linear PEG chains formed significantly ($p<0.001$) more compact NP than unmodified PEI siRNA NP. At N/P 7 and 10, PEI-L-PEG(10kDa) siRNA NP exhibited highly significant levels of cellular internalisation. For PEI siRNA NP nebulisation appeared to significantly diminish their ability to elicit GAPDH knockdown, with no knockdown observed at N/P=15 post-nebulisation. In contrast, PEI-L-PEG(10kDa) siRNA NP appeared to be much more resistant to nebulisation effects, with significant knockdown still evident post-nebulisation. Analysis of both siRNA NPs treated rat lungs, as with the PEI group, revealed no significant difference from PBS controls but PEI-L-PEG(10kDa) anti-IL-8 siRNA were found to be capable of eliciting a decrease of 65% in IL-8 mRNA expression compared to LPS treated. However, histopathology analysis revealed high levels of inflammation in both PEI-LPS and PEI-L-PEG(10kDa)-LPS treated models.

Conclusions: These results indicate a successful synthesis of a co-formulated RVG-tagged cyclodextrin nanoparticle, which possesses an enhanced stability, transfection and target specificity for the treatment of brain cancer. Further in-vitro investigations will be required to analyze the ability to cross the BBB and deliver a functional siRNA for knockdown studies.

Acknowledgment: Authors gratefully acknowledge the funding from Irish Cancer Society, Grant No. PCI11ODR.

POSTER ABSTRACTS

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NOVEL POLYMERS FOR THE MUCOSAL DELIVERY OF SIRNA

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Background: Effective delivery remains the most significant barrier between the potential of siRNA technology and its success as a therapeutic approach. Local delivery to the lung offers advantages over systemic delivery, such as its large surface area and direct delivery to epithelial tissues for the treatment of respiratory viruses and cancer. Here, we assess the suitability of a novel cationic biocompatible polymer as a delivery system for siRNA.

Methods: Physiochemical characteristics of siRNA polyplexes were determined as well as their cytotoxicity. Gene silencing was assessed in the lung epithelial cell lines H1299, A549 and Calu-3 against the housekeeping gene GAPDH. Chemical inhibitors of endocytosis were used to study the mechanism of polyplex uptake in each cell line.

Results: Dynamic light scattering revealed siRNA polyplexes had a diameter of 240nm with a surface charge of +31mV, characteristics desirable for cellular uptake. Knockdown of GAPDH was comparable to the commercial transfection agent Lipofectamine in all 3 cell lines. The highest knockdown was achieved in H1299 cells of ~80% with a low siRNA concentration of 50nM, where the highest level of polyplex uptake was also seen. Mechanism of polyplex uptake was cell line dependent and appeared to have a significant effect on subsequent intracellular trafficking. Knockdown in Calu-3 cells could be increased from 30% to 65% with the use of an endosomolytic agent, suggesting that endosomal escape is a limiting factor in efficacy when uptake is mainly via the clathrin pathway.

Conclusions: We have achieved gene silencing efficiencies comparable to commercial transfection agents with minimal toxicity. In conclusion, this novel polymer appears promising for the mucosal delivery of siRNA and is currently being trialled in vivo.

15 HYDROGELS FOR WOUND CARE APPLICATIONS: A NOVEL METHOD OF SYNTHESIS

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Background: Hydrogels have become popular as materials for wound dressings because of their unique properties, easy use and relatively low cost. They simulate living biological tissue more than any other synthetic biomaterial due to their high water content, porosity and softness. They are able to create an optimum environment at the wound site thanks to their so called 'moisture donor' effect, which could provide an easier and faster healing process.

Methods: A novel synthetic method to produce hydrogels starting from ready-made water-soluble polymers has recently been developed at The University of Reading School of Pharmacy. The cross-linking step is conducted using a common autoclave in a safer, faster and cheaper way than all the other methods available.

Results: In the present work, we have developed this method further by synthesising hydrogels from combinations of poly(methyl vinyl ether-alt-maleic anhydride) (PMVEMA) with different grades of poly(vinyl alcohol) (PVA).

Conclusions: The autoclaved hydrogels produced exhibited excellent swelling properties in deionised water and in ion-containing solutions, significantly dependent on the molecular weight and deacetylation degree of PVA. Even the basic formulations have shown very good mechanical properties and conformability to the skin.

16 A STRATEGY TO TARGET DEVICE RELATED INFECTIONS: OFFERING GREATER FLEXIBILITY USING A SIMPLE DRUG LOADING METHOD

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Background: Perioperative bacterial seeding with subsequent biofilm formation has been highlighted as the main cause of bacterial associated infections (BAI) on orthopedic implants. An attractive approach is to impregnate the hydroxyapatite (HA) coating with antibiotic drugs through a simple loading method. In this study, a novel grit blasting technology named CoBlast™ was used to coat titanium (V) metal with a crystalline HA layer Tetracycline hydrochloride (TCH) was used as a model drug not only for its broad bactericidal activity but also for its bone targeting properties. TCH was impregnated onto the HA surface through a simple loading method. The physicochemical properties, release profile as well as its bactericidal and anti-colonisation activity were assessed.

Methods: Characterisation techniques such as FT-ir, XRD, TGA, DSC and SEM were used. Drug-loading and drug release was measured using UV-vis spectroscopy. Surfaces were challenged using S. aureus (ATCC 1447) and clinical isolate strains (MRSA, MSSA and *S.epidermis).*

Results: The TCH had precipitated onto the HA surface in a hydrated form (FT–IR, DSC, TGA analysis). The drug loading was dependent on the loading time and concentration. Promising antibacterial and anti-colonising results were obtained.

Conclusions: The study here is a strategy to target device related infections; by combating bacterial adhesion in the first 24 hr of implantation, a time-frame which is sufficient to prevent biofilm formation and improve the success rate of the implant in a clinical setting.

17 POLY(ALLYLAMINE) MAGNETOMICELLES FOR IMAGE GUIDED DRUG DELIVERY

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Background: Polymeric micelles have received considerable interest for their use as drug delivery vehicles for the solubilisation of hydrophobic agents. Inorganic metallic nanoparticles have been clinically exploited in diagnostics for contrast ability in magnetic resonance imaging. The combination of these two platforms results in a multifunctional drug carrier for image-guided drug delivery. Here we report the synthesis and evaluation of a new class of poly(allylamine) (PAA) polymer grafted with hydrophobic oxadiazole (Ox) pendant group in a 5% molar monomer:pendant ratio. These thiol-containing Ox groups facilitated attachment of hybrid iron oxide-gold nanoparticles (HNPs) via dative covalent bonding.

Methods: Physicochemical characterisation of PAA-Ox_5 and PAA-Ox_5-HNP polymers using elemental analysis, nuclei NMR, FTIR and PCS showed that polymer synthesis had successfully completed. The drug loading ability of the nano-aggregates was investigated, using both direct conjugation of hydrophilic and encapsulation of hydrophobic drugs, respectively. The model hydrophobic drugs 2,6-diisopropylphenol (propofol), (2S,6'R)-7-chloro-2',4,6-trimethoxy-6'-methyl-3H,4'H-spiro[1-benzofuran-2,1'-cyclohex[2]ene]-3,4'-dione (griseofulvin), chemotherapeutic agent bisnapthalamidopropyldiaminooctane (BNIPDaoct) and 6-Thioguanine (6-TG) were used. In vitro assays on human pancreatic adenocarcinoma cells (BxPC-3) showed increased drug uptake and decreased IC_{50} values using the novel formulations compared with free drug.

Results: The data showed that the addition of HNPs onto the PAA-Ox_5 structure resulted in aggregates of approximately 175 nm diameter. The PAA-Ox_5-HNP nano-aggregates were capable of high drug solubilisation capacities (25.79 $mgmL^{-1}$, 1.68 $mgmL^{-1}$ & 0.92 $mgmL^{-1}$) for propofol, griseofulvin & BNIPDaoct, respectively. 6-TG was also successfully conjugated onto the polymer structure (2.8 $mgmL^{-1}$). The novel formulations encapsulating BNIPDaoct were capable of a 10-fold (PAA-Ox_5) and 13-fold (PAA-Ox_5-HNP) decrease in IC_{50} value compared with free drug respectively. This data therefore indicates that the incorporation of BNIPDaoct into PAA nano-aggregates can enhance its therapeutic effect. Drug loading studies showed that after only 4 h, 83-fold more drug had entered the cells in the PAA-Ox_5 compared with free drug. Additionally, a 104-fold drug concentration increase was present inside the BxPC-3 cells when incubated with the PAA-Ox_5-HNP formulation. This significant increase in cellular internalisation is probably the major contributing factor to the large decrease in cell viability and IC_{50} value after 24 h ($p<0.001$).

Conclusions: This study highlights the potential of PAA-Ox_5-HNP as a bi-functional imaging and drug delivery platform. Further work is on-going to further exploit these unique properties for controlled drug delivery.

19 DEVELOPMENT AND OPTIMISATION OF IPP-LOADED NANOPARTICLES INTO AN ORAL DRUG DELIVERY SYSTEM

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Background: Milk derived peptides have been shown to exhibit antihypertensive effects leading to a rowing commercial interest in their potential health benefits. However, the ability of bioactive peptides to exert a physiological effect in vivo over time is dependent on the bioavailability of the peptide at its target site. The tri-peptide Ile-Pro-Pro (IPP) could be taken orally to treat hypertension however their bioavailability is limited due to poor permeability across the intestine. Establishing an oral delivery system for IPP would be significant for these peptides and other naturally-occurring bioactive peptides. To address this, IPP was formulated into nanoparticles with the aim to enhance permeability and have a controlled release at the target site.

Methods: NPs were produced using an ionotropic gelation technique. Empty NPs were designed changing two factors; chitosan (CL113) and tripolyphosphate (TPP) using a central composite design. A Mixture Amount Design was used for IPP-loaded NPs using the same factors, CL113 (0.7mg/ml-1mg/ml) and TPP (0.2-0.15mg/ml) at a fixed IPP concentration (0.1mg/ml) with 5 experiments and two replicates. The encapsulation efficiency was determined using RP-HPLC. The NPs were characterised using Dynamic Light scattering, SEM, FTIR and zeta potential analysis.

Results: No apparent particle size differences were seen between the empty and IPPloaded NPs at the chosen concentration ranges, suggesting the presence of IPP in the core of the NP. Particle sizes were between 100-400nm with zeta potential values above 30mV and polydispersity index value (PDI) below 0.4. The association efficiency of IPPloaded NPs was around 40%. The statistical analysis allowed for an optimal formulation prediction, suggesting that the use of a range of 0.7mg/ml CL113 and 0.15mg/ml TPP for nanoparticles of around 300nm and Zeta potential above 30mV might be appropriate for oral delivery.

Conclusions: Ionotropic gelation of chitosan based NPs has the potential to produce particles of appropriate characteristics for oral peptide drug delivery.

20 SYNTHESIS CHARACTERIZATION AND APPLICATION OF NOVEL AMPHIPHILIC BLOCK COPOLYMERS

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Background: Poly(caprolactone) and poly(lactic-co-glycolic acid) are well established polymers in the field of drug delivery but their applicability can be hindered due to the non-renewable source and poor loading respectively. This highlights an urgent need for development of safe and cost effective polymers obtained from renewable monomers. In this study, we developed a range of novel polymeric materials from non-toxic, cheap, easily accessible renewable lactone monomer, with great potential for biomedical applications.

Methods: Ring opening polymerization of lactone was carried out using 1,5,7-triazabicyclo [4.4.0]dec-5-ene as the catalyst under mild conditions using poly(ethylene glycol) methyl ether and poly(ethylene glycol) as the initiators to make diblock (mPEGPVL) and triblock (PVLPEGPVL) copolymers respectively. The synthesized copolymers were characterized by spectroscopic methods (1H NMR and 13C NMR), Size exclusion chromatography (SEC) and Differential Scanning Calorimetry. Pyrene method was used to determine the critical micelles concentration (CMC). Curcumin and amphotericin B were used as model drugs to establish the encapsulation efficiency and release profile from Poly(lactone) micelles. Empty and drug loaded micelles were prepared by nano-precipitation method and characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM).

