UKICRS Symposium 2019

United Kingdom & Ireland Controlled Release Society

Liverpool John Moores University

Monday 3 June 2019
Industrial Careers Event, Technical Talks
& Symposium Dinner

Tuesday 4 June 2019
Symposium & Industrial Exposition

WELCOME TO LIVERPOOL!

A word from the Symposium organiser

On behalf of the UKICRS committee, it's my pleasure to welcome you to the 2019 UKICRS Symposium! As is always the case, our goal over the next few days is to create a friendly and stimulating environment for early career researchers to present their research, and build networks within the wider controlled release community.

We are thrilled to have Justin Hanes, President-Elect of the Controlled Release Society, kicking off the programme this year with a keynote presentation on Monday morning, followed by a workshop where Chris Thomas, Bianca Price and Eileen McBride will share with us their insights into post-PhD life. This session proved a hit at last year's highly successful symposium in Belfast, and is sure to resonate equally well with this year's delegates. The Industrial Exposition on Monday afternoon also promises to be as entertaining and informative as ever, with the chance to follow up with exhibitors at their stands throughout the symposium.

The symposium dinner takes place on Monday evening at Love Lane Brewery, providing delegates – quite literally – with a taste of Liverpool. More details on the venue are provided later in this booklet. As always, dinner is complimentary for delegates.

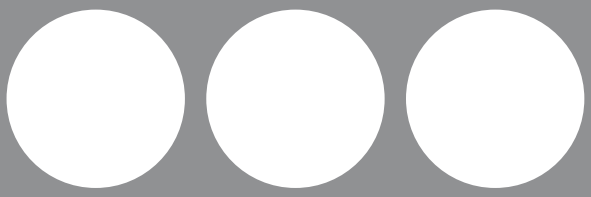
Tuesday's scientific programme will highlight the latest research in the field of controlled release and pharmaceutical sciences. Our keynote speakers Kostas Kostarelos and Andrew Owen will showcase the best of the Northwest, and will be complemented by a diverse series of talks and poster presentations by postgraduate and postdoctoral students. Later on Tuesday afternoon the floor will be opened for a soapbox session – inspired by a similar, highly entertaining session at last year's symposium, this promises to end the scientific programme with a bang.

Further to the symposium itself, I hope you'll find some time during your stay to experience a little bit of Liverpool. This is an exciting time to visit – the city's independent food and drink scene is booming, festival season is in full swing, and as always, there's a Beatles-related attraction or two to be visited. Hopefully the city will also be full of ecstatic football fans by the time you arrive, but regardless, I have no doubt that you'll find Liverpool to be a friendly and open city – entirely in line with the vision of UKICRS.

Enjoy the symposium, and your time in Liverpool!

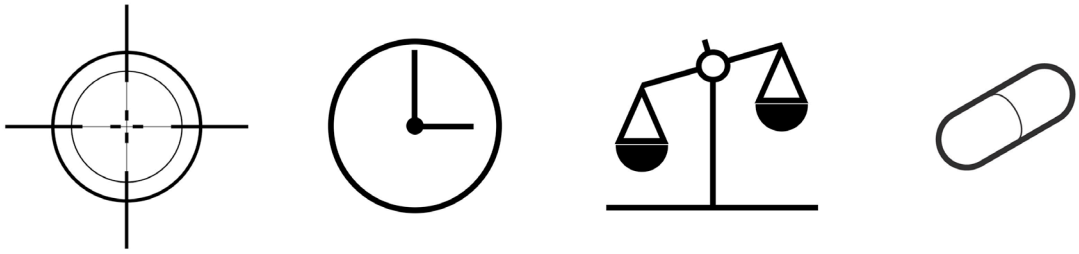
Sarah Gordon

GENERAL INFO



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.

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OUR WORKSHOP SPEAKERS

Chris, Bianca & Eileen

Chris Thomas (Cardiff University) completed his degree in Chemistry with Industrial Experience in 2002 from Cardiff University before moving to the School of Pharmacy to undertake a PhD in transdermal delivery. He then completed a postdoctoral position in Cardiff University School of Medicine before being awarded a Marie Curie Fellowship in 2010 to study at Vanderbilt University School of Medicine, Nashville, TN. In 2013, Chris was awarded a NISHCR/Wellcome Trust fellowship before acting as co-investigator and Research Fellow on an MRC Research Award. He started his current position as Lecturer in the School of Pharmacy and Pharmaceutical Sciences in November 2016.

Bianca Price (University of Manchester) obtained a BSc (Hons) in Biochemistry from the University of Sheffield and then completed a PhD in *Pseudomonas aeruginosa* molecular biology at the University of Cambridge, Emmanuel College. She then moved to the University of Manchester to study the effect of multi-purpose contact lens disinfecting solutions on tear proteins structure and function extracted from worn contact lenses. Since then, Bianca has developed a collagen wound model which can be infected with bacteria, for which she won a Bionow award "Promising Technologist of the Year". She has applied the model to the wound care field, looking at the effect of actives released from medical devices on biofilms and healing. Her research focuses on host and pathogen interactions with medical devices, using clinical studies in conjunction with lab studies in order to increase research impact with a particularly emphasis on chronic wound infections. She is currently a research fellow in the Division of Pharmacy and Optometry.

Eileen McBride (AstraZeneca) has a MPharm degree (2003) and a PhD in Drug Delivery and Biopharmaceutics (2008) from University of Strathclyde. She worked as a post-doctoral researcher for 3 years (until 2011) working across a range of projects developing novel drug delivery approaches and understanding the biopharmaceutics of a range of molecules across different tissues, as well as exploring innovative imaging techniques to measure drug release in vivo. Eileen joined AstraZeneca in 2011 as a senior scientist in biopharmaceutics (in Pharmaceutical Technology & Development; PT&D) working on projects in the later stages of clinical development. In 2015, Eileen became a Team Leader within Global Product Development in PT&D, and currently leads a team of pharmaceutical scientists from a range of backgrounds (formulation, engineering, solid state, materials science and biopharmaceutics). Eileen has expertise and experience in paediatric drug development and leads the development of the internal paediatric strategy from a pharmaceutical development perspective. In 2018, Eileen became the global programme lead for the PT&D Graduate Scheme and is responsible for the management and growth of the scheme, as well as recruitment and development of future leaders through the scheme.

OUR KEYNOTE SPEAKERS

Justin Hanes / Johns Hopkins University, US

Justin Hanes is the Lewis J. Ort Professor of Ophthalmology with joint appointments in Biomedical Engineering, Chemical & Biomolecular Engineering, Environmental Health Sciences, Neurosurgery, and Oncology at the Johns Hopkins University. His degrees are in Chemical Engineering from UCLA (B.S.) and MIT (Ph.D.), and he has completed a postdoctoral fellowship in Oncology and Neurosurgery at Johns Hopkins prior to beginning his faculty position in 1998. He is the Director of the Center for Nanomedicine at the Johns Hopkins University School of Medicine. He is also Director of Therapeutics for the Institute for NanoBioTechnology (INBT), which includes more than 170 faculty members and spans several divisions of the university.

Dr. Hanes is a global leader of research at the interface of nanotechnology and medicine. He is known for designing and synthesizing new biodegradable plastics, with which his research group creates nanoscopic, drug-filled particles capable of targeted and sustained delivery to specific sites in the body. He is an inventor on more than 20 patent families relating to controlled/sustained drug delivery methods. Products based on his drug delivery inventions have been tested in various clinical trials, through and including Phase III. He is the lead founder and Chair of the Scientific Advisory Board of Kala Pharmaceuticals, a company backed by three premiere venture capital groups. Kala is commercializing Dr. Hanes' laboratory's "mucus penetrating particle" drug delivery invention. Dr. Hanes serves on the Scientific Advisory Board for Genentech in the Drug Delivery Division, as a frequent advisor to industry, and as an advisor to government agencies, such as the NIH. Dr. Hanes currently serves as the Principal Investigator on numerous grants from the NIH and other agencies. He directs a laboratory at Johns Hopkins with approximately 50 people under his supervision. He is currently the Associate Editor for Drug Delivery and Translational Research, a official journal of the Controlled Release Society, and has served as Associate Editor for the International Journal of Nanomedicine, Associate Editor for Nanomedicine: Nanotechnology, Biology, and Medicine, and as an Editorial Board Member for both the Journal of Biomedical Nanotechnology and Experimental Biology in Medicine.

OUR KEYNOTE SPEAKERS

Kostas Kostarelos / University of Manchester, UK

Kostas Kostarelos obtained his Diploma in Chemical Engineering and PhD from the Department of Chemical Engineering at Imperial College London, studying the steric stabilization of liposomes using block copolymer molecules. He carried out his postdoctoral training in various medical institutions in the United States and has worked closely with D. Papahadjopoulos (UCSF, USA), G. Sgouros (Memorial Sloan-Kettering, NY, USA) and R.G. Crystal (Weill Medical College of Cornell University, NY, USA). He was Assistant Professor of Genetic Medicine & Chemical Engineering in Medicine at Cornell University Weill Medical College when he relocated to the UK as the Deputy Director of Imperial College Genetic Therapies Centre in 2002. In 2003 he joined the Centre for Drug Delivery Research at the UCL School of Pharmacy as the Deputy Head of the Centre. He was promoted to the Personal Chair of Nanomedicine and Head of the Centre in 2007. The entire Nanomedicine Lab was embedded within the Faculty of Medical and Human Sciences and the National Graphene Institute at the University of Manchester in 2013. Kostas is currently Professor of Nanomedicine at the University of Manchester and Visiting Professor at UCL Faculty of Life Sciences.

He has been invited Fellow of the Royal Society of Chemistry (FRSC), Fellow of the Royal Society of Medicine (FRSM), and Fellow of the Royal Society of Arts (FRSA) all in the United Kingdom. In 2010 he was awarded the Japanese Society for the Promotion of Science (JSPS) Professorial Fellowship with the National Institute of Advanced Industrial Science and Technology (AIST) in Tsukuba, Japan. He has been included in the Highly Cited Researcher 2018 list (https://hcr.clarivate.com) in the Cross-Field category.

He is the Founding and Senior Editor of the journal Nanomedicine and sits on the Editorial Board of:
ACS Nano, Bioconjugate Chemistry (ACS), 2D Materials (IOP), 2D Materials & Applications (NPJ), Archives in Toxicology (Springer), Nanoscale Horizons (RSC), Journal of Visualized Experiments (JoVE), Applied Materials Today (Elsevier), The Journal of Liposome Research (Taylor & Francis).

OUR KEYNOTE SPEAKERS

Andrew Owen / University of Liverpool, UK

Andrew Owen is Professor of Molecular and Clinical Pharmacology at the University of Liverpool, UK. He is Chair of the British Society for Nanomedicine, a fellow of the Royal Society of Biology, a fellow of the British Pharmacological Society, and a fellow of the Learned Society of Wales. His clinical and basic research focuses on understanding mechanisms that underpin inter-patient variability in pharmacokinetics and pharmacodynamics. A major emphasis has been to employ knowledge of these mechanisms to accelerate the translation of technologies to clinical applications for oral and long-acting drug delivery. His ongoing research portfolio is funded by NIH, USAID, EPSRC, EC and pharmaceutical and charitable agencies. He has published over 200 publications, is co-inventor of patents relating to drug delivery and is a co-founder of Tandem Nano Ltd (www.tandemnano.com) and PKTK (www.PKTK.co.uk). Professor Owen also co-leads the UK contribution to the European Nanomedicine Characterisation Laboratory (www.EUNCL.eu) and the modelling core for the Long-acting/Extended release Antiretroviral resource Program (www.LEAPresources.org). He is also an Editor in Chief for the Journal of Interdisciplinary Nanomedicine (www.JOINjournal.com).

INDUSTRIAL EXPOSITION

This year's exhibitors

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 eight companies either sponsoring the event or participating in 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. Thank you!

WYATT TECHNOLOGY

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SYMPOSIUM DINNER

7.30 pm Monday 3rd June 2019

All registered attendees and exhibitors of the UKICRS Workshop & Symposium are invited to attend dinner at 7.30pm on Monday 3rd June at Love Lane Brewery, Bar and Kitchen. The cost of dinner is covered as part of the Symposium registration fee. Delegates will already have received information about the menu. If you have not yet registered to attend dinner and submitted your meal choice but would like to attend, speak to a UKICRS committee member as soon as possible or email us at ukicrs@gmail.com.

Love Lane Brewery, Bar and Kitchen
62-64 Bridgewater Street
Baltic Triangle
Liverpool L1 0AY

ABOUT LOVE LANE

Based in the heart of the Baltic Triangle – the home of Liverpool's independent creative industry –Love Lane Brewery, Bar and Kitchen provides a real taste of Liverpool. Home to a working brewery and distillery, the venue offers a variety of drinks, including its very own craft beers and gin, as well as well as a tasty food menu inspired by great local suppliers. For more information, visit https://www.lovelanebrewing.com

Point your smartphone camera at the QR code below to find Love Lane Brewery on Apple Maps or Google Maps.

MONDAY 3 JUNE

Workshop and Industrial Exhibitor Presentations

8.00 am	**Open for registration / exhibitor set-up / poster set-up** Main Reception, James Parsons Building, Liverpool John Moores University
10.45 am	**Tea & coffee**
Keynote & Workshop: 'Lessons from the other side: life after university' Chaired by Sarah Gordon *(Liverpool John Moores University)* & Yvonne Perrie *(University of Strathclyde)*	
11.00 am	**Justin Hanes** *(Johns Hopkins University, US)* – KEYNOTE SPEAKER Brain penetrating nanoparticles: effective drug and gene delivery to the CNS
11.40 am	**Chris Thomas** *(Cardiff University)* 'I always wanted to be a pharmacist'
11.55 am	**Bianca Price** *(University of Manchester)* 'Industrially funded research in academia: high impact studies'
12.10 pm	**Eileen McBride** *(AstraZeneca)* 'Decisions decisions? . . . . or a world of opportunity?'
12.25 pm	**'On the sofa':** questions to speakers
1.00 pm	**Lunch** / James Parsons Building
Industrial Exhibitor Presentations Chaired by Hugh Giffney *(University College Dublin)* & Sion Coulman *(Cardiff University)*	
2.00–3.30 pm	• **Shilpa Mistry-Patel & Sundip Joshi** *(Chemlink Specialities)* • **tba** *(Cole Parmer)* • **Brian Miller** *(Meritics)* • **tba** *(Mettler Toledo)* • **John Pennington** *(Sotax)* • **Steve Smith** *(Stable Micro Systems)* • **Kevin Jackson** *(Wyatt Technology)* • **Nektaria Servi** *(Surface Measurement Systems)*
7.30 pm	**Symposium Dinner** 'Love Lane Brewery, Bar and Kitchen' / see page 10 for details

TUESDAY 4 JUNE

Research Symposium / Morning session

8.00 am	**Open for registration / exhibitor set-up / poster set-up** Main Reception, James Parsons Building, Liverpool John Moores University
9.00 am	**Welcome & Opening Remarks** Katie Ryan *(UKICRS Chairperson and University College Cork)* Sarah Gordon *(Liverpool John Moores University)*
Session 1: Chaired by Carla Roces Rodriguez *(University of Strathclyde)* & Dimitrios Lamprou *(QUB)*	
9.10 am	**Kostas Kostarelos** *(University of Manchester)* – KEYNOTE SPEAKER Transforming 'Old' and Developing 'New' Nanomaterials for Therapy and Diagnosis: from Liposomes to Graphene
9.40 am	**Kaouthar Bouzinab** *(University of Nottingham)* Enhanced brain tumour targeted delivery of temozolomide analogues using an apoferritin nanocage
9.55 am	**Xinyu Zhao (***Queen's University Belfast)* Multipurpose vaginal rings for HIV prevention and treatment of bacterial vaginosis
10.10 am	**Domhnall Kelly** *(Royal College of Surgeons in Ireland, RSCI)* Comparative assessment of non-viral delivery vectors for the development of an advanced delivery system for RNA therapeutics
10.25 am	**Tea & Coffee**
Session 2: Chaired by Sara Cordeiro *(Queen's University Belfast)* & Jeninfer Mains *(Lonza)*	
10.55 am	**Talat Khan** *(University of Ulster)* Combination of cell penetrating peptide (CPP) and polymersomes (PS) for enhanced effect of photodynamic therapy (PDT)
11.10 am	**Robert Cavanagh** *(University of Nottingham)* Screening of mixed co-poly(ester-carbonate) PEG-based nanoparticles for breast cancer therapy: an *in vitro* and biodistribution based approach
11.25 am	**Swapnil Khadke** *(University of Strathclyde)* M-110P Microfluidizer® technology: Scalable manufacturing of liposomes containing water soluble or water in-soluble drugs

Continued on next page ...

TUESDAY 4 JUNE

Research Symposium / Afternoon session

11.40 am	**Vera D'Aloisio** *(Liverpool John Moores University)* Formulation into chitosan microparticles and stability studies of a truncated version of the CGRP	
11.55 am	**Poster Session 1** / Odd numbers only	
12.45 pm	**Lunch /** James Parsons Building	
Session 3: Chaired by Maria Marlow *(University of Nottingham)*		
1.45 pm	**Andrew Owen** *(University of Liverpool)* – KEYNOTE SPEAKER Title to come	
2.15 pm	**James Flynn** *(University of Limerick)* Injectable hydrogels for the controlled delivery of a broad spectrum bacteriocin, Nisin A	
2.30 pm	**Rosemond Mensah** *(University of Hertfordshire)* Drug-loaded microparticle-based dressing for ocular wound repair	
2.45 pm	**Ebru Altuntas** *(Queen's University Belfast)* Formulation and characterization of dissolving microarry patch loaded with long-acting nestorone nanosuspension for transdermal delivery	
3.00 pm	**Tea & Coffee**	**Poster Session 2 /** Even numbers only
Session 4: Chaired by Carlo Curti *(Aston University)* and Charlie Winter *(Imperial College London)*		
3.50 pm	**Soapbox Session** A series of 2 minute flash talks ... on any topic!	
4.35 pm	**Closing Remarks and Prizes**	

ORAL ABSTRACTS

ENHANCED BRAIN TUMOUR TARGETED DELIVERY OF TEMOZOLOMIDE ANALOGUES USING AN APOFERRITIN NANOCAGE

1

Kaouthar Bouzinab[1], Neil R. Thomas[2], Lyudmila Turyanska[3], Pavel Gershkovich[1], Nicola Weston[4], Marianne B. Ashford[5], Tracey D. Bradshaw[1]

[1] School of Pharmacy, University of Nottingham, NG7 2RD, UK; [2] School of Chemistry, University of Nottingham, NG7 2RD, UK; [3] School of Physics and Astronomy, University of Nottingham, NG7 2RD, UK; [4] Nanoscale and Microscale Research Centre, University of Nottingham, NG7 2QB, UK; [5] AstraZeneca, SK10 2NA, UK

Background: High grade brain tumours like glioblastoma multiforme (GBM) are often treated by surgery, followed by radiotherapy and DNA alkylating temozolomide (TMZ) chemotherapy. Despite the multi-modal approach to treatment, patient prognosis remains poor; in England 5-year survival is < 10%. Resistance to TMZ (inherent or acquired) is a major obstacle thwarting successful treatment. Overexpression of the O6-methylguanine DNA-methyltransferase (MGMT; which removes cytotoxic O6-methylated guanine (O6-MeG) lesions) and deficiency in mismatch repair (MMR; in which case O6-MeG lesions are tolerated) confer resistance to TMZ. To overcome resistance, novel N3-propargyl substituted analogues of TMZ have been developed showing promising activity irrespective of MGMT or MMR status. However, poor brain drug bioavailability, short drug half-life and systemic toxicity remain to be solved. Apoferritin (AFt) is thus proposed as a natural biocompatible drug delivery system. AFt's small size (diameter: 12 nm) and numerous transferrin receptor recognition sites on its surface; alongside enhanced expression of transferrin receptors (TfR1; which sequester AFt) on the membranes of cancer cells, offer a dual targeting approach towards enhanced tumour-selectivity and uptake.

