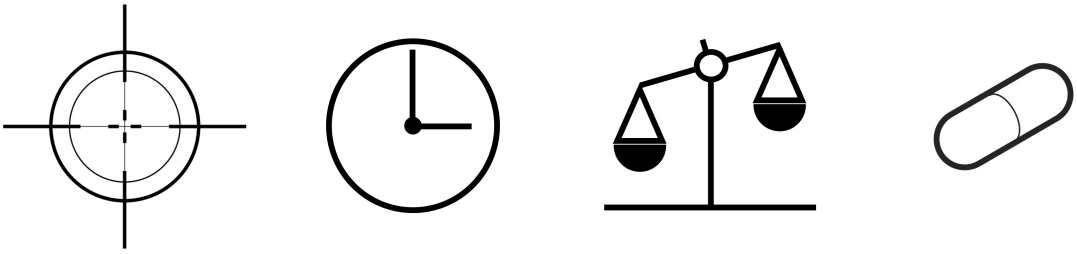
GENERAL INFO

United Kingdom and Ireland Controlled Release Society

2016 UKICRS Newsletter coming this July!

ABOUT UKICRS

www.ukicrs.org



The Controlled Release Society (CRS) was founded in the USA in 1978 to advance the science and technology of controlling the release and delivery of active agents. It is recognised worldwide as the premier professional society in this still developing field. The CRS has established local "chapters" of the organisation around the World and the United Kingdom Controlled Release Society was formed in 1994. In 1998, Ireland joined forces and the United Kingdom and Ireland Controlled Release Society (UKICRS) was formed.

The UKICRS addresses a broad range of research fields based on controlled release which include agriculture, veterinary, food and cosmetic sciences. The UKICRS Committee has drawn up several key aims and objectives, principally:

- To develop the UK and Ireland Controlled Release Society into the primary national organisation for the representation, education, and dissemination of information to scientists from all disciplines who are interested in any aspect of controlled or advanced delivery.
- To broaden the understanding of controlled release science through the organisation of workshops and meetings where lectures presented by scientists drawn from inside and outside the society shall promote discussion and exchange of views.
- To develop links with other scientific organisations and to make representations to government, professional and scientific bodies on issues which may promote the interests of the controlled or advanced delivery community.

ukicrs@gmail.com

https://twitter.com/ukicrs

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WORKSHOPS & EXPOSITION

Thursday 21st April

10.00 am	**Registration** *Room 0.86 Redwood Building* Posters can be hung from 10.00 am
11.00 am	**Graduate Careers** *Paul Spencer Lecture Theatre* Speakers from Pharma will provide insight and advice about careers in the Pharmaceutical Industry • Dr Dan Palmer/ *Midatech* (20 min) • Dr Marie Mc Grath / *GSK* (20 min) • Panel discussion with Dan and Marie (10 min)
12.00 noon	**An ethical debate for research scientists** *Paul Spencer Lecture Theatre* Dr Emma Lane will lead an ethical debate that is related to all of our research
12.45 pm	**Lunch** Redwood Building Room 0.60 & 0.86
1.30 pm	**Industrial Exposition** Exhibitor presentations / *Paul Spencer Lecture Theatre* Industrial Exposition / *Rooms 0.60 & 0.86*
4.00 pm	**Explore the 'Diff** Cardiff (or the 'Diff to locals) is the UK's fastest growing city and has plenty of things to see and do and so delegates have been given a short period of time before the Dinner to explore some of the local interests. Within short walking distance of the Redwood building is the National Museum and Art gallery, Cardiff Castle, Bute Park and the Millennium Stadium.
7.30 pm	**Conference Dinner** 'Zero degrees' on Westgate St in Cardiff City Centre Complimentary for delegates attending Day 1 of the Symposium See page 8 for details.

WORKSHOP SPEAKERS

Thursday 21st April

Dan Palmer (Midatech Pharma) read BSc Chemistry at Heriot-Watt University before undertaking doctoral studies in organo-metallic chemistry & catalysis. He also studied an Executive MBA at Imperial College Business School, and chose to specialise in technology & intellectual-property strategy & entrepreneurship.
He has worked in the biotech sector since 2004, with particular focus on drug delivery and micro/nano-technologies. Dan specialises in management of inter-disciplinary R&D at the interface between SMEs, academia and big pharma. He is named inventor on 15 patent families. Dan is an experienced executive manager. He holds the position of Chief Scientific Officer at Midatech Pharma PLC and previously at Q Chip Ltd.

Marie McGrath (GSK) completed an MPharm degree at King's College London before undertaking doctoral studies in Pharmaceutics at University College Cork. She has worked in the Pharmaceutical sector since 2011 when she began her career with Leo Pharmaceuticals working in the Manufacturing Development of Dermal products. At GSK Marie has both worked as an Oral Solid Dose Formulator for paediatric products and more recently within Drug delivery as an Investigator Scientist specializing in enhanced parenteral products.
Marie specializes in the development and trial of new delivery technologies within R&D working at the interface between early and late development phases. She also works at the external interface with SMEs and academia and is one of the Hosts of the International Microneedle Conference 2016.

Emma Lane completed a BSc in Pharmacology at University College London, and completed her doctorate on the neuropharmacology of Parkinson's disease at King's College London. Following this, she completed a 2 year post-doctoral research post at Lund University, Sweden, developing expertise on the complications surrounding cell transplantation as a therapeutic treatment for Parkinson's disease. She moved to Cardiff School of Biosciences in 2006 before securing a lectureship in the School of Pharmacy and Pharmaceutical Sciences in 2009. Her current research focuses on developing stem cell therapies and working towards improving patient recording outcomes for people with Parkinson's disease.

INDUSTRIAL EXPOSITION

1.30 pm Thursday 21st April

UKICRS has always been passionate about cultivating relationships with UK and Ireland companies working within the pharmaceutical sector. This year, we are delighted to have representatives from seven companies (detailed below) attending our Industrial Exposition and Symposium. Many of these companies are long-term supporters of UKICRS, and their sponsorship is invaluable in ensuring that UKICRS can continue to run a successful symposium each year.

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BÜCHI

SYMPOSIUM DINNER

7.30 pm Thursday 21st April

All registered attendees and exhibitors of the UKICRS Workshop & Symposium are invited to attend dinner 7.30pm Thursday 21st April at 'Zero degrees' on Westgate Street in Cardiff City Centre. The cost of dinner is covered as part of the Symposium registration fee. The menu is buffet style and can cater for those with dietary requirements.

Address: 27 Westgate St, Cardiff CF10 1DD
T: 029 2022 9494
E: cardiff@zerodegrees.co.uk

ABOUT ZERO DEGREES

Situated across the street from the Millennium Stadium, the brewery can be seen from the street as well as from inside the bar. The beers are slightly cooler than cask beers are normally served. A wide variety of pizzas is available, as well as mussels, pasta, risotto, salads and sausage and mash. The upper floors are reserved for diners.

Zero Degrees serves four regular beers:

- Zerodegrees (Cardiff) Black Lager
- Zerodegrees (Cardiff) Pale Ale
- Zerodegrees (Cardiff) Pilsner
- Zerodegrees (Cardiff) Wheat Ale

SYMPOSIUM PROGRAMME

Friday 22nd April / Paul Spencer Lecture Theatre

8.30 am	**Registration** Redwood Building Room 0.86
9.30 am	**Welcome & Opening Remarks** Gary Baxter, Head of School of Pharmacy *(University of Cardiff, UK)* Gavin Andrews, UKICRS Chairperson
Session 1: Chairperson – Sion Coulman *(Cardiff University)*	
9.40 am	**Arto Urtti** *(University of Helsinki)* – KEYNOTE SPEAKER Ocular pharmacokinetic models as tools in drug delivery design
10.20 am	**Sam Tarassoli** *(University College London, UK)* Novel polyglutamate-based indocyanine green nanoparticles for photothermal cancer therapy
10.35 am	**Samuel Bizley** *(The Royal Veterinary College, UK)* Study of the structural and barrier properties of equine skin for the deep tissue delivery of pharmaceuticals in equine therapy
10.50 am	**Tea, Coffee & Welsh Cakes**
Session 2: Chairperson – Katie Ryan *(University College Cork, Ireland)*	
11.10 am	**Affiong Iyire** *(Aston University, UK)* Amino acid facilitated delivery of insulin through buccal cell layers *in vitro*
11.25 am	**Hope Roberts-Dalton** *(Cardiff University, UK)* Thiol-based labelling of prostate-derived exosomes for analysis of cellular uptake and intracellular traffic
11.40 am	**Ali Athab Al-kinani** *(Kingston University London, UK)* Real-time imaging and antioxidant activity of HPMC–coated cerium oxide nanoparticles: a new modality for cataracts

Continued on next page ...

SYMPOSIUM PROGRAMME

Friday 22nd April / Paul Spencer Lecture Theatre

11.55 am	**Poster Session 1** – Redwood Building Rooms 0.60 & 0.86
12.40 pm	**Lunch**
Session 3: Chairperson – Karl Malcolm *(Queens University Belfast, UK)*	
1.40 pm	**Virginia Acha** *(Association of the British Pharmaceutical Industry)* – KEYNOTE SPEAKER Making the most of the biosimilars opportunity for healthcare in the UK
2.20 pm	**Edward Mansfield** *(University of Reading, UK)* Does the nature of a poly(2-oxazoline) coat affect the rate of nanoparticle diffusion through mucus?
2.35 pm	**Ivan Hall Barrientos** *(University of Strathclyde, UK)* Fabrication and characterization of drug-loaded electrospun polymeric nanofibers for controlled release in hernia repair
2.50 pm	**David Walsh** *(Royal College of Surgeons in Ireland, Ireland)* Controlled delivery of DNA from tissue engineered collagen scaffolds using novel, non-viral star-shaped polypeptides
3.05 pm	**Refreshments & Poster Session 2**
Session 4: Chairperson – Dimitrios Lamprou *(University of Stathclyde)*	
3.50 pm	**Sarah Mallen** *(University of Limerick, Ireland)* Oral delivery of nisin via mesoporous silica matrices
4.05 pm	**John Pollard** *(Aston University, UK)* Utilisation of an *in vitro* high-throughput screening assay in the development of orally disintegrating tablets with enhanced delivery capability
4.20 pm	**Daire O'Donnell** *(Dublin City University, Ireland)* Physicochemical characterisation tools aiding oral drug delivery technology optimisation
4.35 pm	Closing Remarks / Poster Prizes / Close of Meeting

KEYNOTE SPEAKERS

Virginia Acha

Virgina Acha is the Executive Director Research, Medical and Innovation and has responsibility for driving the agenda for innovation in the UK.

Dr Acha previously worked for Amgen as Director, Global Regulatory and R&D Policy – Europe, Middle East and Africa. She holds the chair for the Pharmacovigilance working group in the IFPMA Bio therapeutics Committee, and until recently was the Vice-Chair for the European Biopharmaceutical Enterprises (EBE) Biosimilars Task Force and the lead for the EFPIA Regulatory Network supporting Turkey.

Previously, she spent nearly five years at Pfizer working on policy development and engagement in a number of domains, including science, innovation and access and choice in healthcare.

Dr Acha entered the pharmaceutical industry after a decade in academia where she held posts on innovation strategy at Imperial College London Business School, the Science Policy Research Unit (SPRU) at the University of Sussex, the Centre for Research in Innovation Management (CENTRIM) at the University of Brighton, as well as a post-doctoral fellowship at London Business School. She is also a Visiting Researcher in the Innovation & Entrepreneurship Department at Imperial College Business School London.

KEYNOTE SPEAKERS

Arto Urtti

Arto Urtti received his Ph.D. degree in 1986 (University of Kuopio, Finland). He served as Associate Professor of Pharmaceutical Technology and professor of Biopharmaceutics at University of Kuopio.

Dr. Urtti has obtained international research experience as a post-doctoral fellow (Dept. of Pharmaceutical Chemistry, University of Kansas) and visiting professor (Dept. of Bio-Pharmaceutical Sciences, University of California San Francisco and Department of Pharmaceutics, University of Wisconsin).

Professor Urtti has led the Centre for Drug Research (previously DDTC – Drug Discovery and Development Technology Center) since 2005.

Professor Urtti's research programme is presented in about 220 peer-reviewed articles and 20 patents and patent applications.

Arto Urtti has received various scientific awards, including:

- Innovation Award
- American Association of Pharmaceutical Scientists Fellowship
- Honorary Membership of the Finnish Pharmacists' Association
- Albert Wuokko Prize
- Millennium Distinction Award.

He is the editor-in-chief of European Journal of Pharmaceutical Sciences and editorial member in many other journaals. Professor Urtti has evaluated grant applications for the scientific funding bodies of more than 10 countries and the European Union. Professor Urtti's main research fields are drug delivery (controlled release, computational and cell-based methods for ADME research) and nanotechnology (biomaterial structures for drug and gene targeting and for 3-d cell cultures).

ORAL ABSTRACTS

NOVEL POLYGLUTAMATE-BASED INDOCYANINE GREEN NANOPARTICLES FOR PHOTOTHERMAL CANCER THERAPY

1

Sam Tarassoli[1], Sandra Martinez de Pinillos[1], Halla Reinert[2], Hayley Pye[1], Alexander Mosse[1], John Callan[3], Sandy Macrobert[1], Anthony McHale[3] & Nikolitsa Nomikou[1]

[1] Division of Surgery & Interventional Science, University College London, UK; [2] UCL Cancer Institute, University College London, UK; [3] School of Pharmacy and Pharmaceutical Sciences, Ulster University, UK

Background: The application of external physical stimuli to elicit site-specific cytotoxicity and/or enhance the cellular uptake of drugs offers significant potential in cancer therapy. Near-infrared light (NIR) as a stimulus provides deep tissue penetration with minimal effect on tissues. Indocyanine green (ICG), the only FDA-approved NIR-absorbing agent, has attracted attention as it can be safely and efficiently used in tumour imaging, hyperthemic cancer ablation and laser light-induced drug release. However, the wider clinical use of ICG is still hindered by its low stability and poor biodistribution. In this study, we developed ICG-containing nanoparticles that are responsive to the elevated concentrations of cathepsin B in the tumour microenvironment. The photothermal effect of the ICG-containing nanoparticles on a head and neck cancer cell line was investigated and this effect was exploited for the lysosomal release of the cytotoxic protein saporin, under NIR laser light irradiation.

Methods: The enzymatic activity of cathepsin B on polyglutamate was examined by incubation of the mixture and subsequent gel electrophoresis to analyze hydrolysis products. The size of ICG-containing polyglutamate nanoparticles was characterized using DLS. Cellular uptake of ICG-containing nanoparticles was examined using SCC-9 cells and subsequent fluorescence spectrophotometry. The photothermal effect of ICG nanoparticles on SCC-9 cells, following irradiation with 805 nm laser light at 45 J/cm^2 total energy, was determined using a bioluminescence-based cell viability assay. Under these laser light exposure parameters, the photothermally triggered effect of saporin induced by the presence of ICG nanoparticles was further examined using fluorescence microscopy.

Results: Digestion of ICG nanoparticles with cathepsin B resulted in alteration of the mean particle size and a significant increase in ICG cellular uptake. ICG-nanoparticle uptake and toxicity in the absence laser light were minimal. Treatment with laser light resulted in 9%, 37% and 47% cell toxicity in the case of free ICG, ICG-containing nanoparticles and ICG-containing nanoparticles digested with cathepsin B, respectively. In the presence of saporin, the enzyme-digested formulation showed decreased cellular uptake and toxicity under light irradiation. In the case of the undigested nanoparticles, mean cell viability with light exposure was 48% in the presence of saporin and a synergistic effect of 11% in cell toxicity was revealed. On the basis of observations using fluorescence microscopy, the synergistic effect was attributed to the photothermally-induced lysosomal release of the cytotoxic saporin in the presence of undigested nanoparticles.

Conclusions: These results demonstrate that incorporation of ICG in the polyglutamate-based nanoparticles significantly increased light-induced cytotoxicity of the agent on SCC-9 cells. This effect was primarily a result of the improved cellular uptake of the undigested and digested nanoparticulate formulation. Cytotoxicity evaluation and intracellular distribution observations suggested a synergistic effect of NIR-triggered drug release from lysosomes in the presence of ICG-containing nanoparticles. The results suggest that this therapeutic approach offers significant potential for the locoregional treatment of head and neck cancers.

STUDY OF THE STRUCTURAL AND BARRIER PROPERTIES OF EQUINE SKIN FOR THE DEEP TISSUE DELIVERY OF PHARMACEUTICALS IN EQUINE THERAPY

2

Samuel Bizley[1], Roger Smith[1], Jayesh Dudhia[1] & Adrian Williams[2]

[1] The Royal Veterinary College, Department of Clinical Sciences and Services, Hatfield, AL97TA, UK;
[2] Pharmacy Department, University of Reading, Reading, RG66AD, Berkshire, UK.

Background: Background: In comparison to humans, limited characterization has been carried out on the barrier properties of equine skin. Topical drug delivery offers many advantages over routine oral or injectable routes; it is non-invasive and can allow local delivery of pharmaceutical, which avoids first pass metabolism and reduces systemic effects. Equine skin has been reported to differ structurally to human and some other animal tissues in terms of features, such as epidermal and dermal thicknesses and follicular density, though the impact of this on drug permeation across the skin barrier has been rarely discussed and so is the focus of this work.

Methods: Histology of 6 skin sites (distal forelimb, distal hind limb, inguinal region, flank, neck and croup) obtained from horse skin biopsies was carried out via standard cryostat processing, followed by H & E staining and mounting. Optical microscopy was used to identify structural features, image analysis (Image J) allowed epidermal/dermal thicknesses and follicular densities to be calculated. Permeability studies were carried out using static Franz diffusion cells with model hydrophobic (ibuprofen) and hydrophilic (caffeine) drug molecules applied from saturated solutions. Flux and lag times were accessed at each of the skin sites over 24 hours using RP-HPLC. Drug retained in tissue after 24 h was quantified using an extraction process of agitation in heated methanol followed by centrifugation, and analyzed using RP-HPLC.

Results: Full skin thicknesses varied from the thickest sections (croup 1.55mm) to the thinnest (inguinal region 0.60mm). Epidermal thicknesses did not directly correlate to full skin thickness with the thicker sections (croup and flank) showing a reduced proportion of epidermis. Follicular density also showed site to site variation; neck had a high density of small follicles (<0.01 mm^2) compared to the croup with a small density of larger follicles (>0.02 mm^2), though the overall area covered by the hair follicles were similar. Permeability studies showed that whilst both small hydrophobic and hydrophilic drug molecules could permeate through full thickness skin, the hydrophobic molecule had longer lag times compared to the hydrophilic molecule (4.3 h vs <1 h for the flank site) with generally lower fluxes (0.12 µg cm^{-2} min^{-1} vs 4.12 µg cm^{-2} min^{-1}). This was partially attributed to increased retention in the dermal layers where >10% of applied dose was retained.

Conclusions: Structural differences have been identified between equine skin and more routinely studied skin in other species. Site to site variation has also been noted which will help identify suitable application areas for topical delivery. A suitable permeability assay has been developed which allows quantification of drug penetration through equine skin. Comparison of hydrophobic and hydrophilic drug diffusion has shown marked differences in terms of the lag times and fluxes of molecules, with greater retention of hydrophobic molecules within the tissue. Identifying key fundamental parameters will help to develop rational formulation strategies for topical equine therapies.

AMINO ACID FACILITATED DELIVERY OF INSULIN THROUGH BUCCAL CELL LAYERS IN VITRO

3

Affiong Iyire, Maryam Alayedi & Afzal Mohammed

Aston Pharmacy School, Aston University, Birmingham, B4 7ET, UK

Background: The primary driving factor for investigation of non-invasive routes for the delivery of biologicals is built on reducing/removing the need for single/multiple daily injections, which puts a major strain on patient compliance and desired therapeutic outcomes. The aim of this work was to investigate the effect of basic and acidic amino acids on the physicochemical and biological properties of insulin in order to provide alternative safe and effective excipients that enhance buccal insulin delivery, probably by triggering alternative transport mechanisms.

Methods: Solubility of insulin in deionized water and HBSS was established at 25 °C in the presence and absence of model basic and acidic amino acids; and Log P values for insulin determined using octanol and deionized water. Thereafter insulin permeability through TR146 buccal cell layers was assessed in the presence of increasing concentrations of basic (arginine, lysine & histidine) and acidic (aspartic acid & glutamic acid) amino acids; and the surfactant Na deoxycholate. Routes and mechanisms of this transport were investigated using confocal laser scanning microscopy and vectoral transport at suboptimal temperatures.

Results: Basic amino acids were able to significantly improve insulin solubility in water while 200 and 400 µg/ml lysine significantly increased insulin solubility in HBSS. The presence of amino acids was able to increase the lipophilicity of insulin and lower its log P by 0.7 units. However, despite this reduced lipophilicity, amino acids were able to effectively and safely enhance permeation of insulin across TR146 cell layers significantly, pointing to the presence of an active carrier transport system. Permeability data showed a significant improvement in insulin permeation especially for 10 µg/ml of lysine ($p < 0.05$) and histidine 10 µg/ml ($p < 0.001$), 100 µg/ml of glutamic acid ($p < 0.05$) and 200 µg/ml of glutamic acid and aspartic acid ($p < 0.001$) without affecting cell integrity; in contrast to sodium deoxycholate which enhanced insulin permeability but was toxic to the cells. It was hypothesized that at buccal cavity pH, both amino acids and insulin were ionised and able to form stable ion pairs which penetrated through the cells as one entity. Interestingly, investigating the mechanism and route of insulin permeation using increasing concentrations of insulin at suboptimal temperatures, reversed transport direction and confocal microscopy revealed that insulin was transported by an active transcellular process probably provided by the presence of insulin receptors and amino acid nutrient transporters on the cell membrane.

Conclusions: This result obtained for insulin is the first indication of a possible amino acid mediated transport of insulin via formation of insulin-amino acid neutral complexes by the ion pairing mechanism. Amino acids were therefore established as safer, effective and novel penetration enhancers than the commonly used surfactant for insulin using the ion-pairing mechanism.

THIOL-BASED LABELLING OF PROSTATE-DERIVED EXOSOMES FOR ANALYSIS OF CELLULAR UPTAKE AND INTRACELLULAR TRAFFIC

4

Hope Daphne Roberts-Dalton[1], Edel Brown[1,2], Jason Webber[2], Pete Watson[3], Aled Clayton[2] & Arwyn Tomos Jones[1]

[1] Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, CF10 3NB,
[2] Division of Cancer & Genetics, Cardiff University, Velindre Cancer Centre, Cardiff, CF14 2TL,
[3] School of Biosciences, Cardiff University, Cardiff, CF10 3AX

Background: Association of macromolecular therapeutics to vectors that increase cell uptake and protect from extracellular and intracellular degradation is a vital step in the creation of more efficient drug delivery systems. A major goal of these systems is the transportation of cell impermeable macromolecular therapeutics, such as nucleotides, proteins and peptides to the insides of cells. More effective delivery vectors than those currently available are needed to meet the challenge of overcoming diseases such as cancer. Exosomes are extracellularly expelled components of the endocytic system that are often naturally loaded with a variety of macromolecules; some are the very entities that show promise as biopharmaceuticals. They may therefore be viewed as natural vectors capable of delivering signal mediators and macromolecular cargo, and could be manipulated as vectors for therapeutic applications. How exosomes interact with cells and deliver cargo, however, is not fully understood. Cellular uptake of exosomes is typically studied following labelling with fluorescent lipids such as PKH dyes that embed themselves in the exosome lipid bilayer. This may modify their structure and function and thus new labeling systems are needed. The aims of this project were to develop new exosome labelling methods and high-content endocytosis assays to assess their cell interaction, internalization and intracellular traffic in the endolysosomal system.

Methods: Here we describe a thiol-based conjugation method for the attachment of Alexa488 to well characterised prostate cancer (Du145) exosomes. The involvement of different endocytic pathways in HeLa cell uptake was investigated using live cell confocal microscopy of Alexa488-exosomes in cells transfected with siRNA targeting specific endocytic proteins and thus endocytic pathways, together with pharmacological inhibitors of endocytosis.

Results: Alexa488-exosomes retained their function (to differentiate fibroblasts to myofibroblasts), and were effectively internalised into fibroblasts and HeLa cells as highly motile punctate structures throughout the cytoplasm. Depletion of key endocytic proteins via siRNA transfection indicated that proteins implicated in macropinocytosis (a growth-factor activated, actin-dependent form of constitutive fluid-phase endocytosis) may be involved in the uptake of these structures. The inhibitor studies in HeLa cells also highlighted actin-dependent macropinocytosis as a major pathway mediating cell uptake suggesting that exosomes may be activating their own cellular internalisation.

Conclusions: The use of this novel labelling technique in parallel with in vitro endocytosis assays will help characterise how exosomes gain intracellular access to mediate their effects and allow for further assessment of their potential as drug delivery vectors. Complete characterisation of the ways that exosomes gain intracellular access will help to unlock this potential for drug delivery, allowing their exploitation for therapeutic use.

REAL-TIME IMAGING AND ANTIOXIDANT ACTIVITY OF HPMC–COATED CERIUM OXIDE NANOPARTICLES: A NEW MODALITY FOR CATARACTS

5

Ali Al-kinani, Swapn Patel & Raid Alany

School of Life Sciences, Pharmacy and Chemistry, Kingston University London, Kingston Upon Thames, KT1 2EE, United Kingdom.