Results: The number average molecular weight found by SEC are 16249g/mol (PDI-1.15) for PVLPEGPVL and 19556g/mol (PDI-1.17) for mPEGPVL. CMC of mPEGPVL and PVLPEGPVL in water were 3µg/mL and 5µg/mL respectively. The Z-average diameter of empty micelles estimated by DLS was 40 nm with PdI 0.15 for mPEGPVL while PVLPEGPVL exhibited the larger size (32 and 220 nm) with PdI more than 0.20. Curcumin and amphotericin B were loaded successfully using mPEGPVL micelles with the drug content of 3.8wt% and 3.4wt% respectively. The in vitro release behavior exhibits a sustained release profile (55% of drug release in 60 h) with initial burst release.

Conclusion: The loading of amphotericin B into mPEGPVL is 8 fold higher while curcumin showed low loading compared to poly(caprolactone) micelles of same size. These results suggested that synthesized copolymer can be good carrier for highly hydrophobic bulky drugs.

Acknowledgments: Author would like to thank the government of India for financial support.

21 TARGETED GENE DELIVERY FOR PROSTATE CANCER USING A FOLATE TARGETED AMPHIPHILIC CYCLODEXTRIN MOLECULE TO DELIVER SIRNA

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Background: In recent years, modified cyclodextrins (CDs) have emerged as favourable gene delivery vectors by showing efficacy in delivering siRNA in a range of disease models including prostate cancer, Huntington's disease & IBD. In order to improve the performance of these vectors in vivo, it is important that the cationic charge of these molecules is masked and that they contain a specific targeting ligand to reduce side-effects and improve delivery. Our group has previously shown the advantages of using an anisamide-targeted CD delivery vector (CD.AA) versus an untargeted vector for prostate cancer treatment. Many groups have demonstrated the overexpression of the folate receptor in prostate cancer tissues and have designed targeted delivery vectors that exploit this characteristic.

Methods: In this study, DSPE-PEG5000-Folate (DPF) was synthesized from DSPE-PEG5000-amine (Nanocs) and folic acid (Sigma). CD.siRNA complexes were formed by the electrostatic interaction between the cationic CD and the anionic siRNA. DPF was post-inserted into the preformed CD.siRNA complexes at various molar ratios. The size and charge of the resulting NPs was analysed using the Zetasizer Nano Z (Malvern). Gel retardation analyses were used to determine the ability of the targeted nanoparticles to bind and subsequently release siRNA. The ability of these NPs to resist aggregation in a high salt medium (i.e. OptiMEM) was also tested using the Zetasizer Nano Z (Malvern) for up to 48 h. The NPs were incubated in 50 % foetal bovine serum (FBS) for up to 72 h to test their ability to protect siRNA from serum nucleases. For analysis, the samples were incubated with excess heparin for 1 h to displace the siRNA and were run on a 1 % agarose gel. The resulting nucleic acid bands were analysed using ImageJ software (NIH). The morphology of the targeted NPs was determined using TEM.

Results: The incorporation of DPF into preformed CD.siRNA complexes resulted in a significant reduction in zeta potential from 43.80 mV ± 0.61 mV in non-targeted CD.siRNA complexes to 0.98 mV ± 0.16 mV in the targeted particles. The targeted NPs had an average particle size of 260.27 nm ± 10.04 nm. The results of the agarose gels indicated the ability of the targeted NPs to both bind and release complexed siRNA. The incorporation of PEG5000 conferred the ability of the NPs to resist aggregation, with pegylated NPs having an average particle size of 147.03 nm ± 3.61 nm versus 2086.33 nm ± 82.04 nm for the non-pegylated NPs after 48 h. The serum stability studies highlighted the ability of the targeted CD molecules to protect siRNA from serum nucleases when compared with free siRNA. The TEM results indicate the formation of uniform complexes of approximately 300 nm.

Conclusions: The post insertion of DSPE-PEG5000-Folate into preformed CD.siRNA complexes resulted in the formation of nanoparticles that are close to neutral charge, of small particle size (<300 nm) and able to resist aggregation. The TEMs also indicate the formation of uniform particles. These results indicate the formulation of NPs with favourable physicochemical characteristics for gene delivery and forms the basis for further studies in prostate cancer cell lines.

22 VANCOMYCIN HYDROCHLORIDE RELEASE FROM IMPLANT COATINGS TO PREVENT ORTHOPAEDIC RELATED INFECTIONS

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Background: Orthopaedic implants (intra-medullary nails) that provide fixation following long bone fracture are, as any surgical procedure, subject to the risk of infection. Currently systemic, prophylactic antibiotic therapy is administered, but targeted local therapy would significantly improve the post-operative outcome for a number of patients. An implant surface which could control the infection risk associated with the wound site and biofilm formation would be ideal. Polymer/drug coatings have been investigated as an important strategy in this respect. In this study.Vancomycin hydrochloride, a glycopeptide antibiotic commonly used to treat susceptible strains of methicillin-resistant staphylococci (e.g. MRSA).was incorporated in poly(vinyl) alcohol (PVA) films. The impact of polymer concentration and film formulation through excipient addition (propylene glycol) on film properties and drug release was investigated.

Methods: Vancomycin HCl was combined with solutions of PVA and PVA/propylene glycol (PG), coated onto a titanium (Ti) coupon and allowed to dry at room temperature. Drug release studies were carried out in PBS in a shaking water bath at 37°C for up to two weeks, with samples analysed by HPLC. Water contact angle analysis of the surface and FTIR spectroscopy were carried out before drug release studies.

Results: Colourless, transparent films of 0.01-0.05 mm thickness formed on the Ti surface. Burst release of vancomycin HCl was observed within the first 2 hr for all films, with a higher initial release evident from films containing PG. Films containing vancomcyin HCl were more hydrophilic than those without drug; increasing drug content and addition of PG increased hydrophilicity.

Conclusions: Stable polymer/drug films can form on Ti coupons and controlling drug release for up to two weeks. Films are hydrophilic and provide a potential new anti-infective coating for orthopaedic implants.

23 AN INVESTIGATION OF THE IMPACT OF THERMAL ANALYSIS METHOD ON THE CONSTRUCTION OF DRUG-POLYMER THERMODYNAMIC PHASE DIAGRAMS

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Background: Binary thermodynamic phase diagrams for API/polymer systems provide an understanding of the relationship between formulation composition, temperature, and phase separation; providing a framework for the design of optimal amorphous solid dispersions. The most established approach to phase diagram construction is based on endset melt depression accompanying API dissolution in polymer. The objective of this work was to examine the implications of the thermal analysis method selected on endset temperature (T_{end}) determination, and the impact this has on phase diagram construction.

Methods: Thermodynamic phase diagrams for two API/polymer systems (naproxen and HPMC AS LF, and naproxen and Kollidon 17 PF) were constructed. Physical mixtures of API and polymer over a range of compositions were produced. Samples were analysed using two methods: a "dynamic" approach involving heating at a rate of 1 °C/min and an "isothermal" method where samples were held at a temperature above the polymer T_g for prolonged periods, prior to scanning at 10 °C/min.

Results: The T_{end} of naproxen when scanned at 10 °C/min was 160 °C. Endset depression occurred with increasing polymer volume fraction. The Flory-Huggins interaction parameter for naproxen and HPMC AS LF was + 0.72 and + 0.68 for the isothermal and dynamic approach respectively; and for naproxen and Kollidon 17 PF, - 1.6 and - 0.98 respectively. For the miscible system (naproxen and Kollidon 17 PF), a more negative interaction parameter was obtained when the isothermal approach was applied due to the greater time allowed for solubility equilibrium to be reached. In systems where limited miscibility occurs, the discrepancy in the interaction parameter determined may not be significant. However, when systems with greater miscibility are studied, the underestimation of miscibility and solubility of the API in the polymer may be substantial.

Conclusions: The thermal analysis method used to collate data has a deterministic effect on the thermodynamic phase diagram produced. This effect should be considered when designing thermal analysis methods for thermodynamic phase diagram construction.

Acknowledgement: This work was supported by an IRCSET / Eli Lilly S.A. Enterprise Partnership Scheme Postgraduate Research Scholarship, co-funded by the Irish Research Council for Science and Engineering Technology and Eli Lilly S.A.

24 EFFECT OF PHENYL CONTENT ON DRUG ENCAPSULATION EFFICIENCY OF NOVEL PHENYL SUCCINIC ACID-COPOLYMERIZED POLY(DECAMETHYLENE SUCCINATE) POLYMERS

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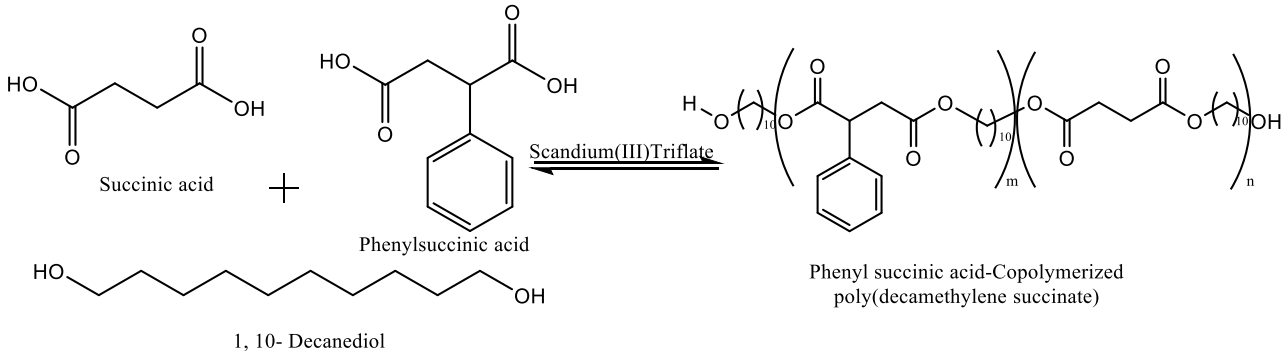

Background: The aim of the study was (1) to synthesize phenyl succinic acid-copolymerized poly(decamethylene succinate) polyester polymers with different phenyl contents in the main chain and (2) to observe the effect of phenyl content on drug encapsulation efficiency of these polymers.

Methods: The polymers were synthesized using polycondensation reaction by reacting 1,10-decanediol with different ratio of succinic acid and phenylsuccinic acid in vacuum to get polymers with different phenyl group contents. The synthesis was tried with two different catalyst i.e. phosphoric acid and scandium (III) triflate. Scandium (III) triflate was found to be a good catalyst while phosphoric acid failed to give the desired conversion. The polymers were characterized by H^1NMR and molecular weights were determined by gel permeation chromatography (GPC). Differential scanning calorimeter (DSC) data revealed the decrement in melting point by increment in phenyl content in the polyester which indicates the higher biodegradability due to decrement in the crystallinity.

Results: Curcumin was used as a model drug as it represents formulation problems while nanoprecipitation method has been utilized to make nanoparticles. The conditions for nanoprecipitation have been optimized. We have observed that polymer having 30% of phenyl content gave the optimum results, showing >80% of drug encapsulation which indicate that a certain amount of phenyl content is essential to enhance the encapsulation efficiency. To the best of our knowledge the effect of phenyl content on encapsulation efficiency has not been published so far but there are few reports available on how phenyl content affect the polymers properties.

Acknowledgement: The authors would like to thank the government of India for financial support.

25 A NOVEL LIPIDIC DISPERSION TO REDUCE THE FOOD EFFECT ON BIOAVAILABILITY OF FENOFIBRATE: IN VITRO, IN VIVO AND IN SILICO ASSESSMENTS

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Background: Altered bioavailability caused by differing prandial states poses difficulties in drug development and can affect drug efficacy and toxicity. Poorly soluble compounds are particularly susceptible to positive food effects. Formulation approaches which increase solubilisation within the GI tract may overcome dissolution limitations of these compounds. A novel lipidic dispersion (LD), combining solid dispersion and lipid formulation techniques, has been developed to reduce food effects on bioavailability. This work aims to assess the feasibility of formulating fenofibrate in this LD, by performing in vitro, in vivo and in silico assessments.

Methods: The LD was prepared by spray drying and characterised by XRD and DSC. Release studies under USP conditions compared the LD to the commercial preparation and crude fenofibrate. Fenofibrate solubility in biorelevant media, both with and without added formulation excipients was quantified. Biorelevant dissolution compared the LD and commercial product in fed and fasted states. In vivo bioavailability was compared in fasted pigs. In silico simulations were performed using Gastroplus™ software.

