12th International NanoMedicine Conference

27th - 29th June 2022
UNSW Sydney, Australia

Abstract Booklet

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Treating Cancer with an Iron Fist

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Cancer cells have a greater demand for iron compared to normal cells, mostly to meed demands for increased metabolism. It has been known for decades that cancer cells tend to up-regulate their iron uptake. More recently, it was discovered that the cancer cells also down-regulate the iron efflux by decreasing expression of ferroportin (FPN), thereby increasing iron retention. Importantly, FPN levels have also been negatively correlated with patient prognosis. This metabolic shift in cancer cells to a higher baseline iron content alo represents a therapeutic opportunity as cancer cells are potentially more vulnerable than noncancer cells. We have found that ferumoxytol (Feraheme; FH), an FDA-approved iron oxide nanoparticle for iron deficiency treatment, can be used as a drug carrier, delivering payload of even several drugs to solid tumors, but also as an anti-cancer therapy alone. Using leukemia cell lines and primary acute myeloid leukemia patient samples (PDX), we show that low expression of FPN in the leukemic cells is a key signature. Reactive oxygen species produced by free ferrous iron lead to increased oxidative stress and cell death. Ferumoxytol treatment results in a significant reduction of disease burden in a murine acute myeloid leukemia (AML) model and patient-derived xenotransplants bearing leukemia cells with low ferroportin expression. Our findings show how a clinical nanoparticle previously considered largely biologically inert could be rapidly incorporated into clinical trials for patients with leukemia with low ferroportin levels. Importantly, we can radiolabel the nanoparticles for imaging and also load additional drugs into them. As AML leads to liver and spleen disease burden, we sought to radiolabel FH to track liver and spleen size by PET as well as assess the therapeutic benefits of FH in AML. We employed chelator free labeled ^{89}Zr-FH to image liver and spleen size in mice as well as track ^{89}Zr-FH distribution outside of these key organs. We found that mice administered ^{89}Zr-FH had an increased mean survival to saline controls and mice that died first had larger spleen sizes, presumably from disease burden. Therapy of tumors with iron oxide nanoparticles can in the right biological setting open an avenue for an entire new therapy approach, termed oxidative ferrotherapy, probably playing into ferroptosis mechanisms. Furthermore, efforts are underway to elucidate the role of nanoparticles in the tumor environment more closely.

Microfluidics for Disease Diagnosis and Monitoring

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Microfluidics has emerged as a powerful technology not only for biological but also clinical research and applications. In fact, microfluidics has also been used in many other areas such as biotechnology, pharmaceuticals, public health and environment. For example, the ability to manipulate and analyse minute volume of fluids and samples has led to a wealth of new biological assays which can provide unique microenvironments for better cell manipulation and capture. Innovative microfluidic-based clinical assays have also been developed to enable more high-throughput rapid testing and readout. Here, I will present several microfluidic technologies that we have developed for both biological and clinical applications relating to diseases such as cancer, diabetes and wound care. These includes microfluidic chips for rare diseased cell separation, for cancer diagnosis, single cell isolation to wearable sensors for physiological signal and wound monitoring.

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Aggregation-Induced Emission: Materials and Biomedical Applications

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The recent years have witnessed the fast grow of fluorogens with aggregation-induced emission characteristics (AIEgens) in biomedical research. The weak emission of AIEgens as molecular species and their bright luminescence as nanoscopic aggregates distinguish them from conventional organic luminophores and inorganic nanoparticles, making themwonderful candidates for many high-tech applications. In this talk, we summarize our recent AIE work in the development of new fluorescent bioprobes for biosensing and imaging.[1,2] The simple design and fluorescence turn-on feature of the molecular AIE bioprobes offer direct visualization of specific analytes and biological processes in aqueous media with higher sensitivity and better accuracy than traditional fluorescence turn-off probes. The AIE dot probes with different formulations and surface functionalities show advanced features over quantum dots and small molecule dyes in noninvasive cancer cell detection, long term cell tracing, and vascular imaging.[3] In addition, our recent discovery that AIEgens with high brightness and efficient reactive oxygen species generation in aggregate state further expanded their applications to image-guided cancer surgery and therapy.

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When nanomaterials meet biology: Understanding what contributes to success and failure of materials in nanomedicine

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Nanomaterials offer unique opportunities to modulate both temporal and spatial delivery of therapeutics owing to the ability to precisely control chemical and physical properties through rational design. However, it is important to understand fully how these properties vary under complex biological conditions, and how nanomaterials behave when exposed to biological cues that might be initiated through both endogenous and exogenous processes. Such complex behaviours requires the development of new approaches for probing biological systems, such that real-time assessment of both function and response can be achieved. Central to this idea is the field of theranostics, where molecular imaging is used to better understand how materials respond to changes in the environment during therapeutic delivery, or to monitor biological changes as a result of therapeutic delivery. Such materials require significant advancements in chemistry, materials science and engineering to ensure that the nanomedicine is both complementary with the biological milieu, but also able to withstand the harsh and often rapid environmental changes encountered upon injection into animals and in subsequent trafficking throughout the body.

In general, the ability to rationally optimise materials for *in vivo* drug delivery is hindered by the inability to directly assess the behaviour of the materials *in vivo*. And while biodistribution of nanomaterials and nanomedicines certainly provides initial evidence for successful delivery, it does not indicate whether a therapeutic has been successfully translocated into diseased tissue or diseased cells. Our work has focussed on developing self-reporting nanomedicines in which the nanomedicine is monitored in real-time using molecular imaging to inform on both delivery of the therapeutic, as well as efficacy of the treatment or cellular localisation of therapy. I will highlight how these studies offer unique insight into why some polymeric and biologic materials are effective, and others are not, as well as discuss the validation of personalised nanomedicines in canine trials with translation to clinical studies.

Graphene – the wonder material!

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The excitement aroused by the discovery of the phenomenal physical properties of graphene – the wonder material- has been tempered by our inability to translate these into practical devices and structures.

Herein lies the frustrating dichotomy in that pristine graphene displays the properties we crave yet is the least processable. Addition of oxygen containing functional groups to pristine graphene renders the material more processable but compromises these properties.

Over recent years we have investigated a number of strategies to tackle this dichotomy.

- Process as graphene oxide (GO): chemical exfoliation results in formation of GO with oxygen contents that render the material dispersible in water. If the GO sheets produced are sufficiently large, liquid crystalline phases are formed.[1] With subsequent control over rheological properties, GO has been used to produce GO fibres via wet spinning.[2]
- Processable reduced graphene oxide (RGO): chemical reduction of GO while controlling the pH of the media results in formation of a conducting graphene with sufficient oxygen content to improve processability e.g. forming films/membranes by LBL deposition, by air brush spraying or filtration.[3]
- Edge functionalised graphene (EFG): our more recent discovery of EFG[4] has enabled us to capture the high level physical attributes of graphene and high processability. This unusual combination of properties enables high dispersity in aqueous or organic media further extending processing options.

These approaches are providing better access to the amazing properties of the wonder material.

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Imaging Using Nanoparticles: From MRI to MPI

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The application of nanoparticles to enhance biological imaging with magnetic fields has a long and rich history. Inorganic materials with notable magnetic properties, when formed with nanoscale dimensions, can be delivered into organisms to permit visualization of specific tissue and disease states. This widespread clinical utility led to iron oxide T2 contrast agents becoming the first FDA-approved nanoparticle in the United States in 2008. Next generation nanoparticle imaging agents face a tough road, however, given the potential toxicity of nanoparticles generally and the growing demand for molecular imaging. This talk will discuss advances in MRI contrast agents, based on gadolinium (T1) and iron oxide (T2) nanoparticles, that exploit the organic surface coatings of the nanoparticles to generate high sensitivity contrast. It will also introduce how exchange-coupled superparamagnets can be designed as high-performance tracers in magnetic particle imaging (MPI).

Gadolinium-containing nanocrystals are interesting alternatives to clinical T1 gadolinium chelates as their elimination route bypasses the highly sensitive kidneys. Additionally, nanoparticles offer the possibility of both intracellular accumulation and active targeting both emerging needs for molecular imaging. Nanocrystal T1 contrast performance has been limited by surface coatings which block water from close interactions with surface gadolinium thereby diminishing T1 contrast. These steric barriers to water exchange can be minimized through shape engineering of plate-like nanocrystals that possess accessible gadoliniums at their edges. Sulfonated surface polymers further promote second-sphere relaxation processes that contribute remarkable contrast even at the highest fields (r_1 = 32.6 mM-Gd^{-1}s^{-1} at 9.4 T). These non-cytotoxic materials release no detectable free Gadolinium even under mild acidic conditions and accumulate in the liver of mice with a circulation half-life fifty percent longer than commercial agents. These features allow these T_1 MRI contrast agents to be applied for the first time to the ex-vivo detection of non-alcoholic fatty liver disease (NAFLD) in mice.

Iron oxide nanocrystals are an FDA-approved and gadolinium free alternative to standard magnetic resonance imaging (MRI) contrast agents. While their magnetic cores are responsible for T2 contrast, the non-magnetic polymers at the particle interfaces can affect the diffusion of bulk water close to particles. This impacts the spin relaxation dynamics of water and consequently makes the nanoparticle surface a powerful design tool for creating T2 contrast agents. We illustrate these effects through the diameter dependent contrast performance of iron oxide nanocrystals with different types of surface coatings. As a group these biocompatible and colloidally stable materials have excellent imaging properties; the largest core diameter (33 nm) coated with an oleic acid bilayer has to our knowledge the biggest T2 relaxivity ever reported (510 mM^{-1}s^{-1}) for an isolated iron oxide nanocrystal. A comparison of the different functional surfaces reveals that permeable surface coatings allow smaller diameter cores to reach the static dephasing limit and maximize T2 relaxivity. Localized water diffusion at IONC interfaces can be an important variable to control in the rational design of highly sensitive T_2 MRI contrast agents.

We turn to schemes to manipulate the susceptibility of magnetic nanoparticles to enhance their value in magnetic particle imaging (MPI). The ability to image superparamagnets at a distance using magnetic fields is an essential physical process in MPT. Moderate exchange interactions between oriented nanocrystals can lead to more sensitive superparamagnets particularly at low field. This property can improve the spatial resolution of magnetic particle imaging as we demonstrate in-vivo.

Artificially Intelligent Medical Nanosensors for Clinical Decisions

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Patients are often diagnosed too late or wrongly as their disease condition progresses or undergo unnecessary treatment due to an inaccurate diagnosis resulting from the limited ability of conventional methods and the limited perspective of human diagnostics. This talk will focus on a new paradigm for the development of advanced clinical decision-support systems (CDSS) based on medical nanosensors to aid decision-makers and help healthcare systems to improve the way they approach the information, insights, and the surrounding contexts as well as to promote the uptake of personalized medicine on an individualized basis.[1-3] The starting point of this paradigm is the deployment of machine learning to allow for Artificial Intelligence (AI) training by combining multiple datasets from past medical records of hundreds of thousands of patients, to identify patterns of the disease conditions within the big data ocean (Figure 1). The obtained CDSS-enable AI algorithm are then enriched with new data from a range of existing and novel tools for enabling accurate pre-screening and diagnosis while providing the most comprehensive information on the sub-type, stage, grade, and genetic mutations, etc. (Figure 1) Relying on these milestones, wearable sensing devices could enable interactive and evolving clinical decisions that could be used for evidence-based analysis and recommendations as well as for personalized monitoring of disease progress and treatment (Figure 1). Ultimately, the outcome is a detailed health status assessment from multiple viewpoints that is systemic and easy-to-use for clinical purposes, leading to improved diagnostic accuracy, increased effectiveness, and enhanced treatment efficacy. The challenges and future opportunities associated with AI-enabled non-invasive medical nanosensors in clinical decisions will be presented and discussed.

Figure 1: Conceptual diagram artificial intelligence (AI) tools and personalized clinical decision support system (CDSS) for integrating a wide spectrum of data and disease diagnosis.

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Dynamic Organ-on-a-Chip models: Bringing Bio-relevance to *In vitro* Evaluation of Nanoparticles

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Microfluidics, manipulation of fluids at the sub-millimetre scale, has given rise to biomimetic systems called organ-on-a-chip to evaluate therapies under conditions mimicking *in vivo* dynamic conditions and the microstructures of biological barriers.[1] My talk will focus on our research on evaluating nanotherapies using dynamic organ-on-a-chip models. We will focus on two novel models developed in our lab. We will highlight recent results from the Labouta Lab on the use of placenta-on-a-chip models for screening safe therapies during pregnancy. We have also developed a vessel-on-a-chip model using vascular endothelial cells subjected to a shear stress within the physiological range, 1 dyne/cm2. Using this model, we examined the effect of wall shear stress on the interaction of nanoparticles with the endothelium in regards to cell viability, cell internalization of nanoparticles, as well as their effect on the cell transcriptome. The results of this work will direct future studies towards the use of in vitro approaches for improving in vitro-in vivo correlation.

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Intelligent Nanomedicines for Tumor Microenvironment Sensing, Targeting and Regulation

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Emerging nanotechnology on precision design and fabrication of intelligent next generation nanomedicine-medical nanorobots hold the great potential to revolutionize the current landscape of drug development. It is also clear that tumor microenvironment plays critical roles on either promotion or restriction on primary tumor rapid growth and metastasis. Those achievements have made targeting and regulation of tumor microenvironment via nanobiomaterials a feasible and fruitful strategy, to improve the therapeutic outcomes for cancer treatment. This presentation will feature our recent development on using DNA and protein based nanorobots as intelligent nanomedicines to regulate tumor microenvironment to block tumor microvessels or re-store the homeostasis of tumor stroma. Robotic molecular systems have great potential as intelligent vehicles to enable the delivery of various potentmolecules, which otherwise never could be used as therapeutics due to numerous limitations. Yet, achieving in vivo, precise molecular-level, and on-demand targeting and delivery has proven extremely challenging. We developed an autonomous nanorobotic system for targeted cancer therapy, programmed to transport molecular payloads and cause on-site tumor infarction. Given the robust self-assembly behavior, exceptional designability, potent antitumor activity and minimal in vivo adversity, the nanorobot represents a promising strategy for precise drug design for cancer therapeutics.

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Social games of tumour and virus on microfluidic device with interconnected microchambers

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Microfluidic devices with heterogeneous microhabitat can be used to recapitulate bacterial social interaction, bacterial resistance, cancer drug resistance, and virus transmission in society.[1-3] We demonstrated that the emergence of drug resistance was accelerated in brain and breast cancers using a cancer drug resistance accelerator (CDRA) chip within 10 days. The CDRA chip consists of 488 interconnected microchambers (200 μm diameter) surrounded with two microchannels, enabling cells to be cultured under gradients of drug and nutrients over two weeks. We observed both normal sized drug-resistant cells and polyploidy giant cancer cells (PGCCs) in microchambers perfused with high concentrations of drug. On the other hand, the chip was used to monitor virus propagation rates by seeding host cells (lung fibroblasts) infected with human coronavirus in the center of a chip filled with uninfected host cells (**Figure 1**). The rate of propagation depended on the initial number of infected cells (I_0), the density of susceptible cells (S_0), and the proportion of immunized cells on the chip. Taken together, it is suggested that our microfluidic platform technology can be utilised for drug screening and disease transmission modelling.

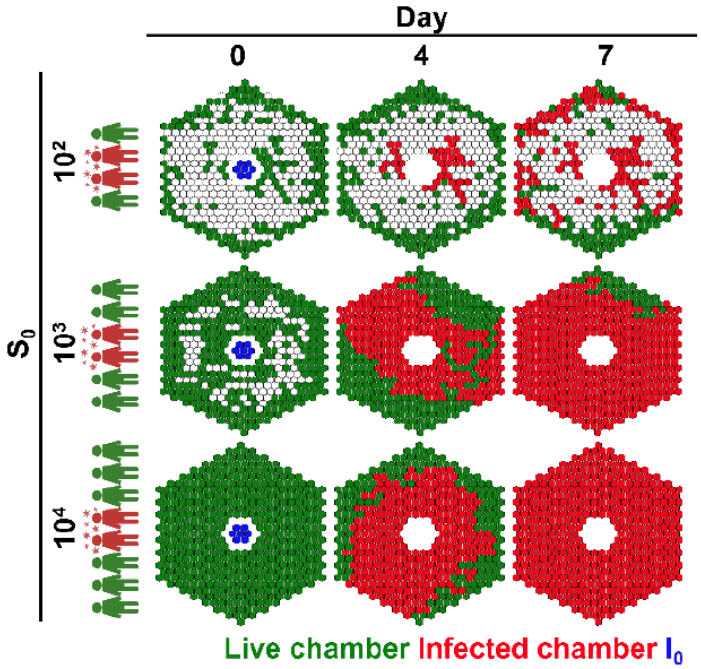

Figure 1: Effect of the density of susceptible cells on virus transmission

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Design of Biohybrid Systems for targeted cancer therapy

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"Biomimicry" derives from Ancient Greek, and it encompasses the fusion of the word *Bios* (life), and *mīmēsis* (imitation), i.e. imitation of Nature. Cells are natural biological entities that secrete small (nano) extracellular vesicles (EVs) as a mean to transport information and biomelcules into other cells. Receiving cells are then equipped with recognition motifs that enable the selective internalization of these EVs.

The imitation of this natural process that enables cells to communicate with both neighbouring and distant cells has inspired the development of new nanotechnological strategies to improve the delivery of drugs at their site of action. Our group has recently conceived nano-biohybrid vesicles, obtained through the fusion of cell-derived components with conventional synthetic materials, which represent an unreported chimeric drug delivery system (DDS) with ideal properties in terms of nano-size (which enables to reach and accumulate at the diseased area), surface cues (which preserve the targeting properties inherited from their original parent cells) as well as ease of loading and functionalization (from the synthetic components), which pave the way towards new advances in the field of nanomedicine.

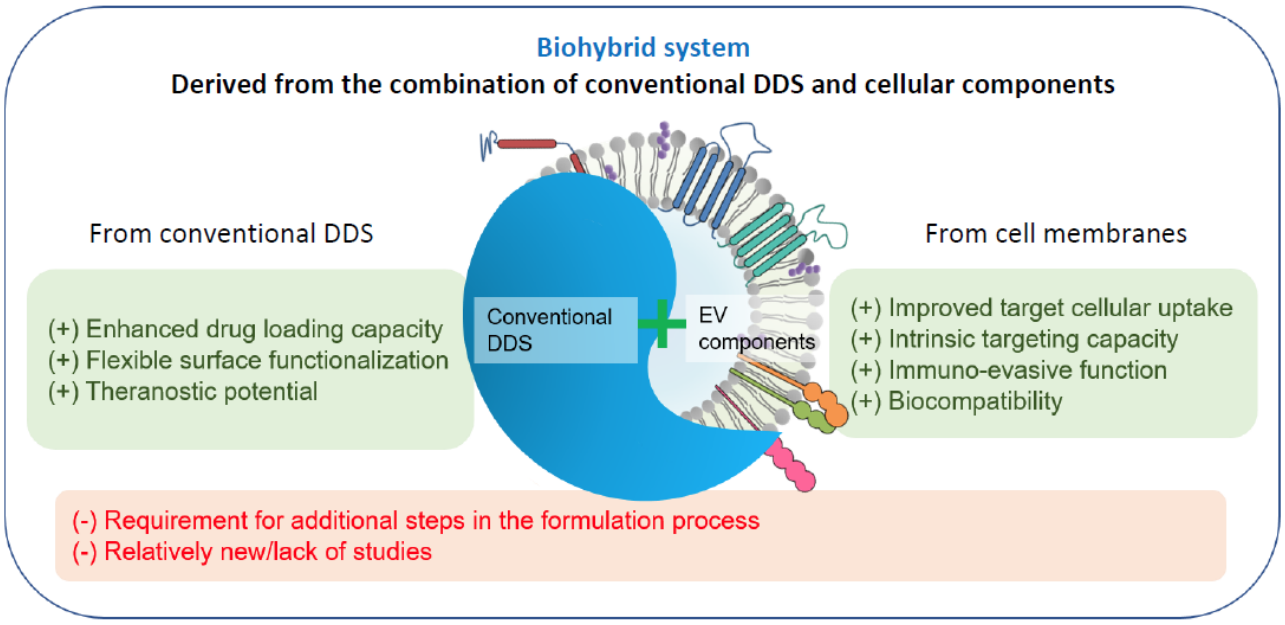

Figure: Schematic representation of a bio-hybrid system, derived from fusing lipids with cell membranes. This system is expected to adopt the beneficial properties from both conventional DDS (i.e. good drug loading capacity, flexibility in functionalization, and theranostic potential) and cellular components (i.e. good biocompatibility, improved cellular uptake and targeting, and ability to prevent premature immune clearance).

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Tissue-Inspired Synthetic Biomaterials

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Improved experimental model systems are critically needed to better understand cancer progression and bridge the gap between lab bench proof-of-concept studies, validation in animal models, and eventual clinical application. Many methods exist to create biomaterials, including hydrogels, which we use to study cells in contexts more akin to what they experience in the human body. Our lab has multiple approaches to create such biomaterials, based on combinations of poly(ethylene glycol) (PEG) with peptides and zwitterions. In this presentation, I will discuss our synthetic approaches to building life-like materials, how we use these systems to grow cells and understand how a cell's environment, particularly the extracellular matrix regulates cancer cell growth, dormancy, and drug sensitivity.

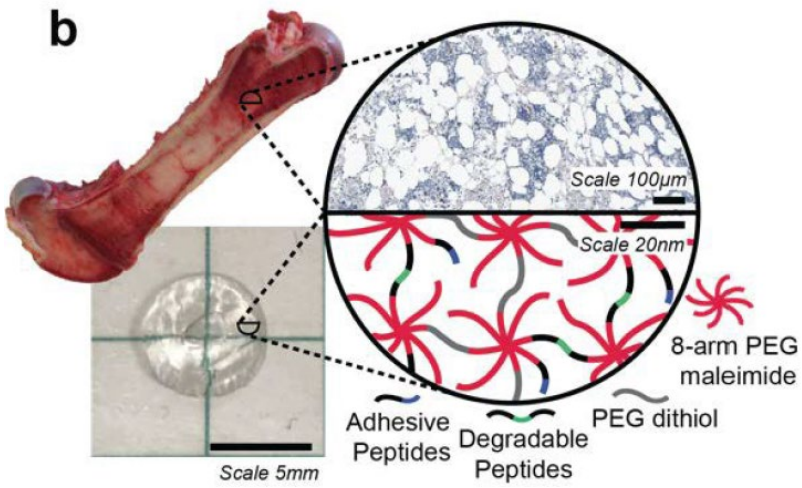

Figure: **(Example of bone marrow-mimicking hydrogel)** we use a combination of mechanical characterization and tissue mass spectrometry to analyze real tissues (bone marrow example shown). Then we approximate those tissue characteristics with combinations of synthetic polymers and peptides for a variety of applications in cancer, tissue engineering, and TBI.

Transcytosic Nanomedicine for EPR-Independent Cancer Drug Delivery

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The enhanced permeability and retention (EPR) effect or so-called "passive tumor accumulation" has been the basis for design of cancer nanomedicine; however, the EPR features are much less characteristic and highly heterogeneous in human tumors, resulting in unsatisfactory clinical efficacies of current nanomedicines. We developed a strategy using nanocarriers to induce transcytosis of tumor endothelial and cancer cells and enable nanomedicines to actively extravasate into and infiltrate in solid tumors, which led to radically increased anticancer activity, i.e., completely eradicating small solid tumors (~100 mm^3) and large established tumours of clinically relevant sizes (~500 mm^3) and significantly extending the survival of mice bearing orthotopic pancreatic tumours. So carrier-induced transcytosis of tumor endothelial cells enables EPR-independent extravasation of nanomedicines and overcomes the inherent extravasation and infiltration dilemmas of nanomedicines.

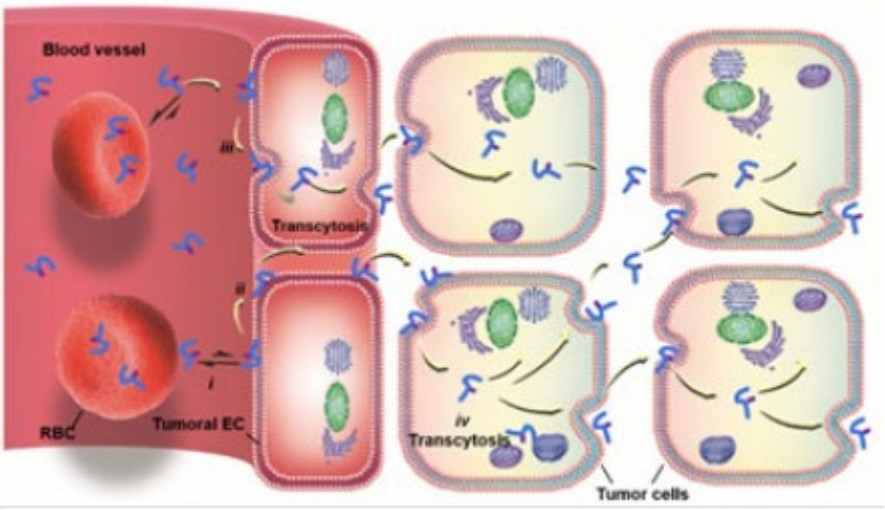

Figure 1: Transcytosis enabled-active extravasation and tumor penetration

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Non-spherical polymersomes and the (new) RNA world

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What do nanomedicine, the recent RNA revolution and the origin of life have to do with each other? Quite a lot! The origin of life remains as one of the biggest, if not the biggest unsolved challenges in Science. In pre-biotic chemistry, significant emphasis has been of late on synthetic cells or "protocells", where the aim is to de novo design of a system that displays all the hallmarks of life: metabolism, reproduction, and compartmentalisation.[1] Compartmentalisation is the separation of a (proto)cell or organelle from its outer environment, which typically is thought of as happening via a lipid bilayer membrane. We entered this field focusing on using polymersomes, as they are more chemically and kinetically inert than liposomes. Importantly, polymersomes are also very useful in drug delivery giving us a twofold motivation for exploring innovative ideas in generating functional polymersomes through the combination of supramolecular and bioconjugation chemistry strategies.

Using this approach we have to date developed polymersomes mimicking key functions of biology ranging from asymmetry in shape (non-spherical polymersomes),[2-3] incorporation of biological components and generating / compartmentalising chemical energy.[4] We have also demonstrated that these polymersomes are an excellent platform for drug delivery in nanomedicine, as exemplified in our work on peptide-functionalized polymersomes targeting medullablastoma as an illustrate example.[8-9]

More recently we moved from looking at polymersomes as organelle mimicks to studying RNA-peptide aggregates as mimicks of biologically occurring RNA-protein based membraneless organelles – often referred also to as condensates or liquid-liquid phase separated droplets (Figure 1).[10] This takes us also back to the pre-biotic question of the RNA world and how RNA and peptides may have co-evolved into the ribosomes. And as in our earlier work, this does is also highly relevant to delivery challenges, in this case with regards to RNA therapeutics, where despite the success of lipid nanoparticles in the current mRNA vaccines, much remains to be done to improve RNA delivery system. To tackle that and other important challenges in RNA science, technology and therapeutics, we at UNSW have to come together to establish the UNSW RNA Institute which I will also briefly introduce here as well.

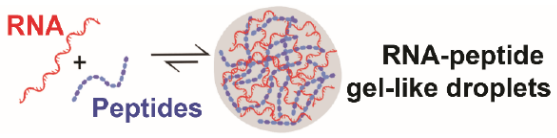

Figure 1: RNA-peptide liquid-liquid phase separated droplets.

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Novel conducting polymer biointerfaces

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Conducting polymers (CPs) have been widely used as electroactive biointerfaces in applications such as electrically stimulated tissue engineering and stretchable organic bioelectronics. In this talk, we will present three different approaches to electroactive biointerfaces. The first approach overcomes the issue of poor solubility and processability of CP by functionalization of CPs with various moieties, in particular by grafting of CP backbones with polymeric side chains [1,2]. That enables modification of optoelectronic, chemical and mechanical properties of the CPs and their use as smart biointerfaces; e.g. responsive to various stimuli, intrinsically stretchable and self-healing. The second approach addresses the issue that electrodes are commonly 2D and as such cannot fully probe the actual 3D cell environment within tissues and organs. Our approach to overcome that is based on a precise fabrication of individually addressable, high aspect ratio, 3D CP-pillar microelectrode arrays by means of 'micro-extrusion printing'. Such 3D microelectrode arrays could be employed in a variety of applications, from biological sensing to recording and electrically stimulating cells and tissues [3], with the design of the arrays being easily adjustable to a particular application. The third approach is based on a design and fabrication, by electrospinning, of flexible, microporous, electrochemically switchable membranes. We demonstrate the use of such membranes for gene sensing and for fast, selective, nondestructive and efficient capture and subsequent release of extracellular vesicles (EVs) [4] and rare cells. In the later case, the membrane concentrates EVs and cells from large volumes of biological samples and into clean and small volumes of buffers, demonstrating a great promise for liquid biopsies.

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NIR-II Fluorescent Probes for in vivo Multiplexed Biodetection

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Fluorescent imaging and sensing with high spatio-temporal resolution and sensitivity allow the direct visualization of dynamic biological interests at different levels of components from the molecules, cells in vitro to the tissues, organs in vivo. Disastrous light attenuation and background autofluorescence in tissue at conventional imaging window of 400-900 nm have limited this technique for in vivo analysis, but they both decrease at progressively longer wavelength. Over the past decade, advances in the development of functional fluorophores operating in the second near-infrared window (NIR-II; 1000-1700 nm) have allowed the investigations of deep anatomical features in vivo with high resolution and sensitivity. However, inhomogeneous signal attenuation due to biological matter hampers the application of multiple-wavelengths NIR-II probes to multiplexed imaging. Here we present lanthanide doped NIR-II nanoparticles with engineered luminescence lifetimes for in vivo quantitative imaging using time-domain multiplexing. To achieve this, we devise a systematic approach based on controlled energy relay that creates a tunable lifetime range spanning 3 orders-of magnitude upon a single emission band. We consistently resolve selected lifetimes from the NIR-II nanoparticle probes at depths up to 8 mm in biological tissues, where signal-to-noise ratio derived from intensity measurements drops below 1.5. We demonstrate that robust lifetime coding is independent of tissue penetration depth, and we apply in vivo multiplexing to identify tumour subtypes in living mice. Our results correlate well with standard ex vivo immunohistochemistry assays, suggesting that luminescence lifetime imaging could be used as a minimally invasive approach for disease diagnosis.

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Role of nanoparticle stiffness in regulating nano-bio interactions

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Mechanical properties play critical roles in many biological processes. For instance, normal human red blood cells (RBCs), which are flexible and deformable, can circulate in vasculatures for 120 days, while aging RBCs become less deformable and thus are prone to elimination by the spleen. Also, the human immunodeficiency virus (HIV) was reported to regulate its stiffness at different life cycle stages. Immature HIV particles are relatively more rigid for budding out of a host, while mature HIV particles become deformable to facilitate their entry into the host cell. Similarly, the physicochemical properties of synthetic NPs (such as size and surface properties) play critical roles in their biological functions. Recently, it has emerged that the mechanical property of NPs (stiffness or elasticity) affect biological functions. However, the effect of this parameter on nano-bio interactions and the underlying mechanism are still not well understood[1]. My lab created a library of nanocapsules (NCs) with a wide range of stiffness (kPa to GPa) that enable isolation of the effects of NP stiffness across four orders of magnitude from other properties, resulting in an unprecedented level of understanding of this vital parameter[2,3.] Our studies demonstrated the critical role of nanoparticle stiffness in regulating the formation of protein corona, immune evasion, receptor mediated nanoparticle-cell interactions and targeted drug delivery[4]. These new insights have significant implications in designing new nanoparticles for enhanced drug delivery.

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Welcome to Magnetic Particle Imaging – A new era in preclinical imaging

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Magnetic Particle Imaging (MPI) and **Localised Magnetic Fluid Hyperthermia** (Localised MFH) are two synergistic technologies that, together, enable a theranostic platform [(1,2)]. MPI is a novel technique that detects the magnetic properties of iron oxide particles (ION) to produce 3D images. This breakthrough technology provides for rapid and efficient imaging timeframes, requires no radiation, accommodates for absolute quantification at any depth, and is 100x more sensitive than MRI with applications in the disciplines of material science and biotechnology. The MPI will be paired with localised hyperthermia whereby magnetic particles generate therapeutic heat in well-defined target regions based on the MPI defined region. Exposure to temperatures of 42 °C damages cancer cells, triggers apoptosis and makes them more vulnerable to standard chemotherapy. *The recently installed MPI/MFH systems at Monash Preclinical Imaging Facility is Australia's only MPI scanner with CT and the world's first commercial MFH system.* I will highlight how these technologies offer a unique theranostic set up for diagnostic and therapy of various diseases.

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From Bench to Boardroom - bringing science to practice

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Medical Director at Amgen and President of APPA

Nanotechnology clearly represents a change in the wind within drug development as modalities of medicines become more personalized and sophisticated. Nanotech is already at the bedside and is attracting attention and investment at a plethora of levels. The paradigm challenge to conventional drug-delivery systems has the potential not only to improve the efficacy of current and future compounds, but also change the way medicine is scheduled and administered which could improve the Quality use of Medicine (QUM). With all this promise comes and imperative to streamline and optimise the commercialization of a new generation of medical products. Without industry backing at a scientific and organizational (boardroom) level, much of this science won't actually get to patients[1,2].

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Nanoplasmonic Material based Optofluidic Biosensors for Immune Functional Analysis towards Personalized Immune Therapy

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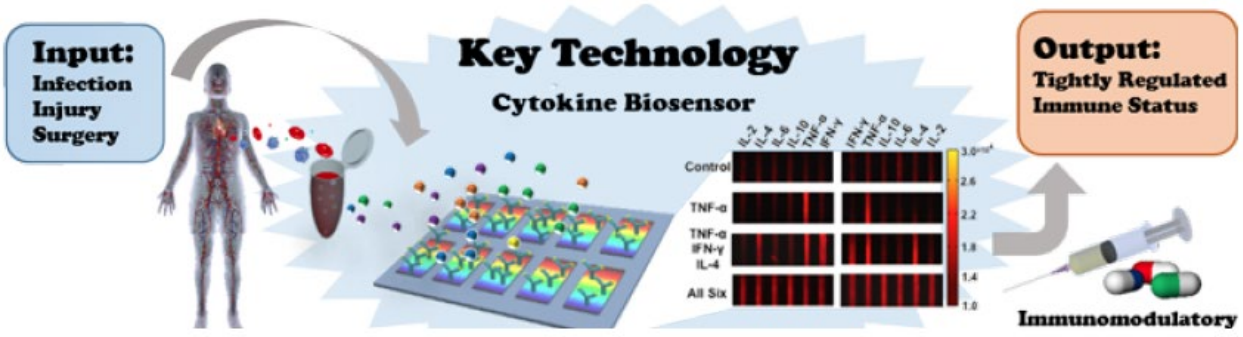
The study of immune functional responses is essential to understand the central role of the immune system in providing immunological host defence and its intercommunication with other systems. Cytokines are one of the key biomolecules acting as intercellular mediators and modulators to regulate the diverse functions in the immune response. Rapid and accurate quantification of cytokine-based immune fingerprints plays a decisive role in effectively treating immune-related diseases especially at point-of-care, where an immediate decision on treatment is needed upon precise determination of individual patient's immune status. Derived from the emerging clinical demands, there is an urgent need for cytokine immunoassays that offers unprecedented sensor performance with high sensitivity, throughput, and multiplexing capability, as well as short turnaround time at low system complexity. In this talk, we will present a number of novel plasmonic nanomaterial based optofluidic cytokine biosensors for immune functional analysis from whole blood to single cell level. The multi-scale research both experimentally and theoretically will bridge the gap in fundamental understanding of immune system and enhance the applicability, diagnosis and prediction power for immune diseases. The developed platforms would ultimately gear the biologists and clinicians with capability to real-time monitor the immune status of patients, a transformative achievement that has immense potential towards safe, effective, and personalized immune therapy.



Nanozymes and polymers for nitric oxide delivery from endogenous and exogenous prodrugs

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Nitric oxide (NO) is a potent biological molecule that contributes to a wide spectrum of physiological systems, including cardiovascular system, immune system, central nervous system, and outflow physiology.[1-4] However, NO delivery technology remains severely limited due to the physiological properties of NO: 1) NO has a short half-life in human tissues (seconds); 2) NO can only diffuse over short distances (~100 μm), thus limiting its action to only areas near the source of delivery; and 3) NO can exert protective or deleterious effects depending on its concentration. Current strategies for NO delivery focus on encapsulation of NO donors into pre-fabricated scaffolds or an enzyme-prodrug therapy approach. The former is limited by the finite pool of NO donors available, while the latter is challenged by the inherent low stability of natural enzymes. Enzyme mimics are attractive substitutes for their natural counterparts in diverse biomedical applications because they have excellent stability against biological degradation, high temperature, and extreme pH conditions compared with natural enzymes. In this work, we present nanoparticles and polymers that can catalytically decompose endogenous and exogenous NO donors (e.g. *S*-nitrosoglutathione, *S*nitrosocysteine, and β-gal-NONOate) to generate NO at physiological conditions.[5-7] With this approach, we envision that sustained NO delivery could be achieved by relying on life-long pools of endogenous NO donors, and when needed, on-demand NO delivery at the desired levels of NO could be realized by externally administered exogenous NO prodrugs. By tuning the concentrations of particles/polymers and NO prodrugs, physiologically relevant NO levels were generated. These materials preserved their catalytic property to generate NO for at least 6 months. The nanoparticles and polymers were immobilized in biomaterials and on surfaces, and we demonstrated the therapeutic activity of NO to inhibit cancer cell proliferation and disperse bacterial biofilms.

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Deconstructing Solid Tumour Heterogeneity: The Stromal Matrix Perspective

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Homeostasis of the extracellular matrix (ECM) is critical for correct organ and tissue function. Both the biochemical and biomechanical properties of the matrix contribute to modulating the behaviour of resident cells and are more than just passive bystanders. In tissue diseases such as cancer, we have shown that the matrix undergoes significant change. These changes, driven by both tumour and local and recruited stromal cells, feed into the pathological progression of the disease[1].

Our studies have shown that the matrix and matrix remodelling can both promote and restrict tumour progression. Through deploying multiple approaches to characterise tumour matrix remodelling, including the development of new technologies to visualise and catalogue the matrix over both time and space, and subsequently recapitulate these microenvironments *in vitro*, we are gaining insight into the factors that shape the development, evolution and cellular heterogeneity of a tumour, as well as its response to a particular therapy.

The non-selective depletion of the matrix has yielded paradoxical results, often accelerating progression. Instead, we have shown that more nuanced approaches to normalising biochemistry and biomechanics, rather than depleting the matrix results in favourable outcomes. As such, co-targeting the changing matrix in cancer, as well as the cellular response to the remodelled tumour matrix offer powerful approaches to improve therapy outcome for patients.

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It's Science, not fiction: the clinical application of AAV gene therapy in child neurology

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Sulfoxide Polymers: A New Class of Low-fouling Polymers for Biological Applications

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Antifouling surfaces are important in a broad range of biological applications such as in medical devices or nanomedicines. An effective approach to antifouling surfaces is to covalently attach antifouling polymer brushes. Poly(ethylene glycol) (PEG) has been used as the standard antifouling polymer for decades. However, recent evidence has shown the emergence of limitations of PEG including its apparent immunogenicity and oxidative instability, which compromises its function as a safe antifouling polymer. This drives the development of other antifouling polymers as alternatives to PEG. The talk will present our nresearch on investigating the potential of an innovative class of sulfoxide polymers as nantifouling polymers (Figure 1). I will talk about how the sulfoxide polymers can modulate the interaction between material surfaces and biological system, and their potential napplications in constructing antifouling surfaces and advanced nanomedicine.[1-4]

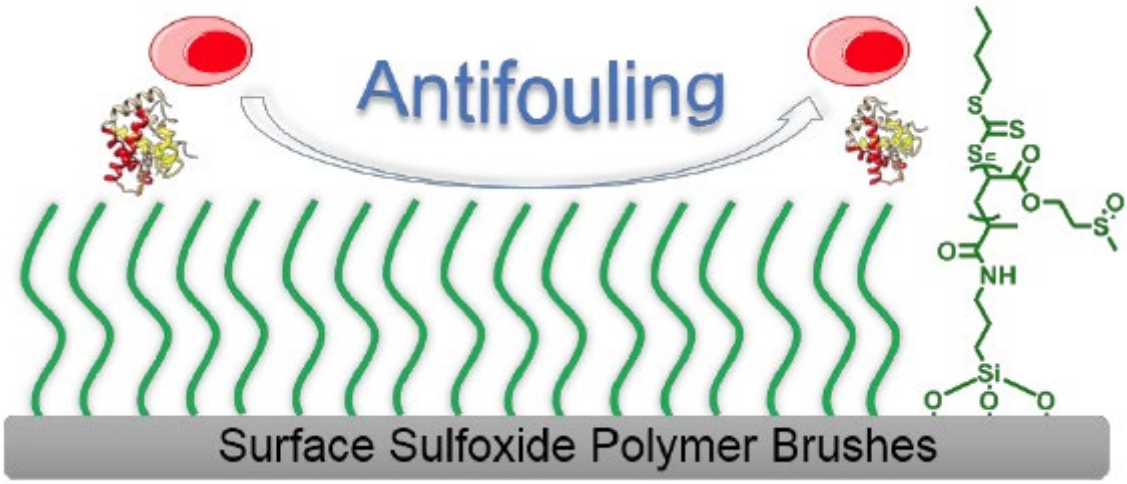

Figure 1: Sulfoxide polymers enable antifouling surfaces that resist unwanted adhesion of proteins and organisms.

