Welcome

On behalf of the organising committee, we extend a warm virtual welcome to all the delegates and guests to the 2021 annual meeting of Drug Delivery Australia. Given the triumphant success of the lipid nanoparticle-based vaccines, this year, we focus on the various materials used for drug delivery applications with a theme “Advanced Materials in Drug Delivery”.

Although we are virtual this year, we are confident that we will have a stimulating gathering where all attendees will be able to learn, collaborate and enjoy the strong scientific content our community has to offer. We are committed to creating an inclusive, accessible, supportive, and harassment-free environment for scientific exchange for every participant, regardless of gender, sexual orientation, disability, physical appearance, race or religion.

Before, during and after the conference, join the conversation about #DDA2021 on Twitter, make sure you tag us in your posts @australianCRS.

Dr Tushar Kumeria & Dr Khay Fong

DDA2021 Conference Co-chairs
ECR organising committee
About CRS

The Controlled Release Society (CRS) is the home for experts dedicated to delivery science, including delivery scientists, engineers, clinicians, and technical professionals. CRS members are creating the future of delivery science and technology through fundamental delivery research, development, regulatory science, and clinical translation. Research published in CRS journals and presented during the Annual Meeting & Exposition offers a breadth of scientific knowledge covering new technologies and science in the multi-disciplinary delivery field.

About the Australian Chapter

The Australian Chapter of the Controlled Release Society (AUS-CRS) was established in 2007, with the aim of providing a forum for science and education for Australian scientists with an interest in delivery of bioactives.

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CONFERENCE PROGRAM

Plenary speakers

Scientia Professor
Martina Stenzel
University of New South Wales

Associate Professor
Natalie Trevaskis
Monash University

Invited speakers

Professor
Palli Thordarson
University of New South Wales

Associate Professor
Bingyang Shi
Macquarie University

Associate Professor
Xin Zhao
Hong Kong Polytechnic University

Associate Professor
Shanta Dhar
University of Miami
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10:00 am-17:20 pm AEDT

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Dr Khay Fong and Dr Tushar Kumeria

Opening Remarks - 10:05 – 10:10
A/Prof. Amirali Popat and Dr Kara Perrow

Plenary lecture – 10:10 – 11:00
Professor Martina Stenzel

Advanced Materials in Drug Delivery

Session One: Soft Matter - Polymer
Session Chair: Dr Samantha Wade

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<td>Professor Shanta Dhar – Invited speaker</td>
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<tr>
<td>11:35</td>
<td>Ms Salma Ahmed</td>
<td>Degradable micelles and polymersomes as attractive pH-responsive platforms for chemotherapeutic drug delivery</td>
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<tr>
<td>11:50</td>
<td>Dr Nirmal Marasini</td>
<td>Development of a Self-Assembled Polymeric-methotrexate Nanomedicine for Rheumatoid Arthritis</td>
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<td>12:05</td>
<td>Ms Parisa Badiee</td>
<td>Intratumoral Delivery and Nanoformulation of an Anti-PD-1 Immune Checkpoint Inhibitor Improves its Biodistribution</td>
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<td>12:20</td>
<td>Dr Lifeng Kang</td>
<td>High resolution photopolymer for 3D printing of microneedle patches to deliver a small peptide</td>
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<td>15:05</td>
<td>Ms Weixi Wu - Engineering Asymmetric Silica Nanoparticles for Plasmid DNA Delivery</td>
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<td>15:35</td>
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09:30 am-16:20 pm AEDT

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**Welcome - 09:30 – 09:35**
Dr Khay Fong and Dr Tushar Kumeria

**Plenary lecture – 09:30 – 10:25**
A/Prof. Natalie Trevaskis

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**Advanced Materials in Drug Delivery**

**Session Three: Soft Matter - Lipid**

Session Chair: Dr Zara Sheikh and Dr Maggie Zhai

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## Session Four: Frontiers in Drug Delivery

**Session Chair:** Mr Pouya Dehghankelishadi and Ms Fatima Abid

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DRUG DELIVERY AUSTRALIA VIRTUAL MEETING  2021

SESSION THEME  SOFT MATTER - POLYMER

LIST of ABSTRACTS for ORAL SESSION
Degradable micelles and polymersomes as attractive pH-responsive platforms for chemotherapeutic drug delivery

Salma Ahmed1,2, James Humphries1,2, Craig Bell1,2, Nicholas Fletcher1,2, Pie Huda1,2 and Kristofer Thurecht1,2
1Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, QLD, 4072, Australia;
2Centre for Advanced Imaging, The University of Queensland, Brisbane, QLD, 4072, Australia.
s.ahmed1@uq.net.au

Purpose: Amphiphilic block copolymers are known for their ability to self-assemble into stable nanocarriers that can be utilised for chemotherapeutic drug delivery. Herein, we report and compare the design and synthesis of degradable, pH-sensitive amphiphilic di-block copolymers that were utilised in forming polymeric micelles and vesicles. Encapsulation of a number of hydrophilic and hydrophobic drug models within these particles is reported, with proper in vitro and/or in vivo comparisons between targeted, non-targeted, crosslinked or non-crosslinked micelles or polymersomes.

Methods: Novel MeO-PEG macro-CTA of different lengths were synthesised to mediate RAFT polymerisation of a number of hydrophobic monomers (namely, vinyl acetate (VAc), 2-methylene-1,3-dioxepane (MDO), and/or vinyl bromobutanoate (VBr)), at different hydrophilic: lipophilic balance. The synthesised amphiphilic blocks were modified with azide end-groups to allow for the incorporation of (1) Cy5 fluorophores (utilised as imaging tracers), and (2) DBCO-PEG4-DBCO crosslinkers. Solvent-switch method was used to self-assemble the blocks into micelles or polymersomes under controlled conditions. Different drugs (doxorubicin (DOX) and camptothecin (CPT)) were loaded into these NP during the self-assembly events. NP sizes, morphologies, stabilities, loading efficiencies and drug release, were assessed and confirmed, via means of dynamic light scattering, transmission electron microscopy and ultraviolet-visible spectroscopy. Targeting bispecific antibodies (α-mEGFR-α-PEG BsAb)2 were conjugated to micelles and polymersomes, and their in vitro behaviour was assessed against MDA-MB-468 breast cancer cells, by means of flow cytometry, confocal microscopy, and MTS cytotoxicity assays. CPT-loaded polymersomes and micelles were used to treat BALB/c nude mice bearing MDA-MB-468 cells, and their ability to inhibit tumour growth was assessed and compared.

Results and Discussion: Polymersomes (~100 nm), and micelles (~25 nm) had consistent sizes, whether were crosslinked or non-crosslinked, loaded with drugs or nascent. Enhanced stability and slower release rates were achieved using crosslinked NP. Targeted NP demonstrated relatively enhanced cellular association and increased drug accumulation within the cellular nuclei. CPT expressed higher cytotoxicity towards MDA-MB-468 cells than DOX, as such was used to assess the in vivo efficacy of NP. Targeted and non-targeted polymersomes showed excellent efficiency in regressing tumour volumes while significantly reducing CPT acute effect on mice health.

Figure 1. Main in vitro characterisation data of polymersomes and micelles.
Conclusions: Our micelles and polymersomes demonstrated sufficient drug loading and release rates, with micelles showing enhanced encapsulation efficiencies (%) and faster release rates. Yet, they demonstrated comparable in vitro performance to polymersomes overall. Nascent NP were safe and showed negligible toxicity; both in vitro and in vivo. Polymersomes proved to be better therapeutic candidates than both, traditional chemotherapy (free drugs) and drug-loaded micelles. Our work demonstrates the obvious advantages of our polymeric vesicles, especially in terms of their high stability, ability to encapsulate wider range of typically difficult to solubilise drugs, in addition to their excellent therapeutic profiles. These findings reinforce the great potential of polymersomes as next-generation cancer therapeutics.

Acknowledgements: NHMRC (APP1148582), ANFF, CBNS (CE140100036), RTP.

References:
r s One of the most successful long-term treatments for rheumatoid arthritis RA is the anti-cancer drug “methotrexate” MTX However MTX is highly toxic to the liver and prolonged use can result in severe liver injury in some patients. The aim of this work was to develop a MTX-conjugated PEGMA hyperbranched polymer that selectively targets rheumatoid joints and limit its exposure to the liver.

PEGMA-based hyperbranched polymers conjugated to unmodified MTX(OH) or alpha-carboxyl modified MTX(OtBu) were successfully synthesized. Approx. 50% NH2-FFK-MTX(OtBu) and 90% NH2-FFK-MTX(OH) cleavage products were released from HBP-MTX(OtBu) and HBP-MTX(OH), respectively. Growth inhibitory effects showed that addition of OtBu functionality increase the IC50 of approx 24h. Biodistribution analysis revealed that less than 5% of the injected dose of HBP-MTX(OtBu) and HBP-MTX(OH) was quantified in the liver, suggesting limited liver biodistribution as expected. Approx. 5% of the injected dose of both nanoparticles was detected after 5 days in the RA induced right knee, with no nanoparticles detected in the left control knee.

An efficient next generation MTX-based nanomedicine was developed by self-assembling of polymer-peptide-MTX conjugates. These nanomedicines avoid liver accumulation, selectively target rheumatoid joints, and provide drug release in the joints which could be beneficial for RA patients on chronic use of MTX.

Intratumoral administration of immune checkpoint inhibitors, such as programmed cell death-1 antibodies (aPD-1), is a promising approach towards addressing the low patients’ responses and high off-target toxicity associated to current systemic infusion. However, good preclinical results have not translated in clinical studies and significant off-target toxicities were still observed. It is hypothesized that the nanoformulation of aPD-1 can positively alter both their loco-regional and systemic distribution following intratumoral administration.

To test the hypothesis, an aPD-1 nanoformulation (aPD-1 NPs) was developed using a custom made 3D printed microfluidic device to produce poly(ethylene glycol)-b-poly(lactide-co-glycolide) nanoparticles that were subsequently conjugated with aPD-1. The biodistribution of the aPD-1 NPs was investigated following intratumoral injection in an orthotopic mice model of head and neck cancer. In addition, a set of comprehensive in vitro biological studies were conducted to confirm the efficacy of the aPD-1 nanoformulation in inhibiting PD-1 receptors and potentiating the cytotoxicity of T-cells against head and neck cancer cells.

Biodistribution analysis demonstrated significantly lower distribution of the nanoformulated aPD-1 in off-target organs compared to free antibodies. On the other hand, both aPD-1 NPs and free aPD-1 yielded significantly higher (more than five times) tumour and tumour draining lymph nodes accumulation than systemically administrated free aPD-1 used as the current clinical benchmark. In addition, aPD-1 NPs effectively inhibited PD-1 expression on T-cells to a similar extent than free aPD-1, and efficiently potentiated the cytotoxicity of T-cells against head and neck cancer cells in vitro by increasing the cytotoxic cytokines and enhancing cancer cells apoptosis and cell cycle arrest.

Altogether, our data warrant further preclinical investigation of intratumoral administration of immune checkpoint inhibitor nanoformulation towards reducing off-target biodistribution and associated toxicity while maintaining the oncological control afforded by this state-of-the art cancer immunotherapy.

This work was supported by the Australian Research Council Center of Excellence in Convergent Bio-Nano Science and Technology.

References:
Acetyl-hexapeptide 3 is an anti-wrinkle small peptide with poor skin permeation. 3D printing of personalised microneedles, that contour to the skin surface, offers an attractive alternative for its skin delivery. In this study, we aim to formulate a liquid resin to print microneedle patches, using two liquid monomers, namely, polyethylene glycol diacrylate (PEGDA) and vinyl pyrrolidone (VP).

PEGDA and VP were mixed at various proportions and tested for critical parameters including mechanical strength of final polymer, rate of polymerisation, rate of swelling of final polymer, 3D printing resolution and safety profile of final polymer. After optimization, a personalised microneedle patch was designed using computer aided design software and subsequently fabricated using a Digital Light Processing 3D printer.

It was shown that the VP:PEGDA ratio of 7:3 was optimal to be used as the printing resin to encapsulate the drug, i.e., acetyl-hexapeptide 3. The drug loaded into the optimal resin remained stable throughout the printing process and there was no effect on the physical properties of final print. In vitro characterisation of fabricated microneedle patch demonstrated its ability to penetrate human cadaver dermatomed skin and the microneedle remained intact after compression. The final polymer also showed minimal cytotoxicity towards human dermal fibroblast.

An optimised photopolymer resin of was developed, with adequate mechanical strength, reasonable polymerisation time and minimal cytotoxicity. High resolution 3D printing of microneedle patches was achieved using the resin.

Cellulose hydrogels with a high swelling ability are potential drug reservoirs for drug delivery to the skin. The limited wood supply has driven the use of alternative cellulose resources such as non-woody biomass as the feedstocks for hydrogel development. However, hydrogel formation and characteristics are heavily affected by the solubilising agents used e.g. alkali solvent. This study aims to develop cellulose hydrogels from oil palm empty fruit bunches (OPEFB) and to evaluate the effect of alkali solvent (NaOH and urea concentrations) on the hydrogel properties and skin permeation performance from ibuprofen (IBU)-loaded hydrogels.

Hydrolysed cellulose powder from OPEFB (2%w/v) were solubilised in different combinations of NaOH (6 – 8%w/v) and urea (4 – 6%w/v) contents before crosslinking with epichlorohydrin to form hydrogels. The gel strength of reswollen hydrogels was measured using Texture Analyser while the swelling ratio of hydrogels was determined after 24 h immersion in distilled water. Ex vivo drug permeation study was conducted using Franz diffusion cells with porcine ear skin over 12 h for hydrogels reswollen in IBU solution.

The cellulose solubility increased with NaOH concentration (Figure 1) by breaking down the inter- and intramolecular hydrogen bonding and inhibiting the reassociation of cellulose. A high NaOH concentration also improved the swelling ratio of hydrogels but gel strength decreased with NaOH content. Urea concentration did not show a major impact on these parameters. Hydrogels with 8%w/v of NaOH were excluded from the drug permeation study because of the fragile structure. Hydrogels prepared from 7%w/v of NaOH and 4%w/v of urea achieved a higher cumulative amount of IBU permeated due to a higher swelling ability.

![](image)

The cellulose dissolution and hydrogel formation are mainly affected by the NaOH concentration. Hydrogels with a higher swelling ratio showed a better drug permeation performance.

Acknowledgements: Purposely added in the paper.

References: Purposely added in the paper. All references are properly formatted according to the journal's style guide.

The authors would like to thank Universiti Sains Malaysia for the funding provided through Research University Individual (RUI) Grant Scheme with Project No: 1001/PFARMASI/8012320, Project Code: UO1822 (Reference No: 2019/0589).

LIST of ABSTRACTS for ORAL SESSION
Oral Abstract - 1

Cellular delivery of plasmid DNA (pDNA) has fostered great success in gene therapy and DNA vaccine over the past decades, where the key challenge still lies in the development of safe and efficient nano-vectors for gene transfection. Distinct from nano-vectors with symmetric morphology, asymmetric structures typically endow unique and intriguing properties benefiting cellular delivery of biomolecules. Different from conventional biomolecules, pDNA possesses rope-like structures with an only 2-3 nm in width but an overall length of several micrometers. Our previous study showed that nano-vectors designed with a spiky surface favored a higher gene transfection rate, where the nanospikes on particle surface acted as hooks to entangle the DNA loops and protected the gene molecules sheltered against nuclease degradation [1]. To our knowledge, taking this specific feature into consideration in custom-designing of next-generation nano-vectors to achieve high pDNA delivery efficacy is rarely reported. Here, we present our recent studies engineering asymmetric silica nanoparticles with either a head-tail morphology or a tailored coverage of spiky surface, which significantly promoted their interaction with pDNA molecules and boosted intracellular transfection.

Asymmetric silica nanoparticles with a head-tail morphology or a tuned spiky surface were synthesized according to our recent report [2, 3]. These particles were characterized using conventional transmission electron microscopy (TEM) and advanced electron tomography (ET) techniques. Cellular uptake and gene transfection assays were conducted in HEK-293T cells with pDNA-EGFP (expressing green fluorescent protein) formulated with these engineered asymmetric silica nanoparticles. Hemolysis assay was tested by using mice blood and the nanoparticle-cell interactions were analyzed using scanning electron microscopy (SEM). Data are shown as mean ± SD (n=3 or 6) with statistically analysis by t-tests (p > 0.05 showing no significant difference (ns), *p < 0.05, **p < 0.01, ***p < 0.001).

Asymmetric silica mesoporous nanoparticles with a head-tail morphology (HTMSNs) were fabricated, where the tail length was tuned from 33 nm to 125 nm as shown in the TEM images in Figure 1a. The asymmetric architecture was visualised in 3D using ET through reconstruction (Figure 1b). Results showed that the asymmetric morphology of nanoparticles promoted pDNA binding and cell internalization, where HTMSNs-66 with a specific tail length of 66 nm achieved the highest transfection efficiency. To trap the rope-like pDNA molecules, a spiky texture was engineered on the silica nanoparticle surface with a tailored coverage of the spiky shell (AMSNs, Figure 1c). Investigations in vitro demonstrated a positive correlation between spiky surface coverage and gene delivery efficiency (Figure 1d). Moreover, a high coverage of spiky surface also benefited a low hemolysis rate, showing good safety profile due to reduced contact between particles and red blood cells.

References
The Role of Silica Intra-wall Microporosity on Abiraterone Acetate Solubilization and In Vivo Oral Absorption

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Purpose: SBA-15 mesoporous silica (MPS) has been widely used in oral drug delivery 1; however, it has not been utilized for solidifying lipid-based formulations and the impact of their characteristic intra-wall microporosity remains largely unexplored. Here, we derive the impact of MPS microporosity on the in vitro solubilization and in vivo oral pharmacokinetics of the prostate cancer drug abiraterone acetate (AbA) when co-encapsulated along with medium chain lipids into the pores.

Methods: AbA in lipid was imbibed within a range of MPS particles with varying microporosity (0 - 247 m²/g) 2, 3. The influence of microporosity on AbA solubilization and metabolism were investigated during in vitro lipolysis studies under simulating intestinal conditions. In vivo oral PK performance was determined by dosing the formulations as suspensions to male Sprague Dawley rats via oral gavage.

Results and Discussion: Drug solubilization studies revealed that microporosity was the key factor in facilitating AbA solubilization by increasing the surface area available for drug-lipid diffusion. Interestingly, microporosity hindered hydrolysis of AbA to its active metabolite, abiraterone (Ab) (Figure 1). In vivo PK studies revealed that MPS with moderate microporosity attained highest relative bioavailability, while poor in vitro-in vivo correlations (IVIVC) existed between in vitro drug solubilization during lipolysis and in vivo AUC.

Figure 1. The influence of varying microporosity on encapsulated AbA solubilization, metabolism and bioavailability.

Our findings suggest that the drug release kinetics, metabolism, and absorption can be modified by controlling small variations in the MPS structure. The tuneable properties of SBA-15 MPS provide attractive benefits for designing intelligent lipid-hybrid systems for the oral delivery of poorly water-soluble drugs.

Acknowledgements: This work was supported by Australian Prostate Cancer (APC) grant (2021/06-QA25111).

References:
Near Infrared light assisted drug release using mesoporous Silica coated upconversion nanostructures

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Purpose: Spurred by advances in material science and nanomedicine, smart stimuli-responsive drug release systems (DRSs) are emerging as a novel nanomedicine platform for diverse diseases therapies¹. To fulfill a highly controllable and biocompatible stimuli-responsive DRS, we developed a near infrared light assisted drug release using mesoporous silica coated upconversion nanostructures.