Results: LD production resulted in the loss of crystalline properties relative to the commercial preparation. Release under USP conditions was enhanced in LD compared to crude fenofibrate. Addition of formulation excipients to biorelevant media resulted in enhanced solubility. Biorelevant dissolution demonstrated similar profiles in both fasted and fed states for LD, whereas the commercial preparation displayed a positive food effect. In vivo bioavailability studies demonstrated a higher C_{max} and lower T_{max} for LD, while absolute bioavailabilities were similar. Simulated plasma profiles demonstrated an increase in AUC for the commercial preparation in the fed state, and predicted elimination of this effect with the LD.

Conclusions: Fenofibrate was successfully formulated in the LD, resulting in elimination of crystalline characteristics. In vitro and in silico predictions indicate that the LD is capable of eliminating the food effect.

26 MULTI-CENTRE CONSULTATION STUDY ON ACCEPTABILITY OF ORALLY DISINTEGRATING TABLETS (ODTS) IN PEDIATRIC AND YOUNG PEOPLE

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Background: There are a number of dosage forms available to administer medicines to patients. All the different formulations have advantages, disadvantages, and the reasons underlying their use. When a pharmacist, a clinician or any other health care professional needs to administer a drug to a patient, they must consider various conditions. One of these is the age of the patient and state of consciousness. Other reasons include time needed for the effect of the drug to be felt, the disease type and even the cost of the medication. There are two major routes of drug administration, including parenteral and oral route. Drug delivery route and choice of an appropriate dosage form are essential when administering drugs to children.

Methods: This study explores the acceptability of the various commonly prescribed dosage forms for children and young adults and the opinion of interviewees regarding their preferences for formulations. The questionnaire consisted of four sections. This study was carried out at three strategic locations around the world namely United Kingdom, Saudi Arabia and Jordan.

Results: Eighty four questionnaires out of one hundred were completed over three different countries. The gender distribution across the three regions was 55.95% male and 44.05% female ranging from 6-18 years of age. The study found that orally disntegrating tablets (ODTs) were the most preferred oral dosage forms (62%) followed by liquids (21%), tablets (12%) and capsules (5%). The preferred colours for these dosage forms were pink and white while the most preferred size was the small size of tablets (< 8mm) with a round shape. Majority of the participants (82.14%) preferred ODTs with a sweet taste as opposed to those with a bitter taste or no taste at all. With regards to flavour, strawberry was the most preferred (28.57%) while vanilla was the least preferred (4.76%).

Conclusions: The primary outcome from the study was that orally disintegrating tablets are a preferred dosage form among children. The benefits that accrue to this dosage form which include safety, higher compliance, dose accuracy and stability make it a good step in the quest to make better drugs.

27 NANOPARTICULATE DRUG DELIVERY SYSTEMS FOR THE LOCAL DELIVERY OF GROWTH FACTORS TO ENHANCE FRACTURE HEALING

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Background: Aquasomes are nanoparticulate carrier systems which comprise ceramic nanocores coated with a polyhydroxyl oligomer to which drug/antigen/peptide can be adsorbed. This delivery system confers lyoprotectant, thermal stabilizing and controlled release properties to the attached moiety. Growth factors are polypeptides that act locally as modulators of cellular activities. They have been exploited in orthopaedics to aid in bone fracture healing as well as increase bone mass density (BMD) in osteoporosis. The present study focuses on the fabrication of bone morphogenetic protein (BMP) - loaded aquasomes for controlled delivery to osteoblasts and pre-osteoblasts.

Methods: Hydroxyapatite cores were coated with trehalose. BMP was then adsorbed onto the surface of the coated cores and lyophilised to fabricate the desired aquasomes. In vitro drug release studies of BMP from BMP-loaded aquasomes to osteoblasts (SAOS-2) and pre-osteoblasts (MG-63) were performed. Alkaline phosphatase (ALP) was used as a marker of MG-63 cell differentiation in response to BMP exposure.

Results: In vitro release profiles of BMP from BMP-loaded aquasomes exhibited controlled release for 8 hr with 5.22 ng (14%) of BMP released. It was found that when BMP-loaded aquasomes were exposed to the MG-63 cells differentiation of the cells was observed as calculated by ALP production. As a control MG-63 cells were exposed to 10-50 ng/ml of BMP- spiked media. A reduction in the production of ALP was observed with cells exposed to concentrations of BMP-spiked media higher than 30 ng/ml. ALP staining of MG-63 cells also indicated the production of ALP from MG-63 cells exposed to BMP-loaded aquasomes.

Conclusions: BMP-loaded aquasomes can be potentially employed for the controlled release of growth factors for osteoinductive properties.

28 REVERSE PHASE HPLC ASSAY FOR THE DETECTION AND QUANTIFICATION OF GEFITINIB: METHOD DEVELOPMENT AND VALIDATION

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Background: Gefitinib is the first selective small molecule epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, which inhibits the growth, migration and invasive capacity of tumors by blocking the tyrosine phosphorylation of the EGFR. At present, gefitinib has only been approved for the treatment of patients with non–small cell lung cancer.

Methods: In order to achieve quantification of gefitinib a simple, specific, accurate and precise reverse phase high performance liquid chromatography method was developed and validated. This method meets the pharmaceutical industry guidelines in accordance with FDA[2]. Two mobile phases were used, acetonitrile (HPLC-Grade) containing (0.05%) TFA as Phase A, and water (HPLC-Grade) containing (0.05%) TFA as Phase B. Sphereclone (ODS-R), 5 μm (100 X 4.6 mm) column (90%Phase A&10%Phase B) at a flow rate of 2 ml/min. The developed method was isocratic and the eluent was monitored by UV detection at 344 nm.

Results: Gefitinib discrete peaks were well resolved to baseline, allowing accurate and consistent quantification of the compound. The typical retention time was 2.4±0.02 min (n=3). The lower limit of detection (LLOD) was 0.25 ppm, and the lower limit of quantification (LLOQ) was 0.50 ppm. The response was linear between 0.25 ppm and 25ppm. The intraday repeatability and the interday precision of the developed method were demonstrated over three consecutive days. Results indicated that the method was reproducible with precision values between 97.9 and 104.1%. Freeze and thaw stability studies were performed to monitor the degradation of gefitinib. The results obtained show good drug recovery 97.1-103.3% (n=3) with no significant degradation of gefitinib.

Conclusions: The developed and validated HPLC method was simple, accurate reproducible and enabled accurate quantification of gefitinib. The developed method will be used for the quantification of gefitinib in permeation studies through the human colorectal cancer cell line CaCo-2.

29 FORMULATION AND CHARACTERISATION OF GEFITINIB SOLID DISPERSION

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Background: Gefitinib is the first selective epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor that blocks signal pathways implicated in solid tumour growth and metastasis. As gefitinib is a very slightly soluble drug, intended to treat the colorectal cancer by oral drug delivery by formulation as solid dispersions to improve the efficacy and to reduce the adverse effects of gefitinib. Solid dispersions are a promising strategy for improving the dissolution and oral bioavailability of poorly water-soluble drugs like gefitinib. Reduction of particle size and the availability of drug in the amorphous form can help to improve the dissolution of a poorly water-soluble drug and hence, the bioavailability may significantly increase.

Methods: Solid dispersions of gefitinib were prepared using polyvinylpyrrolidone (PVP) for enhancing the solubility of the drug, and Eudragit S 100 used for colon targeting by solvent evaporation method in 1:9 weight ratio using spray dryer. In vitro dissolution studies were performed at different PH to observe the drug release from the dispersion. Differential Scanning Calorimetry (DSC), X Ray Diffraction (XRD) used to investigate the crystalline structure changes of gefitinib inside the dispersion and molecular interaction between the drug and polymer.

Results: DSC and XRPD confirmed the formation of amorphous dispersions. A lack of melting peak indicating the formulation of amorphous dispersion. Dissolution studies revealed a clear improvement in drug release for the solid dispersion. After 12hrs (99.7±7.8%) drug release was observed from gefitinib solid dispersions at pH7.2 and (21.1±5.7%) at pH 1.2 and (3.6±0.6%) pH6.5.Therefore, the formation of solid dispersions can increase the dissolution rate of gefitinib.

Conclusions: DSC and XRPD studies have confirmed the drug is dispersed in side the carries to form an amorphous form. In vitro dissolution studies of ternary dispersion, the drug release profile has shown a clear increased at pH7.2. Presence of gefitinib in amorphous form and reduced particle size with formulation of regular spherical size has the effect to increase the rate of drug release.

30 FORMULATION AND DEVELOPMENT OF A NOVEL TRANSDERMAL SYSTEM FOR THE LOCAL DELIVERY OF THE ANTI-CANCER AGENT MTL-004

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Background: Skin cancer is emerging as an increasing public health problem especially in developed countries. The large number of cases diagnosed present as a substantial burden to healthcare services. The majority of skin cancers are treatable, however, poor treatment or particularly malignant forms results in 2,500 deaths annually in the UK. The most common treatment to remove the tumour is surgical excision. This however may not be suitable for all patients thus development of alternative treatments is necessary. MTL-004 is a potent cytotoxic agent which can be used in the treatment of non-melanoma skin cancer. Advantages of MTL-004 include the lack of systemic toxicity and lack of toxicity to normal cells due to it only acting on rapidly dividing cells. This study will propose to apply MTL-004 as a gel directly onto skin cancers and pre-cancerous lesions to deliver MTL-004 directly to the tumour.

Methods: Various silicone gels formulations with drug loadings ranging from 1 to 10% w/w MTL-004 were manufactured. Release studies were carried out into phosphate buffered saline (PBS) pH 7.4. At various time points over 8 hr samples were taken and replaced with fresh PBS. The MTL-004 samples were quantified using HPLC with UV analysis.

Results: Release studies found that MTL-004 was steadily released from the silicone gel over the 8 hr. As the amount of MTL-004 increased the cumulative amount of drug released also increased.

Conclusions: MTL-004 is steadily released from the silicone gel formulations indicating the gel is a suitable medium for developing a transdermal drug delivery system for this drug. Release studies over extended periods of time will be carried out to investigate time required for complete drug release from the gels.

31 EXTENDING THE HUMAN EX VIVO SKIN MODEL FOR ASSAYING LANGERHANS CELL AND DERMAL DENDRITIC CELL RESPONSE TO ANTIGEN DELIVERY

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Background: The ex vivo human skin model is a well-established method of assessing the behaviours of epidermal and dermal immunological cells and has been used to study the response to intradermal vaccine delivery and the invasion of pathogens. Previous studies have shown that epidermal-resident Langerhans cells (LCs) can uptake antigen, become activated, migrate through the dermis towards the draining lymph nodes and subsequently activate systemic immunity. Whilst studying the in situ behaviour of these cells is useful, we aim to extend this system by collecting LCs and the numerous subsets of dermal dendritic cells (dDCs) for characterisation and utilisation outside the limitations of full-thickness skin tissue. An initial objective was to extract cells, phenotype them and test their functionality via infection with a GFP-encoding HIV lentiviral vector, both in their natural state and following inhibition of the antiviral factor SAM domain and HD domain-containing protein 1 (SAMHD-1), which is common to skin-resident LCs and dDCs.

Methods: Human skin samples, obtained with ethical approval and informed consent, were incubated in an enzyme cocktail to facilitate mechanical separation of the epidermis from the dermis. Dermis and epidermis were then incubated separately for 48 hr, after which migratory cells were collected from the media. Migratory cells were then incubated in the presence of the vesicular stomatitis virus (VSV-G) pseudotyped HIV lentiviral vector encoding GFP, with or without pre-treatment with Vpx-encoding simian immunodeficiency virus (SIV3) to inhibit SAMHD-1. Untreated cells were used as a control. Following 5 days incubation, cells were collected, fixed and analysed by flow cytometry for GFP expression within the immunological cell populations.