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Engineering Nanosheets for Disease Diagnosis and Treatment

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Two-dimensional nanosheets, which are featured with large surface areas, biodegradable, and stimuli-responsive properties, have attracted vast interests for high performance biomedical applications.[1] In the first part of my talk, I will present a pH ultrasensitive magnetic resonance imaging (MRI) contrast agent for tumour imaging, which was realized by constructing a Mn-doped layered double hydroxide (LDH) nanosheet.[2] The Mn-LDH could sensitively respond to a very weakly acidic tumour microenvironment (pH 6.5–7.0) with satisfactory tumour imaging performance. The mechanism was revealed to be interaction between protons and unique microstructure of Mn-LDH. Furthermore, cell specific targeting and improved tissue penetration for enhanced MR imaging of tumours was achieved by constructing a cell-derived biomimetic Mn-LDH nanosheet.[3] In the second part of my talk, I will discuss the synthesis and applications of multifunctional monolayer and few-layered LDH nanosheets. The nanosheets were synthesized via a polymer-assisted bottom-up method, and demonstrated high catalytic activity to disproportionate hydrogen peroxide in tumours and consequently in-situ generated a considerable amount of hydroxyl radicals at a high reaction rate to kill tumour cells.[4] We also found that the nanosheet generated oxygen bubbles and promoted the long travel distance with presence of stimuli in the tumour microenvironment.[5] This responsive movement has chemostatic properties, evidenced by the directional movement of the nanosheet toward hydrogen peroxide. Also, the nanosheet-based ultra-high drug loading system exhibited higher therapeutic efficacy and reduced systemic toxicity compared to the free drug and even the high drug loading system. Last but not the least, I will discuss our latest study on ultrasound-responsive Mxene nanosheets for bacterial infection elimination.

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Smart materials for cardiovascular disease therapy

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Cardiovascular disease (CVD) remains the main cause of death worldwide. Novel therapeutics are urgently needed to deliver drugs to unstable atherosclerotic plaques as the underlying cause of CVD. More targeted approaches are also required to treat patients in an emergency (myocardial infarction and stroke) when therapeutics are ideally administered directly in the ambulance to prevent loss of vital tissue. However, the significant adverse effects of current drugs such as bleeding complications are major hurdles to overcome. Nanomedicine plays an increasingly important role in the development of smart and responsive therapies for CVD.

Unstable plaque rupture is responsible for more than two-thirds of fatal myocardial infarctions and the majority of strokes. Matrix metalloproteinase (MMP) 14, a membranetype MMP, has been associated with plaque rupture and plays a pivotal role in activating collagen degradative activity of several other MMPs. To inhibit MMP 14, we have developed targeted 100 nm nanosponges by a metal catalyst polymerisation method and loaded them with the specific blocker Naphthofluorescein. The particles were functionalised with a collagen-binding peptide, an activatable cell-penetrating peptide to facilitate cell entry and aCy3 dye for detection. Our novel construct has an immense translational potential for biologic drug development to stabilise the structural integrity of human plaques to prevent rupture-induced acute thrombosis.

In the event of a ruptured plaque, fast delivery of anticoagulants and/or thrombolytics using nanoparticles can restore blood flow and oxygen supply to affected tissues. We have exploited key biomechanical features specific to thrombosis such as increased blood shear stress and the presence of the pro-coagulant enzyme thrombin to achieve site-directed delivery of drugs. We demonstrated that shear-sensitive phosphatidylcholine based nanocapsules can deliver anti-thrombotic drugs and inhibit thrombus formation selectively under stenotic and high shear flow conditions while leaving thrombus formation under physiologic shear rates unaffected. Furthermore, we have developed state-of-the-art carriers that can deliver thrombolytic drugs such as plasminogen activators and that responds to the thrombus microenvironment to initiate thrombolysis. Our non-invasive and effective agent holds great promise for major progress towards a novel therapy for a large number of patients suffering from thrombotic diseases.

In conclusion, modern nanomedicine is increasingly been used in the CVD field to tackle some of the most pressing therapeutic challenges: the stabilisation of atherosclerotic plaques that are prone to rupture as well as delivery of potent clot busters in a more targeted way with reduced adverse effects. Future success in this area has the potential to benefit many patients with some of the most devastating CVD conditions.

Acknowledgements
This work has been supported by the Australian National Health and Medical Research Council, Australian Research Council and National Heart Foundation.

Barriers of nanoparticle delivery in solid tumours, how do we solve them?

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Systemic delivery of therapeutics and imaging agents including nanoparticles, in tumours relies primarily on accessibility via tumour blood vessels. Whilst some tumours and dysplastic nodules are hyper-vascularised and well-perfused, other aggressive and poorly differentiated tumours show a decrease in vascular function and perfusion. Recently, we established the use of a peptide ligand to target aberrantly expressed extracellular matrix (ECM) in mouse and human tumours[1]. We used this ligand as a tumour-ECM targeting agent to deliver imaging contrast agents[2] and ECM-depleting drug1 in solid cancers. Our targeted nanoparticles (NP) encapsulating imaging agent accumulated significantly in tumour parenchyma, in comparison to low non-specific binding in normal and healthy tissues2. We suggest that this is an effective molecular imaging agent to detect cancer fibrosis. However, the targeted NP is less effective in poorly perfused tumours, such as hepatocellular carcinoma (HCC). This is because HCC with intact ECM is systemically inaccessible to large molecules including NP. Using our ECM-reducing agent1, we recently established that opening of tumour blood vessels by ECM depletion improved tumour perfusion and access of NP[3].

Other NP targeted to poorly perfused tumours may encounter similar perfusion barrier, irrespective of the abundance of targeting moieties unique to the tumours. Our findings suggest strategies to improve systemic access may be particularly important for targeting poorly perfused tumours. **Table 1** below summarises the outcomes of our work, comparing the systemic uptake of small and larger molecules, in moderately and poorly perfused tumours.

Table 1: Systemically injection agents and their uptake in solid tumours[1,2,3].

Agent	Moderately perfused tumour	Poorly perfused tumour (e.g. HCC)
Tumour-targeting peptides (<1 kDa)	✓	✓
Targeted protein therapeutics (<30 kDa)	✓	✓
Lectin (>60 kDa, vessel-binding agent)	✓	low
Untargeted NP (<50 nm)	low	low
Targeted NP (<50 nm)	✓	low
ECM-depletion agent + untargeted NP	✓	✓

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Cell-material interaction *via* controlled mechanotransduction

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The dynamics of cell mechanics and epigenetic signatures direct cell behaviour and fate, thus influencing regenerative outcomes. Cell-to-material interaction is driven by the capacity of cells to adhere to the substrates and react to its features by rearranging nano-sized focal adhesion complex (FA), cytoskeletal elements and activation of mechanotransduction signalling pathways[1]. We investigated how cells respond to nano-islands and 3D polycaprolactone (PCL) fibrous 3D scaffolds.

Mesenchymal stem cells on smaller nanodomain spacings recruit more activated FAK and Src proteins to produce larger, longer-lived, and increased numbers of focal adhesions (FAs), with higher Rac1, cytosolic β-catenin, and nuclear localization of YAP/TAZ and RUNX2, which together biased the commitment of hMSCs to an osteogenic fate.

Another study assessed human primary osteoblast cells' sensing and response to random and aligned PCL scaffolds via the melt electrowriting (MEW) technique. Compared to 2D TCP, 3D MEW fibrous substrates led to immature vinculin focal adhesion formation and significantly reduced nuclear localization of YAP. Notably, aligned MEW fibers induced elongated cell and nucleus shape and highly activated global DNA methylation of 5- methylcytosine, 5-hydroxymethylcytosine, and N-6 methylated deoxyadenosine compared to the random fibers (Figure 1). Our research showed that cells can sense and respond to various materials via nano-scaled FA and downstream mechanotransduction and nuclear epigenetics.

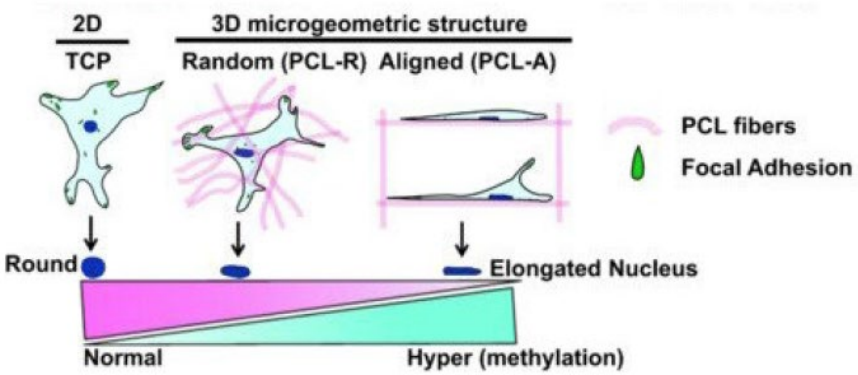

Figure 1: Schematic illustration of osteoblast response to fiber alignment via altered focal adhesion, nuclear mechanosensing, and global methylation.

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New Ways to See Disease via Nanomaterials

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In this presentation I will discuss my lab's use of nanomaterials in medicine. We work both in vitro and in vivo. In vitro work includes diagnostics based on protease detection. I will describe applications in COVID and periodontal disease. Our in vivo work primarily uses photoacoustic imaging, which combines the contrast of optics with the temporal and spatial resolution of ultrasound: It is "light in/sound out" as opposed to traditional "sound in/sound out" ultrasound. I will present results on acoustic-based cell tracking, real-time monitoring of reactive oxygen species, as well as contrast agent-free translational work for wound care and oral health including first-in-man studies.

Biography: Jesse V. Jokerst is a Professor in the Department of NanoEngineering at UC San Diego. Dr. Jokerst graduated *cum laude* from Truman State University in 2003 with a B.S. in Chemistry and completed a Ph.D. in Chemistry at The University of Texas at Austin in 2009. Jesse was a postdoc at Stanford Radiology from 2009-2013 and was an Instructor in that same department from 2013-2015. Jesse started at UCSD in July of 2015, and he has received the NIH K99/R00 Pathway to Independence Award, the NIH New Innovator Award, the NSF CAREER Award, and Stanford Radiology Alumni of the Year Award. He serves on the Editorial Advisory Board of *ACS Applied Nano Materials.*

Cellular and Molecular Mechanbiology in Cardiovascular Health

Lining Arnold Ju

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By linking the mechanical forces behind blood flow and their effects on hematological proteins and blood clotting cells, Dr Ju has established a new field called 'mechanobiology'. His work finds better solutions to help diagnose, treat, and control blood clotting diseases.

To investigate mechanobiology at single cellular to molecular scales, Dr Ju has invented a nanotool called the Biomembrane Force Probe (BFP). It provides precise controls and quantitative readouts in both mechanical and chemical terms, which is particularly suited for live-cell mechanosensing studies over the traditional methods in biochemistry and cell biology that are usually population-averaged and non-real time. He also uses use single-cellular technology to study the mechanism of platelets that produce thrombi in a complex hemodynamic microenvironment.

After that, Dr Ju has continued to upgrade this pioneering technology. To further the platelet mechanobiology studies in a physiological context with dynamic blood flow, Dr Ju complemented the BFP with other cutting-edge technologies building 4Ms: Mechanics, Microscopy, Microfabrication & Mouse model, which integrates the fields of biomechanical engineering, imaging, microfluidics, and molecular biology.

Through his world-leading discovery of a series of mechanosensory ('force-sensing') proteins and by understanding how blood cells utilize these force sensors to process mechanical cues in circulation, Dr Ju has developed novel therapeutic strategies to intervene and prevent diseaseforming blood clots early and efficiently.

Impact of Person-Specific Biomolecular Coronas on Nanoparticle–Immune Cell Interactions

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It is well known that particles interact with a multitude of plasma components, forming a biomolecular corona. This corona is the primary determinant of downstream biological responses of particles, including recognition by and association with human immune cells. The formation of a biomolecular corona is person-specific, given the variation in the blood proteome as a result of genetic background, lifestyle, and underlying health conditions of an individual. Research on person-specific coronas of nanoparticles has focused on diseased-derived plasma variance, whereas variance among healthy donors has been overlooked. However, in clinical trials, healthy volunteers are commonly involved to provide important baseline information about new therapeutics. Therefore, unravelling the effect of blood variance among healthy donors on corona composition and particle immune cell association should help to rationally improve the success of nanomedicines.

In this project, we investigated the formation of personalized biomolecular coronas on particles using plasma from a cohort of healthy donors and their impact on particle–immune cell interactions using an *ex vivo* human blood assay.[1] A matrix of mesoporous silica (MS), poly(ethylene glycol) (PEG)-coated MS particles, and PEG particles (where the MS template is removed) with different sizes (800, 450, 100 nm) were examined and compared with clinically relevant PEGylated doxorubicin-encapsulated liposomes (Doxil). We then performed an in-depth proteomics characterization of the biomolecular protein coronas that was correlated to the nanoparticle–blood cell association results. Our results show that the personalized coronas formed on the MS, PEG-coated MS, and Doxil nanoparticles from plasma of each donor significantly influence the interactions of the nanoparticles with monocytes and B cells (up to a 60-fold difference) regardless of the particle size, dosage, or donors of immune cells. Distinct proteomic fingerprints were observed on the donor-specific coronas, with individual variance in the proteome driving differential association with specific immune cell types. We identify key immunoglobulin and complement proteins that are explicitly enriched or depleted within the corona and that significantly correlate with the cell association pattern observed across the healthy donors. This study demonstrates how plasma variance in healthy individuals significantly influences the blood immune cell interactions of nanoparticles.

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Stealth Poly(2-oxazoline) Nanoparticles and Nanorods for Biomedical Applications

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We report the preparation of functional and biocompatible poly(2-oxazoline) (POx)- based nanoparticles of different morphologies and discuss their bio-nano interactions. Pox are a highly functional polymer class, which provides access to tailor-made polymers with tuneable physicochemical properties. The water-soluble variants are highly biocompatible, non-immunogenic and show a stealth behaviour. Thus, they are highly interesting as shell materials for diverse nanoparticles used in a biological context.

In this presentation we will highlight our recent efforts in fabricating spherical and rod-shaped POx nanoparticles through, e.g. emulsion processes,[1] surface coatings of inorganic nanoparticles[2] and crystallization-driven self-assembly (CDSA).[3] The potential to modulate the interaction of these nanoparticles with biological entities through variation of size, shape and polymer/surface modifications will be discussed (Figure 1).

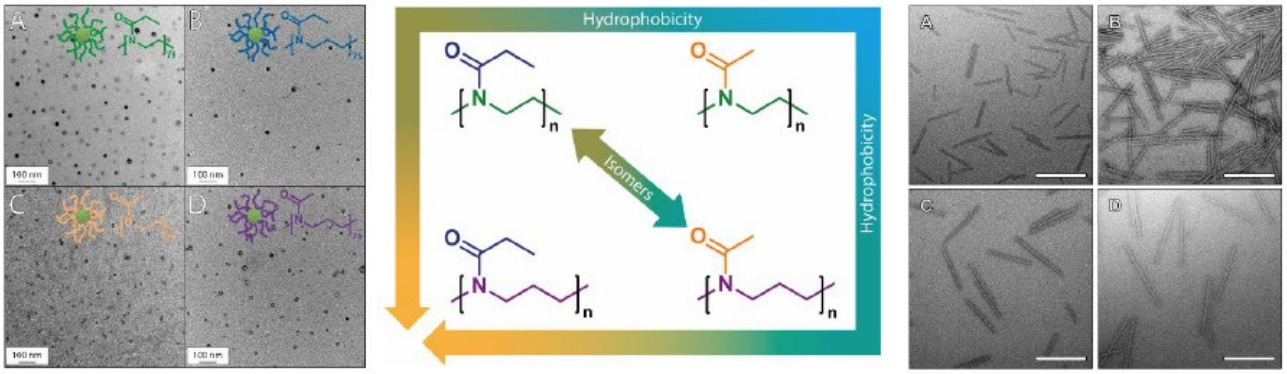

Figure 1: Core-crosslinked micelles[1] and defined nanorods[3] from different poly(2-oxazoline)s.

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‘Inert’-Nanoparticle Sensitization and Remodeling of Tumour

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Metal-based nanoparticles have entered clinical trials and received market approval for enhancing therapeutic effects of radiotherapies. However, thorough mechanistic understanding of the radiobiology remains elusive and published data is often contradictory, let alone definitive.[1,2] It is clear though that radiobiological response depends on properties of the nanoparticle, radiation and biological system. A range of physical, chemical and biological mechanisms may each amplify, or antagonize, a beneficial radiobiological response.

We have been applying a range of single cell analytics for assessing nanoparticle uptake across cell populations in vitro and within the tumor microenvironment in vivo from a 4T1 mouse model for a range of nanoparticles. Various responses to the nanoparticles and radiation have been assessed such as DNA double strand breaks, protein expression, protein translocation and metabolic profiling. These have been correlated to the quantitative analysis of the number of nanoparticles in the same individual cells. The results challenge the idea that any nanoparticle could be considered biologically ‘inert’. Single cell characterization of ex vivo tumors has further identified cell populations that nanoparticles associate with and alter. Our in vivo data challenge the paradigm that sensitization with targeted nanoparticles occurs through enhancement of radiation dose in the cancer cells of the tumour. In-fact we observe that targeted nanoparticles rarely associate with cancer cells, but rather, their fate is with other non-cancer tumour-associated cells. Nanoparticles not only modulate the single cell radiobiology but also remodel the tumor microenvironment which alters radiobiological response.

DNA damage is not the sole mode of action for nanoparticle radiosensitizers and nanoparticle radiosensitization radiobiology is not only due to an interaction between the ionizing radiation and the nanoparticle, but also an interaction of the nanoparticle with the biological system. A more thorough understanding of these mechanisms will promote designing nanoparticle formulations that could not only improve radiotherapy outcomes, but other cancer treatment modalities as well.

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Spatio-temporal control of physical architecture within 3D-bioprinted constructs for enhanced cellular function

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Adequate nutrient and oxygen supply remain an issue in engineering clinically relevant sized tissue constructs. Sacrificial templating, where 3D-printed templates are embedded into matrix biomaterials and subsequently sacrificed to leave open channels[1], is an attractive approach to address this problem. However, the temporal variations in nutrient/oxygen concentrations during native tissue developmental biology[2], cannot be mimicked by current sacrificial inks. Therefore, the aim of this study is to develop sacrificial inks that allow tailorable temporal dissolution, which will affect downstream cellular function and tissue formation.

Sacrificial templates composed of gelatin (10 wt%, termed 'non-crosslinked templates') or gelatin (10wt%) supplemented with Ru (0.1 mM)/SPS (3-10 mM) co-initiators (termed 'crosslinked templates') were printed using a Bioscaffolder (Sys+Eng). Templates were subsequently embedded in a matrix biomaterial composed of allylated gelatin (Gel-AGE) with 18 mM dithiothreitol, 0.1/5 mM/mM Ru/SPS, and finally photopolymerized (30mW/cm2, 180s, 400-450nm). During culture, the timing of channel opening as a result of template sacrificing was assessed daily (1-14 days). The effect of temporal dissolution of sacrificial inks on cell function was assessed via osteogenesis and vasculogenesis. For osteogenesis, mesenchymal stromal cells (MSCs) were encapsulated within Gel-AGE hydrogels containing crosslinked templates, and cultured under osteogenic conditions for 21 days, assessing end-point mineralization (Alizarin Red). For vasculogenesis, MSCs and GFPlabelled human umbilical vein endothelial cells (HUVECs) co-culture was further encapsulated and cultured under endothelial conditions for 10 days, assessing capillary network formation (GFP) using AngioTool[3].

Non-crosslinked templates embedded within Gel-AGE hydrogels dissolved within 4 hours, leaving open channels post dissolution. In contrast, crosslinked templates showed a delayed dissolution behaviour, with channels only opening after 4.3±0.6 to 15±1 days, dependent on the Ru/SPS concentration used. The timing of template dissolution, and subsequent channel opening, greatly affected osteogenic culture: constructs with crosslinked templates exhibited enhanced mineralization throughout the construct. In terms of vasculogenesis, constructs with crosslinked templates demonstrated increased vessel length (271.4±25.1 versus 84.9±14.4μm) and junction density (21.1±11.7 versus 91.2±33.9 junctions mm-1), as compared to constructs with non-crosslinked templates.

We demonstrated that tuning the dissolution time of the sacrificial template to better mimic native tissue developmental biology, is an attractive strategy to promote tissue formation. In this study, we present a novel sacrificial bioink that allows engineering of temporal architectural cues, which will have wide application within biofabrication.

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Elimination of Hepatitis C: from Prison to Laboratory

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The World Health Organisation has set ambitious goals for the elimination of hepatitis C infection as a public health threat by 2030, including an 80% reduction in chronic infection, a 65% reduction in mortality, and a 90% reduction in incident infection. Australia is one of only nine countries worldwide which is on track to achieve these targets by 2030. This progress has been achieved by virtue of universal access to testing, and similarly universal access to the highly curative direct-acting antiviral (DAA) treatments. To date over 100,000 individuals have been treated and cured - representing over half of the affected population. Despite this good progress, there are residual challenges: firstly, there has been a progressive decline in treatment rates reflecting the high marginalised patient population who are predominantly people who inject drugs (PWID) necessitating health service models in non-traditional settings such as prisons and homeless centres. Secondly, the incidence reduction target remains a major challenge with evidence of high rates of infection and reinfection in high risk settings such as prisons, despite existing harm reduction strategies such as opioid agonist therapy (OAT). This has driven investigation of novel approaches to control incidence – the Surveillance and Treatment of Prisoners with hepatitis C (SToP-C) demonstrated that intensive scale-up of testing and treatment in the prison setting was associated with halving of the incidence rate (Treatment as Prevention; TasP)

For global elimination, it is clear that a preventative vaccine will be required as the health infrastructure to achieve outcomes akin to Australia is lacking in the countries with the largest burden of disease. Like HIV, hepatitis C is a highly mutable RNA virus and therefore offers substantial challenges for vaccine development – two decades of effort have been unproductive to date. Two opportunities offer hope – firstly, hepatitis C infection is naturally cleared in one in four cases, and can be repeatedly cleared in a rare subset of high risk individuals (superclearers) - offering an opportunity identify the correlates of protective immunity to inform vaccine design. Secondly, the rapid development, evaluation and deployment of lipid nanoparticle (LNP) vaccine technology in the highly successful vaccines protecting against SARS CoV-2 (another RNA virus) offers hope for comparable developments for hepatitis C infection.

Clinical Challenges in Paediatric Oncology - Opportunities for Nanomedicine?

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This talk will discuss clinical challenges in paediatric oncology, to highlight potential opportunities for application of novel nanomedicine approaches. My current clinical work is focused on neuro-oncology and leukaemia, I am actively involved in development of early phase clinical trials matched to the ZERO Childhood Cancer Precision Medicine Program.

There is an urgent clinical need to develop more effective treatment in high-risk paediatric cancers, with a focus on brain cancers, high-risk extracranial solid tumours and relapsed/refractory haematological malignances. In subtypes of cancers with favourable overall survival, such as acute lymphoblastic leukaemia (ALL) and Hodgkin Lymphoma, there is a need to balance cure rates against treatmentrelated toxicity.

For high-risk paediatric cancers, challenges include i) effective blood brain barrier drug penetrance for brain cancers ii) achieving targeted drug delivery into tumour, without systemic side-effects iii) development of effective combination therapy for high-risk cancers.

Current therapies for paediatric brain cancers are limited. For non-resectable high-grade tumours, further systemic therapy is considered, however is rarely effective. Radiotherapy is avoided in children <3 years old due to the long-term neurocognitive effects. Despite broad advances using chemotherapy in a subset of paediatric brain cancers, including medulloblastoma, poor prognosis subtypes such as Group 3 MYC-amplified medulloblastoma require novel therapeutic approaches. Suggested incorporation of nanomedicine approaches will be discussed for high-risk brain cancers.

My research also aims to understand clinical and germline risk factors for treatment-related toxicities in ALL, the commonest childhood cancer. Five-year event-free survival rates exceed 90% for most children treated for ALL. Significant treatment-related toxicities include severe venous thromboembolism, acute neurotoxicity, pancreatitis, and osteonecrosis. Therapy alterations due to severe treatment-related toxicity can lead to inferior disease outcomes. For example, in our systematicnational retrospective review of 1251 children diagnosed with ALL, omission of intrathecal methotrexate following acute methotrexate-related neurotoxicity led to doubling of central nervous system (CNS) relapse[1]. By predicting and potentially preventing treatment-related toxicities, we may be able to improve survival and reduce the impact of therapy on patient-related quality of life. Is there a role for nanomedicine to reduce toxicity in more treatable paediatric cancer subtypes?

Therefore, by focusing on paediatric brain cancer and leukaemia, opportunities for nanomedicine will be presented for discussion.

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Nanomedicines for use in wildlife applications

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Wildlife comprises a diverse group of animals and delivery of drugs to these populations is challenging. As highlighted by the COVID-19 global pandemic, the ability to deliver therapeutics and treat illness in wildlife is becoming increasingly important. The Australian brushtail possum is the most significant vertebrate pest in New Zealand and disrupting fertility is the most humane method of control. D-Lys6- GnRH is a water-soluble analogue of gonadotrophin-releasing hormone (GnRH), a peptide essential to reproductive function. When administered in slow-release implants, GnRH reduces fertility in brushtail possums[1]. Capture of this widespread, feral animal to administer implants is not feasible and leads to our research question - can nanoparticles be used as a delivery system to deliver fertility controls to the brushtail possum?

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

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

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

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Nanotherapeutic Strategy to Overcome Anticancer Therapeutic Resistance

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Cancer stem-like cells (CSCs), also called tumor-initiating cells, are regarded as one of the determining factors that lead to tumor heterogeneity.[1] CSCs harbor self-renewal capacity, high tumorigenicity and strong therapeutic resistance, which contribute to tumor onset, progression, recurrence and metastasis.[2,3] In this talk, a nanotherapeutic strategy using cell-differentiation-regulated nanoparticle co-loaded with a differentiation-inducing agent, alltrans retinoic acid (ATRA) and a chemotherapeutic drug, camptothecin (CPT) developed by our group will be first introduced to overcome stemness-related chemoresistance.[4] The obtained nanoparticle can adaptively modulate the release of combinatorial drug within different cell phenotypes by responding to their specific cellular biosignals. Such differential release satisfies the demand for enhancing the synergistic anticancer efficacy of ATRA and CPT with different anticancer mechanisms. Treatment with the nanoparticle efficiently suppresses the tumor growth and postsurgical relapse on the CSC-enriched breast tumor mouse models. Furthermore, a liposome-mediated acclimatization approach to combating the stemness-derived resistance to anticancer protein will be presented.[5] The liposome that is coloaded with plasmid DNA encoding tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and salinomycin (Sal) allows cancer cells to serve as protein generators to express TRAIL, and sensitizes resistant CSCs to the TRAIL-induced apoptosis by Sal-triggered upregulating expression of death receptors. We show that the liposome can inhibit the CSCenriched tumor growth and metastasis in the mouse model of the orthotopic colon tumor.

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Two halves equal a whole: the opportunities in translating research

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The medical device field today demands technologies to continuously evolve, becoming better, faster and more cost efficient, whilst still being accessible to the developing and developed worlds. The ongoing pressure on cutting edge research to answer this call, within the context of limited resources and funding, drives the ingenuity behind the translational research in Australia and specifically at SpeeDx.

SpeeDx, invents, develops licences and translates its proprietary technology into products for the detection of diseases and disease-causing pathogens. SpeeDx aims to place the value of diagnostics firmly into best-practice patient management, developing tests that have a true positive impact on human health and clear value to the greater health network.

Our foundational technology, which we call *PlexZymes*, are novel, highly innovative & reliable "sensing" enzymes.[1] The PlexZyme is composed of two DNA oligos that bind adjacently on a target nucleic acid sequence, to form an active enzyme that cuts a reporter oligo bound to the PlexZyme complex. These are cheap and fast to manufacture and can be designed to sense any disease marker. The advantages of the PlexZyme became evident when utilised in real-time PCR, especially when multiple targets are required to be amplified and detected in a single reaction. Early research demonstrated that up to 25 different genetic assays could be combined, without sacrificing the specificity or sensitivity of the reaction.[2]

The process of employing PlexZymes for in vitro diagnostic (IVD) tests, was straight forward on the research side; however, the complexity of the capacity required to take it from the research lab to the pathology lab required additional expertise and the expansion of SpeeDx, made possible only through private and government funding. SpeeDx now successfully manufacture and sell IVD tests for infectious diseases around the world, with a focus on antimicrobial resistance. This novel approach to diagnostics, combining additional information beyond the simple detection of disease-causing agent, is influencing patient management guidelines[3-5] and catalysing further commercial collaborations, expanding access to a wider patient audience.[6] True to SpeeDx ethos, we continue to innovate, aiming to further improve IVD technology so that, if we are to be superseded, it will be by us.

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Wearable Nano-Sensors for Personalised and Preventive Medicine

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Wearable technologies are networked devices equipped with microchips and sensors, capable of tracking and wirelessly communicating information on real time basis. The rapid adoption of such devices in the past decade has placed them as the most attractive innovation in the word of technology. From a fitness activity tracker to Google Glass, miniaturized wearable devices have shown great potential to be embedded in various domains including healthcare, robotic systems, prosthetics, visual realities, professional sports, as well as entertainment and arts. Here, some exciting achievements, emerging technologies, and standing challenges for the development of non-invasive personalized and preventive medicine devices will be discussed. The engineering of wire- and power-less ultra-thin sensors on wearable biocompatible materials that can be placed on the skin, pupil, and teeth will be reviewed, focusing on common solutions and current limitations.

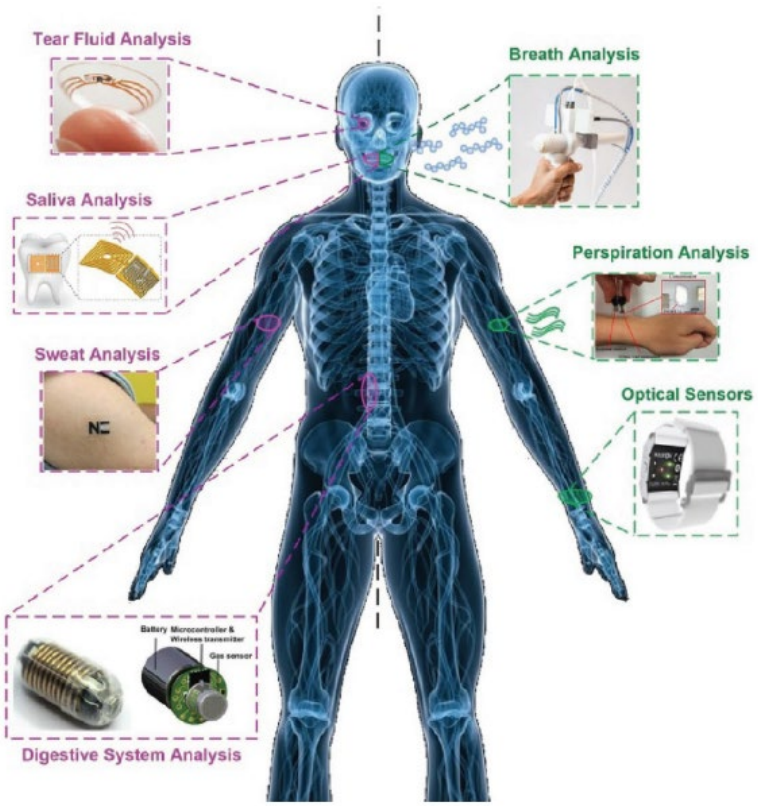

Figure1: Schematic summary of some emerging sensing technologies for personalized and preventive medicine. A major distinction can be made between contact and contactless technologies, such as tear, saliva analysis, sweat, digestive system and optical, breath and perspiration analysis [1].

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Overcoming biological barriers using stimuli responsive silica nanoparticles

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Mesoporous silica nanoparticles (MSNs) have attended frenzy of attention in past 20 years as an emerging biocompatible drug delivery and diagnostic system. Lot of these formulations based on MSNs are now in Phase I and II clinical trials.[1] However, despite use of colloidal silica (Aerosil©) as common tablet excipients since past 50 years, the full translational potential of MSNs as oral drug delivery agents is not realised. Our group focuses on use of MSNs nanocomposites to overcome multiple biological barriers (Gut, Tumour, and BBB). We are particularly interested in effective oral delivery of hydrophobic drugs and macromolecules. For instance, by harnessing the high surface functionality of MSN we have preparedvarious pH and enzyme responsive drug delivery systems based on MSNs for targeting small intestine and inflamed gut. Additionally, we have evaluated the potential of library of silica particles in delivery of variety of small and macromolecules for the treatment of IBD, Diabetes, TB, and recently glioblastoma.[2-7]

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Nanocarriers for Antimicrobials and Antimicrobial Photodynamics

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Pathogenic bacteria and fungi cause many clinical infections, which are becoming recalcitrant due to anti-microbial resistance (AMR). Biofilm forms of such pathogens and intracellular infections are the two most challenging biological barriers that need to be overcome for the more effective application of antimicrobial drugs against infectious diseases. In addition to new conventional drugs, antimicrobial photodynamic therapy (aPDT) is an emerging therapy against AMR. aPDT harnesses the energy of light through photosenstizers to generate reactive oxygen species that inactivate bacteria and fungi with no resistance, but also needs to be designed to overcome the biofilm and cellular barriers which protect pathogens. It is feasible to combined aPDT with antimicrobial drugs to generate synergy against infections.

Nanocarriers composed of either lipids, metals, polymers or porous inorganics can be engineered to overcome the bio-barriers and functionalized for either water soluble or insoluble antimicrobials or aPDT photosenstizers. We describe nanocarriers for improved delivery of antibiotic/antifungal drugs and aPDT agents, and demonstrate the use of specific chemistry and nanostructure to facilitate the crossing of biological barriers and triggered drug release within biofilm and intracellular environments (e.g. low pH and enzyme rich). Case studies will focus on liposomes, lipid liquid crystal nanoparticles (LCNP) and copolymer micelles to:

- Enhance the efficacy of aminoglycosides against P. aeruginosa biofilm infections using a wide range of in vitro, ex vivo and in vivo models[1,2].
- Targeting lipid nanocarrier uptake by specific cell types that harbor intracellular bacteria[3].
- Optimize performance of the novel aPDT photosensitizer, gallium protoporphyrin (GaPP).
- Improve the performance of anti-fungals against C. albicans biofilms, including synergistic effects in influencing the morphologic fungal transitions[4].

Insight into the mechanisms of action of these triggered release nanocarriers will be presented and opportunities for application against clinical infections discussed.

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A Biomimetic Approach Toward Enhancing Angiogenesis & Vascularisation of Biomaterials

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Inadequate or aberrant vascular supply is a hallmark of many ischeamic disorders, including myocardial infarction, stroke, and chronic wound healing.[1] Lack of sufficient and timely vascularisation is also a key obstacle in translating advances in tissue engineering to clinical applications. Thick, critically-sized bioengineered tissues do not vascularise in a timely manner to allow tissue survival and integration once implanted, often resulting in cell necrosis and tissue rejection.[2] Angiogenic therapy involving delivery of pro-angiogenic growth factors to stimulate new blood vessel formation is promising but has seen limited clinical success due to issues associated with the need to deliver supra-physiological growth factor concentrations.[3] Bio-inspired growth factor delivery utilising the native growth factor signalling roles of the extracellular matrix proteoglycans has the potential to overcome many of the drawbacks of angiogenic therapy.[4]

Perlecan is the major extracellular proteoglycan expressed in the vascular basement membrane. Perlecan is known to support angiogenesis though its heparan sulphate chains, by binding and signalling key vascular growth factors, while the protein core supports α2β1 integrin-mediated endothelial cell binding.[5,6] In this study, the potential of the recombinantly expressed domain V (rDV) of human perlecan was investigated as a means of promoting growth factor signalling toward enhanced angiogenesis and vascularization of implanted biomaterials.

rDV was found to promote angiogenesis in established in vitro and in vivo angiogenesis assays by potentiating endogenous growth factor signalling via its glycosaminoglycan chains. Further, rDV was found to potentiate FGF2 signalling at low concentrations that in the absence of rDV were not biologically active. Finally, rDV immobilised on silk fibroin biomaterials remained bioactive and promoted enhanced vascular ingrowth and integration of the implanted scaffolds with the surrounding tissue. Together, these studies demonstrate the important role of this biologically active perlecan fragment and its potential in the treatment of ischemia in both native and bioengineered tissues.

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Developing a platform technology to cross the blood brain barrier and deliver drugs to specific populations

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Prognosis for cancers of the brain, whether primary or metastasis, remains poor, even with advances in treatment for other cancers. This is due to the limited number of drugs that can cross the blood brain barrier (BBB). While methods of overcoming this barrier have been developed and employed with current treatment options, the majority are highly invasive and non-specific treatments, leading to severe neurotoxic side effects. A novel approach to address these issues is the development of therapeutics targeting receptor mediated transport mechanisms on the BBB endothelial cell membranes. We have developed aptamers as targeted delivery agents that can also cross the blood brain barrier. Aptamers are smaller than antibodies, and thus can more effectively deliver drugs into the tumour. Numerous studies have demonstrated that, despite theoretical implications of rapid renal clearance, nuclease degradation, and electrostatic repulsion, aptamers are effective agents for the delivery of cytotoxic agents. These agents can either be used as singular agents, or given their different mechanism of action and suggested lack of drug-drug interaction, alongside antibodies to have a greater efficacy against solid tumours. We have combined two aptamers for the targeted delivery of chemotherapeutics to brain metastases which can cross the blood brain barrier and also specifically target cancer cells. Using this approach, we intercalated doxorubicin into this bifunctional aptamer targeting the transferrin receptor on the blood brain barrier and epithelial cell adhesion molecule on the metastatic cells. The ability of the doxorubicin loaded aptamer to transcytose the blood brain barrier and selectively deliver the drug to epithelial cell adhesion molecule-positive tumours was evaluated in an in vitro model and confirmed for the first time in vivo. We show that co-localised aptamer and doxorubicin fluorescent signals are clearly detectable within the brain lesions 75 minutes post administration. Following a short treatment schedule, brain metastases were shown to decrease following bifunctional-aptamer-doxorubicin treatment, as compared to control or free drug. As well, metastases decreased in bone and ovaries following treatment. Collectively, the results from this study demonstrate that through intercalation of a cytotoxic drug into the bifunctional aptamer, a therapeutic delivery vehicle can be developed for the specific targeting of epithelial cell adhesion molecule-positive brain and systemic metastases. We are now investigating this technology against primary brain cancers with different aptamers targeting other cell surface receptors.

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Smart drug delivery system

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Despite all of the coverage of new drugs, it is not enough to just have an effective drug. To enhance therapeutic efficacy and reduce the side effects, the drugs have to be protected and transported to the right location to get an effect at the right time. Over the past few decades, nano-/microscale materials-enabled drug delivery platforms have made tremendous advancements in preventing and treating human diseases. Especially, the recent great success achieved by the two highly effective mRNA nanoparticle vaccines during the COVID-19 pandemic further highlights the great potential of drug delivery technologies. During the evolution of these drug delivery technologies, materials science innovation has played an important role from drug modification to the synthesis of different drug delivery platforms, which fulfills effective medical applications in various diseases including cancers, cardiovascular diseases, diabetes, infectious diseases, and many others. In this talk, I will introduce our current studies on nano-/microscale materials-enabled drug delivery technologies with the promise to improve health care, as well as our efforts in accelerating their translation into the drug development pipeline.

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Fibroblast Activation Protein specific MRI improves tumour mapping in a preclinical orthotopic model of prostate cancer compared to PSMA

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MRI guided focal therapies of prostate cancer have the potential to reduce treatment-related side effects without sacrificing oncological outcomes. Molecular MRI with agents targeted to tumour tissues enhances tumour margin detection and consequently treatment guidance/planning. Prostate specific membrane antigen (PSMA) expression is currently the gold standard for molecular imaging of prostate cancer. Fibroblast activation protein (FAP) is an extracellular transmembrane protein expressed on cells of the tumour microenvironment, particularly in stromal and vascular components. FAP has been gaining momentum as a pan-cancer marker as (1) its overexpression often correlates with cancer severity, including in prostate cancer; (2) It has been found to be negligibly expressed in healthy tissue. The objective of this study was to compare MRI contrast of PSMA-targeting and FAP-targeting magnetic nanoparticles (MNPs) in an orthotopic murine model of prostate cancer.

Control (no ligand), FAP- and PSMA-targeted MNPs were prepared with modification of a MRI agent (FerroTrace, Ferronova) currently undergoing a Phase 1 clinical trial. In vitro binding was evaluated in PSMA and FAP positive cell lines. MRI was performed in orthotopic tumour-bearing mice 24 hours after intravenous injection of the contrast agents. Enhancement of MRI contrast in tumours was quantified by determining the proportion of pixels with low signal intensity throughout regions of interest. Accumulation of MNPs in prostate tumours was confirmed ex vivo.

PSMA and FAP-MNPs demonstrated specific binding in vitro. MRI contrast of tumours was increased in FAP ($p<0.001$) and PSMA-MNPs ($p<0.05$) but not control MNPs ($p>0.05$), relative to orthotopic tumours with no injected MNPs. FAP-MNPs provided increased contrast relative to PSMA-MNPs ($p<0.05$). Increased accumulation of FAP-MNPs in prostate tumours was observed on tissue sections compared to PSMA-MNPs. Control-MNPs showed minimal tumour accumulation.

These results suggest that FAP-targeting MNPs could enhance the MRI of prostate tumours and assist in the delivery of precise focal treatment.