Methods: Herein, we have used AFt for the encapsulation of therapeutic agents, TMZ and analogues, via molecular diffusion, through channels in the AFt cage. Protein stability was monitored by dynamic light scattering (DLS) and zeta-potential and encapsulation efficiency measured by UV-vis spectroscopy and corroborated by high performance liquid chromatography (HPLC). In vitro cytotoxicity assays on the encapsulated drugs were then performed (MTT, clonogenic and flow cytometry) and conducted against isogenic GBM cell lines, U373V (vector control) and U373M (MGMT over-expressing), as well as healthy MRC-5 (lung fibroblasts) cells. Environmental scanning electron microscopy (ESEM) was further employed to assess morphological changes to the cells after treatment.

Results: Drugs were successfully encapsulated via diffusion, with around 520 molecules of TMZ/analogues encapsulated per AFt cage and corresponding to encapsulation efficiencies of > 65% of the solution used. MTT assays demonstrated significantly increased activity of encapsulated TMZ and analogues, compared to naked drugs, with 50% growth inhibition (GI50) values < 1 μM, compared to >30 μM for naked drugs. Clonogenic and cell cycle analyses further corroborate these findings. Against MRC-5, visibly less activity was seen, with GI50 values > 50 μM. In addition, assessment of the cell morphology of GBM cells after 24 hr test agent treatment, reveal visible shrinking and blebbing of cells; more so with the Aft encapsulated agents.

Conclusions: The AFt nano-delivery system offers a promising route for enhanced specificity, selectivity and potency of TMZ and analogues; paving the way to overcome the battle of drug resistance.

MULTIPURPOSE VAGINAL RINGS FOR HIV PREVENTION AND TREATMENT OF BACTERIAL VAGINOSIS

2

Xinyu Zhao, Peter Boyd, Karl Malcolm

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

Background: Acquired immune deficiency syndrome (AIDS) is one of the most serious global public health diseases. Vaginal microbicides are vaginally-administered pharmaceutical formulations intended to reduce or prevent sexual transmission of the human immunodeficiency virus, (HIV) virus, the causative agent of AIDS. For the past ten years, antiretroviral-releasing vaginal rings have been prioritized as a female-controlled HIV prevention method. A vaginal ring containing dapivirine (DPV), a non-nucleoside reverse transcriptase inhibitor (NNRTI), is being developed as a long-acting HIV microbicide product by the International Partnership for Microbicides (IPM). Multipurpose prevention technology strategies (MPTs) aim to prevent HIV-1 infection, unintended pregnancy and other sexually transmitted infections using a single product. Bacterial vaginosis is a common lower genital tract disorder in reproductive age women and is characterized by the replacement of a Lactobacillus-dominated microbiota by a variable mixture of anaerobic and facultative bacteria. It has been reported that bacterial vaginosis is associated with an increased risk of HIV acquisition. Metronidazole (MET) inhibits nucleic acid synthesis and is the major treatment of treatment for BV. In this PhD project, we propose to develop a combination drug ring that provides sustained release of both DPV and MET to prevent HIV infection and treat or prevent bacterial vaginosis.

Methods: Matrix-type silicone elastomer vaginal rings comprising different MET loadings (100, 250, 500, 1000 and 2000 mg) and either 25 or 200 mg DPV were manufactured using a Babyplast injection molding machine. Samples of matrix silicone elastomer rings loaded either DPV, MET or combination of DPV and MET were analyzed by DSC to characterize the nature of the drugs in the rings. Four rings were randomly selected from each ring formulation for 28-day in vitro release studies in the 0.2%w/w Tween80 release media at 37˚C and 60 rpm. Mechanical tests containing shore M hardness tests, static and dynamic compression response were conducted on these different VR formulations.

Results: After 28-day in vitro release testing, cumulative DPV release was 3.7 and 11.7 mg for rings loaded with 25 and 200 mg DPV, respectively. Cumulative MET release was 30.7, 54.0, 109, 173 and 251 mg for rings loaded with 100, 250, 500, 1000 and 2000 mg MET. In addition, DPV cumulative release was increased to 5 mg for rings loaded with both 25 mg and 1000 mg MET. The thermal results of mixtures of DPV and MET in physical mixtures and incorporating in silicone elastomers showed reduced melting transitions for both drug components, indicating a eutectic composition at 80% MET and at a temperature of 153 ˚C. Mean Shore M hardness values for DPV formulations and MET (100 and 250 mg) formulations were 36 and 34, respectively. The 500, 1000 and 2000 mg MET formulations produced values ranging from 37 to 45. All VR formulations recovered to 90–100% of their original diameter after 1000-cycle compression.

Conclusions: The data support the continued development of these vaginal ring formulations as multi-purpose prevention technologies (MPTs) for HIV prevention and bacterial vaginosis treatment and prevention.

COMPARATIVE ASSESSMENT OF NON-VIRAL DELIVERY VECTORS FOR THE DEVELOPMENT OF AN ADVANCED DELIVERY SYSTEM FOR RNA THERAPEUTICS

3

Domhnall Kelly[1-3], Rosanne Raftery[1,2], James Dixon[4], Kevin Shakesheff[4], Helen McCarthy[5], Christopher Hobbs[1,2], Caroline Curtin[1,2], Caitriona O'Driscoll[3,6], Fergal O'Brien[1-3]

[1] Tissue Engineering Research Group, Royal College of Surgeons in Ireland, [2] Advanced Materials and Bioengineering Research Centre (AMBER), Ireland; [3] Centre for Research in Medical Devices (CÚRAM), NUI Galway, Ireland; [4] School of Pharmacy, University of Nottingham, UK; [5] School of Pharmacy, Queen's University Belfast, UK; [6] Pharmacodelivery Group, School of Pharmacy, University College Cork, Ireland

Background: Small interfering (si)RNA offers an alternative approach to conventional gene therapies, allowing for the silencing of target messenger (m)RNA sequences and the knockdown of specific proteins. To date, the development of a safe and efficient delivery system remains the major limitation to these therapies. This study investigated the ability of several non-viral vectors to deliver siRNA to mesenchymal stem cells (MSCs).

Methods: Polyethyleneimine (PEI), an amphipathic cell-penetrating peptide (RALA), a glycosaminoglycan enhanced binding domain peptide (GET), a novel layered double hydroxide (LDH), and a cationic cyclodextrin, were investigated for the safe and efficient delivery of siRNA. Dynamic light scattering was used to determine particle size and charge, with transmission electron microscopy (TEM) used to further confirm size, particle morphology and aggregation. Gel retardation studies determined complexation efficiency of siRNA cargo. In vitro transfection of MSCs was used to determine cellular uptake, while transfection efficiency was assessed through the knockdown of green fluorescent protein (GFP) in GFP-expressing MSCs.

Results: Results demonstrate the stable formation of complexes displaying physicochemical characteristics of size within the desirable nanoscale range, and a positive surface charge, as per the literature. TEM further confirmed polyplex size and nanoparticle tracking analysis demonstrated low levels of polydispersity. Importantly, cellular uptake of nanoparticles and efficient GFP knockdown was demonstrated in MSCs, with both cell-penetrating peptides demonstrating the greatest levels of cellular uptake and transfection.

Conclusions: Nanoparticle complexes formulated demonstrated both safe and efficient delivery of siRNA to MSCs resulting in transient knockdown of target genes. This study demonstrates the potential application of these nanoparticle complexes for a range of therapeutic indications.

Acknowledgements: Science Foundation Ireland (SFI) European Regional Development Fund 13/RC/2073 and Irish Research Council Government of Ireland Postgraduate Programme (GOIPG/2018/371).

COMBINATION OF CELL PENETRATING PEPTIDE (CPP) AND POLYMERSOMES (PS) FOR ENHANCED EFFECT OF PHOTO DYNAMIC THERAPY (PDT)

4

Talat Khan & Bridgeen Callan

School of Pharmacy and Pharmaceutical Sciences, University of Ulster, BT52 1SA, Northern Ireland, UK

Background: Photodynamic therapy (PDT) has been widely considered as a safe and effective approach for the treatment and management of superficial conditions for example, cutaneous cancer affecting skin, head and neck and infection of the eyes. PDT requires both a photosensitiser compound, Rose Bengal (RB) and light for activation to transform surrounding molecular oxygen to a cytotoxic species. Given this mechanism of action, PDT does not suffer any issues with patients generating resistance to the treatment, however, its major limitation is due to the necessity of light as an activating agent and the depth of penetration of such. The cell penetrating peptide (CPP) (KLAKLAK)2, a vector for the transportation of small molecules across cell membranes, when conjugated with a photosensitizer i.e. RB-CPP, has been shown to significantly enhance the photo dynamic effect on targeted cells requiring less light activation. Polymersomes (PS), a polymeric nano-carrier can be used for dispensing of therapeutics (eg RB) inside cells through enhanced permeation and retention (EPR) effect. It is formulated from an amphiphilic polymer comprised of a hydrophilic monomer unit, polyethylene glycol, and a hydrophobic moiety, a blend of both decyl chains and Cholesteryl. This research combines, for the first time, the use of CPPs and PSs to enhance the effect of PDT on the site of action, with the quest of expanding the current use of PDT by reducing the limitations of light.

Methods: Synthesis of the peptide (KLAKLAK)2 and all conjugates of such was achieved utilizing solid phase peptide synthesis followed by purification using RP-HPLC. The purified product was lyophilized for later use. Decyl and cholesteryl monomers were synthesized to include an olefinic group for polymerisation, they were purified using column chromatography and characterised by NMR and MS. Polymerisation was achieved via a Michael addition reaction using a free radical initiator to generate random copolymers of known configuration. PS were formulated using said polymers and a reverse phase evaporation method. The drug incorporated PS were evaluated for size and polydispersity index by Dynamic light scattering; encapsulation efficiency, release kinetics and cellular uptake were achieved utilizing uv-vis spectroscopy. MTT assay was conducted to establish the cell viability of healthy and cancer cell lines to study the effect of drug combination and PS on PDT in the presence and absence of light.

Results: Mass and NMR spectra of monomers confirmed the presence of required products, polymer spectra confirmed the disappearance of the olefinic protons. Formulated PSs had a size range 200-400 nm, appropriate for cellular uptake, and a PDI of less than 0.5 suggesting a mono-disperse system. PS showed encapsulation of 85% and in-vitro release of 64% of RB after 24h. Initial PDT experiments illustrate that the free drug conjugate of RB-CPP displays a significantly enhanced effect on cell viability compared to RB alone. RB and RB-CPP alone does not cause cell-toxicity in absence of light indicative of being non-toxic to healthy cells.

Conclusions: PDT is a non-invasive, patient compliant method, the effects of which can be improved several fold via a combination of photosensitizer and a peptide-based biomolecule. The use of nano-delivery, such PS, will provide a more targeted approach. Therefore, the combination of CPP, PS and PDT has the potential to expand the usefulness of PDT by removing its limitation to superficial therapy.

SCREENING OF MIXED CO-POLY(ESTER-CARBONATE) PEG-BASED NANOPARTICLES FOR BREAST CANCER THERAPY: AN IN VITRO AND BIODISTRIBUTION BASED APPROACH

5

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Background: Triple negative breast cancer (TNBC) is diagnosed in ~ 200,000 patients per year and is a class of disease associated with local recurrence and poor prognosis. Moreover, unlike oestrogen- and progesterone-receptor positive cancer subtypes targeted therapies are not available. Consequently, chemotoxic are generally used and applied at their maximum tolerated dose and associated with serious side effects as a result of off target, systemic toxicity. The present work investigates the utilisation of oxidative responsive, self-immolative nanoparticles comprised of amphiphilic mixed co-poly(ester-carbonate) PEG-based polymers. The aim of the work is to develop such 'smart' nanoparticles for the delivery of chemotoxics for the treatment of TNBC. Through the use of targeted radiotherapy, tumour site-specific release of drugs can be achieved and thus limit systemic toxicity of therapy while, in tandem, provide dual therapy that may circumvent radio/chemo-resistance in certain tumour populations.

Methods: In vitro assessment of responsive nanoparticles is performed in the TBNC cell line MDA-MB-231 and luminal MCF-7 breast cancer cells cell line. Biocompatibility of nanoparticles is tested using LDH release and Prestoblue assays. Cellular is internalisation studied via microscopic and spectrophotometric means, in addition, the use of inhibitors has been employed to determine endocytotic pathway(s). Moreover, uptake in murine macrophages (RAW 264.7) has been performed to investigate immune evasive capabilities of nanoparticle formulations. In vivo biodistribution experiments were performed on healthy mice in order to assess distribution, organ accumulation and clearance of Cy5-labelled nanoparticles of different architectures.

Results: A series of amphiphilic mixed co-poly(ester-carbonate) PEG-based polymers have been synthesised via friendly ring opening polymerisation and assessed based on in vitro and in vivo uptake characteristics to enable the selection of a lead polymer formulation for functionalisation/drug conjugation. Modification of the PEG based initiator while maintaining the same subset and ratio of monomers enabled different polymeric architectures (polymeric micelle and 'flower-like' micelles) and nanoparticle sizes (82, 86 and 126 nm) to be generated. All polymer formulations demonstrate good biocompatibility. In both breast cell lines tested, size appears to be the most prominent factor in determining rate of uptake, with the nanoparticles < 100 nm internalised most effectively. Through the use of endocytosis inhibitors, it has been observed that nanoparticles < 100 nm exploit both clathrin- and caveolin-mediated pathways, while only the clathrin-mediated route is available to nanoparticles > 100 nm. Assessment of uptake in RAW264.7 macrophage cells demonstrates that polymeric architecture / PEG length, is the determining factor in the immune evasive capabilities of nanoparticles; 'flower-like' micelles show greater tendency for immune cell uptake than polymeric micelles. This observation is then confirmed in vivo via biodistribution experiments in healthy mice. Conjugation of the chemotherapeutic doxorubicin is then performed on the lead polymer nanoparticle formulation and achieves a 10-fold increase in potency relative to free drug alone.

Conclusions: The work performed here outlines an effective approach to high throughput in vitro screening methods for nanoparticle selection. Furthermore, the enhanced potency achieved via drug conjugation to the selected polymeric nanoparticle highlights the potential application of this delivery system for breast cancer therapy.

M-110P MICROFLUIDIZER® TECHNOLOGY: SCALABLE MANUFACTURING OF LIPOSOMES CONTAINING WATER SOLUBLE OR WATER INSOLUBLE DRUGS

6

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Background: Liposomes offer a variety of advantages for the delivery of drugs including flexibility in size, bilayer composition and ability to incorporate and protect drugs from degradation and hence assist delivery. However, despite these advantages, their use as clinically approved products remain limited due to manufacturing costs and scalability. To address this, we have investigated the Electric Benchtop Laboratory M-110P Microfluidizer processor that requires no compressed air and no hydraulic cooling water to achieve up to 2000 bar (30,000 psi) operating pressure produces product flow rate up to 120 mL/min with guaranteed scalability. The purpose of this study was to develop a scalable method to generate small unilamellar vesicles (SUV) liposomes loaded with water-insoluble or water-soluble APIs.

Methods: Multilamellar vesicles were formed containing appropriate amount of phospholipids and cholesterol based on the established thin-filmed hydration film method. To entrap drug within the bilayer, the required amount of anti-inflammatory water insoluble active pharmaceutical ingredient (API) was added to the solvent mixture and subsequently hydrated (Formulation I). The formulation with water-soluble anti-bacterial API was dissolved in an aqueous buffer (Formulation II). To prepare SUV, 60 mL of multilamellar vesicle (MLV) were subjected to high pressure homogenisation and optimised for minimum required number of passes to generate less than 100 nm sized liposomes. Liposomal drug content was analysed by UV-HPLC after purification using tangential flow filtration in order to remove the free drug. The z-average diameter and polydispersity (PDI) of liposomes after reconstitution were determined.

Results: The initial objective was to study the effect of the number of homogenisation passes on liposome particle size and PDI. Placebo formulations were optimised and five passes produced liposomes which were 50-60 nm in particle size liposomes with 0.20 PDI and a single particle size population (total lipid concentration of 10 mg/mL). Formulation I with water-insoluble API was formulated with and without cyclodextrin (CD). Our results showed that lead liposomal formulation loaded with water-insoluble API can be manufactured in absence of CD with 52.53 ± 0.70 nm particle size, 0.19 PDI and 88 ± 4 % drug loading. Formulation II with water-soluble API offered 29 ± 2 % drug loading with 50.20 ± 0.59 nm and 0.22 PDI. Micro-dilution minimum inhibitory concentration (MIC) assay as per CLSI guidelines showed liposomal formulation II improved antibacterial efficacy by 2-fold (E coli), 4-fold (Salmonella), 6-fold (Pseudomonas aeruginosa) and 4-fold (Staphylococcus aureus) in comparison with free API.

Conclusions: Liposomal formulations containing selected water-insoluble anti-inflammatory or water-soluble antibacterial API were successfully screened and manufactured using M-110P Microfluidizer processor with uniform particle size distribution and high drug loading.

FORMULATION INTO CHITOSAN MICROPARTICLES AND STABILITY STUDIES OF A TRUNCATED VERSION OF THE CGRP

7

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Background: The intranasal route is useful for the delivery of drugs to the brain via the blood brain barrier and/or the olfactory nerve. The purposes of this research were to develop a dry powder, mucoadhesive drug carrier for the nasal administration of small peptide CGRP (calcitonin gene-related peptide) antagonists to treat migraine and to assess the stability of the peptide in serum.

Methods: A truncated version of CGRP, Pro34Phe35CGRP(27-37), was synthesized using SPPS procedure. Peptide-containing microparticles (MP) were prepared by spray drying peptide (1%) and low molecular weight (LMW) chitosan (2%) from a solution of 0.5% acetic acid using a Büchi B-290 spray dryer. Moisture content was determined by thermogravimetric analysis (TGA Q5000). The morphology and diameter of the MPs were observed using scanning electron microscope (SEM). The average diameter of the particles was calculated measuring 100 particles for each sample. MPs (10 mg) were suspended in 1 ml of deionized water and the mixture was left mixing at 20 rpm and 37 °C to assess the release of the peptide. Three samples were prepared and, after 24 hr, were centrifuged at 13200 rpm for 10 min. The supernatant was analyzed by RP-HPLC, utilizing the unloaded MPs as blanks. Peptide stability in blood was investigated using human serum. Here, 10µL of aqueous peptide stock solution (2 mg/mL) was added to 190 µL of 25% pooled aqueous human serum to make a final peptide concentration of 50 µg/ml. The mixtures were thermostated at 37 °C and the initial time was recorded. At known time intervals (0, 6, 10 and 30 min) three samples were taken and precipitated by the addition of 200 µL of a 6% TCA aqueous solution. The cloudy reaction samples were cooled in ice for 15 min and then spun at 13,200 rpm for 2 min to pellet the precipitated serum proteins. The supernatants were analyzed using an RP-HPLC.