Methods: As illustrated in scheme 1, the unique DRS consists of two key components: (i) UCNPs and photosensitizer responding to NIR light (980 nm) irradiation, and (ii) a 3D cubic mesostructured silica shell as nanocarrier. The photosensitizer was loaded into the pores together with drug molecules. At the irritation of the 980 nm light, the UCNPs and photosensitizer would be stimulated for configuration change, which would push the drug molecules out to trigger release. The release rate could be slowed down by modifying silica with protein via electrostatic interaction.

Results and Discussion: Fig. 1(a) exhibits the silica coated UCNPs nanostructures. Doxorubicin was loaded to demonstrate the applications for chemotherapy. Firstly, drug loaded mesoporous silica without protein coating were measured under the condition of with and without NIR irradiation. A significant increased release of DOX was observed upon release with NIR on compared to NIR off. The release profile is characterized as a burst release followed by incomplete release of the total loaded DOX within 6 hours (Fig. 1(b)) with NIR off, while the drug release with NIR on achieved 92% within 3 hours. The lysozyme coating slowed down the burst release of DOX and NIR light irradiation still could increase the DOX release rate with lysozyme coating.

Conclusions: We designed and fabricated a novel multifunctional DRS of AMS-6@UCNP responsive to NIR light irradiation. Drug release tests show the feasibility and feasible control of the NIR-induced drug release. The effective cancer cell suppression with the DRS demonstrates the potential controlled drug release for chemotherapy to breast cancer treatments.

Acknowledgements: We would like to acknowledge the financial support from JDRF Innovative Grant and Australia Foundation for Diabetes Research.

References:
Upconversion Nanoparticle-Mediated Lipid Delivery System for Light-Responsive Nitric Oxide and Carbon Monoxide Combination Cancer Therapy.

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Abstract

Gas therapy has gained remarkable attention within the last decade. The advent of gas-releasing molecules has remarkably increased the interest in using these molecules for therapeutic purposes driven by nano-based drug delivery systems. Herein, we present a lipid delivery system to shuttle two therapeutic gases (nitric oxide (NO) and carbon monoxide (CO)) conjugated to their respective gas-releasing molecules. Upconversion nanoparticles (UCNPs) were designed to produce photons at 360 nm upon 808 nm irradiation. These photons trigger gas release from UV-sensitive S-nitrosoglutathione (GSNO) and C30H49N3Mn(CO)3 Br CO-releasing molecule (CORM), respectively. The surface of UCNPs were modified with GSNO and DOPA lipid, and subsequently co-loaded with amphiphilic CORM into lipid bilayers to produce Lipid/UCNP/GSNO/CORM/FA, named LUGCF nanoparticles). The nanoparticles obtained had desirable particle size and zeta potential, and could release both gases in a responsive manner under 0.5 W/cm² NIR light. These gases were detected in HCT116 cells and ROS production was assessed by in vitro intracellular studies. LUGCF nanoparticle was capable of instigating apoptosis in HCT116 cells and CT26 colon cancer cells. The
combination nanoplatform offered an effective therapy for *in vitro* and *in vivo* apoptosis-inducing capability in tumours. Overall, we successfully incorporated two therapeutic gas-release molecules in one NIR light-responsive system for dual-gas cancer therapy, which has a potential for future biomedical applications.
Purpose: Global prostate cancer (PC) statistics show a significant rise in patients over the past few years. Despite several treatment options available to manage PC, the overall patient outcomes have not improved drastically. One of the primary reasons for a disheartening rate of success of therapies like chemotherapy is the non-specific drug release which results in severe side effects due to the cytotoxic nature of drugs. Here, we report nanodelivery system based on a biocompatible lipid bilayer supported mesoporous silica nanoparticles (MSN) for delivering drugs to PC.

Methods: Core-shell MSN were synthesized by a soft templating approach using a three surfactants system. The particle size and morphology were tuned by varying different synthesis parameters. Cabazitaxel drug was conjugated to silanol through an acid cleavable bond by chemical conjugation ensuring the drug release occurs only after giving an acid stimuli. The biomimetic activity was induced through lipid bilayer coating of MSN.

Results and Discussion: Spherical monodispersed MSN with particle size <160 nm were prepared. The MSN were characterized by XRD, BET and FTIR. It was found that MSN displayed a type IV nitrogen adsorption isotherm, indicating the presence of mesopores. The ordered mesostructure of the particles was confirmed by powder XRD and HRTEM analysis. SEM image reveal that the particles are spherical in shape with the diameter less than 160 nm. The prepared MSN exhibits the pore diameter of 2 nm and specific surface area of 800 m²/g. FTIR confirmed the successful grafting of levulinic acid derivative of the drug was conjugated to MSN through a hydrazone bond. The drug conjugated MSN was further coated with lipid bilayer by fusion method.

Conclusion: The current experimental plan and the design strategy have helped to synthesize highly reproducible monodispersed spherical MSN with a size <160 nm by varying the parameters such as solvent ratio and amount of templates in the synthesis process. The drug conjugated system exhibited a stimuli-responsive drug release in the acidic environment, which is significant in cancer treatment and is used to support the targeted drug delivery. This unique MSN based system will serve as a precise delivery platform for the treatment of prostate cancer.

Reference:
LIST of ABSTRACTS for ORAL SESSION
Purpose: Growing concerns of bacterial resistance against conventional antibiotics has shifted the research focus toward antimicrobial peptide (AMP)-based materials. Most AMPs kill gram-negative bacteria by destroying their inner membrane, but have to first pass the outer membrane covered with lipopolysaccharides (LPS). Their interplay with the LPS is crucial for bactericidal activity, but is yet to be elucidated in detail.

Methods: In this study, self-assemblies of Escherichia coli LPS with the human cathelicidin AMP LL-37, free and encapsulated into glyceryl monooleate (GMO) lipid nanoparticles, were analyzed using synchrotron small angle X-ray scattering (SAXS), dynamic light scattering, and cryogenic transmission electron microscopy. Circular dichroism spectroscopy was used to study modifications in the secondary structure of LL-37.

Results and Discussion: LPS was found to form elongated micelles and the addition of LL-37 induced their transformation into multilamellar structures. The addition of LPS to GMO-based cubosomes triggered the swelling of the internal cubic structure, while in multilamellar GMO/LL-37 nanocarriers, it caused transitions into unstructured particles (Figure 1). The balance of hydrophobic and electrostatic attractions between the amphiphilic LPS and LL-37 was attributed to the changes in the self-assemblies.1,2

Figure 1. SAXS profiles of the LL-37/GMO self-assemblies with XLL-37/XGMO ratio of 40/60 in the presence of different concentrations of LPS. Bragg peaks of the lamellar phase are marked with black arrows.

Conclusion: The insights on the interactions among LPS and LL-37, in its free form or encapsulated in GMO dispersions, may guide the design of LPS-responsive antimicrobial nanocarriers. The findings may further assist the formulation of antimicrobial nanomaterials with enhanced penetration of LPS layers for improved destruction of bacterial membranes.

Acknowledgements: This work was supported by the Swiss National Science Foundation through the National Center of Competence in Research Bio-Inspired Materials.

References:
The study aims at developing a cancer selective molecularly targeting permanently charged cationic lipids for the gene therapy of drug-resistant cancers.

Methods: Chemical synthesis, DLS, Gel-retardation assay, MTT assay, Western blotting, in vivo studies in syngenic B16F10 melanoma model.

We have successfully designed and synthesized a novel cationic lipid, C12M (Figure 1) which is known to specifically inhibit hLigI a DNA damage repair enzyme[1]. The cationic lipid was further formulated into liposomes with cholesterol as a co-lipid and survivin siRNA[2] is entrapped at N/P charge ratio of 4:1 and 8:1. Gel retardation assay results indicated that at 4:1 and 8:1 lipid/siRNA charge ratios the C12M liposomes completely bind siRNA. Endogenous gene-silencing study (Figure 2) indicated that either survivin siRNA or C12M liposomes alone failed to inhibit the expression levels of survivin completely, but when complexed with C12M liposomes survivin levels in PC-3 cells were significantly decreased. In vitro cytotoxicity studies (Figure 3) revealed that no toxicity is observed with liposomes alone and free survivin siRNA. In vivo studies in syngenic B16F10 melanoma model are in alignment with the in vitro data, the survivin siRNA loaded C12M liposomes suppressed the tumor growth whereas C12M liposomes or free survivin siRNA showed no significant tumor inhibition.

From the results we conclude that overexpression of survivin, an anti-apoptotic protein, is responsible for the drug resistance and C12M liposomes were efficient in transfecting anti-survivin siRNA to cancer cells both in vitro and in vivo, thus silencing the expression of survivin leading to the inhibition of tumor growth in vivo due to the anti-hLig I action of C12M molecule.

Acknowledgements: I thank CSIR and AcSIR-RMIT for the fellowship

References:
Peptide-phospholipid nanocomplexes for efficient targeted delivery of oligonucleotides

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Purpose: The development of safe and efficient gene delivery systems is highly important for successful transformation of gene therapies into clinic. In this present work, a peptide/phospholipid hybrid system for successful delivery of oligonucleotides (pDNA or siRNA) through Gastrin Releasing Peptide Receptor (GRPR) was developed.

Methods: A multicomponent peptide system, R9K(GALA)-BBN(6-14) has been synthesized successfully, using bombesin peptide (BBN(6-14), a gastrin releasing peptide receptor ligand for receptor mediated gene delivery; cationic nona-arginine (R9) to bind and condense oligonucleotides, endosomal disrupting peptide to assist endosomal release. A phospholipid oligonucleotide delivery system (1:1 1,2-dioleoylsn-glycero-3-phosphoethanolamine and 1,2-dioleoyl-3-trimethylammonium-propane) has been formulated and combined with the peptide system to investigate their effect on pDNA delivery siRNA delivery in terms of complex size, toxicity, receptor-targeted delivery and gene expression or knockdown efficiency.

Results and Discussion: This peptide/phospholipid hybrid system has demonstrated synergistic improvements in transgene expression (pDNA) and knockdown (siRNA) when compared with either (peptide or phospholipid) system alone. The optimized formulation demonstrated high levels of EGFP expression and EGFP knockdown, target specificity of the system towards GRPR, enhanced endosomal release and minimal toxicity.

Figure 1. EGFP knockdown associated with different RLP formulations in PC-3 cells.

Overall, this system demonstrated GRPR-targeted delivery, minimal cytotoxic effect, enhanced endosomal release and greater transgene expression and knockdown, thus suggesting that this system is a potential candidate for DNA/siRNA delivery.

Acknowledgements: This work was supported by Australian Research Council (ARC) discovery project grant DP130100952.

Anjuman A. Begum., Istvan Toth., Peter M. Moyle. Nanomedicine (Lond.) (2019) 14(9), 1153–1171
High-Density Lipoprotein Nanoparticles for Repurposing Simvastatin as Radiosensitiser; An in vitro-in vivo Investigation in Head and Neck Squamous Cell Carcinoma

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Purpose: Advanced radiotherapy modalities with high anatomical precision are not accessible for most head and neck squamous cell carcinoma (HNSCC) patients. Alternatively, the repurposing of already approved drugs as radiosensitisers is a promising strategy with high potential to cost-effectively boost the efficacy of standard radiotherapy ¹. In this study, we developed a high-density lipoprotein nanoparticle (HDL NP) formulation for repurposing simvastatin (SIM) and investigate its radio-sensitisation activity. This parenteral formulation is designed to address the low bioavailability associated with oral SIM formulations, a key challenge to achieve the required radio-sensitising dose in the tumour.

Methods: SIM-HDL NPs were prepared using a 3D printed microfluidic chip. The radio-sensitisation profile was characterized in the UM-SCC-1 HNSCC cell line in terms of metabolic activity, DNA damage response, apoptosis and cell cycle pattern. The radio-sensitisation properties were also assessed in cocultured HNSCC spheroids. Finally, the in vivo efficacy of SIM-HDL NPs was evaluated in a heterotopic MOC-1 HNSCC tumour bearing mice model. ¹⁸F-fluorodeoxyglucose (¹⁸F-FDG) positron emission tomography (PET) and functional analysis of immune cells in tumour tissues were performed to further elucidate the in vivo radio-sensitisation mechanisms.

Results and Discussion: Herein, we report a scalable production method for SIM-HDL NPs using a 3D printed microfluidic mixer. Litre scale amounts of SIM-HDL NPs with minimal batch to batch variation could be produced. SIM-HDL NPs effectively impaired the metabolic activity of 2D/3D HNSCC in vitro models. The SIM-HDL NPs induced metabolic disruption was comparable to that of 10- and 5-times higher doses of free drug, in 2D and 3D cultures, respectively. SIM-HDL NPs and radiotherapy combination significantly hampered tumour growth in vivo compared radiation alone control group. In vivo ¹⁸F-FDG PET imaging confirmed SIM-HDL NPs induced metabolic disruption in tumour. Analysis of tumour associated macrophages and tumour infiltrating T cells showed no significant changes in radiation induced immune response by SIM-HDL NPs radio-sensitisation.

Figure 1. Microfluidic preparation (A) and in vitro-in vivo radio-sensitisation properties of SIM-HDL NPs (B-D).

Conclusion: In this study, we successfully developed a scalable method for the preparation of SIM-HDL NPs with minimal batch to batch variation. The in vitro-in vivo profiling of radio-sensitising properties of SIM-HDL NPs showed potent effects which pave the way for next level preclinical studies.

Acknowledgements: This work was supported by the Australian Research Council Center of Excellence in Convergent Bio-Nano Science and Technology.

References:
Introduction: Chronic inflammation of the nervous system, or neuroinflammation, is a contributing factor to Alzheimer's disease and Parkinson's disease. It is characterised by enhanced secretion of pro-inflammatory cytokines and neurotoxic mediators that could impair the endothelial monolayer function and result in neurodegeneration. Phospholipid-based therapies are considered safe and have shown potential therapeutic efficacy in several inflammatory conditions. In this study, the anti-inflammatory properties of a liposomal nanoformulation of a synthetic phospholipid (1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC)) and cholesterol (referred to as UTSL) was investigated in the brain in vivo and in human endothelial cells in vitro.

Methods: Inflammation was induced via intraperitoneal administration of lipopolysaccharide (LPS) (250 ug/Kg, 7 days) to male C57BL/6 mice. Following one- or two-weeks administration of UTSL to LPS-induced animals, its efficacy was evaluated by measuring the cortex mRNA expression levels of the following cytokines and mediators interleukin (IL)-6, IL-1β, tumour necrosis factor (TNF)-α, toll-like receptor (TLR)-4, NADPH oxidase (NOX)-4 and inducible nitric oxide synthase (iNOS), using quantitative real-time polymerase chain reaction (rt-PCR). In vitro, the anti-inflammatory properties of UTSL were determined by analysing the level of inflammatory cytokine IL-6 and chemokine IL-8 in TNF-induced human cerebral microvascular endothelial cells (hCMEC/D3).

Results and Discussion: In vivo, the mRNA expression levels of all the mentioned markers in the cortex were increased following LPS administration, while treatment with UTSL decreased their expression level (P<0.05 for TNF-α, TLR4, IL-1β and iNOS). In addition, UTSL treatment resulted in decreased IL-6 level (p<0.05) in TNF-induced hCMEC/D3 cells. Taken together, the current results suggest that UTSL can alleviate the brain and endothelial cells inflammation by reducing the inflammatory markers.

Conclusions: Considering the anti-inflammatory properties of UTSL, future studies should focus on further evaluating the potential of UTSL in treatment or prevention of neuroinflammation.

References:
SESSION THEME - FRONTIERS IN DRUG DELIVERY

LIST of ABSTRACTS for ORAL SESSION
A Wild Frontier for Nanomedicines: Drug Delivery for Wildlife
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Purpose: Wildlife comprises a diverse group of animals and delivery of drugs to these populations is challenging. As highlighted by the COVID-19 global pandemic, the ability to deliver therapeutics and treat illness in wildlife is becoming increasingly important. The Australian brushtail possum (Fig. 1) is the most significant vertebrate pest in New Zealand and disrupting fertility is the most humane method of control. D-Lys<sup>6</sup>-GnRH is a water-soluble analogue of gonadotrophin-releasing hormone (GnRH), a peptide essential to reproductive function. When administered in slow-release implants, GnRH reduces fertility in brushtail possums<sup>1</sup>. Capture of this widespread, feral animal to administer implants is not feasible and leads to our research question - can nanoparticles be used as a delivery system to deliver fertility controls to the brushtail possum?

Methods: Poly(ethyl cyanoacrylate) (PECA) nanoparticles containing D-Lys<sup>6</sup>-GnRH were prepared by interfacial polymerization of water-in-oil microemulsions and characterized. In vitro release was quantified using RP-HPLC. D-Lys<sup>6</sup>-GnRH-loaded nanoparticles were administered directly into the gut of brushtail possums. The concentration of luteinizing hormone (LH) in plasma was quantified using a radioimmunoassay to indicate fertility status.

Results and Discussion: Encapsulation efficiency of D-Lys<sup>6</sup>-GnRH was high with 95 ± 4.1% of the peptide entrapped. PECA nanoparticles released approximately 60% of D-Lys<sup>6</sup>-GnRH after incubation in brushtail possum plasma following an initial burst release of 20% (Fig. 2). Following i.v. administration of D-Lys<sup>6</sup>-GnRH, LH serum levels increased within 15 min and was dose dependent. Importantly, D-Lys<sup>6</sup>-GnRH-loaded nanoparticles resulted in a significant biological response to reduce fertility in vivo.

Conclusions: We have demonstrated that nanomedicines have application for the delivery of bioactive agents to wild animals.

References:
To investigate the potential of liquid crystalline nanoparticles (LCNP) to improve the antimicrobial activity of the photoactive antimicrobial agent gallium protoporphyrin (GaPP) against bacterial biofilms.

LCNP loaded with GaPP were fabricated by the hydrotrope dilution method. The influence of LCNP on the ability of GaPP to generate ROS was evaluated using uric acid as a chemical probe. The antibacterial activity of the formulation was tested against two antibiotic-resistant strains of *Staphylococcus aureus* (*S. aureus* 25923 and MRSA USA 300) in planktonic and biofilm modes of growth, respectively. Following 1 h pre-incubation with LCNP-GaPP, photoactivation was performed using mounted LED at 405 nm, total irradiance (0.013 W/cm²) for 1 min and 2 min against planktonic and biofilm modes, respectively. The viability of bacteria following the treatment was determined by enumeration of colony forming units. Finally, the biosafety of photoactivated GaPP-LCNP against dermal fibroblasts was evaluated by the MTT assay.

Antimicrobial photodynamic therapy (aPDT) has emerged as an innovative strategy to combat antibiotic resistant microbes, yet aPDT efficiencies against biofilms are rarely reported and not optimized [1]. LCNP are smart, biocompatible and triggerable delivery system, that can promote efficient biofilm delivery and activity of photosensitizers [2]. GaPP was successfully entrapped in LCNP (size 184 ± 2.7 nm), with 3.3% GaPP load and encapsulation efficiency of 99.4 ± 2.3%. Entrapment of GaPP in LCNP improved the photodynamic activity by 72% based on uric acid quenching data. The improved photodynamic activity correlated with the antibacterial activity as GaPP-LCNP completely eradicated both *S. aureus* and MRSA in planktonic mode of growth. Furthermore, LCNP improved the antibiofilm activity of GaPP by 2-fold, reducing the viability of *S. aureus* and MRSA by 8 and 5 log₁₀ compared to 4 and 2.5 log₁₀ by unformulated GaPP (DMSO solution), respectively. On the other hand, the biocompatibility of GaPP-LCNP was confirmed as neither photoactivated GaPP nor GaPP-LCNP showed any reduction in the viability of dermal fibroblasts within the effective concentration range against biofilms.

Figure 1. Total viable cells of *S. aureus* following treatment with GaPP & GaPP-LCNP both in planktonic and biofilm mode.