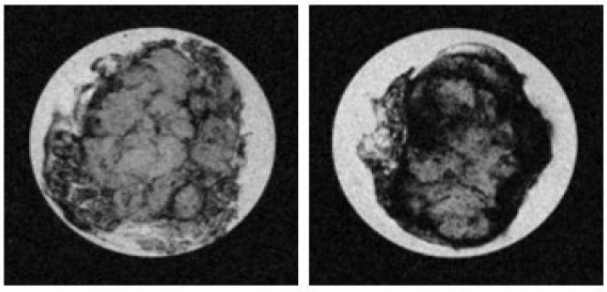

Figure 1: Ex vivo T2-weighted 11.7T MRI of murine LNCaP tumours 24 hours after intravenous injection of PSMA MNPs and FAP1 MNPs (representative image).

Lipid conjugated materials that harness the lymphatics to enhance immunity and metabolism

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Conjugation of materials to lipids can facilitate integration into the natural biological trafficking pathways for lipids – including association with lipoprotein and serum albumin[1]. Conjugation to lipids can thus be used to control the disposition and pharmacokinetics of valuable therapeutics[1,2]. For example, therapeutic peptides such as insulin and GLP-1 agonists have been conjugated to lipids to promote albumin binding and prolong serum half-life[3]. Lipidation of orally administered small molecule drugs can increase drug permeability, and facilitate association with intestinal lipoprotein trafficking pathways into lymph[1,4]. Recently, lipid conjugation to adjuvants and antigens has been shown to enhance vaccine efficacy by increasing exposure to immune cells in lymph nodes by enabling 'hitchhiking' onto albumin trafficking pathway into lymph following injection[5]. Our group has been working on a range of lipid conjugated materials, including orally administered lipid prodrugs of small molecule drugs and lipid conjugated oligonucleotides, peptides and brush PEG polymers administered via injection. Lipid conjugation alters the biodistribution and pharmacokinetics of these therapeutics in surprising ways, and in particular, promotes lymphatic uptake by facilitating integration into endogenous lipoprotein and albumin trafficking pathways into lymph. In this presentation I will highlight the relationships between the nature of the conjugated lipid and the pharmacokinetic outcomes, and the attendant benefits of lipid conjugated materials for vaccines and therapeutics to treat diseases involving the lymphatics, with a focus on immune and metabolic diseases.

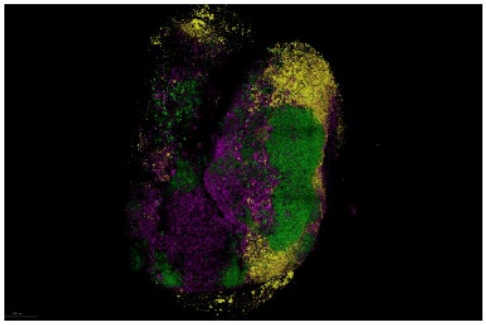

Figure 1: Image showing the lymph node disposition of an orally administered glyceride lipid prodrug (yellow) relative to T cells (pink) and B cells (green) from reference 6

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Boosting Vaccine Efficacy via Controlling Nano-Bio Interactions

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Despite remarkable successes of immunization in protecting public health, safer and more effective vaccines against a number of life-threatening pathogens such as HIV, ebola, influenza, and SARS-CoV-2 remain urgently needed.1 In this talk, I will present my group recent work on engineering nanoparticles for vaccine delivery.[1-3] In particular, we found that the size, surface property and type of nanoparticles (polymer or lipid) dictate their interactions with immune cells.[2,3] We also developed a simple, scalable and reproducible method to functionalize hemagglutinin (HA) immunogens on the surface of nanoparticles via stable metal chelation chemistry.[1] The resulting HA-functionalized nanoparticles display enhanced antigen deposition into germinal centers within the draining lymph nodes (Figure 1), driving increased HA-specific B cell, and follicular helper T cell responses and ultimately, enhanced protection against highly pathogenic influenza virus.[1]

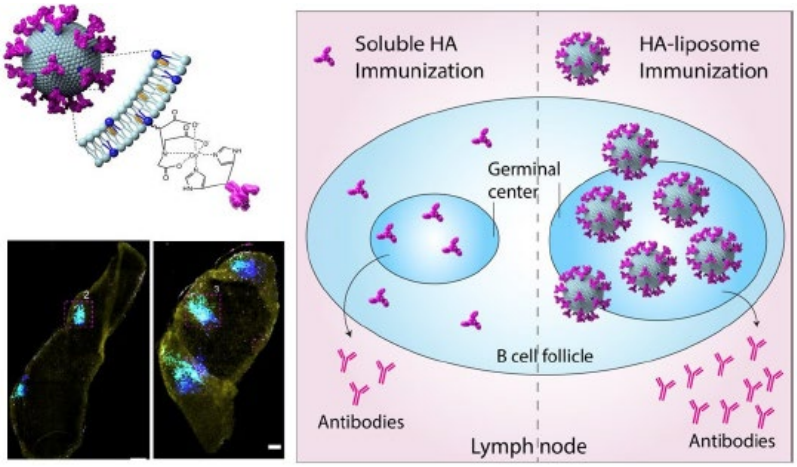

Figure 1: Engineering nanoparticle surface for enhancing antibodies production efficiency.

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A multimodal targeted nanodiamond-based theranostic drug delivery system: Precision therapy of triple negative breast cancer

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Triple-negative breast cancer (TNBC) represents 15–20% of all breast cancers and tends to behave more aggressively with the highest metastasis rate than other breast cancer subtypes.[1] Chemotherapy is the mainstay of treatment to which TNBC cells exhibit a high level of intrinsic/acquired resistance.[2] The presence of breast cancer stem-like cells (BCSCs), which are intrinsically resistant to chemotherapy[3] and the expression of P- glycoprotein (P-gp) play a crucial role in conferring resistance to cytotoxic and targeted chemotherapy in TNBC.[4]

We aimed to develop a novel nanotheranostic involving an iron-dopped nanodiamond core. This nano-construct specifically co-delivers doxorubicin to TNBC cells via a novel luteinising hormone-releasing hormone (LHRH) peptide derivative that targets LHRH receptors together with an anti-BCSC agent. The anti-BCSC agent was dasatinib, that has alsobeen shown to inhibit P-gp overexpression in TNBC cells, potentially overcoming therapy resistance in TNBC patients and improving their survival.

The new LHRH receptor targeted nanotheranostic technology showed increased uptakein LHRH receptor-overexpressing TNBC cell lines and increased cytotoxicity (up to six-fold higher compared to the chemotherapy agent alone). Furthermore, the synergistic anticancer activity of the two combined agents was observed in both targeted and untargeted nanotheranostic constructs with a combination index of 0.3 and 0.2 for doxorubicin and dasatinib, respectively. In a syngeneic preclinical mouse model of TNBC, we observed a significant increase in the antitumour efficacy of the targeted nanoconstruct with a 100% survival rate following discontinuation of the treatment (Figure 1).

The successful development of this targeted nanotheranostic technology might offer several benefits in the aggressive and fast growing TNBC tumours via early detection and destruction of tumour cells invading distal organs, preventing resistance to chemotherapy, and reducing the side effects.

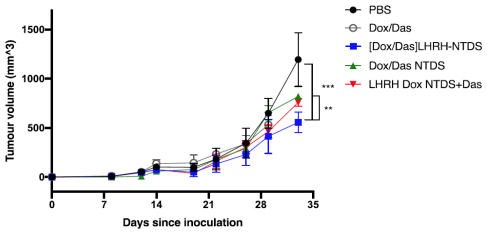

Figure 1: The antitumour efficacy of the targeted nanotheranostic technology in a syngeneic TNBC mouse model.

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Cancer therapy using porous silicon nanocarriers with stimulus-cleavable linkers

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Porous silicon nanoparticles (pSiNPs) have been widely utilized as drug carriers due to their excellent biocompatibility, large surface area and versatile surface chemistry. However, the dispersion in pore size and biodegradability of pSiNPs arguably have hindered the application of pSiNPs for controlled drug release. Here we describe a step-changing solution to this problem involving the design, synthesis and application of three different linker-drug conjugates comprising anticancer drug doxorubicin (DOX) and different stimulus-cleavable linkers (SCLs) including the photo-cleavable linker (*ortho*-nitrobenzyl), pH-cleavable linker (hydrazone) and enzyme-cleavable linker (β-glucuronide). These SCL-DOX conjugates are covalently attached to the surface of pSiNP *via* copper (I)-catalyzed alkyne-azide cycloaddition (CuAAC, *i.e.* click reaction) to afford pSiNP-SCL-DOXs. The mass loading of the covalent conjugation approach for pSiNP-SCL-DOX reaches over 250 μg of DOX per mg of pSiNPs, which is notably twice the mass loading achieved by non-covalent loading. Moreover, the covalent conjugation between SCL-DOX and pSiNPs endows the pSiNPs with excellent stability and highly controlled release behavior. When tested in both *in vitro* and *in vivo* tumor models, the pSiNP-SCL-DOXs induces excellent tumor growth inhibition.

Molecular Imaging of Activated Platelets: Cardiovascular and Malignant Diseases

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Platelets play a significant role in both cardiovascular disease and cancer. Glycoprotein (GP) IIb/IIIa is the most abundant platelet surface receptor, responsible for adhesion and aggregation. We have developed a conformation-specific single-chain antibody (scFv) binding specifically to activated GPIIb/IIIa on platelets.

By conjugating the scFv with the appropriate contrast agents, we have shown i*n vivo* imaging of thrombosis and myocardial infarction across several imaging platforms: 1) microbubbles for ultrasound, 2) near-infrared dyes for fluorescence imaging, 3) iron oxides for MRI and 4) radiotracers for PET/CT. After the administration of clinical used fibrinolytic drugs, we directly visualized the thrombus size reduction. More recently, we have shown that specific targeting of our scFv within the tumour microenvironment using PET/CT, optical and ultrasound imaging. Activated platelets are ideal targets for molecular imaging of atherothrombosis and cancer.

Bleeding complications hamper current pharmaceutics treatment for thrombosis. By genetically engineering our scFv with anti-coagulant or anti-thrombotic drugs, we have achieved side-effects free targeted delivery of these agents to blood clots *in vivo*. Using a low systemic dose of thesefusion constructs, we have demonstrated the prevention of thrombosis and the preservation of heart function post-infarction, without an increase in bleeding.

Building on these diagnostic and therapeutic approaches to attain a novel theranostics strategy, we have shown successful thrombolysis in vivo using an acute thrombosis model. Overall, the targeting of activated platelets provides an opportunity to diagnose and treat a range of cancer and cardiovascular diseases.

Biomimetic Vascular Nanomaterials For Next Generation Medical Devices

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Medical devices that contact the blood range from temporary catheters and heart-lung machines to permanent stents and artificial hearts. However, these devices are still limited by the body's recognition of the foreign materials they are made from, resulting in side effects such as blood clots (thrombosis), inflammatory based reactions or a lack of endothelialisation, causing the devices to fail, the consequences of which can be fatal for patients. Drugs provided to limit these effects, like anticoagulants and anti-platelet drugs to prevent thrombosis, often result in further complications such as fatal bleeding[1].

Our lab uses biomimetic approaches to both create tools to evaluate materials, and to design next generation materials to improve medical devices. We are developing a range of microfluidic and organ-on-a-chip based tools to mimic aspects of the host environment, providing physiologically and pathologically relevant conditions. These allow us to evaluate existing materials to better understand failure mechanisms of medical devices1, andmechanisms of actions of newly developed medical device nanomaterials aimed to improve the function and reduce complications of medical devices.

We are also developing a range of biomimetic approaches to generate new materials including mimicking the local host environment to integrate implants, mimicking nature to impart specific functions to devices and mimicking biological processes or structures to createnew devices. The ultimate aim of these biomimetic tools and novel nanomaterials is to promote integration of the device with the host to improve outcomes for patients receiving medical devices.

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Biomimetic Formulation Engineering for Anticancer Therapy

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Owing to the extreme complexity in vivo, the performance of elaborately designed anticancer formulation in the final clinical trials is often compromised from the previous experimental results. Such an inconsistency apparently reduces the druggability of the research objects and increases the risk of new drug development. Therefore, it is urgent to change traditional ideas of research and development for anticancer formulation.

In order to solve this problem, we have integrated the structure, function and program of biological systems into the design, and developed new preparation processes to construct a series of biomimetic anticancer formulations. we developed in situ drug loading technology based on the unique hollow-porous structure of cage proteins. The high expression of corresponding receptors on the surface of tumor cells further enabled us to achieve targeted drug delivery. Meanwhile, a new hydrothermal process was developed to precisely regulate the bacterial structure for the accommodation of tumor antigen. Through natural infection, a large amount of antigen could be delivered to the dendritic cells, leading to a potent immune response. In addition, we also developed a new membrane emulsification process to coat the nanoparticles with cell membranes, thereby endowing them with excellent in vivo fate, such as long circulation and tumor penetration. Such a camouflage approach could significantly improve the performance for cancer imaging, diagnosis and chemotherapy. As aforementioned, biomimetic formulation followed the intrinsic transport route in vivo, and precisely delivered drug, antigen or probe to the target site as expected. We believe these candidates will lead to the slightest adverse reaction, obtain the optimal application effects, reduce the risk of research and development, and promote the clinical conversion.

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Protein Biohybrid Materials for Degradable and Functional Nanoparticles

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Nature's polymers, such as proteins and polysaccharides show a remarkable versatility as multifunctional biomaterials. Both biopolymers can be easily modified with the toolkit of bioorganic chemistry and are particularly attractive because of their degradability and biocompatibility. The presentation will focus on our latest examples of dynamic protein materials. Conjugation with synthetic and bio-derived polymers results in biohybrid materials that are responsive and show a triggered disassembly and payload release.

We will highlight a universal approach for the preparation of a new class of protein-based nanoparticles for the delivery of therapeutic payloads. We preserve the native structure of the proteins, and the particles are stable without denaturation or crosslinking. The method can be universally applied to any protein and enzyme of choice. These protein nanoparticles can successfully encapsulate small hydrophobic or large hydrophilic drugs like the antibacterial enzyme lysozyme.[1,2]

One example is the surface-modification of cytochrome *c* with acid-degradable polyethylene glycol. pH-sensitivity was obtained through vinyl ether moieties distributed in the polyether backbone. When PEGylated, cytochrome *c* has a different solubility behaviour in organic solvents, which allows for particle preparation using an emulsion-based solvent evaporation method. The resulting particles are stable under physiological conditions but degrade in acidic conditions.[3]

The second part of the presentation will highlight a novel amphiphilic protein polysaccharide conjugate that self-assembles into well-defined nanoparticles with narrow size distribution. It has a dual responsive behaviour for a full degradation and payload release upon cellular uptake.

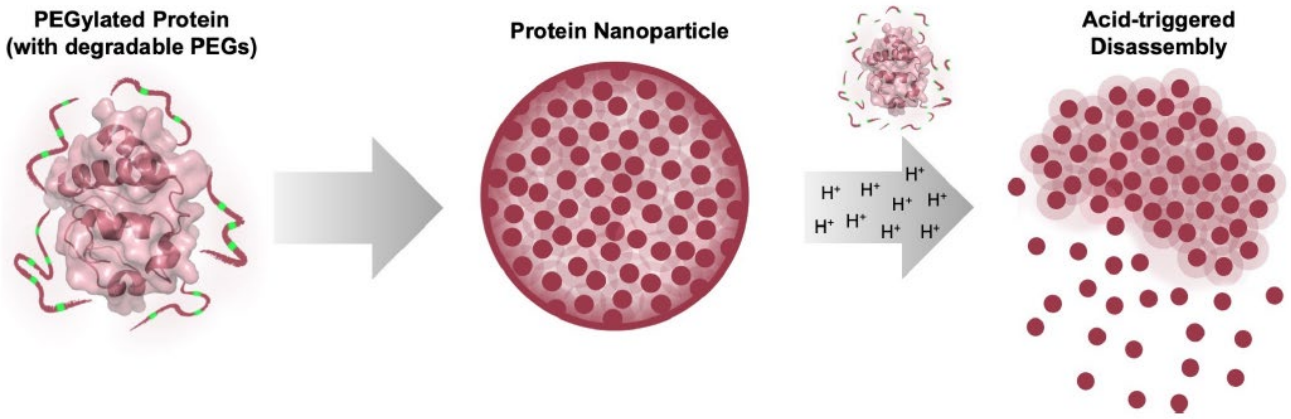

Figure 1: pH-Responsive protein nanoparticles via conjugation of degradable PEG to the surface of protein

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Biological Applications of Plasma Dust Nanoparticles

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Translation of nanocarriers into the clinic has been hampered by complex, time-consuming and expensive synthesis and post-functionalization protocols, mostly focusing on linker chemistry to tether different classes of molecules into a single, multifunctional nanostructure. We were the first to collect and characterize the 'plasma dust', which has long been known to form in particulate-rich 'dusty' plasmas, for biological applications[1]. Plasma dust nanoparticles retain many of the favourable physical and chemical properties known for plasma thin films[2], including hydrophilicity and linker-free biomolecule binding. We have now defined dusty plasma conditions which reproducibly produce a new class of plasma polymerized nanoparticles (PPN)[3], employing them to develop new therapeutics.

Synthesis of nanoparticles via plasma polymerization allows fine control of size distribution, surface roughness, charge and chemical composition, giving rise to a versatile nanoparticle platform. Functional groups formed in the plasma bulk are spontaneously incorporated and preserved onto PPN during synthesis. We have shown that PPN can robustly bind a wide range of clinically relevant molecules including small drugs and peptides, proteins, genetic material (siRNA, miRNA and plasmids), antibodies and imaging probes, in a fast, costeffective and linker-free process. The addition of multiple functionalities is achieved simply by co-incubation in solution. Comprehensive *in vitro* and *in vivo* experiments show that PPN are safe at concentrations well above their therapeutic dose[4]. Our first published efficacy study demonstrates synergistic action of dual functionalised PPN carrying paclitaxel and SiRNA targeting VEGF, significantly reducing tumor growth in a mouse orthotopic breast tumor model[5]. Our latest unpublished work shows that PPN are retained at regions of vascular injury, increasing the efficacy of therapies aiming to enhance vascular repair.

PPN eliminate the trade-off between additional functionality and complexity/cost and could facilitate the upscaling of nanoparticle-based therapies into the clinic, particularly for cardiovascular disease and cancer applications.

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Analysis of circulating extracellular vesicles for liquid biopsy using novel biosensors and bioassays

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Liquid biopsies are revolutionizing cancer management by allowing molecular analysis of tumours using circulating biomarkers in blood and other bodily fluids, in a minimally invasive and repeatable way. However, the challenge that needs to be met is to allow liquid biopsy to be integrated into routine clinical workflow, given current tests for analysing liquid biopsy biomarkers are expensive (hundreds of dollars) and slow (several weeks). This leads to a new research field of using innovative biosensors and bioassays for rapid, sensitive and costeffective molecular diagnostic tests for biomarker detection in cancer. In this talk, I will discuss several analytical technologies we have developed for the detection of circulating extracellular vesicles (EVs), which are promising biomarkers for liquid biopsy. One example is an ultrasensitive digital assay for EV detection using lanthanide-doped upconversion nanoparticles, which achieved a limit of detection two orders of magnitude lower than standard ELISA. The other example is an advancement of an imaging-based nanoplasmonic biosensor system, which allows a rapid, simple, and multiplexed detection of multiple targets simultaneously.

An α-terminal lipid dictates hitchhiking on endogenous lipid trafficking pathways, influencing the lymph uptake, plasma half-life and tissue distribution of brush PEG polymers

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Introduction. Conjugation of materials to lipids can facilitate integration into the natural biological trafficking pathways for lipids. This can prolong plasma half-life and enhance lymph uptake[1,2]. Here we describe a range of novel brush PEG polymers designed with different α- terminal lipid elements (a short single hydrocarbon tail (1C2), a single medium-chain fatty acid (1C12), a diacylglycerol moiety containing two C12 medium-chain fatty acids (2C12) and a cholesterol moiety (Cho))[3]. Functionalization of brush PEG polymers with lipids might promote their hitchhiking on albumin and/or lipoproteins, which can be harnessed to enhance drug delivery to lymph to treat or diagnose lymph-derived/resident diseases.
Aims. To determine the impact of different α-terminal lipids on albumin and lipoprotein binding, biodistribution, plasma pharmacokinetics (PK) and lymph uptake of brush PEG polymers.
Methods. Association of the polymers with albumin and lipoprotein was evaluated using an ultracentrifugation-based assay. Plasma PK and/or thoracic lymph uptake were compared after intravenous (IV) or subcutaneous (SC) injection in male Sprague-Dawley rats. Biodistribution of the four polymers was evaluated following SC dosing in male C57BL/6 mice.
Results. 2C12-PEG had the highest association with albumin. 2C12-PEG and Cho-PEG polymers had statistically longer plasma half-life than the other polymers, consistent with their high association with albumin. SC bioavailability was >20% for all brush PEG polymers. The proportion of the bioavailable dose that was lymphatically transported was statistically higher for 2C12-PEG polymer (28.25% ± 5.28) than other polymers (Fig. 1A). Unexpectedly, 1C2-PEG had statistically higher lymph transport compared to Cho-PEG and 1C12-PEG (Fig. 1B). The four polymers also had different distribution patterns in major organs.
Discussion. Conjugation of brush PEG polymers with different lipids affected the polymers' biodistribution, plasma PK and lymph uptake. In general, conjugating brush PEG polymers with lipids that bind to albumin and lipoproteins, such as 2C12, increased plasma half-life and lymph uptake. This has potential value for promoting drug delivery to lymph to treat or diagnose diseases involving the lymphatics such as autoimmune diseases, cancers and acute diseases.

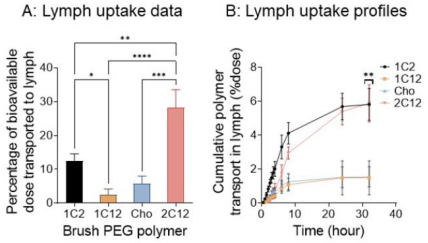

Figure 1: A: Lymph uptake data. B: Lymph uptake profiles. Data are presented as mean ± SD for groups (n=3). Data analysis was performed by one-wayANOVA followed by Tukey's multiple comparisons test. *: P ≤ 0.05, **: P ≤ 0.01, ***: P ≤ 0.001, ****: P ≤ 0.0001

Visualisation and efficacy of nanoparticle delivery for brain cancer

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Introduction: Brain cancers such as glioblastoma persist as an aggressive and lethal tumour group which have disproportionately poor survival[1]. One of the major challenges to therapeutic strategies for brain cancers is the blood-brain barrier (BBB). Therapeutic nanoparticles have shown promise in drug delivery across the BBB. The ability to maximise drug accumulation at the tumour site and reduce collateral toxicity to healthy tissues will directly benefit cancer patients. We have recently demonstrated the efficacy of lactoferrin coated nanoparticles for brain cancer delivery in 2D and 3D cell models[2]. How these nanoparticles enter multicellular tumour models remains unknown. To understand how nanoparticle design impacts function, it is important to assess nanoparticle delivery and efficacy in a 3D tumour environment[3, 4]. Here, we harnessed 3D brain tumour models, combined with our quantitative analysis platform5 and investigated tumour penetration, subcellular localisation and kinetic profiles of nanoparticles for brain cancer delivery.
Methods: Ultra-small, large pore mesoporous silica nanoparticles (MSNs) were developed using a facile synthesis method and subsequently functionalised with lactoferrin coating (Lf-MSNs)[2], and drug loading. Tumour penetration kinetics were first determined in 3D tumour spheroids of glioblastoma (grown up to 500 µm in diameter) using confocal microscopy and quantitative kinetic analysis. Immortalised astrocytes, endothelial cells and pericytes were then used to form BBB organoids. The BBB organoids were then used to investigate the ability of lactoferrin coated MSNs to traverse the BBB and deliver therapeutics *in vitro*, using lightsheet microscopy cellular segmentation techniques.
Results: We identified that Lf-MSNs had enhanced penetration kinetics in live glioblastoma spheroids using quantitative microscopy. The ability of Lf-MSNs to cross an *in vitro* 3D BBB model was confirmed *in situ*, and subsequent investigations using doxorubicinloaded iterations of these nanoparticles revealed elevated efficacy against glioblastoma cells, over their uncoated MSN counterparts.
Conclusions: The functionalisation of MSNs impacts the penetration of live and *in situ* 3D tumour models. The methods developed have broad applicability for fluorescently labelled nanoparticles or drug conjugates to effectively assess the impact of nanoparticle design for future *in vivo* prioritisation and development. These results also hold promise for the continued development of functionalised nanoparticles for brain cancer delivery.

Visible light switchable peptide-based hydrogel for cell culture

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A visible-light switchable peptide was synthesised and shown to form a fibrous hybrid hydrogel with collagen. A short peptide was functionalised with a tetrafluoro-substituted azobenzene. We believe this is the first visible light switchable peptide based supramolecular that shown excellent biocompatibility for stem cells.[1,2] The photoswitching of hydrogel with 520 nm and 410 nm lights results in significant softening and stiffening of the hydrogel, about 50% changes for storage modulus. Although no significant macroscopic difference was observed, the microscopic change was confirmed with cryo-TEM, as the hydrogel fiber diameter decreased from 7.5±0.9 nm to 5.6±0.7 nm during the in-situ softening process. The hybrid hydrogel was used for a stem cell culture, and the cellular responses to visible lightswitched mechanical cues were studied. In-situ softening of hydrogel matrix leads to morphological changes of the cell, *i.e.*, decreased cell area, perimeter and increased circularity. For longer period cell culturing, the cells seeded on hydrogels with initial stiffening shown more obvious neurogenic differentiation compared with the one seeded on hydrogels with initial softening, and the tendency could be modulated by visible light. This peptide-based hydrogel provides a benign platform for the controlled differentiation of stem cells in a noninvasive, accurate and biocompatible way with visible light.

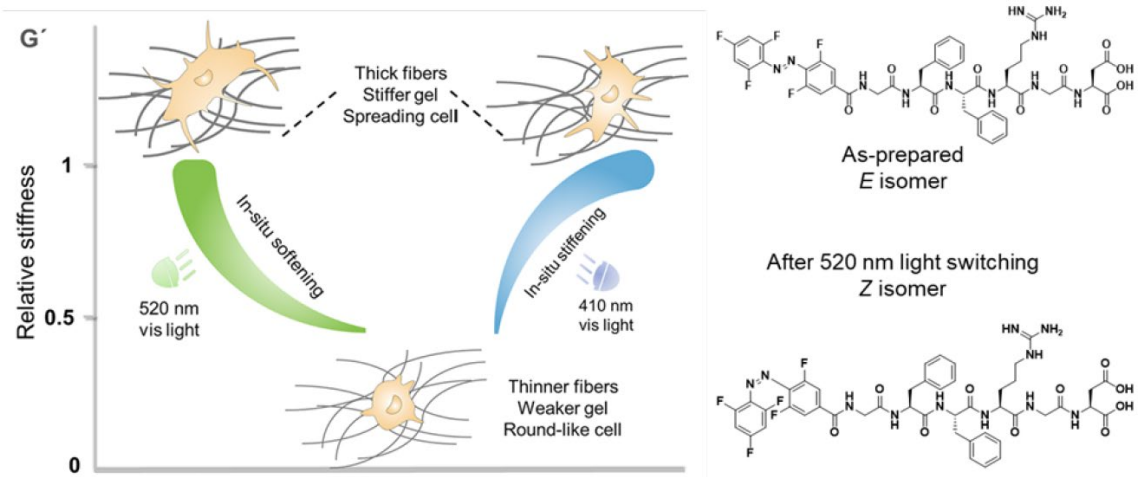

Figure 1: In-situ visible light photoswithing of the supramolecular hydrogel and related changes in fiber diameter, gel stiffness and cellular behavior.

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Harnessing the potential of encapsulin protein nanocages for nanomedicine

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Encapsulins are a new class of prokaryotic protein nanocages that show great promise as tools for nanomedicine[1]. They self-assemble from protein subunits into hollow spherical nanoparticles (≤ 45nm) that exhibit good colloidal properties, robust stability, and excellent biocompatibility[2]. Moreover, encapsulins can be genetically and/or chemically modified to display functional moieties (e.g. fluorophores, drugs, antigens). A key feature of encapsulins is their ability to selectively self-assemble around cargo proteins tagged with an 'encapsulation signal peptide' (ESig). This mechanism can be co-opted to load foreign cargo into encapsulins, reprogramming their functionality for specific applications[3]. Here, I will share our recent work engineering encapsulins to mediate the delivery of protein photosensitizers for *in vitro* photodynamic therapy (PDT), and our ongoing efforts to unravel the dynamic *in vivo* behavior of encapsulins.

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

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

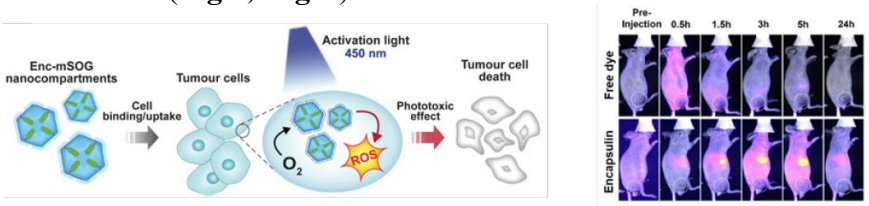

Figure 1. (Left) Schematic of mSOG-Enc mediating *in vitro* PDT. **(Right)** *In vivo* biodistribution of Enc

Conclusions: The natural protein encapsulation system of Enc was co-opted to instead package and deliver a functional protein photosensitizer into lung cancer cells, enabling *in vitro* PDT. Preliminary *in vivo* data shows, that while Enc is safe, it localizes within the liver, suggesting recognition and clearance by the immune system. Our ongoing work aims to better understand the *in vivo* behavior of Encs, including their toxicity, localization, immune interactions, andpharmacokinetic profiles. Collectively, this new information will help advance the development of these promising nanocages for drug delivery and vaccine applications.

Low-fouling Gold Nanorods Enabled by Sulfoxide Polymer Coating

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Au nanorods (GNRs) are particularly attractive as a platform to integrate diagnostic and/or therapeutic capabilities owing to their unique physical properties.[1] However, GNRs without appropriate coatings can be eliminated rapidly by the mononuclear phagocyte system (MPS) upon administration, significantly restricting their *in vivo* applications. Poly(ethylene glycol) (PEG) is commonly used as a low-fouling material for modification of GNRs to improve their biocompatibility and prevent unwanted capture by the MPS. Although PEG is generally known to be safe, recent research has highlighted that PEG itself is able to induce the generation of anti-PEG antibodies and elicit unwanted immune responses.[2] This may compromise the biological performance of PEGylated GNRs. Thus, it is important to develop new classes of GNRs coated with more advanced low-fouling polymers for biomedical applications.

Sulfoxide polymers are highly hydrophilic and show superior low-fouling characteristics compared to PEG counterparts.[3,4] In this study sulfoxide polymers were employed for surface modification of GNRs and to impart stealth properties. GNRs coated with sulfoxide polymers displayed much reduced cell uptake by macrophages compared with PEGylated GNRs, potentially contributing to a longer blood circulation and an improved biodistribution profile. Moreover, GNRs coated with sulfoxide polymers demonstrated excellent photothermal properties able to effectively ablate 4T1 breast cancer cells upon nearinfrared (NIR) light irradiation. Therefore, the sulfoxide polymers coated GNRs are expected to be a promising therapeutic agent with superior low fouling properties to achieve higher efficacy in the treatment of diseases.

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Understanding the interface between liquid metal droplets and bacterial, fungal and mammalian cells

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Gallium based liquid metals (LMs) have recently shown considerable promise for a wide range of biomedical applications. This is due to their unique combination of liquid and metallic properties, low toxicity and their ability to form a thin (~3 nm), self passivating solid oxide "skin", which enables the synthesis of nano- and micro-sized liquid-core, solidshell droplets. Research using LMs and LM droplets has been conducted in the areas of drug delivery, antimicrobials, molecular imaging, cancer therapies and as components in medical devices. Notably, in many of these applications, the LM is in direct contact with cells and other biological materials. Yet, despite the emerging research into LMs and LM droplets, the interactions between LMs and biological entities has not been well characterized or understood. In contrast, the interactions between solid metal particles and cells have been thoroughly investigated.

Here, we describe the dynamic cell-LM interface. A combination of confocal, electron and atomic force microscopy, as well as molecular dynamics simulations, were used to gain an understanding of the interactions between LM-droplets and bacterial, fungal, and mammalian ells. Single-cell force spectroscopy enabled quantification of the adhesion forces between cells and LM. We report that wrinkles form on the surface of the LM droplets, emanating from the point of adhesion. In many instances, the solid oxide skin is also broken. Additionally the cell wall deforms to intimately contact the LM droplets. This phenomena is a unique metal particle cell interaction that has not been previously described.

We propose a novel material-cell interaction whereby the flexible nature of the cell enables multiple adhesion sites with the LM droplets. This imparts tensile forces on the LM droplet surface, which results in surface wrinkling and breakage of the oxide layer. This work will help to underpin and establish an understanding of biological interactions with LMs.

A CRISPR/Cas12a-assisted on-fibre immunosensor for ultrasensitive small protein detection in complex biological samples

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Tracking trace amounts of analytes directly from low volumes of complex biological samples remains an ongoing challenge in precision diagnostics, as the commonly used immunosorbent assays have limited sensitivity. Herein, a CRISPR/Cas12a assisted on-fibre immunosensor (CAFI) was developed based on an antibody-analyte-aptamer sandwich structure, in which a single strand DNA aptamer was applied to detect the analyte while triggering the CRISPR/Cas12a fluorescent detection system to amplify the analyte signal. This novel CAFI biosensing system was fabricated on a glass fibre surface with an antifouling PEG polymer brush modified for the detection of a spectrum of small molecules from complex media. In comparison with a conventional ELISA system, CAFI has a 1,000-fold higher sensitivity with the limit of detection for IFN-γ down to 1 fg mL^{-1} (58.8 aM). It also has a tuneable linear detection range that can be easily adjusted within the range 1 fg mL^{-1} to 100 pg mL^{-1} (5 orders of magnitude), meeting the requirements of the demanding diagnostic scenarios. CAFI has successfully been demonstrated by detecting IFN-γ from a diverse complex biological sample type, including human serum, whole blood, perspiration, and saliva. Moreover, CAFI is applicable for the detection of other analytes by simply modifying the capture antibody and detection aptamer, demonstrated here with insulin. All these superior capabilities associated with CAFI make it a great solution to measure proteins from low (100 μL) volume complex biological samples.

A 3D bioprintable hydrogel with tuneable stiffness for exploring cells encapsulated in matrices of differing stiffnesses

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The emphasis in the development of *in vitro* models of *in vivo* cellular systems has undergone a shift from two-dimensions to three-dimensions as these better reflect the native 3D microenvironment. The advances and new materials available to the field have opened an opportunity to transition this research to a high-throughput platform. 3D bioprinting is an exciting technology with the potential to provide this throughput and produce cell-laden structures with high fidelity. Recently, our team and collaborators have developed a bespoke drop-on-demand printer and demonstrated its capability as a platform to quantitatively study a cells in a high-throughput manner.[1-3] Here, we utilise this technology to develop and print a bioink system with tunable stiffness to expand the limited range of stiffnesses seen in 3D bioprinting. We used a 4-armed polyethylene glycol with maleimide functionalized arms that are crosslinked using thiolate species. Tuning was achieved by employing an MMP-cleavable peptide flanked by cysteines and a 4-armed thiolated polymer. The ratio was adjusted to control the stiffness. This system allows for an elegant method of controlling stiffness while still allowing for the addition of biological motifs. The application of this system was explored using MCF-7 cells for their viability, proliferation, migration, and morphology in printed matrices of different stiffnesses. This work explores the versatility of this bioink in conjunction with a high-throughput bioprinter and demonstrates its capability as a platform for studying cell behavior in a range of environments.

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Colorimetric Detection of a Neurodegenerative Biomarker Using a Metal Organic Framework-based Nanozyme

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Neurodegenerative disorders (NDDs) are a leading cause of movement and mental functioning impairment worldwide, with the most common ones being Alzheimer's (AD) and Parkinson's disease (PD). Recently, it was reported at the Alzheimer's Association International Conference (AAIC, 2021) that an estimated 350,000 individuals per year will develop early onset dementia.[1] Somber statistics like these have led to significant research towards developing sensing assay for NDD biomarkers.[2] One of the most common markers investigated for AD is the β-amyloid (Aβ) peptide. To date, a variety of methods to detect NDD biomarkers have been developed, including enzyme-linked immunosorbent assay (ELISA), surface plasmon resonance (SPR), electrochemical impedance spectroscopy (EIS), and mass spectrometry. While effective, these techniques are expensive, time-consuming, and require extensive sample preparation, sophisticated equipment, and trained personnel. These factors limit their applicability in pointof- care diagnostics. As such, there is a need for a simple and rapid diagnostics test. In the last few decades, metal-organic frameworks (MOFs) have emerged as an attractive enzymemimicking catalytic nanomaterial (nanozyme) for biosensing applications, mainly due to theirlarge surface area, high porosity, and tunable structure.[3]

Herein, we present a metal-organic framework-based nanozyme for colorimetric Aβ detection (Fig.1). The assay relies on metal-doped MOFs, which exhibit peroxidase-like activity, to catalyze the oxidation of colorless 3,3′,5,5′-tetramethylbenzidine (TMB) to a blue product. In the presence of Aβ, the catalytic activity of the MOF nanozyme is inhibited as the peptide strongly binds to the surface, blocking the active sites and resulting in no blue colored product forming. This rapid and simple MOF-based colorimetric assay shows great potential for point-of-care diagnostics of AD.

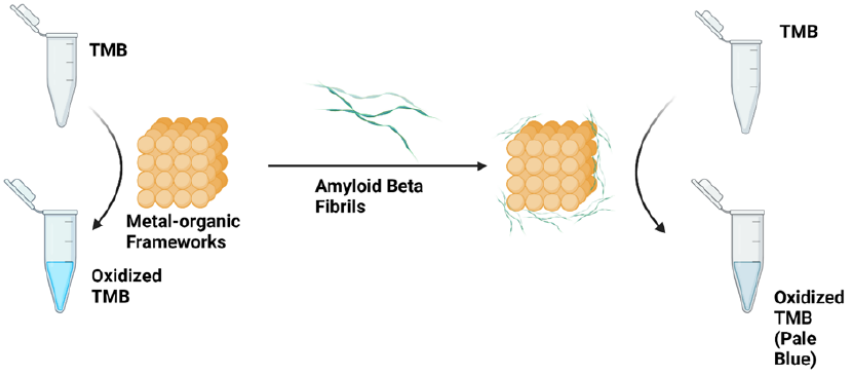

Figure 1: Schematic of MOF-based assay to detect the Aβ peptide via inhibition of TMB oxidation.

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Together or apart: Optimising the pre-targeting of polymeric nanocarriers

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One approach to enhance nanomedicine therapeutic delivery is to incorporate a targeting agent within the nanomedicine construct, such as an antibody, which specifically binds to antigens presented by the target tissue. However, development of stable conjugates between biomolecules and nanomaterials is non-trivial, limiting development in this space. Recent work in our group has engineered bispecific antibodies (BsAbs) with dual specificity, whereby one portion binds to methoxy polyethyleneglycol (mPEG) epitopes present on synthetic nanomedicines, while the other binds to molecular disease markers of interest.[1] In this way noncovalent complexes of nanomedicine core, comprising a hyperbranched polymer (HBP) of primarily mPEG, decorated with targeting ligands is able to be produced by simplemixing.[1] Studies of this targeting platform have now shown enhanced nanomedicine accumulation and therapeutic efficacy against multiple cancer targets including EGFR+ breast cancer in mouse models.[2]

While this targeting approach shows promise for enhancing effectiveness of nanomedicines, the coating of "stealthy" polymeric materials with proteinaceous targeting ligands not only improves interactions with target cancer cells, but also enhances recognition by mononuclear phagocytic system (MPS) components, leading to accelerated clearance from circulation.[3] The current work now focuses on approaches to overcome this dichotomy by combining the stealthy carrier and targeting components *in situ*. We report in vitro flow cytometry and confocal microscopy studies of HBP and BsAb association on the cell surface. This is then coupled with preclinical ^{89}Zr-PET imaging studies in to optimize the administration of targeting ligands and nanomaterial carriers sequentially (Figure 1), resulting in enhanced tumour uptake with reduced hepatic accumulation. Such a pretargeting approach to modulate bio-nano interactions allows the complimentary benefits of targeting and reduced MPS clearance to be realised to improve nanomedicine efficiency.

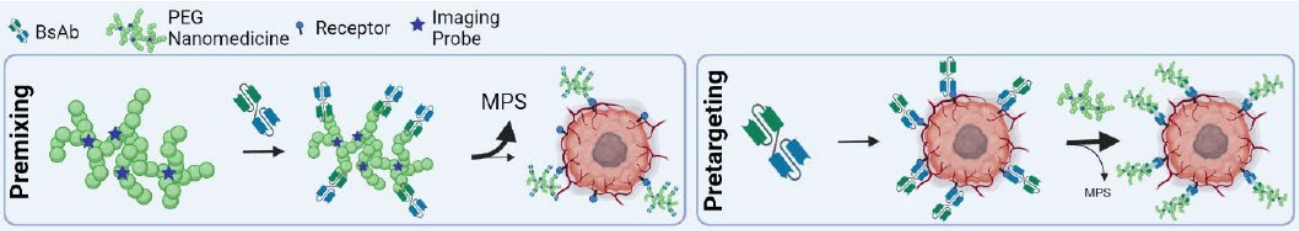

Figure 1: Schematic outline of BsAb pre-targeting strategy in contrast to conventional premixing method to generate nanomedicine targeting approach

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Inkjet printing of polydiacetylene quick response codes on packaging for food spoilage detection

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Food contamination and food waste are pressing global issues that affect human health, the environment, and the economy.[1] There is a critical need for developing a cheap, fast, and accurate sensor to monitor food quality in real-time that can significantly reduce food waste and foodborne illnesses. Polydiacetylene (PDA) is a class of conjugated polymers derived from diacetylene (DA) monomers that polymerize via a 1,4-addition reaction upon UV irradiation. The polymerization results in the formation of blue-phase PDA, which can be detected by UVVis spectroscopy and by the naked eye. A blue-to-red chromatic transition of PDA can be induced by stimuli such as pH, temperature, humidity, mechanical stress, and ligand-receptor interaction.[2] PDA can be easily constructed as thin films or coated onto solid substrates. Collectively, these properties make PDA an attractive candidate as colorimetric sensors. Herein, we developed a PDA-based colorimetric sensor to detect food spoilage in real-time. Specifically, we developed a DA monomer formulation and performed inkjet printing in the format of quick response (QR) codes on common food packaging materials, including baking paper and sandwich bags. Five common biogenic amines (BAs) released from spoiled food, including putrescine, cadaverine, spermidine, histamine, and tyramine were successfully detected by our developed PDA-based QR code printed sensor. The sensitivity of QR codes towards five Bas exhibited in the order of spermidine>putrescine>cadaverine>tyramine>histamine. We then demonstrated this sensor to monitor chicken meat deterioration in real-time when stored at room temperature and 4 °C (Figure 1). In conclusion, PDA-based QR codes can be incorporated intofood packaging. The sensors are stable and can be produced at low cost, showing great potential in providing consumers with information about food spoilage and minimizing foodborne illness.