Results: Low molecular weight chitosan was selected due to its biocompatibility, mucoadhesiveness and non-toxicity. Chitosan MPs containing 5 mg of Pro34Phe35CGRP(27-37) in 0.5 g of LMW Chitosan were prepared with a yield of 45%. The water content of the powder was 8.2%. Microparticles had an average diameter of 10.7 µm and were spherical, some with a rough and wrinkled surface others with a smooth surface. In water 70% of the peptide was released over 24 hr. The stability of the peptide over 30 min was analyzed by RP-HPLC and LC-MS indicating a linear peptide concentration decrease from 38.3 µg/ml at time 0 to 13.8 µg/ml at time 30 min.

Conclusions: Chitosan microparticles loaded with Pro34Phe35CGRP (27-37) were successfully prepared by spray drying, aiming a size suitable for the nasal delivery. The release of the peptide from the microcarriers showed a release up to 70% in 24 hr. The stability studies showed a peptide degradation of 43% after 30 min.

INJECTABLE HYDROGELS FOR THE CONTROLLED DELIVERY OF A BROAD SPECTRUM BACTERIOCIN, NISIN A

8

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Background: The need to improve current ineffectual antimicrobial therapeutics has reached a level of very high importance. A potential alternative therapy to combat drug resistant bacterial infections is the use of antimicrobial peptides called bacteriocins. However, bacteriocins are susceptible to proteolytic cleavage and degradation both in vivo and in vitro. The aim of this work is to develop polymeric hydrogels to encapsulate, and effectively release these peptide drugs at the site of infection, preventing degradation and treating the infection. The bacteriocin nisin was used during this study.

Methods: Hydrogels were synthesized based on the Schiff base formation via the cross- linking of two functionalized polysaccharides; dextran di-aldehyde (with a degree of functionalization of 34%), and alginate functionalized with hydrazine groups. Glycol chitosan (GC) was added to the gels at varying concentrations as a means of controlling release of the entrapped nisin (1 mg/gel). Release was studied by submerging the nisin gels in 1 ml of fasted state simulated gastric fluid (FaSSGF)(pH 1.2). The stability of the gels was determined by weighing the gels over the duration of the release and measuring their diameter.

Results: The gels were formed by dissolving the functionalized dextran and nisin in a KCl/HCl buffer (pH 2) and dissolving the alginate and chitosan in PBS. The polymer solutions were injected into a mold in a double-barreled syringe through a 21 G needle. The cross-linked gels were stable and swelled in low pH media up to 2 weeks before degrading. The higher the percentage GC content in each gel, the higher the degree of swelling (before degradation) over a range of pH conditions. The rate of release of nisin from the gels was studied into FaSSGF (pH 1.2). Perfect sink conditions were maintained throughout all release studies. Addition of GC appeared to induce control over the initial release pattern. The rate of release of nisin was slowed at higher GC content while conversely lower GC content appeared to give a faster release. All nisin was released after a period of 20-25 days. Both the nisin entrapped gels and the release media (FaSSGF) exhibited inhibitory activity toward Staphylococcus aureus and Enterococcus faecium.

Conclusions: The addition of glycol chitosan increases the swelling of the gels by reducing the level of polymer entanglement between the dextran and alginate chains. The GC, with an abundance of amine groups, may interact with the OH groups on the dextran and alginate chains, preventing a higher degree of polymer chain entanglement and hydrazone bond formation, which is observed with the increased swelling at higher GC content. Nisin is a cationic peptide carrying a positive charge in the gel matrix which will interact with the abundance of OH groups on the dextran and alginate polymers. Contrary to the expectation that higher degrees of swelling would result in higher release rates, the lower degree of polymer chain entanglement means that there are more OH groups accessible for nisin to interact with, resulting in a slower release rate. Thus, the release of nisin appears to be dependent on the polymer chain interactions, and thus the GC content, within the gel network. These interactions will be analyzed using SEM-Raman and SS-NMR in future work. Results indicate that glycol chitosan may be an effective additive for controlling the release of cationic antimicrobial peptides from injectable dextran-alginate hydrogels.

DRUG-LOADED MICROPARTICLE-BASED DRESSING FOR OCULAR WOUND REPAIR

9

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Background: Chicken eggshell membrane (ESM) is a unique biomaterial in nature. The ESM contains a large number of bioactive components such as collagen type I, V, and X, fibronectin, proteoglycans and other glycoproteins. These components determine the exceptional biocompatible, biodegradable, cell-adhesive and wound-healing properties of the ESM. Based on these characteristics, the ESM can be exploited as a potential wound dressing for ocular applications. Present approaches for ocular wound healing comprise the use of dressing combined with repeated administration of therapeutic eye drops. The major problem with eye drops is poor bioavailability, patient compliance and, in some case, adverse effects that could include wound-healing complications. Polymer-based microparticulate systems are often used in therapeutic applications as controlled and sustained release carriers for drugs and growth factors to improve the bioavailability and efficiency. Poly lactic-co-glycolic acid (PLGA); a biocompatible, biodegradable, and non-toxic polymer was utilized in the present study to fabricate drug-loaded microparticles (MPs). The aim of this study was to generate and characterize a potential novel dressing consisting of ESM and drug-loaded PLGA microparticles for ocular surface wound-healing applications.

Methods: ESMs were extracted using an optimised acid- and/or chelator-based technique and sterilised accordingly thereafter. The mechanical and biological properties of the ESM were evaluated using a combination of texture analyses, water content analysis, water contact angle test, visual tests for transparency, scanning electronic microscopy (SEM), and thermogravimetric analysis (TGA). Drug–loaded PLGA MPs were created using an established single oil in water emulsion method. The particle sizes were determined using laser diffraction with a HELOS equipped with a RODOS/ ASPIROS dry dispenser (SymPatec, Germany). The morphological analysis of the PLGA MPs was conducted using SEM and chemical composition using FT-IR. The loading efficiency, loading bioavailability, and in vitro release profiles of the MPs were determined. A simple surface adsorption and attachment technique was used to deposit the MPs onto the ESM. A combination of in vitro tests i.e. human cornea cell culture, Franz diffusion/permeability cells and the chorioallantoic membrane (CAM) assay were used in assessing the toxicity, biocompatibility, release profiles and pro-angiogenic responses of the dressing generated.

Results: The ESMs generated using the extraction and sterilisation methods had suitable physical, mechanical and biological behaviors crucial to their performance as an ocular bandage. The characterization protocols and in vitro tests of the generated drug-loaded PLGA MPs exhibited suitable entrapment bioavailability, tailorable release profiles and pro-biological effects required for the therapeutic applications. The MPs were successfully attached onto the ESM. The presence of the loaded MPs did not compromise the transparency of the dressing.

Conclusions: The sterilised membranes demonstrated high biocompabiltiy, durability, flexibility, water absorption capacity and thermal stability. Drug-loaded PLGA-MPs, 10–50 µm, were successfully fabricated and deposited on the ESM. The final dressing produced has potential for ocular wound healing applications.

FORMULATION AND CHARACTERIZATION OF DISSOLVING MICROARRY PATCH LOADED WITH LONG-ACTING NESTORONE NANOSUSPENSION FOR TRANSDERMAL DELIVERY

10

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Background: The fact that approximately half of unintended pregnancies in the United States occurs among users of contraception indicates that the current contraceptive methods are not meeting the needs of all couples. Oral contraceptives are widely used but incorrect and inconsistent use remain common. Non-oral delivery methods have been developed to offer a long-period effect with good compliance and tolerance. However, all these methods demanded professional staff to conduct, which restricted the usage. Therefore, in this study, we aimed to develop a novel long acting contraceptive nanosuspension (NS) loaded dissolving microneedles (DMNs) to deliver a highly potent 19-norprogesterone derivative, Nestorone (NES), efficiently in controlled and sustained manner for monthly application.

Methods: NES loaded NS was prepared by a sonoprecipitation method. The process parameters and the formulation of NES NS was optimized for particle size and polydispersity index (PDI). Drug content, short term stability and in vitro drug release of NES NS was investigated in detail. Subsequently, NES NS-loaded DMNs were prepared by pressure chamber technique. The developed NES NS loaded DMNs were tested for drug content, particle size, mechanical strength. Optical coherence tomography (OCT) was used to assess the successful insertion and scanning electron microscopy (SEM) was used to study the morphology of the DMNs.

Results: The optimized freeze-dried NES NS was obtained with 50 mg drug content, 2% w/v of PVA (10 kDa) as stabilizer and 2.5% of PVP K29-32 as cryoprotectant. The solvent to antisolvent ratio of 1:3 (v/v) and 80% of sonication amplitude for 5 min were selected as the optimized production parameters. The particle size and PDI of the optimized NS were 800.9 ± 11.8 nm and 0.180 ± 0.036, respectively. 10 mg lyophilized NS was found to contain 2.66 ± 0.93 mg NES. There was a significant decrease in mean particle size (from 732.2 ± 12.3 nm to 546.2 ± 71.1 nm) with liquid NS stored at under refrigerated storage condition at the end of 4th week. The lyophilized NS showed almost 1.6 fold higher release than that of NES suspension. The bilayer DMNs consist of a first layer (MN tips) containing NES NSs and the second layer with a mixture of 20% (w/w) PVP K29-32 (M.W: 58 kDa) and 15% (w/w) PVA (M.W: 31–50 kDa) hydrogels. The resulting DMNs were approximately 900 µm in height, with pyramidal tips measuring 600 µm, 300 µm side at the baseplate and top column body measuring 300 µm, and the fabrication process resulted in MNs with sharp needle tips, essential for insertion into skin. Each NES NS loaded DMNs contains approximately 2100.84 ± 15.35 µg/mL drug. The particle size and PDI values of the NES NSs remained stable after casting into MNs. Following application of the 32 N axial load, the NES NSs loaded DMN showed good mechanical strength with 5.25% ± 0.9 height reduction and sufficient insertion depth in Parafilm M®.

Conclusions: This new contraceptive delivery system is promising for improving user compliance by eliminating the daily use obligation of oral contraceptives, also providing users to apply contraceptive themselves easily by solving the necessity problem for professional staff.

Acknowledgements: We thank Population Council for providing Nestorone. This research was supported by International Post Doctoral Research Fellowship Programme (2219) of The Scientific & Technological Research Council of Turkey (TUBITAK).

POSTER ABSTRACTS

DEVELOPMENT OF FITC-BSA LOADED PHOTO-CROSSLINKED INTRAVITREAL IMPLANTS FOR SUSTAINED PROTEIN DELIVERY

11

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Background: Delivery of therapeutic proteins to the posterior segment of the eye has been a major challenge due to the barrier properties of the eye combined with large size and short half-life of proteins. Biodegradable intravitreal implants has advantage for protein delivery due to its ability to overcome multiple barriers of the eye, provide controlled drug release over an extended period, and minimal amount of surgical intervention. In this study, photocrosslinked biodegradable implants were fabricated and characterised from poly(ethylene glycol) diacrylate (PEGDA) and poly (lactic-co-glycolic acid) (PLGA)-based polymers for ocular delivery of a model protein molecule, fluorescein isothiocyanate labelled bovine serum albumin (FITC-BSA).

Methods: Fabrication of Implants: Our optimised gel formulation was composed of 2.5% w/w FITC-BSA, PEGDA Mw 700 Da, PLGA, polyethylene glycol (PEG), and 0.1% w/w Irgacure® 2959. The gels were loaded into tubular silicon moulds and photocrosslinked at 365 nm using Fusion UV LightHammer 6 high power UV curing system (Maryland, USA). Implants were cut to form rod implants with 7.5 x 0.52 mm dimension. In vitro drug release was conducted in 2 mL of phosphate-buffered saline (PBS) (pH 7.4 ± 0.2) at 37 °C. At predetermined time intervals the entire medium was removed and replaced with fresh medium. The concentration of released FITC-BSA was analyzed using a validated fluorescence spectrophotometry assay. Protein distribution inside the implant: Implants were immersed in 5 mL phosphate buffer saline (PBS) with NaN_2 0.05% pH 7.4 for 30 days at 37 °C ±.0.5 °C. At predetermined intervals images of the implants were taken using a Leica DM5500 widefield fluorescence microscope to determine protein distribution.

Results: All implants showed an initial burst release of FITC-OVA ranging from 10 to 20 % within first 24 hr. This was followed by reduction of drug release. All photo-crosslinked implant formulations were able to sustain FITC-BSA release for 63 days. Addition of PEG to the formulation will increase the release rate of FITC-BSA. For e.g. % release was increased from 33.18 % to 49.13 % at day 63, which was due to the leaching of the water-soluble PEG in the release media. However, addition of PLGA will reduce the release rate of FITC-BSA (27.80% cumulative release compared with 33.18% for implants without PLGA) at day 63 due to hydrophobicity of PLGA. Fluorescence imaging shown that with the addition of PLGA, protein maintains its homogeneous distribution inside the implant compared with other formulations after 30 days release.

Conclusions: Photo-crosslinked PEGDA implants can provide sustained delivery of FITC-BSA over 63 days. This study shown that addition of PEG and PLGA can alter the protein release profile from the implants.

USE OF HYBRID IRON OXIDE-SILVER NANOPARTICLES FOR IMAGE GUIDED DRUG DELIVERY IN PANCREATIC CANCER

12

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Background: Pancreatic cancer is the 4th most aggressive cancer in the Western world. An estimated 426,000 people were diagnosed with pancreatic cancer globally in the year 2015 alone. The age range of pancreatic cancer mostly happens in the 65-75 year age group. Nanoparticles (NPs) with easily modified surfaces have been considered widely in recent years. Nanoparticles which can work as drug delivery systems have huge potential for the treatment of different kinds of cancer such as pancreatic cancer. Silver nanoparticles are the most significant nanoparticles used in foods, drugs and other industries. Silver nanoparticles may eventually extend the treatment of various diseases. Their extremely wide surface area permits the coordination of a huge number of ligands. The study of silver nanoparticles applicable to human treatments is widely under investigation in assessing potential efficacy, laboratory and animal studies, toxicity, and costs. However, use of silver coated iron oxide has not yet been reported for pancreatic cancer therapy. In this work we focus on the design, synthesis, and characterization of hybrid iron oxide-silver core-shell nanostructures, which maintain strong magnetism and surface Plasmon resonance (SPR) for use as multimodal drug delivery vehicles.

Methods: HNPs were characterized by coupled plasma-optical emission spectroscopy (ICP), UV-Vis spectrometer, photon correlation spectrometer, transmission electron microscopy (TEM) and laser. Drug quantification was achieved using by reverse phase high performance liquid chromatography (HPLC) using a fluorescent detector.

Results: We successfully synthesized and characterized HNP with different instruments. After laser irradiation at 1064 nm for 60 sec, we demonstrated that the nanoparticles reduce the risk of surrounding tissue damage because the laser beam only focuses on the treated area and the temperature will not increase outside the laser beam. Inside the laser beam temperature changes up to 20 degrees were observed. Bisnaphtalimide spermine (BNIPDSpm) (pancreatic cancer drug) was attached on HNP surface. the amount for drug loading and releasing quantified using HPLC. We successfully loaded the drug in HNP over than 90% in ratio 10:1. In drug release studies, rising temperatures resulted in increased the amount of drug releasing over 65% in first 1 hr.

Conclusions: This study highlights the potential of these technologies for drug delivery, further work is underway to assess their thermally responsive nature and in vitro characteristics.

PRODUCTION OF FULLY SYNTHETIC LIPOSOMES USING MICROFLUIDICS

13

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Background: Liposomes have become valuable tools as adjuvants within the field of vaccine development. However, they are difficult to manufacture and protein encapsulation is dependent on the nature of the phospholipid, (charge, chain length, saturation). The addition of cholesterol is an important factor in stabilising lipid bilayer integrity for encapsulation efficacy and release of antigen or protein to the target. This research seeks to demonstrate the ability to formulate fully synthetic liposomes using SyntheChol™ a non-animal derived lipid using microfluidic processes.

Methods: Liposomes were prepared using Nanoassemblr™ Benchtop (Precision Nanosystems Inc., Vancouver, Canada) and a 300mm staggered herringbone Micromixer. Liposomes were prepared from hydrogenated soy phosphatidylcholine (HSPC) and Cholesterol or SyntheChol™ (2:1 wt/wt). The lipids were dissolved in ethanol and ovalbumin (OVA; 200µg/mL OVA) was dissolved in an aqueous buffer. The flow rate ratio (FRR) between the aqueous and solvent stream was set at a FRR of 2.5:1 and a total flow rate of 15mL/min. solvent and non-incorporated protein was removed using 12 wash cycles pf phosphate buffered saline (pH 7.4) by tangential flow filtration. Liposomes were characterized by measuring size, PDI and zeta potential by dynamic light scattering (Malvern Panalytical, Malvern, UK). Protein encapsulation was determined using Micro BCA assay. All formulations were made in triplicate.

Results: Our results show that liposomes can be easily and rapidly manufactured using microfluidics. Liposomes were prepared from HSPC:Chol initially at a range of flow rates between 3:1 and 2:1 and a flow rate of 2.5:1 was selected based on liposome size and drug loading. At a flow rate of 2.5:1 liposomes were produced that were 76 ± 17 nm in size (PDI <0.25) with protein loading of 34 ± 13%. Using these optimized parameters, liposomes were also prepared where Cholesterol was replaced with SyntheChol™ (non-animal derived cholesterol), produced liposomes of the same size (76 ± 6 nm; PDI 0.27) and comparable drug loading (25 ± 9 nm) demonstrating that fully synthetic liposomes can be manufactured without the use of animal derived lipid components.

Conclusions: Within the study we demonstrate that we can effectively formulate and manufacture small (< 100 nm), high protein loading (20–30%) liposomes using fully synthetic, non-animal derived lipids using a rapid and scale-independent microfluidics process.

TRANSFERSOMES FOR BUCCAL DELIVERY OF LOCAL ANAESTHETIC

14

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Background: Transfersomes (deformable liposomes) have enhanced properties in comparison to standard liposomes, such as their ability to pass through small pores and greater stability due to the reduced risk of bilayer rupture that can happen with rigid liposomes. Transfersomes acquire their elasticity from the presence of an edge activator (EA) or surfactant in their composition. Loading transfersomes with a local anaesthetic (LA) such as lidocaine and using it as a non-invasive buccal delivery system could be an approach to improve patient compliance, reduce side effects and achieve the required localised anaesthesia. Therefore, this research aims to prepare a non-parenteral dosage form to deliver local anaesthetic (LA) that could be used to treat dental and buccal neural pain.

Methods: Lidocaine-loaded transfersomes were optimised and formulated using a simple lipid film hydration method. Taguchi design of experiment (DOE) was used to optimise the loaded transfersomes in terms of phospholipid, EA and phospholipid: EA ratio. Transfersomes were characterised for size, polydispersity index (PDI), charge, entrapment efficiency (%EE), in vitro release and ex vivo toxicity using normal oral keratinocyte (NOK) cells

Results: Transfersomes were 200nm in size, with a PDI ≤0.3. To determine the entrapment efficiency of lidocaine, a new HPLC method was developed. The proposed method was validated for linearity, accuracy, sensitivity, intermediate precision and repeatability, and was shown to be suitable for the analysis of lidocaine free-base according to ICH guidelines. Characterisation of transfersomes showed good %EE (44-56%) and complete drug release over 24 hr. Analysing the data by Taguchi DOE showed the effect of factors were in the following rank order: lipid: EA ratio >EA type >lipid type. Toxicity testing verified their acceptable safety profile.

Conclusions: DOE can help in understanding the effect of formulation parameters on the preparation of transfersomes. Transfersomes loaded with LA were successfully prepared and have the potential be efficiently used as a buccal delivery system.

