Nordic POP Annual Meeting 2025

Copenhagen, Denmark
20th - 22nd of August 2025

Einar Lundsgaard Auditorium
Panum building
Faculty of Health and Medical Sciences
University of Copenhagen
Blegdamsvej 3B
2200 Copenhagen N

NORDIC POP

KØBENHAVNS UNIVERSITET

The open-source LaTeX template, AMCOS_booklet, used to generate this booklet is available at
https://github.com/maximelucas/AMCOS_booklet

Contents

About Nordic POP

Nordic POP (patient oriented products) is a Nordic university consortium coordinated by the University of Copenhagen. It aims to provide the scientific foundation for the next generation pharmaceutical products - patient oriented products- by strengthening the use of interdisciplinary approaches within Nordic pharmaceutical sciences.

Prescribing medicine today is based on a One Size Fits All Principle. However, there is a strong need for more personalized solutions in several critical therapy areas. Recent development within genomics and diagnostic field have enabled development of new innovative medicinal products relying on a combination of diagnostic tools and personalized dose, paving the way towards future health care system based on personalized medicines, where a new type of treatment strategies, taking into account the individual variations between patients, can be designed.

Nordic POP aims to deliver innovative patient oriented products with the use of new product design principles, novel methods for understanding of the product performance, and a paradigm shift in the manufacturing of these products We believe, that this could be reached by the the intensive use of interdisciplinary approaches within Nordic pharmaceutical sciences. Our strategy is, thus, based on working together and sharing the existing research infrastructures and expertise and on increasing mobility actions including senior scientific staff mobility and joint PhD programs between different participating units.

The key elements that Nordic POP will focus on are:

- Facilitating the mobility of PhD students and senior staff in the consortium;
- Publishing of the research findings in high impact scientific journals
- Securing innovation and entrepreneurship within the network
- Mentoring younger scientists and supporting the European Research Council (ERC) applications
- Assuring industrial dissemination and safeguarding the strong global position of Nordic pharmaceutical companies
- Positioning this network in an leading role in Europe within pharmaceutical sciences.

Nordic POP is an unique example of collaboration involving 10 Nordic partner universities and 5 affiliated Baltic universities.

Funding and Meeting Sponsors

Nordic POP is supported by the NordForsk program Nordic University Hub (project #85352) for the period 2018-2025.

Meeting Sponsors

Useful Information

Talks will be held at the **Einar Lundsgaard Auditorium** in the Panum Building.

Coffee breaks and lunches will be held outside the auditorium

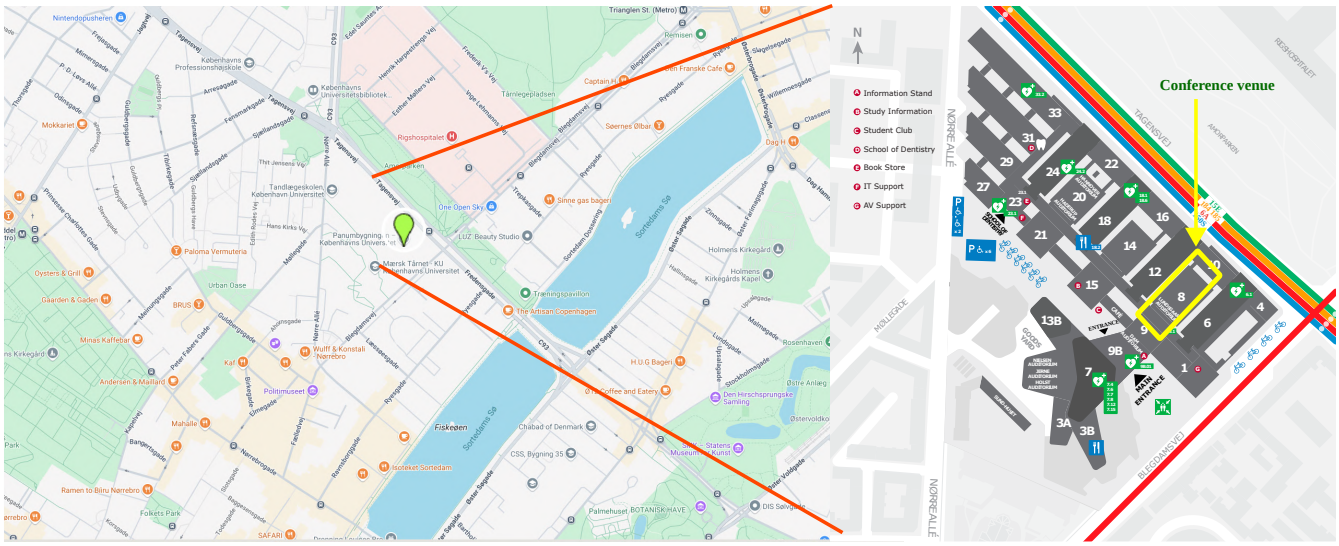
The **conference reception** on Wednesday will be held on the 15th floor of Mærsk Tower at 19:00. The **poster session** is scheduled for Wednesday evening (18:00) and Thursday afternoon (13:00), and will take place outside the auditorium. Posters with even-numbered abstracts will be presented on Wednesday, and those with odd-numbered abstracts on Thursday. Please see the poster abstract list in the corresponding work-package for your poster number.

The **conference dinner** will be held at "Tivoli", located just next to the central train station in central Copenhagen (see next page for further details).

How to get to the Auditorium

The Auditorium is located in the Panum building (8) the address is:
Panum, Building 8
Blegdamsvej 3,
2200
Copenhagen N

Enter the building through the main entrance (located on Blegdamsvej), from there the auditorium will be singposted. Blegdamsvej can be reached by bus 6A, and the perpendicular road Tagensvej can be reached by bus numbers 150s, 6A , 184/185 and 15e (See map below), it is then a (very) short walk to the main entrance.



Conference Dinner

Delighted to announce this year's conference dinner will be held at Brdr. Price in Tivoli Gardens!

Date: 21.08.2025

Time: 20:00

Location: Brdr. Price - https://www.brdr-price.dk/en/restauranter/tivoli
Number 8 on the official Tivoli map (See Below).

Directions: Tivoli Gardens is located between City Hall and the Central Train Station with easy access to public transport. Please use Google Maps for the fastest route on the day and install the DOT Billetter app on your phone for more convenient ticket purchases. If you wish to cycle there are also many rental bike options including Donkey Republic, and Lime.

Instructions: If you signed up for the conference dinner when you registered, you should find a ticket in the back of your name badge. Keep this safe as you will need it to enter the park. Please contact joanne.heade@sund.ku.dk before 17:30 on Thursday 21st if you did not receive or have lost your ticket.
You may enter the park any time before the dinner but please note if you wish to leave the park and re-enter you need a re-entry stamp which you can get by exiting via the Tivoli Food Hall.

For more information, please visit the Tivoli Gardens website - https://www.tivoli.dk/en

Central train station entrance (Bernstorffsgade)

Main entrance (Vesterbrogade)

Timetable

Wednesday, 20th of August

Time	Speaker/Session
15:00–16:00	**Registration and Poster mounting**
16:00–16:30	Welcome and Introduction *Jukka Rantanen and Andrea Heinz, University of Copenhagen*
	Work Package 5 – Synchrotron Session Chair: Samuel Lenton
16:30–17:00	Keynote lecture *Reidar Lund, University of Oslo, Norway* Antimicrobial Peptides and Their Mode of Action: What Can We Learn from Scattering Techniques?
17:00–17:20	*Lea Wurr, University of Helsinki, Finland* Investigating the behaviour of amorphous solid dispersions with stimulated Raman scattering microscopy
17:20–17:40	*Matteo Guidetti, University of Southern Denmark, Denmark* Unravelling the elusive nature of crystalline Form S of posaconazole
17:40–18:00	*Inês C. B. Martins, University of Copenhagen, Denmark* Exploring amorphous *diversity* in pharmaceuticals using combined analytical methods with computational modelling
18:00–19:00	**Poster session, Outside the auditorium**
19:00	**Reception, 15th Floor Mærsk Tower**

Thursday, 21st of August

Time	Speaker/Session
	Work Package 2 – Product Session Chair: Natalja Genina
09:00–09:30	Keynote lecture *Alexandra Teleki, Uppsala University, Sweden* Theranostic inorganic nanoparticles for local treatment of gastrointestinal diseases
09:30–09:50	*Maria Pereverzina, Deparment of Pharmacy UCPH and Novo Nordisk, Denmark* *In situ* solubilization of poorly soluble small molecule APIs
09:50-10:10	*Mai Soliman, University of Helsinki, Finland* Sweet Encounters: Studying nanoparticle-glycan interactions by metabolic labelling and click chemistry
10:10–10:30	*Ana Ćuk, University of Oslo, Norway* Development of an Injectable Hydrogel for Local Drug Delivery by Exploiting Synergism between Polysaccharides and Thermoresponsive Block Copolymers
10:30–11:00	**Coffee break, outside the auditorium**
	Work Package 3 – Engineer Session Chair: Mette Klitgaard
11:00–11:30	Keynote lecture *Daniel Markl, University of Strathclyde, UK* A Self-driving Drug Product DataFactory for Accelerated and Sustainable Development
11:30–11:50	*Petteri Parkkila, Chalmers University of Technology, Sweden* Unveiling structure and biomarker heterogeneity of single extracellular vesicles using waveguide scattering microscopy
11:50–12:10	*Xuedan Sun, University of Copenhagen, Denmark* Sustainable electrospun protein nanofibers for wound healing
12:10–12:30	*Yuming Zhang, Uppsala University, Sweden* A magnetic heating triggered SPION-wax capsule for colon drug delivery with ultrasound imaging assistance
12:30–13:00	**Lunch, outside the auditorium**
13:00–14:00	**Poster session, outside the auditorium**
	Work Package 4 – Analytics Session Chair: Andrea Heinz
14:00–14:30	Keynote lecture *Christian Janfelt, University of Copenhagen, Denmark* Seeing the drug – pharmaceutical applications of mass spectrometry imaging by DESI-MSI and MALDI-MSI
14:30–14:50	*Melissa Hendrén, University of Helsinki, Finland* Development and Validation of Imaging Methods for Intracellular Drug Delivery of Oligonucleotide-Based Drugs
14:50–15:10	*Jakob Tobias Lynnerup,University of Southern Denmark, Denmark* Untangling "Dissolved" Drug Species from Various Formulations of a Poorly Soluble Drug: Sampling Methods, Mechanistic insight, and IVIVC
15:10–15:30	*Caroline Lööf, Chalmers University of Technology, Sweden* Investigating the chemical signature of prostate cancer using mass spectrometry
15:30–16:00	**Coffee break, outside the auditorium**

Thursday, 21st of August continued

Time	Speaker/Session
	Work Package 1 – Barrier Session Chair: Mie Kristensen
16:00–16:30	Keynote lecture *Emma Sparr, Lund University, Sweden* The stratum corneum barrier - from molecular scale to macroscopic properties
16:30–16:50	*Dinh Son Vo, Uppsala University, Sweden* 3D human ileal organoids – a potential screening tool for advanced oral drug delivery systems
16:50–17:10	*Janni Stovring Mortensen, University of Copenhagen, Denmark* Ex vivo mucus-covered Caco-2 cell monolayer model – Compatibility studies
17:10–17:30	*Felix Paulus, University of Southern Denmark, Denmark* In situ formation of stable indomethacin-calcium nanoparticles with enhanced permeation properties in lipolysis buffer
19:00	**Conference dinner at Tivoli**

Friday, 22nd of August

Time	Speaker/Session
	Work Package 6 – Modelling & simulations Session Chair: Inês C. B. Martins
09:00–09:30	Keynote lecture *Mark Coles, University of Oxford, UK* Combining Experimental & Systems Approaches to Accelerate and De-risk Immuno-Therapeutic Development
09:30–09:50	*Xiaoxiao Liang, University of Copenhagen, Denmark* Unravelling Conformational Diversity and the Energy Landscape of Osimertinib Polymorphs through 3D Electron Diffraction and Computational Modelling
09:50–10:10	*Jörg Huwyler, University of Basel, Switzerland* Gene delivery using lipid nanoparticles
10:10–10:30	*Osman Gani, University of Oslo, Norway* Integrating deep learning into Alchemical Free Energy Calculations for lead discovery and optimization
10:30–11:00	**Coffee break, outside the auditorium**
11:00–12:00	**Work package meetings**
12:00–12:30	Closing session, including awards *Jukka Rantanen, Andrea Heinz & Nordic POP 2025 organizing team*
12:30–13:30	**Lunch and poster demounting**

Work package 1: Barriers

WP1 aims to design and apply in vitro barrier models resembling the variable barriers in the human body investigating barrier properties, transporters, solute carriers and enzymes. These barriers may be cell based mimicking epithelial or endothelial barriers. Or, they may be physico-chemical barriers estimating drug release, local concentrations or interactions with physiological constituents such as mucus or colloidal structures.

The design of these in vitro models will be based on physiological-based knowledge, computational design and simulation of flow patterns in restricted geometries, followed by the actual barrier investigations. In these models, the drug transfer across and within these barriers are characterized using diverse analytical tools, as well as high-resolution imaging and synchrotron-based methods.

Validity of these models will be preclinically tested by in vivo pharmacokinetic studies in animals. We aim to develop the future predictive barriers of pharmaceutical science.

Invited speaker talk:
Emma Sparr, Lund University, Sweden
The stratum corneum barrier - from molecular scale to macroscopic properties
Emma Sparr is a professor of physical chemistry and colloidal biology at Lund University, Sweden. Her research is focused on applying physical chemistry principles to biological systems, and the experimental studies include both well-controlled model systems and biological samples with complex compositions. Over many years she has studied lipid-protein interactions and co-assembly, as well as lipid-protein membranes in non-equilibrium conditions. Emma Sparr is a Member of the Royal Swedish Academy of Engineering Sciences and the Director of the Excellence Center COMMONS - Commonalities in biomembrane molecular interactions (funded by the Swedish Research Council (VR).

Selected talks:

Dinh Son Vo, Uppsala University, Sweden
3D human ileal organoids – a potential screening tool for advanced oral drug delivery systems
Janni Støvring Mortensen, University of Copenhagen, Denmark
Ex vivo mucus-covered Caco-2 cell monolayer model – Compatibility studies
Felix Paulus, University of Southern Denmark, Denmark
In situ formation of stable indomethacin-calcium nanoparticles with enhanced permeation properties in lipolysis buffer

The stratum corneum barrier – From molecular scale to macroscopic properties

Emma Sparr[1], R. Qie[1], M. Gunnarsson[1,3], Q.D. Pham[1,3], E.H. Mojumdar[1,4], B. Stenqvist[1], D. Topgaard[1]

[1] Division of Physical chemistry, Department of Chemistry, Lund University, Sweden
[2] TreeToTextile, Gothenburg, Sweden
[3] Gillette Reading Innovation Centre, Reading RG2 0QE Berkshire, UK
[4] CR Competence AB, P O Box 124, 221 00 Lund, Sweden.

Abstract

The outer layer of human skin, stratum corneum, makes up a large interfacial barrier film that protects the body from desiccation and uptake of hazardous chemicals. The healthy skin has low very permeability to most molecules. It is also soft and pliable and it tolerates deformation. Taken together, the stratum corneum fulfills a range of very different requirements, and its special material properties can be related to its molecular scale organization and dynamics with respect to both its lipid, protein and small molecule components.

In this talk I will discuss how stratum corneum can respond on a molecular level to changes in its environment and when it is exposed to solvents or other small molecules. We use polarization transfer solid-state NMR on natural abundance ^{13}C on intact stratum corneum. We characterize the molecular dynamics added molecules when present inside intact stratum corneum and we simultaneously monitor the effects caused by the added solvent on stratum corneum lipids and protein components with close to atomistic resolution. The molecular effects can then be related to changes in the macroscopic properties of barrier function, swelling and mechanical properties. Deepened understanding of molecular effects of foreign compounds on stratum corneum fluidity can have strong impact on the development of skin products in pharmaceutical, cosmetic, and sanitary applications.

Key papers:

[1] E. Sparr, S. Björklund, QD Pham, EH Mojumdar, B Stenqvist, M Gunnarsson, D Topgaard: The stratum corneum barrier – From molecular scale to macroscopic properties, Current Opinion in Colloid & Interface Science, (2023) 67, 101725.
[2] B Stenqvist, MB. Ericson, S Gregoire, B Biatry, G Cassin, M Jankunec, J Engblom, E Sparr: Membrane permeability based on mesh analysis. J Coll Interf Sci (2022) 633, 526.
[3] M Gunnarsson, EH Mojumdar, D Topgaard, E Sparr: Extraction of natural moisturizing factor from the stratum corneum and its implication on skin molecular mobility. J Coll Interf Sci (2021) 604, 480.

3D human ileal organoids – a potential screening tool for advanced oral drug delivery systems

Dinh Son Vo[1], Alina Meyer[1], Foteini Tzioufa[1], Ana Lopes[2], Jens Eriksson[2], Kajsa Björner[3], Johan Vessby[3], Mikael Sellin[2], Per Artursson[1], Madlen Hubert[1]

[1] Department of Pharmacy, Uppsala University, Uppsala, Sweden

[2] Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

[3] Uppsala University Hospital, Uppsala University, Uppsala, Sweden

Introduction: Two-dimensional (2D) *in vitro* cell culture systems have been widely utilized for drug screening due to their simplicity and cost-effectiveness[1]. However, the development of advanced delivery systems, such as nano-sized formulations, demands more sophisticated intestinal models. Bridging the gap between conventional 2D cell-based systems and in vivo models, 3D organoids are able to partially recapitulate a cellular phenotype closer to the normal human intestinal epithelium[2].

Aim: To establish an *in vitro* 3D human organoid model derived from stem cell-containing crypts from ileal biopsies and characterize the model in terms of morphology, differentiation and protein expression.

Method: Culture conditions were established to generate 3D organoids by isolating stem cell-containing crypts from human ileal tissue. Organoids were initially derived as basal-out cultures in an extracellular matrix (ECM). To access the apical surface of the epithelium, the polarity of the organoids was reversed by removing the ECM[3,4]. Apical-out organoids were characterized for structural morphology (size, budding formation, shape) using different microscopy techniques. Mass spectrometry-based proteomics was utilized to evaluate cell composition (differentiation and immaturity marker expression), functionality (tight junction protein expression), and the expression of proteins involved in the cell uptake of drug delivery vehicles and intracellular transport.

Results: Microscopic analysis revealed that the apical membrane of the organoids faced the suspension environment, confirming that the polarity was successfully reversed (Fig 1A). Scanning and transmission electron microscopy demonstrated apical microvilli and typical tight junction structure of single cells in developed organoids (Fig 1B). Apical-out organoids maintained consistent morphological structures over a period of eight weeks (eight passages). Proteomic analysis showed stable protein expression of ileum-specific biomarkers, characteristic features of the intestinal epithelium during these passage numbers. Especially, presence of proteins involved in cell uptake as well as intracellular trafficking were revealed from organoid proteome, presenting an opportunity to utilize the developed model as a screening tool for advanced drug delivery systems.

Conclusion: Apical-out ileal organoids were successfully established with physiology-similar properties and will in the next step be evaluated as a screening tool for advanced drug delivery systems.

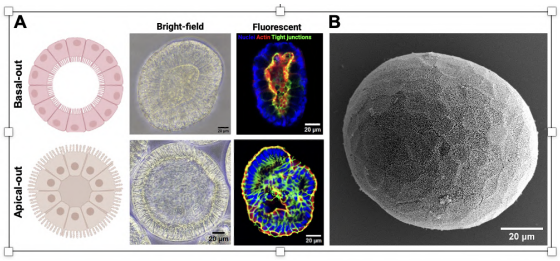


***Fig 1. (A)** Scheme of organoid formats (left), brightfield (middle) and immunofluorescence (right) microscopy images of ileal organoids. **(B)** Scanning electron microscopy showed microvilli structure in apical-out organoids.*

Acknowledgement: The authors are grateful to the European Union for financial support (GENEGUT, GA 101057491).

References: [1] Chu, J. N. et al. Nat. Rev. Gastroenterol. Hepatol. 19, 219–238 (2022) **[2]** Gunti, S. et al. Cancers 13, 874 (2021) **[3]** Co, J. Y. et al. Cell Rep. 26, 2509-2520.e4 (2019) **[4]** Ceylan, M. & Tzioufa, F. et al. bioRxiv (2025)

Ex vivo mucus-covered Caco-2 cell monolayer model – Compatibility studies

J. S. Mortensen[1,2*], **F. Bohr**[1,2*]*, **E. E. M. Nielsen**[2], **N. S. Hatzakis**[2], **H. M. Nielsen**[1]

[1] Department of Pharmacy, University of Copenhagen, Denmark.

[2] Department of Chemistry, University of Copenhagen, Denmark

[*] Shared contribution

Introduction:

The Caco-2 cell monolayer lacks a protective mucus layer, making it rather sensitive to biorelevant drug delivery conditions. Overlaying the monolayer with in vitro mucus models have shown to reduce this sensitivity[1], however these models do not accurately mimic native mucus properties. In contrast, *ex vivo* mucus, better mimicking the native mucus, has rarely been studied, probably due to availability, its composition, and especially the high osmolality, challenging its biocompatibility with the Caco-2 cell monolayer[2].

Aim:

By using orthogonal new approaches, we aimed to investigate if *ex vivo* mucus diluted to isosmotic levels was biocompatible with the Caco-2 cell monolayer.

Method:

The Caco-2 cell monolayer's biocompatibility with hyperosmotic and isosmotic ex vivo porcine intestinal mucus (HYP-PIM and ISO-PIM) as well as porcine gastric mucin solution (PGMII) was investigated by assessing metabolic activity, epithelial integrity (TEER) and nuclear morphology with live cell microscopy. Barrier properties of the mucus-covered monolayer were assessed by permeation of the peptide cyclosporin A.

Results:

For mucus-covered monolayers it was necessary to assess metabolic activity from the basolateral side of the monolayer to prevent damage to the monolayer's integrity from removing the apically applied PIM. It was possible to derive a correlation between apical and basolateral metabolic activities, and we found that none of the mucus preparations had negative effects on the monolayers' metabolic activity or TEER after 3 hours exposure with no difference between HYP-PIM and ISO-PIM. Interestingly, exposure to HYP-PIM led to small but statistically significant changes in nuclear height and roundness, whereas the nuclear morphology of ISO-PIM- covered monolayers was like that of buffer-covered monolayers. While the presence of any mucus preparations significantly reduced the transmucosal permeation of cyclosporin A, HYP-PIM and ISO-PIM significantly reduced the permeation by an additional 15 % and 17 %, respectively compared to PGMII.

Conclusions:

Both HYP-PIM and ISO-PIM were well tolerated by the Caco-2 cell monolayer and constituted a significant barrier to the permeation of the peptide cyclosporin A compared to the commonly used PGMII, highlighting the advantage of this model over *in vitro* alternatives.

Acknowledgements:

The Novo Nordisk Foundation is acknowledged for financial support; BioDelivery center (NNF16OC0021948), COE center (NNF23OC0081287) and 4D cellular dynamics center (NNF22OC0075851). Also, the Swiss National Science Foundation (310030M 204518) is acknowledged for funding.

References:

[1] Birch D. *et al.* Eur J Pharm Sci (2018) 118:144-53.

[2] Boegh M. *et al.* Eur J Pharm Biopharm (2014) 87(2):227-35.

In situ formation of stable indomethacin-calcium nanoparticles with enhanced permeation properties in lipolysis buffer

Mikkel Højmark Tønning[1], **Annette Bauer-Brandl**[1], **Martin Brandl**[1], **Felix Paulus**[1,*], **Ann-Christin Jacobsen**[2]

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[2] Department of Pharmaceutics and Biopharmaceutics, Kiel University, Kiel, Germany

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Introduction:

Microdialysis sampling can differentiate truly dissolved drug from drug associated with colloids, which is not possible with conventional sampling (e.g. filtration)[1]. In this study, we developed a microdialysis method for the weak acid indomethacin (IND) to be used in buffer with calcium, an essential component of in vitro lipolysis buffer. Especially for in vitro lipolysis, where various colloids form, microdialysis sampling could be advantageous over conventional techniques. Surprisingly, we discovered very small IND-Ca nanoparticles, so small only microdialysis could separate them.

Methods:

Using the standard 20 kDa probe, microdialysis conditions were optimized by varying the flow rate and the perfusion medium composition. The size of IND-Ca nanoparticles, revealed by microdialysis sampling with the 20 kDa probe, was measured by DLS. Permeation properties were investigated in side-by-side cells with PermeaPad®

Results:

In the absence of calcium, high recovery (80 %) and fast response (< 10 min) was found using a flow rate of 5 µL/min and 2% polysorbate 80 as perfusion medium. In the presence of calcium, no IND was detected in the perfusate due to the formation of nanoparticles of a poorly water-soluble IND-calcium salt. The nanoparticles, which could not pass the 20 kDa microdialysis probe, had a diameter below 2 nm, which was stable for at least one week. Interestingly, the nanoparticulate salt significantly enhanced permeation across Permeapad®, likely due to charge neutralization. This finding is in line with in vivo observations [2] and highlights the biomimetic properties of the PermeaPad®. With microdialysis probes of 100 kDa cut-off, microdialysis sampling of indomethacin is possible in the presence of calcium.

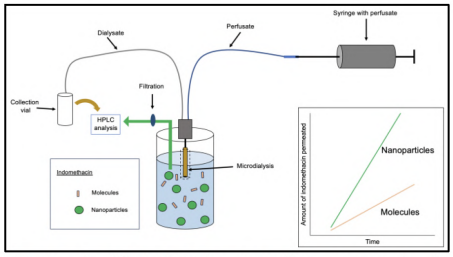

Figure 1 *Microdialysis sampling approach for nanoparticle analysis and schematic comparison between permeation of nanoparticles and unassociated molecules.*

Conclusions:

IND spontaneously forms nanoparticles (diameter < 2nm) with excess of calcium ions at pH 6.5, enhancing permeation in vitro (Permeapad®) and in vivo. These nanoparticles would go undiscovered by centrifugation or filtration, but microdialysis can separate molecules from nanoparticles. The study highlights the impact of buffer composition and analytical methods used on in vitro lipolysis results

Acknowledgements:

The Phospholipid Research Center (MBR-2023-109/2-1) and NordForsk program Nordic University Hub (85352) are acknowledged for funding

References:

[1] Fong, S. Y. K., et al. Eur. J. Pharm. Sci. (2017).

[2] Ogiso, T., et al. J.Pharmacobiodyn, (1983).

WP1 Posters

Abstract ID	Presentation Day	Name
1	Thurs	Anna Klose
2	Wed	Anton Liebezeit
3	Thurs	Bergthora Snorradottir
4	Wed	Camilla Hald Gregersen
6	Wed	Evgeniya Mickols
7	Thurs	Fan Jia
8	Wed	Foteini Tzioufa
9	Thurs	Freja Bohr
10	Wed	Hannah Kolberg
11	Thurs	Jessica Rosenholm
12	Wed	Jonas Burmester
13	Thurs	Jonna Laitinen
14	Wed	Joseph Azumah
15	Thurs	Katariina Mäkiniemi
17	Thurs	Kuldeep Bansal
18	Wed	Leila Rostami
19	Thurs	Maja Nikolajsen
20	Wed	Marco Tjakra
21	Thurs	Marta Mantegna
22	Wed	Mengjie Wang
23	Thurs	Mikołaj Czajkowski
24	Wed	Mikkel Højmark Tønning
26	Wed	Moamin Alkakaei
27	Thurs	Nils Krafft
28	Wed	Nisrin Doudouh
29	Thurs	Prosper Emeh
30	Wed	Rebekka Anna Trenkle
31	Thurs	Saahil Baghel
32	Wed	Seyed Hamed Maljaei
33	Thurs	Shuai Zheng
34	Wed	Simon Dinh
35	Thurs	Teemu Sorsa

Influence of polymeric coating and a targeting peptide on the uptake of DNA origami nanoparticles into an ocular cell line

Anna Klose[1*], Zahra Gounani[1], Sergei Raik[1], Sonja Korhonen[2], Mika Reinisalo[2], Tatu Lajunen[2], Veikko Linko[3,4], Timo Laaksonen[1]
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[2]School of Pharmacy, University of Eastern Finland, Kuopio, Finland
[3]Institute of Technology, University of Tartu, Tartu, Estonia
[4]Department of Bioproducts and Biosystems, Aalto University, Espoo, Finland

Introduction

DNA origami nanoparticles are promising nanocarriers for drug delivery, but face challenges due to their limited stability in blood.[1] DNA origami nanoparticles might be though a great fit for locally delivering therapeutics to the eye, where they are shielded from enzymes in the blood and, due to their inherent negative charge, are expected to be able to diffuse to the back of the eye. Yet, the uptake of plain DNA origami nanocarriers is very limited in retinal ARPE-19 cells.[2] Hence, we explore two strategies to increase the uptake of DNA origami nanoparticles into ocular cells: Firstly, we employ an electrostatically assembled cationic polylysine-PEG5K coating onto the DNA origami nanoparticles that has previously improved uptake in other cell lines.[3] Secondly, we aim to direct the uptake by incorporating targeting peptide moieties into the DNA origami nanoparticle.[4]

Aim

To investigate the impact of a polymeric coating and a targeting peptide on the uptake of DNA origami nanoparticles into the Y-79 retinoblastoma cell line.

Method

The DNA origami nanoparticle called 24-helix bundle (24HB) was prepared to carry Atto488-fluorophores and the targeting peptide PL3. Y-79 retinoblastoma cells were treated with coated 24HB carrying the peptides. Cell uptake was evaluated via confocal microscopy and flow cytometry. Further, the toxicity of the polymer was investigated via AlamarBlue assay. The effect of the coating on particle mobility in the vitreous was investigated by single-particle tracking *ex vivo* in porcine eyes.

Results

Targeting peptides can successfully be attached to the 24HB nanostructure. Upon 24 h exposure, the polymer-coated 24HB is uptaken by Y-79 cells: the 24HB with coating and targeting peptides was more effectively taken up than the coated control samples without the peptide. However, without the polymer coating, the uptake of 24HB with peptides was very limited. The free cationic polymer itself exhibits some toxicity, but upon complexing with 24HB is well-tolerable to the cells. The polymeric coating does not seem to impact the mobility of the 24HB in the porcine vitreous compared to the uncoated structure.

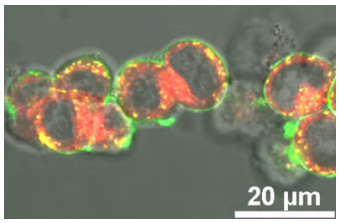

Figure 1 *Confocal image of Y-79 cells treated for 24 h with polymer-coated 24HB carrying 6 peptide targeting moieties and 12 fluorophores (green) and stained lysosomes (red).*

Conclusion

The polymeric coating is essential for promoting cell uptake of DNA origami nanoparticles into ocular cells and does not negatively impact cell viability or particle mobility in the porcine vitreous. Incorporating targeting peptides into the DNA origami in addition to the coating further enhances the cell uptake.

Acknowledgement

The author thanks the Academy of Finland flagship GeneCellNano.

References

1. Keller A, Linko V. Angew. Chem. Int. Ed. (2020) 59(37), 15818-15833.
2. Klose A. *et al.* Nanoscale (2024) 16(37): 17585-17598.
3. Ponnuswamy N. *et al.* Nat. Commun. (2017) 8(1), 15654.
4. Korhonen S. *et al.* Eur. J. Pharm. Sci. (2024) 201, 106866.

Optimization of pH-sensitive liposomes with ionizable cationic lipids

Anton Liebezeit[1*], Shihab Ud Dowla[1], Irem Namli[2], Anna Klose[1], Timo Laaksonen[1], Zahra Gounani[1]
[1]Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, Finland
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Introduction
Liposomes are versatile drug delivery systems that offer several favorable properties, in particular protection of the drug from degradation, prolonged circulation time and improved biocompatibility.[1] To further enhance their performance, various strategies have been explored, including passive targeting mechanisms such as pH-responsiveness. This approach is particularly interesting for facilitating endosomal escape and targeting acidic environments like cancerous and infected tissues. Most pH-sensitive liposomes developed to date rely on the protonation of the ionizable anionic lipid cholesteryl hemisuccinate (CHEMS) under acidic conditions. In contrast, our research focuses on ionizable cationic lipids, which may provide a more effective strategy for delivering drugs to negatively charged bacterial membranes.

Aim
To optimize and characterize a pH-sensitive liposomal formulation using ionizable cationic lipids, aiming to maintain stability in physiological pH while maximizing drug release under acidic conditions.

Method
The ratios of three ionizable cationic lipids DODAP (pK_a=5.6)[2], ALC-0315 (pK_a=6.09)[3] and DODMA (pK_a=6.59)[2] were systematically varied within a liposomal formulation composed of ionizable cationic lipid:DOPE:Cholesterol:DSPE-PEG_{2000} in the molar ratio of x:70-x:25:5, where x ranged from 0 to 70. To further investigate the role of DOPE, helper lipids with differing structural characteristics were substituted in its place to assess their impact on release behavior. Calcein was used as a drug model to determine drug release from 30 min to 24 h of incubation in pH 7.4, pH 6.0 and pH 5.0. Additional characterization was performed using TEM, DSC and DLS.

Results
Optimized formulations for both DODAP and DODMA were identified, showing minimal leakage in pH 7.4 and over 50 % release in pH 5 as illustrated in Figure 1. In contrast, ALC-0315 formulations already demonstrated substantial leakage in pH 7.4 along with broader particle size distribution and high PDI. Formulations containing DOPE showed superior pH-sensitivity compared to lipids with larger hydrophilic head groups and saturated lipophilic chains.

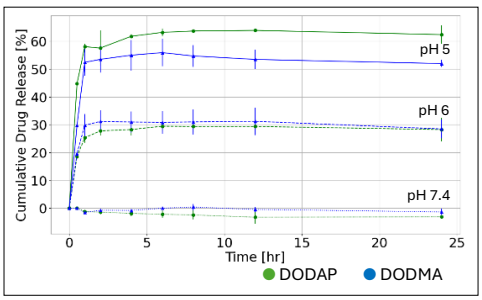

Figure 1: *Cumulative calcein release for optimized DODAP and DODMA liposomes in pH 7.4, 6.0 and 5.0 over 24h with standard deviations*

Conclusion
These results demonstrate that ionizable cationic lipids can effectively be used for pH-sensitive liposomes with a drug release comparable to formulations based on CHEMS.[4] Additionally, insights were gained into the structural interactions between helper and ionizable cationic lipids.

Acknowledgement
The authors would like to thank the Research Council of Finland for financial support (Grant No. 361647).

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An Active Pharmaceutical Ingredient Formulation with Chitosan Products as Permeation Enhancers for Transdermal Drug Delivery

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Introduction

Transdermal drug delivery has gained significant attention for its ability to improve patient comfort and bypass first-pass metabolism. However, the stratum corneum acts as a robust barrier that prevents many drugs from reaching therapeutic levels required for effective treatment[1]. To address this issue, various strategies have been developed, for example the use of penetration enhancers. Common examples include alcohols, fatty acids, and surfactants[2]. Additionally, polysaccharides such as pure chitosan and its derivatives have emerged as promising penetration enhancers due to their ability to interact with skin structures which leads to enhanced drug permeation through the stratum corneum. When incorporated into formulations such as hydrogels, they help improve drug absorption across the skin barrier, making them valuable components in the design of more effective and patient-friendly transdermal therapies[3].

Aim

Investigate whether different chitosan (CH) products enhance skin permeability, and their potential in enhancing the delivery of API through pig skin.

Method

Different CH products, including pure chitosan (80-93% DDA and Mw 121-299 eða 120-300 kDa) and/or trimethyl chitosan (TMC), were incorporated into gel formulations to evaluate their effect on skin permeability. Pig ear skin was used as a model for human skin, and *in vitro* permeation studies were conducted using Franz diffusion cells over a 24h period at 32 °C. Samples were collected at defined time points and analyzed using UHPLC. The amount of diclofenac sodium salt that permeated through the skin was subsequently quantified.

Results

The results from permeation studies and statistical analysis demonstrated that CH products, particularly those with low molecular weight significantly ($p < 0.05$) enhanced drug delivery through pig skin (Figure 1). While a few formulations appeared to result in lower drug release compared to the control, these data sets contained multiple outliers.

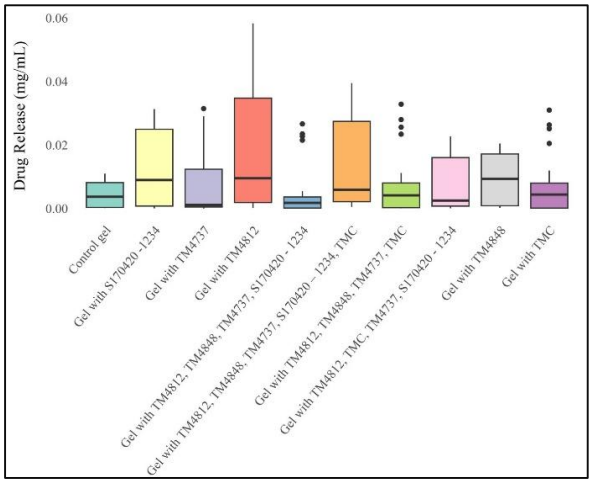

Figure 1 Drug release (mg/mL) from gel formulations containing different chitosan products and their combinations over a 24h period (n = 3). The dots indicate outliers.

Conclusion

Overall, the findings support the potential of specific chitosan products to act as effective permeation enhancers in transdermal drug delivery. Although further research is needed.

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Exploiting film-forming properties of Poly(N-vinylcaprolactam) for drug delivery to the skin

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Introduction
Poly(N-vinylcaprolactam) (PNVCL) is a thermoresponsive polymer gaining focus for its potential in biomedical applications. This is due to its lower critical solution temperature (LCST) being tunable in the range of physiological temperatures, making it a candidate for temperature-responsive drug delivery [1]. Film-forming properties of PNVCL have been suggested but rarely studied, and this attribute could make it suitable for drug delivery to the skin. Film-forming formulations can help improve skin hydration of diseased skin by reducing transepidermal water loss (TEWL) while simultaneously delivering a relevant drug to the skin [2].

Aim
The aim of the study is to synthesize and characterize PNVCL according to its thermoresponsive properties as well as to evaluate PNVCL as film-forming agent for reducing TEWL and delivering a relevant drug to impaired skin. The JAK-inhibitor tofacitinib citrate was chosen as a relevant drug for the treatment of the skin disease atopic dermatitis (AD).

Method
PNVCL was synthesized by free radical polymerization from NVCL. Thermoresponsive properties of PNVCL were evaluated using UV-Spectroscopy, Raman spectroscopy and SAXS/WAXS. Ex vivo skin studies were performed in Franz diffusion cells mounted with porcine dorsal skin impaired by tape stripping to mimic AD-like skin. PNVCL and tofacitinib citrate were dissolved in ethanol and applied to the skin surface, where evaporation of the volatile solvent leads to film formation. TEWL and drug diffusion across the skin were monitored continuously for 24 hours, and drug retention was evaluated after 24 hours.

Results
Characterization of the thermoresponsive behavior of PNVCL was extensively studied in aqueous solution, hydrated state, and dry state using complementary methods, revealing that the thermoresponsive behavior of PNVCL is highly dependent on water content, emphasizing the importance in studying thermoresponsive behavior in relevant biological conditions. PNVCL displays excellent film-forming properties on the skin surface and facilitates delivery of tofacitinib to impaired, AD-like skin.

Conclusion
PNVCL holds potential as a film-forming system for topical delivery of tofacitinib citrate to treat AD.

Acknowledgement
The author would like to thank the LEO Foundation for financial support.

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Primary human hepatocyte spheroids for studies of drug disposition in the human liver

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In drug development, *in vitro* models are used to assess specific aspects of *in vivo* Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties of the drugs. Relevant *in vitro* assays play a crucial role in bringing safe and efficacious compounds to the market, and contribute to the Replacement, Refinement and Reduction (3Rs) of animal experiments.

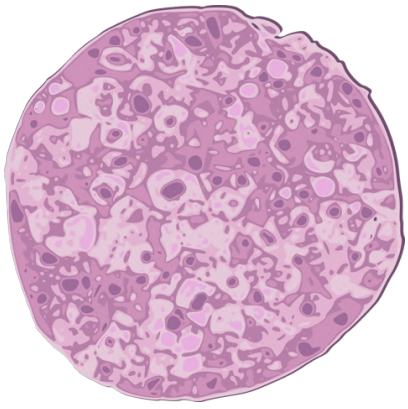
Much effort is now being directed to the development of different physiologically relevant advanced *in vitro* models. One of such models is three-dimensional ***spheroids of primary human hepatocytes (3D PHH)***. These 3D PHH closely resemble the *in vivo* liver at the transcriptome, proteome and metabolome levels. However, 3D PHH are cultured under different conditions and the reproducibility of these cultures varies greatly across laboratories. In our papers we contribute to harmonization of 3D PHH culture approaches.

First, the effect of the ***cell culture medium*** on 3D PHH was evaluated [1,2]. We compared various commercially available media with undisclosed or known content, and also assessed the influence of commonly used medium components such as glucose, insulin, zinc and foetal bovine serum. The choice of cell culture medium had a pronounced effect on the hepatic phenotypes. Importantly, we demonstrate that 3D PHH could be successfully cultured in the animal-serum free physiologically relevant medium with fasting levels of insulin and glucose [3].

Further, we appraised the effect of ***ultra-low attachment culture plates*** on the performance of 3D PHH, and demonstrated that Corning and Biofloat plates facilitate the formation of spheroids with most physiologically relevant phenotypes [4].

Throughout all projects ***mass-spectrometry based global proteomics*** served as indispensable tool for phenotypic description of 3D PHH. However, the choice of ***workflow*** for this analysis has a significant impact on biological interpretation. We compared twelve different proteomics workflows for phenotypic description of 3D PHH, and these results will aid researcher in our field in making an informed decision on the approach to the phenotypical screening of liver spheroid cultures [5].

In conclusion, we provide an improved understanding and optimization of 3D primary human hepatocyte spheroid cultures, and deep integration of this *in vitro* model into drug development pipelines.



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Enhancing Nanomedicine Translation– Evaluating NP transport using Vessel-on-a-chip

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Introduction

Nanoparticles (NPs) offer promising potential for targeted drug delivery, but challenges such as transport efficiency, tissue targeting, and stability in the bloodstream remain[1]. 2D cell culture models produce false positives of NP transport efficiency, and animal models often fail to replicate human responses due to physiological, limiting their predictive value.

Aim

To develop and utilize a vessel-on-a-chip (VoC) model to mimic human vascular conditions to investigate the transport of polymeric NP under different vascular conditions, with the goal of improving the predictive accuracy of NP-based drug delivery systems.