Background: Cloudiness in the lens of the eye due to oxidative stress is a major cause of cataract which could be thwarted by administering antioxidants. Cerium oxide, due to its ability to switch between two valance states, acts as an antioxidant. L-2-Oxothiazolidine-4-carboxylic acid (OTZ) coverts enzymatically to cysteine an important lens antioxidants. In this study, hydroxypropyl methylcellulose (HPMC) coated cerium oxide nanoparticles (CNPs), loaded with OTZ, were formulated to synergistically deliver two antioxidants to the eye for the cataract prophylaxis.

Methods: CNPs were synthesised then coated with HPMC. OTZ was entrapped in the fabricated nanoparticles which were characterised for particle size and zeta potential. HPMC coating of the CNPs was investigated using FT-IR, XRD and Raman spectroscopy. Radical scavenging properties of CNPs against super oxide anions (O-2) were studied in a bovine epithelial lens cells. The in-vitro release mechanism of OTZ from the CNPs and ex-vivo permeation of OTZ through excised bovine sclera/cornea was investigated. The Sulforhodamine B colorimetric (SRB) and Neutral Red uptake (NRU) assays were used to assess the cytotoxicity of CNPs to human lens epithelium cell line (HLEC). The effect of CNPs on HLEC was monitored using real time imaging technology; this allows monitoring the cell morphology and shape in addition to calculating the cell growth in real time. The GSH levels in human lens epithelium cell line were measured before and after the administration of CNPs loaded with OTZ.

Results: CNPs particles size was 15±1.19nm. The X-ray diffractograms of coated nanoceria confirmed the formation of an HPMC coat. FT-IR spectra further confirmed the presence of polymer coating on the surface of the nanoceria by showing peaks at 2900 and 1330cm^{-1} representing hydroxypropyl and epoxy groups of HPMC respectively. Cerium oxide nanoparticles showed zeta potential of -1.67± 2.07mV (with HPMC) and -34.67 ± 2.33mV without HPMC coating. Raman spectrum of coated nanoceria displayed a characteristic peak near 462cm-1, which is usually assigned to T2g mode of Ce-O vibrational unit with (OH) symmetry. In-vitro release studies demonstrated a sustained release with 61.90 ± 0.83% of loaded OTZ was released over 5 h. High coefficient of correlation (R2= 0.94) obtained with data fitted to the Higuchi model indicated diffusion controlled release. The ex-vivo permeation studies revealed that the scleral permeation of OTZ is 10 times higher than the corneal permeation. Bovine lens cells demonstrated bright red color indicating the formation of superoxide (O_2^-) in the bovine epithelial lens cells. The same cells emitted a blue color upon treatment with CPNs. This indicates that the cerium oxide has captured the (O-2). The prepared CNPs showed no sign of cell toxicity on HLEC at the used concentration range over 24 h treatment period, suggesting that the prepared CNPs were well tolerated by HLEC. Real time imaging results and cell growth show that CNPs had no significant effect on the cell line used, the cell shape and morphology was normal compared with the negative control. The results are in agreement with those obtained from the SRB and NRU assays. A significant increase in GSH levels was observed in HLEC after treatment with CNPs loaded with OTZ.

Conclusions: HPMC coated-CNPs have been formulated loaded with and controlled the in-vitro release and ex-vivo permeation of OTZ. They demonstrated synergistic antioxidant activity and were well tolerated by the ocular tissues; they could be further investigated for cataract prophylaxis.

DOES THE NATURE OF A POLY(2-OXAZOLINE) COAT AFFECT THE RATE OF NANOPARTICLE DIFFUSION THROUGH MUCUS?

6

E. Mansfield[1], V.R.de la Rosa[2], R. Hoogenboom[2], K. Sillence3, P. Hole[3], A.C. Williams1, V.V. Khutoryanskiy[1]

[1] School of Pharmacy, University of Reading, UK; [2] Supramolecular Chemistry group, Department of Organic and Macromolecular Chemistry, Krijgslaan 281 S4, B-9000 Ghent, Belgium; [3] Malvern Instruments Ltd, Wiltshire, UK

Background: Nanoparticles are becoming increasingly important in the field of drug delivery, as they can rapidly cross biological barriers. However, due to specific surface properties, some nanoparticles can become trapped in the gastric mucosa. Previously (Nanoscale 2015, 7, 13671-13679) it has been shown that by functionalising the surface of nanoparticles with poly(ethylene glycol) or poly(2-ethyl-2-oxazoline) it is possible to reduce these interactions, thus making a more diffusive particle. Here we explore how the nature of the side-chain in poly(2-oxazoline)'s affects the rate of nanoparticle diffusion.

Methods: Thiolated silica nanoparticles were synthesised by reacting 0.5 mL NaOH (0.5 M) with 0.75 mL 3-mercaptopropyltrimethoxysilane in 20 mL of DMSO for 24 hours, and purified using dialysis. The resulting 50 nm particles were then functionalised with poly(2-methyl-2- oxazoline) (PMOZ), poly(2-ethyl-2-oxazoline) (PEOZ), or poly(2-n-propyl-2-oxazoline) (PNPOZ) and fluorescently labeled with Alexa546 maleimide, or fluorescein -O-methacrylate. Following synthesis and functionalisation, all particles were characterised for size, using DLS and NTA, and functionality using FTIR and Raman spectroscopy. Diffusion studies were carried out using two techniques; particle tracking, using Nanoparticle Tracking Analysis (Malvern, UK), and fluorescence microscopic techniques. For NTA, Alexa-labeled particles were diluted into porcine gastric mucin type II (Sigma), and their diffusion monitored over 6x60 second videos for each reading from which their diffusion coefficients were determined. For microscopic analyses, fluorescein labeled particles were applied from aqueous suspensions onto the surface of fresh porcine mucosal membrane and left at 37 °C for different time periods (0, 15, 30, 45, and 60 minutes). Tissue sections were then fixed in OCT, stored on dry ice, and sectioned on a cryostat. Images were taken on a Leica MZ10F stereomicroscope, and analysed using ImageJ in order to evaluate the degree of penetration for each type of particle into the membrane.

Results: The DLS and NTA showed that the particle size increased from 52±1 (unfunctionalised silica) to 60±3 nm for POZylated silica. This, in combination with a decrease in thiol groups content (Ellman's assay and Raman spectroscopy), and the emergence of peaks in the IR spectra representing POZ, show that the particles were successfully functionalised. The diffusion studies showed that PMOZ-silica was significantly more diffusive in gastric mucin than PEOZ, PNPOZ, or the unfunctionalised silica. This trend confirmed in the penetration study, where images showed that PMOZ nanoparticles penetrated into the tissue to greater levels that seen for the other particles. Previously we have shown that PEOZ is a good enhancer of nanoparticle diffusion, but the data here shows that the methyl derivative enhances nanoparticle mobility to a greater extent.

Conclusions: The work presented here shows that the nature of a polymer coat affects the diffusion of functionalised nanomaterials through mucus. This is an important finding for the design of novel drug delivery systems for transmucosal administration.

FABRICATION AND CHARACTERIZATION OF DRUG-LOADED ELECTROSPUN POLYMERIC NANOFIBERS FOR CONTROLLED RELEASE IN HERNIA REPAIR

7

Ivan J. Hall Barrientos[1,2], Eleonora Paladino[2,3], Sarah Brozio[2], Richard A. Black[1], Clive G. Wilson[2] & Dimitrios A. Lamprou[2,3]

[1] Biomedical Engineering, University of Strathclyde, Glasgow, UK; [2] Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, UK; 3EPSRC Centre for Innovative Manufacturing in Continuous Manufacturing and Crystallisation, University of Strathclyde, Glasgow, UK

Background: Hernia repair is one of the most common operations that a general surgeon will perform, but not without its complications. The mechanical properties of the mesh and the compatibility between the materials and the abdominal wall layers are critical in healing. This is especially important if the risk of graft failure is high and complications due to infection are experienced. This stimulates development in new methods of fabrication incorporating biomaterials and the study of drug encapsulation and drug release in these novel mesh matrices. The purpose of the present study is to examine any potential effects, chemical and mechanically, of collagen (type I) with drug-loaded electrospun nanofiber scaffolds. Polycaprolactone, a biodegradable polyester commonly used in biomedical applications for controlled release and targeted drug delivery, was loaded and electrospun with/without collagen and two different types of drugs (triclosan or levofloxacin) in order to investigate bioavailability and drug release.

Methods: Electrospinning of solutions of varying concentrations of type I collagen, with PCL and irgasan (IRG) or levofloxacin (LEVO) was conducted. The fibres produced were then characterised with regard to fibre size and morphology using scanning electron microscopy (SEM) and atomic force microscopy (AFM). Further physicochemical examinations were conducted to determine mechanical behaviour, and surface and biological properties were examined using contact angle goniometry (CAG), Raman spectroscopy, time-of-flight secondary ion mass spectrometry (ToF-SIMS), cell assay studies, anti-bacterial studies, Brunauer-Emmett-Teller (BET) and in vitro drug release measurements (T3).

Results: Imaging of the collagen-PCL-drug fibres indicated that composition caused a difference on morphology, and a decrease in fibre diameter due to the addition of collagen. The rheological examination indicated that the addition of both IRG and LEVO increased the linear viscoelastic regions for the solutions, suggesting a dispersed and stable polymer-drug system. The release of irgasan from the PCL-IRG scaffold followed a sustained release time profile. The IRG reached a plateau of around 70% released after 140 h. The PCL-LEVO scaffold exhibited a burst release behaviour– the antibiotic was almost entirely released within the first 15 min. The antibacterial studies confirmed a high efficacy inhibiting the growth of E. coli and S. aureus, and also demonstrated a sustain release with PCL-IRG due the decreased inhibition zones.

Conclusions: This research successfully demonstrated that drug-loaded electrospun scaffolds can be fabricated, with the ability to release the antibiotic load at a high rate, and inhibit bacteria such as E. coli and S. aureus. Further work is now undertaken to examine the effect of the drugs on polymeric structure, as the rheological data were indicative of changes in viscoelastic behaviour.

CONTROLLED DELIVERY OF DNA FROM TISSUE ENGINEERED COLLAGEN SCAFFOLDS USING NOVEL, NON-VIRAL STAR-SHAPED POLYPEPTIDES

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David Walsh[1,2,3,5], Robert Murphy[4], Joanne Ramsey[1,3,5], Andreas Heise[4,5], Fergal O'Brien[1,2,5], Sally-Ann Cryan[1,2,3,5]

[1] Tissue Engineering Research Group, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland; [2] Trinity Centre for Bioengineering, Trinity College Dublin, Dublin, Ireland; [3] School of Pharmacy, RCSI; [4] Department of Medicinal and Pharmaceutical Chemistry, RCSI; [5] Centre for Research in Medical Devices (CURAM), RCSI.

Background: Star-shaped poly (L-lysine) polypeptides (star-PLLs) represent attractive, bio-derived non-viral vectors with an inherent ability to complex and condense pDNA into nano-sized cationic complexes. Approximately 2.2 million bone graft surgeries are performed each year with autologous bone harvesting representing the current gold standard of therapy despite recognized limitations. Within our group, highly optimized collagen scaffolds have been loaded with nucleic acid molecules thereby enhancing bone tissue regeneration. Such loaded constructs have been termed a Gene Activated Matrix (GAM). This study aims to develop a next generation GAM via the incorporation of bio-inspired star-PLLs loaded with the therapeutic genes Bone Morphogenetic Protein-2 (BMP-2) and Vascular Endothelial Growth Factor (VEGF) into these scaffolds, thereby facilitating controlled release and expression of the transgene with an associated increase in tissue regeneration.

Methods: Star-PLLs, manufactured with varying core generation and number of polymeric arms were characterized utilizing a combination of nanoparticle tracking analysis, zeta potential measurement and MTT cell viability assays. Collagen scaffolds were fabricated via a well-established freeze-drying process. Star-PLLs complexed with two reporter plasmids, luciferase (pGLuc) and green fluorescent protein (pGFP) as well as the therapeutic plasmids BMP-2 & VEGF were utilized to determine transfection capability of lead star-PLLs in 2D Mesenchymal Stem Cell (MSC) monolayer. Finally, lead star-PLL-pDNA polyplexes were soak loaded onto collagen scaffolds and utilized to transfect MSCs in 3D using pGLuc & BMP-2 thereby forming a functional GAM. Three control vectors, Superfect®, Polyethyleneimine (PEI) and linear-poly-L-(lysine) (L-PLL) were utilized throughout.

Results: Three star-PLLs (01,02 & 03) were utilized to successfully prepare star-PLL-pGLuc polyplexes of varying N/P ratios forming nano-sized cationic complexes from N/P ratios 2-100. Cell viability assays determined a significant decrease in cell viability above N/P10. Transfection of monolayer MSCs identified star-PLL (03) at N/P5 with a 2µg pDNA cargo as a lead non-viral delivery system (size 190.2±42.4nm, charge +30mV, transfection efficiency 23%). Star-PLL 03 was capable of producing nanogram quantities of BMP-2 & VEGF proteins in 2D. 28 day 3D transfection studies demonstrated that this lead star-PLL resulted in a prolonged 28 day expression profile which was statistically better than L-PLL ($P<0.01$).

Conclusions: The N/P ratio of the formulation was the most important factor in determining the resultant polyplex size, charge, cell viability and transfection capability in both 2D and 3D environments. A single lead system from a potential 96 formulations was identified as star-PLL 03 N/P5 2ug pDNA. This formulation was capable of efficiently delivering both reporter (pGFP & pGLuc) and therapeutic (VEGF & BMP-2) plasmids. Star-PLLs demonstrated transfection capability in a 3D environment with a prolonged expression profile of 28 days which is highly desirable in the field of tissue engineering.

ORAL DELIVERY OF NISIN VIA MESOPOROUS SILICA MATRICES

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Sarah Mallen[1,2], Jakki Cooney[2,3] & Sarah P. Hudson[1,3]

[1] Department of Chemical and Environmental Sciences, Synthesis and Solid State Pharmaceutical Centre, [2] Department of Life Sciences, [3] Materials and Surface Science Institute, University of Limerick, Ireland.

Background: Bacteriocins are peptides produced by one bacterial species to inhibit another and could potentially be the next line of antibiotics in the battle against resistance. To date, bacteriocins have not been routinely developed into new antibacterial therapies. The main reason for this lack of exploitation is the poor stability of these entities in the hostile host environment. Therefore, good delivery systems are needed to protect peptides from enzymatic, hydrolytic or thermal degradation without altering their biological activity, while also controlling the rate of release over time to treat persistent infections. This study aims to deliver peptides to the gut, where pathogens such as C. difficile cause infections. A model peptide, nisin, which is a 34-residue, cationic bacteriocin, with broad spectrum activity including against C. difficile, is used to assess the viability of mesoporous silicates as the delivery matrix. Nisin is adsorbed into the mesoporous silicate matrix in an attempt to prevent degradation of nisin by proteases and provide controlled release at the site of infection, i.e. the gut.

Methods: The dimensions of nisin, trypsin and α-chymotrypsin were determined using PYMOL, a molecular visualization tool. The solubility of nisin was determined by adding an excess of nisin to aqueous buffers at pH 2, 5 and 7 which were stirred for 24 h at 37 °C. Antimicrobial activity assays were carried out using Lactobacillus delbrueckii subsp. bulgaricus as an indicator strain. Nisin concentrations were determined by HPLC using a gradient method (buffer A (0.1% TFA) and buffer B (ACN with 0.1% TFA)). Mesoporous silica (SBA-15) was synthesized by condensation of a silica precursor (tetraethoxysilane) around the micelles of a surfactant (P123) followed by removal of the surfactant by Soxhlet extraction. SBA-15 was characterized by X-ray diffraction, nitrogen adsorption analysis and scanning and transmission electron microscopy. Adsorption of nisin onto SBA-15 was carried out at pH 3, 4, 5 and 7. The supernatant was analysed for nisin content by HPLC. Enzyme degradation of free nisin and nisin adsorbed onto SBA-15 was determined by pre-incubating nisin with trypsin and α-chymotrypsin before carrying out an antimicrobial activity assay.

Results: SBA-15 had a surface area of 578 m^2/g, an ordered hexagonal pore system and an isoelectric point of 3.9. SBA-15 particles were rod shaped with a length of ~2 µm, a width of less than 1 µm and an average pore diameter of ~7 nm. Nisin is a long peptide, ~5 nm in length but less than 2 nm at its widest point. It is most soluble at pH 2 (30 mg/mL). Solubility falls to ~10 mg/mL at pH 7. A MIC of ~25 µg/mL against L. bulgaricus was found. After incubation with trypsin and α-chymotrypsin for 3 h at pH 8, nisin exhibited approximately 50% and 25% loss activity respectively. The optimum pH for adsorption of nisin onto SBA-15 was found to be pH 4 and loadings of 77 µg/mg of SBA-15 were obtained. Nisin retained antimicrobial activity following adsorption onto SBA-15. However, SBA-15 did not protect nisin from enzyme degradation by trypsin or chymotrypsin.

Conclusions: Nisin can successfully be adsorbed onto SBA-15 at pH 3, 4, 5 and 7 with maximum adsorption at pH 4. While SBA-15 did not protect nisin against protease degradation, activity against L. bulgaricus was retained after adsorption. Mesoporous silicates with a smaller pore size, e.g. MCM-41 (pore size of ~2.8 nm), are currently being investigated for protection of nisin from proteases. Nisin may fit lengthwise into the pores of MCM-41 while the trypsin (spherical diameter of ~4 nm) and α-chymotrypsin (spherical diameter of ~4-5 nm) should not be able to enter the pores.

UTILISATION OF AN IN VITRO HIGH-THROUGHPUT SCREENING ASSAY IN THE DEVELOPMENT OF ORALLY DISINTEGRATING TABLETS WITH ENHANCED DELIVERY CAPABILITY

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John Pollard[1], Ali Rajabi-Siahboomi[2], Raj Badhan[1], Afzal Mohammed[1] & Yvonne Perrie[1]

[1] Drug Delivery Group, School of Life and Health Sciences, Aston University, Birmingham, B4 7ET, UK; [2] Colorcon, Harleysville, PA, 19438, USA

Background: Following a high-throughput screening (HTS) program to identify and quantify the biological effects of commonly used excipients against P-glycoprotein (P-gp, ABCB1), the following study focuses on our attempts to produce an orally disintegrating tablet (ODT) formulation with the potential for enhanced delivery of drugs which are efflux substrates.

Methods: Caco-2 cells known to express the ABCB1 transporter were grown for 7 days on 96 well plates in the absence of antibiotics, with media renewed every 48 h. ABCB1 function was then assessed in the presence of the specific fluorescence probe Rhodamine 123 (R-123, 2.5 µM) with various concentrations of test excipients, 24 in total. The quantity of excipient required to reduce transporter activity by 50 % (IC50) was determined using sigmoidal curves plotted on GraphPad Prism 6.0 software. The lead candidate, Pluronic F127, was then selected for formulation studies initially at 10 % weight (295 mg convex tablets, 1 MT compression). Further parameters were examined including superdisintegrant type, ethyl cellulose content, and composition of the multifunctional excipients Starch 1500 and StarCap 1500.

Results: Using the HTS assay, the IC50 of Pluronic F127 was determined to be 9.77 x 10^{-4} % (w/v), roughly equivalent to 0.79 µM, and so was chosen for incorporation in to OTDs. Various grades of Kollidon® superdisintegrant were examined (CL and CL-F) on the initial tablet formulation, and the CL-F grade was found to be superior to the CL grade in terms of both increased hardness and decreased disintegration times. Ethyl cellulose, incorporated as a binder, was found to increase hardness although with an increased disintegration time, and was optimised at 2.5 % weight. Next, the multifunctional excipient Starch 1500 was evaluated against a material of similar composition, StarCap 1500, and the latter proved superior in terms of hardness and disintegration times. The level of Pluronic was then optimised at 2 %. At this middle point of the development phase, the current lead formulation has a disintegration time of 30 sec, conforming to the regulations outlined by the USP.

Conclusions: This work combines an in vitro screening method with the development of an ODT platform. It is expected that the formulation outlined herein will be able to realise the potential of an enhanced delivery system designed with the purpose of increasing the oral uptake of drugs which fall victim to efflux.

PHYSICOCHEMICAL CHARACTERISATION TOOLS AIDING ORAL DRUG DELIVERY TECHNOLOGY OPTIMISATION

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Dáire O'Donnell [1,2], Ivan Coulter[1], Anne Shanahan[2], Mónica Rosa[1], Hugh J.Byrne[2]

[1] Sigmoid Pharma Ltd., The Invent Centre, Dublin City University, Dublin 9, Ireland
[2] FOCAS Research Institute, Dublin Institute of Technology, Camden Row, Dublin 8, Ireland

Background: SmPill® is an innovative drug delivery platform developed by Sigmoid Pharma applicable to a wide range of Biopharmaceutics Classification System (BCS) class drugs. SmPill® enables the oral delivery of a pre-solubilised Active Pharmaceutical Ingredient (API) in an integrative manner, helping overcome formulation issues such as solubility, permeability, stability and targeting. SmPill® takes the form of seamless minispheres formed by extrusion process of an oil in water emulsion. In order to perform an in depth characterisation of the technology a range of physico-chemical characterisation techniques were utilized, namely, Optical Microscopy, Scanning Electron Microscopy (SEM), Dynamic Light Scattering (DLS), Fourier Transform Infrared (FTIR) and Raman Microspectroscopy coupled with chemometric analysis. Emulsion quality investigation and coating film analysis were performed. Oil droplet size measurements within the finished SmPill® minisphere and when performing dissolution testing were also investigated. Information on emulsion component spatial distribution on the cross sectioned SmPill® surface was collected using chemometric analysis. This study demonstrates that the use of physical chemical characterization tools can aid in oral drug delivery technology assessment and optimization.

Methods: Emulsion Optimisation: Optical microscopy enabled emulsion quality assessment (e.g. component precipitation) in early stage formulation development. Spectroscopy, both FTIR and Raman, enabled precipitate and optimal emulsion component identification. Droplet Size Characterisation: Analysis of emulsion droplet size formed by various formulations and released during SmPill® dissolution testing was performed using DLS. SEM images of SmPill® minispheres taken during dissolution testing were captured to determine the mechanism of release from the applied SmPill® delayed release coating. Spectral Imaging and Chemometric Analysis: FTIR and Raman spectral mapping of the SmPill® cross section and chemometric analysis to visualise the chemical component distribution across the SmPill® cross sectional surface was carried out to aid optimal formulation development.

Results: A number of physical-chemical characterization tools were utilised to support the optimization of SmPill®-based emulsions. Changes in the formulations like incorporation of new excipients, were evaluated using these tools. Oil droplet size released by original and optimised formulations was measured, highlighting a difference between both. Improvements on emulsion quality and drug dissolution profile were observed. Chemometric analysis of FTIR and Raman spectral datasets indicated the bulk matrix biopolymer was preventing the visualisation of the minor SmPill® components; further work is required to assess the possibility of overcoming this technical difficulty encountered.

Conclusions: Physical-chemical characterization tools can be very useful during oral drug delivery technology development and optimisation. In this work, a number of characterisation techniques were used at different stages of the delivery system manufacturing process to facilitate technology optimisation. Oil droplet size analysis has been shown to allow early screening of formulations and ensure the desired emulsion quality is achieved. Chemometric data analysis of the FTIR and Raman data sets highlighted inherent issues in the characterization of the SmPill® technology, particularly regarding the identification of minority components. Further work is required to be done to investigate this finding.

POSTER ABSTRACTS

ENHANCED CRYOPRESERVATION OF PLACENTAL EXTRACELLULAR VESICLES USING ICE RECRYSTALLIZATION INHIBITING POLYMERS

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Alexandra Burdujan[1], Vuyane Mhlomi[1], Wei Zhang[1], Carolina Motta Mejia[1], Neva Kandzija[1], Gavin Collett[1], Robert Deller[2], Matthew Gibson[3] & Manu Vatish[1]

[1] Nuffield Department of Obstetrics & Gynaecology , University of Oxford, Oxford, OX3 9DU, UK; [2] School of Cellular & Molecular Medicine, University of Bristol, Bristol, BS8 1TD, UK; [3] Department of Chemistry, University of Warwick, Coventry CV4 7AL, UK

Background: Human Placental Extracellular Vesicles (PEVs) are released by the feto-placental unit into the maternal circulation and permit extracellular communication. Enhanced release of PEVs occurs in diseases such as preeclampsia (a major cause of maternal morbidity and mortality). Characterizing PEVs as potential biomarkers for disease or therapeutic targets is attractive since they allow insight into placental function via a peripheral blood sample. Current storage methods result in conformational changes to PEV structure which has important implications for downstream analysis. We attempted to use a previously reported polymer PolyVinyl Alcohol (PVA) to stabilize PEV membranes prior to cryopreservation in order to maintain their conformational state.

Methods: PEVs were isolated using Dual Lobe Placental Perfusion and differential centrifugation. PEVs were immediately characterized by Flow Cytometry (FACS) and Nano-Sight (NTA). PEVs were frozen either as standard (in PBS) or in 0.4 mg/mL or 0.6 mg/mL of PVA. All samples were cryopreserved at –80 °C. Upon recovery/thawing PEVs were re-characterized using FACS/NTA as previously described and findings pre and post cryopreservation were compared.

Results: Analysis of PEVs cryopreserved according to standard protocols revealed significant alterations in the expression of known placental markers, suggesting the cryopreservation process resulted in membrane fracture with subsequent reforming of vesicles. PVA treated PEVs showed pre-cryopreservation FACS/NTA characteristics suggesting they had retained their original conformation.

Conclusions: Use of PVA in cryopreservation of extracellular vesicles may allow their conformation to remain similar to the freshly isolated state. This approach may assist future attempts at characterizing PEV function.

