QMB Abstracts Drugs, Devices & Diagnostics

D1: Inhibition and Conformational Modulation of the Multifunctional Tissue Transglutaminase

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Tissue transglutaminase (TG2) is a remarkable multifunctional enzyme. It was first discovered for its ability to catalyse the cross-linking of structural proteins in the extracellular matrix (ECM), through a transamidation reaction between a glutamine side chain on one protein, and a lysine side chain on another. This acyl-transfer reaction is mediated by an active-site catalytic triad that resembles that of the calpain-type cysteine proteases. The specificity for the amine substrate is very low, so a wide range of primary amines can be incorporated into the glutamine side chain, in a variety of post-translational modification reactions. We have studied the catalytic mechanism of these transamidation reaction extensively.

More recently, TG2 has also been shown to bind guanosine nucleotides and in fact to be identical to Gαh. As such, it modulates intracellular signal transduction in cytosolic G-protein signalling, in pathways upregulated in cancer stem cells. The key to TG2's diverse functionality is the subcellular context dependence of a dramatic conformational change. In the ECM, TG2 binds calcium, adopts an extended linear ("open") tertiary structure and catalyses transamidation. In the cytosol, where calcium concentration is low, the enzyme adopts a "closed" conformation and binds guanosine nucleotides.

In collaboration with a number of different experts, we have recently begun to explore the ligand dependence of this conformational equilibrium, the functionality associated with each conformer, and how we may be able to modulate this function through intervention with judiciously designed inhibitors. To that end we have developed FRET- and CE-based conformational assays, adopted acyl-transfer and GTP-binding activity assays, and carried out structure-activity inhibition studies. Our latest results will be discussed, demonstrating our ability to suppress signalling pathways in cultured cancer stem cells, and validating the breadth of our approach.

D2: Improved healing and scar quality in surgical wounds following treatment with a viral protein combination.

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Introduction. Due to medical advances, a widening spectrum of surgical procedures are accessible to more people. Despite these advances, even minimally invasive surgery can result in cutaneous scarring. The resulting scars are often fragile, painful, prone to damage, and can be disfiguring. With limited available therapies, the results of which are unsatisfactory or inconsistent, a dire need exists for treatments that effectively reduce scarring following surgery. Our specific focus is the poxvirus, orf virus, and the potential of the proteins it encodes as wound therapeutics. This virus produces homologues of our own vascular endothelial growth factor (VEGF) and interleukin (IL)-10, and we have shown that the viral proteins, in combination, accelerate closure of excisional skin wounds in mice and horses.[1-3]
Aim. To evaluate the efficacy of the viral protein combination in preventing surgical wound dehiscence and scarring.
Methods. Incisional wounds in mice were treated immediately, or 48 hours, post-surgery with the protein combination or a saline control, then were allowed to heal by primary intention. The rate of wound closure was assessed macroscopically and through histological analysis. Scar strength was measured using skin tensiometry, with collagen content determined by hydroxyproline, western blot and histological analyses.
Results. A single protein treatment at the time of surgery accelerated closure, with increased wound re-epithelialisation over that of mock-treated wounds. Relative to controls, the closed incision had a reduced scar area, greater collagen content and collagen I/III ratios, and an increased breaking strength following protein treatment.
Discussion. We have demonstrated that the combination treatment of viral VEGF and IL-10 at the time of surgery accelerates healing, reducing both wound dehiscence and scarring. This therapy may therefore have therapeutic utility in the reduction of postoperative wound complications in humans.

1. Wise L et al. (2014) Wound Repair and Regeneration 22(3): 356-67.
2. Wise L et al (2012) Cell Microbio 14(9): 1376-90.
3. Bodaan C et al (2016) Wound Repair and Regeneration 24(6): 966-980.

D3: Inhibitors of Menaquinone Biosynthesis for Control of Pathogenic Bacteria

Furkert, D.P.,[1,2] Ferris, S.,[1,2] Brimble, M.,[1,2,3] Dawes, S.,[1,3] Nigon, L.,[1,3] Jiao, W.,[1,4] Bashiri, G.,[1,3] Lott, J.S.,[1,3] Cook, G.,[1,5] Johnston, J.[1,6]

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The bacterial electron transport chain, responsible for ATP production, has recently emerged as an important drug target for development of new therapeutic agents against *Mtb* and other clinically relevant pathogenic bacteria.[1] This strategy offers potential for treatment of both active and latent states of *Mtb* via their continued need for ATP production for metabolic energy. Within the electron transport chain menaquinone (MQ) plays an important role in transferring electrons to terminal oxidases and eventually to ATP synthase. Interruption of MQ biosynthesis has been demonstrated to significantly affect bacterial viability and strongly potentiate the activity of other drugs (*e.g.* bedaquiline) acting on essential electron transport chain components such as ATP synthase.

Our recent work towards the development of small molecule inhibitors of MQ biosynthesis has been enabled by the ongoing structural biology research programme of Dr Jodie Johnston into the enzymes of the biosynthetic pathway. In particular, a series of crystal structures revealing important events in the first committed step of the sequence, catalysed by MenD, have been used to initiate the drug design process.[2]

Complementary application of organic synthesis/molecular design, crystallography, *in silico* docking/virtual screening and biochemistry, using the diverse skillset of the research collaboration, has enabled a rational approach to novel inhibitor development. The demonstration of *in vitro* activity of compounds produced through this approach will be discussed, along with important new insights into the structure and function of *Mtb*, the implications for the design of succeeding generations of candidate inhibitors, and their potential for future use in multidrug therapy.

1. Bald, D.; Villellas, C., Lu, P., Koul, A. *Targeting Energy Metabolism in Mycobacterium tuberculosis, a New Paradigm in Antimycobacterial Drug Discovery*. (2017) mBio 8:e00272-17. 10.1128/mBio.00272-17
2. Jirgis, E. N. M.; Bashiri, G.; Bulloch, E. M. M.; Johnston, J. M.; Baker, E. N. *Structural Views along the Mycobacterium tuberculosis MenD Reaction Pathway Illuminate Key Aspects of Thiamin Diphosphate-Dependent Enzyme Mechanisms*. *Structure* **2016**, *24*, 1167-1177. 10.1016/j.str.2016.04.018

D4: Microbial biofilm prevention with silver nanoparticle-modified glass ionomer cements

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Glass ionomer cements are fluoride-releasing restorative materials used for a wide range of applications in dentistry, which includes their use as dental fillings for the treatment of caries. Therapeutic procedures designed to treat caries do not always eliminate microorganisms from the remaining dental tissue; furthermore, bacterial micro-leakage can occur in marginal gaps between the tooth and GIC, leading to recurrence of the infection. As a result, there is an evident need for improved GICs with inherent, lasting antibacterial activity.

While claims of anti-cariogenic properties are often made with respect to GICs due to the release of fluoride, numerous studies have revealed that the fluoride release is insufficient to inhibit bacterial growth and prevent the formation of secondary caries. Our team has developed a silver nanoparticle formulation that can be added to a range of existing commercially-available GICs, resulting in the prevention of microbial biofilm formation on the surface of GICs, in contrast to the non-modified materials (Figure 1). The silver nanoparticles were specifically functionalised to enable them to participate in ionic cross-linking within the cement, which we believe to be advantageous in terms of the mechanical properties of material, which will be discussed. Furthermore, due to the chemical bonding of the surface-modified silver nanoparticles within the GICs, no silver leaching from the cement has been detected, offering the potential for ongoing antimicrobial effect at the GIC surface while minimising systemic toxicity concerns. Regardless, the toxicity of our silver nanoparticle formulation has been evaluated against human gingival fibroblast cells and determined to be non-cytotoxic at a silver concentration of ≤ 5.0 μg mL^{-1} (IC_{50} = 10.4 μg mL^{-1}), a concentration above that required to impart antibiofilm effects to silver nanoparticle-modified GICs. The results of our toxicity study will be presented, and compared with those obtained for clinically relevant silver-containing caries treatments.

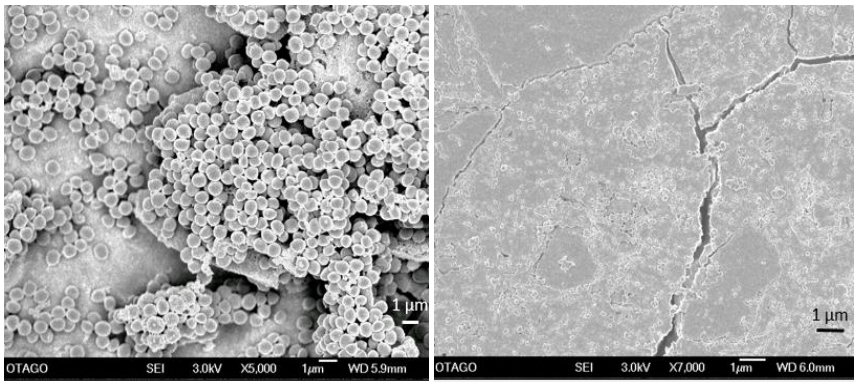

Figure 1. SEM images comparing biofilm development of *Streptococcus sanguis* on the surface of an unmodified GIC manufactured by GC Corporation, Japan (left), and a silver nanoparticle-modified GIC (right).

D5: Towards temporal and spatial control of drug delivery within the brain – application to Parkinson's disease and focal seizures

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L-Dopa is the mainstay therapy for Parkinson's disease (PD), however, its duration of effectiveness without side effects is limited to a few years. Much focus on improving treatment has involved smoothing L-Dopa blood levels to stabilise slow fluctuations in brain dopamine, but this is effective in only select individuals. We believe that optimising PD treatment requires restoration of natural patterns of dopamine receptor activation, involving phasic micromolar pulses of dopamine of a few seconds in duration, upon nanomolar background levels. We therefore designed technology to emulate the natural timing of dopamine release and reinforce only useful movements[1] with the aim of avoiding unwanted movements (dyskinesias) and other side effects of L-Dopa.

Our team has been developing a liposome-based system to deliver dopamine receptor agonists at optimal timing to therapeutic target areas. Coupled with a brain non-invasive controller system utilising focussed ultrasound, we have applied electrophysiology, electrochemistry and behavioural approaches to measure agonist release and evaluate cellular effects in target brain areas. In this talk I will overview our proof-of-concept work delivering dopamine agonists, demonstrating excitatory and neuromodulator effects of phasic receptor activation in neurons recorded in vitro and in vivo in urethane-anaesthetised rats. Finally, I will show preliminary data from behaving rats and our sheep neurotoxin model, that will facilitate scale-up to a brain with similar architecture to humans.

