

UKICRS Symposium 2020

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Wednesday 14 October 2020

- Online conference via Zoom
- Two Keynote speakers
- Vandan Patravale (Institute of Chemical Technology, Mumbai, India)
- Mark Saltzman (Yale University, New Haven, Connecticut, USA)
- 10 talks from selected abstract submissions
- Online poster session

FORMULATION OF CATIONIC ANTIRETROVIRAL NANOCRYSTALS AND SUBSEQUENT INCORPORATION INTO A DISSOLVING MICRONEEDLE ARRAY

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Background: At the end of 2019, 38 million people were living with human immunodeficiency virus (HIV), which is recognised by the World Health Organization as a major global health problem. Of these 38 million cases, 1.7 million were new diagnoses, three times higher than the UNAIDS 2020 target. Current HIV treatments are usually delivered orally or *via* intramuscular injection. This project involves formulating nanosuspensions (NS's) of two antiretroviral drugs, cabotegravir (CAB) and rilpivirine (RIL), into separate dissolving microneedle (MN) arrays which when applied to the skin offer a patient-friendly alternative to hypodermic needles for long acting delivery.

Methods: To increase drug loading and increase the efficiency of drug delivery, both RIL and CAB were formulated into nanocrystals *via* wet bead milling on a small scale. 7 mL glass vials were used as a closed vessel with 0.1-0.2 mm yttria stabilised zirconium beads employed as the milling media. Energy was provided to the system with the use of a magnetic stir plate rotating at 1250 rpm alongside two 25x8 mm stir bars in the vessel. For the RIL NS, 444.2 mg of drug was placed in the vessel and the CAB NS was formulated with 400 mg of drug. In both cases the surfactant solution used to stabilise the system was 5 mL of a mixture of d- α -tocopheryl polyethylene glycol succinate 1000 (TPGS) 1.02% and chitosan medium viscosity 0.05% w/w. After 20 hours of milling the NS was recovered through a 200-mesh sieve. Particle analysis was conducted using a Brookhaven Nanobrook Omni. NS obtained were lyophilised to concentrate the drug and increase stability. This was done by freeze-drying the NS in a 1:1 weight ratio with a solution of poly (vinyl alcohol) (PVA) 9-10 kDa 2% and poly (vinyl pyrrolidone) (PVP) 58 kDa 2% w/w which was used as a cryoprotectant to preserve nanocrystal characteristics. MN arrays were formulated in a two-step process. 100 mg of lyophilised NS was combined with 200 μ L of water in a SpeedMixer at 3000 rpm for 5 minutes. An excess of this formulation was spotted onto the centre of silicon MN moulds. Each mould contained 600 pyramidal needles with a height of 750 μ m, base width of 300 μ m and 50 μ m needle interspacing at the base. These moulds were placed under positive pressure of 4.5 bar for a total of 10 minutes, after which the excess formulation was removed, and these were left to dry at room temperature for 20 hours. A second layer was then added as the baseplate, 300 mg of PVP 58 kDa 30% w/w, and centrifuged for 10 minutes at 3500 rpm then left to dry for 20 hours at room temperature. Following removal from the moulds, MN were characterised using a light microscope. Drug content analysis was performed using HPLC-UV analysis.

Results: Prior to lyophilisation, RIL NC had a particle size of 163.13 ± 5.46 nm and a PDI of 0.109 ± 0.006 (n = 3). Particle size was retained during lyophilisation and zeta potential was determined to be $+16.87 \pm 0.53$ mV (n = 3). CAB NC had a particle size of 194.79 ± 2.70 nm and a PDI of 0.129 ± 0.037 (n = 3). Particle size was again retained during lyophilisation and zeta potential was $+17.29 \pm 0.26$ mV (n = 3). Both sets of MN arrays were well formed and had good mechanical strength on insertion. Each RIL MN array was found to contain 2.47 ± 0.39 mg of drug while CAB MN contained 3.09 ± 0.15 mg (n = 3).

Conclusions: NS of RIL and of CAB were successfully formulated with the desired characteristics *via* wet bead milling. These NS were lyophilised and incorporated into dissolving MN arrays which were well formed, had good mechanical strength and high drug loading. Further studies are needed to assess the *in vitro* release and delivery of the drug from these MN arrays.

FORMULATION AND EVALUATION OF ORODISPERSIBLE FILMS OF NITROGLYCERINAlaa Abd-Albas¹, Ruaa H. Al-Gburi² and Mohammed H. Mahdi¹

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Background: The aim in present study is to develop Oral dispersible Films which can be use for either buccal or sublingual. Oral film is one of the new dosage forms and fast dissolving drug delivery system. They are used as an alternative traditional dosage forms (tablets and capsules) for pediatric and geriatric patients who experienced difficulty in swallowing traditional oral dosage forms. Buccal drug absorption through oral mucosa is greater than that of the skin regarding to the permeability; however, it is less than that of the intestine. Therefore, the oral-mucosa delivery act as a good site for the absorption of drugs that have poor dermal absorption and for drugs suffer from first pass metabolism [1]. Sublingual absorption through sublingual route however, 3-10 times greater than oral route. Oral dispersible film of loaded with nitroglycerin using a novel hydroxypropyl starch polymer were prepared.

Methods: The film was prepared by a solvent casting method in which hydroxypropyl starch was used as a film forming polymer. Briefly, sorbitol and other excipients were added to distilled water followed by adding polymer and stirred that for 5 minutes. The resulting oral film where evaluate using in vitro disintegration time. Petri dish with 6.5 cm was used to determine the disintegration time, which is similar to the sublingual area with diameter 3-4 cm. Moreover, the volume of liquid is comparable to volume of saliva. The time that the film takes before breaking down was recorded as a disintegration time.

Results: The disintegration time of the oral dispersible films was an average of 25 second which is nearly 8 times faster disintegration than sublingual tablet (results not shown). To mimic different saliva flow rate in the oral cavity, the retained drug in the formulation using different flow rate of artificial saliva. There is a significant different in drug release has been obtained from different flow rate. The drug retained indirectly proportion with flow rate. Zero percentage of drug retained after 15 min with flow rate 2 mL/min where about ~ 20 % of drug been retained at 0.5 mL/min rate.

Conclusions: In this study we have demonstrated that the prepared oral film disintegration time efficient and the release profile of nitroglycerin from Hydroxypropyl starch film is dependent on the saliva amount and flow rate in the oral cavity. This highlights the potential application of hydroxypropyl starch polymer in formulation fast dissolving oral film. Furthermore, this work illustrates the importance of understanding that subtle differences in patient physiology could impact on the release from such formulations, and a realization of this is very important especially when designing medicines for elder group of patients.

TOWARDS ORAL DELIVERY OF ARTIFICIALLY INTELLIGENCE-PREDICTED PEPTIDES

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Background: Research into oral delivery of peptides has been encouraged by the FDA-approval of an oral peptide tablet formulation of the GLP-1 agonist, semaglutide (Rybelsus®, Novo Nordisk). The preparation of oral peptide formulations is challenging and is challenged by gastrointestinal enzymes and the intestinal epithelial barrier leading to poor oral bioavailability. Production of stable potent peptides of a molecular weight < 5 kDa is a key factor in progress of oral formulations. A sustainable production by employing machine learning and *in vitro* laboratory approaches has created a library of food source-inspired proprietary peptides with potential health benefits at Nuritas Ltd. The novel bioactive peptides are still subject to the challenges of low intestinal permeability and require investigation for strategies to improve intestinal permeability and oral bioavailability. This study focusses on screening a selection of bioactive peptides using the *in vitro* Caco-2 monolayer assay in Transwells® and *ex vivo* rat jejunum assay using Ussing chambers.

Methods: The Caco-2 monolayer assay is typically performed after incubating the cells on polycarbonate Transwell® filter inserts for 21 days in 12 well plates. Male Wistar rats weighing 250-350 g are used to source tissue sections of jejunal mucosae. The muscle-stripped tissues are mounted on Ussing chambers bathed in oxygenated Krebs buffer on the apical and basolateral sides. Peptides are added to the donor side of monolayers and mucosae and are sampled over 120 min. Samples collected from the assays are analysed by LC-MS/MS.

Results: Transepithelial electrical resistance (TEER) data have been recorded to see effects on epithelial integrity and tight junction openings. Selected peptides have been studied for fluxes in combination with an established intestinal permeation enhancer.

Conclusions: Artificial intelligence-predicted novel bioactive peptides have potential for oral administration for nutraceutical and pharmaceutical applications. Combination of permeation enhancers with selected peptides in *in vitro* studies can offer an indication for specific formulation approaches to take for rat intestinal instillation and oral gavage experiments.

TRANSDERMAL LONG ACTING DELIVERY OF FLUPHENAZINE DECANOATE USING NOVEL POLYMERIC DISSOLVING MICRONEEDLE SYSTEMS.

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Background: Schizophrenia is a severe form of mental illness affecting about 7 per 1000 adults globally. Schizophrenia can be defined as a disturbance that must last for six months or longer, including one month of delusions, hallucinations, disorganized speech, grossly disorganized or negative symptoms. This mental disorder can be managed using medication, psychological and supportive treatment. Despite the critical importance of medication, nonadherence has been recognized as a problem worldwide and maybe the most challenging aspect of treating patients with schizophrenia. Cognitive impairment, lack of illness insight and symptom severity are part of the unique factors of nonadherence in schizophrenia. Nonadherence to medication results in relapse, rehospitalization, longer time to remission, and attempted suicide. Enhancing adherence to antipsychotic medications has the potential to reduce psychiatric morbidity and costs of care substantially. Fluphenazine Decanoate FLU-D is one of the long-acting antipsychotics used as a maintenance treatment of schizophrenia, it is administered as an intramuscular injection every 14-35 days. This long-acting injectable delivery system has overcome plasma drug fluctuations associated with the oral daily intake of the drug. However, FLU-D requires a professional health care provider and specific administration procedures. In addition to the pain and inconvenience of the typical hypodermic needle. Transdermal long-acting delivery of FLU-D using dissolving microneedle MN system has the potential to provide convenient noninvasive long-acting delivery of this lipophilic antipsychotic.

Methods: This research project started with developing a method of preparing dissolving MNs loaded with FLU-D. Firstly, an aqueous blend of 20% Polyvinylpyrrolidone PVP 29-32 KDA, 20% Polyvinyl Alcohol 10KDA and 10% w/w FLU-D was mixed for 15m at 3000 rpm by speed mixer. Secondly, almost 500 mg of this formulation was cast into LTS MNs moulds (600 MN, 750 μ m). Followed by applying 5 bar pressure for 15m using the pressure chamber. The excess formulation was removed and MNs were lifted to dry for 24h at room temperature. To form the free-drug baseplate, 500 mg 50% PVP 360 KDA was added, followed by centrifugation for 15m at 3500rpm and dried for 24h at room temperature. To evaluate the physical ability of the formulated FLU-D loaded dissolving MNs, a compression force test was performed by attaching the MNs to a cylindrical probe of a TA.XT2 Texture analyser moving toward the steel baseplate and was held for 30 seconds at the force of 32 N per patch. The height of individual MNs was measured using Leica EZ4W stereo microscope before and after the test to determine the reduction of MNs height. Besides, to evaluate the insertion ability, MNs were inserted into full-thickness porcine skin manually and Optical coherence tomography OCT images were taken. Further, drug content in MNs was using a validated HPLC method.

Results: FLU-D loaded dissolving MNs showed good physical and insertion properties, the mean of %height reduction is 5.23 ± 0.91 and OCT images showed that the mean of inserted length is $528.05 \pm 20.48 \mu$ m. In terms of drug content in MNs 0.99 ± 0.18 mg per array was found.

Conclusions: A promising FLU-D loaded dissolving microneedles system has been developed. The viable physical and insertion properties of this system have been proved in addition to the drug content. Further in-vitro studies are required to address the drug release.

NOVEL CHITOSAN POLYMERIC MICELLES AS A DELIVERY VEHICLE OF HYDROPHOBIC ANTICANCER DRUGS

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Background: The synthesis of new chitosan derivatives with amphiphilic properties allows the production of polymeric micelles, which have the ability to encapsulate hydrophobic drugs as camptothecin (CPT). Once encapsulated, the drug is protected from the body fluids, its aqueous solubility is increased as well as its intestinal permeability and therapeutic activity.

Methods: Chitosan was chemically modified with O-methyl-O'-succinylpolyethylene glycol and with oleic acid by a carbodiimide reaction to produce micelles by self-assembly. Characterization of the copolymer included ¹H NMR, FTIR, GPC, DSC/TGA and XRD. Size, zeta potential and morphology were determined by DLS and TEM, respectively. The CPT association efficiency was determined by HPLC as well as its *in vitro* release and lactone ring protection from hydrolysis, both performed in simulated gastrointestinal fluids. Cytotoxic studies were performed against Caco-2 and HT29-MTX intestinal cell lines. CPT intestinal permeability was tested in three different *in vitro* cell models and biodistribution and pharmacokinetic studies were performed by gavage in male Balb/c nude mice colorectal cancer induced.

Results: The success of the synthesis and the purity of the new copolymer were demonstrated by ¹H NMR, FTIR and GPC and, after the grafting, the copolymer showed an increase on its thermal stability and crystallinity. The CMC revealed a good stability of the system after dilution and DLS revealed an average size of 140 nm, a positive superficial charge and CPT association efficiency of 78%. TEM analysis demonstrated a round and smooth shape for both empty and CPT-loaded micelles. The CPT *in vitro* intestinal release showed a low release in gastric media and a controlled release in intestinal fluids, suggesting a pH-dependent behaviour. Also, these micelles were able to protect CPT from hydrolysis up to 75% of its initial lactone form, exhibiting a good system stability. Regarding the safety profile, copolymer did not present a cytotoxic effect against colorectal cancer cell lines in concentrations equal or below 10 mg/mL. More importantly, CPT improved significantly its *in vitro* intestinal permeability, as compared with free CPT. Moreover, CPT-loaded micelles showed 5% tumor accumulation, a distribution volume of 0.3 L, an elimination half-life of 7.1 h and a clearance rate of 0.02 L/h.

Conclusions: CPT-loaded chitosan micelles proved to be a potential vehicle of hydrophobic anticancer drugs, as CPT.

DEVELOPMENT OF ANTIBIOTIC HYDROGEL-FORMING MICRONEEDLE ARRAY PATCHES FOR IMPROVED TUBERCULOSIS TREATMENT

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Background: Tuberculosis (TB) is one of the leading causes of death worldwide. Suggests one-quarter of the world's population has been infected by *Mycobacterium tuberculosis*. The current available treatment for TB consists of rifampicin (RIF), isoniazid (INH), pyrazinamide (PYR) and ethambutol (ETH). However, this regimen has been reported causing hepatotoxicity as the most frequent adverse effect triggered by daily oral administration for at least 6 months. Transdermal drug delivery using microneedle array patches (MAP) technology is considered as a promising alternative in delivering TB drug regimen. This route offers a non-splanchnic circulation, thus, pre-systemic metabolism in the liver is avoided. A drug reservoir combined with hydrogel-forming MAP used in this present work which aims to deliver a high dose of TB drugs. Hydrogel-forming MAP are formed through a cross-linking of two or more polymers. This system consists of micron-sized needles that can be used to penetrate the deeper layer of skin, thus, allowing the drug permeated and reached the microcirculation for the systemic absorption.

Methods: The hydrogel-forming MAP (11 × 11 needles and 600 µm in height) were prepared from an aqueous blend containing Gantrez[®] S-97 (co-polymer of methylvinylether and maleic acid), polyethylene glycol (PEG) 10,000 and sodium bicarbonate. An individual reservoir containing each drug was prepared using a directly compression method. Crospovidone was used as a disintegrant in the direct compressed tablet (DCT) formulations of RIF, INH and PYR. In the specific case of ETH, based on the preliminary result, the DCTs were prepared by compressing only the pure drug without any disintegrant. A central composite design (Design Expert Software version 12, State-ease, Minneapolis, USA) was applied to optimise the reservoir formulations by using two parameters: dissolution time and tablet hardness. The optimised formulations were then investigated in *in vitro* permeation studies to quantify the amount of drugs that permeated across the dermatomed neonatal porcine skin through the hydrogel-forming MAP over 24 hours.

Results: All optimised formulations of DCTs exhibited homogeneous and robust properties. The dissolution time and hardness values of the optimised formulations were in the range of 10-12 sec and 33-58 N, respectively. Following the *in vitro* permeation studies, TB drugs were able to permeate across the dermatomed neonatal porcine skin from the DCT reservoirs combined with hydrogel-forming MAP. The result showed that a 0.5 cm² MAP delivered 0.69 mg, 30.96 mg, 25.71 mg and 46.99 mg in 24 hours for RIF, INH, PYR and ETH, respectively. The permeation profiles suggest a promising transdermal delivery of TB drugs.

Conclusions: In this present work, the reservoir formulations of TB drug regimen combined with hydrogel-forming MN arrays were successfully developed. Based on the *in vitro* permeation studies, the developed MAP system was able to deliver the TB drugs across the dermatomed neonatal porcine skin. Therefore, this approach could potentially contribute to the TB treatment improvement.

POLYMERIC MICRONEEDLE-BASED 'DRY' ELECTRODES FOR WEARABLE CARDIAC MONITORING

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Background: Microneedles (MNs) are minimally invasive devices consisting of single, or multiple micron-sized needles. Originally conceptualized and patented in the 1970s, MNs were considered a novel approach to transdermal drug delivery. Over the years, MNs have become more versatile and are now utilised in many fields, including physiological signal monitoring. Electrocardiography (ECG) is a clinical procedure used to help diagnose and monitor cardiovascular conditions. Wet electrodes used during ECG contain conductive gels to maintain a low impedance skin-electrode contact. Whilst effective in the short-term, dehydration of these gels can reduce the quality of recorded signals. MN-based 'dry' electrodes appear to be a promising alternative as they negate the need for conductive gels but also offer the potential to improve the signal fidelity of ECGs. We aim to use ECG signal acquisition, as an exemplar, to assess the wearability and performance aspects of MN electrodes for remote cardiac monitoring.

Methods: To assess MN electrodes and define those parameters which influence performance, a suitable ex-vivo skin model was developed. To account for signal loss resulting from the setup, simulated ECG signals were recorded at multiple stages of the model. Following data normalisation, the signal-to-noise ratio (SNR) and Pearson's correlation coefficient were calculated with respect to the original emitted signal. Following MN removal, penetration was assessed using methylene blue staining, optical coherence tomography (OCT) and histology. A subsequent study compared results in our model with in-vivo ECGs. Following ethical approval, both wet and MN electrodes were applied to the torso of healthy volunteers. ECGs were recorded simultaneously from both types of electrode, at the same sampling frequency, in a lead II configuration over three minutes.

Results: Correlation coefficients and SNR values were determined by analysing fifteen different simulated ECG waveforms recorded over three minutes. Overall, as the signal travelled from its source through to individual electrodes, correlation and SNR values declined, whilst the level of powerline interference increased. Interference was found to be present in substantial amounts at 50Hz. Following the application of a digital notch filter, results improved with the initial output signal achieving a near perfect recreation with an average correlation of 99.9% and SNR of 31.7dB. MN electrodes were comparable with wet electrodes producing SNR values of 27.48dB and 27.57dB respectively post filtering. Upon removal of MN electrodes from ex-vivo skin, application of methylene blue dye suggested MN penetration, which was subsequently corroborated with OCT. Visual analysis of the in-vivo ECGs highlighted that wet electrodes were less susceptible to motion artefacts when compared to the 'dry' MN electrodes.

Conclusions: Our ex-vivo model was successful at generating and acquiring simulated ECG signals through ex-vivo skin. This model can now be utilised for data simulations to assess parameters which affect MN electrode performance. Recording ECGs from healthy volunteers not only helped inform the development of the model, but importantly highlighted both the promise and limitations of our current microneedle design. We are now testing an adapted electrode containing a mechanism to improve MN retention in skin.

DEVELOPMENT OF DRUG-LOADED NANOPARTICLES SUITABLE FOR INHALATION AS TARGETED TREATMENTS FOR *MYCOBACTERIUM TUBERCULOSIS*

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Background: Tuberculosis (TB) is an infectious disease caused mainly by *Mycobacterium tuberculosis* (*Mtb*). It primarily affects the lungs but in some cases, other organs can be affected. According to the World Health Organization (WHO), millions of cases of TB are recorded yearly making it the one of the top causes of death worldwide. Nowadays, there is a rise of multi-drug resistant TB (MDR-TB), whereby patients become resistant to first-line therapy. MDR-TB cases are related to patient non-compliance to medications, due to lengthy dosage regimens and side effects. The ultimate goal of this study is to prepare Poly Lactic –co-Glycolic acid (PLGA) nanoparticles (NPs) suitable for delivery into the lungs via inhalation. Nanoparticles with a range of particle sizes were prepared and studied to improve targeting to the alveolar macrophages (AM), which are the host cells for *Mtb*, and enhance its biodistribution.

Methods: PLGA NPs loaded with a fluorescent dye (Rhodamine B) were formulated in three different sizes 200, 300 and 500 nm approximately by varying drug to polymer ratio 1:100, 1:10 and 1:50 (w/w%), the organic phase volume and stirring speed. The cellular uptake of the Rhodamine B PLGA NP was investigated using THP-1 derived macrophages, as an *in vitro* model of AMs and was confirmed by confocal microscopy using Hoechst 33342 and CellMask green dyes to stain nucleus and cytoplasm, respectively. Then, Rifampicin, as a model TB drug, was loaded into PLGA NPs using nanoprecipitation method.

Results: For Rhodamine B loaded PLGA NPs, a significant effect was seen on the particle size and PDI of the NPs when process parameters were altered including the organic phase volume, stirring speed and the dye content amount. Our results confirm the successful formulation of Rhodamine B-loaded PLGA NPs of sizes circa 200, 300 and 500nm for 1:100, 1:10 and 1:50 dye to polymer ratio (w/w%), respectively. Extensive cellular uptake of all Rhodamine B-loaded PLGA NPs was evident in THP-1 derived macrophages with significant distribution within the cytoplasm for all NPs. RIF loaded PLGA NPs with a particle size <200nm were then prepared. Polydispersity index (PDI) was consistent between batches and <0.2. Zeta potential was almost neutral for all formulations (-0.8 - -2.1 mV). The encapsulation efficiency (EE) of RIF, however, within the all prepared NPs was very low and current work seeks to enhance this using alternative manufacturing methods.

Conclusions: PLGA NPs within the size range 200-500nm demonstrated effective cellular uptake in AMs. Data obtained from confocal microscopy indicates no difference in the uptake among different sized particles. However, further quantitative analysis, using techniques such as high content cell analysis currently underway will better determine the correlation between the size of NPs and cellular uptake. Alternative manufacturing methods including double-emulsion solvent evaporation (DESE), microfluidics and supercritical fluid-based approaches are being explored to increase drug encapsulation efficiency and scale-up the process.

BIOFILAMENTS DERIVED 3D PRINTED MEDICATED SKIN PATCH: DESIGN TO DELIVERY

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Background: Quercetin in combination with polyvinylpyrrolidone (PVP) was found to limit the spreading of necrosis to unaffected tissues in tuberculosis infected mice. Therefore, we hypothesized that 3D printed medicated skin patch incorporated with quercetin-PVP concentration would provide an appropriate therapeutic drug concentration with desired sustained release profile.

Methods: We fabricated quercetin-PVP bio-filaments by hot melt extrusion (HME) technique along with Eudragit® RSPO and tri-ethyl citrate as plasticizer and further 3D printed it to make medicated skin patches using fused deposition modelling (FDM). Various characterizations were performed to optimize the 3D printed patch formulation.

Results: *Ex-vivo* skin permeation study with optimized T₁ patch showed tri-phasic release pattern governing the Higuchi based diffusion mediated release kinetics. Additionally, optimized patch was assessed using SEM, DSC, and XRD studies to confirm the conversion of crystalline quercetin into amorphous form. Finally, pharmacokinetic profile of this optimized patch was studied in rats showed prolonged T_{max}, lowered C_{max}, and reduced fluctuations in plasma concentrations till 18 days with single skin application of 3D printed medicated patch.

Conclusions: Overall data confirmed the feasibility of developing 3D printed medicated skin patches to provide plasma levels for continued 18 days in rats after a single application.

A PLATFORM FOR POLYMERIC NANOPARTICLES AS GENE DELIVERY SYSTEMS IN 2D AND 3D GLIOBLASTOMA MODELS

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Background: Glioblastoma multiforme (GBM) is one of the most aggressive brain tumors classified as grade IV of malignancy by the WHO. Standard-of-care multimodal treatment combines surgical resection, chemotherapy and radiotherapy. However, such interventions result in severe side-effects and only offer a median survival of 15 months from diagnosis. Gene therapy holds great promise due its capacity to target specific pathways within glioblastoma cells by the introduction of exogenous tumor suppressor sequences that are rendered therapeutically effective by using delivery systems. Our research group has been exploring a platform of polymeric nanoparticles (NPs) as gene delivery systems for the treatment of glioblastoma due to an intrinsic ability to encapsulate and protect different nucleic acids, high biocompatibility and delivery efficacy.

Methods: Polymeric NPs with different ratios (8:0:1, 8:4:1, 4:1) were formulated by an ionic complexation method using protamine, polyethylenimine and chitosan as cationic polymers and dextran and a recently synthesized polyphosphazene as anionic elements. The physicochemical and structural properties of the NPs were studied by Photon Correlation Spectroscopy, Laser Doppler Anemometry and Transmission Electron Microscopy. Long- and short-term stability was examined for one month under storage (4°C) and for 4 hours in physiological conditions (37°C, pH=7.4), respectively. The association of different nucleic acids to the NPs was studied by agarose gel electrophoresis. *In vitro* cytotoxicity studies were initially optimized using U87MG glioblastoma 2D monolayers and 3D spheroids followed by assessment using a panel of primary patient-derived glioblastoma cells (GIN-8, GIN-28, GCE-28), by proliferation and cell death assays. The 2D and 3D cellular uptake of 4:1 protamine:dextran (Pr:Dx) NPs was studied using fluorescently labelled-nanosystems by confocal microscopy and quantified by Flow Cytometry. Moreover, their transfection with different concentrations of pDNA was carried out with a plasmid encoding the enhanced Green Fluorescent and Luciferase Proteins (pEGFP-Luc) by fluorescence microscopy.

Results: The NPs present spherical morphology, size below 150 nm, positive surface charge, high encapsulation of nucleic acids and stability under storage and physiological conditions. The cell viability studies indicated low/non-toxicity of the NPs in U87MG 2D cells and spheroids, but showed limited toxicity in glioblastoma patient-derived cells, except for the 4:1 Pr:Dx system. The cellular uptake studies of 4:1 Pr:Dx NPs revealed a transfection efficiency of 99%, with internalization of this nanosystem reaching the inner cell compartments. In addition, the transfection assay analyzed by green fluorescence emitted by EGFP showed an efficient capacity of 4:1 Pr:Dx NPs to transfect glioma cells for doses greater than 1 µg/well.

Conclusions: The physicochemical properties of the NPs make them suitable for the association and protection of different genetic cargos. Among all the nanosystems, the 4:1 Pr:Dx NPs presented the lowest cellular toxicity and were efficiently internalized in 2D and 3D cell models. Finally, these NPs promoted an efficient transfection of model pDNA in glioblastoma cells, indicating that this proof-of-concept formulation could be considered a promising gene nanocarrier for glioblastoma treatment by gene therapy.

IN VIVO ADMINISTRATION OF LONG-TERM RELEASE RISPERIDONE MICROPLATES RESTORES TEMPORAL ORDER RECOGNITION MEMORY IN A MURINE MODEL OF SCHIZOPHRENIA

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Background: Schizophrenia is a serious psychiatric disorder characterized by psychotic symptoms, as well as impaired cognitive symptoms. Symptoms tend to fluctuate over time, with periodic relapses and remissions. The standard treatment for schizophrenia consists in the long-term administration of antipsychotics to reduce symptom severity. However, medication adherence is often poor leading to high rates of relapse and hospitalization. Here, we propose a long-term drug delivery system based on PLGA microparticles for the controlled release of risperidone, a second-generation antipsychotic prescribed to schizophrenic patients.

Methods: PLGA micronized particles were fabricated using a top-down approach to realize square hydrogel microplates (μ PLs). μ PLs were engineered in terms of geometry and polymer content to modulate the release of risperidone over a prolonged period of time (months). μ PLs were characterized for properties relevant to drug delivery, including morphological, physico-chemical, and pharmacological features. Subsequently, μ PLs were tested for their *in vivo* efficacy in a murine model of schizophrenia. μ PLs were i.p. injected once at the beginning of the study, while free risperidone was injected every day as control. Subsequently, 2, 4, 8, and 12 weeks post injection the temporal order recognition (TOR) test was carried out.

Results: The use of a top-down approach allowed for the precise control of particle geometrical features. Specifically, SEM and volume impedance analysis highlighted a square geometry for μ PLs with an average dimension of 20 μ m. Risperidone release from μ PLs was analyzed via HPLC, and results showed a prolonged release up to 100 days. TOR test carried out 2 weeks after the injection showed a discrimination index (DI) for mice treated with risperidone-loaded μ PLs significantly higher than the DI of the untreated ones and comparable to the DI of mice treated with free risperidone. Moreover, the DI of the treated mice was still higher than the one of untreated group even after 4, 8, and 12 weeks, suggesting that the prolonged and constant release of risperidone from μ PLs helps restore the temporal order memory impairment.

Conclusions: The precise control of μ PL geometry and polymer content allowed us to precisely tailor the kinetics of risperidone release. The therapeutic efficacy of risperidone- μ PLs was demonstrated *in vivo* where they helped restore the impaired memory in a murine model of schizophrenia.

BIOMIMETIC LIPOSOMES AS DELIVERY SYSTEMS FOR ANTIMICROBIAL PEPTIDES

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Background: Drug-resistant bacterial infections continue to represent one of the biggest public health challenges of our time. There is therefore an ongoing need for both novel anti-infective agents, and innovative strategies for effective delivery of these agents. In the former case, antimicrobial peptides (AMPs) are promising, broad-spectrum anti-infectives that may provide alternatives to conventional antibiotics. In the latter instance, biomimetic nanocarriers, such as liposomes composed of cell membrane-relevant phospholipids, are of considerable current interest. In this work, the synthesis and antimicrobial activity of the ranalexin analog RN7IN6 was investigated. Liposomes composed of bacteria membrane-relevant phospholipids were additionally prepared, and preliminarily investigated as a delivery platform for RN7IN6.