Method

Human Umbilical Vein Endothelial Cells (HUVECs) were seeded into a microfluidic chip and incubated overnight under standard culture conditions to ensure proper attachment and confluency. Following cell attachment, a peristaltic pump was connected to the chip to generate continuous laminar flow, applying shear stress (10 dynes/cm2) for 24 hours to the endothelial layer to promote cell alignment, mimicking physiological capillary conditions. Subsequently, fluorescently labeled polymer nanoparticles (d =110 nm) were introduced into the chip along with fluorescent cell stains. Z-stack images were acquired to assess NP uptake, distribution, and transport across the endothelial layer using confocal microscopy (for 2 hours).

Results

Application of shear stress through flow promoted the expression and development of VE-cadherin tight junctions in the HUVEC monolayer, indicating enhanced cell–cell adhesion. This flow-induced alignment of endothelial cells contributed to the formation of a functional vessel-like barrier, as demonstrated by the controlled transport of polymer NPs across the cell layer. Quantitative analysis revealed that the fluorescence NPs was significantly higher within the endothelial layer compared to beneath it, suggesting effective retention and limited permeability of the barrier under flow conditions, where the NPs transport easily across the cell layer under no-flow condition.

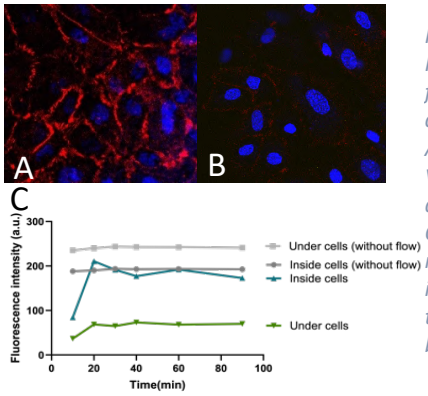

Figure 1. Confocal images of HUVEC monolayers under flow conditions (A) and static conditions (B), stained with Alexa Fluor 647-conjugated VE-cadherin (cell junctions) and Hoechst 33342 (nuclei). Quantification of nanoparticle fluorescence intensity within and beneath the endothelial layer under both conditions is shown (C).

Conclusion

The VoC platform offers a more physiologically relevant model to advance the translation of nanomedicine.

Acknowledgement

This study was made possible by support from Forska Utan Djurförsök.

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Human 3D jejunal organoids as a pre-clinical screening tool of intestinal drug transport

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Introduction

Efficient intestinal drug absorption is an important step in the pre-clinical development of orally administered compounds. Traditionally, immortalized cell lines such as the colorectal adenocarcinoma derived Caco-2 cells have been used to model small intestinal drug absorption *in vitro*[1]. However, despite their usefulness, they have limited physiological relevance in regards to region specific structural characteristics of the small intestine and membrane transporter expression. On the other hand, 3D organoids derived from small intestinal crypts, also called enteroids, show promise as a next-generation pre-clinical *in vitro* model for drug absorption and transport, because their epithelial architecture and transporter abundance are closer to the *in vivo* mucosa[2]. Moreover, their polarity can be manipulated into exposing their apical side into the surrounding media, by everting from basal-out (BO) into apical-out (AO)[3].

Aim

To evaluate jenunal enteroids as an *in vitro* pre-clinical screening tool for intestinal drug transport.

Method

Human apical-out (AO) and basal-out (BO) jejunal enteroids were established from intestinal crypts. The enteroids were diffrerentiated in suspension and characterized. Their maturation and optimally differentiated eneroids was charcterized by quantitative global proteomics and a range of advanced imaging techniques. Jejunal epithelial architecture was confirmed using electron microscopy as well as immunofluorescence. Intact barrier integrity, nutrient absorption and clinically relevant drug transporter activity (P-glycoprotein, Pgp) was further assessed with live-cell microscopy using a hydrophilic integrity marker and different fluorescent transporter substrates.

Results

Enteroids were successfully differentiated and everted from BO to AO polarity, showing all important characteristics of jejunal structure and physiology[4]. They express barrier proteins, influx and efflux transporters similar to native levels and were comprised of optmilally differentiated villus tip enterocytes. Barrier function was demonstrated by exclusion of the fluorescent hydrophilic probe lucifer yellow (LY). Polarized nutrient uptake via fatty acid transport protein 4 was validated by localization of C1-BODIPY-C12 into intracellular lipid droplets in AO enteroids. Pgp activity was also confirmed through apical efflux of substrate rhodamine 123, showcasing a functional pump and successful inhibition by Pgp inhibitor elacridar.

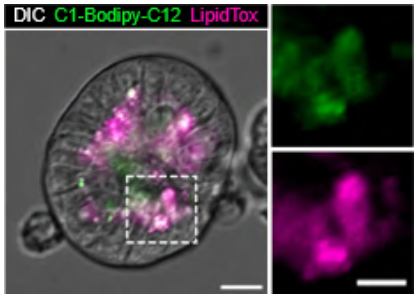

Figure 1. *Co-localization of fatty acid C1-BODIPY-C12 with lipid droplet marker LipidTOX Deep Red after incubation in AO enteroids. Scale bar 20 µm, insert scale bar 10 µm.*

Conclusion

AO and BO jejunal enteroid suspensions show key structural and functional intestinal epithelial characteristics, making them a promising *in vitro* screening tool for oral drug delivery.

Acknowledgements

This research was funded by the Swedish Research Council grant no. 2020-05186 and 2017-01951

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Cell morphology fingerprinting: A machine learning framework to extract mechanisms of morphological changes and enhanced permeation induced by permeation enhancers

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Introduction
The barrier integrity of intestinal Caco-2 monolayers is significantly influenced by permeation enhancer drugs. Despite the effects on barrier function are well characterized, the underlying mechanisms by which these perturbations impact cellular morphology remain poorly understood and the connection between these functional changes and the observed morphological alterations remains to be elucidated[1-3]. Traditionally, cytotoxicity of various drugs and excipients are evaluated based on a quantitative endpoint estimate potentially masking important temporal information, cellular variability and fail to capture the exact mechanism of perturbation.

Aim
To develop a method to capture single cell morphological changes to study the morphological effect of the permeation enhancers L-penetramax, C10 and SNAC for the Caco-2 monolayer model using live-cell microscopy and machine-learning.

Method
Here, we use state-of-the art fluorescent microscopy to capture the morphological changes of cellular compartments at a single cell level under various stimuli. We introduce a machine-learning methodology designed to segment hundreds of individual cells from a single image, enabling the extraction of over 40 key morphological features linked to observed changes across various conditions. To classify these features, we employ a simple logistic regression classifier combined with permutation importance.

Results
We achieve an overall prediction accuracy of 89.11 ± 2.65% through five-fold cross-validation. This result indicates a clear distinction between the different conditions and highlights the significant key morphological differences that contribute to the separation of classes.

Conclusion
Cell Morphology Fingerprinting is a versatile method that can extract and infer valuable insights across a wide range of different cellular system and under various conditions that might alter the cellular morphology.[4,5]

Acknowledgements
The authors would like to thank the NNF Center for Optimized Oligo Escape and Control of Disease (NNF23OC0081287) and the NNF Center for Biopharmaceuticals and Biobarriers in Drug Delivery (NNF16OC0021948).

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A Dual Salicylic Acid Prodrug Approach for Topical Administration: Impact of Thermodynamics on Flux

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Introduction

The skin is a challenging barrier limiting the number of drug compounds that can be administered topically. This work seeks to increase dermal delivery through a dual prodrug approach *in vitro*. Salicylic acid (SA) and SA ester prodrugs are used as model compounds. Assuming that supersaturation is not occurring, the maximum flux of a prodrug is attained when the system is saturated, i.e. in aqueous crystalline solid suspensions. This limits the flux that can be attained with a single prodrug. In a two-prodrug system, the highest thermodynamic activity can be obtained in a system exhibiting ideal behavior, where the prodrugs do not interact with each other. However, if the two prodrugs interact in solution or solid phases, the chemical potential, and thus the thermodynamic activity, will be affected by the presence of a second prodrug [1, 2]. This could be in the case of two solid prodrugs forming a eutectic system, resulting in the formation of an organic liquid phase, or upon the formation of a solid solution. This work aims to investigate how the physical states of the SA prodrugs affect the flux upon simultaneous administration of two prodrugs.

Method

The SA prodrugs investigated were phenyl (A), 4-tolyl (B), phenethyl (C), 2-phenyl-2-oxoethyl (D), and 2-(4-tolyl)-2-oxoethyl (E). The flux across silicone membranes was determined using side-by-side diffusion cells at pH 5.00 and 32 °C. The donor compartment contained saturated systems of either a single prodrug or two prodrugs (mole ratio 1:1) with excess prodrug present throughout the experiments. The acceptor compartment contained 6% Brij O20 (sink conditions). Samples were quantified by HPLC.

Results

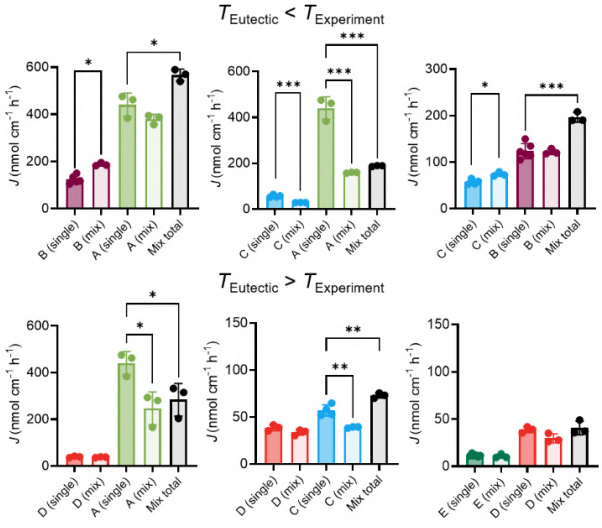

Figure 1: Flux of salicylic acid prodrugs from saturated systems comprising a single prodrug or a two-prodrug system (mole ratio 1:1) across silicone membranes at pH 5.00 and 32 °C.

Prodrug A, B, and C have melting points of 38-43°C while D and E melts at 110-116°C. All two-prodrug systems (Figure 1) formed eutectic systems upon mixing. When excess amounts of system A/D, C/D, and D/E were added to buffer, suspensions were formed as the eutectic temperatures exceed 32°C. However, the eutectic points of A/D and C/D were only 4-7°C above experimental temperature. When excess amounts of A/B, A/C, and B/C were prepared in buffer, a separate liquid phase was observed, consistent with the eutectic point being below 32 °C.

The solubilities of the SA prodrugs in the transport buffer in systems A/D, B/D, and D/E were unaffected upon the addition of a second prodrug. It was expected that individual fluxes in these systems would be unaffected by simultaneous administration with a second prodrug. However, in some cases the fluxes decreased (Figure 1). The solubilities in systems A/B, A/C, and B/C decreased when a second prodrug was added. This is consistent with a decrease in chemical potential. In these systems, the fluxes were expected to decrease by simultaneous administration of two prodrugs. Only in system A/C the fluxes decreased. In systems A/B and B/C the fluxes were unaffected or increased upon co-administration with a second prodrug (Figure 1). Deviations from ideal behavior may be explained by freezing point depressions and solid-state form changes.

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NAP4DIVE: Non-Animal Platform for Nanoparticle-Based Delivery across the Blood-Brain Barrier Interface with Vehicle Evolution

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Introduction

The blood-brain barrier (BBB) is a major obstacle in treating diseases of the central nervous system (CNS) such as Parkinson's, Alzheimer's, Schizophrenia and brain cancer, affecting 180 million Europeans with less than 5% of current candidate drugs effectively reaching the brain. NAP4DIVE strives to revolutionize the traditionally expensive and inefficient drug development for these diseases by establishing advanced non-animal alternatives for testing and predicting nanoparticle (NP)-based drug delivery across the human BBB. This approach aligns with EU and global initiatives to reduce animal testing and advance human-based biomedical research models.

Aim

NAP4DIVE offer new opportunities to deliver effective but as of now undeliverable drugs to the brain and develop new therapeutic, expanding scarce treatment options for CNS disorders such as Alzheimer's, Parkinson's, Huntington's, and Schizophrenia. After the end of the project, NAP4DIVE tools can be adapted and used for personalized disease diagnosis, treatment, and monitoring – when extended with patient data, the computational model, for instance, holds the potential to be expanded for personalized treatment guidance. NAP4DIVE thrives to reduce 95% of animal use in development of drug and delivery systems for CNS diseases, which will also save 30% of costs per drug tested, among other impacts.

Method

The project will develop two complementary non-animal tools: a high-throughput BBB-on-Chip and an *in silico* model based on machine learning ("NP Design Simulator"). A digital repository of optimized nanoparticle designs "NP Design Library" will be created to gather publicly available and newly obtained NP characterisation data, specialised for BBB delivery. The Design Simulator screens thousands of NP designs to recommend the most promising ones, which will be tested in vitro on the microfluidic BBB-on-Chip with real-time measurement of barrier integrity. The accuracy and physiological relevance of both tools will be validated by the pharmaceutical partner through comparison with clinical and preclinical data.

NAP4DIVE tools will reduce animal use in CNS drug development by up to 95% while saving 30 % of costs. By identifying nanoparticles for cross-BBB drug delivery and offering avenues for new effective treatment options, NAP4DIVE addresses one of the most pressing healthcare challenges of the century. A comprehensive HTA will demonstrate market readiness and cost-effectiveness of the tools, an ethical assessment will analyse harm reduction and engagement with regulators and policy makers will promote non-animal alternatives in preclinical testing on a larger scale.

Acknowledgement

This project has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No 101155875, project NAP4DIVE.

References

www.nap4dive.eu

BioLure: An enlightening approach for cytosolic RNA delivery quantification

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Introduction
RNAs offer a huge potential for therapeutic applications in medicine and pharmacy[1]. Despite promising characteristics of RNAs, few RNA-based pharmaceutical products are commercially available, because of RNA's chemical instability and inefficiency in cytosolic delivery. Due to unreliable and/or labor-intensive methods for cytosolic RNA delivery quantification[2], accurate results for RNA delivery are scarce.

Aim
The BioLure project focusses on establishing a method for cytosolic delivery quantification in which a small molecule, D-cysteine, is covalently bound by the RNA of interest. In the reducing environment in the cytosol the D-cysteine is cleaved from the RNA. By addition of NCBT, D-aminoluciferin is formed. As a substrate of Luciferase, D-aminoluciferin causes photon emission in luciferase expressing cells. The concentration of D-aminoluciferin in the cytosol, and thus the amount of delivered RNA, can then be quantified via the light emission.

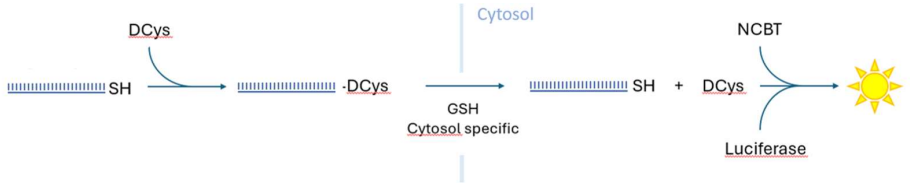

***Figure 1**: 5' SH labelled RNA is conjugated with D-cysteine. After transfection, D-cysteine disassociated from the RNA in the cytosol in the presence of GSH. NCBT and D-cysteine form D-aminoluciferin which enables a bioluminescence reaction in the presence of luciferase.*

Method
RNA is synthesized via in-vitro transcription. Co- or post-transcriptionally, a modified nucleotide is incorporated into the RNA allowing for D-cysteine conjugation. RNA is transfected into cells via electroporation (in later stages other delivery methods will also be explored). The luminescent BioLure signal for RNA delivery success is measured by a plate reader. A GFP based reporter system is used to confirm the transfection success.

Results
Functional siRNA and mRNA have been generated. Thiol labelling has been investigated. Bioluminescent signal can be observed for luciferase expressing cells in the presence of D-cysteine and NCBT.

Conclusion
Preliminary results suggest D-cysteine causes bioluminescence in the presence of NCBT in luciferase expressing cells. Further investigation on the labeling strategy of RNA will be necessary.

Acknowledgement
The Author acknowledges the financial support from the European Union (ERC, BioLure, 101115752).

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Permeability of small molecular weight compounds and macromolecules through *ex vivo* rabbit ocular tissues

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Introduction: Currently, intravitreal injections (IVT) are the standard treatment for retinal diseases. However, these invasive injections require frequent visits to health care which often leads to poor patient compliance. This study explores the potential of topical drug delivery to the retina through the non-corneal route.

Aim: The aim is to determine the apparent permeability (P_{app}) of small molecular weight compounds as well as macromolecules through *ex vivo* rabbit conjunctiva, sclera and sclera-choroid-RPE (SCR).

Methods: 13 compounds were cassette dosed into a Ussing diffusion chamber system in which freshly dissected rabbit ocular tissues were placed between the two chambers. Balanced salt solution (BSS plus) was used as an experiment buffer for conjunctiva and sclera, and Neurobasal medium supplemented with 1 % N2 and 0.4 % GlutamMAX for SCR. The chambers were mixed by bubbling with a gas mix of 5 % CO_2, 10 % O_2, 85 % N_2 (sclera and SCR) or 5 % CO_2, 10 % O_2 for conjunctiva, and maintained at 37 °C for the 6-hour (conjuncitiva and sclera) or 24-hour (SCR) experiment. Permeation of atenolol was used as a tissue integrity marker. The same experiment was also conducted with a Franz cells with sclera. Sample analysis was performed using liquid chromatogram mass spectrometer triple quadrupole (LC-MS QQQ) and the resulting data were analyzed with MassHunter QQQ quantitative analysis program. The results of bevacizumab were analyzed with in-house ELISA.

Results: The P_{app} values of the cassette compounds through sclera ranged from 1.86 ± 2.42 $*10^{-6}$ cm/s (n=11) to 7.79 ± 2.19 $*10^{-6}$ cm/s (n=12) propranolol being the least and atenolol the most permeable. In the Franz system, Papp values were 2-4 times higher ($p<0.01$). P_{app} through conjunctiva were on a range from 6.38 ± 5.54 *10-6 cm/s (propranolol) to 13.39 ± 8.24 *10-6 cm/s (acetazolamide) (n=7). Corresponding values through SCR were 0.030 ± 0.013 and 1.05 ± 0.41 $*10^{-6}$ cm/s (n=5) for propranolol and fluconazole respectively, indicating that sclera and conjunctiva are significantly more permeable to small MW drugs than the RPE ($p<0.05$). The Papp of the anti-VEGF peptide was at the same range with the small molecular weight compounds. For bevacizumab the corresponding Papp values were approximately 10 times lower than the Papp of the cassette compounds ($P<0.01$). Tissues were confirmed to be intact.

Conclusions: In summary, comparing the Ussing chamber with the Franz cell is challenging due to experimental setup differences. Also, the Papp values showed minor variation within the small molecular weight compounds considering the diverse compound properties. The results give valuable information about the rate-limiting barrier RPE in topical drug delivery and for the future development of less invasive administration routes to treat retinal diseases.

Acknowledgments: This study was financially supported by Johnson & Johnson Innovative Medicine and Government of Flander by VLAIO (Vlaams Agentschap Innoveren & Ondernemen, HBC.2021.1130).

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Preparation of stable hyaluronic acid coated liposomes with the one pot method with focus on polymer molecular weight and concentration, amount of charged lipids and the type of hydration medium

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Introduction

Oral health is recognized as a critical part of overall health and quality of life, with the WHO identifying oral diseases including carries and teeth erosion as a public health issue affecting 3.5 billion people globally (1). Some of these oral health disorders is caused by dry mouth. Dry mouth can lead to difficulties in speaking and swallowing, increased risk of dental decay and gum disease, alterations in taste, and negatively impact the overall quality of life for affected individuals. Research on bioadhesive and nanoparticulate formulations, particularly liposomes complexed with biopolymers, shows potential to enhance the administration of therapeutic agents and rehydration of the oral cavity to prevent and reverse oral disorders.

Aim

The overall aim of this study was to develop and characterize hyaluronic acid liposome (HA-lip) complexes using a newly developed one-pot method (2). Additionally, the study aimed to identify key formulation parameters such as HA molecular weight (MW) and amount of charged lipid (DOTAP) that influenced the characteristics and stability of the produced HA-lip complexes. Finally, the impact of the incorporation of glycerol, sodium fluoride (NaF) and paracetamol in the complexes on the stability of the produced complexes.

Method

The HA-lip complexes were prepared by hydrating a dried lipid film of soybean phosphatidylcholine and DOTAP followed by extrusion. The studied formulation factors included HA MW (8-15, 30-50, 90-130 kDa), the amount of DOTAP (10, 18, 25 mol%), the concentration of the coating polymer (0.04 or 0.08 % w/v), and the inclusion of glycerol. The polymer liposome complexes were characterized based on average size, polydispersity index, zeta potential, and storage stability. Alginate coated liposomes were prepared for comparison.

Results

The HA MW combined with the amount of charged lipid were crucial in producing stable HA-lip complexes, where the HA MW 90-130 kDa combined with 25 mol% DOTAP was most successful. Also, the type of charge of the complexes (pos. or neg.) was found to be important for the interaction of the complexes with hydroxyapatite where positive uncoated liposomes were the most adhered followed by HA-lip complexes of MW 90-130 kDa. When glycerol was included in the hydration medium, the stability of the HA-lip complexes was not impacted. The incorporation of NaF, however, led to the formation of large aggregates of the HA-lip complexes (MW HA 90-130 kDa) but not for the Alginate liposome complexes or the uncoated liposomes. Furthermore, it was observed that the incorporation of paracetamol (a neutral drug) in the polymer-liposome complexes did not adversely affect the stability of the prepared formulations.

Conclusion

The study demonstrated the successful production of stable coated liposomes using the one-pot method, underlining the importance of selecting appropriate combinations of the amount of charged lipids, type of polymer, and hydration medium.

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Target discovery for ocular RNA aptamers using proteome integral solubility alteration (PISA) assay

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Introduction: Our previous research has demonstrated that short, synthetic nucleic acid molecules known as RNA aptamers can selectively bind to ocular tissues when administered systemically and locally [1,2]. This tissue-specific binding suggests that RNA aptamers hold promise as targeted drug delivery vehicles for ocular therapies. By enabling more precise delivery, they have the potential to enhance treatment efficacy for eye disorders, extend dosing intervals, and provide a more patient-friendly alternative to the current standard of care, which often relies on intravitreal injections.

Aim: Here we aim to identify the ocular targets of our previously selected RNA aptamer using the recently developed Proteome Integral Solubility Alteration (PISA) assay [3].

Methods: In this study, the employed aptamer employed had a molecular weight of 26 kDa and the nucleotide sequence was 5′-GGGGCCACCAACGACAUUUUGACUGAAUACGCACAUUCGCCAAAUUGCCGGCCCGUUGAUAUAAAUAGUGCCCAUGGAUCCGCGGGUGUCGGG-3′. The supernatant of Long-Evans rat eye tissue homogenate was first incubated for 30 min at 37°C with and without aptamer. Then, samples were subjected to a 3 min thermal treatment at 10 different temperatures (43.9, 44.9, 46.5, 48.5, 50.5. 52.5, 54.5, 56.5, 58.1 and 59.1°C), followed by 6 min incubation at room temperature. Equal aliquots of each temperature were combined, and trypsin/Lys-C mix was used to digest proteins. Analysis was performed using LC-ESI-MS/MS on Evosep One (Evosep, Odense, Denmark) coupled to TimsTOF fleX mass spectrometer (Bruker, Bremen, Germany), equipped with a CaptiveSpray nano-electrospray ionization source. Data-independent acquisition (DIA) was used for analysis, with protein identification and quantification performed in Spectronaut (Biognysos, v19.4) using the Rattus protein database. The fold changes and the p-values of each protein were calculated and plotted in the volcano plot representing the candidate proteins.

Results: Of the 4,205 proteins identified, 54 showed statistically significant upregulation to the aptamer ($p<0.001$). Notable candidates, which are expressed in eyes, included Rho, Mip and RPE65. Rho is localized in rod outer segments and is essential for low-light vision. Mip, a lens-expressed water channel, may help maintain osmotic balance. RPE65, expressed in the retinal pigment epithelium, functions as an isomerohydrolase in the visual cycle.

Conclusions: The PISA method was successfully applied to identify potential target proteins for the selected ocular-targeting aptamer. Future studies will focus on specific ocular tissues incubated with various aptamers, alongside evaluation of aptamer-target binding kinetics using both in silico (molecular modelling) and in vitro (surface plasmon resonance, microscale thermophoresis) approaches. These findings may advance the development of targeted ocular therapies and support the discovery of novel molecular targets for treating eye diseases.

Acknowledgements: This study was supported by the Research Council of Finland (Grant No. 359266). We thank Ph.D. Henri Leinonen for gifting the rat eyes.
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CoE in Materials-Driven Solutions for Combatting Antimicrobial Resistance (MADNESS)

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Introduction

Antimicrobial resistance (AMR) poses a serious threat to public health, as it makes it difficult or even impossible to treat infectious diseases and World Health Organization (WHO) listed AMR as one of the top 10 global health threats. Furthermore, biofilm-forming bacteria pose significant challenges for antimicrobial therapy and are associated with the majority of chronic and device-related infections. For pharmaceutical companies, development of new antimicrobials is not a top priority as it requires enormous economic and labor investment with poor commercial return. Thus, alternative approaches are urgently required to efficiently treat infectious diseases and combat AMR. Our Center of Excellence (CoE) in Materials-driven Solutions for Combatting Antimicrobial Resistance (MADNESS) at Åbo Akademi University was established to generate alternative solutions for AMR, by joining expert forces from the fields of pharmacy, materials chemistry and engineering, as well as software engineering and artificial intelligence (AI).

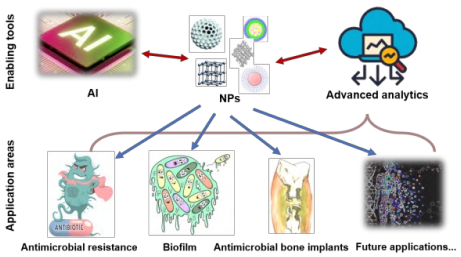
Aim

The aim is to create materials-centered therapeutic strategies against microbial infections with a specific emphasis on addressing AMR.

Methods

We employ several methodologies to create novel materials that are either inherently antimicrobial or have been loaded with antimicrobial agents to augment their therapeutic effects. We will achieve the objectives with the following approaches:

1. Integration of AI approaches in materials design for predicting and maximizing the antimicrobial activity and to shorten the design life cycle.
2. Synthesis of nanoparticles (NPs) – (a) Woody polyphenols as inherently antimicrobial NPs, (b) Functional polymeric NPs as antimicrobial drug carriers and (c) Inorganic NPs as carriers for genetic constructs.
3. Developing antimicrobial medical textiles for combinatorial AMR therapy utilizing flexible cellulose nanofibers (CNFs) with polypyrrole nanocoatings.
4. Development of antimicrobial, functional composite materials for tissue regeneration.
5. Implementing new real-time label-free analytical methods for measurements of bacterial adhesion and biofilm growth kinetics via multi-parametric surface plasmon resonance.



Expected Results

Throughout the study, we will deepen our knowledge on the impact of distinct materials on AMR. We expect to create a “toolbox” for treating infectious diseases where traditional antimicrobials are ineffective.

Conclusion

We envision to develop specifically engineered solutions with substantial potential for practical implementation, with the capacity to exert a significant impact on pharma industries and paving the way for startup companies. We believe to aid people in managing prevalent microorganisms in a financially viable manner in the future.

Acknowledgement

Antimicrobial and Biofilm Eradication Potential of Amine-Functionalized Polymeric Micelles

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Introduction

The rise of antimicrobial resistance (AMR), particularly in biofilm-associated infections, poses a major health threat globally. Biofilms protect pathogens like *Staphylococcus aureus (S. aureus)* and its resistant form, MRSA, from antibiotics lethal effects, making infections harder to treat and potentially life-threatening [1].

Aim

This study investigated the use of diclofenac (DF) as repurposed drug against *S. aureus* infections, using an amine-terminated polymer, mPEG-b-PJL-NH_2, in the form of micelles and assessing the polymer's intrinsic antimicrobial activity.

Method

Micelles were prepared by mixing DF with mPEG-b-PJL-NH_2·HCl in the presence of sodium bicarbonate (to neutralize HCl), and characterized for drug loading, release, size, and zeta potential [2]. Minimum Inhibitory Concentration (MIC) was determined by broth microdilution, and MBEC via an in vitro biofilm model and visualized using crystal violet staining. Further, biofilms grown on cell-derived matrices (CDMs) were also developed to assess formulations efficacy in simulating infected wounds [3]. A cytotoxicity assay was also conducted to assess the biocompatibility of micelles.

Results

Free DF showed antibacterial activity (MIC = 160 ± 33 µg/mL), while blank micelles also possess antimicrobial effect (MIC =148 ± 18 µg/mL), suggesting the advantages of having amine groups on a polymer chain. Micelles having size 46±2 nm enhanced DF solubility by ~70-folds. MBEC assay suggested that free DF had minimal impact (91.5% ± 14.25%), while DF micelles (51.5% ± 9.05%), and blank micelles (28.5% ± 3.79%) are capable of eradicating biofilms. Preliminary tests on the CDM model confirmed these findings, with blank micelles effectively disrupting biofilms. Cytotoxicity assay on HDFn cells suggested that blank micelles were safe up to 100 µg/mL, while DF-loaded micelles showed toxicity at the same concentration.

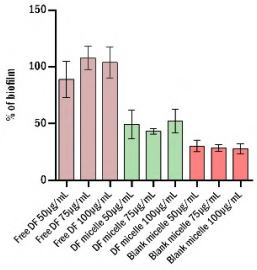

Figure 1 *Biofilm Eradication Assay Plate Reader Results.*

Conclusion

These findings suggest mPEG-b-PJL-NH_2 micelles are capable of treating biofilm-associated infections. DF demonstrates antibacterial activity and can be an interesting candidate against resistant strains. Future studies include testing these formulations against MRSA and comparing their efficacy to the standard antibiotic ciprofloxacin.

Acknowledgement

The authors acknowledge funding support from NordForsk for the Nordic University Hub project #85352 (Nordic POP, Patient Oriented Products).

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Influence of water content on the structure and performance of CAGE for cutaneous drug delivery

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Introduction

Drug delivery across the skin barrier remains highly challenging due to the barrier properties of skin, yet it offers a significant opportunity, both for localized treatment and as an attractive alternative to oral or injectable routes[1]. Ionic liquids (ILs), particularly CAGE consisting of choline and geranic acid (1:2 molar ratio), have emerged as promising strategies to enhance drug permeability of small molecules[2]. However, to fully harness the potential of ILs in cutaneous drug delivery, it is crucial to understand the physicochemical properties of the delivery system and its impact on the skin barrier. Additionally, the presence of water plays a vital role, as skin hydration increases the mobility of skin components[3]. Therefore, understanding how water content influences the drug delivery system and the resulting effect on drug transport across the skin barrier is essential for the development of effective cutaneous drug delivery systems.

Aim

To investigate how varying water content in CAGE affects its molecular structure, drug solubility, rheological properties, and drug permeability across porcine skin.

Method

CAGE was prepared as described by Zakrewsky et al.[2] and combined with varying amounts of water (7,12,17,33,45,55 and 67 vol%). The samples were loaded with drugs of various physicochemical properties (Metronidazole, Baricitinib, Lidocaine, Tacrolimus, Clotrimazole). Drug solubility was quantified by HPLC. Structural changes were analyzed using small-angle X-ray scattering (SAXS), and rheological behavior was evaluated across formulations. Skin permeation studies were conducted using Franz diffusion cells using porcine skin, with HPLC used for quantification.

Results

Varying water content in CAGE led to distinct structural phases: low water levels preserved the neat IL structure, while intermediate concentrations (17–33 vol%) induced lamellar phase formation, and high water content (67 vol%) resulted in more disordered, likely sponge-like phases. Water-loaded CAGE generally showed superior drug loading capacity compared to water itself, with no clear correlation between drug loading efficiency and lipophilicity of the tested drugs. Rheology revealed distinct flow behavior at 17 and 33 vol% water. In pure CAGE, lidocaine and tacrolimus localized in the epidermis and stratum corneum, while baricitinib was retained in the dermis. Permeation studies with varying water contents are ongoing.

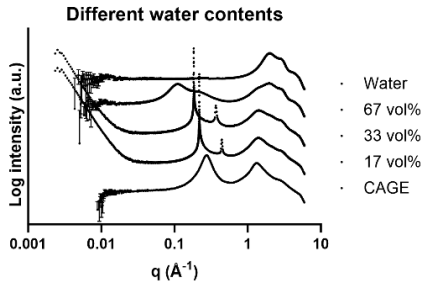

Figure 1 *Small-angle x-ray scattering of CAGE sample with different water contents (0, 17,33,67,100 vol% water in CAGE).*

Conclusion

In conclusion, water content in CAGE plays a key role in modulating its structural organization, drug solubility, and rheological behavior. These findings provide a foundation for further investigation into how water content may influence drug permeation, supporting the development of optimized IL-based systems for cutaneous drug delivery.

Acknowledgement

This work was supported by the LEO Foundation grant no. LF15007 and LF-FE-23-700013.

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Human artificial colonic mucus development for drug delivery study: building on the canine and porcine model

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Introduction

Colonic mucus is a specialized environment compared to other mucus which was present in the mucosal lining of gastrointestinal tract. It hosts the largest number of human microbes, while keeping the inner part close to epithelial cells sterile. However, there are still plenty of different characteristics of human colonic mucus which have not been studied. For example, structural analysis and zeta potential which would influence particle diffusion and drug interaction with the mucus. Human colonic mucus characteristics will be investigated based on previous study[1]. Our research will be focusing on the ascending/proximal colonic mucus. The proximal colon has greater number of microvasculature which indicating more absorption process compared to distal colon[2].

Aim

To study human colonic mucus properties and make interspecies comparison with animal models (dog and pig), of importance from translational aspects.

Method

Samples from the colon, small intestine (adults < 40 years) and also the stomach and small intestine (children < 18 years and adults when possible) are taken from patients undergoing investigation for suspected IBD at one of the participating units. The patient group < 40 years represents a large proportion of newly diagnosed patients who have not yet received treatment and usually do not suffer from other, underlying diseases. Structural analysis of mucus with Cryo-SEM and zeta potential of mucus with DLS were performed based on previous study[3].

Results

An important factor impacting the diffusion of molecules and particles is the zeta potential. The mean value for porcine colonic mucus was −18.2, while the values for canine colonic mucus was −32.1, for adult healthy colonic mucus was −24.5, for child healthy colonic mucus was −18.5, and child inflamed colonic mucus was −19.2.

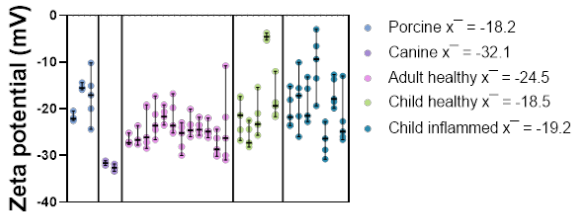

Figure 1 *Zeta potential of interspecies' colonic mucus suspension with standard deviation (error bars)*

Conclusion

It could be shown that the zeta potential of porcine colonic mucus showed closer similarity towards human colonic mucus compared to canine colonic mucus.

Acknowledgement

Marie Skłodowska-Curie grant No 956851 and VINNOVA (2019-00048).

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The Colonic-PVPA: a cell-free *in vitro* permeability model for mucosal colonic drug delivery

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Introduction
Colonic drug delivery has been gaining more interest as a way of treatment for both local and systemic diseases. However, due to the mucus layer's barrier properties, predicting a drug's absorption through the mucosal tissue can be challenging. Thus, during the initial phases of drug development, a straightforward *in vitro* permeability model able to mimic the colonic mucus layer is essential. Based on the already established easy-to-use, cost-effective, and cell-free *in vitro* model PVPA (Phospholipid Vesicle-based Permeation Assay), the Colonic-PVPA was developed, comprising the Porcine Artificial Colonic Mucus (PACM)[1] to investigate the effects of the biosimilar mucus on drug permeability in the colon.

Aim
Development of the Colonic-PVPA model to better mimic the *in vivo* environment of the colonic site to predict the absorption of drugs through the colonic mucosal tissue.

Method
The preparation of the PVPA barriers was based on the previously reported method[2], resulting in a layer of E80 liposomes in a filter support. An additional barrier mimicking the colonic mucus was obtained by incorporating the biosimilar PACM into the barrier. The permeability values of the studied compounds across the Colonic-PVPA were compared to those obtained from the PVPA in the absence and presence of mucin type III dispersion in PBS pH 7.4 (Mucus-PVPA[2]).

Results
The barrier's integrity was assessed by studying the permeability of calcein in the presence of the PACM. The permeability of five model drugs across the novel Colonic-PVPA was tested, showing a significant decrease in the drugs' P_{app} compared to the PVPA and the Mucus-PVPA according to their physicochemical properties (LogP, pKa, molecular weight and charge) and their interaction with the PACM (Figure 1).

Conclusion
The Colonic-PVPA barriers' tightness and, therefore, their integrity was maintained in the presence of the biosimilar mucus. The Colonic-PVPA has shown the potential to mimic the colonic mucus's effect on the selected drugs' permeability. An overall decrease in drug permeability was detected, the extent of which was depending on the physicochemical properties of each drug.

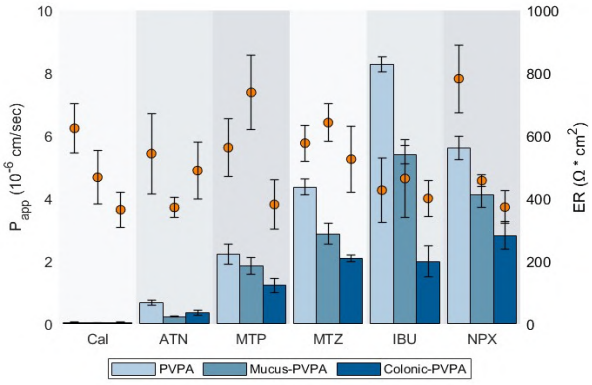

Figure 1. P_{app} values for calcein (CAL), atenolol (ATN), metoprolol (MTP), metronidazole (MTZ), ibuprofen (IBU) and naproxen (NPX), and electrical resistance (ER) of the PVPA barriers, (n=6). The P_{app} of the compounds were investigated in absence of mucus layer (PVPA), presence of mucin from porcine stomach type III dispersion 10 mg/mL (Mucus-PVPA) and presence of Porcine Artificial Colonic Mucus (Colonic-PVPA).

Acknowledgements
The authors are funded by UiT The Arctic University of Norway. The generosity of Lubrizol for the donation of CARBOPOL® 974P NF is highly appreciated and acknowledged.

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Investigation of Endosome escape of Lipid nanoparticles and immunogenicity

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Introduction
Lipid nanoparticles (LNPs) are used as delivery vehicles for nucleic acid-based therapeutics. While they have shown great potential, their unknown immunogenicity remains a key challenge for broader clinical applications beyond vaccines[1]. The endosomal escape plays a dual role—on one hand, it enhances delivery efficacy; on the other hand, it may contribute to toxicity by damaging endosomal and lysosomal membranes[2]. Whether the balance between efficacy and toxicity is determined by endosomal escape remains unclear.

Aim
To investigate the correlation between endosomal escape efficiency and pro-inflammatory responses induced by LNPs.

Method
We formulated LNPs encapsulating in vitro transcribed NanoLuciferase mRNA using ionizable lipids with varying pKa values (DOTAP, DODAP, DLin-MC3-DMA, DLin-KC2-DMA, DLin-DMA. Then the transfection efficiency was measured using the nanoluciferase kit. Immunogenicity was measured using Quanti-Blue assay in RAW-Blue cells. To further minimize the influence of the lipid hydrophobic tail on immune activation, we are also synthesizing a library of ionizable lipids with controlled tail structures and systematically varied pKa valuesEndosomal escape efficiency will be assessed using a high-throughput screening method in GAL-9-mCherry HEK293 cells, where Galectin-9 puncta formation indicates endosomal membrane disruption[3].

Results
The resulting LNPs were negatively charged, with sizes ranging from 119–160 nm and an encapsulation efficiency of approximately 80%, which is suitable for further transfection. The apparent pKa values of the LNPs were generally higher than those of the free ionizable lipids. LNP made from DOTAP and DOTAP always shows a lower transfection efficiency. LNP made from MC3 shows the highest transfection efficiency in RAW264.7 cells. The transfection efficiency of LNPs when N/P =8 is always higher than N/P =3. After 48 h incubation, MC3 LNPs show the highest immunogenicity, whereas KC2 LNPs became more immunogenic over time.

Conclusion
Endosomal escape location and efficiency may be relevant to immunogenicity.

Acknowledgement
The author would like to thank the iCANDOC for financial.

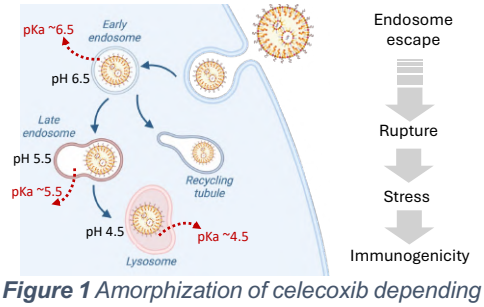

Figure 1 *Amorphization of celecoxib depending on particle size after microwaving with standard deviation (error bars)*

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Biopharmaceutical Assessment of Drug-rich Colloids Formed in Biorelevant Media from Phospholipid-based Amorphous Solid Dispersions.

