Design of porous lipid-silica nanocarriers for local enzyme-controlled drug administration

AIMS & OBJECTIVES

1. To analyse the intricate interplay and structure-activity relationship between immobilized lipase molecules and triglycerides within mesoporous carriers, based on variations in nanostructure and surface chemistry.
2. Develop a predictive model, based on the elucidated structure-activity relationships, that can be used to control and optimise lipase activity within nanostructured materials.
3. Utilise the developed predictive model to design and fabricate an oral drug delivery system that enhances the biopharmaceutical performance of a model poorly-water soluble drug, based on a lipase-provoked release mechanism.

PROJECT PROGRESS

Significant focus over the past 12 months have been attributed to Research task 1 & 2, which has led to a number of promising findings and insights regarding the lipase-mediated digestion process. These insights will be harnessed to derive novel silica-lipid systems for improved drug solubility.

Research task 1 – Interfacial characterization studies of the lipid-lipase interactions.

Accomplishments

The interfacial mechanisms that regulate the lipase-mediated digestion process was investigated using QCM-D in conjunction with TIRF microscopy. Specific focus was attributed to probing the role of Ca2+ ions on the lipid digestion mechanism. Through biophysical analysis, it was established that Ca2+ indirectly altered the accessibility of the lipid-in-water interface by interacting with anionic phospholipid that stabilize the interface. In doing so, this increased the surface area and bioaccessibility for lipase to adsorb and digest the lipids at the interface. The impact that Ca2+ had on the subsequent drug release during lipid digestion was studied by encapsulating the poorly water-soluble and autofluorescent drug, felodipine, into the lipid nanoparticles. It was established that Ca2+ not only controlled the release of lipid digestion products, but also controlled the subsequent release of encapsulated drug molecules. The insights derived from these studies have been essential for improving the understanding of the lipase-mediated hydrolysis process and are also integral to improving *in vitro* lipolysis techniques for lipid-based drug formulations. Additionally, we have demonstrated that TIRF microscopy can be harnessed to study lipid:drug interactions during simulated lipid digestion studies, to investigate if the drug preferentially solubilizes within the triglycerides or the digestion products – a fundamentally important consideration when formulating drugs within lipid systems. This work is currently being prepared in manuscript form and will be submitted in the first half of 2020.

Additional research task associated with Task 1

An important aspect of assessing a drugs ability to be administered via the oral route is to understand how it permeates the small intestine. Currently, there are limited *in vitro* tools that replicate this permeation process, which restricts the ability to predict how the drug, or the drug formulation, will perform *in vivo*. To overcome this, TIRF microscopy was used to deposit a supported lipid bilayer on to a

mesoporous silica thin film. By doing so, it introduced the ability to monitor drug permeation across the lipid bilayer, into the pores of the thin film and thereby quantify only the drug that was transported across the membrane. To validate this approach, felodipine was formulated with lipid vesicles that are typically found in the small intestine, with and without the conventional permeation enhancer, caprylate. We evidenced the caprylate-mediated drug transport across the lipid bilayer, thereby demonstrating the applicability of this new analytical approach for studying formulation-induced changes to drug permeation. The manuscript containing this work is currently under review for publication.

Research task 2 **– Elucidate the structure-activity relationship for immobilized lipases/lipids.**

Accomplishments

Mesoporous silica particles with defined particle sizes and porosities were designed and engineered for the purpose of immobilizing lipids. Their impact on lipid digestion was investigated using QCM-D and TIRF microscopy, whereby the triglycerides were labelled with a fluorescent probe. It was established that mesoporous silica particles significantly enhance the lipolysis mechanism compared to bare lipid droplets. Waveguide microscopy was utilized to further probe the mechanism of lipase:lipid interactions in mesoporous silica. This technique allowed us to study the co-localisation of lipid and silica nanoparticles, by labelling the lipid with a fluorescent dye and monitoring the silica nanoparticles in scattering mode. We demonstrated that lipid preferentially adsorbed to porous silica nanoparticles, compared to non-porous silica nanoparticles, and this preferential binding was hypothesised to trigger an increase lipase activity due to the increase in silica surface area for lipase activation.

Felodipine was encapsulated within the lipid nanoparticles, to allow us to also study the co-localisation of triglycerides and drug, when adsorbed onto porous silica particles. It was established that porous silica nanoparticles triggered enhanced lipolysis kinetics, but retarded drug release, indicating that the drug preferentially adsorbed to the silica nanoparticles rather than be solubilized in the digestion products. This work is being prepared in manuscript form for publication.