Methods: RN7IN6 was synthesised via automated Fmoc-SPPS, characterised and purified. Biomimetic liposomes (LPs) made of POPE (1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine), POPG (1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phospho-(1'-rac-glycerol)) and CL (cardiolipin) were manufactured using the thin film hydration method followed by extrusion. Preliminary biomimetic LP formulations were also prepared via microfluidic mixing techniques. RN7IN6-adsorbed-LPs were prepared by incubating empty liposomes with a RN7IN6 solution with constant mixing. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of RN7IN6 were determined by broth microdilution, using resazurin dye as a marker of cellular viability.

Results: RN7IN6 was successfully synthesised at approximately 90% purity, and noted to be effective against both Gram-positive and Gram-negative bacteria. MBC studies further showed that the AMP had a bactericidal action. Empty biomimetic liposomes prepared via thin film hydration showed a size of 159.75 ± 1.35 nm, a PDI of 0.18 ± 0.01 and a zeta potential of -28.6 ± 1.9 mV. No appreciable difference in size was noted following RN7IN6 incubation; the surface charge of liposomes was however noted to decrease in magnitude, indicative of successful peptide adsorption.

Conclusions: Synthesised RN7IN6 showed a promising, broad-spectrum bactericidal activity. Preliminary studies indicated that RN7IN6 could be surface adsorbed to bacteria-relevant liposomes, to produce a biomimetic delivery platform for a novel anti-infective agent. RN7IN6 loading and antibacterial activity of AMP-adsorbed liposomes is currently under investigation.

NEXT GENERATION OF 3D-PRINTED DRUG ELUTING MESHES FOR TISSUE ENGINEERING APPLICATIONS

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Background: Surgical meshes have been widely employed since the start of 900s for the treatment of pathological conditions, including hernia and pelvic floor dysfunctions, wound healing and breast reconstruction after mastectomy.

Up to now, commercial meshes are mainly made of polypropylene (PP), with their use often associated with several side effects such as infections and pain, which in most of the cases lead to mesh removal. Moreover, stress shielding related to the employment of stiffer meshes must not be underestimated due to the subsequent erosion of the surrounding tissue that eventually could result in the device explantation.

Considering these issues, new material-based strategies should be implemented to design and develop mesh implants with better biomechanical properties and antibacterial potential. Additive manufacturing (AM), and particularly extrusion-based 3D printing, can be a powerful tool to produce safer surgical meshes, guaranteeing customisation of the final product and to allow a direct inclusion of antibacterial agents in the raw material formulation.

Methods: The purpose of the current study is to produce antibacterial loaded meshes by means of hot-melt extrusion (HME) 3D printing in order to resemble biomechanical properties of the pelvic floor and to manage infections, avoiding any future removals. To find the most suitable mesh prototype, meshes with two different fibre patterns, made of Polycaprolactone (PCL, biodegradable) and Thermoplastic Polyurethane (TPU, non-biodegradable), were printed. Morphological and chemical characterisation were carried out using a variety of techniques (e.g. SEM, μ CT, FTIR and DSC/TGA). Tensile tests were performed in order to assess the effects of the materials and of the meshes' pattern on the mechanical properties of the final product. Results were compared with data obtained from the literature to validate the findings.

Results: Two types of mesh patterns were tested, 90° and 45°. In terms of morphology, SEM and μ CT analysis showed that PCL meshes had a defined and precise fibre's shape in respect to TPU. In terms of mechanical properties, results highlighted that 45° PCL meshes performed better in respect to all the others. Specifically, the values of the Young Modulus, ultimate tensile strength (UTS) and maximum strain were very close to the ones of the human vaginal tissue. Moreover, the evaluated tensile stiffness was smaller than the one of some currently used commercial meshes and very similar to the tensile stiffness of rat vagina.

Conclusions: In this study, HME 3D printing was used to produce surgical meshes for the treatment of pelvic floor dysfunctions. Among the two chosen materials and the two tested types of fibres' patterns, 45° PCL meshes, thanks to their improved mechanical properties, seem to be the most promising ones to be employed for this kind of applications.

TARGETED CATHELICIDIN NANOMEDICINES AS NOVEL GLUCOREGULATOR FOR DIABETES THERAPY

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Background: Type 1 diabetes (T1D) results from autoimmune destruction of the insulin-producing β cells. Given that cathelicidin antimicrobial peptide has shown to improve β cell function and neogenesis, this work aims to use a cathelicidin-derived peptide, LLKKK18, loaded on targetable PLGA nanoparticles and assess the ability of the formulation to recover β cell mass and pancreatic function, reverting T1D. For targeting, functionalization of NPs with exenatide, an agonist of the glucagon-like peptide-1 receptor (GLP-1R), which is expressed by β cells, is proposed.

Methods: NPs were characterized using DLS, NTA and TEM. The association efficiency (AE) and drug loading (DL) of LLKKK18 were determined using fluorescamine. Peptide cumulative release from the NPs was studied in phosphate buffer (PB) at 37°C, up to one week. Functionalization of the nanoparticles was performed using the thiol–maleimide “click” chemistry and conjugation efficiency determined using fluorescamine. The effects from blank, LLKKK18-loaded and exenatide-functionalized NPs on the cell viability of L929 and INS1E cell lines were evaluated using MTT.

Results: The obtained results from NP characterization indicate a mean size around 100 nm, for blank, LLKKK18-loaded and exenatide-functionalized NPs and a narrow size distribution (Pdl of 0.10). After loading of LLKKK18, zeta potential of -3.1 mV, with stability up to 20 days at 4 °C in PB. NP functionalization decreased the surface charge of NPs to -12 mV, which may contribute to further stability. LLKKK18 AE and DL of 88 % and 0.9 %, respectively, with a sustained *in vitro* release. The conjugation efficiency of exenatide to the surface of NPs was around 80%, as determined by indirect method, using the molar ratio of 2:1 of maleimide:exenatide. Furthermore, 125 $\mu\text{g}/\text{mL}$ of nanoformulation showed no cytotoxic effects on both cell lines and will be used in the future for the following assays.

Conclusions: In the near future, the bio-functionality of NPs will be evaluated, to address the ability of the NPs to favor interaction with β cells and the ability of the formulation to promote glucose-mediated insulin release and improve β cell replication.

SYSTEMATIC EVALUATION OF PHOTOPOLYMER FORMULATIONS FOR STEREOLITHOGRAPHY 3D PRINTING OF SOLID ORAL DOSAGE FORMS

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Background: Among the most novel technologies emerging in the pharmaceutical field, 3D printing has proved to be a frontrunner technique to manufacture solid oral dosage forms targeting personalised treatments. 3D printing offers a flexible platform to produce medicines directly at the point of care; drug dosage, release profile and tablet geometry can be tailored on the needs of individual patients with the aim to optimize therapeutic outcomes. Stereolithography (SLA) 3D printing, a vat photopolymerization technique already used to fabricate controlled release tablets and polypills, is an attractive technique yet suffering from limitations related to the lack of biocompatible photopolymer formulations that could be used. Therefore, in this work we selected a pool of photopolymers and liquid fillers to prepare 156 formulations to be screened, with the view to identify ideal candidates for future drug loading studies.

Methods: A total of 156 photopolymer formulations were prepared by mixing different ratios of reactive mono/oligomers (polyethylene glycol diacrylate -PEGDA- MW 250, 575 and 700, N-vinyl-pyrrolidone), photoinitiator (diphenyl 2,4,6-trimethyl benzoyl phosphine oxide) and liquid fillers (polyethylene glycol 300, propylene glycol, glycerol). Photoinitiator was added at a concentration of 1%, 0.5%, 0.1% and 0.05% (w/w); liquid fillers were added at a concentration of 12.5%, 25% and 50% (w/w). Formulations were loaded in a modified Form 2 3D printer and cylindrical tablets (12 mm diameter – 4 mm height) were printed at a resolution of 25, 50 and 100 μm . Printability outcomes were evaluated according to an arbitrary scale assigning a Printability Score (PS) from 1 to 6, with 5 being the target value.

Results: Out of the initial 156 formulations tested, 60 were identified as reaching a PS=5 or showing a defined rim when printing a cylindrical tablet at least at one resolution (positive formulations, n=60). Out of these 60 formulations, 35 were identified as reaching a PS=5 and showing a defined rim when printing a cylindrical tablet at least at one resolution, while 5 formulations were identified as ideal candidates because providing the best results at every printing resolution (optimal formulations, n=40). The effect of PEGDA molecular weight was investigated; PEGDA 250 was present in 43.4% and 47.5% of the positive and optimal formulations, respectively. However, PEGDA 700 showed the highest ratio (80%) between optimal and positive formulations, proving to be highly performing when used. The impact of the liquid fillers' concentration was also studied, and the best results were observed at a concentration of 12.5% w/w. Finally, the relation between photoinitiator's concentration and printing resolution used was investigated; according to the results, the most effective photoinitiator concentration was 0.05% w/w used for printing at a resolution of 100 μm . As a result of printing at 100 μm , the number of layers is reduced up to 4 times, with a significant impact on production time.

Conclusions: 5 formulations out of 156 were identified as suitable candidates to be drug loaded and 3D printed as controlled release dosage forms. The investigation of different liquid fillers provides information on which species and which concentration could be used to tune the release profiles in SLA 3D printed tablets. Finally, the use of low concentration of photoinitiator at lower printing resolution is recommended. This not only would result in better printability outcomes but could also have significant implications in reducing toxicity concerns and formulation costs. Furthermore, printing at a lower resolution will decrease the number of layers needed thus speeding up manufacture time.

STEREOLITHOGRAPHIC APPARATUS EVOLUTION: ENHANCING THROUGHPUT AND EFFICIENCY OF PHARMACEUTICAL FORMULATION DEVELOPMENT

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Background: Pharmaceutical additive manufacturing, also known as 3D printing, is a rapidly evolving set of technologies used to produce revolutionary drug delivery devices overcoming the limitations of conventional tableting techniques. The flexibility of 3D printing opens the way to personalised dosage forms, thus shifting from a one-size-fits-all approach to patient-centric medicine. Stereolithography (SLA) 3D printing, a vat photopolymerisation technique, has emerged as an attractive tool in pharmaceuticals for the fabrication of controlled release tablets and polypills and offers a range of advantages over other technologies. However, the limited number of biocompatible photopolymers suitable for SLA coupled with the large amount of material required for a single print and its related cost hold back the further development of such technology. Hence, in this work we aimed to develop a novel Stereolithography apparatus specifically designed for high-throughput screening of pharmaceutical photopolymer formulations; an analysis on the cost effectiveness of the new SLA apparatus was also performed.

Methods: A novel build platform and resin tank inserts prototypes were designed using TinkerCAD and fabricated with a Form 2 SLA 3D printer using Clear photopolymer resin. The final build platform was manufactured through CNC milling of aluminum. The modified parts were subsequently assembled on the Form 2 3D printer and tested to fabricate cylindrical tablets previously designed using TinkerCAD. Three tablet batches, each of 10 units, were printed both on the original and the modified platform and evaluated according to uniformity of weight and thickness. Batches of the same tablet design were printed both with and without supports in order to assess printability outcomes and related material wastage. A time-dependent investigation on the photopolymer resin's loss due to adherence on the build platform after printing was carried by measuring the amount of resin adhering on the platform at different time points between 0 and 3600 seconds. An estimate of the cost implications of such waste was eventually calculated.

Results: The original resin tank having a capacity of 200 mL was modified to operate with only 10 mL of photopolymer resin. Moreover, the novel tank can contain up to 12 different resin formulations. As a result, the novel SLA apparatus we developed will allow us to maximise the formulation development process efficiency. Tablet uniformity data obtained from the modified build platform were comparable to the original platform. The use of supports to print tablets was found to significantly impact the generation of waste. Finally, the recovery of photopolymer resin adhering onto the build platform was found to reach a plateau at 2700 seconds for the original platform and at 1200 seconds for the modified platform. The percentage of resin recovered from the original and the modified platform at such time points was 63.04% and 44.77%, respectively.

Conclusions: A novel stereolithography apparatus was developed to carry a high efficiency screening of pharmaceutical photopolymer formulations. Such SLA apparatus was successfully tested to prove its reliability. Potential areas of wastage were investigated and mainly identified in relation to the use of printing supports and loss of resin adhering onto the build platform after printing. The latter issue was addressed by waiting for an hour of time allowing the maximum resin recovery. This could have significant implications in clinical applications of SLA 3D printing where cost-efficiency and high production rates must be met.

CHITOSAN-COATED PLGA NANOPARTICLES OF TRIAMCINOLONE ACETONIDE: FORMULATION OPTIMIZATION FOR OCULAR DRUG DELIVERY
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<p>Background: Age-related Macular Degeneration (AMD) is a disease of the posterior segment the eye and the most common cause of vision loss in elderly people. There is a rapid increase in the disease population partly due to ageing (~20% of Europe's population are now over 65). The number of Europeans suffering from early and late-stage AMD will have increased to 21.5 and 4.8 million, respectively, by 2040. The existing treatment regimen involves repeated intravitreal injections of anti-VEGF (vascular endothelial growth factor) agents. Such injections are reportedly associated with serious side effects such as retinal detachment, retinal haemorrhage and endophthalmitis. Therefore, there is an urgent need for the development of a non-invasive treatment option for AMD, in order to reduce or eliminate the need for frequent intravitreal injections, enhance therapeutic efficacy and improve patient compliance. The present study aims to develop a topically applied nanoparticulate system exhibiting extended drug release for the treatment of AMD. The corticosteroid encapsulated into the nanoparticulate system has the potential to help in managing this complex disease.</p>
<p>Methods: Triamcinolone acetonide (TA)-loaded chitosan-coated poly (lactic-co-glycolic acid) (PLGA) nanoparticles were prepared using the thin-film hydration technique. The particle size, zeta potential and polydispersity index (PDI) of the nanoparticles were analyzed using dynamic light scattering. High-performance liquid chromatography was used to quantify TA to determine encapsulation efficiency and percentage drug release. The stability of nanoparticles was assessed using thermogravimetric analysis (TGA) and dynamic scanning calorimetry (DSC) techniques.</p>
<p>Results: The particle size of uncoated and chitosan-coated PLGA nanoparticles ranged from 411 ± 2.83 nm to 456 ± 67.89, with an encapsulation efficiency of $63.13\% \pm 6.44\%$ and 24.16 ± 10.29. The zeta potential of uncoated PLGA nanoparticles was -4.1 ± 1.36 mV which increased to $+44.05 \pm 5.02$ mV following chitosan coating indicating the formation of uniform and stable particles with polydispersity indices ranging from 0.08 and 0.19. TGA and DSC results indicated that nanoparticles were thermally stable and in a mono-dispersed form. The <i>in-vitro</i> TA release from the nanoparticles was $27.48 \pm 0.65\%$ in 32 hours, subsequently reaching a plateau suggesting the controlled release of the drug. The cytotoxicity study on human corneal epithelial cell lines revealed the components and the nanoparticles resulted in at least 90% cell viability, an important first step in demonstrating biocompatibility.</p>
<p>Conclusions: The polymer matrix of PLGA aids in controlled diffusion of encapsulated drug, while the mucoadhesive property of chitosan, along with the lipophilic nature of TA may help in better permeation across the barriers of the eye. The size of the nanoparticles in conjunction with the biodegradable and biocompatible properties of the polymers used suggest the prepared chitosan-coated PLGA nanoparticles might be promising for topical ocular drug delivery.</p>

Optimization of ternary complexes as gene delivery systems

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Background: Gene delivery systems are essential for gene therapy to protect the gene from enzymatic degradation and facilitate membrane translocation. PEI (polyethylenimine) is a widely used cationic polymer in gene delivery, however, its usage has been limited due to high cytotoxicity. One strategy to reduce this toxicity is to use a negatively charged polymer coating to reduce the surface charge and thus membrane interaction. Poly (glutamic acid) (PGA), is a polyanionic, hydrophilic, biocompatible and biodegradable polymer which fits these criteria. Furthermore, polyplex surface charge is one of the determining factors for cell internalization. However, due to the increased complexity of these formulations containing a third component, we sought to systematically evaluate the effects of particle size and surface charge of PEI/DNA/PGA (N/P/C) polyplexes under different preparation conditions.

Methods: Polyplexes were prepared via two different methods, direct pipetting and slow addition using a syringe pump. In both cases binary polyplexes (i.e. DNA and PEI only) were prepared with branched PEI ($20 M_w = 20 \text{ kg mol}^{-1}$) and model calf thymus DNA at N/P = 20:1 in HEPES (20 mM). For the pipetting method, 500 μ l of DNA and 500 μ l of PEI solution (0.2 mg/ml) were thoroughly mixed by pipetting. Additional experiments were performed to study polyplex formulation under different pH buffers. For the syringe pump method one solution (either DNA or PEI) was fed into a stirred vial of the other solution (either DNA or PEI) at a variety of flow rate. The effects of adding either PEI to DNA or vice versa, stirring rate (100-1000 rpm) and rate of addition (over 1,3 or 10 min) were systematically evaluated. Preparation of ternary complexes: DNA/PEI/PGA ternary complexes were constructed with different N/P/C charge ratios by adding PGA to pre-prepared polyplexes prepared above. Then the different concentrations of PGA were added to the prepared polyplex by syringe pump or pipetting with different ratios, and the resulting stability of these complexes were monitored by DLS and zeta potential.

Results: These experiments confirmed that the pH and ratio of each material (i.e. PEI, PGA and DNA) and the fabrication procedure (pipetting or syringe pump) influence polyplexes. At N/P ratio of 20, PEI condensed and entrapped the DNA and the 78 nm polyplexes formed with zeta potential of 41.6mV. The pH value of 5 formed a larger polyplex because of the more DNA binding ability of PEI at the low pH. A small, monodispersed and reproducible polyplexes were gained by syringe pump synthesizing method. Slowly DNA addition reduced the polyplexes size, however, this change was statistically insignificant. Additionally, high-speed mixing of DNA and PEI mixture prevented polyplexes aggregation and consequently, we could have the smaller polyplexes. Adding PGA to polyplexes reduced the surface charge up to 30.7mV. The method of adding PGA (syringe or pipetting) didn't affect the particle size. By adding the PGA up to a value of 2.5 the size didn't change dramatically and after that, the size increased rapidly. Because the more amount of PGA couldn't interact with PEI to form ternary complexes.

Conclusions: In conclusion, to achieve small ternary polyplexes the syringe pump method is the most viable route to add DNA to the polymer slowly (in 3 min) and mix gently with high speed (1000rpm). Low pH (pH=5) and optimal N/C/P (20:1:1) are important in polyplex preparation. Additionally, coating the polyplex with PGA can reduce the surface charge.

LAMELLASOME Technology: Off the bench and into production

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Background: LAMELLASOME™ technology is being engineered to deliver active payloads in the treatment of pulmonary conditions including idiopathic pulmonary fibrosis and cystic fibrosis. Two key challenges exist in relation to the production of LAMELLASOME vesicles: 1) The use of scalable downsizing procedures and 2) the commercial compatibility of production methods. The implementation of a simple, solvent-free method produced LAMELLASOME vesicles with desirable characteristics while eliminating hazardous waste and reducing energy requirements.

Methods: LAMELLASOME vesicles were prepared at an optimised concentration using a solvent free process whereby, components were combined and homogenised with pre-warmed Tris buffer (70 °C; pH 7.4) for 1 h at 8750 rpm (Silverson Homogeniser). The Microfluidizer (M110P; 25k psi) was used as a scalable method to downsize lipid vesicles to express desirable characteristics as determined using Dynamic Light Scattering (DLS; Malvern Panalytical). Lipid recovery was monitored over time using ELSD-HPLC. With an ultimate goal of formulating a pulmonary delivery system, the impact of nebulisation on physicochemical characteristics was further investigated.

Results: Downsized LAMELLASOME vesicles produced using scalable methods expressed similar characteristics to those produced at the lab-scale. As expected, LAMELLASOME vesicles were formulated to express an anionic surface charge, low PDI (< 0.3) and average vesicle size between 50 and 60 d.nm. Depending on the nebulisation method, the average aerosol size was found to be between 4 and 6 µm and was not significantly altered based on total lipid concentration. Further physicochemical characterisation supports the generation of stable formulations which may successfully deliver cargo to the lung.

Conclusions: The implementation of a solvent-free method of LAMELLASOME vesicle production generated vesicles with mapped physical properties compared to those prepared using traditional methods, eliminating the use of solvents. In addition, desirable characteristics were achieved using scalable processes. These data support the ability to translate a nebulised pulmonary delivery system from R & D lab-scale production to an industrial-scale manufacturing setting.

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CONTROLLING THE RELEASE KINETICS OF PHARMACEUTICALS *IN VITRO* USING LIPID CUBIC GELS AND DISPERSIONS

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Background: Lipid cubic phase formulations have gained considerable interest as controlled delivery systems for a range of active pharmaceutical and biological agents on account of their desirable physiochemical properties, and ability to encapsulate both hydrophobic and hydrophilic molecules. Their formation is driven by a hydrophobic effect, whereby, under specific environmental conditions, the amphiphilic molecules spontaneously self-assemble in a bid to shield their hydrophobic moieties from an aqueous environment.

In vivo, the biodegradable formulations are susceptible to lipolysis by a variety of biological enzymes, including lipases and esterases, likely influencing the release of the actives from their network. In particular, the release of poorly soluble molecules residing in the lipid membrane portions of the phase is limited by the breakdown of the matrix; thus presenting a means for gaining further control and a more sustained release by targeting the matrix stability and its rate of degradation is desirable.

Methods: The aims of the present study were twofold: to evaluate an approach to regulate the rate of degradation of lipid cubic phase drug delivery systems by targeting the enzyme interactions responsible for their demise using a known potent lipase inhibitor; and to study the subsequent drug release profiles from bulk lipid cubic gels using model drugs of contrasting solubility by means of UV-vis spectroscopy and HPLC .

Small-angle X-ray scattering was utilized to study the effect, if any, of incorporating the inhibitor into the lipid membrane of the lipid cubic gels. Standard gravimetric approach was taken to track changes in mass of the gels owed to lipolytic hydrolysis by lipases as a means of quantifying their stability in solution.

Results: Hybrid materials consisting of cubic phases with monoacylglycerol lipids of different chain lengths formulated with a potent lipase inhibitor tetrahydrolipstatin were designed. The structural properties of the novel inhibitor-cubic phase formulations were extensively studied using synchrotron SAXS and demonstrated no negative effect on the internal nanostructure of the phase. To demonstrate the inhibitor effect, modulation of the release of two model pharmaceuticals were studied. It was shown that the stability of the lipid gels in the presence of enzyme could be tuned from approximately 1 week to beyond 4 weeks by increasing the concentration of inhibitor loaded into the gel. Subsequently, the release of the hydrophobic agent could be controlled in this way simply by addressing the digestion of the lipid envelope.

Conclusions: We have demonstrated a novel working system for addressing the susceptibility of these lipid formulations to control the stability and subsequent delivery of hydrophobic molecules without negatively impacting the structure of the phase.

M. Dully, C. Brasnett, A. Djeghader, A. Seddon, J. Neilan, D. Murray, J. Butler, T. Soulimane, S.P. Hudson, *Modulating the Release of Pharmaceuticals from Lipid Cubic Phases using a Lipase Inhibitor*, *Journal of Colloid and Interface Science* (2020), doi: <https://doi.org/10.1016/j.jcis.2020.04.015>.

ENDOCANNABINOID PATHWAYS IN INFLAMMATORY BOWEL DISEASE

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Background:

Inflammatory bowel disease (IBD) is a debilitating, poorly controlled disease affecting 1 in every 210 people in the UK. IBD remains a significant unmet medical need, with most patients ultimately submitting to surgery. The best current treatment agents, such as anti-TNF- α , therapies, come with black box warnings due to potential catastrophic infections and yet only benefit of subset of patients. My PhD research is focused on defining how the balance of pro- and anti-inflammatory lipids in the colon has become disrupted in IBD. Recent data has pointed to the role of hepoxilin A3 (HxA3) and the N-acylethanolamine (NAE) family of endocannabinoids in regulating the state of intestinal inflammation (Szabady et al, 2018, J Clin Invest; <https://doi.org/10.1172/JCI96817>).

Methods:

Levels of both pro-inflammatory HxA3 and anti-inflammatory NAEs were determined in human intestinal samples. Colonic scrapings were taken from ulcerative colitis and healthy patients. For ulcerative colitis patients, both disease affected and un-inflamed colonic samples were analysed. Organically soluble analytes were extracted by solvent extraction and separated using UPLC prior to analysis by electrospray ionisation QTOF MS.

Due to the inherent instability of HxA3, identification of its receptor has remained elusive. I am therefore using a novel protein internalisation method which aims to identify cell surface receptors that are endocytosed upon application of HxA3. The internalised proteins are then identified using mass spectrometry. This innovative method overcomes the major hurdle for baiting a receptor. An orthogonal approach using transmigration assays will validate findings.

Results:

Quantitation of the pro- and anti-inflammatory lipids in human intestinal samples was determined. The ability to measure and correlate HxA3 and NAEs levels with inflammatory status provides the first-ever possibility to monitor and understand the processes of remission and relapse in the inflammatory diseases related to these lipids. Preliminary data show higher levels of HxA3 in inflamed tissues, whilst levels of NAEs show a decrease.

Conclusions:

This research has multiple potential applications, from the development of safer and more effective drug treatments for IBD, to increasing the wider understanding of epithelial cell structure and function in health and disease, which is important for the delivery of pharmaceuticals across the intestinal barrier. Understanding the balance of HxA3 and NAEs lipid levels as they relate to inflammatory status will provide clinical hallmarks that could be used to identify new biomarkers for early diagnosis of IBD.

TACROLIMUS-LOADED CHITOSAN NANOPARTICLES BY A MODIFIED IONIC GELATION TECHNIQUE FOR THE MANAGEMENT OF PLAQUE PSORIASIS

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Background: Polymeric nanoparticles, especially charged ones, were proven to be superior compared to lipidic ones in dermal deposition and skin retention, particularly in inflamed skin(1). Chitosan is a polymer of natural origin and hydrophilic cationic biocompatible nature. Tacrolimus is a natural macrolide that exhibits an anti-proliferative action by T-lymphocytic cells inhibition (2). Hence, it was tested as a potential topical treatment to improve and control psoriatic plaques. Risk of associated systemic side effects embraces the need for efficient topical drug delivery systems to enhance local disposition with minimal systemic absorption. In our study, we successfully incorporated for the first time the lipophilic Tacrolimus into the hydrophilic chitosan nanoparticles in order to achieve the desired therapeutic response and minimize systemic absorption by means of dermal retention of the synthesized nanoparticles using a modified ionic gelation technique that suits the hydrophobic nature of the drug without using any hazardous organic solvents.

Methods: Chitosan nanoparticles were prepared by an initial solubilization of the drug in a water-miscible organic solvent, propylene glycol at 60 C. Then, mixing the solution with tripolyphosphate aqueous solution. Afterwards, the aqueous solution was injected in chitosan solution under vigorous stirring at rpm, 1100. The synthesized colloidal suspension was tested in terms of particle size, zeta potential, entrapment efficiency, rheological behavior, FT-IR, XRD, in vitro drug release, ex-vivo skin permeation and deposition using rat skin on franz diffusion cells. Therapeutic efficacy was tested on imiquimod (IMQ) mouse model and compared to the standard marketed product (Tarolimus[®] ointment).

Results: The hydrophobic drug, Tacrolimus, was successfully encapsulated into the synthesized round-shaped positively-charged particles with particle size (140.84 nm ± 50), Zeta potential (22.2 mv ± 4.06) and EE of (65.45 % ± 1.3). The drug release profile followed the Higuchi diffusion model. Local skin deposition of the drug was significantly enhanced with 82%±0.6 of the drug retained in the skin compared to 34% ±0.9 From the marketed product. This was confirmed with the lower flux rate from the nanoparticles formula (0.932 Ug/cm²/hr) compared to (4.32 Ug/cm²/hr) from the market product. The determined FT-IR spectrum denoted successful drug encapsulation. XRD confirmed the formation of nanoparticles due to the appearance of an amorphous-like chart with no sharp distinctive peaks. The formulation showed superior therapeutic response in vivo compared to the marketed product, in terms of PASI (psoriasis area severity index) score, total drug deposition in skin, spleen to body weight ratio and histopathological examination of skin samples. All results were statistically significant ($p < 0.05$).

Conclusions: The modified ionic gelation technique is successful in formulation of chitosan nanoparticles loaded with hydrophobic drugs by a fast and non-tedious method without using any hazardous organic solvents. Tacrolimus-loaded chitosan nanoparticles are a promising drug delivery system in the management of chronic plaque psoriasis. Further experimentation on human volunteers is suggested as a future perspective.

References: 1. Abdel-Mottaleb et al., Eur. J.Pharm.Biopharm.. 2011;79(1):36–42.
2. Dheer et al.,Eur. J. Pharm. Sci. 2018;114:217–27.

Tuning the Strength & Swelling of an Injectable Polysaccharide Hydrogel, and the Subsequent Release of Nisin, a Broad Spectrum Bacteriocin

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Background: Controlling the release, swelling, mechanical properties and inhibitory activity of an antimicrobial peptide (AMP) from an injectable hydrogel could enable prolonged local treatment of infections. Here, it was hypothesized that tuning the composition of a dextran alginate crosslinked hydrogel via addition of glycol chitosan (GC) while reducing the concentration of alginate-hydrazine would control the release of the AMP from the resulting porous gel network, while preserving the gel strength and integrity.