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The majority of the new chemical entities in the pharmaceutical pipeline are poorly soluble [1]. This led to the emergence of an amorphous solid dispersion (ASD) as an enabling formulation for poorly soluble drugs. The ASDs proved to be a reliable delivery platform with more than 40 marketed products utilizing this technology. The ASDs' popularity paved the way for many *in vitro* studies where a phenomenon was discovered, namely the formation of colloidal amorphous drug-rich particles (ADRP) [2]. In this study, we further elucidate the formation and morphology of ADRP formed upon dissolution of hydrogenated phospholipid-based (HPL) and polymer-based ASDs in biorelevant media [3].
The tested ASDs were composed of copovidone, hydrogenated phosphatidylcholine (0, 0.5, 20, 80% m/m), and fenofibrate (FEN) as a model compound with a set drug loading of 20%. The ASDs were manufactured using hot-melt extrusion. The extrudates were crushed and triturated with a mortar and pestle. The powder was dispersed in fasted state intestinal fluid (FaSSIF) and vortexed for 30 seconds, and subsequently centrifuged, filtered (0.2µm), or nanofiltered (0.02µm). The FEN was quantified in each fraction. Additionally, a dynamic light scattering measurement was carried out in each fraction. Next, a small-scale dissolution study was carried out. A samples were withdrawn at predetermined intervals, centrifuged, and subsequently nanofiltered. To asses the formation of the colloidal particles single particle optical sizing (SPOS) was utilized. The permeation was assessed using a 96-well PermeaPlain plate.
The FEN concentrations in each fraction varied, with the highest detected amounts in the filtrate and nanofiltrate of polymer-based ASD. In contrast, the concentrations of FEN were lower in the HPL-based ASDs regardless of the separation method. The size of the colloids present in the fractions was ca. 70 nm, irrespective of the formulation type and separation method used. In the dissolution study, the ASDs and raw FEN reached similar FEN concentrations, with the exception of ASD with 20% m/m of HPL, where a boost in solubility was observed. Simultaneously, the colloids formed and asses by SPOS displayed growth in size and particle counts over the course of the dissolution experiment, with absence of those particles in raw FEN. The permeation study displayed a robust flux for the ASDs. On the contrary, the raw FEN did not exhibit quantifiable amounts of FEN in the acceptor compartment.
FEN ASDs, when dispersed in FaSSIF, generate colloidal species of the same size, irrespective of the separation method. However, the varying concentration of FEN in the nanofiltrate indicates the formation of small ADRP, and the lack of size difference may be the result of the masking effect of FaSSIF micelles. At the same time, ADRP formed in the dissolution study and grew considerably during the dissolution study. The absence of those colloids reflected on the lack of flux value of FEN, even though the dissolution profile indicated an increase in apparent solubility.
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Understanding the transport of drugs across biomimetic barriers of various phospholipid compositions using a combined experimental and computational approach

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Introduction

Permeapad®, a biomimetic barrier using only the phospholipid phosphatidylcholine (PC) and therefore differs from the enterocytes' brush border, which contains diverse phospholipids such as phosphatidylethanolamine (PE) and phosphatidylglycerol (PG). Metoprolol's lower Permeapad® permeability compared to Caco-2, and PAMPA studies that demonstrated ion-pair transport with anionic phospholipids, suggest PC alone is inadequate [1]. Novel for biomimetic barriers, Molecular Dynamics (MD) simulations may mechanistically explain drug behavior within the Permeapad® [2].

Aim

To gain a deeper understanding of the functionality of the Permeapad® barrier and its transport pathways, which could be affected by the phospholipid composition.

Method

The content of the phospholipids, PE, PG, in addition to PC where systematically varied in the Permeapad® barrier, and the permeability of three model compounds of roughly similar molecular sizes, metoprolol (weak base), hydrocortisone (non-ionizable) and naproxen (weak acid) across those Permeapad® variants was studied using side-by-side cells. Additionally, MD simulations were performed using Gromacs software and CHARMM36m force field, to model drug transport through those Permeapad® variants, and to evaluate how changes in composition affected its performance.

Results

The permeation experiments showed that only the permeation of metoprolol was affected by the phospholipid composition, and was significantly increased across the Permeapad® variants with 50 % PE, 6.25 % PG and 12 % PG. The MD simulations unearthed several descriptors of membrane properties and predicted membrane permeability. An almost inverse relationship was observed between experimental and simulated permeability results.

Conclusion

It was hypothesized that attraction of the drug molecule to the surface of liposomes and the likelihood of internalization were crucial for the permeation mechanisms of the Permeapad® barrier. Combining permeation experiments and MD simulations can aid designing new Permeapad® variants, possibly with in vivo relevant phospholipid compositions.

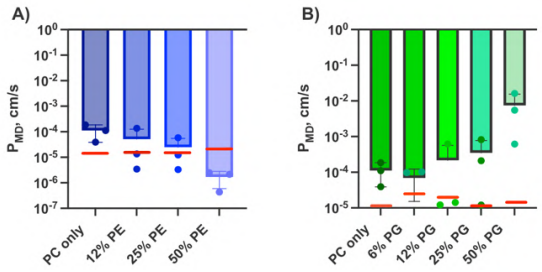

Figure 1 Permeability values (P_{MD}) for metoprolol passing through membranes with various compositions. A) Phosphatidylethanolamine (PE) and B) Phosphatidylglycerol (PG) membranes. For both A) and B) the remainder of the phospholipid composition was phosphatidylcholine (PC). Red lines depict the experimental P_{app} values for the respective membrane compositions. Dots are permeability values of each replicate coming from triplicate simulations.

Acknowledgements

Lipoid GmbH is thanked for phospholipids. Dr. Simon Drescher is acknowledged for initial project discussions. Simulations used resources from NAISS (funded by Swedish Research Council grant no. 2022–06725). NordForsk's Nordic University Hub project #85352 (Nordic POP) is acknowledged for fostering collaborations.

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Serum-free Caco-2 cell cultures for reliable drug permeability studies

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Introduction

In drug development, Absorption, Distribution, Metabolism, and Excretion (ADME) in vitro models commonly rely on fetal bovine serum (FBS) to support cell growth. However, the geographical and seasonal variability of FBS is known to compromise data reproducibility and accuracy. In 2023, Rafnsdóttir et al. introduced an animal serum-free medium (SFM) for 2D and 3D cell culturing, which shows promise for improving in vitro testing reliability and ethics. Here, we demonstrate that culturing Caco-2 cells in the SFM described by Rafnsdóttir et al. maintains cell viability and functionality in a manner comparable to those grown with FBS, supporting improved data reproducibility without compromising model integrity.

Aim

To determine whether the Caco-2 permeability model maintains functional and structural integrity under serum-free culture conditions.

Method

Caco-2 cells were cultured in standard conventional medium (CM) and SFM following established protocols. Growth rate, morphology, and permeability were assessed under each culture condition, focusing on tight monolayer formation and drug transport via low permeability markers, as well as BCRP and MDR1 substrates. Further, global proteomics was used to quantify and compare the overall proteomes with a focus on proteins involved in drug ADME and tight junction integrity.

Results

Caco-2 cells cultured in SFM exhibited comparable growth and morphology to those in conventional FBS-supplemented media. Apparent permeability (P_{app}) values for tight junction markers remained below 1×10^{-6} cm/s in both conditions, confirming monolayer integrity. Efflux ratios for MDR1 and BCRP substrates were significantly higher in SFM, indicating active transporter function. Global proteomic analysis revealed upregulation of MDR1, BCRP, and CYP2S1, while CYP2W1 expression decreased in SFM cultures. No changes were observed in tight junction protein levels between SFM and CM.

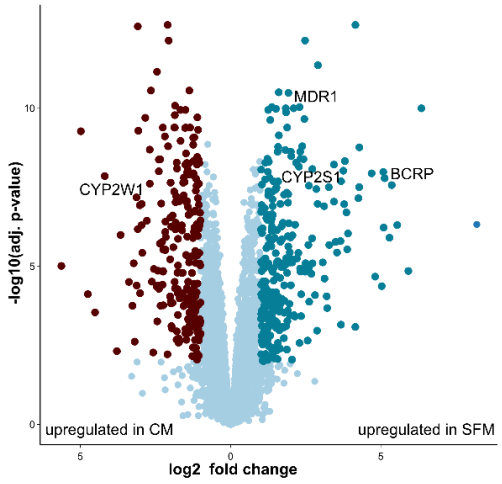

Figure 1. *Differential expression analysis of global proteomes was conducted on Caco-2 cells cultured in serum-free medium (SFM) and conventional FBS-supplemented medium (CM). Stringent analysis criteria were applied (fold change ≥ 1.5; multiple hypothesis-adjusted p = 0.01). The results revealed a marked upregulation of the efflux transporters MDR1 (P-gp) and BCRP, as well as the enzyme CYP2S1, in Caco-2 cells maintained under SFM conditions compared to those cultured in standard CM. These proteomic findings are consistent with permeability study outcomes, indicating enhanced transporter expression and activity in the SFM culture environment.*

Conclusion

These findings suggest that SFM maintains cellular integrity and functional expression of key ADME proteins, supporting its application as a reliable, reproducible, and ethically favorable alternative to serum-based culture systems.

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Nanoscale Probes for Visualization of Cartilage Degradation in Joint Diseases

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Introduction
Early detection of cartilage degradation is limited by the lack of precise imaging techniques for diagnosing diseases such as osteoarthritis (OA) and rheumatoid arthritis (RA). Applications are further limited by poor drug penetration explaining the absence of curing treatments.[1] In addition, rapid turnover of synovial fluid contributes to the quick elimination of traditional drugs from the joint cavity before they reach their intracellular targets. These factors have hindered the development of effective drug-based treatments and imaging techniques for OA.

Aim
To functionalize and evaluate nanoprobes for visualisation of cartilage degradation in articular diseases.

Method
The nanoscale probes were constructed by conjugating fluorescein isothiocyanate and poly(ethylene glycol) monomethyl ether to fifth-generation polyamidoamine (PAMAM) dendrimers.[2] Conjugation and particle size distribution were determined with DLS and various spectrographic techniques, while TLC combined with UV–Vis assessed the relative efficiency of two distinct reaction pathways. After confirmed modification, the nanoparticles were tested on porcine cartilage tissue in vitro using a 3D printed poly lactic acid-based platform with chambers for cartilage explants.[3]

Results
The confocal fluorescence microscopy visualized binding of the functionalized nanoparticles to cartilage tissue treated with collagenase. In contrast, untreated cartilage showed negligible nanoparticle binding, and control experiments with free fluorescein showed no binding, emphasizing the role of the PAMAM-based probe.

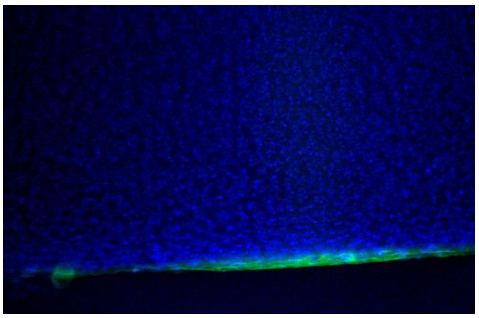

Figure 1 *Confocal image of enzyme treated cartilage with added PAMAM modified particles.*

Conclusion
Collectively, the data suggests that the developed functionalized PAMAM-probes can be used to differentiate between healthy and enzymatically degraded cartilage. Further optimization of these probes, including targeted ligand attachment, holds promise for improving diagnostic specificity and establishing a foundation for the early detection of cartilage degenerating diseases.

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From Liposal®1.0 to Liposal®2.0. Optimizing biopolymer-coated liposomes for enhanced stability and efficacy in the oral cavity

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Introduction

Polymer-coated liposomes are interesting as a drug delivery system and may have potential in relieving the symptoms of dry mouth as well as being a drug delivery system for the oral cavity. Dry mouth is a common issue with a prevalence varying from 22% and 39% in the general population[1, 2]. The condition can lead to challenges in chewing, swallowing, and speaking, as well as an increased risk of dental caries, dental erosion, and candida infections [2]. Despite numerous available saliva substitutes, there is a lack of convincing evidence supporting their effectiveness in relieving dry mouth symptoms. This shortfall highlights the challenge of maintaining drug delivery systems in the oral cavity due to poor retention [3]. Studies have shown that certain biopolymer-coated liposomes exhibit excellent mucoadhesive characteristics, addressing this challenge [4]. In 2024, a double-blind prospective clinical study on Liposal 1.0, a biopolymer coated liposomal spray product, demonstrated safety but fell short in effectiveness. The concentration of polymer coated liposomes, which must be low for stability, may be one reason for this outcome.

Aim

To enhance the efficacy of the polymer-coated liposome spray by examining various polymers and formulations for improving the stability and the bioadhesive properties.

Methods

The liposomes will be prepared using the thin film method. A reproducible method for coating liposomes with biopolymers has been developed by our group, which will be used. In brief, when charged liposomes are introduced into a biopolymer suspension with an opposite charge, the biopolymer can create a coating on the liposome surface [5]. Various biopolymers will be used for coating, and the coated liposomes will be tested for stability and bioadhesive properties. In addition, they will be characterized based on physicochemical properties, such as size, size distribution, and zeta potential. A hydrogel will be created to incorporate the coated liposomes. The rheological properties of the formulation will be examined to explore its ease of application and ability to spread across oral mucosal surfaces.

Expected results

We expect to identify the ideal polymer type, concentrations, and liposome compositions for maximum efficacy in dry mouth treatment. We also expect that several different biopolymer-coated liposomes can be formulated and administered into a mouth spray and a hydrogel, demonstrate stronger adherence to the oral mucosa, and still be stable. The ultimate goal is that Liposal 2.0 is much more acceptable in terms of stability, efficacy and viscosity than Liposal® 1.0.

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Title: Effect of lipidation, chemical structure and formulation composition on the luminal stability and intestinal absorption of peptides

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Introduction

The oral delivery of therapeutic peptides is commonly limited by poor gastrointestinal permeability and susceptibility to digestion by gastrointestinal proteases. Formulation with a permeation enhancer (PE), which can transiently open epithelial tight junctions and increase permeability is the most studied approach for enabling oral peptide delivery (1). Peptide engineering approaches such as amino acid substitution and lipidation have been employed to stabilize peptides against luminal digestion and also prolong their circulatory half-lives respectively (2). We have utilised a series of GLP-1 receptor agonists (RAs), that have undergone similar amino acid substitutions from the native GLP-1 but are different in their degree of lipidation: J211 (non-lipidated), J229 (mono-lipidated) and MEDI7219 (bis-lipidated) and have also include the well-studied semaglutide (mono-lipidated) as a control (3,4). In this study, we explored how the physicochemical properties of these peptides, particularly their degree of lipidation, enzymatic stability and formulation compositions influence their intestinal absorption.

Method

The peptides formulated with and without sodium caprate (C10) were administered as an intraduodenal bolus (ID) or intravenously (IV) in anaesthetized male Wistar Han rats (as previously described in (5)) and blood samples were collected during a 2-hour window. Pharmacokinetic and deconvolution analyses were carried out to determine the rate and extent of peptide absorption in the presence and absence of C10 respectively.

Results

In the absence of C10, all peptides except semaglutide were absorbed at a slow and continuous rate but the cumulative fraction absorbed for each peptide after ID administration is less than 1%. The fraction absorbed increases by around 20 to 25-fold in the presence of C10 for these peptides. However, the fraction absorbed for Semaglutide increased approximately 300-fold to around 2% after co-formulation with C10. The enzymatic stability of MEDI7219 (representative of J221 and J229) and semaglutide was investigated using a modified in vitro pancreatin stability assay. MEDI was relatively stable against pancreatin with a half-life of 4.3h whereas semaglutide was degraded rapidly and completely within the first sampling point of 10 minutes. Interestingly, presence of C10 increased the enzymatic stability of Semaglutide resulting in 15% remaining at 10 mins and an estimated half-life of 3.3 minutes.

Cultivation of the human intestinal cell lines Caco-2 and HT29 in a defined, serum-free cell culture medium

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Introduction
To study oral drug delivery, the intestinal mucosa can be mimicked *in vitro*, with the human colorectal cancer cell line Caco-2 as the most commonly used model. Traditional cell cultures use fetal bovine serum (FBS) as medium supplement, which, however, raises concerns regarding the unethical treatment of animals and batch-to-batch variations leading to poor experimental reproducibility. Therefore, efforts are initiated to replace FBS in cell culturing with serum-free cell culture medium (SFM) such as the one recently suggested by Weber et al. [1].

Aim
To characterise the human intestinal cell lines Caco-2 and HT29 upon culture in a defined, SFM and assess their suitability to study oral drug delivery.

Method
The SFM was prepared as previously described in [1]. Cell growth was determined by analysing confluency of brightfield microscopy images using ImageJ. The cell monolayer barrier properties were assessed by real-time trans-epithelial electrical resistance (TEER) measurement using a CellZscope. The cellular metabolic activity was evaluated using the resazurin assay. Mucin secretion was confirmed by DotBlotting with an antibody directed towards MUC5A. The cellular morphology was investigated by 2D confocal imaging and subsequent image analysis.

Results
Cells cultivated in SFM grow slower compared to cells grown in FBS containing medium. For Caco-2, no difference in cell morphology was observed using brightfield microscopy, while HT29 appeared to grow in differently shaped colonies. TEER measurements confirmed the formation of an epithelial barrier under serum-free conditions. The secretion of mucins was confirmed under all investigated conditions which included HT29.

Conclusion
The investigated cell lines could successfully be cultivated in the SFM.

Acknowledgement
This work was supported by a grant from the Novo Nordisk Foundation Grand Challenge Program: NNF16OC0021948.
We thank Maria Karlgren and Moamin Alkakaei, Uppsala Universitet, Uppsala, Sweden, for guidance and sharing of experiences.

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Incorporation of short and long chain fatty acids in Ionic liquids for transdermal drug delivery

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Introduction
Skin diseases, including fungal and bacterial infections, affect billions worldwide, yet effective treatment is often limited by the skin's outer barrier, the stratum corneum. Transdermal drug delivery (TDDD) offers a non-invasive alternative, though it's constrained by strict drug physicochemical requirements. Chemical permeation enhancers (CPEs) improve permeability but may cause irritation, driving interest in alternatives like Ionic Liquids (ILs). Ionic liquids provide a promising approach for transdermal drug delivery due to their ability to enhance barrier permeability and possess excellent solvation properties for poorly soluble drugs[1]. These functional salts remain liquid at room temperature due to the large asymmetry between the organic cations and anions[2]. Among these, the choline and geranic acid ionic liquid (CAGE) shows strong biocompatibility and permeation enhancement.

Aim
To investigate the incorporation of decanoic (C10) or stearic acid (C18) in CAGE based ionic liquid to form a dual-phasic ionic liquid (IL) system that could enhance transdermal drug delivery of metronidazole and clotrimazole compared to CAGE alone.

Method
Two batches of CAGE were prepared with different stoichiometric ratios of 1:2 and 1:4. (choline:geranic acid) using salt metathesis reaction. Furthermore, decanoic and stearic acids were added in increasing concentrations from 10-100 mg/ml and solubility was assessed using physical visualization and bright field light polarized microscopy. DSC analysis was done to confirm the solubility of fatty acids in CAGE systems. Clotrimazole and Metronidazole were added and solubility was assessed using HPLC quantification.

Results
CAGE 1:4 (choline:geranic acid) significantly improved clotrimazole solubility (35.767 mg/mL with C10) compared to CAGE 1:2, highlighting the influence of IL stoichiometry. Shorter-chain C10 outperformed C18 in solubilization. Rheological analysis confirmed all formulations were liquid-like (G" > G'), suitable for topical use, and DSC showed thermal stability from -70°C to 80°C. Polarized light microscopy indicated possible vesicle formation, aligning with solubility data.

Conclusion
This study highlights the potential of tunable IL-fatty acid systems for transdermal drug delivery of poorly water-soluble drugs. Future work should refine analytics and assess permeation using Franz diffusion cells, advancing IL-based transdermal platforms.

Acknowledgement
The author would like to thank the LEO Foundation for financial support. (grant no. LF-FE-23-700013)

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NOVEL MICROGLIA-TARGETED PRODRUGS LEVERAGING ORGANIC ANION TRANSPORTING POLYPEPTIDE 2B1 (OATP2B1) FOR NEURODEGENERATIVE DISORDERS

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Introduction

Organic Anion-Transporting Polypeptide 2B1 (OATP2B1) is one of the first cloned members of the SLCO solute carrier family with a broad tissue expression including hepatocytes, enterocytes, placenta, retina, and microglia.[1] It is involved in the cellular uptake of a wide variety of drugs, as well as endogenous compounds such as neurosteroids into the human microglia.[2]

Aim

In the present study, the utilization of OATP2B1 for the microglia-selective brain-targeted drug delivery of anti-inflammatory drugs was explored.

Method

Novel prodrugs of several nonsteroidal anti-inflammatory drugs (NSAIDs), including ketoprofen, salicylic acid, flurbiprofen, and naproxen, were designed by incorporating aminosalicylic acid—a known OATP2B1 substrate—as a promoiety. To explore structural diversity, analogs of the aminosalicylic acid promoiety were synthesized with different substitution patterns at the ortho, meta, and para positions using various chemical linkers. The OATP2B1 AlphaFold model was utilized to examine the interactions between the prodrugs and the transporter.[3] *In-silico* studies showed that compounds with a carboxylic acid group at the para and meta positions exhibited stronger interactions and better docking scores. Eight compounds were selected out of these studies for synthesis. The chemical stability and enzymatic bioconversion of the synthesized prodrugs were evaluated using high-performance liquid chromatography (HPLC) in OATP2B1-expressing microglia. Cellular uptake of both the prodrugs and the parent compound was assessed using mass spectrometry.

Results & Conclusion

The combined *in-silico* and *in-vitro* experimental studies identified the optimal substitution pattern and chemical linker structure. The most promising prodrugs demonstrated higher cellular uptake compared to their parent drugs. Additionally, optimal compounds exhibited chemical stability and were successfully bioconverted to release the parent compound. This study enabled the identification of structurally optimized prodrugs that could effectively enhance uptake via OATP2B1 and release the active drug.

Acknowledgement

The author would like to thank the Academy of Finland and Finnish Pharmaceutical society for financial support.

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Drug-Carrier Integrated Fatty Acid Prodrug Microspheres for Synergistic Therapy in Inflammation

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Introduction

Chronic inflammatory diseases and cancers pose significant therapeutic challenges due to low drug loading and poorly biocompatiblity of traditional excipients. Prodrug strategies combined with smart delivery systems offer a promising solution.[1] Natural fatty acids (FAs), with excellent biocompatibility and bioactivity, can act as both drug carriers and therapeutic agents: unsaturated FAs modulate immunity for anti-inflammatory synergy, while saturated FAs induce ROS to enhance tumor microenvironment heterogeneity.[2,3] This research introduces an excipient-free microsphere system using FA-drug prodrugs to achieve high loading, biocompatibility, and synergistic effects.

Aim

To develop and characterize an excipient-free fatty acid prodrug microsphere system for enzyme-responsive drug release and fatty acid-mediated synergistic therapy in chronic inflammation.

Methods:

Dexamethasone-FA prodrugs (myristic, palmitic, oleic, linoleic, α-linolenic acids) were synthesized and formed into ~20 μm microspheres via microfluidics. Particle properties (size/morphology, thermal/chemical stability) were analyzed using SEM, DLS, XRD, DSC, and HPLC under physiological conditions. In vitro release was evaluated in a simulated inflammatory microenvironment. Future work （ongoing）includes transcriptomic/metabolomic analyses, single-cell RNA sequencing, and multimodal imaging to explore mechanisms, efficacy, and biodistribution.

Results

Prodrug microspheres showed excellent biocompatibility. Esterase-responsive drug release occurred over ~50 days, with saturated fatty acids (e.g., palmitic) accelerating release and linoleic acid prodrugs exhibiting the slowest release among unsaturated types. Robust chemical stability was observed across physiological pH. Preliminary data suggest fatty acids modulate ROS/inflammatory pathways, highlighting the system's excipient-free, high-loading, and tailored-release potential.

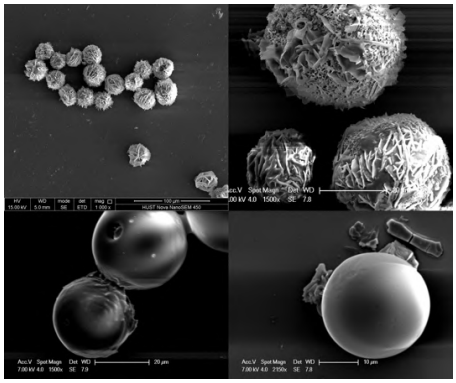

Figure 1 *SEM images of dexamethasone fatty acid ester prodrug microsphere*

Conclusion

This “drug-carrier” integrated microsphere system overcomes traditional formulation limitations, offering tunable release and synergistic bioactivity for personalized chronic inflammation. Ongoing animal studies and multi-omics analyses will further validate mechanisms and efficacy.

Acknowledgements

The authors would like to thank the Research Council of Finland for financial support.

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A Novel Phosphate Prodrug of Sorafenib: Synthesis, Characterization and In vitro Biopharmaceutical Evaluation of Fossorafenib

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Introduction

Sorafenib (SFB) a multi-kinase inhibitor approved for several cancers but with poor aqueous solubility, resulting in low oral bioavailability (38-49%) despite its high permeability. As a BCS Class II compound, its absorption is limited by dissolution[1]. Prodrug strategies, such as phosphate esterification, can improve solubility and promote absorption upon enzymatic cleavage by intestinal alkaline phosphatase (IALP), abundantly expressed on the intestinal brush border and present in the lumen. This cleavage may lead to a transient supersaturation of the parent drug, enhancing absorption before precipitation occurs[2]. Traditional in vitro models like Caco-2 cells are commonly used to study ALP-mediated bioconversion of oral phosphate prodrugs [3], [4]. In contrast, cell-free systems - such as side-by-side diffusion setups eventually combined with microdialysis - enable real-time, non-destructive sampling of molecularly dissolved drug, making them ideal for studies of the complex dynamics under (simulated) human gastrointestinal conditions[5].

Aim

This study aimed to synthesize a water-soluble phosphate prodrug of SFB (fossorafenib, F-SFB) in sufficient yield and purity for in vitro evaluation. Further objectives included assessing F-SFB's solubility, chemical and biological stability, bioconversion, and permeability; comparing externally added bovine IALP and Caco-2 cells for mimicking bioconversion; and developing a microdialysis method to monitor real-time enzymatic cleavage and its interplay with micellar solubilization and permeation.

Methods

F-SFB was successfully synthesized, and its solubility was assessed in HBSS (pH 7.4) and SGF (pH 1.2). Chemical, metabolic and serum stability was evaluated in HBSS, SGF, human liver microsomes, and human serum, respectively. Bioconversion was studied using bovine IALP and Caco-2 cells, which were also used to measure permeability. Microdialysis was employed with bovine IALP to monitor real-time conversion and investigate supersaturation. The influence of bile salts and phospholipids on F-SFB bioconversion was tested using FaSSIF-supplemented HBSS.

Results

F-SFB showed ~585-fold higher solubility in HBSS (pH 7.4) than SFB but poor solubility in SGF (pH 1.2). It was stable in HBSS, liver microsomes, and serum. Enzymatic cleavage with bovine IALP and IALP in the apical chamber of the Caco-2 cell monolayers resulted in supersaturation of SFB. F-SFB was slightly lipophilic (logD 0.39) and moderately permeable across Caco-2 cells (Papp 3.87 - 5.83 × 10^{-6} cm/s. A microdialysis method enabled real-time enzymatic monitoring without ALP inactivation. Bile salts and phospholipids reduced bioconversion, potentially affecting absorption.

Conclusion

This study demonstrated the potential of F-SFB as a phosphate prodrug to enhance solubility. Caco-2 cells captured both bioconversion and permeation, while microdialysis enabled real-time monitoring and revealed how bile salts and phospholipids influence conversion rates. Further research is needed to better understand the intraluminal behavior to further develop and optimize predictive in vitro tools for the evaluation of phosphate prodrugs.

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Title: Hot-melt extruded implants loaded with brinzolamide for intravitreal drug delivery

Authors: Teemu Sorsa[1], Astrid Subrizi[1], Arto Urtti[1] and Matthieu Garin[2]
[1]University of Eastern Finland, [2]Johnson & Johnson Innovative Medicine

Introduction: Intravitreal (IVT) injections are the main administration route for the treatment of retinal diseases. However, the duration of action is limited due to short half-lives of the drug compounds. This is especially the case with small molecules, for which the half-life can be only hours [1,2].

Aim: Our aim was to develop injectable IVT implants using hot-melt extrusion (HME). The challenge was to ensure adequate drug loading in implants small enough to fit through a 25G needle.

Methods: Brinzolamide (PharmaBlock, China) was chosen as the model API. Haake™ MiniCTW micro-conical twin screw compounder (ThermoFisher Scientific, USA) and M22 transport conveyor were used to produce the implant rods. Five grams of polymer and API blend were fed to the extruder at 20 rpm, circulated for 2 minutes at 50 rpm, and extruded through a 0.5- or 1.0-mm extrusion port. Five different polymers with 50wt% and 75 wt% API were screened: Viatel™ DLG 5005E, Purasorb® PDL 05, Purasorb® PDLG 7507, Purasorb® PDLG 8503, and Resomer® RG752H. The crystalline systems were manufactured at 95°C and the amorphous ones at 140°C.

The API, polymers and rods were analyzed using differential scanning calorimetry (DSC) in triplicate. Burst and daily release were studied in triplicate in 0.41% NaCl in water at +37°C, and the samples were analyzed by ultra-high performance liquid chromatography (UPLC). The rods were also analyzed for impurities using UPLC.

Results: All studied polymers were suitable to produce implants with a diameter < 0.21 mm at 50 wt% API content. The extrusion methodology was developed to limit the exposure of API to elevated temperatures and ensure the desired extrusion diameter of the rod. Drug loading of 50 wt% was feasible for all polymers, and 75wt% could be achieved with DLG5005E. Amorphous systems presented only single glass transition between 30.86 to 35.59°C (Tg') as a combination of Tg' of both the API (17.64 °C) and polymer (42.36 to 48.34 °C). This combination Tg' confirmed that the system maintained the amorphous state. On the contrary, the crystalline systems presented only Tg' of the polymer, and the API was fully crystalline.

All implants demonstrated burst release less than 10 % of the total drug amount in one day. The daily release ranged between 4.70 to 22.44 µg/day during the 8-day screening period. No impurities were found in the implants.

Conclusions: Small injectable intravitreal implants were manufactured using hot melt extrusion. The API was incorporated into the polymer as crystalline or amorphous dispersion. This difference and multiple polymers enable us to fine-tune the daily drug release and the release timeframe. For IVT implant, the release timeline should be months to reduce the number of injections required. Developing implants that fit through small needles allow for easier experimentation in smaller animals such as rats and rabbits and might reduce the risk of adverse effects associated with IVT injections.

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Acknowledgements, disclaimers, funding and other disclosure of conflict of interest statements: This study was financially supported by Johnson & Johnson Innovative Medicine and Government of Flander by VLAIO (Vlaams Agentschap Innoveren & Ondernemen, HBC.2021.1130)

Work package 2: Product

Several current challenges in the drug development can be solved by identifying the optimal product design strategy. Within our consortium there are expertise within a broad range of different formulations strategies from nanomedicine to standard tablets. To utilize all of this the focus of WP2 is "enabling formulations".

There are three essential areas where the Nordic POP will aim for providing scientific excellence and new innovative solutions for drug delivery systems: biologicals, representing a category of protein/peptide based drugs that have rapidly gained momentum (specifically, this WP will focus on product design strategies aiming for non-invasive administration of large molecules), site specific drug delivery, covering more efficient and patient friendly products especially for patients with specific need, e.g., in pediatric and geriatric area (WP2 has a special focus on microbiota, mucoadhesion and nanotechnology),and poorly water soluble compounds, representing a major challenge in the drug development, which will be addressed by lipid-based and amorphous drug delivery systems.

Invited speaker talk:
Alexandra Teleki, Uppsala University, Sweden
Theranostic inorganic nanoparticles for local treatment of gastrointestinal diseases
Alexandra Teleki is an Associate Professor in Pharmaceutical Nanotechnology and Drug Development at the Department of Pharmacy and SciLifeLab at Uppsala University, Sweden. She received her MSc in Chemical Engineering from the Royal Institute of Technology (KTH) Stockholm, Sweden and her PhD in Mechanical and Process Engineering from ETH Zurich, Switzerland. Then she joined the Formulation and Application R&D department at DSM Nutritional Products in Basel, Switzerland before she was recruited to Uppsala University and SciLifeLab. Her research focuses on engineering of functional nanomaterials and advanced drug delivery systems for the diagnosis and treatment of gastrointestinal and women's health-related diseases.

Selected talks:
Maria Pereverzina, Novo Nordisk, Denmark
In situ solubilization of poorly soluble small molecule APIs
Mai Soliman, University of Helsinki, Finland
Sweet Encounters: Studying nanoparticle-glycan interactions by metabolic labelling and click chemistry
Ana Ćuk, University of Oslo, Norway
Development of an Injectable Hydrogel for Local Drug Delivery by Exploiting Synergism between Polysaccharides and Thermoresponsive Block Copolymers

Theranostic inorganic nanoparticles for local treatment of gastrointestinal diseases

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Abstract

Superparamagnetic iron oxide nanoparticles (SPIONs) are versatile theranostic nanomaterials for biomedical applications. We have engineered SPIONs via scalable flame synthesis for oral delivery to diagnose and trea gastrointestinal (GI) diseases. SPIONs functionalized with targeting ligands towards inflammation-specific biomarkers can facilitate diagnosis of inflammatory bowel disease (IBD) by magnetic resonance imaging (MRI). Complementarily, SPIONs embedded in wax or hydrogel matrices, can facilitate localized drug delivery and treatment of colorectal cancer (CRC) by magnetic hyperthermia. Overall, these strategies aim to enable patient-friendly and local treatment options for GI diseases. SPIONs were synthesized using a flame aerosol reactor and their physicochemical properties were characterized.[1,2] SPIONs were functionalized with targeting antibodies (e.g. ICAM1) by click chemistry.[3] SPIONs were homogenized into molten wax and shaped into hemispherical capsules using 3D-printed molds. Capsule integrity was tested in biosimilar GI fluids. The magneto-responsive drug release was evaluated in vitro and in vivo using an alternating magnetic field (AMF) and the capsule was imaged in vivo in the colon by ultrasound. Magnetic hydrogels were prepared by mixing SPIONs with PF127 gel. Rheological properties, tissue adhesiveness, and magnetic heating performance were assessed. The thermal ablation potential of the magnetic hydrogel was evaluated *ex vivo* and *in vivo* in mouse MC38 xenografts under an AMF. SPIONs were successfully functionalized with targeting ligands via click chemistry and their targeting efficacy was evaluated using healthy and inflamed Caco-2 cell models. The antibody conjugated SPIONs bound to inflamed tissue in an *in vivo* model for acute colitis and were imaged by MRI. For local drug delivery, SPIONs were successfully incorporated into a docosane/eicosane wax matrix. The capsules exhibited excellent integrity in simulated GI fluids. Upon exposure to an AMF, the capsules melted and rapidly released their cargo, demonstrating their potential as an externally-triggered oral drug delivery platform. Finally, a magnetic hydrogel was successfully developed, that remained in a liquid state at room temperature and immediately gelled at 37°C enabling strong adhesion to tissue. The effective thermal ablation therapy of CRC tumors was demonstrated both *ex vivo* and *in vivo*, with a significant reduction in tumor size after AMF exposure. In conclusion, we have demonstrated the potential of SPIONs as a theranostic platform to enable novel treatment strategies for GI diseases.

Acknowledgement

The authors acknowledge funding from SciLifeLab, the European Research Council (grantagreement no. 101002582), and the EMBO Scientific Exchange Grant.

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In situ solubilization of poorly soluble small molecule APIs

Maria Pereverzina[1,2], Ragna Berthelsen[2], Johan Bøtker[2], Stephen Buckley[2], Jukka Rantanen[1], Drago Sticker[2], Natalja Genina[1]

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Introduction

The *in situ* solubilization of poorly soluble small molecule drug products offers a novel approach to address the challenges of poor solubility and poor oral bioavailability [1], [2]. This study introduces a new approach aimed at enhancing the solubility of poorly soluble drugs through the utilization of heat generated by the exothermic reaction between an inorganic molecule and water. The heat produced during this *in situ* reaction is enough to speed up the dissolution rate of poorly soluble small molecule drugs, and, potentially, lead to their improved oral bioavailability.

Aim

To investigate the influence of the *in situ* heat generation on the on the dissolution properties of poorly soluble drugs.

Method

Ibuprofen (IBU) was used as a model poorly water-soluble active pharmaceutical ingredient (API), which has a melting point at 75-78 °C. The inorganic salts, commonly used in pharmaceutical and food production, were screened as the source of heat generation. The exothermic reactions between inorganic salts and water were quantitatively assessed through calorimetric studies. The effect of the ratio between the inorganic salt and water, the rate of water addition, and the surrounding environment was studied to find the optimal conditions for maximum heat generation. In vitro dissolution studies were conducted with crystalline IBU versus molten IBU, using a MicroDISS Profiler™ (Pion Inc, UK) with inline drug quantification capabilities.

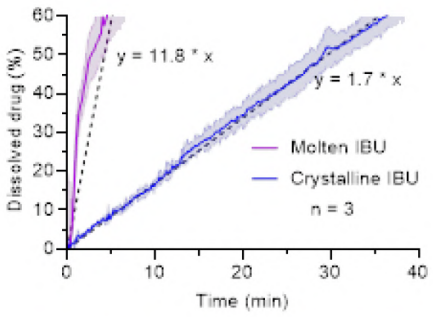

***Figure 1** In vitro dissolution of crystalline ibuprofen versus heated to 80 °C ibuprofen*

Results

The calorimetric studies revealed that the maximum detected change in temperature during the exothermic reaction between inorganic salt and water was equal to Δ80 °C. It was found that the ratio between the inorganic molecule and water should be around 0.9 for the maximum response. A ratio that is too high results in incomplete reaction, while a very low ratio causes the water to absorb excess heat, reducing the overall temperature change. It's crucial to add the water quickly to ensure rapid dissolution of the salt and efficient heat release. In vitro dissolution studies showed that when heated to 80 °C IBU dissolved faster in the used dissolution medium than its crystalline starting material (Fig. 1).

Conclusion

It could be shown that *in situ* heat generation has a positive effect on the dissolution of poorly soluble small-molecule drugs.

Acknowledgement

The author would like to thank Novo Nordisk novoSTAR Programme and Innovation Fund Denmark for funding the project.

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Sweet Encounters: Studying nanoparticle-glycan interactions by metabolic labeling and click chemistry

Mai Soliman[1], **Stefania Garbujo**[2], **Gianni Frascotti**[2], **Davide Prosperi**[2], **Miriam Colombo**[2], **Timo Laaksonen**[1], **Shiqi Wang**[1]

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[2] Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy.

Introduction: Cellular uptake remains key for the successful delivery of RNA nanoparticles (NPs). The glycocalyx is the first contact point for incoming NPs[1]. However, studies face limitations due to glycan complexity and use of fluorescent linkers, which lack physiologic mimicry[2]. We introduce a novel live, quantitative assay combining metabolic labeling and click chemistry with NanoLuc (a bioluminescent enzyme formed of two complementary peptides, HiBiT and LgBiT) so signals can be rapidly read in physiologic conditions

Methods: To verify conjugation, DBCO-HiBiT reaction was tested using ESI-Q-TOF-LC-MS/MS, conjugated HiBiT structure integrity was tested by western blot using Anti-HiBiT antibody, and functional integrity by a calibration curve. 4T1 cell studies were done where metabolic labeling was performed using GalNaz and optimized by DBCO-Cy5. A DBCO-HiBiT proof-of-concept was done using DBCO-GFP assay assessed by flow cytometry. This was followed by assessing the assay ability to detect glycan endocytosis using TAT by a plate reader, which was further verified using CHO-K1, pgsD677, and pgsB618.

Results: For NanoLuc labeling we used a di-functional DBCO linker. As in Fig.1A, the linker clicks with both HiBiT and metabolically labeled azido glycans. Complementary LgBiT is then added for luminescence. LgBiT is membrane impermeable, so it is surface glycan specific and the signal decreases proportional to glycan endocytosis. DBCO-HiBiT conjugation was robust with structure and luminescence integrity retention (results not shown). In cellular assays, a proof-of-concept DBCO-GFP assay using the same linking technology of this project was tested (Fig.1B). Next, DBCO-HiBiT assay was done. To test glycan interactions, we used TAT, a cell penetrating peptide used to improve NP uptake and endocytosed by glycans as model for NPs. (Fig.1C). The signal decreased corresponding to TAT-glycan endocytosis. This was validated by comparing CHO-K1 and its glycan deficient knockouts, pgsD677 (heparan sulphate deficient) and pgsB618 (glycan deficient) cells (Fig.1D).

Conclusions: This assay is a promising technique for the rapid quantification of surface glycoprotein mediated endocytosis in live cells. Prospects include NP-glycan evaluation.

Acknowledgements: Funded by the Research Council of Finland (Grant 1354421).

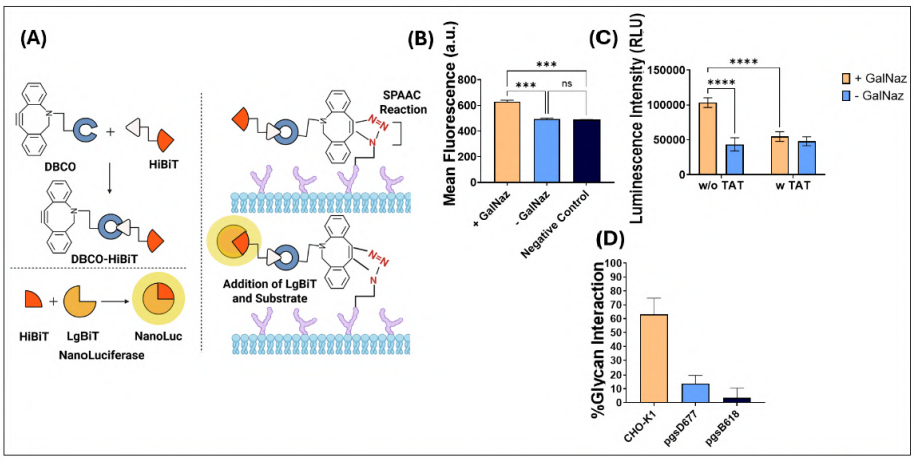

Figure 1. Assay concept and validation. ***A****: Assay Principle.* ***B:*** *Proof of concept DBCO-GFP assay.* ***C****: DBCO-HiBiT assay w or w/o TAT.* ***D****:DBCO-HiBiT assay in CHO-K1 vs pgsB618 vs pgsD677 w TAT*

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Development of an Injectable Hydrogel for Local Drug Delivery by Exploiting Synergism between Polysaccharides and Thermoresponsive Block Copolymers

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Introduction

Poloxamers, PL, are block copolymers of hydrophilic poly-ethylene oxide (PEO) and hydrophobic poly-propylene oxide (PPO). The ability of amphiphilic molecules to form micelles in aqueous solutions have laid foundation for their extensive use in pharmaceuticals[1]. In recent years, they are increasingly attracting attention in the development of injectable hydrogels. The concept is based on micellar entangling which occurs in response to shift from ambient to body temperature, and which macroscopically manifests as sol-gel transition. Blending with polysaccharides, such as alginate, Alg, is employed as a strategy to improve the properties of the in situ formed gels in terms of longer retention at the administration site and better drug retention capacity[2].

Aim

To explore possibilities for physical blending of poloxamers with Alg, without compromising the injectability and the *in situ* gel formation, with the purpose of improving drug retention capacity.