LCNP promoted the photodynamic activity of GaPP and enhanced its delivery to antibiotic resistant *S. aureus*. GaPP-LCNP showed a positive safety profile in dermal fibroblasts and promoted cell proliferation.

### References


Encapsulin Protein Nanocages: Emerging Tools for Nanotherapies

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Introduction: Encapsulins are a new class of prokaryotic protein nanocages with a distinct set of functional features that make them attractive platforms for targeted therapies1. They self-assemble from identical protein subunits into hollow spherical nanoparticles (≤ 45nm) that exhibit good colloidal properties, robust stability, and excellent biocompatibility2. Moreover, encapsulin surfaces can be genetically and/or chemically modified, permitting the attachment of functional moieties (e.g. fluorophores, drugs, antigens). A key feature of encapsulins is their ability to selectively self-assemble around cargo proteins tagged with an encapsulation signal peptide (ESig). This mechanism can be co-opted to load foreign cargo into encapsulins, reprogramming their functionality for diverse practical applications3. Here, I will share our recent work engineering encapsulins to mediate the delivery of protein photosensitizers for in vitro photodynamic therapy (PDT), and our efforts to unravel the dynamic in vivo behavior of encapsulins.

Experimental Methods: Using genetic engineering, encapsulin (Enc) was loaded with ESig-tagged mini-Singlet Oxygen Generator (mSOG), a protein photosensitizer that produces toxic ROS under blue-light irradiation. The resulting miniSOG-loaded Enc (mSOG-Enc) was recombinantly produced in E. coli and purified by chromatographic methods. mSOG-Enc was biophysically characterised by microscopy (TEM), spectroscopy, and protein gel analysis. Light-induced ROS generation from mSOG-Enc was quantified using a ROS-sensitive optical probe. Next, the uptake of mSOG-Enc by A549 lung cancer cells was visualised via fluorescent microscopy. The effect mSOG-Enc (with and without light-activation) had on the cells was assessed by measuring intracellular oxidative stress and cellular viability with a ROS sensor and MTT assay, respectively. Concurrently, BALB/c mice were injected with dye-labelled empty Enc, and the in vivo biodistribution of Enc imaged over 24h.

Results and Discussion: Our results showed that Enc stably packaged mSOG, and mSOG-Enc generated significant amounts of ROS under blue laser light. In contrast to free mSOG, Enc-mSOG was observed entering and accumulating inside lung cancer cells. Internalized Enc-mSOG was successfully activated with blue-light to generate ROS, inducing intracellular oxidative stress which significantly decreased tumour cell viability i.e. in vitro PDT (Fig. 1). Moreover, in vivo imaging indicated that Enc slowly accumulates within the liver over a 24 h period, however, no adverse effects were observed in mice following Enc administration (Fig. 2).

Conclusions: The natural protein encapsulation system of Enc was co-opted to instead package and deliver a functional protein photosensitizer into lung cancer cells, enabling in vitro PDT. Preliminary in vivo data shows, that while Enc is safe, it localizes within the liver, suggesting recognition and clearance by the immune system. Our current work aims to better understand the in vivo behavior of Encs (e.g. immunogenicity) and to use this new information to further engineer these promising nanocages for drug delivery and vaccine applications.

References:
1. Sandra F., Khaliq NU., Sunna A., Care A. Nanomaterials. 2019 (9) 1329.
The blood brain barrier (BBB) and blood tumour barrier (BTB) remains a major obstruction for delivering therapies to treat brain cancer. Amongst the various brain cancers, glioblastoma (GBM) is the most difficult to treat. Treatment of GBM is challenging due to difficulty delivering chemotherapeutic drugs across the BBB and into the tumour microenvironment. Consequently, GBM has high rates of tumour recurrence and poor patient survival. So far, only limited numbers of chemotherapy drugs are available that can cross the BBB to treat GBM. Nanomedicines have potential to overcome both BBB and BTB especially if they have the ability for tumour homing and meet the small uniform particle size (<40 nm) restrictions and large loading capacities. Silica nanoparticles are an attractive solution for treating GBM, with many clinical trials demonstrating its safety and versatility.

Nanoparticles were developed using one-pot, biphasic sol–gel method. Physiochemical properties such as particle size, surface charge and porosity were characterised. Efficacy of nanoparticle delivery platform across BBB and GBM were analysed using 2D and 3D tumour models (Figure 1).

In this study, we report the first-time synthesis of ultra small, large pore silica nanoparticles (USLP) which are; 1) synthesised at room temperature, 2) reproducible and uniform small size of ~30 nm and, 3) large porous structure (>7 nm). This is the first study, that reports loading high concentrations of chemotherapeutic drug doxorubicin and the large protein lactoferrin (80 kDa) as a tumour targeting moiety using silica nanoparticles. Through systematic studies we show:

- USLP can efficiently penetrate in vitro BBB and efficiently internalise into 3D GBM spheroid model
- USLP- delivery systems can be used to deliver doxorubicin to GBM in vitro in both 2D and 3D models.

This work highlights the use of novel ultra small-large pore silica nanoparticles to significantly improve the utility of chemotherapeutic drugs such as doxorubicin, which cannot otherwise cross the BBB and thereby improve penetration of chemotherapy deep into the tumour. This delivery platform can be expected in future to improve survival and quality of life of patients with brain cancer and other CNS disorders.
Development of a Pharyngeal Air-Liquid Interface Cell Model to study Drug Transport

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Abstract Summary: An in vitro air-liquid interface (ALI) model of the immortalized pharyngeal cell line Detroit 562 attains epithelial barrier integrity after 18 days in ALI culture, at an optimum seeding density of \(1.8 \times 10^5\) cells/cm\(^2\). This model could be used to study drug transport and predict the therapeutic efficacy of oropharyngeal deposition of drugs and toxins.

Introduction: An in vitro air-liquid interface model (ALI) of human respiratory epithelial cell lines is an invaluable tool that phenotypically mimics the in vivo airway epithelium and is extensively used to study drug transport and predict therapeutic efficacy [1]. Although several studies have utilized the human pharyngeal cell line Detroit 562 using the ALI model, no studies have yet been performed to optimise the ALI culture conditions and determine whether the ALI model could be used to study drug transport. Therefore, this study aims to determine the appropriate in vitro ALI culture method required to establish the epithelial barrier properties of the Detroit 562 cell line and investigate drug transport using local anaesthetic throat spray, Lidocaine as a model drug.

Methods:

ALI model

To establish an in vitro ALI model of Detroit 562 cells, transwell cell culture inserts were used as previously described [2]. To determine the appropriate seeding density for Detroit 562 cell line, three different seeding densities were chosen: 30,000 cells/well (c/w) (0.9 \(\times 10^5\) cells/cm\(^2\)), 60,000 c/w (1.8 \(\times 10^5\) cells/cm\(^2\)) and 80,000 c/w (2.4 \(\times 10^5\) cells/cm\(^2\)) in between passages 51-57. Briefly, Detroit 562 cells were seeded within the apical chamber in MEM media supplemented with 10% v/v FBS and the same media was added to the basolateral chamber and incubated at 37 °C with 5% CO\(_2\) for 24 hours (h) until confluency was achieved. To initiate ALI conditions, media in the apical chamber was removed after 24 h indicating Day 0 and the cells were maintained under ALI conditions for 21 days. Differentiation media in the basolateral chamber was replaced every 2 days. All experiments were performed on Day 7, 14, 18 and 21 post ALI induction.

Transepithelial Electrical Resistance (TEER)

Re-warmed media was added to the apical chamber, allowed to equilibrate for 30 mins at 37 °C under 5% CO\(_2\) and TEER was measured using EVOM2® epithelial voltohmmeter (Sarasota, FL, USA) attached to STX-2 chopstick electrodes for the ALI cultures, corrected by subtracting the blank inserts, and multiplied by the area of the Transwell inserts (0.33 cm\(^2\)).

Sodium Fluorescein Permeability Assay

Sodium fluorescein (2.5 mg/mL) was added to the apical chamber and pre-warmed HBSS was added to the basolateral chamber and then incubated for 4 h at 37 °C with 5% CO\(_2\). Basolateral samples collected every 30 min for the first 2 h and then every hour for the final 2 h. For analysis, the collected basolateral samples were diluted (1:20 v/v) and fluorescence was measured using the SpectraMax M2 plate reader (excitation: 485 nm; emission: 538 nm).

Transepithelial Electrical Resistance (TEER)

Prior to the analysis, the data was normalized to 100% at Day 0 values. The data was fitted to a two phase exponential model with a decreasing phase to determine the rate constant and half time for TEER reduction. The fitting was done by using a non-linear least squares regression (Levenberg-Marquardt algorithm) to minimize the sum of squares of the differences between observed and predicted values.

Sodium Fluorescein Permeability Assay

The permeability of sodium fluorescein was determined by measuring the fluorescence of the basolateral compartment. The fluorescence was measured at 530 nm and the permeability coefficient was calculated using the following equation:

\[
P_d = \frac{\text{flux}}{\text{concentration gradient}} = \frac{\text{AUC}_{basolateral}}{\text{AUC}_{apical}}
\]

The permeability coefficient was determined for each condition and compared to the control condition.

All results are reported as mean ± standard error of the mean (SEM) and statistical significance was determined using one-way ANOVA followed by Tukey’s post-hoc test. Differences were considered statistically significant at \(p < 0.05\) for all comparisons.
**Transport of Lidocaine across Detroit 562 ALI culture**

Lidocaine transport across the Detroit 562 ALI cultures was conducted on Day 18 post ALI formation. Samples 100 µL were taken from the basolateral chamber every 30 min for the first 2 h and then every hour for the final 2 h, with samples being replaced by fresh, warm HBSS. After the 4 h assay, the apical chamber was washed twice with HBSS to collect any residual drug using a pipette denoted as On and the cell layer was then scraped from the insert membrane and lysed using CelLytic buffer Invitrogen to quantify the amount of drug inside the cells denoted as In. All the samples were subsequently analysed using a High-Performance Liquid Chromatography HPLC system equipped with SPD-20A UV–Vis detector, an LC-20AT liquid chromatograph, a SIL-20A HT autosampler Shimadzu and a Phenomenex Kinetex C-18 column 250 × 4.6 mm, 5 µm, Torrance, California, USA, according to a validated method [3].

**Statistical analysis**

All results are expressed as mean ± standard error of the mean (SEM) of at least three biological replicates. Statistical software, GraphPad Prism (version 8.2.1) was used to test for significance using One-Way or Two-Way ANOVA for each experiment. Significance was determined as p < 0.05.

**Results and Discussion:**

TEER measurements and permeability of the known paracellular marker flu-Na were tested at days 7, 14, 18 and 21 of the ALI culture period. TEER values significantly increased from Day 7 to 18 with no significant changes in TEER values between Day 18 and 21 for all the 3 densities, thus indicating progressive tight junction formation till Day 18 of ALI culture period (Figure 1A). Correspondingly, a significant decrease in apparent permeability (Papp) of flu-Na was observed at Day 18 compared to Day 7 and 14 for 60,000 c/w and 80,000 c/w ALI models, but not for 30,000 c/w (Figure 1B), suggesting that the cell layers have developed functional tight junctions in ALI culture at Day 18 for those two seeding densities. Therefore, the two densities 60,000 c/w and 80,000 c/w are termed as Low and High density, respectively, for the subsequent experiments.

![Figure 1](image_url)

Figure 1. Air-liquid interface (ALI) culture model of Detroit 562 cell line over a culture period of 21 days. A. Transepithelial electrical resistance measurements (TEER) and B. Apparent permeability (Papp) of sodium fluorescein (flu-Na) across Detroit 562 ALI cultures at Day 7, 14, 18 and 21 of ALI culture period (n = 3, mean ± SEM) * p < 0.05, ** p < 0.01, *** p < 0.001 **** p < 0.0001 (using two-way ANOVA with Tukey’s post-test).

A transport study was conducted to determine if the developed ALI models of Detroit 562 cells could be used to study drug transport, using Lidocaine as a model drug on Day 18 post ALI. The cumulative mass of lidocaine transported increased with time for both Low and High density with no significant differences between the two densities Figure 2A. At the end of the transport study, no lidocaine was found inside the cells shown as In,
Figure 2B for both the densities, suggesting that lidocaine may have been transported predominantly through the paracellular route.

![Graphs](image)

Figure 2. Transport of lidocaine across ALI models of Detroit 562 cells on Day 18 of ALI culture period. A Cumulative mass of Lidocaine transported over 4 h period at Low density (grey circles) and High density (black circles) cells of Detroit 562 ALI models. B Percentage of the total mass of lidocaine transported across the ALI cultures (shown as Transported), remaining on the apical layers (On) and inside the cells (In) in Low (grey bars) and High density (black bars) cells at the end of the experiment (4 h).

**Conclusions:** The present study indicated the suitability of the Detroit 562 cell line at a seeding density of 60,000 cells/well \((1.8 \times 10^5 \text{ cells/cm}^2)\) as a representative in vitro air-liquid interface cellular model to study drug transport on Day 18 of the ALI culture period. Future studies should focus on characterizing these cellular models by immunostaining with markers of tight junction proteins and determine differentiating features such as mucus production and response to inflammatory stimuli.

**References:**


Influence of PEGylated porous silicon nanoparticles on permeation and efflux of an orally administered antibiotic

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INTRODUCTION

Porous silicon nanoparticles (pSiNPs) have demonstrated excellent properties to enhance the stability of drugs in the gastrointestinal (GI) tract, improved thermostability, and enhanced oral bioavailability and therapeutic efficacy of both hydrophilic and hydrophobic drugs [1]. Meropenem (MER) is one of the last resort antibiotics and is only available in the parenteral formulation because of low oral bioavailability and an inability to develop any commercially viable formulations. MER has different challenges related to its oral delivery such as gastric degradation and low drug permeability due to p-gp efflux pump [2]. Research into hydrophilic, poorly permeable, and unstable drugs such as MER has largely been ignored and focus is always given to hydrophobic drugs while the hydrophilic drugs still pose a significant drug delivery challenge. Herein, MER-loaded PEGylated porous silicon nanoparticles (TPGS-pSiNPs) were tested for their in-vitro drug permeation across the Caco-2 monolayer system to modulate transmembrane efflux pumps for efficient oral delivery of MER.

EXPERIMENTAL METHODS

Caco-2 cell model was used for permeability study. Caco-2 cells with TEER ≥ 500 Ω cm² were recorded within 6 days, which is an indicator of monolayer development. To investigate the permeability (A to B), the medium from each well was replaced with preheated HBSS and equilibrated at 37 °C for 20 min. After that, the dosing (A) chamber was replaced with 200 μg/mL of MER alone or an equivalent concentration of MER-TPGS in HBSS and 1.5 mL of HBBS only in receiving chamber (B). Samples (0.2 mL) were taken out from the receiving chamber after 3 and 6 h. The volume of the receiving chamber was maintained constant by replacing the withdrawn samples with a similar volume of HBSS. FTIR and XRD studies were performed for the characterization of the MER-pSi-TPGS nanosystem.

RESULTS AND DISCUSSION

The electron microscopy (Figure 1a) and FTIR results showed that the pSi particles were successfully functionalized with PEG. FTIR data (Figure 1b) further confirmed the successful loading of the MER into the pore of pSi-TPGS. The disappearance of the sharp drug crystal peaks in XRD data indicates that MER loaded onto pSi-TPGS existed in amorphous form (Figure 1c). The loading capacity of the pSi-TPGS, as determined by UPLC, was approximately 27 wt %.

We assessed the in-vitro permeability of MER across the Caco-2 monolayer culture model. The data showed that MER permeability was improved significantly using pSi-TPGS in comparison to the pure MER (Figure 1d).

Figure 1. (a) Transmission electron microscopy image of MER-pSi-TPGS, (b) FTIR of pSiNPs, pSi-NH₂, MER and, MER-pSi-TPGS (c) X-Ray Diffractogram of pSi NPs, pSi-NH₂, MER and, MER-pSi-TPGS. (d) In-vitro permeability (Papp) of MER across Caco-2 monolayer culture using pure MER and MER-pSi-TPGS formulation.

CONCLUSION

This study demonstrated that PEGylated MER loaded pSi NPs presents high loading capacity and an innovative approach for efficiently delivering hydrophilic drugs with efflux pumps mediated restricted intestinal permeability.

REFERENCES


The purpose of this study was the development of pH and thermo responsive mesoporous nanocarriers for the encapsulation and delivery of doxorubicin (DOX) anticancer drug for improved anticancer activity against liver cancer cells.

Pure iron oxide (IO) nanoparticles were synthesized using a chemical coprecipitation method. SBA-15 (S15) was prepared by using tri-block copolymer Pluronic P123 (P13) as a structure-directing agent. Tetraethoxysilane was added as a silica source. The final IOS15 nanocomposites were calcined at 550 °C for 6 h. pH and thermos sensitive Pluronic F17 (PF) was used to modify the surface of IOS15. The obtained nanocarriers were loaded with DOX. Several characterization instruments were used to further analysis the properties of the DOX loaded nanocarriers.

The prepared nanocarriers were superparamagnetic with saturation magnetizations of IOS15 and IOS15@PF being 76.3 and 72.1 emu/g, respectively. Small-angle neutron/X-ray scattering (SANS/SAXS) studies displayed that the prepared nanocarriers are temperature-responsive and contain hexagonally arranged microstructures. The integral above theta averages the form factor over all possible orientations of the cylinder with respect to \( q \). The core radius = \( R_p \), core length = \( H_p \) (the mean core radius is \( R_0 \)). It can be seen that the diffraction peak at approximately \( q = 0.07 \) Å\(^{-1}\) corresponds to the scattering of cylindrical mesopores of radius \( R_p = 7 \) nm (standard deviation \( \sigma_p = 0.2 \) nm), thickness \( H_S = 128.1 \) Å (standard deviation \( \sigma_p = 0.9 \) nm), and length \( H_p = 359.3 \) Å (standard deviation \( \sigma_p = 61.3 \) nm), with an ordered 2D hexagonal structure. The results are consistent with the BET analysis, which underscored that H1 hysteresis is a typical feature for cylindrical microporous materials. Cell viability studies confirmed that nanocomplexes induced more apoptosis or necrosis. A temperature (69% release after 48 h) and pH (70% release after 48 h)-dependent DOX release was observed, whereby more DOX was released at a high temperature of 42 °C and pH value of 5.4. At 48 h, considerable toxicity effects were observed compared with 24 h, and this occurrence may be the result of a relatively long period of treatment. Compared with free DOX, IOS15@PF@DOX-AMF nanocarriers revealed stronger in vitro apoptosis and anti-proliferation of cancer cells, making them suitable for future in vivo studies.

The DOX-loaded nanocarriers successfully induced cell apoptosis or necrosis. The dual-responsive nanocarriers under combinative pH and temperature conditions could prompt more drug release, thus making the nanocarriers promising anticancer drug candidates for clinical applications.

References:
DRUG DELIVERY AUSTRALIA VIRTUAL MEETING  2021
List of Abstracts

POSTER SESSION ONE
Poster Theme: INORGANIC
Optimization of Mesoporous Shell on Iron oxide Nanoparticles for Theranostics application

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Purpose: Theranostics is a fast-evolving strategy that integrates therapeutics and diagnostics into a single package for an accurate and targeted treatment. In this study, mesoporous silica coated iron oxide nanoparticles (IONPs) were fabricated for multi-modal imaging that includes MRI and photoacoustic modalities, while the mesopores will enable loading and release of a therapeutic payload. The biocompatible mesoporous shell is made from inorganic mesoporous silica due to its unique properties such as large surface area, low density, surface functionalization, high drug loading capacity, tunable morphology, size, and incorporation of multifunctional core material.