Methods: Hydrogels of different compositions of dextran (Dex), alginate (Alg) and GC were made via injection of the two polymers through a double-barrelled syringe with crosslinking *in situ* occurring in an attached 21 G needle into a mold. Nisin was encapsulated such that 1 mg was contained in each gel. The release of nisin was studied into mFaSSGF (pH 1.6) and quantified using rp-HPLC. The swelling of the gels was studied in the same FaSSGF media where gels were submerged, taken out and blotted dry at different time points and weighed. Young's modulus was calculated using compression testing with a force of 100 kPa applied to each gel. The *in vitro* biocompatibility was determined using MTT assay on human embryonic kidney cells (HEK293), and *in vitro* antimicrobial activity was determined against *S. aureus* (20231 DSM).

Results: Gels were successfully formed with concentrations of 0, 3 and 6 % GC, when combined with 3, 1.5 and 0.5% Alg (respectively) and 6% Dex. Increasing the concentration of GC and subsequently reducing the concentration of Alg modulated the Young's modulus of the gels from 0.18 kPa at 0% GC to 19.8 and 37.3 kPa at 3 and 6% GC respectively. It was also found that the degree of swelling increased as GC increased, where 0% GC gels didn't swell, whereas 6% GC gels swelled by 250%. Increasing GC also slowed the release of nisin into FaSSGF from a max release of 70% (0% GC, day 12) to 25% (6% GC, day 12). Gels showed inhibition of *S. aureus* for at least 10 days. Interestingly, nisin and GC showed synergistic antimicrobial activity. The addition of GC didn't impact the *in vitro* biocompatibility of the gels (HEK293).

Conclusions: The incorporation of glycol chitosan into an injectable polysaccharide gel allows for modulation of the gels swelling and mechanical properties without sacrifice of physiologically appropriate conditions for an encapsulated antimicrobial peptide. In previous investigations, the mechanical strength of hydrogels has been modulated through means of varying ionic concentrations, rendering the gels unsuitable for sensitive biologics. By introducing GC into these gels, the mechanical strength of the gels was increased through varying GC concentrations. The incorporation of glycol chitosan also does not affect the biocompatibility, and it has been found to act synergistically with nisin in the inhibition of the growth of *S. aureus*. This study introduces a highly tuneable platform for the encapsulation and subsequent release of the AMP nisin for at least 10 days.

Flynn, J., Durack, E., Collins, M. and Hudson, S., 2020. Tuning the strength and swelling of an injectable polysaccharide hydrogel and the subsequent release of a broad spectrum bacteriocin, nisin A. *Journal of Materials Chemistry B*.

MICROSCOPIC SHAPE-DEFINED POLYMERIC DEPOTS FOR PROLONGED AND LOCALIZED DELIVERY OF DEXAMETHASONE AND SIRNA NANOPARTICLES IN POST-TRAUMATIC OSTEOARTHRITIS

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Background: Osteoarthritis (OA) is the most prevalent joint disease and a common cause of pain, functional loss, and disability in older adults. It results from a combination of biomechanical factors and genetic predisposition, affecting the whole joint. In addition to macroscopic features, such as cartilage degradation, subchondral bone remodeling and osteophytes formation, joint capsule hypertrophy, OA is characterized by several cellular and molecular alterations resulting in a chronic low-grade inflammation. Nowadays, there is no treatment for curing this chronic disease by halting or reversing its progression. The only therapeutic options can provide transient relief from the symptoms and allow enhancing temporarily joint mobility and function and joint replacement continues to be ultimately the sole option.

Methods: Within this context, a top-down approach was employed for synthesizing shape-defined poly (D,L-lactide-co-glycolide) (PLGA) microPlates (μ PLs) for local and sustained release of anti-inflammatory molecules, such as Dexamethasone (DEX) and matrix metalloproteinase 13 (MMP-13) RNA interference nanoparticles (siMMP13-NPs). Both formulations were physico-chemical and pharmacological characterized. Their therapeutic efficacy was assessed in a mechanically-induced OA mouse model (PTOA).

Results: μ PLs (square prisms of $20 \times 10 \mu\text{m}$ size), made out of 15 mg of PLGA, exhibited an apparent Young's modulus of $\sim 3 \text{ MPa}$ value of about of $3.1 \pm 0.9 \text{ Pa}$, similar to that of cartilage. Also, they showed a high damping capability ($\tan\delta = 0.3$). Indeed, under confined conditions mimicking the joint capsule, this resulting formulation was able to guarantee a continuous drug release for several months, with $\sim 20\%$ of DEX released in 1 month. The anti-inflammatory activity of DEX-loaded μ PLs was tested *in vitro* on LPS-stimulated chondrocytes (ATDC5). Results demonstrated that DEX- μ PLs reduced the expression of pro-inflammatory cytokines on stimulated ATDC5 at both concentrations tested. At the same time, the therapeutic efficacy of an intraarticular injection of DEX- μ PLs in a murine overload injury model was assessed. Results showed that a single injection of DEX- μ PLs decreased the expression of IL-1 β , TNF- α , IL-6 and MMP-13 by approximately half compared to free DEX at 4 weeks post-treatment. DEX- μ PL treatment also reduced load-induced histological changes in the articular cartilage and synovial tissues relative to saline or free DEX treated knees. At the same time, gene silencing efficiency was obtained by siMMP13-NPs released from μ PLs for the whole duration of the study (5 weeks), preserving 50% silencing after 4 weeks.

Conclusions: Top-down fabrication strategy allowed us to synthesize shaped-defined μ PLs ensuring a sustained drug release for several weeks, to alleviate pain, inflammation, and favor tissue regeneration, and mechanical support of the joint, to minimize wear, cartilage laceration and improper bone remodeling

ARTIFICIAL INTELLIGENCE GUIDED GREEN TECHNIQUE: DEVELOPMENT OF LIPID NANOPARTICLES AND ANTI-PSORIATIC ACTIVITY

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Background: Colloidal lipid nanoparticles (CLN) has become the mainstay for the dermal drug delivery. CLN have established their mark as a biocompatible and biodegradable carrier, convenient for the loading of both the hydrophilic and hydrophobic drug candidates. However, CLN is less explored for the treatment of psoriasis, and the process of production is tedious and expensive, associated with a possibility of contamination and requirement of sophisticated instruments. In the present study, the CLN was prepared by greener and safer alternative technique using microwave irradiation. Tretinoin (TRN), a retinoid, was selected to be entrapped into the CLN. TRN-CLN acts on the fibroblast cells aiding the increase in collagen and simultaneously reducing the progression of inflammation, thus being a valuable treatment for psoriasis.

Methods: The microwave irradiation technique was optimized by the application of artificial intelligence (AI) and machine learning (ML), to reduce the requirement of surfactants and prevent the permeation of TRN into the systemic circulation.

Results: The optimized CLN had a particle size of < 80 nm, and showed a narrow size distribution, had a surface charge of < -35 mV and entrapment of > 98% for TRN. The AI/ML guided process produced CLN within 2 min, the CLN was stable for 12 months and limited the permeation of TRN into the bloodstream with skin retention of 87%. The *in vivo* studies showed a complete absence of irritation associated with the parent TRN and decreased the epidermal thickness, and the hyperkeratosis a hallmark of psoriasis. The histological features showed a remarked decrease in the accumulation of neutrophils, further confirming the non-irritancy of TRN in CLN.

Conclusions: The results of the study demonstrate the utility of microwave irradiation as a greener process for the preparation of CLN and the encapsulation of anti-psoriatic drugs.

THE *IN VITRO*, *EX VIVO*, AND *IN VIVO* EFFECT OF POLYMER HYDROPHOBICITY ON CHARGE-REVERSIBLE VECTORS FOR SELF-AMPLIFYING RNA

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Background: RNA vaccines have surfaced over the last decade as a cost-effective, readily manufacturable vaccine platform able to rapidly respond to any emerging disease threat. They elicit immunity by utilising synthetic RNAs encoding for a specific antigen, hijacking the host cells protein synthesis machinery to trigger vaccine production *in vivo*. However, due to its susceptibility to endogenous nucleases and negative charge, RNA must be packaged and delivered to ensure maximum antigen expression upon administration. Cationic polymers represent a commercially scalable and chemically versatile vector platform, but conventional structure-property relationships found in other gene therapy platforms may not be fully translatable to the unique vaccine setting (administration route, immunogenicity requirements etc.). In this work, we systematically evaluated the effect of polymer hydrophobicity on the expression of a model self-amplifying RNA vaccine in appropriate *in vitro*, *ex vivo* and *in vivo* models. The polymer platform chosen exhibits charge-reversal through self-activated hydrolysis to induce RNA release during delivery.

Methods: A library of six copolymers (P1-6) DP50 containing 50% cationic monomer and varied the content of hydrophobic and hydrophilic monomers to tune polymer lipophilicity were synthesised via RAFT polymerisation. Polyplex formulation was optimised with both saRNA and model DNA and the size and zeta potential measured. Transfection studies were performed *in vitro* on HEK293T cells, in an *ex vivo* skin model and *in vivo* after intramuscular injection using saRNA encoding for luciferase or saRNA encoding for GFP.

Results: All six prepared polymers showed excellent molecular weight control and narrow molar mass distributions ($\mathcal{D} < 1.3$; Table 1). When formulated with DNA, polyplexes with above than 20% HEA content hydrolysed significantly faster than more hydrophobic analogues evidenced by the negative zeta potential (-10 mV) and low particle size over the 7 d experiment in pH 7 buffer (Figure 1). Transfection studies revealed that more hydrophobic delivery vectors (P1 & 2) exhibited 30 fold higher activity than hydrophilic vectors and PEI *in vitro* albeit with relatively high cytotoxicity. When applied *in vivo* however, the hydrophilic vectors (P6) performed best. Surprisingly, we observed that charge reversal plays a small role, and transfection efficiency is largely driven by membrane interaction.

Conclusions: Overall our results indicate that *in vitro* and *ex vivo* transfection follows the expected hydrophobicity trend compared to the literature, however *in vivo* we observed the opposite trend likely due to the increased toxicity of more hydrophobic vectors. We anticipate these findings will help direct future design of polymeric materials designed to efficiently deliver self-amplifying RNA vaccines.

FORMULATION AND EVALUATION OF CLARITHROMYCIN BEADS FOR THE TREATMENT OF HELICOBACTER PYLORI INFECTION

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Background: In the last decade, the number of cases suffering from stomach disease such as peptic ulcer and gastric cancer due to *Helicobacter pylori* infection has increased by far. An objective of the present study was to develop chitosan/gellan based floating-mucoadhesive beads of clarithromycin to provide prolonged contact time of antibiotic to treat stomach ulcer. Floating-mucoadhesive beads were prepared ionotropic gelation of chitosan/gellan beads and characterized for in vitro performance.

Methods: 1% of chitosan solution was made and then clarithromycin was added. Chitosan beads were made by simultaneous cross-linking in anionic 1% La gellan solution.

Results: The beads were spherical and roundness in the range of 0.4 to 0.6, but no significant difference was observed in these parameters. The mean particle size of beads obtained was in the range of 0.70 to 1.1 mm. Chitosan beads clarithromycin coated with were evaluated for drug release in 0.1 N HCl, pH 1.2. the prepared beads show less than 30% drug release at the end of 1 hour. The delay may be due to thick gellan coating that slows down the drug diffusion.

Conclusions: In this study we have demonstrated that the prepared controlled release drug therapy for the treatment of *H. pylori* using chitosan beads coated with gellan. gellan-coated chitosan beads containing clarithromycin showed complete growth inhibition of *H. pylori*. Thus, gellan-coated clarithromycin chitosan beads might be a promising drug delivery system for the treatment of *H. pylori* infection.

ENHANCING TRANSDERMAL DELIVERY USING A ONE-STEP HYDROGEL FORMING MICRONEEDLE DEVICE: A CONCEPT STUDY

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Background: Hydrogel-forming microneedles (MN) consist of drug-free, micron scale polymeric needles situated in perpendicular orientation on a base plate to which a separate drug containing reservoir is attached. In its current form, this MN device requires the addition of water (10 μL) prior to skin insertion both *in vitro* and *in vivo* to permit the adhesion of the drug containing reservoir to the MN. Evidently, this current method of application would not be feasible in a clinical setting. For this reason, this concept study aims to develop a hydrogel-forming MN device which can be inserted into the skin in a one-step process.

Methods: In this study, a 3D printed TPU 95A housing was created, into which the MN, lyophilised wafer containing a model compound and a neatly single folded water-containing reservoir were added. These components were then secured in place using a 3D printed lid. Parafilm[®] M and polyethylene were considered appropriate polymeric films for the production of water-filled reservoirs. Using a heat-sealing method, individual sheets of both polymeric films were cut to size (60 x 26 mm) and folded over once. The two adjacent sides were heat sealed for 10 secs, followed by the addition of 600 μL of water. The top opening was folded and heat-sealed to produce a water-tight polymeric pouch. The volume of water released from both films was quantified following application of a 32 N force for 30 secs. MN insertion into an artificial membrane was also tested using two different hydrogel-forming MNs, comprised of 400 μm and 600 μm needle heights respectively.

Results: To quantify the volume of water released from Parafilm[®] M (PR) and polyethylene (PER) reservoirs, the mass of a single 3D printed MN device containing the polymeric pouch was recorded before and after application of a 32 N force. In this case, the mass difference represented the volume of water released. Although both reservoirs were of the same size and encased the same volume of water, PR was observed to release a statistically greater volume of water ($383 \pm 26.7 \mu\text{L}$) compared to PER ($294 \pm 32.2 \mu\text{L}$), equivalent to a total % water release of $63.86 \pm 4.45\%$ and $48.97 \pm 5.37\%$ respectively ($p = 0.0209$). To determine the ability for MNs to insert into an artificial skin membrane using a one-step application, two different MN types comprised of different needle heights, namely 400 μm and 600 μm were tested. After applying a 32 N force for 30 secs to a 400 μm MN, 100% needle insertion into the first layer (126 μm) of Parafilm[®] M was observed with all four setups, namely PR design, PER design, MN & wafer and MN alone. Furthermore, there was no significant difference between all four setups in the second layer and third Parafilm[®] M layer ($p > 0.05$). 100% insertion in layers 1 and 2 was observed with all four setups using 600 μm MNs. Although the PR design displayed the highest percentage insertion in layer 3, there was no significant difference between all four setups ($p = 0.2527$). Again, in layer 4, representing an insertion depth of 504 μm , no significant difference in needle insertion was observed between all four setups ($p = 0.3912$).

Conclusions: With the end user at the forefront of this study, a 3D printed MN housing was designed to permit a one-step application process. Comparing 2 different polymeric films, it was observed that PR released a greater volume of water following application of a 32 N force. In addition, it has been shown that this delivery device does not adversely affect needle insertion into an artificial skin membrane. The next phase in this study will include *in vitro* testing of model compounds to determine whether the greater volume of water released from this design enhances or adversely affects permeation across dermatomed neonatal porcine skin when compared to the current two-step application process.

DEVELOPMENT OF A NOVEL DRUG DELIVERY PLATFORM TO TARGET NLRP3 INFLAMMASOME AND ITS TREATMENT IN POST-TRAUMATIC OSTEOARTHRITIS

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Background: Post-traumatic Osteoarthritis (PTOA) occurs after a joint injury, such as a fracture, cartilage tear or ligament damage. Chondrocytes respond to joint injuries by releasing inflammatory mediators which drive PTOA. Activation of Nod-like receptor family protein 3 (NLRP3) inflammasome through NF- κ B signalling induces Interleukin-1 β (IL-1 β) secretion and mediates inflammatory cell death. MCC950 is a selective NLRP3 inflammasome inhibitor and prevents IL-1 β secretion. Current pharmacological treatment options for PTOA primarily focus on symptomatic improvement of the pain, yet targeting immune mediators using MCC950 may enhance therapeutic outcomes or prevent/reverse injury. Here, we hypothesise that a local drug delivery device capable of delivering MCC950 to the joint space will demonstrate therapeutic efficacy in PTOA.

Methods: 3% Poly Lactic-co-Glycolic acid (PLGA) 75:25 (molecular weight 4000-15000) and 1mM MCC950 were spray-dried using dichloromethane. MCC950 release was measured in PBS (37°C) using HPLC. Macrophages were primed with lipopolysaccharide (LPS) for 3 hours. Cells were treated with 50mM MCC950 and MCC950 released from particles for 40 mins and then stimulated with ATP for further 45 minutes. Cells were tested for cytotoxicity and cultured supernatants were assayed for the levels of secreted IL-1 β by ELISA. To explore the crosstalk between inflamed macrophages and chondrocytes, supernatants from inflamed macrophages treated with/out MCC950 were added on healthy chondrocytes for 24 hours. PCR on chondrocyte markers was used to analyse chondrocyte functionality (collagens, aggrecan, MMP13, ADAMTS4).

Results: Spray-dried PLGA microspheres provided sustained release of MCC950 with 30 μ g released by day 4. LPS is a potent activator of macrophages, and triggers the release of various cytokines including IL-1 β . MCC950 inhibits LPS-induced IL-1 β secretion in macrophages. Both 50 μ M MCC950 and MCC950 released from PLGA particles inhibited pyroptosis significantly and reduced cytotoxic effects on inflamed macrophages. Furthermore, IL-1 β release from inflamed macrophages inhibited chondrocyte collagen formation and increased inflammatory protein level MMP-13. MCC950 (both pure drug and MCC950 released from PLGA particles) reversed these effects.

Conclusions: Spray-dried PLGA microspheres providing sustained release of MCC950 were successfully developed. MCC950 released from PLGA particles remain strongly bioactive and inhibited LPS-induced IL-1 β release from inflamed macrophages. At the same time, MCC950 protected chondrocytes from the adverse effect of IL-1 β released from inflamed macrophages. Our study highlights the potential of MCC950 as a potential drug delivery candidate for targeting IL-1 β , which is believed to contribute to the pathogenesis of PTOA. Future work will test these particles in an in vivo PTOA model.

**DESIGN, PREPARATION AND OPTIMIZATION OF ELASTIC TRANSFERSOMES :
A NEW TECHNIQUE FOR TRANSDERMAL DELIVERY OF CLOTRIMAZOLE****Rashad Kaoud¹, Mohammed H. Mahdi², Jamal Alkrad³, Eman Heikal¹**¹Pharmacy Department, Ashur University College, PO Box 10047, Bagdad, Iraq.² School of pharmacy, University of Ahal Bayt, Karbala, Iraq, 11003³Faculty of Pharmacy, Isra University, PO Box 22 and 23, Amman, Jordan

Background: Clotrimazole (CTM) is an imidazole anti-fungal drug which can be used in treatment of candidiasis. Poor aqueous solubility and hepatic first pass metabolism result in low systemic efficacy of CTM. The aim of the present study was to formulate and evaluate Clotrimazole transfersomes (CTM-TF) gel to increase skin permeability and enhance antifungal activity of CTM -TF gel. Canesten® cream 1% where used as a reference.

Methods: CTM-TF different formulae (F1 – F8) were prepared by lipid film hydration technique with slight modification. Different formulations were characterized for vesicle size, zeta potential, polydispersity, entrapment efficiency (EE %) and elasticity measurement. The time required to release 90 % of CTM from TF (t_{90}) was calculated for each formula. The formula showed the least value of (t_{90}) was selected as the optimized formula as it exhibited long duration of action. The optimized formula (F3) was formulated as gel using Hydroxypropyl Methyl Cellulose (HPMC E15) 2% and examined in comparison with a marketed product (Canesten® cream 1%) for spreadability, homogeneity, viscosity measurement, drug content, in-vitro permeation, in-vitro anti-fungal activity, in-vivo anti-fungal activity and Pharmacokinetic study.

Results: The formulated CTM-TF had EE % from (68.55 ± 0.45) to (90.56 ± 0.62) with vesicle size ranged from (64.52 ± 0.24) to (85.42 ± 0.78). The in-vitro release study showed an inverse relationship between EE % and in-vitro release. The kinetic analysis of all in-vitro release formulations followed Higuchi's diffusion model. The optimize formula showed higher antifungal activity than marketed product. Therefore CTM-TF gel can penetrate the skin, overcoming stratum corneum barrier to treat deep fungal infections.

Conclusions: From this study, Formulated CTM-TF have high EE %, low vesicle size and high in-vitro release. So, it could be formulated as gel to penetrate stratum corneum and show high anti-fungal activity compared to marketed product.

DEVELOPMENT OF A GENE-ACTIVATED SCAFFOLD FOR THE DOWNREGULATION OF ANTI-CHONDROGENIC SIGNALING PATHWAYS IN THE PROGRESSION & DEVELOPMENT OF OSTEOARTHRITIS

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Background: Biomaterial-mediated delivery of siRNA therapeutics provides a method of silencing specific genes known to hinder the tissue regeneration process while also providing a structural support for cell proliferation and matrix deposition [1]. The objective of this study was to use a cell penetrating peptide with a glycosaminoglycan-binding domain (GET) as a non-viral gene delivery vector [2], combined with a previously optimised collagen-hyaluronic acid (coll-HyA) with proven chondrogenic benefit [3] for the development of an advanced delivery system. The overall goal of this study is the manipulation of the early osteoarthritic microenvironment in 2D and 3D cell culture systems, allowing for favourable conditions more conducive to successful stem cell recruitment and chondrogenic differentiation.

Methods: Varying formulations of GET-siRNA complexes were analysed based on size, charge, morphology and encapsulation efficiency using dynamic light scattering, transmission electron microscopy, nanoparticle tracking analysis and gel retardation assays. A fluorescent transfection indicator was used to assess cellular uptake using live cell imaging and flow cytometry. Reporter gene knockdown was assessed using RT-qPCR. Optimised formulations of GET-siRNA were used to deliver siRNA against the p65 subunit of the NF-κB pathway (RELA/p65) to human MSCs in a simulated pro-inflammatory environment (Media + IL-1β/TNF-α). RT-qPCR was used to assess expression of downstream chondrogenic and OA-associated mediators. GET-p65 siRNA formulations were incorporated into porous coll-HyA scaffolds and scaffold architecture was assessed using SEM. hMSCs were cultured on gene-activated scaffolds (3D *in vitro*) in the presence of inflammatory cytokines and activity of downstream mediators was assessed.

Results: An optimized formulation of GET with siRNA resulted in nano-complexes of favourable size (146.0 ± 23.63 nm), zeta potential (32.32 ± 5.5 mV), and encapsulation efficiency ($87.8 \pm 0.1\%$). *In vitro* (2D) screening demonstrated efficient cellular uptake of GET-siRNA nano-complexes and successful reporter gene knockdown in a sustained yet transient manner. Successful knockdown of the p65 subunit attenuated cytokine mediated activation of the NF-κB pathway, preventing nuclear translocation, dampening downstream catabolic mediator expression (MMPs 3, 9, 13), and recapitulating chondrogenic transcription factor activity (SOX9). SEM imaging and release assay highlighted successful incorporation of GET-siRNA nano-complexes within the coll-HyA scaffolds. hMSCs seeded (3D) on gene-activated scaffolds in the presence of inflammatory cytokines further demonstrated successful silencing of the NF-κB pathway ($71.11 \pm 0.22\%$ @ Day 3) and a significant reduction in downstream catabolic mediators (MMPs 3, 9, 13), corroborating results observed in 2D culture systems.

Conclusions: This study demonstrates the successful development of an advanced gene-activated scaffold delivery system capable of manipulation of the early OA microenvironment through the controlled delivery of therapeutic siRNA, creating a promising environment more conducive to stem cell recruitment and chondrogenic differentiation.

MANUFACTURE OF DISSOLVING MICRONEEDLE LOADED WITH NANOSUSPENSION: POTENTIAL FOR PROLONGED LOCAL ANTI-INFLAMMATORY EFFECTS

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Background: Nonsteroidal anti-inflammatory drugs (NSAIDs) are some of the most commonly used drugs at present. Approximately 30 million people consume NSAIDs every day around the world. NSAIDs block the effect of cyclo-oxygenase (COX) enzymes to ease the pain and inflammation. Diclofenac (2-(2,6-dichloranilino) phenylacetic acid) belongs to NSAIDs and it is prominently used in osteoarthritis, ankylosing spondylitis, rheumatoid arthritis treatments and some post-operative pain management. This project involves loading diclofenac nanosuspensions into dissolving microneedles (MNs) to achieve anti-inflammatory effects.

Methods: Diclofenac nanosuspension (NS) was manufactured by beads milling methods. A 7 mL glass vial contains 5 mL polymer blends composed of 9-10 kDa poly (vinyl alcohol) (PVA) and K-29/32 poly (vinyl pyrrolidone) (PVP). 200 mg of diclofenac drug powder and 2 mL of 0.1 mm beads were added into the glass vial. Four 12x6 mm stir bars in the vessel rotated at 1200 rpm. The particle size of diclofenac was measured at different time points. After 20 hours, the milling process was stopped and the final particle size of NS was measured by DLS. Following the milling process, water was removed from the NS by freeze-drying the formulation. Dissolving MNs were cast through a two-step process. The first layer formulations of MNs were freeze-dried diclofenac NS mixed with water. The second layer was a drug-free polymer baseplate which could be formed by RS PRO PLA or 40% K90 PVP gel. Moulds used to make MNs were round silicon moulds contained 600 pyramidal needles with a height of 750 μm , 14x14 moulds with conical needles (500 μm), 19x19 moulds with pyramidal needles (500 μm) and 16x16 silicon moulds with pyramidal needles (850 μm). Formed MNs were left at room temperature to dry. Dried MNs were dissolved in 5 mL water and sonicated until uniform solutions were obtained. Then 100 μL solutions was diluted to 1 mL by ACN and filtered into HPLC vials by 0.2 μm PTFE membranes. The drug contents of MNs were quantified by HPLC. At the same time, 1 mL dissolved MN solutions were diluted to 3 mL by water in cuvettes. The particle size of diclofenac was measured by DLS. In the insertion study, MNs were placed into 37 °C oven for more than 30 mins and then were applied on the full-thickness porcine skin for 30 seconds. Insertion performances were observed under OCT.

Results: The diclofenac drug powder had a particle size of 114 microns. During the milling process, after 3 hours, diclofenac NS had a particle size of 281.97 ± 21.99 nm (n=10) and after 6 hours had a particle size of 241.53 ± 11.87 nm (n=10). After 20 hours, the particle size of diclofenac NS decreased to 192.85 ± 12.86 nm. Particle size was retained during freeze-drying. All four types of MNs were well-formed and had good insertion performances. The MNs with PLA baseplate dissolved quicker. Also the particle sizes of diclofenac in dissolved MNs with PLA baseplate retained as the original sizes. However MNs with PVP baseplate were difficult to dissolve and particle sizes increased compared with original sizes. The drug content of round MNs (750 μm) was 2.3mg (n=5) and it could increase to 3.1mg (n=3). For 14x14 MNs (500 μm), the drug content was 1.3mg (n=6). 19x19 MNs (500 μm) had 1.2mg (n=6) drug loading and 16x16 MNs (850 μm) had 2.3mg (n=6) drug loading.

Conclusions: NS of diclofenac were successfully fabricated *via* beads milling method and significantly decreased the particle size of diclofenac. Four types of dissolving MNs with two different baseplates were well-formed and showed good insertion properties. The MN contained 600 pyramidal needles (750 μm) had the highest drug loading. Further studies are now needed to assess the *in vitro* release and delivery of the drug from these MNs.

Dissolving microneedle-mediated long-acting drug delivery of rotigotine formulations

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Background: Parkinson's disease (PD) is one of the most common neurodegenerative central nervous system (CNS) diseases currently affects approximately 10 million of people all over the world. It is characterised by the loss of dopaminergic neurons. Rotigotine (RTG) is a typical non-ergoline dopamine agonist with a preference for D3 receptors and the first approved transdermal medication for the treatment of PD. It has a half-life of 5-7 hours and 37% bioavailability. LogP and pKa values are 4.3 and 10.03, respectively. RTG transdermal patch (Neupro[®]) is a once-daily administered treatment with the dose ranging from 2-18 mg/day). Thus, potential improvement in frequent administration and relatively low bioavailability are two focuses in future RTG researches. Microneedle (MN) arrays are painless transdermal drug delivery systems, which enables the direct penetration of *Stratum Corneum* (SC). The aim of this project is to develop dissolving MNs loaded with RTG formulations, which intends to form an intradermal depot under the skin and further be released into the systemic circulation.

Methods: A quantification method for RTG was first developed. A 'top-down' beads milling method was applied to prepare the RTG NS. A 7 ml glass vial was utilised as the milling chamber. Two types of magnetic stirring bars, namely 25 x 8 mm and 12 x 6 mm bars were compared in terms of resulted polydispersity index (PDI) and particle size. PVA (9-10K Daltons), PVP (K-29/32), Poloxamer 188 and Tween 80[®] were screened based on their influences on PDI and particle size. Three types of beads with the diameter of namely 0.1 mm, 0.4 mm and 1.1 mm were compared as well. The milling chamber was placed on a magnetic stirrer for 24 h with the speed of 1200 rpm. Prior to lyophilisation process, PVP (K-29/32) was added to the NS as the cryoprotectant in a 1:1 weight ratio with stabiliser content. Dissolving MNs were manufactured by a two-layer casting technique. The mould contained 16 x 16 needles with the height of 850 µm. An excess of drug-containing layer, which composed of 95 mg lyophilised RTG-NS powder mixed with 200 µL deionised water with the aid of SpeedMixer at 3000 rpm for 5 mins, was spread over the moulds center and pressure chamber was applied for 2 mins to push the formulation towards the needle tips. After the removal of the excessive first layer formulation, another 30 mins of 5 bar pressure further ensured the accumulated drug content in the needle tips. The second layer was cast by addition of 850 µL 30% w/w PVP (K-90) and 1.5% w/w glycerol mixture on the top, followed by centrifuge at 3500 rpm for 2 mins. The MN was then left to dry in the room temperature for 48 h and characterised by the light microscope.

Results: A specific and sensitive HPLC-UV quantification method for RTG was successfully developed and validated according to International Council of Harmonisation (ICH) Q2(R1). Limit of quantification (LoQ) was 2 µg/ml and limit of detection (LoD) was 0.67 µg/ml. The RTG-NS was manufactured by 5 ml 1% w/w PVA (9-10K Daltons) solution as the stabiliser, 200 mg RTG and 2 ml 0.1 mm beads. The NS particle size was 307.17 ± 1.25 nm with the polydispersity index (PDI) of 0.174 ± 0.02 (n=3). The manufactured RTG-NS MNs were well-formed in terms of morphology and possessed good mechanical strength for insertion.