DESIGN OF VACCINE DELIVERY SYSTEMS: THE EFFECT OF CPG-ODN-PROTEIN CONJUGATES ANCHORED TO LIPOSOMES

15

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Background: Over the last decades, vaccine development has focused on the design on multifunctional non-viral vaccines with enhanced efficiency, broader coverage and minimal undesirable side effects. These vaccines, are peptide or protein-based vaccines which use a portion of the pathogen (antigen), which in many cases itself is very weakly immunogenic. Thus, adjuvants which are able to boost the immunogenicity of them are needed. The aim of this research is the development of a nanotechnology-based delivery system for subunit vaccines based on modification of protein antigen with TLR9 agonist (CpGODN), a very promising class of adjuvants, followed by anchoring to liposomes. This presentation is expected to increase the potency of the immune response by multivalent presentation of the antigen as well as the TLR9 agonist combined to the adjuvant effect of liposome.

Methods: A TLR9 agonist CpGODN was conjugated to model protein using maleimide-thiol conjugation chemistry. Unreacted CpGODN was removed by centrifugation and conjugate was recovered in PBS (1x) pH 7.4. The success of the conjugation as also the number of CpGODN chains introduced on protein were evaluated by the performance of gel electrophoresis (SDS-PAGE), size-exclusion high-performance liquid chromatography (SEC-HPLC) and UV spectroscopy. In vitro NF-jB luciferase reporter assays were performed by incubating conjugated protein with HEK293-mTLR9 cells for 6h. Cationic liposomal formulations DSPC: Cholesterol: DDA 10:40:50% were prepared using microfluidics and characterized in terms of size, zeta potential and polydispersity index (PDI) by dynamic light scattering. Dialysis was used for the purification of liposomes and the liposome recovery was determined by fluorescence spectroscopy.

Results: Three CpGODN chains were introduced on model protein as was proved by SDS-PAGE and UV spectroscopy. Conjugate showed a faster elution as was expected due to the higher molecular weight compared to unconjugated protein and CpGODN alone. The conjugation of protein to CpGODN did not alter the ability of CpGODN to induce TLR9 activation as was demonstrated by luciferase assays. However, CpGODN conjugated on a model protein has shown ~30-fold higher induction compared to CpGODN alone in the same concentration used. Cationic liposomes were ~90nm in diameter with a unimodal size distribution (PDI ~0.25) and ~25mV zeta potential. Dialysis was the ideal method for purification of these formulations with almost 100% recovery.

Conclusions: CpGODN-protein conjugate and DSPC: Cholesterol: DDA cationic liposomes were prepared successfully.

GOLD NANOROD-LOADED MICRONEEDLE ARRAYS FOR PHOTOTHERMAL TREATMENT OF BASAL CELL CARCINOMA

16

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Background: Basal cell carcinoma (BCC) is one of the most common forms of non-melanoma skin cancer and the most common cancer in the UK, with approximately 53,000 new cases being diagnosed every year. Despite being typically non-fatal, due to its very limited metastasizing abilities, BCC can be locally destructive to great extents. The most common treatment is surgical excision, even though this is an expensive procedure that may lead to functional and cosmetic defects. Plasmonic photothermal therapy (PPTT) comprises the use of particles, such as gold nanorods (GNR), and laser radiation to induce local hyperthermia, leading to cell death and potentially tumour ablation. This innovative approach can represent a more cost-effective alternative for scar-free removal of deep nodular BCC lesions. Therefore, we propose in this work the use of GNR-loaded microneedle (MN) arrays for a PPTT approach to the treatment of this disease.

Methods: GNRs obtained through an optimized seed-mediated growth method were imaged by transmission and scanning electron microscopy. To confirm the potential of these particles for PPTT, their UV absorbance spectrum was recorded. Hydrogel-forming 5x5 MN arrays (with 1.2 mm-long conical needles) were prepared from aqueous blends of 25% w/w Gantrez® S-97, 10% w/w poly(ethylene glycol) and 27.5% w/w aqueous GNR suspension. Gels were degassed and cast into MN moulds in two steps, using centrifugation to allow accumulation of GNRs in the tip of the needles. Following this, the arrays were allowed to dry at room temperature for 3–4 days and cross-linked overnight via esterification at 80oC. The resistance of the arrays to compression and their insertion in skin models such as Parafilm® and neonatal porcine skin were evaluated. For heating studies, MN arrays were irradiated with a 2 W laser at 809 nm and temperature changes were recorded using a thermal camera. To evaluate the heat diffusion from the MN arrays into the surrounding tissue, these were inserted into agar blocks (tissue phantom) and irradiated as previously described.

Results: Rod-shaped particles (GNRs) with approximately 60x15 nm (length x width) were prepared, and showed maximum UV absorption at 760–780 nm. When irradiating GNR-loaded gels with a laser source, similar UV absorption peaks were obtained, evidencing the stability of the GNRs in the chosen formulation. Incorporation of GNRs in the tips of the hydrogel-forming MN arrays was successfully achieved through 2-step casting mediated by centrifugation. Using Parafilm® as a skin model, an insertion of approximately 85% of the needle height was achieved. Upon laser irradiation of the MN arrays, the needle tips registered a temperature increase of 38oC. When inserted in an agar block used as a tissue model, the temperature increase at the needle tips was not as striking. In this case, the temperature increased only by approximately 8oC, which is a value that would be more appropriate for tumour ablation and consequent BCC treatment.

Conclusions: The preliminary results here described show the potential of our approach for BCC treatment, since irradiation of MN arrays inserted in a tissue model led to temperature increases similar to those necessary for hyperthermia-based tumour ablation. Further studies are now required on the diffusion of heat through skin models and the in vivo efficacy of this strategy.

NEXT-GENERATION VACCINES: SELF-ASSEMBLING FERRITIN NANOPARTICLES PRESENTING CHIMERIC ANTIGENS

17

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Background: Modern vaccinology faces the challenge of developing improved subunit antigen vaccines that demonstrate enhanced immunogenicity, broad protective immunity and ease of formulation. Studies have investigated the ferritin nanoparticle as a potential self-assembling multivalent antigen display system to enhance antigen presentation. Here a structural vaccinology approach has been adopted toward the rational design of a ferritin nanoparticle system surface-displaying a chimeric antigen composed of epitopes deriving from two bacterial serogroup B Neisseria meningitides (MenB), antigens. The design of the chimeric antigen confers broad coverage against MenB infection while simplifying the vaccine formulation. The work presented herein is a validation of the structural vaccinology approach toward antigen design, showing that high resolution structural data can be harnessed to design an improved vaccine antigen that can be stably expressed and that optimally presents immunogenic epitopes.

Methods: Beginning with a rational in silico design of the nanoparticle system based on X-ray diffraction data, a DNA construct was designed to enable successful expression and purification of the ferritin nanoparticle presenting the chimeric antigen. Experiments were performed to characterise the epitope presentation of purified nanoparticle, with the aim to confirm that chimeric antigen was able to optimally present immunogenic epitopes. To achieve this, ultrastructural analyses in the form of transmission electron microscopy (TEM) and cryo-electron microscopy were performed in addition antibody-based assays including FACS and SPR.

Results: Structural analysis by TEM indicated that the chimeric antigen could trimerize successfully when the ferritin nanoparticle self-assembled. Cryo-EM was performed to obtain a high-resolution structure of the ferritin expressing the chimeric antigen. The high degree of flexibility of the elongated chimeric antigen clearly created a cloud of lower electron density signal around the ferritin scaffold determined to 3.3 Å resolution. Immunoassays confirmed that the rationally designed chimeric MenB antigen presented on the ferritin surface was able to optimally present immunogenic epitopes.

Conclusions: High resolution structural data has enabled the rational design of a nanoparticle system that can be stably expressed and present a chimeric antigen as had been envisioned. Future work in vivo will look to investigate the central hypotheses, namely that displaying the chimeric antigen on ferritin.

SCREENING AND EVALUATION OF PHOTOINITIATOR SYSTEMS FOR STEREOLITHOGRAPHY 3D PRINTING OF SOLID ORAL DOSAGE FORM

18

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Background: Three-dimensional printing has found novel application in solid oral dosage form production; the inherent flexibility of the technology allows the fabrication of tablets whose properties can be adjusted by digital design, with no need to change the formulation or the manufacturing line. As a result, dosage forms can be printed with different dosages, release profiles, geometries and sizes, thus allowing for personalised products. Several 3D printing technologies have been investigated for pharmaceutical applications in recent years; in this work, we selected stereolithography 3D printing (SLA) based on advantageous characteristics over other techniques. However, SLA has not been extensively investigated for oral dosage form production due to the lack of GRAS-listed excipients - especially photoinitiators - that can be used.Therefore, the aim of this research is to formulate novel SLA printable resins for tablet manufacture by screening and evaluating photoinitiator systems, with the view to enhance SLA 3D printing for future clinical applications.

Methods: SLA 3D printing is based on photopolymerisation; such a process involves photosensitive resins containing suitable monomers, oligomers and photoinitiators cured in the desired geometry by using a laser beam. In this work, a modified Form 2 SLA 3D printer (FormLabs Inc, USA) equipped with a 405nm laser was used to evaluate the printability of the developed formulations. Poly(ethylene glycol) diacrylate (PEGDA 700) was selected as photocurable oligomer in all the formulations. Diphenyl (2,4,6-trimethyl benzoyl) phosphine oxide, 2-hydroxy-2-methyl propiophenone, 2-hydroxy -4-(2-hydroxyethoxy) -2- methylpropiophenone, acetophenone, camphorquinone/triethanolamine and riboflavin/triethanolamine were chosen as photoinitiator systems to screen due to their low toxicity profile. Photoinitiators were added individually to PEGDA 700 at concentrations of 0.1% w/v and 1% w/v. The screening was carried out by printing cylindrical tablets designed on TinkerCAD (Autodesk Inc., USA); printability was eventually evaluated based on an six- point arbitrary scale, targeting a score of 5 indicating a successful printing.

Results: Diphenyl(2,4,6-trimethylbenzoyl) phosphineoxide and acetophenone resulted in successful printing of the CAD generated geometry when used at concentrations of 0.1% w/v. Camphorquinone/triethanolamineand riboflavin/ triethanolamine resulted in partial printing, with gelation of the whole resin formulation when camphorquinone was used. 2-hydroxy-2-methyl propiophenone and 2-hydroxy-4-(2-hydroxyethoxy)-2- methylpropiophenoneproved unsuitable for printing, as no polymerisation was observed.

Conclusions: Printability of six photoinitiator systems at two different concentrations was evaluated. Diphenyl(2,4,6-trimethylbenzoyl) phosphineoxide and acetophenone were found to be suitable to formulate PEGDA based resins for SLA 3D printing of solid oral dosage forms when used in a concentration of 0.1% w/v.

DESIGN, DEVELOPMENT AND CHARACTERISATION OF STEREOLITHOGRAPHYC 3D-PRINTED CONTROLLED RELEASE THEOPHYLLINE SOLID ORAL DOSAGE FORMS

19

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Background: 3D printed drug products are emerging as attractive and innovative tools in personalised medicine. The wide flexibility of three-dimensional printing makes it possible to manufacture solid dosage forms using digital design to adjust dosages, release profiles, geometries and sizes, without altering the formulation and with no need for different production equipment. Several 3D printing technologies have been applied to pharmaceutical manufacture, producing solid oral dosage forms with a wide range of properties;in this work, stereolithography 3D printing (SLA) was used, based on advantageous characteristics over other techniques, to produce controlled-release theophylline tablets. A limitation of SLA has been the lack of GRAS-listed materials suitable to formulate printable feedstock resins; therefore, the aim of this research is to develop and evaluate SLA 3D printed solid oral dosage forms using biocompatible materials to enhance the application of SLA in pharmaceutical manufacture.

Methods: Photosensitive resins for SLA 3D printing consist of mixtures of reactive monomers and oligomers in combination with a photoinitiator that generates the free radicals needed to start the polymerisation reaction. Therefore, a printable resin was formulated using poly(ethylene glycol) diacrylate (PEGDA 700) as a reactive oligomer and 0.1% w/v acetophenone as a photinitiator. Acetophenone was selected due to its low toxicity profile as an inactive ingredient. Theophylline was loaded in the liquid resin at concentrations of 5% w/v, 50% w/v and 85% w/v. Tablets were designed on TinkerCAD (Autodesk Inc., USA) and printed using a modified Form 2 SLA 3D printer (FormLabs Inc, USA) equipped with a 405 nm laser. Tablet printability was assessed through an six-point arbitrary scale, targeting a score of 5 indicating a successful printing. USP monographs were followed to characterise tablets properties. A validated HPLC method was used for drug loading and drug release studies.

Results: PEGDA 700 and acetophenone were found suitable to formulate a photosensitive resin for pharmaceutical applications. Tablets were successfully printed and subsequently characterised. Effect of drug loading on printability and release profile was evaluated. Dissolution testing over eight hours showed that drug release from the photocrosslinked matrix was achieved.

Conclusions: Formulations developed and printed using SLA demonstrated the feasibility of the technique to produce solid oral dosage forms with the view to enhance the potential of 3D printing in personalised pharmacotherapies.

MAXIMISATION OF LIPOPHILIC DRUG LOADING WITHIN LIPOSOMES USING MICROFLUIDIC PROCESSING

20

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Background: A key factor, limiting the clinical availability of liposomes, is the lack of industrial-scale manufacturing processes. There are a number of disadvantages associated with current liposomal down-sizing techniques. For example, extrusion is limited by the use of small volumes, while ultra-sonication subjects samples to overheating and potential metal contamination in the instance of probe sonication (Taylor et al., 2005). The aim of this research is to test a scale-independent processing method for liposomes loaded with lipophilic drugs. To achieve this, we utilised Microfluidizer® technology and optimised the processing parameters.

Methods: Triplicate batches of amphotericin B (AmB)-liposomes (Soya phosphatidylcholine (PC)/ distearoyl phosphatidylglycerol (DSPG)/ cholesterol, 53: 21: 26 mol. %) were synthesised using the Thin Film Method (Baillie et al., 1985). Subsequent processing was performed using a Microfluidizer processor (M-110P) at pressures ranging from 15–30K psi, for up to 5 cycles, with and without heating at 55 °C. The effect of pressure, cycle number and temperature on size, polydispersity index (PDI) and zeta potential was determined using the ZetaSizer (Malvern Panalytical). AmB loading was determined using UV-HPLC (Shimadzu).

Results: Increasing Microfluidizer processing cycle number significantly reduced AmB-liposome size and PDI until a plateau was reached. Only the final particle sizes, after processing at 30k psi, were significantly lower than the lower pressures tested, whereby after 5 cycles at 30k psi AmB-liposomes were 163 ± 18 d.nm in size with a PDI of 0.3 ± 0.05. After cycle 1, there was no significant effect of pass number on zeta potential. The final zeta potential of AmB-liposomes processed at 30K psi for cycles 5 was -51 ± 3 mV. The results also show that pressures as low as 15k psi were able to effectively reduce particle size and improve homogeneity. Given the high transition temperature of the lipids, the influence of processing at 55 °C was tested. This increase in temperature led to further reductions in average particle size and PDI which was 135 ± 2 d.nm and 0.3 ± 0.01, respectively, after 5 cycles at 30K psi. The zeta potential was reduced to -45 ± 1 mV while AmB loading remained high, ranging from 99–102 %, across all conditions tested.

Conclusions: Homogenizer technology using the Microfluidizer processor offers a method to control liposomal particle size and improve loading of lipophilic drugs such as AmB.

EVALUATION OF MUCOADHESIVE POLYMERS FOR INCORPORATION OF INSULIN LOADED TRANSFEROSOMES FOR DIABETES THERAPY

21

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Background: The current study aims to develop and evaluate several mucoadhesive polymers, in combination with the cryoprotectant sorbitol, for the incorporation of insulin in a novel carrier system, known as transferosomes. This system will be used to deliver and potentially enhance insulin permeation via the buccal mucosa for diabetes therapy.

Methods: To study, without bias, the influence of two polymers [low molecular weight (LMW) chitosan or sodium alginate in combination with hydroxypropyl methylcellulose (HPMC)] and sorbitol on mucoadhesion; the program Minitab was used to generate a factorial design. The percentage ratios of each polymer/sorbitol, as suggested by the study design,were dissolved in aqueous media (water or 1% v/v acetic acid) and 2 ml aliquots transferred to wells in 12 well plates.The plates were frozen overnight before being freeze-dried for 48 hr. The patches were then tested for mucoadhesiveness using a TA.XT *plus* Texture Analyser produced by Stable Micro Systems. The patches were attached on a p/10 probe and tested for detachment from porcine buccal mucosa and/or 1% w/v gelatin.

Results: The results of this study suggests, with all the polymers studied, increasing the concentration of the cryoprotectant in the formulation leads to a decrease in mucoadhesion. This was also observed in appearance with the product being porous and soft. It was found increasing concentrations of LMW chitosan and sodium alginate leads to increase in adhesiveness. Unpredictably with HPMC 0.5% w/v was found to be the optimum concentration of the polymer to produce the highest force of mucoadhesion. The influence of the factors were found to be comparable testing on either porcine buccal mucosa or 1% w/v gelatin. 1% w/v LMW chitosan together with equal ratios of HPMC (0.5% w/v) and sorbitol (1% w/v) displayed greater adhesiveness compared to 1% w/v sodium alginate.

Conclusions: The results suggest LMW chitosan would form patches with greater mucoadhesiveness compared to sodium alginate. The optimum ratio of HPMC to include in the formulation is 0.5% w/v. Separate designs were created using Minitab for sodium alginate and LMW chitosan to analyse the influence of HPMC and sorbitol. For future studies to enable a better comparison a more concise factorial design would be created with both polymers in the design. As it will be important to include sufficient concentrations of a cryoprotectant in the formulation further studies are required to enhance mucoadhesion (and appearance of the patch) in the presence of sorbitol (e.g. 1–5 % w/v) possibly with the use of plasticizers.Once the mucoadhesive patch has been finalised the insulin loaded transferosomes will be incorporated in the patch and tested for permeability studies using a modified Ussing chamber with porcine buccal membrane.

DEVELOPMENT OF A DUAL MICRORNA/PDNA NON-VIRAL DELIVERY SCAFFOLD SYSTEM FOR CARTILAGE TISSUE ENGINEERING

22

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Background: The use of gene therapy to induce the chondrogenic differentiation of autologous cells has shown potential as an alternative to current cartilage repair approaches. However, successful differentiation into healthy, stable cartilage has yet to be achieved, in part due to the lack of an efficient and safe gene-delivery system, but also due to a lack of suitable genetic targets. The aim of this project is to develop a non-viral nucleic acid platform system capable of efficiently co-delivering pDNA, to trigger differentiation of human Mesenchymal Stem Cells (hMSCs) towards cartilage, and microRNA (miRNA), to further support chondrogenesis and minimize hypertrophy. This co-delivery system will then be incorporated into previously optimized chondrogenic 3D scaffolds.

Methods: Dual delivery nanoparticles (NPs) were formulated by mixing miRNA and/or pDNA with GET (Glycosaminoglycan Enhanced Transduction) peptide. Nucleic acid encapsulation and NP size were characterized. Collagen based scaffolds were used for 3D gene delivery in vitro. NP-mediated miRNA transfection efficiency was evaluated using a miR-mimic designed to target the mRNA of the housekeeping glyceraldehyde phosphate dehydrogenase (GAPDH). GAPDH mRNA expression levels in hMSCs were measured by PCR.