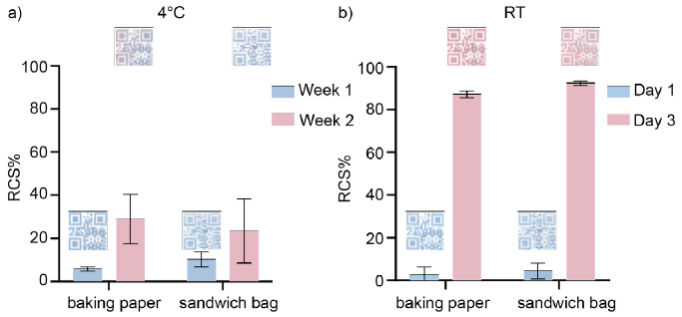

Figure 1: Colorimetric response and red chromatic shift (RCS) of PDA-based QR codes printed on baking paper and sandwich bag when exposed to chicken meat stored at a) 4°C and b) room temperature (RT). n=3, error bars represent standard deviation.

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Sustained Drug Release from Nanoparticles Functionalized by a Neural Tracing Protein for Treatment Respiratory Dysfunction of Spinal Cord Injury

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Respiratory dysfunction is a major cause of death in people with spinal cord injury (SCI).[1] A remaining unsolved problem in treating spinal cord injury is the intolerable side effects of the drugs to patients.[2] In a significant departure from conventional targeted nanotherapeutics, which primarily focus on overcoming the blood-brain barrier, this work pursues a drug delivery approach that uses neural tracing retrograde transport proteins to bypass the blood-brain barrier and to deliver an adenosine A1 receptor antagonist drug, 1,3-dipropyl-8-cyclopentyl xanthine (DPCPX), exclusively to the respiratory motoneurons in the spinal cord and the brainstem. Here, we synthesise, characterise the purity and quality of synthesis and investigate stability, drug release properties and therapeutic outcome of the neural tracing protein (wheat germ agglutinin chemically conjugated to horseradish peroxidase-WGA-HRP)-coupled gold-DPCPX nanoconjugate. We determine that the nanoconjugate formulation is capable of sustained drug release lasting for days in physiologic pH, a prerequisite for long-distance transport of the drug from the diaphragm muscle to the brainstem. The nanoconjugates were stable, the drug release profiles follow both first-order reaction and the Noyes-Whitney diffusion models and the particles do not accumulate in the tissues at the injection site. A single intradiaphragmatic injection at one thousandth of the native drug dosage induces prolonged respiratory recovery in a hemisection animal model. Thus, this study supports further development of neural tracing protein-enabled nanotherapeutics for treating respiratory problems associated with SCI and translation the discovery into new treatments for respiratory dysfunction.

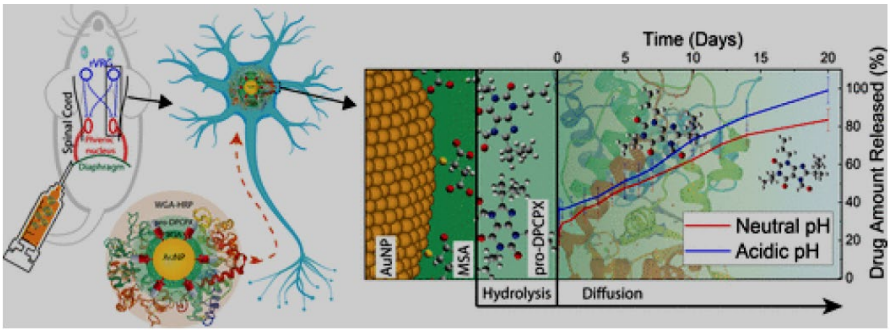

Figure: Graphical presentation of transporter protein and drug conjugated gold nanoparticles passing through neuron (retrograde direction) and drug release kinetics and mechanism through the surface of the gold nanoparticles to the release media by different pH (Gold-DPCPX-WGA-HRP).

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Reactive Oxygen Species Scavenging and Improvement Effect of Inflammatory Disease Treatment by Interaction of Nanoparticles and Intracellular Organelle

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Intracellular oxidative stress is induced pathologic factor in generally inflammatory disease. Previous researched CeO_2 nanoparticles are well known to function as strong and recyclable Reactive Oxygen Species (ROS) scavengers by two oxidation states of Ce^{3+} and Ce^{4+}. Interestingly, ceria-zirconia nanoparticles (CZNPs) are more effective to ROS scavenging than CeO_2 nanoparticles. That's faster conversion from Ce^{4+} to Ce^{3+}. Currently, variety nanoparticles are like ROS scavenging nanomaterials intermittently report because they affect the cellular reaction. Interaction of nanoparticles with cellular functions, such as repression/activation of genes and activation of specific cell organelle. In the present work, we studied the intracellular organelle response with exposure to ROS scavenging nanoparticles in the inflammatory disease model.

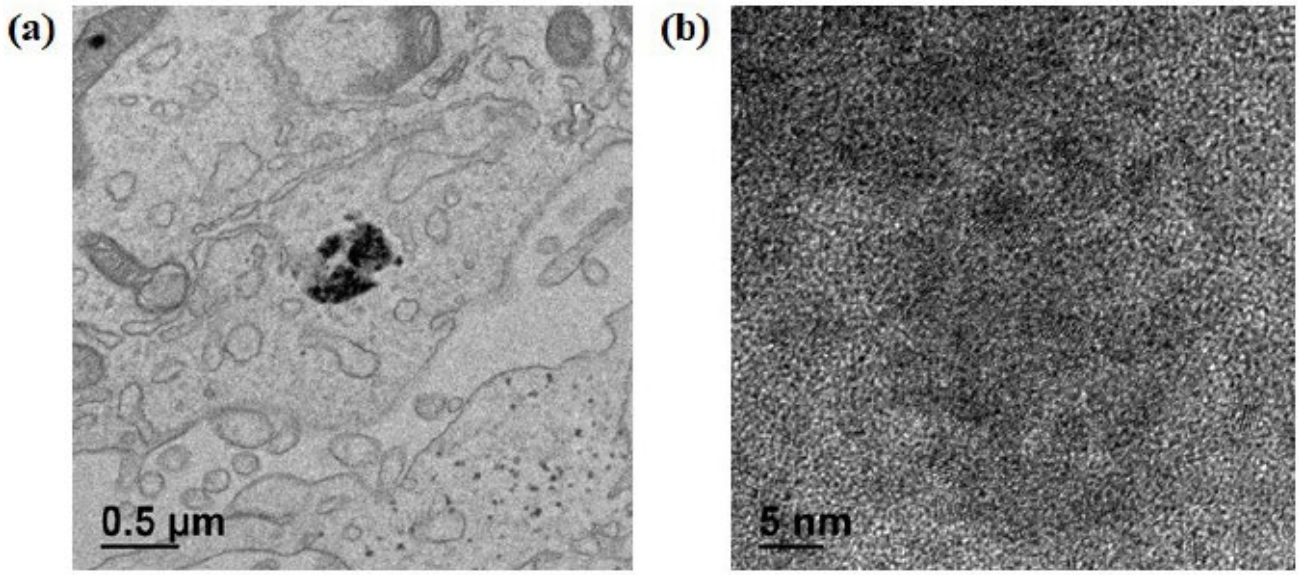

Figure 1: Cells were grown to 80% confluence, then were exposed for 24h in complete culture medium to 2 µm/cm2 CZNPs. The presence distribution of nanoparticles intracellular by Bio-HVEM. (a) and (b) Ceria-Zirconia nanoparticles in the lysosome of human podocytes.

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Oral Nanotherapeutic Formulations of Insulin

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Nanotechnology, particularly quantum dots (QDs), demonstrate rapid intestinal uptake and drug delivery to the liver following oral administration (1). Previously we have shown drastic improvements in drug oral bioavailability following surface attachment to QDs, these changes were facilitated by changes in intestinal and hepatocyte uptake pathways (2).

We aimed to investigate the oral bioavailability of larger peptides such as insulin (that must be given via SC injection) and examine if QDs could act as transporters for insulin to develop an oral therapeutic. We also aimed to investigate glycopolymers of chitosan and glucose to provide functional targeting of hepatocytes with by pH dependent aggregation and glycosidase dependent enzymatic cleavage to control the release of insulin in vivo.

Silver sulfide (Ag_2S) QDs were conjugated to regular insulin and incapsulated within a chitosan/glucose glycopolymer. Bioavailability and biodistribution were investigated in WT C57BL/6J using ^{14}C radiolabeled insulin, with pharmacodynamic data investigated using oral glucose tolerance testing. Finally, we investigated insulin tolerance testing of our QD-insulin in animal models of type 1 diabetes.

QD-insulin demonstrate a 4% systemic bioavailability compared to SC-insulin. However, biodistribution within the liver was 3-fold higher than blood. Both SC-insulin (2 IU/kg) and QDinsulin (20 IU/kg) produced similar reductions in oGTT AUC in WT C57BL/6J mice. In diabetic NOD mice and STZ treated rats both SC-insulin and QD-insulin demonstrated similar reductions and time frame of action in ITT AUC compared to WT mice.

This work investigated the effectiveness of QDs to facilitate delivery of the non-bioavailable peptide insulin. We found that QDs promoted an increase in bioavailability with insulin distributing to the liver and promoting reduction in blood glucose.

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Bone-cartilage interfaces via printing of ceramic ink in stem cell-laden microgel suspensions

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We have developed a 3D bioprinting technique to more accurately recapitulate the bonecartilage interface. These interfaces play a pivotal role in the function of articular joints and vertebrae. The complex biophysical characteristics and biochemical crosstalk between viscoelastic cartilage and the stiff integrated bone are poorly understood. As such, strategies to repair damage to these interfaces, due to the onset of conditions such as osteoarthritis and intervertebral disk degeneration, have had limited long-term success. To address this, we aim to create a three-dimensional in vitro model of the bone-cartilage interface using a multiphasic approach which combines soft and hard materials. We combined photo-crosslinkable gelatin methacryloyl microgel suspensions optimized for inducing chondrogenic differentiation of adipose derived mesenchymal stem cells (ADSCs) with a hard inorganic bone-mimetic ink optimized for 3D printing. We showed that this ceramic bio-ink deposited omnidirectionally inside the microgel suspensions induced osteogenic differentiation of ADSCs in proximity to it. Separately, by modifying the filler content in ADSC-laden microgel suspensions, we were able to optimize them for chondrogenesis. The gels showed increased compressive moduli and expression of chondrogenic marker Sox9 after 21 days. We anticipate that by combining these two approaches, we not only will be able to generate more physiologically accurate bonecartilage interfaces, but the ceramic bio-ink's ability to be printed into complex geometries inside microgel suspensions will allow recapitulation of an array of physiologically relevant bone-cartilage interfaces.

Molecular and cellular insights into the role of gut-bacteria for neurodegeneration

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The gut microbiota has recently been discovered for its role in extra-intestinal diseases such as neurodegeneration, cancer and emotional disorders. As numerous reports clinically support the link between gut-dysbiosis and inflammatory responses in the brain or gut itself, the exact molecular mechanisms for this pathogenic communication are missing. Protein aggregation and fibrillization, called amyloidosis, is a ubiquitous phenomenon that covers a variety of biological processes ranging from physiological storage of hormones in the cell to the pathological paradigms of Alzheimer's and Type-2-Diabetes. Microbial colonies make amyloid fibers to support their biofilms and to survive environmental threats. In this presentation, I will discuss the results from our research that help in understanding how the biofilm components of gut bacteria can exploit the protein aggregation and fibrillization paradigm to cross-talk and trigger the toxic aggregation of proteins in the brain. We have demonstrated that the biofilms forming protein of *Pseudomonas aeruginosa*, *i.e.,* FapC, can seed the aggregation of Amyloid-β (Aβ)monomers into neurotoxic oligomers by cross-β stacking. FapC seeds displayed a suitable binding affinity for Aβ and induced a transition in the native mesoscopic structure of end-stage Aβ fibers. *In-vivo* studies in zebrafish demonstrated early Aβ burden in cerebral tissues and behavioral pathologies. This research provided the first structural and biochemical evidence that peptide cross-seeding can induce mesoscopic inheritance and pathological correlation. Establishing the molecular basis for the catalytic role of pathogenic gut-bacteria can provide new pharmacological targets and pharmaceutical tools against neurodegeneration diseases. The talk will present novel results relating to the pioneering work of Javed et. Al.,[1]

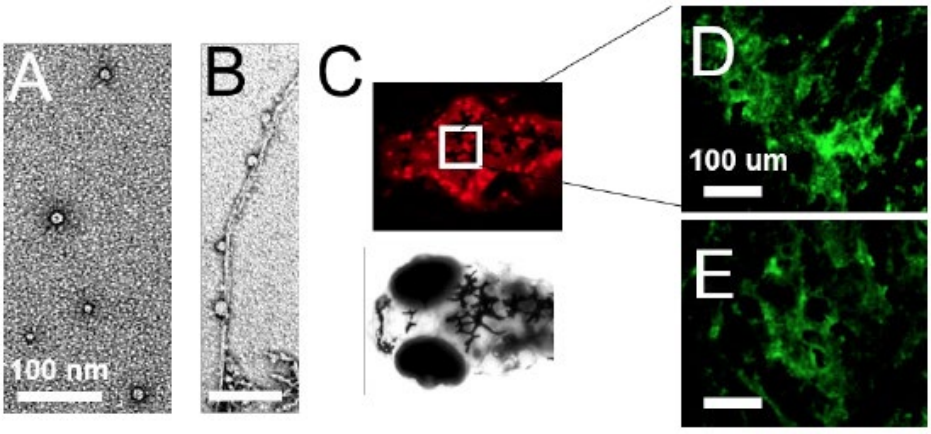

Figure 1: The sonicated nanosized fragments of FapC (A) that accelerated the fibrillization of Amyloid β and adsorb on the sides of Aβ fibrils (B). In zebrafish model of Aβ toxicity, FapC fragments together with Aβ induced higher aggregation into fibrils (C, as detected by in-vivo ThT staining) and higher Aβ cerebral burden (D: Aβ + FapC fragments, E: Aβ only, immunostaining 4 days after treatment).

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A high-throughput 3D bioprinted cancer cell migration and invasion model with versatile and broad biological applicability

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Metastasis is the leading cause of cancer-related deaths, and thus an understanding of the underlying molecular and cellular processes continues to be a key focus of cancer research. The current understanding of the mechanisms underlying these cancer processes is predominately based on flat 2 dimensional (2D) cell culture models due to their simplicity. In addition, these 2D culture models are frequently used in high throughput (HTP) drug discovery although they do not always accurately predict drug response. Therefore, there is an urgent need to develop *in vitro* 3D tumor models that can mimic physiological cell-cell and cell-extracellular matrix interactions, with high reproducibility and that are suitable for high throughput (HTP) drug screening. To achieve this, we recently developed a bespoke drop-on-demand bioprinter (1, 2). Here we leveraged this 3D bioprinting technology and tunable hydrogel system to develop a versatile HTP 3D bioprinting platform for studying cell migration and invasion. As cell models, we selected a combination of cancer cell lines with non-invasive and invasive phenotypes, which are known to express distinct epithelial and mesenchymal markers. This HTP 3D bioprinting platform enables (i) the rapid encapsulation of cancer cells within *in vivo* tumor mimicking matrices, (ii) *in situ* and real-time measurement of cell movement, (iii) detailed molecular analysis for the study of mechanisms underlying cell migration and invasion, which are critical for metastasis, and (iv) the identification of novel therapeutic options. This work demonstrates that this HTP 3D bioprinted cell migration platform has broad applications across quantitative cell and cancer biology as well as drug screening.

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LyP-1 conjugated Methylene blue encapsulated mesoporous silica nanoparticles for targeted photodynamic therapy in breast cancer

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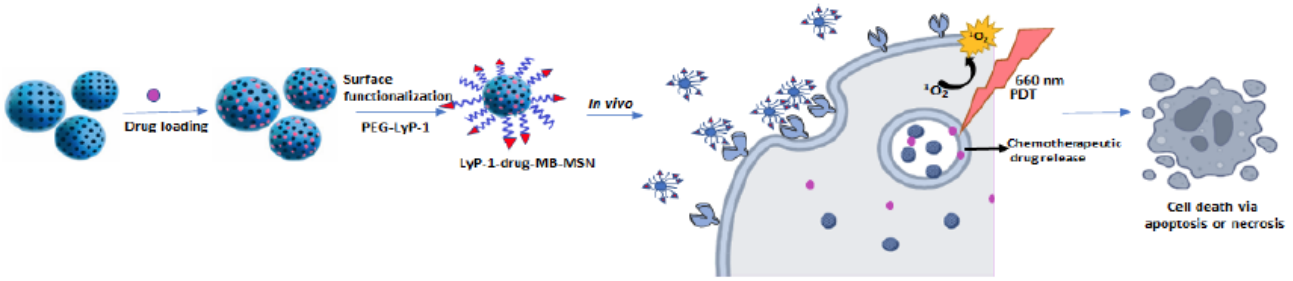
Photodynamic therapy is an approach to treat malignant tissues using a photosensitising drug. It relies on the efficient generation of cytotoxic reactive oxygen species (ROS) at the targeted site upon exposure to a suitable light source. Lately, targeted PDT has been gaining interest as a therapeutic approach to treat different cancer tumours and appears to be a propitious alternative in localised breast cancer treatment. Methylene blue (MB), a phenothiazine-based photosensitiser, has been extensively studied for its antibacterial and antitumour PDT applications. However, unwanted biological interaction and poor tumour localisation impede its biomedical applications. One approach to overcome these limitations is the use of nanoparticles for targeted delivery of MB to specific target[1]. Recently, we developed a silane derivative of methylene blue[2] that enabled the covalent encapsulation of MB inside the matrix of silica nanoparticles (MSNs). The results show that the covalently encapsulated MB silica nanoparticles do not exhibit dye leaching and have higher photosensitisation than nanoparticles encapsulated with MB via physical adsorption. Nonetheless, the use of these nanoparticles requires functionalisation for targeted delivery strategy, particularly if they are administered as systemic agent.

LyP-1 is a tumour homing peptide proven to bind selectively to certain tumour cells, tumour associated lymphatic vessels and macrophages[3]. Nanoparticles conjugated with this tumour targeting ligand can accumulate predominantly at the pathological site and reduce unwanted biological interaction[4]. Therefore, in our lab, we are developing MB encapsulated mesoporous silica nanoparticles, multivalently tagged with LyP-1 peptide (LyP-1-MB-MSN). We will use the LyP-1-MB-MSN to target them in the 4T1 breast cancer cells *in vitro* and *in vivo*. Using PDT to active photosensitizing effect of MB, we will assess the effect of our treatment in killing the tumour cells. This targeted PDT approach to kill tumour cells, may potentially be a new treatment solution for treating breast cancer.



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Synthetic and hybrid analogues of biopolymer networks for tissue engineering applications.

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Reconstituted networks of natural extracellular matrices (ECMs), such as collagen or fibrin show a large increase in stiffness upon externally applied stress or deformation.[1] Recently, a new biomimetic hydrogel, based on oligo(ethylene glycol)-grafted polyisocyanopeptide (PIC), was developed in our group.[2] These extremely stiff helical polymers form gels upon warming at concentrations as low as 0.005 %-wt polymer, with materials properties almost identical to those of intermediate filaments and natural ECMs.[2,3] The application of these materials in cell growth and drug therapeutics revealed the importance of polymer nonlinear mechanics.[3]

The importance of sourcing reproducible materials is exemplified by the fact that the biophysical cues of the extracellular matrix (ECM), including topology, bulk stiffness, stress relaxation, stress stiffening and matrix geometry, have been identified to guide cellular behaviors. Another shortfall of some natural polymers, such as collagen, is their slow process of network reconstitution, which makes the encapsulation of cells in three-dimensional volumes challenging. With the recent focus on the field on three-dimensional cell studies, this has become of concern.

To specifically address the mechanical reproducibility and slow network reconstitution of collagen networks, we implemented our well-defined synthetic biopolymer analogue – PIC, to provide mechanically controlled interpenetrating networks (IPNs) of collagen and the biomimetic PIC polymer. Unlike other synthetic polymers, PIC forms a fibrous network structure at physiological temperatures and responds nonlinearly to mechanical loads analogous to natural fibrillar polymers. Using a custom-designed confocal microscope–rheometer setup,[4] we observe, *in situ*, the formation of collagen networks in PIC on a microscopic scale, while simultaneously recording the mechanical synergy between both fibrillar materials.

This confocal–rheometer system can be applied across a range of synthetic and natural biopolymers, and provide insightful information about the relationship between the structural and mechanical properties of these three-dimensional networks.

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Confined growth directs fibroblasts plasticity and induces 3D bio-assembly through epigenetic reprogramming

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The development of induced pluripotent stem cells (iPSC) has revolutionized the field of regenerative medicine and disease modelling in recent decades[1]. However, the stochastic nature of reprogramming process, limited efficiency and the usage of oncogenes and viral factors has hindered its clinical viability. Here I will present a vector free approach to induce plasticity in fibroblasts with confined substrates and direct subsequent 3D bio-assembly. A combination of extracellular matrix (ECM) proteins, fibronectin, laminin and collagen are covalent conjugated on polyacrylamide (PA) gel to control cell microenvironment and topography. Confined growth of fibroblasts forms spheroids that are then integrated in UV crosslinked GelMA fishing line channels. Controlled stiffness, interfacial topography and ECM protein coupling promote epigenetic reprogramming of primary mouse embryonic fibroblasts (PMEF) into a pluripotent phenotype in the absence of exogenous factors. The stem-like fibroblasts express both pluripotent and germ layer markers, forming a contractile outer layer to maintain tissue integrity. Primed PMEFs lift up upon confluency, forming spheroids that are prone to self assembly in various biomaterial substrates. Early onset of reprogramming is accompanied with heightened autophagy activities, potentially facilitating rewiring of the genome.

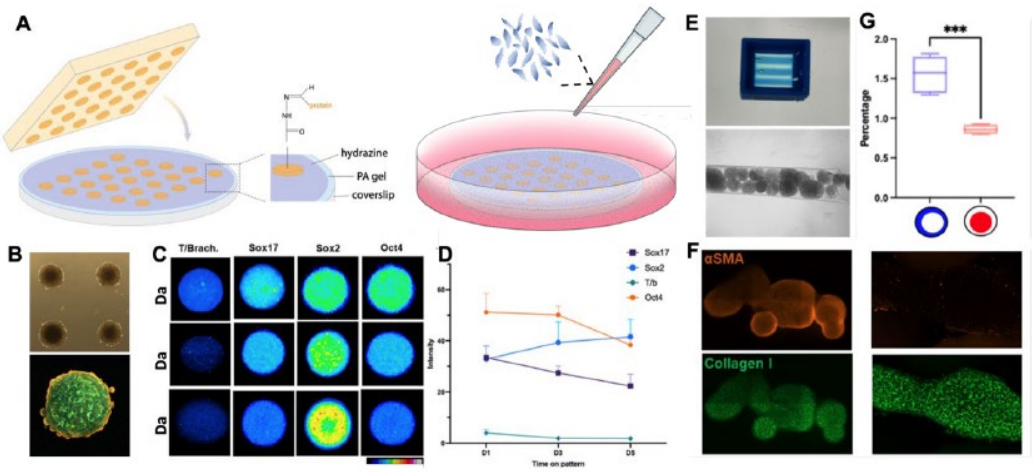

Figure 1: (A) Schematic representation of PA gel patterning. (B)PMEF spheroids formation and contractile outer layer stained with α-SMA (orange) and actin (green). (C) Heatmap of pluripotent and germ layer markers of primed cells on pattern and (D) time course analysis. (E) Fishing line channels and spheroids integration. (F) α- SMA and actin expression of self-assembled spheroids in fishing line channel (left) and control (right) and (G) segment quantification of self-assembled tissue.

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Targeted delivery of immune-boosting peptides using polymeric nanoparticles to manage lung cancer

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Lung cancer is one of four leading cancers causing a high death rate globally. Approximately 85% of lung cancers are non-small-cell lung cancers (NSCLCs), and the average 5-year survival is 15-20%. The dominant factors contributing to the high death rate for NSCLCs are: 1) Advanced stage diagnosis with metastatic disease for most patients, 2) Heterogenicity and low antigenicity of NSCLCs. Thus, novel therapeutic options for long-term treatment of NSCLCs are necessary for patients who have poor prognosis and immune-therapeutic outcomes. InterK Peptide Therapeutics have developed peptides with immune-boosting functions, re-invigorating exhausted T cells and potentially serving as a novel therapeutic agent against human lung cancer. The ability to produce a site-specific response will be the major hurdle to translate these therapeutic peptides for clinical use. Rapid growth in nanotechnology provides an opportunity to improve the pharmacokinetic and pharmacodynamic behaviour of drugs due to their physical and chemical characteristics. Thus, nanocarriers are the ideal platform for the targeted delivery of InterK peptide and increasing their concentration at the tumour site. This project investigates the targeted delivery of InterK's novel immune-boosting peptides to the tumour microenvironment utilising nanomedicines, and evaluating pharmacokinetics, biodistribution and tumour suppression properties of the peptides (Figure 1).

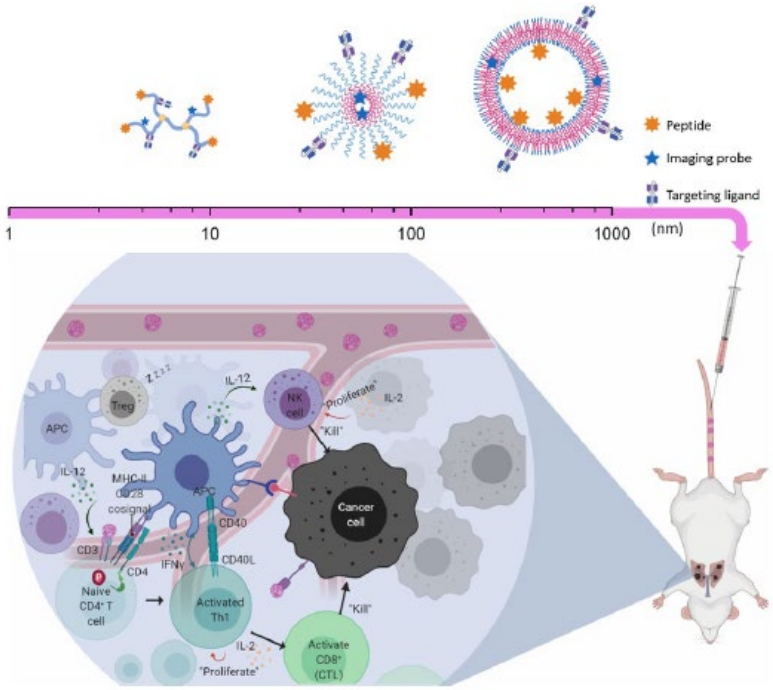

Figure 1: Schematic design of nanocarriers (NPs and cells are not drawn to scale)

Tunable Nitric Oxide Delivery by Nanomaterials and Polymers

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Nitric oxide (NO) is well-recognized as a gaseous signaling molecule in the cardiovascular, nervous, and immune systems. Due to its therapeutic potential, NO is a promising candidate for varied biomedical applications such as anticancer, antibacterial, and wound healing. However, therapeutic NO delivery is not trivial due to NO's short half-life (<5 s), diffusion distance (40–200 μm), and concentration-dependent biological functions *in vitro* and *in vivo*. Extensive efforts have been made to develop systems that can scavenge[1] or deliver[2] NO in a controlled and sustained manner such as polymeric platforms. Ceria NPs are well-known to exhibit NO-scavenging activity[3], but we hypothesize the possibility of ceria NPs to induce NO release by acting as a catalyst to decompose NO donors due to its catalytic and Ce^{3+}/Ce^{4+} switching activity. Herein, we reported the first studies documenting ceria NPs and amine-containing polymers to enable controlled NO generation from endogenous NO donor *S*-nitrosoglutathione (GSNO).[4] By varying the concentrations of ceria NP/polymers and GSNO, biologically relevant NO levels can be tuned. Ceria NP and amine-containing polymers retained NO-generating catalytic potency for at least 5 NO generation cycles. The NO generation mechanism of ceria NPs was deciphered to be attributed to the oxidation of Ce^{3+} to Ce^{4+} on their surface (Figure 1) by X-ray photoelectron spectroscopy and density functional theory (DFT) calculations. And the amine groups on polymers was found to contribute to their NO generation capability. The therapeutic effect of ceria NP-induced NO generation was evaluated by the suppression of cancer HeLa cells, resulting in a 93% reduction of HeLa cell viability. In addition, the biofilm prevention functionality of aminecontaining polymers was demonstrated.

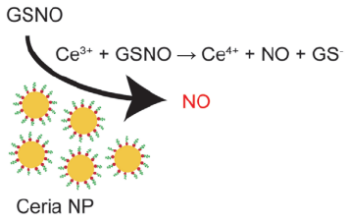

Figure 1*:* Schematic illustration of the mechanism of NO generation from GSNO by the catalytic activity of ceria NP.

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Wearable Platform for Therapeutic Nitric Oxide Delivery

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COVID-19 is known to cause mild to severe respiratory symptoms, with the latter case leading to a substantial strain on healthcare systems (e.g. expensive ventilators). This has resulted in a need for alternative preventative strategies for respiratory symptoms. Among the approaches considered, inhaled nitric oxide (iNO) shows great promise.[1, 2] Nitric oxide (NO) is a gaseous molecule reported to have therapeutic benefits when inhaled at low doses, specifically dilating blood vessels in lung segments involving gas exchange.[3] However, commercially available NO delivery systems involve expensive and large pressurized cylinders, and rely on robust supply chains. Moreover, patients need to attend a hospital to gain access to NO treatment, increasing exposure risk.[4] Other currently researched options involve electric generators and chemically induced NO generation; however, these require high-voltage electrical charges, trained personnel, and toxic chemicals.[4]

Herein, the therapeutic benefit of nitric oxide is combined with an accessible delivery platform, a face mask (Figure 1). NO releasing nanoparticles are synthesized and introduced onto the mask, where the high humidity environment triggers the release of NO. An average release profile of 2 ppm per minute was achieved over 1.5 hours. The NO-mask can be stored safely for extended periods of time without requiring pressurized vessels, and can be easily transported due to its light weight. This platform should reduce the cost associated with NO delivery, increase the accessibility of NO treatment, and allow for point-of-care use, whilst reducing exposure of patients to hospital settings. This is especially important considering the current pandemic. We envision it can be easily acquired at a pharmacy and used for other diseases with medical prescriptions. More importantly, it will have significant implications towards alleviating the strain currently affecting healthcare systems worldwide.

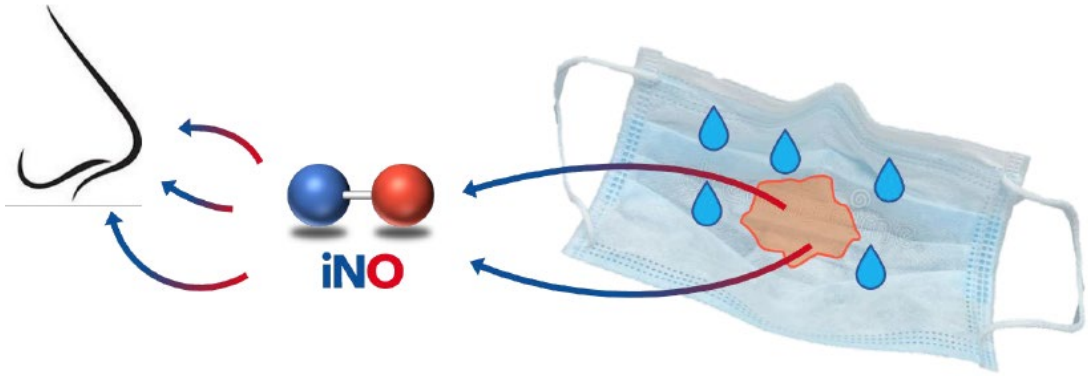

Figure 1: Accessible iNO delivery system. High humidity environment in mask triggers the release of NO, achieving a sustained average profile of 2 ppm per minute for 1.5 hours.

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Targeted delivery of PEGylated liposomal doxorubicin by bispecific antibodies improves treatment of high-risk childhood leukaemia

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High-risk childhood B and T cell acute lymphoblastic leukaemia (high-risk B- and T-ALL) has a poor prognosis due to treatment failure and toxic side effects of therapy.[1] Similarly, children diagnosed with B-ALL and acute myeloid leukaemia (AML) both harbouring *MLL* gene rearrangements have >50% relapse rates and poor overall survival. For these aggressive subtypes of leukemia, there are only limited treatment options with most intensive chemotherapy regimens having reached the limit of tolerability.[2] Drug encapsulation into liposomal nanocarriers has shown clinical success at improving biodistribution and tolerability of chemotherapy.[3] However, enhancements in drug efficacy have been limited due to a lack of selectivity of the liposomal formulations for the cancer cells.

Here, we focus on the development of a dynamic and flexible "mix-and-match" approach for the targeting of liposomal drugs to high-risk childhood leukemia. This approach allows for the rapid adjustment of therapy based on the specific cell surface receptors expressed on leukaemia cells. Specifically, PEGylated liposomal drugs are non-covalently complexed with an interchangeable panel of bispecific antibodies (BsAbs) that simultaneously bind to methoxy polyethylene glycol(PEG) on the nanoparticle surface and CD19, CD20, CD22 or CD38 receptors on leukaemia cells.

BsAbs improved the targeting and cytotoxic activity of a clinically approved and low-toxic PEGylated liposomal formulation of doxorubicin (Caelyx) toward leukaemia cell lines and xenografts that are immunophenotypically heterogeneous and representative of high-risk subtypes of childhood leukaemia. BsAb-assisted improvements in leukaemia cell targeting and cytotoxic potency of Caelyx correlated with receptor expression and were not detrimental toward healthy peripheral blood mononuclear cells or haematopoietic progenitors. Targeted delivery of Caelyx using BsAbs further enhanced leukaemia suppression and extended overall survival by up to three-fold in clinically-relevant patient-derived xenograft models of high-risk childhood leukaemia developed at our institute. Our methodology employing BsAbs therefore representsan attractive targeting platform to potentiate the therapeutic efficacy and safety of liposomal drugs for improved treatment of high-risk leukaemia.

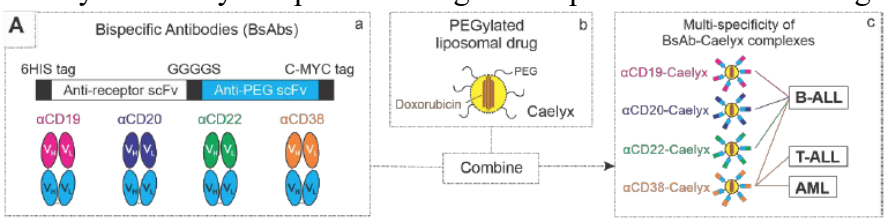

Figure A: Schematic illustration of BsAb expression vector and BsAb products targeting PEG and a leukaemiacell surface receptor (CD19, CD20, CD22 or CD38)(a), BsAbs complexing with Caelyx (b), and targeting of resulting BsAb-Caelyx complexes toward major leukaemiasubtypes (c).

Design and development of novel peptide hydrogels as biomimetic organoid matrices

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Pluripotent stem cell and organoid cultures rely heavily on natural hydrogels as cell culture substates. However, the protein content of natural hydrogels like Matrigel is poorly defined and suffer from batch-to-batch variations, which confound biological readouts[1]. Synthetic peptide hydrogels have emerged as promising alternatives as they enable the decoupling of biophysical properties of the microenvironment from cell behaviour and fate. Certain peptide motifs can self-assemble into nanofibers that form entangled networks reminiscent of native tissue matrices. However, the sequencing space of the twenty canonical amino acids is near infinite and identifying new self-assembling peptides from sequence or experimentation alone remains highly challenging.

Here, we will present our combined computational and experimental approach to identify novel peptide sequences that form hydrogels via a tryptophan zipper (trpzip) motif. High performance computing was used to run coarse-grain molecular dynamics (MD) simulations of hundreds of trpzip peptide monomers within a physiologically solvated simulation box. Simulations revealed promising gelator candidates with high aggregation propensities along with fibrillar morphologies. Experimental studies confirmed the gelation of chosen candidates, while original trpzip peptide controls remained liquid. The nanostructure and molecular interactions of peptide aggregates were investigated using circular dichroism and transmission electron microscopy. The viscoelastic properties of our gelating trpzip variants were characterised using parallel plate rheology, where we found that our 1 wt% peptide hydrogel reaches a stiffness of 16 kPa within an hour. We also observed the self-healing of our hydrogel to a stiffness of 10 kPa within 10- minute time frames. We anticipate this novel trpzip peptide hydrogel system will provide a well-defined yet tunable and biomimetic environment for pluripotent stem cell differentiation and organoid growth and morphogenesis.

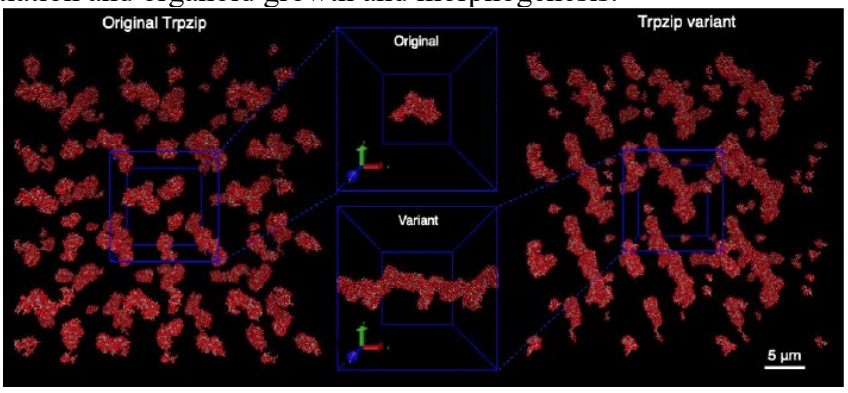

Figure 1: **Snapshot of the final frame of 100 µs coarse-grain MD simulations for original trpzip peptide sequence (left) and the trpzip variant peptide (right).** Middle boxes show the largest peptide aggregates of each simulation aligned against the same axis for comparison.

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Ligand-free Immune Cell Targeting by Lipid Nanoparticles

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Lipid nanoparticles (LNPs) are an emerging technology for the effective delivery of nucleic acid therapeutics, particularly as vaccine platforms.[1] However, little is known concerning specific immune cell interactions of LNP formulations; furthermore, current commercial platforms lack exclusive delivery to lymphoid organs, which may necessitate higher doses to achieve effective vaccination. We developed an ionizable lipid-based formulation (KC2 LNPs) and a formulation additionally incorporating a permanently cationic lipid (DOTAP LNPs) with similar size (~100 nm) while differing in charge at physiological pH. Both LNPs demonstrated high, time-dependent levels of association with antigenpresenting cells in the blood *ex vivo*. Furthermore, after intravenous injection into mice, IVIS *in vivo* imaging showed both LNPs mediated high transfection of a nanoluciferase pDNA cargo in the spleen after 24 h, with little transfection observed in any other organs. Flow cytometry analysis revealed both formulations interfaced with splenic dendritic cells in addition to red pulp and CD169+ macrophages, with DOTAP LNPs demonstrating significantly higher association than KC2. Significantly, high transfection was observed exclusively in the CD169+ macrophages with both LNPs, demonstrating for the first time a non-targeted, ligand-free LNP platform able to effectively transfect this antigen-presenting immune cell type *in vivo*. Together, our study provides insight into how altering the physicochemical properties of a vaccine vehicle can allow effective immune cell and organ delivery without the need to introduce specific targeting ligands, and provides further fundamental understanding into LNPs at the interface of immunity.