Conclusions: A specific and sensitive quantification method was successfully developed and validate. The manufacture method for RTG NS was developed and successfully loaded into dissolving MN by a two-layer casting technique. In the next stage, optimised RTG-NS and RTG powder will be loaded into MN for comparison in terms of drug loading efficiency, MN characteristics and *in-vitro*, *in-vivo* release profiles.

BIOMOLECULAR CORONA AND THERAPEUTIC LIPID NANOPARTICLES: A WORKFLOW FOR HIGH-THROUGHPUT CORONA ISOLATION AND ANALYSIS

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Background: Lipid nanoparticles (LNPs) as an excellent delivery platform for gene therapy, are increasingly utilized into routine clinical practice. To optimize the delivery efficacy in various diseases, it is necessary to acquire a mechanistic understanding of how LNPs adapt to the biological systems in both normal physiological and pathological states. A key aspect of how the biological systems affect LNPs performance, and vice versa, is the formation of a corona around the nanoparticles when they contact biological fluids. Most nanoparticles acquire a corona of biomolecules derived from the biological context they are exposed to. The formation of the biomolecular corona is believed to create a new biological identity for nanoparticles, and this is what biological systems actually perceive, rather than the pristine, uncomplexed nanoparticles. However, a solid method for rapid and high-throughput corona isolation for clinically relevant LNPs from plasma proteins, extracellular vesicles, and lipoprotein particles is still absent.

Methods: A clinically relevant lipid nanoparticle formulation was employed to understand their corona composition within the lean and obese animal plasma. To address the separation of LNPs from lipoproteins, extracellular vesicles and plasma proteins, a novel affinity-based magnetic isolation protocol was developed, without modifying the LNPs formulation. Following corona isolation, physiochemical characterization and proteomics procedures were used to evaluate the efficacy of the corona isolation.

Results: The corona harvest of up to 96 samples were achieved within 50 minutes, requiring a minimal amount of LNP formulation. A clear separation between the LNPs/corona complex from plasma components was successfully achieved. To date, this workflow identified significant differences in coronal apolipoproteins, glycoproteins, and lipids between lean and obese conditions.

Conclusions: The workflow became our handle to tailor LNPs for specific therapeutic contexts. It allows us to quantify the interactions between nanoparticle features (e.g. formulation methods, novel lipids selection, helper lipids selection, components ratio matrix), and hundreds of coronal components and, how this affects the delivery system function. It holds great promise to improve LNPs' efficacy, reduces side effects, and lower costs.

DEVELOPMENT OF IN VITRO TRANSCRIBED mRNA THERAPEUTICS FOR CYSTIC FIBROSIS

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Background:

Cystic fibrosis (CF) is a recessive disease that affects approximately 10,000 people in the UK. The disease is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). Absent/non-functional CFTR leads to an imbalance of sodium, chloride and bicarbonate ion transport, and production of thick, sticky mucus in the lung which results in chronic bacterial infection and inflammation. Gene replacement therapy with viral/non-viral vectors has been explored in the last 25 years but all failed to show significant clinical efficacy. *In vitro* transcribed (IVT) mRNA has emerged in the last few years as a new approach for protein replacement. IVT mRNA offers potential advantages of greater efficiency of expression as mRNA delivery is only required to the cytoplasm rather than the nucleus, and safety as there is no risk of genomic integration.

Methods:

We are developing CFTR IVT mRNA therapy for CF to replace to wild type CFTR protein using receptor-targeted nanocomplex (RTN) formulations. RTNs consist of liposomes, and lung epithelial cell specific receptor targeting peptides and nucleic acid. We have optimised the ability of RTNs to transfect primary cystic fibrosis bronchial epithelial (CFBE) cells at submerged culture, air-liquid interface (ALI) culture and mouse lung using luciferase and GFP reporter IVT mRNAs. CFTR mRNA was also delivered and the transfection efficiency was assessed by Western blot.

Results:

We first optimised the RTN formulation, comparing combinations of three different cationic liposomes and five peptides. As a result, we identified a novel formulation for mRNA delivery that achieved almost 100% cellular uptake efficiency, and 90% transfection efficiency, compared to a maximum of approximately 20% with a plasmid reporter. The optimised formulations were able to deliver luciferase and GFP mRNA in ALI cultured cells and mouse lungs. CFTR IVT mRNA was successfully delivered to a lung epithelial cell line. 16HBE14o-, primary normal epithelial cells (NHBE) and CF epithelial (CFBE) cells. In addition, we found co-delivery of the commercial drug, corrector of CFTR: Lumacaftor (VX-809) improved the expression or stability of CFTR protein in CFBE cells and non-CF 16HBE14o- cells in submerged culture. Moreover, CFTR protein expression was shown to be upregulated in ALI culture of CFTR cells transfected with RTN CFTR mRNA by Western blot.

Conclusions:

IVT mRNA of CFTR delivered by RTNs is a promising novel therapeutic for cystic fibrosis. In addition, the flexibility of lipoplex allows co-delivery of CFTR mRNA with Lumacaftor, which leads to significantly improved CFTR expression.

***In Situ* Gelation of Low acyl Gellan Gum powder for nasal drug delivery**

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Background: Recently, nasal drug dosage form has gained a great attention over the last few decades because of its great potential utility for both local and systemic drug delivery. However, the protective feature of the nasal cavity make intranasal delivery challenging. Therefore, in this study an attempts was made to design a novel drug delivery to sustain drug action at nasal cavity using *in situ* LA gellan gum and caffeine as powder formulation. Caffeine was used as model drug in this study.

Methods: The formulations were prepared by adding different concentration (0.1, 0.25, 0.5 and 1%) of LA acyl gellan gum to deionized water heated to 85 °C while stirring. Once fully dissolved the solution was cooled to ~60 °C and then, caffeine (100 mg/ml) was added. The formulations were then dried by spray drying. The rheological behavior of the rehydrated samples were evaluated in terms of the elastic (storage) modulus (G') and the viscous (loss) modulus (G'') as a function of angular frequency (0.1–100 rad s⁻¹ angular frequency) to produce mechanical spectra of the samples. Measurements were taken at 34 °C and performed at 1% strain (strain amplitude chosen was within the linear viscoelastic region of the sample). Bespoke mucoadhesion apparatus was used to predict drug retention time from dry powder formulations. PBS was then perfused over the dialysis tube surface at flow rate of 1 mL/min. The PBS perfusate was collected at time points upto 60 min and caffeine content was measured using spectrophotometer at wavelength of 272 nm.

Results: Dynamic small deformation oscillatory measurements of G' and G'' highlight the viscoelasticity of the 0.25, 0.5 and 1% with G' slightly greater than G'' across a range of frequencies; this is typical 'weak gel' rheological behavior. To investigate the mucoadhesion properties of rehydrated gellan powder, the release of caffeine from formulations at different concentration were studied. 0.1 % LA gellan shows almost 96% of drug released after 20min; whereas 1% LA gellan shows only 40% drug release at the same time point with the 0.25 and 0.5 % of these two polymers releasing 80 and 60 % respectively after 20 min.

Conclusions: In this study we have demonstrated that a mucoadhesive property of gelling nasal spray has the potential to be formulated using gellan gum powder and the elasticity of the rehydrated gel after dispensing great enough to adhere to the mucosal membrane.

TARGETING POLYMERIC CONJUGATES WITH BIOLOGIC-RESPONSIVE PROPERTIES TO MANUFACTURE DOCETAXEL-LOADED NANOPARTICLES FOR GLIOBLASTOMA CHEMOTHERAPY

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Background: Glioblastoma is the most lethal brain cancer, with a median survival time of only 15 months. Docetaxel is one of the most effective chemotherapeutics against glioblastoma, although it presents pharmacokinetic constraints mainly due to its low solubility and poor blood-brain barrier (BBB) permeation. This project proposes a targeted, biologic-responsive nanomedicine to circumvent these inadequacies based on docetaxel-loaded nanoparticles for glioblastoma treatment. The developed nanomedicine comprises a poly(lactic-co-glycolic) acid (PLGA) core and a polyethylene glycol (PEG) shielding of long- and short-length. The long-length PEG possesses an Angiopep-2 moiety for BBB targeting (binding to the low-density lipoprotein receptor) and is able to dissociate in the acidic pH of BBB endosomes, hence sterically de-protecting the short-length PEG coupled with L-histidine for further glioblastoma targeting (binding to the L-type amino acid transporter 1) upon brain arrival.

Methods: Chemical strategies based on carbodiimide, hydrazone formation via Schiff base reaction and Thiol-Michael addition were employed to synthesize the **PLGA-acid-cleavable, long-length PEG-Angiopep-2** and **PLGA-short-length PEG-L-Histidine** polymeric conjugates that will constitute the nanoparticle matrix. These polymeric conjugates were further characterized by different techniques such as nuclear magnetic resonance (NMR), Fourier-transform infrared spectroscopy (FTIR) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Preliminary work has focused the production of docetaxel-loaded nanoparticles post-polymer synthesis through a scale-up microfluidic manufacturing technique.

Results: The chemical synthesis achieved a total conjugation efficiency value of around 70% and 90% for the **PLGA-acid-cleavable, long-length PEG-Angiopep-2** and **PLGA-short-length PEG-L-Histidine** polymeric conjugates, respectively, as demonstrated by NMR calculations. FTIR confirmed the successful reactions by elucidating the formation of intermediary bonds between the constituents of the polymeric conjugates, and MALDI-TOF confirmed the different ionization behaviors and proved the presence of PLGA and PEG monomers in the structure of the polymeric conjugates. The physicochemical characterization of docetaxel-loaded nanoparticles manufactured through the microfluidic technique demonstrated around 100 nm average size, 0.1 polydispersity index and 10% drug loading.

Conclusions: Overall, this work has allowed, so far, the synthesis of targeted, biologic-responsive polymeric conjugates with high conjugation efficiency (>70%) and suitable to manufacture low size, monodisperse and highly loaded docetaxel nanoparticles in a microfluidic technique with potential to scale-up the batch size. Future work will be dedicated to test the efficacy of the developed targeted, biologic-responsive nanomedicine in vitro and in vivo. The current need to accelerate drug delivery to glioblastoma, bypassing the BBB and targeting tumor tissue of brain, places this system in a privileged position in the field of translational nanomedicines. This work also lays foundation for future targeted, biologic-responsive delivery of other therapeutics to a range of pathologies.

ANTI-INFECTIVE CATHETER FABRICATION THROUGH ADDITIVE MANUFACTURING

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Background: Catheter associated infections (CAI) are one of the most common issues associated with patients on dialysis. The present approach to prevent CAI involves the use of coated catheters, which are often coated with heparin, silver nanoparticles or pyrogallol. Coatings add another step to the manufacture process and also require a balance between thickness of coating and sufficient drug release. In this project, we set out to create catheters using additive manufacturing (AM) for the first time to our knowledge, specifically by Fused Deposition Modelling (FDM) 3D Printing. AM offers the potential to create patient specific catheters, with the potential to varying the drugs loaded in the filaments through hot-melt extrusion (HME).

Methods: Antimicrobial filaments were created using HME, which is a common process used to create filaments for FDM by combining pressure and heat to melt polymer pellets, with the possibility of addition of drug. Tetracycline (TC) was mixed with thermoplastic polyurethane (TPU) pellets and extruded to form filaments with antimicrobial properties. TC concentrations of 0.25, 0.5 and 1% were used. Designs were made using computer aided design (CAD) and sliced using Cura software. The antimicrobial filaments at varying concentrations were used to print the catheter designs using the Ultimaker 3 FDM printer. Release studies were carried out using the printed catheter constructs in phosphate buffered saline (PBS), microbiology studies, thermal analysis, contact angle goniometry (CAG), attenuated total reflection-Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM and X-ray microcomputer tomography (μ CT) analysis were conducted on the printed catheters and TC containing TPU materials.

Results: Results showed that there was sustained release of TC from the catheters over a 2-week period with around 4% of the total drug load being released after 10 days and the drug was evenly distributed throughout the TPU matrix. Microbiology results showed that the catheters had an inhibitory effect on *Staphylococcus aureus* NCTC 10788 bacteria and prevented the bacteria adhering to their surface. Furthermore, catheters containing 1% TC maintained inhibitory effect after 10 days of releasing TC in PBS and also presented a reduction in bacterial adherence of up to 99.7%.

Conclusions: The results in this study shows that TC can be effectively added to TPU to produce 3D printed antimicrobial catheters. Only one application of this process is outlined above, different drug eluting materials can be created using the same method and later used to created medical devices by 3D printing.

TRANSLATIONAL POTENTIAL OF MICROARRAY PATCHES CONTAINING AMOXICILLIN SODIUM: A PRIMARY PACKAGING STUDY

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Background: With the recent advances in microarray patch (MAP) technology and a potential move towards commercialisation of MAP products, there is a need to address issues surrounding the translation of MAP technology from the laboratory setting to that of the end-user. One important aspect of MAPs moving forward is appropriate primary packaging. This research focuses on amoxicillin (AMX)-containing MAPs. These MAPs are currently being explored for their potential role in the treatment of neonatal sepsis in humid and hot countries. In this work, a MAP consists of a hydrogel-forming microneedle (MN) and a drug-containing reservoir. Improper primary packaging in humid and hot countries may result in degradation of active drugs, with the use of substandard medicines a major health concern. AMX is inherently unstable, as hydrolysis readily occurs, due to the presence of a labile β -lactam ring. The research presented here, seeks to investigate the effects of primary packaging on MAP integrity, MAP physical characteristics and AMX recovery from AMX-containing MAPs.

Methods: MNs were fabricated from aqueous blends containing 15% w/w poly(vinyl alcohol), 10% w/w poly(vinyl pyrrolidone) and 1.5% w/w citric acid. Using a manual hydraulic press, reservoirs were prepared using 95% w/w AMX and 5% w/w sodium starch glycolate. MAPs, MNs and reservoirs were then packaged and stored under accelerated storage conditions (40°C and 75% RH) for 168 days, in accordance with international guidelines. The control cohort was MAPs, MNs and reservoirs left unpackaged. At pre-defined intervals, the insertion capabilities of MNs were investigated, using a previously-validated skin simulant, Parafilm M[®]. Physical characterisation and AMX recovery from the reservoirs were also conducted.

Results: Major causes of drug instability are moisture and temperature. To avoid unnecessary degradation, two semi-impermeable primary packaging, in terms of a barrier to moisture and heat was sought. MAPs in Protect(470) foil demonstrated that measurable amounts of AMX didn't migrate into attached MNs. At all-time intervals tested, MNs packaged in Protect(470) foil could insert into 3 layers of Parafilm M[®]. For example, after 168 days of storage, $21.42 \pm 6.80\%$ holes were created in layer 3 of the skin simulant, which is consistent with results from previous studies. Reservoirs demonstrated uniform physical dimensions when packaged in Protect(470) foil. This wasn't the case for reservoirs packaged in Poly(ester) foil. After 168 days, the % of AMX recovered from reservoirs packaged in Protect(470) foil was $103.51 \pm 7.03\%$. However, packaged in Poly(ester) foil, the AMX content significantly ($p < 0.0001$) decreased, which is likely due to the degradation of AMX by the imbibed moisture.

Conclusions: Primary packaging is imperative in maintaining the efficacy and stability of labile medicines and MAPs. For the first time, this study evaluates AMX-containing MAPs in different primary packaging. Of the two different types of primary packaging investigated, the results are very promising for MAPs packaged in Protect(470) foil in terms of moisture barrier function and temperature resistance. This work indicates the importance of investigating the storage stability of other drug-containing MAPs, to ensure they are 'fit for purpose' when they reach the end-user.

Developing a novel drug delivery system utilising a spray device, pectin hydrogel and etoposide and olaparib-loaded nanoparticles for the local delivery to Glioblastoma Multiforme

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Glioblastoma multiforme (GBM) is a WHO grade 4 tumour carrying a dismal average survival time of 14 months from prognosis and a 16% 2-year survival rate. Current treatment consists of debulking surgery and oral temozolomide followed by concurrent chemoradiotherapy. Relapse from residual disease cells is inevitable, with 80% of tumours recurring within 2 cm of the primary tumour. This gives high precedence for a local drug delivery system (DDS) to target the local residual disease. A spray device and formulation (polymer-coated nanoparticles (PCNPs) and hydrogel) have been developed to achieve further penetration of drug into this 2 cm target area.

Pectin hydrogels have been repurposed for use within the brain parenchyma, having found to be non-toxic *in vitro* and *in vivo* at 200 μ M for up to 2 weeks. Fluorescence from Cy5-pectin reduced to background levels after 2 weeks *in vivo*.

PCNPs were generated by creating etoposide and olaparib nanocrystals in aqueous medium before coating with a PEGylated lactide-based polymer. PCNPs were characterised using HPLC, DLS and TEM. PCNP stability was also evaluated after storage at 4 °C and at 37 °C in DMEM, PBS and artificial cerebrospinal fluid (aCSF) using DLS. The PCNPs were then sprayed from an Aptar Pharma device and characterised using TEM and DLS.

PCNP drug loadings of 44.0 \pm 0.2% and 25.5 \pm 0.8% (mean \pm SD) for etoposide and olaparib, respectively, were achieved. Formulations were stable at 4 °C for up to 6 weeks and up to 48 hrs at 37 °C in DMEM, PBS and aCSF. Drug release was rapid, showing an initial burst of \sim 10% in 30 minutes, followed by \sim 85% at 24 hrs. The addition of hydroxypropyl- β -cyclodextrin was required to ensure the stability of the PCNPs when sprayed from the device.

The hydrogel, PCNPs and a spray device have so far proven they are fit-for-purpose, warranting testing in pre-clinical brain tumour models. This DDS is currently being progressed to *in vivo* tolerability and efficacy studies, which are due to be completed soon.

MICROARRAY PATCHES FOR THE DELIVERY OF THE HYDROPHOBIC DRUG OLANZAPINE

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Background: Olanzapine (OLP) is classified as an atypical, second-generation antipsychotic agent with indications for the treatment of schizophrenia, mania and bipolar disorder [1]. As with 70% of emerging therapeutic agents, OLP displays poor aqueous solubility, and therefore reduced oral bioavailability [2]. The resultant high side effect incidence observed when OLP is delivered orally may be circumvented by parenteral administration. However, this route of administration is often considered unsuitable due to needle phobia and the associated high level of risk when working with vulnerable patients. With the socioeconomic burden of mental health disorders growing rapidly, there is an undeniable requirement for improved alternatives to conventional treatment methods. Microarray patches (MAPs) are one such alternative. It is proposed that the rate-controlled delivery of the hydrophobic drug OLP *via* minimally-invasive, pain-free, hydrogel-forming MAPs will demonstrate reduced side effect incidence and increased patient acceptance [3].

Methods: An initial screening process of four cyclodextrin (CD) molecules indicated that hydroxypropyl- β -CD (HP- β -CD) had the greatest ability to enhance the aqueous solubility of OLP. Subsequently, directly compressed tablets (DCTs) containing OLP and HP- β -CD, along with excipients, were formulated and their performance optimised with the help of a central composite design software. Finally, *in vitro* delivery of OLP *via* MAPs across dermatomed neonatal, porcine skin was investigated using modified Franz cell apparatus.

Results: OLP solubility ($104.87 \pm 2.15 \mu\text{g/mL}$) was enhanced approximately 6-fold when in the presence of HP- β -CD at a concentration of 50 mMol/L. Further processing of OLP and HP- β -CD *via* spray drying and co-evaporation techniques produced 42-fold and 45-fold solubility enhancements, respectively. Drug-containing DCTs were designed to ensure that they could be easily secured atop MAPs allowing the delivery of OLP through the swollen hydrogel matrix of the MAPs. DCTs were optimised to consistently produce dissolution times of 41.41 ± 0.46 sec and hardness of 31.0 ± 6.81 N. Subsequent investigations confirmed the successful delivery clinically-relevant doses of the potent drug OLP *via* multiple formulations of hydrogel-forming MAPs *in vitro* using modified Franz cell apparatus.

Conclusions: The goal of delivering poorly soluble therapeutics for the treatment of psychotic disorders is one with many hurdles. Treatment must be safe and effective, whilst ensuring low side effect incidence and maximum patient acceptance. MAPs are an example of a highly promising drug delivery system, equipped with multiple unique and advantageous characteristics that are able to bypass such hurdles and may be revolutionary in the delivery of such therapeutics.

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DISSOLVING MICROARRAY PATCH-MEDIATED INTRADERMAL DELIVERY OF LONG-ACTING ANTIRETROVIRAL NANOSUSPENSIONS

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Background: Despite the recent up-scaled use of antiretroviral (ARV) treatment and preventative measures, HIV remains a global pandemic, affecting approximately 37.9 million people worldwide in 2018. Sub-optimal adherence to oral multi-drug regimens has emerged as the primary cause of therapeutic failure and development of drug-resistant virus. Accordingly, there is an urgent need for the development of self-administered long-acting (LA) delivery methods to overcome existing issues with daily oral adherence. Alternatives in clinical development include two LA intramuscular (IM) injectable ARV nanosuspensions of rilpivirine (RPV) and cabotegravir (CAB). However, their administration requires regular access to healthcare resources and sharps disposal facilities. As a result, this proof-of-concept study was designed to evaluate the potential intradermal delivery of LA RPV and CAB *via* dissolving microarray patches (MAPs). As such, MAPs are utilised simply as a tool to deposit the ARV nanosuspension within the viable skin layers in sufficient amounts to afford sustained administration, thus avoiding the need for adherence to daily oral treatment.

Methods: Dissolving ARV MAPs were prepared by a simple micromoulding process. Aqueous blends of polymer were mixed with lyophilized RPV LA or concentration-enhanced CAB LA nanosuspension, respectively. The resulting formulations were then poured onto MAP moulds (19x19 array, 500 µm height, 300 µm base width), and a preformed polymeric baseplate positioned on top, proceeded by the application of positive pressure. MAPs were then air dried for 24 h at room temperature, resulting in two MAP systems containing 1.73 mg RPV LA and 2.81 mg CAB LA, respectively. The backs of Sprague-Dawley rats were then shaved and in the treatment cohort 4 MAPs were applied (2 RPV and 2 CAB), and in the control cohort rats received 10 mg/kg of RPV LA IM and 5 mg/kg of CAB LA IM, respectively. Mean plasma concentrations of each ARV were simultaneously quantified by RP-HPLC-MS and pharmacokinetic profiles were established over the following 12 weeks.

Results: ARV MAPs applied to the back of the rats in the treatment cohort were removed after 24 h, and despite a high content of hydrophobic drug particles, complete MAP dissolution was achieved in all cases. Therapeutically relevant concentrations of RPV and CAB above the relevant IC₉₀ were observed in the MAP treatment cohort following 1 h and 1-day sampling, respectively. Interestingly, mean plasma concentrations in the treatment cohort continued to rise following removal of the MAP for each ARV, as RPV displayed a C_{max} of 203 ± 183 ng/mL at a T_{max} of 2 days, and CAB displayed a C_{max} of 12,800 ± 5200 ng/mL at a T_{max} of 9 days, respectively. Therapeutically relevant mean plasma levels were still detectable to 70 and 28 days for RPV and CAB, respectively. This indicates that drug is still being released from intradermal micro-depots of solid-drug nanocrystals, further prolonged in the systemic circulation, while concurrently being cleared from the body.

Conclusions: This proof-of-concept work outlines the formulation of novel LA ARV nanosuspensions within dissolving MAP systems for intradermal delivery affording a sustained drug administration. This is the first time that an investigational ARV treatment regimen has been incorporated into a dissolving MAP format, and illustrates the potential of the platform for two or more agents. Thus, future use of MAPs in the needle-free delivery of ARVs for the prevention and treatment of HIV infection deserves exploration. Formulation optimisation, comprehensive preclinical pharmacokinetic evaluation, biodistribution, physiologically-based pharmacokinetic modelling and patient acceptability studies are now necessary to fully realise the potential of these novel delivery platforms.

DEVELOPMENT OF INJECTABLE INTRATHECAL CANNABIDIOL FORMULATIONS

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Background: Neuropathic pain leads to decline in normal functioning and quality of life. Cannabidiol (CBD) has been reported to have a therapeutic action manifesting as pain relief, however, the main drawbacks are extensive first-pass metabolism, low oral bioavailability (logP 6.33) and poor penetration into the central nervous system (CNS). Recent developments in intrathecal drug delivery allow for this technique to administer drugs directly to the site of action in the spine. The aim of this project is to develop solid polymer coated CBD nanoparticles (NPs) and a lipid CBD nanoemulsion for intrathecal delivery to treat neuropathic pain.

Methods: CBD NPs were produced by a double nanoprecipitation method using a 3 arm PEG₁₀₁₄-(LA)₁₀₀ polymer. Free drug was removed by size exclusion chromatography and NPs were freeze-dried to allow quantification of CBD by HPLC. Particle size and zeta-potential were characterised using dynamic light scattering (DLS). Microfluidizer technology was used to manufacture oil-in-water nanoemulsions (composed of soybean oil, lecithin and glycerol). Density gradient ultracentrifugation using various densities of saline solutions was used to load CBD into the lipid droplets and then separate out unbound drug. These formulations were injected intrathecally after exposure of the spinal cord via a laminectomy in rats. Following mechanical stimulation, electrophysiology of pain pathways was recorded. The biodistribution of formulations in the CNS was then measured by HPLC.

Results: Polymer coated CBD NPs displayed a monodisperse population, 110.7 nm with 0.1 PDI, zeta-potential of -62.9 and were stable for 7 days at 4 °C. High encapsulation efficiency of 90.68% and drug loading of 30.2% were achieved. Incubation followed by density gradient ultracentrifugation approach was successful (65.1% and 90.95% association with commercial and microfluidized nanoemulsion respectively). The addition of CBD did not affect particle size < 300 nm or zeta-potential -55 mV which remained stable for 70 days post incorporation. Both nanoemulsions exhibited a PDI of < 0.2. Following intrathecal injection in rats, CBD nanoemulsion was preferentially retained within the lumbar segment of the spinal cord (2545 µg/g) with minimal concentrations reaching the brain (129.6 µg/g). The formulation lead to the inhibition of electrophysiology activity to noxious stimuli within 10 minutes of injection.

Conclusions: These findings pose critical considerations for the development of formulations for small lipophilic molecules for IT delivery, by allowing optimisation of nanoparticle properties to attain widespread distribution within the CNS.

DEPOT FORMING MICRONEEDLES FOR THE LONG ACTING DELIVERY OF A MODEL DRUG; ATORVASTATIN

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Background: Sustained release dosage forms are designed to achieve an extended therapeutic effect by continuously releasing medication over a prolonged period after the administration of a single dose. This has many advantages over the conventional drug delivery methods, such as reducing the undesired fluctuations of drug levels in plasma, reducing the doses frequency and thus improve patients' compliance to their treatment regimens. However, to achieve sustained release over a week or more, injections must be used. This possesses many disadvantages including the difficulty to terminate the treatment in case of drug toxicity. Moreover, the sterility and pyrogen-free requirements for a parenteral product can potentially increase the manufacturing costs of these products. Therefore, in this project, microarray patches (MAPs) are used to deposit a model hydrophobic drug; atorvastatin (ATR), intradermally, to provide a sustained release from a depot in the skin over a prolonged period of time.

Methods: MAPs were fabricated from aqueous blends containing 20% w/w Gantrez[®] S-97, a copolymer of methyl vinyl ether and maleic acid (PMVE/MA) with 7.5% w/w poly(ethylene glycol) 10,000 and 3% w/w sodium carbonate (Na₂CO₃). They were centrifuged casted into moulds consisting of 361 microneedles (19x19) on a 0.5 cm² area, dried at room temperature and crosslinked in an 80°C oven. The drug reservoirs were prepared using the cosolvent method, where a high molecular weight polymer (PEG 6,000 25% w/w) was added to a mixture containing a low molecular weight polymer (PEG 200 75% w/w) and a drug (ATR). The mixture was then casted into square tablets moulds, each had an area of 1cm², a mass of 0.25g and contained 15 mg ATR. To test the drug release *in vitro*, Franz cells were used. Dermatomed neonatal porcine skin (~350 µm thick) was attached to glass donor compartment using cyanoacrylate glue. MAPs were the inserted using manual pressure. The drug reservoir was placed on top of the MAP, then a metal weight of 5 g was put on the top to hold the set still. The receiver compartment contained 12 mL of the release media. The donor compartment was placed on top of the receiver compartment and they were clamped. Samples were taken at predefined time points and were replaced by fresh media. After 24 hours, the cells were dissembled, and the drug was extracted from skin, MAPs and remaining drug reservoirs samples and analysed using HPLC.

Results: Samples of the Franz cells were taken at predetermined time points of 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 24 hours and analysed using a validated method on HPLC to calculate amount of ATR in each sample. The mean amount of ATR delivered after 24 hours was 3.73 ± 1.65 mg per patch, which stands for 25.01% of ATR amount in the reservoir (n=3). The mean amount of drug recovered from the MAPs was 2.01 ± 1.5, which accounts for 2.68% of the initial amount of drug loaded into the reservoir. Whereas 0.49 ± 0.05 mg was obtained from the skin samples, accounting for 0.66% of the total amount of drug. Furthermore, samples obtained from the remaining drug reservoirs contained 4.99 ± 2.2 mg ATR, and that accounts for 6.65% of the total amount of drug. To sum up, a total amount of 11.34 ± 0.14 mg ATR was recovered after analysing all Franz cells samples, which represents 75.61± 1.14% of the drug loaded into the reservoirs.

Conclusions: The formulation of ATR in PEG reservoirs, to be used in a combined MAPs system, proved both possible and effective in facilitating transdermal delivery of this hydrophobic compound. Further skin deposition studies are to follow to assess the amount of ATR that can be deposited in the skin for a sustained release from this depot.