Research task 3 **– Engineer mesoporous silica particles with immobilized lipase or lipid and quantify digestion kinetics.**

Accomplishments

Bioactive hybrid nanomaterials were designed and synthesized by combining medium-chain triglycerides with mesoporous carriers with varying nanostructures and surface chemistries. The fabricated biomaterials were characterized in terms of their ability to modulate digestive enzyme function in stimulated intestinal conditions. The confinement of lipid within hydrophilic porous silica matrices was shown to optimize lipase activity, whereas encapsulation of lipid within hydrophobic porous materials restricted digestion. This study contributed to a publication in *Eur. J. Lipid Sci. Technolog*.

The findings from the above work aided in elucidating the lipase:lipid interaction within porous materials; however, more strategic investigations were required to analyze the impact of fundamental material characteristics on lipase function. This was accomplished by synthesizing an array of mesoporous silica particles with varying pore size, surface morphology and microporosity, while maintaining equivalent surface chemistries. By using a systematic approach to manipulate lipid digestion, it was possible to derive the structure-activity relationship within porous silica-lipid hybrid particles. The novel insights acquired are fundamental for controlling poorly water-soluble drug performance, and thus have high importance for the pharmaceutical industry. This work is currently under review for publication. Furthermore, the

findings from this work triggered a new study in which the impact of silica microporosity on drug solubilisation was investigated for the anti-cancer drug, Abiraterone Acetate, when encapsulated within silica-lipid hybrid microparticles. It was established that microporosity positively correlated to increased drug solubilisation in *in vitro* simulated intestinal conditions, revealing that the increased lipase-mediated digestion promoted drug solubilisation. This work is being finalized and is predicted to be complete within the first quarter of 2020 and will then progress to publication.

An innovative application of this study, was to combine mesoporous silica particles with bioactive fish oil droplets to enhance the stability and lipase-mediated bioaccessibility of omega-3 fatty acids. This novel delivery system provided several key insights into optimizing the delivery of functional and bioactive lipids, which is highly relevant to the nutraceutical industry. The work acts as a 'proof-of-concept' for the use of silica-lipid hybrid systems to control the bioactivity of encapsulated compounds and has led to publication in *Functional Foods*.

Additional work, not specifically relating to the aforementioned Research Tasks

Mesoporous silica nanoparticles were prepared with varying particle sizes and surface chemistries to investigate these properties on their intracellular uptake in Caco-2 cells infected with *S. aureus*. It was found that silica particle size between 40 – 80 nm had no impact on cellular uptake, but rather surface chemistry was the driving force for improving cellular uptake, since mesoporous silica particles with greater hydroxyl density promoted phagocytosis to a greater degree. When the model antibacterial drug, rifampicin, was encapsulated within the mesoporous silica particles, the efficacy against intracellular pathogens was greatest for those with higher hydroxyl densities, leading to a direct correlation between particle uptake and antibacterial efficacy. This study highlighted the potential for mesoporous silica particles to treat intracellular infections – an important finding, considering the therapeutically challenging nature of intracellular infections and urgent demand for new formulation approaches. This work is currently being prepared in manuscript form and will be submitted for publication in the first half of 2020.

RESEARCH OUTPUTS

Journal Publications & Manuscripts

- Joyce, P., Jõemetsa, S., Isaksson, S., Hossain, S., Larsson, P., Bergström, C., Höök, F. Investigating poorly water-soluble drug permeation across a lipid membrane supported on mesoporous silica **2019**, *under review*.

- Joyce, P., Gustafsson, H., Prestidge, C.A., Garcia-Bennet, A. Microporosity, surface morphology and pore size modulate lipolysis kinetics of medium-chain triglycerides adsorbed into SBA-15 mesoporous silica particles. **2019**, *under review*.

- Joyce, P., Dening, T.J., Kovalainen, M., Gustafsson, H., Prestidge, C.A., Nanostructured clay particles supplement orlistat in inhibiting lipid digestion: An *in vitro* evaluation for the treatment of obesity. *European Journal of Pharmaceutical Sciences* **2019**, 135, 1-11.

- Joyce, P., Dening, T.J., Meola, T., Schultz, H., Holm, R., Thomas, N., Prestidge, C.A. Solidification to Improve the Biopharmaceutical Performance of SEDDS: Opportunities and Challenges. *Advanced Drug Delivery Reviews* **2018**, 142, 102-117.

- Joyce, P., Gustafsson, H., Prestidge, C.A. Engineering intelligent particle-lipid composites that control lipase-mediated digestion. *Advances in Colloid & Interface Science* **2018**, 260, 1-23.