Results: Gel retardation assays confirmed the complexation of miRNA by GET. GET formed homogeneous NPs with miRNA and pDNA/miRNA of 31 and 52 nm diameter respectively. Simultaneous delivery of the two genetic cargoes in hMSCs did not have an effect on cell viability or NP uptake. GAPDH mRNA expression was significantly reduced (0.1 fold change) when hMSCs were transfected with the pDNA-miRNA-GET formulation. Additionally, pDNA transfection efficiency determined by Gaussia luciferase expression was also significantly improved when pDNA was delivered with the pDNA-miRNA-GET formulation both in 2D and 3D.

Conclusions: We have successfully developed a NP formulation for the co-delivery of pDNA and miRNA both in 2D and 3D. This represents the first stepping stone in developing an efficient gene therapy that promotes successful, long-term cartilage repair by inducing stable chondrogenesis and inhibiting hypertrophy.

ANTIMICROBIAL NANO-NINJAS AS CHAPERONES OF GEMCITABINE FOR PANCREATIC CANCER

23

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Background: Current chemotherapeutics utilized in the therapy of pancreatic cancer are ineffective for most patients. Theories behind this include the presence of bacteria within tumors that change the drug into a form that can no longer affect the cancer. The aim of this work is to generate tiny particles that can act as a dual treatment for pancreatic tumors by both carrying drugs into tumors, thereby protecting them from destruction by bacteria, and providing an antibacterial effect. We hope that this will increase the chance of success of this treatment.

Methods: The aim of this work is to generate tiny particles that can act as a dual treatment for pancreatic tumors by both carrying drugs into tumors, thereby protecting them from destruction by bacteria, and providing an antibacterial effect.

Results: The particles are spherical and are comprised of two layers, where the internal layer allows these nanoparticles to be visualized by magnetic resonance imaging (MRI). MRI will allow for the tracing of these nanoparticles, ensuring they react the correct target and reduce systemic side effects, as well as being used for further visualization of metastasis and assessment of pancreatic premalignant cysts. The external layer allows these hybrid nanoparticles to heat up at a certain wavelength of light and release the drug attached to the nanoparticles, allowing it to carry out its function.

Conclusions: We aim to show that these particles are advantageous over current drug regimens for pancreatic cancer which ultimately will give hope for better patient treatment and life expectancy after pancreatic cancer diagnosis.

CHARACTERISATION OF POLYMERIC NANOPARTICLES PRODUCED VIA HOT-MELT EXTRUSION

24

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Background: Polymeric nanoparticles (PNPs) are an emerging technology in a rapidly developing area of research. A key focus for this work is the targeting of tumour cells via stimulus-responsive targeted release. The microenvironment of a tumour is acidic; thus pH-dependent polymers can be employed for stimulated release. Additionally, the majority of chemotherapy agents are poorly water soluble. Therefore, amorphous solid dispersion (ASD) technology can be applied in order to deliver anticancer drugs, dispersed in a pH-dependent polymer, for targeted drug delivery. These ASDs can be formulated as PNPs whereby the drug is encapsulated within a polymer at the nanoscale level for targeted delivery. Currently, cancer nanoparticles demonstrate both low drug loading and low productivity, in addition to requiring a complex manufacturing process that requires the use of solvents. We propose a novel way to create PNPs through the use of hot-melt extrusion (HME) technology. We can apply our understanding of ASD and phase diagrams to accurately predict drug loading and processing parameters, resulting in PNPs with a high drug loading that are capable of pH-responsive drug release and can be produced using an efficient, continuous solvent free process.

Methods: ASDs of Naproxen (NPX) and Eudragit® EPO (EPO) were produced using HME with the stability of the system investigated using Raman and Atomic force microscopy (AFM). The overall aim was to create PNPs of NPX and EPO with a Xylitol (XYL) sugar alcohol carrier. The ASD concentration of 40%NPX_EPO was selected based on its excellent stability, the glass transition of the system and its phase diagram using the Flory Huggins (FH) model. Processing parameters, excipient concentrations and the addition of surfactant were altered for the successful production of PNPs in a novel way using HME. The PNPs were then characterized using thermal analysis, namely differential scanning calorimetry (DSC), and X-ray diffraction to determine the crystallinity of the complex. Additionally, a range of microscopy techniques were used, including polarized light microscopy (PLM) transmission electron microscopy (TEM) and scanning electron microscopy (SEM), in order to confirm the presence of successful PNPs and, secondly, to determine their shape, size and morphology. Dynamic light scattering (DLS) was also used to measure particle size, in addition to UV-VIS to determine encapsulation efficiency of NPX within the ASD.

Results: Based on the FH models, 40% NPX drug loaded PNPs were successfully produced in a novel way using HME. The addition of two mixing zones in the extruder resulted in a successful formulation, although with a relatively large particle size of 400 nm. Surfactant PEG 3350 was added to the formulation, effectively reducing particle size, as verified by DLS, to <200 nm. This was visualized and confirmed using the various microscopic techniques. DSC and XRD confirmed the presence of amorphous drug with no interaction between the ASD and the XYL carrier. Furthermore, UV-VIS showed a drug encapsulation of >75% within the polymer.

Conclusions: PNPs with a high drug loading have been developed using continuous manufacturing in a novel way. Extrusion parameters, concentration alteration and addition of surfactants resulted in enhanced production and optimization of PNPs. The PNPs of poorly water soluble NPX and pH-dependent EPO have been characterized using SEM, TEM and PLM. DLS showed PNPs of a particle size less than 200 nm and with a high encapsulation efficiency of amorphous drug within the polymer.

IMMUNOGENICITY OF CHITOSAN COATING ON PLGA NANOPARTICLES

25

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Background: Nanoparticles (NPs) made from polymers can function as delivery vehicles for a number of applications including vaccines. Chitosan has shown potential as a versatile and effective coating material on nanoparticles (NPs) for a number of applications including vaccines. It is thought that chitosan has numerous benefits for vaccine applications by increasing immunogenicity through mechanisms such as through the cGAS-STING pathway, increased cell uptake and mucoadhesion. Numerous variations of chitosan molecules with differing properties have been produced but it is unclear how each modified chitosan affects the immunogenicity on dendritic cells (DCs). This study sought to investigate the effects of different chitosan on the coating properties on PLGA NPs and subsequent immunogenicity on DCs.

Methods: Chitosans with varying properties such as salt form, degree of quaternisation, molecular weight, oligomerisation, carboxymethylation, and from non-animal sources, were coated onto PLGA NPs using the solvent evaporation method. The resulting NPs were characterised for size and zeta potential by dynamic light scattering. Quantification of chitosan adsorption on the NP surface was determined by the fluorescamine assay. Immunogenicity of NPs were evaluated in vitro by incubation of JAWS II cells with NPs over 24 hr, subsequent staining with antibodies for CD40, CD86 and MHC-II, and analysis using flow cytometry. Toxicity of particles was also evaluated by staining the cells with 7AAD during analysis.

Results: PLGA NPs with different chitosan coatings exhibited differing particle characteristics, as well as differing degrees of adsorption and subsequent surface adsorption of model antigen protein. The relationship between the amount of chitosan adsorbed onto the particle surface and the resulting NP size increase differed for each chitosan. As expected, the surface charge also varied greatly based on the type of chitosan used. In terms of immunogenicity, the chitosans generally exhibited a concentration dependent immunogenicity and could increase the up-regulation of cell activation markers.

Conclusions: Coating PLGA NPs with differing forms of chitosan, including salt form, degree of quaternisation, molecular weight, oligomerisation, carboxymethylation, all contribute to NPs containing unique characteristics and immunogenicity.

HIGH-THROUGHPUT MICROFLUIDICS: A FAST AND EFFICIENT MANUFACTURING TACTIC TO MAXIMISE DRUG LOADING INTO LIPOSOMES

26

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Background: The properties of liposomal formulations can vary depending on the composition. However, a standardised preparation method can be used for all lipid vesicles regardless of composition. The general steps of the procedure are: preparation of the lipids for hydration, hydration with agitation, and sizing to a homogeneous distribution of vesicles. Several downsizing techniques have been established in order to make the heterogeneous vesicles more uniform. One major drawback of this method is the difficulty in achieving high drug loading. Furthermore, the process becomes more time and cost intensive because additional processing is required for a defined liposome suspension. To overcome some of the problems identified above, manufacturing of water-soluble and water-insoluble active pharmaceutical ingredients (APIs) liposomal formulations using high-throughput microfluidics technology were investigated.

Methods: To prepare liposomes, the Nanoassemblr™ Benchtop (Precision Nanosystems, Inc., Vancouver, Canada) was used with a 300 mm Staggered Herringbone Micromixer. The lipids along with water in-soluble API, at the appropriate ratio, were dissolved in solvent (Formulation I) and the water-soluble API was dissolved in an aqueous buffer (Formulation II). The flow rate ratio (FRR) between the aqueous and solvent stream was optimised along with and the total flow rate (TFR). Liposomal drug content was analysed by UV-HPLC after purification using tangential flow filtration in order to remove the free drug. The z-average diameter and polydispersity (PDI) of liposomes after reconstitution were determined. Assessment of in-vitro efficacy of anti-inflammatory formulation I is currently ongoing using ELISAs and antibiotic efficacy of formulation II was evaluated using micro-dilution minimum inhibitory concentration (MIC).

Results: Placebo formulations were optimised by modification of flow rate ratios using Nanoassemblr™ Benchtop and characterised. Based on these results, a 3:1 flow rate was selected for further studies. Effect of API to lipid ratio on drug loading was evaluated. Our results showed that drug loading of the API into optimised formulations changed liposome size characteristics. Our results demonstrate that formulation I containing water-insoluble API showed 100 % drug loading, formulation II with water-soluble API offered 30% drug loading. In-vitro cell culture assay to evaluate efficacy of formulation I is currently undergoing using ELISA. Micro-dilution MIC assay as per CLSI guidelines showed liposomal formulation II API decreases MIC by 3-fold (E coli), 7-fold (Salmonella) and 2-fold (Pseudomonas aeruginosa) in comparison with free API.

Conclusions: Liposomal formulations containing selected water insoluble or soluble APIs were successfully designed and manufactured with high drug loading using high-throughput microfluidics technology. These newly developed liposomal formulations offer the potential to promote efficient manufacturing approach to maximise drug loading and ultimately efficacy of APIs delivery.

CARRIER PARTICLE MEDIATED STABILIZATION AND ISOLATION OF API NANOPARTICLES

27

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Background: Approximately 40% of active pharmaceutical ingredients (APIs) in the development pipeline fall into the category of Biopharmaceutics Class II (low solubility-high permeability) and Biopharmaceutics Class IV (low solubility-low permeability). API solubility, dissolution rate, and gastrointestinal permeability are the key determinants of oral bioavailability. For APIs that exhibit poor water solubility enhancing the dissolution rate and/or solubility in the gastrointestinal system can often improve their bioavailability. Nanoparticles (NP) formulation strategies can address these problems of low solubility and slow dissolution rates. The greater surface to volume ratio of nanoparticles (NPs) can result in an improvement in dissolution and bioavailability as well as enhanced permeability. Among the different techniques to form nanosuspensions, liquid antisolvent precipitation is fast, easy and cost-efficient. However, isolation of the resulting NPs to the dried state is a major challenge, due to agglomeration and Ostwald ripening resulting from the high surface energy of the NP. In this work, montmorillonite clay particles (MMT), as received and with slight surface modification, have been employed as carriers to stabilize and isolate NPs of four different APIs directly from suspension without the use of any API-specific soluble stabilizers.

Methods: Antisolvent precipitation was used to generate nanosuspensions of valsartan, carbamazepine, curcumin, and clozapine. Particle sizes and zeta potentials of the NPs and carrier particles were measured using a Malvern Zetasizer Nano ZSP system. Process parameters were optimized to generate NPs of these APIs in suspension. Carrier-mediated stabilization and isolation of these NPs from suspension into a solid form was then achieved by adding MMT carrier particles before/after precipitation followed by simple filtration and air drying. Dissolution studies of the resulting NP-carrier composites and of suspended nanoparticles were then performed in de-ionized water at 37 °C.

Results: Nanosuspensions of all four APIs were obtained using the antisolvent precipitation method. Analysis of these nanosuspensions revealed that particles smaller than 170 nm were obtained in each case. A panel of carrier particles was screened to identify the best carrier-API combination for stabilization and isolation of the respective API. From these screening procedures, MMT was identified as the most promising carrier. The dissolution rate of these NP-carrier composites was comparable with or surpasses those of the stabilized nanoparticles in suspension. API nanoparticles with zeta potentials of greater than ca. –20 mV required the MMT surface to be modified with protamine (PA) (a cationic protein) in order to preserve the dissolution rate at higher API loadings. The optimal loading of PA on MMT was consistently around 4 mg/g, limiting nanoparticle aggregation and thus maintaining the enhanced dissolution rate of these API nanoparticles at higher loadings from the dried state. API nanoparticles with zeta potentials of less than ca. –20 mV did not require the MMT modification with PA in order to maintain the enhanced dissolution rate at high API loadings.

Conclusions: An antisolvent precipitation method successfully precipitated NPs of four APIs which then underwent carrier particle-mediated stabilization using MMT. The resultant NP-carrier composites were readily isolated using a simple filtration process into dry, solid powders. The carrier particles serve a dual purpose, namely (i) to stabilize the suspended nanoparticles, and (ii) to facilitate their ready isolation to the solid state via a simple filtration process. Thus, different BCS Class II APIs were formulated into fast dissolving, solid-state nanoparticle composites using a simple one-step approach, without the need of any API-specific soluble stabilizers. The MMT carrier particle system needed a surface charge modification that was dependent on the zeta potential of the API nanoparticles in suspension.

REPURPOSING PECTIN FOR USE WITH A MECHANICALLY-ENGINEERED SPRAY DEVICE TO INCREASE BRAIN PENETRATION OF CHEMOTHERAPEUTIC NANOPARTICLES IN THE TREATMENT OF HIGH-GRADE GLIOMAS

28

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Background: Glioblastoma multiforme (GBM) is a grade 4 primary brain tumour and is the most common and malignant of the glial tumours. Despite current standard-of-care treatment methods of maximal surgical resection, followed by concurrent chemoradiotherapy, there is still a dismal 2-year survival rate of 16.9%. Following surgical resection, the tumour consistently reoccurs, often within 2 cm of the resection margin due to the highly infiltrative nature and the heterogeneity of the tumour cells. Design and implementation of innovative local drug delivery systems (DDS) may overcome current limitations in GBM treatment, such as the lack of therapeutic drug concentrations reaching residual GBM cells following surgery. The novel DDS developed in this group utilises a bespoke spray device, delivering a mucoadhesive gel, pectin, and chemotherapy-encapsulating nanoparticles into the resection cavity using force from pressurised air.

Methods: To determine if Low methyl-esterified Citrus fruit Pectin (Pectin) is biocompatible, it was incubated at up to 200 µM with U87 cells and primary human astrocytes; viability was assessed using a presto blue assay and a RealTime-GloTM assay respectively. Pectin degradation was monitored using gel permeation chromatogahy (GPC) following 4 weeks of incubation in artificial cerebrospinal fluid (aCSF) at 37 °C. Gels were also incubated with human blood to assess haemolysis levels colorimetrically, alongside further in vivo analysis where 200 µM pectin was injected orthotopically into mice and incubated for two weeks; immunohistochemistry (IHC) was carried out using an anti-caspase-1 antibody to measure local inflammation. Applicability to the spray device was evaluated by assessing gel integrity after spraying (vial inversion method), alongside assessment of pectin's bioadhesiveness to rat brain slices using a TA-XT texture analyser. Minimum gel concentrations of Pectin with brain levels of calcium were found by spraying Pectin onto calcium soaked TLC plates at 45°.

Results: Pectin was found to be biocompatible with U87 cells up to 200 µM and with astrocytes up to 100 µM. No significant haemolysis was found with human red blood cells and it was also found to degrade to 2% of its Mw in aCSF over 4 weeks. In vivo work showed no neurological deficit as a result of Pectin within the brain for up to two weeks. Ongoing IHC work is evaluating cleaved caspase 1, an inflammatory marker, within brain slices of mice after 1 and 14 days. A viscous solution of Pectin can survive the shear stress of being sprayed at 50–200 µM, forming a solid gel within a vial containing calcium. It also gelled at the low concentrations of calcium found in the brain (1–2 mM). Significant bioadhesive properties were found when rat brains were placed onto pectin gels before removing them with a texture analyser.

Conclusions: Pectin is suitable for use within the brain as demonstrated by all biocompatibility methods, both in vitro and in vivo, undertaken herein. It is also suitable to be sprayed by the spray device as it survives the shear stress and then forms a stable gel upon contact with calcium concentrations which are found locally in the brain. Future work will now incorporate chemotherapeutic containing nanoparticles into this gel to study compatibility and efficacy.

DEVELOPMENT OF INJECTABLE INTRATHECAL FORMULATIONS FOR POORLY SOLUBLE DRUGS

29

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Background: Neuropathic pain leads to a decline in normal functioning and quality of life. Current pharmaceutical interventions only result in an average of 20–30% reduction of pain intensity, therefore the need for novel analgesic treatments is expanding as the incidence of chronic pain increases. Cannabidiol (CBD) has been reported to have a therapeutic action on TRPV1 and TRPA1 receptors. However, the main drawback associated with the use of cannabinoids in analgesia are the side-effects and extensive first-pass metabolism. CBD is an extremely lipophilic drug (logP 6.33) and its oral bioavailability is approximately 13 - 19%. Therefore, these properties make it an excellent candidate for formulation in a lipid-based nanoemulsion. Recent developments in intrathecal drug delivery systems (such as the success of intrathecal baclofen for spasticity) allow for this technique to administer drugs at therapeutic levels directly to the site of action in the subarachnoid space thus reducing adverse effects and by-passing first pass metabolism. To date, there are limited controlled release formulations for intrathecal delivery in pain management. The aim of this project is to expand on the formulations available for intrathecal delivery.

Methods: Microfluidizer technology was used to create two oil-in-water nanoemulsions. An emulsion Intralipid® composed of soybean oil, lecithin and glycerol (used commercially for parenteral nutrition) and a third generation lipid emulsion (Lipoid) containing an additional emulsifier – Sodium Oleate B. Density gradient ultracentrifugation using standard solutions of phosphate-buffered saline of various densities was used to incorporate CBD in commercially available Intralipid® nanoemulsion and microfluidized nanoemulsions. Lipid droplets loaded with CBD were separated by ultracentrifugation and the drug-containing upper lipid layer was collected. Reverse-phase HPLC was used to quantify the amount of CBD encapsulated within the nanoemulsion. Particle size analysis using dynamic light scattering (DLS) technology and zeta-potential measurements were carried out to monitor the physical stability of the nanoemulsion.

Results: Incubation followed by density gradient ultracentrifugation approach was successful in incorporating 1.28 mg/mL (65.1% association) CBD within the oil phase of the commercially available Intralipid® and 1.61 mg/mL (90.95% association) in microfluidized Intralipid® emulsion as demonstrated by HPLC. These concentrations achieve therapeutic potency in rats as evidenced by current literature. The addition of CBD to Intralipid® did not affect particle size (<300 nm) or zeta-potential (–55mV) which remained stable for at least 70 days post incorporation in commercially available Intralipid® and microfluidized Intralipid® emulsion. Lipoid emulsion displayed a significant increase in particle size after day 30. Both Intralipid® nanoemulsions exhibited PDI of <0.2 whereas Lipoid PDI was 0.2–0.3 over 70 days.

Conclusions: CBD was incorporated in the lipid phase of a commercially available Intralipid® and microfluidized nanoemulsion by the use of density gradient ultracentrifugation and remained stable for up to 70 days.