Methods: Iron Oxide nanoparticle (IONP) was selected as core and synthesized via co-precipitation method\textsuperscript{1}. Briefly, Iron (II) and Iron (III) chloride solutions were combined in basic \textit{pH} solution, centrifuged, washed, and dried to obtained IONPs. Next, to prepare mesoporous silica shell, two different sol-gel synthesis methods (A\textsuperscript{2} & B\textsuperscript{3}) were utilized yielding nanoparticles with different size and dispersity. Furthermore, these synthesized nanoparticles were characterized using dynamic light scattering (DLS), transmission electron microscopy (TEM) techniques.

Results and Discussion: The size of synthesized IONPs were found to be 10-20 nm in range (Figure 1a, 1d). The mesoporous silica coated iron oxide nanoparticles were synthesized successfully and was found to be in the range of 100-110 nm for Method A and 190-220 nm for Method B, characterized using (TEM) (Figure 1b, 1d). Furthermore, these fabricated nanoparticles were responsive towards magnetic field (Figure 1c).

Figure 1: TEM images of a) IONPs, b) Core shell nanoparticles, and c) magnetic properties and d) a table summarizing the TEM and DLS particle size of the core-shell NPs prepared using the two methods.

Conclusions: In this study, mesoporous silica-coated iron oxide nanoparticles were systematically synthesized with controlled size and retained their magnetic properties after mesoporous shell coating. This research provided a platform for theranostics application with a modular approach on mesoporous silica shell architecture, which can be a reservoir for therapeutic payloads. MRI imaging, while thorough characterizations are currently underway.

Acknowledgements: The authors acknowledge the Bedegal people on whos land UNSW-Kensington campus is located. We also acknowledge the MWAC and BRIL facilities at UNSW for access to the microscopy and bioimaging equipment.

References:
Title: Lipid Coated Mesoporous Silica Nanoparticle Mediated Brain Targeted Delivery of Berberine: Preparation, Characterization, In vitro and In vivo Evaluation

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Introduction: Drug delivery to the brain is limited due to the high selective permeability of blood brain barrier (1). This study was aimed to deliver berberine (a benzylisoquinoline alkaloid having low bioavailability, F = 0.68%) via lipid coated mesoporous silica nanoparticles (L-MSNs) to accomplish better anti-amyloidogenic effects in Alzheimer’s disease (AD).

Methods: In the first step, MSNs were synthesized by modified Stober’s method followed by drug loading (2). In the second step, liposomes were synthesized followed by lipid coating of drug loaded MSN particles by ultrasonication with liposomes. The liposomes were prepared by film hydration method (3). The physicochemical characterization of blank L-MSNs and berberine loaded L-MSNs (BBR-L-MSNs) was executed by evaluating entrapment efficiency, particle size, zeta potential, FT-IR, PXRD TEM, HR-TEM, SEM and in vitro release study. The BBR-L-MSNs were also evaluated for ex vivo and in vivo pharmacokinetics study in Wistar rats.

Results: The mean particle size of L-MSNs and BBR-L-MSNs were 50-55 nm and 90-95 nm, respectively. The average entrapment efficiency of BBR-L-MSNs was 88%. In vitro release studies indicated early flow, followed by a slow lasting and constant release (biphasic pattern). BBR-L-MSNs were identified as less hemolytic compared to BBR through hemolytic studies. Low thioflavin T fluorescence of BBR-L-MSNs in contrast to L-MSNs and BBR indicates its anti-amyloidogenic effect. Percent AChE (acetylcholinesterase) inhibition (p<0.05) values were 82 ± 4.98% (BBR- L-MSNs), 32 ± 3.25% (BBR) and 42 ± 4.25% (L-MSNs). Expression of amyloid-β and BACE-1 proteins showed down regulation in the expression of BBR-L-MSNs group as compared to the scopolamine induced AD mice model. Plasma drug concentration AUC₀-2₄ (ng h/ml) for BBR-L-MSNs were prolonged (2550.54 ± 0.432) as compared to pure BBR solution.
BBR-L-MSNs raised the maximum plasma concentration ($C_{\text{max}}$) and dropped the time to reach maximum plasma concentration ($T_{\text{max}}$) contrary to BBR. The promising outcome of BBR-L-MSNs indicated enhanced BBR delivery in Wistar rat’s brain (almost 2 folds).

**Conclusion:** L-MSNs effectively improved the bioavailability of BBR and can be suggested as a promising carrier in brain drug delivery for the therapeutic agents with low bioavailability.

**Acknowledgements:** The authors are thankful to the Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi and Central Instrument Facility Centre, Indian Institute of Technology (Banaras Hindu University), Varanasi for providing research facilities.


**Presenter biography:** Anurag Kumar Singh is a doctoral research scholar at the Centre of Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India. He earned M. Pharm degree from the School of Chemical Sciences and Pharmacy, Central University of Rajasthan, Ajmer, Rajasthan, India. His scholarly interests range widely to develop novel nano-particulate systems for brain targeted drug delivery. He has published several research papers in various peer reviewed National and International journals.

**Learning Objectives**
Understand the process used to synthesize lipid coated mesoporous silica nanoparticles as a promising drug carrier for brain delivery.
Understand the effect of various formulation variables on the performance of lipid coated mesoporous silica nanoparticles as drug carrier.
Explain various evaluation parameters for brain targeted drug delivery nanocarriers.
Silicon microparticles for enhanced topical delivery
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Purpose: Managing various skin disorders at the site of action results in high treatment efficacy and eliminates off-target effects. Localized topical delivery into the skin is quite challenging, specifically for large therapeutic molecules, due to the strong barrier properties of the skin. In this study, we report silicon microparticles that can be applied by massaging onto the skin to create perforations for the diffusion of loaded therapeutics into the skin.

Methods: Silicon microparticles were prepared by controlled milling of reduced silica microparticles and characterized by X-ray diffraction analysis, and electron microscopy. The microparticles were evaluated for surface area, in vitro degradation, and in vitro release. Cytocompatibility was assessed using immortalized human keratinocyte cells and penetration into excised human skin was examined by confocal and photoacoustic imaging.

Results and Discussion: High surface area rough microparticles featuring porous surface were produced. The rough silicon microparticles had a surface area of over 150 m²/g and showed a biphasic degradation behavior with 30% degradation over 30 days. Different-sized fluorescent dyes loaded onto the particles showed burst release followed by slow sustained release for up to 24 days. Cytocompatibility studies showed no effect on cell viability and no induction of inflammatory response in keratinocytes. Confocal and photoacoustic imaging in excised human skin demonstrated that silicon microparticles penetrate the stratum corneum upon simple massaging.

Conclusions: The work presented here reports biocompatible silicon microparticles as promising biodegradable topical delivery-enhancing carriers for managing various skin diseases. Ongoing work is focused on testing the efficacy of therapeutics-loaded silicon microparticles using in vitro and ex vivo models.

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Poster Theme: LIPIDS
The chemotherapeutic drugs delivery to cancer treatment via developing multifunctional lipid nanoparticles

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Abstract summary: Lipid-based cubosomes have emerged as a novel material for the next generation of nanomedicine. In this work, we successfully synthesized smart lipid-based cubosomes, these can respond to tumour microenvironment: low pH and high hydrogen peroxide (H₂O₂) concentration. The results showed that these cubosomes have desirable particle size, high drug loading capacity, excellent mesophase changes under different conditions, sustained release characteristics, high inhibitory effect to the proliferation of AGS and L929 cancer cells.

Introduction: Effects are being made to develop ‘intelligent’ nanocarriers, such as cubosomes, liposomes and hexosomes, by synthesizing and using stimuli responsive materials. Among these lipid nanoparticles, cubosomes are receiving much attention due to the high loading capacity, enhanced bioavailability, long circulation time and the ability to encapsulate both hydrophilic and hydrophobic drugs¹. It is worth noting that tumour sites have low pH value (around 5.5-6.5)², and high H₂O₂ concentration (100 uM) when compared with normal cells and healthy tissues³. Nanoparticles that respond to pH and H₂O₂ stimuli could provide improved delivery of anti-cancer drugs into cancer cells with a controlled rate⁴.

Purpose: We synthesized and used two novel amphiphilic block copolymers (ABCs) to stabilize monoolein-based cubosomes loaded with paclitaxel. These ABCs contains one or two responsive groups: poly(2-(dimethylamino) ethyl methacrylate) (PDMAEMA) and/or poly(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) benzyl acrylate) (PTBA). Our hypothesis is that these ABCs will act as steric stabilizers to make cubosomes and provide pH and/or H₂O₂ responsiveness for enhanced paclitaxel (PTX) delivery to tumour sites.

Experimental methods: Cubosomes loaded with PTX were prepared by sonication method, and cubosomes were characterised by dynamic light scattering to measure particle size, polydispersity (PDI) and surface charge, small angle X-ray scattering for mesophase and structure identification, drug loading, encapsulation efficiency, in vitro drug release and in vitro cytotoxicity.

Results and Discussion: High throughput small angle X-ray scattering studies demonstrated that the synthesized ABCs could simultaneously stabilize cubosomes and provide internal particle nanostructure responsiveness to changes of H₂O₂ concentration and pH. The particle size of all samples was less than 300nm. The PDI of them was around 0.25. The encapsulation efficiency of them was in the range of 50-65%. Additionally, in drug release study, cubosomes presented a desirable sustained release behaviour (Figure 1). And in vitro cytotoxicity study indicated that these nanoparticles could inhibit the proliferation of AGS and L929 cancer cells in a concentration-dependent manner. Nanoparticles with a pH and H₂O₂ responsiveness as described in this study may be useful as drug delivery carriers for the treatment of cancers.

![Figure 1: In vitro drug release of PTX-loaded nanoparticles in pH 5.5, pH 7.4 and 50 mM H₂O₂.](image)

Conclusion: In this work, lipid-based cubosomes with stimuli responsive features had been successfully synthesized and characterised by the measurement of particle size, PDI, encapsulation efficiency, in vitro
drug release and in vitro cytotoxicity. Based on these results, lipid-based cubosomes with stimuli responsiveness as described in this study may be useful as drug delivery carriers for the treatment of cancers.

Acknowledgements: We thank Dr. Bo Fan and Prof. San H. Thang from Monash University for providing the ABCs and their general discussion.

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Biocompatible ionic liquids as designer solvents for the formation of non-lamellar lyotropic liquid crystalline nanoparticles as drug delivery vehicles

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Abstract summary: The interest in lyotropic liquid crystalline nanoparticle (LCNPs) as drug delivery vesicles continue to grow owing to their unique structural features. Controlling the structure of these particles by rational design to suit specific applications is paramount. Ionic liquids (ILs) are designer solvents known to support the self-assembly of amphiphiles, and have been used as designer solvents for LCNPs. We employed small angle X ray scattering (SAXS) to examine the phase behavior of monoolein-based ionic liquid-water solvents. Particles with a variety of nanostructures were obtained by altering the anions in the ILs. This study will help elucidate the role of solvent design on these particle’s characteristics and functionality.

Introduction: Ionic liquids (ILs) have emerged as a remarkable class of green solvents with unique characteristics, and feasible task-specific tailoring of their properties. The application of ILs has extended to facilitate amphiphile self-assembly. ILs not only support the self-assembly of amphiphiles, they can also be used as designer solvents1. Lipid amphiphiles can assemble into a wide range of lyotropic liquid crystalline mesophases possessing unique highly ordered multidimensional structures. The bulk phases can be further broken into nanoparticle dispersions (LCNPs), for examples cubosomes and hexosomes, that are characterized by their high surface to volume ratio. These particles are receiving growing interest due to their great potential as drug delivery vehicles for both hydrophilic and hydrophobic drugs2. The structure-function relationship of non-lamellar LCNPs is still an area of tremendous interest as the internal nanostructure of these particles influence many factors such as stability, drug loading, drug release/diffusion, cytotoxicity, and pharmacokinetics/biodistribution3-4. Therefore, tuning the internal mesophases of non-lamellar LCNPs is a real need for rational design to suit specific applications.

Purpose: We aimed to investigate the phase behavior of Monoolein (MO)-based LCNPs in choline amino acid ionic liquids (ILs)-water solvents using a combinatorial high throughput synthesis and characterization method. A total of 14 choline ILs were added to LCNPs formulations to investigate whether using anions with different characteristics (side chain structure, charge, pKa, hydrophobicity) can induce changes in the internal structure of MO LCNPs.

Methods: High throughput synchrotron small angle X ray scattering (SAXS), cryogenic transmission microscopy (cryo-TEM), and dynamic light scattering (DLS) were employed to analyze the internal nanostructure, and the size of the nanoparticles.

Results and Discussion: We examined the internal nanostructures of MO LCNPs in 14 choline ILs mixed with water at six different concentrations. The partial phase diagram of a total of 84 nanoparticle samples is represented in Figure 1. The results show the formation of LCNPs with different nanostructures including the inverse primitive bicontinuous cubic (Q2) phase with the Im3m crystallographic space group, the inverse hexagonal (H2) phase, and the micellar cubic phase Fd3m (Figure 1). Figure 1 shows that the internal nanostructures of the
MO nanoparticles depend on three main factors, the anion group of the IL, the IL concentration, and pH, which is related to the IL species in the system.

![Diagram](image)

**Figure 1:** Partial phase diagrams of MO LCNPs prepared in various choline IL-water solvent conditions with an IL concentration range of 0.16 wt% to 20 wt%, derived from the 1D SAXS scattering patterns. The pH range for the samples is also provided.

**Conclusions:** The structure function relationship of LCNPs has prompted a field of research dedicated for their preparation by rational design. Solvent manipulation has emerged as a promising mean of tailoring LCNP nanostructures, and in that context, ILs, referred to as tailorable green solvents, are excellent candidates. In our study, we generated LCNPs with tuneable structures using different IL-water solvents. Particles with nanostructures exhibiting the cubic Im3m phase, the H_{II} phase and the rarely reported Fd3m phase were obtained by varying the anions of the ILs. Further experiments will examine the cytotoxicity and drug release mechanism of these particles with different nanostructures and explore any potential role of the solvent composition on the functionality of these particles. This study will elucidate the potential application of IL solvents as powerful tools to develop next-generation non-lamellar LCNPs as drug delivery system.
References:
Peptide-based multicomponent oligonucleotide delivery system – a delivery tool for receptor targeted delivery.

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Abstract Summary: A library of non-viral peptide-based multicomponent oligonucleotide delivery systems were synthesized for investigating the performance of cooperating a receptor targeting bombesin peptide ligand and a poly-L-lysine dendron (8+ charge) in the tumour targeting oligonucleotide delivery systems.

Introduction: Gene therapy has the potential on treating and preventing hereditary and acquired genetic disorders.1 One of the major limitations of gene therapy is lacking a safe and efficient delivery system that can deliver the oligonucleotide to the specific target site.2 A receptor targeting non-viral peptide-based multicomponent oligonucleotide delivery system is a promising strategy for delivery of oligonucleotide to a targeting site. It has the advantage of improved efficacy, reduced off-target effects, and the ability to administer lower, more cost-effective doses of oligonucleotides.3 Bombesin (BBN) peptide has been studied on the targeting ability to the gastrin-releasing peptide (GRP) receptor, which is overexpressed in multiple common cancers cells (e.g. breast, lung, pancreatic, prostate and colon).3 Full length and truncated bombesin peptides with a poly-L-lysine dendron(8+ charge) at the N-terminus were synthesised and characterised for plasmid DNA(pDNA) delivery.

Methods: 1) The components of the peptide-based non-viral multicomponent oligonucleotide delivery systems consist of a short length bombesin peptide (BBN (6-14)) and a full-length bombesin peptide (BBN) with a cationic poly-L-lysine dendron attached (8+ charge). A 5(6)-Carboxyfluorescein fluorescent (FAM) were attached to the BBN 6-14 and full BBN peptide ligand to investigate the receptor targeting and mediated internalisation ability. Stepwise Fmoc solid-phase peptide synthesis processes were used to synthesise the library of compounds. 2) Gel retardation assays were performed to investigate the oligonucleotide complexing ability of the gene delivery systems. 3) Cell uptake studies and confocal microscopy assay were performed to investigate the receptor targeting and mediated internalisation ability of the BBN 6-14 and full BBN peptide ligand.

Results and Discussion: According to the results of flow cytometry (FC) and confocal microscopy analysis, both peptides have demonstrated significant uptake into GFP overexpressed PC-3 cells. Wild type BBN peptides were used to compete with the FAM-BBN in a competition assay to show that the uptake of the delivery system is receptor-mediated. Both poly-L-lysine-BBN and poly-L-lysine-BBN (6-14) have shown an ability to condense pDNA in gel retardation assay. Statistical comparisons were performed using ANOVA followed by Tukey post hoc test.

Conclusions: Full-length and truncated bombesin peptide containing delivery system has shown no significant difference in cell uptake and pDNA complexing ability. This suggested the truncated bombesin peptide (BBN 6-14) could be used in the targeted delivery of oligonucleotides into tumour cells.

Acknowledgements: This was being supported by ARC discovery project DP 130100952

References:
**Architecture and composition of lipid nanoparticles on intracellular delivery: A comparison between macrophages and endothelial cells**

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**Purpose:** Nanocarriers are emerging as a promising approach for the delivery of antibiotics to treat intracellular infections. However, successful eradication of intracellular bacteria remains challenging and mechanisms are poorly understood [1]. Therefore, in this study, the cellular uptake behavior of liposomes and cubosomes (two widely used lipid-based nanoparticles) were evaluated in macrophages and lung epithelial cells. The influence of PEGylation/presence of surfactant molecules in nanoparticles, as well as their interaction with serum proteins in biological fluid were evaluated.

**Methods:** Cyanine7 (Cy7)-labelled liposomes composed of (i) didodecyldimethylammonium bromide (DDAB) and phosphatidylcholine (PC) (DDAB/PC), and (ii) distearoylphosphatidylcholine (DSPC) and 1,2-distearyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (DSPE-PEG2000) (DSPC/DSPE-PEG2000) were fabricated using microfluidics (NanoAssemblr®). Cy7-labelled cubosomes (with and without Pluronic F127) were synthesized using the hydro trope dilution method using glyceryl monooleate as the lipid component [2]. Cellular toxicity was evaluated using MTT assay and cellular uptake was quantified using fluorescence-activated cell sorting (FACS) in A549 lung epithelial cells and RAW264.7 murine macrophages.

**Results and Discussion:** Liposomes and cubosomes around 200 nm were produced, as measured using dynamic light scattering (DLS). The non-PEGylated liposomes had a preferential uptake in epithelial cells, whereas cubosomes showed higher uptake in macrophages. However, in the presence of serum, cubosomes had significantly higher uptake in lung cells. This highlights the importance of understanding the influence of serum in modulating the surface hydrophobicity of nanoparticles to promote internalization. PEGylated liposomes showed no internalization in either cell line. Interestingly, cubosomes without (rather than with) Pluronic were more highly internalized in macrophages. Previous studies have shown the role of Pluronic as an adjuvant, which may support the current observation.

![Diagram showing uptake of lipid-based nanoparticles in alveolar cells](image)

**Conclusions:** The internalization properties in non-phagocytic and phagocytic cells differ significantly depending on the lipid composition of the formulation. Further studies to correlate the uptake of nanoparticles to intracellular delivery of antibiotics and efficacy against bacterial killing is being undertaken.

**References:**
Development of pH-responsive Hexosomes and Cubosomes as nanocarriers for cancer therapy

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Purpose: The objective of this study was to identify a suitable pH-responsive drug delivery vehicle, which exhibits slow-release hexosomes (H₂) at physiological pH and fast release cubosomes (Q₂) at the acidic tumour site for controlled release of anticancer drugs. For this purpose, we designed and synthesized a library of ionizable lipids containing amine headgroups and fabricated them into MO (Monoolein) dispersed nanoparticle system.