Method

The samples were prepared by dissolving PL407 and its more hydrophilic analogue, PL338, in water, each in concentration of 10 wt%. A model drug, ropivacaine (RPVC) was dissolved in water prior to the polymer addition. Alginate (Protanal LF10/60, Mw 180kDa) was added in concentrations of 0.25-0.75 wt%. Rheological characterization was performed on MCR702e Multi Drive rheometer (Anton Paar, Austria), equipped with the cone plate (CP25-4) for viscosity measurements, or the parallel plate geometry (PP12) for determination of the gelling temperature, T_{gel}. Drug retention capacity was evaluated in an *in vitro* study with the Prolabo dissolution instrument.

Results

Viscosity was obtained by subjecting liquid samples to a stepwise increase in shear rate at 20°C. Alg had a notable effect on viscosity, and a critical concentration of 0.5 wt% was identified above which the formulations were too viscous for injection. With respect to T_{gel}, temperature ramps under small amplitude oscillatory shear revealed a sharp increase in complex viscosity when the PL solution was heated above 30°C. The T_{gel} of pure PL was increased by Alg 0.75 wt%, while the opposite was observed for Alg 0.5 wt%. Interestingly, the lowest Alg concentration was associated with the most beneficial effect on the PL's drug retention capacity, where the time needed for release of 80% of RPVC was almost doubled. This suggests that facilitated micellar packing may act synergistically with the electrostatic interactions between the oppositely charged RPVC and Alg molecules to sustain the drug release, probably by shielding the uncharged portion of RPVC.

Conclusion

Addition of Alg did not seem to interfere with the gelling of PL in the investigated temperature range. Despite somewhat increased viscosity, a concentration range was identified in which injectability was well preserved. Facilitated gelling, observed in formulations with low Alg amounts, correlated with the superior drug retention capacity, which leads to conclusion that addition of Alg is beneficial if it supports micellar packing, in this way enhancing an additional drug retention mechanism.

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WP2 Posters

Abstract ID	Presentation Day	Name
37	Thurs	Alma Karlsson
38	Wed	Arvindh Seshadri Suresh
39	Thurs	Asbjørn Rasmussen
40	Wed	Bárbara Sánchez-Dengra
41	Thurs	Berglind Eva Benediktsdóttir
42	Wed	Erika Espo
43	Thurs	Erna María Jónsdóttir
44	Wed	Ezgi Özliseli
45	Thurs	Fadak Howaili
46	Wed	Giulia Torrieri
47	Thurs	Hamza Yakubu
48	Wed	Hanna Mårtensson
49	Thurs	Hanne Cecilie Winther-Larsen
50	Wed	Hilke Lösing
51	Thurs	Jiaying Shi
52	Wed	Jonas Autenrieth
53	Thurs	Konsta Koskelainen
54	Wed	Krisztina Juriga-Toth
55	Thurs	Laura Giorgi
56	Wed	Lidia Habtemikael
57	Thurs	Loise Råberg
58	Wed	Maddhusja Nalliah
59	Thurs	Madlen Hubert
60	Wed	Maha Fawzi Emam Naser
61	Thurs	Maria Pereverzina
62	Wed	Mariana Mazetto de Carvalho
63	Thurs	Marianna Boraschi
64	Wed	Mats Rune Pettersen
65	Thurs	Melvin Wostry
66	Wed	Mette Mølgaard
67	Thurs	Mikkel Højmark Tønning
68	Wed	Paarkavi Udayakumar
69	Thurs	Pall Thor Ingvarsson
70	Wed	Passant M. Al-Maghrabi
71	Thurs	Puja Gangurde
72	Wed	Randi Hamre Svendsen
73	Thurs	Ratish Rajgopalan Nair
74	Wed	Rebekka Hovd
75	Thurs	Salah Eddine Ghellab
76	Wed	Thomas Adams
77	Thurs	Tuomas Kilpeläinen
78	Wed	Vanessa Chivere
79	Thurs	Wei LI
80	Wed	Wuzhong He
81	Thurs	Yu Cheng

Formulated Fluorescent RNA Studied with Spectroscopy and Scattering Techniques

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[1]Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Sweden

RNA has emerged as a promising biomolecule in drug development for diseases currently lacking effective treatment. However, its poor stability and low bioavailability necessitates advanced delivery systems, such as lipid nanoparticles (LNPs). RNA therapeutics and their delivery vehicles are often studied using fluorescence-based techniques, typically by labelling the RNA with conventional fluorophores like cyanine-5 (Cy5). Yet, due to its bulky structure, Cy5 may perturb RNA behaviour, leading to nonrepresentative results. To address this, we have developed minimally perturbing fluorescence base analogues (FBAs), such as tC^O, which can be incorporated directly into RNA via transcription. In this study, we investigate if and how fluorescence labelling of RNA alters LNP structures by formulating RNA labelled with tC^O and Cy5, respectively, and compare it to unlabelled RNA, using small angle X-ray and neutron scattering (SAXS and SANS). Furthermore, using absorption spectroscopy we reveal that Cy5 dimerizes within the double-stranded RNA used here, which could perturb the lipid-RNA self-assembly. This is supported by our scattering data which shows that LNPs containing the dimerising Cy5 RNA form particles with reduced physical and hydrodynamic radii compared to those with unlabelled and tC^O-labelled RNA.

Additionally, we are exploring the concentration dependent fluorescence lifetime of another fluorescent base analogue, 2CNqA, for its potential versatility in fluorescence lifetime imaging. We observe that a decrease of RNA concentration in buffered solution results in a decreased fluorescence lifetime of 2CNqA. We suggest that this is due to less RNA strand interactions. On the contrary, the lifetime of LNP-encapsulated 2CNqA-labelled RNA is unaltered, owing to the constant local concentration within the LNP irrespective of a change in overall concentration. When decreasing pH we detect a time-dependent decrease in lifetime, suggesting LNP rupture and RNA release. Our results suggest that 2CNqA-labelled RNA may in the future be used in fluorescence lifetime imaging for tracking RNA uptake into cells via endocytosis and monitoring endosomal escape – an essential but poorly understood step in RNA delivery.

Influence of humidity and storage on the physical stability of hot melt extruded HPMC-Naproxen amorphous solid dispersions

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Introduction
Amorphous solids dispersions (ASDs) are a promising approach to overcome the challenges faced by the poor aqueous solubility of many drugs used in modern pharmaceutical industry[1]. However, these systems are susceptible to instability issues and the drugs have a tendency to recrystallize over time. Recrystallization happens due to thermodynamic instability, molecular mobility and environmental conditions such as change in temperature and humidity[2]. Among these factors, humidity plays a crucial role, especially when the polymeric carriers used are hydrophilic polymers such as hydroxypropyl methyl cellulose (HPMC). Therefore, it becomes important to understand the drug recrystallization kinetics of the ASDs during storage in high humidity conditions.

Aim
To investigate the influence of humidity on the physical stability and drug recrystallization tendency in hot melt extruded HPMC-Naproxen amorphous solid dispersions.

Method
Amorphous solid dispersions of HPMC HME 100 LV and Naproxen at different drug loadings were prepared using hot melt extrusion. The extrudates were subjected to different humidity conditions (75% and 98% RH) for a period of 14 days. Samples were removed at different time intervals (Day 1, 2, 3, 4, 7, 10, 14) and analyzed for their physical stability using Differential Scanning Calorimetry, Infrared Spectroscopy and Wide-angle X-ray scattering. Dissolution tests were performed on 5 and 30 wt.% samples before and after storage.

Results
Under ambient and moderately humid conditions (up to 75% RH), both systems maintained their amorphous character over 14 days. However, exposure to high humidity (98% RH) significantly compromised the stability of naproxen-based dispersions. Moisture uptake by HPMC under these conditions resulted in water-induced plasticization, as confirmed by FTIR and DSC, which accelerated molecular mobility and triggered partial recrystallization of naproxen. Additionally, the extent of recrystallization was also strongly influenced by drug loading. Low drug-load formulations (e.g., 5 wt.% naproxen) remained physically stable even under high humidity, while higher-loaded systems (20–30 wt.%) were markedly more susceptible to moisture-induced crystallization.

Conclusion
The study reflects the diminishing ability of the polymer to inhibit crystallization at elevated API concentrations, where water further enhances molecular mobility. In conclusion, this work demonstrates that HPMC-based ASDs of naproxen can be effectively prepared with hot melt extrusion in the fully amorphous state and exhibit excellent physical stability under dry or moderately humid conditions. Nevertheless, these formulations are vulnerable to recrystallization upon exposure to high humidity.

Acknowledgement
The author would like to thank the Swedish Research Council for financial support.

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Cocrystal Solubility Prediction: a novel approach using solubility of individual components

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Introduction: Cocrystals are useful as an enabling formulation principle for poorly soluble drugs, which has led to the implementation of cocrystals in FDA-approved drug products. Cocrystals of the same drug compound with different coformers have different solubilities, leading to distinct dissolution profiles that can be used to tailor the dissolution behavior of the formulations. Knowing the cocrystal equilibrium solubility is critical for predicting and comparing the dissolution behavior of cocrystals of the same drug compound. The cocrystal solubility is experimentally determined from drug and coformer concentrations at the eutectic point [1]. However, reaching this point is time-consuming and requires extensive sampling.

Aim: This work presents a new method to predict cocrystal eutectic points and cocrystal solubility from individual drug and coformer solubilities which are accessible with limited experimental effort.

Results: The proposed approach is based on the solubility of compounds in two different media and is derived from the fact that the free energy difference between a cocrystal and its components is independent of solvent:

$$\frac{K_{sp\,CC}\,(org)}{S_{API}\,(org)\cdot S_{Coformer}(org)} = \frac{K_{sp\,CC}\,(aq)}{S_{API}\,(aq)\cdot S_{coformer}\,(aq)}$$

Where K_{sp} is the cocrystal solubility product in organic (org) or aqueous (aq) media, and S indicates the solubility of the pure components. The method was evaluated using two examples of cocrystals (carbamazepine–benzamide and cilnidipine–*p*-toluenesulfonamide), both comprising neutral components in a 1:1 stoichiometric ratio. The solubilities of both cocrystals in buffer were predicted using experimental values obtained from the organic solvents in which they were synthesized. Despite enormous differences of solubility and solubility advantage ($S_{cocrystal}/S_{API}$) between the two cocrystals, the predicted values aligned well with the experimental measurements at the eutectic point (Figure 1).

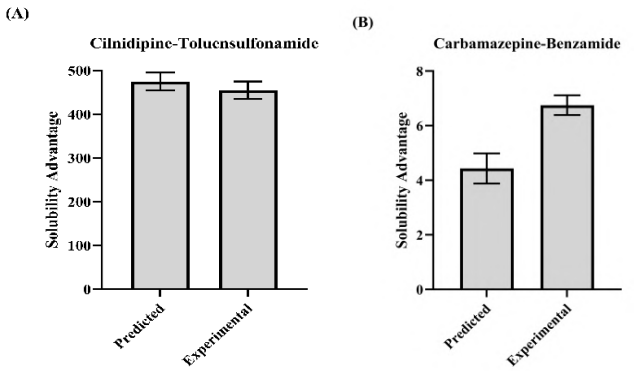

Figure 1: Cocrystal solubility advantages of (A) CILP-TSA and (B) CBZ-BZA in buffer (pH 6.5). The eutectic point was reached by adding solid API and cocrystal to coformer solution and confirmed by solid state analysis.

Conclusion: A novel method to predict cocrystal equilibrium solubility while minimizing experimental work was presented and evaluated. Unlike many alternative predictive tools, it accounts for compound activity at higher concentrations, a factor that is difficult to capture by thermodynamic modeling or molecular descriptors.

Acknowledgements & References:

Naír Rodríguez-Hornedo, Tatiane Machado, Susan M. De Paul and Fritz Blatter are acknowledged for fruitful discussions made possible through Nordic PoP (Nordforsk grant number 85352), and Tina Christiansen for excellent laboratory support.

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Can we use dissolution tests to predict a bioequivalence failure for a BCS class IV drug? Combination of dumping test data and PBBM, a promising approach.

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Introduction
According to the Biopharmaceutics Classification System (BCS) and regulatory agencies criteria (EMA and FDA), when drugs have a high solubility, they belong to BCS class I or BCS class III and *in vitro* dissolution tests can be conducted to compare different reference and generic products. Nonetheless, when solubility is not high and drugs are classified as BCS class II or class IV, then an *in vitro-in vivo* correlation (IVIVC) must be established based on *in vivo* bioequivalence studies and only after its validation the *in vitro* tool can be used to evaluate similarity among formulations [1].

Aim
To develop a physiologically based biopharmaceutics model (PBBM) to establish a valid IVIVC for three different products of Valsartan (VALS), BCS class IV, using as *in vitro* input the dissolution profiles obtained from a dynamic two-stage one-compartment *in vitro* tool (dumping test).

Method
VALS products were tested in the USP II (paddle) apparatus at 50 rpm, in two phases (FaSSGF + FaSSIF). Then, the dissolution profiles were described with a Weibull equation during the first stage and with a first-order model after adding FaSSIF (second stage). Drug disposition was explained by a two-compartment model. A linear relationship was established to scale in terms of time *in vitro* dissolution to *in vivo* ($t_{scaled} = a \cdot t + b$), as the *in vivo* dissolution process tends to be slower than the *in vitro* one, and a scaling factor (m) was defined to include the influence of excipients in the absorption ($ka_{formulation} = m \cdot ka_{API}$), as VALS is a BCS class IV.

Results
The combination of the *in vitro* dissolution data and the PBBM was able to predict the plasma profiles obtained from the *in vivo* bioequivalence study (figure 1) and all the percentages of prediction error (% PEs) were all below the established limits (15 % for each drug product and 10 % for the average).

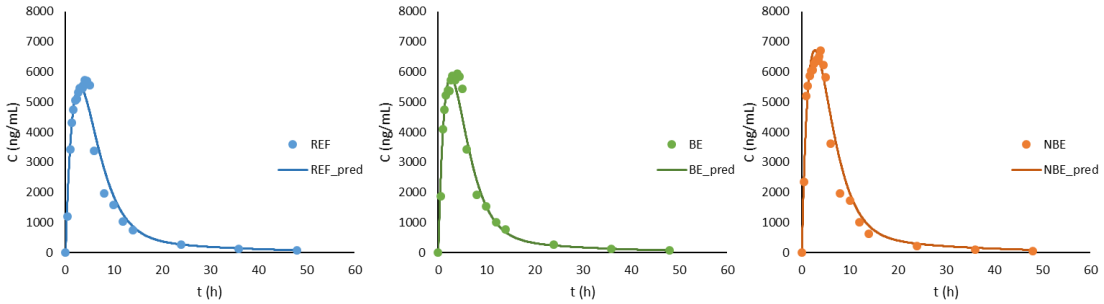

Figure 1 *Experimental (dots) and predicted (line) plasma profiles for VALS reference and test products.*

Conclusion
A valid and biopredictive IVIVC has been obtained after using a two-stage one-compartment dissolution system and adding a scaling factor to simulate the influence of excipients in permeability. This IVIVC may be used in quality control or in the development of another generic formulation.

Acknowledgement
Funding information: GVA Conselleria de Educación Universidades y Empleo (Grant No. #CIAICO/2022/082) and MCIN/AEI/10.13039/501100011033/FEDER, UE (Grant No. #PID2021-123888OB-I00).

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Development of Fab-streps for immunoaffinity isolation of extracellular vesicles for their use as nanodrug carriers: Comparison with commercial Fab-strep

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Introduction
Efficient extracellular vesicle (EV) isolation for downstream nanoparticle therapeutic applications requires a method that preserves EV size or activity. Immunoaffinity capture using commercial CD9 or CD81 Fab-streps has previously been shown by our group to yield EVs with minimal protein contamination and preserved integrity. The Fab fragments contain a Twin-StrepTag which are immobilized to a column containing StrepTactin affinity matrix, allowing for quasi-immunocapture of EVs. However, the commercial CD9 and CD81 Fab-streps were recently discontinued by the vendor.

Aim
To develop in-house Fab-streps to enable continuous availability and reduce costs of Fab-streps to enable further optimization of the isolation process for academic use.

Methods
Bl21(DE3) *E.coli* cells were transformed in the cytosol with the pET11a plasmid encoding codon optimized for CD9 or CD81 Fab-strep ORFs, gifted by IBA-Life Sciences®, and cultured at either 18 or 25°C in glass shake flasks. For pET11a the heavy (H) and light (L) chains were expressed under a single T7 promoter. For comparison a pETDuet vector was also employed where the H and L chains were expressed on separate T7 promoters. Cells were harvested, lysed and clarified for affinity purification using ÄKTA Pure 25 FPLC, with a Strep-Tactin® 4Flow® high capacity column and desthiobiotin for elution. SEC was used to measure MW of CD9 Fab using MW standards. MEXi HEK293E cells were cultured at 37°C in serum-free medium. At cell density of 2-6*10^6 cells/ml (viability at least 90%), 25 mL of conditioned medium was collected for each EV isolation and clarified. EVs were isolated using Strep-Tactin®TACS agarose using either commercial (IBA®) or newly generated in-house CD9 or CD81 Fab-strep (0.05 mg/mL). After elution with biotin (1 mM) and concentration using Amicon (100 kDa MWCO), EVs were analysed using NanoSight.

Results
In-house CD9 and CD81 Fab-strep were achieved in concentrations of 5-20 mg/L LB culture, with >95% purity. MW for CD9 of~50 kDa (theoretical MW 50.3 kDa) with both monomeric and dimeric forms detected via western blotting. CD9 Fab-strep expression was 4-5x greater than CD81. Preliminary EV isolation using in-house CD81 Fab-strep yielded 1.76 ± 0.08*10^{11} particles/ml while commercial Fab-strep yielded 4.44 ± 0.4*10^{11} particles/ml (2.5x higher). EVs from in-house CD9 Fab-strep reached 1.48 ± 0.05*10^{11} particles/ml compared to 2.23 ± 0.05*10^{11} particles/ml using commercial Fab-strep (1.5x higher). The WB EV markers Alix and CD9 were both present in EVs isolated with the in-house CD9 Fab-streps whilst only faint Alix signal was detected for the EVs isolated using the in-house CD81.

Conclusion
In-house CD9 Fab-strep was more efficient in isolating EVs than CD81 Fab-strep whilst not reaching the particle amount using commercial Fab-streps. Further optimization and characterization of the purity and activity of the Fab-streps are now underway to improve efficiency.

Acknowledgement
The authors acknowledge funding support from the cancer awareness fund “Göngum saman”, the National Cancer Society Science fund, the STAFN grant and the University of Iceland Research fund.

Poly(2-oxazoline)-based polymers as universal stabilizers for nanosuspension formulations

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Introduction

Nanosuspensions are a promising formulation technique to address challenges related to the poor solubility of drugs. Nevertheless, the primary challenge in nanosuspension formulation is the selection of an applicable stabilizer[1]. The stabilizer is the most critical excipient in this formulation technique, as it ensures the maintenance of small particle size. Poly(2-oxazoline)-based polymers have shown promising results in several formulation techniques with various drug models, suggesting potential for their application in nanosuspension formulations as well[2,3].

Aim

To explore two poly(2-oxazoline)-based polymers (P1 and P2) as stabilizers for itraconazole, celecoxib, furosemide and naproxen nanosuspension formulations.

Method

Nanosuspensions were prepared using a wet-ball milling technique. The amount of drug varied based on pre-optimization studies with Pluronics (F68 or F127, depending on compatibility). The stability of nanosuspensions was evaluated by measuring particle size and polydispersity index (PdI) for 28 days. Solid-state characterization was performed using differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR) and X-ray powder diffraction (XRPD). Dissolution profiles were assessed under non-sink conditions to distinguish formulations and compare them to the untreated drug.

Results

The P1 polymer performed well with all drug models and demonstrated excellent stability properties for 28 days. The P2 polymer also performed well with all drug models, althought some challenges were observed with celecoxib and furosemide. With furosemide, P2 had some stability challenges, as the particle size (initially around 200 nm) nearly doubled after one week of storage. In the case of celecoxib, the initial particle size (around 400 nm) remained relatively high compared to the other drug models. Solid-state analysis confirmed that no changes occurred in the crystalline state or decomposition of the drug models after the sample preparation. The dissolution behavior of nanosuspensions improved significantly compared to the untreated drug.

Conclusion

Overall, both P1 and P2 polymers paired well with all drug models used in this study. All nanosuspension formulations showed significant particle size reduction and good stability properties. The dissolution properties of model drugs presented a remarkable improvement in the solubility and performed significantly better than the untreated drug.

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Engineering extracellular vesicles for targeted therapy in EGFR1+ triple-negative breast cancer

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Introduction
Triple-negative breast cancer (TNBC) poses a significant challenge due to the lack of specialized treatment options that are available for other subtypes. Targeting the epidermal growth factor receptor (EGFR1) is a promising approach for targeted therapy in triple-negative breast cancer (TNBC), as it is overexpressed in approximately one-third of patients. Extracellular vesicles (EVs), derived from human cells, have great potential as nanosized drug delivery systems in targeted anti-cancer therapy due to their enhanced stability and engineering possibilities, compared to liposomes.

Aim
The main objective was to engineer HEK293E cells to produce EVs with EGFR1 targeting ligands and load them with the anti-TNBC agent doxorubicin (Dox).

Method
Suspension-adapted HEK293E cells were transfected to constitutively express EGFR1-targeting ligands and the green fluorescent protein (GFP). EVs were isolated from the conditioned media using Strep-Tactin CD81 Fab-TACS®. Size distribution was determined with nanoparticle tracking analysis (NTA) and EV protein markers were assessed using capillary western blotting (WB). An Amplex Red Cholesterol assay was also used to confirm the identity of EVs. Dox loading was achieved through either passive or active loading of EVs. For active loading, the conditioned medium (100 kDa centrifugal filtration) was buffer exchanged to 0.3 M ammonium sulphate buffer followed by sonication, then EVs were isolated as above. Dox was incubated with EVs for 30 min at RT and unbound Dox was removed with ultrafiltration (100 kDa). For passive loading of Dox, EVs were incubated with Dox for 30 min at RT followed by excess Dox removal with ultrafiltration. Dox quantification was performed using fluorometry (λ_{ex} 470 nm / λ_{em} 595 nm) and encapsulation efficacy (EE) calculated.

Results
The average mode size for both original and engineered EVs was similar (116.5 ± 3.6 nm and 116.2 ± 7.1 nm, respectively). EV isolation was confirmed by the presence of EV protein markers Alix and Synthenin-1, along with the absence of the negative marker calnexin. Significantly higher cholesterol content was observed for EVs compared to CL ($p<0.001$), providing conclusive evidence of the successful isolation of EVs. The successful engineering of EVs was confirmed with GFP in WB. Active Dox loading resulted in higher EE (63%) compared to passive loading (17.5%).

Conclusion
Engineered EVs retain similar properties to original EVs in terms of size and protein markers, besides GFP. Furthermore, active loading of Dox inside EVs proved superior to passive loading. These EVs will be further used to assess their binding affinity towards both low and high EGFR1-expressing breast cell lines with the ultimate objective of directing them against TNBCs.

Acknowledgement
The author acknowledges funding support from the cancer awareness fund “Göngum saman”, the Icelandic National Cancer Society Science fund, the University of Iceland Research fund, the STAFN research fund and the UI PhD fund. The presenting author is a Fulbright and Leifur Eiríksson fellow.

Real-time label-free sensing platforms for characterizing nanoparticle-based gene/drug delivery systems

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Nanoparticles-based delivery systems (NP-DSs) transform therapeutic approaches by enabling precise gene and drug delivery. Nanoparticles (NPs) can effectively deliver genetic material or drug molecules to treat specific medical complications. However, only 1% of NP-DSs have reached clinical use due to limitations in the available analysis tools. These methods are often invasive, time-consuming, and may require labeling, and frequently fail to detect particles smaller than 50 nm in complex biological fluids. This project addressed these limitations by developing and evaluating an advanced, multiparametric surface plasmon resonance (MP-SPR™) detection technology for label-free, real-time monitoring and characterization of NP-DSs.

Different types of NPDSs, including polymeric (micelles), biological (viral capsid), and inorganic (mesoporous and solid silica NPs) were synthesized in-house [1,2] and characterized in physiological buffers by MP-SPR™ to determine their size and concentration. The NPs were dispersed in physiological buffer and injected onto a polymer-coated gold sensor surface with the flow rate of 20µL/min using a serial concentration series. This characterization involves simultaneously utilizing surface plasmon resonance at two wavelengths of 670 and 785 nm, each with a different sensing depth, and analyzing the ratio of their responses to determine the particle size attached on the surface of MP-SPR gold sensor .The bulk concentration was determined, followed by calculation of the refractive indices of the deposited NPs on the sensor surface using LayerSolver™ software and Fick's equation. The results were all confirmed and evaluated furthur with other characterization methods including: Transmission Electron Microscopy(TEM), Atomic Force Microscopy (AFM), Nanoparticle Tracking Analysis (NTA), Dynamic light scattering (DLS), and Quartz Crystal Microbalance (QCM) [3].

The developed sensing platform demonstrated high sensitivity and accuracy in determining the size and concentration of various NP types under physiological conditions, using real-time, label-free, and single-injection analysis at low concentrations. By correlating MP-SPR™ data with results from conventional techniques, the method was shown to be both reliable and complementary.

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Surface functionalized core/shell drug crystal nanoparticle platform for targeted delivery

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Introduction
Nearly 70–90% of the classical drug modalities belong to the Biopharmaceutics Classification System class II or class IV, which are characterized by poor water solubility and high or low permeability, respectively.[1] Drug nanocrystals have emerged as promising fomulation technology option among others to overcome the delivery of poorly water-soluble drugs with significant commercial success.[2] In the light of cancer therapy, to date, no drug nanocrystals have been approved by FDA in clinical use mainly due to uncontrolled drug release, easy clearance by the mononuclear phagocytosis system, lack of active targeting.[3] More importantly, major challenges remain, as state of the drug crystal nanoparticle (NP) technology lack the surface functionalization strategies to enable attachment of targeting ligands for active tumor targeting.

Aim
To develop a platform for surface functionalizing core/shell drug crystal nanoparticles (NPs) for targeted drug delivery. The platform will be based on customized microfluidics chip design to enable All-in-One manufacturing for core/shell drug crystal NP conjugated with targeting ligands.

Method
Targeted core/shell drug crystal NPs were produced by glass capillary microfluidics using model compounds from Bayer. Conjugation of AlexaFluor®647 (used as surrogate for the targeting moiety) on the surface of the polymer coated drug NPs was performed both off chip and on the same chip by adding another capillary system for feeding of the conjugation reagents (Figure 1A). Strain-promoted azide-alkyne [3+2] cycloaddition (SPAAC) click chemistry was used to achieve fast and efficient conjugation of the dye on the surface of the produced core/shell systems.

Results
By tuning the flow rates and solvent/antisolvent compositions, we were able to achieve stable core/shell drug crystal NPs with size comprised between 100 and 200nm (Figure 1B). Transmission electron microscopy (TEM) images (Figure 1C) showed the presence of a solid drug core covered by a polymer layer. Furthermore, off-chip surface modification of purified NPs was successful as indicated by the presence of a fluorescence signal.

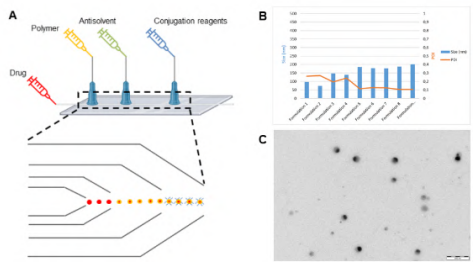

*Figure 1 (**A**) Overview and close-up of the used 3D glass capillary device (**B**) Size and PDI of different formulations (**C**) TEM image of produced core/shell drug crystal NPs*

Conclusion
It could be shown that we can manufacture core/shell drug crystal NPs in an All-in-One microfluidic platform. Surface modification of the obtained core/shell drug crystal NPs within the same chip will be tested in the near future.

Acknowledgement
The author would like to thank the Finnish Research Impact Foundation (Vaikuttavuussäätiö) for financial support.

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Superparamagnetic Iron Oxide Nanoparticles as Dual Functional T1 and T2 contrast agents for Enhance Magnetic Resonance Imaging

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Introduction

Imaging cancer cells is crucial for accurate diagnosis, and Magnetic Resonance Imaging (MRI) offers a non-ionizing imaging modality. However, current gadolinium-based contrast agents, which enhance MRI image quality, are associated with toxicity concerns, including nephrogenic systemic fibrosis (NSF), gadolinium deposition in the brain, and potential nephrotoxicity prompting the search for safer alternatives [1]. Superparamagnetic Iron Oxide Nanoparticles (SPIONs) are promising due to their biocompatibility and unique magnetic properties [2]. This study focuses on developing monodisperse SPIONs (nanocubes, nanospheres, and nanostars) using the solvothermal method [3]. The nanoparticles were functionalized with dopamine to create biocompatible and functional dual T1 (bright signal) and T2 (dark signal) contrast agents for enhance Magnetic Resonance Imaging of tumour cells, offering a safer alternative to gadolinium-based agents.

Aim

To develop superparamagnetic iron oxide nanoparticles as safer alternative to gadolinium base contrast agents for enhance Magnetic Resonance Imaging.

Method

SPIONs were synthesized via the solvothermal method, adapting the approach reported by Gavilán et al. [3]. Briefly, a homogeneous solution of 1-octanol, hexadecyl amine, and oleic acid was prepared in a glass vial under magnetic stirring (1000 rpm) at 60°C for 30 min. After cooling to room temperature, iron pentacarbonyl and benzaldehyde were added, and the mixture was stirred (1000 rpm) for 30 min. The solution was then transferred to a 25.0 mL solvothermal autoclave reactor and heated at 200°C for 4 h. By varying the ratio of benzaldehyde (shape-directing agent) to iron pentacarbonyl, nanoparticles with different shapes were achieved.

Results

The synthesis of nanoparticles with distinct shapes was achieved by adjusting the ratio of iron pentacarbonyl to benzaldehyde. Ratios of 1:0.6, 1:0.4, and 1:0.2 yielded nanostars, nanocubes, and nanospheres, respectively (Figure 1). This approach demonstrates the importance of controlling reaction conditions to tailor nanoparticle morphology.

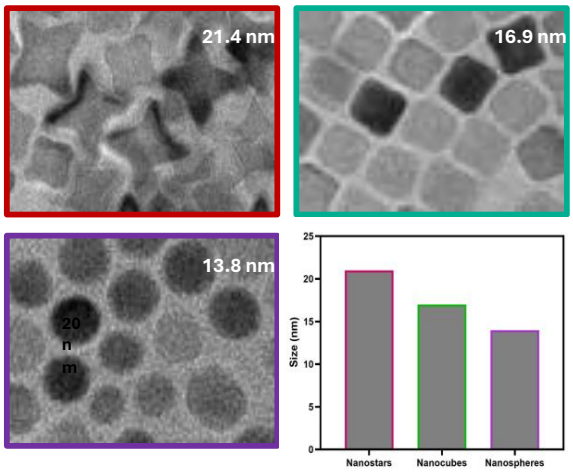

Figure 1: *TEM images of iron oxide nanoparticles including Nanostars, Nanocubes, Nanospheres and their sizes.*

Conclusion Iron oxide nanoparticles of different shapes such as nanocubes, nanostars and nanospheres were synthesized. The surface of the nanoparticles was functionalised with dopamine to enhance their biocompatibility.

Acknowledgement

The authors would like to thank Chalmers Area of Advance Nano for financial support.

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Modified Glycogen Nanoparticles for Nucleic Acid Delivery Targeting Immune Regulation

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Nucleic acid therapies modulate gene expression by delivering nucleic acids including DNA and mRNA to cells with applications in areas of vaccines, gene therapies, and immunotherapies. However, nucleic acids are limited by their instability in biological systems. Efficient delivery systems are essential for the success of nucleic acid therapies, and polymer-based nanoparticles have emerged as promising delivery vectors.

This study develops and evaluates naturally occurring glycogen nanoparticles, as delivery vehicles for nucleic acid therapies. To enable complexation with anionic nucleic acids, the glycogen is modified with different polyamines to introduce cationic properties. The particles are evaluated based on their loading and delivery efficiency of in-house synthesized mCherry-encoding mRNA into cells. Additionally, a polysaccharide coating is added to enhance transfection efficiency and enable targeting of specific immune cells. Improved delivery vehicles could significantly advance nucleic acid therapies, enabling safer and more effective treatments for patients.

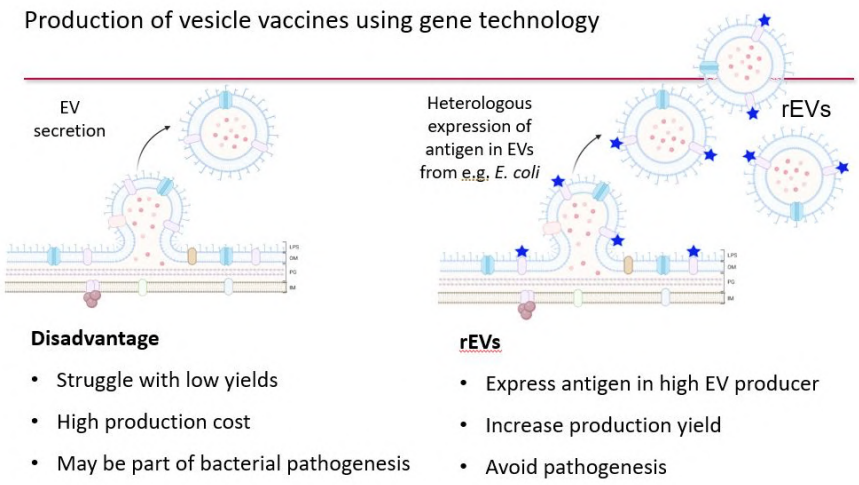
Introduction.
Extracellular vesicles (EVs) are nano-sized membrane enclosed entities that are secreted from the bacterial body. Their release is associated with a range of phenotypes including cell-cell communication, host-pathogen interactions and antimicrobial resistance. EVs also elicit an immune response after immunization of a host making them an interesting vaccine candidate.
We have investigated the use of EV as a potential vaccine against several bacterial pathogens creating problems in farmed animals including fish. Immunization with EV revealed protection against some diseases, but not in others where we hypothesized that the EV was part of the microbial pathogenesis.

Objectives
To circumvent possible negative side effects of using natural EVs, we have explored the possibility to use recombinant EV (rEV) produced in non-pathogenic in *E. coli* expressing antigens from other infectious agents (se Figure).

Methods
We have expressed antigens from the fish bacterial species *Yersinia ruckeri* in *E. coli*. The rEV was isolated using tangential flow and ultracentrifugation before analysis by transmission electron microscopy (TEM) and nano-particle tracking analysis (NTA). Antigen expression was confirmed using SDS-PAGE and proteomics analysis, while their potential toxicity was tested on fish cell lines.

Findings
We could isolate rEV form E. coli using tangential flow and ultracentrifugation which was confirmed by TEM analysis. rEV particle size was from 120 – 180 nm pending on *E. coli* strain used and the expressed antigen. The number of antigen expressed seem to effect the *E. coli* viability. The rEVs did not elicit any toxicity when exposed to fish cells in culture.

Conclusion.
These results show that *E. coli* has the potential to be utilized as a vector for production of EVs expressing antigens from other bacterial species. These rEVs will hopefully circumvent the negative side effects of native EVs isolated from the pathogenic bacteria when used as vaccines and may enhance the repertoire of EVs based vaccines within animal health.



Itraconazole co-crystals for inhalation: Physicochemical properties and dissolution performance

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Introduction

Poorly soluble drugs pose special formulation challenges, especially if only limited amounts of the API can be administered. One example is inhalation therapy, where doses exceeding one milligram are considered high.[1] Consequently, the formulations must comprise to the lowest amount of excipients possible. A promising strategy for achieving high drug loads and simultaneously improved dissolution performance is the use of co-crystals (Figure 1).

Aim

To investigate an Itraconazole co-crystal system for physicochemical and aerodynamic characteristics, with a particular focus on the dissolution behaviour.

Methods

Co-crystals (Co-Itra) of Itraconazole (Itra) and Succinic acid (SA) were prepared by solvent-assisted ball milling in a molar ratio of 2:1 (adapted from[2]). XRPD and DSC were utilised for identification and physicochemical characterisation. The dissolution process was monitored in real time using a Rainbow R6 instrument. The optical probes were immersed in 50 ml of phosphate buffer (pH 7.4), with stirring at 300 rpm. The calibration and quantification of Itra was conducted in a wavelength range of 244 - 262 nm.

Results

The successful production of co-crystals of Itra and SA was confirmed by DSC and XRPD measurements. It had been demonstrated that Co-Itra has enhanced dissolution in acidic environments or in the presence of surfactants.[3,4] Our study demonstrated that Co-Itra also has a faster dissolution in the absence of surfactant and within the neutral pH range. After 10 h, the concentration of Co-Itra was found to be 3.5 times higher than that of Itra (Figure 1, blue box). In powder dissolution, it is also important to consider the particle size distribution, with x_{50} being 4.42 µm for Itra and 5.00 µm for Co-Itra underlining that the co-crystal formation is substantial for this behaviour.

Conclusion

The hypothesis, that the co-crystal of Itra and its co-former SA leads to enhanced dissolution in the absence of any surfactant and within a neutral pH range was confirmed over time. The next step is to conduct *in vitro* dissolution testing of the inhalable fraction.

Acknowledgement

This work was supported by NordForsk Project #85352: Nordic POP, Patient oriented Products. The Institute of Inorganic Chemistry in Kiel is kindly acknowledged for the XRPD measurements.

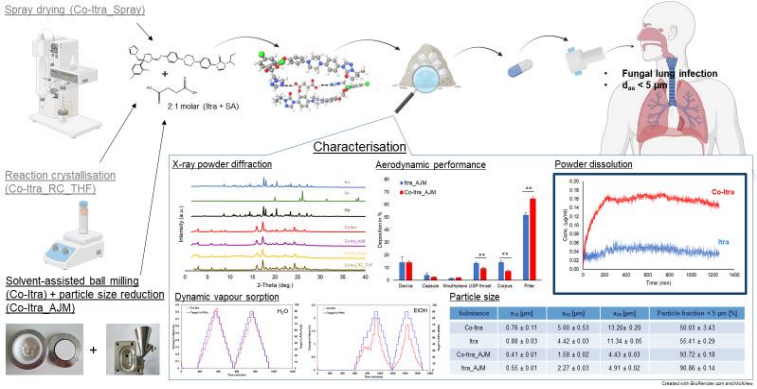

Figure 1: Project overview: Different preparation strategies for Co-Itra (left), characterisation methods (data shown is for solvent-assisted ball milling) and intended use in inhalation.

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Exploring co-administration of amorphous solid dispersions (ASDs) and self-nanoemulsifying drug delivery systems (SNEDDS) to improve the *in vitro* performance of nilotinib

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Introduction

Nilotinib (NTB) is a 'brick dust' compound (log P =4.5, melting point (T_m) = 236 °C). Although its oral bioavailability rises markedly with a high-fat meal (> 80%), low drug solubility limits its dissolvable dose in lipid-based formulations (LBFs). Addition of the drug in the form of an ASD to a SNEDDS can raise its apparent solubility in the SNEDDS and delay drug precipitation in the resulting super-SNEDDS. However, direct incorporation of ASD into SNEDDS may cause phase instability or agglomeration (due to limited solubility of the ASD polymer in the SNEDDS). We therefore adopted a capsule-in-capsule (CIC) approach that co-administers ASD (in the outer capsule) and a blank SNEDDS (in the inner capsule), hypothesizing that (i) SNEDDS droplets can help solubilize the NTB and (ii) the polymer from the ASD would inhibit precipitation during intestinal digestion.

Aim

1. To evaluate whether an ASD is essential for maintaining NTB solubility during *in vitro* lipolysis.
2. To assess how varying SNEDDS loads influence NTB solubilization during *in vitro* lipolysis.

Materials and Methods

Solid phase (30 mg NTB): a), Crystalline NTB (NTB30) b), Ball-milled amorphous NTB (AmorNTB30); c), Physical mixture AmorNTB: Eudragit L100/55 (EL) 30:70 w/w (AmorNTB30-EL70 Mix); d), Ball-milled ASD 100mg containing NTB: EL 30:70 w/w (ASD100(NTB30)).
Lipid phase (0, 150, 200, 250, 300, 600 or 900 mg): Blank SNEDDS (Kolliphor RH40/ Capmul MCM / Captex 300/ Absolute ethanol = 35.0/36.7/18.3/10.0 %, w/w)[1].
CIC preparation: Size 00 HPMC capsules were filled with SNEDDS, sealed, then placed inside size 000 HPMC capsule containing the solid. Each solid was paired with 300 mg SNEDDS; All seven SNEDDS loads were paired with ASD100(NTB30).
In vitro lipolysis: CIC was dispersed for 15 min in 25 mL intestinal media (pH 6.5, 37 °C), after which 5 mL pancreatic extract was added and pH-stat digestion continued (pH 6.5) for 60 min.

Results

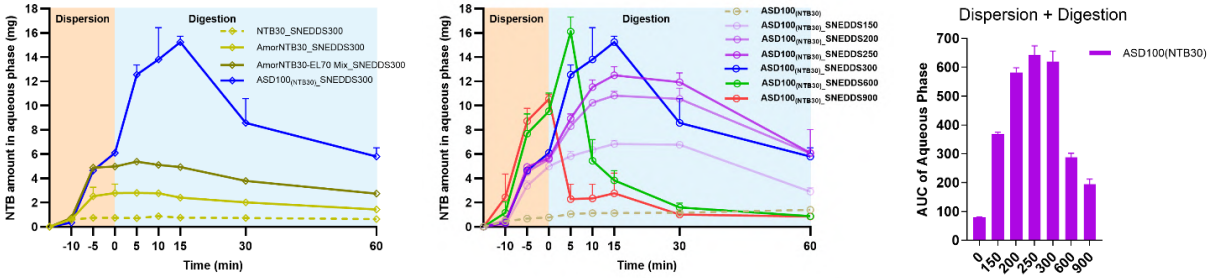

Figure 1 *Left: Amount of NTB in aqueous phase during lipolysis for formulations: NTB30/ AmorNTB30/ AmorNTB30-EL70 Mix/ ASD100(NTB30) + SNEDDS 300 mg; Middle & Right: Amount of NTB & area under curve of amount of NTB over time in aqueous phase during lipolysis for formulations: ASD100(NTB30) + SNEDDS 0/150/200/250 300/600/900 mg;*

Conclusion

ASD is essential to stabilize NTB supersaturation. Co-administration with a moderate SNEDDS load (~250–300 mg) maximizes solubilization, whereas higher lipid doses trigger precipitation.