Because of the targeted non-invasive nature of the technology, it can also be used to dampen epileptic foci in the brain where seizures originate. I will also show preliminary application of the technology to on-demand seizure suppression.

1. Fisher, S.D., Robertson, P.B., Black, M.J., Redgrave, P., Sagar, M.A., Abraham, W.C., & Reynolds, J.N.J. (2017). *Reinforcement determines the timing dependence of corticostriatal synaptic plasticity in vivo.* Nature communications 8: 334.

D6: The development of a device for the long term measurement of pressure

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Pressure within different sections of the body is fundamental in the process of exchange and supply in tissues. Although we understand the criticality of maintaining pressure within certain ranges in organs such as the brain, bladder, lungs and vascular system, it is surprising that currently the only chronic measurement made of pressure is blood pressure. The primary reason for this is the current inability to make long term accurate measurements of pressure from implanted devices where there is no ability to recalibrate the device. This presentation introduces our research program to solve this issue. Funded by a large scale MBIE program grant ($13M from 2017-2022) we are developing miniature pressure sensors that exhibit very low drift and are suitable for chronic implantation. Target applications include the monitoring of heart failure and hydrocephalus. Our spectrum of approaches includes ASIC design, microfabrication, pressure drift testing and animal testing. This program aims to establish the team as a world-class centre for the development of pressure sensing for medical applications. Success will be measured against our ability to commercialise.

D7: Pulse-Modulation Drug Delivery from Liposomal Nanostructures

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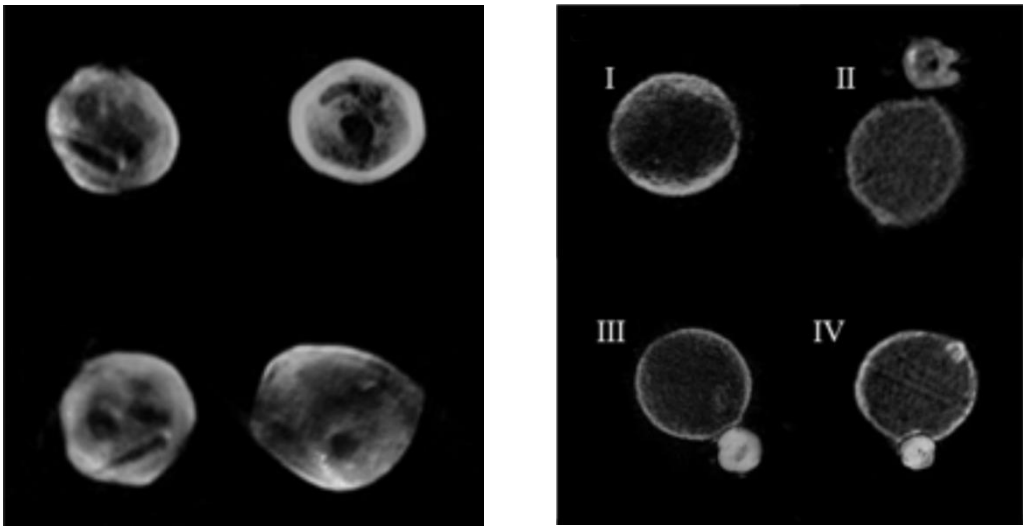
Nanoscale drug delivery systems that can be controlled to release bioactive agents with both spatial and temporal precision are increasingly being recognised as the future of chemical intervention in clinical medicine. This precise on-demand control allows for drug release to be achieved in a fashion that can emulate natural patterns of chemical release thereby mimicking normal bio-functionality. This presentation will describe drug delivery systems we have investigated[1-3] that are based on liposomal drug carriers that allow controlled pulsatile release of neurochemicals or neurochemical-like agents such as dopamine and dopaminergic agonists. In one of these systems liposomes are surface-functionalised to enable tethering to hollow gold nano-shells (HGNs), which act to sensitise the system to low energy irradiation of both light and ultrasound. This system allows for precise neurochemical release both *in vitro* and *in vivo* that resembles endogenous neurochemical dynamics. The synthesis, preparation and characterisation of these liposome-based systems will be presented along with some preliminary evidence for their potential applications in neurobiological research and the treatment of neurological diseases.



Electron tomography of hollow gold nano-shells (HGNs) (left) and of HGN-tethered liposomes (right), showing a control liposome (I), and liposomes with an attached HGN at different times after conjugation (II-IV).

1. Hegh, D. Y.; Mackay, S. M.; Tan, E. W. *Pulsatile release from pH triggered imidazoline switchable surfactant liposomes* RSC Advances, 6, 56859-56866, (2016).
2. Nakano, T.; Mackay, S.M.; Tan, E. W.; Dani, K. M.; Wickens, J. *Interfacing with neural activity via femtosecond laser stimulation of drug encapsulating liposomal nanostructures* eNeuro, 3(6), e0107-16, ENEURO.0107-16.2016 (2016).
3. Nakano, T.; Chin, C.; Myint, D. M. A.; Tan, E. W.; Hale, P. J.; Krishna M., B. M.; Reynolds, J. N. J.; Wickens, J.; Keshav M, D. *Mimicking subsecond neurotransmitter dynamics with femtosecond laser stimulated nanosystems*, Sci. Rep., 4, 5398-5403, (2014).

D8: Building a biosensor for roadside testing of illicit drugs using DNA aptamers

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The use of illicit drugs has plagued the society on many levels. In New Zealand, methamphetamine and cannabis are used in very high quantities. Annually, New Zealand spends approximately $1.8 billion on illicit drug related harm and the use of illicit drugs is correlated to an increase in crime rate and accidents.

Aptamers are custom designed synthetic oligo nucleotides that are evolved using *in vitro* selection techniques to bind a target of choice. Using this technique, aptamers were developed to specifically bind meth and THC in salivary conditions. After evaluation of target binding characteristics, the aptamers were utilised in a variety of bio-sensing platforms to evaluate their performance.

An electrochemical format was selected to develop a portable bio-sensor that could be utilised in workplace and roadside testing of the two aforementioned illicit drugs. After optimisation of the sensor using AuramerBio's gold standard estradiol binding aptamer, meth and THC binding aptamers will be applied and tested in field trials.

D9: Gut Instincts: Explorations in Intestinal Physiology and Drug Delivery

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Gastrointestinal (GI) physiology from its anatomy, pH, fluid composition and motility have long been explored for the scope of colon drug delivery. But truly, how deep and relevant is our understanding of the dynamic and complex nature that is the gut? In light of the development of modern and challenging research, we aim to unearth GI physiology that will shape our future opportunities of colonic drug delivery; this ranges from the power of the microbiome, the influence of food, the effects of mucoadhesion, the GI in its disease state and even sex. Although some parameters are more considered than others, their true variability and distinctions are not fully appreciated in intestinal dosage form design or in vitro testing. The only certain phenomenon in this area is that there are still many unknowns. As pharmaceutics moves forward into the molecular era, an understanding of the role of cellular mechanisms of transporters and metabolic enzymes is important – however, the basics must not be forgotten. As oral drug delivery will continue to be the most widely administered drug dosage form in pharmaceutics, a better understanding of our fundamental knowledge will pave the way for the future of gastrointestinal drug delivery.

D10: Photo-curable thiol-ene Gelatin-Heparin Hydrogels for 3D-biofabrication of functional cartilage

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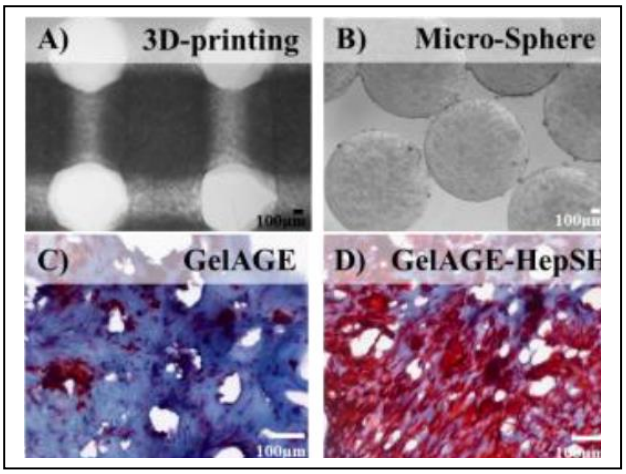

Introduction. A major challenge in biofabricating living cartilage tissue lies in designing a bioink that allows high-resolution 3D-printing, tailorability and is cell-instructive all-in-one. This study thus aimed to develop a thiol-ene bioink by polymerising allylated gelatin (GelAGE) with thiolated heparin (HepSH); systematically studying its biofabrication compatibility and capacity to facilitate chondrogenesis.

Methods. GelAGE and HepSH were synthesised[1,2], 3D-plotted (12x12mm), biofabricated[3] (micro-spheres Ø1mm), and polymerised (450nm, 5.4 kJ/cm2, 1/10mM Ru/SPS, 20wt.-%GelAGE, 30-120mM DTT, 0.5wt%HepSH). Mechanical- and rheological properties, and HepSH retention were recorded. Cellular health (Live/dead®, alamarBlue®) and tissue formation (GAG, DNA, SafO, IHC, RNA) of cell-laden constructs (15x106 chondrocytes/ml, tgf-β, 5w) were studied.

Results. GelAGE and GelAGE-HepSH was successfully biofabricated into constructs with high shape fidelity (Fig.A-B) using two distinct, high throughput, biofabrication platforms. In addition, the physico-chemical properties were tailorable by varying the thiol concentration (7-98kPa) while the viscosity was not significantly altered. All constructs displayed good viability (>80%) and allowed efficient conjugation of bioactive HepSH (>96%), yielding significantly greater differentiation and increase in mechanical properties (AGE:SH-GAG/DNA-stiffness: 1:1.5-184g/g-79kPa, 1:3-134g/g-129kPa and 1:6-84g/g-156kPa) compared to GelAGE alone (AGE:SH-GAG/DNA-stiffness: 1:1.5-16g/g-54kPa, 1:3-24g/g-100kPa and 1:6-20g/g-118kPa, Fig.C-D).

Discussion. GelAGE-HepSH is a new generation multifunctional bioink concurrently demonstrating high shape fidelity, tailorable physico-chemical properties and improved bioactivity for functional cartilage repair.

[1] Bertlein S (2017). Advanced Materials 29(44).
[2] Lindberg G (2007). Macromolecular Bioscience. 17(12).
[3] Serra S (2007). Langmuir 23:7745-50.