Results: Numbers of migratory ('walkout') primary dDCs varied between donors (n=4) from 24-39% of the total cells collected. The majority were HLA-DR+/CD45+ and showed the previously described DC subsets based on CD11c and CD141 expression. CD141+ dDCs also expressed CD14. LCs represented 4-16% of the total cells collected from the epidermis of the skin donors tested (n=4). LCs showed expression of CD1a, HLA-DR and Langerin (CD207). Cell viability was typically over 80% following a 48-hr walkout period. Inhibiting SAMHD-1 with Vpx led to a statistically significant increase in infection in both dermal and epidermal cells (Fig 2 $p \leq 0.05$ and $p \leq 0.001$ respectively using Student's t-test). The mean increase was significantly greater in DCs than in LCs (11.1 and 2.5-fold increases respectively; $p \leq 0.05$ using Student's t-test).

Conclusions: Suitable numbers of functional immunological cells could be obtained from ex vivo skin samples. The collected cells were phenotypically distinguishable as epidermal LCs and dermal DCs. The ability of cells to express GFP showed that as well as being viable, they remained functional following walkout from the tissue. The results also agreed with previous work, indicating that LCs possess a SAMHD-1-independent antiviral factor. This ex vivo skin cell model represents a potentially useful tool in the study of skin immunology. Cells can be isolated at a greater purity than resident in the skin and could therefore be utilised in studies with intradermal vaccine candidates to allow uptake, processing and presentation to be studied in a simpler model system. The model might also be useful for to assess the effects of any topically applied compounds on the immunological cells of the skin, given the wealth of data that can be obtained via flow cytometric analysis.

32 CHARACTERISATION OF A MODIFIED DI-LYSINE CYCLODEXTRIN FOR THE DELIVERY OF SIRNA TO THE CENTRAL NERVOUS SYSTEM

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Background: RNA interference (RNAi) holds great potential to treat neurodegenerative diseases presently classified as incurable. The presence of the blood-brain barrier (BBB) prevents short interfering RNA (siRNA) from gaining entry to the central nervous system. However, the potential exists to overcome the BBB through the use of targeted nano-carriers. Cyclodextrins (CDs) are starch molecules with low toxicity that can be chemically modified to effectively complex siRNA and, protect from degradation. The aims of this study are; to characterize complexes formed between siRNA and dilysine cyclodextrin (Lys2CD) co-formulated with adamantine-PEG500 (AD-PEG500) and adamantine-PEG500-RVG (AD-PEG500-RVG), and to investigate their uptake and toxicity of the resulting complexes in an endothelial cell model.

Methods: Lys2CD and siRNA were formulated at different mass ratios (MR) (CD:siRNA) with different molar ratios of AD-PEG500 and AD-PEG500-RVG. The nanoparticles were characterized for size, polydispersity surface charge and aggregation by using a Malvern Zetasizer Nano ZS. Gel retardation assay was performed to determine the ability of nanoparticles to bind and release intact siRNA. In vitro, uptake and cytotoxicity studies using various formulations of Lys2CD.siRNA were performed on b.End3, murine brain microvascular endothelial cells.

Results: The Lys2CD.siRNA nanoparticles prepared had size a of <200 nm with a positive charge of 10-25 mV. The incorporation of AD-PEG500 and AD-PEG500RVG into Lys2CD.siRNA nanoparticles showed a non-significant increase in size and a decrease in surface charge with enhanced stability against aggregation. A molar ratio of 1:0.5:0.5 for Lys2CD:AD-PEG500:AD-PEG500-RVG was identified as the optimal formulation with highest uptake and minimal toxicity, in vitro.

Conclusions: These results indicate that RVG targeted cyclodextrin.siRNA nanoparticles can be formulated and are worthy of further investigation as potential delivery systems for permeability across the BBB.

33 QUALITY BY DESIGN (QBD): INVESTIGATING CRITICAL PROCESS PARAMETERS TO PRODUCE FUNCTIONALISED PARTICLES USING DRY POWDER COATING

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Background: QbD is a systematic methodology in product development that starts with defined objectives and focuses on product and process clarification as well as control. Production of functionalised particles (FP) using dry powder coating as a one-step environmentally friendly process is employed to develop particles with targeted properties and favorable functionalities. The method is based on homogeneous dispersion of fine (guest) particles over the surface of larger (carrier) particles upon the application of high shear and impaction forces. QbD was adapted in this study to optimise the formation of FP with extended release profile using dry powder coating.

Methods: Initially, the quality target blend profile was determined based on the desired properties. Risk assessment was employed to identify high-risk critical process parameters (CPPs). Similarly, critical quality attributes (CQAs) of the final blend were studied to determine the impact of process parameters on the powder blend. DOE (design of experiment) with D-optimal design was applied to investigate the impact of the CPPs (speed, processing time, air flow and batch size) on the CQAs (content uniformity, dissolution profile, particle size analysis (PSA) and the FTIR spectrum intensity at wavenumber 1708cm-1 for the model guest (ibuprofen) that is attributed to C=O bond.

Results: The results showed that the associative interaction between speed and batch size was the most significant process parameter on the formation of FP. When the speed of processing vessel was increased, high shear force is generated into the system that increases the adhesion of the fine guest particles to the surface of the insoluble carrier and hence reduces the dissolution rate (<80% released at 60 min of FP compared with 100% release at 15 min of physical mix). Additionally, it resulted in reduction in the amount of fines (an increase in particle size at the X10 region in PSA) and reduction in the intensity of FTIR spectra at 1708 cm-1 indicating the formation of hydrogen bond between guest (ibuprofen) and carrier microcrystalline cellulose. The increase in batch size showed an antagonistic effect on dissolution with a dissolution rate reaching 100% within 60 min resembling the physical mix. Optimum regions for FP production were capable of predicting powder homogeneity within the targeted range of RSD <5%. The revised model demonstrated reliable predictive capability for dissolution response and moderate for the others. The model demonstrated good validity and reproducibility for all CQA.

Conclusions: The QbD with DOE provides an expedient and cost-effective tool in optimising the dry powder coating process parameters. DOE results enabled the identification of design space for optimal CQAs.

34 SPATIAL AND TEMPORAL CONTROL OF PROTEIN RELEASE FROM MICROSPHERES-HYDROGEL COMPOSITE SCAFFOLDS

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Background: The diffusion of growth factors and signalling molecules is important for many vital processes such as embryogenesis. The ability to control the spatiotemporal profile of growth factors within tissue engineered scaffolds is important for understanding and inducing desirable differentiation of stem cells.

Methods: We have studied the effect of gel matrix on the diffusion of fluorescein isothiocyanate-labelled bovine serum albumin as model protein using a compartmentalized diffusion model. We measured the gradient profile at 20 μm resolution using Leica CM1100 cryostat and Infinite® 200 micro-plate reader. Further control over gradient profiles was achieved via spatial localization of FITC-BSA loaded Poly (DL-lactide-co-glycolide) microspheres as depot within the hydrogel matrix.

Results: The composition and the nature of gel matrix showed strong impact on the microscopic structure of the gel matrix and hence the diffusion characteristics of FITC-BSA. Upon replacement of the sink compartment with spatially localized protein depots, concentration gradients were successfully established and maintained over time. Moreover, altering the hydrophobic nature of PLGA by addition of 10% w/w PLGA-PEG-PLGA triblock copolymer to the composition of microspheres reflected the influence of depot release characteristics on the diffusion profile.

Conclusions: The model employed could be used to generate gradients of multiple growth factors in a stem cell-laden matrix.

35 NANO CERAMIC DPI FORMULATION FOR THE DELIVERY OF PROTEIN AND PEPTIDES

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Background: Aquasomes are one of the recent delivery systems for protein/peptide-based pharmaceuticals. Aquasomes are self-assembled three-layered carrier systems, which consist of three layers: an inner solid core, a middle polyhydroxy carbohydrate coating and an outer drug layer.

Methods: 100 mg of hydroxyapatite was added to 10 mL of 0.15 M solution of trehalose under stirring at 4^{o}C for 1.5 hr. The sample was then centrifuged, washed and freeze-dried. 15 mL of BSA (1 mg/mL) was added to the freeze-dried sample under stirring for 1.5 hr at 4^{o}C. The sample was then centrifuged, washed and freeze-dried. The aerosolisation properties of BSA loaded aquasomes were investigated using a NGI. A quantity of 20 mg in a size 3 capsule was introduced to the 1-7 stages of the impactor (flow rate 60 L/min). The samples were redistributed in 10 mL of simulated lung fluid and placed in a shaking water bath at 37^{o}C and 100 rpm. A quantity of 0.3 mL was taken for analysis at hourly time points up to 6 hr.

Results: The aquasomes had an average size of 1.5 ± 0.96 µm. Zeta potential values were calculated after the coating (trehalose, -11.6 ± 1) and loading (BSA, -1 ± 0.5) stages to ensure the aquasomes consisted of the three layers. The deposition of BSA loaded DPI aquasomes at each stage of the NGI at the time of manufacturing and after 6 months at 25^{0}C/60% RH (n = 3). Approximately 70% of the delivered dose has a cut-off diameter of 2.82 µm. The BSA release is controlled over the 6 hr time period.

Conclusions: Aquasomes with an aerodynamic diameter of 0.94 µm released 430 µg of BSA. This is very encouraging for potential protein/peptide delivery using aquasomes via the pulmonary route. Cell culture studies will be performed to complement the study using Clau-3 cell lines.

36 SOLUBILITY ENHANCEMENT OF A POORLY WATER-SOLUBLE DRUG BY ADSORPTION ONTO MESOPOROUS SILICA USING SUPERCRITICAL CARBON DIOXIDE

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Introduction: Solid dispersions are one of the most successful strategies employed to improve the drug release/dissolution performance of poorly soluble drugs whilst stabilizing amorphous compounds. In this work, solid dispersions are formed using supercritical carbon dioxide. Attempts to increase the dissolution rate of poorly water-soluble drugs have been made in several studies by approaches such as decreasing the particle size (e.g. by milling), precipitation of micro- or nanoparticles and producing micronized particles using supercritical CO_2 ($scCO_2$). Supercritical carbon dioxide is an important commercial and pharmaceutical industrial solvent because of its high dissolution capability, low toxicity, low environmental impact and its mild critical conditions (7.38 MPa and 31.0 °C). This research aimed to characterise the solid-state properties, physical stability and solubility/dissolution properties of amorphous solid dispersions containing porous high surface area silica produced via $scCO_2$.

Methods: Indomethacin (IND) was used as a model drug with two grades of high surface area silica (average particle sizes 3µm and 7µm)**.** The thermal stability of the materials was analysed using a TA Instrument *Q*500 TGA (Leatherhead, U.K.). Material crystallinity was evaluated using powder X-ray diffraction (PXRD, Rigaku™, miniflex II, Japan) and high-speed differential scanning calorimetry (Hyper-DSC 8000, Perkin Elmer, USA). Materials were placed in a high-pressure vessel consisting of a CO_2 cylinder, a Thar™ Technologies P50 high-pressure pump and a 750 ml high-pressure vessel (Thar, USA). Physical mixtures were exposed to CO_2 at different pressure and temperature values above its critical conditions, different parameters were investigated (loading ratios of drug, experiment time, pressure and temperature inside the vessel), and Hydrophilicity was determined by contact angle measurements using FTÅ 200 (Virginia, USA). Drug release was examined using USP type II dissolution tester (Caleva™, UK).

Results: Raw materials had acceptable thermal stability. The two grades of silica were found to be amorphous using PXRD and Hyper-DSC. Contact angle measurements showed a significant increase in the hydrophilicity of the produced formulations. Using PXRD, formulations containing approximately 20 % drug load were found to be completely amorphous. The drug release properties were significantly influenced by silica type, chamber temperature and pressure and experiment duration. The amount of drug release was higher than that of the pure drug alone under non-sink conditions at pH 1.2.

Conclusion: The incorporation of high surface area silica with $scCO_2$ technology demonstrated that a significant enhancement in drug release of poorly water-soluble drugs could be achieved. The technique was affected by many parameters such as: drug load, temperature, and pressure and experiment time. The previous parameters affect the final formulation morphology and the percentage of drug load. This project has shown that silica carrier platform may be used as an alternative approach to generating solid dispersions of amorphous drugs.