Methods: Synthesis was conducted using oleic acid and amino alcohols, purified by column chromatography and performed FT-IR, NMR, and GCMS analysis. We used high-throughput formulation techniques to fabricate nanoparticles by blending various amounts of aminolipid with MO. Using Synchrotron small-angle X-ray scattering (SAXS), phase diagrams were determined to understand the effects of mixing MO and aminolipid on self-assembled internal particle structure, and pH effect was studied by pH values ranging from 4 to 10. Particle size (PS), polydispersity index (PDI), zeta potential (ZP) were measured using Zetasizer. pH-responsiveness of the system was further evaluated by loading six different anticancer drugs.

Results and Discussion: A total of nine oleic esters were successfully synthesized, purified and analyzed. The lipids were formulated to nanoparticles with various concentrations of MO. These nanoparticles appeared stable, and their particle sizes were around 200-300 nm with a PDI range of 0.05-0.27. At physiological pH (7.4), the lipid headgroup charge is neutral, and the nanoparticles exhibit characteristics of slow-release phases such as H₂. At lower pH (4.0-6.5), the headgroups are protonated with a positive charge, which drives the system to cubic phase Q₂. We observed eight out of nine systems induced hexagonal to cubic transition at pH<7.4[1]. SAXS data for drug-loaded formulations reveals that the developed nanoparticles maintain their pH responsiveness and can be used to encapsulate both hydrophilic and hydrophobic drugs.

Figure 1. Aminolipid structure, R=Oleyl chain. Figure 2. Partial phase diagram of aminolipid doped nanoparticles.

Conclusions: This study is the first report of a library of MO-based nanoparticles exhibiting a phase transition from a "closed" H₂ at physiological neutral pH to an "open" Q₂ at an acidic pH. The nanosystem investigated herein has the potential therapeutic role for the controlled release of drugs in solid tumours.

Berberine loaded Microfluidics liposomes shows in vitro anticancer activity
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Purpose: Breast cancer is the most prevalent cancer and the main reason for death in women. About 10-20% of the breast cancers are sorted as triple-negative breast cancer (TNBC) (1). Berberine (BBH), a well-known phytochemical compound with an isoquinoline structure, attracts considerable interest due to its pharmacological effects, including antibacterial, anti-cancer, and anti-inflammatory responses. Despite these characteristics, berberine’s poor solubility (2) is one of the limitations of its clinical usage. Our work outlines a new approach to manufacture berberine-loaded hybrid liposomes with highly reproducible and controllable properties using a novel 3D printed microfluidic chip (3) to obtain an anticancer effect on MDA-MB-231 cells as triple-negative breast cancer (TNBC) cells. This project aims to improve the uptake and the anti-cancer efficacy of berberine on triple-negative MDA-MB-231 breast cancer cells.

Methods: For the liposome preparation by microfluidic method 3 mg/ml of BBH were dissolved in 10 ml of water at 60 °C; meanwhile, the total concentration of 8 mg/ml of sucrose ester (SS), Phospholipon 90-G (P90), and Cholesterol (Chol) with the weight ratio of 3:1:2 was dissolved in the ethanolic phase. The ethanolic and water phase were mixed through 3D printed chip with 2:1 and 14 ml/min FRR and TFR, respectively. The ethanol in the final formulation was discharged with one round of centrifuge at 17200 RCF for 60 min in 4° C. Thereafter, the pellets were resuspended by a precise amount of water to reach the primary concentration. In order to investigate the liposome’s physical characteristic stability of the formulation was evaluated by measuring their size and EE for 3 months using Malvern Zeta sizer Nano S instrument and HPLC, respectively. The biocompatibility and antiproliferation effects of the formulations including free BBH, liposome without BBH and SS (NL), liposomes with SS and BBH (NL+BBH), liposomes with SS and no BBH (HL), and liposomes with SS and BBH (HL+BBH) were investigated in vitro with Sulforhodamine B (SRB) assay on Human Cardiomyocyte Cell Line (AC16) and MDA-MB-231 cancer cells, respectively. The cell viability test was done with both SRB and water-soluble tetrazolium (WST)-8.

Results and Discussion: BBH loaded liposomes display 8% encapsulation efficacy. Based on the size of, PDI the formulation shows considerable stability at 4°C after 3months. The EE% has 2.5% decreases in 4 °C. Biocompatibility studies that were performed on AC16 cardiomyocyte cells show liposomes biocompatibility on the normal cell line. Additionally, the cell growth percentage of the cancer cells was 100, 60, and 35 for free BBH, NL+BBH, and HL+BBH, respectively which shows the enhanced effect of berberine in loaded form. Moreover, SS enhances the nanoparticle uptake by the cancer cells and increases the antiproliferative effects of the HL+BBH

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>size</th>
<th>PDI</th>
<th>EE%</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>173.7±0.45</td>
<td>0.06±0.004</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>173.9±0.34</td>
<td>0.059±0.01</td>
<td>-</td>
</tr>
<tr>
<td>60</td>
<td>174.2±0.25</td>
<td>0.075±0.002</td>
<td>-</td>
</tr>
<tr>
<td>90</td>
<td>178.5±0.17</td>
<td>0.06±0.03</td>
<td>decrease 2.5%</td>
</tr>
</tbody>
</table>

Table 1) the stability of the formulation in 3 months considering different parameters including size, PDI and EE%
Figure 1. a) Cell viability of the free BBH, NL, HL, NL+BBH, HL+BBH with the on AC16 cells with both WST-8 and SRB method, b) antiproliferative effects of the mentioned formulations which shows a dose-dependent effect of the formulation and berberine using SRB.

Conclusions: Taken together our findings represent berberine-loaded liposome with considerable anticancer effect on MDA-MB-231 cells with negligible cytotoxicity on normal cell lines with a reproducible and controllable method.

References:
Poster Theme: POLYMERS
Development of peptide based subunit vaccine against tuberculosis

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Purpose: Vaccination has been critical in saving lives and reduce the burden of many infectious diseases in the world. Peptide based subunit vaccine is a promising approach to combat Tuberculosis as it minimizes microbial components, still elicits the desired immune response and avoids pathogenic reversion which is possible in live or attenuated vaccines [1]. Bacillus Calmette–Guérin (BCG) is currently the only licensed vaccine against TB. The early secreted antigenic target 6 kDa (ESAT-6) is encoded in the chromosomal locus of RD1, an essential determinant of mycobacterial virulence but absent in BCG. However, ESAT-6 has inherently low immunogenicity and would require a suitable adjuvant or delivery system to evoke sufficient immune response. As the currently available adjuvants are toxic with adverse reaction potentials, we aimed to incorporate ESAT-61-20 into novel self-adjuvanting polyhydrophobic or polymer-based delivery systems.

Methods: Peptides were synthesized using manual stepwise solid-phase peptide synthesis via Fmoc chemistry. Preparative HPLC, analytical HPLC, ESI-MS were used to purify and analyze the crude peptides.

Results and Discussion: We were able to synthesize and purify all vaccine candidates (>98% purity). The compounds were able to self-assembled into nanoparticles with consistent characteristics (Figure 1 and Table 1). We will carry out in vivo study with both vaccine candidates.

<table>
<thead>
<tr>
<th>Vaccine candidate</th>
<th>Size (nm)</th>
<th>PDI</th>
<th>Zeta potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>E6- Polyacrylate</td>
<td>374.4 ± 96.6</td>
<td>0.5 ± 0.1</td>
<td>+ 34.1 ± 1.2</td>
</tr>
<tr>
<td>E6- Polyleucine</td>
<td>280.7 ± 5.2</td>
<td>0.3 ± 0.02</td>
<td>+ 12.7 ± 1.0</td>
</tr>
</tbody>
</table>

Figure 1. HPLC of E6-Polyleucine. Table 1. Physical characterization of vaccine candidates.

Conclusions: Novel delivery systems for ESAT-61-20 were developed with stable physical properties

Acknowledgements: This work was supported by the National Health and Medical Research Council, Australia (NHMRC Program Grant: 1132975).

References:
An Overview of Chitosan Nanoparticles’ Application for Pulmonary Drug Delivery

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Purpose: Among polymeric nanoparticles, chitosan-based group is one of the most used for drug delivery systems, mainly because of wide and smooth availability of its resources in nature, low cost, various available derivatives, and proportional safety and non-toxicity. Notwithstanding more acceptable biocompatibility and biodegradability of chitosan in comparison to other biopolymers, still the safety or toxicity of chitosan nanoparticles in the human respiratory system is a matter of concern. A series of chitosan-based nanoparticles were previously formulated and used in lungs for targeted and rate-controlled delivery of encapsulated therapeutic agents for both local and systemic usage. However, there is a distinct lack and strong motivation for developing new derivatives of chitosan with demanded properties.

Results and Discussion: Due to its mucoadhesive and permeation enhancer properties and some exclusive features such as adjustable physicochemical characteristics, chitosan has been used for pulmonary delivery of a vast group of therapeutics including genes and vaccines. The mucoadhesive property plays a key role in extended release and decrease in required dose frequency. Additionally, smaller particles have a bigger chance of penetration into the mucosa. On the other side, the positive surface charge of chitosan increases transcellular transport and paracellular penetration in lung epithelium and alveoli. Because of its amphipathic nature, it is possible to encapsulate both hydrophilic and hydrophobic therapeutics into chitosan nanoparticles. Poor solubility of chitosan in aqueous entity in neutral and basic pH and hence biological environment (with pH 7.4) is a major physical barrier against its usage in drug delivery systems. Its insolubility can be optimized by lowering pH less than 5, which is mainly depending on molecular weight of chitosan. However, keeping pH at acidic level raises the chance of damage to respiratory tissue. Recently, several derivatives of chitosan have been formulated in different studies owing to achieve more suitable chemical structures with demanded physicochemical characteristics and biocompatibility. A long series of polymers were previously blended, or graft copolymerized with chitosan to obtain a variety of derivatives with enhanced chemical stability and loading capacity.

Conclusions: Nonetheless, there is a long-lasting controversy and a serious shortage regarding published results and executed human studies about biocompatibility and toxicity of chitosan nanoparticles. Moreover, it is required to formulate more suitable derivatives of chitosan aiming at pulmonary delivery.

References:
Development and Characterization of 5-Fluorouracil Loaded Inulin Hydrogels for Colon Targeted Delivery
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Abstract Summary: Development and physicochemical characterization of the 5FU Loaded inulin hydrogels

Introduction: 5-fluorouracil (5-FU) is one of the chemotherapeutic agents used in the treatment of colon cancer. However, the clinical application of 5-FU is limited by unwanted systemic side effects and off-target problems. Consequently, there is a dire need to develop a suitable drug delivery platform that can help reduce the toxicity of 5-FU by providing localized and targeted delivery of 5FU to the colon.

Methods: Drug-loaded 5FU inulin hydrogels were prepared by crosslinking inulin with pyromellitic dianhydride (PMDA) using triethylamine as a catalyst[1] followed by loading of the 5-FU using the swelling method. To get a deeper insight and better understanding of the drug-loaded materials, the drug-loaded 5-FU gels were characterized using different techniques such as Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Scanning electron microscopy (SEM), in-vitro release, degradation, and cytotoxicity studies.

Results and Discussion: The encapsulation of 5-FU into the hydrogel was confirmed with FTIR with absorption band at 3071, 1246, 1433, 750 cm⁻¹. The change in the crystalline structure of 5FU within the hydrogel was confirmed with XRD. The encapsulation of 5FU within the pores of the hydrogels was further confirmed by the SEM (Figure 1A and B). PMDA concentration in the crosslinking reaction was used to tune the loading of 5FU loaded into the inulin hydrogel with 8.2-18.0 % 5FU Loading depending on the ratio of PMDA crosslinker. The in-vitro release was pH-dependent and by varying the ratio of the crosslinker the release of 5FU from the hydrogels can be tuned. Despite the crosslinking, the hydrogels display excellent enzymatic biodegradability. The 5FU-loaded hydrogels demonstrated concentration dependent efficacy against HCT116 colon cancer cells

Conclusions: The preliminary results make 5FU Loaded hydrogels a promising system for the localized delivery of 5-FU to the colon

References:
Pharmacological effects of bio-nanotechnological tissue-engineered transplants on an inflammatory disease model

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Purpose: Sodium alginate is a known medium for cell and drug delivery. However, some physicochemical properties including degradation in biological systems and low mechanical strength, limit alginate’s use as a transplant matrix. The addition of an amphiphilic compound to alginate could remedy its low mechanical integrity (1-3). Octyl β-D-glucopyranoside is a sugar-based surfactant, with the ability to form hydrogen bonds with other molecules (4). Based on this, it is a prospective candidate for the enhancement of biomaterials. Different concentrations of octyl β-D-glucopyranoside were added to sodium alginate. Hydrogels were then seeded with NIT-1 cells. After incubation, viability and bioenergetics of cells were assessed.

Methods: Hydrogels were made with biomaterials stated in table 1. in sterile conditions. Pancreatic beta-cell line (NIT-1) were incubated in 20 µl of hydrogel H1, H2 or H3 for 24 hours. Afterwards, cells were subjected to MTT test for cell viability test; and Seahorse XF Cell Mito Stress Test to assess mitochondrial function.

Table 1. Hydrogel formulations.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Sodium Alginate</th>
<th>Octyl β-D-glucopyranoside</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>2.1 %</td>
<td>0.15%</td>
</tr>
<tr>
<td>H2</td>
<td>2.1 %</td>
<td>0.35%</td>
</tr>
<tr>
<td>H3</td>
<td>2.1 %</td>
<td>0</td>
</tr>
</tbody>
</table>

Results and Discussion: There were no significant differences in survivability amongst cells immersed within different hydrogels (Figure 1a). However, the bioenergetic profile shows differences in oxygen consumption rate (OCR). Cells in H2 consume the most oxygen, more than alginate control (H3), while cells in H1 show a significant decrease in OCR (Figure 1b). Complete energetic profile with OCR and extracellular acidification rate (ECAR) shows that both H2 and H3 have energetic metabolism related to high viability. H1 has decreased metabolism, with retained viability, suggesting cellular adaptation to a lower oxygen environment. An anaerobic shift in H1 metabolism is not present, suggesting that cells are able to meet their oxygen requirements within H1 hydrogel (5).

Figure 1. Pancreatic beta cells viability (a), oxygen consumption rate (b) and bioenergetic profile (c).

Conclusions: The octyl β-D-glucopyranoside, a sugar-based surfactant, could be used as a properties enhancement for cell delivery matrices for pancreatic beta cells, as a major negative impact on cells was not detected within examined concentration range.
Acknowledgements: For their support, the authors acknowledge Australian Postgraduate Award & Curtin Research Scholarships.

References:
Cholesterol and Morpholine Grafted Cationic Amphiphilic Copolymer for Delivering CRISPR/Cas9 Ribonucleoprotein for the Genome Surgery to Treat Wet-Age Related Macular Degeneration

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Purpose: The major cause of vision loss in wAMD is the overexpression of VEGF-A-mediated angiogenesis. Anti-VEGF injections are routinely done as a first-line treatment, but patients have developed resistance to them [1], necessitating the development of a new treatment modality. As a corollary, we proposed using CRISPR/Cas to knock out the VEGF-A gene. The applicability of CRISPR/Cas9 is limited due to its high molecular weight, hydrophilicity, supranegative charge, degradation, and short half-life. As a result, we worked to develop a lipopolymeric nanocarrier that could deliver Cas9 to the retinal dystrophic cells in vivo.

Methods: An amphiphilic polycarbonate copolymer was synthesized using ring-opening polymerization between mPEG and a cyclic monomer i.e 2-methyl-2-benzyloxyacarbonylpropylene carbonate (MBC) and then grafted with functional ligands such as cationic chains (provides +ve charge), morpholino groups (eases endosomal/lysosomal escape), and cholesterol (facilitates intracellular fate). Using the w/o/w emulsion method, the copolymer was used to make cationic nanoplexes, which were then incubated with RNPs to produce RNP nanoplexes. The RNP nanoplexes were examined for particle size, zeta potential, TEM analysis, complexation, transfection efficiency, nuclear localization, gene editing, and retino-ocular distribution in vivo.

Results and Discussion: Developed RNP nanoplexes having particle size and zeta potential of 117.3 nm and +6.17 mV respectively. TEM images confirmed their morphology and mobility shift assay demonstrated the maximum complexation at a 1:10 ratio of RNPs to polymer (w/w). Moreover, after 6 h incubation, the nanoplexes were able to transfect almost 70 to 80 % of ARPE-19 and NIH3T3 cells with 42% gene editing. After 48h of transfection, the RNPs enter the nucleus with intact DNA binding activity, as per CasFISH assay findings. Further, the RNP nanoplexes were capable to diffuse through the vitreous to transfect the retinal layer in vivo.

Conclusions: A non-viral lipopolymeric nanocarrier containing CRISPR/Cas9 RNPs was developed and characterized in terms of its Cas9 carrying capacity to retinal cells in vitro and in vivo. Now, we can make this nanocarrier actively targeted using ligands (cRGD, Retinoic acid, folate) and utilize it for the treatment of various disease-seeking gene editing in vivo.

Acknowledgements: We sincerely thank the Department of Biotechnology (BT/PR26897/NNT/28/1489/2017) and the Indian Council of Medical Research (ICMR-SRF-45/66/2019-Nan/BMS) for their financial support.

References:
Direct powder extrusion 3D printing of polyhydroxybutyrate implants for prolonged drug release

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Purpose: The aim of the present work was to investigate the feasibility of polyhydroxybutyrate (PHB), as alternative biomaterial, to produce adsorbable implants for prolonged drug release. In addition, we explored the innovative technology of direct powder 3d-printing. For this purpose, square shapes implants were printed in different sizes and loaded with increasing percentages of paracetamol (model drug). The resulting implants were characterized, focusing on the chemical structure (Fourier transformed infrared spectroscopy), thermal behavior (thermogravimetric analysis) and release profile.

Methods: Polymeric devices were produced by mixing PHB pellets (the polymer) and paracetamol (the model drug), using mortar and pestle, until homogeneity was ensured. Any other solvent, excipient, or plasticizer were necessary. The resulting mixture was directly fed into the printing unit to form the designed devices. Square shape implants were printed in 3 different sizes (side x height: 12x2 mm, 18x2mm, and 24x2 mm) each of which was loaded with increasing percentages of paracetamol (10%, 20%, and 30% w/w). Each product was obtained following the same printing parameters except for the temperature that was set accordingly to the percentage of paracetamol present in the blend. The sustained drug release profile of the formulations was tested for 21 days, in phosphate- buffered saline (PBS) at pH 7.4, under stirring (100 rpm) at 37°C. After each timepoint, collected samples were analyzed with high-performance liquid chromatography (HPLC). Thermal gravimetric analysis (TGA) and (FTIR) were performed to investigate possible interactions between the materials. For TGA, scans were run at room temperature to 500°C at a speed rate of 10°C/min under a nitrogen flow rate of 30 mL min while FTIR measurements were performed at 450–4000 cm⁻¹ with a resolution of 4 cm⁻¹ and a total of 64 scans.

Results and Discussion:

The continuous evolution of 3D printing proceeded in parallel with the search for suitable and sustainable materials, with increasing interest in biopolymers. Polyhydroxybutyrate (PHB) is a thermoplastic aliphatic polyester of biological origin. Since it is biodegradable, biocompatible, and non-toxic, it can find rich applications, including drug delivery, being an alternative to conventional polymers.¹ In this study we manufactured subcutaneous implants for prolonged drug release, using direct powder 3d-printing. The design of the implants was developed using CAD software, Figure 1 shows the 3d-printed squares, produced in three different sizes (side x height: 12x2 mm, 18x2mm, and 24x2 mm) and with different blends of PHB and paracetamol (P). The printed implants showed good uniformity in dimensions and weight.