D11: Evidence for an allosteric drug binding site in the lipid kinase PI3Kα

Flanagan, J.U..[1,2,3], Gong, G.Q.[3,4], Masson, G.[5], Lee, W-J.[4], Dickson J.M.J.[3,6], Buchanan, C.M.[3,4], Kendall, J.D.[1,3], Rewcastle, G.W.[1,3], Denny, W.A.[1,3], Shepherd, P.R.[1,3,4], Williams, R.W.[5]

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The class IA phosphatidylinositol-3 kinase PI3Kα, phosphorylates the membrane embedded phospholipid phosphatidylinositol-4,5-phosphate ($PI(4,5)P_2$) making $PI(3,4,5)P_3$, which is a secondary messenger that promotes the membrane localisation of proteins with specialist domains that recognize the phosphorylated inositol unit. To perform this reaction, PI3Kα needs to bind its ATP substrate and interface with the cells plasma membrane to find its $PI(4,5)P_2$ substrate. The gene encoding PI3Kα is one of the most mutated in cancer, and many of the mutations create hyper-activated enzymes. Based on this, there is a huge effort to find inhibitors that are selective for the PI3Kαenzyme, with the current molecules all blocking the ATP binding site. Despite this, the effect of these inhibitors on membrane binding has yet to be investigated, even though they bind to the region of the protein most involved in membrane binding and substrate recognition.

Using a Forester resonance energy transfer (FRET) reporter system and biolayer interferometrry, we have investigated the effect of ATP blockers on the interaction between PI3Kα and a synthetic membrane using mixed lipid liposomes.

We identified one molecule that had a dramatic effect on wild type PI3Kα membrane binding compared to a common oncogenic form that has better membrane binding capability. We also observed a similar effect using biolayer interferometry to follow protein-liposome interactions with immobilised liposomes. In dissecting the function of some of the drug-ATP binding site interactions, we developed a reactive probe molecule that led to the unexpected identification of a new binding site outside of the active site.

We hypothesise that this new site is responsible for the membrane binding effect via an allosteric mechanism. These new data provide evidence that some PI3K inhibitors have two modes of action and further exploration of this could be used to develop new types of PI3Kα inhibitor.

D12: *In vitro* dissolution behaviour of respirable anti-tubercular drug particles

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The dissolution behaviour of respirable moxifloxacin and ethionamide drug particles was evaluated using a custom-made dissolution apparatus, which mimics lung conditions. Solubilities of moxifloxacin and ethionamide in phosphate buffered saline (PBS) were 17.68 ± 0.85 mg/mL and 0.46 ± 0.02 mg/mL whereas in the presence of lung surfactant (0.4% Curosurf® in PBS) solubilities were 20.76 ± 0.35 mg/mL and 0.56 ± 0.03 mg/mL, respectively. A fine particle dose (~50 µg) of moxifloxacin or ethionamide with a geometric particle size <5 µm was collected onto a glass coverslip using a modified Twin Stage Impinger. The dissolution behaviour of the fine particle dose of well separated particles was evaluated at various perfusate flow rates (0.2, 0.4 and 0.8 mL/min of PBS, pH 7.4), mucus simulant concentrations (1.0, 1.5 and 2.0% w/v polyethylene oxide, PEO in PBS) and volumes (25, 50 and 100 µL of 1.5% PEO), and in the presence of lung surfactant (0.4% w/v Curosurf®). The dissolution behaviour of the respirable size particles was observed under an optical microscope and the dissolved drug diffused into the perfusate was quantified by HPLC. The moxifloxacin particles showed faster dissolution (<30 min) compared to the ethionamide particles at all the dissolution conditions evaluated. This study demonstrated the differences in the dissolution rates of moxifloxacin and ethionamide particles and may be useful to estimate the residence time of the inhaled dry powder particles in the lungs.

D13: Targeting BCL-2 family proteins to treat cancer: expanding the repertoire of small molecules BH3-mimetics

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Apoptosis, or programmed cell death, is a highly conserved biological process required for the removal of unwanted, damaged or infected cells[1]. The BCL-2 family of proteins governs the intrinsic apoptosis cascade. A delicate interplay between members of this family that promote cell survival (e.g. BCL-2, BCL-X_L, MCL1) and those that induce cell death (e.g. "sensors" BIM or BAD and "executioners" BAX, BAK) dictates whether a cell will live or die.

Down-regulation of apoptosis, often through over-expression of pro-survival BCL-2 proteins is a hallmark of most if not all cancers. Due to their key role in the initiation and maintenance of tumours, the BCL-2-family proteins have become attractive, yet challenging, targets for drug discovery[2].

BH3-mimetics, small molecules targeting the pro-survival BCL-2 proteins, are a new class of drugs that functionally replicate the tumour suppressor activity of sensor proteins such as BIM or BID [2]. This presentation will highlight our decade-long efforts towards the discovery and characterisation of such agents including developments targeting MCL1[3]. From design to application in models of cancers, this work highlights the breakthrough that these compounds may represent in the fight against cancer.

1. Czabotar PE, Lessene G, Strasser A, Adams JM. *Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy.* Nat Rev Mol Cell Biol 2014; 15: 49-63.2. Lessene G, Czabotar PE, Colman PM. *BCL-2 family antagonists for cancer therapy*. Nat Rev Drug Discov 2008; 7: 989-1000.

3. Kotschy A, Szlavik Z, Murray J, Davidson J, Maragno AL, Le Toumelin-Braizat G et al. *The MCL1 inhibitor S63845 is tolerable and effective in diverse cancer models*. Nature 2016; 538: 477-482

D14: Bioorthogonal Prodrug Activation

Matikonda, S.S.[1], Fairhall, J.M.[1], Dadhwal, S.[1]; Gibson, B.; Garden A.[1]; Hook, S.[1], Gamble, A.B.[1]

[1]School of Pharmacy, University of Otago, Dunedin, NZ, [2]Department of Chemistry, University of Otago, Dunedin, NZ

Bioorthogonal chemical reactions have been utilised in bioconjugation and imaging strategies. While these reactions could be considered as “click-and-stick” type reactions, our group is interested in taking advantage of a “click-and-release” type strategy for controlled drug delivery applications. To achieve sufficient activation *in vivo*, the reaction must be fast and the drug must be released in a high enough concentration to exert its biological response. We have identified a new and general bioorthogonal activation strategy based on the 1,3-dipolar cycloaddition of an azide-substituted self-immolative linker (SIL) and *trans*-cyclooctene (TCO). Substituents on the SIL can influence the rate of the bioorthogonal reaction, the subsequent hydrolysis of intermediates, and elimination of the linker. In this presentation I will discuss our *in vitro* and *in vivo* proof-of-concept studies demonstrating how we have applied our approach to prodrug activation strategies. The kinetic and mechanistic investigations provide insight into future stimuli-responsive prodrugs and drug delivery systems.

D15: 3D-printed shells for drug delivery

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[1] Drug Delivery Research Group, School of Science, Faculty of Health and Environmental Sciences, Auckland University of Technology, Auckland, New Zealand; [2] Bio Design Lab, School of Engineering, Computer & Mathematical Sciences Auckland University of Technology, Auckland, New Zealand; [3] Counties Manukau District Health Board, Auckland, New Zealand; [4] Department of Surgery, University of Auckland, Auckland, New Zealand; [5] School of Interprofessional Health Studies, Faculty of Health and Environmental Sciences, Auckland University of Technology, Auckland, New Zealand.

Introduction: The incapability for customisation of conventional drug production has created the opportunity to cater to the individual treatment needs of some patients. Currently, new technology, such as fused-deposition modelling (FDM) 3D printing (3DP), has the capability to fabricate structures of precise geometries from digital designs (Boetker et al., 2016). The advantages of this are: low-cost, time efficiency for small batch numbers and the capability to easily fabricate complex hollow objects (Chai et al., 2017). To study the drug release profile from a FDM 3D printed shell, this study aimed to: i) Design and print a shells of differing wall thickness using poly lactic acid (PLA). ii) Inject a chemically cross-linked composite hydrogel system into the 3DP shell cavity to study the drug release profiles. Iii) Study the drug release profile between different shell wall thicknesses.

Methodology: i) An external circumscribing sphere model with an internal regular sphere cavity was designed by computer aided design (CAD) and 3D printed. A small hole with a diameter of 1 mm was then drilled at the thinnest part of the PLA shell. ii) The spherical shell thickness was changed to study the different drug release profiles. iii) Cross-linked drug loaded hydrogel was injected into the cavity of the 3D printed sphere to study the drug release profile.

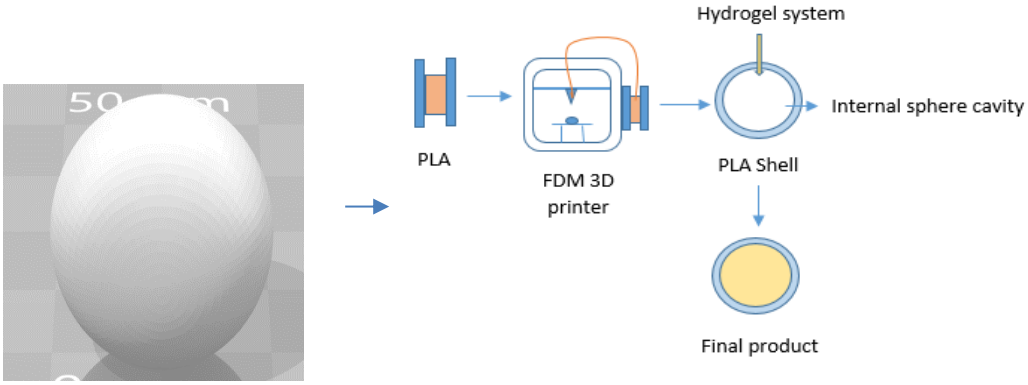

Fig1: The process of fabricating PLA sphere.

Conclusion: All of the 3DP spheres produced sustained-release in all formulations. The maximum release rate occurred in the sphere with 1 mm wall thickness where 18.8% Dex within 2 h and progressed to 92.4% after 6days (Fig. 2). Release was slower with the sphere with a 3 mm wall thickness with 11.5% Dex being released in the first 2 h and slowly

progressed to 71.8% after 6 days, showing the potential of 3DP technology to facilitate future applications for personalised 3DP drug delivery devices.