37 P-GLYCOPROTEIN AND MRP1 ARE FUNCTIONALLY EXPRESSED IN NCI-H441 CELL LINE

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Background: The respiratory tract offers great potential for effective drug delivery, however, due to its complex nature, the fate of the drug after reaching the pulmonary mucosa is poorly understood. The human bronchiolar epithelial cell line, NCI-H441 was recently suggested as an *in vitro* model of the distal lung air-blood barrier. This study aims to determine whether similar levels of expression and function of the ATP-powered efflux pumps, P-glycoprotein (P-gp) and multidrug resistance-related protein-1 (MRP1) in NCI-H441 are observed as in freshly isolated human distal lung epithelial cells in primary culture.

Methods: P-gp and MRP1 expression level was determined by immunoblot using mouse monoclonal antibodies against the respective antigen. Confocal laser scanning microscopy (CLSM) was used to investigate transporter localisation. Uptake and release experiments with rhodamin 123 (Rh123, P-gp substrate) and 5,6-carboxyfluorescein-diacetate (CFDA, MRP1 substrate) were carried out to assess transporter function *in vitro*. Furthermore, the pharmacological effects of verapamil (50 µM broad-spectrum inhibitor), LY-335979 (0.5 µM, specific P-gp inhibitor) and MK-571 (20 µM, specific MRP1 inhibitor) on Rh123 and carboxyfluorescein (CF) release were investigated. Apical-to-basolateral and basolateral-to-apical transport studies with Rh123 and CFDA were carried out across NCI-H441 monolayers grown on Transwell filters to confluence for 10-12 days.

Results: Expression of both efflux pumps was observed by means of Western blot in NCI-H441 cell monolayers. CLSM data confirmed these findings, and, additionally, revealed localisation of the transporters along apical (P-gp) and basal (MRP1) membranes and in perimembranous vesicles. Rh123 and CF were both released from NCI-H441 monolayers in a time-dependent manner. This release was significantly inhibited by the relevant pharmacological agents. In bi-directional transport studies, a net-secretive flux of Rh123 and CF was observed, further supporting functional evidence from release experiments.

Conclusions: P-gp and MRP1 expression and activity in NCI-H441 cell monolayers were found to be comparable with data previously reported from human alveolar epithelial cells in primary culture. Hence, the NCI-H441 cell line can be suggested as an *in vitro* model to investigate efflux pump effects at the air-blood barrier.

38 EVALUATION OF SMALL SCALE PRODUCTION METHODS TO ASSESS DRUG-POLYMER MISCIBILITY IN AMORPHOUS SOLID DISPERSIONS

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Background: The increased solubility of amorphous APIs relative to their crystalline counterparts has been exploited in the formulation of poorly aqueous soluble compounds as amorphous solid dispersions (ASDs). The higher apparent solubility of the amorphous form is attributed to the relatively higher thermodynamic state in comparison to the crystalline but also causes the amorphous form to be unstable. Solid dispersions thermodynamically and kinetically stabilise the drug in the amorphous form however for an ASD to be stable the drug and polymer should be miscible as a single amorphous phase is preferred. Small scale differential scanning calorimetry (DSC) studies are often used to assess drug-polymer miscibility and provide measurements which can be used in further thermodynamic calculations. However the inherent viscosity of polymers and reliance on diffusive mixing without the action of external forces make the conditions in a DSC pan very different to industrial scale production methods. In this study we used modulated DSC (MDSC) and Raman spectroscopy to compare ASDs produced in a DSC (melt quench (MQ) method) to those produced using solvent evaporation (SE), hot melt extrusion (HME) and supercritical fluid impregnation (SFI).

Methods: Indomethacin (INM) was chosen as a model poorly-soluble and PVP K15 and K90 and Soluplus were used as solubilising excipients. Various compositions of drug and polymer were ballmilled then subjected to two heat-hold-cool DSC cycles with two different hold times. The glass transition (T_g) region was investigated for the presence of one or two transitions as well as the value and width (temperature range over which the transition occurs) of the transition(s). Methanol was the solvent used for SE. HME was carried out using a Thermo Minilab co-rotating twin-screw extruder at 130-150ºC. SFI was carried out at temperatures of 60-90ºC and a pressure of 150 bar for 2 hr.

Results: INM-K15 showed a single T_g across all preparation methods, the value of which corresponded to that predicted by the Gordon Taylor (GT) equation for composition dependence of T_g and was independent of production method. QM T_gs measured for INM-SOL and INM-K90 systems were statistically different to T_gs produced by the other methods. The $\Delta Cp/\Delta T$ signal of MDSC showed a good sensitivity for amorphous phases by displaying either a shoulder or two resolved peaks in mixtures containing two phases, corresponding to two glass transitions which were confirmed by Raman spectroscopy. Separate phases were found in both K90 and SOL QM ASDs.

Conclusions: INM and PVPK15 can be successfully mixed in a DSC to create a homogenous mixture however the higher molecular weight, T_g and viscosity of PVPK90 meant that separate drug- and polymer- rich phases remained after the DSC routine. Separate phases were found in INM-SOL QM ASDs using Raman spectroscopy which were presented in MDSC traces as a single Tg, however the transition which took place over a much wider temperature range. Therefore both glass transition value and width are important for the characterisation of amorphous solid dispersions.

39 ENHANCEMENT OF THE RHEOLOGICAL PROPERTIES OF A STIMULI-RESPONSIVE DEPOT DRUG DELIVERY SYSTEM

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Background: Stimuli-responsive polymers are used extensively in drug delivery systems. Smart polymeric materials respond sharply to small chemical or physical changes in surrounding conditions, such as temperature and pH. These systems are advantageous in drug delivery as they can provide controlled release of therapeutic agents at localised sites. The main responsive polymers of interest are poloxamers (Pluronics®) and poloxamines (Tetronics®). Pluronics® are the linear temperature responsive polymers and Tetronics® are the bifunctional, branched counterpart, providing both temperature and pH responsiveness.

Methods: Aqueous 20% w/w Pluronic® F127 formulations were prepared via the cold method and allowed to equilibrate for a minimum of 24 hr before analysis. Three different types of Tetronics® (901, 904, 908) were added to the 20% w/w Pluronic® F127 formulations at differing concentrations (10%, 15%, 20% w/w). Using an AR1500 EX rheometer (T.A. Instruments), flow rheograms were obtained to determine the flow properties and oscillation completed to determine the storage modulus (G'), loss modulus (G") and the sol-gel temperature of the formulations. Flow and oscillatory rheology were conducted at both 20^{o}C and 37^{o}C, with triplicates completed for all experiments.

Results: The flow rheograms obtained indicated that all systems are shear thinning or Newtonian at 20^{o}C and 37^{o}C. The power law mathematical model was employed to determine viscosity of the formulations. From the data obtained, it is evident that an increase in temperature from 20^{o}C to 37^{o}C results in an overall increase in viscosity of all formulations. The rheological results of Pluronic®-Tetronic® formulations demonstrated an increase in viscosity with the addition of all three types of Tetronics® in comparison to the control formulation containing no Tetronic® (20%w/w Pluronic® F127). The effect of increasing the concentration of each Tetronic® showed an increase in viscosity up to 15% w/w, where the viscosity decreased when 20%w/w Tetronic® was added. Oscillation results for aqueous Pluronic®-Tetronic® formulations indicated the systems are more solid-like at 37^{o}C according to the storage and loss modulus and are generally frequency independent. The addition of Tetronic® to the control formulation enhances the storage modulus at 37^{o}C. Oscillation data was supported by the temperature ramp completed to determine the sol-gel temperature (~27^{o}C). The addition of Tetronic® 901, hindered the temperature-responsiveness of the formulations, the increasing concentration of 904 shifted the sol-gel temperature closer to body temperature and the addition of increasing concentration of 908 enhanced the response, with the sol-gel temperature remaining ~27^{o}C.

Conclusions: These formulations illustrate promising rheological properties for the production of a stimuli-responsive injectable depot system. It was evident from the rheological data obtained that the combination of a temperature responsive poloxamer (Pluronic® F127) and dual-responsive (temperature and pH) poloxamines (Tetronic® 901, 904 and 908) enhance the rheological structuring of the formulation resulting in an injectable depot system that will promote localised controlled release. Further work is required and will be completed to understand the intermicelle interactions and how this will affect drug release.

40 DEVELOPMENT OF A NOVEL STRATEGY TO MINIMIZE BIOMATERIAL RELATED INFECTION USING SPRAY COATINGS OF CHLORHEXIDINE BASE AND COUNTER IONS

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Background: Nosocomial infections not only impact patient morbidity and mortality but also place a great burden on healthcare finance. A major contributor to the risk of acquiring a nosocomial infection involves the use of biomedical devices. Biomaterial-centered infections vary depending on the biomedical device employed; for example, urinary catheters have an infection risk approaching 90-100% after three weeks of implantation. Furthermore, the development of biofilm microenvironments represents an additional barrier that must be tackled in order to fully treat the patient. As such, novel strategies are necessary in order to ameliorate infection risk thereby minimising patient harm.

Methods: Fatty acids (FAs) were investigated as potential candidates for drug delivery systems for chlorhexidine (CHX) that could be used in the spray coating of biomaterials; capric acid (CA), myristic acid (MA) and stearic acid (SA). Ratios of CHX:FA (90:10, 85:15, 80:20 and 75:25) were milled together for 2 min at 20 Hz to ensure homogenous mixing. 10mg samples of each ratio were accurately weighed out into DSC pans, crimped and placed in a TA Q100 DSC. The program consisted of a heating rate of 10^{o}C/min from 20-160^{o}C. The viscosity of each FA at elevated temperatures was investigated using an AR1500 ex TA rheometer. A steady state flow procedure was employed measuring the viscosity against shear rate over time for each FA at 70^{o}C, 75^{o}C, 80^{o}C, 85^{o}C and 90^{o}C.

Results: Each DSC thermogram exhibited three distinct melting endotherms; a melt for the FA, a melt for CHX and a melt of the CHX-FA mixture at around 80^{o}C. The heat of fusion (ΔH, J/g) was then calculated for each of the CHX melting endotherms and plotted against CHX base loading (%). There appears to be a correlation between FA carbon chain length and CHX solubility; CHX solubility is ranked as follows: $CA_{10C} > MA_{14C} > SA_{18C}$. The mean viscosity ($\eta$) for each FA at each temperature was then plotted against temperature (^{o}C). The temperature region whereby viscosities of all FAs were similar was determined to be between 80-90^{o}C.

Conclusions: Viscosity at elevated temperatures has no impact on the dissolution of chlorhexidine in each of the investigated counter ions.

41 DRUG-POLYMER PHASE DIAGRAMS AND THEIR APPLICATION TO HOT MELT EXTRUSION

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Background: Amorphous drug-polymer solid dispersions have been found to enhance the bioavailability of various poorly water-soluble drugs. The rationale behind the selection of suitable polymeric excipients for particular APIs remains unclear. In a recent study, the application of the Flory-Huggins (FH) theory to construct thermodynamic drug-polymer phase diagrams has been performed. Calculated limits of drug-polymer miscibility in these plots can be utilised to screen excipients. The positions of the binodal and spinodal curves can be used to probe the thermodynamic miscibility of particular drug-polymer systems. The aim of this study was to select various hot melt extrusion (HME) conditions based on these phase diagrams and characterize the extrudate produced.

Methods: Felodipine (FD) was the model drug used in this research along with the amorphous polymers Polyvinylpyrrolidone (PVPK15) and polyvinylpyrrolidone/vinyl acetate (PVP/VA64). Drug-polymer phase diagrams were produced using melting point depression data and application of the FH theory. Various drug loadings and processing temperatures were selected based on the positions of the liquid-solid transition line and the binodal and spinodal curves. Extrudate characterization was performed using High speed Differential Scanning Calorimetry (HDSC), Powder X-Ray Diffraction (PXRD) and Raman Microscopy. Solubility studies for each drug-polymer system have also been completed.

Results: Extrudates produced using the temperatures identified by the calculated liquid-solid transition curves were found to be completely amorphous for each drug-polymer system. The drug release profiles demonstrated a greater enhancement in FD solubility in FD-PVP/VA64 formulations when compared to FD-PVPK15 amorphous solid dispersions.

Conclusions: This study has demonstrated how drug-polymer phase diagrams, produced using the FH theory can be applied to a processing technique, such as HME.