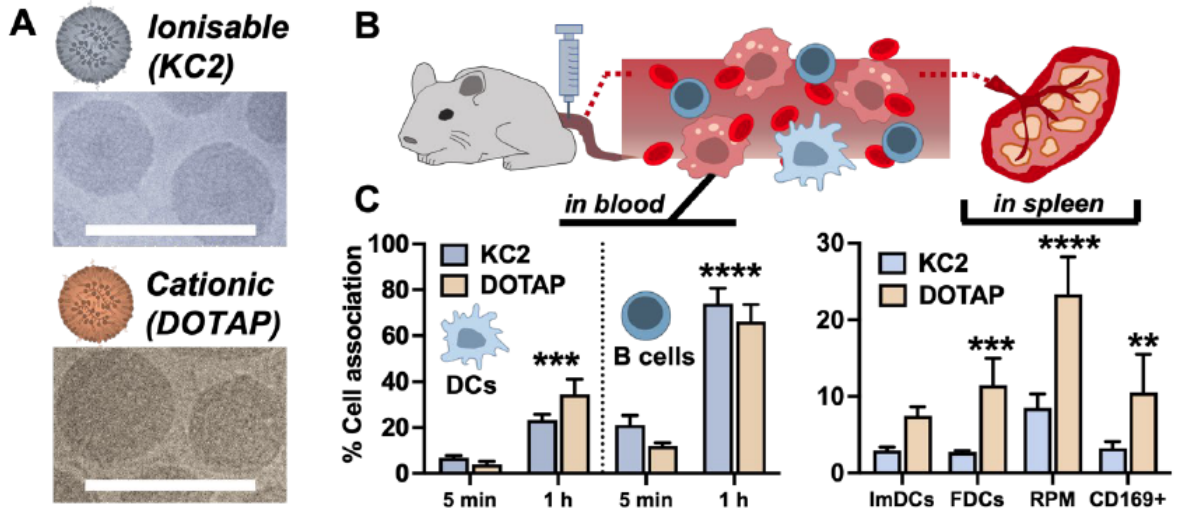

Figure 1: A. Scheme and cryo-electron microscopy of KC2 (above) and DOTAP (below) formulations, scale: 100 nm. B. Scheme showing intravenous (IV) injection of LNPs into mice and immune cells present in the blood and spleen. C. Left graph: time-dependent LNP association of dendritic cells (DCs, left) and B cells (right) in blood; right graph: LNP association with murine splenic immature (ImDCs) and follicular (FDCs) DCs, red pulp (RPM) and CD169+ macrophages (CD169+) 24 h post IV injection. ** p < 0.01, *** < 0.001, **** < 0.0001, error: SEM.

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Understanding nanoparticle accumulation in a complex *in vitro* tumour-on-a-chip model

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Microfluidic devices for cell biology research have revolutionized the way we can analyse nanoparticles (NPs) and novel drugs over the past 20 years.[1] These devices have shown great promise in being highly tunable, high throughput systems that can be used for a wide variety of biological experiments to better understand NP interactions on the cellular level.[2] In my work, I have designed a polydimethylsiloxane (PDMS) microfluidic device with the capability to culture multicellular tumour spheroids (MCTS) that can be imaged and analysed in real time to gain detailed understanding of NP interactions at the tumour site.[2] These MCTS are designed to incorporate cancer (SKOV-3), fibroblast (NIH/3T3), macrophage (RAW264.7) and endothelial (HUV-EC-C) cell lines to create a complex 3D model that better replicates the tumour microenvironment. Flow rate and sheer stresses can be appropriately scaled in this tumour-on-a-chip model, allowing for extravasation and accumulation of NPs to be understood through real time imaging in a physiologically relevant environment. This work will look at a library of NPs with various surface chemistries and characteristics to gain insight into what properties allow greater accumulation and penetration into the tumour site. Through this research, we hope to show how a complex *in vitro* dynamic model such as the tumour-on-a-chip can better recapitulate the tumour microenvironment and therefore enhance our understanding of biological interactions with NPs.

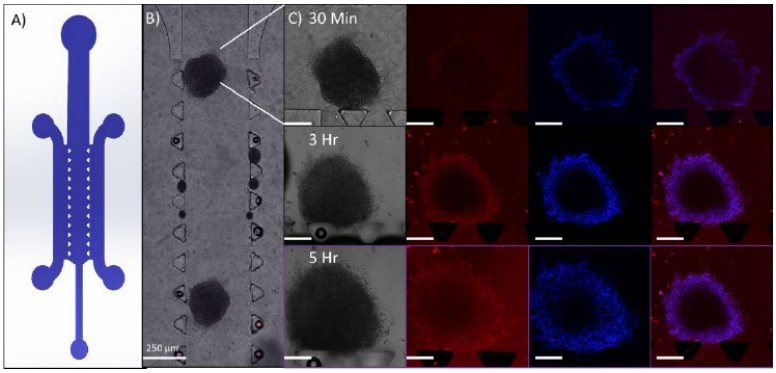

Figure 1: Tumour-on-a-chip microfluidic device design created with CAD software (A). Tile scan image of tumour spheroids present in the microfluidic device after being incubated overnight (B). NP uptake into tumour spheroid at various timepoints (C) with NP tagged with Cy5 shown in red and Hoechst stain of live cells in blue. Images taken using a Leica SP8 confocal microscope at 10x magnification. Scale bar denotes 200 µm.

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Linking the intracellular journey of gold nanoparticles to protein expression changes in prostate cancer cells

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Many nano-pharmaceuticals have been granted FDA approval or, are in clinical trials. Metal-based nanoparticles such as NBTXR3(a hafnium oxide nanoparticle), a radio enhancer and silica-gold coated with PEG for thermal ablation of solid tumors have been used in clinical trials for treatment of cancer[1].

However, while the mechanisms of action by these nanoparticles are inspired by physical interactions of the radiation with the nanoparticle[2], little research has been conducted to identify how nanoparticles interact with the cells. Here, we proposed that nanoparticles alter protein regulation by cells as an additional, or alternate, means by which nanoparticles enhance therapeutic response. This was tested through proteomic approaches to identify which, of thousands, of proteins were up or down regulated as a function of time andnanoparticle exposure.

Through the observational analysis of nanoparticle transport and fate in cells by TEM (Fig 1a), along with observations of other changes in cell attributes (e.g., mitochondria), the temporal effects of ~18nm gold nanoparticles on PC-3 cell expression have been quantified (Fig 1b). Several pathways have been identified to be significantly up or down regulated within 2 hours of exposure to nanoparticles. A number of these pathways are related to cellular metabolism and stress response which can assist in explaining how nanoparticles sensitize cells to therapeutic treatments. The data also highlight potential genotoxic stress on cells by nanoparticles despite no indication of toxicity to the cells measured by conventional assays.

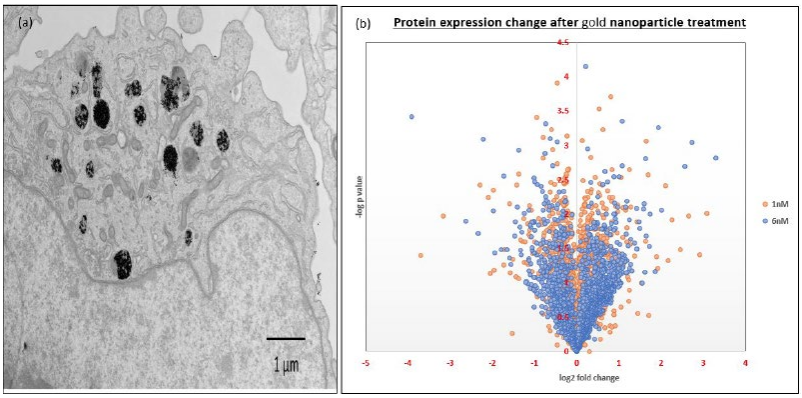

Figure 1: Protein expression changes and major pathways (a)Volcano plot, (b) TEM of gold nanoparticle in cells

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Computational Analysis, Fabrication, and Optimization of Phytoextracts based nanoemulsion system for the neuroprotective activity

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The prevalence and distribution of Central nervous system (CNS) disorders are increasing globally and until 2021, it was estimated that 55 million people worldwide were already affected by dementia or Alzheimer's disease (AD), with additional 10 million new cases diagnosed each year1. AD is widely regarded as the most difficult class of dementia to treat, and it is also marked as one of the most alarming global demography aging phenomena. It causes a person's cognitive ability and orientation to deteriorate dramatically with debilitating behavioral and social changes. Pathological alterations in AD include a gradual loss of cortical neurons and pyramidal cells, disruption of synapses and neural circuits, expanse of degenerative changes to the neocortex, and aggregation of Aβ proteins (Perl, 2010). Fixing such diseases is difficult due to a variety of unavoidable physiological limitations, such as circulation obstructions, drug permeability, degradation, and so on. This limits the drug distribution to the affected areas of the brain, preventing the passive release of therapeutic compounds into the CNS. Therefore, the concept of nanotherapeutics has evolved as a viable tool for drug delivery in CNS3 (Lee & Minko, 2021). In our study, we have computationally evaluated the efficacy of Ayurvedic herbs (*Withania somnifera* and *Bacopa Monierri*) for their neuroprotective targets in the CNS4,5. Thereafter, they have been formulated for the nano-emulsions system and optimized to attain the nanometric size range[6,7]. The computational results showed that the formulated extracts have a better binding affinity than their standard drugs towards the biomarkers involved in CNS diseases. Subsequently, an oil-in-water nano-emulsion system was developed for both *Withania somnifera* (Linseed oil, Cween 20, Ethanol) and *Bacopa monierri* (Almond oil, Triacetin, Ethanol) with an aqueous titration method. The characterization results exhibited the nanometric size range of the optimized nano-formulations with comparatively better neuroprotective ability than their extracts.

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Biologics as Peptide Delivery Vehicles for Immunotherapy

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Lung cancer is the leading cause of cancer-related deaths in the world. Conventional treatments for non-small cell lung cancers (NSCLCs) include surgery and chemotherapy but these treatments are associated with undesirable side effects. InterK Peptide Therapeutics have developed immune-boosting peptides as an alternative treatment strategy. Currently, the accumulation of these peptides in the tumour microenvironment is limited as they undergo rapid renal clearance. This talk will discuss the use of antibody fragments specific to various tumour and immune cell surface receptors for targeting the tumour microenvironment. These antibody fragments have been linked to InterK's peptides, creating biologics which have a longer blood circulation time. As a result, the antibody fragments can guide InterK's peptides to the tumour microenvironment and increase their site-specific accumulation. The peptides then boost the immune system by re-invigorating exhausted T cells and mounting an immune response to suppress lung cancer. Validation of these constructs have been undertaken in mouse models. InterK's peptides are a promising new class of immunotherapeutics that could serve NSCLC patients who do not respond to conventional therapies. The use of a delivery platform, such as the biologics in this project, are beneficial to ensuring these therapeutic peptides are directed to the tumour microenvironment. With a decrease in the likelihood of undesirable side effects, the quality of life for patients suffering from NSCLCs is likely to improve. While this project focusses on NSCLC treatment, the findings from this study can be applied to other types of cancer.

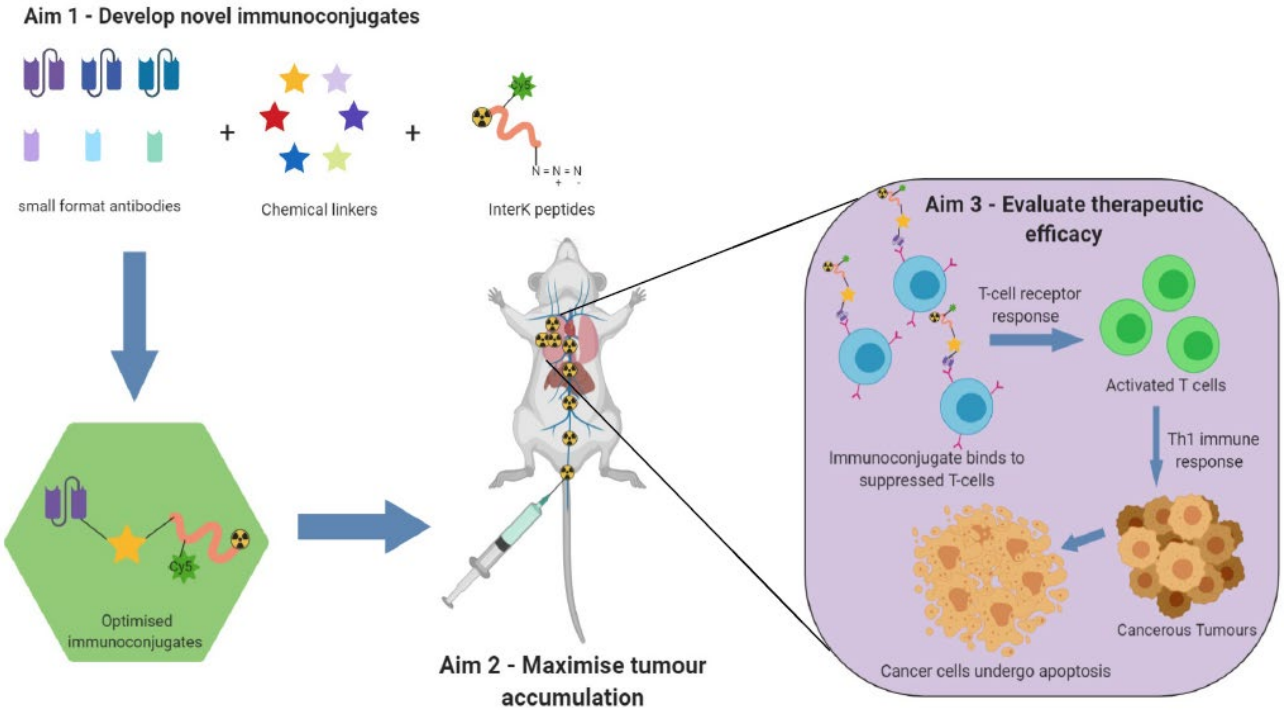

Figure 1: **Schematic illustration of talk.** Aim 1 will evaluate the in vitro stability and binding affinity of

Pulmonary delivery of lipid-based nanoparticles: Identification of proteins that mediate cellular uptake kinetics

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Intracellular infections are challenging to treat with antibiotics owing to their poor penetration through host cell membrane. The use of nanoparticles as carriers for antibiotics (nanoantibiotics) are becoming of growing interest, however successful eradication of bacteria remains challenging to achieve[1]. Several challenges that limit successful clinical translation of nanoantibiotics include (i) the heterogeneity of pulmonary cells present as bacterial reservoir and (ii) poor understanding of the role of biological fluid on cellular interaction of nanoparticles in the lung. In this study, we explored the dynamics of cellular uptake of two widely used lipid-based nanoparticles, i.e., liposomes and liquid crystalline nanoparticles (cubosomes). We investigated their interaction in two types of pulmonary cells, RAW264.7 macrophages, which are known for their phagocytic function and A549 alveolar epithelial cells, which play important role in regulating various pulmonary functions. Liposomes composed ofdidodecyldimethylammonium bromide(DDAB) and phosphatidylcholine (PC) were fabricated using microfluidics (NanoAssemblr®). Cubosomes using glyceryl monooleate were synthesized via hydrotropic dilution method as described previously[2]. A selective preference was observed for liposomes in A549 cells in comparison to cubosomes, but the trend was opposite for RAW264.7 macrophages (Figure 1). To further evaluate the influence of biological fluid on cellular uptake, the nanoparticles were treated with fetal bovine serum (FBS) and murine bronchoalveolar lavage fluid (BALF). Interestingly, the presence of proteins significantly enhanced the internalisation of cubosomes in A549. Further analysis with proteomics revealed unique proteins from the protein corona of the individual nanoparticles with specific properties that mediate cellular uptake. The outcome of this research highlights the importance in understanding the role of biological fluids in mediating cellular interaction of nanoparticles and subsequently their efficacy against pulmonary infections.

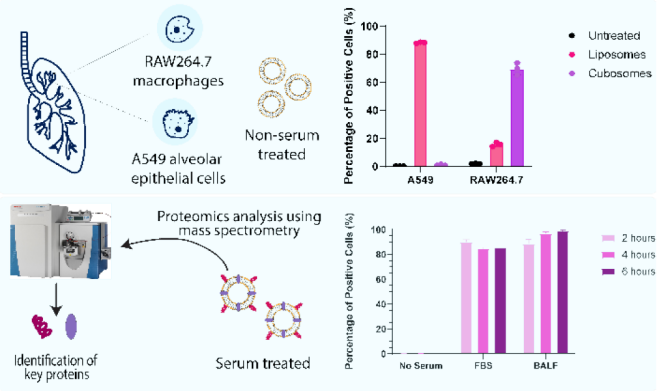

Figure 1: Selective preference of uptake in pulmonary cells and the influence of serum

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Structurally tailored drug-free layered double hydroxide nanoparticles for synergized photothermal/photodynamic/chemodynamic therapy

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Photodynamic therapy (PDT) has shown promise in cancer therapy. However, the therapeutic effects are seriously limited by oxygen reliance. Photothermal therapy (PTT) and chemodynamic therapy (CDT) as the oxygen independent treatment modalities can kill tumor cells by local hyperthermia and reactive oxygen species (ROS) generated via Fenton or Fentonlike reactions. On this ground, the combination of PTT, PDT and CDT shows the potential to overcome the restrictions and achieve more efficient inhibition of tumor progression.

In this study, a nanoplatform based on layered double hydroxide (LDH) nanoparticles was tailored structurally for effective combination breast cancer therapy. Cu-containing LDH nanoparticles loaded with indocyanine green (ICG) were artificially engineered with more Cu-coordination defects (d-Cu-LDH/ICG) for combined photothermal/photodynamic/chemodynamic therapy (PTT/PDT/CDT) of 4T1 tumors. Acid generated OH vacancies on the LDH surface and photo-stable ICG molecules in the LDH interlayer significantly enhanced photothermal transduction and singlet oxygen (1O2) generation under the same laser irradiation. Moreover, d-Cu-LDH/ICG nanoparticles generated hydroxyl radicals (·OH) in the presence of H_2O_2 as Fenton catalysts, which was further enhanced by Cu(II) reduction with glutathione (GSH). Consequently, such d-Cu-LDH/ICG nanoparticles significantly suppressed 4T1 cell proliferation in the presence of H_2O_2 under single 808 nm laser irradiation. Furthermore, this multifunctional LDH-based nanoformulation showed effective inhibition to the tumor growth in vivo through combined PTT/PDT/CDT only once under very mild laser irradiation, i.e., at the laser power output of 0.23 W cm^{-2} for 5 min. Altogether, the fabricated d-Cu-LDH/ICG nanoplatform offers a new and safe treatment option for combination cancer therapy without using any anti-cancer drugs.

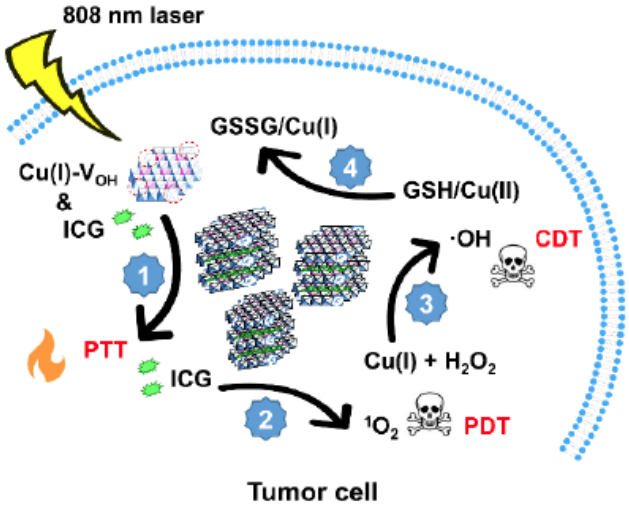

Figure 1: Schematic illustration of near infrared-induced combined PTT/PDT/CDT using tailored d-Cu-LDH/ICG.

Polydiacetylene-based colorimetric sensor array for rapid volatile organic compounds detection to diagnose early lung cancer

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This talk will present the novel application of polydiacetylene (PDA) based colorimetric sensor array (CSA) to detect volatile organic compounds (VOCs) that are found in human breath and are indicative of early lung cancer. Cancer is the leading cause of global deaths and early diagnosis is known to improve survival rate.[1] However, current screening tests such as imaging, biopsies, blood, and chromatography are expensive, bulky, invasive and require extensive experts involvement. PDA is a class of conjugated polymers with unique optical properties and exhibits blue-to-red colorimetric transition that can be monitored by the naked eye.[2] PDA as colorimetric sensors has great potential to overcome the inherent limitations of current VOC measurement techniques by enabling simple and low-cost qualitative and quantitative VOC detection.[3]

This work presents the potential of PDA-based CSA to detect VOC lung cancer biomarkers (e.g. ethylbenzene, 2-ethyl-2-hexanol, hexanal, 2-butanone, undecane). Responses against common breath interferents including acetone, isoprene and ethanol were also evaluated. All sensors were evaluated within normal breath conditions at 35°C and relative humidity of 60% and 90% to ensure that color changes were not due to temperature change and water interferents. **Figure 1** depicts the colorimetric response of PDA in its unmodified and modified form to evaluate its selectivity when exposed to target VOCs. Pattern recognition can be used to identify outcomes to predict the likelihood of lung cancer.

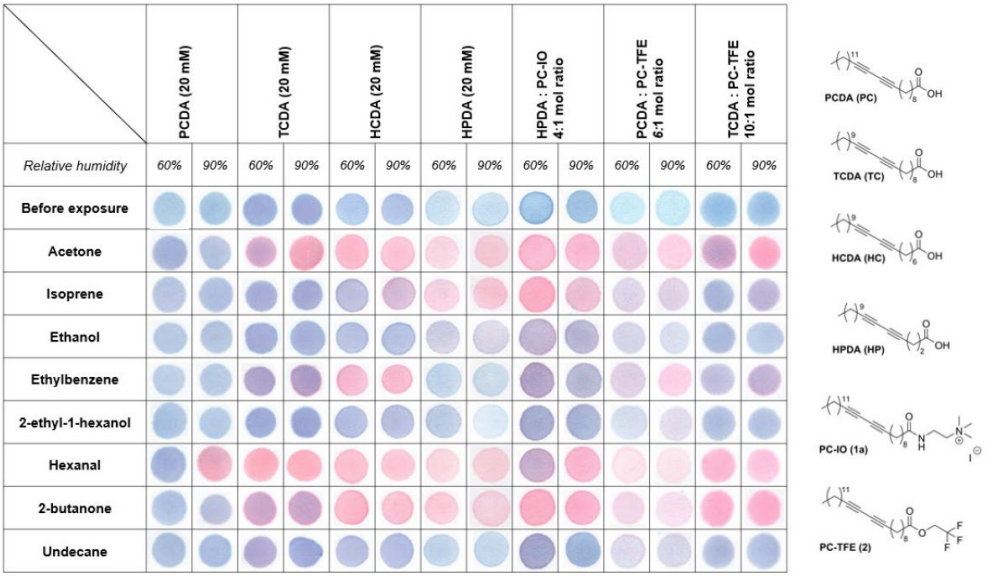

Figure 1: Colorimetric response of polydiacetylene-based sensor array when exposed to VOC biomarkers indicative of early cancer at 35°C and relative humidity of 60% or 90%.

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Cell migration in well-defined 3D biomimetic extracellular matrices

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Cells migrate through the extracellular matrix (ECM) to perform many fundamental functions such as immune response, cancer metastasis, and wound healing. Cell migration is influenced by the interaction between cell and its ECM. However, the ECM *in vivo* has complex properties and thus, it is difficult to determine how each component affects cell migration. One way to deconvolute the different parameters is through reducing the ECM *in vivo* to its essential components *in vitro*. A major challenge in developing biomimetic ECM is achieving a synchronous control over variables such as stiffness, porosity, and presentation of biochemical cues. In this work, we studied 3D cell migration in well-defined biomimetic ECMs based on polyethylene glycol (PEG) hydrogels. Using bioconjugation of 4-arm PEG maleimide and 2-arm organothiol crosslinker, we achieved a synchronous control of stiffness and presentation of bioactive cues of the PEG-based ECMs without changes in the porosity. The PEG-based ECMs have stiffnesses ranging from 0.7 to 2.3 kPa, which mimic a wide variety of native soft tissues such as brain, lung, liver, pancreas, thyroid,[1] and median pore diameters of 10.6 μm, which is close to most cell diameters. Three-dimensional cell migration assays were performed in highthroughput manner using 3D bioprinter, time-lapsed fluorescence microscopy, and computational tools. With this well-defined biomimetic ECMs and high-throughput assay, we studied the effect of ECM stiffness to migration of immune cells such as cytotoxic T lymphocytes and Jurkat T cells. We learned that cytotoxic T lymphocytes migrate fastest at 1.3 and 1.8 kPa, whereas Jurkat T cells migrates faster with increasing ECM stiffness.

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Photocrosslinked silk hydrogels for biomedical applications: understanding complex gelation mechanisms

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Covalently crosslinked silk fibroin hydrogels have gained popularity over their physically crosslinked (via beta-sheet formation) counterparts due to their elastomeric nature, transparency and ability to support cell encapsulation. Covalent crosslinking is achieved via dityrosine bond formation between tyrosines natively found in silk, without the need for any modification of the polymer chain. This reaction can be mediated through three broad strategies, including enzymatic-, Fenton reaction-, and photocrosslinking-based approaches. Interestingly, beta-sheets can spontaneously form in covalently crosslinked hydrogels resulting in a rapid transition from soft elastomeric to opaque and stiff gels. This transition mechanism, its implications and control over it are not well understood and hamper the use of covalently crosslinked silk hydrogels in biomedical applications.

We have found that silk photocrosslinking using a photoinitiator ruthenium, unlike enzymatic HRP crosslinking, supports rapid gel formation, high density cell encapsulation and is compatible with biofabrication. Interestingly it can also inhibit/delay beta-sheet formation, an effect we found to be highly dependent on gelation conditions. In this study, we explored the effect of silk concentration and molecular weight, gelation buffers and photoinitiator concentration on gel formation and kinetics, mechanical and optical properties of silk hydrogels and formation of beta sheets. We optimised conditions that support rapid crosslinking of silk at concentrations as low as 1% wt/v and can modulate compressive modulus between ~1-100 kPa. The optical properties of the gels were silk and photoinitiator concentration dependent and could be modulated to >90% light transmittance. Interestingly, gelation buffer (all physiologically relevant buffers) played a key role in the ability of silk hydrogels to form beta sheets, offering an avenue for generation of hydrogels with controllable and on-demand stiffening.

This study offers new insights into di-tyrosine bond formation and silk gelation with implications for use in tissue engineering, in vitro models and medical devices.

Developing nanostar systems for sustained endosomal release Of a neurokinin-1 receptor antagonist to provide long-lasting relief of chronic pain

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Chronic pain is experienced by 1 in 5 adults during their lifetime and is often poorly managed with opioids that have unacceptable side-effects. Pharmacological agents that can inhibit paintransmitting receptors have long been pursued as alternative drug targets, but with limited success. One potential reason for this lack of effective targeting is that many of these receptors are stimulated by chemical "pain" signals at the cell surface and subsequently internalise into endosomes to promote cell signalling and neuroexcitability. By doing so, these receptors are 'hidden' from untargeted therapeutics.

We have previously shown that pH-responsive soft polymer micelles can enhance analgesic potential of therapeutics, via endosomal drug delivery of an inhibitor for the pain-transmitting Neurokinin 1 Receptor (NK_1R). These particles disassemble and selectively release a drug payload into the endosomal network of spinal neurons, and provide efficacious yet transient relief from chronic pain.[1] However, the duration of analgesia may have been limited due to the instability and rapid cargo release from these micellar nanoparticles. Star polymer "nanostars" are an attractive platform for advanced drug delivery due to their modular nature and the potential to conjugate multiple drugs through various linkers, attach targeting moieties and readily adjust the hydrodynamic diameter. This talk describes our recent efforts to develop new delivery systems, utilising stable core-crosslinked nanostars. Using the NK_1R antagonist aprepitant, the drug cargo was conjugated to the star arms via a range of hydrolytically unstable linkers, to provide slow release profiles. Nanostars continually released cargo for 24 h, trafficked through the endosomal system, and disrupted NK_1R endosomal signaling. After intrathecal injection, Cy5-labelled nanostars accumulated in endosomes of spinal neurons. Using 2 different chronic pain mouse models, drug-loaded nanostar-aprepitant reversed 'spontaneous' and 'evoked' pain behaviours more effectively than free drug and was maintained for >10 h. The sustained endosomal delivery of antagonists from slow-release nanostars provides effective and long-lasting reversal of chronic pain and provides valuable proof-of-concept for other pain-transmitting and trafficking receptors.

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Can the Shape of Nanoparticles Enable the Targeting to Cancer Cells over Healthy Cells?

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Macropinocytosis is a consequence of oncogenic alterations of cancer cells while most healthy cells are non-macropinocytic. It is currently unclear whether macropinocytic cancer cells can be targeted rather than healthy cells, by adjusting the shape and size of nanoparticles. Herein, the endocytosis of two differently shaped nanoparticles; nanorods and nanospheres are compared in cancer and healthy cells. The cells are breast epithelial cancer cells (MCF7) and breast epithelial healthy cells (MCF10A) and pancreas cancer cells (PANC-1 cells) and non-tumourogenic patientderived cancer-associated fibroblasts (CAFs). MCF7 cells use clathrin-mediated endocytosis and micropinocytosis to take up the nanorods while MCF10A cells use predominantly clathrinmediated endocytosis. Based on the comparison of endocytic behavior of cancer and healthy cells, MCF7 cells can be induced to take up more nanorods and suppress the metabolism and endocytosis of nanorods in MCF10A cells. The nanorods allow targeting to breast cancer MCF7 cells and pancreas cancer PANC-1 cells over their healthy cell counterparts. Furthermore, it is shown that the nanorods can selectively deliver doxorubicin to the nucleus of breast cancer cells and to the cytoplasm of pancreatic cancer cells. This study opens exciting possibilities of targeting cancer cells based on the material shape rather than targeting antibodies.

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Low-Fouling Fluoropolymer Grafted Metal Organic Framework Nanotheranostics for ^{19}F MRI-guided Cancer Therapy

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Cancer theranostics that combine cancer diagnosis and therapy are a promising approach for personalized cancer treatment.[1-3] However, current theranostics strategies suffer from low imaging sensitivity for visualization and an inability to target the diseased tissue site with high specificity, thus hindering their translation to the clinic.[4] In this study we have developed a tumor microenvironment-responsive theranostic agent by grafting an innovative class of water-soluble, low-fouling fluoropolymers to pH-responsive zeolitic imidazolate framework-8 (ZIF-8) nanoparticles. The conjugation of the fluoropolymers to ZIF-8 nanoparticles not only allows sensitive *in vivo* visualization of the nanoparticles by ^{19}F MRI, but also significantly prolongs their circulation time in the bloodstream, leading to improved delivery efficiency to tumor tissue. Moreover, the designed nanotheranotics can respond to an acidic tumor microenvironment and attenuate the "stealth" performance for enhance specific uptake by cancer cells. This enables efficient tumor-targeting delivery of chemodrugs, such as doxorubicin, and a resulting enhancement of anticancer therapeutic effect.

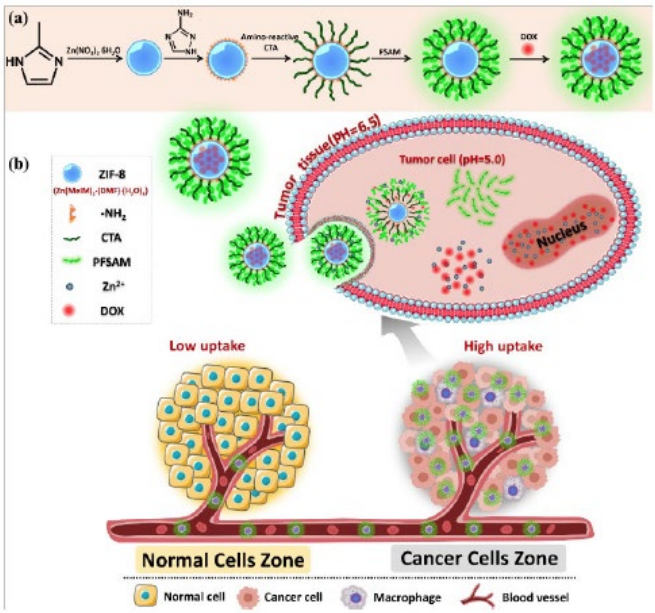

Figure 1: (a) Synthetic route of ZIF-8-PFSAM-DOX. (b) Schematic illustration of magnetic resonance imaging-guided chemotherapy of cancer.

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Controlling the axonal transport of gold nanoparticles with neural circuit tracing proteins

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This presentation will show how to control the retrograde transport of gold nanoparticles in axons *in vitro*. Nanoparticles generally move in both directions in axons of neurons[1], and the purpose of this research is to give nanoparticles a bias in transport direction, allowing them to move retrogradely (toward the nucleus of neuron) to deliver drugs to the nucleus. Dorsal root ganglion (DRG) primary neurons were used as the cell model. To drive nanoparticles moving retrogradely, two neural circuit tracing proteins (NCTs) were chemically conjugated to gold nanoparticles. The NCTs are wheat germ agglutinin (WGA) and horseradish peroxidase (HRP), which are known for retrograde movements in neurons[2]. The axonal transport of the gold-based nanoparticles in DRG primary neurons was tracked and analysed using advanced quantitative microscopy, revealing that (1) gold nanoparticles move slowly in the retrograde direction; (2) NCTs move rapidly in the retrograde direction consistent with active axonal transport; (3) NCTs-conjugated gold nanoparticles move faster than gold nanoparticles alone but slower than NCTs alone. These findings suggest that NCTs conjugation enables active axonal transport of gold nanoparticles. This work demonstrates the potential of neuronal cell-based assays in understanding axonal transport of nanoparticles and helping the design of next-generation of neuron-targeting nanomedicines for treating a variety of neurological disorders.

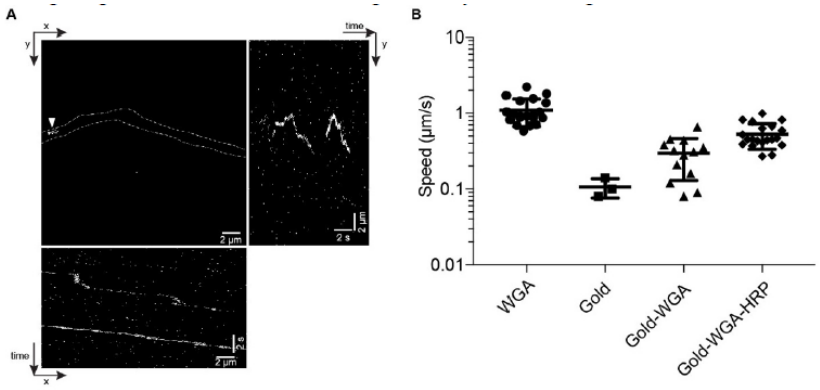

Figure: (A) Confocal image from a time-lapse of WGA-HRP conjugated gold nanoparticles (white arrowhead) in an axon (white dash line) of neuron (top left) and the corresponding kymograph (bottom left and top right). (B) The retrograde transport speed of WGA, Gold (gold nanoparticle), Gold-WGA (WGA conjugated gold nanoparticle), and Gold-WGA-HRP (WGA-HRP conjugated gold nanoparticle).

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Defined 3D fibrin networks for 'scarless' wound healing studies

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Scarless wound healing has been observed in mammalian fetuses but has not been achieved in adults due to its unknown mechanisms and the limited ability to achieve the desired healing phenotypes[1,2]. Generally, the study on fetal scarless wound healing is hampered due to a lack of in vitro models. However, a comprehensive study on fetal-specific healing has its translational significance in regenerative medicine.

Fibrin is the provisional matrix after injury, delivering key biophysical cues to promote wound healing in a timely and coordinated manner[3]. Nonetheless, the precise role of the fibrin 3D biophysical cues on wound healing and scarring remains unclear. Though fibrin has been used as a scaffold for 3D cell culture because of its inherent advantages, such as biocompatibility, biodegradation, high affinity to proteins and unique mechanical properties, its uncontrollable and unstable structural and mechanical properties have hindered its wide use[4,5].

Here, we have established a novel snake venom-controlled fibrin platform with precisely and independently controlled structural, mechanical properties and ligand density. Utilizing this defined in vitro system, we precisely mimic the key events of fetal coagulation. We reveal that the differential fetal-mimicking biophysical cues of the fibrin networks have prevailing control over cell behaviors. These findings implicate matrix biophysical cues as key triggers of fetal wound healing and provide new biophysical strategies in the design of biomaterials to promote scarless wound healing and regenerative tissue engineering.

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Quantum Dot Nanomedicine Formulations Dramatically Improve Pharmacological Properties and Alter Uptake Pathways of Metformin and Nicotinamide Mononucleotide in Aging Mice

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Orally administered Ag_2S quantum dots (QDs) rapidly cross the small intestine and are taken up by the liver (1). Metformin and nicotinamide mononucleotide (NMN) target metabolic and aging processes within the liver.

This study examined the pharmacology and toxicology of QD-based nanomedicines as carriers of metformin and NMN in young and old mice, determining if their therapeutic potency and reduced effects associated with aging could be improved.

Pharmacokinetic studies demonstrated that QD-conjugated metformin and NMN have greater bioavailability, with selective accumulation in the liver following oral administration compared to unconjugated formulations. Pharmacodynamic data showed that the QD-conjugated medicines had increased physiological, metabolic, and cellular potency compared to unconjugated formulations (25× metformin; 100× NMN) and highlighted a shift in the peak induction of, and greater metabolic response to, glucose tolerance testing. Two weeks of treatment with low-dose QD-NMN (0.8 mg/kg/day) improved glucose tolerance tests in young (3 months) mice, whereas old (18 and 24 months) mice demonstrated improved fasting and fed insulin levels and insulin resistance. High-dose unconjugated NMN (80 mg/kg/day) demonstrated improvements in young mice but not in old mice. After 100 days of QD (320 µg/kg/day) treatment, there was no evidence of cellular necrosis, fibrosis, inflammation, or accumulation. Ag_2S QD nanomedicines improved the pharmacokinetic and pharmacodynamic properties of metformin and NMN by increasing their therapeutic potency, bypassing classical cellular uptake pathways, and demonstrated efficacy when drug alone was ineffective in aging mice (2).

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Liquid biopsy nanodiagnostics for monitoring cancer and the human immune System

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Precision medicine is regarded as one of the most promising approaches to treat or even cure many severe diseases including cancer. Although precision medicine has delivered new and individualised treatment plans such as targeted therapy or immune checkpoint therapy, it has not yet lived up to its full promise. One reason that has limited the advancement of precision medicine is the requirement for a specific molecular profile to tailor the therapy. The creation of such a molecular profile is difficult and requires highly sensitive and specific technologies that can detect multiple biomarkers in readily accessible biofluids. Nanomaterial- and nanostructured-based systems have attracted interest due to their unique physico-chemical properties that can be explored as nanodiagnostics for molecular profiling in precision medicine. This presentation will highlight examples of nanodiagnostics for (1) monitoring targeted therapy in melanoma[1], (2) early detection of melanoma[2], and (3) monitoring the immune system with single cytokine precision[3].

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Gold Nanocluster-induced Immunomodulation for Tissue regeneration via Mitophagy Regulation: A Perspective on Materiobiology

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Recent advances in biomaterials have revealed the importance of material-biology interplay, which is termed as "materiobiology" to describe the regulatory effects of biomaterial properties on biological functions at cell, tissue, organ, and the whole organism levels. A typical example of materiobiology is material-induced host immune response, the earliest biological behavior after material implantation in vivo, which plays a determinant role in material application, especially for tissue engineering/regeneration. It is therefore necessary to dissect the cellular and biochemical mechanisms under material-immune cell interaction, which will facilitate the design/development of biomaterials with the capacity to induce an ideal immune environment for tissue regeneration. In the current study, we found that dihydrolipoic acid-gold nanoclusters (AuNCs), a type of fluorescent materials for biolabelling and bioimaging, effectively regulated macrophage inflammatory response. AuNCs made up of 10 to 100 atoms have ultra-small size (< 3 nm) and therefore could efficiently accumulate in macrophages. The results showed that under inflammatory stimulation, AuNC-uptake effectively suppressed macrophage response by inducing the phenotype switch from inflammatory M1 towards tissue-regenerative M2 in a dose-dependent manner, which was achieved through activation of autophagy in inflammatory macrophage. The activated autophagy facilitated the clearance of damaged mitochondria (termed as mitophagy), an effect not only preventing intracellular accumulation of reactive oxygen species (ROS), but also shifting the energy metabolism pattern from glycolysis into oxidative phosphorylation (mitochondria-dependent). This reprogramed macrophage response was found to facilitate both osteogenic differentiation and bone regeneration in vitro and in vivo, suggesting that AuNC-application could generate a favourable immune microenvironment for tissue regeneration. Therefore, our study has discovered a novel mechanism under nanomaterial-induced immunomodulation on macrophage response, which also provides a potential approach for translational tissue regeneration in the future.

Antifouling and Antibacterial Surfaces Grafted with Hydrophilic and Hydrophobic Copolymers

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For applications in biosensing, diagnostics, and medical devices, the precise control of the interactions between the surface of materials and the biological environment is an important but challenging task. Antifouling surfaces capable of reducing the non-specific binding of proteins, cells, and microorganisms are important for a variety of biomedical applications. In addition, biofilm formation due to bacterial adhesion is a problem in many healthcare-related devices. Therefore, it is desirable to develop surfaces with dual antifouling and antibacterial activity.

Herein, underpinned by our previous research,[1] a series of surface-attached copolymer brushes with varied compositions of hydrophilic sulfoxide monomers and their hydrophobic counterpart sulfide monomers have been synthesized by surface-initiated photoinduced electron/energy transfer-reversible addition-fragmentation chain transfer (PET-RAFT) polymerization. The structure-antifouling activity relationship of the copolymer brushes was investigated. The antifouling effect of the surface polymer brushes can be enhanced by increasing the molar ratio of the hydrophilic monomers in the copolymers. The surface copolymer brushes can be further ionized to become positively charged to impart antibacterial activity as demonstrated by a significant extent of bacterial cell death when the bacteria make contact with the surfaces. In summary, this hierarchical copolymer brush system provides the basis for the development of biocompatible and antibacterial surfaces useful for various biomedical applications.