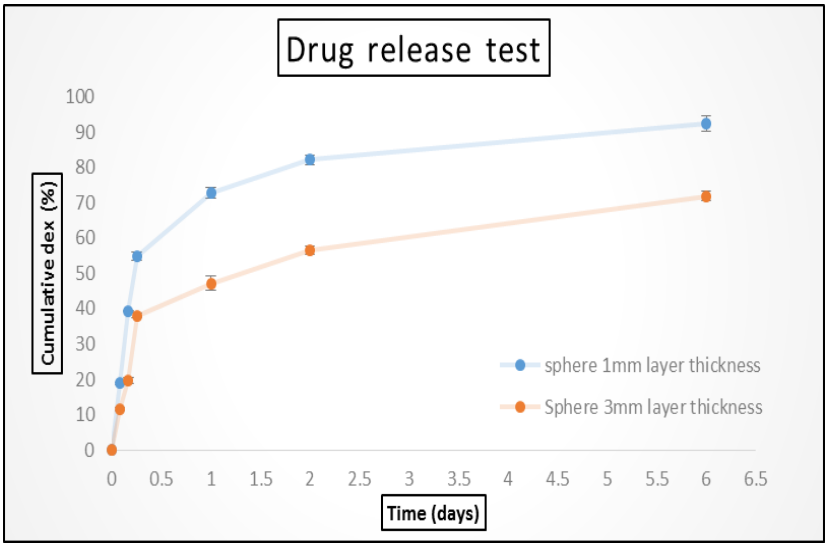

Fig 2: *In-vitro* drug release profile from 3D printed sphere.

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D16: Optimization of TiO2 nanotube formation and dimensions on Ti6Al4V alloys influences osteogenic differentiation of human mesenchymal stromal cells

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Anodization, which generates nanotubes on titanium surface, is proved to enhance osteogenesis and promising for bone implant modification. However, few studies have applied this onto Ti6Al4V most commonly adopted for bone-interfacing implants. In this study, we optimized nanotube (NT) fabrication on Ti6Al4V in ethylene glycol based electrolyte and investigated osteogenic differentiation of human mesenchymal stromal cells (hMSCs) on NT surfaces.

Anodized Ti6Al4V surfaces were characterized by scanning electron microscopy (SEM). Computational Fluid Dynamics (CFD) was employed to simulate electrolyte flow profiles under various stirrings, and their correlation to NT formation. Polished Ti6Al4V disks (240 grit) were anodized at 20, 40 and 60 volts under optimal electrolyte flow conditions for comparison of NT surface-controlled osteogenic differentiation and mineralization of hMSCs over 21 days culture in osteogenic media.

Ti6Al4V surfaces anodized at 20, 40 and 60 volts resulted in NT with average diameters 39, 83 and 104 nm, respectively (Fig 1). Hydrodynamics (shear stress) significantly influenced uniformity of NT formation, i.e. parallel shear flow impaired mature NT formation whereas uniform perpendicular shear flow promoted homogeneous NT distribution. After 1 day culture, hMSCs showed filopodia-like cellular extension on anodized samples and sheet-like morphology on controls. At D21, significantly higher osteocalcin, collagen I expression and greater mineralized deposits were observed on V20 NT surfaces (Ø39 nm) than V40 and controls (Fig. 1).

Our study provides for the first time a clear view about the hydrodynamic effect to NT formation on Ti6Al4V and the potential application of anodization to enhance bone-implant surface modification.

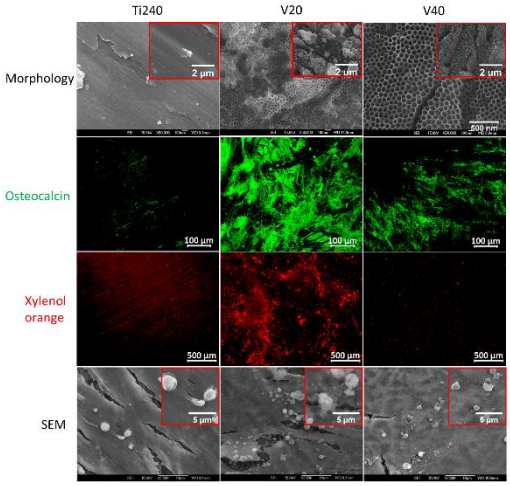

Figure 1: Nanotube morphology of 20 and 40 volt anodized samples, and osteogenic differentiation analysis of hMSCs after 21 days culture. Osteogenic differentiation was evaluated by fluorescent staining of osteocalcin, xylenol orange staining of mineral deposits and SEM/EDS for mineralized nodules. Few cells existed on V60 samples and were excluded from osteogenic differentiation assay.

D17: 3D printed ballistic drug delivery system for wildlife applications

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Introduction: The increasing populations of pest wildlife present considerable threats to ecosystem and public health. The current methods for delivering contraceptives to wildlife suffer from serious limitations such as the lack of target specificity and stability issues. To address the above problems, we have designed a 3D printed ballistic drug delivery system (BDDS) to load and deliver specific dosages of drug(s) as below:

a) A prototype 3D printed bio-bullet with poly lactic acid (PLA) for ballistic delivery of contraceptive drug progesterone (P4)
b) Prototype optimisation using a microsphere and hydrogel system for prolonged release of levonorgestrel for 2-5 years
c) Modified prototype for multi-compartmental delivery of multiple drugs (anaesthetics and anti-inflammatories) with both immediate and sustained release profiles

Methodology:

a) P4 was loaded into PLA matrix by hot melt extrusion and 3D printed by fused deposition modelling (FDM) as a projectile for .177 air-rifle.
b) Levonorgestrel was encapsulated in chitosan microspheres and coated by freeze-thaw PVA hydrogel
c) Lidocaine was loaded into chitosan-pectin thermosensitive hydrogel and 3D bio-printed as a mesh scaffold; Dexamethasone was formulated with chemically cross-linked PVA hydrogel.

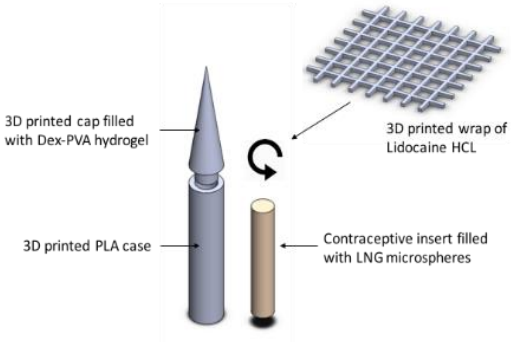

Figure 2. 3D printed BDDS with multiple drugs.

Conclusion: Multiple drugs (progesterone, levonorgestrel, lidocaine and dexamethasone) were formulated with different release profiles. Each formulation can be used individually and in an assembly as BDDS.

3D printed P4-PLA bullets showed a long-term release of 6 months. Dexamethasone incorporated into hydrogel matrix presented a sustained release of 33 days. A 3D printed hydrogel scaffold containing lidocaine HCL exhibited complete release within 6 hours. Further development of these bio-bullets with prolonged release of high-dose contraceptive could make an important contribution to the maintenance of pest wildlife and public health.

D18: PEDOT membranes for electrically tuneable drug delivery

Bansal, M.[1], Yasin, MN.[1, 2], Sharma, M.[1], Rupenthal, I.D.[2], Waterhouse, GIN.[3], Svirskis, D.[1]

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Template directed polymerisation can be used to change the bulk properties of conducting polymers and alter the electrochemically available surface area. In this research we aimed to prepare both porous and non-porous poly(3,4-ethylene dioxythiophene) (PEDOT) thin films by vapour phase polymerisation. The PEDOT films were applied to electrically-tuneable drug delivery both as rate controlling membranes and as ion-exchange membranes.

To prepare 3-dimensinally ordered macroporous (3DOM) PEDOT films polystyrene colloidal crystals were used as a template to direct polymerisation, filled with the Fe(III) tosylate based oxidant solution. Non-porous PEDOT films were prepared without the template present. The substrates were exposed to EDOT monomer in a vacuum oven to form PEDOT layers.

3DOM and non-porous freestanding PEDOT films were prepared. The films were around 2 µm thick, smooth, uniform and mechanically robust when dry. All films were conductive and redox active. The 3DOM porous films had a 2.9 fold increase in electrochemically accessible surface area compared to non-porous PEDOT, as determined by cyclic voltammetry. The non-porous PEDOT films were applied as rate controlling membranes using a custom-modified Franz cell setup. The membranes were highly permeable to nicotine and dexamethasone phosphate, however, large lactoferrin molecules could not diffuse through the PEDOT membranes. The permeability of dexamethasone phosphate could be controlled electrically with an increase in flux observed when the membranes were maintained in the oxidised state compared to the reduce state. Both 3DOM and non-porous PEDOT films were applied as ion-exchange resins of the anionic dexamethasone phosphate. The extended surface area of the 3DOM porous materials was more efficient for both drug loading and release. Electrically triggered release of drug was demonstrated.

The highly conductive thin PEDOT films are exciting materials to explore for molecular separation and drug delivery applications.

D19: DYNAMIN STUCTURE AND FUNCTION HINGING ON RYNGOS

Cardoso, D.A.,[1,2], Abdel-Hamid, M.K.,[3], McCluskey, A.[3], Robinson, P.J.[1,2]

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Dynamins are multi-domain GTPase enzymes capable of performing the final scission of invaginated plasma membrane prior to the completion of endocytosis. Pharmacological targeting of dynamin in relevant mouse models has been shown to provide therapeutic relief for ailments as diverse as chronic kidney disease and epilepsy. We have generated a series of small molecule modulators (Ryngos) which 'lock' dynamin into a 'ring' oligomeric state that structurally differs from the 'helical' state required for endocytosis. However, these compounds exhibit different activities on enzyme activity *in vitro* (Ryngo-1: mixed-mode / Ryngo-3: stimulation). Due to their chemical similarity, it can be surmised that these pharmacological agents share a common binding pocket. To establish the binding site of Ryngos and allow for targeted drug design and dissection of dynamin residues responsible for inhibition or stimulation of activity, advanced computer modelling was initially employed. Lead compounds, Ryngo-1-23 and Ryngo-3-32, were predicted to independently localize to, and differentially interact with Hinge 1, located between middle domain and bundle-signalling element of dynamin. A partial overlap of implicated residues between Ryngo-1-23 and Ryngo-3-32 suggests drug binding to different sub-regions of Hinge 1 may be capable of imparting different actions (stimulation/inhibition) on dynamin activity *in vitro*. To validate this model, mutagenesis of implicated Hinge 1 residues was carried out and resultant mutants characterised. Biochemical assays largely support these predictions (i.e. single mutations specifically lost drug action) as well as highlight a broader role for Hinge 1 in dynamin characteristics (e.g. activity, oligomerisation, and endocytosis). To account for allosteric effects of mutation, a chemically dissimilar dynamin-targeting compound (Dynole-34-2) was employed and revealed loss of Ryngo action to be specific to Hinge 1. The data supports the proposed model of these compounds differentially interacting with a flexible hinge within dynamin, an exceptionally rare binding site for pharmacological agents.