42 CONTROLLED DELIVERY TO THE POSTERIOR SEGMENT OF THE EYE USING SOLVENT-INDUCED IN SITU FORMING GELS

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Background: Drug delivery to the posterior segment is extremely difficult. Current methods have downfalls especially recurrent intravitreal injections. A resolution may lie with solvent-induced in situ forming gels (IFPG) that are injected as low viscosity solutions, which solidify in an aqueous environment such as the vitreous.

Methods: Formulations composed of PLGA and organic solvent (NMP/ novel solvent) were investigated for implant forming ability, in vitro release, rheology and syringeability. Phase-inversion behaviour was examined in porcine vitreous and rabbit eyeballs using optical coherence tomography (OCT). Fluroscein sodium (FS), triamcinolone acetonide (TA) and BSA *in vitro* release was investigated in PBS. Effect of hydrophilic polymers on the release of FS, TA and BSA from PLGA/NMP implants was examined. HPLC with UV was used to quantify release of FS and TA with a microassay used for BSA. Syringeability of the gels was determined through 27G needles by using a Texture Analyzer with rheological behaviour studied by a Rheometer. Implant morphology was examined through SEM.

Results: OCT imaging showed IFPG form spherical implants upon injection. In PBS, PLGA/NMP implants released FS, TA and BSA for around 50 days. Addition of hydrophilic polymers showed varied results with a 1.5% HEC (Mw 1.3 x 10^6) showing an 18 % increase in burst release, whereas 0.5% HPMC (Mw 90,000) resulted in a 20% reduction in NaFl burst. HEC and HPMC addition resulted in a greater burst of TA and BSA. Syringeability studies indicated that the force required to expel 0.1ml of formulation through 27G needles was between 16.795 and 79.402 N.mm, which correlates with rheological data. IFPG made with the novel solvent showed a biphasic release profile for TA, initial burst followed by zero order release for over 60 days.

Conclusions: The IFPG systems show the ability to release drugs for a prolonged period and may be key in improving delivery to the eye.

43 A LAMINATED POLYMER FILM FORMULATION FOR ENTERIC DELIVERY OF LIVE VACCINE AND PROBIOTIC BACTERIA

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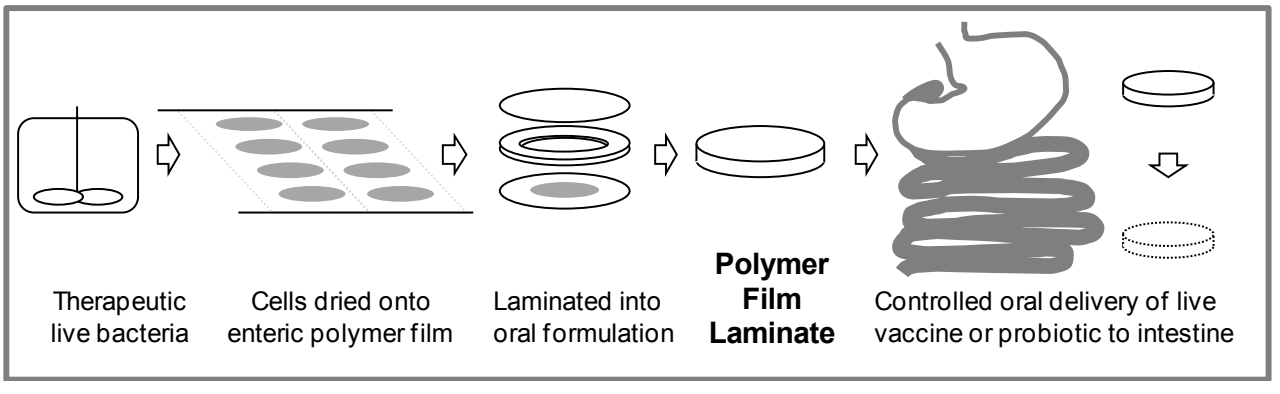
Background: Live bacterial cells (LBC) are administered orally as attenuated vaccines, for the *in situ* production of biopharmaceutical agents, and as probiotics to improve gastrointestinal health. However, LBC represent a complex biological agent presenting many unique formulation challenges, and after oral administration LBC must survive gastrointestinal antimicrobial defenses such as gastric acid. We present a new and simple oral delivery formulation concept, termed Polymer Film Laminate (PFL).

Methods: LBC are ambient dried onto cast acid-resistant enteric polymer films that are then laminated together to produce a solid oral dosage form with dimensions similar to a tablet. Two examples of therapeutic LBC – an attenuated vaccine (*Salmonella* Typhimurium SL3261) and a probiotic (*Bifidobacterium breve* NCIMB 8807) – were dried directly onto a cast film of enteric polymer. Acid resistance, cell controlled release and viability of PFL formulations were investigated during *in vitro* gastro intestinal conditions.



Results: The effectiveness at protecting dried cells in a simulated gastric fluid (pH 2.0) depended on the composition of enteric polymer film used, with a blend of ethylcellulose plus Eudragit L100 55 providing greater protection from acid than Eudragit alone. However, although PFL made from blended polymers films completely released low molecular weight dye into intestinal conditions (pH 7.0), they failed to release LBC. In contrast, PFL made from Eudragit alone successfully protected dried probiotic or vaccine LBC from simulated gastric fluid for 2h, and subsequently released all viable cells within 60min of transfer into simulated intestinal fluid. Release kinetics could be controlled by modifying the lamination method.

Conclusions: These studies demonstrate the feasibility of a new oral formulation concept for simple, effective, controlled enteric delivery and protection from acid of sensitive complex biologic payload such as live bacteria for vaccine and probiotic applications.

44 UNDERSTANDING THE ROLE OF MILLING TEMPERATURE AND COMPOSITE TG IN PROCESS INDUCED AMORPHISATION

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Background: Active pharmaceutical ingredients (APIs) can undergo solid state changes as a result of milling, leading to the formation of surface and bulk amorphous regions, thus potentially altering the dissolution profile and/or physical stability of the API. The level of amorphisation induced during milling is known to depend on the glass transition temperature (T_g) of the material relative to the temperature of the process, with milling temperatures at or near T_g promoting the re-crystallisation of generated amorphous material. The purpose of this work is to investigate the effect of milling temperature and composite T_g on the ability of low T_g crystalline excipients to prevent process induced disorder in a model API.

Methods: Sulfadimidine (T_g= 78°C) was milled alone and co-milled with glutaric acid (T_g = –14°C) in a planetary ball mill (PBM) and a vibrational ball mill (VBM) at different temperatures (room temperature (RT) and –196°C (cryo-temperature, CT)). The solid state properties of the milled systems were monitored by PXRD, DSC, TGA and FTIR.

Results: The rate and extent of milling induced amorphisation of sulfadimidine was found to depend on both the type of ball mill (PBM vs VBM) and the temperature of milling. PBM milling of SDM for 10 hr at RT resulted in an amorphous content of 82% (by PXRD), whereas VBM milling induced the same level of amorphisation in 36 min and 18 min at RT and CT respectively. When co-milled with 50% w/w glutaric acid at RT, sulfadimidine amorphisation could be completely mitigated in both the PBM and VBM. In contrast, co-milling of glutaric acid with sulfadimidine at cryo temperatures resulted in amorphisation of the API.

Conclusions: Milling induced amorphisation can be prevented by co-processing API with a low T_g excipient at room temperature; however at cryo temperature amorphisation is promoted.

45 SELF-ASSEMBLED FOLATE NANO-CARRIER FOR CONTROLLED DELIVERY OF CHEMOTHERAPEUTIC DRUGS

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Background: Nanoparticle mediated drug delivery for chemotherapeutics has gained much attention owing to potential for sustained release and lowered toxicity. The present study reports the use of a novel class of materials – *Chromonics* – in cancer drug delivery. Folic acid as a chromonic material has been explored to develop nanocarrier for sustained release of chemotherapeutic drugs *in-vitro*.

Methods: The study included understanding of folate self-assembly from low to moderate concentrations, developing the method for synthesis of nanoparticles and designing strategies to control the size and release of drugs from these nanoparticles. Each of these was significant studies since a number of chemotherapeutic drugs behave similarly as the carrier molecules. The effect of different factors like cross-linking agent, relative polymer and folate concentration, on the size distribution was investigated in detail. The present study then explored the release of drugs from these nanocarriers.

Results: We have investigated the release of folic acid carrier from these nanoparticles as well as release of methotrexate and cytarabine as an anticancer drug for the period of 60 days. It was observed that release of drug was dependent on nanoparticle size, release medium, pH of the release medium, crosslinking agent and its concentration. A method to study the efficacy of these nano-carriers on cancer cell line was also developed.

Conclusions: We show that folate nanoparticles can be a potent nanocarrier for the sustained release of cancer drugs.

46 DEVELOPING FLUORESCENTLY-LABELLED POLYSACCHARIDES FOR MUCOADHESIVE STUDIES

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Background: Mucoadhesives are widely used in pharmaceutical applications to improve the retention of drugs on mucosal surfaces thus enhancing their delivery and bioavailability. The factors that influence mucoadhesion are complex but include the characteristics and properties of the polymers and the mucosal surface studied. Many studies previously have used buccal tissue and other, lower parts of the gastrointestinal tract when determining the retention of dosage forms and drug permeability. This study will measure the retention ability of various natural polysaccharides, in hydrated and solid states, on various oral mucosal surfaces. Particularly, porcine tongue tissue will be used as a novel ex vivo model of oral retention. Investigating the mucoadhesive properties of food safe, GRAS, polymers could lead to the development of potential additives to modulate the organoleptic properties of food products.

Methods: A method for labelling food safe polysaccharides with fluorescein amine was developed. Pectins, which were kindly provided by Herbstreith & Fox KG (Neuenbürg, Germany), with different esterification and amidation degrees, sodium alginate and sodium carboxymethyl cellulose were chosen to be labelled. Labelling was achieved by 1 equivalent of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide to fluorescein- amine and 2 equivalents of N-Hydroxysulfosuccinimide sodium salt. Labelling efficiency was determined by fluorometry with standard curves. Polysaccharides were labelled fluorescently to enable visualization under fluorescent microscopy. Polysaccharides will be prepared either into films or to viscous solutions and applied to ex vivo porcine oral tissues. They will be washed off with artificial saliva until fluorescence is no longer visible. The retention of polysaccharides on the oral tissue will be determined by the fluorescence detected.

Results: NMR and TLC results suggest no unbound dye was present in freeze dried polysaccharide solutions after labelling. Fluorometry standard curves suggest >0.01% of carboxylic acid groups are labelled for each polysaccharide. Viscometry measurements of the various polysaccharides were carried out. Each polysaccharide's viscosity was measured in a buffer solution with and without pig gastric mucin. Viscometry results show an increase in viscosity of polymers in a buffer solution containing mucin compared to polymers in buffer without mucin.

Conclusions: The labelled polysaccharides are suitable to use for retention experiments with no unbound fluorescein. Viscometry results can be used to make sure polysaccharide solutions are made up to a similar viscosity to control for variation in polysaccharide retention which could be accountable to different viscosities of solutions, by making sure all polymers are of similar viscosity when hydrated.

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PENETRATION ENHANCERS IN OCULAR DRUG DELIVERY

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Background: Riboflavin is used as a drug for ultraviolet induced collagen crosslinking for the treatment of keratoconus. It is a poorly water soluble compound and stromal saturation of the solution is hampered by barriers that the cornea offers. The main one being the corneal epithelium which is a lipophilic layer with tight junctions and this structure strongly resists ingress of aqueous drug solutions. Cyclodextrins are container molecules that can improve aqueous solubility of lipophilic or poor water soluble compounds by forming guest – host complexes. This study has shown that they can also render the epithelium more permeable to aqueous solutions by extraction of cholesterol. Tight junctions are dependent on an undetermined amount of calcium availability in order to function. Calcium sequestering compounds such as ethylenediamine tetraacetic acid (EDTA), ethylene glycol-bis(2-aminoethylether)-N,N,N'N'-tetraacetic acid (EGTA) and ethylenediamine-N,N'-disuccinic acid trisodium salt (EDDS) are able to extract calcium ions from cellular environments. This research compared enhancement of riboflavin penetration into the cornea when using cyclodextrins or calcium sequestering compounds.