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Snake venom protein-based hydrogel wound sealant for rapid and stable haemostasis

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Uncontrolled bleeding is the second leading cause of preventable death from traumatic injury. The ability to rapidly control bleeding is the primary step to reduce fatality. Following traumatic injury, the physiological balance between blood clot formation (coagulation) and blood clot breakdown (fibrinolysis) is often lost and results in a catastrophic bleeding. Limitation of the current haemostatic agents is that they require a functioning coagulation system to control the bleeding and they lack in restoring the physiologic balance between coagulation and fibrinolysis. A unique aspect of this study, compared to existing haemostatic agents, is that we employed two recombinant snake venom proteins; the procoagulant snake venom protein (PSVP), to rapidly initiate coagulation even in coagulopathic state (a state of impaired blood clotting), and the antifibrinolytic snake venom protein (ASVP), to prevent premature blood clot breakdown, a key characteristic of trauma induced coagulopathy. These proteins, a procoagulant PSVP, and the antifibrinolytic ASVP was bioconjugated to a synthetic thermoreversible polyisocyanopeptide polymer, result in a highly efficient hydrogel based wound sealant. *In vitro* human whole blood coagulation and fibrinolysis evaluation of the wound sealant showed that the blood clot formation was rapidly initiated, and blood clot lysiswas effectively inhibited. Clot stability provided by the antifibrinolytic snake venom protein was comparable to the clinically employed tranexamic acid and aprotinin. *In vivo*, bleeding was rapidly and stably controlled in a warfarin-induced coagulopathic mice tail amputation injury model. Significant reduction in both bleed volumes and time were achieved. Neither the hydrogel nor the developed wound sealant induced an inflammatory immune response in mouse skin wound incisional model. In summary, this work highlights the effectiveness of a new class of potential haemostatic agents employing snake venom proteins to address the current clinical shortfalls in treatment of trauma induced bleeding.

Cell-derived biomimetic 2D nanoparticles to improve cell-specific targeting and tissue penetration for enhanced magnetic resonance imaging

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A critical challenge of nanoparticle-based imaging diagnosis of cancers is the limitation of particle accumulation and tissue penetration at the biological target.[1] Here, a cancer cell derived membrane coated 2D layered double hydroxides nanoparticle (CM-PEG/MnLDH) has been developed to realize both cell-specific targeting and deep tissue penetration for enhanced magnetic resonance imaging of tumors.[2] Using 4T1 breast cancer cells as a model, the biomimetic CM-PEG/MnLDH exhibits excellent cell-specific targeting ability with significantly higher accumulation in homologous cancer cells than the non-homologous cells. Using 3D tumor spheroid cultures, the CM-PEG/MnLDH demonstrates highly selective binding and deep tissue penetration ability. In vivo imaging reveals that intravenous injection of CM-PEG/MnLDH provides clear MR imaging of tumor tissues in a 4T1 mouse model. Particularly, the CM-PEG/MnLDH has enhanced imaging performance in the central region of tumors, owing to the cell homing and tissue penetration ability of the biomimetic nanoparticle. In vivo biosafety evaluation shows that the CM-PEG/MnLDH has a high degree of biocompatibility, signifying its suitability for further clinical applications. The biomimetic CM-PEG/MnLDH nanoparticle featured with cell-specific targeting ability and deep tissue penetration is a promising MR imaging contrast agent for precise cancer diagnosis.

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Tracking Epithelial-Mesenchymal Transition in liquid biopsy using Surface-Enhanced Raman Scattering Nanotechnologies

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Epithelial–mesenchymal transition (EMT) is a primary mechanism for cancer metastasis. Detecting the activation of EMT can potentially convey signs of metastasis to guide treatment management and improve patient survival. Hallmark of EMT is characterized by dynamic changes in cellular expression levels of epithelial and mesenchymal markers, including the downregulation of epithelial markers (e.g. E-cadherin) and upregulation of mesenchymal markers (e.g. N-cadherin). With the phenotypical and morphological changes of epithelial cancer cells at primary tumor sites, EMT results in the genesis of circulating tumor cells (CTCs) and promotes the metastatic capability of CTCs in circulation. In this presentation, two liquid biopsy approaches based on surface-enhanced Raman scattering (SERS) nanotechnology will be introduced for EMT detection and monitoring: (i) A microfluidic immunoassay (termed "SERS immunoassay") was developed for sensitive and simultaneous detection of soluble E-cadherin and soluble N-cadherin proteins in plasma.[1] The duplex SERS immunoassay enabled the monitoring of EMT process through detecting the cadherin shift of soluble fragments in breast cancer patients.[1] (ii) A 4-plex SERS nanotechnology was developed for comprehensive characterization of EMT-associated CTC phenotypes from patient's blood.[2] The technology detected downregulation of epithelial markers (EpCAM and E-cadherin) andupregulation of mesenchymal markers (N-cadherin and ABCB5), and also revealed the heterogeneity evolution of these markers in CTCs. Overall, these two approaches provide new means for monitoring the EMT process in cancer, insights into the detailed mechanistic progress of the diseases, and have potential for detecting the early occurrence of cancer metastasis.

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Tunable Gasotransmitter Generation and Delivery using Nanozymes

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Gasotransmitters such as nitric oxide (NO) and hydrogen sulfide (H2S) are gaseous signalling molecules extensively researched due to their vasodilating, antibacterial, antiinflammatory, and tumoricidal properties.[1,2] They have demonstrated therapeutic potential towards many diseases, including cancer, cardiovascular disease, as well as bacterial and viral infections.[1] Many efforts have been devoted to developing transport systems that can deliver these gases in a controllable and safe manner, for example, by encapsulating NO or H_2S donors into stimuli-responsive biomaterials. However, the amount of gasotransmitter released and the longevity of the delivery systems principally rely on the finite reservoir of the drug donors. To overcome these challenges, herein, we synthesized a Cu-doped zeolitic imidazolate framework (Cu^{2+}/ZIF-8) nanozyme (Figure 1), in which copper acts as a catalyst that enables *in situ* NO generation from endogenous NO donors, such as *S*-nitrosoglutathione (GSNO) and S-nitrosocysteine (CysNO).[3] By tuning the Cu doping percentages, we achieved controlled NO generation from GSNO and CysNO at the physiologically relevant range. The developed Cu^{2+}/ZIF-8 retained its catalytic potency after 5 NO generation cycles and produced 10-times more NO compared to previous reports. The co-administration of Cu^{2+}/ZIF-8 and GSNO resulted in a 45% reduction in biofilm biomass, making them attractive candidates for antimicrobial applications. Following this study, we further developed a nanozyme/hydrogel system to co-deliver NO and H2S for potential biomedical applications such as enhancing angiogenesis.

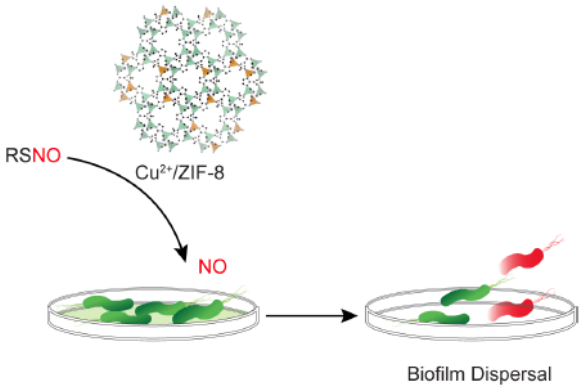

Figure 1: Schematic illustration of Cu_{2+}/ZIF-8 decomposing RSNO to generate NO for biofilm dispersal.

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Magnetic Particle Imaging with Tailored Cobalt-Doped Ferrite Nanoparticle Tracers

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Biomedical imaging is an integral part of the diagnosis and treatment planning of a wide range of chronic diseases.[1,2] Magnetic particle imaging (MPI) is an emerging noninvasive imaging modality that directly visualises the distribution of superparamagnetic iron oxide nanoparticle tracers.[1,3] In MPI, tailoring the magnetic properties of tracers is paramount to generating high intensity signal and achieving good sensitivity and spatial resolution.[4,5] To date, the magnetic core of MPI tracers have been designed based on pure magnetite (Fe_3O_4) nanoparticles (NPs).[6] In this study, Co doping was employed to enhance magnetic properties and MPI performance of magnetite cores. To do so, we synthesised two series of monodisperse Co-doped ferrite ($CoFe_2O_4$) NPs with different Co:Fe ratios (1.5:10 and 3:10) and various sizes ranging from 13-16 nm to 23-24 nm. The crystal structure, size, and composition of synthesised NPs were studied using XRD, TEM, and ICP, respectively. The results showed that NPs were highly monodisperse with single crystal phase and their XRD patterns can be indexed with the spinel $CoFe_2O_4$. The magnetic properties of NPs were characterised by using vibrating sample magnetometry (VSM). Hysteresis loops were registered at 300◦K as the magnetic field was cycled between -30 and 30 KOe. The saturation magnetisation (MS) of NPs with Co:Fe=1.5:10 ratio was measured as 109, 123, 143 emu/gFe for particles with the average size of 13-16 nm, 21-22 nm, and 23-24 nm, respectively. By increasing the Co:Fe ratio from 1.5:10 to 3:10, the MS was increased to be 143, 150, 155 emu/gFe for each particle size range, respectively. The MPI signal intensity was measured for noncoated NPs with Co:Fe=3:10 ratio and average particle size of 16 nm because of their high MS, low coercivity and small particle size. We observed a 1.65-fold enhancement in MPI signal intensity compared to Vivotrax, a commercially available tracer, thereby demonstrating the potential of Co-doped NPs for high performance MPI.

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Design and evaluation of degradable, pH-sensitive camptothecin-loaded micelles and polymersomes from RAFT-based diblock polymers

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Developing safe and effective nanoscale drug delivery systems for cancer treatment is an ongoing priority for researchers from different scientific backgrounds. The therapeutic performance of these systems, especially when used to improve the solubility of hydrophobic drugs, depends on their shapes, sizes and dynamic behaviour.[1] Herein, we report the synthesis of novel degradable amphiphilic diblock copolymers, by RAFT-terpolymerization of 2-methylene- 1,3-dioxepane (MDO), vinyl acetate (VAc) and vinyl bromobutanoate (VBr) monomers. By varying the lengths of the hydrophobic blocks, we developed synthetic platforms for the production of pH-sensitive polymeric nanoparticles; namely, micelles (~25 nm) and polymersomes (~100 nm) (**Figure 1**).[2] We selected the antineoplastic drug, camptothecin, as a model drug to be delivered by our systems due to its high toxicity and poor water solubility, which typically restrain its application in chemotherapy. We thoroughly investigated the morphologies, stabilities, drug encapsulation, release profiles, cellular association and dose-dependent cytotoxicities of our systems when tested against MDA-MB-468 breast cancer cells. Our micelles and polymersomes demonstrated good drug loading efficiencies and sustained release rates, with micelles showing enhanced encapsulation efficiencies (> 80%) and relatively faster rates of release compared to polymersomes. Both systems however demonstrated pH-sensitivity and comparable *in vitro* cytotoxicities and cellular uptake. We then assessed the *in vivo* efficacy of micelles and polymersomes when used to interrogate BALB/c nude mice bearing xenograft MDA-MB-468 breast cancer tumours. Polymersomes were far-superior to micelles in terms of their tumour accumulation, tumor regression, and reduction of side-effects, despite both systems showing 100% survival rates by the end of the therapeutic study, compared to free drug. Our work overall confirms the advantages of these nanoparticles to deliver lipophilic and difficult to solubilize drugs.

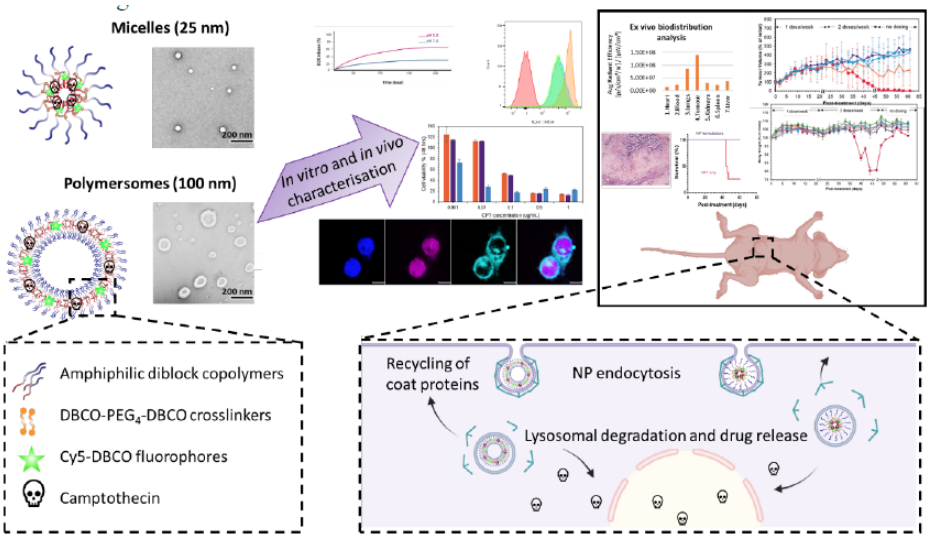

Figure 1: Self-assembled micelles and polymersomes and their application as chemotherapeutic delivery systems for camptothecin.

Design And Fabrication of Surface Enhanced Raman Spectroscopic (SERS) Based Barcoding System for Spatial Transcriptomics

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Spatial transcriptomics is a molecular profiling technique that can map all the gene activity in each cell in complex tissue. It is significant both for diagnosing disease and monitoring its progression and therapeutic effects.[1,2] Microwell arrays are particularly designed for performing the spatial transcriptomic analysis by capturing the messenger RNA (mRNA) from each single cell.[3] In this study, we coupled the surface-enhanced Raman spectroscopic (SERS) barcoding system with a micro-well array chip that provides more specificity and accuracy in identification by providing the SERS barcodes for spatial transcriptomics analysis. The SERS based barcoding system was fabricated by the combination of SERS nanoparticles and the host polymeric microbeads. SERS nanoparticles were prepared through the surface modification of citrate capped spherically shaped AuNPs (10 nm) using the Raman scattering molecules. Different Raman molecules with a unique signal band that does not overlap with one another were selected to prepare different kinds of SERS nanoparticles. These SERS nanoparticles were then encapsulated into the host polymeric microbeads that results barcodes which is a collection of well-separated signal bands.

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Rapid customisation of high-aspect ratio 3D microneedles using 3D printing

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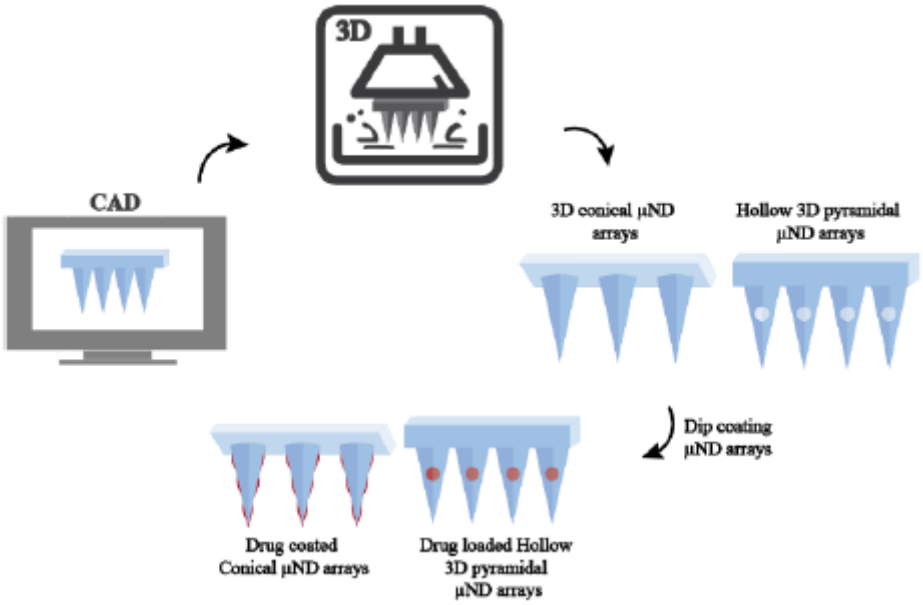
Here we present a simple and customizable 3D printed microneedle fabrication technique using a low-cost desktop SLA 3D printer. As opposed to conventional moulding-based microneedle fabrication methods, this technique neither requires complex and expensive manufacturing facilities nor expertise in microfabrication. Low cost 3D-printed microneedles to date have displayed low aspect ratios and poor tip sharpness. Studying first the effect of varying design input parameters (microneedles aspect ratio) and print settings (print layer height), our conical 3D printed microneedles have shown shorter dimensional aspects than specified. Modifying the resin formulation have improved on the print quality. As we decreased the input microneedle height, needles began to display a greater than specified needle base diameter. Both factors contributed to low aspect ratio needles when attempting to print micron height needles. By setting input height, it is possible to print needles with high-aspect ratios and tip radii of 50–100 µm. A skin insertion study was performed to demonstrate the functional capabilities of the printed arrays. Our next aim is to make both trigonal pyramidal hollow 3D printed microneedles using the same resin.



Engineering a Modular Nanocage System into a Vaccine Against Alzheimer's Disease

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Active immunotherapy aims to harness a patient's immune system to recognise and selectively clear pathological entities from the body. This approach is particularly promising for treating Alzheimer's Disease (AD) as it bypasses the need to deliver a therapeutic directly into the brain. The main contributors to the spread of AD throughout the brain are abnormal forms of amyloid beta (Aβ) and tau proteins.[1] As such, a number of newly developed vaccine formulations targeting abnormal variants of Aβ or tau have undergone clinical trials. Whilst some of these vaccines enable the targeted clearance of abnormal Aβ or tau from the brain, their capacity to halt cognitive decline was not observed, possibly due to the low immunogenicity of antigens and/or the propensity to focus on a single pathogenic protein.[2] Therefore, given the complex and highly heterogenous nature of AD pathophysiology, a vaccine that simultaneously targets both abnormal Aβ and tau species may improve treatment efficacy.

To this end, we are investigating encapsulin protein nanocages as a vaccine platform for the dual-display and delivery of both abnormal Aβ and tau epitopes for the combinatorial treatment of AD. Encapsulins are an emerging class of protein nanocages found in nature that self-assemble into hollow cage-like structures. They have demonstrated early promise as a vaccine platform and have been shown to elicit both humoral and cellular immune responses *in vivo*.[3] Furthermore, the outer and inner surfaces of encapsulins can be readily engineered to display and/or package peptide/proteins[4], thus representing an exciting and versatile system for the rational design of vaccines with multiple antigen modalities.

Herein, I will present a number of different conjugation strategies we are exploring for the modular attachment of Aβ and tau epitope variants, including enzymatic ligation, electrostatic interactions, and isopeptide bond formation (SpyCatcher/SpyTag) **(Fig. 1)**. I will also present the characterisation of encapsulins' unique structural properties, stability, and the safety and toxicity of the vaccine platform in mouse models. Future work will focus on understanding the *in vivo* bio-nano interactions of encapsulins and assessing the capacity of Aβ and tau epitope-displaying encapsulins to induce functional immune responses in an AD mouse model.

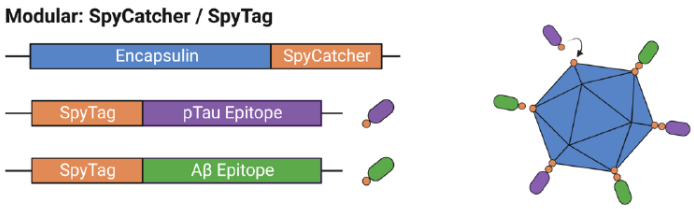

Figure 1: Schematic of pTau and Aβ epitope modular attachment to an encapsulin nanocage using isopeptide bond formation between SpyCatcher and SpyTag peptides.

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Integrated Platform Addressing the Finger-Prick Blood Processing Challenges of Point-of-Care Electrical Biomarker Testing

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Point of care (PoC) testing aims to produce accurate, fast, and diagnostically relevant results for on-site diagnostic/screening testing, through compact and portable devices. Among various biosensing technologies relevant to PoC testing, label-free electrical biosensor within the field-effect-transistor (FET) platform presents significant advantages including low power consumption, multiplexing capability, high-sensitivity, rapidity and quantitative measurement capability that enables direct signal transduction as well as on-chip sample processing and integration. To date, countless research in the FET biosensor landscape emphasized on sensor development and bioassay/detection elements with only limited attention being brought to the equally significant issues associated to sample processing that is typically required prior/during testing.

Whilst blood is the most used biofluid in PoC biomarker testing, various difficulties in testing with electrochemical sensors are yet to be solved. Poorly or unprocessed samples may lead to failure of the assay, resulting in inaccurate measurements and therefore misdiagnosis. Although various sample processing approaches for different biofluid have been investigated, current practices typically rely on manipulation by trained staff, sophisticated analytical equipment, and dedicated laboratory facilities to perform plasma separation and sample delivery to the sensors. It is typically challenging to implement these approaches in PoC settings as they are not readily translatable where space, time, trained personnel, and equipment are the limiting factors.

We present here a simple, yet robust autonomous finger-prick blood sample processing platform integrated with nanoscale FET biosensors and demonstrate the feasibility of measuring the SARS-CoV-2 nucleocapsid protein. The 3D-printed platform incorporates a high-yield blood-to-plasma separation module and a delay valve designed to terminate the assay at a specific time. The platform is driven by hydrostatic pressure to efficiently and automatically dispense plasma and washing/measurement buffer to the FET sensors. Our model study demonstrates the feasibility of detecting down to 1.4 pg/mL of the SARS-CoV-2 nucleocapsid protein within 25 min and with only minimal operator intervention.

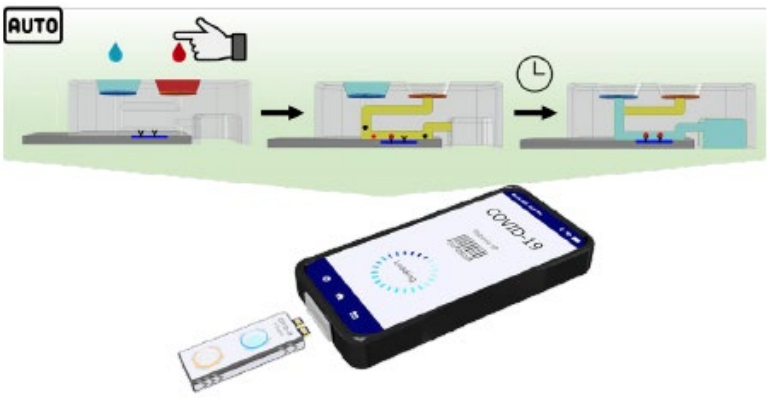

Figure 1: Schematic of the PoC finger-prick blood processing platform.

Improvement on the Current Tracer for Magnetic Particle Imaging (MPI) by using Zinc Doped Iron Oxide Nanoparticles

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Magnetic Particle Imaging (MPI) is an emerging non-invasive imaging modality that uses superparamagnetic nanoparticles as tracers.[1] MPI promises to deliver high quality images, no radiation, a signal direct from the tracer, high depth penetration and nearly no background from tissues.[2] Superparamagnetic iron oxide nanoparticles (SPIONs) are the most commonly used tracers.[3] They are non-toxic,[4] biodegradable,[1] and below the critical size of 27 nm (dia.), they exhibit superparamagnetic behaviour.[5] However, the current available commercial tracers, such as Resovist, have poor MPI performance and extremely short blood half-lives due to their polydispersity. Additionally, it was also reported that only 3% of these commercial tracers contribute substantially to MPI signal due to the presence of a large fraction of nanoparticle with an average dynamic size of over 60 nm.[6] Therefore, it is evident that there is a current need for the development of tracers that can deliver high quality images and have prolonged circulation time *in vivo*. In this project, we used zinc and iron to synthesise zinc doped iron oxide nanoparticles to optimise the magnetic properties of magnetite. The zinc ferrite samples showed an improvement in magnetic properties with maximum 2.3 times better than magnetite of 16 nm. These nanoparticles (NPs) also exhibited excellent MPI performance with a higher signal intensity and better spatial resolution compared to Resovist. To improve the biodistribution and water dispersity of these nanoparticles for biomedical applications, the zinc doped iron oxide tracers were encapsulated with a poly (maleic anhydride-*alt*-1-octadecene) (a.k.a. PMAO) polymer. The hydrodynamic size for these NPs was determined to be less than 200 nm and was also found to be stable in biological media. The small hydrodynamic size also enables these NPs to be used for cerebrovascular imaging with MPI because they are small enough to cross the blood brain barrier (*i.e.,* <200 nm). Therefore, these zinc ferrite nanoparticles present great potential as MPI tracers that can deliver high signal intensity and spatial resolution in MPI as well as possessing excellent biocompatibility.

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Development of ubiquitin specific protease 5 (USP5) inhibitors to treat high-risk Neuroblastoma

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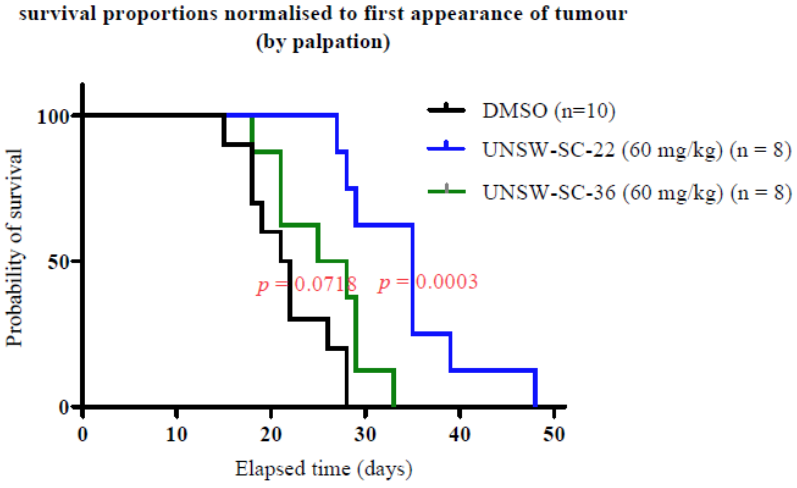
High-risk neuroblastoma is a therapeutic challenge as the long-term survival rate is less than 50% and the MYCN oncogene is a key driver gene in neuroblastoma. Histone deacetylases (HDACs) are transcriptional repressors that are dysregulated in many cancers, making them attractive therapeutic targets. Several HDAC inhibitors have been FDA-approved for the treatment of cancer but have shown limited efficacy as single agents due to induced treatment resistance. We recently described a novel pyrido-benzimidazole analogue, SE486-11, which enhanced the therapeutic efficacy of HDAC inhibitors by increasing MYCN ubiquitination.[1] Subsequent investigations revealed that USP5 is a mediator of the effects of the combination treatment and a co-factor for MYCN oncogenesis. Using structure-activity relationships, we have now developed 64 substantially more potent analogues of SE486-11, with IC_{50} values as low as 26 nM for the most potent compound. Here we report the anti-cancer evaluation of these novel and more potent inhibitors of the USP5 enzyme. The hit compounds, UNSW-SC-22 and UNSW-SC-36 were found to be the most promising among a series of compounds with excellent single agent *in-vitro* activity in MYCN-amplified SK-N-BE(2)-C and Kelly neuroblastoma cell lines. These compounds synergistically enhanced *in-vitro* cytotoxicity of HDAC inhibitors, SAHA and panabinostat, in neuroblastoma cells, when used in combination. Moreover, knockdown of USP5 and MYCN in treated neuroblastoma cells showed that USP5 and MYCN expression were necessary for the cytotoxic activity of these compounds. Most importantly, UNSW-SC-22 displayed significant *in-vivo* efficacy in neuroblastoma-bearing homozygous transgenic TH-MYCN mice, thus providing a clinically relevant rationale for further development of these compounds.



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Platelet membrane-coated bioactive glass for reduced cellular uptake and enhanced osteoimmunomodulation

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Phagocytes are key cellular participants that determine the therapeutic efficacy of nanoparticles through identification, uptake and clearance. The cell membrane coating technique has emerged as an ideal surface modification approach to bypass immunocyte uptake and mononuclear phagocytic system clearance. Herein, we enclosed bioactive glass (BG) in the platelet membrane to bestow BG with unique cell surface functions for immune evasion and immunomodulation. Compared with uncoated particles, the platelet membrane coated BG (PBG) has reduced cellular uptake by macrophages and appeared to modulate the immune environment by activating efferocytosis. The PBG and PBG-mediated immune environment triggered robust osteogenic differentiation of bone mesenchymal stem cells, suggesting the synergistic effect of platelet membrane and BG in bone regeneration. These results collectively imply that the cell membrane coating is a promising strategy to provide stealth ability and improve therapeutic competence in biomedical applications.

Polydiacetylene-based colorimetric sensor for bacteria detection

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Antimicrobial resistance has emerged as a major public health threat, with an estimated 1.2 million people died in 2019 from antibiotic-resistant bacterial infections.[1] To reduce bacterial infection and the occurrence of antibiotic resistance, rapid and portable bacteria detection is critical in the fields of clinical diagnosis, food safety and environmental monitoring. Conventional bacteria detection methods such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) are the current gold standards.[2] However, these methods are all lab-bench techniques, requiring well-trained personnel and specialized instruments, which limit their applicability for on-site detection. As such, developing a point-of-care (POC) device is of great importance. Polydiacetylene (PDA) is considered as an attractive candidate for portable colorimetric sensors. When exposed to external stimuli such as heat, pH, mechanical strength, and/or specific binding processes, these self-assembled polymerized vesicles change in colour from blue to red.[3]

Taking advantage of the easily observed colorimetric response of PDA, we propose a PDA-based sensor for bacteria detection. Lipids are also incorporated in the vesicles to increase fluidity and stability. To achieve portable detection, a sandwich lateral flow assay (LFA) was developed (Figure 1). Briefly, on the conjugation pad, the target bacteria binds to antibody, which is conjugated on PDA/lipid vesicles to form a complex, which then migrates through the nitrocellulose membrane, further being captured by the detection antibody and forming a sandwich-like structure. The colour change intensity of the PDA/lipid vesicles is proportional to the bacteria concentration, thus being reflected by the colour of the test line. Compared to conventional LFAs, which utilize metal nanoparticles (e.g. AuNPs and AgNPs) as signal transducers, PDA leads to reduced costs and potential commercialization.

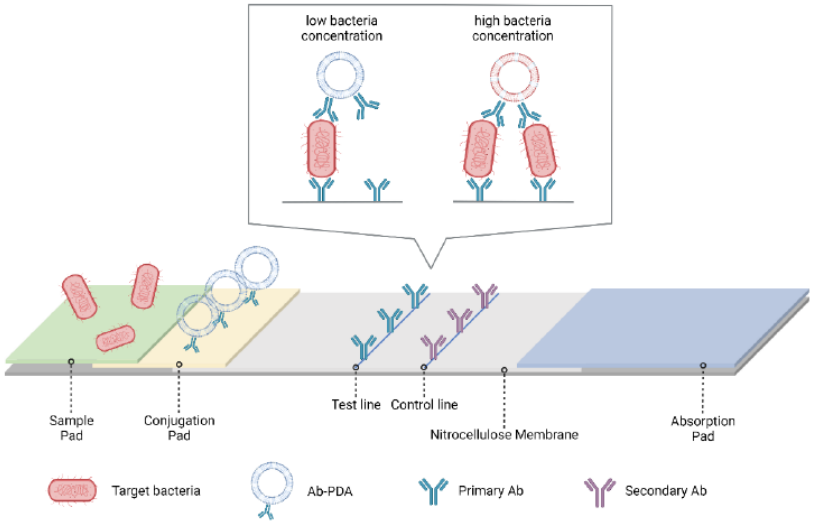

Figure 1: Schematic diagram of PDA based sandwich LFA for bacteria detection

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The effect of radiosensitizing gold nanoparticles on cancer cells' metabolic reprogramming at the single-cell and cell-population level

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High-Z metal nanoparticles (MNPs) are used as tumor tissue radiosensitizers in clinical trials as well as clinical practice. MNP-induced radiosensitization was originally motivated by enhancing the physical interaction of radiation in tumors with MNPs. Our recent investigations1 as well as findings from other studies, however, are indicative of another important underlying mechanism caused by MNP-cell interactions and the resulting intracellular biochemical changes. Cancer cell metabolism appears to be one of the biochemical entities affected by MNPs. This could be inferred from the changes we have observed in the expression level of metabolic proteins and the level of MTT reduction in prostate cancer cells treated by gold NPs[2], as well as previously reported gold NP-induced metabolomic alterations in cancer cells.

As metabolic reprogramming promotes tumor progression, cancer relapse, and resistance to various treatments, we hypothesized that the observed NP-induced metabolic changes may also contribute to the radiosensitization effect of MNPs. To test our hypothesis, we conducted this study to assess the effect of gold nanoparticles coated with polyethylene glycol (PEG) and transferrin (Tf) on metabolic reprogramming at the single-cell level and to investigate how this effect may change the sensitivity of cancer cells to harsh insults such as radiation. In this regard, we applied Fluorescence Lifetime Imaging Microscopy (FLIM) to measure the ratio of free to protein-bound NAD(P)H (reduced nicotinamide adenine dinucleotide (phosphate)) inside prostate cancer cells (PC-3 cell line) at different time points following continuous and non-continuous exposure to 0.6 nM of gold-PEG-Tf nanoparticles. A change in the observed free/bound NAD(P)H ratio can be representative of a shift between glycolysis and oxidative phosphorylation, which is a hallmark of metabolic reprogramming in cancer cells. Using the FLIM-derived data, we also measured the effect of nanoparticle treatment on the NADPH/NADH ratio which can be an indicator of changes in antioxidative defense in cancer cells and their sensitivity to cancer treatments. We also aimed to assess if there is any correlation between nanoparticle uptake and metabolic changes at the single-cell level.

Our single-cell FLIM measurements indicated heterogeneity of metabolic state among both non-treated and NP-treated PC-3 cells. Exposure to gold-PEG-Tf nanoparticles altered the average as well as the statistical distribution of metabolic parameters among the single cells. These nanoparticle-induced metabolic changes can affect the sensitivity of cancer cells to the cytotoxic effects of various cancer treatments including radiation therapy by changing the antioxidative defense state. This data provides new insights into the cell-NP interaction and how it can affect the safety and efficacy of MNPs in regards to their broader medical applications.

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Glypican-1 targeting immuno-PET imaging tracers for detection of glioblastomas

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Glioblastoma (GBM) is the most aggressive form of primary brain cancer, accounting for ~85% of all primary tumors of the central nervous system. Despite advanced treatment strategies like surgery, radiation and chemotherapy, the median survival time of patients following diagnosis with GBM is only ~15 months. Poor prognosis of GBM is also associated with a very high chance of tumor recurrence, indicating a dire need for improved therapeutic alternatives for this disease. Radioimmunotherapy holds great promise in treating GBM, combining the medical imaging and therapeutic properties of radioisotopes with the target specificity and high affinity of antibodies. My research (in collaboration with industry partner GlyTherix, Ltd., Australia) explores *Miltuximab®*[1], a clinical stage antibody that recognizes Glypican-1 (GPC-1), a heparin sulphated proteoglycan highly expressed in GBM (and other cancers) but poorly expressed in normal tissue. The ongoing project involves developing a multi-format antibody platform for GBM theranostics. The platform would include full-length Miltuximab® (MIL38), Fab'2, Fab and single-chain variable fragments (scFv) as constructs with high affinity, but exhibiting variable pharmacokinetic properties. In the present study, all four formats were radiolabeled with 89Zr to validate their potential as diagnostic probes for non-invasive positron emission tomography (PET) imaging. These agents are concurrently being tested in 3D-tumour spheroids and preclinical mouse GBM tumour models to determine their safety and effectiveness using fluorescence and PET/CT imaging, respectively.

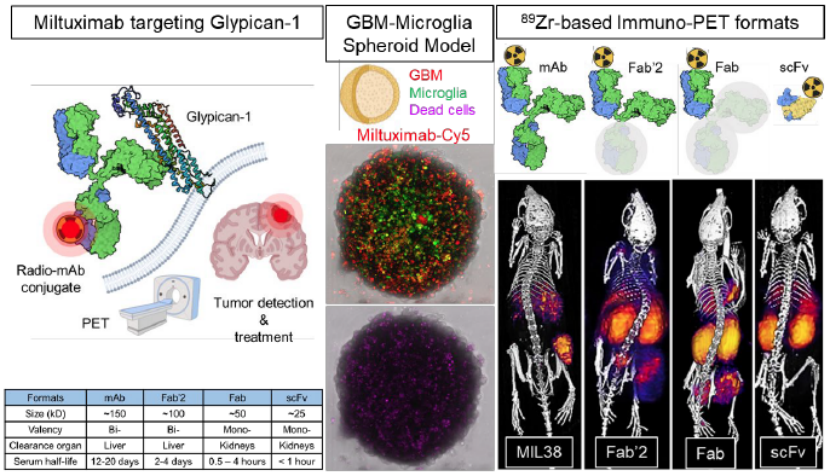

Figure 1: Radio-antibody-conjugates as targeted theranostics for GPC-1 overexpressing GBM

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Optical voltage sensing using new biocompatible materials

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Fluctuations in Ion concentrations are the initiator of many cellular activities, from contraction due to the release of Ca2+ ions in muscles to the generation of action potentials in neural and cardiac cells. Measurement of these ion fluxes can provide information on cellular mechanisms and cell health. Multiple optical sensors have been developed that respond to changes in ion concentration in their local environment by either binding, conformational changes, or electric configuration shifts. None of the commercially available sensors have the combined capability of high spatial resolution and real-time sensitivity. On top of that, they usually operate in the visible range of the spectrum, which limits their in vivo applicability to superficial tissue layers.

Research in this field is focused on increasing the resolution and the in vivo efficiency. One of the most promising new materials in this field are semiconductor nanoparticles. These display changes in their photoluminescence spectrum – peak wavelength and emission intensity – in response to changes in the electric potential in their environment. Some semiconductor nanoparticles have emission bands in the near-infrared, which allows collecting information at larger tissue depths.[1]

In our work, we have studied the response of near-infrared-emitting semiconductor nanoparticles to induced electric fields. We use Ag2S nanoparticles in our experiments since they have a strong, narrow emission peak in the near-infrared together with a large surface-tovolume ratio, facilitating the conjugation of ligands for targeting.[2]

The particles were placed in an artificial cell membrane capable of inducing an electric field. The measured outcome shows changes in the emission characteristics of the nanoparticles in physiologically accurate conditions. These findings indicate that Ag2S nanoparticles can be used as an indicator of electrical potential shifts induced by ion fluxes.

The use of near-infrared-emitting reporters, such as Ag2S nanoparticles, can provide high resolution, non-invasive, and real-time imaging to monitor changes in electrical potential. Further research in the coating of the particles is needed to increase the safety and efficiency of their use as voltage indicators.

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The effect of avidity on nanobody-based formats for theranostic applications

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Nanobodies (Nbs) can be engineered to be multi-specific and multivalent to improve their binding capabilities through functional affinity (avidity). Together with their small mass and rapid clearance, the high specificity of Nbs is a particularly attractive feature for diagnostic imaging and radioimmunotherapy in oncology. There is a frequent assumption that engineering antibodies or fragments (e.g. Nbs) in this way will be synonymous with greater diagnostic or therapeutic efficacy, but some studies have shown that this is not the case *in vivo*. My research aims to elucidate the effect of increasing avidity through engineered Nb formats and how it affects the biodistribution and penetration into solid tumours.

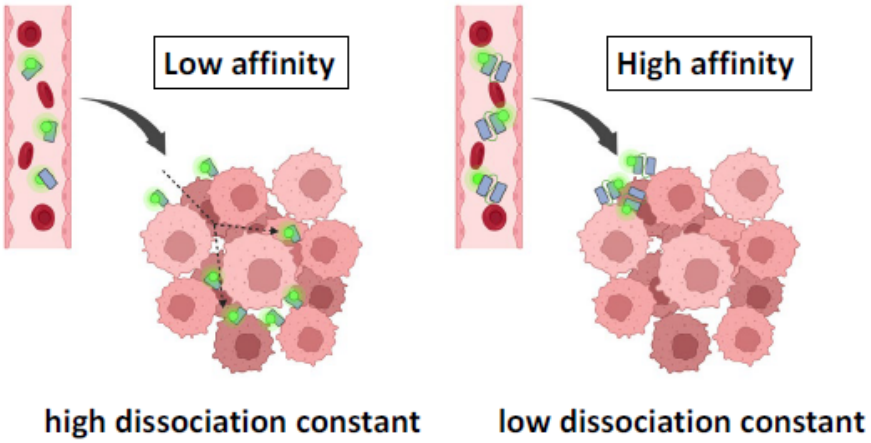

Figure 1: Nb avidity leads to lower therapeutic efficacy.
High affinity is a key determinant for antibodies and drug effectiveness and can be achieved through increasing avidity on antibody fragments such as nanobodies. Studies have shown that some antibodies, antibody fragments and small targeting proteins[1,2] show reduced tumour penetration and surface localization. It is hypothesized that A) low avidity nanobodies have better coverage due to being able to perfuse into the tumour, whereas, B) high avidity nanobodies bind their first encountered epitope, causing patchy binding on the surface, ultimately leading to reduced tumour penetration.[3]

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Understanding acquired immunity to polymer nanomedicines – management strategies and potential benefits.

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The antibody mediated clearance of synthetic polymers presents a number of unmet challenges to the field of nanomedicine. Despite being widely reported, usually in response to some nanomedicines featuring the synthetic polymer poly(ethylene glycol) (PEG) in both preclinical and clinical settings[1], strategies to address this phenomenon have been sparingly investigated. It has long been thought that the development of synthetic materials with similar properties to PEG (but different chemical identities) can be used to overcome these obstacles, however it is currently unknown if this is the case in practice.

In this talk a rational, materials-driven approach to overcoming acquired immunity against various synthetic polymers will be demonstrated, revealing that altering the hydrophilic polymeridentity used in a model nanomedicine is capable of restoring a naïve equivalent biodistribution in immunologically-relevant animal models. Expanding further on this, a series of novel anti-poly(2-methyl-2-oxazoline) and anti-poly(2-ethyl-2-oxazoline) monoclonal antibodies have been successfully isolated, and pertinent information such as their typical binding kinetics and specificity will be discussed in order to inform on the potential clinical applicability of these materials as PEG alternatives. These antibodies also present new opportunities to develop sensitive diagnostic assays and *in vivo* theranostics, and examples of these applications will be presented.