DEVELOPMENT OF LONG CIRCULATING PEGYLATED LIPOSOME OF DISULFIRAM FOR COLORECTAL CANCER THERAPY

30

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Background: Disulfiram (DS), an anti-alcoholism medicine, shows strong anti-cancer activity in the laboratory but the application in the clinic has been limited by its prompt metabolism. The anticancer activity of DS is Cu dependent as the reaction between DS and Cu generates cancer-targeting reactive oxygen species (ROS) and DDC-Cu which is also toxic to cancer cells. The aim of this study is to design, formulate and characterize liposome of (DS) to improve drug stability and provide a proposed long circulation to target cancer cells.

Methods: Liposomes were prepared by using ethanol injection method by dissolving lipids consisting of various ratios of phospholipids, namely: hydrogenated soya phosphatidylcholine (HSPC) (or dipalmitoyl phosphatidylcholine; DPPC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (DSPE- PEG(2000)) with cholesterol (Chol; 0.9:0.1:1, respectively) in ethanol. DS was dissolved in the alcoholic solution in different lipid mol% ratios. Ultra-pure water (HPLC grade) was added, followed by vigorous vortexing to produce large multilamellar vesicles (MLV). The size of MLVs were reduced by high-pressure homogenization (Nano DeBee, 10 cycles at 21,000 PSI). Size analysis, zeta potential measurements were performed using Laser Doppler Velocimetry. Drug loading efficiency and stability studies in horse serum were performed and DS was detected using a validated HPLC method. *In vitro* cytotoxicity was evaluated in both the wild-type H630 and the chemoresistant H630R10 (chemoresistant to 10μM 5-fluorouracil) colorectal cancer cell lines by MTT assay.

Results: Overall, small unilamellar vesicles (SUVs, nanoliposomes) were generated with a size of approximately 80 to 120 nm with a polydispersity index (PDI) ranging from 0.10 to 0.35. Zeta potential of all vesicles negative, and the surface charge tended to increase by PEGylation. PEGylated liposomes were smaller in size (80–90 nm) and had significantly lower PDIs than naked ones. However, all liposomes showed similar loading efficiencies (around 60%) without any significant impact for the lipid type (HSPC or DPPC) or the PEGylation. PEGylated liposomes showed slower drug release compared to that of naked liposomes and provided the highest drug biostability among all formulations in horse serum; for example, PEGylated DPPC liposomes had t1/2 58 min compared to 8 min for free DS. All liposome formulations of DS demonstrated high cytotoxicity against both the wide type and chemoresistant colorectal cancer cells and PEGylated DPPC liposomes had the lowest IC_{50} (less than 100 nM) for both cell lines.

Conclusions: PEGylated liposomes of disulfiram have been developed, formulated and characterized. The PEGylation resulted in lipid vesicles that are smaller than traditional (non-PEGylated) liposomes. Furthermore, PEGylated liposomes of DS were monodispersed and provided a good level of protection to DS in horse serum. Finally, showing higher cytotoxicity against colorectal cancer cells, PEGylated liposomes made of DPPC showed great potential to be used as an anticancer carrier for disulfiram.

SPRAYABLE IN SITU GELLING FLUTICASONE SUSPENSIONS: NASAL DEPOSITION UPON BI-DIRECTIONAL DELIVERY *IN VITRO*

31

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Background: Nasal corticosteroids are the first-line therapy for nasal inflammatory disorders, however, superior effect of systemic over local corticosteroids shows room for improvement of local corticosteroid delivery. In situ gelling system presents advantageous nasal delivery platform. It can be easily sprayed as liquid in the nasal cavity and prolong contact time with nasal mucosa due to rapid gelation. It could be expected that effective nasal deposition of such innovative formulation platform could significantly improve local therapeutic effect of corticosteroids. One of the latest approaches to improve corticosteroid deposition within the targeted nasal region refers to bi-directional nasal delivery. The aim of our study is to assess the nasal deposition pattern of sprayable in situ gelling fluticasone suspensions upon simulated bi- directional delivery.

Methods: In situ gelling suspensions were prepared using fluticasone propionate (Carbosynth Ltd., UK), pectin CF 025 (Herbstreith&Fox, Germany), gellan gum (Phytagel, Sigma-Aldrich, USA), sodium hyaluronate (Contipro, Czech Republic), Tween 80 (Sigma- Aldrich, USA) and mannitol (BDH Prolabo, UK). The formulations were characterised in terms of rheological properties (Rheometer MCR 102, Anton Paar, Austria), spray cone angle (measured by a virtual protractor after spraying against a dark background) and sprayability/droplet size distribution (Malvern Spraytec, UK). For the determination of deposition pattern of in situ gelling systems, the inner surface of a nasal cavity model (Koken Co Ltd., Japan) was evenly covered with Sar-gel® (Arkema Inc., Sartomer Americas, USA), a water-indicating paste. To simulate exhalation in bi-directional delivery, the model was connected to respiratory pump (Harvard Apparatus, USA). The suspensions were administered by using Spray Pump 3K (Aeropump, Germany) under different angles against horizontal and vertical (i.e. nasal septum) plane.

Results: All formulations showed suitable rheological properties. Under formulation and administration parameters employed, appropriate window of droplet size distribution and spray angle was reached. The concept of bi-directional nasal delivery was successfully employed resulting in targeted deposition pattern within the nasal cavity.

Conclusions: This research confirmed bi-directional nasal delivery as a useful way to deliver in situ gelling fluticasone suspensions to the targeted area within the nasal cavity.

Acknowledgements: This work has been supported in part by Croatian Science Foundation under the project UIP-2017-05-4592.

IN VITRO PULMONARY CYTOTOXICITY OF NOVEL POLYMERIC NANOCARRIERS FOR DRUG DELIVERY

32

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Background: Biodegradable polymers are fueling the development of drug delivery systems due to their biocompatibility, biodegradability, and ease of fabrication. Poly (lactic-co-glycolic acid) (PLGA) is one of such biodegradable polymer and is Food and Drug Administration (FDA) approved for a wide variety of drug delivery systems administered via different routes apart from the pulmonary route. Thus, novel materials are under development for pulmonary drug delivery systems. Poly glycerol adipate-co-ω-pentadecalactone (PGA-co-PDL) is such a new polymer that has been developed and characterized in Liverpool John Moores University (LJMU) laboratory and under intensive investigations and has shown promising results for pulmonary vaccination and macromolecules drug delivery. The aim of this study is to evaluate in-vitro cytotoxicity profile of PGA-co-PDL nanoparticle (NP) carriers in comparison to PLGA NPs for pulmonary drug delivery.

Methods: NPs were formulated by single emulsion-solvent evaporation method using Poly Vinyl Alcohol (PVA) as emulsifier and 1,2-dioleoyl-3-trimethylammonium-propane (chloride salt); DOTAP as a cationic emulsifier, and characterized for size, shape and charge using the Transmission Electron Microscopy (TEM) and Zetasizer Nano ZS. In-vitro cytotoxicity was evaluated by Alamar Blue and Reactive Oxygen Species (ROS) assays. The NPs were re-suspended with serum starve-cell culture media prior to cell culture assays. Calu-3 cells were seeded at a density of 40 x103 per well in 96 well plates for 48 hr prior to treating with serial concentration of NPs (0.0.0195–1.25 mg/ml) in triplicate for another 24 hr then colorimetric/floroumetric evaluation using the plate reader.

Results: PGA-co-PDL NPs were less toxic in comparison with PLGA NPs confirmed by Alamar Blue assays. The negatively charged particles (using PVA) were more compatible than positively-charged (using DOTAP) particles. ROS assay showed that PGA-co-PDL NPs had antioxidant activity in low concentrations and less scavenging activity was detected with the positively charged NPs

Conclusions: PGA-co-PDL polymer was successfully formulated into NPs with a suitable size range for pulmonary drug delivery. The results showed a good toxicity profile of PGA-co-PDL NPs in comparison with the PLGA NPs confirming future suitability for pulmonary drug delivery.

TRANSFECTION AND UPTAKE MECHANISM ANALYSIS OF A RANGE OF NON- VIRAL pDNA VECTORS FOR TISSUE ENGINEERING APPLICATIONS

33

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Background: Following a challenging safety history of viral vectors for gene delivery, non- viral vectors have gained popularity in tissue engineering and regenerative medicine. However, despite the superior safety profile of non-viral delivery vectors, efficacy remains a significant challenge. Our lab has previously optimized several non-viral plasmid (p)DNA delivery vectors, such as chitosan, polyethyleneimine (PEI), and nanohydroxyapatite (nHA) for gene delivery in bone repair indications. Building on this work, this study compares two novel cell penetrating peptides, glycosaminoglycan enhanced transfection (GET) peptide, and RALA (ExphectTM), designed by our collaborators, with the more established vectors described above. Additionally, we sought to understand the uptake mechanism of each nanoparticle. The overall objective of this study was thus to compare the range of non-viral delivery vectors described above, in order to determine the optimal pDNA delivery vector for tissue engineering applications.

Methods: Dynamic and electrophoretic light scattering, nanoparticle tracking analysis, and transmission electron microscopy were carried out to characterize nanoparticle size and morphology. Transfection efficiency was evaluated by transfecting rat mesenchymal stem cells (MSCs) with nanoparticles delivering reporter plasmids. Cell uptake of nanoparticles was evaluated by carrying out transfections at lower temperatures, in reduced serum media, and in the presence of endocytosis pathway inhibitors.

Results: Size and morphology characterisation showed that all nanoparticle formulations had appropriate properties for cell uptake, although the nanoHA nanoparticles were significantly larger than other groups. All of the nanoparticles had a spherical morphology, conducive to cellular uptake. All nanoparticles transfected MSCs successfully, increasing from Day 1 to Day 7. Furthermore, GET and RALA cell penetrating peptides had the highest transfection levels in MSCs. Large quantities of GET and RALA nanoparticles co- localized with cell nuclei and cytoplasms. The uptake of GET and RALA nanoparticles was energy dependent, as reduced transfection levels were observed when cells were transfected with RALA nanoparticles at lower temperatures, and with GET nanoparticles in the absence of serum.

Conclusions: These results demonstrate that novel cell penetrating peptides GET and RALA were optimal non-viral delivery vectors, and that their uptake mechanism is heavily energy dependent. GET and RALA nanoparticles will be utilised in future work to deliver therapeutic plasmids from collagen nanohydroxyapatite scaffolds for bone repair.

Acknowledgements: Funding: Health Research Board, Grant Number ILP-POR-2017-032

THE MANUFACTURE OF DOXORUBICIN-LOADED LIPOSOMES AND THE ABILITY TO DE-RISK THE TRANSLATION FROM BENCH TO CLINIC

34

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Background: Nanomedicines are considered a powerful and promising platform for the amelioration of many diseases and their passive targeting make them particularly effective as anti-cancer agents. Despite the fact that liposomes have been extensively studied as drug delivery systems, only few liposome products have reached the market. This is in part due to the challenges in the manufacturing process of these compounds. Batch processing is the current production method for liposome-based products. However there are a range of issues associated with this form of production including scalability, down-time between batches and increased product waste and cost. Therefore, new techniques based on fluid control are emerging to address these issues.

Methods: Liposomal formulations containing HSPC, cholesterol and DSPE-PEG2k at a molar ratio 56:39:5 were formulated using microfluidics (NanoassemblrTM, Precision Nanosystems). Briefly, lipids dissolved in ethanol at the desired concentration and ammonium (sulphate or citrate) buffer were injected into the system. Tangential flow filtration (TFF) was used to remove organic solvent and to establish a pH gradient between the interior and exterior phase of the preformed liposomes. Subsequently, liposomes were loaded with doxorubicin hydrochloride (DOX) at a 8:1 w/w lipid:drug ratio and non-entrapped DOX was removed by TFF. Physicochemical characteristics of the liposomes were measured using dynamic light scattering (Malvern nano ZS, Malvern PANanlytical). DOX loading was quantified by measuring the absorbance at 490 nm with a plate reader. DOX release profiles were carried out using a USP-4 apparatus (SOTAX®) and dialysis. Scalability and reproducibility of the system was evaluated using the Blaze system which produces up to 1 L. Cryo-TEM was used to study the morphological structure of the produced vesicles either empty or DOX-loaded.

Results: The influence of the microfluidics process parameters (total flow rate, flow rate ratio, temperature, buffer composition, lipid choice and lipid concentration) were studied. Liposomes were highly homogenous in nature, with a size range between ~50 to 160 nm. Effect of the incubation temperature and time on DOX loading was also evaluated, demonstrating that high encapsulation efficiencies (EE) can be achieved (>90%) in 10 min at 60 °C or at a longer at reduced temperatures (>1 hour at 40 °C). Therefore, results showed that HSPC:Chol:DSPE- PEG2k liposomes can be tightly controlled by adjusting the above-mentioned parameters and comparable attributes to Doxil® can be obtained. Moreover, USP-4 drug release studies have shown the ability to distinguish between formulations produced using different lipid composition whereas dialysis studies showed more marked differences between the formulations tested. Morphological studies showed homogeneous populations of unilamellar spherical vesicles for the empty liposomes. On the other hand, DOX-loaded liposomes gave rod- like structures with the crystallised DOX in the interior. The translation of the Nanoassemblr results to the Blaze was successful in terms of physicochemical characteristics, DOX loading and morphological evaluation.

Conclusions: Here we present a scalable manufacturing method for the production of liposomal doxorubicin which demonstrates the ability to de-risk the translation from bench to clinic. Comparable physicochemical characteristics to the marketed Doxil® were obtained using this method, achieving high drug loading efficiencies (>90% EE). However, differences in drug release and morphological structure were observed.

Acknowledgements: The project was established by the Centre for Process Innovation (CPI) as part of the foundational capability of the National Formulation Centre (NFC) alongside contributions from the project partners.

PREPARATION OF miR-146a-LOADED CHITOSAN NANOPARTICLE AIMING TO OVERCOME CISPLATIN RESISTANCE IN NSCLC.

35

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Background: Lung Cancer is the leading cause of cancer death in the world, accounting for more than 1.4 million deaths per year, 80-85% of which are due to non-small cell lung cancer (NSCLC). Cisplatin has been the most common used chemotherapy agent for the past 40 years, even though its use has only resulted in small and incremental declines of mortality rates. After continuous or multiple administration, the development of resistance to the cisplatin-based therapy leads to treatment failure. MicroRNAs (miRNA) are small, endogenous, non-coding RNA involved in post-transcriptional regulation of gene expression and it is believed that miRNA overexpression and down-regulation are associated with the development and progression of various tumours. They have been discovered to play a crucial role in developing resistance to Cisplatin. However, the delivery of miRNAs faces many obstacles such as cellular uptake, RNA's stability and degradation in blood serum and immunogenic response. Nanoparticles represent a promising tool to solve this challenge, given that they can either adsorb or encapsulate miRNAs within various polymers. Chitosan is a biocompatible, biodegradable polymer that has been widely used as nanocarrier delivery system, due to its capability to bind, absorb and carry other compounds such as drugs, genes, peptides and proteins.

Methods: Nanoparticles made of a chitosan water-soluble derivative (CHT NPs) were manufactured using the NanoAssemblr™ bench-top instrument (Precision NanoSystems, Inc., Vancouver, Canada), based on microfluidic mixing technology. Chitosan and TPP, used as cross-linking agent, were dissolved in nuclease-free water. Different amounts of miR-146a (5, 7 or 10 ng) were added to the TPP solution to manufacture miR-146a-loaded NPs. Size and charge of the NPs were measured by means of Dynamic Light Scattering (DLS), while the miR-146a Encapsulation Efficiency (EE%) was measured by subtracting the amount of miR-146a in the supernatant after NPs centrifugation.

Results: Analysis of blank CHT NPs indicated particle sizes of 186.15±27.32 nm and PDI of 0.26±0.03. Analysis of miR-146a-loaded CHT NPs showed no differences in nanoparticle size from the blank formulation when adding 5 or 7 ng of miR-146a to the TPP (174.13±18.12 nm and 161.45±32.31 nm, respectively), while the size increased significantly when 10 ng of miR-146a, reaching the micrometer size range (1375.01±70.71 nm). Encapsulation Efficiency (EE%) of miR-146a within the nanoparticle ranges from 75.85±1.11% to 91.38±1.03%.

Conclusions: The study showed that a water-soluble derivative of Chitosan can be employed to prepare particles in the nanometer size range using the microfluidic mixing technique, that allows the manufacture of nanoparticles in a faster and more reproducible way compared to traditional methods. Moreover, it has been demonstrated that the polymer is suitable for encapsulating miR-146a, and further studies will be performed to investigate the ability of the particles to deliver miR-146a in cancer cells.

PREPARATION OF THE CATIONIC LIPOSOMAL VACCINE ADJUVANT CAF09B USING A MICROFLUIDIC METHOD

36

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Background: Subunit vaccines often require adjuvants to induce robust immune responses. The cationic liposomal adjuvant CAF09b consists of the lipids dimethyldioctadecylammonium bromide (DDA) and monomycoloyl glycerol (MMG) with the TLR3 agonist polyinosinic:polycytidylic [poly(I:C)] electrostatically adsorbed to the liposomes, and is capable of inducing robust cytotoxic T-cell responses. The microfluidics technique was applied to manufacture CAF09b, as it enables formulation of the adjuvant at ambient temperature, which is not possible with the previously employed thin film method. In a quality-by-design study, microfluidics parameters identified as critical for particle size and colloidal stability were varied systematically to assess the optimal settings to obtain a stable formulation with particle sizes of less than 100 nm.

Methods: Liposomes were prepared using the NanoAssemblr (Precision Nanosystems) microfluidics system. Weighed amounts of DDA and MMG were dissolved in EtOH, 99% while the poly(I:C) was diluted in Tris-buffer (10 mM, pH 7.4) with different amounts of DMSO. A face-centered response surface model (RSM) design was set up with the variables; EtOH content (20-40% v/v), final lipid concentration (6–12 mg/mL) and DMSO concentration (0-10% v/v). The total flow rate was 12 mL/min, and the samples were stored at 4 °C. Particle sizes, polydispersity indexes and zeta-potentials were measured using DLS. The encapsulation/adsorption of poly(I:C) was determined by the Ribogreen assay and agarose gel electrophoresis with different concentrations of SDS (0–3% w/v) using ethidium bromide for development.

Results: All formulations were highly cationic (zeta-potential: 50–70 mV). The three factor RSM for the formulations at day 0 resulted in a satisfactory model showing that the EtOH and DMSO concentrations had a significant impact on the resulting particle sizes. Thus, a low EtOH concentration and a high DMSO concentration resulted in the smallest particle sizes at approx. 70 nm. No free poly(I:C) was detected in untreated samples with the Ribogreen assay, while almost all poly(I:C) could be detected after treatment with 1 % w/v SDS. The level of detected poly(I:C) was RSM modelled, and the levels of EtOH concentration and final lipid concentration were highly significant for the amount of detectable poly(I:C). To further characterize the incorporation of poly(I:C) into the liposomes selected samples were subjected to agarose gel electrophoresis after treatment with 0–3 % w/v SDS. In untreated samples, no poly(I:C) could be detected after staining with ethidium bromide, indicating all poly(I:C) is completely encapsulated inside the liposomes. After treatment with SDS, poly(I:C) could be detected, both remaining adsorbed to the liposomes and displaced into the gel. At the highest SDS concentrations, all poly(I:C) was displaced from the liposomes.