Methods: Riboflavin at 0.1 mg mL^{-1} was added to PBS solutions of α-, β-, γ- and hydroxypropyl-β-cyclodextrin up to 30 mg mL^{-1}, and to solutions of EDTA, EGTA and EDDS at 1 mg mL^{-1}, a solution of riboflavin in PBS at 0.1 mg mL^{-1} was also prepared for comparison. Bovine corneas were exposed to these solutions using Franz diffusion cells and samples were taken for HPLC analysis every 30 min for 3 hr to determine any enhancement to riboflavin permeation through the corneas compared to riboflavin in PBS. Microscopy analysis was used to determine any histological changes to the exposed corneas.

Results: For all riboflavin solutions there was an initial lag time of 60-90 min before permeated riboflavin could be detected, at 180 min the receiving solution contained riboflavin at 5.4 ng mL^{-1} for RF in PBS, this was slightly less for the solution with γ-CD at 5.1 ng mL^{-1}, and with α-CD it was 7.9 ng mL^{-1}, with HP-β-CD it was 9.4 ng mL^{-1} whilst with β-CD there was a significantly higher amount of 15.9 ng mL^{-1}. Permeability enhancement of riboflavin using calcium chelators were found to be; with EDDS 6.83 ng mL^{-1}, EGTA 7.06 ng mL^{-1} and EDTA 8.21 ng mL^{-1}. Microscopy has shown epithelial disruption for all solutions except riboflavin in PBS. Disruption tends to be in the superficial layers.

Conclusions: Cyclodextrins and calcium chelators have been shown to disrupt corneal epithelia and increase permeability to riboflavin; HP-β-CD and β-CD proving to have the best performance overall. The mechanism for this enhancement has been shown to be due to cholesterol extraction for CD's and due to Ca^{2+} extraction for the chelators. Both classes of compounds loosen tight junctions by disrupting corneal epithelia.

48 EVAPORATIVE ANTISOLVENT PRECIPITATION OF ENTERIC ITRACONAZOLE NANOPARTICLES

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Background: Itraconazole (ITR) is a typical BSC class II drug with solubility less than 1 µg/ml. ITR displays pH-dependent solubility with a very low solubility at high pH. In this work, we report on the use of cellulose acetate phthalate (CAP) for the synthesis of enteric ITR nanoparticles (NPs) using heat induced evaporative antisolvent precipitation.

Methods: NP preparation: 2 ml of the solvent phase (6.4 mg/ml ITR and 1 mg/ml CAP in acetone maintained at 50°C) was rapidly injected into 20 ml of deionised water (antisolvent) at 80°C with continuous stirring at 13,000 rpm. This mixture was then transferred to a thermostated cylinder and allowed to cool to 25°C. Size, shape and the solid state properties of resulting NPs were examined using dynamic light scattering, scanning electron microscopy, X-ray diffraction (XRD) and differential scanning calorimetry (DSC). Dissolution studies were performed for 1 hr in FaSSGF (pH 1.6) followed by a media shift to FaSSIF (pH 6.5) for another 3 hr.

Results: Spherical NPs with a mean particle size of 197.1 ± 2.6 nm (polydispersity index = 0.058 ± 0.005) were produced. XRD and DSC data revealed that these NPs were liquid crystalline. The dissolution profile indicated a pH-sensitive release of ITR. The amount of soluble ITR was minimal in FaSSGF and was enhanced following pH shift (FaSSIF). The greatest amount of soluble ITR (60% increase compared to the maximum amount released in FaSSGF) was achieved 45 min following the pH change.

Conclusions: The precipitation of ITR in the presence of CAP produced spherical liquid crystalline NPs which were able to delay the release of ITR in the acidic media and promote dissolution in FaSSIF.

49 PARTICLE ENGINEERING STRATEGY FOR CONTROLLED POROSITY, SURFACE HABIT AND FUNCTIONALITY OF PHARMACEUTICAL EXCIPIENTS

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Background: Future medicines and drug delivery technologies cannot be developed with yesterday's excipients [1]. Fabrication of excipient particles with predefined shape, porosity, surface habit and mechanical properties is highly sought-after in drug delivery arena. The current study aims to develop a new excipient particle (modified mannitol) for the preparation of Orodispersible dosage forms. The work utilizes spray drying particle engineering capabilities to induce fast disintegration and high mechanical strength into mannitol particle, through understanding the kinetics of particles formation.

Methods: D-mannitol Mod I (10-70%, w/v) was spray dried from aqueous feed using a lab-scale spray dryer B-290 after incorporating 5-20% (w/v) of leavening agent (NH_4HCO_3). The processing parameters selected based on a previous DOE study were: 90% aspirator rate, 10% feed rate, 670 NormL/hr air flow rate whereas inlet temperature was varied between 110-170°C. Engineered mannitol was examined via SEM before and after compression into tablets (at 20 kN). Preformulation assessment included Helium Pycnometry for porosity measurements, laser diffraction for particle size, flow behavior & out-of-die Heckel analysis. Hot stage microscopy (HSM) was used to simulate the process of spray drying by monitoring particle formation kinetics and crystallization behavior. This was coupled with thermal and polymorphic profiling of the particles using DSC, X-ray powder diffraction & FTIR. Texture profile analysis (TPA) was also used to investigate particles plastic/elastic relationship.

Results: Spherical mannitol particles were formed with surface submicron gaps and high mechanical strength as envisaged. Porosity of powder particles increased from 0.77 to 0.90 while that of tablets from 0.39 to 0.53 due to NH_4HCO_3 leaving pores behind during spray drying. Consequently, disintegration time of tablets was fast (< 60 sec) due to the wicking nature of porous particles. The mechanical hardness of engineered mannitol tablets was nearly double (120 N) that of pure excipient (57 N). The resultant particles have approximately 8 fold higher plasticity than unmodified mannitol indicated by Heckel analysis (Py = 244 compared to 2000 MPa respectively). Flow properties of the produced powder were indifferent to that of pure material and exhibited a cohesive behavior (VMD < 20 μm).HSM simulation showed the phenomena of pores generation and simultaneous particle crust formation to be dependent on leavening agent decomposition together with conductive heat exchange in the spray drying chamber. At higher temperatures (150°C), a smooth crust was formed around the spherical droplet before NH_4HCO_3 decomposed into NH_3, CO_2 and H_2O which subsequently leave the preformed crust. At 110°C, crystallization of mannitol was much faster thus microparticles showed irregular crystallites distribution. Surprisingly, no amorphous material was formed after spray drying confirmed by DSC, XRPD and FTIR. The results of XRPD, however, indicated the formation of a mixture of Mod II in addition to the original Mod I polymorph. The mixture of polymorphs resulted in a better radial hardness due to superiority of compaction properties of Mod II [2]. The functionality of formed particles was further elucidated using TPA which showed an increased axial elasticity of mannitol after spray drying due to inherent elastic energy stored in the polymorphic mixture.

Conclusions: Multi-functionality engineered into mannitol particles resulted in both superior hardness and fast disintegration. Spray drying of material-leavening agent feeds could be beneficial in formulating porous frameworks for controlled release applications.

50 REMOTELY TRIGGERED SCAFFOLDS FOR CONTROLLED RELEASE OF PHARMACEUTICALS

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Background: Scaffolds fabricated for tissue engineering purposes require controlled pore structure and size, allowing diffusion of nutrients and toxins to in-growing tissue. Biodegradable scaffolds that collapse after a fixed time period, either naturally or after external stimulus, offer further control for long term use. Fe_3O_4-Au hybrid nanoparticles (HNPs) have shown increasing potential for biomedical applications such as image guided stimuli responsive drug delivery. Here we demonstrate the incorporation of HNPs into a biocompatible scaffold in order to allow an external switch on scaffold characteristics. Using a laser to irradiate the particles in situ, a localised heating effect causes thermally-responsive scaffold materials to deform mechanically. Additionally, growth factors or drug molecules can be incorporated, being thermally released into the surrounding tissues upon irradiation. In conjunction, the dense nanoparticle cores will also provide contrast in MRI imaging which can be used to visualize and monitor scaffold position and degradation.

Methods: Here we successfully fabricated biodegradable scaffolds based on thermo-responsive poly(N-isopropylacrylamide) (NipAM) polymers. Nanoparticles acting as the trigger for controlled degradation and release were synthesized using a wet chemical precipitation method followed by electrochemical gold coating, and incorporated into the intrinsic structure of the scaffold. The constructs were fully characterised using TEM, SEM, DSC, PCS, FTIR and microscopy. We investigate the potential of such scaffolds as thermally triggered systems using a Q-switched Nd:YAG laser. pNiPAM-HNP composites were prepared containing methylene blue (MB) dye as a model drug, chosen for its intense absorption in the UV/Vis range allowing visualization during expulsion from the scaffold after thermal deformation. Biological safety was determined using live/dead fluorescence staining and cellular stress levels deduced via ROS and LPO quantification.

Results: The scaffold-hybrid constructs were successfully synthesized. The results showed the ability to remotely control switching of the hybrid scaffold materials to cause thermal deformation, resulting in release of a model drug compound. Upon laser irradiation samples were again found to deform, expelling solvent carrying MB out of the polymer network. The constituents did not show any cytotoxic effect or adverse cellular response on exposure to 7F2 cells.

Conclusions: These studies show that incorporation of HNPs resulted in scaffold deformation after very short irradiation times (seconds) due to internal structural heating. Our data highlights the potential of these hybrid-scaffold constructs for exploitation in drug delivery, using methylene blue as a model drug being released during remote structural change of the scaffold. Further work is on-going to further exploit these unique properties for controlled drug delivery.

51 THE HPMC MATRIX "POST-PRANDIAL" EFFECT: HOW MATRIX FAILURE APPEARS TO BE INDEPENDENT OF POLYMER CONTENT IN THE PRESENCE OF FOOD SALTS

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Background: Hydroxypropyl methylcellulose (HPMC) matrices are a popular extended release (ER) drug delivery technology. Their drug release profile is determined by formulation composition. The drug release rate for example can be increased by lowering the polymer content. This can be necessary to achieve pharmacokinetic (PK) targets if the current formulation extends drug release over excessive *in vivo* time periods, resulting in poor drug absorption in the colon. A reduction in polymer content is thought to render the matrix more susceptible to processing and environmental factors but this hypothesis has not been extensively investigated in the published literature. Another environmental factor that may affect low polymer content matrices is the presence of food in gastric media. This "postprandial effect", whereby *in vivo* drug release rate varies between a fed or fasted environment, has already been reported with HPMC matrices. It is also known that very high concentrations of food components such as salt and citrate buffers can induce failure of 30% w/w HPMC matrices (Williams, HD et al. 2010). It is important for pharmaceutical formulators to understand the implications of reducing matrix polymer content and this study aims to investigate how low polymer matrices respond to the challenge of food components.

Methods: Matrices containing 5 to 30% w/w HPMC (Methocel K4M), 10% w/w caffeine, and a 2:1 ratio of Lactose (FastFlo) to Avicel (PH102) were manufactured by direct compression at 8kN (250 mg fill weight and 8 mm flat, round image). Matrix drug release behaviour was studied by USP II (paddle) dissolution tests (in water, 0.05 to 0.2M trisodium citrate (TSC) and 0.2 to 1.0M sodium chloride (NaCl)), confocal laser scanning microscopy, photographic imaging and texture analysis studies.

Results: Surprisingly, it was found that the salt concentrations required for matrix failure did not change with polymer content, but remained at 0.15M TSC or 0.8M NaCl. Although the presence of solute resulted in increased matrix swelling and gel layer thicknesses, there was little change in drug release rate until these failure concentrations were reached. At these concentrations particles failed to coalesce into a gel layer, the failure to form a limiting diffusion barrier resulted in enhanced liquid penetration of the core.

Conclusions: In this study matrices with reduced polymer content were no more affected by these soluble food components than normal matrices containing 30% w/w HPMC. A reduction in polymer matrix content did not alter the sensitivity of a formulation to food salts, and therefore the presence of postprandial effects cannot be attributed to insufficient polymer content. Further study is required to fully elucidate what formulation factors result in a food sensitive formulation.