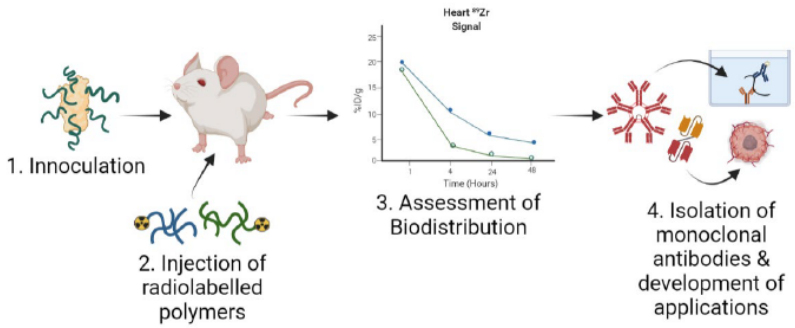

Figure 1: Schematic overview of investigation of antibody mediated clearance of different polymer systems and the development of new applications from specific anti-polymer monoclonal antibodies.

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Acid-Responsive Micellar Nanoparticles for the Delivery of Self- Amplifying ROS-Responsive Prodrugs

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Antibody-decorated selective SPIONs for capturing circulating tumor cells – new approach of preventing prostate metastasis

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Despite the dynamic progress of medicine, cancer is still among the most dangerous civilization diseases. The main reason of death in neoplastic diseases, rather than primary tumor, is its metastasis to other organs, spread by the malignant cells circulating in the blood. A rapid increase in the number of cancer cases observed in recent years is one of the main reasons for the intensified search for new, better methods of cancer diagnostics and treatment. Current research focuses ontwo approaches to reducing the risk and extend of metastasis. First of all, the efforts are made to understand the mechanisms of metastasis at the genetic and epigenetic level, which will allow the improvement of the currently used preventive methods and drugs[1], such as the neoadjuvant method. On the other hand, the scientists aim at preventing the spread of circulating tumor cells which can initiate the formation of secondary tumor. The best-known methods include CellSearch (based on magnetic capture) and ISET (based on the differences in the cell size).[2]

Prostate cancer is the second most-common type of malignant tumor. It is estimated that 1 in 8 men will be diagnosed with prostate cancer in their lifetime. The aim of our research was to develop surface-modified superparamagnetic iron oxide nanoparticles (SPIONs) for the effective magnetic capture of prostate cancer cells with metastatic potential). The obtained nanoparticles were stabilized with the cationic derivative of chitosan and decorated with carefully selected antibodies that would allow to target tumor cells. Three prostate cancer cell lines were studied: PC-3, DU 145 and LNCaP, differing in the degree of malignancy and in the expression of surface proteins. By analyzing the differences in the expression of surface proteins, three antibodies were selected and covalently attached to the surface of nanoparticles: anti-N-cadherin[3], anti-P-cadherin and anti-PSMA antibodies. The physicochemical properties (including size and colloidal stability) and magnetic properties of the antibody-decorated SPIONs were studied, as well as their interactions with all three prostate cancer cell lines mentioned above. The negligible toxicity of nanoparticles was demonstrated based on the colorimetric tests, their distribution and effective attachment to the cell surface were confirmed and visualized, and their selective attachment to the cell surface was proven using flow cytometry. The effective magnetic capture of cells in the static conditions was also confirmed.

Karolina Karnas acknowledges the support of InterDokMed project no.POWR.03.02.0000I013/ 16 and MNiSW no. N41/DBS/000597.

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RBC coated camouflaged nanoparticles for drug delivery application

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Background: The red blood cell (RBC) membrane coated nanoparticles (NPs) are very attractive drug carriers due to their exceptional characteristics such as ability to escape immune cells, long blood circulation time, and specific surface molecules for recognition and in cell targeting[1]. Because of the combined merits of the synthetic core (as drug reservoir) and natural membrane coating platforms (biomimetic), these systems have been widely studied for drug delivery, detoxification, vaccination, and cancer therapy applications[2]. Here, we reported the fabrication of RBC membrane coated (3-aminopropyl) triethoxysilane (APTES) modified MSN as well as their characterization using dynamic light scattering (DLS) and transmission electron microscopy (TEM).
Methods: MSN were prepared using modified Stöber method[3] and conjugated with APTES using reported protocol[4]. Fresh RBCs were obtained from Australian Red Cross Lifeblood and their membrane were isolated using hypotonic PBS buffer. RBC membrane coated NPs were then fabricated by passing them through series of polycarbonate membrane (1 µm, 0.4 µm and 0.2 µm) using mini extruder. These nanoparticles were then characterized for their particle size and zeta potential using dynamic light scattering (DLS) and transmission electron microscopy (TEM).
Results: The synthesis of MSN and APTES modified MSN were confirmed using FTIR (Figure 1A) and TEM (Figure 1B, left hand side image). The average particle size of MSN was found to be 110 nm, while the APTES modified MSN was 120 nm (Table 1). The zeta potential of APTES modified MSN showed shift from -15.89 mV in non-modified MSN to +14.96 mV (Table 1). For RBC coated NPs, APTES-MSN showed increased in particle size to 193 nm after membrane coating. Furthermore, APTES modified MSN with RBC coating demonstrated negative (-15.55 mV) zeta potential (Table 1). This indicates the successful coating of RBC membrane on APTES modified MSN NPs. The formation of RBC coated nanoparticles was then confirmed using TEM images (Figure 1B, right hand side image).

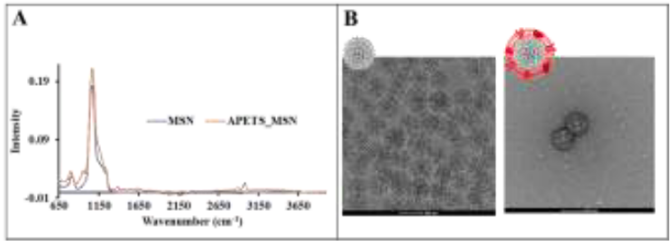

Figure 1: Characterization of RBC membrane coated NPs using A] FTIR, B] TEM.

Table 1: RBC coated NPs size and zeta potential analysis using DLS techniques

NPs	Hydrodynamic diameter (nm)	Zeta potential (mV)
MSN	110	-15.89 (±0.66)
APTES modified MSN	120	+14.93 (± 1.20)
RBC coated APTES MSN	193	-15.55 (± 0.93)

Conclusion: The RBC membrane coated NPs hold a greater potential due to their immune escaping and long blood cocirculation abilities. Thus, in this study we explored and optimized their fabrication methods. Additional characterization including drug loading, time dependent release and *in vitro* studies are currently under investigation.

Acknowledgements: T. K. acknowledges the support from the National Health and Medical Research Council of Australia (NHMRC) for Early Career Fellowship (GNT1143296) and the University of New South Wales for support and Scientia Grant. T.K. and G. R. K. also thank Australian Research Council (ARC) for the Discovery project grant (DP200102723).

Single cell profiling of tumour remodeling and heterogeneity of nanoparticle fate in radiosensitization of a syngeneic triple-negative breast cancer mouse model

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Metal-based nanoparticles are clinically available for radiosensitization of tumour and a number of others are proceeding through clinical trials. However, their mechanisms of action remain controversial. and are likely a combination of increased local radiation dose deposition along with other chemical and biological mechanisms[1-3]. Furthermore, questions exist about what cells, and how many cells, even take up nanoparticles within tumour which can greatly influence therapeutic outcomes. Tumor microenvironment (TME) is constructed by cellular components such as immune cells, stromal cells, and extracellular matrix[4]. Therefore, the heterogeneity of cell populations and gold nanoparticle uptake on those heterogeneous subsets in the TME needs to be evaluated holistically. We conducted single-cell analysis on fresh tumor tissues obtained from a mouse model. Single-cell mass cytometry (CyTOF) was used to investigate the heterogeneous cell populations and a semi-quantification of gold nanoparticle uptake across the populations simultaneously. Our formulation was injected into the bloodstream and revealed that gold content was present in the tumor cells, cancer stem cells, CD45 cells, endothelial cells, epithelial cells, tumor-associated fibroblasts, NK cells, dendritic cells, granulocytes, and macrophages for all time points (24h, 48h and 20 days), however the maximum was observed at 24h. As expected, direct injection of the formulation into primary tumors resulted in a greater uptake of gold in those cell populations than injection into the bloodstream of animals' tail vein. Remodeling of the TME due to nanoparticles can also enhance therapeutic response to radiotherapy. The data provide unique insight into which tumour cell populations take up nanoparticles and how this modulates response to additional treatments.

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Design of FeBi Nanoparticles for Imaging Applications

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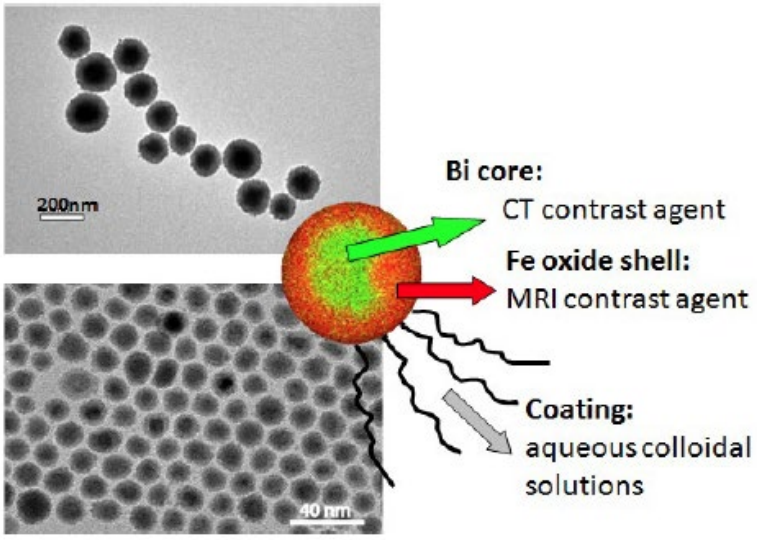
A variety of imaging technologies are now routinely used in the medical field, their use being continuously enlarged through the development of contrast agents. Recently nanoparticles (NPs) proved efficient to improve imaging in vivo by increasing contrast and improving targeting capabilities. The current trend is now focused on the development of dual contrast agents combining two or more functionalities on the same NP.[1] Motivated by this new challenge we developed FeBi NPs as new nanomaterials with potential application as contrast agent for MRI and CT imaging.[2] In addition to the well-known use of iron in the development MRI contrast agents,[3] we chose Bi as CT imaging agent rather than the more documented gold, because it possesses a larger X-ray attenuation coefficient, has low toxicity and is much less expensive.[4] The NPs were synthesized using a well known organometallic approach. They are spherical, and contain distinct domains of Fe and Bi, and a hydrophobic coating. The development of the coating chemistry to afford aqueous colloidal solutions will be discussed.



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A CRISPR/Cas12a-associated ultrasensitive immunoassay for the measurement of single microorganisms

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The recently established CRISPR/Cas-based biosensing schemes exhibit excellent sensitivity and specificity for the detection of nucleic acids, and they have also been rapidly adapted to sensing other types of analytes, including proteins, small molecules, ions, etc. However, so far, CRISPR/Cas biosensing applications have not been able to directly detect larger biological structures, such as an intact single microbe. Here, a novel ultrasensitive CRISPR/Cas12aassociated immunoassay scheme was proposed for targeting the whole Cryptosporidium parvum (Crypto) oocysts. To this aim, a specific antibody and ssDNA conjugate were developed for the recognition of the targeting Crypto oocysts, which is also capable of triggering the downstream CRISPR/Cas12a trans-cleavage for amplification of fluorescent signal. Our thus developed Crypto immunoassay has a three-log linear detection range (6.25 – 1600 oocysts/mL), and it is capable of reaching single Crypto detection from 100 µl sample after an extended CRISPR/Cas12a reaction time. Unlike gold-standard microscopy-based identification, this approach uses a plate reader and it does not require high level expertise or time-consuming staining and counting procedures. In addition, it is effective in various complex sample matrices with a simple dilution step, and it has been shown to detect 10 Crypto from water source-related mud samples. The single oocyst sensitivity achieved in this work is critical important because Crypto has an extremely low infectious dose, and public health screening must meet the detection threshold of a single contaminating microorganism in each tested sample. As the first attempt to realize CRISPR/Cas-based biosensing in directly targeting complex single microorganism structures, our work expands the actual application scenarios of CRISPR/Cas biosensing for a broad range of complex biosensing targets.

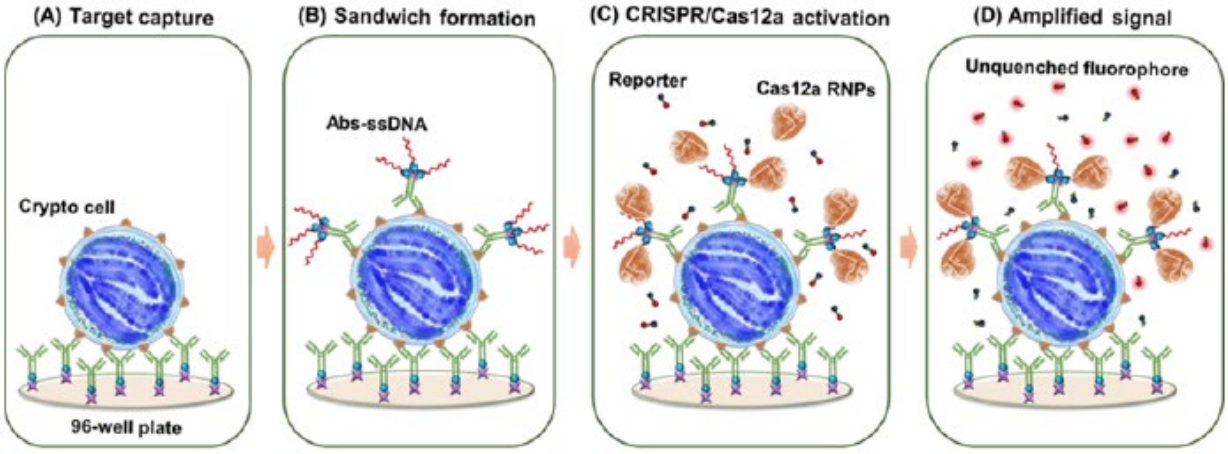

Figure 1: *The standard procedure of the CRISPR/Cas12a-associated immunoassay for whole microorganism detection. It includes 3 major steps: target capture, sandwich structure formation, and CRISPR/Cas12a-based signal amplification.*

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CRISPR/Cas12a-based signal amplification in immunoassay schemes for attomolar level small protein diagnostics

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Recent advances in CRISPR/Cas biosensing have led to impressive performance in sensitivity, specificity, and speed for nucleic acid detection. However, the remarkable advantages (such as universality, ultralow, attomolar detection limits) of CRISPR/Cas biosensing systems are limited in testing non-nucleic acid targets. Herein, by synthesizing a functional hybrid conjugate of antibody and single strand DNA oligonucleotide, we had successfully demonstrated the capability to integrate CRISPR/Cas12a-based signal amplification into different types of well-established immunoassay schemes without the need for any additional recognition molecule or molecular synthesis during the detection process, thus providing a simple but generally applicable approach to improve the conventional immunoassays with attomolar sensitivity for small protein detections, referred as the CRISPR-based Universal Immunoassay Signal Enhancer (CRUISE). CRUISE is capable of being integrated into various immunoassays either through the primary antibody or the secondary antibody, with sensitivity down to 1 fg mL^{-1} (~50 aM) and 6 logs of linear range for detecting small proteins, such as IFN-γ and EGFR, under 3~4 hours. It has a 10^3 times higher sensitivity compared to a commercial IFN-γ ELISA kit, but uses the same experimental setup. The same 1 fg mL^{-1} sensitivity along with 6 logs of linear range was realized for IFN-γ detection in plasma diluent samples. We are expecting that our CRUISE provides an alternative but simple, user-friendly, and effective strategy for those who rely on the use of immunoassays, while struggling with the limits of their sensitivity or detection ranges.

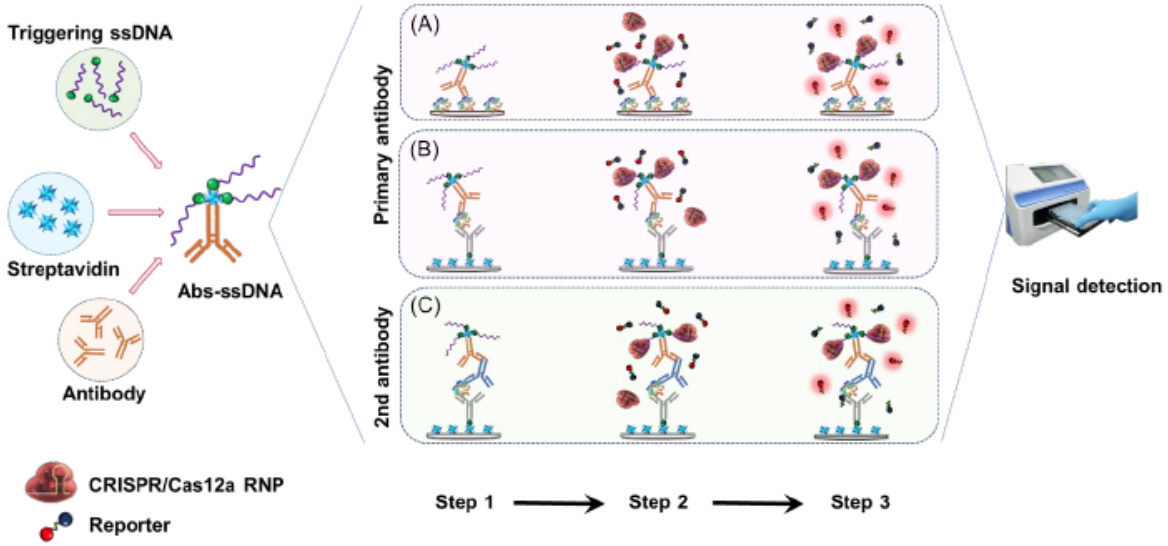

Figure 1: Schematics of CRUISE platform. The key elements for CRUISE to bridge the CRISPR/Cas12a and immunoassays is the Abs-ssDNA conjugate, which has 3 components, including the selected antibody, streptavidin, and biotinylated triggering ssDNA. The prepared Abs-ssDNA conjugate can be used either as primary antibody or secondary antibody in different immunoassay schemes.

References: unpublished works

A versatile hyaluronic acid nanoparticle drug delivery system for cancer treatment

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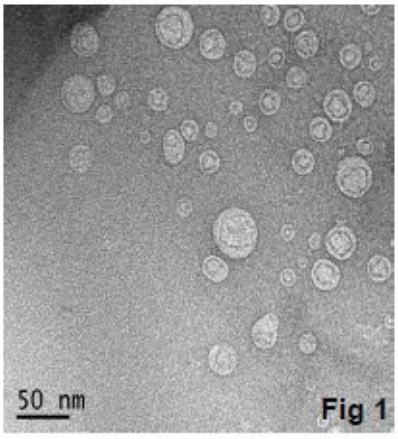
Cancer is a leading cause of death due to the continuing challenges in achieving efficacious delivery to tumour and sparing of normal tissues.[1] Advanced materials and cutting-edge nanotechnology constructs are developing promising drug delivery profiles to improve efficacy of treating cancer.[2,3] Hyaluronic acid, a natural and nontoxic polymer, is a promising material for developing 'soft' carriers. The repeated-units of soft carriers offer abundant functional components and space for covalent packaging of drug.[4] Moreover, it has affinity to the CD44 receptor which is highly expressed in many cancer cells.[5] The biological (targeting CD44+ cell specifically), physio-chemical properties of particles (~100 nm) and tumour microenvironment (offering biodegradable and cleavable stimulus) can be taken into consideration to optimise a drug delivery system. The carrier is designed to provide better tumour control outcomes and lower side effects due to: controlled drug release mediated by a hypoxic tumour environment rather than freely distribute to healthy tissues; prolonged drug retention in tumour; good biocompatibility; and altered drug pharmacokinetics and biodistribution. Data are presented on the nanoparticle characterization (Fig 1), uptake across multiple cancer and normal cells, biodistribution (Fig 2) and delivery of drugs and prodrugs in 2 mouse models representing triple negative breast cancer and multiple myeloma.



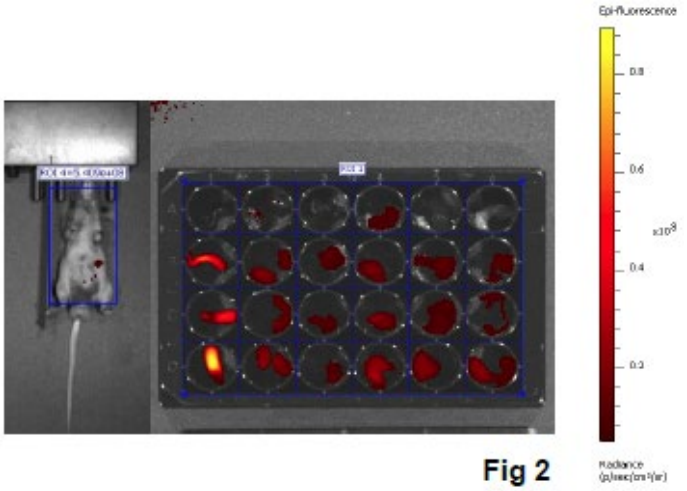


Figure 1: TEM image of hyaluronic acid nanoparticles.
Figure 2: Representative IVIS images of nanoparticles in organs after injecting Cy7 Hyaluronic acid NPs.

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A nanomedicine approach to treat brain metastases from breast cancer

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Brain metastases have dismal prognosis. Although chemotherapy extends life for patients with systemic spread, the dosage required for an effective outcome is toxic with debilitating adverse effects. There is a lack of targeted treatment for brain metastases. Nanomedicine can circumvent off-target toxicities and factors limiting efficacy of conventional chemotherapy as well as providing diagnostic option for earlier detection of brain metastases. We investigate PEG-based polymeric nanocarrier that targets HER2 and HER3 receptors, expressed in a subset of breast cancer brain metastases, and be internalised into cancer cells; and, controlled-release of a cytotoxic drug, doxorubicin (DOX). We have (1) developed bi-specific antibody (bsAb) fragments against HER2/3 and PEG; (2) functionalise nanocarriers with targeting bsAbs, and a Cy5 fluorophore for biodistribution studies; (3) attached HER2/3-targeted-nanocarriers with doxorubicin via an acid-labile hydrazone linkage for pH-dependent release in tumour microenvironments, or endosomes after internalization; and (4) evaluated therapeutic outcomes with cell and animal model. We found that in acidic endosomal condition (pH5.5), doxorubicin-release from nanocarrier was double compared to blood serum condition (pH 7.4). The HER2/3-targeted nanocarriers induced receptor internalisation and doxorubicin uptake in BT474 cancer cells, contributing to a reduced cell viability. In contrast, untargeted nanocarrier was less cytotoxic at higher doses, highlighting low off-target toxicity. Therapeutic study using a brain metastasis mouse model showed that all nanomedicine treatment groups improved survival over free DOX. PET/MR study demonstrated accumulation of nanocarriers in brain metastasis region over uninvolved brain. In particularly, HER3-targeted nanocarriers displayed greater efficacy over other groups and treated mice have smaller brain tumours with less proliferative tumour cells. Notably, nanomedicine groups were well-tolerated with no severe chemotherapy associated toxicity observed in free DOX group. Heart wall and oocyte studies showed reduced cardio and gonado-toxicities. In conclusion, the targeted nanocarrier has the potential to reduce chemotherapy-associated toxicities by delivering a higher concentration or more potent choice of chemotherapy to cancer cells selectively. The imaging feature is ideal for early diagnosis of brain metastases and to monitor progression.

Variability in Nanodrug Uptake is Influenced by Melanoma Heterogeneity in Tissue Engineered Microtumors

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Nanomedicine treatment for advanced cancer has been ofgreat interest, with the promise of selective cell localisation and targeting. As the field of nanomedicine develops, more physiologically relevant model systems are required to streamline promising formulations into clinical use. One such technique is the use of micropatterned hydrogel matrices as a simple system to control the biophysical and biochemical cues experienced by cells. This approach involves soft lithography to pattern proteins and grow cells into confined geometries which mimics aspects of the tumour microenvironment while having a reproducible and spatially addressable format. Interfacial geometry controls the heterogeneity of the confined cells, where subpopulations with stem cell characteristics are predisposed to periphery regions of high stress, analogous to the *in vivo* microenvironment of the invasive niche.[1] A new nanoparticle system which shows promise for future clinical application scaffolded around curcumin, polydopamine and fructose was then used to treat the melanoma microtumours.

This study aimed to determine the uptake and localisation of a model nanoparticle on cells with varying metastatic potential, which was inferred by measuring expression of the putative cancer stem cell marker, ABCB5. Results showed increased nanoparticle uptake on cells with greater ABCB5 expression with the population influenced by geometric stress at 104% and native high metastatic population at 47% more uptake on average compared to the non-primed low metastatic population (Fig 1). These results suggest there is strong correlation between high expression of putative cancer stem cell markers in melanoma cells and uptake of the model nanoparticle system. Therefore, by using a model with physiologically relevant features, it was found this nanoparticle had selectivity, localising in more metastatic populations.

Overall, the results show there is strong correlation between expression of putative cancer stem cell markers and uptake of the model nanoparticle system. Together, these findings suggest that different subpopulations within a tumour mass may be more susceptible to nanoparticle treatment, thereby providing a new approach for selectively targeting based on cell state.

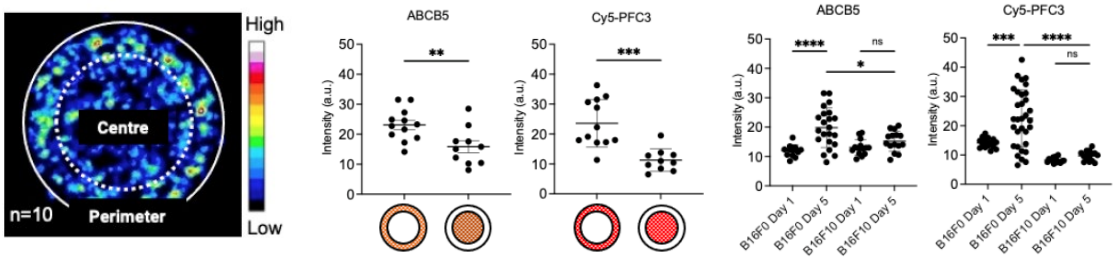

Figure 1: Mechanical cues prime a population of cells with increase stemness which increase uptake of Cy5-PFC3

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Targeting Lipid Nanoparticles for Improved Delivery of siRNA to Neuroblastoma

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High-risk neuroblastoma is an aggressive and difficult to cure childhood cancer. The toxic side effects of conventional therapies remain a major clinical challenge and there is a clear need for effective and less toxic therapies. One approach is to silence the expression of genes that promote tumour growth, such as polo-like kinase 1 (*PLK1*), using short-interfering RNA (siRNA). Whilst lipid nanoparticles (LNPs) are the most clinically successful siRNA delivery vehicle, clinical LNPs contain polyethylene glycol (PEG)-lipids on the surface that are quickly lost in circulation, leading to rapid uptake by healthy organs, primarily the liver[1,2]. Hence, delivering siRNA to extrahepatic sites, such as neuroblastoma, remains challenging. The addition of neuroblastoma targeted antibodies, such as epidermal growth factor receptor (EGFR), on the LNP surface may aid uptake. Here, we explore if the combination less diffusible lipids and antibody targeting enhances siRNA delivery and *PLK1* silencing in neuroblastoma cells. This strategy aims to improve outcomes for patients with neuroblastoma.

Three PEG-lipids with decreasing diffusion rates from the particle surface (DMG-PEG, DSGPEG and DSPE-PEG respectively) were used to create siRNA-LNPs via microfluidic mixing. We then combined siRNA-LNPs with bispecific antibodies (BsAbs) that recognise and bind to EGFR as well as PEG on the LNP surface to yield targeted, siRNA-loaded particles (BsAbsiRNA- LNPs). Association and internalisation of BsAb-siRNA-LNPs in neuroblastoma cells was examined *in vitro* using flow cytometry and confocal microscopy respectively. *In vitro* PLK1 silencing following treatment with BsAb-siRNA-LNPs was examined at the mRNA level using RT-PCR, and studies investigating protein knockdown via western blot are underway.

Untargeted siRNA-LNPs made with the more diffusible DMG-PEG had greater cell association and internalisation than DSG-PEG and DSPE-PEG LNPs. The addition of 0.04 EGFRBsAbs/ PEG significantly ($p<0.001$) increased DSG-PEG and DSPE-PEG siRNA-LNP association with EGFR-positive SH-SY5Y and SKNBE(2) neuroblastoma cells, whilst BsAb addition had minimal impact on uptake and cell association of the DMG-PEG formulation. EGFR BsAb addition also improved *PLK1* gene silencing of DSG-PEG and DSPE-PEG siRNALNPs more than 3-fold when delivered at a concentration of 90 nM siRNA.

Whilst exchanging DMG-PEG with less-diffusible PEG-lipids reduces non-specific siRNALNPs uptake, such changes may assist in avoiding non-specific uptake of siRNA-LNPs by healthy organs, including the liver. BsAbs against EGFR or any neuroblastoma antigen of choice can then be used to improve uptake and gene silencing of DSG-PEG and DSPE-PEG siRNA-LNPs in neuroblastoma cells. Hence, there is great potential in the combination of reduced PEG-diffusivity and BsAb targeting to improve siRNA delivery to tumours.

Advanced materials to inhibit pancreatic enzymes in the gut to treat organ failure

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Introduction: Acute and critical illnesses (ACIs) are typically managed in emergency and intensive care settings in hospitals1. Recently, the 'gut-lymph model' has demonstrated that 'toxic factors' from the gut, including pancreatic protease, lipase and lipase generated lipotoxins, enter the lymph and the blood circulation in ACIs to promote systemic inflammation and organ dysfunction/failure2, 3. Currently there are no specific and effective treatments for organ failure in ACIs. Therefore, we investigated if delivering adsorbent materials into the gut lumen could bind to and reduce pancreatic enzyme activity in the intestine fluid.
Aims: To determine the optimal properties of adsorbent materials to bind to and inhibit the activity of lipase (from rhizomuscor miehei) and protease (e.g. trypsin from bovine pancreas) enzymes *in vitro*. To compare the *in vivo* effect of different adsorbent materials on pancreatic enzyme activity (e.g. analysed by BAPNA substrate) after intestinal administration to rats.
Methods: The *in vitro* loading and inhibition of pancreatic enzymes by different adsorbent materials, including MSP with different pore size (4 – 12 nm), and activated charcoal, were tested in Tris buffer by BCA assay. The *in vivo* pancreatic enzyme inhibition was assessed following infusion of adsorbent materials into isolated intestinal segments in rats.
Results: MSP (SBA-15) with a pore size of 8 nm was found to have the highest trypsin binding (128.9 mg/g). Activated charcoal was found to be an extremely effective platform to adsorb both trypsin (101.4 mg/g) and lipase (183.9 mg/g) in buffer (Figure 1). Both MSP and activated charcoal reduced pancreatic enzyme (trypsin) activity in rat intestine.
Discussion: Adsorbent materials can bind to and load pancreatic enzyme *in vitro* and reduce pancreatic enzyme activity *in vivo*. It appeared that pore size but not particle size was the critical factor that influences enzyme loading, where the highest binding which is likely linked to pore size (8 nm) being not too small or too large to bind the enzyme. These materials may provide a novel medical approach to reduce the progression of ACI to organ dysfunction and failure. Figure 1: (A) Trypsin binding (mg/g) over time, and (B) Lipase binding (mg/g) over time to adsorbent materials in buffer. Data are mean ± SD (n=3).

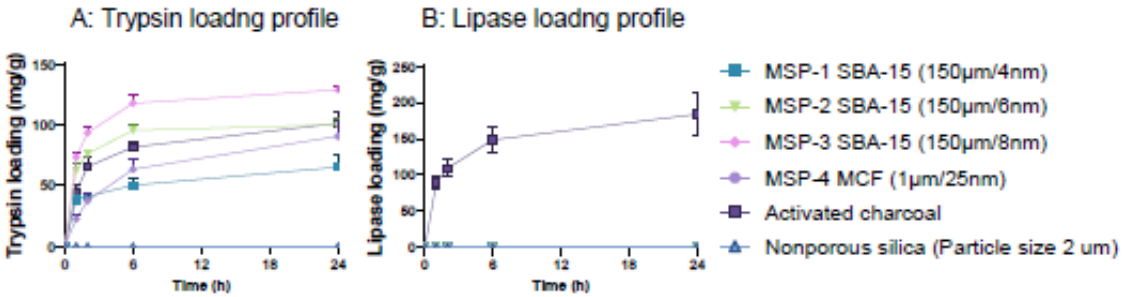

Figure 1: (A) Trypsin binding (mg/g) over time, and (B) Lipase binding (mg/g) over time to adsorbent materials in buffer. Data are mean ± SD (n=3).

Functionalized porous silicon nanovectors for lysosomal-targeted delivery system to treat triple-negative breast cancer

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Viable targeted treatment strategies against triple-negative breast cancer (TNBC) – an aggressive subtype of breast cancer – remain elusive. Chemotherapy is the standard therapeutic approach, but it is often associated with tremendous collateral damage to normal tissues. An actively targeted aptamer-conjugated drug complex can efficiently kill malignant cells and spare healthy cells. However, an aptamer-conjugated drug complex without a nanoparticle system has a limited payload capacity and is not fit for sustained-release therapy. The present research work overcomes these limitations and aims to develop aptamer-conjugated porous silicon nanoparticles (pSiNPs) that carry chemotherapeutic drugs to deliver to TNBC.

A highly stable colloidal suspension of pSiNP-aptamer was created by employing a salt-aging technique during the attachment of aptamer on the functionalized pSiNPs. The study demonstrates an efficient loading of doxorubicin (Dox) as a model chemotherapeutic drug (179 ± 5 μg/mg of pSiNPs). In vitro studies showed a feasible targeting approach towards the MCF10Ca1h TNBC cell line. Next, an intracellular trafficking investigation confirmed the lysosomal-targeted delivery system to treat TNBC.

The targeting properties enabled the pSiNP-aptamer conjugate to selectively deliver Dox and kill TNBC cells while sparing normal cells. These findings laid the solid foundation for further investigating the pSiNP-aptamer conjugate's potential as a targeted treatment strategy in preclinical TNBC models.

In Vitro Evaluation of a Novel Antibody Fragment-Drug Conjugate Targeting Paediatric Philadelphia-Like Acute Lymphoblastic Leukaemia

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Paediatric Philadelphia-like acute lymphoblastic leukaemia (Ph-like ALL) cases that overexpress cytokine receptor-like factor 2 (CRLF2) exhibit poor clinical outcomes associated with chemotherapeutic drug resistance[1]. Despite recent advances in the development of targeted therapeutics, Ph-like ALL patients treated with currently available antibody-drug conjugates may undergo relapse due to the possibility of antigen loss[2]. Since CRLF2 signalling is vital for the survival of leukaemia blasts, we hypothesis that it provides an ideal target for drug delivery with less probability of relapse associated with antigen loss. Therefore, and for the first time, we have developed an antibody fragment-drug conjugate targeting CRLF2 (CRLF2-FDC). The selective cytotoxicity of CRLF2-FDC for targeting Ph-like ALL cells was confirmed *in vitro* using CRLF2- knockdown cells representing isogenic controls. Two wildtype (WT) Ph-like ALL cell lines, MHH-CALL-4 and MUTZ-5, were lentivirally transduced to enable the inducible knockdown of CRLF2 (KD-CALL-4 and KD-MUTZ-5, respectively), in which CRLF2 expression was determined using flow cytometry. The transduced KD-CALL-4 and KD-MUTZ-5 cells showed significant reduction in CRLF2 expression compared to their WT counterparts (90% and 44% respectively, $P<0.0001$). CRLF2-FDC elicited significantly higher selective cytotoxicity towards the $CRLF2_{positive}$ population compared to the $CRLF2_{negative}$ population in KD-CALL-4 (30% difference in viability, $P \leq 0.05$) and KD-MUTZ-5 (70% difference in viability, $P \leq 0.001$) cells. We then assessed the internalisation of CRLF2-FDC using confocal microscopy. Orthogonally viewed images captured from different planes revealed the selective intracellular uptake of CRLF2-FDC in MHH-CALL4 compared to KD-CALL-4 cells, confirming successful intracellular drug delivery and highlighting the promising therapeutic efficacy. The future direction of this work is to evaluate the preclinical therapeutic and targeting efficacy of the CRLF2-FDC *in vivo* using an orthotopic patient-derived xenograft mouse model.

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Controlled Spatial Differentiation of Mesenchymal Stem Cells in Gradient Microgel Suspensions

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During tissue development, progenitor stem cells form functional tissue with high cellular diversity and intricate micro and macro architecture. Current approaches have attempted to replicate this process with materials cues or through spontaneous cell self-organization. However, it has become increasingly clear that cell-directed and material-directed organization are both required to achieve native structure and function.[1] Here, I will present a biomanufacturing method to deposit stem cells in freeform within a granular matrix while spatially guiding their differentiation. The directed combination of varied microgels establish a 3D bioprinting support medium with gradients of mechanical stiffness and physical size. The packed suspension of gelatin methacrylate microgels crosslink together under near UV light, locking the cells and gradients in place. Microgel matrices composed of small particles were found to enhance adipogenic differentiation, while larger particles fostered increased cell spreading and osteogenic differentiation. After 21 days of culture in osteogenic media, significant mineralization of calcium phosphate was found within the individual microgels, leading to an order of magnitude in stiffness change. Attenuating the cytoskeleton of adipose derived stem cell (ADSCs) with small molecule drugs suggest initial morphometrics guided by particle size direct lineage choice. Controlled 3D printing of ADSCs across size and stiffness gradients enables guided differentiation to osteogenic, adipogenic, or chondrogenic phenotypes. And variations in the printed cell architectures nudged cells down either adipogenic or osteogenic lineages. We anticipate this material platform will open new avenues for regenerative medicine and fundamental studies on the role matrix cues play in stem cell differentiation.

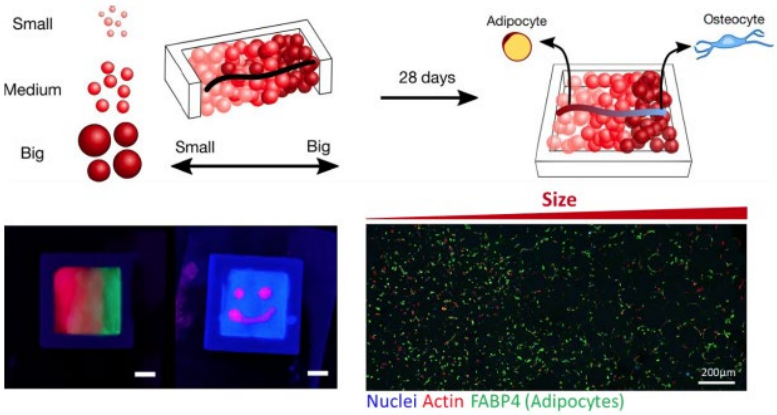

Figure 1: Schematic representation of the formation of cell-laden gradient suspensions (top). Optical images of gradient microgel suspensions (Bottom left). Confocal image of spatially differentiated ADSCs into adipocytes after 21 days (Bottom right).

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PEG functionalised chitosan based targeted nontherapeutic system for bone metastasized breast cancer

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Chitosan (CHI)-based materials have a long history as drug delivery systems due to their biodegradability, high drug carrying ability, and multi-functionality.[1] In the present project, we are working on the modification of CHI with PEG and alendronate-PEG (ALd-PEG) to be used for the encapsulation of curcumin (Cur) and siRNA, thus developing Cur/siRNA encapsulated particle systems. Medical grade CHI was modified using PEG and ALd-PEG at different molar ratio of PEG and CHI by carbodiimide coupling. ^{1}H NMR verified the coupling of mPEG-COOH and Ald-PEG-COOH to CHI with a DS of 2-20 and 8-15 %, respectively. The solubility of CHI, mPEG-CHI and Ald-PEG-CHI was evaluated at a range of pH (4-9), and it was found that modified chitosan polymers had high solubility especially above pH 6 compared to chitosan. Nanoparticles were prepared by an ionic gelation method.[2] The particle diameter was measured by dynamic light scattering (DLS) and further confirmed by transmission electron microscopy (TEM). The size of the siRNA loaded particles was controlled to be < 100 nm and the zeta potential was between -10 to 10 mV. Nanoparticle stability was evaluated in 50 % serum, and it was found that modified chitosan particles were more stable in terms of size and zeta potential as compared to chitosan particles. Furthermore, the therapeutic effect of the encapsulated nanoparticle systems in breast cancer cells was confirmed by western blot and other biological assays. The results confirmed the potential of these modified targeted chitosan particle systems in breast cancer anticancer applications with a combinatorial approach using anticancer (using CUR) and gene silencing effects.