THE DEVELOPMENT OF A TEMPERATURE RESPONSIVE HYDROGEL FOR THE DELIVERY OF LIPOPHILIC MOLECULES AND STEM CELLS TO THE DISTAL AIRWAYS

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Christina Payne[1,2], Sally-Ann Cryan[1,2,3], Helena M. Kelly[1,2]

[1] School of Pharmacy, Royal College of Surgeons in Ireland, Dublin, Ireland; [2] Tissue Engineering Research Group (TERG) Royal College of Surgeons in Ireland, Dublin, Ireland; [3] Centre for Research in Medical Devices (CÚRAM), National University of Ireland, Galway, Ireland

Background: Limitations associated with current treatment of chronic obstructive pulmonary disease has resulted in the need for new and efficient systems to enable delivery of therapies to, and extend the duration of action at, damaged pulmonary alveolar tissue. To address this need, a novel methylcellulose (MC) based thermoresponsive hydrogel has been developed, which has the potential to act as both a cell support matrix and a drug delivery system. This thermoresponsive hydrogel system is being explored for its utility in relation to both human mesenchymal stem cell (hMSC) and drug delivery with a view to supporting airway regeneration. The drug molecule being assessed for delivery is all-trans Retinoic Acid (atRA), which has shown potential in alveolar regeneration. Due to the poor water solubility of atRA, solid lipid nanoparticles (SLNs) were prepared for encapsulation of the molecule, for subsequent loading into the hydrogel.

Methods: A MC, collagen and beta glycerophosphate (βGP) hydrogel was formulated using established methods. Initial characterisation was performed via rheological analysis, gel disintegration and diffusion testing. Mechanical "pushability" testing of the gel through a syringe, needle, and catheter was investigated. atRA-SLNs were formulated and optimised based on particle size, zeta potential, polydispersity index and encapsulation efficiency. atRA-SLNs were imaged using transmission electron microscopy (TEM). atRA release kinetics from the SLNs was determined via HPLC. Both the gel formulation and the atRA-SLNs were assessed for their ability to sustain viability and proliferation of hMSCs. Preliminary assessment of the immunomodulatory effect of the atRA-SLN formulation on a human respiratory cell line (A549) in vitro was determined by ELISA.

Results: MC is able to form a thermoresponsive hydrogel in combination with collagen and βGP which undergoes sol-gel transition at approx. 37 °C, and demonstrates a strong 3-dimensional structure. "Pushability" testing of the gel showed the gel can be delivered through a range of devices. In vitro cell studies showed the gel is capable of supporting hMSC survival and growth over a 14-day time period. atRA-loaded SLNs were 150–200 nm in size, had a zeta potential of -19.25 mV and polydispersity index of 0.28. Encapsulation efficiency was 43.82 ± 2.87%. atRA release from SLNs was sustained over a 24-hour time period. Cell viability and proliferation studies showed the atRA-SLNs are capable of supporting hMSC survival and growth, and initial assessment of immunomodulatory effects shows an increase in anti-inflammatory IL-10 levels.

Conclusions: We have demonstrated that a MC/collagen/βGP gel displays a suitable thermoresponsive profile for use as a delivery vehicle for drugs or stem cells into the body. We have also shown that all-trans retinoic acid (atRA) can be encapsulated within SLNs for incorporation into these gels. Results to date indicate the potential therapeutic applicability of these formulations in vivo.

OPTIMISATION OF THE ENCAPSULATION EFFICIENCY OF SELENOMETHIONINE LOADED CHITOSAN ZEIN NANOPARTICLES

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Giuliana Vozza[1,2], Minna Khalid[1,2], Hugh J. Byrne[2], Sinéad M. Ryan[3], Jesus M. Frias[1]

[1] School of Food Science and Environmental Health, Dublin Institute of Technology, Cathal Brugha Street, Dublin; [2] FOCAS Research Institute, Dublin Institute of Technology, Camden Row, Dublin 8;
3 School of Veterinary Medicine, University College Dublin, Belfield, Dublin.

Background: Selenium (Se) is an essential micronutrient both in human and animal nutrition. The putative health benefits of Se administered at an optimum concentration include cancer prevention, increased immunological status and increased fertility. As a result of diverse geological conditions, the Se status of humans varies considerably across the globe. This is why the current recommended daily amount (RDA) for Se has a broad range of 70–350 µg (World Health Organisation). In many parts of the world, average consumption of Se is less than the lower nutritional recommendation level of 40 µg/day. Dietary Se supplementation is therefore challenging due to a narrow therapeutic index range, low bioavailability and a high susceptibility to oxidation. A Selenomethionine (SeMet) nanoparticle (NP) delivery system has the potential to overcome these obstacles whilst maintaining the health benefits.

Methods: Through ionotropic gelation, SeMet can be encapsulated in the biodegradable polymer chitosan (Cs) via complexation with the polyanion tripolyphosphate (TPP). In this work, the SeMet NP formulations were optimized utilising a Box-Behnken experimental design, with the goal of maximising the encapsulation efficiency (EE%) while attending to the physicochemical constraints needed for oral delivery (~300 nm particle size, PDI < 0.5 and ZP > 30 mV). The 3 independent variables studied were the ratio of Cs:TPP, pH of the Cs solvent (based above and below the isoelectric point (pI) of SeMet) and the load concentration. The dependent variables were loading efficiency, particle size, polydispersity (PDI) and zeta potential (ZP). Further to this experimental design, two formulation improvement experiments were performed in a sequential manner: 1) a study of the optimal pH of the ionotropic solution components and 2) Zein, a prolamine rich protein derived from maize was employed as a co-formulation component in order to increase the EE%.

Results: The results of this study indicate that low concentrations of SeMet (50–100µg/ml), with the Cs solvent held at pH 5 (0.5 units distance below the pI of SeMet) were the optimal conditions for ionotropic gelation within the range studied, producing a maximum loading efficiency of 45 ± 9 %.EE% was subsequently increased to 65 ± 2 % by exploiting ionizing groups on both SeMet and Cs during the ionotropic gelation process. The use of Zein resulted in a slight increase in NP size from 290 ± 55 nm to 385 ± 50 nm whilst the EE% increased from 65 ± 2 % to 80 ± 1.5 %. The other physiochemical properties (PDI 0.151, 0.239 and ZP 32, 39) were invariant.

Conclusions: SeMet-loaded Cs/Zein NPs were successfully produced via ionotropic gelation techniques. The pH of the formulation component medium was shown to have a beneficial effect on the EE% by influencing the ionisation state of the NP components. Furthermore, the addition of Zein in the formulation significantly enhanced he EE%, thus demonstrating itself as a useful component in the formulation. The physiochemical properties of these NPs indicate promising potential for the improved delivery of SeMet through the oral route.

NEW BIOCOMPATIBLE INORGANIC-BASED DRUG DELIVERY SYSTEMS

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Abdessamad Y. Kaassis & Gareth R. Williams

UCL School of Pharmacy, University College London, 29-39 Brunswick Square, London, WC1N 1AX, UK

Background: It has been determined that, for many conventional pharmaceutical therapies, the efficacy may be improved and the side effects reduced if the therapy is administered continuously (although at potentially variable rates), rather than through conventional burst release techniques. Modern controlled release systems are usually polymer based; much less attention has been paid to inorganic delivery systems, even though have a number of promising features. but many are cytotoxic and often the drug release profile is very rapid.

Methods: In this work, new biocompatible inorganic-based drug delivery systems have been developed to deliver commonly used drugs in a tuneable manner. The sodium salts of naproxen, diclofenac and valproic acid were loaded into biocompatible inorganic carriers through an ion- exchange route. The products were characterised by XRD, FTIR, NMR and EDX. The drug release studies were performed at pH 1.0, 6.8 and 7.4.

Results: X-ray diffraction and modelling studies showed that the drug ions formed bilayers arrangement. According to infrared and NMR spectroscopy analyses, the drugs remained intact after intercalation. The materials obtained were formulated and drug release studies were carried out under conditions that mimic the human gastrointestinal tract. The inorganic-based formulations were compared to commercial formulations currently available on the market. The new systems showed similarity to commercial diclofenac formulations and better release profiles than sodium ibuprofen formulations.

Conclusions: It proved possible to design and develop efficient new drug-delivery systems with tuneable release via the use of inorganic materials.

MODIFYING RELEASE OF AN ANTIMICROBIAL DRUG BY ALTERING THE PHYSICOCHEMICAL PROPERTIES WITHIN POLY-HEMA

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Bridgette McGeever, Gavin Andrews & David Jones

School of Pharmacy, Queen's University Belfast, Belfast, BT9 7BL, County Antrim.

Background: Indwelling medical devices often succumb to bacterial colonization and as a consequent patients can suffer from severe infection. With the expectation that these devices will remain in situ for periods of greater than one month, the possibility that the device itself may be coated and provide local therapeutic activity shows promise. By incorporating the antimicrobially active chlorhexidine (CHX) into a polyHEMA based platform this could be achieved. CHX in its basic form is poorly soluble in water: in order to improve release from the hydrophilic platform, the natural surfactant cholic acid (CA) has been included at various concentrations. Furthermore, to investigate the change in release profile, various methods of characterization have been employed to assess the nature of the components within the hydrogel film.

Methods: Hydrogels were formed via thermal polymerization as per Jones et al using 5% CHX drug loading, 0.5,1 and 2:1 molar (M) ratios of CA:CHX. Specific dimensions were cut and CHX release measured in citrate buffer pH 7, 37 °C, 40 RPM. Timed aliquots were removed and analyzed using a derived HPLC assay with UV-detection. Samples of these films were analyzed using x-ray diffraction (XRD), dynamic mechanical analysis (DMA) and Raman spectroscopy.

Results: The presence of CA in poly-HEMA alongside CHX shows a statistical improvement in release profile of the drug. It is observed that by varying the concentration of CA present the release can be modified; there is a correlation between increasing concentration of CA present and enhancement of drug release. It can be hypothesized that the presence of CA improves solubility of CHX at the surface of the polymer, allowing for this greater release. Analysis has proven that there are no crystalline structures present within the hydrogel film suggesting that the components exist within the film as either a single phase or an amorphous dispersion. Examination of glass transition temperatures calculated via DMA suggests single-phase formulations however, further analysis may be considered to confirm these findings through methods such as DSC. Raman spectroscopy suggest the possibility of amorphous drug and indicates there are potential interactions between the drug and polymer backbone based on both peak broadening and shift in wavenumber respectively at the identifying CHX peak.

Conclusions: Incorporating a natural surfactant with a well established antimicrobial drug into a hydrophilic polymer system can alter the release of said drug. Analysis of the hydrogels can conclude that within the polymeric system, the components are dissolved however not necessarily in a single phase with the polymer. This alteration in structure along with the surfactant effect of CA may contribute to the modified release of CHX.

ENGINEERING OF LIPOSOMES AS VACCINE ADJUVANTS USING MICROFLUIDICS

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Carla B. Roces Rodriguez[1], Dennis Christensen[2] & Yvonne Perrie[1]

[1] Aston Pharmacy School, Aston University, Birmingham, B4 7ET, United Kingdom;
[2] Statens Serum Institut, Copenhagen, 2300, Denmark

Background: Liposomes are well reported for their activity as vaccine adjuvants, which are a prerequisite for the subunit vaccine formulation in order to induce potent and persistent immune responses. The cationic liposomal adjuvant formulation (CAF) 01 which is based on the cationic surfactant dimethyldioctadecylammonium (DDA) bromide and the immunopotentiator trehalose 6,6'-dibehenate (TDB) from Mycobacterium tuberculosis has previously been shown to be a strong adjuvant system against several diseases such as tuberculosis. Controllable technologies such as microfluidics have several advantages compared to traditional methods for liposome preparation such as uniform flow and mixing, high efficiency, continuous operation, easy control and low cost. Therefore the aim of this study was to investigate the use of microfluidics to prepare liposomal adjuvants.

Methods: Liposomes were prepared by two different methods: 1) the traditional method of thin lipid film (Bangham et al., 1965) followed by hydration and high shear mixing (HSM) and 2) microfluidics. Using these processes, four liposomal formulations were prepared: DDA and TDB concentrations were fixed at 3.96 mM and 0.5 mM respectively and cholesterol was added to the CAF01 formulation at two different concentrations (18 and 31 mol %) in order to increase the fluidity of the membrane bilayer. In the fourth formulation, DDA was replaced by 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and kept at the original concentration. A model antigen (ovalbumin) was added on the surface of the preformed liposomes in a concentration of 2 µg per vaccine dose (50 µL).

Results: Our results demonstrate that cationic liposomes formulated by microfluidics can be formulated in sizes down to 65 nm depending on the parameters adopted. This is in contrast to vesicles subject to HSM, where the minimum size was ~200 nm. However, liposomes produced using microfluidics tended to be more heterogeneous in nature. Addition of cholesterol was shown to facilitate the production of liposomes by microfluidics with formulations containing 31 % cholesterol providing small, reproducible liposome suspensions. This might be due to the decrease of transition temperature in the formulation when cholesterol is incorporated. Given their highly cationic nature, all CAF formulations gave high protein loading (>90%) whilst the neutral DMPC:TDB formulation was not able to electrostatically bind OVA.

Conclusions: Microfluidics allows for the large-scale and reproducible preparation of liposomal adjuvants. These vesicles can be produced in a range of sizes depending on the control parameters selected.

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CAN EQUILIBRIUM ADSORPTION ISOTHERMS BE USED AS A PREDICTOR FOR IN-VITRO DRUG DISSOLUTION FROM SILICA CARRIERS?

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Carol McCarthy, Katie Ryan & Abina Crean

School of Pharmacy, University College Cork, Cork, Ireland

Background: : Drug loading onto a silica substrate has been investigated as a method to improve the aqueous solubility of BCS Class II drugs. There are a number of silica-based solid dispersions in clinical development but no commercial formulation has reached the market to date. A number of studies have reported incomplete release from drug-loaded silica carriers which has implications for formulation development. This work investigates whether this incomplete release can be explained by the drug's equilibrium adsorption behaviour onto silica in the dissolution medium.

Methods: A model poorly water-soluble drug (sulphamethazine (SZ)) and two model silica substrates (SBA-15 (mesoporous) and Aerosil®200 (non-porous)) were identified. Part A: Drug dissolution from the silica material was investigated by first loading SZ onto the silica carrier by solvent impregnation. This was quantified by thermogravimetric (TGA) analysis. Three dissolution experiments were conducted to examine drug dissolution in both sink and non-sink conditions (n=6). For two dissolution experiments, 20 ml of pre-warmed medium (pH1.2 at 37oC, 100rpm) was added to glass vials containing the SZ-loaded silica formulation. The third dissolution experiment was conducted using USP I basket apparatus in sink conditions (150 ml medium at pH1.2, 37 °C, 100 rpm). Samples were taken at defined time points over 120 min, filtered and analysed by HPLC. Part B: Adsorption was studied in 20 ml glass vials containing 100 mg of silica in a range of defined drug concentrations in water (pH1.2). Adsorption studies were conducted for 24h at 37 °C in a shaker water bath (100 rpm). At defined time points, samples were removed from the supernatant solution, filtered and analysed by HPLC. Pore size analysis of SZ loaded silica formulations before and after dissolution was conducted using a Gemini VI Surface Analyser (Micromeritics). The solid state properties of the SZ loaded silica samples were examined using Differential Scanning Calorimetry (DSC) and Scanning Electron Microscopy (SEM) before and after dissolution.

Results: Incomplete dissolution of SZ from silica was observed for porous and non-porous silica formulations in both sink and non-sink conditions. Equilibrium adsorption of SZ onto Aerosil®200 and SBA-15 was described using adsorption isotherms. The Langmuir Plot fitted the SZ-loaded non-porous Aerosil®200 isotherm (R^2= 0.9919) while the multilayer BET Plot best described the SZ-loaded mesoporous SBA-15 data (R2= 0.9224). The ratio of milligrams of SZ adsorbed to grams silica (qe) and the concentration of adsorbable species (Ceq) at 120 min were calculated for each of the dissolution experiments. The results were compared with the adsorption isotherm data. After 120 min of dissolution the ratio of drug adsorbed onto Aerosil®200 agreed with the ratio determined during the adsorption study. For SBA-15 samples the ratio of SZ adsorbed deviated from the isotherm data.

Conclusions: Incomplete drug release from non-porous drug-silica solid dispersions can be explained by the drug's equilibrium adsorption behaviour onto the silica carrier. The results suggest the adsorption isotherm could be used as a predictor of drug release from non-porous silica. Adsorption behaviour alone cannot explain drug dissolution from mesoporous systems at the drug loading ratios investigated in this study. Future work should focus on investigating the deviations observed for mesoporous systems.

THE ROLE OF PROTEIN SURFACE INTERACTIONS FOR APPLICATIONS IN CONTROLLED DRUG DELIVERY

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David Mallinson[1], Alexander B. Mullen[1] & Dimitrios A. Lamprou[1,2]

[1] Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS), University of Strathclyde, Glasgow, UK; [2] Continuous Manufacturing and Crystallisation (CMAC), University of Strathclyde, Glasgow, UK

Background: The study of protein-surface interactions represents one of the most important topics in the field of biomaterials and is critical for targeting drug delivery. Proteins can be used as adapters conjugated to nanoparticles (e.g. chitosan, gold, liposomes, silica, self-assembly) for targeted drug delivery. Furthermore, the interaction between the protein molecules with drug carriers and cell surfaces is crucial, since the cell adhesion to surfaces depends on the availability of specific protein-binding (e.g. integrins) sites. Protein-surface interactions also play a significant role in biosensors as a diagnostic tool since proteins such as ligand are immobilised on the probe surface, and can be used to analyse the corresponding integrin. Anastellin is a fragment of the first FnIII type domain of fibronectin that can polymerise with whole fibronectin to make a special form called superfibronectin. Polyurethane (PU) and poly (methyl methacrylate) (PMMA) are polymers that are used for ureteral catheters and bone cement but also for drug delivery particles (e.g. microspheres, lipid-fibronectin adsorption) and for drug-releasing materials (e.g. scaffolds). Studying anastellin adsorption to these surfaces should help understand the adsorption mechanism.

Methods: To determine whether the protein fragment retained the expected secondary structure it was analysed with circular dichroism (CD) with a Chirascan Plus spectrophotometer (Applied Photophysics). Force of adhesion (Fad) was determined using a Multimode 8 atomic force microscope (AFM; Bruker Nano). Fad measurements were manufactured with probes that silanised with APTES and/or anastellin protein fragment (silanisation was required for protein attachment).

Results: The CD spectra suggest that the protein fragment did retain its expected structure of anti-parallel beta sheets which is indicated by a negative maximum peak at 218 nm. AFM results appear to show that anastellin-functionalised probes give higher adhesion on all surfaces than silanised probes with no protein. Early results show that mean Fad to PMMA, PU and silica increased by 25.3 %, 86.1 % and 10.7 %, respectively. Though more work will need to be done in order to confirm, Fad may be able to be correlated with biocompatibility.

Conclusions: Anastellin functionalisation increases adhesion of the probe to all surfaces. This difference is greater for the polymers, especially for PU coated surfaces. This means that such proteins can be adsorbed onto these surfaces to extend the lifetime of particles in the body or confer properties upon surfaces such as drug eluting stents.

TIME OF FLIGHT SECONDARY ION MASS SPECTROMETRY (TOF-SIMS) IN PHARMACEUTICAL SCIENCES

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Eleonora Paladino[1,2,3], David G. Watson[1], Melissa K. Passarelli[3] & Dimitrios A. Lamprou[1,2]

[1] Strathclyde Institute of Pharmacy & Biomedical Sciences, Strathclyde University, Glasgow, UK; [2] EPSRC Centre for Innovative Manufacturing in Continuous Manufacturing and Crystallisation, University of Strathclyde, Glasgow, UK; [3] National Physical Laboratory, Hampton Road, Teddington, Middlesex, UK.

Background: Structure-property relationships are often poorly defined in advanced continuous pharmaceutical manufacturing processes and products, and hence it is difficult to control the final product performance to the required degree to deliver advanced functionality. The dynamics of particles within complex mixtures and the effect of processes and storage on their disposition and microstructure is also challenging to measure. Hence, there is a clear need to have techniques for analysis and measurement of composition, dynamics and structure with increased spatial and temporal resolutions. Secondary Ion Mass Spectrometry (SIMS) is a technique that enables the analysis of the ionized species (secondary ions) ejected from a sample's surface after it is bombarded by energetic primary ions. SIMS spectra typically contain cluster ion, molecular ion and ion fragments of the compounds present on the surface of the sample. In imaging mode, at every pixel of the image a complete mass spectrum is collected. An ion image shows the distribution of ions of a single m/z. Moreover, cluster ion beam opens up the possibility of molecular depth profiling and imaging in three dimensions. In a dual beam configuration, 3D images are produced by sequentially imaging the surface with a high lateral resolution Bismuth source, then sputtering material with an Argon clusters beam. As the beams step across the surface, the spectrum describes how the total count of each ion (m/z) varies over the sputtering time (linked to the depth). This technique guarantees high molecular specificity, high spatial resolution and high sensitivity, and permits to acquire a three dimensional image of the distribution of Active Pharmaceutical Ingredients (APIs), impurities or excipients in the formulated product. Therefore, to apply this technology to the study of Pharmaceutical Products would be extremely interesting in order to investigate the impact that disposition of components could have on the product's final performance.

Methods: ToF-SIMS analyses were preformed using an IONTOF ToF-SIMS V (Munster, Germany) based at Wolfson Foundation "Pharmaceutical Surfaces Laboratory" within the national CMAC Centre. The instrument is unique in UK and the only one in Scotland. It is equipped with four different ion sources: Bismuth (Bi^+ Bi^{3+} Bi^{3++}), Cs^+, O_2^+, and Argon Clusters. Each ion source has a particular application, and the variety of ion sources allows the analyst to select the most suitable and appropriate analysis beam for the task.

Results: Evaluation and discussion of the ToF-SIMS capability in Pharmaceutical Sciences, by presenting data from our research lab and from the recent literature concerning the use of ToF-SIMS on different Pharmaceutical finished products (e.g. extrudates, tablets, granules).

Conclusions: The aim of the study is to investigate the potentiality of ToF-SIMS for analysing Pharmaceutical finished products.

DEVELOPING A MICRONEEDLE DELIVERY SYSTEM FOR ANTIGEN-SPECIFIC IMMUNOTHERAPY IN TYPE 1 DIABETES

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Farah Arikat [1,2], Ravinder Kaur Singh[2], Kathryn Haskins[3], Luciano Vilela[4], Colin M Dayan[2], F Susan Wong[2], Stephanie J Hanna[2], Sion A Coulman[1] & James C Birchall[1]

[1] School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, UK; [2] School of Medicine, Cardiff University, Cardiff, UK; [3] Department of Immunology and Microbiology, School of Medicine, University of Colorado, Denver, CO 80206, USA; [4] Biomm S.A., Belo Horizonte, Brazil

Background: Antigen-specific immunotherapy (ASI) involves generation of tolerance to autoantigens. One such autoantigen is proinsulin (PI), the precursor of insulin and a key factor in type 1 diabetes (T1D) development. Microneedles (MN) provide advantages for delivery of PI, including targeted delivery to immune cells in the skin and little/no skin damage or inflammation. This study aims to develop a PI-coated MN system and investigate its potential to generate tolerance. Tests were conducted to develop a quantification method for PI and a biological readout for PI activity.

Methods: An absorption spectrum was obtained (190–800 nm) using UV-Vis spectroscopy for PI (Biomm S.A.) in PBS. At the identified discriminatory wavelength (276 nm), the absorbance of PI (1000-31.25 ug/mL) was measured to generate a calibration curve. For a biological readout, PI was co-cultured for 48 or 72 h with antigen-presenting cells (APCs) and G9 CD8 T cells, which recognise the B15-23 segment of PI. T cell markers were then analysed by flow cytometry and IFNg and MIP-1β were measured in the supernatant using ELISA. In vivo studies used NOD FIR mice (mouse model of T1D), which were given an intravenous transfer of CFSE-labelled G9 CD8 T cells +/- intradermal injection of 100ug PI. Lymph nodes were harvested 4 days later and CFSE proliferation and T cell markers were analysed. BDC4.38vb12 T cell clones, which recognise the B9-23 segment of PI, were also cultured with APCs and PI, after which IFNg was measured in the supernatant using ELISA.

Results: The UV-Vis absorption spectrum indicated that the λmax for PI was between 209 and 225nm. However, the narrowest (most discriminatory) absorbance peak was seen at 276nm. Thereafter, the calibration curve generated an R2 value of 0.99871. For the in vitro assays with G9 CD8 T cells, CFSE proliferation and T cell activation markers showed no response to any doses of PI, even as high as 25 ug/mL and 100 ug/mL, respectively. Measuring the chemokine MIP-1β using ELISA was found to be the most sensitive readout, showing some response to PI at doses above 25 ug/mlL. The G9 CD8 T cells in vivo experiments showed no clear difference in proliferation of cells or CD44 (T cell activation marker) expression between PI-treated and control mice. In the in vitro assays with BDC4.38vb12 T cells, IFNg was produced in response to PI, the highest concentration detected being 688.8 pg/mL to 100 ug/mL of PI.

Conclusions: UV-Vis spectroscopy has been established as a potential method for quantifying PI in MN coating studies. Immunological assays showed that both cell types show promise as a biological readout for PI activity but the readout sensitivity has not been optimised. Further work will include developing a PI MN coating formulation and investigating coating efficiency as well as delivery efficiency in both (ex vivo) human and (in vivo) murine skin.