Reference

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Selective Laser Sintering of Distinct Drug and Polymer Layers as a Novel Manufacturing Strategy for Individually Dosed Tablets

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Introduction

Conventionally, drugs are produced in large batches with consistent doses. However, patients in the pediatric or geriatric populations often require modified doses because of their body weight or comorbidities. Selective Laser Sintering (SLS), a subdomain of 3D printing, offers potential to bridge this gap between mass production and patient-specific needs. This method creates a solid structure in a layer-by-layer approach by fusing powder particles together with the help of a laser. In other studies, powder blends of different API concentrations have to be prepared for each individual dose.[1] The aim of the present study was therefore to simplify the process by enabling direct printing of the pure raw materials. In addition, it was sought to combine the separated printing of API and excipient into a one-step procedure to form a finished tablet.

Aim

To simplify current approaches in SLS-based 3D-printing for the production of individually dosed tablets by combining distinct layers of pure API and pure excipient.

Method

Indomethacin was used as API and polyvinyl alcohol (PVA) as an excipient. SLS printing was performed using a SnowWhite 2 printer (Sharebot, Nibionno, Italy). With the help of separated powder tanks, the printer alternated between spreading out layers of either pure indomethacin or pure PVA. The dose was controlled by varying the total number of API layers in a tablet. Tablets were analyzed in accordance with standards set by the European Pharmacopoeia. This included mass uniformity, content uniformity and dissolution.

Results

Tablets with varying API content between 10 % and 25 % were successfully printed. Weighing of the prints showed that tablet mass slightly increased with higher doses of indomethacin. When measuring the content, the results were close to the expected value based on the amount of API that was added into the print. However, the dose accuracy seemed to increase with higher doses. For the three higher doses, all content measurements of five batches per tablet type lied within uniformity limits. Dissolution tests showed evidence that the sintered tablet matrix facilitated release, with faster dissolution and improved solubility compared to API powder.

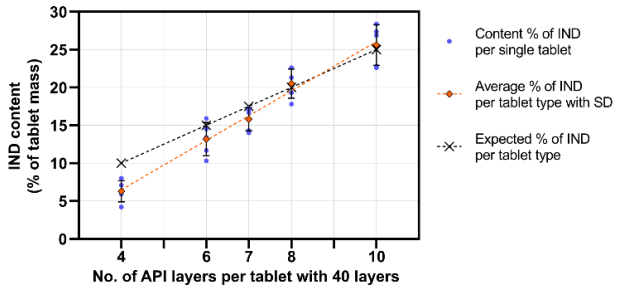

Figure 1 *Expected and measured API content in tablets depending on number of API layers*

Conclusion

We successfully 3D-printed individually dosed tablets using SLS. A key achievement was the printing of pure API layers, although SLS is designed for the processing of polymers. We simplified the process by eliminating powder blending steps.

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Hemicellulose as a direct compression excipient: impact on quality attributes of tablets

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Introduction

Hemicelluloses are a heterogeneous group of polysaccharides which are present in a plant cell wall structure located between cellulose microfibrils. Despite being the second most abundant biomass after cellulose, these materials have attracted limited interest and have been the focus of only a few studies within the pharmaceutical sector. Certain hemicelluloses have been identified with promising features such as, hydrophilic nature, solubilizing effects, gelling and emulsifying properties[1, 2]. Thus, there is an untapped potential in utilizing them for pharmaceutical applications, particularly as functional excipients in pharmaceutical formulations.

Aim

The aim was to investigate selected quality attributes of direct compression tablets which were produced from binary mixtures of paracetamol and hemicellulose.

Method

A 2-level full factorial experimental design with 3 variables (Hemicellulose type (HemiT), quantity of drug (QoD) and compaction cycle time (CT)) was implemented to study their effects on the responses: tensile strength (TS), elastic recovery (ER), cumulative dissolved drug portion (at 0.5h) and area under the cumulative dissolution curve (AUC, 0–180min). Hemicellulose types were arabinoxylan (AX) and galactoglucomannan (GGM), QoD levels were 20% (m/m) and 60%, and CT levels were 500 ms and 1800 ms. Round 10 mm diameter flat-faced tablets were made with a compaction simulator from binary mixtures of paracetamol and hemicelluloses. Targeted compaction force and tablet mass were 15 kN and 300 mg, respectively. Dissolution study was conducted in a phosphate buffer (pH 7.2). Model fitting (Multiple linear regression, MLR) and data analysis was performed by using MODDE® Pro – software (13.0.2, Sartorius AG, GER).

Results

In all the design points within the experimental design, coherent tablets were produced. Edited MLR models for TS, ER and cumulative dissolved drug portion contained only main factors, while an interaction term (CT*QoD) was included in the AUC model (Figure 1). Model statistics R^2 (goodness of fit) and Q^2 (prediction precision) indicated fairly accurate models except with the ER model. With GGM, tablet TS could be increased and ER decreased compared to using AX as an excipient. All the GGM tablets had over 1.2 MPa TS values, while AX tablets with 60 % paracetamol had TS values well below 1 MPa. Surprisingly, QoD was the only significant factor in cumulative dissolved drug portion (0.5h) model. It had the most significant effect on the AUC response, as well.

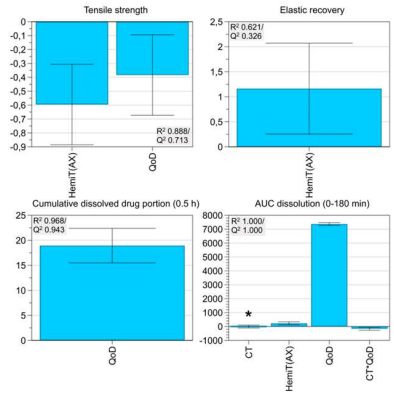

Figure 1. *Scaled and centered coefficients for edited MLR models presented by bar plots. (*=insignificant model term, p > 0.05)*

Conclusion

GGM as an excipient provided stronger, denser and less elastic tablets compared to those made with AX. The QoD in the formulation had the greatest effect on both dissolution responses.

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Doxycycline-containing biodegradable membranes to support periodontitis treatment

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Introduction

Guided tissue regeneration is a well-known treatment to regenerate bone surrounding teeth and dental implants affected by periodontitis. The optimal barrier membranes should be capable of providing a sealed space for bone regeneration by preventing the migration of unwanted cell types to the location of interest. Commercially available barrier membranes exist, however, there are bottlenecks regarding the properties of the ideal membrane, such as biocompatibility, structural integrity, optimal shelf life and easy clinical application[1].
Electrospinning is a cost-effective, versatile, and easy-to-use technique to produce dense fibrous structures. By this technique, various resorbable and non-resorbable, natural or synthetic polymer membranes can be produced depending on the nature of the applied polymer[2].

Aim

In this work, we aim to develop a dense membrane structure with sustained doxycycline release from polycaprolactone (PCL), poly(lactic-co-glycolic acid) (PLGA) and poly(lactic acid) (PLA) polymers loaded with an antibiotic drug doxycycline (DXY), which additionally has anti-inflammatory and anti-bone resorption effects.

Method

For the production of fibers, the electrospinning process was finetuned regarding the solvent and the polymer concentration. The high voltage and the flow rate of the polymer solution were adjusted by monitoring the Taylor cone during the procedure. The morphology and the diameter of the fibers were visualized and quantified by scanning electron microscopy and ImageJ software. The physicochemical properties of the membranes were investigated by Fourier-transformed infrared spectroscopy, X-ray diffraction analysis, Thermogravimetric analysis and Differential scanning calorimetry. The drug release was investigated in phosphate-buffered saline solution at body temperature by µDiss UV-monitoring system.

Results

The polymer concentration and the incorporated DXY amount can significantly alter the fiber diameter, that affects the release kinetics. In case of PCL, fibers with a diameter around 1 µm provide burst release, while PLGA and PLA fibers, with diameters around 0.5-1 µm, provide prolonged drug release. The physicochemical characterization suggests that in PCL and PLGA the interactions between the polymer and the drug are stronger compared to PLA.

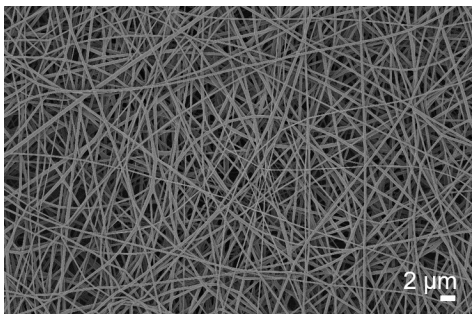

Figure 1 *Scanning electron microscopy image of electrospun DXY-containing PLGA fibers.*

Conclusion

These resorbable fibrous membranes are promising candidates to provide sealed space for bone tissue regeneration while releasing doxycycline to enhance the healing process of periodontitis.

Acknowledgement

We would like to thank the Swedish Pharmaceutical Society and the Elisabeth and Alfred Ahlqvist Foundation for financial support (Grant No. 2023-0367-HT-PD).

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Investigation of the Effect of Gliflozins on Lecithin:Cholesterol Acyltransferase

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Introduction

Lecithin:cholesterol acyltransferase (LCAT) is a key enzyme in lipoprotein metabolism, being responsible for the synthesis of plasma cholesteryl esters. LCAT activity is required for the growth and maturation of high-density lipoproteins (HDL) and is involved in the reverse cholesterol transport [1]. In this context, LCAT-activating compounds, or positive allosteric modulators (PAMs), have been explored as potential therapies for coronary heart diseases and LCAT deficiencies. Previous *in silico* and *in vitro* experiments conducted in our laboratories have identified two antidiabetic drugs that belong to the gliflozins class, as new potential LCAT activators [2]. Gliflozins are FDA-approved compounds for the treatment of diabetes type 2 that have recently shown promising cardioprotective effects [3]. However, the exact cardioprotective mechanism of these molecules is not clear, highlighting the need for a deeper understanding of their effects on the lipoprotein metabolism.

Aim

This research aims to investigate the effect of gliflozins on LCAT activity. The possible correlation between LCAT activity and gliflozins' mechanism may provide a more specific clarification about the cardiovascular protection effect, enhancing our understanding of these versatile class of drugs.

Methods

LCAT activity in plasma is assessed using a colorimetric assay [1]. To determine the EC_{50} values of Canagliflozin and Dapagliflozin, increasing concentration of each drug are added to plasma. The EC_{50} values indicates whether the plasma concentration of gliflozins in the antidiabetic treatment (1-3µg/mL) is enough to activate LCAT. Then, LCAT activity will be measured in *ex vivo* plasma samples from patients treated with Gliflozins.

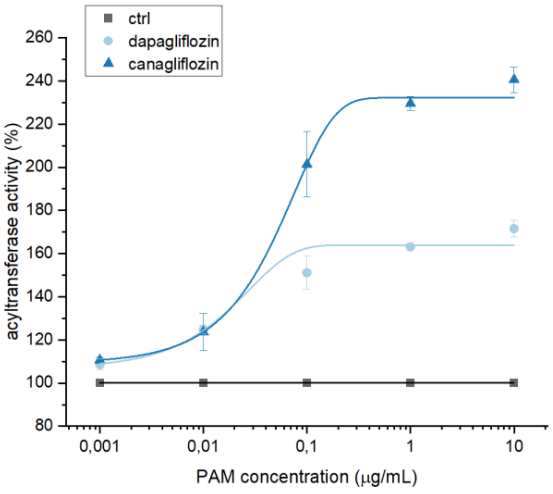

Figure 1. Dose response curves of Canagliflozin (dark blue) and Dapagliflozin (light blue). Control in grey refers to the basal activity of LCAT in plasma without drugs. Data are reported as mean±SEM (n=3) of triplicate measurements.

Preliminary results

As shown in Figure 1, Canagliflozin and Dapagliflozin increased LCAT activity by 240% and 160% respectively, with EC_{50} values in the range of 0,7-1µg/mL. *Ex vivo* experiments on plasma samples from diabetic patients treated with Gliflozins are still ongoing.

Conclusion

The antidiabetic drugs Canagliflozin and Dapagliflozin were identified by previous *in silico* and *in vitro* data as new potential LCAT activators. The dose response curves obtained in plasma samples suggest that the blood concentration of the antidiabetic treatment should be enough to improve LCAT activity. *In silico* and *in vitro* data will be further validate by *ex vivo* experiments.

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3D-printed Carvedilol Tablets vs a Marketed Product: A Comparison of the Performance *In vitro*, *In vivo*, and *In Silico*

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Introduction

Physical alterations of oral dosage forms are often required when administering age-appropriate doses of therapeutic agents to children [1]. For poorly water-soluble compounds, the alterations may implicate a risk of inaccurate dosing as well as changes in solubility, bioavailability and stability [2,3]. By combining lipid-based formulations (LBF) with 3D printing approaches, the risks can be avoided and child-friendly dosage forms with flexible doses can be produced in a controlled manner. To date, a limited number of studies have utilized the combination of LBF and 3D printing to develop child-friendly dosage forms, and to our knowledge, no studies have evaluated the performance of the developed product against available marketed products.

Aim

To compare the *in vitro* digestion and permeation, and the *in vivo* exposure of the poorly water-soluble compound carvedilol from 3D-printed tablets versus a marketed product. Additionally, the project aimed to better understand the permeation of carvedilol using molecular dynamics simulations.

Method

Tablets were 3D-printed using semi-solid extrusion of emulsion gels comprised of drug-loaded LBF and cellulose-based polymers. *In vitro* digestion-permeation assays were performed to evaluate the flux across an artificial lipid (LiDo) membrane. The carvedilol exposure *in vivo* was investigated with a crossover study design in juvenile pigs. Coarse-grain molecular dynamics simulations were used to evaluate the impact of LBF components on carvedilol transport across a phosphatidylcholine membrane model, mimicking the LiDo membrane used in the in vitro model.

Results

The *in vivo* study suggests comparable carvedilol exposure of the 3D-printed tablets and the marketed product. Preliminary results suggest a slightly higher mass transfer for the 3D-printed product *in vitro*. The *in silico* simulations suggest that the components of the LBF in the 3D-printed tablets formed structured colloidal aggregates that enhanced carvedilol's interaction with, and permeation across, the membrane.

Conclusion

Given the possibility of controlling the 3D printing manufacturing process, the overall results suggest that 3D-printed tablets from emulsion gels can serve as an alternative approach to develop personalized and child-friendly dosage forms. The combination of *in vivo*, *in vitro*, and *in silico* approaches provides a platform for rational formulation design, with molecular-level insights guiding future optimization.

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Sialic Acid-binding Nanoparticles Modulate Inflammation

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Introduction
Modulating immune cell function remain a key challenge in treating diseases such as cancer, cardiovascular, autoimmune disorders and osteoarthritis (OA). Advances in the field of glycobiology have highlighted the crucial role of sugars – particularly the monosaccharide Sialic acid (Sia), located at the terminal ends of glycans – in orchestrating immune cell communication and disease progression.[1] Sia contributes to both homeostatic and inflammatory processes. We hypothesize that targeting surface-associated Sia using polymeric nanoparticles (NPs) can modulate aberrant immune cell recruitment and mitigate chronic inflammation, such that is found in OA. Due the multivalency and spatial configuration, NPs can enhance binding affinity to Sia and offer a novel immunomodulatory strategy.

Aim
Our aim was to develop biocompatible polymeric NPs with high affinity to Sia that can modulate immune cell recruitment and communication.

Method
We synthesized Sia-targeting NPs by conjugating phenylboronic acid (PBA) to a cationic polymer decorating a NP-core of PLGA, formulated by nanoprecipitation (termed PLGA-PBA). NP size and charge were evaluated using dynamic light scattering and scanning electron microscopy. PBA conjugation was confirmed using nuclear magnetic resonance. Sia-binding affinity was evaluated using isothermal titration calorimetry. For the biological assessment, cell viability was evaluated in L929, U937, and RAW264.7 cell lines. We subsequently used primary monocytes from healthy donors and performed flow cytometry, ELISA and trans-well migration studies.

Results
Our findings demonstrate that PLGA-PBA NPs exhibited an average size of 236nm and demonstrated a pH-dependent high-affinity binding to Sia. They were biocompatible, as confirmed by minimal impact on cell viability and cytokine release. Biological binding specificity was validated by enzymatically removing Sia from cell surfaces, resulting in reduced NP attachment. Importantly, Sia-binding NPs significantly reduced monocyte migration in a concentration-dependent manner (Figure 1).

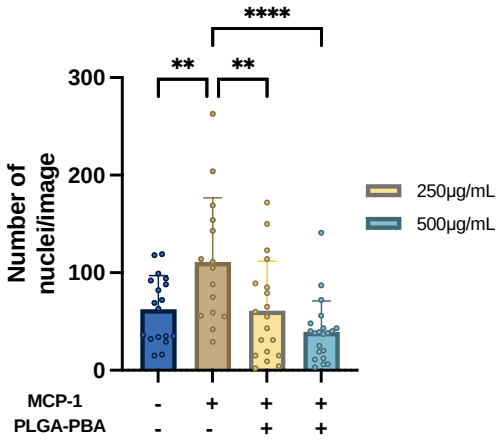

Figure 1. *Number of nuclei per image indicated how many cells migrated through the trans-well membrane towards the chemoattractant MCP-1*

Conclusions
Our findings demonstrate that PLGA-PBA NPs selectively bind Sia with high affinity without compromising cell viability. These NPs effectively reduce immune cell migration, suggesting that targeting Sia is a promising strategy to modulate inflammation in diseases like OA.

Acknowledgement
The author would like to thank the Swedish Research Council for financial support (Grant No. 2021-01870)

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A Novel Liposomal Delivery of Abrocitinib: A Nanocarrier-Based Strategy for Enhanced Topical Anti-Inflammatory Therapy in Atopic Dermatitis

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Introduction
Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by eczematous lesions, erythema, intense itching and impaired skin barrier function, all of which significantly impact patients' quality of life (1). Topical JAK inhibitors are a novel class of drugs well-suited for the treatment of AD, offering localized efficacy with potentially fewer systemic side effects than topical steroids (2).

Aim
This study aimed to develop and evaluate a novel liposomal formulation of the JAK inhibitor, Abrocitinib (Ab), to enhance targeted skin delivery, improve anti-inflammatory effects and reduce cytotoxicity in AD.

Methods
Ab-Liposomes were prepared and characterized, followed by an *in vitro* drug release and *ex vivo* skin penetration study using Franz diffusion cells. Anti-inflammatory effects were measured by nitric oxide (NO) production in LPS-induced macrophages and interleukin-6 (IL-6) production in Poly(I:C)-induced primary human epidermal keratinocytes. Cytotoxicity was assessed in murine macrophages, human dermal fibroblasts and keratinocytes using CCK-8 assays.

Results
Ab-Liposomes demonstrated high entrapment efficiency and controlled release with low skin permeability. Ab-Liposomes significantly reduced NO levels and effectively suppressed IL-6 production, indicating strong anti-inflammatory activity. Cytotoxicity studies showed high cell viability and good tolerability at the tested lipid concentrations.

Conclusion
Our novel formulation offers a promising approach for improving topical delivery and anti-inflammatory therapy in AD. However, further optimization is needed to understand its anti-inflammatory efficacy and improve its applicability for dermal drug delivery for AD skin. Our final formulation will consist of the incorporation of liposomes into a hydrogel to develop a patient-friendly treatment for improved compliance.

Acknowledgment
This project was funded by UiT The Arctic University of Norway. The authors thank Pfizer (New York, USA) for providing Abrocitinib and Lipoid GmbH (Ludwigshafen, Germany) for gifting Lipoid S100. Special thanks to Iva Pitelkova and the Proteomics and Metabolomics Core Facility, Department of Medical Biology, UiT, for LC-MS/MS support.

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3D-printed tablets containing liquid crystal nanoparticles for oral antibiotic delivery

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Introduction

Oral drug delivery is favored for its high patient compliance and cost-effectiveness.[1] However, many antibiotics suffer from low bioavailability due to poor solubility, permeability, or instability in the GI tract. High doses required for efficacy increase the risk of antimicrobial resistance. Liquid crystal nanoparticles (LCNPs), with their amphiphilic lipid matrix and high surface area, offer a promising platform for improving oral delivery.[2] We previously developed LCNPs that could encapsulate the antibiotics vancomycin and clarithromycin and demonstrated effective loading, intestinal stability and protection for commensal bacteria such as *E. faecalis* and *E. coli*.[3] Despite their advantages, incorporating lipid-based systems into solid oral dosage forms remains a key formulation challenge.

Aim

To incorporate antibiotic-loaded LCNPs into a 3D-printable formulation suitable for semi-solid extrusion (SSE) technology for oral tablet development.

Method

LCNPs were composed of phytantriol and α-tocopherol in a 4:1 ratio, resulting in an inverse hexagonal internal structure.[3] 3D-printed tablets based on LCNPs were produced by developing and optimizing a 3D-printable gel through the integration of the polymeric gelling agent methyl cellulose (MC) and the disintegrant croscarmellose sodium (Ac-Di-Sol), followed by a series of printing processes. Small-angle X-ray scattering (SAXS) and cryo-transmission electron microscopy (cryo-TEM) confirmed structural integrity of the LCNPs during printing and after dissolution. LCNPs were loaded with vancomycin, and tablets were evaluated for disintegration time and dose accuracy.

Results

Tablets containing inverse hexagonal LCNPs were successfully 3D-printed using SSE with a formulation comprising 3 wt% MC A4C, 3 wt% MC A4M, and 8.4 wt% Ac-Di-Sol. These tablets demonstrated well-defined mass (m = 67.9 mg ± 1.5 mg) and disintegrated within 32 minutes. Importantly, the internal hexagonal phase of the LCNPs remained stable throughout gel production, 3D-printing as confirmed by SAXS and cryo-TEM, and was also observed upon rehydration of the tablets. Dose accuracy showed strong linearity ($R^2 > 0.9$), enabling reliable estimation of vancomycin content.

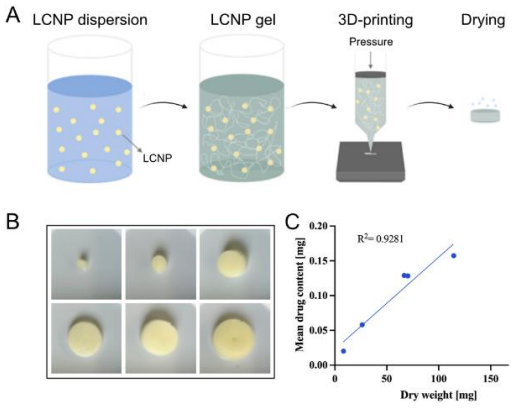

Figure 1. (A) *3D-printing workflow.* ***(B)*** *Examples of 3D-printed tablets based on LCNP ink in different sizes.* ***(C)*** *Correlation of drug content vs. dry weight.*

Conclusion

This study demonstrates a proof-of-concept for 3D-printing LCNPs into solid oral dosage forms, preserving their nanostructure and enabling controlled antibiotic dosing, an innovative step toward enhancing oral bioavailability of poorly absorbed drugs.

Acknowledgement

The author would like to thank Uppsala Antibiotic Center and SweDeliver (VINNOVA, 2019-00048) for financial support.

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Title: Semi-Solid Extrusion 3D Printing of Indomethacin-Nicotinamide Cocrystal-Loaded Oral Fast-Dissolving Films

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Introduction

Personalized medicine for vulnerable patients necessitates innovative drug delivery with precise control. Semi-solid extrusion (SSE) 3D printing, operating at ambient temperatures, is uniquely suited for thermosensitive drugs and enables on-demand, point-of-care fabrication of patient-specific doses (1). Many new drugs face poor water solubility, limiting oral absorption and bioavailability. Cocrystals, formed by combining a drug with co-former, offer a promising strategy to enhance physicochemical properties of poorly soluble drugs (2). While high-temperature 3D printing of cocrystals like Fused Deposition Modeling exists (3), SSE 3D printing for the fabrication of cocrystal-loaded dosage forms remains unexplored, highlighting this study's novel, gentler approach.

Aim

To pioneer the SSE 3D printing of personalized, cocrystal-loaded oral fast-dissolving films (OFDFs). This involves optimizing ink formulations for superior printability while confirming cocrystals integrity within the final formulations.

Method

Indomethacin-Nicotinamide (IND-NIC) cocrystals were prepared using liquid-assisted ball milling (2). Printing inks were then formulated with HPMC, glycerol, sodium starch glycolate (SSG), and varying levels of cocrystals (2%-10% w/w IND loading). All components were precisely mixed using a mortar and pestle. For 3D printing, films were CAD-designed as 25x10x2.5mm rectangular-shaped. Printability, including extrudability and shape fidelity, was assessed on freshly prepared-inks using an Allevi SSE 3D bioprinter. The content uniformity was quantified via UV-Vis spectrophotometry. Cocrystal solid-state characterization within films were performed using Differential Scanning Calorimetry (DSC), X-ray Powdered Diffraction (XRPD) and Polarized Light Microscopy (PLM).

Results

Ink formulations with varying levels of IND-NIC cocrystals loaded and 2% (w/w) SSG showed optimal extrudability and shape fidelity, indicating successful printing and suitable rheological characteristics for smooth and consistent flow. The 3D printed OFDFs exhibited low thickness (0.23-0.26 mm) and weight variability (RSD max 4.39%), suggesting excellent printing quality and process repeatability. Drug content analysis was within 90.02-93.71%, indicating a high drug homogeneity. Comprehensive solid-state characterization (DSC, XRPD and PLM) confirmed the successful incorporation and integrity of the cocrystals within the 3D printed formulations.

Conclusion

SSE 3D printing was successfully demonstrated for fabricating personalized IND-NIC cocrystal-loaded OFDFs with excellent printability, shape fidelity, and drug homogeneity.

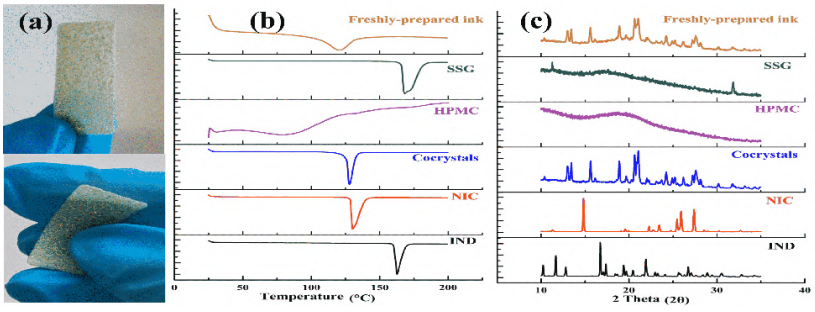

Figure 1 : (a) Successful 3D printed films; (b) DSC scans and (c) XPRD patterns of powders, cocrystals and 3D film.

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Preparation of stable β-glucans coated liposomes - the role of the chemical structure of the coating polysaccharides

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Introduction

The present project proposes the application of β-glucans, a class of polysaccharide presenting immunomodulatory properties[1], as source material for the development of polymer-coated liposomes. β-glucans compose structurally diverse polysaccharides[1]. The polymer chemical structure plays an important role in the colloidal system properties, as reported for ionic polysaccharides[2]. However, β-glucans are neutral polysaccharides, and the role of their chemical structure related to the coating process remains to be investigated.

Aim

The aim of this study was to investigate the role of the β-glucans chemical features on the size and stability of coated liposomes based on these polysaccharides.

Methods

β-glucans fractions from the mushrooms *Pleurotus eryngii*, *Lactarius deterrimus* and *Lactarius scrobiculatus* were extracted. Commercially available laminarin (LMN) and yeast β-glucans (YBGS) were also employed. Linkage patterns were determined through methylation analysis and nuclear magnetic resonance spectroscopy. Molar mass (M_w) was determined using size exclusion chromatography coupled to laser light scattering. Liposomes composed of 75% SoyPC and 25% DOTAP were prepared using the thin film method and then added to the polysaccharide solutions to obtain the β-glucans coated liposomes. The coating was evaluated according to zeta potential, size, size distribution (PDI) and size increment up to 3 months.

Results

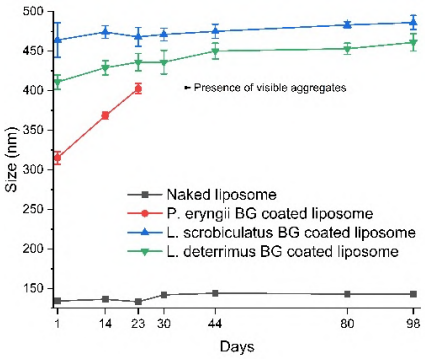

Figure 1. Liposome size along the days

The P. eryngii fractions were composed of 3- and 6-linked β-glucans branched by one glucose residue and M_w ranging from 7-36 kDa[3]. From *Lactarius sp*., fractions of 3-linked-β-glucans branched by a short glucan side chain and M_w range of 13-86 kDa were obtained[4]. LMN is a 3-linked β-glucan branched by glucose and M_w of 5kDa, while YBGS is a 3-linked β-glucan branched by a long glucan side chain and M_w populations of 8, 35 and 240 kDa[4]. The size and PDI of the β-glucan coated liposomes decreased according to the increase of M_w[3]. The β-glucans from *Lactarius sp*. presented the lowest size increment up to 3 months, suggesting that short oligosaccharides as side chains may stabilize the system through steric hindrance. However, long side chains may impair the process, since YBGS was not suitable for the process. LMN was not suitable to the coating either, which was attributed to its low M_w.

Conclusions

The obtainment of stable β-glucans coated liposomes depended on a minimum M_w, suggested to be around 30 kDa[3], and on the length of the side chain of the coating polysaccharide.

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Co-spray dried Piperacillin-Tazobactam inhalation powder for the management of Pseudomonas aeruginosa pulmonary infections in non-cystic fibrosis bronchiectasis

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Introduction

The absence of inhalation therapies specifically designed for pulmonary infections frequently results in the off-label administration of antibiotics dosage forms, originally intended for parental use[1]. Dry powder inhalers (DPIs), consisting of an inhalable powder with a delivery device, offer the potential to achieve elevated drug concentrations at the site of action in the lungs, while reducing systemic exposure and associated side effects[2]. In this study, the semisynthetic β-lactam antibiotic Piperacillin was co-spray dried together with the β-lactamase inhibitor Tazobactam, to prepare a combined inhalation powder.

Aim

Investigation of a co-spray drying formulation approach for the development of a combined inhalation powder, targeting pulmonary infections in non-cystic fibrosis bronchiectasis (NCFB).

Method

Piperacillin (Pip) and Tazobactam (Tzb) were co-spray dried at an 8:1 weight ratio with a mini spray dryer (Büchi, Switzerland). The feed solution was prepared at a solid concentration of 0.5% w/v in a 70/30 v/v methanol/water solvent mixture. The solid state of the combined powder was characterized by X-ray diffraction (XRD), modulated differential scanning calorimetry (MDSC) and Fourier-transform infrared spectroscopy (FTIR), to investigate potential intermolecular interactions that could enhance the physical stability of the amorphous system.

Results

XRD analyses revealed that Tzb was initially amorphous after spray drying but recrystallized within 24 hours, while Pip maintained as amorphous material for at least 3 weeks. No evidence of recrystallization was observed in the co-spray dried Pip-Tzb powders over a period of 5 weeks at RT. FTIR analyses didn't reveal any chemical interactions between the two APIs. These findings suggest that co-spray dry of Pip and Tzb results in the formation of an amorphous material, which may contribute to the physical stability of combined dry powders.

Conclusion

Co-spray drying of piperacillin and tazobactam at an 8:1 weight ratio resulted in an amorphous powder, in which tazobactam is molecularly dispersed within an amorphous matrix formed by piperacillin. The presence of hydrogen bonds in the combined dry powders is hypothesized to prevent tazobactam recrystallization during storage, thereby enhancing its physical stability. Further studies will be focused on investigating the spray dried process parameters that may influence the critical quality attributes of the co-spray dried powders.

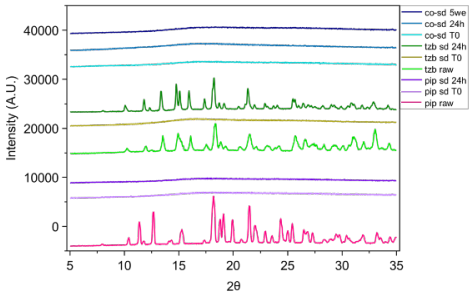

Fig 1. XRD patterns of co-spray dried powders, APIs raw and spray dried APIs.

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Lipid Nanoparticles for delivery of peptides and DNA nanostructures

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Introduction

Antimicrobial peptides [AMPs] have gained attention as promising alternatives to conventional antibiotics [1]. Tetrahedral DNA nanostructures [TdNs] have demonstrated anti-inflammatory effects, potentially beneficial in treatment of microbial infections as virulence factors from bacteria can trigger excessive inflammation [2]. However, the stability and aggregation of AMPs have been a challenge in antimicrobial treatment [3]. In this study LNPs were utilized to encapsulate and deliver both AMPs and TdNs.

Aim

To investigate the loading of antimicrobial peptides and DNA nanostructures into lipid nanoparticles. Characterization of the nanoparticles and the impact of the nanoparticles on *Staphylococcus aureus*.

Method

The nanoparticles were generated by microfluidic mixing. The nanoparticles were characterized based on their size, polydispersity index, zetapotential, and morphology. The loading of the nanoparticles with peptides/DNA nanostructure was measured to determine the encapsulation efficiency. The interaction between nanoparticle and microbe (*Staphylococcus aureus*) was measured to determine the %uptake, the co-localization, and the antimicrobial effect.

Results

The five nanoparticles generated were all around 100-150 nm with a low poly disperity index. The Zeta potential of LP and TLP were positive whereas BL, TL, and TLP(h) were negative. There was an enhaned antimicrobial effect when loading the lipid nanoparticle with the tetrahedron DNA and L12 peptide.

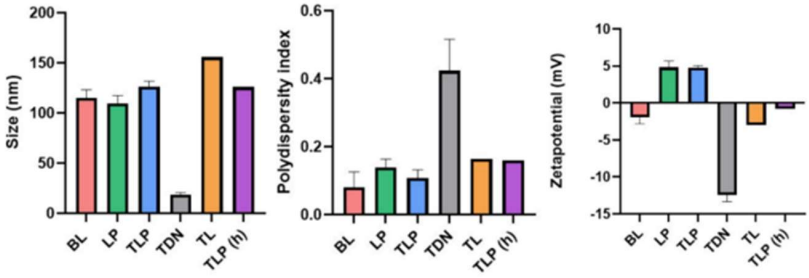

Figure 1: Characteristics of the nanoparticles by dynamic light scanning.

Conclusion

AMPs may be enhanced by adding them to lipid nanoparticles. DNA nanostructures may also further enhance the antimicrobial effect.

Acknowledgement

Research supported by Tromsø Forsknings-Stiftelse (TFS, Grant No. 20_SG_SO).

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3D-Printed Complex Carrier Particles for Inhalation

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Introduction
In modern dry powders for inhalation, the carrier-based approach of interactive powder mixtures is the most used formulation technique. While the micronised drug has to reach the lung tissue as final target, the carrier particle improves powder characteristics such as flowability and dispersibility. α-lactose monohydrate in its crystalline form is used in most commercial inhaler devices as excipient, nevertheless, recent studies have investigated alternative carrier particles. In this project, a special focus was given on the influence of the carrier particle's geometry, investigated with a combination of new approaches such as in-silico modelling of particular interactions and dispersion behaviour[1,2] as well as the exploration of additive manufacturing techniques for experimental assessment.

Aim
The effect of different complex geometries for the carrier particle in an interactive mixture was evaluated with precisely 3D-printed carrier particles.

Method
Four different carrier particle geometries (**Fig. 1**) were 3D-printed using a two-photon polymerisation printing platform[3]. Approximately 3 million particles per geometry were produced. Each powder bulk was then used to create an interactive powder mixture with the drug budesonide in 2 % (w/w) in a miniature scale low shear mixer at 42 rpm. The interactive mixtures were tested for aerodynamic performance using the Fast Screening Impactor at 80 l/min flow rate. Drug was collected and dissolved in ethanol; the quantification was processed using high performance liquid chromatography with external calibration.

Results
The results of the aerodynamic assessment showed significant differences between the four powder batches based on different carrier geometries. While Sphere and Soccerball performed similar with a fine particle fraction (FPF) of 4 %, the Rollingknot was inferior with only around 2 %. The best performance was achieved by the Pharmacone with 17 % in FPF.

Conclusion
The influence of the carrier particle geometry on the performance of the respective powder blend during inhalation was shown. The best geometry, namely the Pharmacone, achieved a 4-times higher FPF compared to the next-best geometries. The enhanced performance likely stems from the spike structures, which promote chaotic motion and increase further collisions which are key to drug dispersion[4]. These results, combined with the correlating in-silico models, enable further optimisation of carrier particle geometries with a parametric design approach following the goal to better understand beneficial geometry features for the aerodynamic performance.

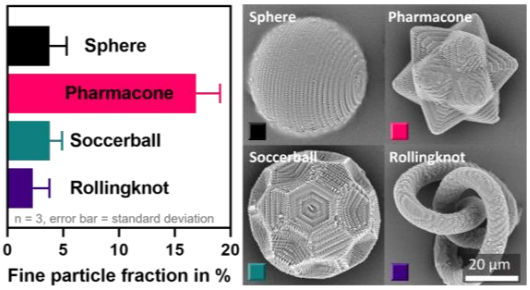

Figure 1 Results of aerodynamic assessment of four powder batches (left) and scanning electron microscope images of 3D-printed particle geometries (right)

Acknowledgements
The authors would like to thank the Karlsruhe Institute of Technology for the support and preparation of the 3D-printed particles.

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The influence of solubilizing agents on the molecularly dissolved drug concentration of co-crystals

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Introduction

Pharmaceutical co-crystals represent a promising strategy to improve drug's bioavailability by enhancing the solubility of the active pharmaceutical ingredient (API) without altering its chemical structure[1]. The use of solubilizing agents can affect co-crystal apparent solubility differently compared to the pure drug (Figure 1). However, there is no experimental data on the molecularly dissolved drug behavior of co-crystals in the presence of solubilizing agents. Determination of the freely dissolved drug concentration is crucial, as this form readily permeates the gastrointestinal tract barrier[2].

The method of microdialysis can be employed to determine the freely dissolved concentrations of different APIs, providing insights into their dissolution mechanism.

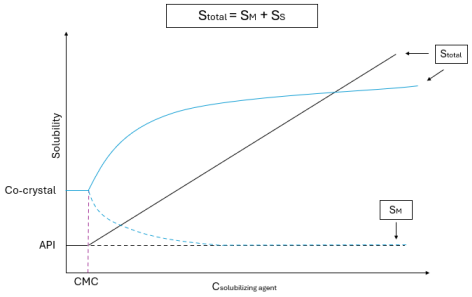

***Figure 1**: A theoretical illustration of the apparently dissolved drug (S_{total}, solid line) vs. molecularly (freely) dissolved drug (S_M,* dash line*) of both a crystalline API and a co-crystal in the presence of increasing concentrations of solubilizing agents.*

In this study, the freely dissolved drug concentration of Indomethacin (IND) and a pharmaceutical co-crystal, Indomethacin-Saccharin (IND-SAC) was investigated in the presence of the non-ionic surfactant, polyethylene (20) oleyl ether (Oleth-20).

Aim

To determine the freely dissolved drug solubilities of IND and IND-SAC in the presence of varying concentrations of the non-ionic surfactant Oleth-20.

Method

A 50 mM phosphate buffer (pH 2.1) was utilized to make different medias consisting of increasing concentrations of Oleth-20, along with an access amount of powder of IND or IND-SAC. Solubility of IND in the media was determined by shake flask method, while co-crystal solubility was determined at the eutectic point. Each media was sampled both via the microdialysis method and manual sampling (via filtering). All samples were analyzed by use of HPLC.

Results

The molecularly dissolved solubility of IND was determined to remain the same, despite increasing Oleth-20 concentrations. Comparatively, the concentrations determined from direct sampling (apparently dissolved drug concentration), were increasing steadily with the increase in Oleth-20 concentrations.

Conclusion

This study indicates that the molecularly dissolved solubility of IND is not affected by increasing concentrations of the non-ionic surfactant Oleth-20, while the molecularly dissolved solubility of IND-SAC approaches the one of the pure drug at high surfactant concentrations.

Aknowledgement
The authors would like to thank Nordic POP (Nordforsk grant number 85352).

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Understanding the transport of drugs across biomimetic barriers of various phospholipid compositions using a combined experimental and computational approach

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Introduction

Permeapad®, a biomimetic barrier using only the phospholipid phosphatidylcholine (PC) and therefore differs from the enterocytes' brush border, which contains diverse phospholipids such as phosphatidylethanolamine (PE) and phosphatidylglycerol (PG). Metoprolol's lower Permeapad® permeability compared to Caco-2, and PAMPA studies that demonstrated ion-pair transport with anionic phospholipids, suggest PC alone is inadequate [1]. Novel for biomimetic barriers, Molecular Dynamics (MD) simulations may mechanistically explain drug behavior within the Permeapad® [2].

Aim

To gain a deeper understanding of the functionality of the Permeapad® barrier and its transport pathways, which could be affected by the phospholipid composition.

Method

The content of the phospholipids, PE, PG, in addition to PC where systematically varied in the Permeapad® barrier, and the permeability of three model compounds of roughly similar molecular sizes, metoprolol (weak base), hydrocortisone (non-ionizable) and naproxen (weak acid) across those Permeapad® variants was studied using side-by-side cells. Additionally, MD simulations were performed using Gromacs software and CHARMM36m force field, to model drug transport through those Permeapad® variants, and to evaluate how changes in composition affected its performance.

Results

The permeation experiments showed that only the permeation of metoprolol was affected by the phospholipid composition, and was significantly increased across the Permeapad® variants with 50 % PE, 6.25 % PG and 12 % PG. The MD simulations unearthed several descriptors of membrane properties and predicted membrane permeability. An almost inverse relationship was observed between experimental and simulated permeability results.

Conclusion

It was hypothesized that attraction of the drug molecule to the surface of liposomes and the likelihood of internalization were crucial for the permeation mechanisms of the Permeapad® barrier. Combining permeation experiments and MD simulations can aid designing new Permeapad® variants, possibly with in vivo relevant phospholipid compositions.

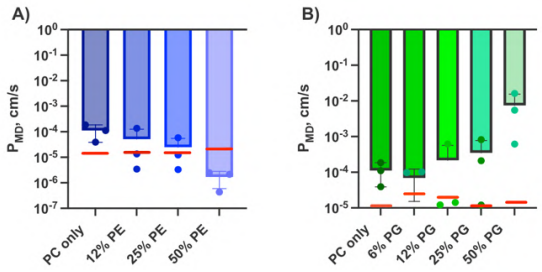

Figure 1 Permeability values (P_{MD}) for metoprolol passing through membranes with various compositions. A) Phosphatidylethanolamine (PE) and B) Phosphatidylglycerol (PG) membranes. For both A) and B) the remainder of the phospholipid composition was phosphatidylcholine (PC). Red lines depict the experimental P_{app} values for the respective membrane compositions. Dots are permeability values of each replicate coming from triplicate simulations.