D20: Drug delivery for wildlife

McDowell, A.

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Wild animals comprise a diverse group of animal patients and delivery of drug to these populations is challenging. These situations extend the challenges of conventional veterinary delivery because bioactive compounds must often be remotely delivered to the animal. The specific example of using nanotechnology for the delivery of fertility control agents for the brushtail possum in New Zealand will be discussed. Our work has shown that there is opportunity for pharmaceutical formulation science to provide valuable input into the efficacious delivery of therapeutic agents for wildlife[1]. The recent establishment of The Wildlife Hospital in Dunedin provides an exciting platform to further develop the area of wildlife drug delivery.

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D21: Biological strategies for promoting bone and tendon healing

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University of Auckland

Skeletal regenerative medicine or tissue engineering is a significant leap forward in clinical practice and a new frontier in skeletal medicine. It is an exciting multidisciplinary approach aimed at improving the surgical outcomes of skeletal repair, through the collaboration of biologists, engineers and orthopaedic surgeons. The demand for orthopaedic repair is huge due to the ageing population, lifestyle changes with increased expectations regarding the quality of life, an increase in sports-related injuries and poor posture leading to spinal problems. The worldwide sales of orthopaedic products is greater than $48 billion pa.
Providing a scaffold or medical device that encourages appropriate cell attachment, growth, and ultimately tissue regeneration could improve the clinical outcomes from injuries.
We have tested many scaffold materials of both natural and synthetic origin to evaluate their biomaterial properties and their potential utility in musculoskeletal regenerative medicine. Attachment and growth of primary osteoblasts and tenocytes on the scaffolds were analyzed using live-dead fluorescence and alamarBlue® staining. The morphological phenotype and cell differentiation was evaluated by differential gene expression to better understand the biocompatibility of potential tissue engineering products. Importantly, we have also tested the materials for their immunogenicity to assess the potential foreign body response.

In addition, we have developed preclinical models to address clinical problems. We have identified a number of scaffold biomaterials that show potential for use in bone and tendon regeneration. Much time and expense can be saved by such *in vitro* evaluation of biomaterials prior to embarking on *in vivo* studies.

D22: Exploring the Btk Active Site

McCone, J.,[1,2], Teesdale-Spittle, P.,[1,3], Flanagan, J.[1,4] Harvey, J.[1,2]

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Bruton's tyrosine kinase (Btk) is a validated drug target for diseases associated with B-cell receptor (BCR) signalling, such as mantle cell lymphoma (MCL) and chronic lymphocytic leukaemia (CLL). There is considerable interest in Btk for drug development to complement the successful drugs Ibrutinib and Dasatinib already used in the clinic.

Development of an accurate model of the Btk active site would enable the design of better drug leads. To this end, we have performed native and cross docking of the available crystal structures of Btk with inhibitors (and decoys) to build a cluster of effective models for ensemble docking.

We have applied the docking model to predict the binding modes of a new Btk inhibitor based on the fungal natural product TAN-2483B, and are currently synthesising analogues to probe the model further.

D23: Designing Bio-ink and Bio-resin platforms for 3D Bioprinting and Bioassembly

Woodfield, T.[1], Lim, S.S.[1] Brown, G.,[1] Schon, B.S.,[1] Mekhileri, N.V., [1] Hooper, G.J., [1] Levato, R.,[2] Malda, J.,[2] Groll, J.[3]

[1] Christchurch Regenerative Medicine and Tissue Engineering (CReaTE) Group, Department of Orthopaedic Surgery, Centre for Bioengineering & Nanomedicine, University of Otago Christchurch, New Zealand [2] Department of Orthopaedics, University Medical Center Utrecht, Utrecht University, The Netherlands. [3] Department for Functional Materials in Medicine and Dentistry (FMZ), University of Würzburg, Pleicherwall 2 97070 Würzburg, Germany.

Biofabrication technologies, including 3D bio-printing and bio-assembly, enable the generation of engineered constructs that replicate the complex 3D organization of native tissues via the automated hierarchical placement of cell-laden bio-inks, tissue modules, growth factors and/or bioactive agents. The major challenge for translational regenerative medicine is that the biofabrication window and processing requirements of current bio-inks are narrow and vary significantly between multiple biofabrication methods. This requires the optimization of bioinks for each individual biofabrication technique and tissue type.
We describe the development of a platform visible light photoinitiator system for gelatin-based bioinks using ruthenium/sodium persulfate (Vis + Ru/SPS) [1, 2, 3, 4] and demonstrate efficacy across a range of biofabrication techniques. Furthermore, we describe versatile photo-clickable step-growth thiol-ene based hydrogels based on allylated gelatin (GelAGE) and gelatin-norbonene (GelNOR) combined with Vis + Ru/SPS crosslinking as platform bio-inks and bio-resins successfully applied to 3D Bioplotting, 3D Bioassembly and high resolution DLP biofabrication technologies [2].
Finally, automated 3D bio-assembly technology platform for biofabrication of complex hybrid constructs for regenerative medicine or 3D *in vitro* models for high throughput screening using chondrogenic, osteogenic and cancer microtissues as well as cell-encapsulated photocrosslinked microspheres are introduced [5, 6].

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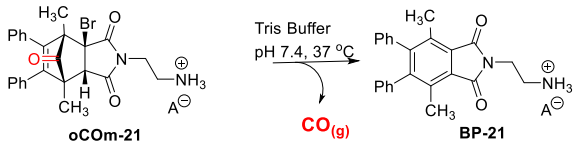
D24: Norborn-2-en-7-ones as Physiologically-Triggered Carbon Monoxide-Releasing Prodrugs

Larsen, D.S.[1], Kueh, J.T.B.[1], Harrison, J.C.[2], Sammut, I.A.[2]

[1]Department of Chemistry, University of Otago, Dunedin, NZ, [2]Department of Pharmacology, University of Otago, NZ.

A prodrug strategy for the release of the gasotransmitter carbon monoxide (CO) at physiological pH, based upon 3a-bromo-norborn-2-en-7-one Diels-Alder cycloadducts of 2-bromomaleimides and 2,5-dimethyl-3,4-diphenylcyclopentadienone will be discussed.[1] Examples possessing pendent protonated amine and diamine groups showed good water solubility and thermal stability. Half-lives for CO-release in TRIS buffer at pH 7.4 ranged from 19 to 75 mins at 37 °C and 31 to 32 h at 4 °C. Increased intracellular CO levels following **oCOm-21** exposure were confirmed using a CO specific fluorescent probe. Bioavailability in rats was demonstrated by oral gavage **oCOm-21** showed dose-dependent vasorelaxant in pre-contracted rat aortic rings with an EC_{50} of 1.6 ± 0.9 µM.



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D25: Controllable needle-free jet injection: from the laboratory to the clinic

Ruddy, B.P.[1,2], Brunton, P.A.[3], Bullen, C.[4], Chase, J.G.[5], Chu, J.T.W.[4], Jeong, S.H.[6], Madadkhahsalmassi, B.[1], McKeage, J.W.[1], Pearson, L. H.[5], Svirskis, D.[7], Tingle, M.D.[8], White, D.E.[9], Xu, J.[1], Taberner, A.J.[1,2]

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Drug delivery by hypodermic syringe can be uncomfortable and painful, leading to poor patient compliance with therapies involving injection. Jet injection is a promising needle-free substitute for hypodermic needles, delivering a narrow stream of liquid drug through the skin at high speed, such that it penetrates to the desired depth using only the momentum of the jet. This method can be inconsistent and acoustically noisy, however, leading us to develop[1,2] a form of jet injection powered by an electric motor, which can be precisely controlled for consistency and gently accelerated to reduce noise.

In this talk, we will discuss our work on three emerging clinical applications for controllable jet injection, and the key adaptations needed to bring this technology from the engineering lab to the clinician's office. First, we will discuss the use of controllable jet injection for the delivery of dental anaesthetic, a common procedure whose needles are a leading cause of dental phobia. In this application, currently undergoing initial *ex-vivo* testing, the injection system must be tailored to reach the molars without patient discomfort; we have established that jet injection with a remotely-located nozzle is feasible and overcomes this challenge. Second, we will discuss jet injection of nicotine for smoking cessation, where a needle-free method would be essential for patient acceptance. Here, we will discuss our initial *in-vivo* animal testing and show that jet injection can achieve functional equivalence with needle injection. Finally, we will discuss an integrated approach to needle-free diabetes management, including jet injection of insulin, and our initial clinical testing of the method in humans.

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D26: Perforin inhibitors: A twist in the tale

Julie A. Spicer,[1,2] Jiney Jose,[1] Christian K. Miller,[1] Patrick D. O'Connor,[1] Anna C. Giddens,[1] Eleanor Leung,[3] Raymond S. Norton,[3] Ruby H. P. Law,[4,5] James C. Whisstock,[4,5] Hedieh Akhlaghi,[6] Joseph A. Trapani.[6,7]

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The pore-forming protein perforin is a key component of the human immune response, performing a crucial role in the granule exocytosis pathway employed by natural killer (NK) cells and cytotoxic T lymphocytes (CTL) to eliminate virus-infected and transformed cells. CTLs and NK cells have also been implicated in several autoimmune diseases (e.g., insulindependent diabetes) and therapy-induced conditions (e.g., allograft rejection, graft-versushost disease). Conventional immunosuppressive treatments indiscriminately depress immune function, but since perforin is expressed exclusively by cells of the immune system, inhibition of this target should be a highly selective strategy for the treatment of these conditions.

In this presentation I will report our initial identification of compounds that bind perforin protein using an NMR-based screen of small fragment molecules. I will then describe their optimisation to nanomolar inhibitors of human NK cells using structural data obtained by our collaborators in Melbourne. Finally, I will discuss the unexpected mode of action of these compounds and the implications of our findings for the design of future generations of perforin inhibitors.

D27: Forays in Drug Discovery

Baell, J.B.