INVESTIGATION OF DRUG RELEASE FROM POLY(ETHYLENE-CO-VINYL-ACETATE) HOT MELT EXTRUDATES

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Georgina Procter, Gavin P. Andrews & David S. Jones

School of Pharmacy, Queen's University Belfast, BT9 7BL, UK

Background: Despite advances in the instrumentation of the urinary tract, catheter associated urinary tract infections remain a fundamental contributor to patient morbidity and mortality in the secondary care setting1. This research aims to tackle this problem by developing a strategy utilising a multilayered drug delivery platform composed of poly(ethylene-co-vinyl acetate) (EVA) to improve release kinetics. Hot melt co-extrusion provides an ideal manufacturing technique for such a device. In this study nalidixic acid (NAL) and triclosan (TRIC) were selected as model antimicrobial agents that are hydrophilic and hydrophobic, respectively. Monolayer extrudates were fabricated using hot melt extrusion and preliminary drug release was conducted. The purpose of this study was to evaluate the impact of VA grade, drug loading and incorporation of pore formers (hydroxypropylcellulose, HPC and polyvinyl alcohol, PVOH) on the release of NAL and TRIC.

Methods: Hot Melt Extrusion: Extrusion was performed on a twin-screw extruder (Dr. Collin, Germany). A series of formulations were fabricated with¬ a range of properties: VA grade (5.5%, 28% VA), drug loading (5, 10, 20% w/w) and pore formers (5, 10% w/w HPC or PVOH). Triclosan Drug Release: Release was conducted in tris buffer (pH 7.2, 37°C) under sink conditions. HPLC (λ=288 nm, ACN:H2O 70:30) was used to quantify TRIC at predetermined intervals. Nalidixic Acid Drug Release: Samples were placed in PBS (pH 7.2, 37°C) under sink conditions. Quantification of NAL was conducted using a Carey 300 UV spectrometer (334 nm) at appointed time points. Results: The extent of TRIC release was lower from the 28% VA compared to 5% VA. Increased VA segments increase the polymer hydrophilicity and this may diminish the diffusivity of lipophilic TRIC and therefore limit the amount of drug release. In contrast, NAL is more hydrophilic and the increase in VA led to the expected increase in NAL released. For both the NAL and TRIC systems, higher drug loadings resulted in greater drug release. The inclusion of pore formers did not elicit greater TRIC release. In the case of NAL, the incorporation of PVOH and HPC resulted in an increased amount of drug release. HPC and PVOH are hydrophilic pore formers, hence they induce greater water ingress into the polymer. The pore former may then be removed and consequently form water filled channels, facilitating greater drug release. However, if the pore former is not removed from the system, it may swell and fill the area in the polymer with a hydrophilic gel. If this is the case the hydrophobic TRIC will be unable to diffuse through the gel and the pore formers will therefore decrease the amount of drug released.

Conclusions: This study demonstrated that by altering the drug loading, EVA grade and incorporation of pore former, drug release may be modified. The hydrophilic/hydrophobic nature of the drug plays a significant role in how these parameters influence drug release. These results have implications for the design of the middle, drug-containing layer of the multi-layered system, and future research will examine the release from bi- and tri-layer devices.

SYRINGEABILITY STUDIES FOR THE DEVELOPMENT OF INJECTABLE MULTI-PURPOSE PREVENTION TECHNOLOGY FORMULATIONS

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Clement M. Haeck, Thakur Raghu Raj Singh & R. Karl Malcolm

School of Pharmacy, Queen's University Belfast, Belfast BT9 7BL, UK

Background: There is growing interest in the development of multi-purpose prevention technology (MPT) products for women that combine contraceptive activity with prevention of human immunodeficiency virus (HIV) and/or other sexually transmitted infections. Recently, use of in situ implant forming gels has gained incredible interest. However, prior to application of gels, it is important to evaluate their syringeability so that it is clinically usable. Therefore, in this study, in situ forming implant (ISFI) gels containing PLGA in NMP (without drug) were formulated and evaluated to determine ideal gel concentrations that will be suitable for intramuscular injection. Syringeability tests were conducted to select optimum gel formulations that can be easily expelled using standard syringes, which are commonly employed in clinical setting. In this study, care was taken to select clinically relevant rate of expulsion and suitable needle type. Thus, impact of polymer type, polymer concentration and expelled volume was investigated.

Methods: Poly-lactic-co-glycolic acid (PLGA) 5050 (50% lactic acid, 50% glycolic acid monomers) and 7525 (75% lactic acid, 25% glycolic acid) (referred to as PLGA5050 and PLGA7525 respectively) were purchased from Corbion Purac Biomaterials (Gorinchem, The Netherlands). N-methyl-2-pyrollidone (NMP) was obtained from Sigma Aldrich (Gillingham, England). ISFI formulations were prepared by dissolving the required amount of PLGA 5050 or PLGA 7525 in NMP to produce concentrations of 10, 20 and 30 % w/w, respectively. The formulations were left to stir for at least 24 hours, at room temperature, to ensure complete dissolution of the polymers in the NMP. Work of syringeability (WoS) and maximum force (MF) of expulsion were investigated using needles of different diameter, i.e. 22G, 25G and 27G. Using a Texture Analyser, 0.1 or 0.5 mL of formulation was expelled from a 1 mL syringe at different rates (0.5, 2 and 5 mm/s). The resultant force-distance plot was used to determine the WoS and the MF to expel the formulations. Five replicate measurements were made in each case and the solvent NMP (without PLGA) was used as control.

Results: ISFI formulations made from these different concentrations of PLGA5050 or PLGA7525 dissolved in NMP were syringed through three different needles to investigate the ease at which they could be expelled from these narrow bore needles. They were then compared to one another and to the control. An increase in the gauge number of the needle, i.e. a decrease in the needle outer diameter, resulted in an increase in the WoS and MF exerted on the syringe in order to expel the fixed volume of formulation. For each set of parameters, the greatest WoS and MF were recorded with the 27G needle, with 22G showing the lowest. WoS and MF increased with increasing polymer concentration and were higher with PLGA7525 compared to PLGA5050. More force was required to expel a higher volume of formulation and with an increasing rate of expulsion. Furthermore, it was not possible to syringe some highly concentrated formulations (at 30% w/w) using the smallest needles (25G and 27G) and at high speeds (2 and 5 mm/s).

Conclusions: This study helped to define optimum ISFI formulations. After incorporating a combination of an antiretroviral and a contraceptive drug within these ISFI formulations, we aim to use them to develop injectable multi-purpose prevention technology (MPT) products for simultaneous hormonal contraception and HIV prevention therapy.

FORMULATION AND PROCESS DEVELOPMENT OF PEDIATRIC ODTS: A SYSTEMATIC INVESTIGATION INTO THE ROLE OF MOISTURE CONTENT AND MICRO/MACRO PROPERTIES OF PHARMACEUTICAL EXCIPIENTS

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Hamad Alyami, David Terry & Afzal R Mohammed

Aston Pharmacy School, Aston University, Birmingham B4 7ET, UK.

Background: The amount of water related with solids may meaningfully affect physical and mechanical properties of pharmaceutical materials. Properties such as powder flow, powder coating, AFM, nanoindntaion, compaction, hardness, friability, disintegration time are influenced by moisture content. This study aims to examine the effect of moisture sorption of microcrystalline cellulose (MCC) on powder blending/coating using a prototype dry powder coater and consequent tableting by direct compression.

Methods: The moisture content of MCC was optimized using spray water approach and analysed using thermogravimetric analysis (TGA). Selected particle sizes of both mannitol (guest particle size ≤ 38 µm) and MCC (carrier particle size ≥ 250 µm) were obtained by sieving and confirmed by particle size analyzer. Two different blends of the mixture of MCC and mannitol were studied: interactive mixtures obtained by blending the mix at 300rpm for 5 minutes and particle coated mix obtained by blending at 1500 rpm either with or without air flow. Consequential ODTs of powder blend (interactive mixture) and powder coating (with and without air) were investigated for hardness, friability, disintegration time and porosity.

Results: The results showed that as moisture content of MCC increases, the flow properties improve dramatically. MCC demonstrated its best flowability at moisture content of 11.2%, (low angle of repose 29.6±0.86) when compared to control comprising of 3.9% moisture (angle of repose 38.52±0.67). This difference in flow could possibly be attributed to the lubricant activity of moisture that reduces the friction between particles and the solid surface. The next sets of investigations were focused at developing interactive and dry particle coated (mannitol coated on to MCC) mixtures of MCC with different moisture content (3.9% and 11.2%). The results showed that formation of interactive mixtures resulted in a drop of moisture content from 3.9% to 1.6and from 11.2% to 2.6 Similarly, upon dry coating without air the moisture content dropped from 3.9% to 1.7 and from 11.2% to 4.12 Interestingly, inclusion of air flow within the process of dry coating reduced the moisture content from 3.95 to1.7 and 11.2% to 1.5. These differences in moisture content of the powder blends, due to variations in processing conditions demonstrated significant variations in ODT properties. Stronger compacts were obtained from interactive mixtures for the two different levels of moisture content for MCC whereas dry coated powder blends (with and without air) yielded weaker ODTs. These differences in ODT properties are due to the differences in the moisture content which cements the particles and impacts on mechanical properties as well as particle layering with mannitol coated MCC presenting fragment able surfaces which result in weaker tablets.

Conclusions: Moisture considerably affects the consolidation characteristics of blended powder. The extent of consolidation and the bonding of particles not exclusively on moisture content but also on the powder processing conditions.

DEVELOPMENT OF A PRAGMATIC STRATEGY TO OVERCOME MANNITOL FRAGMENTATION DURING DIRECT COMPRESSION EMPLOYING PARTICLE SIZE REDUCTION FOR USE IN ODTS

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Jasdip Koner[1], Ali Rajabi-Siahboomi[2], James Bowen[3], Yvonne Perrie[1], Dan Kirby[1] & Afzal Mohammed[1]

[1] Aston Pharmacy School, Aston University, Birmingham, B4 7ET, UK; [2] Colorcon® Ltd, Harleysville, Philadelphia, PA 19438, USA; [3] School of Chemical Engineering, The University of Birmingham, Birmingham, B15 2TT, UK

Background: Mannitol is a common excipient employed in ODT's due to its sweet taste and cooling effect within the mouth. However its major drawback is its fragmentation during compression producing tablets that are weak and friable. The key aim of this study was to understand the fracture behaviour of crystalline mannitol, utilising ball milling as a method of particle size reduction.

Methods: Powders were prepared utilising different milling parameters to allow an analysis of the energy input on resultant particle characteristics. Several different methods were employed to analyse the powder characteristics, including; SEM, DVS, XRD, DSC and laser diffraction. Compressibility was also analysed using Heckel analysis. ODTs were prepared using 99.5% mannitol and 0.5% magnesium stearate, and subsequently analysed for hardness and disintegration.

Results: Results indicated that the predominant plane of fracture for the mannitol crystal was the (011) plane, as was observed using SEM images, alongside DVS surface energy data also pointing to fracture at the same plane, as the dispersive surface energy of the milled powders had decreased. In addition, ODTs prepared from milled powder indicated that mannitol fracture occurred at the (011) plane, thereby exposing the hydrophilic plane which decreased disintegration time. Heckel analysis showed that milling improved the compressibility of mannitol, as the lower yield pressures obtained revealed that the milled powders compacted through plastic deformation with minimal or no fragmentation. Evaluation of the crystal state using XRD and DSC showed that mannitol largely retained the β polymorph at the different milling conditions.

Conclusions: Results obtained in this study conclude that mannitol exhibits extensive fracture at the (011) plane upon exposure to external pressure, which is similar to the environment encountered during tabletting, and that particle size reduction is to be a pragmatic strategy to overcome the current limitation of mannitol fragmentation.

OPSISPORIN – SUSTAINED RELEASE OCULAR DRUG DELIVERY

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Katherine Bamsey, Rhian Groves, Paul Seaman & Daniel Palmer

Midatech Pharma, Cardiff, CF24 0AA, UK.

Background: While there are treatments available for patients suffering from non-infectious uveitis of the posterior segment of the eye, none are ideal. Topical steroid treatment is often ineffective, while systemic treatment regimens are limited due to significant side effects associated with prolonged use. We have sought to develop a product that will bring a significant benefit to patients suffering from this sight-threatening disease, via the local sustained delivery of cyclosporin A (CsA)1. The complexities of intravitreal injection necessitated a formulation that provided high drug loading, controlled release of the drug to allow 3-monthly dosing, precise control of residual solvents and injection via 27–30 gauge needles whilst avoiding local tolerance and toxicity problems.

Methods: Utilizing the Q Sphera platform, cyclosporin A was encapsulated into bioresorbable polymers via a method that is free from emulsions, harsh solvents, sheering forces, high temperatures and is completely compatible with aseptic production. The process has been proven for sterile manufacture and filling of clinical trial materials, as is necessary for parenteral products of this nature. Microspheres of poly-lactide and CsA were produced by co-dissolution of the API and polymer, before droplet generation via piezo-actuator. Extremely rapid and sequential solvent extraction of each droplet at very high frequencies (120 KHz) produced solid microspheres of precise size, loading and excellent residual solvent profile. A range of PLAs and post-process conditions were investigated, allowing for optimization of drug loading and drug release kinetics. Batches of microspheres were thoroughly characterized before assessment of efficacy in a murine model of experimental auto-immune uveitis (EAU). Following intravitreal injection of CsA-loaded microspheres, efficacy was assessed by scoring the extent by which the inflammatory response was attenuated against placebo controls.

Results: An in vivo study has illustrated the efficacy of OpsiSporin in the treatment of experimental auto-immune uveitis (EAU) in a murine model. Mice receiving an intravitreal injection of OpsiSporin showed a significant reduction in EAU severity compared to those animals that received vehicle alone. Topical endoscopic fundal imaging (TEFI), a technique that monitors the progression of retinal degradation in ocular disease, measured a reduction in the rate of disease severity and development, specifically a significant reduction post day 15 of the study. In vitro modelling of cyclosporin release kinetics, indicate that the rate of release that was shown to be efficacious in vivo is maintained for 3 months, therefore facilitating a 3 monthly product.

Conclusions: OpsiSporin manufactured via Q Sphera was shown to be efficacious in the treatment of EAU, and represents a potential therapy for the treatment of posterior uveitis in man. The formulation enabled intravitreal injection via 33 gauge needle, whilst other properties indicate that local tolerance and toxicity problems should be avoided. The unique platform of Q Sphera aligned with local delivery of sustained release depots to the eye, represents a new and exciting method of treating chronic and/or recurring eye diseases.

SUSTAINED RELEASE OF OVALBUMIN FROM PHOTOCROSSLINKED IMPLANTS FOR OCULAR DRUG DELIVERY

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Kathryn McAvoy, David Jones & Raghu Raj Singh Thakur

School of Pharmacy, Queen's University Belfast, Belfast, BT9 7BL, Northern Ireland, UK

Background: Delivery of drug molecules to treat visually impairing ocular diseases has been an extremely challenging task to the pharmaceutical scientists. This study aims to fabricate and characterise poly(ethylene glycol) diacrylate (PEGDA)-based photocrosslinked implants for ocular delivery of a model protein molecule (ovalbumin, OVA).

Methods: i) Formulation and preparation of gels: Formulation composition was optimised to contain 2.5% w/w OVA, 10% w/w of various pore forming agents and 0.1% w/w photoinitiator in required quantity of PEGDA Mw (250 or 700 Da). ii) Implant formation: The prepared gels were poured into moulds and photocrosslinked at 365nm using a UV crosslinker, Fusion UV LightHammer 6 high power UV curing system (Maryland, USA) to form implants of 10 x 5 x 0.5mm dimensions. iii) Implant characterisation: Prepared implants were evaluated for dynamic and equilibrium swelling, mechanical strength (compressibility) and surface porosity (via scanning electron microscopy, SEM). iv) Drug release: In vitro drug release was conducted in phosphate-buffered saline (PBS) (pH 7.4±0.2) and quantified using a validated size exclusion chromatography (SEC) assay.

Results: All implants, regardless of PEGDA Mw or pore forming loading, experienced an initial burst release of drug within the first 24hours, followed a reduction in release in the following days. The effect of PEGDA Mw on release of OVA is less significant than the effect of pore forming loading. For example, PEGDA Mw700 implants loaded with sodium carbonate exhibited 54.5% release at day 14, compared to 19.1% without pore-former. PEGDA Mw250 implants loaded with sodium bicarbonate exhibited 41.6% release at day 14, compared to 17.3% without pore-former. Release data is supported by SEM images that show increased porosity in implants composed of pore forming agents. The effect of varying pore former and Mw of PEGDA on implant swelling was also investigated, it was observed that increase in Mw of PEGDA has significantly increased percentage swelling. For example, sodium carbonate loaded PEGDA Mw 700 implants experienced approx. 40% swelling, compared to 1.5% swelling with PEGDA Mw 250 implants. Mechanical strength of the implants measured after 24 h in PBS, was affected by both PEGDA Mw and pore-former loading. For example, mannitol loaded Mw250 implants required 8.70 N of force compared to Mw 700 implants that required only 0.27 N of force.

Conclusions: Photocrosslinked PEGDA implants can provide sustained delivery of OVA. This study showed varying PEGDA Mw and/or the addition of pore forming agents can affect drug release from the implant system and alter its mechanical and swelling properties. Thus indicating that this delivery system has the capability to control the drug release by varying the implant composition.

FORMULATION DEVELOPMENT OF AN ELASTIC LIPOSOME FORMULATION FOR THE DERMAL DELIVERY OF ACTIVE AGENTS

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Mandeep Marwah, Yvonne Perrie & Deborah Lowry

Life and Health Sciences, Aston University, Birmingham, B4 7ET, UK.

Background: This study aims to formulate controlled release liposomes loaded with the model drug naringenin. Naringenin is the predominant flavanone in grapefruit. It is an antioxidant, free radical scavenger, anti-inflammatory agent, and immune system modulator. The aim of the study was to investigate the effects of various loadings of surfactants (Tween 80, Tween 20 and sodium cholate) on the deformability of drug loaded liposomes and to quantify drug release over an 8 h period. Permeation of naringenin into cells was tested by applying formulations to a dermal cell line.

Methods: Drug loaded liposomes were formulated from phosphatidylcholine and cholesterol with varying quantities of surfactant (0-10%). Naringenin (0.00025% w/v) was incorporated in the lipid mixing stage. Liposomes were characterized and release across a semi permeable membrane into PBS was quantified. At each time point, supernatant was removed and quantified. Formulations were applied to cultured dermal fibroblast cells and drug release over 8 hours was quantified. All samples were quantified using HPLC with UV analysis.

Results: The addition of surfactant to the lipid bilayer resulted in the formation of smaller liposomes. The surfactant destabilizes the vesicle bilayer by reducing the amount of work required to expand the interface allowing the liposome to have elastic properties. Naringenin loaded liposomes were found to have a greater size when compared to blank liposomes. A release study was carried out on liposomes formulated with various loadings of Tween 20 and naringenin. A steady release with a maximum of 73% over 8 h was observed. As the loading of surfactant increased, a slower rate of release was observed.

Conclusions: As surfactant loading in the bilayer is increased, liposome size decreases. The presence of naringenin in the bilayer increases the liposome diameter, however, the inclusion of surfactant decreases the diameter. It appears elastic liposomes are useful in enhancing drug penetration into dermal cells and furthermore may be useful in the development of a controlled release formulation.

TUNING POLYMERS FOR INTRACAMERAL CONTROLLED DRUG DELIVERY

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Mariam Badawi[1], Clive G. Wilson[1] & Peter Cormack[2]

[1] Strathclyde Institute of Pharmacy and Biomedical Science, University of Strathclyde, G4 0RE, UK
[2] Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, G1 1XL, UK

Background: The eye is prone to a wide range of sight threatening diseases. The physical vulnerability and physiological function limits the residence time of applied topical formulations. Controlled release polymeric matrices might provide a solution for such shortcomings. Intracameral polymeric implants have been utilized in the treatment of open angle glaucoma. However, the risk of distortion on hydration causing endothelial contact, the biological fate of the polymeric system and the physico-chemical properties of the active compound are all issues that need to be integrated into the design. The FDA approved polymer, Poly-Lactide-co-glycolide (PLGA), has been widely investigated in intraocular delivery systems. The effect of the copolymer composition and molecular weight has been addressed in attempts to control hydrophilicity and drug-polymer interaction. In particular, understanding the effect of endcapping of PLGA polymers on tuning drug delivery is being investigated. The current study investigated the effect of end capping to achieve desired drug release kinetics and implant mechanical profiles upon intraocular exposure.

Methods: Ring open polymerization and NHS/EDC chemistry were employed to generate a series of ester and amide end capped PLGA analogues with varying hydrophilic/hydrophobic balance. This was carried out to optimize pharmaceutical attributes in terms of drug incorporation efficiency, release pattern, degradability and swelling properties. Polymers were characterized for their chemical structure using 1H-NMR, 13C-NMR; thermal properties using DSC, TGA; and swelling behavior using dynamic vapor sorption (DVS). Brimonidine- loaded films were prepared by either solvent casting or melts. In vitro drug release behavior was examined by an HPLC developed method.

Results: NMR results provided comprehensive data on the polymer microstructure and confirmed successful endcapping of the polymers (2D-NMR). Thermal analysis revealed that the endcapping effect depends predominantly on the nature of the end group. Capping PLGA with a dodecyl ester resulted in a significant decrease of the recorded glass transition temperature (T_g) when compared with the non-endcapped starting material (from 43.22 to 37.07 °C). This suggests that the end group acts as a plasticizer. Since the implants will be subjected to an aqueous environment when injected intracamerally, examining the swelling properties is essential in the selection of polymers with tailored water-uptake profiles. Hysteresis evident in the nonendcapped polymers were attributed to the changing hygroscopicity of the samples during water adsorption and desorption. In contrast, the reversibility noted in the endcapped polymer profile indicates that the moisture is adsorbed on the surface of the material rather than the structure due to the hydrophobic nature of the endcapping function. Non-endcapped, brimonidine loaded PLGA films showed a predominant burst release over the first 6 h which was decreased by 5% and 7% upon endcapping the matrix with benzyl and dodecyl amide chains respectively. Similarly, there was a reduction in the diffusion coefficient of the drug through polymer matrix, supporting the contention that the slower water ingress decreased drug release.

Conclusions: Preliminary results show that end group chain modification can be exploited to manipulate well explored polymers such as PLGA. This provides exploration of a new array of parameters that fine tune drug release profiles and affect mechanical properties, allowing optimization of implant performance.

PREDICTION OF FELODIPINE MISCIBILITY IN LIPIDS USING FLORY–HUGGINS THEORY

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Mazin Al-bujasim, Yiwei Tian, David S. Jones & Gavin P. Andrews

School of Pharmacy, Queen's University Belfast, BT9 7BL, UK.

Background: It has been reported that many new chemical entities coming directly from synthesis are poorly water–soluble. Recently, solid lipid particulate-systems-have been shown to improve the oral bioavailability of these poorly water-soluble drugs. However, due to low miscibility between poorly water-soluble-drugs and commonly used lipids, drug tends to crystallize out from the initially homogenous drug–lipid solution during storage. This is especially favourable when drug loading is high. Consequently, it is useful to construct a phase diagram including the liquid-solid phase transition curve to predict the-equilibrium felodipine-solubility in lipids at room temperature. This curve is obtained from the TM endpoint measured using conventional differential scanning calorimetry (DSC) of different drug-lipid combinations, and used to understand the drug-lipid interaction parameters and construct temperature-composition diagrams using Flory-Huggins theory. From melting point depression measurement of samples with different drug compositions, we can obtain the drug-lipid interaction parameter (X) values at different temperature.

Methods: Felodipine was obtained from (AstraZeneca, UK), Glycerol monostearate (Alfa Aesar, USA), Glycerol behanate (Gattefosse SAS, France) were both used in purchased form. The melting depression of felodipine in different lipids mixtures at various compositions was determined using differential scanning calorimetry DSC 4000 (Perkin Elmer, USA) from 0°C to 160°C at a scan rate of 5°C /min. Thermal stability was investigated using a Q500 TA instrument (Leatherhead, U.K.).

Results: GB was found to be less miscible with felodipine as evidenced by the low degree of melting point depression with felodipine. In contrast, GMS was miscible to a greater extent with felodipine, exhibiting a greater degree of-melting point depression in all experimental ranges. The interaction parameter was calculated for each composition and plotted against the inverse of temperature. The interaction parameter was then extrapolated to lower drug loading compositions based on the experimentally determined values. These values were then substituted into Flory-Huggins equation to determine the solid-liquid line which represents felodipine equilibrium solubility in lipid. At 20°C, the predicted equilibrium solubility of felodipine is 3 %w/w ($\Phi = 0.0275$), while in GMS the predicted solubility of drug is 12 %w/w ($\Phi = 0.097$). It is proposed that the partial glycerides content within the GMS may interfere the formation of GMS crystal lattice and hence offers more space for accommodation of felodipine. Behenic acid, a saturated fatty acid containing 22 carbon atoms, represents the main fatty acid present in GB (>87%). The differences in fatty acid chain length may contribute to the number of imperfections created in these lipids (2).

Conclusions: The solubility of felodipine in glycerol monostearate and glycerol behanate is assessed using Flory-Huggins temperature-composition phase diagrams. It has been shown that GMS is more miscible with felodipine than GB and the equilibrium solubility of felodipine in GMS is 12% w/w whilst 3% w/w in GB at 20 °C.

DEVELOPMENT OF ISOLEUCINE-PROLINE-PROLINE CHITOSAN NANOPARTICLES COATED WITH ZEIN TO HELP INCREASE THE LOADING EFFICIENCY FOR AN ORAL DRUG DELIVERY SYSTEM

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Minna Khalid[1,2], Giuliana Vozza[1,2], Hugh J Byrne[2], Jesus M. Frias[1] & Sinéad M. Ryan[3]

[1] School of Food Science and Environmental Health, Dublin Institute of Technology, Cathal Brugha Street, Dublin 1; [2] FOCAS Research Institute, Dublin Institute of Technology, Kevin Street, Dublin 8; [3] School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4.