Acknowledgements

Lipoid GmbH is thanked for phospholipids. Dr. Simon Drescher is acknowledged for initial project discussions. Simulations used resources from NAISS (funded by Swedish Research Council grant no. 2022–06725). NordForsk's Nordic University Hub project #85352 (Nordic POP) is acknowledged for fostering collaborations.

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Microneedle enabled dual drug delivery system for vaginal infections using magnetic hyperthermia

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Introduction:
About 75% of women are susceptible to vulvovaginal candidiasis (VVC), and majority show a recurrent form of the infection. Current vaginal therapies not only lack sufficient mucus penetration and drug retention, but also show increasing drug resistance. Addressing these barriers, clotrimazole loaded lipid based nanocarriers have been incorporated into microneedles (MNs) (1). Here, we explore the dual action of drug-releasing microneedles with magnetic hyperthermia to combat VCC. The combined action can have a synergistic effect on the eradication of *C. albicans* biofilms (2).

Methods:
Lipid-based formulation (LBF) was employed for the delivery of clotrimazole (1). Superparamagnetic iron oxide nanoparticles (SPIONs) were used to produce heat in the presence of an alternating magnetic field (AMF) (3). SPIONs were incorporated into the non-dissolvable backing layer of the MNs and LBF in the dissolvable tips. Hyperthermia efficiency of the MNs was evaluated at 14 mT using Magnetherm™ for 3 min, where an infrared (IR) camera was used to track the heating of MN patches over time. The thermal stability of clotrimazole was tested using thermal shaking, followed by high-performance liquid chromatography analysis. The antifungal efficacy of the dual-functionality MNs was evaluated against *C. albicans in vitro* and *ex vivo* by fungal growth curve analysis (1,4).

Results:
Clotrimazole was formulated in the LBF at a drug load of 44 mg/g and the formulation had a hydrodynamic diameter of 310 nm. SPIONs were successfully incorporated into the non-dissolvable backing layer of dissolvable MN patches with clotrimazole-loaded MN tips of height 800 µm. (Fig 1a) Rapid dissolution in water within 5 minutes was demonstrated. (Fig 1b,c) The SPION loaded MN patches could reach the target temperature range of 45-52ºC within 3 minutes. The IR image indicates a homogenous heating of the patch. (Fig 1d, e & f)

Conclusion:
Dual-action MNs with clotrimazole loaded LBF and SPIONs for magnetic hyperthermia were developed, reaching a target heating range of 45-52°C. Ongoing research explores the synergy of hyperthermia and antifungal action. This approach could enhance treatment of VVC by overcoming vaginal barriers, reducing drug doses, and combating antimicrobial resistance.

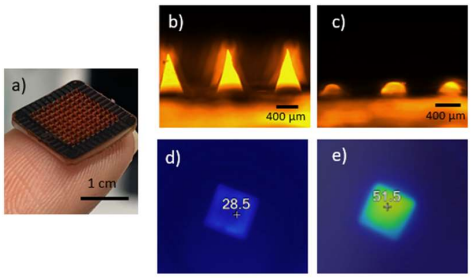

Figure 1. SPION loaded MNs a) Phone camera image of the MN patch, b),c) optical microscope imaged of the MN tips before and after dissolution, d)&e) thermal images of the patch with AMF turned off at t=0 min and t=3 min.

Acknowledgements: This work is supported by the Swedish Research Council (grant #2023-03057).

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Developing Dry Powder Inhalable Cystic Fibrosis Treatment

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Introduction
Cystic fibrosis (CF) is caused by CFTR gene mutations, leading to thick mucus, lung infections, and reduced function. Key treatments include nebulized rhDNase to thin mucus and oral azithromycin for its antimicrobial and anti-inflammatory effects. However, rhDNase needs refrigeration and nebulization, while long-term azithromycin poses microbial resistance and side effect risks. Overall, this project aims to create a spray-dried inhalable powder combining both drugs, offering room-temperature-stable rhDNase and localized low dose azithromycin delivery to reduce systemic exposure and side effects.

Aim
This study aimed to evaluate the feasibility of co-spray drying azithromycin with ovalbumin (as a model protein for rhDNase) using a three-fluid nozzle, and to apply design of experiments to optimize the resulting particles for inhalable dry powder delivery.

Method
Spray drying was performed using a Büchi S-300 with a three-fluid nozzle. Both liquid feeds ran at 2 ml/min, with an inlet temperature of ~110 °C, outlet temperature of 70 ± 2 °C, and drying gas flow at 23 m³/h. The inner phase contained equal concentrations of ovalbumin and trehalose with 0.1% polysorbate 80 in water; the outer phase contained azithromycin in methanol/water (5:2 v/v). The azithromycin-to-protein weight ratio was fixed at 28, signifying inhaled doses of 70 mg azithromycin and 2.5 mg rhDNase. A central composite face-centered design tested the effects of spray gas flow (700–1300 l/min) and solids concentration (16.5–23.5 mg/ml) on yield, mass median aerodynamic diameter (MMAD, the aerodynamic particle size where half of the mass is in smaller particle sizes), and fine particle fraction (FPF, the percentage of mass in particles below 5 µm, thus considered inhalable) in a total of eleven experiments.

Results
All generated models were deemed good based on linearity, predictability, validity and reproducibility. MMAD was significantly influenced negatively by spray gas flow and positively by solids concentration. Opposite effects were observed for both process parameters on FPF. Yield was only affected by the spray gas, positively for the primary factor with a negative quadratic term. A sweet spot plot was created with criteria of MMAD 2-4 µm, FPF >70% and yield >75%. The sweet spot is formed in a large part of the tested area, primarily limited by the FPF criterion (Fig. 1).

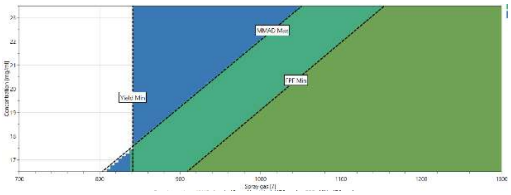

Figure 1 *Sweet spot plot for all three tested responses. Spray gas flow on x-axis and concentration on y-axis. Green colored area signifies the sweet spot where all criteria are met.*

Conclusion
Spray drying equipped with a three fluid-nozzle can be used to optimize particle attributes for lung delivery. The smallest MMAD achieved in the study was 3.06 µm showing that the tested ranges achieve particles suitable for depositing in the conducting airways, which is the target area for CF, whereas other strategies may be required for alveolar deposition.

Acknowledgement
The authors would like to thank NordForsk via the Nordic University Hub Project #85352 (Nordic POP – Patient Orientated Products), for the support.

Enabling Oral Delivery of Salmon Calcitonin via SNEDDS: Effects on Protection and Activity

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Introduction
Oral peptide delivery is hindered by enzymatic degradation and poor permeability. Self-nano emulsifying drug delivery systems (SNEDDS) can enhance absorption, but hydrophilic peptides require prior lipidization via hydrophobic ion pairs (HIP)[1]. Here, salmon calcitonin (sCT), a plasma calcium regulator, was complexed with anionic surfactants (AnS), sodium decanoate (C10) and sodium docusate (SDOCS), then loaded into SNEDDS and evaluated for proteolytic protection and pharmacological activity.

Methods
Anionic surfactant solutions were added dropwise to sCT (1:1–1:8 sCT:AnS molar ratios). After centrifugation, supernatants were analyzed for complexation efficiency%, and precipitates are lyophilized. Then 1:4 sCT:AnS complexes were incorporated into SNEDDS (30% MCT, 30% MGDG, 30% RH40, 10% PG). Free sCT, complexes, and SNEDDS-loaded complexes were tested for proteolytic stability against trypsin (pH 6.8, 37 °C, 120 min). Plasma calcium levels were measured after oral gavage in rats (1 mg sCT/kg), compared with subcutaneous injection (0.1 mg/kg), and pharmacological activity (PA) was calculated.

Results
Complexation efficiency% for both AnS reached ≥89% at a 1:4 ratio and decreased or plateaued thereafter, indicating optimal neutralization at that ratio (Fig.1a). sCT:AnS complexes in SNEDDS provided superior proteolytic protection. free sCT and C10 complex degraded within 30 min, while C10 complex loaded SNEDDS retained >55% after 2 hr. SDOCS complex alone retained >60%, further increased to 70% when loaded in SNEDDS (Fig.1b). *In vivo*, oral C10 complex reduced calcium to 85% (1.7% PA), but C10 complex in SNEDDS reduced it to 65% (5% PA). SDOCS complex alone reduced calcium to 72%, and SDOCS complex in SNEDDS to 49% (8.4% PA) (Fig.1c). *In vitro* results correlated well with *in vivo* data (Fig.1d).

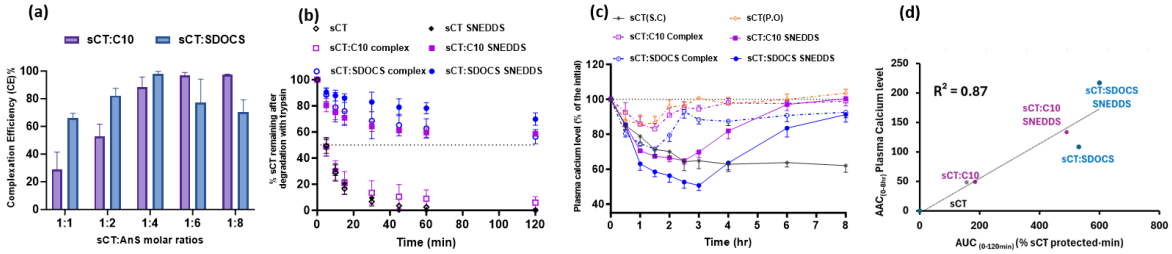

Figure 1: (a) Complexation efficiency %, (b) %sCT after proteolysis (mean±SD, n=3), (c) Plasma calcium % (mean±SE, n=6), (d) *IVIV* correlation.

Conclusions
At a 1:4 molar ratio, Complexation efficiency% reached ~90%, confirming effective pairing. Both AnS complexes showed enhanced proteolytic protection when loaded in SNEDDS, with SDOCS outperforming C10. *In vivo*, sCT:SDOCS in SNEDDS significantly lowered calcium levels, emphasizing the combined role of SNEDDS and AnS type for oral peptide delivery.

Acknowledgments
Passant M. Al-Maghrabi thanks the Egyptian government scholarship for financial support.

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Remote-Controlled Light Activated Drug Delivery Implant with Wireless µLEDs

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Introduction: Poor patient compliance is considered one of the leading causes of failure in the treatment of chronic diseases, mainly in elderly patients. Repeated dosing is necessary to maintain adequate levels of drugs within the therapeutic window to treat chronic diseases.[1] There is a need to develop implantable drug delivery systems with temporally and spatially controlled drug release capabilities to achieve precision and personalized medicine. We developed a smart light-activated drug delivery implant system, containing a tiny light source in the capsule containing liposomal hydrogel. This light source can be easily connected to the user's mobile phone via Bluetooth. Duration and intensity of light activation can be controlled to modulate the drug release using the application on a mobile phone.

Aim: To develop a remotely controlled implant system for drug delivery.

Method: The implant consists of three main components. 1. A 3-D printed capsule that serves as a holder for both the drug reservoir and the light source. 2. A nanocellulose hydrogel that functions as the drug reservoir, incorporating drug-encapsulated light-sensitive liposomes. 3. A light source, which is made up of six micro-LEDs and a rechargeable battery. For the release studies, calcein-loaded liposomes were mixed with the nanocellulose hydrogel and placed in the 3-D printed capsule. The study was conducted in a 50 mL buffer solution, and at scheduled timepoints, 100 µL samples were taken to measure calcein fluorescence.

Results: Light activation at 1.97 mW (level C) effectively enhances and sustains calcein release, showcasing the potential for spatiotemporal control in drug delivery systems. The light-treated implant shows a rapid increase in release within the first 5 hours (~11%) and continues to rise, reaching ~25% at 48 hours, while the control remains below 10%. The photosensitizer in the liposomes produces reactive oxygen species upon light exposure, oxidizing unsaturated lipids and triggering calcein release. Additionally, the hydrogel serves as a reservoir for the liposomes and helps maintain a steady release rate after activation, avoiding any sudden surges.[2]

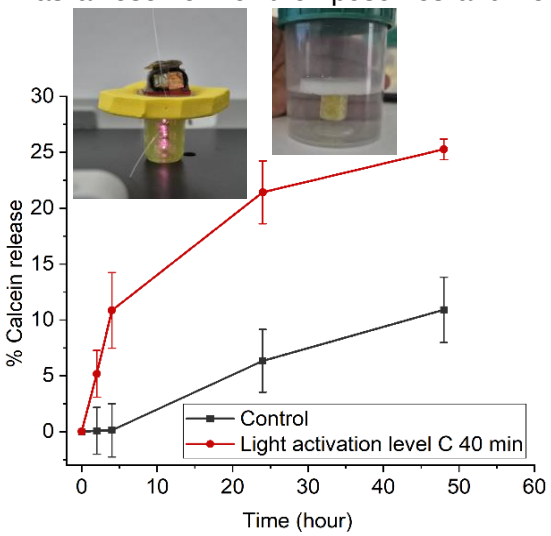

Figure 1: Calcein release after light activation at 1,97 mW (690 nm) with 40 min exposure time.

Conclusion: The main limitation of light-activated drug delivery systems is their tissue penetration depth. To overcome this challenge, we have successfully developed an implant that contains both a light source and a drug reservoir. This design allows for controlled drug release, which can be tailored to the patient's needs.

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Dose accuracy after manipulation of prednisolone tablets compared to a compounded suspension for paediatric patients

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Introduction
Tablets are frequently manipulated to achieve an appropriate dose or to ease administration when administering to paediatric patients[1]. Manipulation is often done *off-label*, and common methods of manipulations include crushing, splitting and/or dispersing a tablet in water. While this might be common in clinical practice, information about the safety and dosing accuracy after manipulation is limited[2]. Extemporaneous compounding by a pharmacy could provide a safer alternative to *ad hoc* manipulation on the wards and at home but comes with higher cost and lead times.

Aim
To investigate the dose accuracy and precision attained after manipulating two brands of prednisolone tablets, and to compare with a pharmacy compounded suspension to establish the best practice for adjusting the dose of prednisolone in paediatric care.

Methods
Tablets with prednisolone (2.5 mg and 5 mg) from two manufacturers (Prednisolon Orifarm and Prednisolon Alternova) were manipulated to reach a dose of 1.25 mg either by splitting tablets in half or quarters and dispersing the fragment in an oral syringe or by dispersing a tablet in 10 ml water and extracting a partial dose (25 % of a whole tablet). The pharmacy compounded suspension of prednisolone 1 mg/ml was tested by dispensing doses of 0.5 mg with an oral syringe. Dose accuracy and precision for both tablets and suspension were determined by quantification with a validated HPLC-UV method. Results within ±20 % of target dose have previously been defined as adequate dose accuracy after manipulation[3].

Results
Dose accuracy was within ±20 % of target dose after splitting tablets in both half and quarter fragments (Figure 1), but splitting into quarter fragments showed lower precision due to less accurate tablet splitting. Dispersing tablets in water was less accurate than splitting in quarter fragments, but with higher precision and could be an alternative if doses outside of half or quarters are needed. Compounded suspension had a dose accuracy of 108.5 % (low-high 107.1-110.2 %) and provided the best option if high precision is required, e.g. upon tapering doses.

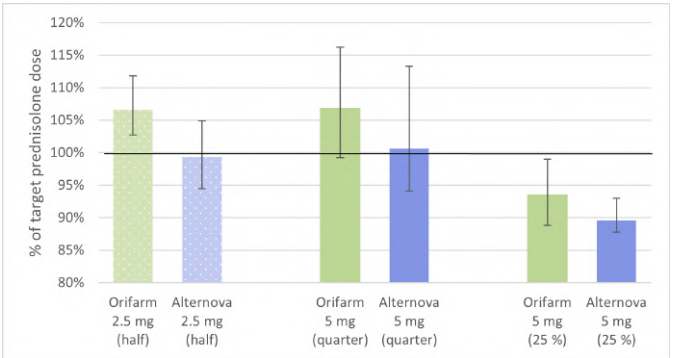

Figure 1. Dosing accuracy (% of target dose, average, low-high, n=6) of Prednisolon Orifarm and Prednisolon Alternova (2.5 mg and 5 mg) after dispersing half or quarter fragments in an oral syringe, or by extracting 25 % of a whole tablet dispersed in a medicine cup to achieve doses of 1.25 mg.

Conclusion
The highest dose accuracy was achieved by splitting the tablets in half before dispersing it in water in an oral syringe. Dispersing prednisolone tablets in water could be an adequate alternative for doses outside of half or quarters. Compounded suspension was the most precise method of administering prednisolone.

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Development of array-based fluorescent nanohybrids for discrimination between different glycans

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Accurate monitoring of sugar levels is essential for many fields from food industry to human health. Array-based fluorescence sensing employs cross-identification among analytes and various sensing units to identify substances or complex systems. In our work, we have employed functionalized magnetic nanohybrids as fluorescent array-based sensors for simultaneous detection and differentiation between different sugar units. The sensing array is prepared by capitalizing on the binding affinity saccharides for boronic acids. We have proposed multiple boronic acid functionalized magnetic nanohybrids, capable of identifying different sugars and providing signals based on their interactions. By incorporating various saccharides, it regulates the properties of the single sensing unit at the molecular level, altering its interaction with the analyte. This modulation leads to the generation of multiple distinct detection signals for the target, effectively facilitating the goal of array sensing. This approach streamlines the design and construction of the array sensor, while simultaneously enhancing detection efficiency. This research will provide a novel approach that simplifies the construction of array sensors and simultaneously furnishes a potent tool for diagnosing diseases resulting from sugar imbalance.

APC24-7 Covalently Combines Boronic Acid and Chelator Moieties to Restore β-lactam Efficacy Against Metallo- β-lactamase-Producers

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Introduction
β-lactam/β-lactamase inhibitor combinations have significantly improved treatment outcomes for infections caused by serine β-lactamase (SBL)-producing pathogens. However, the continued emergence and spread of metallo-β-lactamases (MBLs), for which no clinically approved inhibitors currently exist, poses a serious threat to the long-term effectiveness of β-lactam-based therapies. To overcome this therapeutic gap, the boronic acid transition state analogue taniborbactam (Venatorx Pharmaceuticals) was developed, targeting both SBLs and widespread MBLs such as NDM-1 and VIM-2. However, taniborbactam-escape variants have been detected among various MBL enzymes, including members of the NDM- and IMP families.

Aim
We aimed to investigate APC24-7 which covalently combines a Zn^{2+} chelator moiety and the boronic acid structural moiety found in taniborbactam to explore the covalent linking of inhibitory moieties with complementary modes of action, as a proposed strategy in novel inhibitor design.

Method
Clinical isolates of SBL- and MBL-producing *Escherichia coli* and *Klebsiella pneumoniae* were used to determine the minimum inhibitory concentration (MIC) of meropenem (MEM) in combination with APC24-7 or taniborbactam (TAN). Isogenic *E. coli* strains were used to allow study of single β-lactamases and determination of MICs of MEM-APC24-7 and MEM-TAN as well as checkerboards for relevant taniborbactam-escape variants. The effects on MICs were also studied in an excess of exogenous Zn^{2+}, to determine the contribution of chelation.

Results
Compound APC24-7 successfully sensitized clinical isolates of SBL- and MBL-producing *E. coli* and *K. pneumoniae* to meropenem. While APC24-7 demonstrated similarities in inhibitor behavior to taniborbactam against a wide range of isogenic *E. coli* expressing single SBLs, it showed the ability to also affect MBL-producing strains expressing taniborbactam-escape variants NDM-9 or IMP-26.

Conclusion
Compound APC24-7 represents a hybrid inhibitor with a proposed complementary mode of inhibition which exemplifies how covalently combining complementary inhibitor motifs, such as chelators and boronic acid transition state analogues, can produce hybrid SBL- and MBL inhibitors with a broadened inhibitory spectrum.

Acknowledgement
The authors would like to thank The Research Council of Norway (project number 333270), Eurostars part of Eureka network (project number 336720), the Centre for new antibacterial strategies (CANS) at UiT, the Northern Norway Regional Health Authority (HNF1722-24) and NordicPop (project number 85352) for their financial support.

Designing Smart Active Colloids for Future Drug Delivery and Imaging Systems

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Introduction

Targeting nanoparticles to malignant tissues for improved diagnosis and therapy is a widely explored strategy [1]. However, biological barriers significantly limit delivery efficiency. In fact, studies report that only a median of 0.7% of the administered nanoparticle dose reaches solid tumors, leading to unintended accumulation in non-target tissues [2]. To overcome this challenge, *active colloids* have emerged as promising tools due to their ability to be remotely activated and precisely controlled [3]. These smart particles hold the potential to navigate biological obstacles and enhance targeted delivery. Designing such intelligent active colloids for both diagnostic and therapeutic purposes could substantially improve delivery efficiency, reducing required dosages, side effects, and associated costs.

Aim

Designing smart systems that can navigate biological barriers for diagnosis and therapy, by being remotely controlled and engineered to target only the desired area.

Method

TPM (3-trimethoxysilyl propyl methacrylate)-Au Janus particles were fabricated by coating TPM colloids with gold using ion sputtering and detaching them via sonication. Particle sizes ranged from ≈0.9 to ≈2.6 µm. Hematite cubes were synthesized via a sol–gel method and then coated with hydrolyzed TPM to form Fe_2O_3/TPM composite particles (≈2.4 µm). This involved ammonia-catalyzed polymerization of TPM followed by heating with AIBN. Gold coating was subsequently applied using the same sputtering technique as for TPM-Au particles. These composite particles serve as building blocks for microswimmers. Microscopy was performed using an Olympus IX73 with a 60× oil objective and xiQ camera, capturing bright-field and fluorescence images at 5 fps. Particle tracking used a standard algorithm and ImageJ. A dual-control system combined photoactuation (UV/blue/green LED) and magnetic steering via orthogonally mounted Helmholtz coils, controlled by a wave generator, enabling dynamic manipulation of active colloids.

Results

The resulting microswimmer functions similarly to a vehicle: its speed is controlled by light intensity, forward and backward motion by switching UV light, emitted light color by frequency, and rotation by a magnetic field. This multi-responsive control strategy offers a promising platform for future micro/nanorobotic applications in targeted delivery and remote sensing.

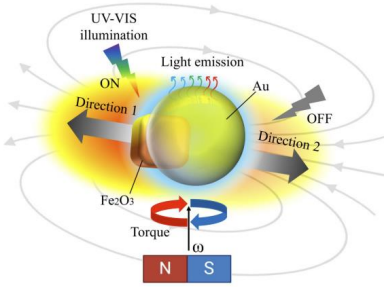

Fig. 1 Schematic illustration of the microswimmer functionalities

Formulation and Pharmacological Validation of Next-Generation Fluorescent Lipid Nanoparticles (LNPs) for mRNA Delivery

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Despite the clinical effectiveness of recently approve mRNA vaccines, current research is focused on optimizing the biodistribution of LNP formulations and the potential for targeted delivery to specific cell types. The promise of *in-vivo* CAR-T cell therapy, for instance, relies on understanding biodistribution and pharmacokinetic properties of mRNA-LNPs [1]. Further, challenges remain in optimising inefficiencies in LNP-mediated cytosolic delivery of mRNA. Frequently cited figures suggest as little as 2% of the total mRNA taken up by cells is translated to functional protein [2]. Addressing these inefficiencies and continued development of targeted LNPs could allow for significant dose reductions; limiting toxicities and improving translational potential of mRNA therapeutics and vaccines.

In this work, we investigated a novel approach to fluorescent labelling of mRNA-LNPs. Fluorescent base analogues (FBA) are a novel class of fluorescent triphosphate molecules, offering an advantage over existing fluorescently labelled RNA as they more closely model in-situ mRNA biochemistry [3]. A recent study also found that mRNA LNPs co-loaded with triphosphates were able to deliver functional mRNA more effectively than traditional LNP counterparts [4].

We hypothesised FBA-mRNA-LNPs could offer physiologically relevant fluorescent based tracking of LNPs and potentially improve transfection effectiveness (Fig 1). In this work, we use screening and optimisation approaches to develop lead formulation candidates. We characterise these formulations compared to a current gold-standard' LNP formulation (*Pfizer*) using advanced analytical techniques. We also assess the transfection efficiency and cytotoxicity to determine pharmacological activity and safety and understand the biophysical and chemical interactions precluding protein translation. Overall, this research contributes to the development of the next generation of effective fluorescent LNPs for mRNA delivery with improved safety, physiological relevance and utility.

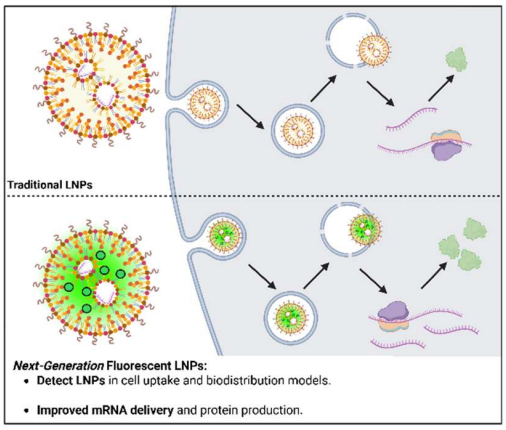

Figure 1: *Next-Generation* Fluorescent LNPs design principle and strategy.

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Evaluating Hemicellulose–API compatibility using melting point depression

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Introduction
Hemicelluloses are branched heteropolysaccharides and represent the second most abundant biomass component after cellulose [1]. Their shorter polymer chains, compared to cellulose, result in favourable properties such as enhanced aqueous solubility and stabilization capacity, making them attractive candidates for amorphous solid dispersions (ASDs) [2]. Despite these advantages, hemicelluloses remain underexplored in pharmaceutical applications. To understand if a polymer works as ASD carrier it should be sufficiently miscible with active pharmaceutical ingredients API [3]. One widely used indicator of such miscibility is the depression of the melting point (T_m) of the API in the presence of a polymer.

Aim
To examine the thermal behaviour of two hemicellulose-based polymers—one derived from spruce (SH) and the other from oat (OH)—to evaluate their potential as carriers in ASDs with model APIs.

Method
Polymer-API mixtures were prepared with different hemicellulose matrices, containing API concentrations ranging from 50% to 95% by weight. Differential scanning calorimetry (DSC) was used to measure melting point (T_m) depression and glass transition temperatures (T_g). The weight ratios were transformed to volume ratios using densities determined with pycnometer. The thermal data, combined with Flory–Huggins theory, were then used to estimate the free energy of mixing and to construct phase diagrams for each polymer–API system.

Results
SH exhibited a high glass transition temperature (T_g) and did not induce Tm depression in two of the APIs, indicating immiscibility. In contrast, T_m depression was observed with a third API possessing a significantly higher Tm, suggesting improved miscibility with SH. Conversely, OH consistently depressed the Tm of all model APIs, indicating greater compatibility across the systems. The Flory–Huggins interaction parameters further suggested that the ASDs remain in a single-phase region only near the solubility temperature across all compositions. Consequently, at room temperature, hemicellulose-based systems are expected to accommodate only limited amounts of API.

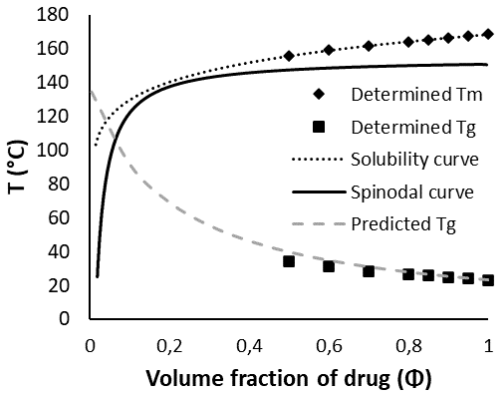

Figure 1 *Phase diagram predicted for arabinoxylan – paracetamol system.*

Conclusion
This study demonstrate that both hemicelluloses can form stable ASDs when paired with suitable APIs. Furthermore, the results underscore the importance of the relative thermal properties of the components: for the Tm depression method to indicate miscibility, the Tm of the API should exceed the Tg of the polymer.

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Biocompatible Stabiliser for Liposomes

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Introduction
Polyethylene glycol (PEG) is commonly employed in pharmaceuticals to boost stability and therapeutic performance. The addition of PEGylated molecules into drug delivery systems such as liposomes, lipid nanoparticles, and polymer nanoparticles offers several advantages, including improved drug solubility, greater stability, extended circulation time, and enhanced targeting capability. While PEGylated compounds have attracted considerable interest and widespread use, there is also increasing evidence pointing to the immunogenicity of PEG.[1] Our project aims to introduce a more biocompatible stabiliser as an alternative to PEGylated stabilisers used in drug delivery platforms, by utilising host-guest chemistry to stabilise liposomes. Supramolecular host molecules generally have hydrophobic cavities and hydrophilic exteriors and are available in a wide array of different cavity volumes and geometries. This allows encapsulation of diverse guest molecules, forming stable host-guest complexes with varied structures and properties.[2,3]

Aim
To explore a supramolecular approach for stabilising liposomes.

Method
We synthesised a series of novel functionalised lipids with a guest molecule attached. The guest acts as the binding site for the supramolecular host. These functionalised lipids, with a guest molecule tethered, were synthesised via a two-step edc/nhs coupling. They were then used to fabricate liposomes stabilised with host. A range of liposomes were formulated with varied amounts of the newly functionalised lipid, DSPC and cholesterol.

Results
By varying functionalised lipid/cholesterol/helper lipid ratios and other process parameters we were able to obtain liposomes with average particle size of 102 nm, with a polydispersity index (PDI) below 0.3, indicating high uniformity, this was characterised using dynamic light scattering (DLS). Control liposomes formulated without host showed significant aggregation, with average particle size > 300 nm and PDI > 0.4.

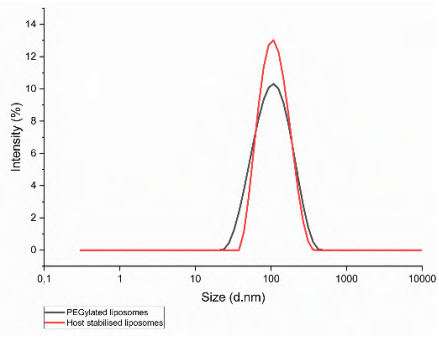

Figure 1 *Dynamic light scattering (DLS) results, showing Z-average (nm) comparison between host stabilised liposomes (red) and PEGylated liposomes (black)*

Conclusion
The functionalised lipid was used to formulate a range of host stabilised liposomes, which showed comparable size and PDI to that of analogous PEGylated liposomes. On the other hand, the control liposomes formulated with no host stabiliser aggregated rapidly. We now aim to further optimise the liposome formulation, load cargo and carry out drug release and *in vitro* toxicity assays.

Acknowledgement
The author would like to thank Kristina Stenborgs Stiftelse and Vetenskapsrådet Grant for financial support

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Polymeric microspheres with both high drug loading and controlled drug release

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Introduction

High drug-loaded polymeric microspheres hold promise in biomedical fields due to reduced excipients administration, minimized side effects, and enhanced therapeutical efficacy[1, 2]. Although thermodynamic factors like drug-carrier material compatibility are well-known to influence the drug loading capacity of microspheres[3], they fail to explain the huge difference in drug loading degree observed for polymers and drugs with similar interactions. Here, based on the droplet microfluidic platform, we investigated the single droplet solidification process. The results indicated that amorphous polymers can hinder drug diffusion during droplet solidification compared to crystal polymers, resulting in a higher drug loading degree. Overall, our results offer insights into the impact of polymer crystallization on droplet solidification kinetics, which consequently affecting the drug loading capacity.

Aim

To investigate the influence of the kinetics factor on the droplet solidification and drug loading process using microfluidics.

Method

The drug loading capability of commonly used biocompatible polymers was measured and corresponding thermodynamic interactions were calculated. Moreover, the solidification process was observed through polarizing microscope to explore the crystallization performance of polymers. To further demonstrate the effectiveness of the kinetics factor to improve the drug loading capacity of crystalline polymers, amorphous random co-polymer poly(caprolactone-co-L-lactide) (PCL-PLLA) was selected as a control against crystalline polymers polycaprolactone (PCL) and poly(L-lactide) (PLLA).

Results

The results indicated that amorphous polymers can hinder drug diffusion during droplet solidification compared to crystal polymers, resulting in a higher drug loading degree. Next, we applied this principle to improve the drug loading capability of crystal polymers (PCL and PLLA) by random co-polymerization (PCL-PLLA), achieving 6.2–22.2 times increased drug loading degree.

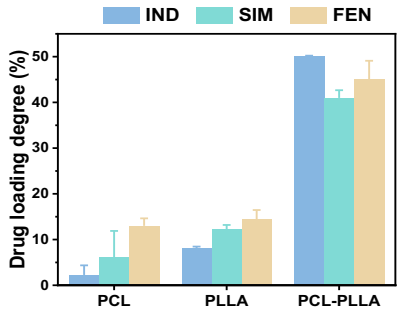

Figure 1 *Drug loading degree of drug loaded PCL, PLLA, and PCL-PLLA microspheres.*

Conclusion

It could be shown that the amorphous polymers offer greater potential for achieving high drug loading with reduced drug molecules diffusion from droplets.

Acknowledgement

The authors would like to thank the Research Council of Finland, Finnish Cultural Foundation and Chinese Scholarship Council for financial support.

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Customized hydroxypropyl methylcellulose-based solid foams loaded with poorly soluble drugs by tunable modular design

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Introduction
Carvedilol, which is indicated for the treatment of high blood pressure, is often self-administered by patients with frequent dose manipulations[1]. This can easily result in patients not taking the correct dose. One potential solution to this challenge is to fabricate personalized oral dosage forms by inkjet printing the carvedilol-containing ink onto designed edible porous substrates[2]. Freeze-dried hydroxypropyl methylcellulose (HPMC)-based porous foams with open-cell structure have been considered as the most favorable substrate for inkjet printing pharmaceuticals due to superior mechanical and ink absorption characteristics[2].

Aim
To propose a roadmap for developing bespoke HPMC-based drug products using the tunable modular design (TMD) approach to enable flexible doses and tailored release profiles of poorly soluble APIs like CAR.

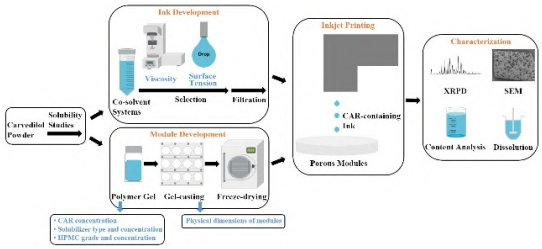
Method


Figure 1. A diagram of the research study flow and methodology. CAR = carvedilol; HPMC = hydroxypropyl methylcellulose; XRPD = X-ray powder diffraction; SEM = scanning electron microscopy.

Results
The limitation of poor CAR solubility was overcome by designing pharmaceutically approved co-solvent systems. This approach ensured printable inks of a high drug content, and sturdy and flexible modules with uniform distribution of CAR to achieve effective and accurate doses of CAR. The tailored release rate of CAR from TMD products was succeeded by varying the composition, particularly, the content and grade of HPMC, and physical dimensions of modules.

Conclusion
The TMD approach holds potential for designing bespoke high-quality products, containing hydrophilic cellulose ethers such as HPMC and poorly water-soluble APIs.

Acknowledgement
Wuzhong He acknowledges the China Scholarship Council (202207940015) and CurifyLabs (Helsinki, Finland) for financial support. The PhD student Ilari Ahola is thanked for lab assistance. The team of Primera Technology is thanked for technical assistance with their inkjet printer.

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Rheological and drug release properties of low-solid content cellulose nanofiber hydrogel

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Introduction

Controlling drug release is a central theme in pharmaceutical research[1]. Hydrogels have been widely studied in the field of controlled drug release due to their good biocompatibility, superior adsorption properties and porous structure[2,3]. As a flexible drug-release trigger for advanced drug delivery systems, light can be used to trigger spatially and temporally controlled release from for example hydrogel-incorporated liposomes[4]. Here, we explored a new low-density hydrogel for the storage and release of active cargo.

Aim

To investigate polysaccharide-based double-network hydrogels (DN hydrogels) composed of TEMPO-oxidized cellulose nanofibrils (TCNF) and mixed-linkage glucan (MLG) with low solid content as a novel drug delivery system.

Method

Different concentrations of TCNF and MLG were mixed to optimize the low solid content hydrogel formulation, and rheological measurement and scanning electron microscopy (SEM) imaging were used to characterize the mechanical properties and morphology. In order to evaluate drug release, 4 drug molecules with different charge and molecular weight, including 4 kDa and 40 kDa FITC-DEX, ketoprofen (KETO), and nadolol (NAD), were selected as model compounds. Drug-loaded hydrogels were incubated in HEPES buffer at 37°C under constant shaking. Samples were collected at different time points and measured using high performance liquid chromatography (HPLC). Two types of liposomes were incorporated into the hydrogels to evaluate the nanoparticle release.

Results

Spontaneous hydrogel formation resulting from intrinsic interactions between MLG (0.2% w/w) and TCNF (0.2% w/w) occurred, exhibiting viscoelastic solid-like behavior. SEM imaging revealed the highly porous structure of freeze-dried DN hydrogels. Drug release studies revealed size- and charge-dependent release kinetics. Small molecule (4 kDa FITC-DEX) or negatively charged molecule (ketoprofen) showed rapid release (>95% in 6–8 h), while larger molecule (40 kDa FITC-DEX) or positively charged molecule (nadolol) exhibited slower release (~70% in 24 h). Liposomes were strongly retained within the hydrogel over 10 days.

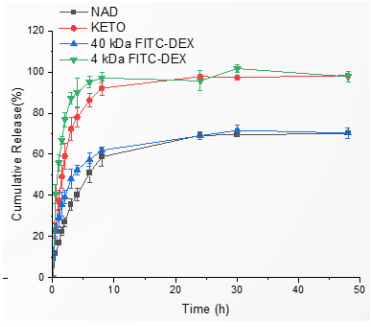

Figure 1 *Cumulative release profiles of NAD, KETO, 40 kDa FITC-DEX and 4 kDa FITC-DEX.*

Conclusion

TCNF and MLG showed efficient gelation at low solid contents (0.4% w/w). The DN hydrogels exhibited great potential for the incorporation of drug cargo and light-sensitive liposomes.

Acknowledgement

The author would like to thank the Research Council of Finland for financial support (Grant No. 360110).

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Work package 3: Engineer

Pharmaceutical manufacturing and processing has been identified as a rate-limiting step in translating new and innovative product ideas into new medicinal products. A key in enabling modern pharmaceutical production will be the implementation of additive manufacturing and continuous manufacturing principles: continuous manufacturing also leads to accelerated production processes, flexibility of scale, smaller facilities with lower costs, lower consumption of energy and materials, lower amount of waste production and improved process reliability and flexibility.

In the WP3, we focus on both traditional powder based processing and additive manufacturing preferably in a continuous mode using 2D/3D printing,

Invited speaker talk:
Daniel Markl, University of Strathclyde, Glasgow, UK.
A Self-driving Drug Product DataFactory for Accelerated and Sustainable Development
Daniel Markl is a Professor in Pharmaceutical Product Engineering at the University of Strathclyde and Associate Director at CMAC. His research aims to develop cyber-physical and predictive systems and associated, innovative methods for drug product development and manufacturing that accelerate the pace at which new medicines are developed and delivered across important therapeutic areas. He is Training Director of the Centre of Doctoral Training in Cyberphysical Systems for Medicines Development and Manufacturing and leads the new UK Centre of Excellence in Regulatory Science and Innovation (CERSI) for the digital transformation of medicines development and manufacturing.

Selected talks:
Petteri Parkkila, Chalmers University of Technology, Sweden
Unveiling structure and biomarker heterogeneity of single extracellular vesicles using waveguide scattering microscopy
Xuedan Sun, University of Copenhagen, Denmark
Sustainable electrospun protein nanofibers for wound healing
Yuming Zhang, Uppsala University, Sweden
A magnetic heating triggered SPION-wax capsule for colon drug delivery with ultrasound imaging assistance

A Self-driving Drug Product DataFactory for Accelerated and Sustainable Development

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Abstract

Traditional methods of developing drug products for a new active pharmaceutical ingredient are time-consuming, costly and often inflexible. The selection of the right excipients in tablets and process conditions are crucially important as they can impact manufacturability, performance and stability of the drug product. Formulation optimisation studies are conducted to identify a robust formulation that can meet manufacturability criteria (e.g. flowability, tensile strength) while fulfilling the desired performance targets, e.g. release of > 80% of the drug in less than 30 min. This is a multidimensional optimisation problem with a high degree of interdependence between raw material attributes, process parameters, and drug product properties. These complex relationships cannot be fully captured by first principle models and it is not feasible, in a reasonable time, to experimentally optimise these multidimensional formulation (type of excipient, concentration, drug loading) and process parameter (e.g. compression force, dwell time) spaces following traditional experimental planning and methods. This talk will present a self-driving, data-intensive micro-scale tablet formulation development system – the Tableting DataFactory - that can automatically dose and prepare desired blends, and produce and test single tablets. By employing robots, the system combines an automated multi-dosing and blending unit, a dedicated powder transportation unit, near-infrared spectroscopy for evaluating powder blend homogeneity, a compaction simulator, and an automated testing system for measuring tablet properties. The data is automatically structured and fed into a database for the development of a hybrid system of models, including mechanistic and data-driven (AI) approaches, to predict critical powder blend (e.g. flowability) and tablet attributes (tensile strength, porosity) from raw material properties. This talk will further discuss the combination of hybrid modelling approaches with model-based optimisation and the micro-scale tablet development system. This approach substantially reduces hands-on-lab time (> 80%), material, and waste, offering significant potential for accelerated and sustainable drug product development.

Key papers:

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Unveiling structure and biomarker heterogeneity of single extracellular vesicles using waveguide scattering microscopy

Petteri Parkkila[1], **Mattias Sjöberg**[1,2], **Erik Olsén**[1], **Björn Agnarsson**[1], **Ulla Impola**[3], **Kai Härkönen**[3], **Petra Ilvonen**[3], **Saara Laitinen**[3], **Fredrik Höök**[1]

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Introduction

Deciphering EV heterogeneity is essential for understanding their biological functions and enhancing their use in drug development and medical diagnostics. Assessment of sample purity in terms of various physicochemical parameters is needed to advance the use of EVs from lab to clinic. Here, we present label-free waveguide scattering microscopy (WGSM), coupled with simultaneous fluorescence readout, for multiparametric EV profiling.