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Selective radiofrequency ablation of cancer cells by Folic acid decorated gold nanoclusters

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One of the stimuli that has been used for triggering drug release 'on-demand is radiofrequency (RF) mediated heating. Gold nanomaterials have been employed as hyperthermia agents because they are energy-transducing materials that can be heated under RF. Because of their unique physical and chemical properties, gold nanoclusters (AuNCs) have attracted much attention from researchers as a novel class of fluorophores. These characteristics include easy synthesis, ultra-small size, exceptional fluorescent properties, excellent optical/colloidal stability, and biocompatibility. The AuNCs have been utilised as RF susceptible agents that can generate heat. In this study, we have synthesised a drug delivery system that releases drug under RF irradiation. Inspired by the ability of DNA to hybridise with the complementary strands and melt at a specific temperature, we fabricated AuNCs coated with single-stranded DNA able to hybridise with its complementary strand functionalised with folic acid (FA). We demonstrate that the resulting targeted DNA-coated AuNCs (AuNC@dsDNA-FA) can encapsulate doxorubicin (Dox) given its DNA intercalation ability and release the drug ondemand upon RF exposure. Confocal microscopy images showed that AuNC@dsDNA-FA were internalised into the MCF-7 cells at a higher level when compared to non-targeted AuNCs. More specifically, FA-modified AuNCs are mainly internalised by the cell via clathrinmediated endocytosis. When incubated with cells, Dox-loaded AuNC@dsDNA-FA had the lowest cell viability in comparison with any other groups when exposed to RF irradiation, confirming the triggered release of Dox. This theranostic nanoplatform provides an innovative strategy for the treatment of cancer.

Dynamic Protein Corona of Gold Nanoparticles with An Evolving Morphology

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Understanding the interactions between nanoparticles and blood proteins has become essential for the advancement of nanomedicine. The protein corona is a ubiquitous biophysical phenomenon associated with nanoparticles in biological environments, yet little is known about its formation on nanoparticles which undergo spontaneous surface-energy minimization, a common phenomenon occurring in the synthesis and during the shelf life of nanomaterials. Here we have employed gold nanoparticles (AuNPs) possessing evolving shapes of spiky, mid-spiky and spherical shapes and determined their acquisition of human plasma protein coronae with label-free mass spectroscopy.[1] Different AuNPs collected coronal proteins differing in abundance, physicochemical parameters, and interactive biological network. The biophysical characteristics of the coronal proteins matched the morphology of the AuNPs, where small globular and large fibrillar proteins were abundant on spiky AuNPs, while large proteins were enriched on spherical AuNPs. The AuNPs were able to induce endothelial leakiness at different degree and the protein corona formation partially hindered the leakiness created by the AuNPs as confirmed by confocal fluorescence microscopy, *in vitro* and *ex vivo* transwell assays, and signaling pathway assays. This study revealed the dynamic protein corona formation of gold nanoparticles possessing an evolving morphology and demonstrated their implications in harnessing the paracellular pathway for nanomedicine delivery.

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Preparation and characterization of aluminum hydroxide nanoparticles for nanomedicine applications

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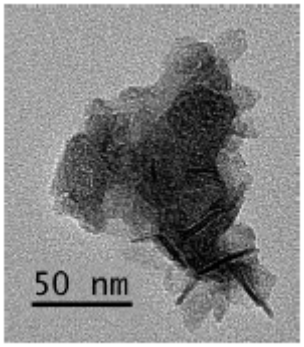
Aluminum (Al) salts are used as adjuvants in numerous human and veterinary prophylactic vaccines because of their well-tolerated profile compared to other adjuvants. Due to their clinical approval and generally regarded as safe status, they offer potential to be applied in other areas of medicine, including nanomedicine. The clinically used adjuvants agglomerate into microparticles of heterogeneous sizes. Recently however, literature indicates that moving from micron dimensions towards the nanoscale can modify the adjuvanticity of Al, no longer leading to induction of type 2 responses used in vaccination [1-3]. In this context we are interested to establish a stable, well characterized Al-based nanoparticle which can be applied to nanomedicine applications.

Here, we present preparation and characterization of a range of $Al(OH)_3$- NPs with three different sizes (10, 50 and 100 nm), and suspended in distilled water. Characterization of the nanoparticles is presented for hydrodynamic size and Zeta potential. Morphology and elemental composition of the nano-formula are evaluated by transmission electron microscope and energy dispersive X-ray spectroscopy, respectively. Next, the nano-formula is coated with either (dicarboxylic) polyethylene glycol or poly acrylic acid (co-maleic acid) to prevent aggregation and shield the positive surface charge. Finally, targeting moieties can be linked to provide targeting ability towards specific cell types.



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Development of Bispecific Antibody Deliveries Against Intracellular Targets

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Approved therapeutic antibodies currently only target cell surface molecules or extracellular proteins. Despite high antigen specificity and affinity, the use of antibodies in targeting intracellular proteins is challenging. This is because of their inability to cross the plasma membrane and their susceptibility to proteolytic degradation by endolysosomes. Development of strategies that enable antibodies to internalise into cells and to escape from endosomes would substantially expand the number of therapeutic targets.

Here, we used bispecific antibodies (bsAbs) in a tandem single-chain Fv (scFv) format conjugated to endosomolytic peptides as a protein-based drug delivery system. These bsAbs are a combination of a cell receptor binding scFv and an intracellular target binding scFv. The former scFv targets epidermal growth factor receptors (EGFR) that enable cytosolic delivery via receptor-mediated endocytosis. Once inside the cell, the therapeutic scFv interacts with its antigen, the SOX18 transcription factor to block subsequent gene activation and cell proliferation. The combination of therapeutic and cell surface targeting moieties not only ensures selectivity and cellular uptake.

The use of endosomolytic peptides to enhance intracellular translocation and endosomal escape efficiency of bsAbs were evaluated via luciferase-based reporter assay. In addition, the measurement of binding affinity and kinetic analysis of antibody-antigen interactions against EGFR and SOX18 proteins were assessed. Flow cytometry and confocal laser scanning microscopy analysis were also employed to ensure that the antibodies can bind to and internalise into cancer cells via EGFR. Our findings may lead to an early prototype of an effective cytosolic delivery and release system.

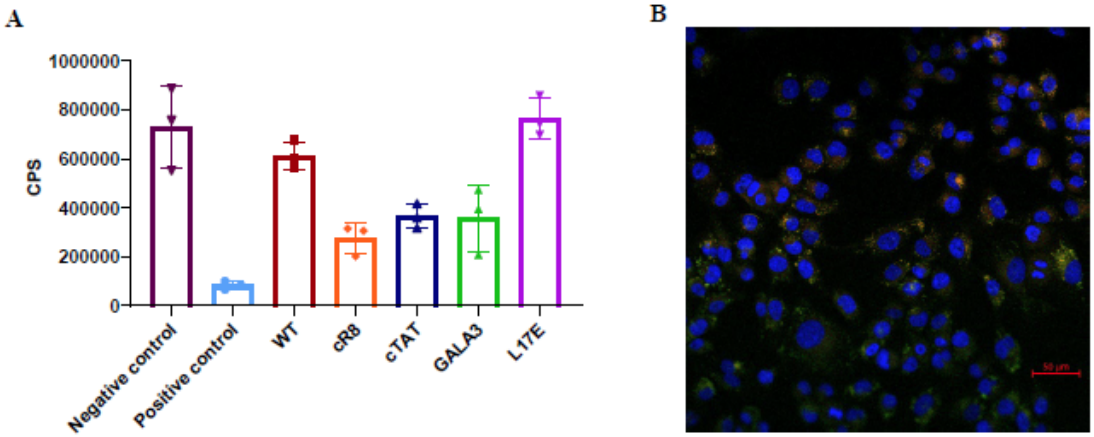

Figure 1 (panel A and B): Panel A. Luciferase reporter assays of a bispecific antibody (WT) and its derivatives genetically attached to endosomolytic peptides – cR8, cTAT, GALA3, and L17E. Bar graph to indicate the Vcam1 (SOX18 target) promoter activity. A negative control was measured from promoter activity of Vcam1 promoter fused to the firefly luciferase reporter gene without bsAb treatments. Promoter activity of coexpressing of Vcam1 promoter and aSOX18 scFv were also measured as negative controls. Luminescence of each bsAb treatment were measured, showing that bsAbs conjugated to endosomolytic peptides are capable to inhibit SOX18 endogenous function. Panel B. Exemplary image of bispecific antibody fused to GALA3 internalisation by confocal microscopy in MDA-MB-231 cells. Nuclei were stained with DAPI (blue); GALA3 was stained with a FITC labelled anti-myc antibody (green); endosomal and plasma membranes were stained with Nile Red (red). Green signals in merged image represented successful delivery of bsAb to the cytoplasm. (Scale bar equaled 50 µm.)

Piperlongumine-loaded human serum albumin nanoparticle: synthesis, characterization and biological evaluation

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Colorectal cancer (CRC) was among the top three diagnosed cancers and ranked second in terms of cancer mortality according to GLOBOCAN 2020 report, by International Agency for Research on Cancer (ICAR). There is a pressing need of the new therapeutic molecules which can help in managing CRC effectively. Piperlongumine (PL) is active plant alkaloid with the range of biological activities including anti-inflammatory, antibacterial, antifungal, antidiabetic, antiatherosclerotic, and anticancer activity. Poor water solubility limit its biological application, nanotechnology based approach can help improving the water solubility. In present study we have formulated PL-loaded human serum albumin nanoparticles (PLNP) by the desolvation method. Characterization of formed particles exhibited the particle size of 116 ±8 nm with the zeta potential in the range of –34.7 ± 2.0 mV. The TEM images of the PLNP confirmed the production of spherical nanoparticles. The produced nano particles were characterized using FTIR, DSC, and powder XRD analytical techniques. When compared to the pure drug, the developed PLNP increased the potential of PL against human colorectal cancer HCT 116 cells. The effectiveness of the prepared PLNP was confirmed by induction of apoptosis via AO/EtBr and Hoechst 33342 assays, colony formation and study of mRNA expression in HCT 116 cells. Cellular uptake studies indicated that HSA nano particles can carry hydrophobic molecules inside the cells. Hence, the results of the study demonstrated that PLNP could be a viable carrier for the delivery of PL, thus providing a therapeutic option.

Keywords: Piperlongumine, HSA nanoparticle, colon cancer, drug delivery.

Magnetic Enrichment of Immuno-Specific Extracellular Vesicles for Mass Spectrometry Using Biofilm-Derived Iron Oxide Nanowires

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Extracellular Vesicles (EVs) naturally released from cells offer non-invasive insights into various physiological and pathological processes, and also offer promise towards establishing novel non-invasive diagnostic methods. Immuno-magnetic separation based on EV membrane protein expression is the gold standard for enriching specific EV subtypes. Herein, we report on a novel EV immuno-enrichment method using bacterial biofilm-derived iron oxide magnetic nanowires (NWs) (Figure 1A). These NWs were modified with poly(acrylamide)-based diblock polymers (*p*AAm) and conjugated with EV membrane marker-specific placental alkaline phosphatase (PLAP) antibodies to efficiently enrich PLAP+ve EVs specific for placental EVs (Figure 1B). The enrichment performance of the NW-PLAP was comparable with that of commercially available Dynabeads™, both showing high recovery of PLAP proteins enriched from a cell line model (83.7 ± 8.9% and 83.2 ± 5.9%, respectively, Figure 1C), high particle-to-protein ratios (7.5 ± 0.7 × 10_9 and 7.1 ± 1.2 × 10_9, respectively), and low non-specific binding of PLAP-ve EVs (7 ± 3.2% and 5.4 ± 2.2%, respectively). Subsequent mass spectrometry-based proteome profiling of EVs enriched by the NWs and Dynabeads identified respectively 2518 and 2545 protein groups with excellent reproducibility (Pearson correlation 0.986 and 0.988, respectively). The proposed enrichment method of immuno-specific EVs using naturally produced iron oxide magnetic NWs provides a low-cost, highly scalable yet efficient, high-throughput alternative approach for downstream proteomic studies.

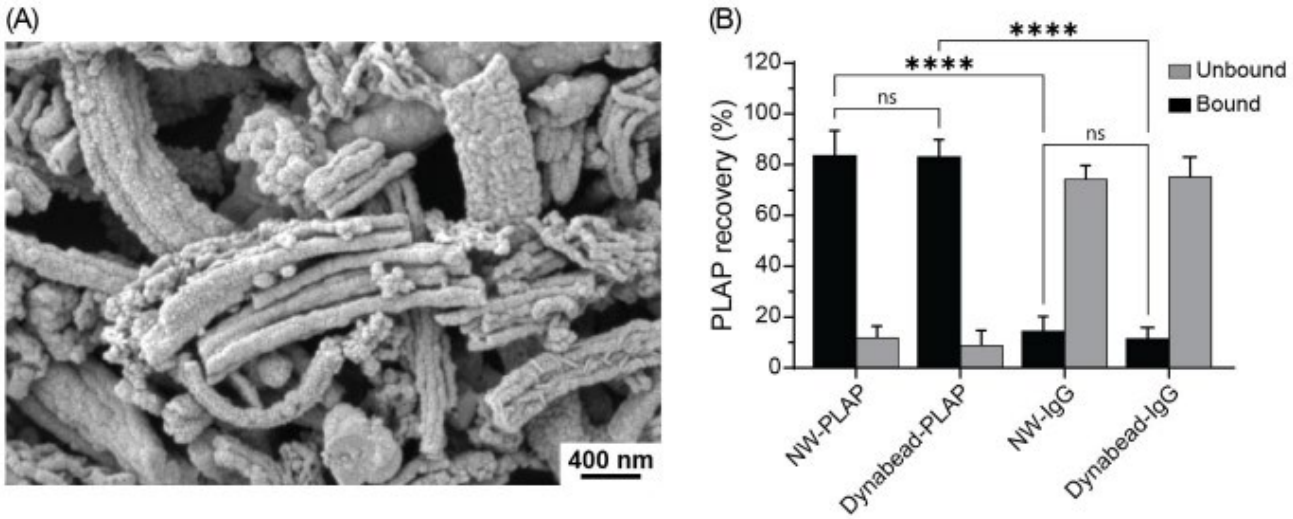

Figure 1: A) Scanning electron microscopy image of NWs. B) Recovery efficiency of PLAP protein extracted from EVs captured by NWs and Dynabeads conjugated with either anti-PLAP or an irrelevant IgG (bound) and in the corresponding supernatants (unbound). Data are presented as means + SD (n = 3, one-way ANOVA with Tukey's post-test, ns = not significant and ****p < 0.0001).

Cytoskeleton remodeling during stem cells adaptation to substrate stiffness and volume changing stresses

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Cytoskeleton regulates stem cells adaptation and responses to local mechanical environment, underpinning the emergence of structure-function relationship during tissue development and regeneration[1,2]. Understanding how cytoskeleton adaptation at nanoscale influences the global macroscale tissue function is key to deciphering mechanism of structure-function emergence and to improve engineering of tissues, materials and/or devices for regenerative medicine. We modulated the microtubule of C3H/10T1/2 murine embryonic stem cells using Paclitaxel (PAX), a microtubule stabilizing agent, and examined their structure and function adaptation and mechanical properties. Simultaneously we modulated stem cells local mechanical environment through introducing high seeding density, previously shown to induce local compression[3], and compliant substrates. Stabilization of microtubule with PAX preserves cell viability, however, reduces their proliferation. Adaptation to perturbed microtubule function involve changes in cell and nuclei shape and increase in cell volume in time and dose dependent manner. Quantitative microscopy reveals thick, bundled microtubules and highly aligned, thicker and longer actin stress fibers concomitant with increase in cells young's moduli; indicative of adaptive actin ordering as a means for cells to redistribute forces as microtubules are unable depolymerize. When seeded on substrates of 10 and 100 kPa, actin alignment reduces despite the cells young's moduli nearly match those of the substrates and increase with PAX treatment. Given microtubule serves as compression resisting filament, compression introduced via seeding at high density (HD) compensate for the PAX-mediated cell volume increase while exhibiting higher bulk modulus than those of low density (LD). Cells proliferated to HD seeded on 10 and 100 kPa substrate exhibit bulk modulus larger than those of LD and match closer to that of the substrate, although smaller difference is found between control and PAX-treated cells (than on glass), indicating that HD overrides PAX effect during adaptation to compliant substrates. We conclude that upon stabilization of microtubule, enhanced actin polymerization and alignment mediate stem cells mechanoadaptation to maintain adhesion and force balance depending on the substrate compliance and compression from high seeding density. Future correlation of cytoskeletal spatiotemporal responses with cell fate may provide insights on development of nanoengineered materials and drug delivery tools for directed cytoskeleton remodeling and targeted tissue neogenesis.

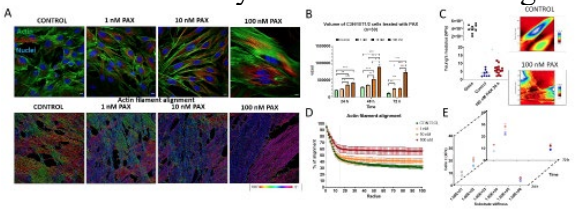

Figure 1: Stem cell mechanoadaptation during perturbation of microtubules. *A,D)* PAX Dose-dependent actin alignment facilitates B) cell volume and C) young's moduli increase, can be modulated with E) substate stiffness

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Optimisation of Mesoporous Shell on Iron oxide Nanoparticles for Theranostics application

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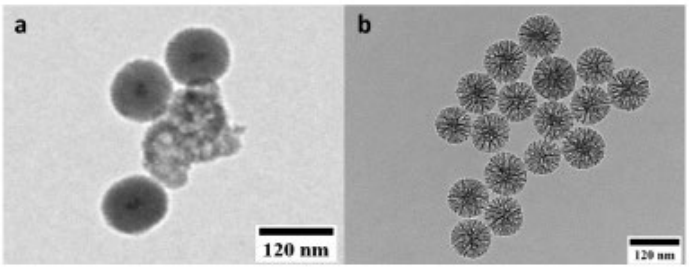
Theranostics is a rapidly developing field of research that integrates both therapeutics and diagnostics for accurate and targeted treatments. In this study, mesoporous silica coated iron oxide nanoparticles (IONPs) were fabricated for multi-modal imaging with magnetic resonance imaging (MRI) and photoacoustic (PA) modalities, whilst simultaneously enabling the loading and release of a therapeutic payload. The biocompatible mesoporous shell is made from inorganic mesoporous silica due to its unique properties such as large surface area, low density, surface functionalisation, high drug loading capacity, tuneable morphology, size, and incorporation of a multifunctional core material.

Iron Oxide nanoparticles were selected as the core and synthesized via a co-precipitation method[1]. Briefly, Iron (II) and Iron (III) chloride solutions were combined in basic *pH* solution, centrifuged, washed, and dried to obtained IONPs. Next, to prepare the mesoporous silica shell, two different sol-gel synthesis methods (A^2 & P^3) were utilised yielding nanoparticles with different sizes and dispersities. Furthermore, these synthesised nanoparticles were characterized using dynamic light scattering (DLS) and transmission electron microscopy (TEM) techniques.

The size of synthesised IONPs were found to be in the 10-20 nm range (Figure 1c). The mesoporous silica coated IONPs were synthesised successfully and were found to be in the range of 100-110 nm for Method A (Figure 1a, 1c) and 110-210 nm for Method P (Table in Figure 1c), characterised using TEM. Furthermore, these fabricated nanoparticles were responsive towards a magnetic field. In this study, mesoporous silica-coated IONPs were systematically synthesised with controlled size and retained their magnetic properties after mesoporous shell coating for MRI and PA imaging. This research provides a strong platform for theranostics applications through a modular approach to the mesoporous silica shell architecture, allowing the particles to act as a vehicle for therapeutic payloads.



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Sample Name	TEM size range (nm)	DLS size range (nm)	PDI
IONP	10-20	-	-
Method A	100-110	80-180	0.348
Method P	90-110	110-210	0.332

Figure 1: TEM images of Core shell nanoparticles using a) Method A, b) Method P and c) a table summarising the TEM and DLS particle sizes of the core shell NP's prepared using the two methods

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Porous Silicon- Gold Hybrid as Peroxidase Enzyme Mimetics

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Simple nanostructured materials (e.g. gold nanoparticles; AuNPs) that exhibit enzyme mimicking catalytic activity are termed as nanozymes. Nanozymes have attracted pervasive interest in recent decades as alternatives to naturally occurring enzymes attributable to some advantages including low cost and easy mass production, controllable and tuneable catalytic activity, straightforward functionalization, high stability, and robustness even in harsh environments [1-4]. Porous silicon (pSi) with features such as large specific surface area and good biocompatibility [1], and the capability of gold nanomaterials to mimic enzyme activity open new opportunities for biomedical diagnostics and treatment. Herein, a nanohybrid of pSi and AuNPs is generated through spontaneous reduction of gold by pSi and demonstrated as peroxidase nanozyme activity. Firstly, porous silicon was synthesized using the electrochemical etching method in which the porous layer was removed from silicon substrate using lift-off method and converted into microparticles by sonication. After that gold chloride solution ($AuCl_3$) was mixed with pSi to form pSi-Au. To demonstrate the peroxidase-like activity of pSi-Au, a set of optimization experiments were conducted on 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of hydrogen peroxide (H_2O_2). The nanozyme activity of pSi-Au nanohybrid was tested under various pH and temperature conditions. The effects of TMB, H_2O_2, and ratio of pSi:Au on the nanozyme activity were investigated. The surface morphologies of pSi and pSi-Au were examined by TEM as shown in **Fig. 1a** and **1b**, respectively. As shown in Fig. 1a, pSi with vertically running pores can be clearly seen, whereas dark sold gold nanoparticles were formed after mixing of pSi with Au salt solution confirming spontaneous reduction of Au by pSi nanoparticles. Peroxidase-mimicking activity of pSi-Au was investigated through catalytic oxidation of TMB in the presence of H_2O_2 by monitoring the colors as illustrated in **Fig. 1c**, as well as by taking the UV-visible (UV-vis) readings. **Fig. 1d** shows the TMB oxidation reaction kinetics for pSi-Au nanohybrid under different TMB concentrations, while **Fig. 1e** shows a graph with linear relation between the concentration of TMB and nanozyme activity (extracted from **Fig. 1d** at 30 min time point).

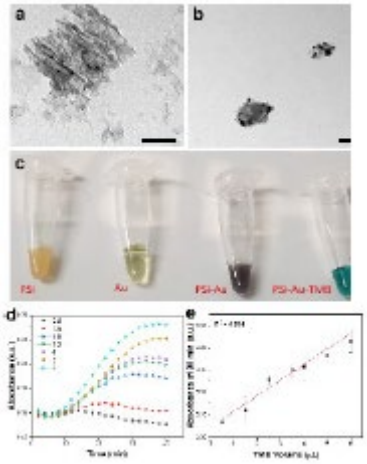

Figure 1: The TEM images: a) pSi and b) pSi-Au. c) TMB oxidation reaction shown in stepwise manner using pSi-Au. pSi (yellow), Au solution (light yellow), pSi-Au (purple), and pSi-Au-TMB (blue). d) TMB oxidation reaction carried out with different volumes of TMB (10 mM stock solution) measured at 40 °C and a pH of 3.5 for 40 minutes at 652 nm using a UV-Vis plate reader. e) Absorbance vs TMB volume trace extracted by plotting Abs values at 30-minute time point from figure 2d.

Effect of Silica Nanoparticles Morphology on Cellular Uptake

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Purpose of research: Silica nanoparticles have attracted substantial attention in targeted drug delivery[1]. Their popularity is fueled by the proven cyto-compatibility, large surface area, ease of surface modification and ability to load a range of payloads[2]. In this study, we systematically investigate effect of silica nanoparticles morphology on cellular uptake using four types of silica nanoparticles with different porosity.

Methods: Silica nanoparticles (solid and porous) were synthesized using modified Stöber method. The particle sizes and morphology are determined by varying the conditions such as reactant concentrations, surfactant, catalysts, and temperature. Briefly, solid, MCM41, MCM48 and Dendritic silica nanoparticles were prepared and characterized using DLS, FTIR and TEM. The cytotoxicity was studied using MTT assay and trypan blue assay on HeLa (cervical adenocarcinoma cells), MCF-7(breast cancer) and RAW.

Results: The synthesized silica nanoparticles were subjected to TEM and DLS analysis for particle size and morphology determination. The particles size was found in the range of 200-250 nm with different shapes as shown in **Figure 1**. The cell viability assay revealed that these particles were non-cytotoxic towards HeLa (Fig.2), MCF-7 and RAW (macrophages).

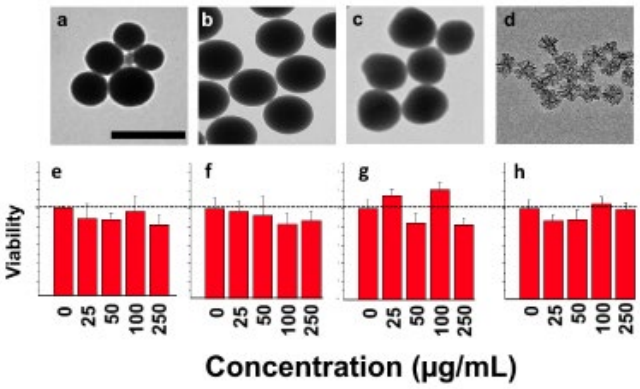

Figure 1: TEM images of various silica particles (a) Solid, (b) MCM-48, (c) MCM -41, and (d) Dendritic . Scale bar = 500 nm. Cell viability of HeLa cells upon exposure to different concentrations (0-250 µg/mL) of various silica nanoparticles (e) Solid, (f) MCM-48, (g) MCM -41, and (h) Dendritic. The black dotted line represents 100 % viability level.

Conclusion: The fabricated nanoparticles with different shape, and sizes shape. These silica particles are found to be biocompatible and non-toxic toward cells. The effect of morphology on cellular uptake is under investigation using the trypan blue assay and AnnexinV-PI staining. The pre-incubation with these particles is expected to increase the drug uptake by the cells via creation of transition pores or activation of biomolecular mechanism.

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Boronic acid-Antibody-Functionalized Analyte Capture Surface

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Obtaining an enriched, phenotypically-pure cell sub-population from heterogeneous cell mixtures like blood is important for diagnostics and biosensing. Existing techniques such as fluorescent activated cell sorting (FACS) and magnetic-activated cell sorting (MACS) require pre-incubation with antibodies (Ab) and time-consuming preparations. Cell affinity chromatography (CAC) removes the need for pre-incubation and shows high resolution and specificity of separation. The majority of the available "Antibody-Mediated Analyte Capture" techniques require a modification on Abs (e.g. Oxidation or Biotinylation) to make them attachable to a surface's cross-linker[1]. In this work, no antibody modification is needed because we take advantage of the carbohydrate chain in the Fc region. We have used Phenylboronic acid (PBA) as a crosslinker to bind the Ab to a modified Polystyrene surface. Phenylboronic acid binding was used because it allows functional orientation and cleavable binding of the immobilized Ab. Immobilized Abs' efficiency to detect and capture a biomolecule was examined by capturing a human lymphocyte cell type (e.g. Raji cell) through the targeted cell surface antigen. Specificity of cell capturing was tested with another human lymphocyte cell line which does not express the targeted antigen on their surface.

We are currently focused on quantifying non-specific protein binding and developing a process for cleaving the Ab and its captured cell from the surface. Together, the goal of this work is to deliver a cleavable Ab binding surface with low non-specific binding of biomolecules to provide an enriched population of a specific cell type or analytes that can be detached for further research and characterization.

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Silibinin encapsulated bovine serum albumin nanoparticles as a drug delivery system for breast cancer treatment

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The majority of phytochemical drugs are less effective due to pharmacological drawbacks such as poor aqueous solubility, low bioavailability, low absorption, drug instability, and rapid excretion, and so forth. Hence, a promising drug delivery system is required to overcome these limitations. Silibinin (SBN), a natural substance extracted from the seeds of the milk thistle, has an anticancer efficacy against many cancers, such as breast, liver, lung, and skin. So in this study, Bovine Serum Albumin (BSA) has been used as a nano carrier and Silibinin (SBN) was encapsulated within it forming SBN loaded BSA nanoparticles (SBN-BSA NPs). Further, the therapeutic efficiency of SBN-BSA NPs as a targeted drug delivery system for breast cancer treatment was evaluated. SBN-BSA NPs were synthesized by the desolvation method and physiological characterization was performed using the DLS, FESEM, TEM, FTIR, XRD, DSC, and TGA. DLS and FESEM results exhibited that particles were smooth, spherical in shape and size was within the range suitable for efficient drug delivery. The FTIR and XRD analysis corroborated the successful encapsulation of SBN within BSA NPs. Furthermore, DSC and TGA analysis revealed the amorphous nature of synthesized nanoparticles. Interestingly, controlled and prolonged drug release was observed in the medium having physiological pH. To evaluate *in vitro* anti-cancer activity of SBN-BSA NPs, a MTT assay was subsequently performed using the MDAMB-231 and MCF-7 breast cancer cell lines. *In vitro* studies exhibited an enhanced anti-cancer efficacy of SBN when delivered to cells as SBN-BSA nanoparticles. Additionally, the AO-EtBr staining assay, colony formation assay, and *in vitro* cellular uptake study conferred higher internalization and cytotoxic effects of SBN-BSA NPs in breast cancer cells compared to pure SBN. All together the physicochemical characterization and *in vitro* validation directed that the therapeutic efficacy of SBN was enhanced when delivered via BSA nanoparticles. Hence, validating SBN-BSA-NPs as an innovative avenue for availing benefits of natural anti-cancer compound-SBN with enhanced bioavailability as a propitious breast cancer treatment.

Keywords: BSA Nanoparticles, Silibinin, Breast Cancer, SBN-BSA NPs

High-throughput *in vitro* analysis of engineered tumour microenvironments using 3D bioprinting

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Traditional 2D flat cell cultures of cancer cells and *in vivo* animal experiments remain the most commonly used techniques as *in vitro and in vivo* platforms for cancer research, drug screening and toxicity tests prior to entering human clinical trials. This is due to their low cost, efficient workflows and optimised downstream analysis techniques. Evidence suggests that these traditional cell culture techniques do not accurately replicate the complexity of human tumours. For example, these models have limitations in mimicking tumour stromal heterogeneity and tumour cell-extracellular matrix (ECM) interactions, thus potentially limiting the ability to mimic *in vivo* tumours realistically. This has led to the development of three-dimensional (3D) *in vitro* models which have been shown to reflect the cellular responses *in vitro*.

Synthetic gels have been used extensively over naturally derived biomaterials, such as collagen without the complexity of cell-matrix interactions, as they offer opportunities to control and modulate cells. Herein, we developed an electrostatically crosslinked PEG-based gels system for creating high-throughput 3D *in vitro* models using a 3D drop-on-demand bioprinter to mimic the extracellular matrix cancer environment. First, the 3-arm PEG-based polymer backbones were conjugated with various degrees of cell adhesive RGD motifs (0%, 25%, 75% and 98%) to study the influences of cell adhesive motifs on breast cancer (MCF-7) spheroid formation. Formation, stability and mechanical properties of gels were tested with and without RGD motifs in different conditions to evaluate cellular response to materials parameters in 3D environment. Biocompatibility was tested with MCF-7 breast cancer cells by encapsulating cells within a gel for 7 days, where high cell viability of approximately 99.0 ± 1.4% was observed. Notably, electrostatically crosslinked gels can be degraded in the presence of salts at room temperature by breaking the interaction of oppositely charged polymer chains. As such, both MCF-7 cells and spheroids were released by simply exposing a gel in a 2 M NaCl solution for 5 min. The released MCF-7 breast cancer cells and spheroids remained highly viable with no significant differences before and after release. Finally, the released MCF-7 cells/spheroids were analysed with flow cytometry to characterise cellular responses and behaviours in more detail.

In summary, this PEG-based tuneable gel system shows great promise as a 3D ECM mimic of cancer microenvironments with controllable biophysical and biochemical properties. The ease of gelation and dissolution through salt concentration provides an innovate means to engineering tumour microenvironments, where the cells can be quickly harvested for analysis. The speed in which cells and aggregates can be reclaimed provides scope for integrated multimodal analysis, where 3D immunofluorescence imaging of the gel can be supplemented by parallel molecular analysis at the protein and transcript level after dissolution and cell collection.

Magnetic Particle Imaging using Trastuzumab Targeted Nanoparticles for Cancer Imaging

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Magnetic Particle Imaging (MPI) offers a new approach to rapid and sensitive molecular imaging.[1] The modalities ability to directly detect Super Paramagnetic Iron Oxide Nanoparticles (SPION) with exceptional contrast and sensitivity outcompetes that of clinically used systems such as MRI and PET. Further, the capability of MPI for absolute tracer quantification and its lack of depth attenuation affirms its applications across a variety of disciplines, with an increasing focus on cancer imaging. However, current cancer imaging applications are hindered by the SPION's limited tumour accumulation and rapid sequestration by the hepatic system, causing sparse success. To offset this issue, antibody targeted SPION are a more powerful approach leading to active pursuit and specificity to the tumour. Such systems have shown immense success in imaging of cancer in different preclinical settings.[2,3] In this project we describe the modification of a 25 nm SPION with Trastuzumab, a monoclonal antibody specific to the human epidermal growth factor receptor 2 (HER2), over expressed in a variety of cancers. We describe the suitability of the novel alpha-HER2 conjugated SPION for MPI with a detection limit of a single microgram of iron and no impact to the antibodies binding functionality *in vitro*. We also demonstrate its superior performance and blood circulation time in comparison to a non-targeted SPION for *in vivo* imaging. The results highlight the strengths of antibody targeted tracers for enhanced cancer imaging using MPI, putting it forward as a favourable preclinical system. The study presents the first application of an antibody targeted SPION for cancer imaging using the newly installed MPI with CT scanner at Monash's preclinical imaging facility.

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Polymer-metal oxide nanoformulation to absorb hydrogen sulfide and destabilise sulfide-producing bacteria for improved bowel health

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Globally, colorectal cancer (CRC) is the third most common cancer and second deadliest[1]. Increased levels of hydrogen sulfide (H2S) in the bowel have been linked to disorders such as irritable bowel syndrome and diseases such as CRC[2]. H_2S in the bowel is mainly produced endogenously through enzymatic pathways and also through bacteria. Fusobacterium is a sulfur-producing gut bacteria that has been linked to CRC due to its ability to induce CRC progression, metastasis and chemoresistance[3]. This poster will present the synthesis of a polymer-metal oxide nanoformulation to absorb hydrogen sulfide and destabilise sulfideproducing bacteria for improved bowel health. Porous polymethyl methacrylate (PMMA)- based microparticles (~100 µm diameter with pore sizes of ~20 µm) were synthesized using using W/O/W double emulsion technique[4-6] (Figure 1) and encapsulated copper oxide (CuO) nanoparticles, with the latter functions to absorb H_2S (Equation 1). The polymeric microparticle is designed to be sufficiently large enough to pass through the colon and prevent CuO nanoparticle organ absorption and toxicity and is also large enough to be captured by wastewater treatment plants. Using the methylene blue assay, we confirmed that clinically relevant amounts of H_2S can be absorbed by 50 nm CuO nanoparticles (Figure 2). These particles may prove useful for CRC prevention and treatment of excess H2S in the gut.

Equation 1: $H_2S + CuO \rightarrow CuS + H_2O$ (1)

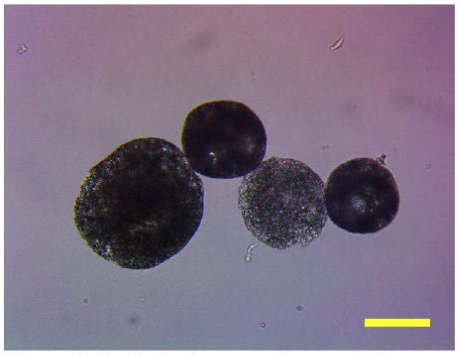

Figure 1: Optical microscope image of gigaporous microparticles, scale bar is 70 µm.

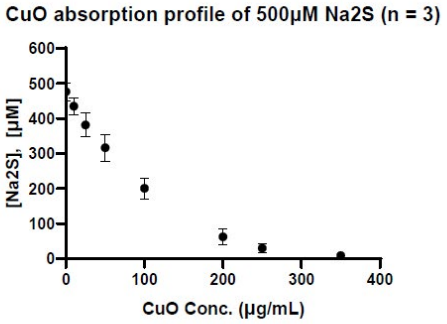

Figure 2: CuO absorption profile of 500uM Na_2S (H_2S donor) in aqueous solution.

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3D Bioprinted Silk Fibroin-Based Hybrid Hydrogels for Cardiovascular Applications

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Cardiovascular disease (CVD), including heart attack and heart failure, is the leading cause of death worldwide, with one heart attack every 10 minutes in Australia. Following the sustained damage caused by a heart attack (or "myocardial infarction"), healthy cardiomyocytes die and are replaced by a fibrous scar tissue. This irreversible damage leads to a failing heart. The alternative to death for heart failure patients is a heart transplant, which comes with several risks for the patient. Current studies focusing on novel approaches to overcome the shortage and limitations of a heart transplant include the transplantation of patient-derived stem cells. However, due to their limited survival following their delivery in a failing heart, a more physiological 3D microenvironment has been suggested to provide the optimal delivery of these cells. 3D bioprinting has emerged in the past ten years as a potential optimal approach to biofabricate viable and functional cardiac tissues to promote regeneration in patients. Our laboratory has recently developed a novel approach to engineer vascularized bioprinted cardiac tissues using bioinks containing alginate (Alg) and gelatin (Gel) for optimal cell viability, printability and durability[1,2]. Given its tunable properties and low immunogenic response in the human body, we have investigated the potential use of silk fibroin (SF) as biomaterial for cardiac bioink formulations[3]. SF protein is extracted from the *Bombyx mori* silkworm thread, in order to obtain an aqueous solution. Then, hybrid hydrogels were generated by mixing SF to Alg/Gel hydrogels. Our preliminary studies showed that addition of SF does not affect the printability and durability of bioprinted hydrogels up to 30 days. Current studies are evaluating the use of SF hybrid hydrogels with cardiac cells and will be tested for both *in vitro* and *in vivo* cardiovascular applications.

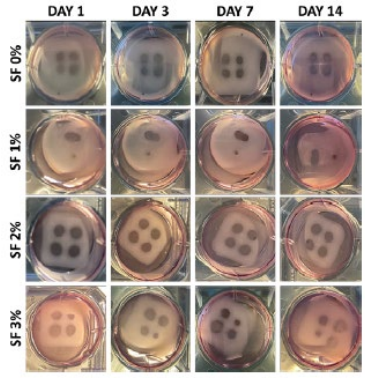

Figure: **Hybrid Hydrogels containing SF are durable over time**. Representative images of 3D bioprinted structures fabricated with hybrid 4%Alg/8%Gel hydrogels with varying concentrations of SF (n=6)

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Understanding of catalytic ROS generation by metal-free carbon catalysts for therapeutic effects in tumor microenvironment

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Cancer is one of the world's largest health problems, accounting for nearly 10 million deaths in 2020[1]. Although metal-based catalysts have become an exciting research frontier to combat tumors, expensive metal catalysts suffer from several sustainable issues. Owing to their unique physicochemical properties, controllable structure, biocatalytic behaviors, and biocompatibility, carbon nanomaterials have been investigated as safe and low-cost alternatives to metal-based catalysts for biomedical applications[5,6]. While considerable effort and progress have been made in the mechanistic understanding of metal-free carbon catalysts (C-MFCs) for non-biomedical applications, their bio-nano interactions and catalytic mechanism have rarely been investigated. This presentation will feature our recent study on defect-rich graphene quantum dots for efficient ROS generation, specifically in the H_2O_2-rich tumor microenvironment to cause multi-level damages of subcellular components. While a desirable anti-cancer performance was achieved, the catalytic performance was found to strongly depend on the defect density. In this talk, the first defect-induced catalytic generation of ROS by C-MFCs in the tumor microenvironment and the associated catalytic mechanism will be discussed.

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Drug delivery to the brain-optimizing nanomedicines for blood-brain barrier crossing

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INTRODUCTION

The current treatments for neurological diseases are mainly limited by the presence of the BBB which prevents the delivery of drugs to the brain. Nanomedicines have shown to be a novel promising technology as they can promote BBB penetration and target the diseased regions in the brain with minimized potential side effects. However, the essential parameter of designed nanomedicine such as ligand density differed between studies and was often not well characterized. Furthermore, the size of nanomedicine can influence greatly on the BBB transportation efficiency while there is a lack for systemic comparison of this property.[1] Therefore, a systematic comparison of particle surface density and size for BBB penetration using well-defined assays is an urgent need to advance the development of nanoparticles for drug delivery to the brain.

SUMMARY

We utilize porous silicon nanoparticles (pSiNPs) as a platform to systematically investigate particle surface content and size for blood-brain barrier (BBB) penetration capability. Using bifunctional poly(ethyleneglycol)-(PEG) linkers (with carboxyl and amine terminal groups) we generated a library of pSiNPs with different transferrin surface densities (Figure 1a). Immortalized human brain microvascular endothelial cells (hCMEC/D3) were exploited in confocal microscopy, flow cytometry, a static Transwell model, and a dynamic BBB-on-chip model to compare the influence of these parameters on cellular association, uptake, and transcytosis (Figure 1b).

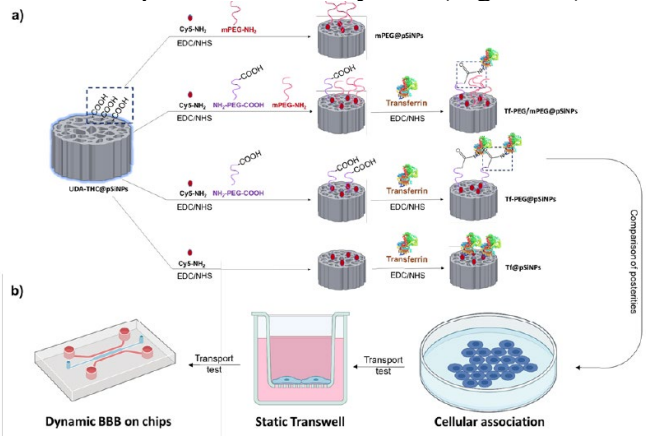

Figure 1: (a)Fabrication of different Tf modified pSiNPs using a combination of mPEGNH2 and NH2-PEGCOOH. (b)Schematic of methodology in comparing effect of surface properties and size of pSiNPs in three hCMEC/D3 set up models.