Background: The milk derived tri-peptide Ile-Pro-Pro (IPP) has potential to treat hypertension. IPP has previously been shown to have angiotensin-converting enzyme (ACE) inhibitory potency of 5 µM in vivo and may contribute to hypertension treatment if presented as an oral pharmaceutical. However, it has low oral bioavailability due to poor permeation across the intestinal wall. To address this, IPP was formulated into nanoparticles (NPs) based on a mucoadhesive polymer (chitosan). In addition, a prolamine protein (zein) was coated into the NP with the aim of increasing the encapsulation efficiency and producing a controlled release at the target site.

Methods: NPs were produced using ionotropic gelation. A Mixture Amount Design (MAD) was initially used to determine the best ratio of chitosan and a crosslinker, TPP at which the IPP is encapsulated. This was done using variable concentrations of chitosan (1.4 mg/mL – 1.7 mg/mL) and TPP (0.2–0.5 mg/mL) at a fixed IPP concentration (0.1 mg/mL) over 5 experiments and three replicates. Zein was then coated onto the prepared IPP-chitosan nanoparticles at 1:1 ratio (chitosan: zein) using a dropwise method. The encapsulation efficiency was determined using reverse phase high performance liquid chromatography. The NPs were characterised using Dynamic Light Scattering, Scanning Electron Microscopy (SEM) and Fourier Transform Infrared (FT-IR).

Results: A ratio of 3 (chitosan/TPP) produced the highest encapsulation efficiency of 20 ± 2% without zein addtion. Particle sizes produced were 124 ± 5nm, Zeta potential values 36±8mV and polydispersity index values (PDI) 0.259 ± 0.012. Particle size differences were seen between the coated and uncoated zein/chitosan IPP NPs, being 124 ± 5 nm and 350 ± 19 nm respectively. Using a 1:1 ratio (chitosan: zein), the encapsulation efficiency increased to 85 ± 4% of IPP-loaded NPs. Spherical nanoparticles for both coated and uncoated were observed using SEM. FT-IR showed changes in absorbance for IPP-loaded chitosan nanoparticles compared to the unloaded chitosan nanoparticles.

Conclusions: Chitosan nanoparticles may constitute an optimal delivery system for small food peptides like IPP with appropriate physical properties for oral delivery drug development. Zein is a biopolymer which can help increase the encapsulation of bioactives.

INVESTIGATION OF THE EFFECT OF LIPOSOMAL CHOLESTROL CONTENT ON THEOPHYLLINE DRUG ENCAPSULATION EFFICIENCY AND RELEASE CHARACTERISTICS

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Mohammad Alyami, Lindsay Marshal, Mandeep Marwah & Deborah Lowry

School of Life and Health Science, pharmacy, Aston University, Birmingham B4 7ET

In today's pharmaceutical industry, liposomes as a vehicle are considered as one of the leading controlled release delivery systems available to deliver a wide variety of active agents as they can carry hydrophilic and hydrophobic compounds. However, the properties and applicability of liposomes as carriers are largely dependent on the physicochemical characteristics of the membrane. The purpose of this study was to develop vehicles of liposomes that are able to enclose high content water-soluble drug particles for a controlled release pulmonary delivery system. Various ratios of L-a- phosphatidylcholine phospholipid (PC) and cholesterol were prepared as the following 16:2, 16:4 and 16:8 using the lipid film hydration method. The study showed that sonication resulted in a large reduction on liposome size from 2527+37 nm to 94.36+0.17nm. Importantly, in the absence of cholesterol, the liposomes content leaked easily; but this could be largely inhibited by the inclusion of cholesterol. The entrapment studies revealed that the correlation between the amount of cholesterol added and percentage entrapment efficiency was directly proportional, theophylline loading increased from 7.7% and 9.3% to 29.4% when the amount of cholesterol was increased. The in vitro release data exhibited a fairly constant release of theophylline over 24 h. Significantly, a high cholesterol content highlighted its capability of controlling drug release over extended periods of time releasing only 20% of theophylline after 7 h, 16:4 released 65% at 10 h, while 16:2 released 80% after 10 hrs. To conclude, the cholesterol is a substantial component of liposomes that helps improve liposome stability, enhances the loading of water-soluble compounds and provides sustained release of theophylline.

PACLITAXEL LOADED LIPID NANOEMULSIONS FOR THE TREATMENT OF BRAIN TUMOUR

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Mohammad Najlah[1], Alisha Kadam[2], Ka-Wai Wan[2], Waqar Ahmed[2] & Abdelbary Elhissi[3]

[1] Faculty of Medical Science, Anglia Ruskin University, CM1 1SQ, UK; [2] Institute of Nanotechnology and Bioengineering, School of Pharmacy and Biomedical Sciences, University of Central Lancashire, PR1 2HE, UK; [3] College of Pharmacy, Qatar University, Doha, Qatar

Background: Lipid nanoemulsions have been increasingly used as carriers for poorly soluble drugs owing to their biocompatibility and biodegradability. Paclitaxel (PTX) is an anticancer drug with wide activity against many types of cancer. However, the poor solubility in water is a serious limitation of this drug. Taxol is an established marketed formulation of PTX, which represents the drug dissolved in a vehicle consisting of ethanol and Cremophor EL (polyoxyethylated castor oil). Unfortunately, the toxic effects of Cremophor EL (nephrotoxicity, neurotoxicity, hypersensitivity, etc.) represent a significant drawback. In this study, we investigated commercially available Total Parenteral Nutrition (TPN) nanoemulsions, namely Intralipid 20% (Fresenius Kabi, Germany) and Clinoleic 20% (Baxter Healthcare, USA) nanoemulsions as vehicles and solubilizers of PTX and studied the efficacy of formulations against glioma cell lines and normal glial cells.

Methods: PTX was loaded into the nanoemulsions via vortex-mixing for 5 min followed by bath-sonication for 2 h at 40°C (Drug concentrations of 0-6 mg/mL). Size analysis and zeta potential measurements were performed using dynamic light scattering and electrophoretic mobility respectively. The entrapped fraction of PTX was calculated using UV by subtraction of the non-entrapped fraction from the total drug amount after forcing the emulsions through 400 nm syringe filters and quantifying the drug retained in the filter. MTT studies were conducted to investigate cytotoxicity of the formulations against U87-MG (grade 4 glioma) and SVG-P12 (normal glial) cell lines.

Results: Size was highly dependent on nanoemulsion type, being in the range of 254 – 264 nm for Clinololeic and 283 – 295 nm for Intralipid, depending on PTX concentration and polydispersity was generally higher for the Intralipid emulsion. Zeta potential values were negative for both emulsions with more intense charge for the Clinoleic formulations. Drug entrapment values were in the range of 70–80% and 44– 57% using for the Clinoleic and Intralipid formulations respectively. PTX-loaded Clinoleic decreased the viability of U87-MG glioma cells to 6.4%, compared to only 21.29% using PTX-loaded Intralipid nanoemulsion. Both nanoemulsions were less toxic to normal glial cells (SVG-P12), indicating selectivity of the emulsions against malignant cells. The higher entrapment in the Clinoleic emulsion correlated with the higher activity of its formulations against malignant cells. The difference in activity between the two emulsions is attributed to their different composition.

Conclusions: Nanoemulsions are applicable vehicles for solubilizing PTX and acting selectively against malignant glioma cells. Moreover, the enhanced cancer targetability of nanoemulsions might be attributed to the nutritive value of lipids present in the nanoemulsions.

AMINO ACID PRODRUGS OF LINIFANIB:LOW MOLECULAR WEIGHT GELS FOR LOCALISED CANCER THERAPY

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Mohammed Al-Ameedee, Maria Marlow & Michael Stocks

School of Pharmacy, University of Nottingham, Nottingham, NG7 2RD, UK

Background: In recent years, great effort has been focused on the innovative role of low molecular weight gelators (LMWGs) in drug delivery and tissue engineering. This interest is driven by their inherent biocompatibility as these LMWGs can self-assemble into a 3D fibrillar network, forming a hydrogel with large amounts of associated water and a low solid content (i.e. less than 2% w/w).2 One of the applications in this field is the design of hydrogels for localised controlled release, at a tumour resection site, for cancer therapy. For the studies described herein, we have selected linifanib, which is a potent multi-targeted tyrosine kinase inhibitor, with potential as a cancer therapeutic, as a candidate to create self-assembling prodrug gelators. To explore these potential self-assembling linifanib prodrugs we have linked amino acids and dipeptides to linifanib. Also some features of linifanib such as the urea functional group (hydrogen bond donor and acceptor) and aromatic rings (π–π stacking) may enforce the promotion of self-assembly into a linifanib-based prodrug hydrogel.

Methods: Linifanib was synthesised by a 3-step route as reported in the literature. Linifanib amino acid prodrugs were synthesised using liquid phase peptide synthesis. The amino acids were linked to the indazole ring of linifanib. Vial inversion was performed using 5 to 25% v/v DMSO/water mixtures using 0.25 and 0.5% w/w linifanib amino acid prodrug concentrations for preliminary screening of gelation. Oscillatory rheology was then carried out using an Anton Paar rheometer at 37 °C to confirm gel strength. Cleavage of the linifanib amino acid prodrugs to linifanib, at 37 °C in DMSO/water mixtures was also monitored for 45 days using HPLC.

Results: The Amino acids (glycine, alanine, proline, tyrosine, serine, and threonine) were linked to linifanib, and the resulting analogues were evaluated to test their potential as prodrugs. In addition, dipeptide analogues (proline-prolinyl, proline-alaninyl, proline-glycyl, phenylalanine-prolyl, alanine-glycyl, glycine-glycyl and tyrosine-glycyl) were also synthesised with yields ranging from 40-60%. It was found that the serine analogue was the optimal gelator when analysed by vial inversion in all DMSO/water ratios. Initial structural analysis suggests that the hydroxyl group, lipophilicity and length of side chain are believed to play an important role in the gelation behaviour. Oscillatory rheology confirmed the gel network. We studied the cleavage of the amino-acid analogues and demonstrated single amino acid analogues to be the most stable. Interestingly, the proline-containing dipeptides cleaved faster in 20% v/v DMSO/water at 37 °C with the highest cleavage rate being observed for the proline-proline analogue which cleaved cleanly to linifanib in 7 days. Interestingly the proline-proline analogue sample was initially in solution but then formed a linifanib gel at this 7 day time point. Proline is hypothesised to promote enhanced cleavage through the "Thorpe Ingold effect".

Conclusions: In this study we have undertaken the synthesis of a novel series of amino acid and dipeptide linifanib prodrugs and showed their self-assembling properties. We have demonstrated that the serine derivative is a promising gelator at low concentrations in DMSO/water for localised cancer therapy.

CHARACTERISATION OF SELF-ASSEMBLING PEPTIDES FOR DRUG DELIVERY

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Monica Mistry[1], Nicola Cardy[2], Helen Dearie[2], Barrie Kellam[1], Maria Marlow[1] & Stephanie Allen[1].

[1] School of Pharmacy, The University of Nottingham, Nottingham, NG7 2RD, UK;
[2] Boots Alliance, Nottingham, NG7 2SD, UK

Background: A new range of biomimetic and bioinspired research is emerging1 using peptides as building blocks and molecular self-assembly. One such category of materials are peptide amphiphiles (PA), which have been explored as materials for use within tissue engineering, 3D cell culture, controlled drug release and skin care1. Their unique, biocompatible properties make them ideal candidates for a drug delivery system. The Matrixyl® peptides (C16-KTTKS, C16-GQPR and C16-GHK) fall within this category. These short palmitoylated peptide sequences have been shown capable of permeating the skin to reach dermal layers and exert significant facial skin improvement. Numerous authors have reported the self-assembling ability of these PA into supramolecular constructs such as extended nanotapes and micelles. We have further explored their self-assembly on a model anionic substrate, to crudely simulate skin adsorption in the studies reported herein. We have also evaluated their potential as a drug delivery platform, by determining the encapsulation of a model hydrophobic drug molecule, pyrene.

Methods: Atomic force microscopy (AFM) was used to image PA (C16-KTTKS, C16-GHK and C16-GQPR) self-assemblies of varying concentrations (wt%) in solution. AFM sample solutions were prepared by dissolving PA in freshly distilled water and sonicating for 15 min. 20 µL aliquots were dispensed directly onto freshly cleaved mica surfaces, allowed to equilibrate for ten minutes, with surplus solution being removed prior to imaging. Tapping and peak force QNM modes in solution were performed using Multimode1 AFM with NanoScope V controller, equipped with Bruker model MSNL-10 and ScanAsyst-Fluid+ cantilever tips. All AFM images were flattened to the first or third order and the NanoScope Analysis Software v1.50 was employed for further image analyses. To determine the encapsulating ability of our PA self-assemblies, pyrene spectrofluorimetry was performed using Varian Cary Eclipse fluorescence spectrophotometer. Samples were made by dispensing aliquots of $2x10^{-6}$ M pyrene/acetone solutions into vials, from which the solvent was then evaporated. Dried pyrene films were re-suspended using PA solutions maintaining $2x10^{-6}$ M pyrene concentration; solutions were sonicated for 15 min in order to promote encapsulation. Pyrene fluorescence emission spectra were measured from 360–600 nm, using λ_{ex} = 335 nm

Results: Our results complement self-assembly studies previously reported for 1 wt% pal-KTTKS samples, whereby nanotape assemblies were observed. However, pal-KTTKS micellar assemblies were noted at concentrations as low as 0.003 wt%, with increasing concentration (up to 0.3 wt%) resulting in a honeycomb arrangement, and individual structures measuring 4-6 nm in diameter. Similar micellar constructs were observed for both pal-GHK and pal-GQPR (Matrixyl 3000® actives; 0.3-0.003 wt%). Interestingly, nanotape assemblies with distinct lamellar layers (6.6 nm) were observed by the unreported pal-GQPR peptide amphiphile at 1 wt%. Preliminary spectrofluorimetry data for PA-pyrene solutions showed increased fluorescence intensity, suggesting encapsulation of this model drug by micellar constructs.

Conclusions: We have successfully shown low concentrations of palmitoylated peptides are able to self-assemble in water to form micellar structures, with pyrene encapsulating capabilities. As such, our PA's demonstrate potential to be exploited as a drug delivery platform.

EFFECT OF NON-CROSS-LINKED CALCIUM ON CHARACTERISTICS, SWELLING, DRUG RELEASE AND MUCOADHESION PROPERTIES OF CALCIUM ALGINATE BEADS

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Mouhamad Khoder[1], Amr ElShaer[1], Ayman Karam[2], Mohammad Najlah[3] & Raid G. Alany[1]

[1] School of Pharmacy and Chemistry, Kingston University, KT1 2EE, Surrey, UK; [2] Institut de Chimie des Milieux et Matériaux de Poitiers, CNRS, Université de Poitiers/ENSIP, France. [3] Faculty of Medical Science, Anglia Ruskin University, Chelmsford CM1 1SQ, UK

Background: Alginates are natural, safe, cheap, and biodegradable polymers. They form gel in the presence of divalent cations, such as Ca^{2+}, without the need for toxic reactants. Due to these unique properties and the gelation simplicity, calcium alginates beads (CABs) are widely used in many pharmaceutical applications, namely in controlled release delivery systems. The Ca2+ retained by CABs can be either strongly cross-linked with polysaccharide's chains (CL-Ca) or non-crosslinked (NCL-Ca); having a weak interaction with the alginates. NCL-Ca is normally removable by a washing process whereas the CL-Ca is not washable. In this study, ibuprofen-loaded CABs with the same degree of crosslinking and different amounts of NCL-Ca were prepared and the influence of NCL-Ca on beads properties, mucoadhesiveness, swelling and drug release was explored in two different simulated intestinal fluids.

Methods: Ibuprofen-loaded CABs were prepared by ionotropic gelation using $CaCl_2$ as a cross-linker. Washing process involved soaking the freshly prepared beads in deionized water. The number, duration, and volume of washes were varied. Beads were then collected and dried at a temperature of 40 °C for 48 h. CABs swelling and drug release studies were performed in phosphate or maleate based simulated intestinal mediums using a USP rotating basket apparatus. The mucoadhesion properties of CABs were evaluated on sheep intestinal mucosa by the in-vitro wash-off method.

Results: This study showed that increasing the number or duration of washes resulted in significant decreases in the amount of NCL-Ca. However, the impact washes volume was not significant. Approximately 70% of the initial amount of Ca^{2+} was NCL-Ca which was removable by washing while only 30% was cross-linked (CL-Ca). Ca^{2+} release from the CABs was bimodal where the NCL-Ca was firstly released followed by a slower release of CL-Ca. Washing methods and, thus, the amount of NCL-Ca had significant influences on the CABs characteristics, swelling and mucoadhesiveness, and the drug release profile in SIFs.

Conclusions: This study highlights the importance of washing methods and the amount of NCL-Ca to establish CABs properties and understand their behavior in the SIFs. It demonstrates also that the composition of the SIFs is of great significance in order to perform reliable and consistent CABs swelling and release studies.

EFFECTS OF TOTAL FLOW RATE AND FLOW RATE RATIO ON LIPOSOME SIZE AND STABILITY IN A MICROFLUIDIC FABRICATION METHOD

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Muhammad Taufiq Bin Mohd Jailani[1], Katharine Carter[1] & Dimitrios A. Lamprou[1,2]

[1] Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS), University of Strathclyde, Glasgow G4 0RE, UK; [2] EPSRC Centre for Innovative Manufacturing in Continuous Manufacturing and Crystallisation (CMAC), University of Strathclyde, Glasgow, UK

Background: The biocompatibility and the flexible nature of lipid nanoparticles have been useful in producing various drug-loaded derivatives of lipid nanoparticles with desirable characteristics. Both in vitro and in vivo applications have shown that they can enhance the therapeutic efficacy of a drug by passive accumulation of the drug at the target site and reduce drug toxicity by reducing systemic exposure to the drug. Active targeting by adding a specific ligand (i.e. stealth ligands) can further improve bioavailability at the targeted site. Current evidence suggests that combination of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phospho-rac-(1-glycerol) (DSPG) and cholesterol can produce liposomes with a mean diameter of 110 nm, which can be used for encapsulation of anticancer drugs. Microfluidic technology, using flow focusing method, is one of the most suitable techniques for producing liposomes in large scale rather than using traditional batch production. In this study, the effect of manipulating aqueous-to-lipid volumetric flow rate and ratio within the microfluidic device on liposome characteristics (i.e. size, zeta potential, vesicle stability) was determined so that liposomes with a specific size could be produced.

Methods: Combinations of phospholipids (i.e. DSPC, DPPC, DSPG) and cholesterol with varying molar ratio of the phospholipids and phospholipid-to-cholesterol molar ratio were produced by NanoAssemblr™. Different total flow rates (TFR) and flow rate ratios (FRR) of aqueous-to-lipid were used in order to determine what effect they had on size and stability of formulations. The size of liposomes was determined weekly for a period of 5 weeks using Zetasizer Nano ZS. The stability of the liposomes was determined by physical observation of the product over time and by monitoring the Polydispersity Index (PDI) during the study.

Results: FRR of 3:1 and 5:1 yielded smaller-sized liposomes (53–179 nm) and the most stable liposomes at all TFRs, except for the liposomes produced using formulation with phospholipid-to-cholesterol molar ratio of 90:10 and a TFR of 0.5 mL min-1. FRR of 1:1 yielded larger-sized liposomes (220–1148 nm) compared with higher FRRs and mostly unstable products at all TFR, except for formulations that contained cholesterol molar ratio of at least 40. At TFR of 4 mL min-1, increasing FRR from 3:1 to 5:1 produced liposome that were significantly smaller. The same effect was not observed at the highest TFR of 16 mL min-1 except for the formulation with a phospholipid-to-cholesterol molar ratio of 90:10. This effect was also observed with TFR of 0.5 mL min-1, albeit at much lower magnitude compared with TFR of 4 mL min-1.

Conclusions: Increasing FRR has a significant size-decreasing effect on fabrication of liposomes using flow-focusing microfluidic technique. However, this effect is limited by TFR utilised in the fabrication process itself. FRR of 1:1 produced larger sized liposomes which were usually unstable. However, if the molar ratio of cholesterol in the formulation is increased then it is possible to produce stable liposomes.

GLUCOSE SENSITIVE SYSTEMS FOR DELIVERY OF ANTIDIABETIC DRUGS

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Negeen Kargar, Raid G Alany, Alex J Sinclair & Amr M ElShaer

Drug Discovery, Delivery and Patient Care (DDDPC), School of Life Sciences, Pharmacy and Chemistry, Kingston University London, Kingston Upon Thames, Surrey, KT1 2EE, UK

Background: Diabetes mellitus is one of the oldest healthcare conditions reported in the history. A description of the disease goes back to a 3000-year old Egyptian manuscript. In 2000, around 171 million of the world population suffered from diabetes and the number is expected to increase to 642 million by 2040 in the world. According to Diabetes UK the number of people living with diabetes in the UK has tipped over the 4 million mark for the first time in January 2016. The aim of the current project is to synthesize glucose sensitive polymer used as smart system for insulin delivery. Phenylboronic acid derivatives characterised and tested for the glucose sensitivity.

Methods: Polyglycerol synthesis carried out initially as microwave assessed acid catalyst hydrolysis, and the product characterization was carried out by nuclear magnetic resonance spectroscopy (NMR), Fourier Transform Infrared Spectroscopy (FTIR). ^{1}H and ^{13}C NMR and correlation spectra were recorded in D_2O using standard parameter sets, on a Bruker Avance III 400 MHz FT-NMR spectrometer equipped with a 5 mm PABBO BB-1H/D Z-GRD probehead. The chemical shift are reported in parts per million and are referenced to the solvent lock signal. In addition, chemical modification of the OH bonds of the polygrlycerol forming ether bond with phenylboronic acid protected with pinacol, consequently deprotection of pinacol and glucose sensitivity would be measured. Constitution of the polymer will be characterized whether it is linear, branched or cross-linked polymer. Chemical, physical, mechanical and thermal properties of the synthesized polymer will be evaluated. Different polymers in addition to proposed polymer will be evaluated to determine how polarity, charge, pH, temperature and solution viscosity would influence glucose responsiveness.

Results: Coupling ^{1}H and ^{13}C NMR demonstrated the formation of a new oligomer as new peaks appeared at 3.4–3.5 ppm for ^{1}H NMR and 65–73 ppm hydroxyl groups, 71–73 pmm polyether chains at 60–62 ppm terminal units of polyglycerol are characterized. FT-IR spectra showed peaks at 980–1200 cm^{-1} reflecting the formation of new ether linkages between glycerol monomers. Obtained spectra are similar to previously reported polyglycerol spectra in literature.

Conclusions: Synthetic chemical ligands are considered to be more stable, easier to handle and cost effective compared to enzyme-based systems. In addition, boronic acid is considered as the most promising chemical ligand. Glucose sensitiveness is demonstrated by complexation of the hydroxyl group of boronic acid with the hydroxyl groups of glucose. This can be measured quantitatively.

COCRYSTAL MECHANOCHEMICAL MANUFACTURE VIA GREEN TECHNOLOGY: HOT MELT EXTRUSION

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Niamh Heron, Jones David S. Jones & Gavin P. Andrews

Pharmaceutical Engineering Group, School of Pharmacy, Queens University Belfast, BT9 7BL, UK

Background: The solubility of a drug within the gastrointestinal tract has a direct impact upon its oral bioavailability. Currently numerous strategies have enhanced drug solubility; however, these approaches are unreliable as long term solutions due to physical instability of the drug form. Crystal engineering via cocrystallisation allows for the optimization of physicochemical properties of the drug, in particular solubility and improved physical stability. Cocrystals incorporate pharmaceutically acceptable neutral guest molecules (coformers) into the crystal lattice of the API, most commonly via hydrogen bonding. Traditionally, cocrystals have been manufactured via solvent evaporation, solid-state and solvent-assisted grinding. Mechanochemical solid state grinding can be utilized as a solvent free method for cocrystal formation, as supramolecular interactions between drug and coformer can be broken and reformed under mild mechanical conditions, however it lacks ease of scale up. More recently there is a desire for an environmentally friendly solvent free approach that also has an ease of scale up. Hot melt extrusion is a green method that has gained popularity within pharmaceutical manufacturing of cocrystals. This work proposes the use of, hot melt extrusion, for the manufacture of indomethacin (IND) and saccharin (SAC) cocrystals.

Methods: Hot Melt Extrusion (HME), an equimolar (1:1) mixture of IND and SAC were extruded using a co-rotating twin screw extruder (Rondol Microlab Twin Screw 10mm). A range of screw speeds, temperatures and screw configurations were tested to optimise cocrystal yield. Samples were weighed (7–10mg) and crimped into Perkin-Elmer DSC pans. Melting points were measured in a heat-flux DSC (DSC4000, Perkin-Elmer, United Kingdom, TA Instrument). In Vitro Dissolution Studies. Testing was carried out using a Copley dissolution USP II paddle apparatus (Copley DIS 8000), with 900 mL of 0.1N hydrochloric acid solution (pH 1.2) maintained at 37 ± 2°C, a paddle speed of 100 rpm for 4 h. Samples were analysed using UV spectroscopy at an absorbance of 318 nm.

Results: DSC scans for IND-SAC extrudates, across a range of processing parameters, exhibited various melting transitions characteristic of individual components and cocrystal. A low screw speed and a high temperature within the mixing zones significantly enhanced the ability to obtain a single melting transition corresponding to the IND-SAC cocrystal. Kneading elements introduced to the screw configuration in three separate mixing zones had a significant effect upon cocrystal yield. Kneading elements generate high friction between the barrel and screws inducing excellent mixing of the materials. Also at a higher extrusion temperature IND has melted and has the added advantage of a solubilising effect on SAC. Bulk IND exhibited a slow dissolution rate whereas cocrystal extrudates exhibited a faster dissolution rate; with an approximate 3-fold increase. Interestingly, solvent evaporated cocrystals superseded extruded cocrystals, with an approximate 5- fold increase relative to bulk IND.