Aim

To disseminate EV heterogeneity in terms of size, refractive index, structure, biomarkers, and presence of lipoproteins.

Method

The WGSM setup combines an upright microscope with a planar waveguide chip for time-resolved surface-sensitive nanoparticle imaging. Our surface functionalization approach consists of capturing with cholesterol self-insertion into the EV membrane. The optical contrast between blood-derived EVs and the surrounding medium is varied using membrane-permeable and -impermeable solutes, allowing to resolve EV size and refractive index (RI), distinguish between vesicles and non-vesicular particles using the glycerol-available volume, and calculate the concentration of biomolecules in the EV lumen. In addition, dual-color time-resolved staining against CD markers is performed.

Results

RIs for platelet EVs (PEVs) and red blood cell (RBC) EVs were similar (1.41) but RI distribution was wider for PEVs, and the presence of low-RI subpopulations (RI <1.38) was found for both EV types. Concentration of cargo biomolecules was high (∼0.4 g/mL) for both EV types. Monitoring time-resolved antibody binding allowed to calculate number of CD markers per EV and rates of antibody binding to individual EVs. Median numbers of CD63s per PEV (∼70) are lower than number of CD9s (100), and wide distribution in binding rates for anti-CD63 suggest heterogeneity in antibody-CD63 protein interaction. Fractions of CD9-positive and CD63-positive PEVs were measured as 48% and 23%, respectively.[1]

Conclusions

WGSM provides low sample consumption (100 µL at a concentration of $\sim 1 * 10^{10}$ particles/mL) and moderate measurement times (60 to 120 minutes) for analysis of a few thousand EVs in a single experiment. Therefore, we see potential for the method to aid in assay standardization, liquid biopsy analysis, and other tasks that benefit from multiparametric EV assays beyond size and surface marker profiling.

Acknowledgement

We acknowledge Knut and Alice Wallenberg Foundation, # 2019-0577, Swedish Research Council, #2018-04900, The Research Fund of the Finnish Red Cross Blood Service, NordForsk Nordic University Hub project Nordic POP (#8 5352) for funding the project.

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Sustainable electrospun protein nanofibers for wound healing

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Introduction

Electrospun fibers attracted a great interest in diverse applications due to their high surface area, porous structure, and controlled drug release capabilities. Protein-based nanofibers, like the ones based on α-lactalbumin (ALA) and soy protein isolate (SPI), demonstrate excellent biocompatibility and degradability. ALA nanofibers have shown high tunability and enhanced wound-healing effect[1,2], while SPI nanofibers exhibit distinct aqueous stability[3]. Combining different proteins presents a promising strategy for developing eco-friendly, multifunctional electrospun materials with tailored properties.

Aim

To rationally design a green, eco-friendly, multifunctional electrospun material that can act as a protective barrier and promote tissue regeneration for wound healing.

Method

With polyethylene oxide (PEO) employed as an assisting polymer, nanofibers were produced by electrospinning aqueous solutions of ALA/SPI/PEO. The resulting samples were characterized using various techniques. Scanning electron microscopy (SEM) was employed to examine fiber morphology. The stability of the nanofibers was assessed in aqueous and *ex vivo* tissue environments. Dynamic mechanical analysis (DMA) was performed to evaluate the mechanical properties of the fibers and protein release was quantified using the bicinchoninic acid (BCA) assay. Finally, an *in vivo* study was conducted to investigate the wound healing efficacy of the nanofibers.

Results

1) Various nanofibers with different content of ALA, SPI, and PEO can be fabricated by electrospinning with high reproducibility using water as the sole solvent. 2) Nanofibers with tunable water resistance and mechanical properties were achieved by precise modulating ALA, SPI, PEO content. 3) ALA and SPI displayed different release behavior from nanofibers. 4) ALA/SPI/PEO nanofibers demonstrated wound healing effects on rat models.

Conclusions

We provide a highly tunable and eco-friendly platform for designing and producing functional nanofibers, with a protein content of up to 90% (w/w), through electrospinning of aqueous solutions containing ALA and SPI. The ALA/SPI/PEO nanofibers, with adjustable morphology, hydrophobicity, and mechanical properties have potential as wound-healing materials.

Acknowledgement

We acknowledge financial support from the VILLUM FONDEN and Novo Nordisk Foundation

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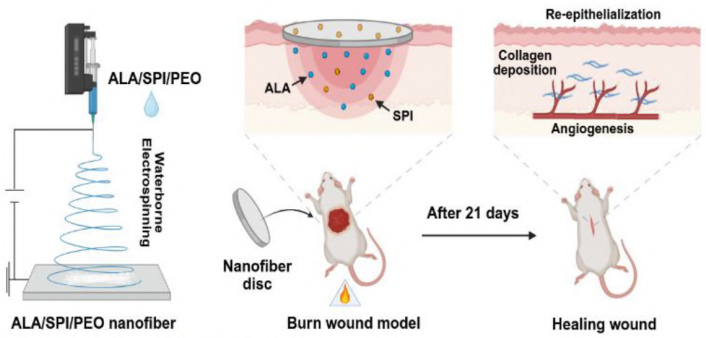

Figure 1 *Graphic abstract*

A magnetic heating triggered SPION-wax capsule for colon drug delivery with ultrasound imaging assistance

Yuming Zhang[1,2], Kai Zhang[1], Krisztina Juriga-Tóthy[1], Christina Paraskeva[3], Peter Thelin Schmidt[4], Vasiliki Koliaraki[3], Alexandra Teleki[1,2]

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Introduction

Targeted drug delivery to the colon remains a significant challenge. To address this, we developed an oral capsule designed for site-specific drug release in the colon, utilizing magnetic field and ultrasound guidance. The capsule comprises superparamagnetic iron oxide nanoparticles (SPIONs) embedded in a low-melting-point wax matrix. Upon exposure to an alternating magnetic field (AMF), the capsule melts, triggering a burst release. This non-invasive system enables controlled release, precise localization, and minimizes off-target effects.

Aim

To develop a SPION-wax capsule for oral colon drug delivery that achieve on-demand release and can be visualized by ultrasound to facilitate precise drug release at the disease site.

Method

Capsules were fabricated using a 3D-printed mold to shape the SPION-wax mixture into hemispheres. A Design of Experiments (DoE) approach optimized SPION concentration and capsule thickness to achieve efficient heating performance and mechanical strength exceeding 2 N, ensuring rapid and precise drug release while withstanding peristalsis. Cytotoxicity was evaluated via the extraction method. Digestion studies in simulated gastrointestinal (GIT) fluids assessed capsule stability and integrity. AMF-triggered capsule melting was tested under a magnetic field strength of 14 mT at 590.6 kHz. Capsule melting and localization were visualized *in vivo* in mice using endoscopy and ultrasound, respectively.

Results

SPION/wax capsules were successfully fabricated with reproducible quality (mass variability: standard deviation <10% across 20 capsules). DoE optimization mapped capsule thicknesses ranging from 0.3 to 1.5 mm at a fixed SPION concentration of 10 wt%, meeting the required criteria. Cytotoxicity studies showed no toxicity following overnight incubation at 37°C with colorectal cancer cell lines (Caco-2, SW480). Digestion studies confirmed capsule stability in GIT fluids, with no drug release detected after 72 hours of sequential incubation. Under AMF exposure, capsules melted and released their payload within 30 seconds. *In vivo* studies demonstrated successful capsule melting under AMF in the mouse colon, and ultrasound effectively detected capsule localization.

Conclusions

We developed an oral capsule for targeted colon drug delivery that enables ultrasound detection and AMF-triggered drug release. This system holds promise as a non-invasive local colon drug delivery platform

Acknowledgement

The authors would like to express gratefully gratitude for the financial sup-port from the Science for Life Laboratory and European Research Council

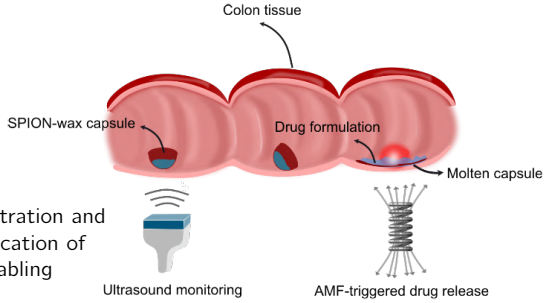

Figure 1 Schematic illustration of the SPION-wax capsule designed for oral administration and targeted delivery to the colon. Ultrasound imaging is used to monitor the precise location of the capsule, while an alternating magnetic field (AMF) triggers capsule melting, enabling controlled payload release at the desired site.

WP3 Posters

Abstract ID	Presentation Day	Name
82	Wed	Aleksandra Słaba
83	Thurs	Ehsanollah Moradi
84	Wed	Oddny Björgvinsdottir
85	Thurs	Yao Huang

Phospholipids show a plasticizing effect on polymer-based amorphous solid dispersions (ASDs) containing celecoxib: a DSC-study into glass-transition

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Phospholipids are amphiphilic molecules with favorable physicochemical properties, making them versatile as functional excipients e.g. as solubilizers, wetting agents, emulsifiers, and structural components within drug delivery systems [1]. We have recently suggested the use of hydrogenated phosphatidylcholine for amorphous solid dispersions with poorly water-soluble drugs [2] and demonstrated their processability during hot melt-extrusion [3]. Still, there remains a lack of systematic studies examining their role as plasticizers. This study aims to comprehensively assess the plasticizing effect of various phospholipids on solid dispersion to support their use in the hot-melt extrusion.

The plasticizing effect of three phospholipid blends with varying amounts of hydrogenated phosphatidylcholine (Phospholipon P90H; P90H, Lipoid P75-3; P75-3, egg yolk phosphatidylcholine; E80), BCS type II drug (celecoxib), and polymers (polyvinylpyrrolidone-vinylacetate, Kollidon VA64, hydroxypropyl methylcellulose acetate succinate, HPMCAS) was evaluated by differential scanning calorimetry (DSC). The physical mixtures at selected ratios were prepared by ball milling using glass beads in Eppendorf vials and transferred to pierced aluminum pans. The DSC program consisted of three heating and cooling runs from 20 °C to 180 °C with a constant rate of 10 °C/min. Samples were held at the endpoints for 2 minutes to ensure equilibration. Subsequently, thermograms of the third heating runs were analyzed.

DSC analysis confirmed that all tested phospholipids (E80, P90H, P75-3) exhibited a plasticizing effect in both CXB-PVPVA and CXB-HPMCAS matrices, as evidenced by a progressive decrease in glass transition temperature (Tg) with increasing phospholipid content (0.5–10%). The most pronounced Tg reduction was observed for 5% P75-3 in both polymer systems. The most consistent and linear Tg decrease with increasing phospholipid concentration was seen for E80 in the CXB-HPMCAS matrix. This trend was consistent across all formulations studied.

Phospholipids act as effective plasticizers in both PVPVA64- and HPMCAS-based amorphous formulations by significantly lowering the glass transition temperature (Tg). Due to their generally recognized as safe FDA status, they offer a promising alternative to traditional plasticizers used in hot-melt extrusion.

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Proteomic Profiling of Extracellular Vesicles Derived From Primary Human Hepatocytes with a Focus on Drug Metabolism Biomarkers

Ehsanollah Moradi[1], Päivi Järvinen[1], Jaakko Teppo[1], and Tiina Sikanen[1]
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Introduction

Extracellular vesicles (EVs) are membranous structures, delimited by a lipid bilayer that are secreted into body fluids via various cell types in normal and pathological conditions. EVs carry cellular cargo (proteins, noncoding RNA, mRNA, and lipids), which is representative of the individual's physiological condition and facilitate the transfer of these bioactive molecules, influencing various biological processes like immune responses, tissue repair, and cell growth [1,2]. Proteomic and transcriptomic profiling of EVs derived from primary hepatocytes in vitro has revealed the presence of various proteins and RNA species related to absorption, distribution, metabolism and excretion (ADME) [3].

Aim

To study the potential of the primary human hepatocytes-derived EVs for Drug Metabolizing Enzymes (DME) biomarker applications.

Method

In this study, we have cultured PHHs in 3D spheroid format and have isolated EVs with the help of commercial ExoEasy kit from the media in different days of the culture. Control samples included EVs from Day 4 of Huh7 and HepG2 3D spheroid cultures, and Day 11 of human induced pluripotent stem cell-derived cardiomyocytes differentiation cultures. The proteomic profling of EV samples was conducted to comprehensively evaluate their protein cargo. EV protein samples were purifed, digested to peptides, labelled with Tandem Mass Tag (TMT) reagents and fnally analyzed with Orbitrap LC-MS/MS for detection and quantifcation of the proteins (Figure 1).

Results

Comparative analysis with Vesiclepedia Top 100 EV proteins confirmed successful EV enrichment, with over 70% of abundant EV proteins present in the samples. A focused analysis identified 30 DME proteins, revealed increased abundance in phase I metabolism proteins in PHH-derived EVs. Gene Ontology analysis using the KEGG pathway tool highlighted significant enrichment in drug metabolism and cytochrome P450 pathways in PHH-derived EVs, underscoring their potential for studying drug metabolism. The presence of cytochrome P450 enzymes is crucial for assessing drug metabolic fate.

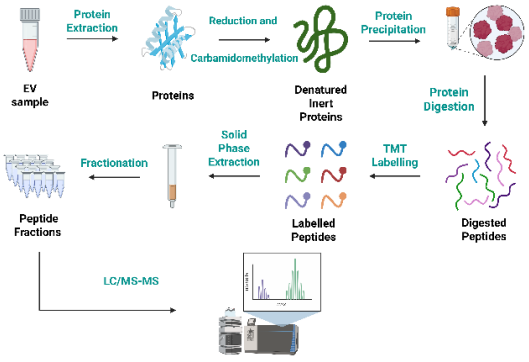

Figure 1 *Proteomic profling of the EV samples. A schematic overview of the sample preparation for LC-MS/MS-based proteomics.*

Conclusion

The ability of PHH-derived EVs to refect the metabolic activity of the parent cells positions them as a promising tool for non-invasive monitoring of liver function in vitro. Moreover, the identifcation of specifc metabolic pathways and enzymes within EVs opens avenues for their use in personalized medicine, where EVs could serve as indicators of individual metabolic capacity and drug response.

Acknowledgement

The author thanks the Research council of Finland, ERC and Finnish Cultural Foundation for their support.

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Fiber-elastomer anchoring through direct electrospinning

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[2]Faculty of Medicine, University of Iceland, Iceland
[3]Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark
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Introduction
Proper adhesion of electrospun materials to elastomers is critical for various tissue engineering applications such as skin, vascular and neural regeneration[12]. Additionally, it is an important step in the integration of fibers to medical devices or their surfaces. The combination of these two materials usually serves as a scaffold with a functional bearing, or as a reinforcement of the elastomer[2]. Most adhesion methods involve additional chemicals that may be toxic in vivo. Here, the objective is to develop an adhesion method without adverse chemical processes that physically binds the fibers to the elastomer during the electrospinning process.

Aim
To create an adhesion method that binds electrospun fibers to an elastomer.

Method
The elastomer was spin coated and cured at 60°C for 1 h. When fully cured, a metal layer was sputter coated onto the elastomer and another layer spin coated on top. Then a polycaprolactone electrospinning solution was spun onto the elastomer until it rose above the elastomeric surface (Figure 1). After electrospinning the elastomer was cured at room temperature for 24 h. The mechanical properties of the resulting materials were tested and compared to elastomer only. Both silicone elastomer and polydimethylsiloxane (PDMS) elastomers were tested.

Results
An adhesion method that combines fibers with silicone elastomer or PDMS was established. Sputter coating was a necessary step to create enough voltage difference to drive the spinning process. After increasing the conductivity of the elastomer, polycaprolactone fibers could be directly spun onto an uncured elastomeric layer, resulting in mechanical bonding of the two materials. Comparison of the mechanical properties showed increased stiffness of the fibrous material compared to the elastomer alone.

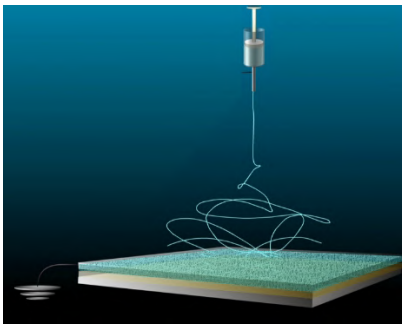

Figure 1 *Schematic drawing of fiber anchoring to an elastomer via direct electrospinning.*

Conclusion
An adhesion method was developed that mechanically anchors electrospun fibers to an elastomeric film. Tensile testing of the combined material showed increased stiffness.

Acknowledgement
The author would like to thank the "Stiftung PROPTER HOMINES - Vaduz / Fürstentum Liechtenstein", the "Schwyzer-Winiker Stiftung" and the ETH Zurich Foundation for financial support.

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Work package 4: Analytics

Precise, sensitive, and selective (molecular) analysis is the basis for monitoring of the quality of pharmaceutical products. To ensure safety and efficacy of drug products, a variety of in-process and end product analyses as well as in vitro and in vivo analysis of drug compounds and their biological and chemical degradation products is needed. With the promise of improved throughput, microfluidics and miniaturized sample preparation and separation devices are emerging to supplement the well-established chromatographic separaration systems spectroscopic techniques.

WP4 aims to provide both new analytical methods (using established instrumentation) and novel analytical tools (new instrumentation) ensure high quality drug products and to provide us with new insights into relevant complex processes and molecular interactions including but not limited to membrane transport, formulation pathways, and production line.

Invited speaker talk:
Christian Janfelt, University of Copenhagen, Denmark.
Seeing the drug – pharmaceutical applications of mass spectrometry imaging by DESI-MSI and MALDI-MSI
Christian Janfelt is an associate professor at the Department of Pharmacy, University of Copenhagen. He obtained his Ph.D. from the Department of Chemistry, University of Copenhagen, in 2008. Since 2010, he has been dedicated to the development and application of mass spectrometry imaging (MSI) through the DESI-MSI and MALDI-MSI techniques. He is the author of more than 65 publications on MSI. In his lab, these two MSI technologies have been extensively utilized in numerous studies covering various fields, including pharmacology, drug delivery, drug metabolism, plant biology, biotechnology, and cancer research.

Selected talks:
Melissa Hendrén, University of Helsinki, Finland
Development and Validation of Imaging Methods for Intracellular Drug Delivery of Oligonucleotide-Based Drugs
Jakob Tobias Lynnerup, University of Southern Denmark, Denmark
Untangling "Dissolved" Drug Species from Various Formulations of a Poorly Soluble Drug: Sampling Methods, Mechanistic insight, and IVIVC
Caroline Lööf, Chalmers University of Technology, Sweden
Investigating the chemical signature of prostate cancer using mass spectrometry

Seeing the drug – pharmaceutical applications of mass spectrometry imaging by DESI-MSI and MALDI-MSI

Christian Janfelt[1]

[1] Department of Pharmacy, University of Copenhagen

Abstract

Mass spectrometry imaging (MSI) provides compound-specific images of hundreds of compounds in one single experiment without any use of labeling by fluorescence or radioactivity. Drugs and metabolites are easily distinguished and can be imaged relative to endogenous compounds, e.g. lipids, which may serve as biomarkers of different tissue types for histological classification. Also, in some cases the pharmacodynamic response to drug treatment can be observed in images of endogenous compounds.

The presentation will give an introduction to MSI by DESI (Desorption Electrospray Ionization) and MALDI (Matrix-assisted Laser Desorption Ionization), and the possibilities for quantitative MSI will be discussed along with instrumental developments in the field.

To illustrate the broad applicability of MSI in the pharmaceutical sciences, the presentation will feature examples including imaging of drugs and metabolites in whole-body mouse sections, vaccine delivery to the lungs, drug penetration through the skin, and comparison of different administration routes within a single animal.

Finally, the potential role of MSI in future cancer treatment strategies will also be highlighted.

Key papers:

[1] Granborg, Jonatan Riber, Anne Mette Handler, and Christian Janfelt. Mass spectrometry imaging in drug distribution and drug metabolism studies–Principles, applications and perspectives. TrAC Trends in Analytical Chemistry 146 (2022): 116482.

[2] Jacobsen, S. C., Speth, N. R., Xiong, M., Herth, M. M., Kristensen, J. L., Palner, M., & Janfelt, C. (2021). Desorption Electrospray Ionization Mass Spectrometry Imaging of Cimbi-36, a 5-HT 2A Receptor Agonist, with Direct Comparison to Autoradiography and Positron Emission Tomography. Molecular Imaging and Biology, 1-10.

[3] Morineau, L., Jacobsen, S. C., Kleberg, K., Hansen, H. S., & Janfelt, C. (2018). Delivery of amitriptyline by intravenous and intraperitoneal administration compared in the same animal by whole-body mass spectrometry imaging of a stable isotope labelled drug substance in mice. Expert Opinion on Drug Delivery, 15(12), 1157-1163.

Development and Validation of Imaging Methods for Intracellular Drug Delivery of Oligonucleotide-Based Drugs

Melissa Hendrén[1], **Elina Vuorimaa-Laukkanen**[2], **Shiqi Wang**[1],

[1] Division of Pharmaceutical Chemistry and Technology, University of Helsinki, Finland

[2] Tampere University, Finland

Introduction

Currently, less than 2% of administered oligonucleotide-based drugs encapsulated in lipid nanoparticles (LNPs) reach the cytoplasm[1]. This makes the delivery of the oligonucleotides for translation and transcription highly inefficient. One of the main bottlenecks contributing to the inefficient delivery is endosomal escape. Here, we propose a new method to detect endosomal escape, by utilizing fluorescent lifetime imaging. By labeling the payload, we can combine the methods of lifetime imaging and protein markers to obtain a better understanding of how and when the payload escapes the endosome.

Aim

To develop and validate the use of fluorescent lifetime imaging to detect endosomal escape events.

Method

Plasmid DNA (pDNA) was used as the model payload and labeled with FITC via covalent binding to the oligonucleotides. pDNA–FITC was then encapsulated in LNPs and transfected to the cells. HEK293 cell line was used, which had been modified to continuously express mCherry–Galectin9 fusion protein (mCherryGAL9) for endosomal rupture detection[2]. The main contribution for a lifetime change for pDNA–FITC is expected to be a pH change from endosomal 5.5 to cytoplasm 7.4. Therefore, also time-correlated single photon counting (TCSPC) measurements of pDNA–FITC in pH buffers (pH 5.5–7.4) were measured to compare TCSPC results with the cell fluorescent lifetime imaging (FLIM).

Results

Imaging of HEK293-mCherryGAL9 after pDNA-FITC:LNP transfection, shows an increase of high intensity spots of mCherryGAL9, indicating endosomal rupture. At the one-hour timepoint, the mono-exponential fitting was more appropriate than multi-exponential fitting of the FLIM decay, indicating that the majority of the pDNA-FITC are fully protonated in cytosolic pH. From FLIM data we can see a clear shift in lifetime over one hour, indicating a change in the particle environment (Fig 1).

Conclusion

Initial FLIM results from the cell model imaging indicate that there is clear increase in the lifetime of the pDNA–FITC:LNP molecules over time during internalization. Based on the supporting measurements from TCSPC, we can suggest that the increase in lifetime could be due to endosomal escape of the pDNA–FITC:LNP particle.

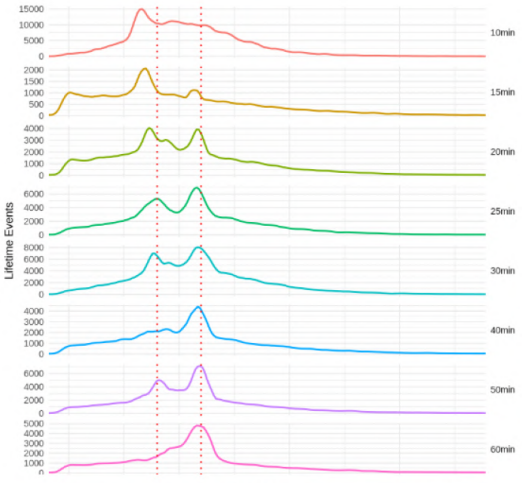

Figure 1 Fast FLIM lifetime of pDNA-FITC:LNP over one hour.

Acknowledgement

The author would like to thank the Doctoral Education Pilot in Precision Cancer Medicine Program iCANDOC for funding the research.

References:

[1] Gilleron et al., Nat Biotechnol 31, 638–646 (2013).

[2] Munson et al., Commun Biol 4, 211 (2021).

Untangling "Dissolved" Drug Species from Various Formulations of a Poorly Soluble Drug: Sampling Methods, Mechanistic insight, and IVIVC

Jakob Tobias Lynnerup[1,2], **Tim Lillotte**[2], **Maximilian Feldmueller**[3], **Johanna Anlahr**[4], **Annette Bauer-Brandl**[1], **Martin Brandl**[1], **Uwe Münster**[4], **Maximilian Karl**[4]

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Introduction

As many new drug compounds exhibit poor aqueous solubilities and poor dissolution characteristics, enabling formulations are of interest to overcome these challenges for oral drug delivery. There is growing evidence that it is imperative to differentiate between apparently and molecularly dissolved states to assess formulation performance in vitro.[1] Microdialysis and nanofiltration recently allowed to distinguish between colloid-associated and molecularly dissolved drug states, and that this can improve the in vivo prediction of oral absorption.[2–4]

Aim

Our study aimed to both provide comprehensive mechanistic enlightenment of the dissolution, supersaturation, and precipitation behavior of different formulations using various sampling approaches, such as 2nd derivative in situ UV spectroscopy, bench-top centrifugation, micro- and nanofiltration, and microdialysis. Additionally, the predictive abilities of the various sampling methods were evaluated by in vitro in vivo correlations (IVIVC).

Methods

A non-sink, single-vessel, two-stage, biomimetic dissolution setup was employed to compare the sampling methods using a diverse set of formulations and dose strengths of emodepside; conventional crystalline tablets (5, 20 mg), polymeric amorphous solid dispersion (ASD) tablets with either HPMCAS or PVPVA (5, 15 mg), and a 1 mg mL^{-1} PEG-based solution (5, 20 mg).

Results

The sampling methods captured various sizes of drug-containing colloidal species based on their size-excluding properties. Observations indicate that microdialysis captures the molecularly dissolved drug fraction, whereas 0.02 µm filtration additionally captures small drug-associated colloids (polymer-bound, small micelles). Both bench-top centrifugation and 0.45 µm filtration capture larger drug-containing species (mixed micelles, drug-rich sub-micron particles). In situ 2nd derivative UV spectroscopy substantially overestimated the "dissolved" drug concentration of the 20 mg PEG-based oral solution, possibly due to high turbidity caused by the formation of sub-micron particles. This formulation was underestimated by all sampling approaches in the IVIVC, due to early onset of precipitation. Excluding this formulation from the IVIVC, the formulation ranking correlated with the size-excluding properties of the sampling methods from best to worst; microdialysis (R^2 0.90), nanofiltration (R^2 0.79), bench-top centrifugation (R^2 0.60), filtration (R^2 0.38), 2nd derivative UV spectroscopy (R^2 0.29).

Conclusion

It was demonstrated that assessing enabling formulations using bench-top centrifugation, 2nd derivative UV spectroscopy, filtration, and nanofiltration obviously capture various sizes of colloid-associated drug states as dissolved, whereas microdialysis is, based on recently published results,[2–4] assumed to report exclusively the molecularly dissolved drug concentrations. The IVIVC was improved by evaluating the molecularly dissolved drug concentration whereas inclusion of various colloid-associated drug species led to poor differentiation of enabling formulations forming sub-micron particles.

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Investigating the chemical signature of prostate cancer using mass spectrometry

Caroline Lööf[1], Auraya Manaprasertsak[2], Kenneth J. Pienta[3], Jmma U. Hammarlund[2], Per Malmberg[1]

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[2] Tissue Development and Evolution (TiDE) Group, Department of Experimental Medical Science, Lund University, Lund, Sweden

[3] The Cancer Ecology Center, Brady Urological Institute, Johns Hopkins School of Medicine, Baltimore, MD, USA

Introduction

Prostate cancer is the second most common solid-organ cancer in men worldwide, and there is a critical need for more sensitive and specific diagnostics to enable early detection and individualized treatment.[1] Cancer cells rely on metabolic reprogramming to enhance their proliferative activity, altering biochemical processes and tissue morphology.[2] While most cancer biomarker discovery efforts focus on omics approaches targeting genes, proteins, and metabolites, these methods face challenges such as biological variability and limited reproducibility.[3] To overcome these challenges, our research explores the potential of stable isotope analysis as an alternative strategy for cancer detection. The relative abundance of stable isotopes reflects underlying metabolic fluxes and the preferential incorporation of lighter or heavier isotopes in cancer cells to meet the energy demands of altered biochemical pathways, potentially offering a more sensitive and mechanistically insightful chemical signature of malignancy.[4]

Aim

We investigate variations in the relative abundance of isotopes between cancerous and healthy prostate tissue, with the goal of identifying patterns linked to altered metabolic fluxes in cancer.

Method

Prostate tissue samples were obtained via the Institutional Review Board (IRB) approved Hopkins School of Medicine protocol. Cryosectioning was performed and the sections were analyzed using Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS).

Results

Mass spectrometry data were analyzed using principal component analysis (PCA), which revealed a distinct separation between the data groups for cancerous and healthy prostate tissue, indicating differences in chemical profile or ionization. Key contributors to this separation included cholesterol and phosphatidylcholine fragments. Further analysis of cholesterol fragments showed altered isotopic patterns in tumor tissue, with the fractional abundance of ^{13}C-containing peaks either increased or decreased relative to healthy tissue.

Conclusions

Our findings suggest measurable chemical and isotopic differences between cancerous and healthy prostate tissue. Ongoing work using Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS) will enable higher resolution analysis of isotopic fine structures, offering deeper insight into isotopic patterns in prostate cancer.

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WP4 Posters

Abstract ID	Presentation Day	Name
86	Wed	Dennis Thorsen Thisted
87	Thurs	Elina Harju
88	Wed	Helena Hamzehpour
89	Thurs	Helga Helgadóttir
90	Wed	Marta Miotke-Wasilczyk
91	Thurs	Philipp Hans
92	Wed	Tapani Viitala
93	Thurs	Ziyang Mao
94	Wed	Karin Korelc

Characterization of recrystallization behavior of amorphous materials via multimodal nonlinear microscopy

Elina A. Harju[1], Jani Ikonen[1], Niklas G. Johansson[1,2], Teemu Tomberg[1,2], Anneleen van Rijckeghem[1], Leo Zabihian[1], Lea Wurr[1], Jukka Saarinen[1], Bert van Veen[3], Clare J. Strachan[1]

[1]Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland
[2]Department of Chemistry, Faculty of Science, University of Helsinki, Helsinki, Finland
[3]Orion Corporation, Espoo, Finland

Introduction
The study of crystallization processes is important in pharmaceutical sciences, and developing better analytical methods to image them could improve our understanding of these phenomena. Lactose, a widely used pharmaceutical excipient, is a particularly interesting model for investigating crystallisation behaviour due to its ability to exist in two anomeric forms (α and β). Amorphous lactose can contain varying ratios of these anomers and recrystallize into forms composed of α, β, or both within the same crystal lattice.

Aim
To investigate the use of multimodal nonlinear microscopy in imaging the recrystallization of amorphous materials, using lactose as a model system.

Method
Building on our previous work, where SRS was shown capable of differentiating between five crystalline forms and an amorphous lactose form[1], we applied the method to study recrystallization of amorphous lactose (spray-dried/milled) at different relative humidities. SRS provides chemically and solid-state specific imaging based on vibrational spectra, whereas SFG is specific for non-centrosymmetric crystalline structures, and as such efficiently differentiates between crystalline and amorphous lactose. Recrystallization was followed either *ex situ*, after storage over saturated salt solutions, or *in situ* using a humidity-controlled imaging chamber. Additional characterization techniques, including X-ray powder diffraction (XRPD), were used to support the microscopy findings.

Results
SFG microscopy enabled detection of early-stage crystallinity at levels far below the XRPD detection limit and allowed analysis of crystallization kinetics. SRS, in turn, allowed identification of the recrystallized forms and visualization of their distribution.

Conclusion
Multimodal nonlinear microscopy is a powerful tool for characterization of the recrystallization behaviour of amorphous materials.

Acknowledgement
The project has been financially supported by Tandem Industry Academia Professor funding from the Finnish Research Impact Foundation (decision number 392), the Research Council of Finland (decision numbers 331837 and 327732) and HiLIFE, University of Helsinki. The Quantitative Chemically-Specific Imaging Infrastructure for Material and Life Sciences (qCSI) and HiLIFE LifeSpec infrastructure, University of Helsinki, was utilised in this research.

References
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Precision in dermal Pharmacokinetics: Analyzing systemic drug distribution in multi-layered skin tissue

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[2]Integrated Bioanalysis, Clinical Pharmacology and Safety Sciences, AstraZeneca, Gothenburg, Sweden.

Introduction
Skin diseases represent a significant global health burden, often requiring systemic treatment for severe conditions. While topical administration has been extensively studied, the inverse penetration of systemically administered drugs into the skin is less understood. To improve our knowledge of drug distribution and pharmacokinetics (PK) in the skin following systemic administration, we are developing advanced analytical workflows suitable for the complex multi-layer structure of the skin. These methods enable us to obtain more precise data to support the development of physiologically-based pharmacokinetic (PBPK) models, predicting drug behavior in skin tissues.

Aim
This work aimed to develop an analytical workflow to quantify drug concentrations and describe PK of systemically administered drugs in murine skin tissue samples and isolated epidermis and dermis.

Methods
Diphenhydramine, fluconazole, metoprolol and sulfamethoxazole were administered intravenously to C57BL/6N female mice (1 mg/kg each). Plasma and skin tissue samples (back and tail) were collected at multiple time points, ranging from 5 min to 2 hours post-injection. Back skin tissue samples were subsequently incubated at 56 °C for 4 min prior to a 30 s rest to separate the skin tissue layers. Both unseparated and separated back skin tissues were stained using hematoxylin-eosin (H&E) to evaluate morphological integrity and identify the layer of separation. After tissue homogenization the drugs were extracted from the matrix. Drug levels in skin and plasma were quantified by using liquid chromatography coupled to tandem mass spectrometry. PK parameters for plasma and back skin samples were calculated using a non-compartmental and a one-compartment model with 1st order kinetics, respectively.

Results
H&E staining revealed that the back skin tissue samples contained not only the dermis and epidermis but also the panniculus carnosus muscle layer (PC) and adipose tissue. A heat-assisted tissue separation method was successfully established, allowing reproducible isolation of the skin layers, epidermis and dermis, from the underlying compartments. The protocol for drug extraction from the skin homogenate was optimized. All drugs were quantified in plasma, back and tail skin tissue. The hydrophilic compounds showed higher t_{max} and c_{max} in back skin tissue compared to the hydrophobic compounds. Higher drug levels were observed in the PC compared to epidermis and dermis. Tail skin tissue results were likely influenced by its dual role as the administration and sampling site.

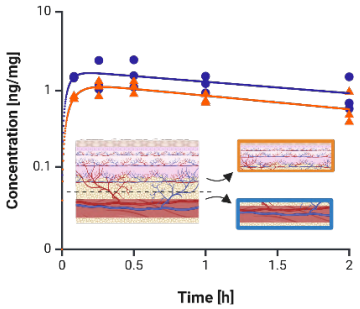

Figure 1 Kinetic drug profiles for fluconazole in different parts of back skin tissue (created in Biorender.com).

Conclusion
Sampling technique as well as sample structure and composition are critical factors to consider when interpreting bioanalytical results and should also be taken into account alongside to gender and species differences in PK studies and PBPK model development.

Acknowledgement
This work was supported by AstraZeneca and the Nordic POP foundation.

Optimization and Validation of a UPLC-QDa Method for the Simultaneous Quantification of Tazarotene and Tazarotenic Acid in Porcine Skin

Helena Hamzehpour[1], Kristófer H. Hauksson[1], Helgi Jónsson[2], Sveinbjorn Gizurarson[1] and Bergthora S. Snorradottir[1*]

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[2]Faculty of Medicine, University of Iceland, Reykjavik, Iceland

Introduction

Genetic studies linking the retinoic acid pathway to hand osteoarthritis (HOA) have sparked interest in retinoids as potential therapeutic agents for HOA.[1] Tazarotene, a third-generation retinoid, shows promise as a transdermal treatment.[2] To assess its suitability, a reliable method is required to quantify both tazarotene and its active metabolite, tazarotenic acid, in biological samples.

Aim

To develop and validate a robust UPLC-QDa method for the simultaneous detection and quantification of tazarotene and tazarotenic acid in porcine skin samples.

Method

In vitro permeation studies were performed using unjacketed Franz diffusion cells with porcine skin. Method optimization was carried out using the design of experiment (DoE) approach. Central composite orthogonal (CCO) design was used to optimize variables affecting retention time, peak area, peak resolution, and peak width. The method validation followed FDA guidelines and included ketoconazole as the internal standard (IS).

Results

A DoE screening revealed the ideal mobile phases for chromatographic separation, acetonitrile and 0.1% formic acid with 5 mM ammonium acetate. The remaining factors were optimized using CCO design, and the final method parameters included 1.6 min gradient steepness, 70% organic solvent, 0.5 mL/min flow rate, 35 °C column temperature, and 1.2 kV capillary voltage and a cone voltage of 15 V. These parameters resulted in a resolution greater than 1.5 between tazarotene and tazarotenic acid, with clear chromatographic separation from the internal standard (Figure 1).

The method was validated for selectivity, sensitivity, accuracy, precision, matrix effect, dilution integrity and stability. No interfering peaks were observed. Linearity was confirmed from 0.4–18,750 ng/mL for tazarotene and 13.32–12,500 ng/mL for tazarotenic acid ($r^2 \geq 0.99$). The method met precision and accuracy criteria (<15%) and showed analyte stability for up to 7 days at -20 °C.

Conclusion

This validated method provides a reliable and accurate approach for the simultaneous analysis of tazarotene and tazarotenic acid, facilitating further exploration of their therapeutic potential in treating HOA.

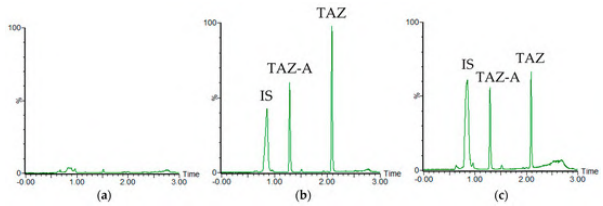

Figure 1. *UPLC-QDa chromatograms of porcine skin samples. (a) blank; (b) skin sample with tazarotene (TAZ) and tazarotenic acid (TAZ-A) spiked with the IS; (c) blank spiked with tazarotene, tazarotenic acid, and the IS.*

Acknowledgement

The authors would like to thank the Technology Development Fund Iceland for financial support (Grant No. 2215525-0611).

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Relationship Between Plasma and Saliva Concentrations of Acetylsalicylic Acid for Therapeutic Drug Monitoring

Anna Helga Kristjánsdóttir[1], Arndís Sue Ching Löve[1], Helga Helgadóttir[1*]
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Introduction
Therapeutic drug monitoring typically relies on the analysis of plasma samples, which is an invasive method for patients and therefore not always feasible. Saliva offers a non-invasive alternative matrix, but its utility depends on establishing a consistent and predictable correlation with plasma concentrations. Acetylsalicylic acid is rapidly metabolized to the active metabolite salicylic acid. This study explored whether the saliva-to-plasma concentration ratio of salicylic acid could support the use of saliva as a reliable matrix for therapeutic drug monitoring following administration of acetylsalicylic acid.

Method
A high-performance liquid chromatography method with ultraviolet detection (HPLC-UV) was adapted for the quantification of salicylic acid in plasma and saliva. The HPLC-UV method was validated for linearity, accuracy and precision, specificity, and limit of quantification.
A clinical study was conducted with nine healthy adult volunteers, each receiving a 500 mg oral dose of acetylsalicylic acid. Plasma and stimulated saliva samples were collected at three points: baseline (pre-dose), 1-hour, and 2-hour post-dose. Sample preparation was performed using filtration and protein precipitation followed by HPLC-UV analysis.

Results
The method validation met the set criteria and sensitivity of the method was adequate for the analysis of salicylic acid in plasma and saliva samples.
Salicylic acid was detectable in both plasma and saliva after acetylsalicylic acid administration. Peak concentrations were observed at 1-hour post-dose. Plasma salicylic acid levels remained relatively stable across time points, while salivary concentrations showed greater variability over time and between individuals. Saliva/plasma ratios ranged from 5–17%, and correlations between matrices were weak and non-significant. Contributing factors to the lack of correlation may be related to the sample preparation, stimulated saliva collection, and matrix-specific variability.

Conclusion
While salivary salicylic acid is detectable following acetylsalicylic acid administration, the variability observed in saliva limits its current reliability for therapeutic drug monitoring. Further studies are needed to identify the factors affecting salivary variability and to establish robust correlations between saliva and plasma concentrations for clinical application.

Optimizing a Photoreactive Hyaluronic Acid Platform for Transdermal Drug Delivery: A Spectroscopic Analysis of Azobenzene-Drug-Environment Interactions

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Optimising and improving transdermal drug delivery systems is a key objective in the treatment of skin diseases. One promising solution is to provide local therapy on demand in response to a selected stimulus, which increases the bioavailability of the drug. However, it is necessary to optimise the exposure time while maintaining the highest possible delivery efficiency. The goal of the project is to develop a photoreactive platform based on hyaluronic acid (HA) hydrogel, a polymer well suited for skin applications due to its ability to moisturise the skin and facilitate penetration[1]. The mechanism of action of the platform is based on the incorporating azobenzene photoswitches, which undergo reversible *trans-cis* isomerisation when exposed to light. This geometric change is designed to disrupt specific and non-specific interactions (such as hydrogen bonds and dipole-dipole interactions), which potentially bind the drug molecule to the photoswitch or matrix[2]. This disruption provides a mechanism for controlled, light-triggered drug release[3]. To optimise this system, a basic spectroscopic analysis was performed to understand how three key factors influence the photoisomerization process: the molecular structure of azobenzene derivatives (different functional groups), the molecular structure of selected drug molecules with specific transdermal activity, and the local microenvironment (solvent *vs*. polymer matrix).