Medicinal Chemistry Theme, Monash Institute of Pharmaceutical Sciences, Parkville 3052, Melbourne

In this talk we will explore a variety of our forays in recent years into drug discovery, from antiparasitic to anticancer agents with screening, design or the literature as starting points. On one hand, we view high throughput screening (HTS) as a central science for such ambitions, so that we can find new starting points for medicinal chemistry against targets of interest. For example, we identified a screening hit from HTS that targeted a protein kinase of interest to us – LIMK1 - and for which we had genetic evidence of its relevance to metastasis. This ultimately led to an intriguing outcome – an unexpected but necessary twist in the tail. On the other hand, in a separate project, we took a compound from the literature and successfully engineered a heterocyclic isostere with potent cell-based activity that we showed was mechanism-based and acting on the pro-survival proteins Bcl-xL and Bcl-2. We also took the structure of protein ligands to Bcl-xL to design active peptidomimetics of the interfacial binding epitope. Finally, using HTS we have discovered potent antiparasitics that target Trypanosomes – the causative agents of neglected diseases such as Sleeping Sickness and Chagas' Disease.

D28: Twisting tails and curious channels – the phosphoribosyltransferases

Parker, E.J.[1], Moggré, J.[2], Mittelstädt, G.[2] Jiao, W.1, Kundu, P.[1], Cookson, T.[2] Lott, S.[3]

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Phosphoribosyltranferases (PRTs) catalyse the transfer of the sugar, ribose 5-phosphate to a nitrogenous base. We have examined catalysis by two PRT enzymes, both of which play key roles in amino acid biosynthesis, and have been identified as new targets for antimicrobial therapeutics.

ATP phosphoribosyltransferase (ATP-PRT) catalyses the first step of histidine biosynthesis resulting in the transfer of a phosphoribosyl unit to ATP. We have carried out kinetic isotope effect measurements to determine the mechanism of this reaction for ATP-PRT enzymes the pathogens *Campylobacter jejuni* and *Mycobacterium tuberculosis*. We are currently using this information to design transition state analogues as inhibitors of this enzyme.

Anthranilate phosphoribosyltransferase (An-PRT) catalyses the formation of phosphoribosyl anthranilate in the biosynthetic pathway for tryptophan. Our results with substrate analogues and inhibitors of An-PRT from *Mycobacterium tuberculosis* reveal how a substrate binding channel both protects an enzyme-bound reactive intermediate and acts as an Achilles heel by providing a mechanism for inhibition.

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D29: Development of Transfection and Drug Delivery Vehicles.

O'Byrne,S.[1], Lopez-Gonzalez, R.[2], Sewell, A.L.[1], Silva Nigenda, E.[1], Power, B.J.[1], Petersen, J.[3], Belougne, J.[4], Zhang, X.[4], Flachbartova, Z.[5], Carrick, E.[5], Selzer, P.M.[6], Asada, M.[7], Mullen, W.[5], Britton, C.[5], Christie, J.M.[3], Kaneko, O.[7], Marquez, R.[1,8*]

1. School of Chemistry, University of Glasgow, Glasgow, G12 8QQ, U.K. 2. Department of Chemistry, Xi'an Jiaotong-Liverpool University, Suzhou, 215123, P.R. China. 3. Institute of Molecular Cell and Systems Biology, University of Glasgow, Glasgow, G12 8QQ, U.K. 4. Centre d'Immunologie de Marseille-Luminy, Aix Marseille Univ, CNRS, INSERM, Marseille, France. 5. Institute of Biodiversity Animal Health and Comparative Medicine, University of Glasgow, Glasgow, G61 1QH, U.K. 6. Boehringer Ingelheim Vetmedica GmbH, Germany. 7. Nagasaki School of Tropical Medicine, Nagasaki, Japan. 8. School of Physical and Chemical Sciences, University of Canterbury, Christchurch, 8140, N.Z.

Selective drug delivery in parasitic and bacterial infections is a cornerstone of medicinal chemistry. There have been entire drug research programmes that have been shut down due to toxicity issues that have arisen from unselective targeting and drug delivery.
However, drug treatment has a number of associated problems, including the development of drug resistant parasites, poor drug efficacy against some parasite species and life-cycle stages, and side-effects on host cells due to non-selective drug delivery. Development of new drug classes is prohibitively expensive in both time and money, and there is therefore an increase in research aimed at prolonging the lifespan and effectiveness of current drugs.
We have developed a set of tuneable vehicles able to deliver selectively both small molecular weight compounds as well as large biomolecules into nematodes, kinetoplastids and the intracellular stages of apicomplexan parasites. Our delivery vehicles can be easily generated and do not target uninfected mammalian cells, and as such, are non-toxic.

D30: EMA401 – A novel analgesic in clinical development for the treatment of neuropathic pain

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Neuropathic pain is an area of large unmet medical need. Although many preclinical studies have produced promising efficacy data in rodent models of neuropathic pain, most have failed to translate into positive Phase 2a clinical trial outcomes. An exception is EMA401, a small molecule angiotensin II type 2 (AT2) receptor antagonist that has 10,000-fold binding selectivity over the angiotensin II type 1 (AT1) receptor. Specifically, EMA401 at 100 mg twice daily for four weeks evoked pain relief in patients with post-herpetic neuralgia (PHN), a type of peripheral neuropathic pain that is often intractable. This presentation will focus on preclinical investigations of the role of angiotensin II (Ang II) signaling via the AT2 receptor in the pathobiology of peripheral neuropathic pain. Specifically, in the widely used chronic constriction injury (CCI) rodent model of peripheral neuropathic pain, small molecule AT2 receptor antagonists with >1000-fold selectivity over the angiotensin II type 1 (AT1) receptor evoked dose-dependent pain-relief. In CCI-mice null for the AT2 receptor, pain relief was abolished with intermediate effects in the heterozygotes, thereby confirming the AT2 receptor as the drug target. In sections of ipsilateral lumbar dorsal root ganglia (DRGs) from CCI-rats as well as similar sections from the corresponding group of sham-animals, e*x vivo* immunohistochemical and molecular biological methods have been used to gain insight on the cellular and molecular mechanisms of pain relief and these data will be presented.

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Summary of Abstracts for the Poster Session

No.	Title	Presenter	Institutions
D43	New Isothiocyanates: Covalent inhibitors of Macrophage migration inhibitory factor	Putha, L.	School of Pharmacy, University of Otago, Dunedin, NZ
D44	Preformulation studies for the development of a novel suppository to deliver amoxicillin	Purohit T. J.,	School of Pharmacy, University of Auckland, Auckland, NZ
D45	Probing the reaction mechanism and inhibition of Mtu-AnPRTase enzyme: a potential target to novel antimicrobial drug design	Kundu, P.	Ferrier Research Institute, Victoria University of Wellington, NZ.
D46	Fabrication of minimally invasive biodegradable microneedle arrays for the delivery of anti-inflammatory drug	Duarah, S.,	School of Pharmacy, Faculty of Medical and Health Sciences, University of Auckland, NZ
D47	Synthesis of bioactive natural products	Badart, M.	Department of Chemistry, University of Otago, Dunedin, NZ
D48	Identification of transporters involved in drug-drug interaction during gout treatment in primary rat hepatocytes	Bahn, A.	Department of Physiology, University of Otago, Dunedin, NZ
D49	Stimuli-responsive delivery of drug to tumor improves the survival in a mouse model	Dadhwal, S.,	School of Pharmacy, University of Otago, Dunedin, NZ
D50	Structure-Activity Relationships and Binding Optimization of Chlamydia HtrA Inhibitors	Agbowuro, A.A.	School of Pharmacy, University of Otago, Dunedin, NZ
D51	Comparison of the uptake of rutin-loaded nanoparticles in a zebrafish model	Vu, H. T.H.	School of Pharmacy, University of Otago, Dunedin, NZ
D52	Safety of a Wireless Power Transfer System for Powering Heart Pumps	Au, S.L.C.,	Auckland Bioengineering Institute, University of Auckland, NZ
D53	Preformulation studies to determine the physiochemical properties of L-Glutathione	Liu, M.	School of Pharmacy, Faculty of Medical and Health Sciences, University of Auckland, Auckland, NZ
D54	Synthesis and Evaluation of Menaquinone D (MenD) Inhibitors as Potential TB Therapeutics	Ferris, S.	School of Chemical Sciences, The University of Auckland, NZ
D55	New Enzyme Architecture: An Investigation on the Evolution of Super-Long form ATP-PRTase	Khajeaian, P.	Ferrier Research Institute, Victoria University of Wellington, NZ

D43: New Isothiocyanates: Covalent inhibitors of Macrophage migration inhibitory factor

Putha, L.[1], Kok, L. K. [1], Chen, K. [1], Sagatova, A. A. [2], Wilbanks, S. M.[2], Gamble, A. B.[1], Vernall, A. J.[1], Tyndall, J. D.[1]

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ABSTRACT: Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine that plays an important role as a pro-inflammatory component of numerous inflammatory diseases and cancers. MIF is overexpressed in numerous cancers, including colorectal cancer, prostate cancer, breast cancer and leukaemia as well as inflammatory diseases such as atherosclerosis, rheumatoid arthritis. Isothiocyanates (ITCs) are small molecule inhibitors of MIF, with several examples found in cruciferous vegetable. MIF has tautomerase activity that is inhibited by covalent attachment of ITCs to the N-terminal, catalytic proline. We produced recombinant human MIF (rhMIF) in *Escherichia coli* to assay inhibition of tautomerase activity by novel ITCs. However, this rhMIF is heterogeneous, with initiator methionine cleaved from only a fraction of recovered protein. MIF lacking the N-terminal methionine was able to react with ITCs while MIF retaining the methionine did not. Building on our previous work on benzyl ITC derivatives with sulphonamide and benzodioxole moieties, we investigated the effects of linker length along with modification of aromatic substituent to further expand the structure activity relationships. We synthesized 12 new compounds and rhMIF biological assays revealed IC50 values of 0.72 ± 0.06 and 0.44 ± 0.06 μM for two of the best compounds. The activity of ITCs with a sulfonamide substituent increased with an increase in linker length. However, the increase in linker length of other ITCs (benzodioxole series) did not show any increase in MIF inhibitory activity. Further investigation has identified a lead compound with lower nanomolar activity. Further optimisation of the substituents is being carried out with a view to testing lead compounds in cancer cells.