Conclusions: HME has been shown to be effective in producing IND-SAC cocrystals. This study highlighted that extrusion temperature, screw speed and screw configuration are all critical processing parameters in order to manufacture a high yield of pure cocrystals.

CHITOSAN BASED IN SITU GELLING SYSTEMS FOR INTRAVESICAL DRUG DELIVERY

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Oluwadamilola Kolawole, Wing-Man Lau & Vitaliy Khutoryanskiy

Department of Pharmacy, University of Reading, Reading, RG6 6AP, United Kingdom

Background: Bladder cancer chemotherapeutics given orally or systemically are ineffective due to the poorly vascularized urothelium. Site specific drug delivery offered by intravesical administration has been limited by urine drug dilution and wash-off. Chitosan/β-glycerophosphate (CH/ß-GP) systems have not been explored for intravesical drug delivery despite applications in other areas of pharmaceutics and tissue engineering. The mucoadhesive properties of chitosan, in addition to its gelation in the presence of ß-GP would generate formulations with improved performance.

Methods: CH/ ß-GP solutions were formulated using low, medium and high molecular weight chitosan (LCH/ß-GP, MCH/ ß-GP and HCH/ ß-GP). Their gelation properties were evaluated at 20 °C and 37 °C using vial inversion method. Glass vials containing samples incubated in water bath (37 °C) for 1 h were used to determine the gelation time. Rheometer scanned from 10 °C to 65 °C as well as maintained at 37 °C determined their gelation temperature / strength and time, respectively.

Results: Rheological analysis showed that HCHI/ ß-GP formed gel most readily, with stronger gels than LCH/ ß-GP and MCH/ ß-GP. They all had final pH from 7.0-7.26 but only HCH/ ß-GP (1.5wt%: 50wt%) maintained in water bath formed gel at 6 min and remained in a gel state after 30 mins. However, LCH /ß-GP (2wt%: 60wt%) formed gel at 5 min but underwent syneresis (expulsion of water by the gel) within 30 min. MCH /ß-GP (1.5wt%:80wt%) only formed gel after 1h.

Conclusions: Findings suggest that chitosan's molecular weight is the critical parameter for gelation of such systems at 37 °C. HCH/GP systems may be viable delivery vehicles for the intravesical administration of anticancer drugs for bladder cancer treatment.

INVESTIGATING THE FUNCTIONAL CYTOSOLIC DELIVERY OF PEPTIDES USING SPLIT GFP

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Pamela Riester[1], Arwyn Jones[2] & Peter Watson[1]

[1] School of Biosciences, Cardiff University, Cardiff, CF10 3AX, Wales, UK;
[2] School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, CF10 3NB, Wales, UK

Background: Macromolecular biopharmaceuticals hold great potential as therapeutic agents to cure diseases. Despite this potential, it is still a major challenge to deliver macromolecules such as proteins or peptides across cellular membranes to access therapeutically relevant targets in the cytosol. Drug Delivery Systems (DDSs) such as Cell Penetrating Peptides (CPPs) can facilitate the uptake and delivery of cargo across the plasma membrane. Many of these systems exploit the uptake and internalisation of extracellular cargo into membrane bound vesicles through the process of endocytosis. In order to affect a cytosolic target, it is crucial that the biological therapeutic must avoid degradation in the lysosome, preferably by escaping from the endolysosomal system. For this reason it is essential that we are not only able to assess cell binding and uptake, but also functional delivery of the cargo into the cytosol in order to evaluate the delivery efficacy of DDSs. One system that holds great promise to detect functional cytosolic delivery is based on the complementation of split Green Fluorescent Protein (GFP). It relies on the separation of GFP into two non-fluorescent components, a large fragment (GFP1-10) and a small fragment (known as M3). When present in the same compartment, these fragments are able to reassemble to full length GFP and restore the characteristic green fluorescence properties to the protein. Here we describe the generation and testing of a cellular readout for the functional delivery of biologicals into the cytosol utilising split GFP.

Methods: A strategy was developed to define the effectiveness of CPP based peptide delivery systems. The suitability of a number of split GFP constructs was assessed for their ability to generate a quantitative readout of cytosolic delivery of functional peptides, detected by widefield microscopy. Stable cell lines were generated to improve consistency within the assay.

Results: CPP based peptides were investigated for their ability to deliver M3 into GFP1-10 expressing cells. Functional cytosolic delivery of M3 was shown to be CPP dependent and increased in a concentration dependent manner. Delivery was also shown to be highly influenced when a fluorescent dye was attached to the CPP-M3. Real time delivery of a CPP-M3 peptide can be visualized using time-lapse microscopy.

Conclusions: The Split GFP system is a robust method to assess the functional delivery of the M3 peptide by CPPs. Concentration as well as time dependency of delivery can be monitored using this method. Detection of GFP complementation with a microscope can give detailed information about real time delivery and intracellular localization of the cargo but also holds great potential for high throughput application to evaluate novel DDSs.

FORMULATION OF SONICATED BILAYER LOADED LIPOSOMES

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Peter Stone[1], Alex Rozhin[2], Jerome Kroonen[3] & Yvonne Perrie[1]

[1] School of Life and Health Sciences, [2] School of Engineering and Applied Science, Aston University, Birmingham, B4 7ET, UK. 3Diagenode, Liege Science Park, [3] Rue bois Saint- jean, 4102 Seraing, Ougrée, Belgium.

Background: Bath sonication has been shown to be useful for the rapid small scale production of bilayer-loaded liposomes. The purpose of this study was to investigate and develop a bath sonication protocol using the Bioruptor® Standard to prepare liposomal solubilisation systems for propofol.

Methods: Liposomes were formed using the lipid hydration method; weighed amounts of lipids and propofol were added to a final concentration of 1, 2-dimyristoyl-sn-glycero-3- phosphocholine (DMPC) and propofol (8:6 µmoles; 5:2 w/w) or DMPC in combination with cholesterol and propofol (4:4:6 µmoles; 10:7:4 w/w). The organic solvent was extracted using roto-evaporation and the lipid film hydrated using 2 mL of 10 mM Tris buffer at pH 7.4 at above 45 °C. Multilamellar vesicles (MLV) were then sonicated in the bath sonicator (Bioruptor® Standard, Diagenode, Liege, Belgium), at 45°C for 15 minutes. The size and polydispersity (PDI) were measured using a Brookhaven Nanobrook 90 plus Zeta (Brookhaven Instruments, New York, USA) and drug loading confirmed by HPLC (Luna 5µm C8, Phenomenex, pore size of 100 A).

Results: Initially DMPC liposomes prior to sonication were over 3343 ± 362 nm in size, with the addition of cholesterol to the formulation approximately doubling vesicle size to 6167 ± 2674 nm. Sonication reduced the liposomes in size to 213 ± 26.6 nm for DMPC liposomes and to 576 ± 123 nm with the addition of cholesterol. The addition of propofol to these liposomes resulted in a reduction in vesicle size to 1435 ± 236 nm for DMPC liposomes and 1142 ± 104 nm for DMPC liposomes in combination with cholesterol. Interestingly the presence of cholesterol within these MLV formulations reduced propofol loading from 39.5 ± 4 % to 11.5 ± 1 %. Sonication of all formulations resulted in a reduction in vesicle size, as would be expected; however, sonication did not reduce drug loading with DMPC liposomes of 135 ± 13.5 nm being able to incorporate similar drug concentrations compared to the MLV formulation with 36.4 ± 4 % drug concentration. With the addition of cholesterol, sonicated liposomes were 185 ± 3.4 nm in size and incorporated a drug concentration of 9.9 ± 2 %. This would suggest the bilayer composition rather than vesicle size is the dominant factor controlling drug loading.

Conclusions: MLV and sized-reduced DMPC and DMPC: cholesterol liposomes were loaded with propofol successfully and it was found that the vesicle composition rather than the vesicles size was the controlling factor in drug loading.

HAEMATOCOMPATIBILITY ASSESSMENT OF SILK AND SILICA NANOPARTICLES

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Manfred F. Maitz[1], Claudia Sperling[1], Thidarat Wongpinyochit[2], Manuela Herklotz[1], Carsten Werner[1] & F. Philipp Seib[1,2]

[1] Leibniz-Institut für Polymerforschung Dresden e.V., Max Bergmann Centre for Biomaterials Dresden, Hohe Strasse 6, 01069 Dresden, Germany; [2] Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow, G4 0RE, UK

Background: Nanoparticles often are designed as potential nanomedicines for parenteral administration. However, emerging evidence suggests that haemocompatibility is highly particle- and test bed-dependent. Thus, the knowledge of bulk material properties does not enable the accurate prediction of nanoparticle haemocompatibility.

Methods: Therefore, here the haemocompatibility of both silk nanoparticles and silica reference nanoparticles was tested using human whole blood under both quasi-static and flow conditions.

Results: We demonstrate, for selected nanoparticles, substantial differences between quasi-static and dynamic haemocompatibility studies. In particular, the inflammatory response towards silk nanoparticles was significantly lower under flow conditions than under quasi-static conditions. We also demonstrated very low pro-coagulant properties for silk nanoparticles—an observation that was scalable from the macro- to nano-level. The haemocompatibility studies of nanoparticles were complemented by preliminary live cell measurements to provide insights into the endocytosis and trafficking of these nanoparticles in human blood cells.

Conclusions: Overall, this study demonstrates that a multitude of factors affect nanoparticle hemocompatibility, including the test bed design.

FLEXIBLE FORMULATIONS FOR CHALLENGING COMPOUNDS IN SUSTAINED RELEASE MICROSPHERES

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Rhian Groves, Paul Seaman & Daniel Palmer

Midatech Pharma, Cardiff CF24 0AA, Wales, UK

Background: Background: In clinical ophthalmology, novel drug regimens are in development for the treatment of major eye diseases such as Age Related Macular Degeneration and Posterior Uveitis. Systemic administration of these therapeutics often lead to off target side effects. Utilization of long acting treatments achieved by encapsulating the drug in PLA microspheres is a desirable alternative. However, the hydrophilic or lipophilic nature of these novel APIs make the traditional method of manufacturing these particles unfeasible as they often result in low drug loading efficiency and significant initial burst release. Midatech Pharma's sustained-release technology, Q Sphera, enables flexibility in formulation design providing an opportunity to address these challenges, whilst addressing the challenges of intravitreal administration.

Methods: The Q Sphera microsphere manufacturing platform was utilized to encapsulate a large pegylated polypeptide within a PLA matrix. Formulation development focused on (i) avoiding poorly tolerated organic diluents such as dichloromethane and tailoring the formulation solvents to the therapeutic; (ii) the manufacturing procedure exploits solvent exchange principles to turn droplets of PLA into microspheres thereby avoiding solvent interfaces and shear force. Tuning this desolvation process ensures the drug remains within the microsphere to produce high drug loading; (iii) introducing a secondary processing step which attenuates the initial burst release

Results: The pegylated polypeptide was successfully encapsulated within PLA matrices, achieving the characteristics beneficial for effective drug concentration sustainably released into the intraocular target site.

- A drug loading of between 8–10% w/w
- Monodispersed particles (CV - 5.57 %) of diameter 35 µm
- Provided rapid suspension and were injectable via ½" 27G hypodermic needle
- Linear release rate kinetics maintained for 90–100 days with a release rate of 0.5 µg/mg/day attained

Conclusions: The Q Sphera illustrated the ability to process novel ophthalmic APIs in to controlled release formulations. These data support that the Q Sphera technology represents a transformation in control for microsphere manufacture. The flexibility afforded in formulation development opens up the possibility that notoriously problematic therapeutic molecules – for example poorly soluble, highly hydrophilic or toxic molecules can be encapsulated within PLA microspheres resulting in therapies that previously could not be considered.

OXIDATION-RESPONSIVE DRUG DELIVERY SYSTEMS: IMPROVING THE SENSITIVITY TO REACTIVE OXYGEN SPECIES

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Richard d'Arcy & Nicola Tirelli

University of Manchester, Manchester, M13 9PT, UK

Background: Many of today's prevailing diseases have a pathology associated with inflammation, these include Alzheimer's, Parkinson's, cirrhosis, diabetes, atherosclerosis and cancer, all of which have been heavily linked with the presence of reactive oxygen species (ROS). Polysulfides are sulfur (II)-composing organic polymers which can be oxidized by ROS to the higher oxidation state sulfoxides (or sulfones) resulting in a large increase in polarity and a hydrophobic to hydrophilic transition; accordingly, this transition can and has been exploited to release a drug payload in response to ROS. In this work, tailoring of the payload release was achieved using novel polysulfides with differing degrees of hydrophobicity.

Methods: The novel monomer thioglycidol (TG) was synthesized from its epoxy analogue glycidol and copolymerized with propylene sulfide (PS) via an anionic ring-opening polymerization in differing ratios (i.e. 15:5, 10:10, 5:15 and 0:20) from a PEG-thioacetate initiator and end-capped with propargyl bromide. PEG-polysulfide diblock copolymers were fashioned into micelles through nanoprecipitation in water and core-crosslinked through a radical thiol-yne reaction between a trithiol (trimethylolpropane tris(3-mercaptopropionate)) and the terminal propargyl moieties; AIBN was used as the radical initiator. Nile Red (NR) was loaded into the micelles and used a fluorescent probe to measure the rate of polysulfide oxidation/drug release when exposed to hydrogen peroxide (H_2O_2).

Results: Polymers were synthesized close to their theoretically predicted molecular mass values with low dispersities ($Đ \leq 1.25$) and upon nanoprecipitation into water formed aggregates ranging from 20–200 nm (increasing size with increasing TG content). The rate of NR quenching measured after exposure of micelles to H_2O_2 was found to be greatly enhanced with increasing TG content; additionally, crosslinking of the micelles resulted in a somewhat decreased rate of oxidation; this effect being most significant for samples of high TG contents with time to half maximum NR quenching ($t_{1/2}$) increasing from 42 to 256 min after crosslinking.

Conclusions: Decreasing the hydrophobicity of the polysulfide lead to an increase in the rate of oxidation/payload release in response to H_2O_2, whereas crosslinking of the micelle structure has an opposite effect which is mostly limited in the higher TG-composition polymers; this is most likely due to the higher CMC of these polysulfides.

EXPOSURE TO NON-IONIC SURFACTANT (SOLUTOL HS15) INDUCES TRANSIENT 'METABOLIC SPIKE' AND MITOCHONDRIAL MEMBRANE HYPERPOLARISATION PRIOR TO ONSET OF CYTOTOXICITY IN INTESTINAL EPITHELIAL CELLS

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Robert Cavanagh & Snjezana Stolnik

School of Pharmacy, University of Nottingham, Nottingham, NG7 2RD, UK

Background: Solutol HS15 is a non-ionic surfactant and principle component of CriticalSorb™, a novel drug delivery platform which has been previously demonstrated to enhance the bioavailability of bio-therapeutics in vitro and in vivo. As with other surfactants, Solutol demonstrates a profound effect on the cell plasma membrane. It is this membrane effect that is attributed to increasing epithelial cell permeability via the enhanced transport of molecules along the transcellular pathway of endocytosis or transcytosis. The current work set out to study the kinetics of Solutol induced mitochondrial and membrane effects, in an effect to decipher the succession of events dictating Solutol's action on intestinal epithelium and what influence these may play in Solutol's ability to enhance permeability.

Methods: Caco-2 intestinal cells were seeded at a density of $1x10^4$ cells/well in 96 well plates and were cultured for 48h prior to assaying. Plasma membrane integrity and relative metabolic activity were assessed using the LDH release assay and MTS assay, respectively. Mitochondrial membrane potential was studied using the JC-1 assay. Effect on cellular uptake was probed by measuring uptake of FITC-dextran (4,000 MW).

Results: At exposures ≥ 60 min uptake of FITC-dextran (4,000 MW) is enhanced, however, dose- and exposure-dependent cytotoxic effects also begin to occur, including LDH release, decrease in metabolic activity and depolarisation of the mitochondrial membrane potential. Within the initial 10 min of exposure, however, a dose-dependent increase in metabolic activity is observed, maximal increase 2.3-fold relative to control, alongside a concurrent dose-dependent hyperpolarisation of the mitochondrial membrane potential and dose-dependent decrease in FITC-dextran (4,000 MW) uptake. All three parameters then return to baseline levels by 20 min of exposure.

Conclusions: Current work illustrates that Solutol appears to be inducing some striking metabolic effects prior to the classical plasma membrane destabilizing associated with surfactant exposure. Mitochondrial membrane hyperpolarisation is known to effectively shut off mitochondrial respiration. During this period cells have to rely on aerobic glycolysis, rather than mitochondrial-dependent metabolic pathways for their bioenergetic demands. Glycolysis alone is a rapid, but inefficient pathway to produce ATP. But, per ATP molecule generated, glycolysis produces more NADH than mitochondrial-dependent pathways. Therefore, cells dependent on glycolysis may have higher levels of NADH, which catalyses the conversion of MTS to formazan, the basis of the MTS assay. This may, thus, explain the spike observed in metabolic activity following short (5–10 min) Solutol exposures. Taken together these results indicate that Solutol, and possibly other non-ionic surfactants, may be capable of inducing effects on cellular metabolism almost immediately following application. Furthermore, in addition to possible functions as a cell survival mechanism, these effects may be responsible for the decrease in uptake observed initially and, thus, may play significant roles in the regulation of epithelial permeability.

SYNTHESIS OF NOVEL MUCOADHESIVE NANOGELS VIA POST-POLYMERISATION MODIFICATION

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Ruairí Brannigan[1], Michael Cook[2], Prasopchai Tonglairoum[1] & Vitaliy Khutoryanskiy[1]

[1] School of Chemistry, Food and Pharmacy, University of Reading, Reading, RG66UR, UK;
[2] Department of Pharmacy, University of Hertfordshire, Hatfield, AL109AB, UK

Background: Mucoadhesive microgels have been of great interest in recent times owing to their potential application as dosage forms for trans-mucosal drug delivery. Furthermore, the synthesis of nano/microgels have been extensively studied offering a unique platform for the facile generation of drug carriers. In this presentation, we discuss recently published work in the synthesis of novel thiolated microgels as well as some current work on the synthesis of a new generation of mucoadhesive microgel materials.

Methods: Synthesis and characterisation of microgels: Microgels were synthesised via the thermally-initiated free-radical emulsion polymerisation of functional monomers using a bifunctional acrylic crosslinking agent. The microgels were purified by centrifugation followed by dialysis against deionised H_2O. Size distributions of the microgels were determined by dynamic light scattering (DLS), nanoparticle tracking analysis (NTA) transmission electron microscopy (TEM). The functionality of the microgels were determined by FT-IR spectroscopy. Mucoadhesion: Microgel mucoadhesion was determined by fluorescence microscopy using *ex-vivo* animal tissue and an in-house developed technique under simulated physiological conditions.

Results: Synthesis of microgels: Microgels were synthesised and modified post-polymerisation and purified to yield thiol, acrylate and malaimide functional materials. Microgel characterisation: All microgels were found to have a monomodal size distribution with polydispersity index (PDI) of <0.2 and a relatively good colloidal stability, as measured by zeta-potential measurements. FT-IR spectroscopy confirmed the presence of the respective functionalities.

Conclusions: The post-polymerisation modification of microgels offers a facile route to the generation of mucoadhesive materials which can be used as delivery mechanisms for therapeutics.

HOT MELT EXTRUSION FOR THE PRODUCTION OF NOVEL FIXED DOSE COMBINATIONS

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Saeed Marji, David Jones & Gavin Andrews

School of Pharmacy, Queens University Belfast, BT9 7BL, UK

Background: Fixed dose combination (FDC) products are commonly used in the treatment of chronic diseases such as hypertension, diabetes and human immunodeficiency virus (HIV). They allow for the combination of two or more drug molecules in a single dosing unit for optimization of treatment. Polymeric platforms are commonly used as a dispersion matrix where the properties of the final dosage form are influenced by the polymer properties. Recently, polymeric mixtures were employed to enhance the solubility of APIs and/or modify their release behavior. Hot melt extrusion has been used to manufacture innovative FDC for hypertension, incorporating the calcium channel blocker felodipine (FD) and angiotensin receptor blocker losartan (LS). This FDC presents formulation challenges as FD is a poorly water-soluble drug whilst LS is highly water soluble but has a short half-life. Thus a formulation with the capability of enhancing FD solubility and sustaining LS delivery is ideal. Soluplus was used as a dosage form matrix, which enhances the solubility of FD. Hydroxypropyl cellulose (HPC) was used to modify the release behavior of LS and FD.

Methods: Solid-state characterization was performed using differential scanning calorimetry, DSC 8000 (Perkin Elmer, USA) and powder x-ray diffraction (PXRD) (RigakuTM, miniflex II, Japan). Thermal stability was investigated using Q500 TA instrument (Leatherhead, U.K.). Physical mixtures of formulations were extruded using a co-rotating twin-screw extruder (Microlab, Rondol Technology Ltd., France). Drug release was performed in 500 mL PBS (pH 6.8) using USP type II dissolution tester operated over a period of 7 h (Copley, Copley Scientific Ltd., UK).

Results: DSC results exhibited a glass transition (Tg) with absence of any endothermic peaks relating to the melt of crystalline FD. PXRD patterns of extruded samples showed no distinctive peaks relating to crystalline FD however, peaks relating to crystalline LS were visible. This was indicative of the formation of an amorphous solid dispersion of FD and soluplus with crystalline LS dispersed in this matrix. Dissolution results showed a significant enhancement in the solubility of FD as a result of the drug being formulated into its amorphous form. FD release was prolonged over the experimental period of 7 h with greater than 85% drug released. The use of HPC enabled the release of both drugs in a sustained manner.

Conclusion: Hot melt extrusion was successfully employed to produce FDC using different hydrophilic polymeric matrices. Release behavior of the formulations based on polymeric mixtures was manipulated to provide a sustained-release formulation whilst also enhancing the solubility of FD.

ARTIFICIAL NEURAL NETWORK: PREDICTION OF ENHANCEMENT OF THE APPARENT AQUEOUS SOLUBILITY OF INDOMETHACIN BY HYDROTROPES

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Safa Damiati, David Barlow, Norman Smith, Luigi Martini & Jayne Lawrence

Institute of Pharmaceutical Science, King's College London, London SE1 9NH, UK

Background: Approximately 40 % of drug candidates fail to reach the market due to problems of poor aqueous solubility. It is well known that poor aqueous solubility can significantly limit a drug's efficacy and ultimately its bioavailability, thereby necessitating the administration of higher doses and increasing the chance of side effects as well as costing more per patient. Solubilisation using hydrotropes is a possible solution to address this problem. A hydrotrope is a compound that can enhance the apparent solubility of poorly water-soluble (hydrophobic) compounds. Like surfactants, hydrotropes are amphiphilic in nature but unlike surfactants they do not self-assemble into micelles. This is because although they consist of both hydrophilic and hydrophobic parts, the hydrophobic part is 'too small' to cause micellisation. Despite their potential for drug solubilisation, relatively little work has been performed exploring the ability of different hydrotropes to increase solubility. The aim of this work was therefore to use an artificial neural network (ANN) to predict the ability of a range of potential hydrotopes to improve the aqueous solubility of the poorly water-soluble drug, indomethacin.

Methods: Indomethacin was selected as a poorly-water soluble drug together with a set of structurally related potential hydrotropes (e.g, sodium benzoate, sodium salicylate and nicotinamide). First, excess indomethacin was added to aqueous solutions of hydrotrope prepared at a range of concentrations. After equilibrium had been reached, excess drug was removed from the solution and the amount of indomethacin dissolved as a function of hydrotrope concentration, quantified by UV spectroscopy coupled with high performance liquid chromatography. ANNs were then trained using the drug solubility as output, and input furnished by the experimental data generated, together with various computed physicochemical descriptors for the hydrotropes. The trained ANN was subsequently used for in-silico screening of untested hydrotrope systems in a search for compounds that might afford a greater enhancement of indomethacin solubility.

Results: The trained ANN was found to give highly accurate predictions of indomethacin solubility in the presence of hydrotropes and was thus shown to provide a valuable means by which hydrotrope efficacy could be screened in-silico. Interrogation of the network weights also allowed quantitative assessment of the relative importance of the various hydrotrope physicochemical properties in determining the extent of the enhancement in indomethacin solubilisation.

Conclusions: *In-silico* screening of drug/hydrotrope systems using artificial neural networks has afforded new insights into the phenomenon of hydrotropy, and is shown to have the potential to reduce the need for extensive laboratory testing of these systems, thereby providing an economy in terms of reduced costs and time in formulation development.

USING FROG FOAM AS A NOVEL DRUG DELIVERY SYSTEM

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Sarah Brozio[1], Dimitrios Lamprou[1,2] & Paul Hoskisson[1]

[1] Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS), University of Strathclyde, UK
[2] EPSRC Centre for Innovative Manufacturing in Continuous Manufacturing and Crystallisation (CMAC), University of Strathclyde, Glasgow, UK.

Background: Drug delivery systems offer an opportunity for enhanced therapeutic effect and among the variety of delivery systems that have been developed there is still room for improvement; as many systems fail to release the concentration required for effective treatment. Engystomops pustulosus is a frog species found in Trinidad, and lay their eggs in highly stable foam nests which consist of six proteins called Ranaspumins (RSNs). RSN-2 is the main surfactant protein; it is non-toxic to mammalian cells. The foam can uptake dyes or antibiotics and is currently being investigated as a novel drug delivery system. In this research a model release system was produced to investigate the foams drug release potential.