Conclusions: RSM could be used to model the impact of EtOH content, lipid and DMSO concentration for microfluidics formulation of the liposomal adjuvant CAF09b. Poly(I:C) was completely encapsulated inside the liposomes, but could be detected after treatment with SDS.

OPTIMISING THE PROPERTIES OF THERMOGELLING MATERIALS USING A DESIGN-OF-EXPERIMENTS APPROACH

37

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Background: Thermogelling polymers exhibit a solution to gel transformation above a critical temperature. Materials undergoing this transition in a physiological temperature range have been drawing attention for their use in biomedical applications as in-situ gelling systems, where the polymer solution may pass through an applicator before forming a gel upon contact with the body. Poloxamer 407 (or Pluronic F127) has been widely investigated due to its "thermogelling" properties and has been previously used in FDA approved medicines. However, there are acknowledged drawbacks for thermogelling poloxamer solutions that limit their performance, including: low gel strength, instability, and rapid dissolution. To improve gel strength, researchers have studied blends of poloxamer with other hydrophilic polymers. However, these studies are typically limited to binary blends, and only a handful of additives have been explored. The aim of this study is to explore complex thermogelling polymer blends containing poloxamer 407.

Methods: In order to modify complex polymer blends containing poloxamer, a Taguchi Orthogonal Array (OA) design-of-experiments approach was taken. This approach allows for the optimization of complex mixtures with a lower number of experiments required. OA-L18 was performed at 8 factors (polymers) and different levels (concentration) using Design Expert® software. We examined the addition of poly(vinyl alcohol), polyacrylic acid, polyethylene glycol (each at two different molecular weights) and poloxamer 188 at three levels (0, 0.1 and 1 % w/w) to P407 at 15 and 20 % w/w. The properties of the blends were evaluated by measurement of the gelation temperature and the storage modulus (G`), which have been studied by rheometry.

Results: Our results showed some of the polymer additives, namely poly(acrylic acid), poly(ethylene glycol) and poly(vinyl alcohol), had a significant effect on the gel strength. PEG addition showed an increase in gel strength with the increase of PEG concentration from 0.1% to 1.0%. PVA showed a decrease in gel strength at lower concentration (0.1%). However, it increased with the increase of PVA concentration to 1.0%. On the other hand, poly(acrylic acid) had a negative effect with the increase of concentration. Only P407 negatively influenced the gelling temperature significantly.

Conclusions: Improving the gel strength of poloxamer formulations is highly important for their success in drug delivery applications. In this research project, DOE has been used to further understand the effect of polymer additives in complex polymer mixtures with P407. This technique may be used to develop thermogelling materials with improved performance, using safe polymer additives.

USING LICENSED TOTAL PARENTERAL NUTRITION NANOEMULSIONS AS DRUG CARRIERS FOR CIPROFLOXACIN

38

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Background: The good bioavailability of ciprofloxacin (CP), a fluoroquinolone antibacterial drug, is accompanied with a short half-life and a high percentage of protein binding in human blood circulation. These obstacles, in addition to its sparingly water solubility level rationalise the need to develop controlled release drug delivery systems for CP. The aim of this work is to investigate the physicochemical properties and stability profiles of two total parenteral licensed nutrition (TPN) lipid nanoemulsion formulations, Clinoleic® and Intralipid®, loaded with different concentrations of CP.

Methods: The CP-nanoemulsion formulations with concentrations from 1 to 10 mg/mL were prepared by mixing for 5 min and sonicating for 2 hr at 40 °C. The physicochemical characteristics were evaluated by measuring the particle size, poly dispersity index (PI), zeta potential, pH, entrapment efficiency and in vitro dissolution release. The short-term stability study was performed at 4 °C for six months and at room temperature (RT) for two months to assess the feasibility of this loading methods in clinical applications and determine storage conditions.

Results: In general, both nanoemulsions recorded mean droplet sizes between 220 and 250 nm with polydispersity index (PI) around 0.250. Also, the zeta potential was in negative range between -38 and -53 mV, whereas the pH values were at neutral range (6.5–7.7). The percentage of entrapment efficiency (EE%) is relatively high for both types of nanoemulsions, for example, at 3 mg/mL drug concentration EE% was 87% ± 12 for Clinoleic and 72 % ± 12 for Intralipid. The dissolution profile from dialysis bags showed a sustained drug release reaching approximately 70% for both nanoemulsions after 24 hr. At 4 °C for six months, both Clinoleic and Intralipid formulations at a range of drug concentrations (1–10 mg/ml) showed high stabilities measured periodically by the average droplet sizes, PI, pH and zeta potential values. Similar results, but pH values, were shown when the formulations for both nanoemulsion stored at 25 °C for 2 months. The pH values for both formulations, stored at RT, recorded a significant drop to acidic range after 6 weeks of storage. However, this acidic drop became less significant for high drug concentrations (above 3 mg/mL) in both nanoemulsions.

Conclusions: Overall, this study has shown that CP was successfully loaded into clinically licensed TPN lipid nanoemulsions with high stability profiles at 4 °C, the required storage temperature for these formulations.

DIETHYLDITHIOCARBAMTE COPPER II SOLUBILITY ENHANCEMENT VIA CYCLODEXTRIN PARENTRAL FORMULATIONS

39

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Background: the anticancer activity of disulfiram, anti-alcoholism drug, results from the combination of its metabolite, diethyldithiocarbamate (DDC), with copper to give a very stable insoluble compound, $(DDC)_2Cu$. Nanomedical formulations, such as liposomes, were not success in solubility enhancement. The aim of this work is to improve the solubility of $(DDC)_2Cu$ by dissolving in cyclodextrin (CD) solutions and freeze drying the content to make injectable formulations that could be applicable to human.

Methods: the phase solubility study of $(DDC)_2Cu$ at different concentrations of two types of beta-cyclodextrins; 2-hydroxypropyl- beta -cyclodextrin (HP-CD) and sulfa butyl ether beta cyclodextrin (SBE-CD) solutions, was applied by shaking flask method. The solubility results were used to prepare freeze dried formulations characterized by FTIR, DSC, TGA and PXRD . The precipitation study for one month at room temperature were applied for all solubility preparations and reconstituted freez-dried samples. Finally, the cytotoxicity studies were performed on triple-negative breast cancer cell lines, MDA-MB-231 and those which have acquired chemoresistance to paclitaxel 10 nM concentration (MDA-MB- 231P10).

Results: the phase solubility results showed a significant enhancement of the $(DDC)_2Cu$ solubility in both CD solutions, reaching to 4.482 ± 0.094 mg/mL and 3.661 ± 0.085 mg/mL at 20% w/w of SBE-CD and HP-CD solutions, respectively. The exponential relationship between the solubility and both types of CD concentrations were also confirmed. The percentage of residual water was less than 3 % for all lyophilized formulations and the reconstitution time for freeze-dried formulations were less than one minute. The endothermic peak of pure $(DDC)_2Cu$ at 201 °C was completely disappeared in lyophilized formulations, and the peaks for the pure drug of both FTIR and PXRD signals was completely disappeared. This confirms the enactment of the solubility of the lyophilized formulations. The precipitation studies for both parentral and reconstituted lyophilized formulations for one month at room temperature recorded more than 90% at 15% w/w and 20% w/w concentrations for both CD types. Finally, the cytotoxicity of both CD formulations was similar to that of the positive control $(DDC)_2Cu$, recording IC_{50} values less than 250 nM for the sensitive cell lines and less than 150 nM for the paclitaxel resistance cell lines.

Conclusions: the prepared parentral formulations of DDC_2Cu in HP-CD and SBE-CD solutions offered an excellent solution to the solubility problem of DDC_2Cu and confirmed the anticancer activity of the resulting formulations. The cyclodextrin formulations of DDC_2Cu might have a great potential for further in vivo studies.

TRANSDERMAL DELIVERY OF ONDANSETRON HYDROCHLORIDE-LOADED BILOSOMAL GEL SYSTEMS VIA HIGH FREQUENCY SONOPHORESIS

40

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Background: Ondansetron hydrochloride (OND) is a 5-HT receptor antagonist which is commonly prescribed for the control and treatment of emesis following chemotherapy and operations. Following oral administration, OND suffers from rapid elimination ($t_{1/2}$= 3–4 h) and low bioavailability (60%) due to the hepatic first-pass metabolism. Furthermore, the frequent dosing of intravenous or intramuscular OND injections usually results in poor patient compliance. To surmount such limitations, the current work aimed to explore the potential of high-frequency ultrasound (HFU) waves to promote transdermal delivery of OND-loaded bilosomal gel systems (BGS).

Methods: OND-loaded bilosomal systems (BS) were prepared and mixed with Carbopol®-based coupling gel (0.5% w/v) to develop OND-loaded BGS. The latter was characterized for pH and rheological properties. The variables influencing HFU were optimized, including; bilosomal system: coupling gel ratio (1:2 or 1:3), ultrasound application period (5, 10 or 15 min), intensity (half or full), mode (continuous or pulsed), duty cycle (20, 50 or 100%). *Ex vivo* permeation and confocal laser scanning microscope (CLSM) studies were conducted. The best performing BGS was subjected to histopathological and permeation studies in rats.

Results: OND-loaded BGS were shear thinning having suitable pH values (5.5–7) for transdermal application. The optimized HFU variables were the bilosomal system: coupling-gel ratio of 1:3, and application of full intensity, continuous, ultrasound waves for 10 min at 100% duty cycle. *Ex vivo* permeation and CLSM studies revealed superiority of BGS on HFU-pretreated rat skin. BGS10-HFU exhibited minor histopathological changes. Compared to OND oral solution, a non-significant ($p > 0.05$) difference in t_{max} and significant ($P< 0.05$) higher AUC_{0-24} and MRT_{0-24} values were revealed.

Conclusions: BGS10-HFU showed promising capability to initiate a rapid onset of action, to achieve a more prolonged drug-plasma profile, and to increase OND bioavailability.

FINE-TUNING MICROFLUIDIC PARAMETERS FOR THE MANUFACTURE OF LIPOSOMAL VACCINES

41

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Background: Microfluidics is a flexible process that offers scale-independent manufacturing processes for liposomes which allows us to circumvent current complex and time-consuming manufacturing methods. Microfluidics offers higher drug loading and better physico-chemical attributes compared to other liposomal production processes. The aim of this study was to optimise microfluidic parameters for the manufacture of liposomes.

Methods: Hydrogenated soy L-α- phosphatidylcholine (HSPC) and cholesterol were dissolved in ethanol at 2:1 wt/wt (final lipid concentration 1 mg/mL). Liposomes were produced using the NanoAssemblr® Benchtop system from Precision Nanosystems. Flow rate ratios (FRRs) ranging from 2:1 to 3:1 aqueous:lipid phase were used at a total flow rate of 12 mL/min. Ovalbumin dissolved in phosphate-buffered saline (PBS) was added to the aqueous inlet (200 µg/mL) while PBS was added when 'empty' liposomes were produced. After production, liposomes were purified using tangential flow filtration (Krosflow Research Iii system with a 750 kDa mPES column). Liposome size, charge and PDI was measured using a Malvern Zetasizer Nano ZS. Protein encapsulation was quantified using MicroBCA (Pierce™ BCA Assay Kit, Sigma Aldrich, Poole, UK) following manufacturers recommendation.

Results: Our results show that lower FRRs (2:1) produced liposomes 93 ± 4 nm in size and protein loading of 38 ± 2 %. Increasing the FRR to 3:1 reduced the size and loading of the vesicles with the size reducing to 64 ± 5 nm with a corresponding loading of 23 ± 3 %. These results suggest that small changes in the flow rate ratio can be used to further optimise the manufacture of liposomes incorporating proteins.

Conclusions: Microfluidics offers the ability to formulate liposomes with good protein loading (20 to 38 % depending on the FRR) and low vesicle sizes (<100 nm) across a range of FRRs, with the ability to fine-tune these parameters in terms of sizes down to 64 nm.

EFFECT OF DENSITY ON DAPIVIRINE RELEASE FROM THERMOPLASTIC VAGINAL RINGS FABRICATED BY ADDITIVE MANUFACTURING

42

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Background: Droplet deposition modelling (DDM) is an additive manufacturing (AM) process employed by the Arburg Freeformer. A piezo controlled shut-off nozzle is used to discreetly control the exiting of material from the nozzle, creating polymeric droplets of defined geometry, providing precise levels of control over an object's design and morphology. Properties including density, geometry and surface area can be manipulated in ways that cannot be achieved using conventional thermoplastic processing techniques. Here, the DDM process and injection moulding were used to manufacture dapivirine (DPV) loaded vaginal rings using a pharmaceutically relevant, life science grade thermoplastic polyurethane.

Methods: Vaginal rings (outer diameter 54.0 mm, cross sectional diameter 4.0 mm) were manufactured by injection molding or Arburg Plastic Freeforming – a proprietary DDM process, using a hydrophobic TPU (T87 or T60) loaded with 10% w/w dapivirine. Using the DDM process, rings of 100, 50 and 10% matrix density were produced. Rings were evaluated for *in vitro* drug release over 29 days in an aqueous release media and assessed for thermal characteristics.

Results: Raw materials were thermally stable with a weight loss (%) equal to or less than 0.70% at the maximum processing temperature. DSC thermal traces for TPU plus 10% w/w DPV showed that the DPV melt endotherm was absent, suggesting that it was fully solubilised within the TPU at the experimental conditions. Daily DPV release from all ring designs ranged between 387–8666 µg (Day 1) and 193–992 µg on Day 29. T87 and T60 DDM printed vaginal rings with 10% infill density (62 and 68 mg DPV load respectively) exhibited up to a seven fold increase in DPV release rate compared to injection moulded rings containing 192 mg DPV. For DDM printed rings, there was significant correlation between decreasing ring density and increasing DPV release rate as a percentage of total drug loading.

Conclusions: Vaginal rings with an infill density of 100% manufactured by IM and DDM released up to 4 and 10% of their total DPV loading, while rings with an infill density of 50 or 10% (DDM) released up to 56 and 79% of their total DPV loading after 29 days. DDM printing on an Arburg Freeformer has therefore provided a new potential to either increase the release rate of poorly water soluble compounds or reduce the loading required to maintain a desired release rate.

DEVELOPMENT OF AN ENZYME RESPONSIVE CARVACROL NANOPARTICLE HYDROGEL FOR METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS INDUCED SKIN INFECTIONS

44

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Background: Skin and skin structure infections (SSSIs) have been rising dramatically since last decade. Methicillin resistant Staphylococcus aureus (MRSA) is found to be the principal causative agent in approximately 90% of SSSIs posing a major threat to public health, mainly due to the escalating prevalence of antibiotic resistant isolates. Accordingly, novel treatment approaches are warranted to control these often life-threatening infections. Carvacrol (CAR), a monoterpenoid phenolic compound, has shown significant antimicrobial activity against multiple resistant bacterial strains, including MRSA. Herein, an enzyme responsive CAR nanoparticle (NP) delivery platform was developed to avoid non-specific release of a broad spectrum antimicrobial agent. For this purpose, poly(caprolactone) (PCL) was used which is subject to degradation by bacterial lipases.

Methods: CAR-PCL NPs were developed by a nanoprecipitation method and optimized through response surface methodology. The resultant NPs were characterized in terms of physicochemical properties and their antimicrobial activity was evaluated against linezolid resistant MRSA clinical isolates. To facilitate dermal delivery, CAR-PCL NPs were incorporated into Carbopol hydrogel and further *in vitro* evaluation of gel performed.

Results: Optimized CAR-PCL NPs showed mean particle size (190 nm), polydispersity index (0.046), zeta potential (-17.7mV), entrapment efficiency (83.28 ± 3.62%) and 2-fold reduction in minimum inhibitory concentration of CAR. Characterization of CAR-PCL NPs hydrogel in terms of drug content uniformity, rheological properties, bioadhesion time, spreadability, extrudability and stability manifested its suitability for skin application. *In vitro* release studies demonstrated 85.5% of CAR release in 48 h in the presence of bacterial lipase enzyme in contrast to only 8.9% release in the absence of bacterial lipase, highlighting the potential of the system for differential delivery of CAR. Furthermore, *ex vivo* skin permeability studies demonstrated more skin retention of CAR.

Conclusions: These results indicated the successful formulation, optimization, and *in vitro* characterisation of CAR-PCL NPs hydrogel demonstrating its antimicrobial potential against resistant MRSA strains. This approach may facilitate the differential delivery of CAR in the presence of lipase producing bacteria at infection sites and may provide a novel effective approach alternative to that of conventional antibiotics for treatment of MRSA induced skin infections. Future work will investigate its *in vivo* potential and utilization of microneedle array technology for more effective delivery of these stimuli responsive NPs.

TOWARDS TRANSMUCOSAL PEPTIDE DELIVERY: INCORPORATION OF AN ACTIVE MODEL PROTEIN INTO A MUCODAHESIVE NANOFIBRE PATCH USING UNIAXIAL ELECTROSPINNING

45

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Background: The oral delivery of peptides is challenging because of degradation in the gastrointestinal tract. Transmucosal drug delivery is an attractive alternative due to avoidance of the gastrointestinal tract and hepatic first-pass metabolism, and favourable ease of administration and patient compliance in comparison to subcutaneous parenteral delivery. However, significant obstacles remain for the development of effective formulations including permeation through the epithelial barrier and loss of biological activity. We have developed a biodegradable, mucoadhesive oral patch that demonstrates long residence times *in vivo* and is currently involved in a stage 2 clinical trial. The patches are comprised of a two-layer electrospun polymer system composed of a highly bio-adhesive inner layer and an outer saliva-resistant, durable but flexible protective layer. This research aims to further develop the patch for transmucosal delivery of therapeutic peptides.

Methods: Lysozyme, an antimicrobial enzyme, was incorporated into poly(vinylpyrrolidone)/Eudragit RS100 polymer nanofiber patches as a model protein using a variety of ethanol/water mixtures as solvents and uniaxial electrospinning. Loading rates, bioactivity, and release profile were investigated by soaking the patches in PBS to release the enzyme and then analysing the supernatant using enzyme kinetics and protein assays. The nanofiber morphology was analysed using scanning electron microscopy. The hydrophobic backing layer was produced by electrospinning an additional poly(caprolactone) layer and melting at 65 °C to produce a continuous film. Residence times were evaluated using a simple *in vitro* test and agar disc diffusion assays were used to assess any antimicrobial effect against oral bacteria strains.

Results: For solvent mixtures in the range of 97–40 wt% ethanol, the bioactivity of the released enzyme was above 90 % and there was no significant difference between solvents. The loading efficiencies ranged from 70–100 % with no significant difference between solvents. The average fibre diameter was significantly decreased at 60 and 40 wt% ethanol due to higher solution conductivity and lower viscosity. Samples were taken from different parts of the patches, showing that the distribution of lysozyme is homogenous. The release profile showed that 87 % was released within 1 hr, which is desirable given that the existing patches show residence times of around 2 hr. There was no significant decrease in bioactivity after melting the backing layer at 65 °C.

Conclusions: The resulting protein-loaded patches displayed high bioactivity and clinically relevant release rates making them a promising proof of concept for the delivery of bioactive peptides to the oral mucosa. Additionally, lysozymes' antimicrobial properties may give the patches a potential application as antiseptic dressings for oral wounds.