- Joyce, P., Gustafsson, H., Prestidge, C.A. Enhancing the lipase-mediated bioaccessibility of omega-3 fatty acids by microencapsulation of fish oil droplets within porous silica particles. *Journal of Functional Foods* **2018**, 47, 491-502.

- Dening, T.J., Joyce, P., Kovalainen, M., Gustafsson, H. Prestidge, C.A. Nanostructured Spray Dried Clay Particles as a Novel Treatment Against Obesity. *Pharmaceutical Research* **2018**, 36, 21.

- Joyce, P., Dening, T., Gustafsson, H., Prestidge, C.A. Modulating the lipase-mediated bioactivity of particle-lipid conjugates through changes in nanostructure and surface chemistry. *European Journal of Lipid Science and Technology* **2017**, in press DOI: 10.1002/ejlt.201700213.

Manuscripts in preparation

- Joyce, P., Jõemetsa, S., Ali-Doosti, B., Gustafsson, H., Prestidge, C.A., Höök, F. Probing the Influence of Ca^{2+} Ions on the Interfacial Mechanisms of Lipid Digestion **2019**, *manuscript in preparation*.

- Joyce, P., Subramaniam, S., Maghrebi, S., Jõemetsa, S., Wignall, A., Gustafsson, H., Höök, F., Prestidge, C.A. Nanoparticle size and surface chemistry controls the cellular uptake and anti-bacterial activity of rifampicin-loaded mesoporous silica **2019**, *manuscript in preparation*.

Conference Presentations

- Joyce, P., Jõemetsa, Isaksson, S., Agnarsson, B., Hossain, S., S., Hedge, O., Bergström, C., Höök, F. Total Internal Reflection Fluorescence (TIRF) Microscopy for Oral Drug Delivery Applications. *ARC Centre of Excellence in Convergent Bio-Nano Science Annual Retreat* **2019**, Healesville, Australia.

- Joyce, P., Jõemetsa, Isaksson, S., Agnarsson, B., Hossain, S., S., Hedge, O., Bergström, C., Höök, F. Total Internal Reflection Fluorescence (TIRF) Microscopy for Oral Drug Delivery Applications. *Drug Delivery Australia* **2019**, Brisbane, Australia.

- Joyce, P. Fat Quenchers: Next-Generation Anti-Obesity Therapeutics (Spark Tank Pitch). *Frontiers in Bio-Nano Science* **2019**, Brisbane, Australia.

- Joyce, P., Maghrebi, S., Prestidge, C.A. Development of Polymer-Lipid Hybrid Carriers with Well-Defined Nanoarchitectures for Oral Drug Delivery. *Controlled Release Society Annual Meeting & Exposition* **2019**, Valencia, Spain.

- Joyce, P., Kovalainen, M., Dening, T.J., Gustafsson, H., Garcia-Bennett, A., Prestidge, C.A., Höök, F. Designing Nanostructured Particles that Modulate Fat Digestion and Absorption for the Treatment of Obesity. *International Conference on Advanced Nano Materials* **2018**, Alveiro, Portugal.

- Joyce, P., Kovalainen, M., Dening, T.J., Gustafsson, H., Prestidge, C.A., Höök, F. Designing Nanostructured Particles that Modulate Fat Digestion and Absorption for the Treatment of Obesity. *Gordon Research Conference: Biointerface Science* **2018**, Lucca, Italy.

- Gustafsson, H., Joyce, P., Hook, F., Prestidge, C.A. Design of Porous Silica-Lipid Nanocarriers for Local Enzyme-Controlled Drug Administration. *International Nanomedicine Conference* **2017**, Sydney, Australia.

- Joyce, P., Gustafsson, H, Dening, T.J., Hook, F., Prestidge, C.A. Nanostructuring Biomaterials with Specific Activites towards Digestive Enzymes for Controlled Lipid Hydrolysis. *Euro Fed Lipid* **2017**, Uppsala, Sweden.

- KEYNOTE presentation: Joyce, P., Gustafsson, H., Dening, T.J., Hook, F., Prestidge, C.A. Design of Porous Silica-Lipid Nanocarriers for Local Enzyme-Controlled Drug Administration. *European Colloidal & Interfacial Science Symposium* **2017**, Madrid, Spain.

- Gustafsson, H., Joyce, P., Prestidge, C.A. Design of Porous Silica-Lipid Nanocarriers for Local Enzyme-Controlled Drug Administration. *Drug Delivery Australia* **2017**, Wollongong, Australia.