Methods: FTIR and CD were carried out to analyse the secondary structures present in the foam. A dynamic dialysis method was applied to test the capability of E. pustulosus foam to release vancomycin, calcein and Nile red. Rheology investigations have been carried out using oscillation and time sweeps. Experiments are under way to produce a synthetic foam using expression constructs in E. coli to over produce the Ranaspumin proteins.

Results: Dye release studies demonstrated a stable release over three days, and up to one week. Initial rheology experiments indicate that the foam is stable under shear stress until around 50Pa, and undergoes coarsening during oscillation sweeps. RSN-2 and RSN-3 have been successfully overexpressed in E.coli, expressed RSN-2 produces a short lived foam.

Conclusions: The current results indicate that the foam has potential as a drug delivery system, and that synthetic foam could be created. Future work includes performing toxicology experiments using HeLa epithelial cells, and investigating the release of antimicrobial agents such as silver sulfadiazine. Further, the killing ability of foam released vancomycin will be tested against Staphylococcus aureus and Pseudomonas sp. The final goal is to produce a synthetic foam, which could be utilized as a novel topical drug delivery system.

CONTROLLING THE DISSOLUTION BEHAVIOUR OF POORLY WATER SOLUBLE ACTIVE PHARMACEUTICAL INGREDIENTS

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Pauric Bannigan, Teresa Tierney, Kata Bodnar, Barry Murphy, Vivek Verma, Raquel Arribas Buenos, Ake Rasmuson, Peter Davern, Kieran Hodnett & Sarah P Hudson

Synthesis and Solid State Pharmaceutical Centre, Department of Chemical and Environmental Sciences, Materials and Surface Science Institute, University of Limerick, Limerick, Ireland

Background: Many potential active pharmaceutical ingredient (API) candidates have poor water solubility and slow dissolution rates in vivo, leading to limited bioavailability and failure to reach the market. The rate at which an API is absorbed into the body, be it after oral, subcutaneous, intramuscular or topical administration, depends on the rate at which it dissolves into the surrounding bodily fluids/tissue. However the API can be formulated with excipients (e.g. polymers, surfactants, sugars, salts, etc) to speed up that rate of dissolution or to slow it down. If the API is in solid form when administered to the body, the molecular interactions between the API molecules themselves and their interactions with the excipient molecules can influence the rate of dissolution. In our research group, strategies to enhance apparent solubilities or dissolution rates include nucleation of metastable polymorphs, heterogeneous crystallization onto excipients, generation of multicomponent crystals and nanoparticle generation by antisolvent precipitation. The following poorly water soluble APIs have been recently investigated: carbamazepine (anti-convulsant API), fenofibrate (cholesterol lowering API), mefenamic acid (pain relieving API) and clofazimine (antibacterial API).

Methods: Supersaturated solutions of APIs are generated by evaporative or antisolvent addition methods to nucleate metastable polymorphs or stable polymorphs of smaller particle size. This is done in the presence of both suspended and dissolved excipients and the role of the excipient in controlling the form and particle size of the resulting API is examined. Novel multicomponent crystals are isolated by mixing the API with the appropriate amount of coformer in an appropriate solvent, followed by slow evaporation of the solvent and analysis of the resultant crystals. X-ray diffraction, scanning electron microscopy, laser diffraction particle size analysis and thermal analysis are used to characterize the resultant solid forms. Rate of dissolution into aqueous solutions are measured by HPLC or UV-visible spectroscopy, as appropriate.

Results: Metastable polymorphs of carbamazepine and mefenamic acid are formed in highly supersaturated solutions, generated by rapid solvent evaporation or antisolvent precipitation respectively, in the presence of excipients. Nanosuspensions of fenofibrate and mefenamic acid have been generated by antisolvent precipitation and show enhanced dissolution rates compared to commercial formulations. The stabilization of the nanosuspension during nucleation, drying and isolation is challenging and requires the presence of stabilizing additives. Multicomponent crystals of clofazimine with salts have been isolated and characterized. One of these multicomponent crystals shows a large enhancement in the solubility (temporarily) and the dissolution rate of clofazimine in distilled water compared to its known polymorphs.

Conclusions: Manipulating the solid form, composition or particle size of a poorly water soluble API can enhance the apparent solubility or improve the dissolution rate, thus enhancing the bioavailability, of orally administered poorly water soluble API's.

IMPROVING TARGETING AND CELLULAR UPAKE OF LIPOSOMES TO DEVELOP LYMPHATIC DRUG DELIVERY

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S. Khadke[1], A. Cameron[2], A. Devitt[2] & Y. Perrie[1]

[1] Molecular Biomedical Research Group, Aston University, Birmingham, B4 7ET, UK
[2] Cell & Tissue Biomedical Research Group, Aston University, Birmingham, B4 7ET, UK

Background: Liposomes have been extensively studied as carriers for the delivery of therapeutic and diagnostic agents to the lymphatic system. The lymphatic system is a vital route to the spread of metastasis of most human cancers. To eradicate those metastatic cancer cells from the lymphatic system, efforts have been made to develop new and efficient lymphatic targeting systems in order to achieve high lymphatic uptake and localization. Previous studies suggest that both vesicle size and charge may be major factors in targeting, with cationic liposomes tending to form a depot at the site of injection and limiting drainage from the injection site. Therefore, this study has considered the role of anionic lipids in the development of liposomal targeting systems to the lymphatics.

Methods: Liposomes were formulated from distearoyl phosphatidylcholine (DSPC) and cholesterol (6:4 µmoles) and also supplemented with either the addition of phosphatidylserine (PS) or dimyristoyl phosphatidyloglycerol (DMPG). To track their distribution in vivo 3H-cholestrol was incorporated with the liposomal bilayer. Biodistribution of the radiolabeled liposome formulations was studied in 6-9 week old, BR/NMRI female mice at four time points post injection. Each dose of 50 µL of i.m. injection contained 48 ng PS/DMPG. All in vivo studies were conducted under the regulations of the Directive 2010/63/EU. Macrophage uptake studies were performed in vitro using the human macrophage cell line THP-1.

Results: Results showed that liposomes containing PS showed the highest localisation at the draining lymph node with 27 % at 24 h, compared to neutral DSPC:Cholesterol and anionic DMPG liposomes. This suggests that the choice of anionic lipid within the liposomal formulation is a controlling factor. Moreover, reduction of the size from ~700–100 nm further improved locatisation to draining lymph nodes. Lymphatic localization of the dose of 100 nm PS liposomes was approx. 30 times higher at 24 h post injection compared to 700 nm vesicles. Furthermore, saturation of lymphatic uptake and lymph node localization did not occur over a large liposomal lipid dose range up to 480 µg/dose, illustrating the efficient performance of lymph nodes in capturing i.m. administered PS liposomes. The presence of liposomes at the lymphatics was confirmed by histology of lymph nodes. This enhanced trafficking of small PS liposomes may be the result of macrophage uptake; considering macrophage uptake using differentiated THP-1 cells shows that uptake was promoted by the presence of PS and reduced vesicle size with 80% of the macrophages taking up 100 nm PS liposomes after 180 min of incubation with macrophages. This may be due to the presence of PS serving as a recognition signal for phagocytosis by macrophages.

Conclusions: Inclusion of PS within 100 nm sized liposomes and injection via the i.m. route of administration promoted targeting and retention of the liposomes to the draining lymphatics.

NOVEL METHOD FOR THE EVALUATION OF DRUG RELEASE FROM DRUG-ELUTING BEADS

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Tanya S. Swaine[1,2], Laura Waters[1], Yiqing Tang[2], Pedro Garcia[2], David Grey[2], Alex Henman[2], Sean L. Willis[2] & Andrew L. Lewis[2]

[1] University of Huddersfield, Applied Sciences, Queensgate, Huddersfield, UK
[2] Biocompatibles UK Ltd, a BTG International group company, Watchmoor Park, Camberley, UK

Background: A novel flow through elution method mimicking embolization was developed to quantify drug elution from Drug Eluting Beads (DEB). This study intends to overcome limitations of existing techniques and to provide more in vivo-like drug elution profiles for DEB evaluation. DEB of various sizes loaded with doxorubicin at clinically relevant concentrations were tested. The developed method used a temperature controlled continuous open-loop flow through extraction cell through which the elution medium was driven by a peristaltic pump, and drug elution was monitored by a UV detector.

Methods: Historically, elution rates have been generated largely on a closed-loop system, which are highly dependent upon the method and conditions used, making comparisons very difficult. USP II [1] and IV [2] methods offer comparative or quality control data due to rapid drug extraction and automation, whereas the T-apparatus [3] mimics the in vivo situation correlating to systemic exposure but has some practical limitations. Our method generates reproducible data, allowing more effective DEB elution over a practical time period. Elution remains faster the smaller the beads size but the difference in release rates is less pronounced than the other methods which are highly dependent on bead surface area exposure.

Results: This new method was used to characterize the elution of drug from a number of different DEB for comparison with published in vivo data and offered more clinically-relevant conditions than existing methods.

Conclusions: It allowed for evaluation of various parameters (drug, extraction flow rate, size, geometry, novel DEB materials etc.) ultimately aiding in the development of more sophisticated products for the clinician.

HOT MELT GRANULATION AS A MANUFACTURING PLATFORM FOR AMORPHOUS SOLID ORAL DOSAGE FORMS

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Timothy Brannigan[1], David S Jones[1] & Gavin P Andrews[1]

[1] School of Pharmacy, Queens University Belfast, Belfast, UK

Background: The primary area of research involving twin-screw extruders involves hot melt extrusion (HME) and subsequent solubility enhancement of BCS class II drugs through production of amorphous solid dispersions (ASDs). Several challenges surround ASD formulation such as poor dissolution rates associated with pelleted formulations as well as stability issues, particularly on subsequent processing operations. Hot-melt granulation (HMG) is a technique used to produce granulated formulations in a hot-melt extruder. Continuous production of granulated ASDs is an innovative method of improving processability in addition to maintaining stability of the amorphous form and the subsequent solubility advantage. This work aims to investigate how judicious formulation and processing using a twin-screw extruder may permit production of an enabling formulation for the poorly soluble drug celecoxib.

Methods: Mixtures in various ratios of celecoxib to binding polymer, Hydroxpropylmethyl cellulose acetate succinate (HPMCAS, LF grade AQOAT) and compression aid, Microcrystalline cellulose (MCC, Avicel grade PH101) were weighed and hand mixed using mortar and pestle. Melt granulation was carried out in a twin-screw, co-rotating extruder fitted with an open-ended barrel at temperatures of 114°C to 160°C. Resultant granules were subjected to DSC and PXRD analysis to provide information on the crystallinity of granules. Granule sizing was performed using sieve analysis and tablets were produced in a manually operated press to contain 10 mg celecoxib. Tablet hardness measurements and In-vitro drug release was performed to assess the supersaturating ability of the formulation over 6 hours. Stability of tablets was assessed by DSC, XRD analysis and In-vitro drug release over a 3-month period of storage at ambient temperatures and humidity.

Results: All formulations produced granules and were determined to contain amorphous celecoxib as demonstrated by XRD and DSC analysis. Granule sizing demonstrated significant particle growth and reduction in fines compared to the initial powder formulation. A significant relationship was determined between increased granule size and decreased MCC content. Tablets were successfully produced and hardness measurements demonstrated acceptable levels of resistance to breakage. In-vitro drug release studies exhibited supersaturation of celecoxib across all formulations. The degree of supersaturation was dependent upon drug loading, HPMCAS to drug ratio and the weight fraction of MCC. Supersaturation concentrations of up to 16.52 ± 0.78 µg/mL were achived in the granulated formulations, vastly greater than crystalline celecoxib solubility of 1.61 ± 0.03 µg/mL. Increased MCC provided greater release in the early stages of testing whereas increased levels of HPMCAS provided greater supersaturation after 6 hours. Formulations remained amorphous over 3 months storage.

Conclusions: HMG is an innovative and efficient method for the production of ASDs capable of direct compression into tablets with favourable mechanical properties. Drug release and supersaturating ability was affected by the formulation make up, permitting modulation suitable to application. Formulations were stable over a period of 3 months storage at ambient conditions, this suggests HPMCAS conferred solid state stability to the drug. This work presents a novel method of manufacture that has the potential to provide the benefits of greater processability, continuous manufacture and solubility enhancement to formulations produced using a twin-screw extruder.

DESIGN OF EXPERIMENTS FOR OPTIMISATION OF DROPLET SIZE AND ITS EFFECT ON FILM COAT QUALITY STUDIED USING NON-INVASIVE IMAGING TECHNIQUES

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Tom Dennison[1], Raj Badhan[1], Michael Hofmann[2], Julian Smith[3] & Afzal Mohammed[1]

[1] Aston University, Birmingham, West Midlands, UK; [2] University of Birmingham, Birmingham, West Midlands, UK; [3] Viridian Pharma Ltd, Newport, Gwent, UK

Background: Atomisation of aqueous polymer solutions to produce a spray of fine droplets is required for modern tablet film coating techniques. It is believed finer droplets produce higher quality film coats, benefitting from more rapid water evaporation. If droplet size is reduced too far however (< 10 µm) there is a risk of droplet spray-drying. Control of droplet size within a desired range is therefore important.

Methods: A Spraytec System (Malvern Instruments Ltd, Malvern, UK) was used to determine droplet volume median diameter (VMD) of aqueous solutions of Kollicoat IR (BASF, Germany), using laser light-diffraction technique. The solutions were pumped and atomized using a Mini Coater Drier-2 (Caleva Process Solutions Ltd., Dorset, UK). A Design of Experiments (DOE) approach was employed using MODDE 10 software (Umetrics Inc., USA). Three critical process parameters: atomising pressure, polymer concentration and coating suspension pump rate were identified and altered to assess their effect on VMD. Confocal microscopy was performed with a CLSM TCS SP5 II System (Leica Microsystems GMBH, UK) using a 10x dry objective and riboflavin monophosphate sodium as a fluorescent dye (0.5% w/w) in the film coat solution. XµCT was carried out using a Skyscan 1172 high- resolution micro-CT (Bruker, Belgium) and bismuth oxide (2.5% w/w) as contrast media in the film coat solution.

Results: The data collected was used to create a model to predict droplet size. The model generated demonstrated high strength and robustness, with a high R2 value of 0.977, indicating that the data fitted the model very well. A high Q2 value of 0.837 showed good predictability and the model also proved to have high validity and reproducibility. All three process parameters had an effect on droplet size, whether it was linear or a quadratic relationship, or both. In general, the droplet size decreased with increased atomisation pressure and increased with increased polymer concentration, with the effect of pump rate being more complex. Optimisation revealed process parameters for production of droplets of a desired VMD of 20 µm (small) and 70 µm (large). CLSM and XµCT imaging showed that small droplets formed more complete and uniform coatings with lower porosity. Images of large droplet coatings showed droplet outlines suggesting incomplete water evaporation, although TGA analysis revealed no difference in moisture content between the coats. Image analysis and 3D projections demonstrated greater surface roughness for large droplet coatings.

Conclusions: A DOE approach generated a strong and robust model. The model confirmed that all investigated process parameters significantly impacted droplet size and was able to predict the complex effect of altering these parameters. Optimisation revealed parameters required for production of droplets of a desired size. CLSM and XµCT imaging demonstrated that small droplets are preferable for the production of a uniform and concise film coating. Small droplet coatings would likely carry the benefits of more effective protection and a reduced risk of over-wetting and its detrimental effects. Incorporation of bismuth oxide at a low concentration as a radiopacifier allowed for imaging which would not have been previously possible, due to the similar radiopacities of the coating and core materials.

HOW PEGYLATION AND POZYLATION CHANGE THE PHYSICOCHEMICAL AND MUCOADHESIVE PROPERTIES OF THIOLATED SILICA NANOPARTICLES

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Twana Mohammed M. Ways, Wing Man Lau & Vitaliy Khutoryanskiy

Reading School of Pharmacy, University of Reading, UK

Background: Over the last decade, the area of nanoparticulate drug delivery has expanded rapidly. In addition, nanoparticles have a great potential of applications in mucosal drug delivery. One such nanoparticles is silica nanoparticles which have been studied for their potentials in mucoadhesive drug delivery.

Methods: The aim of this study is to synthesise and evaluate thiolated silica nanoparticles from 3-mercaptopropyltrimethoxysilane and then functionalise them with PEGylation with 5 kDa methoxy polyethylene glycol maleimide and POZylation with 5 kDa alkyne terminated poly(2-ethyl-2-oxazoline) [1]. These nanoparticles were subsequently fluorescently labelled. The particles were characterised in term of their sizes, PDI, zeta potential, FTIR and fluorescence spectra, thiol group content, and thermogravimetric analysis (TGA). Thereafter, the nanoparticles were evaluated for their mucoadhesive properties, i.e. retention on rat intestinal mucosa using fluorescence microscopy.

Results: The sizes of thiolated, PEGylated, and POZylated silica nanoparticles were 54.06 ± 0.49, 69.47 ± 1.20, and 59.33 ± 0.72 nm, respectively. The FTIR spectra confirmed the presence of both PEG and POZ on the surface of these two functionalized nanoparticles. Additionally, TGA revealed that PEGylated and POZylated nanoparticles contained 18.47 % and 26.74 % of PEG and POZ, respectively. Moreover, it was shown that thiolated nanoparticles are more mucoadhesive than both PEGylated and POZylated ones.

Conclusions: Potentially these nanoparticles may be considered as novel drug delivery carriers for designing controlled release dosage forms.

USING FATTY ACIDS TO COMBAT BLINDNESS IN NEW BORNS CAUSED BY GONORRHOEA INFECTION: ANALYTICAL METHOD DEVELOPMENT AND VALIDATION

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Ummara Butt[1], Amr ElShaera[1], Lori Snyder[2] & Raid G Alany[1,3]

[1] Drug Discovery, Delivery and Patient Care (DDDPC), School of Pharmacy and Chemistry, Kingston University London, UK; [2] School of Life Sciences, Kingston University, UK; [3] School of Pharmacy, University of Auckland, Auckland, New Zealand

Background: Fatty acids (FAs) are widely occurring in natural fats and dietary oils. Fatty acids have many important functions in the body, including energy storage and microbicidal effects. The current study is looking at developing a fatty acid-based eye formulation that will prevent eye infections in newborns caused by N. gonorrhoeae. Prior to formulating an ocular delivery system for fatty acids, a fast and accurate analytical method needs to be developed. Fatty acids can be analysed as free fatty acids (underivatised) or as fatty acid methyl esters (derivatised). However, fatty acids in their underivatized form are highly polar, making them difficult to analyse. Derivatization decreases the polarity of the fatty acids and improves peak shape which provides better separation.

Methods: A Design of Experiment (DoE) approach was used to optimise the derivatization of FAs. A Gas chromatographic method (GC-FID) was developed and validated for the identification and quantification of FAs. In this regard, response linearity, sensitivity, precision and accuracy were all determined.

Results: The results were found to be linear over the concentration range studied (200 µg/mL to 25 µg/mL) and the values of R^2 were higher than 0.99 for all the FAs. The results showed highest recovery values and the lowest intra-day and inter-day variation values for all fatty acids.

Conclusions: Thus, the procedure could be an accurate, precise and reliable method for analysing FAs.

PREPARATION, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF FATTY ACID ESTERS

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Ummara Butt[1], Amr ElShaera[1], Lori Snyder[2] & Raid G Alany[1,3]

[1] Drug Discovery, Delivery and Patient Care (DDDPC), School of Pharmacy and Chemistry, Kingston University London, UK; [2] School of Life Sciences, Kingston University, UK; [3] School of Pharmacy, University of Auckland, Auckland, New Zealand

Background: Fatty acids (FAs) were found to have antibacterial and antifungal properties (Bergsson et al 1999). Various unsaturated FAs and their esters (methyl & ethyl esters) have been reported to exhibit antimicrobial activity against various oral microorganisms including Streptococcus mutans, Candida albicans, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, and Porphyromonas gingivalis. It is evident that free fatty acids tend to interact with the calcium ions (Ca^{2+}) in simulated tear fluid, resulting in the precipitation of these fatty acids and thus reducing their lytic activity. So, free FAs can be converted into their methyl and ethyl esters and these ester forms can be used to avoid the precipitation.

Methods: Five FAs namely; Lauric acid, Tridecanoic acid, Myristoleic acid, Palmitoleic acid, α-Linolenic acid were derivatised into their methyl and ethyl esters using BCl3-methanol and ethyl chloroformate respectively. ATR-FTIR and NMR (^{1}H ^{1}D and ^{1}H-^{13}C HSQC 2D NMR spectroscopy) techniques were used to determine the structure of FAs and their esters. The antimicrobial activity of these FAs and their esters was evaluated against N. gonorrhoeae using zone of inhibition and log reduction tests.

Results: The ATR results demonstrated the successful preparation of the FAs' ester forms as the carbonyl stretching bands C=O shifted from 1710 cm^{-1} to 1740 cm^{-1} while NMR showed that the signals of all protons are well-separated, thus permitting the rapid and non-destructive determination of FAs and their ester forms. The results of zone of inhibition and log reduction tests showed that unsaturated FAs and their esters exhibited strong antimicrobial activity against N. gonorrhoeae than saturated FAs and their esters.

Conclusions: The free FAs can be converted into their methyl and ethyl esters and these FAs' ester forms can be used to avoid the precipitation.

DESIGNER (TUNABLE SIZE) MICROSHPERES BY MICROFLUIDICS USING DESIGN OF EXPERIMENT (DOE) APPROACH

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Zahid Nazir[1,2], Robert Shaw[1], Marianne Ashford[1], Maria Marlow[2] & Matthew Ling[1]

[1] Pharmaceutical Sciences, AstraZeneca, SK10 2NA, Macclesfield, UK
[2] School of Pharmacy, The University of Nottingham, NG7 2RD, UK

Background: Fabrication of monodisperse microspheres of tunable sizes is very much desired for localised controlled release drug delivery to achieve different release rates. Various techniques have been described in the literature to make such monodispersed microspheres including, microfluidics, acoustic excitation, spinning oil film, membrane extrusion and replica molding. Microfluidics technology offers a relatively low cost and easy to use platform for this purpose. We have systematically evaluated microfluidics technique by using design of experiment (DOE) approach to make monodispersed tunable size microsphere. The aim is to identify the conditions to make 50–200 µm size microspheres. This size range has been reported to be the most suitable for injectable sustained release systems.

Methods: The microspheres were prepared by an emulsion method. The oil (organic) phase composed of PLGA dissolved in dichloromethane and aqueous phase contained polyvinyl alcohol dissolved in water. Two phase were mixed by using the Dolomite microfluidics system fitted with a droplet junction microchannel chip which results in the formation of precisely controlled emulsion droplets. The droplets were collected in a vessel containing dilute surfactant solution. After collection, DCM was removed by evaporation and the hardened microspheres were washed three times with dilute surfactant solution and dried under vacuum. Two DOE were conducted, first full factorial design to determine the design space and the second fractional (n-1) factorial design to find the optimum conditions. Microspheres were analysed for size and size distribution by laser diffraction, optical microscopy and scanning electron microscopy (SEM).

Results: The first DOE gave microspheres with size ranging from 90–800 µm and total flow rate and the surfactant concentration were identified as the most significant factors that affect the particle size. Increase in the total flow rate resulted in the decrease of the particles size whereas, increase in the surfactant concentration in the aqueous phase resulted in the increase of the final particle size. Microspheres with size ranging from 50–500 µm were formed in the second DOE and flow rate ratio, PLGA concentration in the organic phase and their interaction turned out to be the most significant factors. In general, increase in the flow rate ratio and decrease in the PLGA concentration resulted in smaller particles. In addition, due to the interaction between these two variables impact of flow rate ratio on the particle size was more pronounced at the higher PLGA concentration compared to the low PLGA concentration. In general, all the experiments in the second DOE resulted in the formation of monodispersed microspheres. This data highlights mechanistic differences and superior results of the microfluidics technique compared to commonly used bulk emulsification method.

Conclusions: A design space has been successfully identified to make monodispersed microspheres in the size range of 50–200 µm by emulsion method using microfluidics technology. In addition, a good mechanistic understanding of the process has been developed to tune the final particle size.

THERMORESPONSIVE COPOLYMER: HPMA-CO-(AMPA- CHOLESTERYL) POLYMER SYNTHESIS AND PHYSIOCHEMICAL CHARACTERIZATION

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Ali Alsuraifi[1], Danvir Randhawa[2], Turkan Sentip[2], Anthony Curtis[1,2] & Clare Hoskins[1,2]

[1] Institute of Science and Technology in Medicine, Keele University, ST5 5BG, UK
[2] School of Pharmacy, Keele University, ST5 5BG, UK

Background: Poly(N-(2-hydroxypropyl) methacrylamide)(pHPMA) is a thermo-responsive polymer which undergoes a conformational change at its lower critical solution temperature (LCST); pHPMA has been exploited in drug delivery due to its ability to form nano-sized aggregates in copolymer formation. The aggregates are capable of solubilising hydrophobic compounds such as drug molecules. Above the LCST shrinkage of polymer structure is experienced leading to disruption of aggregates and hence cargo release. The LCST can be tailored depending on the substitution of the polymer. In this work we have synthesized hydrophobicaly modified (N-(3-aminopropyl) methacrylamide (APMA) monomers to incorporate into the HPMA copolymer at varied molar ratio. Characterisation of the novel amphiphiles will be carried out using various techniques to determine success of synthesis and physical properties of the system including aggregate formation, critical aggregation concentration. Drug loading studies will be carried out in order to determine optimal loading parameters such as initial drug: polymer feed, polymer concentration etc. On-going studies are being carried out in order to evaluate the potential of these systems in thermo-responsive drug release.