52 SYNTHETIC MUCOSA-MIMETIC HYDROGELS TO REPLACE ANIMAL TISSUE IN MUCOADHESION RESEARCH

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Background: Mucosal membranes cover most of the gastrointestinal tract, airways and the exterior of the eye. Many drugs require absorption across these mucosal layers if they are to reach their site of action. However, diffusion across these layers can be slow, and some dosage forms may be quickly removed from the intended site of action. In order to increase the uptake of drugs at the intended point of action, a dosage form can incorporate a mucoadhesive material, which sticks to the mucosal surface, holding a dosage form in place. In order to assess whether materials are mucoadhesive, often experiments using mucosal membranes taken from animals are conducted. In two-thirds of cases, these tissues are taken from animals that are slaughtered for this tissue alone. This number could be drastically decreased by the use of a synthetic alternative to mucosal tissue, which could be during preliminary testing of mucoadhesives.

Methods: A number of hydrogels have been synthesised from various monomers which contain moieties with the potential to hydrogen-bond. These hydrogels have been assessed as potential mucosa-mimetic materials by measurement of the force required to detach mucoadhesive tablets from their surface. Modification of the synthesis has allowed for grafting of these hydrogels onto glass surfaces, to ease handling. These glass-bound hydrogels have been taken on for the testing of liquid and semi-solid mucoadhesives.

Results: We have synthesised an array of mucosa-mimetic hydrogels, which could offer an alternative to *ex vivo* tissue. As mucosal layers are coated in mucin glycoproteins, hydrogen bond donors/acceptors were included to mimic the heavily glycosylated regions of mucin. Several hydrogels were identified that were successfully able to mimic mucosal tissues in these experiments. The greatest mucosa-mimetic materials contained a synthetic monomer, *N*-acryloyl glucosamine, which should best mimic the oligosaccharides present in mucin.

Conclusions: Mucosa-mimetic materials are being developed which show promise for the replacement of animal tissue in mucoadhesion assessment. While these materials are unlikely to fully replace animal tissue in this research, they may be able to pre-screen mucoadhesives with a standardized material, reducing the amount of animal tissue required, and therefore the number of animals slaughtered for research.

53 INVESTIGATION OF THE APPLICATION OF INTESTINAL PERMEATION ENHANCERS TO INCREASE ABSORPTION OF FOOD-DERIVED ANTIHYPERTENSIVE PEPTIDES

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Background: Ile-Pro-Pro (IPP) is a bioactive peptide found in bovine milk when casein is hydrolyzed by gastrointestinal enzymes. It has ACE inhibitory potency of 5μM and may have potential to reduce blood pressure as a component of an oral pharmaceutical dosage form. Its stability has been demonstrated in intestinal fluids and in the presence of Caco-2 brush-border peptidases, however it has poor intestinal permeability, which is a limiting factor of its potential as an oral pharmaceutical. Permeation enhancers are added to oral drug formulations since intestinal absorption of peptides, proteins and macromolecules is difficult due to intestinal metabolism, poor membrane permeability and high intra-subject variability. The aim was to investigate the potential of two enhancers to increase permeability of FITC labelled IPP across jejunum and colon tissue.

Methods: Permeation enhancement potential of enhancers were tested using Ussing chambers. Isolated rat colonic mucosae and rat jejunal tissue were mounted and FITC-IPP (500μM) was added apically in the presence or absence of either C10 (10mM) or a novel medium chain fatty acid derivative ('Analogue') (10mM) were tested. Transepithelial electrical resistance (TEER) and apparent permeability (Papp) of FITC-IPP were measured over 120 min.

Results: Both enhancers significantly reduced the TEER within 5 min in colon and from 40 mins in jejunum ($P< 0.001$). Papp of FITC-IPP was significantly increased in colon ($P<0.001$) and jejunum ($P<0.01$). This data suggests that the tight junctions are being opened thereby allowing FITC-IPP to permeate at higher levels than without an enhancer.

Conclusions: Permeability is a major hurdle for oral delivery of food-derived peptides however, permeation was increased using an established and a novel permeation enhancer.

55 FORMULATION AND EVALUATION OF CONTROLLED RELEASE BEADS OF CAPECITABINE

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Background: Capecitabine is an orally-administered chemotherapeutic agent with bioavailability of 40%. It is administered orally at dose of 1250 mg/m_2 given twice a day for colorectal cancer. Such a high frequency and dose leads to serious side effects like hand and foot syndrome, vomiting, pain in stomach, fever or infection.

Methods: Beads were formulated using ionotropic gelation method. DSC and FTIR study confirmed that there was no drug-polymer interaction and formation of calcium alginate has taken place due to reaction of sodium alginate and calcium chloride.

Results: Optical microscopy confirmed the spherical shape of beads. Evaluation of micromeritic properties of beads indicated their good flow property and size range of 1000µm - 1500µm. Optimized batch gave entrapment efficiency of 58.34±1.49 and % cumulative drug release of 62.12±1.74 % in 7 hr. Swelling index was found to be 1203.70 % in phosphate buffer pH 6.8. Mucoadhesivity was measured using in-vitro wash off test method and was found to be 60 %. In case of in-vitro drug cytotoxicity studies, it was seen that the blank beads did not showed any cytotoxicity against HT-29 cells. Beads showed controlled drug release with minimum of 56% cell viability in 7 hr meaning that maximum of 44 % cell death occurred at this time. Stability studies done at 25°C ± 2°C / 60%RH ± 5% and 40°C ± 2°C / 75%RH ± 5% for 3 months indicated no significant change in % drug content and in-vitro drug release from the beads.

Conclusions: Hence, controlled release beads formulation was formulated using sodium alginate, calcium chloride and chitosan. Hence, the developed bead formulation shows great promise as a drug delivery system. However, extended research on the formulation in terms of pharmacokinetic studies is expected to improve our understanding on the performance of the formulated beads in-vivo.

56 IN VITRO UPTAKE STUDIES OF CELL TARGETING AGENTS AND NANOPARTICLES

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Background: In the last 20 years, polymer therapeutics have attracted interest from the pharmaceutical industry and some are already into the market. Most recently, advances in synthetic chemistry have provided new *smart* nanosized polymers that can respond to environmental changes via conformational rearrangements. These new responsive materials may enable a tailored and personalized approach to therapeutic delivery. However, endocytic pathways used by nanoparticles to access cells affect their intracellular compartmentalization and fate. Knowledge of uptake and trafficking pathways is essential information that should be better understood when designing new nanoparticles as drug carriers.

Methods: Human transferrin (Htf) and lactosylceramide (LacCer) were used as clathrin and caveolin endocytic markers; chlorpromazine (CPZ) and methylbetacyclodextrin (MBCD) were used as inhibitors of clathrin dependent and independent endocytosis in 3T3, HCT116 and MGLVA-1 cells. Carboxylated polystyrene beads (C-PB) of 50 and 100nm were characterised and used as a model of uptake of negatively charged 50 and 100nm nanoparticles.

Results: CPZ inhibited the uptake of Htf and MBCD inhibited the endocytosis of LacCer. Moreover, none of the pathway inhibitors showed cross reactivity with unspecific markers of endocytosis. The inhibition of the endocytosis was time dependent and incubation times of the inhibitors were cell dependent. CPZ needed 4h incubation with MGLVA-1 cells to shut down CDE while 1h was necessary for HCT116 cells. 50 and 100 C-PB exposed to cells in the presence of pathway inhibitors showed that 50 C-PB access cells through CDE in HCT116 and MGLVA-1, 100nm C-PB access cells through CDE in 3T3 cells and through a CIE in MGLVA-1.

Conclusions: Non-specific chemical pathway inhibitors can sometime shut one pathway of endocytosis only temporarily before other compensating mechanisms reopen the pathway. In addition, rates of inhibition vary in some cell lines with respect to others. For this reason the incubation time of the inhibitors should be tailored for specific cell lines. Uptake variations were also shown in cell lines when exposing 50 and 100nm C-PB to CPZ and MBCD.

57 ORALLY DISINTEGRATING TABLETS: ENHANCING DRUG PERMEATION THROUGH FORMULATION

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Background: P-glycoprotein (P-gp) is a broad-substrate efflux transporter found on the apical membrane of epithelial tissues such as the intestinal lumen, and therefore represents a major obstacle to overcome for efficient oral drug delivery. Although inhibitors of P-gp have been developed, these often display unfavourable characteristics such as immunosuppression. However, a growing volume of literature has shown a diverse range of compounds, many PEGylated, which have the potential to be exploited in order to enhance drug uptake by inhibiting P-gp efflux. As such, the permeability of the P-gp substrate indomethacin model drug in the presence of two commonly used PEGylated compounds, either PEG 8000 or POLYOX™ N-10, was tested over a clinically achievable range using the caco-2 Transwell® model. These materials were then incorporated into orally disintegrating formulations using direct compression and the post-compression properties of the resulting tablets were tested.

Methods: Permeability studies were conducted using confluent 21-day old caco-2 cells grown on 6-well inserts, with trans-epithelial electrical resistance (TEER) values exceeding 400 $\Omega.cm^2$. Apical to basolateral transport was assessed by twice washing the cells in HBSS, and adding the inserts to fresh serum-free media with 25 mM HEPES (pH 7.4). The apical wells were then refilled using 10 μM indomethacin in serum free-media containing 1.19 $mgmL^{-1}$ of either PEG 8000 or POLYOX™ N-10. Cells were rotated at 100 rpm, and samples taken at discrete time points. The permeability of the test compounds was compared to uptake of drug-only media, and samples were analysed using HPLC. Individual 500 mg ODTs incorporating either 5 % PEG 8000 (w/w) or 1.5 % POLYOX™ (w/w), were made, and these tablets were characterised for hardness and disintegration, with any drug interactions probed using FT-IR. Statistical analysis was performed using the *t*-test on Graphpad Prism, with a P value < 0.05 considered significant.

Results: Both polymeric binders increased the percentage transport of indomethacin, becoming statistically significant in the presence of PEG 8000 after 30 min and in the presence of POLYOX™ N-10 after 60 min ($p < 0.05$) compared to the permeability of indomethacin alone. This is potentially due to the inhibition of the efflux transporter P-gp by lipid membrane interactions and subsequent disruption of the inner-leaflet binding site, as has previously been suggested as the mechanism of inhibition for other PEGylated compounds. This is also seen in the corresponding apparent permeability (*Papp*) of the drug, with statistically significant increases seen in the presence of PEG 8000 ($p < 0.005$) after 30 min and after 60 min for POLYOX™ ($p < 0.005$). When incorporated into ODTs, the hardness values (above 65 N) and the disintegration times (below 30 seconds) for both formulations conformed to USP specifications.

Conclusions: The incorporation of functional additives when reformulating compounds which are substrates of efflux transporters, such as indomethacin, offers the opportunity of increased permeability without additional rigorous safety tests or increased manufacturing costs. The concept of including such additives may also convey additional financial benefits, such as patent extension. These results further support the paradigm of using PEGylated materials to enhance the permeability of substrates of the P-gp efflux transporter.

58 INVESTIGATING DISINTEGRANTS IN A SIMPLE FORMULATION FOR AN ORALLY DISINTEGRATING TABLET

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Background: Dysphagia, a difficulty in swallowing, is a condition experienced by a significant proportion of the population. It creates a substantial problem for compliance with solid oral dosage forms particularly for the young and elderly, institutionalised and infirm patients and those without access to water. Orally disintegrating tablets (ODTs) dissolve rapidly upon contact with saliva, overcoming the issue of dysphagia without the need for concomitant ingestion of water. Direct compression technologies offer the most efficient and economic method for ODT production, however the effectiveness of these tablets relies heavily upon the careful selection of excipients, in particular disintegrants. A number of mechanisms for the action of disintegrants have been proposed, with the most important considered to include swelling, wicking and deformation recovery. Disintegrants commonly work by one or a combination of these mechanisms.

Methods: Different disintegrants and combinations of these disintegrants were investigated in a simple mannitol based ODT formulation in order to determine the most effective disintegrant and to investigate whether combination produced more rapid disintegration. Tablet properties including hardness, and disintegration time were assessed.

Results: Crospovidone alone (4% w/w) performed best when compared to croscarmellose sodium, sodium starch glycolate and starch alone, and also when compared to different combinations of crospovidone, croscarmellose sodium and sodium starch glycolate.

Conclusions: It was concluded that crospovidone demonstrated superior disintegration due to its multi-mechanistic approach to disintegration, in particular its ability to promote wetting through wicking and that crospovidone was a good choice for disintegrant in formulations containing large quantities of water soluble diluent, such as mannitol.

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