Two series of azobenzene derivatives were prepared: a series of 4-hydroxyazobenzene derviatives with various substituents with electron-donating and electron-accepting properties (-CH_3, -CHO, -COOH, -$N(CH_3)_2$, -NO_2) and a series with PEG linkers (to study potential improvement in water solubility and to investigate the effect of chain length on photoisomerization). For model drugs, different substances were selected for the study: sulfacetamide, tofacitinib, terbinafine and dapsone. The analysis was performed using UV-Vis absorption spectroscopy and quantum mechanical calculations. To quantitatively determine the interactions between the photoswitches and the drug in a given microenvironment, stationery and time-resolved spectroscopic studies were performed. The kinetics of photoisomerization were determined by exposing samples to 365 nm radiation and analysing the behaviour of photo-switches in the presence of selected drugs.

All azobenzene derivatives showed effective photoisomerization. As expected, their photophysical properties were strongly influenced by their molecular structure. The kinetics of isomerisation were also dependent on the microenvironment, slowing down significantly in more viscous media mimicking the polymer matrix, indicating steric hindrance as a key factor. These findings provide a rational basis for selecting the most effective photoswitches and drugs to maximise the efficacy of light-triggered release.

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Integrating structure solution by deep learning in Olex2

Philipp Hans[1], Anders Støttrup Larsen[1], Anders Østergaard Madsen[1]
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Introduction
PhAI [1], [2], [3] is a recently developed deep learning approach to solve the phase problem in crystallography. In this contribution we will present our progress on the Neural Network itself and on its implementation into the popular structure solution, refinement, and analysis program Olex2 [4], [5], as an accessible GUI (graphical user interface).

Aim
The aims of this research are twofold: First, it was to solve the phase problem in crystallography, which is an essential part of analysing a crystal structure of an unknown sample, by means of a Deep Learning approach. Second, an implementation of PhAI in the Olex2 software suite was performed. Olex2 is an especially interesting platform for end-users because 75% of all new crystal structures published in the CSD database being reference Olex2 as the GUI, indicating its popularity.

Method
PhAI employs a convolutional, multilayer perceptron architecture and can interpret 3D but also 1D diffraction data. PhAI, was trained on millions of fictive structures containing metal atoms and/or molecular fragments. PhAI was subsequently validated on thousands of structures retrieved from the Cambridge Structural Database for which the structure factor amplitudes were generated at several resolution limits and fed into the trained network to output phases.

Results
Being trained on a set of millions of artificial but physically sound structures, the neural net “learned” diffraction, becoming able to interpret even diffraction patterns with low completeness or resolution (i.e. data reaching only up to approximately 2 Å or having wedges missing). PhAI can retrieve phases with a striking accuracy, leading to correct structure solutions for over 99% of the validation set entries. A prototype implementation in Olex2 will be presented.

Conclusion
Our results indicate that deep learning can be used to obtain electron density maps for structures where data could only be obtained at very limited resolution and are more than challenging to solve by state-of-the-art methods. By integrating PhAI in a GUI such as Olex2, operation without programming skills or any special system setup procedure becomes possible.

Acknowledgement
The author would like to thank the Independent Research Fund Denmark for financial support (Grant No. 3103-00222 B).

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A new unique waveguide-based instrument for single nanoparticle characterization

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Abstract
Synthetic and biological nanoparticles have high potential for targeted and personalized therapeutics. However, a thorough characterization of nanoparticle properties is mostly performed with average-assembled techniques, whereas determining NP interactions with cells and cell membranes are performed with static in vitro assays. Chalmers University of Technology have developed a unique waveguide scattering microscopy (WGSM) technique that provides single-nanoparticle-resolved information on particle size, particle size distribution, refractive index and cargo loading (Figure 1) [1-3]. WGSM also allows to monitor time-resolved NP interactions with cell membrane mimics and surrounding protein solutions. Combined with fluorescence detection the technique also provides the opportunity to simultaneously observe light emitted from fluorescently labeled compounds (e.g., proteins) adsorbed on the particles and changes in the light scattering orig inated from both synthetic and biological NPs, thus measuring Independently the signal from labelled compounds and the NPs.

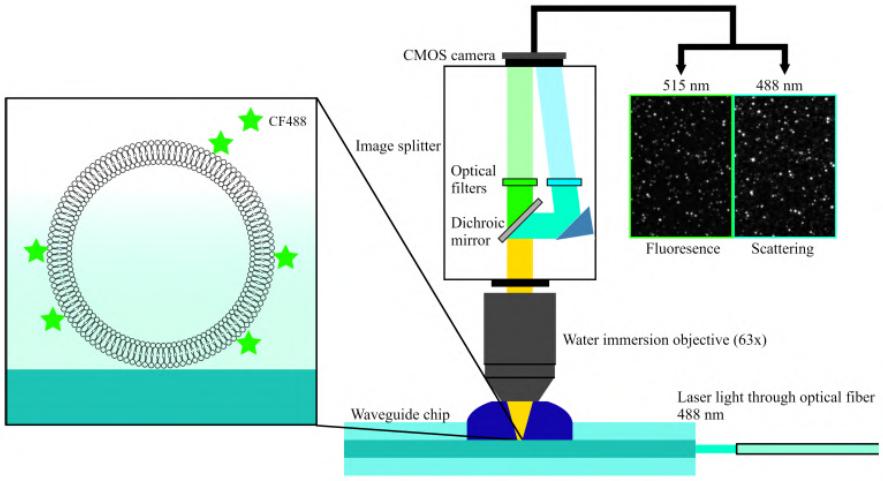

Figure 1. Schematic of the WGSM technique for simultaneous monitoring of the scattering and fluorescence signals of individual nanoparticles. The binding of fluorescently labeled molecules to the nanoparticles can be monitored both in scattering and fluorescence mode. The corresponding micrographs (approximately 20 µm wide) show the signals after protein binding [2].

The WGSM technique provides a unique opportunity for NordicPOP partners to better understand the property-functionality relationship of nanoparticle-based drug delivery systems. With the help of NordicPOP and through knowledge transfer from Chalmers the WGSM technique is now also available at Åbo Akademi University. This enables a wider use of this unique measurement capability within the NordicPOP community. Here we will present the working principle of the WGSM technique as well as a range of demonstrated applications.

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Development of a matrix-based subcutaneous tissue model for evaluating *in vitro* release of diclofenac from liquid crystalline phases

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Introduction: *In situ* forming drug delivery systems based on liquid crystalline phases (LCPs) provide sustained release of drug molecules with different physicochemical properties [1, 2]. *In situ* forming LCP-based injectables are typically administered by subcutaneous (*s.c.*) injection. Predictive *in vitro* models are critical for formulation development as animal models may fail to guide human studies because of their poor predictability of absorption in humans [3]. However, the lack of standardized methods for *in vitro* drug release testing poses a challenge in the development of sustained release injectables for *s.c.* administration. Here, an *in vitro* release testing system named IVR cartridge (*In Vitro* Release cartridge) was investigated for evaluating the release of diclofenac (DIC) from LCP-based injectables.

Aim: This study aimed to develop an *in vitro* release testing system to capture the sustained release properties of DIC from LCPs intended for subcutaneous injection.

Methods: The IVR cartridge is based on a porous based matrix (Sephadex beads) intended for size-exclusion-chromatography. The matrix confines formulations upon injection. Two *in situ* forming LCP-based formulations (F1 and F2) were prepared with a DIC loading of 18 mg/g. F1 was a medium-chain-triglyceride (MCT)-based formulation and F2 was a 1,2-dioleoyl-sn-glycero-3-phospho-rac-glycerol sodium salt (DOPG)-based formulation. A solution of 50% (v/v) human serum in 67 mM phosphate buffer, pH 7.4 was used as the release medium. The effect of the release medium flow rates (0.25, 1 and 2 mL/h) on drug release was evaluated at 37°C. Collected samples were analysed by HPLC. The nanostructures of the two LCPs were characterized by small-angle X-ray scattering (SAXS) analysis. *In vitro* release was compared to *in vivo* DIC absorption obtained by a numerical deconvolution of plasma concentration data of F1 and F2 from an *in vivo* study in rats.

Results: DIC released faster in 50% (v/v) human serum in 67 mM phosphate buffer, pH 7.4 than that in phosphate buffer. Both the F1 and F2 showed sustained DIC release by formation of LCPs depot upon injection. The increase in the flow rates of release medium accelerated the release of DIC from LCP depots. F2 exhibited a faster DIC release than F1. The different release rates from the two LCPs were attributed to the different nanostructures. SAXS analysis suggested that F1 tended to form a hexagonal phase while F2 formulations tend to form cubic phases, which have larger aqueous nanochannels than those in hexagonal phases, facilitating the drug diffusing out of the LCP depot. The *in vitro* release from F2 matched its *in vivo* absorption, while the *in vitro* release from F1 was slower than its *in vivo* absorption in rats. The cause of the *in vitro in vivo* difference for F1 remains unclear. It may be speculated to the differences between rats *s.c.* and *in vitro* conditions e.g., high lipolytic activity in rats *s.c.* tissue, limited availability of free fluid in *s.c.* interstitial fluids, and different shapes of the formed LCPs depot.

Conclusions: Modifications of release medium in the IVR cartridge can alter drug release. The IVR cartridge differentiated the sustained release of DIC from two *in situ* forming LCPs formulations. The IVR cartridge using 50% human serum as release medium did not simulate the rats *s.c.* tissue.

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The effect of substituents of HPMC on their interaction with chitosan surfaces

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[a] co-first author

Introduction

Hydroxypropyl methyl cellulose (HPMC) is one of the important cellulose derivatives. Differences in the degree of substitution and the substitution pattern can affect the release profiles from HPMC matrix tablets (Viridén et al., 2010). Chitosan is a natural cationic linear polysaccharide, widely used in biomedical and pharmaceutical applications (Cheung et al., 2015). Combining the two polysaccharides is interesting in formulation development of various dosage forms, e.g., bilayer films.

Aim

To investigate the influence of the HPMC substituent groups (60SH 4000, 65SH 4000, 90SH 4000) on interaction with chitosan using quartz crystal microbalance with dissipation monitoring(QCM-D).

Method

1% chitosan solution was prepared by dissolving chitosan (Chitinor, Norway) in 1% (v/v) acetic acid. 150 µl of chitosan solution was spin coated on a SiO_2 coated QCM-D sensor (5000 rpm, acceleration 2500 rpm/min, 60s). The spin coated sensors were immersed in 0.5M NaOH solution to neutralize and stabilize the films. 0.1% w/w HPMC solutions were prepared by dissolving HPMC (Shin-Etsu Chemical Co. Ltd, Japan) in water. The spin coated chitosan films were equilibrated in water to obtain a stable baseline. HPMC solutions were injected, monitoring the frequency shift and dissipation. After reaching the equilibrium, water was reintroduced to the flow cell to remove the loosely bound HPMC chain from the chitosan surface. The frequency response corresponded to the irreversibly attached HPMC on the surface. The adsorbed mass of HPMC on chitosan films was computed using Johannsmann's model (Johannsmann, 2008).

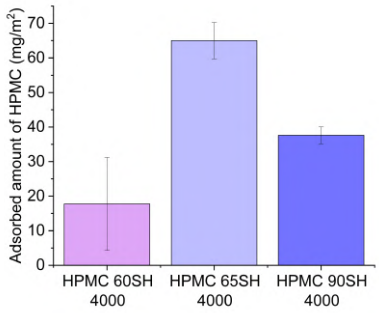

Figure 1: Amount of adsorbed HPMC with standard deviation (error bars).

Results

The injection of HPMC solutions led to decrease in frequency in all cases, indicating the adsorption of HPMC at the interface. Rinsing with water did not result in any increase in frequency for any of the HPMC grades examined, suggesting that HPMC adsorbs irreversibly onto the chitosan surface. Among the tested variants, HPMC 65SH 4000 exhibited significantly higher adsorption compared to HPMC 60SH 4000 and HPMC 90SH 4000 (Figure 1). These results clearly show that the degree and type of substituent groups affect the interaction of HPMC with the chitosan surface.

Conclusion

This study provides a comprehensive investigation into how the degree of substitution in HPMC influences its solubility and their interactions with chitosan surfaces, which can be useful for development of formulations of dosage forms where these are combined, such as bilayer films.

Acknowledgement

The authors would like to acknowledge the support from the NordForsk funded Nordic University Hub project #85352 (Nordic POP, Patient Oriented Products).

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Work package 5: Synchrotron

Innovation within design and manufacturing of complex drug delivery systems (DDS) calls for high-end imaging techniques. Of special interest is the capability of studying these systems in operando – and to study the DDS behaviour in situ, e.g. as a function of time, pressure, humidity, temperature and pH, and in both equilibrium and non-equilibrium situations, and to map out chemical composition as well as physical properties of these structures. For such studies, the large-scale facilities offer special advantages.

The high flux and hard X-rays from the synchrotron translates directly into higher spatial and temporal resolution. Neutrons offer the advantage of non-destructive and bulk analysis in combination with unique isotopic information that yields differing elemental sensitivity to that of X-rays. This WP will provide next generation solutions for the visualization of the structure of biological systems and pharmaceutical products.

Invited speaker talk:
Reidar Lund, University of Oslo, Norway
Antimicrobial Peptides and Their Mode of Action: What Can We Learn from Scattering Techniques?

Selected talks:
Lea Wurr, University of Helsinki, Finland
Investigating the behaviour of amorphous solid dispersions with stimulated Raman scattering microscopy
Matteo Guidetti, University of Southern Denmark, Denmark
Unravelling the elusive nature of crystalline Form S of posaconazole"
Inês C. B. Martins, University of Copenhagen, Denmark
Exploring amorphous diversity in pharmaceuticals using combined analytical methods with computational modelling

Antimicrobial Peptides and Their Mode of Action: What Can We Learn from Scattering Techniques?

Reidar Lund[1]

[1] Department of Chemistry, University of Oslo, Norway.

Abstract

Antimicrobial peptides (AMPs) are effective against wide range of bacteria, even resistant pathogens, due to their ability to disrupt the cell membrane. However, there is still no clear consensus on the molecular mechanism by which AMPs act. While pore formation has been suggested as a potential mechanism, there is currently insufficient experimental evidence to support this hypothesis. Furthermore, AMPs often face the challenge of enzymatic degradation, limiting their circulation in the bloodstream. This project aims to address these challenges in two ways. Firstly, we investigate the mechanism of natural AMPs by utilizing scattering methods in combination with model systems for both bacterial and mammalian membranes. Secondly, we concentrate on nanostructured peptides formed through self-assembly, which exhibit enhanced stability and considerable potential for therapeutic applications.

To investigate the mechanism, we employ scattering methods in conjunction with model systems that represent both bacterial and mammalian membranes. Small-angle X-ray and neutron scattering (SAXS/SANS) techniques enable us to analyze the structure and dynamics of lipids and peptides at the nanometer scale [1–3]. Using SAXS, we can assess the membrane insertion of AMPs and explore potential pore formation. Meanwhile, contrast variation SANS provides valuable insights into the lateral organization of membranes containing lipid nanodomains, so-called "rafts"[4]. Furthermore, time-resolved scattering methods allow us to examine lipid dynamics, including flip-flop and exchange processes[5,6], as well as ion transport across the membrane. Our results indicate that AMPs do not necessarily form well-defined pores or cause significant membrane damage; however, they effectively accelerate lipid flip-flop and facilitate the permeabilization of ions through the membrane. This finding is supported by computer simulations, which suggest that AMPs create transient water channels rather than structural pores, as previously proposed in earlier textbooks. In the final part, we will briefly explore antibiotic nanoparticles formed through self-assembly of peptide-polymer conjugates[7]. In our talk, we will discuss various microscopic mechanisms for antimicrobial peptides, their stability and therapeutic potential.

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Investigating the behaviour of amorphous solid dispersions with stimulated Raman scattering microscopy

Lea Wurr[1], Teemu Tomberg[1], Jukka Saarinen[1], Leena Peltonen[1], Robert Luxenhofer[2], Thomas Rades[3], Bert van Veen[4], Clare Strachan[1]

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[4] Pharmaceutical Sciences, Orion Corporation, Finland

Introduction

Amorphous solid dispersions (ASDs) play an important role in the formulation development of poorly water soluble drugs (most new small-molecule drugs). However, ASDs can exhibit complex phase separation behavior during the dissolution. This can drastically affect the drug release and cause problems in formulation development. To investigate the phase separation behaviour one needs to have adequate analytical techniques. Stimulated Raman scattering (SRS) microscopy is an emerging tool to characterise dynamic phase phenomena of ASDs *in situ*, allowing temporally resolved quantitative, label-free, chemically- and structurally-specific analysis[1].

Aim

To investigate the phase behaviour of ASDs as a function of drug loading and temperature, using SRS microscopy with fast spectral focusing.

Method

In situ SRS microscopy was used to characterize ASDs in the form of thin drug-polymer films with different drug-to-polymer ratios (10, 15, 20, and 30 % drug), using ritonavir (RTV) and poly(vinylpyrrolidone-co-vinyl acetate) (PVPVA). The sample was placed between two microscope cover glasses forming a thin film of an approximate thickness of 40 µm. Phosphate buffer (pH 6.8) was added between the cover glasses with a syringe and tubes. SRS spectra, covering the most characteristic Raman peaks of each component, were measured every 20 seconds at room temperature and at 37 °C. Classical least squares (CLS) analysis was used to create false colour images to visualise different chemical (drug, polymer and water) species in the sample every 20 s.

Results

The imaging showed an initial water penetration front moving into the ASD, followed by both surface-directed and bulk phase separation in the film, depending on drug loading and temperature. Films with a RTV concentration of 15 % and higher showed surface-directed phase separation, with a continuous drug rich layer forming at the matrix-buffer interface during the analysis at room temperature, but at 37 °C only the 30 % drug loaded film showed this behaviour. In addition, SRS microscopy revealed a subsequent liquid-liquid phase separation in the buffer, seen as drug rich droplets, in the samples up to 20 % drug loading.

Conclusion

Overall, SRS microscopy provides new insights into water-induced ASD phase phenomena. In this case, the influence of both drug loading and temperature on phase behaviour was revealed.

Acknowledgement

Lea Wurr thanks the Doctoral Programme in Drug Research at UH for financial support. Clare Strachan and Bert van Veen acknowledge Tandem Industry Academia Professor funding from the Finnish Research Impact Foundation (decision number 392).

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Unravelling the elusive nature of crystalline Form S of posaconazole

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[2] Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark

Introduction

Pharmaceutical compounds can exist in different crystal forms, each with unique properties affecting stability and solubility. Identification of the solid-state landscape is critical for the development of the final drug product. For posaconazole (POSZ), a broad-spectrum antifungal agent with over ten distinct crystal forms,[1] anhydrous Form I is the most thermodynamically stable form under ambient conditions. Interestingly, the commercial oral suspension contains a different form—Form S—whose nature remains unclear[2]. Form S is generated by exposing Form I to water and possibly when used as oral drug substance, however, it quickly reverts to Form I upon drying,[1,2] making its characterization challenging. In this study, we investigate the transformation of Form I to Form S under varying humidity conditions using a custom-built humidity cell and synchrotron radiation, shedding new light on the elusive Form S.

Aim

To study the solid-phase transformation of posaconazole crystal Form I at variable humidity and to identify the nature of the elusive Form S.

Method

POSZ Form I was examined via Powder X-ray diffractometry (PXRD) combined with an adjustable humidity and temperature chamber at the DanMAX beamline at the MAX-IV Synchrotron Facility in Lund, Sweden. Form I was subjected to changing relative humidity (r.h.) conditions at 25°C: starting from 25% r.h., then gradually ramping to 95% r.h., and dropping to 0%.

Results

PXRD experiments under variable humidity revealed that POSZ Form I converts to Form S only at very high humidity levels (90–95% r.h.) and reverts back to Form I as soon as humidity drops below 90% (Figure 1). Complementary Dynamic Vapor Sorption (DVS) experiments showed almost no water uptake of the crystal form up to 95% r.h.[1]

Conclusion

PXRD experiments in the humidity chamber confirmed the conversion to Form S at high relative humidity, while complementary DVS data suggests that Form S is an anhydrous polymorph of POSZ.

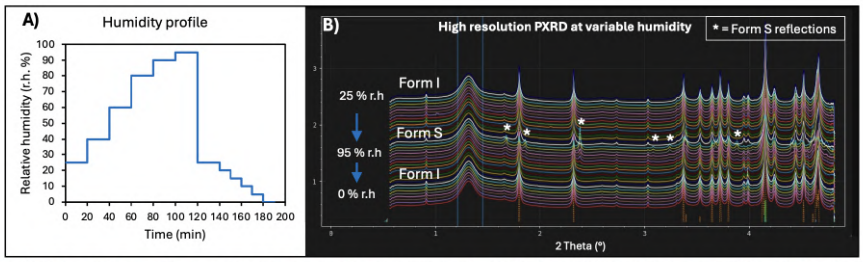

Figure 1 *A) humidity profile investigated during the measurement; B) High-resolution PXRD at variable relative humidity of posaconazole Form I. Phase transformation to Form S is observed at 90 - 95% r.h. (white stars = Form S reflections).*

Acknowledgements

The authors thank the MAX IV Laboratory for beamtime on the DanMAX beamline under proposal 20240766 and Nordic POP (Nordforsk grant number 85352). This project has received funding from the European Union's Horizon 2020 programme under the Marie Skłodowska-Curie grant agreement No 955756 InPharma. Construction of the humidity/temperature cell was supported by the Carlsberg Foundation, grant CF20-0587.

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Exploring amorphous diversity in pharmaceuticals using combined analytical methods

Inês C. B. Martins[1], **Thomas Rades**[1]
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Introduction

Most drug delivery today utilizes solid dosage forms, especially for small molecules. However, the solid state of drugs can take many forms, and increasingly high-energy solids are used, with different degrees of order, ranging from stable and metastable crystalline forms, to amorphous forms. Amorphous forms are ideal candidates to improve the solubility and thus potentially the oral bioavailability of poorly water-soluble drugs. When searching for amorphous forms, only one "amorphous state" is currently considered as a result from the screening process via different preparation methods. However, depending on the preparation conditions, it is possible that distinctly different amorphous forms with distinct molecular-level organizations as well as physicochemical properties can be isolated and even interconverted between each other in a phenomenon termed poly*A*morphism. [1-3] The occurrence of diversity among amorphous forms of the same drug is therefore a topic of great interest in the investigation of oral drug formulations.

Aim

To investigate the occurrence of amorphous *diversity* (specifically, poly*A*morphism) on the pharmaceutical compounds indomethacin, hydrochlorothiazide, valsartan and furosemide.

Method

Ball milling, spray-drying and quench-cooling were used to obtain amorphous forms of various pharmaceutical compounds. The differently prepared amorphous forms were characterized using a range of analytical methods, including X-ray total scattering, powder X-ray diffraction, differential scanning calorimetry and isothermal microcalorimetric analysis.

Results

Amorphous *diversity* (and specifically poly*A*morphism) was found in differently prepared amorphous forms of hydrochlorothiazide, valsartan and furosemide. Distinct physicochemical characteristics properties, such as the glass transition temperature (T_g), structural relaxation times, physical stability and dissolution behavior were obtained. For indomethacin, on the other hand, the reasons underlying the occurrence of differences in the T_g values as well as dissolution behavior of the corresponding amorphous forms were attributed, not to the presence of amorphous *diversity* (or poly*A*morphism), but rather to a neglected and very important aspect in the investigation of amorphous solid forms, the presence of degradation products.

Conclusion

Poly*A*morphism and amorphous diversity offer significant potential to improve drug solubility and physical stability by optimizing preparation conditions to tailor the formation of specific amorphous forms with the most desirable physicochemical properties. However, the presence of impurities, including degradation products, can also substantially impact dissolution behavior and physical stability of amorphous forms, potentially leading to misleading conclusions about poly*A*morphism.

Acknowledgements
Independent Research Fund Denmark, project 0135-00103A; Novo Nordisk Foundation, project NNF23OC0086891 and NordForsk program Nordic University Hub (project:85352).

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WP5 Posters

Abstract ID	Presentation Day	Name
95	Thurs	Dima Abu Zahu
96	Wed	Martina Olsson

Enhancing Antimicrobial Agents: Structural Study of Lipopeptides

Dima Abu Zahu[1], Paraschiva-Catalina Irazi[2], Håvard Jenssen[2,3], Josefine Eilsø Nielsen[1]

[1]Department of Pharmacy, University of Oslo, [2]Department of Science and Environment, Roskilde University and [3]Department of Chemistry, University of Oslo.

Introduction:

Antimicrobial peptides (AMPs) are natural occurring molecules found in various species and form the first line of defense in the human innate immune system (1). Despite their crucial role, AMPs face challenges such as rapid proteolytic degradation (2). To overcome this, structural modifications can be made by attaching lipid tails, resulting in lipopeptides (3). These compounds not only retain antimicrobial properties, but also exhibit the ability to self-assemble into customizable nanostructures (4), making them promising candidates for drug delivery applications. Understanding their structural organization is crucial for optimizing their functionality. In this study, small-angle X-ray scattering (SAXS) was used to characterize the morphology and self-assembly behavior of selected lipopeptides, and their antimicrobial activity was evaluated to assess their potential as novel therapeutic agents.

Methods:

The lipopeptides were synthesized using solid-phase synthesis and the antimicrobial activity was evaluated using MIC assays. SAXS was used to determine structural parameters of the selected lipopeptides, including particle size and morphology. SAXS experiments were conducted at the BM29 bioSAXS beamline at the ESRF, Grenoble. The scattering data was analyzed using models described in (5).

Results:

The SAXS experiments revealed that these lipopeptides have a propensity to self-assemble into micellar structures. Through fit analysis we showed that the morphology of these structures depends on the chemical properties of the lipopeptide, including tail length, number of aromatic groups and charges. Nanostructures were observed, including spherical, ellipsoidal and cylindrical micelles.

Antimicrobial activity studies revealed antimicrobial activity of the lipopeptides dependent on chemical properties and self-assembly (MIC down to 4 µg/ml).

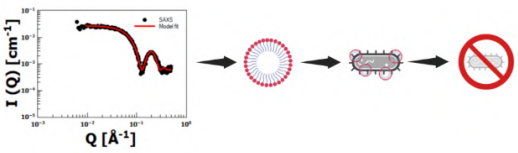

Figure 1. SAXS data of an antimicrobial lipopeptide self-assembling into a micellar structure, plotted together with best fit (red line) using models described in (5).

Conclusions:

This study provides a detailed characterization of lipopeptide self-assembly and antimicrobial activity. The SAXS data confirm their self-assembly into nanostructures, and MIC measurements highlight their potential as antimicrobial components. These findings contribute to the understanding of lipopeptide design for future biomedical applications.

Acknowledgements:

We thank the ESRF for beamtime access and the BM29 staff for technical support.

Work package 6: Modelling & Simulations

The accuracy and applicability of computational tools to model and simulate complex systems (ranging over biological systems, chemical engineering to production lines) has increased significantly during the last decade as a result of the increased computational power available to handle big data. This WP brings together a critical mass with computational competences spanning over a wide range, from classical multivariate data analysis (MVA) and molecular dynamics (MD) simulations (mechanical/quantum) over discrete element, finite element and finite volume methods to physiology-based pharmacokinetic (PBPK) modeling. Our mission is to enable mechanistic insights into complex processes (membrane transport, formulation pathways, production line), visualize molecular interactions (important for crystallization, permeation, solubilization etc.), and facilitate e.g. 3D representation, images as well as templates, for e.g. 3D cell culture production or 3D printed dosage forms.

Invited speaker talk:
Mark Coles, University of Oxford, U.K.
Combining Experimental Systems Approaches to Accelerate and De-risk Immuno-Therapeutic Development
Mark Coles is a Kennedy Professor of Immunology in the Kennedy Institute of Rheumatology and an affiliated professor in the Wolfson Centre of Mathematical Biology, University of Oxford. He is the lead for industrial strategy and entrepreneurship in the Kennedy Institute and a fellow at Reuben College. His research is focused on human first immunology combining systems based approaches with human experimental medicine to develop next generation therapeutics for immune mediated inflammatory diseases and cancer including vaccines, biologics and small molecules. Mark founded 3 SMEs focusing on delivering impact to patients through novel therapeutics, these include Mestag Therapeutics (https://www.mestagtherapeutics.com/) and Lightox Ltd (https://lightox.co.uk/) targeting autoimmune disease and cancer. Mark is the congress secretary for British Society of Immunology. (Funded by the MRC, Chan Zuckerberg Initiative and Kennedy Trust for Rheumatological Research).

Selected talks:
Xiaoxiao Liang, University of Copenhagen, Denmark
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Combining Experimental Systems Approaches to Accelerate and De-risk Immuno-Therapeutic Development

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Abstract

The development of biologic therapies transformed how cancer and immune mediated inflammatory disease are treated through modulating the cellular, humoral and cytokine networks that drive immune pathology and can stimulate efficacious immune responses. This was built on deep mechanistic understanding of immune function using animal models. Recently the development of the Human Cell Atlas (HCA) combined with advances in single cell spatial biology has provided a new tool to understand human immune function in tissues and accelerate therapeutic discovery without relying on animal models. The potent combination of AI/ML based approaches of poly-omic human datasets (including single cell and spatial biology), combined with computational modelling has the potential to accelerate and de-risk the development of a new generation of therapeutic approaches with the potential to cure human diseases. In this talk I will touch on some of the approaches we are using to model human disease using these new approaches where we have gained new and unexpected insights into disease pathways with the longer term ambition of impacting on human disease.

Unraveling Conformational Diversity and the Energy Landscape of Osimertinib Polymorphs through 3D Electron Diffraction and Computational Modeling

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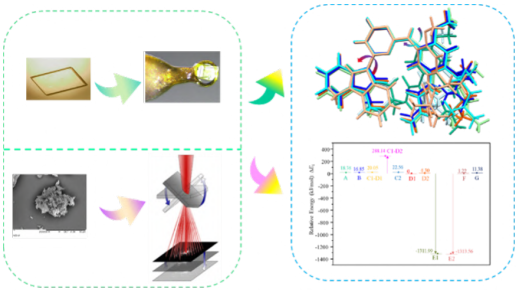
Introduction

Determining the crystal structure of drugs is a fundamental prerequisite for understanding their physicochemical properties.[1] While single crystal X-ray diffraction (SCXRD) has been widely employed to elucidate the structure of materials[2], obtaining large and suitable crystals to be analyzed by this technique, remains a challenge. This is especially true when using preparation methods such as rapid precipitation, high-temperature, or dehydration, which typically yield metastable microcrystals[3] and crystals with defects. To address this, three-dimensional electron diffraction (3D ED) is employed as a powerful tool for comprehensive structure determination of materials. Herein, we used 3D ED and SCXRD, to determine eight new solid forms of osimertinib. Structural analysis revealed that conformational variations and distinct hydrogen bond interactions resulted in packing diversity. Conformational energy calculations and Hirshfeld surface analysis highlighted the crucial role of weak intermolecular interactions in stabilizing high-energy conformations that may be kinetically trapped. Lattice energy landscape calculations provided insights into the relative thermodynamic stabilities of the polymorphs, with theoretical predictions showing a good agreement with experimental observations.

Aim

The aim of this study was to perform 3D ED and theoretical analysis on various new solid forms of osimertinib to reveal how conformational flexibility and weak interactions govern the structural diversity and stability of polymorphs.

Method



Results

We firstly identified eight solid state forms of osimertinib, including three hydrates, two polymorphs, two solvates and one salt. Among them, theoretical and experimental analysis indicated polymorph D to be the most stable form.

Conclusion

Crystal quality significantly affects structure elucidation. By combining 3D ED with computational modeling, we revealed the structural diversity and energy landscape of osimertinib polymorphs. This integrative approach bridges experimental observations with theoretical predictions, contributing to the rational design and control of crystalline pharmaceutical forms.

Acknowledgements

The author would like to thank the National Natural Science Foundation of China and the funding from Guangdong province. Electron diffraction experiments are supported by Novo Nordisk Foundation Research Infrastructure grant n. NNF220C0074439

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Integrating deep learning into Alchemical Free Energy Calculations for lead discovery and optimization

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Abstract

G protein-coupled receptors (GPCRs) are crucial drug targets. Our Alchemical Free Energy Calculations (FEP/TI) on an AlphaFold model yielded an RMSE of 2.15 kcal/mol for specialized pro-resolving modulators (SPMs), but highlighted convergence and workflow challenges. To enhance this, we plan to integrate cutting-edge generative AI. Boltz-2, a structural biology foundation model, uniquely approaches FEP accuracy for binding affinity prediction with over 1000x computational efficiency, overcoming the speed-accuracy trade-off and enabling large-scale virtual screening. Crucially, reliable predictions demand strict adherence to Alchemical Free Energy Calculation (AFEC) best practices, including meticulous system preparation, optimal alchemical pathways, efficient sampling, and rigorous data analysis for transparency and reproducibility.

WP6 Posters

Abstract ID	Presentation Day	Name
97	Thurs	Carita Sallomy
98	Wed	Daniel Haga Hasselstrøm
99	Thurs	Jingwen Chen
100	Wed	Juho Karuaho
101	Thurs	Majd Awad
102	Wed	Mikko Nikunen

Computational study of phenolic compounds with polyethersulfone (PES)

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Introduction
Phenolic compounds are metabolites found mainly in plant species with large structural diversity[1]. Polyphenols can have an important role in a new strategy to combat pathogenic microorganisms. Antibacterial phenolic compounds could be utilized in prevention of undesired formation of bacterial biofilms, which is a problem e.g. in the field of membrane technology, especially in membrane-based water treatment applications[2]. Modification of the membrane with polyphenols such as vanillin and quercetin possessing anti-biofouling potential could offer interesting possibilities to create membranes or surfaces that actively disturb the growth of the biofilms. We have performed a virtual screening (VS) study to identify potential antibacterial polyphenols[3]. VS study successfully identified four potential antibacterial compounds which interactions on polyethersulfone (PES) membrane are studied. UV grafting could be used in coating PES with polyphenols.

Aim
To investigate interactions of antibacterial phenolic compounds on PES membrane, which could help in coating anti-biofouling properties on filter membranes. Studying the mechanism and duration of the interactions is important to examine if the compounds are stable on the surface and the compounds' potential to prevent biofilm formation.

Method
Quantum mechanical calculations with Jaguar were performed to investigate the reactivity, stability, electron densities, and electrostatic potentials of polyphenolics. Molecular dynamics (MD) simulation studies of the antibacterial compounds on PES polymer surface are performed using Desmond in OPLS4 forcefield with simulation times of 100 ns.

Results
Four compounds: baicalein, piceatannol, methyl gallate, and compound 10 showed antibacterial effect against *E. coli* and *S. aureus*,[3] and were selected for MD simulations. The compound adsorption with PES polymer model is studied (Fig. 1). Preliminary studies show PES interacting with phenolics' hydroxides via hydrogen bonds.

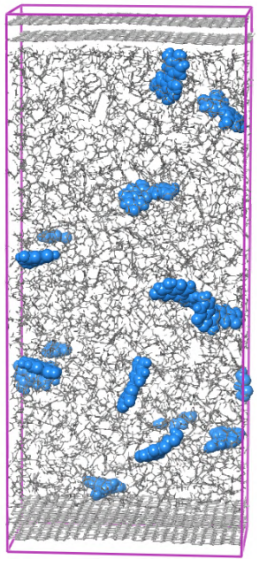

Figure 1 *Modeling compounds (blue) with PES polymer (grey).*

Conclusion
The compounds are promising for antibiofouling membrane modifications. Information from MD simulations of the compounds with PES will help to manufacture the membranes that will be coated with these phenolics.

Acknowledgement
The author would like to thank the Research Council of Finland for financial support (grant no. 339710) and CSC-IT Center of Science for the computational tools. We thank Sari Ukkonen for the microbe testing, and collaborator Jenni Korhonen from the Institute of Public Health and Clinical Nutrition, UEF.

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Development and implementation of computational free-energy protocols for lipid GPCRs in inflammation and pain

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Several lipid-activated G protein-coupled receptors (GPCRs) are central mediators of inflammation and pain, making them high-value targets in drug discovery. These receptors are activated by diverse endogenous lipid ligands, including specialized pro-resolving mediators (SPMs) and endocannabinoids. To understand ligand binding and accelerate hit optimization for these receptors, we have optimized and implemented several computational protocols combining molecular dynamics (MD) simulations with rigorous binding free energy calculations.

In one project, we focused on SPM-GPCR interactions, targeting GPR32, GPR101, and FPR2. Using AlphaFold-predicted GPCR structures refined through MD, we applied Free Energy Perturbation (FEP) methods via the NAMD software to predict absolute binding affinities for 10 SPMs at GPR32. This approach yielded an RMSE of 2.15 kcal/mol, with excellent accuracy for RvD1 (0.45 kcal/mol). We also modeled the affinity impacts of RvD1's metabolic transformation into its active epimer (AT-RvD1) and other metabolites using relative binding free energy calculations with AMBER Thermodynamic Integration (TI). Parallel work on $RvD5_{n\text{-}3\ DPA}$ and analogs, using a recently published cryo-EM structure of GPR101 revealed affinity trends supporting dual optimization strategies for binding affinity and analogs with reduced metabolic turnover. Stable analogs of the FPR2 agonist BML-111 have also been designed and modeled, synthesis is complete, and experimental validation is currently pending.

In a second project, we applied the same protocols to the endocannabinoid receptors CB1 and CB2. Starting from a weakly active anandamide analog (CB1 Ki >10 μM), TI calculations guided the discovery of two high-affinity, selective ligands: one with an impressive 0.04 nM CB1 affinity and >200-fold selectivity over CB2, and another with 24 nM CB2 affinity and 400-fold selectivity over CB1. Free energy predictions strongly correlated with experimental data across an affinity range spanning over eight orders of magnitude (CB1: RMSE = 1.97 kcal/mol, R^2 = 0.86; CB2: RMSE = 1.72 kcal/mol, R^2 = 0.61).

Together, these efforts establish a robust computational strategy for understanding lipid–GPCR interactions and accelerating ligand discovery in inflammation and pain. Future efforts will aim to integrate AI-driven techniques with physics-based free energy calculations to further improve the efficiency, scalability, and predictive power of our protocols.

Amorphous solid dispersions (ASDs) are widely used to enhance the solubility of poorly water-soluble drugs. However, their physical instability, particularly recrystallization, remains a major formulation challenge. To support early-stage screening, we developed an integrated in silico approach that combines molecular dynamics (MD) simulations with machine learning (ML) to predict ASD stability.

ML models were trained on a combination of physicochemical descriptors and a comprehensive set of MD-derived features that capture intermolecular interactions and molecular mobility. Incorporating MD features improved prediction accuracy to 85.95%, with feature importance analysis identifying hydrogen bonding and API–excipient contacts as key stability predictors. These findings highlight the value of MD-informed features in enhancing both model performance and mechanistic interpretability.

Building on this framework, we integrate coarse-grained modeling and imaging data to study phase separation in ASDs across multiple scales. Stimulated Raman scattering microscopy enables real-time visualization of dynamic processes, including water ingress, drug–excipient mixing during dissolution, and both amorphous–amorphous and liquid–liquid phase separation.

Dissipative particle dynamics simulations complement these observations by modeling mesoscale transformations over extended spatiotemporal windows. Coupling simulation and imaging enables construction of multimodal ML models that capture both molecular-level interactions and macroscale transformation patterns, providing novel validation for highly coarse-grained models.

Targeting Neurodegeneration: Molecular Dynamics Insights into PP2A-Alpha4 Protein Complexes

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Introduction

Neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD) are characterized by a progressive neuronal loss and abnormal protein accumulation[1]. These protein aggregates may be cleared via autophagy, and protein phosphatase 2A (PP2A), a serine/threonine phosphatase, has been implicated in this process by modulating autophagy and thereby influencing aggregate degradation[2]. Upregulation of PP2A has been associated with enhanced neuronal autophagy[2], while reduced PP2A activity contributes to increased protein accumulation and is linked to the pathogenesis of neurodegenerative disorders such as AD and PD[2,3].

The catalytic activity and expression of PP2A are further regulated by protein Alpha4, which protects PP2A from polyubiquitination and degradation[4]. This mechanism involves Alpha4 binding to the catalytic subunit of PP2A (PP2Ac) near the active phosphatase site, stabilizing PP2Ac in an inactive state while simultaneously sterically hindering the ubiquitination site of PP2A. However, the molecular-level details of these protein–protein interactions require further investigation. Identifying the interaction hotspots of PP2A and Alpha4 will clarify how these proteins function together as complexes. These insights could aid the development of new therapies for neurodegenerative diseases.

Aim

To elucidate the molecular-level mechanisms underlying the interaction between PP2A and Alpha4, thereby advancing the understanding of PP2A's functional role in the broader context of neurodegenerative disease pathologies.

Method

Protein–protein docking will be performed using tools such as HADDOCK[5], followed by molecular dynamics simulations with the GROMACS software suite on a high-performance computing (HPC) cluster. Additionally, various regulators and ligands will be docked to assess their impact on the structural stability and interaction characteristics of the resulting complexes.

Results

The analysis will focus on the interaction dynamics between PP2A and Alpha4, highlighting key binding features and conformational changes observed during simulations. The study will also evaluate how different ligands affect the stability and binding behaviour of the complexes.

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Effect of Novel Binding Site on Prolyl Oligopeptidase Protein Conformations and Protein-Protein Interaction Mechanisms

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Introduction

Prolyl oligopeptidase (PREP) is a proline-specific serine-type endopeptidase expressed in various tissues in mammals, including the brain.[1] In addition to its role as endopeptidase, PREP has been shown to influence many cellular processes through protein-protein interactions (PPI), such as α-synuclein aggregation as well as reducing autophagy activation and increasing reactive oxygen species via complex formation with protein phosphatase 2A.[2] These PPI-derived functions make PREP a promising drug target for neurodegenerative diseases such as Parkinson's disease. PREP inhibitors have been shown to modulate PPI-derived functions independently of the enzymatic activity inhibition and a novel alternative binding site has been presented in recent studies.[3] However, the effects of inhibitor binding at the site on the protein conformation and PPI modulation remain unclear.

Aim

This study aims to determine how inhibitor binding at the novel site affects PREP's conformational changes and to elucidate the mechanisms affecting its PPI functions.

Methods

Identification of the binding site and the docking of the novel PREP-inhibitors were conducted with tools available in Schrödinger Maestro's package.[4] The docking results served as the basis for the subsequent molecular dynamics (MD) simulations. Binding dynamics and the effects of inhibitors on the protein conformation were studied with MD simulations using the GROMACS release 2025-1.[5] Computational tasks were performed using CSC-IT Center for Science Ltd. resources.

Results

The binding dynamics showed that the lipophilic moiety of the PREP-inhibitors is stabilized in the novel binding site while the polar moiety is stabilized between TYR471 residue and the catalytic triad of the enzyme. Study of PREP conformational changes is ongoing.

Conclusions

The novel inhibitors bind to the novel binding site, and they may affect PREP conformations and thus have impact on its protein-protein interactions.

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