D44: Preformulation studies for the development of a novel suppository to deliver amoxicillin

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Amoxicillin is a broad spectrum, amino-penicillin antibiotic used in the treatment of different infections. There is a lack of child-friendly dosage forms for the treatment of infections such as pneumonia, especially in resource-poor countries. Rectal delivery is suitable for paediatric patients. Amoxicillin suppositories could potentially offer a low-cost, easy-access treatment option. The objectives of the present study were to select suitable materials to formulate a suppository to promote rectal absorption of amoxicillin. To support the formulation development a high-performance liquid chromatography (HPLC) method was required. Interference of bioadhesive polymers (sodium alginate, chitosan and polyvinyl pyrrollidone) on melting and cooling behaviour of potential suppository bases, polyethylene glycol (PEG) 1500 and 4000 were determined using differential scanning calorimetry (DSC). The samples were exposed to 0-70^{o}C at the rate of 5^{o}C/min. The cycle was designed to simulate the formulation and *in vivo* temperature changes. The HPLC method was developed and validated according to the International Council for Harmonisation (ICH) guidelines.
The melting point and cooling temperatures of PEG 4000 were decreased by 2 -3 ^{o}C and 7-10 ^{o}C respectively, when mixed with PEG 1500 and bioadhesive polymer at a ratio of 2-5%. Polyvinyl pyrrollidone showed a cooling range from 25-40 ^{o}C instead of a peak at 32 ^{o}C and 32.8 ^{o}C for chitosan and alginate, respectively. Addition of drug to the base is also expected to affect the thermal behaviour. A simple, rapid HPLC method was validated to be linear within the range of 0.5 – 50 µg/ml (R^2 >0.999), with limit of detection and quantification of 0.02 and 0.07 µg/ml, respectively, and can be used in the drug stability and *in vitro* and *in vivo* studies of the proposed amoxicillin suppository. Bioadhesive polymers would improve retention of the suppository therefore allowing better absorption of amoxicillin.

D45: Probing the reaction mechanism and inhibition of *Mtu*-AnPRTase enzyme: a potential target to novel antimicrobial drug design

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Tuberculosis (TB), which is estimated to affect 2 billion individuals worldwide, is predominately caused by an infection with *Mycobacterium tuberculosis*. The particular concern is increasing the prevalence of TB, which is becoming resistant to the available treatments. A genetic knockout of *trpD* gene, which encodes for anthranilate phosphoribosyltransferase (AnPRTase) was unable to cause disease, even in immune deficient mice[1]. AnPRTase plays an important role in the synthesis of essential amino acids in *M. tuberculosis*. Therefore, this enzyme is a potential drug target for the treatment of TB and other infectious diseases.

Our research explores the synthesis of different substrate and potential transition state analogues in order to understand catalysis and inhibition of AnPRTase enzyme to aid novel drug design. We have managed to achieve effective inhibition of AnPRTase enzyme with a range of different substrate analogues with K_i values of 1.3-15 μM.[2] However, these analogues were shown through X-ray crystal structures to bind to the substrate binding tunnel, with no observed binding to the actual catalytic site of the enzyme. Recently, we managed to solve the structure of a potential transition state inhibitor, which bound at the active site, giving us new insight into the enzyme and allowing us to develop far more specific inhibitors.

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D46: Fabrication of minimally invasive biodegradable microneedle arrays for the delivery of anti-inflammatory drug

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Background:
Anti-inflammatory drugs are prescribed for a wide range of conditions, but their oral or parenteral delivery often leads to several adverse effects [1, 2]. Dexamethasone is a synthetic corticosteroid exhibiting both anti-inflammatory and immuno-suppressant properties and is prescribed at the lowest effective dose for the shortest possible time, but sometimes on a long-term basis [3]. Hence, an alternate route for its effective and minimally invasive delivery is necessitated. Microneedle technology is a promising technique to ensure effective and pain-free drug delivery using multiple micro-sized needles attached to a supporting membrane [4]. Microneedles enhance patient compliance owing to their comparatively effortless application making them further desirable for long-term use [5, 6].

Objectives:
To fabricate and characterize dexamethasone incorporated dissolving microneedle array.

Methods:
Microneedle arrays were prepared by solvent casting method using PDMS molds (15 x 15 array, with 200 µm square base and 500 µm length). A wide range of polymeric mixtures were trialled. The morphological and mechanical properties were assessed using SEM and texture analyser. Drug content and stability attributes of the prepared microneedles were also studied.

Results:
Dexamethasone containing dissolving microneedles showed different morphological and stability characteristics with different polymeric compositions. Each array was loaded with 12 mg of dexamethasone during formulation and it showed appreciable drug loading of 75 ± 0.48 %.

Discussion:
The drug content analysis showed the potential of the microneedles to deliver dexamethasone in therapeutically- relevant doses. This work illustrates a significant progression towards the utilisation of microneedle technology and the further planned *in vitro* and *in vivo* studies on stability, efficacy and safety can ensure its application for effective drug delivery.

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D47: Synthesis of bioactive natural products

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Natural products have been, and remain, the greatest source of medicinally relevant molecules. The synthesis of these compounds allows for the generation of useful quantities in those which are naturally scarce, as well as the prospect of producing structural analogues to increase and further probe biological activity.

Here we report on the synthesis of the natural product anithiactin A, isolated from a Streptomyces sp.,[1, 2] and the synthesis of clavatadine C, isolated from the marine sponge Suberea clavata in 2009.[3, 4] These compounds were then examined for cytotoxicity against colon and breast cancer cell lines and were found to possess moderate cytotoxicity.

Finally, efforts towards the synthesis of spiroaspertrione A will be reported. Spiroaspertrione A is a meroterpenoid, isolated from the fungus Aspergillus sp. TJ23, which exhibited antibacterial activity against methicillin-resistant Staphylococcus aureus (MRSA) with a minimum inhibitory concentration (MIC) of 4 μg/mL.[5] Spiroaspertrione A also displayed a synergistic effect against MRSA when co-dosed with the known antibiotic oxacillin, reducing the MIC of oxacillin against MRSA from 32 μg/mL to 1 μg/mL (spiroaspertrione A was also dosed at 1 μg/mL). Interestingly, no synergism was found with any other antibiotics trialled. This project aims to complete the total synthesis of spiroaspertrione A, producing sufficient quantities of material to then fully investigate its biological activity.

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D48: Identification of transporters involved in drug-drug interaction during gout treatment in primary rat hepatocytes

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Allopurinol is the gold standard therapy for gout, with its urate-lowering effect mainly attributed to its active metabolite, oxypurinol. Hypertension is a common comorbidity of gout and is often treated with diuretics such as furosemide. However, concomitant treatment with furosemide compromises the therapeutic effects of allopurinol. The molecular mechanisms underlying this adverse drug-drug interaction are unknown. Evidence from clinical studies suggests that complex interactions might be occurring in the liver, where allopurinol and oxypurinol act to lower serum uric acid. Based on current knowledge of the transport of furosemide and allopurinol/oxypurinol by transporters in the kidney and the fact that similar transporter setups exist in the liver, we hypothesise that transporters known to translocate these drugs in the kidney are responsible for the drug-drug interaction in the liver. Transporters with affinities for the above mentioned drugs and found to be expressed in human as well as rat liver were chosen as candidates, namely OAT2, OAT3, MRP4, GLUT9, NPT1, NPT4, and ABCG2. RT-qPCR and immunoblotting were performed to confirm expression of the transporters in rat liver. Primary rat hepatocytes were extracted and used as a functional model. Transport of allopurinol and oxypurinol and their effects on each other were examined by measurements of extracellular uric acid concentrations. Additionally, the contribution of candidate transporters to the transport of allopurinol and/or oxypurinol will be investigated by application of specific transporter inhibitors and siRNAs.

D49: Stimuli-responsive delivery of drug to tumor improves the survival in a mouse model

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Toxic side effects of current chemotherapy limits the use of cancer-killing agents. To overcome issues such as nonspecific tissue distribution, drug can be incorporated into particles to specifically target the tumor. Doxil, a liposomal formulation of an anticancer drug doxorubicin (DOX), is an example of clinically used nanomedicine.[1] However, although the use of a liposomal formulation of DOX has been shown to somewhat improve tumor accumulation the effect on patient outcomes has not been impressive. We hypothesize this could be improved using a responsive system whereby drug is specifically released at the tumor site.
Herein we report the design and synthesis of a block copolymer to formulate stimuli-responsive polymer particles (polyersomes) for tumor targeted drug delivery. The designed polymersomes can be triggered by either an exogenous bioorthogonal trigger (*trans*-cyclooctenol, TCO) or an endogenous trigger such as hydrogen sulfide (H_2S), as cancer cells are reported to have a higher level of H_2S due to the overexpression of H_2S producing enzymes.[2-5] *In-vitro* release studies showed slow release of DOX from polymersomes triggered with TCO, whereas release was faster with H_2S. Cytotoxicity assays performed using the B16.F10 melanoma cancer cell line found that the stimuli-responsive DOX-loaded polymersomes demonstrated more cytotoxicity than control polymersomes. The *in-vivo* efficacy of polymersomes was explored in a therapeutic B16.F10 murine melanoma model. Treatment of mice with a single dose of TCO triggered polymersomes did not improve the median survival of the mice and we hypothesise the slow release of DOX from the TCO polymersomes could not maintain a therapeutic concentration of DOX. However, mice treated with H_2S triggerable polymersomes showed improved survival and delayed tumor growth compared to mice treated with non-responsive DOX loaded polymersomes or unformulated DOX. Overall, these novel polymersomes demonstrate the potential to overcome some of the challenges in chemotherapy by improving tumour specific delivery.

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D50: Structure-Activity Relationships and Binding Optimization of *Chlamydia* HtrA Inhibitors

Agbowuro, A.A.[1], Hwang, J.[1], Peel, E.[2], Mazraani, R.[2], Springwald, A.[1], Marsh, J.W.[3], McCaugney, L.[3,4], Gamble, A.B.[1], Huston, W.M.[2] , Tyndall, J.D.A.[1]

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Despite the availability of efficacious antibacterial agents, the management of chlamydiosis remains a challenge. Human chlamydia infections are estimated to have a global incidence of approximately 130 million and clinical treatment failures have been reported, leading to concern over the effectiveness of the current general antibacterial-based treatment. We propose targeting a vital *Chlamydia* enzyme as an avenue to overcome current treatment drawbacks. The *C. trachomatis* high temperature requirement A (CtHtrA) protein is a serine protease that performs both proteolytic and chaperone functions. We previously demonstrated its importance for *Chlamydia* survival using two selective irreversible CtHtrA inhibitors, JO146 (Boc-Val-Pro-Val^{P}$(OPh)_2$) and JCP83 (Boc-Ala-Pro-Val^{P}$(OPh)_2$ in *in vivo* and *ex vivo* chlamydia models. JO146 was also found to be active against koala *Chlamydia* species with negligible toxicity on koala tissue thus validating CtHtrA as a viable drug target.