DEVELOPMENT OF MULTIFUNCTIONAL POROUS SILICA NANOPARTICLES FOR TARGETED AND STIMULI-RESPONSIVE GLIOBLASTOMA DRUG DELIVERY

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Background: Porous silica nanoparticles (PSiNPs) are among the most promising of drug delivery vehicles; made of chemically inert silica, their biocompatibility, large internal pore volumes and surface area, and ease of functionalisation, have long attracted attention in medical research. There is still, however a need for simple, scalable, and cost-effective methods to obtain multifunctional PSiNPs suitable for targeted cancer drug delivery. PSiNPs are most commonly synthesised through a modified Stöber method, a sol-gel process with cytotoxic surfactant CTAB acting as a template for the porous structure, although this method does not typically allow the synthesis of sub-100 nm particles required for optimum cell uptake. Protocols which allow access to the sub-100 nm range typically either lead to poor monodispersity, poor reproducibility, or require complex synthetic protocols. Furthermore, these particles then require post synthetic treatment to remove cytotoxic reagents and incorporate targeting and triggered drug release functionality rendering them unlikely candidates for clinical translation. This work presents a facile, reproducible, scalable and cost effective method for the synthesis of sub-100 nm PSiNPs, which incorporate cancer targeting and stimulated drug release without the need for retrospective functionalisation.

Methods: A novel synthetic pathway was developed where the particle size, uniformity and porous structure were templated by polyelectrolyte complexes formed from poly(acrylic acid) (PAA) and L-arginine (Arg). The addition of silica precursors lead to the formation of monodispersed porous silica nanoparticles functionalised with PAA and Arg. Drug loading capacities and release rates were determined with doxorubicin hydrochloride (Dox) and the efficacy of particle targeting and toxicity were tested *in vitro* using FITC-labeled particles in primary patient derived glioblastoma multiforme (GBM) and non-tumorigenic neural progenitor cells.

Results: The mixing of oppositely charged PAA and Arg led to the formation of monodispersed polyelectrolyte complexes ranging from 50-140 nm, which served as templates for the hydrolysis and condensation of the added silanes. The PSiNPs were readily tuneable from 40-200 nm while maintaining a narrow size distribution (PDI < 0.2), and with disordered pores ranging from 1-2.5 nm in diameter. The presence of PAA within the pores enabled high Dox loading, with a 22% w/w loading achieved, and provided a 4-fold increase in drug release over 48 hours under weakly acidic conditions (pH 5), representative of the endosomal and tumour microenvironments. The use of Arg in the polyelectrolyte complexes not only catalysed the basic hydrolysis and condensation of silane species but also conferred cancer targeting properties towards the cationic amino acid transporters which are overexpressed in GBM cells. The surface presentation of Arg gave significantly increased intracellular accumulation of PSiNPs in GBM1 and GBM20 cells compared to unfunctionalised PSiNP and to a non-tumorigenic control cell line (P < 0.05). The enhanced cellular accumulation effectively translated to a lower IC50 value of 2.9 μ M compared to 8.1 μ M of the non-targeted PSiNPs.

Conclusions: A new pathway for the synthesis of monodispersed, sub-100 nm PSiNPs with intrinsic functionality for stimuli-responsive drug delivery and tumour specific targeting was developed. This method obviates the need for cytotoxic reagents or post-synthetic functionalization associated with the conventional synthesis of PSiNPs. The simplicity and efficacy of this method presents a feasible candidate for progression into clinical trials and point-of-care medicine.

NANOPARTICLE-LOADED CONTACT LENS: A POTENTIAL OCULAR DRUG DELIVERY SYSTEM FOR A CONTROLLED RELEASE

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Background: Treatment of ocular disorders have often relied upon topical delivery strategies, which can be associated with low drug bioavailability. While improving therapeutic bioavailability, intravitreal routes can be associated with side effects (retinal detachment). Developments in nanoscience have resulted in the study of several novel, safe, patient-friendly drug delivery systems. Additionally, the use of therapeutic contact lenses (CLs) has received considerable research focus. This project aims to develop a soft CL impregnated with drug-loaded polymer nanoparticles for controlled drug release.

Methods: Various process parameters: curing conditions, hydration/extraction processes and sterilization methods were investigated in the manufacturing of the CL. Lenses were fully characterised to establish their optical, physical and mechanical properties.

To optimise drug loading, inclusion complexes were prepared using various drug concentrations, solvent systems and complexation methods. Phase solubility studies determined the optimum stoichiometric ratio of drug and cyclodextrin. The complex was characterized by thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), Fourier-transform infrared spectroscopy (FTIR) and high performance liquid chromatography (HPLC).

Chitosan (CS) and Hyaluronic acid (HA)-coated CS nanoparticles were synthesized using an ionic gelation technique at room temperature. These particles were then loaded with naringenin (NAR), a drug chosen because of its wide range of pharmacological activity for ocular therapy. The developed NAR-loaded nanoparticles were examined by dynamic light scattering, TGA, DSC, and FTIR with drug encapsulation efficiency determined by HPLC.

Results: Soft hydrogel CLs were successfully manufactured to commercial standards on-site. These lenses exhibited >99% optical transparency, 78% water content, refractive index of 1.37332, dimension of 14.3 mm and tensile strength of 0.64 ± 0.05 MPa.

Phase solubility studies (25 °C) demonstrated that the optimum drug:cyclodextrin molar ratio was 1:3. Freeze-drying of a tert-butyl alcohol:water co-solvent system was determined to be the best preparation method after characterizing the complexes by TGA, DSC and FTIR, with a resulting complexation efficiency of $98.7\% \pm 0.8\%$. A significant (>6000-fold) increase in hydrophobic drug aqueous solubility was obtained.

Non-drug and NAR-loaded CS NPs were successfully developed and characterised, with a particle size of 360 ± 9.9 nm and 333.3 ± 26.6 nm, a zeta potential of $+38.6 \pm 2.1$ mV and $+22.0 \pm 4.3$ mV, and a polydispersity index of 0.0671 ± 0.0362 and 0.0777 ± 0.0580 , respectively. NAR encapsulation efficiency in CS NPs was measured to be $13.0 \pm 1.9\%$. HA-coated CS NPs (366.3 ± 27.7 nm, -28.6 ± 1.1 mV and 0.1212 ± 0.0216) were also formulated and shown to enhance the stability of CS NPs at pH 6.8-7.4.

Conclusions: The results from this study demonstrated that NAR-loaded NPs for ocular drug delivery (ODD) were successfully prepared by ionic gelation of CS and cyclodextrin. Coating the NP with HA improves NP stability and targeted delivery, as well as enhancing eye comfort for CL wearers. Such nanoparticulate systems will be loaded into the developed SCL of commercial quality. This model can act as a novel ODD system to provide a more sustained, less invasive and controlled delivery of a drug or supplement to the eye.

Development of a novel process to create an effective 'off the shelf' gene therapeutic for incorporation into bioinks for 3D printing

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Background: Articular cartilage facilitates the frictionless movement of synovial joints, however due to its avascular and aneural nature it has a limited ability to self-repair. While some success in the repair of small cartilage defects has been reported with the use of biomaterial scaffolds alone, larger defects often require additional therapeutic intervention. The development of 3D printed biomaterial scaffolds functionalised with cells or nucleic acid loaded nanoparticles (NPs) provide a potential solution and thus there is now an increased focus on the formulation of novel cell-laden and gene-activated bioinks for cartilage repair. The overarching aim of this study is to develop a novel process to create an 'off the shelf' gene therapeutic consisting of a non-viral delivery vector and therapeutic plasmid DNA (pDNA) NP which can be pre-prepared for incorporation into bioinks. This would negate the need to complex fresh NPs immediately prior to each print. This gene-activated bioink will then ultimately be used to 3D print an advanced bio-implant for cartilage repair.

Methods: In order to achieve an 'off the shelf' NP formulation, lyophilisation was investigated as a potential solution. NPs consisting of the non-viral delivery vector glycosaminoglycan enhanced transduction (GET) peptide and pDNA for the reporter protein *Gussia luciferase* (pGLuc) were formulated at increasing ratios of peptide to pDNA based on their electrostatic interactions, defined as charge ratio (CR), of 6, 9 and 12. These NPs were formulated with 5% trehalose as a lyoprotectant, then lyophilised in a Christ Epsilon benchtop freeze dryer and NP size and polydispersity was measured via dynamic light scattering and NP zeta potential was measured via electrophoretic light scattering using the Zetasizer 3000 HS (Malvern, UK). Rat mesenchymal stem cells (rMSCs) were then transfected with both lyophilised NPs (L-NPs) and freshly complexed NPs (F-NPs) to determine if the lyophilisation process affected transfection efficiency of the NPs. Cell metabolic activity was assessed using the alamarBlue® assay (Thermo Fisher Scientific) and transfection efficiency was determined using the Pierce™ *Gussia Luciferase* Flash Assay Kit (Thermo Fisher Scientific) at 24hrs and 72hrs post-transfection respectively.

Results: There was no significant difference in size between L-NPs and F-NPs at all CRs and there was no significant difference in zeta potential between L-NPs and F-NPs at CR 6 or CR 12. A significant difference in zeta potential of -7mV was observed at CR 9, however this did not correlate with a decrease in gene expression. Transfection with L-NPs resulted in a significant increase in rMSC metabolic activity, a finding which may be attributed to the use of trehalose as a lyoprotectant. We were able to obtain comparable levels of transfection efficiency with L-NPs and F-NPs at CR 6 and CR 9. Further optimisation should be carried out for the formulation at a higher CR and future work will also include stability studies.

Conclusions: This study has demonstrated that lyophilisation shows promise in enabling stable storage of gene therapeutic nanoparticles without affecting cellular uptake. (Acknowledgements – Funding: ReCAP: ERC Advanced Grant number 788753).

MONODISPERSE NIOSOMES NANOPARTICLES FORMULATION BY MICROFLUIDIC MIXING

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Niosomes nanoparticles are self-assembling vesicular nano-carriers obtained by hydrating a mixture of non-ionic surfactant and cholesterol or amphiphilic molecules. Niosomes can be unilamellar or multilamellar and are suitable as carriers of both hydrophilic and lipophilic drugs. Furthermore, niosomal vesicles are non-toxic, more stable than liposomes and thus result in lower production costs. One of the methods used for liposome production is through microfluidic mixing, which depends on the rapid mixing of the lipids in solvent with the aqueous phase in microchannels. Using microfluidic systems, a tight control of the mixing rates and ratios between the aqueous and solvent streams can be achieved, with lower liquid volumes required, which facilitates process development by reducing time and development costs. Here we used the microfluidic mixing for the production of niosomes. The particles produced were compared to the particles generated by the thin film hydration (TFH) method followed by extrusion and the particles from both methods were investigated for their physicochemical characteristics, stability, morphology and cytotoxicity.

Materials and Methods:

Monopalmitin was obtained from Larodan Fine Chemicals Sweden, cholesterol and dicetyl phosphate (DCP) were obtained from Sigma–Aldrich Company Ltd., UK.

Formulation using microfluidics

Formulations were prepared on a NanoAssemblr™ (Precision NanoSystems Inc., Vancouver, Canada). Monopalmitin, cholesterol and DCP were dissolved in ethanol at a molar ratios of 50:40:10. Niosomes were manufactured by injecting the lipids and aqueous buffer into separate chamber inlets of the micromixer. The flow rate ratio (FRR; ratio between solvent and aqueous stream) as well as the total flow rate (TFR) of both streams were controlled by syringe pumps.

Formulation using TFH method

Monopalmitin, cholesterol and DCP were dissolved in chloroform at a molar ratios of 50:40:10 in a round bottom flask. The chloroform was then evaporated using a rotary evaporator and the lipid film was then hydrated with PBS to form multilamellar niosomes which were then passed through Avanti-polar miniextruder.

The particles generated from both methods were characterised by dynamic light scattering (DLS), ζ -potential, and atomic force microscopy (AFM). Cytotoxicity assays were also performed on A2780 ovarian cancer cells and A375 melanoma cells

Results: Using microfluidics, monodisperse niosomes were prepared in one step with an average size of 120 ± 10 nm determined by DLS and confirmed by AFM were shown to be spherical in shape. The size of the niosomes was controlled by monitoring the FRR and TFR in both the lipid and aqueous phases. In contrast, niosomes prepared with TFH method were too polydisperse and required a further size reduction step after preparation by extrusion or probe sonication which is time consuming and limited to the bench scale. A stability study was performed for the particles generated by both methods at four different temperatures (4, 25, 37 and 50°C) for 4 weeks and the vesicles were shown to be stable in terms of size and poly disparity index (PDI). Cytotoxicity studies showed that the IC₅₀ was around 625 $\mu\text{g/ml}$.

Conclusions: Stable, charged niosomes, with controlled size can be manufactured by microfluidisation, which has the potential to be scaled up for pharmaceutical production.

Optimizing Delivery Systems via Multiscale Simulations

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Background: Nowadays, physical, chemical, and biological properties of materials can be tailored at the nanometer scale to precisely deliver therapeutics. Computer simulations such as Molecular Dynamics provide a useful tool for predicting and understanding structure-property relations in developing novel nanomedicines while reducing extensive experimental trials.

Methods: Models at different chemical-physical resolution (e.g., coarse-grained, all-atom representation) are employed depending on the temporal and spatial scales spanned by interest phenomena.

Poly-lactic-co-glycolic acid (PLGA) and polyethylene glycol (PEG) polymers' miscibility is evaluated with all-atom simulations. Then, PLGA and PEG mixtures' mechanical strength is estimated under different regimens of miscibility to elucidate the mechanisms regulating nanoparticle deformability.

Next, the free energy profile of dexamethasone (DEX) and curcumin (CURC) molecules translocation across the polymer carrier into the aqueous solvent is derived via Umbrella Sampling method, providing insights on the drug release kinetics dependency on the physical-chemical interaction between the drug and the polymer matrix.

On a higher level of complexity, coarse-grained simulations integrate in vivo and in vitro experiments on the adsorption of blood proteins on the particle surface as a function of the polymer coating.

Results: The addition of PEG amounts comparable to that of PLGA (~50% w/w) results in heterogeneous blends with a level of phase separation that grows with the PEG's molecular weight. At low PEG concentrations, homogeneous mixtures are generated for both low and high PEG's molecular weights. The computed Young's modulus of PLGA/PEG blends is observed to decrease with the PEG content.

The higher free energy barrier associated with DEX translocation from the PLGA matrix to the aqueous solution confirms the higher affinity of this molecule for the PLGA matrix compared to CURC, supporting the experimentally documented slower release of DEX from the PLGA matrix.

Finally, nano-particle surface camouflaging with appropriate proportions of carboxyPEG2000 and methoxyPEG550 can suppress protein binding/intercalation, thereby affecting sequential dynamic processes in complement convertase assembly and nano-particle-mediated complement activation.

Conclusions: Together, these data provide the rationale to optimize delivery systems at reduced cost and experimental workload.

SOLUBILIZATION AND INTERACTION OF BCS II DRUGS WITH AN ENDOGENOUS GASTRIC ENZYME, PEPSIN
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<p>Background:</p> <p>Hydrophobic drugs suffer from poor and variable oral bioavailability due to their poor solubility in the gastrointestinal fluids. They pose a significant roadblock in drug development discovery. However, digestive proteins in gastrointestinal fluids may play a role in the solubilization of hydrophobic active pharmaceutical ingredients through binding to hydrophobic sites on the protein surfaces. To date, the solution concentration of two hydrophobic molecules, clofazimine citrate¹ and an unnamed drug², have been shown to increase in simulated gastric fluids in the presence of pepsin, an endogenous gastric enzyme. In the current investigation, pepsin has been explored for its potential to solubilize two additional BCS class II drugs, ketoprofen and carbamazepine.</p> <p>¹ S. Pinnamaneni et al, "Effect of pepsin on maintaining the supersaturation of the HCl salt of a weakly basic drug: a case study.," <i>Pharm. Dev. Technol.</i>, 2016, 21, 311–20.</p> <p>²P. Bannigan et al, "Delivery of a hydrophobic drug into the lower gastrointestinal system via an endogenous enzyme-mediated carrier mechanism: An in vitro study," <i>Eur. J. Pharm. Biopharm.</i>, 2018, 133, 12-19.</p>
<p>Methods:</p> <p>Equilibrium solubility and dynamic dissolution of hydrophobic drugs individually and with varying concentrations of pepsin were assessed in simulated body fluids. To understand any change in the polymorphic nature of drugs during dissolution, powder X-ray diffraction was performed to understand the polymorphic nature of the drugs during dissolution. Binding energy and inhibitory constants between the ligands (drugs) and receptor (pepsin) were determined by molecular docking in the Autodoc program.</p>
<p>Results:</p> <p>Carbamazepine and ketoprofen both exhibited improved solubility and dissolution profiles in the presence of pepsin by forming a drug-pepsin complex. The nature of the interactions in the drug-pepsin complex was further explored by molecular docking studies which indicated that carbamazepine bound to pepsin near the active site while ketoprofen, with lower binding energies, was found to bind at other locations on the pepsin structure. Powder X-ray diffraction showed conversion of a polymorphic form of carbamazepine during solubility studies while the added polymorph of ketoprofen remained unchanged.</p>
<p>Conclusions: <i>In-vitro</i> studies demonstrated the enhancement of solution concentration of two BCS II drugs in the presence of the endogenous enzyme. It was further shown by molecular interaction simulation in Autodoc. This endogenous enzyme could be further explored as a formulation strategy for improving the solution behavior of other poorly water-soluble drugs for oral administration.</p>

DISSOLVING MICRONEEDLES FOR INTRADERMAL DELIVERY OF AMPHOTERICIN B

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Background: Skin fungal infections are among the most prevalent infectious diseases observed in clinics. Traditional topical treatments suffer from limited permeation. Amphotericin B, an effective antifungal agent with poor solubility, was loaded into a dissolving microneedle system to treat skin fungal infections in this study.

Methods: Amphotericin B dissolving microneedles (ADM) were fabricated by a mould casting technique. The tips were cast by a drug gel mixture of amphotericin B, poly(vinylpyrrolidone) (PVP) and poly(vinyl alcohol) (PVA) (MW 9 – 10 kDa). Excess drug gel was removed after filling the cavities of the moulds. The tips were dried overnight in the microneedle moulds. The baseplates were cast using a gel containing PVP and PVA (MW 31 – 50 kDa). The ADMs were optimized and characterized in terms of height reduction rate after compression of 32 N for 30 s and insertion depth both in the Parafilm[®] layers (PF) and in the porcine skin. The skin deposition and dermatokinetic profiles were evaluated using Franz cell setup. The release profile of ADM tips was obtained. Thermal gravimetric analysis (TGA), differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR) and powder X-ray diffraction (PXRD) were performed for ADM tips, amphotericin B and physical mixtures. Antifungal performances of ADM were demonstrated using a disk diffusion test and in an *ex vivo* model of biofilm on the porcine skin.

Results: The ADM was an array of 16×16 needles arranged on a 0.40 cm² area with 850 μm needle height (600 μm pyramidal tip, 250 μm base column). Amphotericin B was located just in the tips of ADM. ADM contained 2.80 ± 0.34 mg of amphotericin B per patch. The mechanical characterisation revealed the height reduction after compression of 32 N was lower than 10%. The insertion depth of ADM in PF was between 378 and 504 μm and in porcine skin was 301.34 ± 46.86 μm. After 24 hours' application in the porcine skin, the drug deposition of ADM reached 271.40 ± 46.14 μg/cm². Dermatokinetic profile in the epidermis layer of the skin increased rapidly in the first 3 hours, slightly increased until 24 hours, and achieved an AUC₀₋₂₄ of 1008.0 ± 104.7 h.μg/cm². Dermatokinetic profile in the dermis layer displayed a similar trend and the AUC₀₋₂₄ for the dermis was 3562.0 ± 223.2 h.μg/cm². Amphotericin B was released completely from ADM tips in the fourth day and exhibited a 1.6 times faster dissolution rate within 4 days compared to the amphotericin B powder. The results of TGA, DSC, FTIR, and PXRD demonstrated no interaction between drugs and excipients and reduced crystallinity in ADM tips, which explained the release data. ADM showed remarkable inhibition of *Candida albicans* in the disk diffusion test with a zone area of 437.2 ± 135.4 mm², which equals a circle with a radius of 11.6 ± 2.6 mm. Antibiofilm activity of ADM revealed that after 24-hour treatment with ADM, the number of fungi cells inside the porcine skin was reduced from 6 × 10⁶ CFU/mL to 2.7 ± 2.1 CFU/mL. The fungi viabilities were much reduced following 24h of administration of ADM (*p* < 0.00001). The killing rate of ADM against *Candida albicans* in the *ex vivo* porcine model reached 100%.

Conclusions: This study reports the successful incorporation of amphotericin B into bilayer dissolving polymeric microneedle arrays. The ADM was demonstrated mechanically strong and contained amphotericin B only in the tips. Moreover, it demonstrates a high drug deposition of amphotericin B *in vitro*. Dermatokinetic profiles indicated that ADM delivered amphotericin B mainly into the dermis layer and remained at a high level during a 24-hour application. The antifungal effects of ADM were shown effective both in *in vitro* agar plates and in an *ex vivo* infected porcine skin model. Overall, this research shows the promising application of ADM to combat skin fungal infections.

INDIRECT ESTIMATION OF IN-VIVO PLASMA ASSOCIATED/DISSOCIATED FRACTIONS OF DRUGS CARRIED IN NANOPARTICLES

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Background: Lipid-based nanoparticles (LNP) are composed of phospholipids and PEG formulated with drugs for targeted and/or long-acting drug delivery. One major limit in the clinical translation of these nanoproducts is the unknown in-vivo release kinetics of drugs in the carriers. Microdialysis or solid-phase micro-extraction techniques both have important limitations: Microdialysis may not represent the in-vivo condition; Micro-extractions may be confounded by the LNP composition of endogenous lipids. In the absence of a drug release measurement in-vivo, we propose a low-cost pharmacokinetic way to quantify fractions in plasma of drugs associated (i.e. stability in-vivo) and dissociated (i.e. active drug) using typical data generated in the lab.

Methods: We propose two pharmacokinetic methods using the developer's typically available pharmacokinetic data (free and LNP-formulated drugs IV).
Method 1: AUC-based method providing boundaries of time-averaged percentages of associated vs. dissociated in plasma: $max\ dissociated = AUC_{free}/AUC_{LNP} \%$.
Method 2: based on dynamic modeling. Pharmacokinetic (PK) modeling of the physical system, in which LNPs are administered, provides predictions of associated and dissociated species time-courses. PK modeling founds on the conservation of mass-balance principle.
 We computed these two methods with data from LNP formulations in primates intended for long-acting HIV therapy (Drugs: *atazanavir*, *lopinavir*, *ritonavir*, *tenofovir*). These methods were included in several IND and FIH approvals for HIV long-acting therapy.

Results: Collecting primates' pharmacokinetic data for the free and LNP drugs we estimated via Method 1 that the drugs max dissociation from LNP (over 96h experiment) as 2-13% of the dose. Hence, 87-98% was the min dose that remained associated with LNP in-vivo. In particular, hydrophilic *tenofovir* had been estimated as 10% associated with dialysis experiments, while we found it 97% associated in-vivo using our investigational tools.
 Method 2 completed the analysis providing time-course simulations of the two simultaneously circulating species in the plasma. The simulated dissociated fractions, i.e. the active molecule released by LNP, resulted in a 17-20 h half-life compared to the 4-8 half-life of the free control.

Conclusions: These methods are easily and cheaply applicable to all kinds of circulating nanocarriers, such as liposomes and polymeric particles. Taking advantage of mandatory pharmacokinetic studies, the presented methods provide invaluable quantification of the in-vivo stability and the modified pharmacokinetics of the active drug as dissociates with time. **Method 1** is very easily computable to provide indicative boundaries. Although **Method 2** requires modeling expertise, once implemented, it leads to knowledge-gap-filling predictions of plasma time-courses for both associated and dissociated species. If the developer invests in Method 2, i.e. a computer model, his drug development pipeline for long-acting formulations can leap ahead of competitors as it can be used, once validated, to skip costly and ethical concerning study about in-vivo stability and efficiency (i.e. levels of free drug released on target).

ACOUSTICALLY-STIMULATED DRUG CARRIERS FOR TARGETED DRUG RELEASE AND BONE FRACTURE REPAIR

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Background: Impaired fracture healing has a significant physical and mental impact on patients, in addition to a financial burden for healthcare services. The overall aim of our work is to develop acoustically-stimulated microbubbles (MBs) and nanodroplets (NDs) as targeted drug delivery systems for bone repair, in order to overcome pharmacokinetic limitations which, have halted progress of therapeutic agents. In this study, we tested the hypothesis that MB and ND preparations are non-toxic to human cells. We also determined the bioactivity of NDs loaded with an anabolic activator of the Wnt signaling pathway, which is involved in osteogenic differentiation - 6-bromoindirubin-3'-oxime (BIO).

Methods: MB suspensions were prepared with a 9:1 molar ratio of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) to polyoxyethylene(40) stearate (PEG40S). Lipid films were hydrated in PBS and sonicated to form air-containing MBs, or ND emulsions of perfluoropentane (PFP) with and without BIO. Primary, patient derived, bone marrow stromal cells were used to assess MB and ND cytotoxicity with exposure to varying concentrations up to 3×10^7 MBs/ml and 1×10^9 NDs/ml, for up to 72 hours. Alamar Blue®, Picogreen and Live/Dead assays were carried out as an indicator of cell viability. Bioactivity of BIO was assessed by exposure of BIO-containing NDs to 3T3 cell lines transfected with luciferase under the control of a Wnt-responsive promoter.

Results: DSPC:PEG40S MBs induced a dose-dependent decrease in metabolic activity at 72 hours exposure, with a significant reduction at 3×10^7 MBs/ml ($63\% \pm 6\%$, $p < 0.0001$). However, with exposures up to 24 hours, both MBs and NDs did not exhibit any inhibitory effect on cell metabolism. Increasing concentrations of free BIO induced Wnt expression with a peak at $5 \mu\text{M}$. BIO-loaded NDs activated Wnt signaling to 40% of the maximal value with free BIO. Unloaded NDs had no inhibitory effects on Wnt-signaling induced by free BIO.

Conclusions: Microbubbles do not inhibit cell metabolism at concentrations up to 3×10^7 MBs/ml, while nanodroplets have no inhibitory effect on cell metabolism up to 1×10^9 NDs/ml, over 24 hours. These results validate the use of MBs and NDs in future studies as drug delivery agents. BIO-loaded NDs have displayed reduced activity compared to free drug, demonstrating their suitability as a drug delivery agent, maintaining drug activity prior to controlled release upon ultrasound stimulation. Future work will investigate ultrasound activated BIO release.

Design & Development of Curcumin Loaded Zinc Oxide Nanoparticles Decorated Mesoporous Silica Liquid Stitches: A Proof of Concept in Animals

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Background: The present research was aimed to develop an **alternative for sutures, cyanoacrylate adhesives** etc. which are currently used to close the wounds or surgical incisions; As these solutions have a drawback of toxicity and delayed healing. Moreover, the sutures lead to the formation of scar tissue as well as the permanent marks on the skin which is not desirable. The cyanoacrylates produce the local heat and liberates the formaldehyde upon application which leads to damage of tissue. To overcome these problems, we have developed a liquid stitches. The present research was aimed at synthesizing and characterizing curcumin-loaded zinc oxide nanoparticle-decorated mesoporous silica as a tissue adhesive (Liquid Stitches). The mesoporous silica nanoparticles facilitate adhesion to tissues through the nanobridging effect. The curcumin strengthens the antibacterial effect of this novel tissue glue (Liquid stitches), while the zinc oxide nanoparticles enhance the strength of the bonding between two tissues

Methods: The mesoporous silica was synthesized using a sol-gel methodology, and the drug was incorporated using the wetness impregnation method. The platform that was prepared was characterized using infrared spectroscopy, TEM, DSC, XRD, particle size analysis, BET analysis, a tissue model adhesion test, an antimicrobial assay and a wound model in Sprague Dawley rats.

Results: The average particle size was found to be 72.4 nm, while the surface area was found to be 654 m²/g. The tissue model adhesion graphs showed significantly different values for the peak load, work done and deformation at peak load, which reflects a difference between the glue strengths of the mesoporous silica nanoparticles and the Cur-ZnO-MSN and the carrier medium (water). The animal study provided a proof of concept by gluing wounds in less than 1 minute and healing the wound within 5 days.

Conclusions: The desired pore volume, particle size and surface developed an excellent tissue glue. It facilitated an excellent bond between tissue chains with the help of nanobridging effect which was shown by in-vivo and in-vitro experimentation. It facilitated gluing of skin in less than 1 minute & healed the wound in 5 days & proved itself as an excellent substitute to stitches.

TRANSDERMAL DELIVERY OF VANCOMYCIN HYDROCHLORIDE USING HYDROGEL-FORMING MICROARRAY PATCHES

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Background: Vancomycin is one of the most effective antibiotics for the treatment of methicillin resistant *Staphylococcus aureus* (MRSA)-derived infection. For treatment of systemic diseases, it is currently administered *via* intravenous (IV) injection because it is poorly absorbed when administered orally. However, use of an IV injection may result in bleeding, pain and poor patient compliance. Additionally, IV injection of Vancomycin hydrochloride (VCL) has been associated with toxic shock-like symptoms and skin rash. Therefore, other routes of drug delivery should be investigated to prevent and minimize the undesirable effects of IV injections. Transdermal delivery may be an alternative strategy for delivery of VCL. To facilitate a successful transdermal drug delivery, microarray patches (MAP) was employed for enhancing the drug bioavailability *via* this route. Hydrogel-forming microarray patches (HFMAP) are micro-structured patches that penetrate the skin and facilitate drug delivery. This work explores MAPs as an alternative delivery mechanism for VCL.

Methods: In this study, HFMAPs were manufactured from aqueous blends containing poly (methylvinyl ether-co-maleic acid) crosslinked by esterification with poly (ethylene glycol). Moreover, VCL was formulated into two different reservoirs (films and compressed tablets). The reservoirs were fabricated using various polymers at different concentrations. VCL-containing reservoirs were evaluated in an *in vitro* skin permeation study using Franz diffusion cell apparatus. Subsequently, an *in vivo* pharmacokinetic study was performed using female *Sprague-Dawley* rats.