Methods: Thermoresponsive copolymers were synthesized in two steps. First, hydrophobic cholesteryl groups were grafted onto the primary amine group of APMA which was subsequently reacted with N-(2-hydroxypropyl) methacrylamide (HPMA) in a second reaction.

Results: The cholesteryl modification of APMA was successful according to the NMR and FTIR spectra. Incorporation of the modified. APMA into the HPMA polymer was carried out at 0.5% and 1% molar ratios. The NMR and FTIR spectra indicated polymerisation had occurred.

Conclusions: This work highlights the use of hydrophobically modified HPMA polymers for use as drug solubilising agents. Further work is ongoing to exploit the LCST for these polymers for thermo-responsive drug release.

HYBRID GOLD-IRON OXIDE NANOPARTICLES AS A VEHICLE OF GEMCITABINE ADDUCT FOR PANCREATIC CANCER THERAPY

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M.A.M. Alfahad, C. Hoskins & A.D.M. Curtis

Institute for Science and Technology in Medicine, Keele University, Keele, ST5 5BG, UK

Background: Gemcitabine is an analogue of deoxycytidine which has been authorized for the treatment of many types of cancers including pancreatic cancer. However, gemcitabine treatment only proves effective in 23.8% of patients with pancreatic cancer. Extensive first pass metabolism by cytidine deaminase and poor tumour tissue penetration of gemcitabine are one of the main challenges in anticancer therapy. Nanotechnology can play an essential role by delivering drugs in a targeted fashion to the malignant cells that will reduce the systemic toxicity of the anticancer drug. We show a stepwise development of a nanoparticle-based targeted delivery system for in vitro in pancreatic cancer. In this part of the study, we have shown the fabrication and characterization of the delivery system containing hybrid gold-iron oxide nanoparticles as a delivery vehicle together with the synthesis of the Novel prodrugs of gemcitabine which is capable of linkage on to hybrid gold-iron oxide nanoparticles in order to achieve deeper tissue penetration and increase drug efficacy.

Methods: The Fe_3O_4 particles were synthesized by precipitation method and coated with PEI and gold. Gemcitabine was reacted with lipoic acid using established procedures to deliver prodrug. The compound was characterised using a combination of spectroscopic and spectrometric techniques including ^{19}F-NMR

Results: The hydrodynamic radius and zeta potential of Fe_3O_4 core and HNP was determined by photon correlation spectroscopy. Metal content of the HNPs was determined using inductively coupled plasma-optical emission spectroscopy. Novel prodrugs of gemcitabine were isolated which are substituted at the amine in the 4 position of the pyrimidine nucleus The structure of these compounds was confirmed using multinuclear NMR spectroscopy.

Conclusions: Further work is on-going to investigate the prodrug attachment to hybrid nanoparticle surface via dative covalent linkage and to investigate the potential of the prodrug-nanoparticulate constructs in vitro for pancreatic cancer treatment.

THE CO-ADMINSTRATION OF ANTICANCER AND PRO-APOPTOTIC AGENTS AS ANOVEL APPROACH IN LIVER CANCER THERAPY

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Wejdan Al-Shakarchi, David Morgan ,Gabor Varbiro & Clare Hoskins

Institute for Science and Technology in Medicine, Keele University, Keele ST5 5BG, UK

Background: Hepatocellular carcinoma (liver cancer) is characterised by defective or ineffective apoptosis which is considered to be the main cause of cancer progression. Cytochrome-C (pro-apoptotic protein) triggers mitochondrial apoptosis and is responsible for activating the downstream apoptosis pathway during cell death in the tumour cells. It is often difficult to achieve cellular internalisation of large protein molecules at concentrations high enough to exhibit biological effects. Conjugation of Cytochrome-C onto iron oxide-gold core-shell hybrid nanoparticles has shown enhanced cellular permeability and uptake.

Methods: Hybrid nanoparticles-Cytochrome C conjugates in combination with clinically used anticancer drugs. Anti-microtubule drugs (paclitaxel, vincristine and vinblastine) were used to treat HepG2 cell line. Subsequently the cells were treated with combination of these drugs with HNP-Cytochrome C showing a 10% growth inhibition alone in HepG2 cells.

Results: Significant synergistic toxicity of anti-microtubule drugs with HNP-CYT C and hence improves disease treatment by apoptosis.

Conclusions: The results prove the effectiveness of pro-apoptotic proteins in triggering apoptosis to complement the anticancer effect of chemotherapy and to increase their efficiency.

EVALUATION OF AMINO ACID AS COUNTER IONS OF MODEL ZWITTERIONIC DRUG TETRACYCLINE

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Annsar Warraich[1], Yvonne Perrie[1], Ayesha Rahman[2], Majad Hussain[3] & Afzal R Mohammed[1]

[1] School of Life and Health Sciences, Aston University, B4 7ET, UK; [2] School of Applied Sciences, University of Wolverhampton, WV1 1LY, UK; [3] Quest Healthcare Ltd, Birmingham, UK

Background: Oral drug delivery is the most popular route of drug administration due to the convenience it offers. However, high solubility and permeability are two requirements that are limiting the scope of most new chemical entities. Our group has shown that utilizing amino acid as counter ions improves both solubility and permeability of model drugs. Tetracycline (TC) is broad spectrum antibiotic exhibiting activity against both gram-positive and gram-negative bacteria. Its bacteriostatic activity is achieved by inhibiting protein synthesis through the prevention of the association of t-RNA with the bacterial ribosome. TC has a very low solubility and therefore is administered as the more water soluble tetracycline hydrochloride. This study determines the effect of acidic (L-aspartic acid and L-glutamic acid) and basic (L-arginine, L-lysine and L-histidine) counter ions on the solubility of TC.

Methods: Maximum solubility of TC in different concentrations of acidic and basic amino acids was determined. Excess amounts of TC was added to amino acid solutions of different concentrations. The mixture was stirred at ambient temperature for up to 24 h. Solution pH was obtained after which the solution was left to rest for 2 h. The supernatant was filtered through 0.45 µm syringe filter. The filtrate was diluted adequately with the mobile phase and solubility determined through HPLC.

Results: TC formed ion pairs with L-aspartic acid, L-glutamic acid and L-lysine. L-aspartic acid was found to be the most suitable counter ion, representing an enhancement of solubility by 337.22% from control.

Conclusions: This study shows L-aspartic acid to be a suitable candidate for TC solubility enhancement. Next steps include the investigation of the effect of this ion pair formation on the permeability of TC, followed by characterization of ion pair formation whilst investigating the strength of the ion pairs formed.

CONTROLLED RELEASE OF GET PEPTIDES AND ENHANCED INTRACELLULAR DELIVERY OF THERAPEUTICS

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Hosam M. Abu Awwad, Kevin M. Shakesheff & James E. Dixon

School of Pharmacy, Wolfson Centre for Stem Cells, Tissue Engineering, and Modelling, Centre of Biomolecular Sciences, University of Nottingham, Nottingham, NG7 2RD, United Kingdom

Background: Cell penetrating peptides (CPPs) are an attractive and non-genetic means of delivering therapeutic molecules into cells for treatment of many different conditions. Complexes of CPPs and their cargoes are macromolecular in nature, which makes them challenging systems to formulate and deliver in a controlled manner. There is a need for intracellular protein delivery systems that can provide active therapeutic doses and restrict their administration to a specific location. We have developed novel fusion proteins that couple a membrane-docking peptide to heparan sulfate glycosaminoglycans (GAGs) with protein transduction domain (PTD)A. This GET (GAG-binding enhanced transduction) system can be utilised to enhance delivery of different cargos, including proteins (i.e. transcription factors and enzymes), nucleic acids, nanoparticles and drugs. Here, we aimed to develop a controlled delivery system, based on poly (lactide-co-glycolide) (PLGA) microparticles, which would provide a localized release of the encapsulated GET-conjugated therapeutic molecule at the treatment site. In this research, we have investigated all the manufacturing process parameters, which may affect GET–protein molecule activity during encapsulation and release processes. We have demonstrated a successful controlled delivery of GET–protein molecule, retaining the high transduction activity of the GET system.

Methods: A self-reporting fluorescent cargo (mRFP1, mR) was used as a model protein. This protein was fused, by cloning, with GET system peptides (P21 and 8R) to create a recombinant P21mR8R protein that was expressed in E.coli and purified by GST affinity chromatography. Microparticles were formed from poly (DL-lactide-co-glycolide, 50:50, 52kDa), using solid in oil in water (S/O/W) solvent evaporation method. The generated microparticles were characterized for average size, morphology, encapsulation efficiency and protein release profile. The encapsulated and released GET - protein molecule biological activity was tested using mammalian cells (NIH 3T3). Protein was transduced into cells by addition to culture media. Cells were imaged using fluorescence microscope, then harvested and analysed for fluorescence intensity by flow cytometry. Protein transduction activity was compared to the experiment control.

Results: Manufactured microparticles were 40–60 μm with spherical shape. Encapsulation efficiency was in range of 60–80%, the total protein loading was fixed to be 0.5% (w/w). The released protein showed gradual loss of activity over time; however, the released protein activity was enhanced by the addition of a proton scavenger, L-Histidine, which was co-encapsulated with the GET–protein molecule during the manufacturing. The results showed GET peptides sensitivity to different pH conditions. However, use of the proton scavenger, L-histidine, showed enhanced protein stability at lower pH (i.e. pH ≈3).

Conclusions: We presented here an extensive study of the possible factors affecting GET peptide biological activity, during the encapsulation and release from PLGA microparticles. A robust method of fabricating protein-loaded microparticles was optimised, using S/O/W emulsion technique. L-histidine was used as a stabilizer to maintain the protein activity during release from microparticles. The advantage of this delivery system is to provide localised release of GET-tagged therapeutic molecules with minimised risk of systemic dosing that may lead to adverse reactions. This delivery technology has potential to be valuable in the controlled delivery of various potent therapeutic molecules, coupled with GET peptides, which can be used for tissue engineering and regenerative medicine applications.

NOVEL IRON OXIDE-GOLD NANOHYBRIDS WITH HEAT TRIGGERED SURFACE MANIPULATION

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Adeolu Oluwasanmi, Anthony Curtis & Clare Hoskins

School of Pharmacy, Keele University, ST5 5BG, United Kingdom

Background: Iron oxide-gold nanohybrids (HNPs) are core-shell nanoparticles whose properties include inherent magnetism from the iron oxide core and surface plasmon resonance (SPR) from the gold shell [1,2]. Laser irradiation of these HNPs triggers SPR resulting in localised heating to around 45 °C and 70 °C on the HNP surface, which can be used for thermal manipulation. In order to facilitate the transport of cytotoxic drugs, a thermally-labile linker has been developed to attach the cytotoxic drugs to the HNP surfaces.

Methods: The synthesis of a thiolated cycloadduct with gemcitabine was achieved in several steps. Firstly, boc protection of 2-furanmethane thiol, followed by a Diels Alder reaction with 4-maleimidobutyric acid n-hydroxysuccinimide ester, formed a Diels Alder cycloadduct. This adduct was used to perform an amide coupling with previously boc protected gemcitabine, before an acid wash deprotection of the boc groups, yielded our gemcitabine cycloadduct linker. Synthesized cycloadducts were dissolved in a 70:30 (D2O:CD3OD) solution and heated to temperatures of 20, 37, 45, and 70 °C in order to observe the rate of the retro Diels Alder reaction over regular intervals at these temperatures using proton NMR spectroscopy.
The presence of the thiol group on the cycloadduct allowed the formation of a covalent bond between the thiol group and the gold surface of the HNP's.

Results: The thiolated cycloadduct successfully underwent the retro Diels Alder reaction at 45 and 70°C, with measurable levels of starting material being detected after the first 1h interval increasing at a rate of 29% per hour at 70°C. This cycloadduct cleaved via retro Diels Alder reaction at the rate of 0% per hour at 20°C displaying thermal stability at core body temperature.

Conclusions: We have successfully shown that the retro Diels Alder reaction will occur within the temperature range achievable through SPR. Further work involves in-vitro analysis of this thiolated cycloadduct with the use of SPR to deduce the potential of these carriers for thermo-responsive drug delivery.

NOVEL BIOMATERIAL-BASED APPROACHES FOR CONTROLLING SECREATION OF ANGIOGENIC GROWTH FACTORS

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Eseelle Hendow & Richard Day

Applied Biomedical Engineering Group, Division of Medicine, University College London, London, WC1E 6JJ, UK

Background: Cardiovascular disease is a leading cause of death worldwide. Promoting regeneration of tissue damaged by ischemia resulting from cardiovascular disease poses a significant challenge. Therapeutic angiogenesis aims to deliver angiogenic factors to promote the formation of new blood vessels and thus treat ischemic tissues. Biomaterial scaffolds can be an effective way of delivering cells to a target site, as well as utilizing physical characteristics to influence cell behaviour. Surface topography is known to influence cell alignment, morphology and differentiation and rough surfaces have been shown to effect cellular expression of biomarkers and growth factors. By producing substrates with tuneable surface characteristics cellular responses can be controlled. This work aims to investigate the effect of substrates patterned with hierarchical surface topographies prepared from biodegradable polymers (poly-DL-lactide-co-glycolide) on the controlled release of angiogenic factors from adipose derived mesenchymal stem cells (ADMSCs).

Methods: To create hierarchically structured surfaces, substrates were prepared using thermally induced phase separation (TIPS). Surface topographies were characterised using scanning electron microscopy, atomic force microscopy and Dektak surface profilometer. The biological effect of surface topography on ADMSCs was then assessed using a vasculogenesis to angiogenesis array, proteome profiler angiogenesis array, cell viability and proliferation assays and ELISA assays for measuring secretion of angiogenic growth factors.

Results: Roughness measurements show the TIPS process results in a hierarchically structured porous and rough topography compared with control surfaces coated with the same polymer (AFM Ra: TIPS 2000 nm vs. control 0.6 nm ($P<0.0001$), dektak surface profiler Ra: TIPS 6000 nm vs. control 60 nm ($P<0.05$)). Vasculogenesis to angiogenesis array showed ADMSCs grown on TIPS surfaces had increased tubule length (11732±1502 $P<0.05$), number of junctions (547±123 $P<0.05$) and branches (748±66 $P<0.01$) compared to polymer controls (8404±752, 247±47, 405±53 respectively) and tissue culture plastic (8607±389, 339±11, 442±14). Profiling secretion of angiogenic growth factors and ELISAs showed increased secretion of VEGF on TIPS surfaces after 7 days (5760 pg/mL) compared to polymer and tissue culture plastic controls (4750 pg/mL and 1980 pg/mL respectively).

Conclusions: These findings open up the possibility of utilizing hierarchical structured surfaces to control therapeutic angiogenesis.

CONTROLLED DELIVERY OF MYOBLASTS

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Nina Parmar[1], Pauline Guhmann[1], Panagiotis Sofokleous[1], Sara Maffioletti[2], Saverio Tedesco[2] & Richard Day[1]

[1] Applied Biomedical Engineering Group, Division of Medicine, University College London, UK; [2] Department of Cell and Developmental Biology, University College London, UK

Background: Volumetric muscle loss (VML) is the traumatic or surgical loss of skeletal muscle with resultant functional impairment. Autologous tissue transfer, allograft transplantation and prosthetics are currently used for the surgical treatment of critical-sized defects. The use of 3D scaffold materials for cell delivery is a key component of regenerative medicine. The delivery of cells attached to the surface of a substrate is preferential to delivering anchorage-dependent cells in suspension to the affected tissue environment. Porous degradable microcarriers offer features ideally suited for localized cell delivery. However, optimized techniques for cell attachment of anchorage-dependent myoblasts (muscle precursor cells) to the surface of highly porous, biocompatible and biodegradable microcarriers have not yet been characterized. The aim of this study was to investigate methods for achieving improved attachment of human myoblast cells to the surface of highly porous microcarriers and their controlled delivery in vivo.

Methods: Poly-DL(lactide-co-glycolide) was used to fabricate highly porous microcarriers measuring 300 µM. Various incubation conditions were tested to attach human skeletal muscle myoblasts (HSMM) to the surface of the microcarriers and optimized parameters for cell concentration ($5x10^4$ – $5x10^5$), culture volume (125–1000 µL), culture vessel and static-dynamic culture regimes. HSMM attached to the surface of highly porous microcarriers were then delivered to an *in vivo* model of VML.

Results: The results from this study demonstrate a static-dynamic method can be used to efficiently attach 1,000 primary human myoblast cells per microcarrier. The myoblasts attached to the surface of the highly porous microcarriers were successfully delivered to the in vivo model and were retained at the site of implantation after 3 weeks.

Conclusions: Anchorage-dependent myoblast cells can be attached to the surface of porous degradable microcarriers and locally released at the target site in vivo. These data are important for understanding the attachment of cells to porous microcarriers and their planned use in clinical trials as an advanced therapeutic medicinal product for cell therapy.

ENHANCED INTRADERMAL DELIVERY OF DOXYCYCLINE USING MICRONEEDLE ROLLERS

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Abbie Omolu[1], He Li[2], I-Hui Yang[2], Maryse Bailly[2] & Richard Day[1]

[1] Applied Biomedical Engineering Group, Division of Medicine, UCL, London, UK
[2] Department of Cell Biology UCL Institute of Ophthalmology 11-43 Bath Street London, UK

Background: Doxycycline hyclate functions as an inhibitor of matrix metalloproteinase (MMP) activity. Chronic ulcers have upregulated MMP activity, attributable to either upregulated expression, dysregulated inhibition or a combination of both. Controlled delivery of doxycycline intradermally to affected dermis could provide a novel therapeutic strategy for the treatment and/or prevention of early stage ulceration. Microneedles present a novel way of delivering doxycycline across the skin. Doxycycline is a hydrophilic compound of a molecular weight roughly twice that will pass through the pore size of the human skin. Microneedles act as mechanical penetration enhancers which, on application to the skin, create micropores that provide conduits for drug permeation. Once microneedled, the drug of interest can be applied to the skin as a solution, gel or patch for controlled long-term release to a specific skin layer. For larger surface areas, microneedle rollers present a more practical solution than traditional patches. Here we present a novel, highly reproducible in vitro model using a synthetic membrane in place of animal-derived skin to mimic the skin barrier (the stratum corneum, epidermis and upper dermis). This model can be used to test different drug formulations, microneedle parameters and cellular stressors.

Methods: Cellularised collagen gels were created by seeding primary adult human dermal fibroblasts in serum-enriched type 1 collagen. Doxycycline hyclate solution was prepared at sub-antimicrobial doses and delivered to the gels. Basic delivery was achieved by adding drug solution on top of the culture medium bathing the gels. To mimic the skin barrier, cell crowns were used to create a transwell, whereby the gels were separated from the well opening by Strat-M membrane. The Strat-M membrane was treated with different microneedle roller lengths 250, 500 and 750 µm and doxycycline solution pipetted onto its surface. Over a period of seven days, delivery of doxycycline to the gels was monitored by percentage gel contraction and total MMP activity. Several cellular stressors were introduced to replicate the ulcer micro-environment including co-culturing with macrophages and compression of gels with weights.

Results: Our results show firstly, that contraction of gels cellularised with human dermal fibroblasts (an indirect marker of MMP activity) is inhibited by doxycycline and that this inhibition is dose-dependent at sub-antimicrobial concentrations. Secondly, doxycycline significantly inhibits MMP activity over a period of seven days. Strat-M membrane acts as an effective 'skin' barrier to doxycycline permeation; doxycycline's inhibitory effect is reduced since drug flux to the gel is impeded in the presence of the membrane. This can, in part, be explained by the reduced total volume reaching the gel as some drug is retained in the membrane and a prolonged delivery time in the presence of a barrier. Importantly, microneedling enhances doxycycline delivery shown by the gel contraction and MMP activity assay studies. Furthermore, this action is microneedle-length dependent.

Conclusions: This novel in vitro model shows how microneedling can be used to enhance the precise intradermal delivery of larger hydrophilic drugs to injurious skin sites. Our experiments will continue to develop the model to include the effect of inflammatory components and mechanical strain typically associated with pressure ulcers.

LIST OF ATTENDEES

ATTENDEES

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May Abdulrahman (Keele University)
Virginia Acha *(ABPI)*
Jenan Al-Ameri *(Keele University)*
Mohammed Al-Ameedee *(University of Nottingham)*
Mazin Al-bujasim *(Queen's University Belfast)*
Hosam M. Abu Awwad *(University of Nottingham)*
M.A.M. Alfahad (*Keele University)*
Ali Al-kinani *(Kingston University London)*
Wejdan Al-Shakarchi *(Keele University)*
Ali Alsuraifi *(Keele University)*
Hamad Alyam*i (Aston University)*
Mohammad Alyam*i (Aston University)*
Gavin Andrews *(Queen's University Belfast)*
Valeria Annibaldi *(Spraybase)*
Farah Arikat *(Cardiff University)*
Emma Baczkowski *(Cardiff University)*
Mariam Badawi *(University of Strathclyde)*
Katherine Bamsey *(Midatech Pharma)*
Ivan J. Hall Barrientos *(University of Strathclyde)*
James Birchall *(Cardiff University)*
Samuel Bizley *(The Royal Veterinary College)*
Pauric Brannigan *(University of Limerick)*
Ruairí P Brannigan *(University of Reading)*
Timothy Brannigan *(Queen's University Belfast)*
Sarah Brozio *(University of Strathclyde)*
Alexandra Burdujan *(University of Oxford)*
Ummara Butt *(Kingston University London)*
Ellen Byrne *(Spraybase)*
Fabio Cattelan *(Cardiff University)*
Victoria Cavalier-Hirth *(Midatech Pharma)*
Robert Cavanagh *(University of Nottingham)*
Sion Coulman *(Cardiff University)*
Fraser Crofts *(Aston University)*
Richard d'Arcy *(University of Manchester)*
Safa Damiati *(King's College London)*
Anders Davideson *(Cardiff University)*
Tom Dennison *(Aston University)*
Maria Dul *(Cardiff Univerisity)*
Edel Durack *(University of Limerick)*
Anna Maria Fiethen *(Bluestar Silicones Germany)*
Rhian Groves *(Midatech Pharma)*
Benedetta Gualeni *(Extraject Technologies)*
Clement M. Haeck *(Queen's University Belfast)*
Eseelle Hendow *(University College London)*
Niamh Heron *(Queen's University Belfast)*
Affiong Iyire *(Aston University)*
Muhammad Taufiq Bin Mohd Jailani *(University of Strathclyde)*
Tahani Jihad *(Keele University)*
Arwyn Jones *(Cardiff University)*
Guy Jones *(Stable Micro Systems)*
Abdessamad Y. Kaassis *(University College London)*
Negeen Kargar *(Kingston University London)*
S. Khadke *(Aston University)*
Minna Khalid *(Dublin Institute of Technology)*
Mouhamad Khoder *(Kingston University)*
Oluwadamilola Kolawole *(University of Reading)*
Jasdip Koner *(Aston University)*
Dimitrios Lamprou *(University of Strathclyde)*
Emma Lane *(Cardiff University)*
Jayne Lawrence *(Kings College London)*
Richard Lewis *(Biopharma)*
Jenifer Mains *(Encap Drug Delivery)*
Manfred F. Maitz *(Leibniz-Institut für Polymerforschung)*
Karl Malcolm *(Queen's University Belfast)*
Sarah Mallen *(University of Limerick)*
David Mallinson *(University of Strathclyde)*
Edward Mansfield *(University of Reading)*
Saeed Marji *(Queens University Belfast)*
Maria Marlow *(University of Nottingham)*
David Martin *(Buchi Ltd.)*
Mandeep Marwah *(Aston University)*
Laura Mason *(Universtiy of Nottingham)*
Samantha Maurice *(Cardiff University)*
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Carol McCarthy *(University College Cork)*
Bridgette McGeever *(Queen's University Belfast)*
Marie McGrath *(GSK)*
Monica Mistry *(University of Nottingham)*
Mohammad Najlah *(Anglia Ruskin University)*
Zahid Nazir *(University of Nottingham)*
Sarah O'Brien *(Buchi Ltd.)*
Dáire O'Donnell *(Dublin City University)*
Adeolu Oluwasanmi *(Keele University)*
Abbie Omolu *(University College London)*
Eleonora Paladino *(University of Strathclyde)*
Dan Palmer *(Midatech Pharma)*
Andrew Parker *(Juniper Pharmaceuticals)*
Nina Parmar *(University College London)*
Christina Payne *(Royal College of Surgeons in Ireland)*
Yvonne Perrie *(Aston University)*
John Pollard *(Aston University)*
Georgina Procter *(Queen's University Belfast)*
Ben Proudlove *(Merrow Scientific)*
Pamela Riester *(Cardiff University)*
Hope Daphne Roberts-Dalton *(Aston University)*
Carla B. Roces Rodriguez *(Aston University)*
Katie Ryan *(University College Cork)*
Edward Sayers *(Cardiff University)*
Paul Seaman *(Midatech Pharma)*
Simon Stebbing *(PQ Corp.)*
Peter Stone *(Aston University)*
Tanya S. Swaine *(University of Huddersfield)*
Sam Tarassolie *(University College London)*
Priyanka Tripathi *(CSIR-Central Drug Research Institute)*
Arto Urrti *(University of Helsinki)*
Giuliana Vozza *(Dublin Institute of Technology)*
David Walsh *(Royal College of Surgeons in Ireland)*
Annsar Warraich *(Aston University)*
Pete Watson *(Cardiff University)*
Twana Mohammed M. Ways *(University of Reading)*
Charlie Winter *(Imperial College London)*
Paul Whittles *(Sirius)*
Jen Wymant *(Cardiff University)*
Xin Zhao *(Cardiff University)*

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NOTES

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