JO146 (IC_{50} = 12.5 μM) as a lead molecule is currently not potent enough, and may also be susceptible to chemical and enzymatic degradation being a peptidic molecule. The present study employs various strategies to optimize JO146 for potency and selectivity. Herein, we present the structure activity relationships of 23 JO146 analogues tested against human and koala *Chlamydia* species. The compounds span a series of reversible transition state analogues; namely α-ketoheterocycles, α-diketones, N-methylamides, valinol, α-ketoamide, and boronic acid. In addition, the P_1 and P_3 valine residues of JO146 were systematically replaced with other natural and non-natural amino acids with the aim of optimizing binding at the respective CtHtrA subpockets.

Potent inhibitors suitable for *in vivo* pre-clinical studies were identified, notably Boc-Tle-Pro-Ile^{P}$(OPh)_2$ with an approximate 700-fold increase in cellular activity relative to JO146 and the reversible Boc-Val-Pro-Val-benzothiazole with improved selectivity (~3-fold)over the closely related human neutrophil elastase. This study provides a template for obtaining clinically relevant inhibitors, towards the development of the first *Chlamydia*-specific antibacterial agents.

D51: Comparison of the uptake of rutin-loaded nanoparticles in a zebrafish model

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Rutin, a strong antioxidant, has been implicated in the prevention of liver inflammation[1]. However, low solubility and low permeability through gut wall limits the application of rutin as a therapeutic agent. Nanoparticles can improve the oral delivery of such compounds. Zebrafish are a well characterised vertebrate model for biomedical science that can be used to investigate the uptake of nanoparticles[2,3]. The aim of this study was to investigate the uptake of nanoparticles of different compositions *in vivo*, firstly in the whole zebrafish and then specifically into the liver.

To investigate uptake, poly (lactic glycolic acid) (PLGA) nanoparticles, liposomes and phytosomes containing lissamine rhodamine and 0.002 µg/mL rutin were prepared. For uptake into whole fish, nanoparticles were dispersed in 200 µL of media (E3) and zebrafish (wild type, 5 days post fertilization (dpf)) were exposed to the media for 5 h. At different time points, zebrafish were washed with E3 media and anesthetized. Zebrafish were live imaged using a fluorescent microscope (LEIKA M205 FA Leica Microsystems). The corrected total fluorescence in zebrafish was analysed using Fiji software. Uptake of nanoparticles into the liver was quantified using *Tg*(irg1:EGFP) zebrafish at 5 dpf. Following 3 h exposure to nanoparticle suspensions, zebrafish were washed with E3 media and anesthetized, then live imaged using confocal microscopy (NIKON C2+, Nikon Instruments).

A higher uptake of liposomes and phytosomes compared to PLGA nanoparticles was observed in zebrafish after 3 h exposure. Fluorescence was observed to be localised in the gastrointestinal tract and liver of zebrafish, with a higher accumulation of rutin liposomes and rutin phytosomes into the liver compared to PLGA nanoparticles. In this study, rutin loaded nanoparticles were taken up into zebrafish and liver via oral administration.

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D52: Safety of a Wireless Power Transfer System for Powering Heart Pumps

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Implantable heart pumps such as ventricular assist devices (VADs) are commonly used in patients with late stage heart failure as a bridge-to-transplant device until a suitable donor is available. Recently, with technological advancements, VADs are used as a therapeutic device for patients in early stages of heart disease. No implantable battery is capable of meeting the continuous, high power consumption requirements of a VAD. All commercially available VADs rely on a percutaneous driveline to transfer power from an external battery pack. However, the main cause of adverse events for VAD patients is infection around the driveline wound.

A transcutaneous energy transfer (TET) system consists of an external coil coupled inductively to an implanted receiving coil and delivers power wirelessly across the skin, eliminating the risks associated with a driveline. For the safety of the patient, the implant surface temperature and the rate of energy absorption by tissue when exposed to the radio frequency electromagnetic field generated by the coils must be within acceptable levels.

As excessive heat can lead to irreversible tissue damage, the International Organization for Standardization (ISO) sets a maximum implant surface temperature to be no greater than 39 °C. The International Commission on Non-Ionizing Radiation Protection (ICNIRP) limits the maximum specific absorption rate (SAR) to be 2 W/kg averaged over 10 g of tissue. This work shows that the proposed TET system has a peak SAR of 0.118 W/kg over 10 g of tissue and a maximum surface temperature of 38.3 °C, within the acceptable limits for patient use.

D53: Preformulation studies to determine the physiochemical properties of L-Glutathione

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L-Glutathione (GSH) is a tripeptide with antioxidant property (1). GSH scavenges free radicals and protect cells against reactive oxygen species and xenobiotics (2). Furthermore, a decreased GSH level is associated with various diseases like Parkinson's disease (3). Hence, GSH has the potential to be used as a nutritional supplement. Hence prior to formulation development, preformulation studies are necessitated. It is fundamentally important to determine the physiochemical properties of the candidate drug in order to develop a stable and effective formulation (4).

Objectives

To determine essential physiochemical properties useful to the development of a stable formulation for GSH.

- To determine preformulation parameters such as thermal properties, crystalline habit, partition coefficient and solubility.
- To investigate the factors influencing drug stability.
- To evaluate the antioxidant activity and cytotoxicity.

Methods

Thermal analysis was performed using differential scanning calorimetry and thermogravimetry. Crystalline habit was determined under polarized microscope. The partition coefficient, solubility and forced degradation studies were determined using high performance liquid chromatography (HPLC). For cytotoxicity and evaluation of antioxidant efficacy, cell viability was investigated with and without UVA-irradiation in skin fibroblast cells.

Results

GSH exhibits crystalline form with melting point of 195.6 to 200.0 ℃. GSH has high aqueous solubility (252.7 mg/ml), with Log D and Log P of -3.6 and -3.1 respectively. It is stable under acidic conditions as well as in normal light. No cytotoxicity was observed for fibroblast cells in the presence of GSH (0.005 to 10 mg/ml). Moreover, great protective effects were found against UV radiation in the variable concentrations of GSH tested.

Discussion

Preformulation studies are fundamental processes in the selection of an appropriate formulation for the drug candidates and is helpful in developing a stable formulation.

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D54: Synthesis and Evaluation of Menaquinone D (MenD) Inhibitors as Potential TB Therapeutics

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Menaquinone (MQ) is a lipid soluble electron carrier, central to normal function of the electron transport chain of *Mtb* and other gram-positive bacteria, in both latency and active growth. It presents a potentially useful therapeutic target as it is not produced in humans. Building on the essential role of MenD in *Mtb* survival and the established effectiveness of MenA inhibitors, a series of putative MenD inhibitors are being designed to explore the potential utility of MenD as a therapeutic target.[1]

Using a number of complementary approaches, several series of inhibitor candidates are in preparation. These approaches are informed respectively by; the results of commercial and in-house library screening, *in silico* modelling studies, and rational design informed by crystallographic data from key intermediates in the MenD-catalysed transformation of isochorismate. Progress in design and organic synthesis of inhibitor candidates will be presented, with a current summary of information gained through *in silico* compound docking, crystallography and kinetic assay data against MenD.

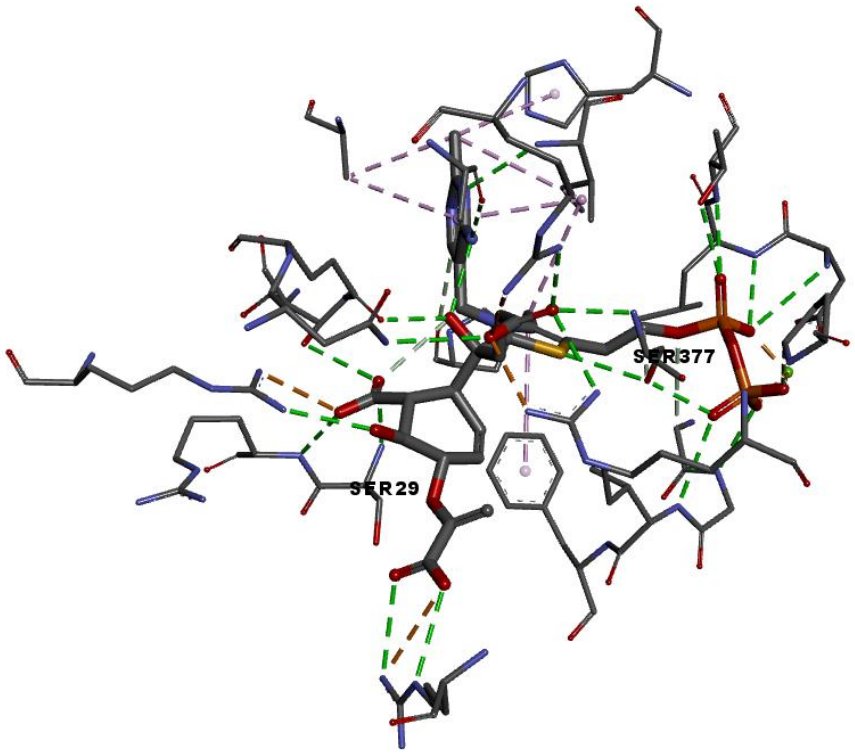

Figure 1. Crystal structure of the final intermediate in the MenD catalysed transformation of isochorismate.[2]

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D55: New Enzyme Architecture: An Investigation on the Evolution of Super-Long form ATP-PRTase

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Adenosine triphosphate phosphoribosyl transferase (ATP-PRT) is involved in the first committed step of histidine biosynthetic pathway. The enzyme is allosterically inhibited by histidine, as a means of controlling the pathway in response to metabolic demant for histidine. Previously, two molecular architectures including the homo-hexameric long form and hetero-octameric short form have been described for ATP-PRT.[1] Recently, a third structure the "super-long" form has been revealed by our examination of protein sequences. This super-long form is homo-tetrameric, resembling the non-covalent hetero-octameric complex of the short form, and has been studied from *Leuconostoc mesenteroides* (denoted *Lme*HisZGFusion). It appears likely that this protein has evolved via the fusion of genes encoding the short form complex and undergoes a large conformational change upon histidine binding.
We are currently in the process of characterisation of this enzyme and solving the X-ray crystal structure. The investigation on the evolution is pursued using molecular biology techniques. Studies are being conducted to assess whether the fission of *Lme*HisZGFusion into its constituents HisZ and HisGs subunits, leads to a functional non-covalent complex which resembles the short form ATP-PRTase.[2] We were able to construct the truncated HisZ subunit and are in the process of exploring its characteristics. Additionally, genetic knockout complementation studies are being employed to assess the essentiality of this gene product to the survival of the strain.

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