Results: *In vitro*, VCL was successfully delivered from films (FD) and compressed tablets (CST) with $36.39 \pm 1.84\%$ and $46.39 \pm 8.04\%$ of drug permeated, respectively. Thus, a VCL-loaded CST (60% w/w VCL) was selected as the most promising reservoir to be integrated with HFMAPs. *In vivo*, VCL peak plasma concentration of $3.29 \pm 1.06 \mu\text{g/ml}$ was achieved at 48 h using CST-HFMAP. The $\text{AUC}_{0-\text{inf}}$ values of CST-HFMAP and oral groups were $162.04 \pm 61.84 \mu\text{g.h/ml}$ and $30.50 \pm 9.18 \mu\text{g.h/ml}$, respectively.

Conclusions: This present work has demonstrated successful transdermal delivery of VCL using HFMAP both *in vitro* and *in vivo*. This system could provide new treatment options which may be useful in conditions such as neonatal sepsis. Furthermore, CST-integrated HFMAP could reduce the adverse drug reactions which is associated with the IV injection of VCL. With respect to outpatient therapy, this type of controlled release platform may be beneficial to maintain plasma level of VCL resulting in a reduced frequency of drug administration. Future work will include a pharmacodynamic study to observe the correlation between plasma concentration and the therapeutic effects using appropriate infection models *in vivo*.

DISSOLVING POLYMERIC MICROARRAY PATCHES LOADED WITH RILPIVIRINE NANOPARTICLES OBTAINED BY BEAD-MILLING

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Background: According to the World Health Organisation, human immunodeficiency virus (HIV) is one of the major global public health issues. Antiretroviral agents are commonly delivered orally or intramuscularly. As a patient-friendly alternative, dissolving bilayered polymeric microarray patches (MAPs) for the intradermal delivery of rilpivirine (RIL) were developed. To achieve high drug loading, a nanosuspension (NS) of RIL was prepared by bead-milling at laboratory scale prior to MAP fabrication.

Methods: The RIL NS was produced in a glass vial (total volume 10 mL) using ceramic milling beads (12 g, 0.1-0.2 mm) and two magnetic stir bars (25 x 8 mm) to facilitate bead movement. RIL (0.25 g) and a surfactant solution (5.5 mL) containing 2% w/w poly (vinyl alcohol) 9-10 kDa and 2% w/w poly (vinyl pyrrolidone) 58 kDa were added to the vial and milled at 1,500 rpm for 24 h at an angle of 75°. The NS was separated from the beads by sieving, made up to a total volume of 6 mL and lyophilised. A NanoBrook Omni Particle size analyser was used for measuring the particle size of obtained nanoparticles.

The lyophilised NS was reconstituted in 710 µL deionised water and immediately used for casting the first layer of MAPs (100 µL/ MAP) using silicone micromoulds (600 pyramidal needles, height 750 µm, base 300 x 300 µm, interspacing 50 µm). After application of a pressure of 5 bar for 15 min, excess formulation was removed and MAPs were dried at room temperature for 2 h. To form a mechanically strong baseplate, 0.7 g of an aqueous poly (vinyl pyrrolidone) 360 kDa blend (30% w/w) was cast on top of the first layer, followed by centrifugation at 3,500 rpm for 15 min.

After further drying for 18 h, MAPs were demoulded, visually inspected using a stereo microscope and tested in terms of their mechanical strength (compression at 32 N against an aluminium surface for 30 s using a Texture Analyser) and insertion efficiency into a previously validated skin model consisting of eight layers of Parafilm M[®]. Drug content was calculated based on the total needle volume of 13.8 mm³ and the composition of the prepared NS.

Results: The NS had a mean particle size of 168 ± 2 nm (PDI 0.18 ± 0.02, n = 9) before and 169 ± 3 nm (PDI 0.20 ± 0.05, n = 9) after lyophilisation and reconstitution. Observation of MAPs under the stereo microscope showed two clearly separated layers with RIL visible in the needle shafts only and a clear baseplate. MAPs were compressed 9 ± 6% (n = 48) of their total needle height of 750 µm and approximately 50.8% of the total needle height could be inserted into Parafilm M[®]. Based on theoretical calculations, MAPs had a total drug load of 2.8 mg/ MAP.

Conclusions: RIL loaded MAPs were mechanically strong and could be easily inserted into a validated skin model. Further studies will now need to be conducted to evaluate in skin dissolution times and RIL deposition.

**INVESTIGATING THE KEY MANUFACTURING PARAMETERS FOR THE
MICROFLUIDIC PRODUCTION OF LIPID NANOPARTICLES**

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Background: In order for genetic drugs to be used clinically, sophisticated delivery systems are required. Lipid nanoparticle systems (LNP) for delivery of small molecule drugs have led to rigorous design criteria. These criteria include a size range of 100 nm or less, highly efficient encapsulation processes, robust, scalable manufacturing processes, and product stability of at least 1 year at 4°C. Here, we assessed the effect of the formulation and production parameters for the formulation of LNP for gene delivery.

Methods: The NanoAssemblr® Benchtop from Precision Nanosystems Inc. was used for the preparation of LNP. Neutral (HSPC, cholesterol, DOPE), cationic (DOTAP and DDAB) and polyethyleneglycol lipids (DMG-PEG2000 and DSPE-PEG2000) were dissolved in ethanol or methanol, and used in different lipid combinations. As aqueous phase, Tris buffer (pH 7.4 10 mM) was used. Both aqueous and solvent phases were then injected into the system and the microfluidic production parameters were evaluated. In order to reduce the final solvent content to acceptable levels, samples were diluted post-production in Tris buffer. Polyadenylic acid (PolyA) and salmon ssDNA were used for method optimization due to their relatively low cost and were loaded in-line (in the aqueous phase) within the microfluidics system.

Results: In general, all formulations tested showed low sensitivity to the total flow rate and lipid concentration and high sensitivity to the flow rate ratio. Solvent selection impacted on the characteristics of the produced particles and the data shown is highly reproducible between laboratories. Regardless of the choice of cationic lipid or PEGylated lipid, the manufactured vesicles showed comparable physicochemical characteristics. Loading of Poly(A) and ssDNA resulted in high loading efficiencies.

Conclusions: Here, we have shown a comprehensive study of the effect of the lipid choice and microfluidic process parameters for the production of LNP loading Poly(A) and ssDNA as surrogates for RNA. This method optimisation and formulation screening could be used as a model for the preparation of LNP for gene delivery.

DEVELOPMENT OF THE BACTERIOCIN, LACTICIN 3147 FROM *LACTOCOCCUS LACTIS*, INTO AN ANTIBIOTIC

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Background: Due to an alarming increase in antimicrobial resistance worldwide, alternatives to traditional antibiotics, such as antimicrobial peptides are being investigated. Lacticin 3147 is a two-component antimicrobial peptide (LtnA1 and LtnA2), produced by *Lactococcus lactis* DPC6577. It is active against many antimicrobial-resistant bacteria at a nanomolar scale, e.g. *Clostridium difficile* and methicillin-resistant *S. aureus*. The use of antimicrobial peptides, such as lacticin 3147, is limited due to their susceptibility to enzymatic cleavage, their propensity to unfold and aggregate and their instability *in vivo*. This project involves (i) the production and purification of lacticin 3147 (ii) the study of its stability and solubility and (iii) the design of a formulation strategy to optimise its activity upon oral administration.

Methods: Lacticin 3147 was produced and purified following a protocol developed by Rea *et al.* Lacticin 3147 production was confirmed by MALDI-TOF spectroscopy using sinnapinic acid as the matrix. The effect of the gastrointestinal enzymes on lacticin 3147s and activity and structure was investigated. Known concentrations of pepsin, trypsin and α -chymotrypsin were added to a 400 nM lacticin solution in a 96 well plate and left for 3 hours. 1 μ l of each was removed and analysed by MALDI-TOF. 150 μ l of *L. monocytogenes* ATCC1916 in TSB (OD=0.1) was added to the remaining solutions. The 96 well plate was incubated in a Biotek ELx808 Ultra microplate reader at 37 °C. Readings were taken every 30 mins for 24 hrs at a wavelength of 590 nm. To investigate lacticin 3147's solubility and activity in a variety of media, solutions of the individual lacticin 3147 peptides (0.5 mg/ml) were made up in PBS buffer (pH 7.4), HCl/KCl buffer (pH 2), FaSSGF (pH 1.6) and FaSSIF (pH6.5). At 0 hrs, 5 hrs and 24 hrs, aliquots of each sample were taken, filtered and analysed by RP-HPLC. 25 μ l of the filtered aliquots were added in triplicate to a 96 well plate. The wells were then filled to 200 μ L with bacterial cell culture and analysed for bacterial growth.

Results: Lacticin 3147 was rendered inactive after incubation with trypsin and α -chymotrypsin indicating that both enzymes degrade it. Pepsin did not have any effect on lacticin 3147's activity despite the presence of pepsin's target amino acids in LtnA1. The lacticin 3147 peptides work synergistically to kill bacteria therefore the structures of the peptides after incubation with trypsin and α -chymotrypsin were examined by MALDI-TOF spectroscopy. The absence of the LtnA1 and LtnA2 peaks in the resulting spectra showed that both peptides were degraded by trypsin and α -chymotrypsin. Both lacticin 3147 peptides showed low solubility in pH 2.2 and pH 7.4 aqueous buffers. Both peptides' solubility increased in FaSSGF and FASSIF but LtnA1 was seen to be unstable over time. As the concentration of lecithin and NaTc is above the critical micelle concentration in FaSSIF the encapsulation of LtnA1 and LtnA2 in micelles may account for the increase in solubility. Despite lacticin 3147's poor solubility in PBS (only 0.34% and 0.55% of the 0.5 mg of LtnA1 and LtnA2 added dissolved), it was still found to be active. This is because <1 μ g/ml of LtnA1 and LtnA2 is active against *L. monocytogenes*.

Conclusions Both lacticin 3147 peptides exhibit some aqueous solubility but it is low and can be unstable in biorelevant media. This coupled with its susceptibility to degradation by enzymes found *in vivo* necessitates the development of a controlled release system for this dual-acting antimicrobial peptide. From these results, two approaches are currently being studied – polymer conjugation and lipid-based delivery systems.

Development and characterisation of a library of dissolving polymeric microneedles for targeted drug delivery for basal cell carcinoma.

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Background: Basal cell carcinoma (BCC) is the most common skin cancer in humans. One of the drugs that is frequently employed in the management of BCC is imiquimod (Aldara™). However, imiquimod possesses physicochemical properties that limit its permeation to reach deeper tumour lesions such as those seen in nodular BCC. Thus, such topical treatment with Aldara™ cream is restricted to the management of superficial BCC. In light of these drawbacks, it is hypothesised that the use of microneedles to disrupt the *stratum corneum* may promote the permeation of imiquimod to reach deeper BCC lesions.

Methods: Microneedle moulds of different microneedle architectures were produced using micromachining. The moulds were then used to manufacture dissolving microneedles from the commercial polymer Kollidon® VA 64 as well as from novel polymers that were synthesised via free radical polymerisation reactions. The polymeric microneedles of different designs and chemistries were characterised via microscopy, fracture test and *ex vivo* skin insertion studies. Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) analysis on microneedles was conducted to visualise drug, polymer and excipient distribution along the microneedles in a label free fashion. Drug release studies in *ex vivo* porcine skin was also conducted in comparison to the commercial cream, Aldara™. The capability of the microneedles to puncture and deliver the payload in human skin tumours was evaluated using full thickness *ex vivo* patient BCC tissue (NHS HRA approval ID: 130880) followed by ToF-SIMS analysis to visualise drug and polymer distribution in human skin tumours. In addition, the efficacy of the formulation was also evaluated *in vivo* using a rodent model for skin tumours.

Results: Eight dissolving polymeric microneedle patches of different designs and chemistries were manufactured. These patches displayed sufficient tensile strength and skin insertion properties. ToF-SIMS analysis on the microneedles showed that the microneedles displayed homogenous drug and polymer distribution along the needle length. Drug release studies from *ex vivo* skin tissues demonstrated that some of the patches were capable of achieving higher intradermal delivery of imiquimod relative to the commercial cream, Aldara™ in a dose sparing manner. In addition, ToF-SIMS analysis of patient BCC tumours treated with imiquimod loaded microneedle patches demonstrated significant intradermal delivery of imiquimod within the skin tumour. Furthermore, the parallel detection capabilities of the ToF-SIMS permitted visualisation of the dermal distribution of unlabeled polymer within the native skin tumour milieu.

Conclusions: The current work highlights that dissolving polymeric microneedles is a viable drug delivery platform for the treatment of nodular BCC. Through judicious selection of microneedle design and chemistry, imiquimod loaded microneedle patches were successfully fabricated and characterised. Some of these patches were capable of achieving higher % intradermal delivery of applied imiquimod dose (>25%) relative to the commercial cream, Aldara™ (5.7%). This is further corroborated by the successful delivery of imiquimod into human BCC tumours demonstrating the potential of the microneedle-based approach to treat BCC.

DEVELOPMENT OF NANOMEDICINES TARGETING INFECTION AND INFLAMMATION

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Background: Targeted therapy is an area possessing wide scope for treatment opportunities, with diclofenac being a potential active that can be used as a targeted therapy, particularly in pain management. Effective and targeted delivery of diclofenac is an issue which has been problematic for both patient and health-care professionals. Star-shaped polypeptides (SPs) are polymers which can be potentially used as a carrier to effectively deliver targeted therapies whilst maintaining safety and uniformity. The aim of this project involved investigating the effectiveness of G5 poly(L-lysine) (PLL) as a carrier for diclofenac.

Methods:

UV/Vis absorbance of diclofenac was determined through a Biochrom Libra S22 UV/Vis Spectrophotometer to generate calibration curves as a basis for concentration determination.

DLS Particle size and Zeta (ζ) Potentials were assessed using a Malvern Zetasizer® Nano ZS. For this analysis, 1mg of diclofenac was weighed, dissolved in 1mL of ethanol and was subsequently vortexed for several seconds. Similarly, a 1mg/mL solution of G5-PLL was also prepared using ethanol as the solvent.

NTA particle sizing was assessed using a Malvern NanoSight 300. Three measurements were recorded per sample (each mass ratio) and were performed in triplicate.

Results: The diclofenac absorbance showed linearity between 0.015625mg/mL and 0.125mg/mL with a correlation coefficient (r^2) value of 0.9964.

The particle sizes of diclofenac/G5-PLL complexes varied with different mass ratios analysed. Pdl values of 0.5-0.7 were obtained, indicating that samples at different mass ratio are polydisperse, i.e. non uniform distribution size. Zeta Potentials ranged between $22 \pm 1.64\text{mV}$ to $9.3 \pm 0.46\text{mV}$ Mean particle size were ranged from 175nm and 300nm.

Results showed the samples displayed polydispersity at different mass ratios of diclofenac:G5-PLL.

Conclusions: These results demonstrate the potential for loading SPs with small molecule therapeutics such as diclofenac. With polymers of known age/ stability, accuracy of future sampling is expected to increase. Furthermore, this data will be used to modify the particle size distribution in order to create more monodisperse sample sizes.

CAN mRNA - LIPID NANOPARTICLE SURFACE COMPOSITION REGULATE APOLIPOPROTEIN BINDING FROM SERUM?
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<p>Background: Therapeutic treatments based on the production of proteins by delivering messenger RNA (<i>mRNA</i>) represent a promising approach to treat many diseases that currently lack other alternatives. One of the major challenges for designing such treatments is the adequate protection of these macromolecules from enzymatic degradation and their safe deliver into the target cells. Lipid nanoparticles (LNPs) are promising vehicles for mRNA delivery and are formed by a cationic ionizable lipid (CIL), DSPC, cholesterol (Chol) and a pegylated (PEG) lipid. Even though some LNPs were recently FDA approved for the treatment of peripheral nerve disease by delivery of small interference RNA (<i>siRNA</i>), there are still concerns about the safety profile of these nanoparticles. A good understanding of the physical and chemical characteristics of the LNPs under study is necessary to progress from pre-clinical testing. In addition, the bio-distribution and cellular uptake of LNPs are affected by their surface composition as well as by the extracellular proteins present at the site of LNPs administration, such as proteins in the plasma. Therefore, it is also important to understand the relation between LNP physical chemical properties and their ability to collect proteins. A common component found in the “protein corona” of LNPs is Apolipoprotein E (ApoE), which is responsible for the transport of fats in the systemic circulation and it triggers the fat uptake by cell-rich in low-density lipoprotein (LDL) receptors. This recognition step is critical to control the LNP’s circulation time and thus its pharmacological efficiency.</p>
<p>Methods: We employed small angle neutron scattering (SANS) to investigate the distribution of components in the LNP and the effect that ApoE could have on the LNP structure. In addition, we have developed a sensor platform based on Quartz Crystal Microbalance with Dissipation (QCM-D) to assess the binding affinity of serum protein to LNPs with different size and surface composition.</p>
<p>Results: Previous studies reported the core shell structure of LNPs, and highlighted the enrichment of the shell with the saturated lipid DSPC. By means of SANS, we reveal the precise location of cholesterol and CIL across the LNPs. Additionally, we determine the extent of ApoE binding to LNP and subsequently the effect that protein binding exerts on the lipid distribution within the LNP particle. The structural studies provided fundamental information to understand how ApoE mediates cell uptake via LDL receptor.</p> <p>Additionally, with the sensor platform based on QCM-D, we show that, in line with what has been reported in literature, ApoE has a higher binding affinity compared to HSA and ApoA1.</p>
<p>Conclusions: The structure of mRNA-LNPs was studied by SANS yielding the localization of the different components across the particle inner core and shell. We developed a tool to assess the ability of the LNPs to bind proteins. Combining these approaches, we can determine how changes in the LNP formulation, and hence structure, affect the protein binding to LNPs.</p>

siRNA DELIVERY BY PORE-CAPPED SILICA NANOPARTICLES FOR NEOVASCULAR AGE-RELATED MACULAR DEGENERATION

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Background: Age-related macular degeneration (AMD), a condition with progressive loss of central visual acuity creates blind spots on the retina with increasing age. Irregular angiogenesis characterized by weak and leaky blood vessels become prominent due to the over-expression of the vascular endothelial growth factor (VEGF). The currently available clinical regimens are commonly invasive, patient incompliant, along with numerous side effects and pharmacokinetic variations in individuals. For this reason, we tried to develop a multifunctional nanocarrier based system to effectively delivery the anti-VEGF siRNA.

Methods: Polyethyleneimine (PEI) modified large-pore mesoporous silica nanoparticles (MSNs) cargo systems have been developed for efficient siRNA loading. Rhodamine B loaded MSNs were first obtained to test the ability of PEI to cap the pores of the MSN system and control the release of its cargo. *In-vitro* release of rhodamine B from PEI-coated MSN was examined in the acidic lysosomal extract obtained from rabbit liver tissue. Additionally, the cytotoxicity of these MSNs was assessed in retinal cells of ARPE-19 cell line. Finally, the siRNA loading into the MSNs pores, its release and the silencing of VEGF was performed to confirm the effectiveness of the nanocarrier system.

Results: The shift in the zeta potential values confirmed the effective coating of the cationic PEI on MSNs surface with sufficient pore size and volume for nucleic acid loading. The triggered release of rhodamine B from MSNs was confirmed and hypothesized to be due to the proton sponge effect activated by the cationic PEI in the late endosomal/early lysosomal environment. The resultant nanocarrier system shows higher toxicity with increase in PEI concentration, and effective gene silencing of VEGF.

Conclusions: The developed porous silica nanocarrier system withholds huge potential as an effective gene delivery vehicle. The toxicity arising with PEI coating can be tackled with further functionalization steps with non-toxic and/ or biocompatible polymers. Thus, such a versatile platform technology has tremendous prospects for further scientific exploration and development.

POLYMER STRUCTURE AND PROPERTY EFFECTS ON AMORPHOUS SOLID DISPERSIONS WITH HALOPERIDOL: POLY(N-VINYL PYRROLIDONE) AND POLY(2-OXAZOLINES) STUDIES

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Background: Amorphous solid dispersions are defined as physical mixtures of poorly-soluble drugs with some hydrophilic materials to improve the solubility and dissolution profiles of hydrophobic drugs. In some studies, the reduction of drug crystallinity in solid dispersions with various polymers has been explored and related to the chemical structure and properties of water-soluble polymers. However, systematic studies into the effects of polymer structures on their ability to reduce drug crystallinity are currently lacking because there are limited opportunities to vary polymer structures in a controlled manner; most studies use commercially available polymers. Poly(2-oxazolines) are an emerging class of polymers, currently attracting substantial interest due to a number of unique physicochemical properties and lack of toxicity. Recently, poly(2-oxazolines) were used to prepare solid dosage forms as individual polymers and also in combination with some other pharmaceutical excipients. Using different water-soluble poly(2-oxazolines) to design solid dispersions offers interesting and previously unexplored opportunities to understand the effect of polymer molecular structure and hydrophilic-hydrophobic balance on the crystallinity of a dispersed drug.

Methods: Poly(2-methyl-2-oxazoline) (PMOZ), poly(2-propyl-2-oxazoline) (PnPOZ) and poly(2-isopropyl-2-oxazoline) (PiPOZ) were synthesized by hydrolysis of 50 kDa poly(2-ethyl-2-oxazoline) (PEOZ) and subsequent reaction of the resulting poly(ethylene imine) with acetic, butyric and isobutyric anhydrides, respectively. These polymers were characterized by ¹H-NMR, FTIR spectroscopy, powder X-ray diffraction, and differential scanning calorimetry. The poly(2-oxazolines) as well as poly(N-vinyl pyrrolidone) (PVP) were used to prepare solid dispersions with haloperidol (HP), a model poorly soluble drug. Dispersions were investigated by powder X-ray diffractometry, differential scanning calorimetry and FTIR spectroscopy. Dissolution studies were carried out on HP and polymer-HP solid dispersions and analyzed by UV-Vis.

Results: Increasing the number of hydrophobic groups (-CH₂- and -CH₃) in the polymer resulted in greater inhibition of crystallinity of haloperidol in the order: PVP > PnPOZ=PEOZ > PMOZ. Interestingly, drug crystallization inhibition by PiPOZ was lower than with its isomeric PnPOZ because of the semi-crystalline nature of the former polymer. Crystallization inhibition was consistent with drug dissolution studies using these solid dispersions, with exception of PnPOZ, which exhibited lower critical solution temperature that affected the release of haloperidol.

Conclusions: Polymer structure and properties were found to influence the crystallinity of the drug and its release from solid dispersions. By synthesizing polymers with equivalent degrees of polymerization, the effects of polymer hydrophobic-hydrophilic properties, their semi-crystalline nature, hydrogen bonding strengths, and lower critical solution temperature (influencing polymer solubility) on the structure of solid dispersions and drug release have been demonstrated. Our studies show that, when selecting a carrier for solid dispersions, it is important to consider not only the hydrogen bonding capabilities of the polymer but also its broader properties including their semi-crystallinity, steric properties and lower critical solution temperatures.

**PHOTOSENSITIZER ENCAPSULATED METALLOCATANIONIC VESICLES FOR
PHOTODYNAMIC THERAPY**

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Background: Developments in the field of photodynamic therapy (PDT) are being made by investigating appropriate photosensitizers (PSs) and enhancing the penetration effect of light by developing new nano-carriers. So, to boost the PDT effect, new metallosurfactant based metallocatanionic vesicles (MCV) were fabricated by a convenient, efficient, green and inexpensive method to encapsulate Rose Bengal as PSs and evaluate their antimicrobial PDT against the drug-resistant bacterium.

Methods: We have prepared from a combination of a double-chained copper-based cationic metallosurfactant (CuCPCII) and an anionic surfactant sodium bis(2-ethylhexyl)sulfosuccinate (AOT). We have prepared the different ratio of CuCPC II:AOT from 10:90 to 90:10 in PBS of 7.4 pH. In this approach, two of the fractions, one each from a cationic rich and anionic rich side, were selected to encapsulate anionic (rose bengal (RB)) PSs. It was characterized by SAXS, AFM, FE-SEM, Zeta-sizer, and conductivity measurements.

Results: These studies reveal that the MCV have dual functionality *i.e.* encapsulate PSs and even show dark toxicity against *S. aureus*. MCV help in enhancing singlet oxygen yield of RB. To study the killing of *S. aureus*, bacterial DNA was extracted and its interactions and conformational changes in the presence of MCV were analyzed *via.*, UV-Visible, and circular dichroism (CD) spectroscopy. Comet assay (single-cell gel-electrophoresis) demonstrated the DNA damage after PDT treatment in an individual cell. The bacterial DNA damage was more with the metallosurfactant rich 70:30 fraction than with the 30:70 fraction, in combination with RB under irradiation

Conclusions: This work provides a new metal hybrid smart material that possesses dual functionality and is prepared by an easy, economical and feasible procedure which resulted in enhanced PDT against a drug-resistant bacterium, thus, providing an alternative for antibacterial therapy.

COMBINATORY THERAPEUTIC STRATEGY TO METASTATIC COLORECTAL CANCER TREATMENT USING FUNCTIONALIZED NANOPARTICLES

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Background: Colorectal cancer (CRC) is one of the deadliest cancers in the world, mainly due to distant metastasis events. Despite the improvements on the chemotherapy, 5-fluorouracil (5-FU), remains one of the most effective drugs commonly used to treat CRC, but is also associated with several limitations due to its high toxicity. To overcome those drawbacks, nanomedicine might be used as a promising strategy to treat those cases providing an effective, controlled and targeted therapy which be useful to decrease the side effects and improve treatments. Carcinoembryonic antigen (CEA) is one of the most interesting candidates to target CRC cells, due to its overexpression at most CRC tumors. Besides, the treatment outcome, could be widely associated with the immunosuppressive tumor microenvironment (TME) which promotes the cancer cell immune escape. In our project, the major aim is to develop an innovative combinatory therapy based on immunochemotherapy targeted NPs. Two different targeted NPs will be developed in this work. The first nanosystem is planned with tropism to CRC cells expressing the CEA, carrying the chemotherapeutic agent 5-FU. The other one will target a molecule in TME highly expressed in CRC tumors, carrying one cytokine.

Methods: Polymeric NPs were produced by double emulsion and loaded with 5-FU followed by functionalization with an engineered antibody (scFv) targeting CEA. Physical-chemical properties were assessed by Dynamic Light Scattering (DLS) and Laser Doppler Anemometry (LDA). Morphology, drug loading (DL) and conjugation efficiency were evaluated by TEM and HPLC, respectively.

Results: NPs with 121.41 ± 5.35 nm were achieved, with about PDI 0.1, confirming the monodisperse population and around of 6% of DL. The surface charge was close of the neutrality (-3.51 ± 0.46 mV) and the spherical shape was confirmed. Conjugation Efficiency is still being evaluated. *In vitro* studies to assess drug release, binding efficiency, cytotoxicity and targeting ability of the NPs against CEA-expressing and non-expressing cells will be further performed.

Conclusions: Through this work, we developed and characterized 5-FU-loaded NPs functionalized with a scfv targeting CEA to improve chemotherapy delivery, decreasing its side effects and enhancing its efficacy. *In vitro* studies will be complemented evaluating the impact of these NPs on immune cell profile and function. Finally, biodistribution and ability to modulate the immune response, impairing tumor progression will be evaluated *in vivo* using the AOM-CRC mouse model. As future work, we intend to develop the functionalized NP carrying a relevant cytokine to target the TME. After performing both functionalized NPs *in vitro* and *in vivo* assays will be performed to assess the distribution and internalization of the NPs, as well as the impact on cancer and immune cell profile. Therefore, this combinatory strategy will have a dual role acting on cancer cells and recruiting immune cells, changing the TME activity, improving the outcome of chemotherapy and modulating the tumor immunosuppressive environment.

AN INTRA-PERITONEAL DELIVERY OF NANOCAPSULE LOADED HYDROGEL TO TREAT ADVANCED OVARIAN CANCER

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Background: The standard treatment for ovarian cancer (OC) is cytoreductive surgery, performed either in between or followed by intravenous. The difficulty in excising all the disease leads to recurrence in many patients. Hence, there is an urgent need for treatment options that can remove microscopic or macroscopic disease effectively. Poor tolerability limits the usage of many therapies for OC. However, loco-regional delivery is being used for the therapeutic index improvement. Nevertheless, intraperitoneal (IP) chemotherapy has not yet been widely adopted for OC treatment due to the risk of local toxicity, catheter-associated infections, bowel perforations and obstructions, and lack of products specifically approved. Nanoparticles (NPs) have been investigated for IP delivery of chemotherapeutics because of their potential to offer higher tumour penetration. However, in general, NPs are rapidly cleared from the peritoneal cavity. Hence, to maximise this NPs loco-regional effect, we have proposed the incorporation of nanocapsules (NCs) within a hydrogel for IP delivery and to provide a more sustained release profile. The first part of this work involves the NCs formulation optimization and incorporation of paclitaxel (PCX), a first-line drug for OC and the focus herein. The second part will be the development and evaluation of a cross-linked polyethylene glycol (PEG) NCs loaded hydrogel for IP drug delivery, for use at the point of surgical resection, and will be the focus of future studies.

Methods: Polymeric NCs were prepared with hyaluronic acid 40 KDa (HA), Tween[®] 80, Cremophor[®] RH 40 and Labrafac in an ethanolic solution by a self-emulsifying method. The physico-chemical properties and stability were characterized in terms of size (Z-Ave) and polydispersity index (PDI), by Dynamic Light scattering (DLS), and zeta potential (ZP), by Laser Doppler Electrophoresis. The NC formulation optimization was supported by Design of Experiments (DoE) to obtain monodisperse and stable NCs around 400-600 nm. Blank NCs were prepared, stored at 2-8 °C and in physiological conditions (37 °C) and then their stability evaluated after 6 days (size, PDI and ZP). After NCs optimization, PCX was encapsulated in the NCs (PCX-NCs).