Figure 1. Showing the top view of the printed implants
TGA measurements were performed to verify the degradation profiles of the components of the implants and the thermal stability of the final products. Thermograms, reported in Figure 2, showed that samples were stable up to at least 200°C; in particular, the drug didn’t show any significant degradation under 250°C. In addition, when paracetamol was combined with PHB, the degradation rate was decelerated. Overall, TGA data confirmed that no degradation is likely to occur during the printing process.

![Figure 2. TGA results of paracetamol, PHB and the 3d-printed implants](image)

To further investigate if any interactions arose between the materials, FTIR was employed. The resulting spectra are illustrated in Figure 3. All the formulations (PHB+30%P, PHB+20%P and PHB+10%P) presented the characteristic peaks of paracetamol (amide group) and PHB (ester group), suggesting that both materials were successfully incorporated in the final products.

![Figure 3. FTIR spectra of paracetamol, PHB and the 3d-printed implants](image)

Finally, dissolution tests of the different designs and formulations were evaluated for 21 days at pH 7.4 (Figure 4). The release profile of all the formulations demonstrated to be dependent on the dimensions of the devices,
indeed, independently on the formulation, by increasing the size, higher concentrations of paracetamol were released. Formulation PHB+30%P, showed a faster release, compared with the other samples. Comprehensively, all implants showed a prolonged release of the incorporated drug.

Conclusions: In this work, we demonstrated, for the first time, the potential role of polyhydroxybutyrate as alternative biopolymer to manufacture prolonged drug release implants that can be personalized based on patient’s needs. In addition, the feasibility of direct powder extrusion 3D-printing technique, was explored; due to the simplicity and the small amount of materials required, it may create and opportunity for pharmacies and hospitals to produce on-demand personalized systems.
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Polysaccharide Based Amphiphiles as Stimuli-Responsive Nanomaterials
Vinod Kumar Kannaujiya\textsuperscript{1}, Matthias Konhäuser\textsuperscript{2}, and Peter R. Wich\textsuperscript{1}

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Purpose: Dextrans are biodegradable, biocompatible, and water-soluble polysaccharides, composed of 1-6 glycosidic-linked glucose units. The acetalation of dextran hydroxyl groups turns the polysaccharide into an acid-sensitive hydrophobic material that can be used as a biopolymer building block for amphiphilic block copolymer synthesis. The acetal-modified dextran has been extensively used for the synthesis of various stimulus-responsive AB-type block copolymers. Here, we present the synthesis of a pH-responsive polysaccharide-based block copolymer of polyoxazoline and acetalated dextran (AcDex). In addition, we are also reporting the first dual responsive amphiphilic protein-polysaccharide conjugate synthesized by covalent conjugation of horseradish peroxidase (HRP) with the hydrophobic AcDex via thiol exchange reaction for enzyme prodrug therapy.

Methods: For the synthesis of dextran-based amphiphiles, we modified a microwave-assisted method by Breitenbach \textit{et. al} \cite{1} and developed a reductive amination strategy in a borate-buffer/methanol solvent system to introduce p-substituted aniline derivatives at the reducing end of dextran. Further, the acetalation of dextran was performed using 2-methoxypropene and a catalytic amount of pyridinium p-toluenesulfonate (PPTS). The end-modified dextran can be used to create different stimuli-responsive amphiphiles \cite{2} via Cu-click or thiol exchange reactions for the synthesis of the block copolymer or protein-polysaccharide conjugates.\cite{3}

Results and Discussion: The combination of a linear hydrophilic polyoxazoline block with a hydrophobic AcDex block resulted in a linear block copolymer with low CMC that self-assembles in water into spherical micellar nanoparticles. The formed particles have a narrow size distribution (z-average) below 150 nm in diameter and disassemble in slightly acidic conditions. On the other hand, the conjugation of HRP with AcDex resulted in a dual-responsive amphiphile that self-assembles into spherical micellar nanoparticles with a size (z-average) 210 nm in diameter. A model prodrug, indole-3-acetic acid (IAA) was encapsulated into the hydrophobic AcDex core of the nanoparticles. The disassembly of the particle system under acidic/reductive conditions facilitates the oxidation of IAA by horseradish peroxidase into cytotoxic radicals, which leads to cellular apoptosis.

Figure 2. (a) PEOZ-b-AcDex block copolymer synthesis. (b) Self-assembled dual-responsive HRP-AcDex conjugate and prodrug activation.

Conclusions: In summary, the biocompatibility, biodegradability, and the stimuli responsiveness of acetalated dextran block make this polymer an ideal candidate for synthesis of both stimuli-responsive block copolymer and protein-polymer conjugate for nanoparticle-mediated drug delivery and enzyme prodrug therapy.

References:
DRUG DELIVERY AUSTRALIA VIRTUAL MEETING  2021

List of Abstracts

POSTER SESSION TWO
A Metabolic Labelling Based Approach for Highly Selective Gastrointestinal Delivery

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Purpose: Non-natural sugars such as acetylated mannose-azide (Ac4ManNAc) can be expressed on the cell surface when ‘fed’ to cells in place of natural sugars.1 After metabolic labelling, the azide group is available for click reactions with an alkyne to attach a probe, such as a fluorophore or drug carrier.2,3 Here we do this the other way around, we label the cells with an alkyne (DBCO) which can spontaneously react with an azide probe. To date, metabolic labelling has not been studied on in vitro intestinal cell model or the intestine in vivo. The project contributes to the literature by understanding the potential to utilise the combination of metabolic labelling of cells and duodenal tissues with unnatural sugars and click chemistry as a tool to achieve site-specific delivery of particles in the intestines.

Methods: In vitro studies (monocultures of caco-2 cells and HT29-MTX cells) and in vivo studies were completed to determine the efficiency of metabolic labelling on the cell lines and tissues. Ac4ManNDBCO was treated to the cells and tissues as the metabolic labelling agent. Azide-cy5 was employed as a dye to indicate the expression of Ac4ManNDBCO. The intensity and expression of cy5 signal were determined using confocal microscopy, flow cytometry and in vivo imaging system (IVIS).

Results and Discussion: Results showed that both cell lines could be metabolically labelled with 75 µM and 100 µM Ac4ManNDBCO within 6h. There was specific binding of azide-cy5 dye as well as azide functionalised bacteria to DBCO modified caco-2 cells. Duodenal tissues were also labelled with DBCO sugar via in vivo perfusion.

Conclusions: Human gastrointestinal epithelial cell lines and duodenal tissues were successfully labelled with synthetic DBCO tagged mannose and could be specifically targeted by the complimentary ‘clickable’ dye. This shows its potential to achieve orthogonal targeting via oral administration.

Acknowledgements: This research was conducted and funded by the Australian Research Council Centre of Excellence in Convergent Bio-Nano Science and Technology.

References:
**Ocular Penetration and Distribution of Hydrophobic Curcumin Eyedrops**

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**Purpose:** Curcumin is a natural alkaloid proposed to have therapeutic application in the management of inflammatory ocular pathologies (1). However, its hydrophobic nature makes it difficult to formulate as an aqueous eyedrop. A non-aqueous eyedrop vehicle may facilitate ocular delivery of curcumin in therapeutically relevant concentrations; however, ocular bioavailability from non-aqueous vehicles is poorly understood. Therefore, in this study, a non-aqueous curcumin solution and a suspension were formulated and their ocular distribution was evaluated ex vivo.

**Methods:** Curcumin eyedrops were prepared either as a solution in medium chain triglycerides or as a microsuspension in a long chain alkane and their ocular penetration was compared using a porcine ex vivo whole eye model. Curcumin penetration and distribution in the cornea, conjunctiva and the sclera was evaluated one hour post-application quantitatively and qualitatively by fluorescence spectroscopy and confocal microscopy, respectively.

**Results:** Curcumin concentrations in the cornea and sclera were slightly higher after application of the suspension; however, this difference was not statistically significant. Considerably higher curcumin amounts were observed in the conjunctiva after application of both eyedrops, with the suspension showing significantly greater conjunctival penetration than the solution. These observations were supported by qualitative data showing preferential localization of curcumin in the corneal epithelium and the conjunctiva; however, the microsuspension especially favours deeper penetration into the underlying cell layers.

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**Figure 1.** Comparison of curcumin penetration in the cornea, conjunctiva and sclera from a hydrophobic solution and suspension.
**Conclusion:** Curcumin eyedrops formulated using hydrophobic liquids can provide a surfactant and preservative free alternative for ocular drug delivery. The curcumin microsuspension formulated using a non-aqueous vehicle showed enhanced ocular penetration and may have therapeutic application in the management of ocular inflammatory diseases.

**Acknowledgement:** PA’s salary is supported by the Health Research Council of New Zealand [20-317].

**References:**
**Size-reduced Clay Materials for Volatile Drug Delivery**

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**Purpose:** Volatile drugs are highly bioactive compounds that evaporate under ambient conditions. The study aims to engineer a scalable biocompatible material for volatile drug delivery. Clay materials are excellent inert thermal insulation compounds widely available. Layered structure clay as phyllosilicates can accommodate the drug molecules among the layers that require extensive protection from heat. It assists the transportation of the heat-labile drug molecules. However, for the performance increment of the drug, the surface-area-to-volume ratio of the drug carrier must be increased by reducing the bulkiness. Our study has selected size-reduced vermiculite (SMV) as the model clay material and oregano oil (OEO) as the volatile antibacterial drug to study clay materials' insulation performance and the drug's performance in clay encapsulation.

**Methods:** Phyllosilicate clay material was size reduced from a top-down approach (ball milling) to produce micron-sized particles. Particles were characterized by Scanning Electron Microscope (SEM), Transfer Electron Microscope (TEM), Energy Dispersive X-ray (EDX) spectroscopy, Fourier-Transform Infrared (FTIR) spectroscopy. The performance of insulation was evaluated via an isothermal test from Thermo-Gravimetric Analysis (TGA). Antibacterial performance was quantified by the Minimum Inhibitory Concentration (MIC) test.

**Results and Discussion:** The low magnification SEM images of the SMV (Figure 1A) show sub-micron-sized particles in the range of 200–1000 nm, with irregular shapes. Energy-dispersive X-ray spectroscopy (EDS) analysis revealed the chemical composition of SMV. Al (yellow), Si (indigo) and O (red), K (orange), Fe (purple), Ti (light blue), and Mg (green) elements were observed in the elemental mapping. Consequently, an increased specific surface area of SMV due to the size reduction is beneficial for loading the active component of OEO and possibly other cargo molecules.

![Figure 1. SEM (A, B), TEM (C) and (D) EDS elemental mapping images of SMV.](image)

SMV was used as a carrier to load OEO by mechanical mixing. FTIR was used to verify the successful OEO loading in the SMV. Figure 2 shows the FTIR spectrum of free OEO (green). Twenty obvious characteristic peaks were found at 638, 696, 718, 751, 810, 935, 993, 1054, 1116, 1172, 1243, 1297, 1356, 1423, 1459, 1507, 1583, 2869 and 2960 cm\(^{-1}\). In the spectrum of OEO-loaded SMV (blue), compared to non-encapsulated SMV (red), characteristic peaks at 1172, 1297, 1243, 1356, 1423, 1459, 1507, 1583, 1620, 2869 and 2960 cm\(^{-1}\) originated from OEO can still be observed, indicating the successful loading of OEO.
An elevated temperature of 60 °C was selected for the isothermal test since OEO is volatile and evaporates at high temperature. The isothermal release profiles of free OEO, SMV and OEO-loaded SMV are shown in Figure 3. The weight loss of SMV came from the adsorbed moisture, consistent with TGA results. Based on the weight loss, it was calculated that 45.31% of the loaded OEO evaporated in the 14 h isothermal release at 60 °C. This data indicates that after loading by SMV, OEO shows a slower and sustained release.

The agar dilution method[1] obtained the minimum inhibitory concentrations (MIC) of OEO and OEO-encapsulated SMV. As shown in Figure 7, the free OEO treated group showed visible growth of bacterial colonies for both E. coli and S. epidermidis at OEO concentrations of 0.16–0.64 mg/mL. The inhibition to the bacterial colonies increased with the OEO concentration, and no visible bacteria growth was observed at an OEO concentration of 1.28 mg/mL, indicating the MIC value of OEO. The OEO-loaded SMV also showed dose-dependent inhibition of the growth of both E. coli and S. epidermidis. A much lower MIC value of 0.64 mg/mL was observed, indicating the OEO-loaded SMV exhibited efficient inhibition to bactericidal growth at this concentration.
Conclusions: A top-down ball milling approach was used to reduce the size of the vermiculite clay to the micron level. The OEO's isothermal release test showed that SMV could slow down OEO release and significantly improve its thermal stability. OEO-loaded SMV also demonstrated enhanced antibacterial performance in in-vitro antibacterial tests against E. coli and S. epidermidis bacteria. The small particle size, higher density, and improved stability of OEO were the crucial parameters for the high antibacterial performance of the OEO-loaded SMV.

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Berberine loaded liquid crystalline nanoparticles attenuate proinflammatory cytokines in human bronchial epithelial cells

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Abstract

Purpose: Human bronchial epithelial cells are the interface between the external environment and the lung and play a significant role in respiratory diseases. Epithelial cells generate a variety of pro-inflammatory mediators, such as interleukin-6 (IL-6) and IL-8, in response to environmental contaminants, allergens and smoking, resulting in airway inflammation and tissue damage. IL-6 is a multifunctional cytokine produced at the site of inflammation by the airway epithelium, macrophages, and other cytokines such as tumor necrosis factor-α (TNF-α) while interleukin-8 (IL-8) is a member of C-X-C chemokine that recruits neutrophils to areas of inflammation and plays an essential role in triggering chronic and acute inflammatory reactions in different respiratory diseases. Therefore our study aimed to investigate whether berberine loaded liquid crystalline nanoparticles (Berberine LCNs) suppress airway inflammation by inhibiting the release of TNFα-induced IL-6 and IL-8 in human bronchial epithelial cells (BEAS-2B) in vitro.

Methods: MTT assay was used to determine the safe dose of Berberine LCNs for BEAS-2B cells. BEAS-2B cells were stimulated with tumor necrosis factor-α (TNF-α) with/without Berberine LCNs. IL-6 and IL-8 levels in cell culture supernatants were measured using enzyme-linked immunoassay (ELISA).

Results and Discussion: Berberine LCNs, at doses of 125 nM, 250 nM, 500 nM and 1000nM reduced the cell viability by 5%, 9.4 %, 17% and 50% respectively when compared to the control (untreated). At 500 and 1000 nM, the viability of BEAS-2B cells was reduced significantly (P≤ 0.001). Therefore, further investigations were performed with doses of berberine LCNs not exceeding 250 nM. There was a significant reduction in the TNF α -induced release of IL-6 & IL-8 levels (P < 0.001) when BEAS-2B cells were pre-treated with berberine LCNs (250 nM), as compared to the negative control (TNF-α only) (Fig. 1).
Fig.1: Effect of berberine-LCNs on IL-6 and IL-8 release induced by TNF-α

**Conclusion:** Berberine-LCNs treatment suppressed TNF-α induced IL-6 and IL-8, indicating a promising therapeutic role for Berberine in the management of airway inflammation in a variety of lung diseases.

**References:**


**Poly hydrophobic vaccine delivery system for intranasal immunization**

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**Abstract:** Since Edward Jenner's invention in 1976, vaccination has proven to be the most successful strategy for preventing pandemics and intractable diseases [1]. Vaccines have saved millions of lives and eradicated the majority of illnesses. Traditional vaccinations, such as inactivated or lived-attenuated vaccines, include the entire organism and can elicit robust immune responses following inoculation, but they can also generate safety concerns or autoimmune difficulties. To optimize vaccination approach, researchers utilize subunit vaccines with the smallest microbial component possible. The component will be synthesized into peptides that will target specific targets and will be multi-epitope against various strains, including pathogens at various phases of their life cycles. Group A streptococcus (GAS) is a gram-positive, aerotolerant bacteria. The antibodies produced following immunization with inactivated GAS vaccine cross-react with human cardiac myosin, which is a key issue during GAS vaccine development [2]. Synthetic antigens lack the danger signals that activate immune responses, necessitating adjuvants to assist boost humoral and cellular responses as well as shield peptides from enzyme destruction. Poly hydrophobic amino acids (polyHAAs) made from natural amino acids are fully defined and biodegrade polymers. PolyHAAs were conjugated to hydrophilic peptide antigen derived from GAS M protein and self-assembled into nanoparticles. These nanoparticles induced strong immune responses after subcutaneous delivery [3].

**Purpose:** To develop a delivery system for peptide-based intranasal vaccine against group A streptococcus (GAS). It is hypothesized that the arrangement of epitopes with 15 copies of leucine in vaccine construct may affect the immune responses upon intranasal immunization.

**Methods:** All the compounds were synthetic peptides using microwave assisted Boc and Fmoc solid phase peptide synthesis (SPPS). The crude compounds were then purified by preparative high performance liquid chromatography (HPLC) and characterized by electronic ionization mass spectrometry (ESI-MS). Hydrophobic moiety and hydrophilic moiety of the synthetic compounds would self-assemble into nanoparticles and/or anchor into liposomes and then analyzed by dynamic light scattering (DLS), circular dichroism spectrometry (CD) and transmission electron microscope (TEM).

**Results and Discussion:** Compound 3 and 4 aggregate into chain-like aggregate of nanoparticles (CLAN) and rod structures and compound 5 showed fibrils structures because the hydrophobic and hydrophilic moieties self-assemble into nanoparticles in water. All the liposomes were positive charge. The sizes of liposome 3 and 4 were 170 nm and compound 5 was around 128 nm. Compound 3-5 were helical with minimum ate 208 nm and 220 nm. The helical structure of compound 4 was more profound compared to the other compounds.
The leading compound liposome 3 and its self-assembled compound 3 were able to induce both IgG and IgA antibody responses against J8 after boost 3.

Combine the DLS and TEM results of compound 3-5, hydrophobic moieties and hydrophilic moieties self-assemble into nanoparticles successfully. The conjugation of poly leucine or C16 to the epitopes enables the compound to be attached to the lipid bilayer of the liposome, instead of just being encapsulated. Due to the positive didodecyldimethylammoniumbromide lipid, all the liposomes were positively charged to load peptide efficiently and increase the membrane permeability. From the ELISA result, commercial adjuvant CTB was poorly effective to stimulate high antibody titers. There was no significant difference in immune response between self-assemble compounds and their respective liposomes. It was assumed that the immune responses between each mice in each group was not consistent due to the outbred nature of these mice.

**Conclusions:** Although the compounds have the same B cell epitope and T cell epitope, different structures arrangements and adjuvants added led to variable morphology and size characteristics. From the DLS and TEM results, compound 4 formed the best α helix confirmation with single size which mimic coiled coil α helix of M protein. However, compound 3 (both self-assembled and in liposomal formulation) elicited the highest IgG antibody titers among all the tested compounds. It is hypothesized that C-terminal position of B-cell epitope exposed itself better to environment, allowing easier recognition by B-cell. This needs confirmation in the future. Compared with compound 5 adjuvanted by C16-LCP system., poly leucine still has the advantage as an adjuvant through intranasal immunization. It quickly induced systemic immune responses after 1st boost.
References:


Validation of the next generation impactor for cellular impaction testing of dry powder inhalers
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Purpose: The purpose of this study was to validate the deposition profiles of dry powder inhalers (DPIs) using the next generation impactor (NGI) modified to include Snapwell™ inserts, in which NCI-H441 cells were grown in the air liquid model, placed in impaction stages. Resveratrol, a natural phenol with promising therapeutic effects against cancer, cardiovascular and respiratory diseases, was used as a model drug [1,2]. In this validation study, the in vitro aerodynamic performance of the model formulation using the RS0 DPIs was evaluated using the modified NGI and compared using the conventional NGI assembly as outlined in the US pharmacopoeia

Methods: The modified impaction plates of the NGI containing Snapwells™ were fitted at stage 7 (cut-off diameter of 0.4 0.55 mm - Figure 1). Aerodynamic performance of resveratrol was performed using the NGI at constant airflow of 60 L/min for 4 s. NCI-H441 cells were used as representative model of the human distal lung epithelia, with the ability to form monolayers with appreciable barrier properties. EVOM Voltohmmeter was used to monitor the transepithelial electrical resistance (TEER) of the epithelial cells during the seeding period. Sodium fluorescein assay (Flu-Na) was employed to assess the integrity of cell monolayer over 4 h post airflow and drug aerosolisation. Significant difference was based on P < 0.05. Data represent the mean ± SD of at least three independent experiments

Results and Discussion: The aerodynamic parameters of resveratrol, including fine particle dose (2696.69 ± 69 μg vs 2599 ± 8 μg), fine particle fraction (52 6 ± 5 25 % vs 5 99 ± %) and MMAD (5 ± 0.9 vs 0 ± 0.04), demonstrated no significant variation in both conventional and modified NGI set-ups (p < 0.05), respectively (Figure 1). Flu-Na assay confirmed that airflow and the model drug had no significant effect on the integrity of H441 cell barrier in stage 7 after deposition (Figure 2), suggesting the great potential for such advanced set-up to be used in drug permeation and transportation studies [3].