PREPARATION, CHARACTERIZATION AND COMPARATIVE IN-VITRO EFFICACY OF QUERCETIN LOADED LIQUID CRYSTALLINE NANOPARTICLES USING IMMORTALIZED BRONCHIAL EPITHELIAL CELL LINE AGAINST FLUTICASONE FOR THE TREATMENT OF ASTHMA

46

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Background: Asthma is a chronic inflammatory lung disease where, the airways become narrow and inflamed which makes breathing difficult. It is estimated that 334 million people worldwide suffer from asthma. It affects people of all ages. The present-day treatment for asthma is primarily focused at suppressing and preventing the disease. This generally requires long term therapy that comes with many sides effects. Therefore, there is a demand for new and more effective treatment strategies. Quercetin, a dietary flavonoid commonly found in fruits and vegetables exhibits a wide spectrum of pharmacological activities. Extensive studies have elucidated the potential therapeutic use of quercetin as an anti-asthmatic agent, mainly due to its potent anti-inflammatory property. However, the poor bioavailability of quercetin limits its use in clinical practice. This present study aimed to formulate quercetin loaded liquid crystalline nanoparticles (LCN) and surface modified liquid crystalline nanoparticles (sm-LCN) as well as investigate its anti-inflammatory activity in human primary bronchial epithelial cell line (BCi-NS1.1) induced with lipopolysaccharide (LPS).

Methods: Quercetin LCN were prepared using ultrasonication method. The formulated LCNs and sm-LCN were characterised in terms of particle size, zeta potential as well as the drug encapsulation efficiency. Furthermore, their morphology and in-vitro release profile were also studied. Finally, the anti-inflammatory activity of quercetin LCN and sm-LCs were evaluated by measuring the concentration of pro-inflammatory markers namely interleukin (IL)-1β, IL-6 and IL-8 in BCI-NS1.1 cell lines via cytometric bead array technique.

Results: The formulated quercetin LCN demonstrated a mean size and zeta potential of 223.9 ± 1.8 nm and -15.6 ± 0.2 mV. For sm-LCN, 236.1 ± 2.12 nm and 15.8 ± 0.29 mV, respectively. The encapsulation efficiency for both quercetin LCN and sm-LCN were 99.4 ± 0.4%. Quercetin LCN and sm-LCN significantly ($p < 0.05$) decreased the production of IL-1β, IL-6 and IL-8 compared to LPS only group. Encapsulation of quercetin into LCN and sm-LCN further enhanced its anti-inflammatory activity compared to quercetin in dimethyl sulfoxide (DMSO). In addition to that, quercetin LCN and sm-LCN also exhibited comparable activity to fluticasone in terms of significantly ($p < 0.05$) reducing the production of IL-1β and IL-6.

Conclusions: LCN and sm-LCN have shown to enhance the anti-inflammatory effect of quercetin and it was observed that the pro-inflammatory markers such as interleukin 6 (IL-6), interleukin 8 (IL-8) and interleukin 1 beta (IL-1β) in BCi-NS1.1 cell line were significantly suppressed. Quercetin loaded LCN and sm-LCN could be a potential therapeutic intervention for asthma as they are efficacious in suppressing the production of key pro-inflammatory markers associated with the development of asthma.

PREPARATION AND OPTIMIZATION OF CEFOTAXIME-LOADED CHITOSAN NANOPARTICLES USING A MICROFLUIDICS MIXING TECHNIQUE

47

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Background: The complex double membrane structure of Gram-negative bacteria presents a considerable barrier to the entry and action of anti-infective agents, and is responsible for mediating the growing level of resistance of Gram negatives to antibiotic therapy. As a further problem, the shrinking pool of effective antibiotics is currently not being refilled by new treatment options. Nanotechnology can be considered as an effective solution to enhance anti-infective trafficking across the bacterial cell envelope. In particular, carriers made of chitosan (CHT) have attracted interest due to the biodegradable and biocompatible nature of this material, as well as its low toxicity and intrinsic antimicrobial activity. An innovative method to produce chitosan nanoparticles (CHT NPs) is the NanoAssemblr™ platform which is an automated microfluidics-based system that eliminates user variability and is capable of reproducible and scalable manufacture of NPs. The aim of this study was to manufacture blank CHT NPs and CHT NPs loaded with cefotaxime, a third-generation cephalosporin antibiotic, using the NanoAssemblr™ bench-top instrument. The influence of various preparation parameters on NP size, charge and encapsulation efficiency was focused on in particular.

Methods: Nanoparticles made of low molecular weight chitosan (CHT NPs) were manufactured using the NanoAssemblr™ bench-top instrument, based on microfluidic mixing technology. Chitosan and TPP (tripolyphosphate), used as cross-linking agent, were dissolved in 1% acetic acid and distilled water respectively at desired concentrations. Taguchi design L18 orthogonal array was constructed through Minitab 16 Statistical Software® and a further investigation of flow rate ratio was used to determine the best blank formulation in terms of size, PDI (polydispersity index) and charge. Different amounts of cefotaxime were added to the TPP and chitosan solution to manufacture cefotaxime-loaded CHT NPs. Blank and loaded CHT NPs were characterized in terms of size, PDI and surface charge. Encapsulation efficiency of loaded NPs was measured using HPLC by quantification of unentrapped drug amounts following NP centrifugation.

Results: Analysis of blank CHT NPs indicated particle sizes of less than 100 nm and a low PDI. Analysis of cefotaxime-loaded CHT NPs showed no marked differences in nanoparticle size from the blank formulation when various amounts of drug were added to the TPP and CHT solutions. Encapsulation Efficiency (EE%) of cefotaxime ranged from approximately 5%, when the smallest amount of drug was added to CHT solution, to approximately 15% when the highest amount of drug was added to the TPP solution.

Conclusions: The study showed that CHT NPs can be easily manufactured and optimized in the nanometer size range using NanoAssemblr™ microfluidics technology. Moreover, it is possible to encapsulate cefotaxime within nanocarriers using the same method, allowing the manufacture of nanoparticles in a faster and more reproducible way compared to traditional methods.

FORMULATION OF PLGA NANOPARTICLES LOADED WITH A NOVEL PEPTIDE TO TREAT MIGRAINE

48

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Background: Nose-to-brain drug delivery is an interesting pathway to treat migraines either by increasing the speed of therapeutic action or reducing systemic side effects. The CGRP (calcitonin gene-related peptide) receptor has been shown to be related to migraine pathogenesis and we have identified a small 10 amino acid peptide, P006, to be a potent CGRP antagonist (patent pending). The aim of this research was to synthesise and encapsulate an analogue of P006 in PLGA nanoparticles (NPs). These NPs will then be embedded within a chitosan dry powder for nasal delivery.

Methods: A modified sequence of P006, P006 (S for T) was synthesized using solid phase peptide synthesis. NPs were prepared by a double emulsion technique using 0.5 mL of peptide solution (2 mg/mL) in the internal aqueous phase (SDS 0.2% w/v). The oil phase was composed of PLGA (50 mg) dissolved in DCM (2 mL) and the external aqueous phase (20 mL) contained PVA (1%). Two methods were compered by including NaCl either in the external aqueous phase (0.5% w/v) or in the internal aqueous phase (saturated solution). Samples were prepared in triplicate and unloaded NPs used as a control. Peptide encapsulation efficiency were determined using RP-HPLC and peptide structure using LC-MS NP size and charge was analyzed using a Malvern Zetasizer. To produce a dry powder for nasal delivery, NPs were suspended in a low molecular weight chitosan solution (2%) in glacial acetic acid (0.5%) and spray-dried (Buchi 209). The morphology of resulting microparticles was observed by SEM and the NP size/charge and peptide structure re-assessed.

Results: Using only SDS and no salt addiction, NPs have a diameter size of 213.2 ± 3.02 nm, 0.262 PDI, charge of -25.89 ± 0.41 mV and an encapsulation efficiency (EE) of 13%. Adding NaCl to the external aqueous phase gave negligible encapsulation but using saturated NaCl in the inner aqueous phase produced an EE of 35%, a NP diameter of 204.9 ± 2.89 nm, 0.237 PDI and a charge of -9.69 ± 0.41 mV. The spray-dried chitosan MPs had a diameter of 1.136 ± 0.048 µm, 0.258 PDI and charge of +41 ± 0.62 mV. Spray-drying yield was 60.37%.

Conclusions: Using a hypertonic solution (saturated NaCl) as an internal aqueous phase creates an osmotic gradient between the inner and the outer phase that increases the tendency of the hydrophilic peptide to remain inside the NPs. The osmotic pressure of the external aqueous phase in addition to the capability of the peptide to interact with both salts enabled an efficient encapsulation of 35%. The chitosan MPs are of a suitable size for aerosolisation prior to nasal deposition.

CAPE-LOADED ALBUMIN NANOPARTICLES ALLEVIATE NF-kβ AND HIF IN AN IN VITRO MODEL OF COLON CANCER

49

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Background: Colorectal cancer is the third most common cancer which kills 800,000 patients annually. Many causes of the disease are documented however the precise mechanisms underpinning the disorder have not been confirmed. We have shown amongst others that the overstimulation of nuclear factor kappa beta (NFkβ) and hypoxia-inducible factor (HIF) are the hallmarks of colorectal cancer. Chemotherapy, immunosuppressive agents, episodes of radiation and nutrition supplement-based therapy are current first line of treatment. However, these therapeutics cause several sides effects due to number of factors such as solubility and selectivity of current therapeutic compounds. Recently, there has been increased interest to explore therapeutic benefit of natural compound which can modulate HIF and NF-kβ pathways in human disease. However, most of the natural medicinal compounds are poorly soluble in aqueous media thus resulting in poor bioavailability and low therapeutic efficacy. Hence, in the current work we have used nanotechnology to fabricate albumin nanoparticles of caffeic acid phenethyl ester (CAPE) to enhance the solubility of CAPE in order increase its therapeutic potential and provide targeted delivery.

Methods:
1.Fabrication of CAPE-loaded albumin NPs by desolvation technique.
2.In-vitro anticancer studies.
3.Qualitative and quantitative analysis of transcription protein p65 and HIF-1α.

Results: The particles of the CAPE-loaded albumin NPs were found in the range of 200-300nm. Cellular localization of CAPE-loaded albumin NPs was confirmed by cellular uptake study. Anticancer studies confirm the significant increase in therapeutic potential of CAPE-loaded albumin NPs in colorectal cancer cell lines.

Conclusions: CAPE-loaded albumin NPs improves the anticancer potential and stabilize the overstimulation of transcription protein p65 and HIF-1α significantly as compare to free CAPE. Therefore, due to decrease in particles and targeted delivery to CAPE nanoparticles the therapeutics action increases significantly.

SALBUTAMOL-LOADED CHITOSAN NANOPARTICLES PREPARED BY MICROFLUIDIC MIXING SYSTEM FOR TREATING LUNG OBSTRUCTIVE PATHOLOGIES

50

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Background: Salbutamol is a short-acting, selective beta2-adrenergic receptor agonist. It is 29 times more selective for beta2 receptors than beta1 receptors, giving its higher specificity for pulmonary beta receptors versus beta1-adrenergic receptors located in the heart. Salbutamol is used to relieve symptoms of asthma and COPD such as coughing, wheezing and feeling breathless. It works by relaxing the muscles of the airways into the lungs, which makes it easier to breathe. Nanoparticles represent a valid drug delivery system, owing to their potential for the mucosal delivery of drugs and antigens, crossing lung biological barriers, and enhancing cellular uptake. These carriers of appropriate size and surface charge can protect drugs from enzymatic degradation, improve their penetration across the mucosal epithelium, modulate drug pharmacokinetics, improve efficacy and reduce drug toxicity. Chitosan is a bioactive polymer with a wide variety of applications. Recently it has been widely studied as nanocarrier delivery system, due to its capability of bind, absorb and carry other compounds such as drugs, genes, peptides and proteins. Also it provides several advantages because of its biodegradability, low toxicity, mucoadhesive and antimicrobial properties. Microfluidic mixing technology is an innovative method to manufacture nanoparticles. Compared to traditional methods, it allows quicker production, easier results reproducibility and scalability and eliminates user variability.

Methods: Chitosan NPs were manufactured using the NanoAssemblr™ bench-top instrument (Precision NanoSystems, Inc., Vancouver, Canada), based on microfluidic mixing technology. Chitosan and TPP were dissolved in water at desired concentrations, and salbutamol was then dissolved in the chitosan solution for the preparation of loaded nanoparticles. Size and charge of the NPs were measured by means of Malvern Zetasizer Nano ZS, while the Salbutamol Encapsulation Efficiency (EE%) was measured by subtracting the amount of Salbutamol in the supernatant after NPs centrifugation. Moreover, the influence of the amount of salbutamol added and of the flow rate on the EE% was investigated.

Results: Analysis of blank Chitosan NPs indicated particle sizes of 97.78±6.65 nm and PDI of 0.29±0.04. Analysis of Salbutamol-loaded Chitosan NPs showed no differences in nanoparticle compared to the blank formulation when 1 mg of Salbutamol was added to the chitosan solution (98.4±3.13 nm), while the size increased when adding 2 mg of Salbutamol (236.1±12.16 nm). Encapsulation Efficiency (EE%) of Salbutamol within the nanoparticle ranges from 67.21±0.32% to 79.29±0.34%.

Conclusions: The study showed that the chitosan water soluble derivate is suitable for the manufacture of nanoparticles. Salbutamol can be encapsulated within the nanocarrier, with a high encapsulation efficiency. The higher EE% is reached when 2 mg of salbutamol is added to the carrier.

PREPARATION, CHARACTERIZATION AND EVALUATION OF THE ANTI-INFLAMMATORY POTENTIAL OF CELASTROL LOADED LIQUID CRYSTALLINE NANOPARTICLES IN ASTHMATIC PRIMARY BRONCHIAL EPITHELIAL CELL LINES

51

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Background: Celastrol, a potent therapeutic compound, is isolated from the Chinese medicinal plant, Tripterygium wilfordii, which had been extensively studied on its various pharmacological activities attributed to its molecular mechanism involving signaling pathways. However, clinical applications are limited by its unfavorable physiochemical and biological properties. Moreover, the recent emerging reports on severe asthma and steroid-resistant asthma, prompting the need to develop novel drugs for the optimal treatment of asthma. Nanoformulations of celastrol may be a promising candidate for this purpose. Therefore, in this study, we aim to formulate celastrol into liquid crystalline nanoparticles, and to evaluate its anti-inflammatory activity on asthmatic primary bronchial epithelial cell lines.

Methods: Celastrol-loaded liquid crystalline nanoparticles were prepared using monoolein as the lipid and poloxamer 407 as the surfactant, through ultrasonication. The physiochemical characteristics of the nanoparticles, that were evaluated include; i) particle size of the nanoparticles, ii) polydispersity index iii) zeta potential, iv) morphology of the nanostructures, which were studied using transmission electron microscopy, v) entrapment efficiency and, vi) in vitro release profile of the formulation. The in vitro release studies were determined by dialysis using Spectra/Por dialysis membrane bag. In addition, to potentially explain the variances observed in the in-vitro results, we also performed molecular simulations namely, static lattice atomistic simulations. The observations were crucial to predict the molecular phases involved in the formation of the liquid crystal nanosystems and the preferential positioning of celastrol with respect to the hydrophobic domains of the liquid crystal network. Finally, the anti-inflammatory effect of the formulation on asthmatic cell lines (BCi-NS1.1) were evaluated using cytometric bead array assay and flow cytometry.

Results: Celastrol-loaded liquid crystalline nanoparticles showed the mean particle size of 194.1 ± 9.78 nm, a polydispersity index value of 0.248 ± 0.04, along with a zeta potential value of -24.27 ± 2.11 mV. TEM visualization revealed a cubical-like structure of the nanoparticles. The formulations had a high entrapment efficiency of 99.1 ± 0.02 % and in-vitro release study demonstrated that the formulations achieved sustained drug release. On the other hand, the reduction of IL-1β cytokines levels was observed when BCi-NS1.1 cell lines were treated with the formulations.

Conclusions: Celastrol-loaded liquid crystalline nanoparticles with the dose of 200 µM had showed significance ($P<0.05$) in lowering the amounts of IL-1β. This suggests that celastrol-loaded liquid crystalline nanoparticles could be used as a promising candidate for the management of asthma, supported by its effectiveness in the suppression of proinflammatory markers that play an important and crucial part in the pathology and pathophysiology of inflammatory conditions like asthma.

STICKINESS IN PROTEIN-SUGAR FORMULATIONS

52

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Background: Due to the significant increase of protein-based therapeutic applications in recent years the for- mulation of proteins has become an area of high interest. A common method is the freeze-drying of proteins embedded in amorphous structures formed by sugars. Although these sugar matrices provide some stabilization, environmental conditions such as temperature and humidity have a strong impact on product performance and storage. An increase in moisture content or tempera- ture may cause a glass transition and crystallization of the sugar resulting in a loss of thermal stability of the protein. The glassy state is rigid, brittle and not sticky but the rubbery state is sticky. For this reason knowledge of physico-chemical properties such as glass transition and water sorption behavior is important. In this study the impact of water sorption and glass transition behavior of various bovine serum albumin (BSA) - sugar formulations has been studied.

Methods: Water sorption experiments have been carried out by Dynamic Vapor Sorption (DVS) at 25°C. DVS is a well-established method for the gravimetric determination of vapor sorption isotherms using a Cahn D200 recording ultra-microbalance. The vapor partial pressure around the sample was controlled by mixing saturated and dry carrier gas streams using electronic mass flow con- trollers. Mannitol, sucrose, and maltose samples were obtained from the same freeze-drying process with varying amounts of BSA: pure sugar, 11% BSA, 20% BSA, 33% BSA, as well as pure BSA. Each sample was initially dried at 0% relative humidity (RH) at the desired temperature. Then, the sample was exposed to a particular RH while monitoring the change in mass. Measurements were carried out both in a step ("isotherm") and ramp mode. The sample mass would initially increase gradually due to surface adsorption. If the material passes through a glass transition, the vapor uptake will increase dramatically as bulk absorption dominates. If the temperature and humidity were great enough to induce a crystallization event, there would be a measurable mass loss. The crystalline phase typically has a lower surface area and affinity, resulting in a lower ca- pacity for water vapor and the decrease in mass.

Results: Figure 1 shows the humidity ramping experiments for a series of maltose-BSA mixtures, including pure maltose and pure BSA. In this experiment the humidity is linearly ramped from 0 to 95% RH at 5% RH per hour. A similar glass transition is observed for all samples containing maltose, however the crystallisation point shifts to higher humidities as the BSA loading is in- creased (see Figure 2), indicating BSA acts as an "anti-plasticizer". Figure 6 displays the humidity ramping experiments (2% RH/hour) for various freeze dried sucrose-BSA samples. There is little change in the glass transition RH, but a drastic change in the crystallization RH as the BSA loading increases. When the BSA loading increases, the crystallization RH increases dramatically, indicating BSA has a stabilizing affect on the freeze-dried sucrose (see Figure 7). This is a similar trend as observed for the freeze-dried maltose samples.

Conclusions: Moisture sorption experiments were performed on a series of BSA-sugar samples. Although the sugar component is supposed to stabilize the protein in the co-lyophilized product it could be shown that BSA had a stabilizing affect on the sugar, too. The crystallisation humidity of sucrose and maltose increases with increasing BSA content while mannitol shows a more complex relationship due to a kinetic effect related to a change in amorphous content.

LIST OF ATTENDEES

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Thank you for your support!

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Cameron Webb *(University of Strathclyde)*
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Vicky-Leigh Young *(Queen's University Belfast)*
Xinyu Zhao *(Queen's University Belfast)*

Keynote and Invited Speakers

Justin Hanes *(Johns Hopkins University)*
Kostas Kostarelos *(University of Manchester)*
Eileen McBride *(AstraZeneca)*
Andrew Owen *(University of Liverpool)*
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80 attendees in total

NOTES

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