Results: The DoE screening showed a major influence of the oil Labrafac on the size, PDI and ZP. Blank NCs were optimized to a proportion of 20:10:20 (% w/w) of Tween[®] 80, Cremophor[®] RH 40 and Labrafac, respectively. These NCs gave a size, PDI and ZP of 357 ± 8 nm, 0.2 ± 0.01 and -20 ± 0.2 mV, respectively. Furthermore, they were stable for at least 6 days under storage conditions (2-8 °C). Incorporation of PCX into NC (PCX-NCs) presented a slight increase in size to 391 nm, and a PDI and ZP of 0.2 and -18 mV, respectively. The drug loading and encapsulation efficiency are expected to be at least 3 mg/mL and 45%, based on previous data for a drug with similar physicochemical properties to PCX. PCX-NCs will then be loaded in a PEG-based hydrogel, developed by chemical crosslinking of a multi-arm PEG-maleimide (PEG-MAL) with a PEG-dithiol.

Conclusions: The Paclitaxel loaded NCs were optimized by using a DoE approach to a maximum size that is also stable. A larger size will increase the sustained release time course from the hydrogel and reduce clearance from the IP cavity. Future studies will incorporate PCX-NCs into a hydrogel formulation.

DEVELOPMENT OF A BIODEGRADABLE SUBCUTANEOUS IMPLANT FOR THE TREATMENT OF HYPOTHYROIDISM

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Background: Deficiency of thyroid hormones (hypothyroidism) is thought to affect more than 1.3 million people in the UK. Thyroid hormones control many physiological processes and deficiency causes symptoms such as chronic fatigue, weight gain and cold intolerance. Treatment with oral levothyroxine (LEVO) is the current standard. However, the effectiveness of oral administration is highly dependent on the co-administration of food and other drugs. As such, LEVO is recommended to be taken at least 30 minutes before food and other medication. These additional directions and the chronic nature of this condition mean that there are concerns with patient compliance. This work aimed to develop a subcutaneous implant for prolonged delivery of LEVO to treat hypothyroidism. This could overcome challenges with patient compliance and co-administration and could improve treatment of this condition. Stability of LEVO sodium in solution is a known challenge and as such a suitable biorelevant medium for extended *in vitro* release studies and an appropriate quantification method needed to be developed.

Methods: Implants were produced by solvent casting mixtures of poly(caprolactone) (PCL), poly(ethylene glycol) (PEG) and LEVO sodium. Formulations were prepared from mixtures of PCL of differing molecular weight, PEG and different LEVO sodium loadings (20% or 40% w/w). Implants were characterised using DSC, FTIR and SEM.

LEVO sodium stability in a range of release media was investigated and the most suitable was chosen to conduct release studies in. *In vitro* release studies were performed in 0.1% bovine serum albumin (BSA) and at predetermined time points the entire release medium was replaced. LEVO sodium release was analysed using HPLC (Zorbax Eclipse plus C₁₈ column (4.6 x 250 mm) with guard column of matching chemistry, a mobile phase of acetonitrile: 0.1% trifluoroacetic acid (50:50% v/v), a flow rate of 0.6 mL/min, an injection volume of 50 µL and a detection wavelength of 225 nm).

Results: Rod shaped implants of 2.5 x 40 mm were successfully produced by solution casting into silicone moulds. Implants prepared containing PEG showed an immediate discoloration and stability studies indicated an instability of LEVO sodium in PEG. FTIR, SEM and DSC results suggest that LEVO sodium is insoluble in the solvent used and is dispersed throughout, but not interacting with, the polymer matrix.

Quantification of LEVO sodium was achieved using RP-HPLC coupled with UV detection. This method was linear in the range 0.012 – 25 µg/mL and had a limit of detection and quantification of 0.03 and 0.09 µg/mL, respectively. LEVO sodium was found to be stable in 0.1% BSA for at least 14 days and as such this was chosen as the release medium for *in vitro* release studies. Release rates ranging from 42.01 ± 3.98 – 109.07 ± 6.17 µg/day and 101.41 ± 15.64 – 106.95 ± 16.77 µg/day were achieved for formulations containing 20% and 40% drug loading, respectively.

Conclusions: The implants produced in this work showed promising *in vitro* release rates for the delivery of LEVO sodium for the treatment of hypothyroidism. Future work will aim to optimise the implant formulation and investigate *in vivo* release rates.

RATIONALLY DESIGNED PEPTIDES ALTER INTESTINAL TIGHT JUNCTION COMPOSITION AND INCREASE PERMEABILITY TO POORLY ABSORBED DRUGS

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Background: Tight junctions (TJs) form a semi permeable barrier between epithelial cells, and in the intestine represent a major barrier to drug absorption. TJs consist of a number of transcellular proteins, such as occludin and claudins, which are anchored to the actin cytoskeleton and interact with TJ proteins from adjacent cells. TJ proteins have different barrier properties and the make up of TJs affects their permeability. TJs can be opened by phosphorylation of myosin light chain (MLC), which is controlled by MLC kinase (MLCK) and MLC phosphatase (MLCP). MLCP is formed by a protein phosphatase subunit (PP1) associating with a targeting subunit (MYPT1) via an interaction at a specific binding motif. We have designed **P**ermeable **I**nhibitor of **P**hosphatase (PIP) peptides that bind PP1 and inhibit MLCP to increase TJ permeability.

Methods: PIP peptides were applied to the apical surface of Caco-2 monolayers in addition to 4 kDa fluorescent dextran (FD4). Basal concentration of FD4 was measured and used to calculate apparent permeability (P_{APP}) with and without PIP peptides. TEER measurements were taken before and after PIP application. At the end of experiments, Caco-2 cells were lysed and levels of tight junction proteins were analysed by western blot. PIP peptides (20mM) were injected into the intestinal lumen of rats with 200 mg/kg exenatide (Ex4) or salmon calcitonin (sCT). Blood samples were taken and EX4 and sCT concentration was measured by ELISA. Injection sites were fixed and stained for visualization of tight junction proteins.

Results: Two lead peptides were tested, PIP250 and PIP640. Both peptides increased pMLC and increased P_{APP} of FD4 from 0.11×10^{-6} cm/s to 0.315 and 0.453×10^{-6} cm/s respectively. Positively charge dextran had a higher P_{APP} with both PIP640 (0.72×10^{-6} cm/s) and PIP250 (0.48×10^{-6} cm/s). PIP250 and PIP640 reduced TEER to 35% and 49% of baseline respectively. PIP640 increased claudin-2 1.6x and PIP250 increased it 1.2x in Caco-2 cells. PIP250 reduced occludin levels to 60% of baseline in caco-2 cells, whereas PIP640 had no effect. Intraluminal injection of Ex4 or sCT alone did not produce detectable serum concentrations. PIP250 increased Ex4 and sCT serum concentrations to 10.4 ng/mL and 10.5 ng/mL respectively. PIP640 increased serum concentrations to 10.14 ng/mL and 14.6 ng/mL respectively. PIP250 treated tissue showed a redistribution of occludin away from TJ localisation to the lateral membrane of epithelial cells.

Conclusions: Both peptides increased permeability in *in vitro* and *in vivo* models, demonstrating potential as permeation enhancers. Both also increase pMLC but also appear to have different permeation enhancement profiles. PIP640 produced a more rapid increase in permeability than PIP250. Permeability mediated by both peptides was preferential to positively charged molecules, but the effect was considerably greater with PIP640. PIP640 also increased claudin-2 levels more than PIP250. Claudin-2 is associated with cation selectivity at tight junctions. This suggests that PIP mediated claudin-2 upregulation contributes to cation selectivity of permeability. PIP250 also causes occludin down-regulation, which increases permeability. PIP640 does not have an effect on occludin. PIP250 occludin down-regulation takes longer to happen than pMLC increase, which may explain why PIP250 mediated increase in permeability is slower than PIP640. We have demonstrated two peptides that can enhance intestinal drug delivery *in vitro* and *in vivo*. The difference in permeability characteristics means there is a potential to use either peptide with specific drugs to enhance oral bioavailability.

LARGE-PORE SILICA NANOCARRIERS FOR ANTIANGIOGENIC TREATMENT AGAINST AGE-RELATED MACULAR DEGENERATION

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Background: Wet age-related macular degeneration (AMD) is a progressive disease that leads to central vision loss due to damaged retinal pigment epithelium (RPE) and photoreceptors in the central area of the retina. Wet AMD occurs in patients who develop subretinal choroidal neovascularization, mostly produced by an abnormal expression in the RPE of the vascular endothelial growth factor (VEGF). Current approaches for the treatment of AMD present considerable issues such as important side effects and low patient compliance. The encapsulation of anti-VEGF drugs in suitable nanocarriers with better penetration, higher retention times and sustained release would be of great interest. The objective of this work is the development of a drug delivery system for the topical administration of anti-VEGF siRNA molecules based on large-pore mesoporous silica nanoparticles (LP-MSNs). siRNA is loaded into the LP-MSNs pores, while the nanoparticles' external surface is functionalized with polyethylenimine (PEI) chains, which act as pore capping ensembles that allow the siRNA controlled release and promote endosomal escape to facilitate its cytosolic delivery.

Methods: LP-MSNs were functionalised to obtain three different sets of materials. The first one, S1, was loaded with the fluorescent dye rhodamine B and capped with PEI chains, to perform cargo release assays and verify PEI capping ability; S2 was covalently functionalised with rhodamine B isothiocyanate through 3-aminopropyltriethoxysilane chains, and externally capped with PEI, and employed to study particles cytotoxicity, cellular uptake and hemocompatibility; finally, S3 was loaded with anti-VEGFA siRNA and capped with PEI, and used for VEGF silencing in ARPE-19 retinal cells. The materials were characterised using standard techniques such as transmission electron microscopy, dynamic light scattering, zeta potential measurements and N₂ adsorption-desorption analysis. siRNA quantification was performed using a NanoDrop 2000 spectrophotometer. Nanoparticles hemotoxicity was tested with red blood cells and in platelet rich-plasma using a Quartz Crystal Microbalance with Dissipation monitoring (QCM-D).

Results: Spherical monodispersed dendrimer-like nanoparticles with an average size of 105 nm and center-radial large pores of about 17 nm were obtained. The release studies demonstrated that the cargo remains protected inside the pores in the absence of an adequate triggering stimulus. The siRNA-loaded nanodevices reduced VEGF expression, demonstrating the developed nanocarrier capacity to provide siRNA protection, endosomal escape and consequent cytoplasmic release. Nevertheless, although bare LP-MSNs showed negligible toxicity in several cell lines and in erythrocytes in previous assays, coated nanoparticles affected cells viability and induced hemolysis and platelet aggregation, probably due to the positively charged external PEI layer.

Conclusions: Our results represent a first step for the development of topically administered nanovehicles based on LP-MSNs for the sustained attenuation of VEGF in the RPE by siRNA. The successful results obtained in VEGF silencing in ARPE-19 cells demonstrate that although further modifications are needed for improving their biocompatibility, the designed nanodevices present a great potential for nucleic acid delivery, holding great promise for the next stages of the project.

DEVELOPMENT OF POLY(CAPROLACTONE) (PCL)-BASED POLYMERIC IMPLANTABLE DEVICES FOR SCHIZOPHRENIA TREATMENT

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Background: Nowadays, the development of implantable devices is widely explored in the pharmaceutical field due to its potential to provide long-acting drug delivery. The use of implantable devices can improve the management of chronic conditions such as schizophrenia as they require long-term pharmacological treatment. Accordingly, in the present work, biodegradable implants loaded with risperidone, a drug used to treat schizophrenia, have been prepared. The drug was combined with poly(caprolactone) (PCL), a biocompatible and biodegradable polymer. PCL offers long degradation times which are suitable for the purpose of implantable delivery system. Additionally, it is an inexpensive polymer. In order to tailor its degradation rate and drug release, hydrophilic and amphiphilic substances were combined with the polymer. This work investigates the use of PCL and its combination with poly(ethylene) glycol (PEG) 600, PEG 3000 and Tween 80 for the development of implantable devices aimed to treat schizophrenia.

Methods: PCL-based implantable devices were prepared following a solvent-casting method using dichloromethane as solvent. Four types of implants were formulated containing different combination of polymer and 50% (w/w) of risperidone. Each of the formulations was subsequently characterised using DSC, TGA, SEM, and FTIR. Moreover, degradation kinetics and *in vitro* drug release studies were conducted using PBS (pH 6.5). Samples were taken at predetermined times and analysed using HPLC. Mathematical models were applied to determine the release kinetic of risperidone from implants.

Results: Implants fabricated using risperidone and PCL were solid and flexible. It was found that the addition of PEG 600, PEG 3000 or Tween 80 to the formulations decreased slightly the melting point of the resulting materials. Additionally, these additives were able to increase the degradation rate of the materials. It is important to note that no chemical interaction was found between polymers and drug. Following the *in vitro* release studies, implants containing PCL were able to control the drug release over 28 days. In contrast, implants containing PEG 600, PEG 3000, and Tween 80 were found to sustain the drug for 14, 4, and 8 days, respectively. The risperidone release pattern of implants made of PCL showed good fitting to the Korsmeyer-Peppas model presenting an average release rate of 1.17 ± 0.07 mg/day, which will be clinically relevant as the recommended dose of risperidone for schizophrenia is 1-2 mg/day.

Conclusions: In this present work, monolithic implants containing risperidone were successfully fabricated using a solvent-casting method. The addition of hydrophilic and amphiphilic compounds modified the properties of implant, including the drug release rate. Based on results obtained in the *in vitro* release studies, it can be concluded that implants made of PCL showed the more sustained release profiles providing up to 28 days.

**A NOVEL ALTERNATIVE “MORNING AFTER” LOCAL ADMINISTRATION
APPROACH FOR POST-EXPOSURE PROPHYLAXIS OF HIV: DEVELOPING A
FORMULATION**

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Background: Since 2013 the number of newly diagnosed cases of HIV has practically remained unchanged. Current post-exposure prophylaxis (PEP) involves an oral administration, often with considerable side effects. Under WHO guidelines, PEP in a sexual exposure (PEPSE) is limited to only high-risk scenarios, thus potentially excluding a significant population exposed in lower to moderate risk scenarios. While the size of this population is unclear, unpublished data from our group shows that it could be as high as 30% of a sexually active population.

Methods: Dolutegravir was selected the antiviral of choice. It was then chemically modified; producing a dolutegravir myristate (MDTG). A two-step nanoprecipitation system was designed. Three testing conditions were assessed; using different coating masses of mPEG5000-LA100. Biocompatibility at 24 hours was on Caco-2 and Raw 264.7 cells. Uptake at 24 hours was then traced quantitatively and qualitatively by substituting 10% of the total nano-carrier with a Cy5 Blue labelled PEG5000.

Results: MDTG can be precipitated into an unstable nanocrystal. All formulations developed contained a significant drug content with a final average of 215nm in size. The coated formulation with 0.22mg was chosen to test further as it carried a significant drug content. In vitro data suggests biocompatibility and internalization of the formulation in target cells. Therefore providing potential for drug delivery in target tissues.

Conclusions: Due to the 14-carbon chain in MDTG, it can be successfully self-assembled into an unstable nanocrystal through a nanoprecipitation set up. Once in this form, it can be coated with a polymer agent, in a second nanoprecipitation.

Nanoformulations with a high drug content (above 50% wt%) of MDTG were developed. In vitro data suggests that uptake is higher in Raw 264.7 macrophage than Caco-2 cells at 24 hours. Target cells for this formulation are macrophages and antigen presenting cells, thus the results are encouraging.

More complex *in vitro* models such as Caco-2/M cells co-culture need to be investigated to provide more physiological relevant uptake data.

DEVELOPMENT OF A SUSTAINED-RELEASE TABLET FORMULATION OF NOVEL ANTIHYPERTENSIVE DRUG MT-1207

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Background: Hypertension is one of the most common chronic cardiovascular disorders. Controlled-release formulations can be used to maintain drug concentration within therapeutic levels throughout the treatment and increase patient compliance. The purpose of the present study was to develop a once-a-day tablet formulation for the novel antihypertensive agent MT-1207.

Methods: A tablet manufacturing method was developed and optimised, including wet granulation of the powder mixture containing the drug and the excipients, prior to compression. The matrix tablets produced were characterised for hardness, friability, uniformity of weight and *in vitro* release of MT-1207. Furthermore, Fourier Transform InfraRed (FTIR), differential scanning calorimetry (DSC) and X ray powder diffraction (XRPD) analyses were carried out as part of the physical characterisation of the tablets. Additionally, *in vitro* drug release studies were carried out in a dissolution medium of 0.1 M phosphate pH 6.8 with 0.2% w/v sodium dodecyl sulfate (SDS) using USP II paddle apparatus. Finally, *in vivo* animal studies were performed in Beagle dogs for the optimised sustained-release and immediate-release tablets.

Results: The tablets containing HPMC K4M could not retard the release of MT-1207. When this polymer was substituted with HPMC K15M which has higher molecular weight and viscosity than HPMC K4M a sustained release of MT-1207 from the tablets was achieved. Formulation F4 containing 31% w/w HPMC K15M gave a 24-hour release of MT-1207 with an almost constant release rate up to 20 hours. *In vivo* studies were then carried out in Beagle dogs for F4 and for the MT-1207 immediate-release tablets. The results showed that a sustained release of MT-1207 from F4 was achieved since the drug $t_{1/2}$ value was 2.5 times higher than that obtained after oral administration of the IR tablets. Moreover, the $AUC_{0-\infty}$ values of both sustained- and immediate-release tablets were identical at the same MT-1207 dose (30 mg) which showed that the same amount of drug was absorbed in each case.

Conclusions: A 24-hour sustained release of MT-1207 from the 30 mg optimised tablet formulation was achieved *in vitro*. However, *in vivo* studies in Beagle dogs showed that the plasma concentration of MT-1207 was not sustained over 24 hours and potentially the drug levels were below the therapeutic window at the 24-hour timepoint. Therefore, further optimisation of the formulation is probably needed, in alignment with pharmacological data that are expected from phase II clinical trials of the immediate-release tablets.

DEVELOPMENT OF RECEPTOR-TARGETED NANOCOMPLEXES FOR IN VIVO DELIVERY OF CRISPR/CAS9 AS A POTENTIAL THERAPY FOR CYSTIC FIBROSIS

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Background: Cystic Fibrosis (CF) is recessively inherited, multi-organ disease, however morbidity and mortality is caused mostly by progressive respiratory impairment due to mucus retention and chronic bacterial infection in the lungs. Gene therapy is an attractive therapeutic option for CF, targeting the underlying cause of the disease, rather than treating symptoms. We aim to develop a novel gene therapy for the respiratory manifestations of the disease based on gene editing with CRISPR/Cas9. This allows for the precise introduction of double strand breaks in the DNA followed, in the absence of a DNA template, by non-homologous end joining (NHEJ), an error-prone but efficient DNA repair pathway functioning in both dividing and terminally differentiated cells.

Methods: For treatment of CF, CRISPR/Cas9 must be delivered with sufficient efficiency to the lung. Our approach is to deliver Cas9/gRNA ribonucleoprotein (RNP) complexes with a non-viral, receptor-targeted nanoparticle (RTN), previously described for in vivo DNA and siRNA delivery to the lung. RTNs comprise a peptide component, mediating targeting of epithelial cells, and lipid components, enabling endosomal escape.

Results: To provide a model, we engineered primary, human bronchial epithelial cells to stably express GFP by lentiviral transduction and used this model to compare nanoparticle formulations for knockout of GFP by CRISPR/Cas9. We achieved levels of up to 75% GFP knockout, as measured by flow cytometry. Complexes had a desirable size (90 nm), charge + 40 mV and polydispersity index (~0.2) appropriate for delivery to the CF lung, where mucus accumulation prevents penetration of larger particles.

We next used RTNs to deliver RNP formulations to primary CF basal epithelial cells to delete a deep intronic CFTR mutation that creates a cryptic splice site (3849+10kb C>T). Following repeat delivery of RTNs, indel frequency was more than 80% by ICE analysis of DNA sequencing. Correct CFTR splicing was restored, while chloride ion transport was shown to be partially restored in Ussing chamber measurements.

Finally, to evaluate the efficiency of in vivo editing, we delivered the RNP nanoparticles to the lungs of Ai9 TdTomato reporter mice, where a loxP-flanked STOP cassette prevents transcription of TdTomato. By using two gRNAs targeting upstream and downstream of the STOP cassette, we aimed to excise the cassette, restoring TdTomato fluorescence. Following oropharyngeal instillation of RTNs, TdTomato expression was evident in the airway epithelium, with no evidence of an inflammatory response.

Conclusions: This work advances potential therapeutic avenues for nanoparticle-mediated Cas9 RNP delivery to the airways for treatment of CF.

IMPLEMENTATION OF A NOVEL MICROFLUIDIC STRUCTURE TO TRANSITION PROTEIN LOADED LIPOSOME PRODUCTION FROM BENCH TO GMP

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Background: Microfluidics is a flexible process that offers scale-independent manufacturing processes for liposomes. Microfluidics offers higher drug loading and better physio-chemical attributes compared to traditional liposomal production processes. These processes are often time-consuming and complex which can be circumvented using microfluidics allowing for easier up-scaling. The aim of this study was to compare different microfluidic architectures and test liposome production from the lab bench to GMP scale.

Methods: 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and cholesterol were dissolved in methanol or ethanol at 2:1 wt/wt (initial lipid concentration 4 or 16 mg/mL mg/mL). Liposomes were produced with different microfluidic architectures using either a staggered herringbone mixer (SHM) or a toroidal mixer (TrM). The flow rate ratio (FRR) used was 3:1 aqueous:lipid phase at a total flow rate ranging between 12 - 200 mL/min. Ovalbumin dissolved in PBS was added to the aqueous inlet (250 – 4000 µg/mL). After production, liposomes were purified using tangential flow filtration (Krosflow Research Iii system with a 750 KDa mPES column). Liposome size, charge and PDI was measured using a Malvern Zetasizer Nano ZS. Protein encapsulation and protein release kinetics was quantified using reversed-phase high performance chromatography (RP-HPLC, Shimadzu 2010-HT, Milton Keynes, UK) connected with a UV detector at 210 nm using a Jupiter 5 µm C5 300A 4.6 mm i.d x 250 mm length or micro-BCA assay. For GMP production a modified HPLC pump and NxGen cartridge 500 with TrM architecture was used at a TFR of 400 mL/min.

Results: Our results show that liposomes produced by both microfluidic mixers gave high protein loading (30-40%) with small sizes (50-60 nm) and homogenous (<0.2 PDI) liposome formulations with comparable protein release rates. Using the TrM system we are able to produce these liposome formulations at up to 200 mL/min giving the facility for high-throughput scale-independent production with flow rate having no impact on formulation attributes.

Conclusions: From our results we have demonstrated the ease of production at high-throughput using the TrM architecture by maintaining liposome properties across different microfluidic architectures. By increasing the flow rate to 200 mL/min it is now possible to scale-up microfluidic production with the same critical quality attributes across a range of production speeds and volumes using process parameters providing a direct path from bench to GMP.

Developing neural transplant cell sprays for traumatic neurological injuries

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Background:

Traumatic neurological injuries to the brain and spinal cord can have devastating clinical consequences with high costs for healthcare systems. Enhancing regeneration following neurological injury represents a major clinical challenge. Neural cell transplantation therapies have been shown to have significant translational potential to promote regeneration following such injuries. However, current cell delivery methods for neural cell transplantation have major drawbacks, including clinical risks associated with surgical microinjection into neural tissue (e.g. embolism, haemorrhage), and high cell loss on microinjection through fine gauge needles into densely packed neural tissue.

Cell spray delivery can offer significant translational benefits in this regard, including rapid and homogenous delivery and the capacity to combine cell therapy with drugs/biomolecules whilst being minimally invasive. Such an approach has been proven efficacious for skin wounds but never been attempted for neural transplantation.

This study aims to investigate whether spray delivery of neural transplant cells is safe. To achieve this, we tested the effects of cell spraying on two major neural transplant populations, proven to have therapeutic potential in neurological injury. Post-spraying, cells were assessed for their viability and key cellular properties (proliferation and differentiation) which underpin their therapeutic potential.

Methods:

Primary rodent mixed glial cultures were used to generate oligodendrocyte precursor cell (OPC) and astrocyte populations which were spray delivered via a commercial spray bottle. Controls were standard delivery by pipetting. Cell viability was assessed using a live-dead assay and cell proliferation using an EdU assay. Immunohistochemical markers GFAP (astrocytes), NG2 (OPCs), and MBP (oligodendrocytes) were used to identify individual cell populations.

Results:

Post spraying, both transplant cell types could survive with high cell viability. They also showed evidence of normal proliferation and differentiation (OPCs), with retention of characteristic cellular markers following spray delivery.

Conclusions:

Our findings show that spray delivery technology could offer a novel clinical solution for cell delivery in transplantation therapies for traumatic neurological injuries. Further refinement requires the identification of optimal spray parameters for clinical delivery.

Targeted non-viral delivery of mini-intronic plasmids for rheumatoid arthritis

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Background: Rheumatoid arthritis (RA) is an autoimmune disorder of the joints characterised by inflammation, synovial hyperplasia and increased vascularisation. Synovial fibroblasts are aggressive cells in RA pathogenesis, contributing to inflammation and cartilage degradation. Current treatment with disease modifying anti-rheumatic drugs increases risk of serious infection. Further, some patients do not respond to drugs whilst others develop resistance leading to loss of efficacy. Hence, we aim to develop a gene therapy that modulates RA disease without life-threatening, systemic immunosuppression.

Our approach involves developing receptor-targeted nanoparticles (RTN) that selectively deliver therapeutic genes to the inflamed synovium, thereby improving efficacy and reducing systemic side-effects. The RTN comprises of a cationic lipid that self-assembles with the anionic DNA backbone and a neutral lipid DOPE to aid DNA endosomal escape. In addition, formulations include a peptide containing a cationic, 16-lysine domain for electrostatic DNA packaging and a synoviocyte targeting ligand, separated by a cleavable or hydrophobic linker to alter RTN stability. Further, we aim to develop a DNA vector that provides high and sustained therapeutic gene expression while minimising inflammatory response based on mini-intronic plasmids (MIPs). MIPs lack bacterial propagation sequences, making them safer and potentially less inflammatory, and have been shown to provide greater and more prolonged gene expression than conventional plasmids.

Methods: Rabbit synovial fibroblasts (HIG-82) were transfected with luciferase or GFP reporter plasmids with RTNs containing different peptides. Transfections to assess cell type specificity were performed with chondrocytes (C28/I2) or hepatocytes (HepG2). Luciferase or GFP expression was measured after 24 or 48-hours incubation, respectively. MIPs contained a luciferase reporter gene with either a CMV or EF1 α promoter and luciferase activity following HIG-82 or HEK293T transfection with RTNs was compared to that of conventional plasmids over time at equimolar ratios.

Results: RTNs with synoviocyte peptides demonstrated equal transfection efficiency in HIG-82 cells than positive control peptides that have previously shown good efficiency in various cell types, regardless of linker type. In comparison, synovial-targeted RTNs yielded much poorer transfection efficiency in hepatocytes and chondrocytes compared to RTNs with positive control peptides, which transfected all cell types indicating a degree of targeting specificity for synoviocytes. Additionally, RTNs with cleavable peptides gave more efficient transfection than hydrophobic in all cell types, presumably due to enhanced RTN disassembly following cell uptake.

When MIPs were delivered with RTNs to HIG-82 or HEK293T cells there was no clear difference in transfection efficiency as compared to conventional plasmids. Additionally, it was predicted that MIPs may be less toxic than conventional plasmids due to their lack of bacterial CpG sequences and their smaller size allowing for a lower amount of DNA to achieve the same gene copy number. However, no difference in cytotoxicity was noted between MIPs or conventional plasmids in either HIG-82 or HEK293T cells. Investigations are ongoing to find a suitable vector that provides the required sustained expression.

Conclusions: This work provides the basis for a targeted RA gene therapy approach that specifically delivers DNA to synoviocytes in the inflamed joint.

Intradermal delivery of long-acting bictegrovir nanosuspension-loaded microneedles for potential treatment of HIV infection

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Background: HIV/AIDS affects approximately 36.7 million people worldwide. Oral administration is one of the commonly used options for HIV treatment. However, one drawback is poor patient compliance given the lifelong, daily dosing required. Transdermal delivery possesses advantages in maintaining stable drug plasma levels by eliminating the first pass effect, resulting in reducing daily dose requirement. Microneedles are transdermal drug delivery devices that painlessly by-pass the *stratum corneum*. The development of long-acting nanosuspension (NS) HIV drugs potentially promises to improve the adherence. Dissolving microneedles (DMNs) can deposit NS in the viable skin layers for absorption by the dermal microcirculation and also possibly uptake by the lymphatic system, an important reservoir for HIV virus. Accordingly, this study aimed to deliver BIC intradermally for sustained absorption.

Methods: BIC NS was manufactured by a wet media milling technique using 1% w/w PVA (10 kDa) and ceramic beads as milling media. The particle size and polydispersity (PDI) of NS was optimized by increasing milling time. Cryoprotectant of PVP (K29-32) was added in optimized NS, which were lyophilized in the freeze dryer for 25 h. Particle size and PDI were measured with dynamic light scattering (DLS). The NS was characterized based on attenuated total reflectance fourier transform infrared (ATR-FTIR). The first layer of DMNs (needle density of 16 × 16, 600 μm pyramidal needles with 250 μm column shaft, 300 μm width at base and 300 μm interspacing) were prepared from the aqueous blend of BIC NS by applying positive pressure in pressure chamber (5 bars, 2.5 min) and 30% w/w PVP (360 kDa) was used to cast the baseplates with the centrifugation (3500 rpm, 15 min). Mechanical study and Parafilm M[®] insertion were also carried out to characterize the DMNs.

Results: DLS reports of BIC NS confirmed that milling time was able to influence the particle size. The NS formulation prepared with a milling time of 24 hours exhibited the smallest particle size (396.11 ± 28.67 nm) and PDI (0.17 ± 0.04). The lyophilized NS gave good resuspension in deionized water after slightly shaking. The particle size and PDI of the NS obtained after lyophilization were 390.34 ± 37.65 nm and 0.192 ± 0.02, respectively. According to ATR-FTIR spectra, the major peaks retained in NS compared with pure drug powder and no additional peaks were observed, indicating that there were no chemical interactions between pure drug and excipients. Digital microscope images clearly showed the DMNs had sharp tips filled with BIC NS and transparent baseplate. The percentage of height reduction of DMNs was less than 10% and the insertion depth into Parafilm M[®], a skin simulant, was up to 504 μm.