Conclusions: In this study, the use of NGI set-up modified to include a functional H441 air-interface model for drug delivery to the lower airways has been described. This novel deposition model can collect aerosolised drug with more representative aerodynamic features for subsequent cell permeation and transport studies.

References:
Novel Cyclic Peptide Against ITGA5 Reduces Tumor Stroma and Potentiates the Effect of Gemcitabine in Pancreatic Cancer

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Purpose: Pancreatic ductal adenocarcinoma (PDAC) is characterized with abundant tumor stroma which not only promotes tumor growth but also prevent chemotherapy leading to treatment failure [1]. Different approaches are being investigated to fulfill the clinical need to resolve the problem. In PDAC pancreatic stellate cells (PSCs) are the origin of cancer associated fibroblasts (CAFs) [2]. Our previous study showed that CAFs overexpress ITGA5 receptor which is linked to poor overall survival of patients with PDAC [3]. The focus of our present study is developing novel cyclic peptide that is envisioned with enhanced stability binding and potency to inhibit CAFs-induced stroma barrier and thereby enhance the effect of chemotherapy.

Methods: Based on former peptide (AV3) from our previous study [3] three more peptides called AV3.3 cyclic AV3 (cyAV3) and cyclic AV3.3 (cyAV3.3) were synthesized custom made. First the stability of peptides was evaluated using high performance liquid chromatography (HPLC) and the secondary structure was evaluated using circular dichroism. To study the effect on CAFs their precursors PSCs were used. The ability of peptides to inhibit PSC adhesion to fibronectin (FN) was examined. Furthermore inhibition of FN binding towards ITGA5 was also examined. The effect of peptides on TGF-β-activated PSCs on was studied using western blot. In vitro 3D heterospheroid model (PANC-1 + PSC and MIA PaCa-2 + PSC) were used for studying the effect of cyAV3.3 on stroma and efficacy of gemcitabine. In vivo we tested the effect of cyAV3.3 (biweekly 20 mg/kg i.p. 3 weeks) on the efficacy of gemcitabine (biweekly 50 mg/kg i.p.) in stroma-rich co-injection tumor models (PANC-1+hPSC and MIAPaCa-2+hPSC).

Results and Discussion: HPLC confirmed AV3.3 higher stability in serum compared to AV3 and also higher stability of cyclic peptides (cyAV3 and cyAV3.3) compared to their linear analogues. Circular dichroism showed changes after cyclization of the peptides which may contribute to the stability of peptides. Next binding affinity of peptide towards ITGA5 was increased as confirmed with cell-free ITGA5-FN binding assay. Both stability and binding evaluation showed cyAV3.3 be the most potent peptide. However the increase in binding does not necessarily improve the biological activity of peptides to same extent as shown in the inhibition of α-SMA and Col-1 at gene level after different regime of treatment. At protein level ITGA5-targeting peptides significantly reduced α-SMA and Col-1 in TGF-β induced PSC differentiation by inhibiting pFAK as shown with western blot analyses. We concluded that cyAV3.3 performed the most potent efficacy in inhibiting PSC activation. Next we trailed cyAV3.3 in 3D heterospheroids (PANC-1 + PSC and MIA PaCa-2 + PSC) and observed an enhanced effect of gemcitabine in heterospheroids treated with cyAV3.3 (56.76% inhibition in PANC-1 + PSC and 76.37% inhibition in MIA PaCa-2 + PSC) as shown in spheroid growth and viability. In vivo cyAV3.3 attenuated the tumor growth by itself and potentiated the effect of gemcitabine in subcutaneous co-injection (73.56% tumor shrinkage in PANC-1 + PSC and 65.83% tumor shrinkage in MIA PaCa-2 + PSC) tumor models. Histologically our novel peptide cyAV3.3 showed significant reduction of extracellular matrix (ECM) component collagen 1 and decompression of blood vessels leading to enhanced tumor perfusion and thereby potentiated the efficacy of gemcitabine.
Figure 1. cyAV3.3 attenuated tumor growth and potentiated the efficacy of gemcitabine in subcutaneous co-injection tumor model of (A) PANC-1 + PSC and (B) MIA PaCa-2 + PSC.

Conclusions: This study represents an important improvement of peptide-based targeting to modulate PSC via ITGA5 receptor. Changes in structural components of former peptide AV3 results in differences secondary structure which may affect the stability of the peptides. Our novel cyclic peptide cyAV3.3 shows high capacity in blocking FN as the native ligand of ITGA5. Biologically it inhibits the activation of PSCs into CAF-like myofibroblasts. Furthermore cyAV3.3 significantly reduced tumor growth which was attributed to the reduced collagen 1 and the decompression of blood vessels leading to tumor penetration of chemotherapy. Altogether this study highlights the evidence that targeting ITGA5 using cyAV3.3 may improve the efficacy of chemotherapy in pancreatic cancer.

References:
Synthesis, Development and Characterization of a Novel TMZ-POH Carbamate Loaded Hollow Gold Nanoparticles for the Treatment of Grade IV Astrocytoma

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ABSTRACT

Purpose: Converging advances in the development of nanoparticle-based drug delivery system and improved understanding of the molecular biology of brain tumors (gliomas) offer the potential to provide physicians with new tools for the diagnosis and treatment of these deadly diseases. Hollow gold nanoparticles (HAuNPs) loaded with chemotherapeutic agents have gained much interest in cancer therapeutics. The poor response of tumors to various types of chemotherapeutic agents are often due to intrinsic drug resistance. Hence, various analogues of chemotherapeutic agents have been studied in an effort to overcome these problems. Here a biocompatible PEG coated hollow gold nanoparticle loaded with TMZ (Temozolomide) POH (Perillyl alccohol) carbamate (TMZ conjugated with POH- NEO212) which is capable of specifically targeting glioma tumors is presented.

Methods: PEGylated HAuNPs were synthesized with some modifications in sacrificial galvanic replacement method. The surface plasmon resonance peak was observed using Ultraviolet spectroscopy (UV) -Schimadzu. The particle size was estimated using dynamic light scattering (DLS) technique whereas the zeta potential of particles was noted using Malvern zeta sizer. High resolution Transmission electron microscopy (HR-TEM) was carried out to examine the inner core diameter and outer shell thickness. On the other hand, chemical synthesis of TMZ POH carbamate was performed as shown in fig 1. TMZ-POH complex was characterized using Nuclear Magnetic resonance (NMR) i.e: ¹H NMR, ¹³C NMR and Fourier Transform Infra-red (FTIR) techniques. PEGylated HAuNPs conjugated with the TMZ-POH complex were also characterized using FTIR and NMR techniques.
Results and discussion: Concentrations of all the chemicals used during the synthesis of HAuNPs plays an important role in maintaining good surface plasmon resonance peak at 810nm and particle size with good homogeneity and sphericity. HR-TEM imaging reveals that the nanoparticles have good shell thickness and inner core diameter. Dynamic light scattering data revealed that HAuNPs with 20nm particle size were obtained. Zeta potential value was found to be -15.5 mV. In addition to this, the concentration and the ratios of the reactants used during the chemical conjugation of TMZ POH carbamate also plays a crucial role in obtaining the final product with maximum yield and negligible impurities. 1H NMR (NH- 8.42 s,1H), 13C NMR (CO stretch 158.52) and FTIR (CONH- 3145.58; COO-1744.64) study reveals that TMZ-POH carbamate was formed with maximum yield. High targeting specificity establish this nanoparticle as a potential platform to aid in the diagnosis and treatment of gliomas and other tumors.

Conclusion: The aim of the present work was to synthesize PEGylated HAuNPs with highest stability. Conjugation of TMZ POH carbamate into PEGylated HAuNPs can prove to be efficacious in treating TMZ resistant gliomas.

Acknowledgment: We are thankful to DST-SERB (Department of Science and Technology- Science and Engineering Research Board- EMR/2017/003086) for funding this research work.

References:

Synthesis of Hollow Gold Nanoparticles: Investigating variables in optimization of route of synthesis

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ABSTRACT

Summary: Hollow gold nanoparticles (HAuNPs) are of growing interest in cancer imaging and therapy. Photothermal therapy involving the use of HAuNPs are being envisaged for delivery of drugs owing to their tuneable size and shape and ability to be delivered at the target site. The native surface chemistry of these particles is simple and can be easily functionalized for subsequent bioconjugation to target receptors on cancer cells. Sacrificial galvanic replacement method is commonly employed for synthesis of hollow gold nanostructures. One of the most challenging aspects in synthesis of these nanostructures is to produce HAuNPs with reproducible shape, size and desired surface plasmon resonance (SPR). In this work, we present the impact of various factors critically involved in synthesis of HAuNPs.

Introduction: Hollow nanoparticles consist of a spherical gold shell filled with the imbedding medium. HAuNPs were synthesized using modifications in the sacrificial galvanic replacement method. Synthesis of HAuNPs is associated with lack of reproducibility owing to variations in concentrations of reactants used, hydrolysis time, and ratio of reactants used or rate of addition affecting size, shape and morphology, wall thickness, core diameter and SPR of the particles.

Experimental method: HAuNPs were synthesized using modified reported procedures (Figure 1). Sodium citrate was used as a capping agent and sodium borohydride (NaBH4) was used to reduce the cobalt core which was then added to gold chloride solution. The SPR spectrum of nanoparticles solution was measured using Shimadzu 1900 UV-Visible spectrometer. Size and polydispersity of particles were measured on Malvern particle analyzer. Hollowness and morphology of particles were observed using High-resolution transmission electron microscopy (HRTEM).
Results and Discussion: Sodium citrate (0.1M), CoCl₂ (0.1M) and NaBH₄ (0.25M) are the chemical reagents that are critically involved in synthesis of cobalt nanoparticles. HAuNPs obtained with this method showed particle size in the range of 50-100nm. TEM images revealed that particles have uniform shell thickness and hollow core.

The ratio, concentration and sequence of addition of Sodium Citrate, CoCl₂ and NaBH₄ help in controlling the growth of cobalt nanoparticles. Low concentration of sodium citrate produces particles that tend to aggregate. Increasing the concentration of cobalt chloride increases the size and homogeneity of particles. HAuNPs in SPR 700-800nm are formed only when sodium citrate: cobalt chloride (4:1) ratio is maintained. Concentration of sodium citrate can be reduced with addition of citric acid. Slow addition of cobalt nanoparticles to gold solution yield particles with thicker shells and broad spectrum whereas fast addition gives uniform spectrum with low poly polydispersity of particles. If the concentration of gold is increased beyond 0.100mM; red shift in SPR is observed. Addition of polyvinylpyrrolidone as stabilizer can affect further functionalization of HAuNPs since the surface is occupied by PVP molecules. Entire synthesis has to carried out under constant supply of nitrogen since presence of any oxygen molecules affect the gold shell growth and hollowness of HAuNPs.

Conclusion: We were able to synthesize lab scale reproducible batches of HAuNPs with uniform shape and size with desired SPR. The size of cobalt particles, thickness of gold shell, size of HAuNPs can be tuned by varying concentration of sodium citrate and cobalt chloride, by changing hydrolysis time, concentration of NaBH₄, by changing the rate of addition of cobalt nanoparticles and gold chloride. These synthesized HAuNPs can be further functionalized using various ligands like peptides, carbohydrates and coupled with photothermal therapy to be used in many biomedical and diagnostic applications.

Acknowledgment

We thank Board of Research in Nuclear Sciences (58/14/26/2019-BRNS for funding this research work. We would like to thank Indian Institute of Technology - Bombay to help us use Transmission Electron microscopy for getting images of our samples.

References:


**Thermosensitive Nanoemulsion-Based Sol-Gel of Clozapine for Nose-to-Brain Delivery**
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**Introduction:** Clozapine is the most effective antipsychotic for treatment-resistant schizophrenia1. It is, however, associated with many peripheral adverse drug reactions (ADRs)2. Nose-to-brain (N2B) drug delivery offers a promising approach to deliver clozapine directly to the central nervous system (CNS) while minimizing peripheral ADRs by avoiding unwanted peripheral tissue distribution of the drug.

**Purpose:** This study illustrates the formulation and characterization of clozapine-encapsulated nanoemulsion in a sol-gel system for N2B delivery to minimize peripheral ADRs.

**Methods:** Clozapine (1% w/w) and oleic acid (1% w/w) were added to 15% w/w surfactant mixture: Smix polysorbate 80:propylene glycol (3:1), and sonicated using a probe sonicator at 100% amplitude (continuous mode) for 5 min. Then the mixture was added to pre-made 20% w/w poloxamer 407 (P407) solution and stirred in an ice bath at 400 rpm until a clear mixture formed. Benzalkonium chloride 0.1% w/w and sodium metabisulfite 0.1% w/w were added as preservative and antioxidant, respectively. The final formulation was adjusted to pH 5.5 with acidic buffer solution containing HCl/KCl (pH 2.0) and alkaline buffer solution containing NaOH/KCl (pH 13.0). Clozapine solution (1% w/w) was used as control, prepared in 7 mL 0.1 M HCl and made up to 10 g with 0.1 N NaOH (final pH made to 5.5).

**Results and Discussion:** The formulation showed mean globule size of 17.5 ± 0.2 nm with PDI 0.1 ± 0.03. The zeta potential of the globules was recorded to be -39.7 ± 1.5 mV. *In vitro* drug release study (n=3) showed 84.2 ± 3.9% of clozapine was released from clozapine solution at 72 h, while only 38.9 ± 4.6% of clozapine was released from the nanoemulsion sol-gel formulation. At 8 h, *ex vivo* drug permeation study (n=6) showed 56.2 ± 2.3% of clozapine was permeated through sheep nasal mucosa as compared to 83.2 ± 2.8% from clozapine solution. The clozapine nanoemulsion sol-gel and solution had comparable turbidity at 6.9 ± 0.04 NTU, and 8.4 ± 0.2 NTU, respectively. The storage modulus (indirect measure of gel strength) of the nanoemulsion sol-gel was 0.03 ± 0.02 Pa at 8 °C (storage temperature), 0.3 ± 0.1 Pa at 25 °C and 11643.2 ± 563.5 Pa to 12285.0 ± 295.6 Pa at 32-34 °C (nasal temperature).

![Figure 1. Sol form at 2-8 °C](image1)

![Figure 2. Gel form at 32-34 °C](image2)

**Conclusions:** The *in vitro* and *ex vivo* studies demonstrated the potential of the nanoemulsion sol-gel formulation for N2B delivery of clozapine. *In vivo* studies are required to investigate the drug targeting efficiency and direct drug transport of the formulation post-intranasal administration to prove its appropriateness in clinical practice for the management of schizophrenia.

**References:**
Electrochemically Nano-Engineered Zirconium Dental Implants Towards Enhanced Bioactivity

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Purpose
Attributed to their excellent physical, mechanical and biological properties, Zr-based implants are rapidly gaining popularity [1]. While Zr-based dental implants have shown promising outcomes, long-term success may be challenged, especially in compromised conditions. To cater to such conditions, conventional Zr implants have been modified via physical, chemical and biological means to augment cellular functions to achieve enhanced osseointegration, soft-tissue integration and local therapy [2-3]. This research demonstrates the optimized fabrication of ZrO₂ nanostructures (ZON) on clinical implant-relevant micro-machined and rough surfaces. Our approach bypasses the conventional implant polishing, thereby preserving the underlying micro-roughness that is crucial for osseointegration and holds great promise towards improving bioactivity and therapeutic potential.

The project aims to:
1. Fabricate ZrO₂ nanostructures (ZON) onto Zr-based implants via anodization.
2. Perform thorough physical-chemical characterization of ZONs.
3. Investigate the functions of osteoblasts on ZONs in vitro.

Figure 1. Schematic representation of fabrication of nano-engineered Zr implants via anodization towards enhanced bioactivity.

Methods
ZONs were fabricated on Zr flat foil and Zr wire (model for abutment) using electrochemical anodization at various voltages/times. Both Rough- (as received) and Micro- (micro-machined) Zr were used as substrates. Characterization was performed using SEM, AFM, XPS, XRD and water contact angle. Further, osteoblasts were cultured on the surface of ZONs in vitro, followed by evaluation of proliferation and adhesion.

Results and Discussion
Randomized ZONs were fabricated on Rough-Zr, while aligned ZONs were formed on micro-machined Micro-Zr. Further, evidence of the effect of anodization voltage was evident, with the preservation of underlying micro-architecture to form dual micro-nano structures. ZONs promoted the proliferation of osteoblasts and aligned ZONs mechanically stimulated cells to align parallel.
Conclusions
Dependent on substrate topography, voltage and time, aligned or random ZONs can be fabricated. Further, nano-engineered implants enhanced the function of osteoblasts and enabled their alignment parallel to the nanostructures. Controlled nanostructures fabricated on complex implant topography are a promising strategy for enhancing the bioactivity of conventional Zr-based dental implants.

Acknowledgements
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References
Engineering nanoexosomes for targeted drug delivery for ovarian cancer

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Purpose: Ovarian cancer is the 8th most common cause of cancer mortality in women globally, the high mortality rate owing to unfavourable biodistribution, low penetration and rapid clearance of therapeutics. Biomimetic extracellular vesicle (EV)-encapsulated mesoporous silica nanoparticles (MSNs) can be ideal therapeutics by combining target-homing capacity of EVs with high drug loading capacity of MSNs. [1] Here, we propose construction of EV-coated MSNs as drug carriers for ovarian cancer therapy.

Methods: EVs were derived from SKOV-3 cell lines using differential ultracentrifugation. Amino-functionalized mesoporous silica nanoparticles (MSNs); 50nm in size; were synthesized using previously established methods. The MSNs were loaded into the EVs using three different methods, extrusion, extrusion followed by incubation: and sonication. The morphology of the EV-coated MSNs was observed with transmission electron microscopy (TEM) and the physical properties were characterized using zeta potential measurement.

Results and Discussion: EV-coated amino functionalized MSNs were successfully synthesized using the stated methods. TEM images showed complete coating of the EVs around the nanoparticles. Zeta potential measurements demonstrated the reversal of positive charge of amino-functionalized MSNs to a negative charge imparted by the EV membrane.

Conclusions: We report the successful synthesis of EV-coated amino-functionalized mesoporous silica nanoparticles using three different methods. These biomimetic particles could significantly enhance the delivery of therapeutics to ovarian cancer tumours owing to their low immunogenicity, biocompatibility, and target homing capacity. Future studies will assess their gene delivery potential and efficacy of the particles in vitro.

Acknowledgements: The authors acknowledge the facilities and assistance of the Microscopy Australia Facility at the Centre for Microscopy and Microanalysis (CMM), The University of Queensland. A.G. also acknowledges the support from University of Queensland RTP post-graduate scholarship.