Conclusions: The NS formulation and NS-loaded DMNs were successfully developed. In future studies, drug deposition, *ex vivo* dermatokinetic study, skin distribution, as well as *in vivo* pharmacokinetics will be explored.

A SPOONFUL OF SUGAR (OR ACID) HELPS THE MEDICINE GO DOWN: A MULTIPURPOSE VAGINAL RING STRATEGY FOR TREATMENT OF BACTERIAL VAGINOSIS.

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Background: Bacterial vaginosis (BV) is a common dysbiosis of the human vaginal microbiome associated with depletion of the normally dominant *Lactobacillus* species and overgrowth of facultative anaerobic bacteria. Although most women diagnosed with BV do not suffer complications, BV can lead to preterm birth, risk of infection after gynecologic surgery, pelvic inflammatory disease, and increased risk of acquiring a sexually transmitted infection, including infection with human immunodeficiency virus (HIV). Following the significant advances in recent years in developing antiretroviral-releasing vaginal rings (VRs) for HIV prevention, there is now considerable interest in developing new multipurpose prevention technology (MPT) VRs aimed at treating/preventing BV in addition to delivering an antiretroviral drug. Here, we describe formulation efforts to develop a MPT VR offering simultaneous release of two or more of the following actives: dapivirine (DPV, a potent experimental antiretroviral); 5-nitroimidazole antibiotic drug, including metronidazole (MET), tinidazole (TNZ), secnidazole (SNZ) and ornidazole (ONZ); sucrose (selectively promotes the growth of lactobacilli), and boric acid (antimicrobial and anti-biofilm properties).

Methods: Matrix-type silicone elastomer VRs containing various combinations of DPV, MET, sucrose and BA were manufactured. In vitro testing of rings included: rheological assessment of cure properties; drug release; thermal analysis (DSC, TGA); mechanical testing (compression, Shore Hardness); swelling studies in aqueous medium; surface imaging using scanning electron microscope.

Results: All of the active agents, both singly and in various combinations, were successfully incorporated into silicone elastomer matrix-type vaginal rings. For rings loaded with 250 mg 5-nitroimidazole drugs, rank order *in vitro* release was SNZ>ONZ>MET>TNZ. The incorporation of sucrose in MET rings increased MET release. DPV release was readily modulated by changing the drug loading or combining with MET. Mechanical properties of these vaginal ring formulations were acceptable. Release of boric acid from rings decreased the pH of the release medium. Incorporation of sucrose and boric acid caused surface roughness of the rings. The incorporation of up to 25% w/w sucrose and boric acid caused the vaginal ring to swell by 70% and 12.5% of the original mass, respectively.

Conclusions: Despite challenges in developing a VR device offering simultaneous release of multiple actives, the data support the further development of combining a 5-nitroimidazole antibacterial drug with sucrose, boric acid and an antiretroviral drug.

Bio/chemoinformatics and formulation insights on the hydroxychloroquine debate on combating COVID-19
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Background: Hydroxychloroquine(HCQ) is undergoing several clinical trials for evaluating its efficacy and safety as an antiviral. Yet, there is still a great debate about their efficacy in combating COVID-19. We hereby, hypothesize the success of the intranasal and the pulmonary routes through a gelatin matrix to overcome a lot of its pharmacodynamic and pharmacokinetic challenges and to increase their local concentrations at the sites of initial viral entry while minimizing the side effects.
Methods: Molecular dynamic simulation of a gelatin matrix was performed. Molecular docking of HCQ on this simulated carrier and on mucin as well as various receptors including Angiotensin-converting enzyme 2 (ACE-2), heparin sulphate proteoglycan and Phosphatidylinositol binding clathrin assembly protein (PICALM), which are expressed in the lung and intranasal tissues and represent initial sites of attachment of the viral particles to the surface of respiratory cells was accomplished.
Results: Molecular docking on the gelatin-simulated matrix proposed high loading and a sustained release profile. Moreover, strong binding to all the investigated receptors was obtained.
Conclusions: The presented data provide insight into the rational for an intranasal or pulmonary HCQ formulation aiming for a sustained prophylaxis effect and/or a treatment strategy against COVID-19 pandemic viral infection.

DEVELOPMENT AND CHARACTERIZATION OF 3D HYDROXYAPATITE-PLGA SCAFFOLDS TO STUDY METASTATIC PROSTATE CANCER IN THE BONE

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Background: Bone-related cancers can arise directly in the bone structure such as primary cancers osteosarcoma or result from metastatic spread from other primary sites. Prostate cancer is a metastatic type of cancer and the second most frequent malignancy in men worldwide, which accounted for nearly 3,8% of all cancer-related deaths in men in 2018. Advancements of new oncology treatments are poor and research has focused on *in-vitro* models to address the need to explain disease pathophysiology and to develop more effective clinical therapies. Still, 70-80% of *in vitro* cancer studies are reported to be conducted using conventional 2D cell-culture models despite the fact that they fail to recapitulate the properties of native tissue and that cancer cells exhibit markedly different behavior *in vivo* compared to in 2D cell-cultures. 3D models which recreate the cues in the biological environment are increasingly of interest.

Here we aim to develop a physiologically relevant 3D model of the bone using PLGA (polylactic-co-glycolic acid 85:15) mixed with nHA (nano-hydroxyapatite), which is chemically similar to bone mineral. The scaffolds will be used to study more effectively metastatic prostate cancer in the bone by conducting 3D culture studies of hFOB 1.19 (human fetal osteoblasts) and PC3 (prostate cancer cell line) in mono and co-culture.

Methods: Polymer microspheres were obtained by electro spraying, a process by which a solution of 3,5% PLGA in DCM is extruded through a nozzle by application of high voltage (15 kV). The microspheres produced were used to obtain different types of scaffolds: A) Plain PLGA B) 2mg nHA/PLGA C) 4mg nHA/PLGA, using NaCl as porogen. After the powder mixture was compressed (1500 psi) and foamed in CO₂ (800 psi, 24 h), the salt porogen was leached to produce a porous scaffold. For cell culture studies, scaffolds were sterilized with multiple washings of 70% ethanol, PBS and FBS and left to dry prior to use. Microspheres and scaffolds were characterized by SEM. The scaffolds porosity was calculated following immersion in deionized water (30 min). Prior to 3D cultures, common 2D mono and co-culture controls were set up: 20,000 cells/well for PC3; 20,000 or 40,000 cells/well for hFOB 1.19. Cell metabolic activity was conducted by performing MTT assay. hFOB 1.19 behaviour was tested by staining ALP and quantified with pNPP. Current 2D co-culture studies (25,000 cells) were conducted at two different ratios (hFOB 1.19: PC,3) of 4:1 and 1:1 at 33.5°C and 37°C.

Results: The SEM characterization of microspheres indicated particles has a collapsed, spherical morphology with a size in the range 2-6 µm. SEM characterization of scaffolds revealed the inner porous structure diameter was approximately 300 µm. The average porosity of the scaffolds was 55%±3.2 (plain PLGA), 48,2%±5,58% (2mg nHA/PLGA) and 60,91%±12,44 (4mg nHA/PLGA). 2D monocultures indicated that both the cells lines were viable after 7 days. Based on ALP staining and pNPP test hFOB 1.19 demonstrated higher differentiation at higher temperature (37°C), cell density (40,000 cells/well) and in co-culture at 4:1 ratio when compared to 1:1. Studies on mono and co-culture in 2D and 3D are undergoing investigation, however, first indicative results suggest hFOB 1.19 display a better growth profile in hHA/PLGA scaffolds compared to the one in plain PLGA scaffolds.

Conclusions: A porous, composite scaffold was produced and sustained hFOB 1.19 and PC3 growth for 7 days. 2D mono and co-culture studies show that cell behavior is influenced by seeding density, ratio of different cells and incubation temperature. This suggests that initially, PC3 could generate an osteoblastic lesion in the bone leading hFOB 1.19 to deposit more bone mineral. 3D co-culture studies are currently under investigation.

SPECTROGRAPHIC MONITORING OF THE RECONSTITUTION OF LYOPHILIZED HIGH CONCENTRATION PROTEIN FORMULATIONS

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Background: Traditional methods for quantifying reconstitution time of lyophilized formulations involves the visual identification of the endpoint, which has led to inconsistent values throughout the literature. The use of Deep Ultra-Violet fluorescence time-resolved spectroscopy as a novel alternative to visual quantification of the reconstitution time of lyophilised biopharmaceutical was investigated.

Methods: Spectrographic information was collected via a novel custom-made setup that allowed the measurement of the reconstitution time in a standard sealed lyophilization vial. The spectra obtained were analyzed via principal component analysis to obtain a time-based representation of the changes in a reconstituting formulation.

Results: At high protein concentration. The variability of the reconstitution time measurements was reduced from 80.4% relative standard deviation obtained via the traditional method to 8.2% for the instrumental method presented in this study.

Conclusions: The methods for reconstitution endpoint detection presented in this study provide an easy-to-use, precise, and reproducible alternative to the traditional visual observation of reconstitution protocol. The instrumental method is customizable for a wide variety of use-cases, affordable, easy to set-up, and easy to use. Increasing the viability of the measurement of the reconstitution time of lyophilised products pre-administration in a clinical environment. Furthermore, the results obtained using this method are easily cross compared, improving the usability of the reconstitution time of lyophilised product as a critical quality attribute.

PREPARATION OF BISPECIFIC ANTIBODY MIMETICS

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Background: Fab-PEG-Fab (FpF) molecules have been shown to act as IgG mimetics [1]. Each antibody Fab is conjugated at both termini of a *di-bis*-alkylation PEG reagent site-specifically by disulfide bridging conjugation. We now wish to examine the binding properties of bispecific FpFs, which would be derived from two different Fabs and are designed to act as bispecific antibody mimetics. The aim of this study was to compare the preparation of bispecific FpFs using the *di-bis*-alkylation reagent and *mono-bis*-alkylation reagents functionalized on the other PEG terminus with a functional group capable of undergoing a copper free 1,3-dipolar cycloaddition (click reaction) between an azide (N₃) and the strained alkyne in dibenzocyclooctyne (DBCO) [2].

Methods: *Synthesis of mono-bis-alkylation PEG "click" reagents:* BocNH-PEG-NH₂ (5 or 10 kDa) was first functionalised with the *bis-alkylation* conjugation moiety using isobutyl 1,2-dihydro-2-isobutoxy-1-quinolinecarboxylate (IIDQ) in acetonitrile and the product purified by precipitation in cold acetone (dry-ice). The Boc was then removed using 30% TFA in dichloromethane, and following roto-evaporation, the TFA-amine salt was precipitated in cold acetone. The second PEG-amino terminus was then functionalised with a DBCO-NHS analogue using 4-dimethylaminopyridine (DMAP) in acetonitrile. The final *mono-bis*-alkylation-PEG-DBCO reagent was isolated after precipitation in cold acetone. The azide partner was synthesised by functionalising NH₂-PEG-N₃ with the *bis-alkylation* conjugation moiety using IIDQ in acetonitrile and isolated after precipitation in cold acetone.

Results: Analysis of H-NMR spectra indicated that the amide coupling reactions occurred in high efficiency for both the *mono-bis*-alkylation PEG DBCO and N₃ reagents with final reagent purities > 90% based on analysis of integrals of the diagnostic peaks due to the functional groups on each termini and the PEG methylenes. Fab conjugation at the *bis*-alkylation moiety for each reagent proceeded in the same manner as observed previously for Fab PEGylation [3]. The Fab-PEG_{5k}-DBCO and Fab-PEG_{10k}-N₃ conjugates were then mixed in phosphate buffered saline for 24 hours, SDS-PAGE indicated the formation of the click product at approximately 120 kDa. Purification by ion exchange chromatography yielded the bispecific FpF. Use of the *di-bis*-alkylation reagent described in [1] and sequential additions of the two Fabs also yielded the desired bispecific FpFs, but the reaction mixture was more difficult to purify.

Conclusions: The *mono-bis*-alkylation PEG-DBCO and PEG-N₃ reagents allow for a simpler preparation of bispecific FpFs than bispecific FpFs prepared using a *di-bis*-alkylation PEG reagent.

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Rheological Characterisation of Sclerosing Foams in Biomimetic Settings Using Clinically Relevant Parameters

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Background: Sclerotherapy is one of the most common and least invasive methods of treatment against varicose veins. While bench-top properties of sclerosing foams (e.g. bubble size distribution and foam half-life) have been characterised previously, their flow behaviour remains largely uncharacterised. Given that aqueous foams exhibit a time-dependent rheology due to ageing phenomena (i.e. liquid drainage, bubble coarsening and coalescence), the identification of appropriate rheological measurement systems is critical to the quantification of clinically meaningful foam properties.

Methods: A biomimetic pipe viscometry apparatus was used to characterise the rheology of polidocanol (1%) sclerosing foams across a clinically relevant range of shear rates (6 s^{-1} – 400 s^{-1}) within polytetrafluoroethylene pipes of different diameters (2.48 mm and 4.48 mm). Foams were formulated using varying liquid-to-gas ratios (1:3, 1:4 and 1:5) and manufactured using the Tessari and DSS (double syringe system) methods. Additionally, end-effect and wall-slip correction methods were utilised to model the nominal rheology of foams. The rheological data were fitted into the power-law model to obtain the fluid flow index (n) and the fluid consistency index (K) of sclerosing foams, followed by systematic statistical analysis of power-law indices.

Results: The observed rheological behaviour of sclerosing foams is shown to be dependent on pipe diameter and liquid-to-gas ratio, while the effect of formulation technique appears to be statistically insignificant. Although wall-slip correction ultimately failed in providing physically meaningful results; nevertheless, end-effect correction was successful. Sclerosing foams behaved as shear-thinning fluids with flow indices ranging $0.24 < n < 0.45$, while the observed consistency indices ranged $2.98 < K < 12.49$. The nominal (end-effect-corrected) rheology of foams were shown to follow similar trends with respect to liquid-to-gas ratio and formulation technique when measured in different tube diameters, although the range of the flow consistency indices were narrowed by end-effect correction ($2.21 < K < 6.30$). In addition to the power-law characterisation of sclerosing foam rheology, the rheograms demonstrated evidence of a quasi-static drainage effect that affected foam viscosity during slower injections.

Conclusions: In this study, a biomimetic set-up was successfully employed to evaluate sclerosing foam rheology. Overall, results suggest a direct correlation between foam dryness and viscosity. Different manual techniques of formulating foams (DSS vs. Tessari) resulted in foams with comparable rheology. Additionally, this study provides a detailed description of the power law indices of different physician compounded sclerosing foams. Findings from this study could inform the optimisation of sclerosing foam's formulation and administration techniques.

CONTINUOUS FEEDING OF A MESOPOROUS SILICA USING A LOSS-IN-WEIGHT FEEDER

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Background: In continuous manufacturing, the initial feeding of raw materials is a critical step. Inconsistencies in the feed stream here may pass variability onto subsequent downstream processes and may negatively affect the overall quality of the final drug product. Silica has been a widely used excipient in pharmaceutical formulations. However, mesoporous silica in particular has gained significant popularity in recent years due to its drug delivery capabilities. Using a loss-in-weight feeder, we investigated the impact of tooling setups on the silica feed rate variability. Additionally, the study examined how the feeding process alters the physical properties of the silica raw material.

Methods: A twin-screw loss-in-weight feeder was operated at varying feed rate setpoints under different tooling setups. Tooling configurations included 2 screw options (fine concave and coarse concave screws) and 3 discharge screen options (fine square, coarse square and no screen). Feed rate data was collected using an independent catch scale for each tooling combination and was analyzed to determine the variability. Post-feeding silica samples were characterized to determine if any physical change occurred.

Results: In general, all feeder tooling configurations produced feed rates with similar relative standard deviations, apart from one combination (coarse concave screw/no screen) which was higher. Bulk density and flow behavior of the silica was altered by the feeding process and this change was dependent on the feed rate and tooling configuration used. Higher feed rates reduced bulk density and improved powder flow whereas the fine discharge screen had the converse effect.

Conclusions: This study describes a methodology to characterize the feeding behavior of a material using a loss-in-weight feeder. In relation to the mesoporous silica investigated, feeder tooling configuration was shown to have a minimal effect on feed rate variability. However, tooling selection was still an important factor as it impacted both the bulk density and flowability of the fed material.

Depot Forming Dissolving Microneedle for Intrasccleral Protein Delivery

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Background: Age-related macular degeneration (AMD) is a chronic progressing degeneration of the macula and is the leading cause of vision impairment among elderly individuals. Currently, the intravitreal injection of anti-vascular endothelial growth factor (Anti-VEGF) agents is a standard approach for AMD treatment. However, owing to the chronic nature of AMD, patients require frequent injections, performed by highly invasive hypodermal needles. Therefore, the intravitreal route is always associated with severe complications, such as retinal detachment, endophthalmitis, and cataract development. Dissolving microneedles (MNs) have been proposed as an alternative to the hypodermic needle, offering minimum invasion and increased patient compliance. Therefore, in this study, ovalbumin (OVA)-loaded dissolving MNs have been fabricated to transport protein to the back of the eye in a minimally-invasive manner. A number of polymers have been selected and assessed for fabrication of dissolving MNs and assessed for its characteristics to develop optimised delivery system.

Methods: OVA was used as a model protein and four polymers hyaluronic acid (HA), polyvinyl acetate (PVA), polyvinylpyrrolidone (PVP) and the mixture of PVA and PVP were selected for MN preparation. The MN arrays contained 9 (3*3) conical-shaped needles, approximately 700 µm in height, with a base width of 300 µm and 50 µm interspacing were fabricated using MN moulds. MNs were characterised for its morphology, mechanical strength, insertion depth, dissolution time and *in vitro* permeation, followed by a cell toxicity assay in human retinal pigment epithelial (ARPE-19) cells lines.

Results: The results of light microscopy and scanning electron microscopy imaging indicated that all polymers investigated were feasible to be fabricated into MN arrays with sharp needles. Optical coherence tomography showed that except for the OVA MN made of HA, which were too soft to penetrate the porcine sclera, all other MNs prepared from PVA, PVP and their mixtures were successfully inserted into the porcine scleral tissue with insertion depth greater than 75% of the total MN height. OVA MNs fabricated from all selected polymers were found to possess the rapidly enough dissolution (< 3 min) within the scleral tissue. OVA was successfully delivered across porcine sclera *in vitro*, with PVP MN delivering the greatest amounts (i.e. 57.87±2.20 µg) in 24 hours, which was three times higher compared to conventional formulations (e.g. topical eye drops and gels). Furthermore, it was found that the viability of ARPE-19 cells was always >76%, which demonstrated that all selected polymers were non-toxic to retinal cells.

Conclusions: This study optimised the polymer for rapidly dissolving MN applied for posterior segment protein delivery via intrasccleral route. Except for the MN composed of HA, MNs fabricated from other polymers were sharp and robust enough to puncture the scleral tissue with limited reduction of height. *In vitro* studies indicated that MN made of all selected polymers could rapidly dissolve within the tissue after insertion and showed an increased degree of permeation of model protein, which demonstrates that dissolving MN is capable of bypassing ocular barriers and delivering high molecular weight proteins in close proximity to the target tissue (choroid/retina). Moreover, the materials used in the fabrication of MNs were found to be biocompatible for human retinal cells. In futures work, OVA-encapsulated nanoparticle will be loaded into dissolving MNs to sustained the release of drugs from rapidly dissolving MNs and ultimately provide a minimally invasive and long-term treatment for the posterior segment of ocular diseases.

Nose to Brain Drug Delivery of Aerosolized NanoparticlesAida Maaz¹, Ian Blagbrough¹, Robert Price¹, Paul De Bank¹¹Department of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY, U.K.

Background: Our research aim is to assess the performance of intranasal nano-formulations *in vitro*, particularly for CNS targeting delivery via the uppermost region within the nasal cavity. For the first time and in order to achieve, deep penetration into nasal cavity, this project is focused on the delivery of nanoparticles via a liquified propellant contained within a pressurized metallic canister. The design of the nanocarriers in terms of their shape, size, surface charge and functionalization will be optimized to enhance the efficiency of carrier/drug *in vitro* deposition in the olfactory region. In addition, aerosol/device related parameters as well as administration instructions (cast tilting and actuator tip orientation) will also be investigated for nasal regional localization.

Methods: Firstly, spherical Au seeds were synthesized: 100 mL of HAuCl₄.3H₂O aqueous solution was boiled for 2h and then the reducing agent sodium citrate dihydrate was added with continuous stirring to produce a rose wine type solution, referring to the citrate capped seed's formation. AuNFs were prepared in a 10 mL vial of ultra-pure water by adding specific amounts of the pre-prepared seeds followed by HAuCl₄.3H₂O, silver nitrate as a shape directing agent, methoxy-PEG thiol as stabilizing agent and dopamine as both a reducing agent and model therapeutic, followed by stirring for two hours. The resulting dark blue color solution reflected the successful growth of gold nanoparticles, which were concentrated by evaporation (F1) or freeze drying (F2) to be further suspended within metallic canister with a hydrofluoroalkane propellant and delivered via a bespoke nasal device. Upon actuation, the spray jet nozzle creates high velocity droplets for transportation of the therapeutic particles into the nasal cavity. Glass collection apparatus (Copley Scientific, UK) was used to test the integrity of the AuNFs formulations after filling within the cans. F1 and F2- filled canisters were tested with adjusted flow rate at 15 ± 0.2 L/min. The samples were firstly primed by shaking the canisters 20 times and sonicating for 90 seconds, 10 actuations were carried out for each sample and finally the spray mist was collected on a mixed cellulose ester membrane located at the top of the tube to receive the fired shot. A piece of mica and carbon grid was stuck on the membrane for SEM and TEM analysis, respectively. *In vitro* deposition studies will be performed using nasal cast model recreated from MRI scans of human nasal airways. The model 3D printed in house to build copies with separated distinct regions.

Results: Both gold nanosuspension F1 and F2 were characterized and compared before and after the filling and spraying by the metered dose device. The concentrated gold nanosuspension was dark blue in color. DLS measurements showed that both formulations had a size range of 132.6 ± 0.95 nm and 137.8 ± 0.7 nm, were highly monodisperse, with PDI of 0.003 to 0.017, and positively charged (Z.P 5.42 ± 0.24 mV). These results were further confirmed with TEM imaging. Interestingly, no changes were observed in terms of the shape and the size of the prepared particles following the filling within the propellant system. However, very few particles could be detected on the TEM grids, so SEM-EDX analysis of the upper membrane was carried out to investigate elemental gold within the receiver membrane. Tiny fluorescent dots distributed throughout the membrane were observed and they belong to Au element.

Conclusions: Aerosolized AuNFs were successfully prepared as a pioneer nasal system for further brain targeting. Their simple preparation, flexible morphologies together with high stability within the propellant based devices make them excellent candidates for nose to brain drug delivery studies.

STEREOLITHOGRAPHY 3D PRINTED LONG-ACTING INTRAOCULAR IMPLANTS FOR POSTERIOR SEGMENT DISEASE

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Background: Drug delivery to the posterior segment of the eye remains challenging because of the anatomical and physiological barriers of the eye. Sustained release ocular implants can minimize the side effects and improve patient compliance. The development of new drug delivery devices requires reliable, reproducible and efficient technologies. 3D-printing technology can aid in fabrication of micron-sized implants with high precision, desired size/shape, and tailored release profiles. To date, the usage of 3D-printing technology to fabricate ocular implants has not been widely developed. In this study, a long acting intraocular implant of triamcinolone acetonide (TA) was fabricated using stereolithography (SLA) 3D-printing technology. Further, the implants were characterized for chemical/physical characterizing and drug content release.

Methods: Implants were fabricated using SLA-3D printer by loading 5% (w/w) of TA into a non-biodegradable polymer (PlasGRAY®). Implants were made in hollow and non-hollow rod shaped. Subsequently, implants were characterized using Texture Analyzer, Different Scanning Calorimetry (DSC), Size Exclusion Microscope (SEM), and Fourier Transform Infra-Red (FTIR). In vitro drug release studies have been carried out using PBS Buffer pH 7.4. Twenty microliters of samples were injected into HPLC system and the detection was set at 236 nm for TA analysis.

Results: Hollow implants (0.9 OD x 0.3 ID x 7L mm) and non-hollow rod shaped (0.9 OD x 7 L mm) implants were successfully fabricated using SLA-3D printing technology. Hollow and non-hollow implants showed no significant difference in mechanical strength (~ 40 N; $p < 0.05$). DSC, SEM and FTIR results showed that TA is soluble in the PlasGRAY® polymer. Non-hollow implant showed only 2.05 ± 0.32 % of TA released, whereas hollow implant released 8.50 ± 1.32 % of TA in 16 weeks.

Conclusions: Micron-sized of TA-loaded PlasGRAY® implants can be fabricated using SLA-3DP technology. Sustained drug release was achieved from these implants, where modification of degree of polymer crosslinking, drug loading, and implant design will have direct influence upon the release rates.

Surface modified Niosomes entrapping Minoxidil: Targeted Drug Delivery platform to treat Alopecia

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Background: Alopecia defined as loss of hair from the scalp and other parts of the body may be involved in some types. It is a term comes from the Greek alope^x, "fox" relating to manage in foxes and may be include a huge variety of conditions such as autoimmune, genetic, environmental and infectious. The appearance of clinical manifestations can diversify depend on the pathogenesis of this disease. Scaring and non-scaring are two types of alopecia. Scarring alopecia is classified into primary and secondary according to cause of destruction of hair follicle, while non scarring hair loss is classified according to the cause into: androgenic alopecia, alopecia areata, traction alopecia, effluvium (anagen and telogen), tinea capitis and trichotillomania. In 1965 scientists discovered the potent antihypertensive effect of minoxidil and since then it has been used as a treatment for hypertension, but later it was found that it could be used for treatment of alopecia "e.g. androgenic alopecia ,anagen effluvium ,alopecia areata and traction alopecia" due to its hpertrichotic effect. Minoxidil entrapped in niosomes coated with chitosan were prepared to develop targeted drug delivery systems with controlled release profile to treat alopecia

Methods: Niosomes composed of span 60 and cholesterol in molar ratio 1:1 (F2) coated with chitosan (F3) were prepared using thin film hydration method and evaluated for particle size, zeta potential using mastersizer, entrapment efficiency, morphology using scanning electron microscope (SEM), and structure by X-ray diffraction. The prepared niosomes were then incorporated in topical cream (F4) and (F5). The formed creams were evaluated for viscosity using Brookfield viscometer , spreading and irritation properties. In vitro release was also investigated for all formulae and compared to drug suspension (F1)

Results: The results revealed that particle size of (F2) and (F3) were 349.9 nm and 674.9 nm, respectively, While entrapment efficiency of (F2) and (F3) were 22.33% and 13%, respectively. Moreover, zeta potential was -40mV for (F2) and +32 mV for (F3). X- ray diffraction showed that minoxidil is crystalline in nature which didn't change and remained crystalline in all formulae. Viscosity of (F5) was 9000-9300 Cp and 6850-7600 Cp at 6 and 12 rpm respectively, with no irritation and good spreading property. In vitro release after 6 hours of F1, F2, F3, F4 and F5 were 100%, 39.11%, 33.56%, 19.66%, and 12.17%, respectively.

Conclusions: In the light of the aforementioned results, chitosan coated niosomes entrapping minoxidil incorporated in cream were successfully prepared to achieve targeted drug delivery and controlled release as well.

Non-woven Electrospun Micro/Nanofibers Inserts for Sustained Topical Ocular Drug Delivery

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Background: Ocular inserts are a promising strategy to achieve sustained drug delivery to the anterior segment of the eye. Common challenges with ocular inserts include corneal abrasion, accidental loss or sticking out due to poor muco-adhesiveness, allergic reaction and inflammation. However, electrospun patches have a high potential to overcome some of these issues due to their softness, strong mucoadhesiveness and improved biocompatibility. Moreover, their ability to achieve high drug loading and tailored thickness make them versatile in both the engineering and medical field. The aim of this research is to develop a small and effective ocular insert in the *cul-de-sac* which can achieve sustained drug delivery for one week in treating anterior segment ocular diseases.

Methods: PVA and PCL nano/microfibers were fabricated to deliver hydrophilic and hydrophobic anti-inflammatory agents such as triamcinolone acetonide and bromfenac sodium. Non-woven inserts were fabricated via the electrospinning process. PVA fibers were further crosslinked by glutaraldehyde after fabrication to avoid the rapid swelling and breakdown. Co-axial fibers were fabricated for further optimization to achieve pre-defined physicochemical properties. TEM and SEM were used to identify the micro-structure of the inserts. Thermal analysis was carried out to check possible solvent residue and polymorph. Thickness was recorded before and after swelling. Content analysis and *in-vitro* release were carried out and analyzed by HPLC. A porcine-gastric model was used for the muco-adhesive test.

Results: PCL fibers tends to be several micrometers thick while PVA fibers are just 200-500 nanometers. Inserts with homogeneous nano-fibers were produced with a drug loading up to 40% by weight for both drugs. All inserts were highly hydrophilic according to the contact angle test despite the hydrophobicity of materials themselves, indicating the abundant capillary-like structures which act as potential tunnels for oxygen and water exchange. Inserts consisted of co-axial fibers and crosslinked fibers could remain a similar dimension after swelling for a week, while inserts made by uncrosslinked uni-axial fibers showed a thickness increase of around 50%. The inserts loaded with triamcinolone acetonide showed a sustained drug release for a week – with a 20-40% burst release followed by a 10-20% release per day. Inserts loaded with bromfenac sodium, on the other hand, showed a burst release higher than 60% with extremely slow follow release less than 0.1% per day. Electrospun inserts showed an average 73% higher rupture force compared with casting films in all cases, illustrating a better mucoadhesive property.

Conclusions: In this study, we developed coaxial electrospun nano/microfibers with predefined physicochemical properties to treat inflammation on the anterior ocular chamber. We demonstrated that co-axial fibers had better swelling properties and drug release pattern compared with normal nano-fibers.