References:
Oral delivery of macromolecules with silica nanoparticles

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Purpose: Diabetes is a chronic disease which impact over 400 million people, and the most effective way to treat diabetes is insulin injection. However, injection pain and injection site infection are responsible for dose skipping in patients. Oral delivery is the most desired drug administration route with the highest patient compliance. However, insulin cannot be administrated orally due to highly acidic condition in the stomach, active hydrolytic enzyme in the intestine and intestinal epithelial1. Silica nanoparticles (SNP) have been widely investigated as a drug carrier for proteins orally because of its excellent biocompatibility, high stability, ease of functionalization, and ability to enhance permeation of biologics such as insulin2. Interestingly, the size of solid SNP could impact its ability to cross the intestinal barrier3, but the influence of pore size has not been investigated.

Methods: Three types of silica nanoparticles (Stöber, MSN, LPSNP) were synthesized with around 100 nm but different pore size. The morphology of silica nanoparticles was observed by Transmission electron microscopy (TEM) and the physiochemical were characterized. The permeability of silica nanoparticles was investigated in Caco2 monolayer model and Caco2/MTX-HT29 co-culture model.

Results and Discussion: SNP around 100 nm with different pore size were synthesized and characterized. In vitro studies proved that silica nanoparticles could successfully open the tight junction of Caco-2 monolayer and Caco-2/ MTX-HT29 co-culture model. The TEER value indicated different pore size SNP had different cellular transportation ability (Figure 1).

Conclusions: The change of pore size driven the shift of surface morphology of SNP, which played a significant role in opening the intestinal epithelial tight junction and may improve the bioavailability of oral insulin.

Acknowledgements: Y.C acknowledges support from the University of Queensland post-graduate scholarship.

References:
Wet milled inhalable Ibuprofen microparticles with improved aerosolization and dissolution

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Purpose: The purpose of the study was to prepare the inhalable Ibuprofen microparticles (<5 µm) for developing dry powder inhaler (DPI) formulations.

Methods: The inhalable sized microparticles were achieved by wet milling and dry milling micronization techniques. The particle size and the morphological properties were examined by mastersizer and scanning electron microscopy (SEM). The DPI formulations were prepared by mixing the milled drug particles with excipients such as L-leucine as a flowability enhancer and Magnesium stearate as a lubricating agent. The drug interactions with the excipients were characterized by using Fourier transform infrared spectroscopy (FTIR), and Using a Twin Stage Impinger (TSI), the in-vitro aerosolization performance of the drug from the DPI formulations were performed at a flow rate of 60 ± 2 L/min and the deposited drug concentrations were quantified by utilizing a validated modified HPLC method. The Ibuprofen release profile was investigated by a Dissolution tester in phosphate buffer solution (pH 7.4) at 37 °C temperature.

Results and Discussion: The fine particle fraction (FPF) of the DPI formulation mixture containing L-Leu (5-6.25%) significantly increased to 38.5 ± 3.8% compared to that of the drug only formulation (FPF as 3.7 ± 0.9%). The dissolution performance of the prepared DPI formulation substantially enhanced with the addition of L-leu owing to the formation of hydrogen bonding with the carboxylic group of ibuprofen (1). The XRD data revealed the crystallinity behavior of the micronized drug. The DSC and TGA analysis provided no significant change in the physical stability of the DPI mixtures No significant interaction among drug and excipients was observed by FTIR.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Formulation Composition (%)</th>
<th>Aerosolization performance</th>
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<tr>
<td></td>
<td>IBF</td>
<td>Lue</td>
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<td>Ibu Original</td>
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<td>DPI formulation with Wet milled IBF</td>
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<tr>
<td>FA</td>
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<tr>
<td>FI</td>
<td>2.5</td>
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<td>DPI formulation with Dry milled IBF</td>
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<td>FJ</td>
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Table 1. Ibuprofen dispersion from DPI formulations.

Conclusions: This study successfully manufactured inhalable sized IBF microparticles by wet milling method for developing DPI formulations. Ibuprofen microparticles from prepared DPI formulations provided better aerosolization in presence of L-leu and Mg-St compared to the drug only formulation. The enhanced solubility, and dissolution of the prepared particles were observed due to the hydrogen bonding. The outcome of this study could be employed for the large-scale production of micronized IBF inhalable particles in future.

Acknowledgements: The authors appreciatively acknowledge Queensland University of Technology, Australia.

References:
The DOX Release and Kinetics Of Temperature Dependent Nanocarrier F127@MIL-88B(Fe)
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Purpose: Metal-organic frameworks MOFs have had varied applications in the biomedical field for decades with considerable attention on intelligence-triggered drug delivery In this study MIL– Fe nanomaterials with gas capture and good biocompatibility were developed in a fusiform shape For optimal drug delivery release tests three Fe sources of MIL– Fe were considered for comparing the crystallinity particle size morphology and surface area or pore size distribution Here a Pluronic F with positive temperature sensitivity was applied during MIL– Fe synthesis in a polytetrafluoroethylene-lined autoclave The pyrolyzed polymer monomers doped into the lattice during the MIL reassembly supported the crystallinity and increased the surface area by four times DLS was used to demonstrate the effectiveness of the changes in particle size distribution The size distribution of F –MIL– Fe was sensitive to the increase in temperature and the size of particles decreased with increasing temperature As the temperature increased from to °C the drug release rate of F –MIL– Fe doubled The MOFs split into smaller crystals with increase in temperature which released the drug loaded in the mesoporous frameworks

Methods: Synthesized MIL-88B(Fe) was obtained from hydrothermal method. The doxorubicin DOX was released at three controlled temperatures namely and °C in simulated veins of humans including × PBS pH = in each isolated system DOX-loaded samples were dispersed in a pre-heated flask with mL PBS and stirred at rpm Samples were collected at the following time intervals: 6 6 and h After data measurement the samples were replenished with the release system Liquid samples were analyzed using UV Vis spectroscopy Hitachi model U to evaluate DOX absorption at 6 nm The raw absorptions were fitted with the DOX calibration curve and calculated using the following equation to obtain the relative release rate:

\[
\text{Drug releasing rate (\%) = 100\% \times } \frac{\text{In situ released amounts (mass)}}{\text{Drug released amounts (mass)}}
\]

Results and Discussion: In our study to model the in vivo environment the dialysis bag and PBS pH = were simulated as blood and vein as a simple and easy device for drug delivery Three isolated devices were set at different temperatures and °C The calibration curve of DOX was collected with 6 nm absorption of UV spectra the R–square value y = a b x of within mg mL The UV absorption of DOX@MIL– Fe mixed with a carrier mg mL and DOX mg mL in PBS mL with pH = was measured and the results of the inner and outer dialysis membranes were divided into I begin and I final to evaluate the concentration of release The DOX absorption value was converted to gram units using mathematical calculations to establish the error of the volume effect Owing to drug release porous material properties have been reported as an important factor in loading and releasing kinetics [ ] nonporous microporous and mesoporous materials showed low encapsulation efficiency and slow-release mechanisms while the macroporous microspheres pore sizes nm displayed a high initial burst release and full release [ ] The MIL– Fe drug release profile is displayed in Fig. 1(a), and the pure drug DOX PBS dilution was referenced as the standard release rate Drug release comparison of free DOX DOX@bare MIL– Fe and three temperatures DOX@F –MIL– Fe are displayed The MIL– Fe type shows a significant gap than free DOX the microporous of MIL– Fe has a delayed-release rate which approached only 6 % at h For the temperature dependence kinetics the drug release profiles of F –MIL– Fe at and °C confirm the thermo–response DOX release and it can be seen that LCST leads to the structural transformation of F –MIL– Fe at °C Therefore F –MIL– Fe had a release rate of approximately % at °C the DOX concentration reversal i.e. concentration of outside > concentration of inside has been suggested as a possible reason After balancing the drug release device a similar long-term release result ~ % of free DOX and F –MIL– Fe was observed Therefore the release rate Fig. 1(a) stabilized after h and the group
F127‒MIL‒88B(Fe) % at °C has increased by % compared to groups at and °C ± % and 6± % respectively. To further analyze the release kinetics, the allometric model \( y = a \times x^b \) was applied to the release kinetic studies at and °C respectively. The calculation duration considered to be the high initial burst release ranged from to h and the release kinetics were simulated using the allometric model in Fig. 1(b). The three temperatures of F127‒MIL‒88B(Fe) have different \( R^2 \) values, which can be used to distinguish the release kinetics reliably. In addition to MIL‒88B(Fe), the temperature-controlled experiments present a reliable index that is higher than \( R^2 = 0 \)

**Figure** Drug release rate analyze with UV spectra has been analyze with math model, the DOX release rate of MIL–88B(Fe) and furthers has been shown in (a). The kinetic study modeling with allometric model simulate the \( R^2 \) value of F127–MIL–88B(Fe) in different temperatures.

**Conclusions:** In this study, the preparation of MIL–88B(Fe) was studied using different iron sources and various purification methods. Structural characterization including XRD, N gas adsorption, ASAP, TEM, and FE–SEM were conducted. Simple drug release tests have proposed a model of temperature acceleration at °C. An increase in temperature causes F127–MIL–88B(Fe) lattice expansion division and leads to rapid drug release. The release rate \( t = \) can be modeled with an allometric model and the temperature interval has an \( R^2 \) value of at °C. In conclusion, this study developed an optimal MIL–88B(Fe) synthesis process with a temperature-sensitive cationic polymer support and it was successfully applied in drug release modeling. Therefore, it is a potential coating technique for MILs or MOFs for drug delivery.

**Acknowledgements:** We would like to thank the Ministry of Science and Technology MOST Taiwan MOST - -E- -MY for funding this study.

**References:**

Development of a Novel SDA321-loaded Self-nanomicellizing Solid Dispersion System for Resistant Acne Infections

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Purpose: Acne vulgaris is one of the most prevalent inflammatory skin conditions worldwide most associated with bacterial infiltration causing the development of skin lesions. The long-term overuse of the existing antibiotics has led to bacterial resistance development indicating a need to develop a new antibacterial. SDA321 is a broad-spectrum antimicrobial agent that has been tested for antibacterial activity against human isolates of Cutibacterium acnes (C. acnes). However, due to its poor aqueous solubility (≤ 40 μg/mL) its translation into an effective anti-microbial agent is challenging. To overcome this challenge, a novel SDA321 formulation (NSF) was developed using the strategy of self-nanomicellizing solid dispersion (SNMSD) to translate SDA321 into an effective anti-acne agent.

Methods: A novel HPLC method was developed using Refractive Index (RI) detector since SDA321 lacks a chromophore making it less susceptible to UV detection. To enhance aqueous solubility of SDA321, three SNMSDs were developed using solvent evaporation method, with polymers namely Soluplus, HPMC-ASLG and HPMC-ASMG; with the drug/polymer ratio 1:5 and evaluated for solubility study. The NSF was characterized using differential scanning calorimetry (DSC), fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), and were also analyzed for particle size, zeta potential, and loading ability. The NSF was also characterised for its antibacterial activity against thirty-one C. acnes isolates, and Staphylococcus aureus (S. aureus) and Staphylococcus epidermidis (S. epidermidis) and further evaluated for cell viability using MTT assay to investigate potential cytotoxicity.

Results and Discussion: A simple, reproducible, precise, and accurate analytical method was developed, shown to be linear between the concentrations 200 -900 μg/mL, with the limit of detection and limit of quantification of 7.1 μg/mL and 21.5 μg/ml respectively. A significant improvement in aqueous solubility was shown by three developed NSF in PBS pH-7.4 with the Soluplus-based formulation (NSF-1) showing the greatest solubility of 117-folds. The characterization results of NSF-1 using DSC (figure 1), XRD, SEM (figure 2) and FTIR showed the uniform entrapment of SDA321 and conversion of crystalline to the amorphous state. The morphology study through TEM (figure 3) and evaluation of micellization property of NSF-1 showed the NSF-1 generates micelles of particle size < 100 nm. The antibacterial study results (MIC <0.008 μg/mL) showed that antibacterial activity of NSF and SDA321 is similar, however, the results of cell viability assay showed that NSF is safer than SDA321.

Conclusions: The results demonstrated that SDA321 is effective against resistant acne bacterial strains and NSF is a promising candidate for the treatment of resistant acne infections.

References:
Eco-evaluation of Analytical Methodologies in Pharmaceuticals Development
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Purpose: Pharmaceuticals’ development is a complex procedure involving several stages, among which analysis plays an integral role. Suitable analytical methods are essential to obtain reliable data for drug loading, uniformity, release, and stability. However, analysis is associated with environmentally harmful aspects such as waste generation, solvent utilization, and energy consumption. Therefore, development and eco-evaluation of some analytical methods were performed to adopt more environment friendly practices.

Methods: The greenness profile of the developed HPLC methods were constructed using several tools; national environmental method index (NEMI), eco scale analysis (ESA), green analytical procedure index (GAPI), and analytical greenness metric approach and software (AGREE)¹,².

Results and Discussion: NEMI provided a general description of the greenness of the analytical methods, nonetheless, weaknesses of the methods were not well displayed. Therefore, NEMI was coupled with ESA, GAPI, and AGREE for thorough assessment as well as obtaining quantitative scores. Accordingly, modifications including the reduction of hazardous chemicals quantity, use of greener solvents, shorter analysis times, and multi-component analysis were applied to ensure the most sustainable procedures possible.

Figure 1. Greenness profiles of HPLC method for the analysis of cisplatin and 5-fluorouracil in release medium using (a) NEMI, (b) ESA, (c) AGREE and, (d) GAPI tools

Conclusions: Formulation scientists aim to improve the quality of human life through tireless attempts to fabricate efficient medications. That being said, research processes are demanding and require considerable trials for optimization and it is very challenging to eliminate all non-green procedures. However, it is worthwhile to consider eco-friendly methods where possible. The evaluation of analysis methods and applying valid modifications can contribute to successful formulation development without compromising the health of researchers in the field and the safety of the environment.

References:
Ultrasonic Atomizer-Driven PLGA-SAHA Nanoparticles to Combat Brain Cancer

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Purpose: Suberoylanilide hydroxamic acid (SAHA), also known as ‘Vorinostat’, is a well-known histone deacetylase inhibitor (HDACi) and has the potential to act as a therapeutic agent against tumorigenesis[1]. Taken into account, our experimental findings indicate that SAHA-PLGA nanoparticles could play a significant role in enhancing the effectiveness, bioavailability and reducing adverse effects of cancer chemotherapy.

Methods: We have fabricated SAHA incorporated into biocompatible and biodegradable PLGA nanoparticles using a facile method of ultrasonic atomization[2] and evaluated their anticancer property (Figure 1 A). Physiochemical properties were characterised using various characterisation techniques and were checked for their anti-tumour properties and efficacy against U87 glioblastoma cells.

Results and Discussion: In this study, SAHA-PLGA NPs synthesized were of average mean size of 105±6.0 nm (Figure 1 B). The In-vitro assessment for their anticancer activity on U87 glioblastoma cells showed cellular cytotoxicity (Figure 1 C). The cellular uptake studies reveal cellular internalization (Figure 1 D).

Conclusions: The work highlights the use of novel approach of using ultrasonic atomizer for the synthesis of SAHA-PLGA-NP’s and significantly enhance the chemotherapeutic efficacy of SAHA targeting brain cancer. The small size particles with biocompatible nature holds the ability for penetration overcoming toughest barrier BBB. It also highlights the inherent potential of these biocompatible entities for chemotherapeutic applications in biomedical and pharmaceutics. They can produce a significant therapeutic effect against brain cancer and may have practical implications towards unreachable drug-resistant tumours.

References:

X-Ray Diffraction and Quantitative phase analysis of developed dry powder inhaler formulation of Puerarin

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Abstract

Pharmaceutical aerosol delivery is undergoing dramatic changes in inhaler devices as well as formulation development. It is best suitable for the drugs which are chemically unstable by oral route of delivery and have poor bioavailability. Puerarin (4,7-dihydroxy-8-d-glucosyl isoflavone) is a Chinese herbal medicine and is commercially available in the market as oral formulations, such as granules, capsules, and pellets. The bioavailability of puerarin from these orally administered formulations is very low and thus requires daily high doses to get the therapeutic benefits. Intravenous administration was thought to be the preferred drug delivery method, but addition of co-solvents causes adverse drug reactions. Therefore, it is essential to administer high doses that consequently leads to severe adverse effects and constrains its clinical application [17]. A new delivery system would be significant to avoid the ongoing limitations of the currently available formulations. To overcome these problems, an alternative delivery strategy for pulmonary delivery as dry powder inhaler (DPI) formulation has been proposed in this study. DPIs are mostly formulated as micronized particles mixed with large carrier particles with the particle size range of 1-5µm. XRD is a powerful tool to characterise the crystalline size, phase composition (Quantitative phase analysis) and crystal structure of active pharmaceutical ingredient. The crystalline size of Puerarin before and after crushing was measured using PXRD and analysed by Rietveld full pattern refinement. The average crystallite size of this drug crystal has been reduced $Rwp = 7.36\%, \chi^2 = 3.96.$, for enhanced drug dissolution and delivery. Different formulation excipients (lactose, leucine, and magnesium stearate) have been utilised to develop the formulation and its phase composition has been verified using QXRD. Their molecular structures were modelled using rigid body and their crystal structure were refined in TOPAS software. A PONKCS phase was built for magnesium stearate to achieve accurate QPA result. Since the refined crystal structure of puerarin monohydrate achieved good fitting to its measured XRD pattern, it enables accurate quantitative phase analysis (QPA) for the API concentration, which is usually the minor phase of low concentration in the formulated mixture.
Figure 1. Rietveld refinement of the puerarin crystal structure using rigid body model to simulate (red line) the measured XRD pattern (black dot) of the pure puerarin sample. Rwp = 7.36%, $\chi^2 = 3.96$.

References:

3D printing in the pharmaceutical field: A versatile technology

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Purpose: The aim of these works has been to deeply explore the possible uses of the fused deposition modeling (FDM) 3D printing technique. Since 1986 when the first 3D printing technology has been patented, this manufacturing field has grown exponentially. During the last decade, the interest in its usage for pharmaceutical related purposes raise dramatically. 3D printing has been explored to produce pharmaceutical forms, medical devices, manufacturing devices, and analytical devices, all personalized based on the user’s needs.

Methods: The FDM technique relies on the extrusion process using a filament obtained from a thermoplastic material (eventually mixed with a drug) that is driven, through a geared system into a heated nozzle in the printing head. Here, becoming softer, it can be deposed layer by layer over the 3D printer building plate. By optimizing the printing parameters, the user can obtain perfectly fused layers in the final object.

Results and Discussion: Here, we would show you the results of our research with the application of 3D printing. We were able to efficiently produce a 3D printed intravaginal ring using thermoplastic polyurethane loaded with clotrimazole, a first-line antifungal treatment. This ring showed a sustained release and an efficient in vitro activity against C. Albicans, the pathogen generating vulvovaginal candidiasis. Moreover, we developed 3D printed microfluidic devices firstly using polylactic acid (PLA) and then polypropylene (PP). Using them, we manufactured a wide library of lipid and polymer-based nanocarriers in a controllable and tunable way. These devices resulted resistant to the manufacturing process with a very lower overall cost compared to commercially available microfluidic systems. Finally, we developed a 3D printed vertical diffusion cell that can be efficiently used instead of glass ones to evaluate both drug release and permeation.

Conclusions: 3D printing has opened a new era in the pharmaceutical field. This has been possible thanks to the versatility of this innovative technology. We strongly believe that in the close future 3D printing will be efficiently integrated in pharmacies to formulate personalized medicines and that this technology will help the diffusion of personalized and low-cost manufacturing and analytical devices in research laboratories.

References: