UKICRS Symposium 2021

United Kingdom & Ireland Controlled Release Society

Wednesday 13th October 2021

- Online conference via Zoom
- Two Keynote speakers
- Seeram Ramakrishna (National University of Singapore)
- Rebecca Brodsky (Population Council)
- 10 talks from selected abstract submissions
- Online poster session

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ENZYME-DEGRADABLE POLYION COMPLEX FOR ANTIMICROIBAL DELIVERY

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Background: Antibiotic resistance microbes will cause 10 million death by 2050 according to the WHO, more than all cancers if no effective treatment found. There are several efforts need by governments and industry to look for new antimicrobial agents. One direction of research is relay on the new formulation of the last few antibiotics that still toxic against pathogenic bacteria may be a good solution faster than looking for new antibiotics. This including the delivery of these antibiotics inside the degradable bacterium enzymes selectively in the site of infections.

Methods: Here, we describe the preparation of novel polyion complex (PIC) particles for the delivery of Polymyxin B (*Pol-B*), an antimicrobial peptide currently used in the clinic as a last resort antibiotic against multidrug-resistant gram-negative bacteria *Pseudomonas aeruginosa*. Towards this end, we have prepared polymer containing peptide sequence (-Glu-Gly-Leu-Ala-) this sequence is selectively degraded by *pseudolysin,* an elastase produced by this pathogenic bacterium.

Results: A range of conditions for the controlled assembly of Pol-B with polymer containing peptides has been identified that result on stable colloidal PIC particles containing different Pol-B:Polymer ratios with size around 120 nm. The prepared nanoparticles showed significant stability under simulated physiological conditions such pH, osmotic pressure, and temperature. Furthermore, preliminary evaluation of the antimicrobial activity of these Pol-B containing PIC particles has been performed, by monitoring their effect on the growth of *Pseudomonas aeruginosa.* The prepared PIC showed very low toxicity to the mutant bacteria that does not produce *pseudolysin* enzyme in the infection site while undergo high degradation rate (80% in 4h) with antibiotic release in the presence of the wild type. In addition to high stability against the Human elastase enzyme that often found in the infection site, this proves the high selectivity of these nanoparticles toward the drug delivery in the presence of specific target.

Conclusions: Bacterial enzyme degradable nanoparticles were prepared and used for the selective fast delivery of antibiotic in the infection site caused by a multidrug-resistant gram-negative bacteria *Pseudomonas aeruginosa.* The presented nanoparticles showed significant stability in biological conditions and high selectivity as well as fast delivery. These polyplex formulation method has promising potential for antimicrobial delivery as it's easy to prepare, present stability, selectivity, and lead to the release of the drug with complete efficiency.

Design of invasomal nanovesicles for improved transdermal permeation and bioavailability of asenapine maleate for treatment of schizophrenia

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Background: Asenapine Maleate (ASPM) is a second generation antipsychotic used for the management of schizophrenia with very limited oral bioavailability due to its extensive first pass metabolism. Transdermal administration of ASPM might offer an excellent alternative to its oral administration in order to enhance the bioavailability and provide a sustained action with subsequent improved compliance especially with the help of nanosystems. Invasomes are considered among the nano-vesicular systems that perfectly enhance the transdermal flux of different drugs.

Methods: ASPM-loaded invasomes were successfully prepared by thin film hydration technique; meanwhile the penetration enhancing effect of terpenes (cineole and limonene) was compared to hydromiscible cosolvent (Transcutol®). The prepared nanovesicles were characterized in terms of mean particle size (PS), polydispersity index (PDI), zeta potential (ZP), entrapment efficiency (EE%), Ex- vivo skin permeation. Selected formulations were stored at 4 ± 1◦C and re-evaluated for the change in particle size and zeta potential after two and six months as an index for their stability status. The optimized formulation was further studied using Transmission electron microscopy and FTIR techniques. In vivo pharmacokinetic study was carried out in rats with the optimized invasomal formulation and the pharmacokinetic parameters such as Cmax, Tmax, AUC $_{0\text{-}t}$, AUC $_{0\text{-}\infty}$, MRT of ASPM upon intravenous, oral and transdermal administration were estimated.

Results: Soft nanovesicles containing Transcutol® displayed smaller particle sizes than invasomes containing limonene and cineole while invasomes showed higher efficiency to encapsulate asenapine. Ex- vivo skin permeation revealed that invasomes with limonene are more efficient than those with cineole for the transdermal delivery of asenapine. Invasomes employed 1% limonene (F2) showed the highest values of both entrapment efficiency and asenapine permeation across rat skin. Transmission electron microscopy showed uniform spherical vesicles with intense outline and lighter core and FTIR study emphasized that ASPM was completely incorporated within the vesicles. The in- vivo pharmacokinetic study revealed that transdermal invasomes achieved 2 folds higher Cmax compared to oral suspension and delayed the Tmax from 1.5h to around 4h. The bioavailability of asenapine loaded invasomes after transdermal application was above 50% exceeding the bioavailability of sublingual tablets currently available in the market and exhibited sustained release kinetics over 72h which permits reduction of dosing frequency to increase patient adherence to medication.

Conclusions: The current work described the successful use of invasomes as a very promising vesicular carrier system for the transdermal delivery of drugs; especially hydrophobic ones. Synergistic effect of ethanol and terpenes is the underlying cause of enhanced permeation of invasomes. The optimized invasomal formulation displayed relatively small particle size (82.03 nm ± 0.62) and considerable ex-vivo permeation of asenapine along with optimum stability on storage for six months. A sustained transdermal delivery of ASPM up to 72 hours was achieved that could reduce dosing frequency, enhance bioavailability and improve patient compliance.

VORICONAZOLE-CYCLODEXTRIN COMPLEX LOADED OCULAR FILMS FOR FUNGAL KERATITIS

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Background: Fungal keratitis is one of the leading causes of ophthalmic mycosis affecting the vision due to corneal scarring. Voriconazole (VRC) is the most preferred azole antifungal agent for treating ocular mycotic infections. Shorter residence time with topical eye drops necessitate the frequent dosing (for every hour) and is associated with several drawbacks such as nasolacrimal drainage, reflex blinking accounting for < 5%bioavailability of topically applied eye drops. Apart from low aqueous solubility, VRC also suffers from stability issues. Complexation of poorly water soluble pharmaceuticals with cyclodextrin (CD) derivatives has shown to increase the solubility and stability of the therapeutic molecules.

Methods: VRC-CD complex was prepared using lyophilization technique. VRC-CD complex loaded ocular films were prepared by the solvent casting method. In vitro release study of the films was carried out using Franz diffusion cells. Ex vivo transcorneal permeation studies were performed using goat eyeball. The antifungal efficacy was carried out on *Aspergillus fumigatus*.

Results: Phase solubility suggested ~17 fold improvement in VRC solubility whereas physiochemical characterization suggested the inclusion of VRC in the cyclodextrin inner cavity. Complex loaded ocular PVA films showed the sustained release of VRC from the films and improved transcorneal permeation by several folds. The developed films demonstrated improved antifungal activity against Aspergillus fumigatus.

Conclusions: The VRC-CD loaded ocular films is a promising delivery approach for providing controlled drug release targeting the ocular tissue and served as an effective treatment regime for fungal keratitis.

THE EFFECTIVENESS OF DERMAROLLERS® IN IMPROVING TRANSDERMAL DELIVERY OF PRIMAQUIEN IN THERMORESPONSIVE-BIOADHESIVE HYDROGEL FORMULATIONS

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Background: Malaria has affected 228 million people in world. Oral administration of primaquine (PMQ) has been applied as a conventional treatment for this infection disease. Nevertheless, numerous drawbacks were reported associated with this conventional therapy. Therefore, the development of new delivery approach is urgently needed. Here, for the first time, PMQ was incorporated into thermoresponsive-mucoadhesive hydrogels for improved and sustained transdermal delivery of PMQ combined with Dermarollers®.

Methods: Thermoresponive-bioadhesive hydrogels were formulated using Pluronic F127 (PF127) and Pluronic F68 (PF68) as thermosensitive polymers and HPMC as bioadhesive polymer. The hydrogels contained 1% w/v PMQ. The formulations were then evaluated for their gelation temperature, bioadhesive properties, *in vitro* release and *ex vivo* permeation behaviours. Finally, the skin permeability of PMQ from thermoresponsive hydrogels was investigate *ex vivo* with the use of Dermaroller®**.**

Results: After various formulation optimizations, formulation containing 15% PF127, 3% PF68 and 0.4% HPMC with 1% PMQ was considered as the optimum formulation. The optimized formulation possessed desired thermoresponsive and biadhesive properties. Importantly, the use of HPMC as bioadhesive agent did not change thermoresponsive characteristic of the formulation. The formulation showed gelation temperature at the skin temperature (32°C) with shear thinning behavior. *In vitro* release study showed biphasic release manner of PMQ from the hydrogel over 24 h. Essentially, in *ex vivo* permeation study, Dermaroller® with the length of 1 mm was able to improve and sustaine the permeability of PMQ through rats' skin by 4-fold compared to untreated skin over 72 h experiments. Following these interesting studies, the efficacy of PMQ from this approach should be evaluated in *in vivo* studies.

Conclusions: Thermoresponsive with bioadhesive properties containing PMQ was successfully developed using PF127, PF68 and HPMC. The formulation possessed an adequate thermoresponsive and bioadhesive characteristic, as well as *in vitro* biphasic release manner. Importantly, Dermaroller® was able to increase and sustain the permeation of PMQ through rats' skin.

TRANSDERMAL DELIVERY OF PRIMAQUINE USING THE COMBINATION OF POLYMERIC PATCH AND SOLID MICRONEEDLES

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Background: Malaria, caused by *Plasmodium vivax*, have been a major health problem worldwide. Primaquine (PMQ) in an effective drug for the treatment of malaria, given through oral route. However, this conventional treatment resulted in several disadvantages, namely causing side effects and undergoing extensive first-pass metabolism in the liver. Accordingly, an alternative delivery route to overcome these issues. In this study, we developed polymeric patch containing PMQ for transdermal delivery, combined with solid microneedles for improved permeation profiles.

Methods: Polymeric transdermal patches were prepared using HPMC as the main polymer with the use of PEG and glycerin as plasticizers. The patches were evaluated for the thickness, uniformity weight, uniformity content, folding endurance and hemocompatibility. Importantly, permeation of PMQ from transdermal patch were also investigated through dialysis membrane for *in vitro* and rats' skin for *ex vivo*. Specifically, in *ex vivo* studies, the effect of solid microneedles (Dermaroller®) on permeation of PMQ was finally assessed.

Results: The results showed that following several optimizations, the optimum patch formulation contained 2% HPMC, 1.75% PEG and 0.5% glycerol with 2% PMQ. The formulation showed uniform thickness and weight with drug recovery around 100%. Importantly, the folding endurance was found to be > 300, indicating an adequate mechanical property of the patch. Moreover, hemocompatibility assay showed that percentage of hemolysis of the formulation was less than 5%, presenting the safety of the formulation. *In vitro* and *ex vivo* permeation studies showed that 31.31% and 22.55% of PMQ released from patch formulation, respectively. Importantly, in *ex vivo* permeation study, the use of Dermaroller® with the length of 1 mm was able to improve the permeation of PMQ, with 45.89% of PMQ permeated after 24 h administration. Based on these promising findings, *in vivo* experiments regarding pharmacokinetic and pharmacodynamic should be performed.

Conclusions: PMQ was successfully incorporated into polymeric transdermal patch using HPMC, PEG and glycerol. The formulation exhibited desired physical and mechanical characteristics. Following *ex vivo* permeation study, Dermaroller® was found to improve the permeation ability of PMQ through rats' skin compared to untreated skin.

THERMOSENSITIVE-MUCOADHESIVE GEL FOR VAGINAL DELIVERY OF CABOTEGRAVIR FOR HIV TREATMENT

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Background: Cabotegravir (CAB) as one of antiretroviral drugs for HIV treatments. It is currently administered via oral and injection route. However, these routes resulted in several drawbacks, especially low bioavailability and painful administration. Additionally, it has been also reported that in terms of source of infection of HIV transmission, vagina is one of the routes for this transmission. Interestingly, vagina is also reported to be a promising drug delivery route for both localization and systemic purpose. Accordingly, here, we formulated thermosensitive-mucoadhesive vaginal gel containing CAB.

Methods: Thermosensitive-mucoadhesive vaginal gel was prepared using Pluronic F127 (PF127) and Pluronic F68 (PF68) as thermosensitive agents, HPMC as mucoadhesive agent, and PEG as permeation enhancer. The gels were characterized for their gelation temperature, mucoadhesive strength, mucoadhesive time, rheological properties, hemocompatibility, *ex vivo* vaginal permeation and retention ability.

Results: The results showed that, following several optimizations, PF127 and PF68 with the ratio 16% and 6% containing 0.5% HPMC showed thermosensitive properties, showing the gelation temperature around vaginal temperature (35^{o}C). Importantly, the mucoadhesive strength was found to be 10888.89 dyne/cm^2 with more than 8 hours mocoadhesion time. The formulation also showed desired rheological properties for thermosensitive preparations. Essentially, after the addition of PEG as permeation enhancer, the thermosensitive and mucoadhesive properties were affected. Furthermore, hemocompatibility exhibited that all formulations did not cause hemolysis with hemolysis percentage values of <5%. Finally, it was found that the use of 10% PEG in thermosensitive-mucoadhesive vaginal gels resulted in improved permeation and retention of CAB in porcine vaginal tissue in *ex vivo* studies with 6.10% and 17.28 µg/g vaginal tissue of CAB permeated and were retained in the vaginal tissue following 8 h administration of our approach. Following these results, *in vivo* animal studies with suitable model must be conducted.

Conclusions: CAB was successfully formulated into thermosensitive-mucoadhesive gel for vaginal delivery in HIV treatment using PF127, PF168 and HPMC. The use of PEG did not affect the physical properties of the formulation and was able to improve permeation and retention of CAB in porcine vaginal tissue in *ex vivo* studies. Further *in vivo* studies should now be carried out.

IMPROVED PERMEATION AND RETENTION OCULAR DELIVERY OF CEFAZOLINE USING THERMOSENSITIVE-MUCOADHESIVE *IN SITU* GELS

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Background: Keratitis bacteria, caused by *Pseudomonas aeruginosa*, is still becoming a significant health problem. Cefazoline (CFZ) is available as liquid eye drops and are used as a treatment for keratitis. However, conventional eye drop shows several limitations, mainly due to short retention time in the ocular tissue, resulting in low ocular bioavalibility of drugs applied ocularly. In an attempt to overcome these drawbacks, alternative drug delivery system should be developed. Here, we incorporated CFZ into thermosensitive-mucoadhesive *in situ* ocular gels.

Methods: Ocular *in situ* gels with thermosensitive and mucoadhesive properties were prepared using Pluronic F127 (P127) and Pluronic F68 (P68) as thermosensitive polymers, as well as hyaluronic acid (HA) as mucoadhesive polymer. The *in situ* gels were further evaluated their thermosensitive characteristics, mucoadhesive properties, rheological properties, hemocompatibility, *ex vivo* ocular permeation and retention ability.

Results: The optimized formulations were *in situ* gel containing P127, P68 and HA with the concentration of 15%, 5% and 0.2%, respectively. All formulations contained 0.35% CFZ. The optimized formulations showed gelation temperature around ocular temperature (35^{o}C), showing desired thermosensitive properties. Essentially, the mucoadhesive strength in ocular tissue was observed to be 9748.69 ± 1184 g with more than 4 hours mocoadhesion time. The rheological evaluation showed that the *in situ* gel possessed desired rheological properties for thermosenstitive preparations. Moreover, no hemolysis was observed in hemocompatibility study. Finally, the incorporation of CFX in this system could enhance permeation and retention of CFZ in porcine ocular tissue in *ex vivo* studies with 1.27 ± 0.13 mg and 1.53 ± 0.16 of CFZ permeated and localized in the ocular tissue following 24 h administration of thermosensitive-mucoadhesive *in situ* ocular gels

Conclusions: Thermosensitive-mucoadhesive *in situ* ocular gels containing CFZ was successfully formulated. The combination of P127, P68 and HA resulted in gels with desired characteristics. Importantly, this approach improved the permeation and localization of CFZ in the ocular tissue, which could potentially improve the treatment of bacterial keratitis.

GEMCITABINE-LOADED METAL-ORGANIC FRAMEWORKS FOR PANCREATIC CANCER

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Background: Gemcitabine is a chemotherapeutic utilized in the treatment of pancreatic cancer, however its use has become more limited due to issues with developing resistance. The aim of this work is to generate metal-organic framework (MOF) nanoparticles and load these with gemcitabine to determine any improvement in cytotoxicity in pancreatic cancer cell lines.

Methods: MOFs were synthesized and subsequently characterized by several techniques, including powder X-ray diffraction, scanning electron microscopy, and nuclear magnetic resonance. After completion of the drug loading protocol, HPLC analysis was used to determine the loading values, then these materials re-characterized using the same techniques used initially. Further, the cytotoxicity of free drug, MOF and drug-loaded MOF were determined in pancreatic cancer cell lines *in vitro*.

Results: The selected MOFs have been successfully synthesized (<200 nm) and loaded with the anticancer drug gemcitabine, with large drug loading efficiency values for each framework. Characterisation techniques, including powder X-ray diffraction, scanning electron microscopy, nuclear magnetic resonance, and gas adsorption have been used to determine that the structural integrity of the MOFs was not corrupted by the loading protocol, as well as to further evidence the presence of gemcitabine in the structures. Finally, *in vitro* testing has been completed to ascertain if there were any differences in the free gemcitabine versus the gemcitabine loaded MOFs.

Conclusions: Overall, the selected MOFs have been successfully synthesized and characterized by several techniques, with successful drug loading of gemcitabine. *In vitro* cytotoxicity experiments have been completed to ascertain the toxicity of the drug loaded MOF versus the free drug.

PREPARATION AND CHARACTERIZATION OF DISULFIRAM β-CYCLODEXTRIN INCLUSION COMPLEXES FOR THE TREATMENT OF TRIPLE NEGATIVE BREAST CANCER

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Background: Breast cancer (BC) is the most frequently diagnosed cancer in women and the second major cause for cancer related death, and Triple Negative Breast Cancer (TNBC) accounts for an estimated 10-20% of all BCs and is characterised by its clinical implications and lack of prognosis(1). Disulfiram (DS), an anti-alcoholism drug, proven to have an excellent anti-cancer activity, remains a focus point in pharmaceutical research because of its low water-solubility and rapid metabolism (1,2). Cyclodextrins (CDs), cyclic oligosaccharides, are biocompatible macromolecules used to improve the solubility of drugs. CDs have a hydrophilic surface and a lipophilic center being able to interact with hydrophobic drug molecules to form inclusion complexes soluble in water (2).

Methods: In order to enhance the solubility of DS, two types β-cyclodextrins, Hydroxypropyl β-Cyclodextrin (HP) and Sulfobutyl Ether β-Cyclodextrin (SBE), were used to form inclusion complexes. Formulations were prepared by mixing DS with five different concentrations of both cyclodextrins (1%, 5%, 10%, 15% and 20% w/w). Solubility of DS was assessed using spectrophotometric analytical method. DS-CD solutions were freeze dried to study the interaction between DS and CD using DSC, TGA, FT-IR and XRD. Finally, the cytotoxic effect of DS-CD inclusion complexes of chemoresistant TNBC cell lines was evaluated using MTT assay.

Results: The solubility of disulfiram increased significantly when combined with β-cyclodextrins, reaching more than 10 mg/mL at 20% w/w. The phase solubility of DS SBE-β-CD showed the aqueous solubility of disulfiram to be a linear function of the CD concentration (A_L type), suggesting that the total amount of drug increases as a function of the CD concentration. Whereas the solubility of DS had a polynomial relationship with the increase of HP-β-CD, deviating negatively from linearity (N_A type). All lyophilized formulations were easily reconstituted indicating instant water solubility of the resulting freeze-dried formulations. DSC studies confirmed the inclusion of the amorphous of the drug in the CD-DS complexes. Finally, CD-DS inclusion complexes reserved the cytotoxic effect of DS on chemoresistant TNBC cell lines. Combined with Cu^{2+}, both CD formulations were of the similar cytotoxic effect to that of DS.

Conclusions: Our results report that the cyclodextrin inclusion complexes are a very practical approach for the enhancement of DS solubility and have a great potential for further in vivo studies against triple negative breast cancer.

References:

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SOLID DISPERSION AND FLOATING GEL *IN SITU* APPROACHES FOR IMPROVED SOLUBILITY AND SUSTAINED RELEASE BEHAVIOR OF β-CAROTENE EXTRACTED FROM PALM OIL

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Background: Free radical has caused numerous serious diseases around the world. β-carotene is one of antioxidant agents used to overcome this issue. However, this compound possesses low aqueous solubility and low bioavailability. Therefore, it is critical to enhance the solubility and sustain the release profile of β-carotene, resulting in high bioavailability. With respect to source, palm oil has been reported to be the rich source of β-carotene. Here, we extracted β-carotene from palm oil using saponification reaction. To improve solubility and sustain the release, β-carotene was further formulated into solid dispersion and floating gel *in situ*.

Methods: Palm oil was obtained using soxhletation method with isopropanol. β-carotene was further extracted using saponification method with KOH. Furthermore, solid dispersion formulation was optimized using central composite design. Solid dispersion was then characterized for their physical and chemical properties, as well as dissolution profile. Solid dispersion was finally incorporated into floating gel in situ which was evaluated for their physical properties and release behavior.

Results: The yield value of β-carotene obtained from the palm oil was 6.36%. Following optimization process, the final formulation of solid dispersion was PVP: PEG: cyclodextrin with the ratio of 0.334: 2: 0.983. The solid dispersion showed that there were no interactions between all compound in the formulation and the form of β-carotene changed from crystal to amorph. Importantly, the dissolution profile of β-carotene was improved from 7.98 ± 0.51 % to 88.81 ± 6.91% following the solid dispersion formulation, showing the successfulness of this approach to improve the solubility of β-carotene. In the floating gel in situ formulation, the formulation containing β-carotene solid dispersion, sodium alginate, sodium bicarbonate, calcium carbonate, and HPMC with the concentrations of 0.875, 1.5, 0.75, 0.5, and 1.5 % exhibited desired characteristics with optimum floating lag time. Finally, the *in vitro* release study showed that the incorporation solid dispersion of β-carotene was able to sustain the release β-carotene around 99.28 ± 17.11% over 24 h compared to solid dispersion formulation.

Conclusions: Solid dispersion-floating gel *in situ* containing β-carotene extracted from palm oil was successfully formulated. This combination approach could improve the solubility and sustain the release of β-carotene over 24 h.

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STEREOLITHOGRAPHY (SLA) ASSISTED FABRICATION OF 3D PRINTED POLYMERIC FILM FOR TOPICAL BERBERINE DELIVERY: IN VITRO, EX-VIVO & IN VIVO INVESTIGATIONS

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Background: A 3D printed skin film intended for topical delivery of berberine (BBR) was developed utilizing stereolithography technology to enhance its local concentration. PEGDMA was used as the photopolymerizing oligomer, with PEG 400 as the inert component to facilitate berberine solubilization and film consistency. The system was designed to provide a controlled berberine release profile and a biocompatible matrix for topical applications. The effect of resin composition on the physicochemical properties and drug release profiles was investigated.

Methods: Three batches of topical films were 3D printed by varying the resin composition. Physicochemical characterizations of the films were performed along with microscopy and ex vivo drug permeation study. In vivo skin irritation studies were conducted to assess the skin irritation potential of topical films.

Results: The films were printed as per the CAD design specification with minimal variation. Microscopic analysis confirmed the layer-by-layer 3D printing, while thermal analysis (DSC) and XRD studies revealed the amorphous nature of the drug in the printed film. The FT-IR results confirmed the photo-crosslinking process. The stability data indicated no discernible variations in the physicochemical profile of the 3D printed film over time. Drug permeation study showed effective diffusion of the drug from the film with a higher PEG 400, leading to high ex vivo drug diffusion up to 344.32 ± 61.20 μg/cm^2 after 24 hours through rabbit ear skin. In vivo skin irritation studies have suggested the non-irritant nature of the printed films.

Conclusions: The results indicated the suitability of SLA to fabricate topical film with desired physicochemical properties intended for topical use to treat skin diseases. The presence of PEG 400 in the films facilitated BBR diffusion resulting in an improved flux in the ex-vivo model. The utilization of biocompatible polymers for the additive manufacturing of the films ensured the non-irritant property in vivo.

POLYDOPAMINE-COATED NANOCOMPOSITE THERANOSTIC IMPLANTS FOR LOCALIZED CHEMOTHERAPY AND MR IMAGING

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Background: Postoperative adjuvant chemotherapy has been proven to improve long-term prognosis compared to surgery alone in the treatment of early and localized tumors. However, systematic chemotherapy often fails to achieve desirable clinical outcomes owing to physiological barriers to drug delivery, or harmful effects on healthy organs. Alternative delivery approaches are required to enhance efficacy. One suitable option is the use of implantable drug delivery (IDD) systems. However, concerns arise associated with the safety and efficacy of IDD systems. It is important to monitor the implants to avoid any risks of displacement and/or deterioration.

Methods: Implantable fibres incorporating methotrexate (MTX) and magnetic resonance imaing (MRI) contrast agents were developed. To prolong the drug release profile and enhance MTX stability over a prolonged period, here MTX was firstly loaded into layered double hydroxide (LDH-MTX) carrier particles by anion exchange. IDD systems, biodegradable PCL-based core-shell fibres loaded with LDH-MTX, were then produced by electrospinning. Unlike the majority of electrospun platforms reported where functional agents are encapsulated inside the bulk of the formulation, MRI contrast agents, superparamagnetic iron oxide nanoparticles (SPIONs) were then incorporated onto the surface of the fibres via post-fabrication PDA coating. This should permit effective interactions with nearby water molecules, so as to provide high local contrast for MR imaging.

Results: Fibres were prepared by co-axial electrospinning and loaded with LDH-MTX nanocomposites in the core, yielding organic–inorganic hybrids with a diameter of 1.48 ± 0.58 μm. After surface coating with polydopamine and SPIONs, the hydrophilicity profoundly increased and the SPIONs were proven to be evenly distributed on the surface, providing high MRI contrast. *In vitro* drug release studies showed the PDA coated fibres gave sustained release of MTX over 18 days, and that the release profile is responsive to conditions representative of the tumor microenvironment such as slightly acidic pH values or elevated concentrations of the reducing agent glutathione (GSH). *In vitro* studies with Caco-2 and A549 cells showed highly effective killing with the PDA coated formulations, which was further enhanced at higher levels of GSH.

Conclusions: The fibres have the potential to act as an implantable drug-eluting platform for the sustained release of cytotoxic agents within a tumor site, providing a novel treatment option for post-operative cancer patients.

A NANOMEDICINE, CHEMISTRY AND TUMOR BIOLOGY THERAPEUTIC RECIPE MADE @ UK-PORTUGAL TO DEFEAT GLIOBLASTOMA

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Background: The 5-year survival of glioblastoma (GBM) patients is limited to a dismal 5%, highlighting the need to advance more effective therapies. GBM tissue abnormally overexpresses the L-type amino acid transporter 1 (LAT1), for which L-histidine (His) is an inexpensive and powerful targeting ligand. Thus, we propose the chemical modification of a conventional chemo-immunogenic drug, docetaxel, into a nanomedicine with surface-His (nano-DTX-His) to target GBM tissue via LAT1 adhesive binding and further augment localized cell death. Since nano-DTX-His cannot be used for IV therapies due its inability to cross the blood-brain barrier (BBB) *per se*, we further propose its modification with an acid-cleavable Angiopep-2 layer (nano-DTX-His-*clv*-Angiopep2) to favor BBB translocation. It is of important note that the choice of DTX is based on its IC_{50}, which is around 20.000-times lower than the standard temozolomide (in https://www.cancerrxgene.org/).

Methods: Carbodiimide and carbamate hydrolysis were employed to synthesize a poly(lactic-co-glycolic) acid (PLGA) and His-functionalized polyethylene glycol (PEG) conjugate, to serve as the nano-DTX-His matrix. Nano-DTX-His was further manufactured through a scale-up microfluidic technique. To evaluate cell uptake and viability, 2D and 3D models of conventional GBM cell lines (U87, U251, U373) and primary lineages isolated from the GBM invasive margin of human tumors (GIN lineages) were used. To investigate a possible immunogenic cell death, a novel 3D high-throughput model of the GBM microenvironment was developed, including U251 cells, buffy coat-isolated monocytes, and brain primary endothelial cells. Finally, nano-DTX-His was modified into nano-DTX-His-*clv*-Angiopep2 though inclusion of a PLGA-acetal-PEG-Angiopep2 conjugate (Angiopep-2 coupled with a PLGA-acetal-PEG polymer via Thiol-Michael addition) into the final nanomedicine formulation. BBB translocation was evaluated in hCMEC/D3 Transwell® systems.

Results: Monodisperse nano-DTX-His was manufactured with c.a. 250 nm and a controlled DTX release over 48 h. The uptake of nano-DTX-His was 3.5-times higher than nano-DTX-ØHis in U87, U251 and U373 cells. In GIN lineages, cell uptake was 8-times higher than the controls. 2D studies of cell viability in GIN lineages demonstrated an anti-cancer potential of nano-DTX-His 50% superior compared to the controls, after 4 h flash and 96 h treatments. In a heterotypic GIN 3D culture, this anti-cancer potential was kept. Moreover, in the 3D GBM microenvironment model, nano-DTX-His presented 1) a 60% cytotoxicity increase compared to the controls, and 2) the capacity to polarize macrophages into an anti-tumor phenotype. Finally, nano-DTX-His-*clv*-Angiopep2 was able to provide higher BBB translocation of DTX compared to nano-DTX-His and nano-DTX-His-Ø*clv*-Angiopep2 in hCMEC/D3 Transwell® systems.

Conclusions: Nano-DTX-His demonstrated better properties of cell uptake and cytotoxicity compared to the conventional therapy, in reliable 2D and 3D models of GBM. Moreover, its modification into nano-DTX-His-*clv*-Angiopep2 allowed the nanomedicine to acquire an ameliorated BBB translocation capacity. Ongoing work focuses on *in vivo* studies.

Probing the impact of protein/peptide adsorption onto solid-substrates on functional behavior and stability.

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Background: Protein adsorption refers to the accumulation and adherence of a protein to the surface of a solid, but without surface penetration occurring. Proteins can adsorb to a variety of materials that are used in bioprocess, manufacture, formulation, and storage and can have unintended consequences such as loss of expensive protein medicines. Protein adsorption to a surface is strongly influenced by the physicochemical properties of the protein, protein-formulation interactions, and protein-surface interactions. Despite increasing knowledge in the field of adsorption, a molecular-level understanding of all aspects of protein adsorption is still incomplete particularly with observed phenomena such as conformation and orientation, cooperativity, and aggregation. To elucidate the influence of protein-formulation interactions on adsorption behavior and its corresponding phenomena, our study investigates the role of buffer choice, temperature, and pH on the adsorption of Lysozyme to type I borosilicate glass.

Methods: Quantification of desorbed proteins was performed using an Agilent 1200 HPLC system equipped with a Diode Array Detector G1315D at 215 nm. Chromatographic separation was performed using a Poroshell 300SB-C8 column. Circular dichroism spectroscopy was measured with a path length of 0.1 mm in the range 185-260 nm. The average of three spectra was obtained and a 5-point smoothing algorithm applied. Isothermal titration calorimetry experiments were performed with a Microcal PEAQ-ITC microcalorimeter at 298 K. Lysozyme solution was loaded in the cell and buffer was loaded in the syringe. With a fixed stirring speed of 1000 rpm, the first drop was set to 0.4µL followed by 19 drops for subsequent 2µL injections.

Results: Lysozyme in PBS at pH 7.4 had the highest amount of protein adsorbed overall, 0.501 mg/ml for 2-5 °C and 0.587 mg/ml for 20-25 °C. The amount of adsorbed lysozyme reduced when PBS was exchanged for histidine buffer with 0.279 mg/ml for 2-5 °C and 0.426 mg/ml for 20-25 °C. Histidine also adsorbed to borosilicate with 0.323 mg/ml at 2-5 °C and 0.194 mg/ml at 20-25 °C. At pH 5.5 in histidine buffer, more lysozyme adsorbed at 2-5 °C than histidine and equal amounts adsorbed at 20-25 °C. To further understand the role of pH we studied lysozyme at pH 3.6 in citrate buffer and glycine-hydrochloride. There was a large increase in the adsorption of lysozyme at 2-5 °C for citrate buffer, 0.882 mg/ml, and a reduction was observed at 20-25 °C, 0.248 mg/ml compared to PBS. This was similar for glycine buffer however, the total amount of protein adsorbed was significantly less. We also studied citrate buffer at pH 5.5 and found that it has a similar adsorption profile to citrate at pH 3.6. The ITC data revealed that lysozyme interacts with histidine buffer at pH 7.4. At pH 7.4 histidine retains a partial positive charge and we surmise that the histidine molecules are interacting electrostatically with pockets of negatively charged residues on the surface of lysozyme. CD Spectroscopy revealed that for lysozyme in pH 3.6, lysozyme was able to adsorb without structural reorientation. For pH 7.4 and pH 5.5, lysozyme underwent conformational changes upon adsorption for all buffers, with loss of α-helix and increased random coil being observed.

Conclusions: These results suggest that pH and temperature have a strong effect on the adsorption profiles of lysozyme, but that buffer choice can aid suppression of protein adsorption to borosilicate. CD Spectroscopy indicated that lysozyme has better structural stability on adsorption at pH 3.6 than at pH 5.5 or pH 7,4.

MICROFLUIDIC-MEDIATED SELF-ASSEMBLY OF PHOSPHOLIPIDS FOR THE DELIVERY OF BOVINE SERUM ALBUMIN AND TRYPSIN

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Background: Biologics play a major role in our current healthcare systems worldwide; for example, insulins and monoclonal antibodies; however, their use is restricted to parenteral delivery with a relatively uncontrolled release profile. Using a novel microfluidic-assisted method, the encapsulation of model biologics within phospholipids (e.g., liposomes), bovine serum albumin (BSA) and trypsin (TRP), have been successfully demonstrated in this study to portray a contemporary microfluidic method for biologic medical formulation.

Methods: Using various unmodified lipids (e.g., DMPC – Dimyristoylphosphatidylcholine, DPPC – Dipalmitoylphophatidylcholine, DSPC – Distearoylphosphatidylcholine, and DOPC – Dioleoylphosphatidylcholine), parameters such as lipid concentration, active pharmaceutical ingredient (API) concentration, total flow rate (TFR) and flow rate ratios (FRR) have been investigated to determine optimal conditions for loaded-liposome formation. To aid further in the characterisation of the liposomes produced, the carriers were subjected to atomic force microscopy (AFM), Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and high-performance liquid chromatography (HPLC) in order to capture the encapsulation efficiency and drug release.

Results: The current studies have shown encapsulation efficiencies greater than previously published data by methods such as sonication or film hydration, whilst maintaining very promising physicochemical properties. In addition, 28-day physical stability studies have been performed under various conditions to demonstrate the formulations' viability for expansive manufacturing. Aiming to maintain liposome diameters ≤500 nm throughout the study, the shorter chained lipids performed exceptionally at both 21°C and 5°C, suggesting towards their proclivity as suitable API carriers. Initial drug release studies have been measured over 72 hours to indicate the controlled release of the API *in vitro*, with a maximum release of *circa.* 80% release occurring within this duration from DMPC formulations. DSC measurements suggest minimal impact on the thermal stability of the liposomes upon encapsulation of the APIs and has been seen to slightly improve it in the cases of TRP-DMPC liposomes.

Conclusions: The results suggest the success of a novel microfluidic method in achieving the synthesis of biologic-loaded liposomes, as well as offering insight into areas for advancing the technique. Factors such as pH and temperature have been observed to influence factors including encapsulation efficiency and physical stability, which could be explored in further studies to help optimise the process.

DIELS-ALDER MEDIATED RELEASE OF COMBINATION THERAPIES FROM HYBRID NANOPARTICLES

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Background: Pancreatic cancer or pancreatic ductal adenocarcinoma (PDAC) is the deadliest type of cancerous malignancy with a 5-year survival rate of only 7%. The historic first line treatment is a drug called gemcitabine, which displays effectiveness in only 22% of patients over 12 months.[1] Combination therapies such as GemCap, has been shown to improve patient outcomes, by operating under different mechanisms reducing the likelihood drug resistance. Hybrid nanoparticles (HNPs) comprised of an iron oxide core and outer gold coat have shown great potential for heat triggered drug release.[2] This work seeks to utilize this knowledge to develop a combination therapy using both gemcitabine and capecitabine.

Methods: Following on from preliminary studies, the cycloadduct is being attached to capecitabine at various potential points such as; the pyrimidine amine group, the 3-OH, and/or the 5-OH ribose hydroxyl groups. All four capecitabine-linkers (Figure 1 (3-6)) will be evaluated for their release rates at elevated temperatures to determine the optimal linker anchor points. The initial use of protecting groups such as tert-butyloxycarbonyl (Boc) may be used to facilitate linker attachment desired locations. The capecitabine-linkers will be attached to HNP's via a gold-thiol bond.[2]

Results: Historically Gem-Mal's cytotoxicity was determined by MTT and trypan blue assay to be 4.6 times lower than gemcitabine. The 11-fold enhanced uptake aided by the HNP's, coupled with rDA mediated release at 44 ˚C, led to an 26% increase in cytotoxicity compared to gemcitabine, confirming its temperature driven activity.[2] Successful capecitabine-linkers synthesis and drug loading onto HNP's will be confirmed by NMR and HPLC. The dual drug loaded HNP's cytotoxicity will be determined by trypan blue exclusion and MTT assay.

Conclusions: The gemcitabine-linker-HNP formulation has already been shown to have improved cytotoxicity compared to gemcitabine alone.[2] This work will focus on determining whether dual attachment of gemcitabine and capecitabine to HNP's as drug delivery vehicles for controlled release improves cytotoxicity in vitro with the MTT assay and trypan blue exclusion. Beyond this, the attachment of deoxy-5-fluorocytidine and 5-fluorouracil, which are metabolites of capecitabine is also planned for future work. Optimal combined drug-HNP formulations determined by *in vitro* analysis will be evaluated *in vivo*.

MULTIFUNCTIONAL HYBRID-NANOPARTICLES TO IMPROVE PANCREATIC CANCER THERAPY

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Background: Pancreatic cancer is the most aggressive common cancer. A major challenge facing current treatments and contributing to the diseases' dismal prognosis is chemotherapy resistance. Silver iron-oxide hybrid nanoparticles are attractive drug delivery systems due to their physiochemical properties and feasibility for controlled delivery and triggered release. Whilst the interior magnetic core can be exploited for imaging, silver possesses unique characteristics which we expect can be exploited to enhance drug efficacy. The objective of this study is to develop novel nanohybrids to tackle drug resistance and enhance chemotherapy drug efficacy in pancreatic cancer.

Methods: Silver iron-oxide hybrid-nanoparticles were synthesised and characterised using UV-Vis spectrophotometry, zeta potential measurement, dynamic light scattering, transmission electron microscopy and superconducting quantum interference device (SQUID) magnetometry. The effect of tailoring the particle's structural composition has been investigated. Optimal drug loading levels have been assessed alone and with polymer coating and drug release profiles under various physiological conditions were evaluated. The heating ability of the particles are currently being assessed.

Results: Silver iron-oxide hybrid-nanoparticles (approximately 50 nm) have been successfully synthesised. Anti-cancer drugs have been immobilised onto the HNP surface with high loading efficiency as determined by high performance liquid chromatography. The particle's ability to act as a thermally triggered delivery system is currently under evaluation.

Conclusions: The optimal composition of the silver iron-oxide hybrid-nanoparticle was selected to progress to drug loading studies. High drug loading efficiencies and drug release profiles show promising results for these systems to be used as drug-carriers. Overall, the proposed systems can progress into *in vitro* studies and can be considered as a potential theranostic agent.

LYSINE CO-HISTIDINE HYPERBRANCHED POLYMERS FOR SIRNA DELIVERY

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Background:

Small interfering RNA (siRNA) has made clinical progress because of its promise for gene silencing. siRNA works via the RNA interference (RNAi) mechanism, where it can potentially degrade any mRNA of interest and inhibit its translation into a protein. Developing safe and more efficient formulation strategies for siRNA remains a complex challenge. Previous work suggested that lysine-co-histidine hyperbranched polymers (pKH) are effective carriers for plasmid DNA. We investigated the one-step synthesis of pKH polymers for possible siRNA complexation.

Methods:

pKH polymers were prepared in a one-pot thermal polycondensation. Lysine (K) and histidine (H) with molar ratios of 4:1 and 4:2 were dissolved in a small amount of water in the presence of KOH and stirred at 170ºC for 8 or 16 h under a stream of N_2 to evaporate water to allow melt polymerisation as the reaction proceeded. The structure of pKH hyperbranched polymers was confirmed with NMR and Mn was obtained by GPC. For complexation, we used a short double strand DNA oligo (21bp) as a siRNA surrogate for proof of concept. Complexation was confirmed with agarose gel electrophoresis.

Results:

NMR analysis confirmed the formation of hyperbranched pKH polymers, however the starting stoichiometry of lysine and histidine was not maintained during the reaction, resulting in different/inconsistent lysine and histidine ratios. Variations in the molecular weight were also observed between batches, suggesting heterogenicity and that reproducibility was process dependent. When complexing pKH polymers with DNA oligos, different pKH batches displayed varying complexation efficiencies.

Conclusions:

The heterogenicity of hyperbranched pKH polymers results in inconsistent complexation efficiency with short DNA oligos. Process dependent factors related to water removal and heat transfer during pKH preparation are difficult to control and may be the cause of batch-to-batch variability. We are currently examining the solution-phase synthesis of pKH to increase the reproducibility of its complexation efficiency.

CONTROLLED RELEASE OF GENE EDITING TECHNOLOGY FOR THERAPEUTIC MANAGEMENT OF CYSTIC FIBROSIS SYMPTOMS

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Cystic fibrosis is one of the most common inherited lethal diseases, caused by mutations in the *CFTR* gene. Small-molecule therapies have revolutionised patient outcomes but are lifelong interventions and cannot correct all *CFTR* mutation variants.

We have demonstrated effective non-viral gene delivery to mice using peptide-nanocomplexes composed of plasmid (p)DNA and GET peptides. Glycosaminoglycan (GET) peptides bind and transduce cell membranes and we have generated mucus-penetrating formulations; allowing enhanced delivery and transgene expression to epithelium when aerosolised. Our formulations utilise endosomal-escaping strategies to deliver gene correction/augmentation strategies, presenting a ‘genetic cure’ which includes treatment of CF patients unaffected by current state-of-the-art therapies.

Our pDNA vector library (lacking CpG dinucleotides, reducing methylation silencing), modified with S/MAR sequences, allows for increases in long-term retention/expression viability. Additional ‘integration sequences’ have been included, enabling us to target stable integration into the ‘safe harbour’ AAVS1 locus. We have confirmed our system’s efficacy with fluorescent-protein encoding *ZsGreen1* and will confirm CF correction in patient-derived lung cells with *CFTR* transgene pDNAs.

Presently, we are comparing strategies: HDR (homology driven repair) and HITI (homology-independent targeted integration) via CRISPR. We are also exploring directed integration with Rep-mediated (exploiting viral mechanisms targeting P5IEE to the AAVS1 locus) systems and *Sleeping beauty* transposase.

Here we present the efficiencies and comparison of different systems. Repeat delivery/transfections using GET technology does not affect cell viability, so we can build integration. Ultimately, an aerosol-based strategy to progressively correct CF patients, converting transient gene expression into stable life-long genetic correction, may be viable.

Design of Novel Antibody-Decorated Nanoparticles Targeting HER2 Positive Breast Cancer

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Background: Every year there are over 50,000 new cases of breast cancer in UK, representing the most common UK cancer (CR-UK). HER2 (Human Epidermal Growth Factor Receptor 2) is a transmembrane oncoprotein encoded by the HER2/neu gene and overexpressed in approximately 20 to 25% of invasive breast cancers. Tumours overexpressing HER2 are more aggressive and carry a poor prognosis; thus, the receptor is a priority therapeutic target. One targeting entity is Trastuzumab (Tz), a monoclonal antibody that has also been attached to anti-cancer agents to form an antibody drug conjugate (ADC). A Tz-ADC (e.g Kadcyla) requires access to the cell interior by endocytosis to be effective. HER2 is, however, resistant to endocytosis and this is likely to significantly affect the efficiency of any Tz-ADC. Our previous studies showed that a process mediating HER2 crosslinking by Tz was able to induce HER2 endocytosis, lysosomal delivery and degradation - *Moody, P. et al. Mol Ther 23, 1888 (2015); Wymant, J., et al. J Cancer 11, 3288, (2020).* This presented work lies under the hypothesis that nanoparticles (NPs) decorated with sufficient numbers of Tz could also cause Her2 crosslinking, endocytosis and degradation to affect cell physiology in the absence of a cytotoxic drug.

Methods: PGLA TZ-NPs was prepared using the oil in water (O/W) emulsion-solvent evaporation method using 1:1 mixture of PLGA and PLGA-COOH in acetone. These were mixed with Povinyl alcohol prior to aceton removed by overnight stirring and resulting NPs isolated by centrifugation and resuspended in PBS. PLGA-COOH activation was via N-hydroxysuccinimide (NHS) and N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride prior to incubation with Alexa488-labelled Tz or Tz alone. The resulting Alexa488-Tz-NPs or Tz-NPs (mean size 230nm) were then analysed on breast cancer cells for cytotoxicity and endocytosis via live-cell confocal microscopy.

Results: The generated Alexa488-Tz-NPs (mean size 230nm) were found to be highly selective for HER2 expressing breast cancer cells over controls. The NPs were observed as large clusters at the plasma membrane, indicative of HER2 receptor clustering and large amorphous structure (possibly endosomes) were also observed inside the cells. Interestingly, Alexa488-Tz-NPs internalization was very rapid (< 30 min) differing considerably to Tz-Aexa488 alone that showed very little evidence of endocytosis even after 7 hrs. In the absence of a cytotoxic drugs Tz-NPs showed higher levels of toxicity compared to Tz or NP alone.

Conclusions: The data shows that there is a high possibility that the generated Tz-NPs are initially inducing plasma membrane Her2 clustering that is then driving endocytosis. Our previous studies using a different crosslinking method suggest that this may be via plasma membrane ruffling and uptake via macropinocytosis. Further work will explore this and also investigate whether different NP structures can be tailored to give the same level of internalisation and higher cell death.

IMPACT OF EXCIPIENTS ON SOLID-STATE FORM TRANSFORMATION OF INDOMETHACIN DURING LIQUID ANTISOLVENT (LAS) PRECIPITATION

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Background: 90% of new drug entities and 40% of approved drugs on the market have poor aqueous solubility which leads to unfavourable bioavailability. A variety of bottom-up and top-down methods exist to produce the crystalline particles. However, existing marketed formulations are generally produced by top-down technologies. These techniques are energy and time inefficient, limited in achievable crystal size, and exhibit lack of control over the resulting crystal structure. Product contamination and amorphization of the active pharmaceutical ingredients (API) during the process has also been reported.

As an alternative, a bottom-up approach can be applied, where particles of the desired size and polymorphic form are formed directly, in suspension. To become industrially viable, a scalable bottom-up technology should be developed that is able to produce a range of crystal suspensions from a large variety of challenging APIs.

Methods: Production of crystalline indomethacin suspensions via bottom-up (liquid antisolvent (LAS) precipitation) approach. The impact of excipients on the relative kinetics of nucleation, particle growth and stabilization of the suspensions after formation were investigated. In order to achieve the stable solid-state form, LAS precipitation was performed with seeding, at different concentrations, and before and after nucleation of the API.

Solid state characterisation, i.e. X-ray Powder Diffraction (XRPD), particle size determination, thermal analysis, FTIR spectroscopy, and Scanning Electron Microscopy (SEM), was conducted on the resulting suspensions.

Results: Particles of indomethacin of the aimed solid-state form were only obtained by using one of the two excipients studied. Additionally, it was only possible to obtain the stable solid state-form when seeds of the desired polymorphic form were added before nucleation. Different solid-state forms of the API were obtained by each technique, which may have a direct impact on dissolution rates.

The particle seeds had a size of approximately 17µm (D50), the particle size distribution (PSD) of the suspension was: 5-10µm, indicating that the size of the initial seeds may not be critical for the final PSD of the suspension.

Conclusions: An alternative, energy efficient bottom-up method for the production of drug microsuspensions with a reduced risk of contamination from milling equipment and fewer processing steps may prove to be comparable in terms of stability and PSD to the current industrially accepted top-down approaches.
Better control over chemical and physical properties of the suspension through selection of appropriate solvents, excipients and process parameters can be achieved via a bottom-up approach.

BONE BIOMIMETIC 3D SCAFFOLDS AS PLATFORMS TO ELUCIDATE THE BEHAVIOR OF HUMAN FETAL OSTEOBLAST CELLS WITH PC3 A PROSTATE CANCER CELL LINE

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Background: Prostate cancer is the second leading cause of death in men worldwide[1]. It arises *in situ* in the prostate but can spread elsewhere in the body with prevalence in the bone. External and microenvironmental stimuli influence cell behavior and responses to drug treatment[2]. 2D cell-cultures are relatively cheap and practical but fail to recapitulate cell behavior *in-vivo*[4]. 3D biomimetic scaffolds more closely resemble the natural composition and cues in the cells' microenvironment. Here, we aim to study the behavioral responses of hFOB 1.19 (human fetal osteoblasts) in mono and coculture with PC-3, a prostate cancer cell line using 3D porous scaffolds composed of PLGA and nano-hydroxyapatite (nHA), a bony mineral.

Methods: Scaffolds are produced following a multi-sequenced process which include: i) tableting of powdery mixtures, ii) CO_2-foaming, iii) leaching of the porogen (NaCl). Three batches of scaffolds were produced: i) Plain PLGA, ii) 2mg nHA/PLGA, iii) 4mg nHA/PLGA. Cell viability was assessed by quantifying the amount of DNA produced (Picogreen) at Day 3 and Day 7. The test was first carried in 24 well-plates in mono ($2x10^4$ cells/well) and coculture ($2.5x10^4$ cells/well hFOB 1.19/PC-3 ratio 4:1) prior to conduct it in 3D scaffolds ($2x10^5$ cells/scaffold, hFOB 1.19/PC-3 ratio 4:1). Scaffold colonization was assessed by performing histology after staining hFOB 1.19 and PC3 with fluorescent dyes DiO and DiI. hFOB 1.19 differentiation behavior was evaluated by staining of alkaline phosphatase (Fast-Blue) prior to imaging. Collagen production in coculture was assessed by staining the different scaffolds slides at Day 7 with 0,5% Fast Green FCF. Ongoing studies are focusing on assessment of PC3 and hFOB 1.19 behavior (RT-qPCR) and quantification of live/dead cells pre- and post-treatment with Docetaxel (10nM).

Results: 3D mono- and cocultures confirmed cell viability over 7 days. 4mg nHA/PLGA scaffolds provided higher viability at Day 7 corresponding to higher amount of DNA released in comparison with the other batches examined. DiO/DiI staining confirmed the cells successfully colonized the scaffolds and migrated deeply in the structure. Fast-Blue staining confirmed the presence of differentiated hFOB 1.19 in the scaffolds. Fast Green FCF staining assessed the cells in coculture produced collagen with the higher amount produced in the scaffolds with the highest nHA loading. Further quantification (RT-qPCR) is ongoing to investigate whether it is the amount of nHA or the presence of PC3 to have impacted on the differentiation rate of osteoblasts. Preliminary results on 3D cytotoxicity (Picogreen) showed Docetaxel is cytotoxic to the cells in coculture after 72h treatment compared to relative controls (no drug applied) in all the batches produced. Ongoing studies are also quantifying the cytotoxic effect between 2D and 3D but first indicative results show that Docetaxel seems less effective in 3D.

Conclusions: nHA/PLGA-mixed scaffolds can be used as biomimetic models to elucidate cells' behavior in metastatic prostate cancer. nHA loadings can impact on the viability of the cells. Additional studies are assessing the impact of nHA loadings on the differentiation of hFOB 1.19 cells. Ongoing studies are focusing on the impact of Docetaxel on cells in pre- and post-treatment phase in both 2D and 3D.

A REVIEW ON THE EFFICIENCY OF ELECTROSPUN NANOFIBERS FOR OCULAR DRUG DELIVERY

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Background: The eye is one most delicate organ in the body and cannot be medicated using the conventional methods. Its inability to hold extra fluids washes out the drug within no time, making the drug delivery system non biocompatible. Other drawbacks are bad permeability of drugs through the cornea, small possibility for drug absorption, preocular retention and sometimes even severe side effects. There is a need of a drug delivery system that can ensure prolonged drug release within the eye without causing drug wastage, corneal abrasion or side effects. Some of the diseases that require treatment are glaucoma, keratomycosis, dry eye the main drug release route of which is through the cornea. One of the potential drug carriers which has massively been a part of recent study are electrospun nanofibers. It is proposed that they have high adaptability and can set well in the cornea because of their soft nature. This puts them at a higher advantage then their semi solid and liquid counterparts, thus overcoming the issue corneal semi permeability. Their high surface area ensures prolonged drug delivery. Some studies also show them in favour of being a replacement for eye drops. This review talks about the impact of several drug polymer combinations used for ocular drug delivery.

Methods: Since the drug delivery must take place through the cornea, the system has to be completely sterile and free of any impurities that might cause irritation. Electrospinning is the process used to generate the nanofibers. The drugs are mostly encapsulated in the polymer solution and are sprayed on a collector plate using a syringe. The driving force is potential difference between the tip of the syringe and the collector plate. Different drugs have different concentration ratios of mixing, some studies even experimented with multiple drugs and other encapsulation methods. Some drugs were even tested with different polymer combinations. FTIR and SEM and drug release studies were carried out on these systems and data were recorded. Some studies were carried in vitro, while some were in vivo (rabbbit's eye).

Results: All the studies conducted with various permutations of drugs and carriers showed prolonged drug release. The ones which involved in vivo studies carried out on rabbit's eye, proved the bio compatible nature of the system by not causing any sort of irritation or toxicity to the cornea. The drug release went from being as low as 24 hours (Dexamethanose loaded ENI) to as high as 30 days (Levoflaxin loaded Polycaprolactone). Other systems showed an approximate drug release of 10 days.

Conclusions: All the studies conclude that the usage of electrospun nanofibers for ocular drug delivery showed a positive result and they can be used as drug carriers in the future. Other necessary conditions like long residence time, sustaining and a constant rate of drug release, betterment of the biocompatibility were all met. On drawing comparisons, Levoflaxin loaded PCL had the best drug release and did not cause any corneal abrasion in the in vivo test, and hence, could be termed as the best candidate for ocular drug delivery.

GREEN SYNTHESIS OF CHICKPEA WASTE (*Cicer arietinum*)- DERIVED SILVER NANOPARTCILE FOR ANTIMICROBIAL APPLICATION

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Background: Nanotechnology is a field of research at nanoscale which involves dimensions between 1 to 100 nanometers. There is a wide scientific interest on the biosynthesis of nanoparticles. Silver nanoparticle have been known to have inhibitory and bactericidal effects. The non-hazardous silver nanoparticle synthesis using the extract of biological organism has gained widespread attention in various field including food sectors. Here, we report on the development of silver nanoparticles by a reduction of aqueous Ag+ ion with the extract of biowaste *Cicer arietinum* peels.

Method: In the present study, a unique stable silver nanoparticles were synthesized by a green route using biowaste of chickpea waste *Cicer arietinum* peel. The presence of biosynthesised silver nanoparticle was confirmed by an analysis of colour variations from pale yellow to reddish brown, as well as the appearance of surface plasmon resonance (SPR) bands at 400-450nm using ultraviolet-visible spectroscopy. Biosynthesised silver nanoparticle was characterized by scanning electron microscopy, transmission electron microscopy, Fourier transform infrared spectroscopy. Biosynthesised silver nanoparticle was evaluated for their significant effect on antimicrobial activity against Gram-positive and Gram-negative bacteria

Results and Conclusion: These results indicate the nanobiocomposite films can potentially be used as active packaging material for food packaging and preservative applications. This study highlights innovative development of AgNP with antimicrobial properties from agricultural waste.

Keywords: Silver nanoparticle, Antimicrobial properties, Nanotechnology, Chickpea waste

SELF-ASSEMBLY OF AMPHIPHILIC DRUG BETA BLOCKERS IN AQUEOUS SOLUTION

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Background:

Some amphiphilic drugs behave like surfactants and self-assemble in aqueous solution and form 'micelle-like' structures when their concentration exceeds a given – critical micelle – concentration[1]. Such micelles can significantly affect the solubility, membrane permeability and ultimately the therapeutic activity of the drugs from which they are formed[2,3]. In the study presented here, we sought to understand, at the molecular level, the structure-function relationship of the β-blocker, propranolol hydrochloride, in relation to its aggregation behaviour in aqueous solution, and thereby to gain insight into the impact of these phenomena on its therapeutic activity.

Methods:

Small angle neutron scattering (SANS) studies of propranolol hydrochloride (propHCl) dispersed have been performed to determine the behaviour of propHCl in aqueous solution. Complementary ^{1}H-NMR dilution studies and full-atom molecular dynamics (MD) studies, with the simulations run over 6000 ns, of the drug self-assembly process have been performed.

Results:

The SANS data clearly show that the propHCl forms aggregates in aqueous solution. The aggregates grow with concentration, and become smaller with increased temperature. The SANS data obtained for propHCl shows the aggregates are well fitted as prolate ellipsoids with a 'dry' core consisting primarily of the naphthyl moieties. MD simulations of the corresponding systems show that the drug forms polydisperse aggregates in aqueous solution, with the shape, size, and internal core-shell structure of the aggregates consistent with those of the aggregates obtained from model-fitting the SANS data. The NMR studies also confirms the existence of aggregates of the propHCl in aqueous solution. NMR in combination to the other two techniques, indicates the dominant interacting moiety of propHCl in the self-assembly process is the naphthyl rings.

Conclusions:

PropHCl forms aggregates which grow larger at higher concentrations, and become smaller with increased temperature. The aggregates are prolate ellipsoids with a 'dry' core consisting of naphthyl rings. The driving force for self-aggregation of propHCl is likely to be π-π stacking.

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ALBUMIN NANOPARTICLES FOR INTRA-ARTICULAR DELIVERY OF CELECOXIB TO TREAT OSTEOARTHRITIS

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Background: Osteoarthritis (OA) is a leading cause of chronic disability and musculoskeletal pain. Oral non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely recommended treatments. Of these, cyclooxygenase type 2 (COX-2) inhibitors have some advantages over other NSAIDs. The only approved COX-2 inhibitor for OA is CELEBREX® (celecoxib) capsules, but it has a black box warning of cardiovascular events and gastrointestinal bleeding. One possible way to avoid these side effects is direct intra-articular (IA) delivery to the OA knee. However, IA-delivered celecoxib is removed from the joints rapidly due to its low molecular weight and high diffusivity. Our hypothesis was to increase IA retention time by loading celecoxib in nanoparticle (NP) delivery system made from human serum albumin (HSA). NPs of size less than 100 nm are known to enter the collagen matrix of cartilage to target chondrocytes.

Methods: We synthesised batches of stable celecoxib-loaded HSA NPs of size less than 100 nm in a novel process without using cross-linking agents, high temperature, oils, or Class I/II solvents. The NPs were characterised using several spectroscopic, microscopic, and scattering techniques. Finally, the cytotoxicity and anti-inflammatory efficacy of the NPs were tested on a lipopolysaccharide (LPS)-stimulated human leukemia monocyte (THP-1) cell line and in primary chondrocytes from OA patients.

Results: The celecoxib-loaded NPs were spherical and had a low polydispersity index of ~0.1. Cell viability assays indicated that the NPs do not have any deleterious effects on cells. *In vitro* anti-inflammatory efficacy studies demonstrated that NPs efficiently reduced inflammatory signals in stimulated THP-1 cells (monocyte chemoattractant protein-1, MCP-1 and prostaglandin E_2, PGE_2) and in primary chondrocytes from OA patients (PGE2).

Conclusions: Having established a reproducible process, the next step is to assess efficacy and joint residence time of the NPs in preclinical animal models of OA following IA administration. These NPs may have the potential to be used for IA delivery of celecoxib to treat OA in humans. The HSA NPs also have the potential to load temperature-sensitive drugs and drugs used to treat other conditions.

CANNABIDIOL AS POTENTIAL GLIOBLASTOMA MULTIFORME TREATMENT: *IN VITRO* ASSESSMENT OF ANTI-CANCER PROPERTIES AND *IN VIVO* DELIVERY

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Background: Glioblastoma Multiforme (GBM) is the most aggressive brain cancer, with a very poor prognosis of <5% 5-year survival. Resistance to the standard treatment, chemotherapeutic temozolomide (TMZ), occurs in 50% of cases, commonly involving mechanisms of the over-expression of O^6-methylguanine DNA-methyltransferase (MGMT) or by a deficiency of mismatch repair (MMR). The anti-cancer properties of the main natural non-psychoactive phytocannabinoid cannabidiol (CBD) have been investigated in a number of cancers including lung, colon and gliomas. Whilst CBD is commonly reported to exhibit anti-cancer properties against GBM, and to work in synergy with other antineoplastic compounds, its ability to overcome the common resistance mechanisms associated with TMZ treatment has not yet been comprehensively investigated. This work studies the anti-cancer properties of CBD and the drug's ability to overcome MGMT over-expression and MMR-deficiency, as well as the assessment of the delivery of CBD to the specific anatomical locations within the brain following oral administration.

Methods: *In vitro* anti-cancer assessment of CBD against human GBM lines U373-M (MGMT over-expressing) and U373-V (non-resistant) and human colon cancer cell line HCT116 (MMR-deficient) were conducted by MTT and cell count assays. *In vivo* assessment of the delivery of CBD to the brain after oral administration in sesame oil and a control lipid-free vehicle (propylene glycol/ethanol/water) was studied in Sprague-Dawley rats. The distribution of CBD within the brain was assessed up to 8 hrs post administration by means of a validated HPLC-UV methodology.

Results: The *in vitro* assessment confirmed the two common resistances to TMZ treatment in the U373-M and HCT116 cell lines with GI_{50} values of 331.61 μM and 609.21 μM, respectively (U373-V control GI_{50}: 10.42 μM). CBD was shown to efficiently overcome both resistances, with GI_{50} values of 23.16 μM (U373-M), 13.37 μM (HCT116) and 33.04 μM (U373-V control). *In vivo* assessment in rats demonstrated the ability to deliver CBD efficiently to the brain after oral administration in both sesame oil and the lipid-free vehicle. After administration with sesame oil, the maximum CBD concentration is observed at the same time as the maximum concentration in the plasma (3 hrs post administration). However, a higher concentration is seen in the brain, 295 ng/g, compared to the plasma, 123 ng/mL. Assessment of the distribution within the brain demonstrates that CBD can be efficiently delivered to areas where GBM is most commonly found (frontal and temporal lobes).

Conclusions: The data suggest that phytocannabinoid CBD has high efficacy against GBM *in vitro*, and can overcome the two common resistances to treatment by TMZ (MGMT over-expression and MMR-deficiency). This work also demonstrates that CBD can be delivered to the relevant anatomical locations (frontal and temporal lobes) within the brain as quickly as 2 hrs post oral administration, and at concentrations similar to the required *in vitro* GI_{50} values, even when administered at a relatively low oral dose.

APPLYING QUALITY BY DESIGN APPROACH IN FLUPHENAZINE DECANOATE NANOEMULSION OPTIMIZATION USING DESIGN EXPERT®

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Background:
In the development of pharmaceutical formulations, there are numerous variables that contributes to a highly variable final product. Various active pharmaceutical compounds with variable excipients combined withs several methods lead to a poor-quality pharmaceutical formulation. To control the pharmaceutical products quality, the United States Food and Drug administration (USFDA) inculcated quality by design (QBD) approach in regulatory practice and pharmaceutical development (2009). QBD refers to understanding various parameters and their interactions to obtain the desired quality. Nanoemulsion (NE)is a colloidal system of nanoscale droplets dispersed in continuous phase and stabilized by surfactants and co-surfactants. Nanodroplets are formed under mechanical extrusion or high shear stress. Due to the various parameters that contribute to NE formulation, QBD was applied to optimize the formulation.

Methods:
The NE formulation was optimized using a 4-factor, 5-level central composite design (CCD) with Design Expert Software version 11 (State-ease, Minneapolis, USA). Oil amount (mg), surfactant to oil ration (SOR), co-surfactant amount(mg) and sonication time(m) were used as factors in the optimization process. Two responses were then observed, namely droplet size and polydispersity index (PDI). Twenty-eight formulations were suggested from the software and their responses of the dependent variables were recorded.

Results:
The results revealed that the droplet size and PDI of FLU-D NE optimization process followed the quadratic models. The F-values of droplet size analysis was 11.24. With respect to the PDI analysis, the F-values was found to 9.35. The *p-value* of<0.001 was found in the case of droplet size and PDI analysis, indicating that the parameters exhibited significant effects on the droplet size and PDI of FLU-D NE. In terms of sonication time, increasing the sonication time from 5m to 15m did not have a significant effect (p>0.05) on both dependent variables. Further, SOR within the pre-selected range didn`t show a significant effect on Droplet size and PDI, (*P value*=0.6996) for droplet size and (*P value=0.1772*) for PDI. On the other hand, oil amount has a significant effect (p=0.01) on the droplet size. Also, co-surfactant amount was found to have a significant influence on the droplet size and PDI of FLU-D NE (p<0.05). Based on the interactions between different parameters and their effect on the final formulation, the software suggested a formulation to match the desired responses. The suggested formulation was tested, and responses were recorded. Responses of the suggested formulation were within the desired ranges.

Conclusions:
From the response surface plots it can be concluded that with respect to parameters observed. QBD approach showed a significant role in time saving and successful development of NE, matching the established target product profile.

NOVEL FLU-D NANOEMULSION LOADED DISSOLVING MICRONEEDLES ARRAY FOR TRANSDERMAL DELIVERY.

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Background: As a chronic treatment of schizophrenia, fluphenazine decanoate (FLU-D) is administered intramuscularly every 2- 4 week. Schizophrenia symptoms and Lack of illness insight boost poor medications adherence. To improve the clinical outcomes of antipsychotics, continues effort is directed toward investigating different dosage forms or routes of administration. Transdermal delivery as a self-administered dosage form has the potential to benefit the medical practice and enhance patients adherence to prescriptions. Nanoemulsion (NE) has been investigated previously as a nanocarrier of lipophilic molecules. NE improves the permeation of lipophilic compounds through the biological membranes. Further, Microneedles bypass the *stratum cornium* (SC) to enhance the transdermal permeation. Combining various permeation enhancers such as NE and MNs can enhance molecules permeation through the skin.

Methods: The FLU-D NE was prepared by sonication method. NE components were screened based on drug solubility, droplet size and polydispersity index (PDI) using HPLC, particle sizer and transmission electron microscopy (TEM) respectively. Further, a quality by design approach was applied to optimize FLU-D NE using Design-Expert® software. Finally, FLU-D NE was loaded to polymeric DMNs formulation to form bilayer DMNs. FLU-D NE loaded DMNs were characterized, and i*n-vitro* skin dissolution and deposition were studied in full-thickness neonatal porcine skin (obtained from stillborn piglets) using Franz diffusion cells.

Results: The optimised FLU-D NE was found to be 210 ± 5 nm in droplet size with PDI 0.14± 0.01, well-dispersed spherical droplets as shown in Figure 1. FLU-D NE was stable for 2 weeks at room temperature and at 8° C in terms of droplet size and PDI. The EE% was 99.81 ±0.1% with LC %of 14.5 ±0.01%. FLU-D NE loaded DMNs showed a sufficient mechanical and insertion properties to bypass the *stratum corneum* (SC). The novel FLU-D NE loaded DMNs formulation has an *in-vitro* skin dissolution < 10 m. This combined system of MNs and NE was able to deliver FLU-D transdermally.

Conclusions: Based on *in –vitro* studies, FLU-D NE MNs was able to bypass the stratum corneum of the skin and deliver FLU-D transdermally as illustrated in figure 8. These findings suggest that this oily lipophilic prodrug can be delivered transdermally using this novel combination of MNs and NE. For future work, the pharmacokinetics evaluation of FLU-D NE loaded DMNs will be performed in an animal model.

Manufacturing & characterisation of lipid nanoparticles by microfluidics

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Background: Applying nanoparticles (NPs) technology for therapeutic purposes, particularly for targeting delivery as Drug Delivery System (DDS), makes a significant development in the drug administration systems for a number of medicinal purposes, including overcoming the limitations of chemotherapy. Between the different platforms of NPs, lipid-based NPs (e.g., liposomes) reported as the less toxic formulation *in vivo*, beside the ability to carry hydrophobic or hydrophilic molecules, and the prolonged half-life. Among the multiple methods of preparing liposomes, microfluidic technology is a promising method for the manufacturing of liposomes. Microfluidics offering a high-level control of the process's various parameters, which support controlling particle size, distribution, and physiochemical properties. Using microfluidic technology to encapsulate hydrophobic drugs (e.g., cancer drugs), has shown a better uptake and potency in several cell lines in comparison to other carriers prepared by non-microfluidic methods.

Methods: Multiple phospholipids (e.g., DMPC, DPPC, DSPC, and DOPC) have been combined with cholesterol, using microfluidic apparatus, obtained empty liposomes as a standard formulation. Different lipids to cholesterol ratios (e.g., 2:1, 3:1) have been investigated independently. All of experiments took place at total flow ratio (TFR) 1, 2, and 3 ml min^{-1}. In addition, every TFR has experimented at three different flow rate ratios (FRR) 1:2, 1:3, and 1:4. Particle size, polydispersity PDI, and zeta potential was investigated in the formulated NPs. Moreover, FTIR and DSC studies were also performed for further analysis of the obtained NPs.

Results: By comparing the phospholipid structure, it can be determined that DPPC, DMPC, and DOPC provide the most relevant result based on the particle size, PDI, and SD values**.** As the increase of the phospholipid acyl length and transition temperature (DSPC > DPPC > DMPC), display increasing of the liposome diameter. The phospholipid to cholesterol composition ratio shows an effect on produced liposomes, as DMPC and DPPC 2:1 phospholipid to cholesterol ratio shows a smaller average of liposome size, lower PDI, and more stable particles comparing to 3:1 phospholipid to cholesterol ratio. DMPC produced the smallest liposomes diameter among the whole samples (147 ± 19 nm). The PDI average of each phospholipid was 0.22 for DPPC, 0.25 for DMPC, and 0.27 for DOPC, which indicates a homogenous formulation, and especially for DMPC and DPPC. From the presented data, it can be shown that DMPC and DPPC 2:1 phospholipid to cholesterol formulation specifically with TFR 1 and 1:4 FFR provide the most suitable liposomes with confirmed size range and homogenous formulation.

Conclusions: The microfluidic system, as a computerized, flexible, and highly controlled system, allows modifying variety of parameters that affect the manufacturing process, such as the TFR and FFR. The change of FFR and TFR impacts the liposome dimensions, uniformity and assists in achieving the most optimum liposomes with desired dimension (< 200 nm) and PDI (<0.25). Most of the results show that increasing the FFR at a given TFR decreased the size of the liposomes. Optimal liposomes with preferred dimension (< 200 nm) could be an excellent carrier beside the capacity to cross tissues and cell barriers and act as an "ideal" a drug delivery system.

Depot-Forming Dissolving Microarray Patches for the Non-invasive Delivery of a Model Hydrophobic Drug

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Background: Prolonged release dosage forms are becoming increasingly attractive to scientists due to their ability to provide continuous release of medications following their administration. This increases patients' compliance to their treatment regimens and thus improve their quality of life. However, to achieve prolonged release over more than 24 hours, injections or implants are required. Those techniques are invasive and difficult to terminate upon the development of toxic or adverse events. Therefore, in this project, microarray patches (MAPs) containing a model hydrophobic drug; atorvastatin (ATR), are being developed to deposit a therapeutic dose intradermally to provide a sustained release over a prolonged period.

Methods: Dissolving MAPs were fabricated in a bilayer-casting technique. The first layer consisted of ATR-containing Poly(vinylalcoho)l (PVA) 9-10 K and Poly(vinylpyrrolidone) (PVP) 29-32 K blends. The second layer comprised of PVP K 90 and glycerol. Those MAPs were firstly characterised in terms of their mechanical strength, drug content, and *in situ* insertion efficiency. Afterwards, *ex-vivo* skin deposition studies for 4 & 24 hours was conducted using Franz-cells set up to quantify ATR amount deposited in the skin after using those MAPs.

Results: MAPs were found to contain a mean of 5.15 ± 0.4 mg of ATR/MAP. Their tips were able to dissolve within 60 minutes *in situ* and were completely inserted into porcine skin. Skin deposition studies showed that 1.3 ± 0.1 mg ATR was deposited in the skin 24 hours after MAPs application. Furthermore, 0.8 ± 0.4 mg ATR was quantified in the receiver compartment of Franz-cells. Therefore, the total amount of ATR delivered was found to be 2.0 ± 0.33 mg after 24 hours, which represents 38.7 ± 6.7% of the initial amount loaded per MAP

Conclusions: Those findings demonstrate the efficiency of MAPs to successfully deposit hydrophobic ATR intradermally for its subsequent release. Oral bioavailability of ATR after 40 mg oral dose was found to be 14%, thus, the amount delivered from one MAP over 24 hours transdermally might be therapeutically sufficient. Future *in vivo* study to be conducted to evaluate ATR delivery over two weeks

Thermal responsive nanofibers for cancer therapy

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Background: Cancer is one of the leading causes of death worldwide. In 2018 alone, 9.6 million deaths were caused by cancer. Mainstay therapies for cancer include radio and chemotherapies. The latter can be very powerful, but without targeting there is significant off-target toxicity. A stimuli-responsive nanocarrier provides a smart system for various therapeutic applications. Generally, stimuli-responsive systems are able to enhance the release of therapeutic drugs in response to triggering signals (intrinsic or extrinsic). The internal signals include pH, temperature in human body, enzymes, and oxidative stress, whereas external stimuli constitute magnetic field, light, or heat. Meanwhile, the thermal response systems have been researched a lot in the field of electrospinning nanofibers. By using the thermal sensitive polymer, the thermo-responsive behaviour is one of the most essential properties, which can be divided in two types: upper critical solution temperature (UCST) and lower critical solution temperature (LCST). A polymer solution below the LCST is a clear, homogeneous solution while a polymer solution above the LCST appears cloudy (leading to LCST also being referred to as cloudy point). On the contrary, some polymers are soluble above the UCST, and become cloudy below the UCST. These behaviours happens because it is energetically more favourable. In this study, both types of polymers were used to compare the different performances between them in the electrospinning formulation.

Methods: For the multi-responsive systems, pH and temperature-responsive NFs are the most studied. In this study, thermal responsive system had been performed used PNIPAm and copolymer of acrylamide and acrylonitrile (AAm-AN). PNIPAm is a commercially available LCST polymer that is a thermo-responsive polymer, which is one of the most common studied polymers in reference to biomedical applications due to its LCST being very close to body temperature at around 32-34°C and it is fast on-off switching. Meanwhile, the copolymer (AAm-AN) of acrylamide and acrylonitrile, synthesized by random RAFT polymerisation, have been studied as a biocompatible UCST polymer. PNIPAm and AAm-AN were both co-dissolved with PCL as carrier polymer in HFIP to achieve the polymer solution. The formulation all used 1.3ml/h as flow rate and 9.5kv as supplied voltage. The characterisation process including DSC, TGA, XRD, NMR and FTIR had been processed to define the physicochemical properties of these fibres and pure polymers. Furthermore, drug release profile was established, using carmofur as model drug.

Results: As the results, these two thermal sensitives systems, using PNIPAm and copolymer of acrylamide and acrylonitrile with PCL as a carrier polymer, performed uniform fibre diameters and smooth surface geometry. Meanwhile, these fibres had been produced with stable thermal behaviour. According to results of the dissolution test that both LCST and UCST system have shown thermal sensitive properties that the drug release diverse by changing the temperature of the buffer (Phosphate Buffer Saline (PBS) pH 7.4). The AAm-AN/PCL fibres released carmofur faster in 42°C buffer rather than in the 25°C buffer. On the contrary, the PNIPAM/PCL fibres behaved in diverse way that it release faster in 25°C condition which both related to the expected thermal sensitive properties.

Conclusions: To sum up, both PNIPAm and AAm-AN with PCL system can be produced uniformed fibres with good physical properties. In the meantime, the fibres were shown thermal responsive under 43°C buffer which means it can be applied for thermal sensitive application in the future.

PREPARATION, STABILISATION, ISOLATION AND TABLETING OF VALSARTAN NANOPARTICLES USING A SEMI-CONTINUOUS CARRIER PARTICLE MEDIATED PROCESS

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Background: Ninety percent of new drug entities and forty percent of approved drugs on the market have poor aqueous solubility which leads to unfavourable bioavailability. It is commonly accepted by the pharmaceutical industry and in academic literature that drug nanoparticles enhance the delivery and bioavailability of drugs with poor aqueous solubility when administered orally. Among the different techniques to form drug nanoparticles, liquid antisolvent precipitation is fast, easy and cost-efficient. However, it is challenging to scale-up in batch mode due to variations in local supersaturation resulting in wide particle size distributions within larger batch processing volumes. Another major challenge is the subsequent isolation of the nanoparticles to the solid state due to issues with particle growth and agglomeration. This work investigated the technical feasibility of preparing, stabilizing and isolating poorly water-soluble drug nanoparticles into nanocomposite powders and tablets with enhanced dissolution profiles, using a novel carrier particle technology.

Methods: A novel semi-continuous process was developed for the carrier particle mediated production, stabilization and isolation of valsartan (Val) nanoparticles into a solid form using montmorillonite clay (MMT) as the carrier particles. Tablets of nanocomposite powders at a range of drug loadings with just 10 % w/w disintegrant were formed. Particle sizing and zeta potential measurements were conducted as well as dissolution studies of powdered and tabletted nanocomposites. Small modifications to the surface of MMT using protamine, a cationic polymer, were made to facilitate high drug loadings.

Results: The semi-continuous process operated robustly for the entire duration of the experiment (~16 min) and steady-state conditions were reached after ~5 min. Nanoparticles of valsartan (51 ± 1 nm) were successfully prepared, stabilized and isolated with the help of montmorillonite (MMT) or protamine functionalized montmorillonite (PA-MMT) up to 33.3 % w/w drug loading into the dried form by this semi-continuous route. The dissolution profile of the isolated valsartan nanocomposite solids and their tablet formulations were similar to those produced via the corresponding laboratory scale batch mode process, indicating that product quality is retained during semi-continuous processing of the nanoparticles, which can be easily compressed into tablets without compromising the dissolution behaviour.

Conclusions: The developed semi-continuous process in this study offers a rapid and robust template for preparing stable drug nanosuspensions, which can then be readily isolated via adsorption (at different drug loadings) onto inert carrier particles. The resulting drug-carrier particle nanocomposite solids are suitable for tableting, affording fast-dissolving tablets for oral dosage forms.

NANOCRYSTALS AS VERSATILE PLATFORM FOR ENHANCING THE DISSOLUTION PROFILE OF RISPERIDONE FOR THE TREATMENT OF SCHIZOPHRENIA

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Background: Schizophrenia is a brain disorder characterized by mental-related symptoms such as delusions, hallucinations, disorganized speech or behavior, and impaired cognitive ability. These symptoms occur due to an imbalance in neurotransmitters including dopamine and serotonin. Antipsychotics are a class of drugs that used to treat schizophrenia. These drugs can be categorized into typical and atypical antipsychotics. Risperidone is an atypical antipsychotic that is used for the treatment of schizophrenia and related disorders as it has antagonistic effect on the serotonin-5HT2 and dopamine-D2 receptors. It is a hydrophobic drug that falls under class II according to the Biopharmaceutical Classification System (BCS). It is practically insoluble in water, freely soluble in methylene chloride, and soluble in methanol. In order to circumvent this limitation, the preparation of nanosuspension may be utilised as a strategy to facilitate solubilisation of lipophilic drugs, such as risperidone. Nanosuspensions can be defined as a biphasic system consisting of poorly water-soluble drug dispersed as nanocrystals (NCs) in aqueous vehicle. Nanosuspensions can be prepared *via* a bottom-up approach where the NCs are prepared by precipitating dissolved drug molecules in an aqueous vehicele. In contrast, the top-down strategy involves applying forces to decrease the size of a coarse drug particle. In the current work, a top-down approach was employed *via* media milling to prepare risperidone nanosupension as this strategy obviates the use of any organic solvent while improving the overall solubility the dissolution profile of the antipsychotic.

Methods: NCs were prepared by wet media milling technique. 300 mg of risperidone, 30% beads (0.1-0.2 mm), 6ml of 2% PVA 9-10 KDa and 4 magnetic bars were placed in a 10 ml vial. The drug was milled at 1250 rpm under room temperature and pressure. The prepared nanosuspension was converted into a powder by lypholisation. The developed NCs were characterized for particle size, polydispersity index (PDI) and the release of the drug was evaluated using an *in vitro* dissolution studies. The release of pure risperidone and the prepared NCs was conducted over a period of 28 days in a water bath set at 37^{o}C. Formulation of pure drug and NC containing 24.2 mg risperidone, were suspended in 1ml PBS (pH 7.4) and pipetted into a Spectra/Por®7 Dialysis Membrane. The loaded dialysis membrane was then placed in a bottle containing 100 mL of PBS spiked with 1% w/w of SLS. At predetermined time points, 1 ml aliquot of the medium was sampled and replaced immediately with the same volume of fresh release medium. The concentration of risperidone in the withdrawn samples was determined by reverse phase HPLC-UV.

Results: The prepared nanosuspension showed a particle size of 298.52 ± 3.72 nm and a PDI of 0.166 ± 0.03. After lyophilization the NCs displayed a particle size of 303.9 ± 3.67 and a PDI of 0.162 ± 0.021. Due to the intrinsic hydrophobicity of risperidone, the dissolution rate of the pure drug was very slow. However, the NCs exhibited significant enhancement in dissolution rate and cumulative release in comparison to the pure drug ($p= 0.0203$). After 28 days, the NCs achieved a cumulative release of 79.59 ± 2.92% relative to the pure drug which only showed a cumulative release of 34.97 ± 7.27% ($p< 0.0001$).

Conclusions: The preparation of risperidone NCs showed an enhancement in the dissolution profile of the drug. Such improvement in the dissolution profile of the drug may lead to enhancement the overall bioavailability of risperidone.

INFLUENCE OF ADDITIVES ON THE ANTISOLVENT PRECIPITATION OF STABLE PHARMACEUTICAL NANOPARTICLES

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Background: Low solubility and bioavailability are two major challenges faced by the pharmaceutical industry. Antisolvent precipitation is a fast, cost and energy efficient method used to prepare nanosize drug particles, to increase the solubility and the dissolution rate of poorly water soluble active pharmaceutical ingredients (APIs). Generally different types of additives such as surfactant and polymeric molecules are used to stabilize the nanoparticles during precipitation and subsequent storage or drying stages. Although nanosuspensions are used in some dosage forms, their short shelf life often necessitates separation of the nanoparticles from the solution to prepare dry powders. Freeze drying or spray drying processes can be used but frequently lead to agglomeration. Additives can act as redispersing agents which help to enhance the dissolution rate of agglomerated dry nano/micro particles.

Methods: A systematic investigation of the effect of different additives, before and after nucleation, and process parameters on the antisolvent precipitation of 2 poorly water-soluble APIs, fenofibrate (FF) and dalcetrapib (DCP), was conducted. The produced nanoparticles were isolated to dryness by freeze drying. PXRD, DSC and SEM analyses were performed on freeze dried particles to confirm the morphology and polymorphic form present. In vitro dissolution rate studies of the as received drug, nanosuspensions and freeze-dried particles were compared to probe the influence of additives during the precipitation and freeze drying processes.

Results: It was observed that lowering temperature helps to stabilize nanoparticles produced by antisolvent precipitation. For both APIs FF and DCP, nanosuspensions in the presence of single additives showed multimodal particle size distributions resulting in D[90] values in the micrometre range. In the presence of mixture of surfactant and polymeric additives (e.g. DOSS and PVA), both APIs produced narrower particle size distributions than in the presence of single additives resulting in D[90] values remaining in nanometer range over time (up to 30 mins). From particle size measurements of freeze-dried nanoparticles, it was observed that agglomeration happened during the drying process resulting in micrometer size particles of various habits. Dissolution rates of freeze-dried APIs prepared with additives showed rapid dissolution profiles compared to ‘as received’ API and freeze-dried APIs without additive. Freeze dried dalcetrapib in the presence of multiple additives underwent rapid dissolution similar to the aqueous nanosuspension which can be contributed to the thin needle like morphology of dried dalcetrapib particles.

Conclusions: Additives help in decreasing particle size and stabilizing nanoparticles of FF and DCP. For both APIs a combination of surfactant and polymeric additives are more efficient to stabilize nanosuspension and produce narrow PSD than single additives. Additives had a significant effect on the resulting morphology of dried particles. Additives present in dried APIs act as redispersing agents to increase the dissolution rate. Particle habits have direct effect on dissolution rate.

3D PRINTED DRUG DELIVERY IMPLANTS FOR INNER EAR THERAPIES

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Background: In the UK, greater than 11 million people suffer from hearing loss. With hearing loss affecting a large proportion of the population, the cost of treatment for the healthcare industry is large. Due to the complicated structure of the inner ear, drug delivery can be quite difficult, with ear drops being used as a common method of treatment. A prolonged method of delivery would be more ideal for patient compliance and ease of use. 3D printing (3DP), in particular Fused Deposition Modelling (FDM), could be a viable method of fabrication, as it provides the potential to create personalized devices catering to the winding anatomy of the inner ear. Previous studies of implantable devices using FDM have also proved the potential for prolonged drug delivery. In this study, we explore the incorporation of drug into Thermoplastic Polyurethane (TPU) using Hot-Melt Extrusion (HME), followed by 3DP of implants for the ear of varying designs.

Methods: Drug loaded TPU filaments were created using HME, by combining TPU pellets along with Levofloxacin drug powder. Filaments were created with 3 drug loads of 0.25%, 0.5% and 1% w/w Levofloxacin. These drug loaded filaments were then used to print 3 different designs of implants for inner ear therapy. Designs were created on Free online, computer aided design (CAD) software (TinkerCAD, USA). Designs were downloaded onto Ultimaker Cura software for pre-processing of designs prior to being sent to Ultimaker 2 FDM Printer (Ultimaker B.V., Geldermalsen, Netherlands) for printing. Designs were printed in triplicate for all further characterization methods. Implants were analysed using Fourier Transform infrared (FT-IR) spectroscopy and Scanning Electron Microscopy (SEM). Drug release studies were also carried out by placing the implants in 5 mL of phosphate buffered solution (PBS) and placing in an incubator at 37°C. Implants were removed, sample of solution taken and implants placed into a fresh solution of 5 mL of PBS. Sampling was done at 1, 2, 4, 24, 48, 72 and 96 hrs. Extracted solutions were analysed under UV-Vis Spectroscopy at a wavelength of 292 nm.

Results: Drug loaded filaments were successfully created using HME, with Levofloxacin Powder well distributed throughout the filament. FDM was able to printer successfully 3 designs of implantable ear devices at 0.25, 0.5 and 1% of drug concentrations. Surface morphology analysed using SEM showed that there were no visible drug aggregates present, indicating that Levofloxacin was well distributed within the TPU matrix. FTIR analysis of Levofloxacin loaded filaments showed no clear peak shift which is due to the low levels of drug added to the polymer. Release studies indicated an initial burst release of drug in the first 2 hrs of release followed by a period of sustained release.

Conclusions: This proof of concept study has been able to show that implants for the inner ear can be successfully printed using FDM. Drug loaded filaments were created with homogenous dispersion of drug within the filament. 3 different designs were successfully printed using these drug loaded filaments. Release studies showed that after an initial burst release in the first 2hrs, sustained delivery was possible for a period of 4 days. Longer release study and further characterization of the implants are required. However, this study shows the potential for a new quick, cheap and efficient method of fabrication of implants for the inner ear.

PRELIMINARY FORMULATION DEVELOPMENT OF SILICONE ELASTOMER VAGINAL RINGS FOR SUSTAINED RELEASE OF METRONIDAZOLE, SUCROSE AND LACTOBACILLUS

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Background: Bacterial vaginosis (BV) is a common dysbiosis of the human vagina in which commensal vaginal lactobacilli are displaced by mixed pathogenic bacterial populations. Current treatments for – including metronidazole (MET) and clindamycin – result in short-term cure but often lead to recurrence. New treatment options are needed. Lactobacilli are being actively developed as a probiotic treatment option for BV, given their ability to inhibit growth of pathogenic microorganisms and to maintain the health and stability of the vaginal tract microbiota. Also, prebiotic sucrose gels have shown promise for treatment of BV in clinical studies. Here, we report preliminary formulation work as part of our efforts to develop sustained-release vaginal ring formulations for simultaneous release of MET, a prebiotic lyoprotectant and lactobacillus. Specifically, the influence of incorporating various lyoprotectants into silicone elastomer rings is investigated.

Methods: Silicone elastomer matrix-type rods (as prototype vaginal rings) were prepared having various antibiotic and prebiotic components incorporated, including: MET, maltodextrin (MD), mannitol (MT), sucrose (SC), polyethylene glycol 4000 (PEG) and 20% freeze-dried sucrose (FDSC). Briefly, MET and lyoprotectant were added to Parts A and B of medical-grade silicone elastomer and mixed (3000 rpm,10 s, SpeedMixer DAC-150 FVZ-K). Rods were prepared by injecting the mix into PVC tubing and then curing in an oven at 40 °C. Manufactured rods were demolded and then cut into 5 cm lengths. Rods containing lyoprotectant only (n=4) underwent in vitro swelling testing in simulated vaginal fluid medium for 14 days. Rods containing MET + lyoprotectants were also tested for in vitro MET release in 200 mL water over 14 days (37 °C and 60 rpm), with quantification by reverse-phase UPLC (ACQUITY UPLC).

Results: Swelling ratio values (Q_{wt}) were calculated following the swelling experiment. The addition of lyoprotectants increased the swelling ratio (hydrophilicity) of the silicone elastomer rods. As the concentrations of lyoprotectants MT, MD and SC increased, the hydrophilicity of silicone elastomer increased. From 5% to 10% of PEG4000, the hydrophilicity also increased. The FDSC rods absorbed more water than the corresponding SC rods (Q_{wt} increase from 0.0 to 0.4). All rods indicated a burst MET release on day 1 followed by a slightly decreasing daily release with time and a linear cumulative release *vs.* square root time (all $R^2 > 0.9$), indicative of a permeation-control drug release mechanism from a polymeric matrix device containing excess solid drugs. In this 14-day release study, cumulative release for all rods increased as the lyoprotectant loading increased, consistent with hypothesis that the prebiotic lyoprotectant can facilitate the release of metronidazole by absorbing fluid.

Conclusions: Incorporation of hydrophilic prebiotic lyoprotectants (for the future purpose of also incorporating live lactobacillus) into silicone elastomer vaginal rings containing MET causes swelling of the rings and facilitates release of the MET. Moving forward, it will be important to minimize swelling of the ring device while still achieving sufficient release of MET, the prebiotic and the lactobacillus. In future work, freeze-dried lactobacillus will also be incorporated into the rings.

ENHANCED UPTAKE OF CRYOPROTECTANT AND ADVANCED THERAPY WITH MAMMALIAN CELLS

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Cryopreservation at very low temperatures has been used extensively to store cells, tissues, embryos and microorganisms for long term use. Challenges with cryopreservation include post-thaw cell viability, growth, maintenance of function and differentiation ability issues, as well as alteration in gene expression. Cryopreservation requires the use of cryoprotective agents (CPAs) that protect cells from damage caused by cooling and warming processes. Commonly used CPAs, such as dimethyl sulfoxide (DMSO), can be toxic and affect cell viability; they must be removed before the clinical administration of the cells. Trehalose, a disaccharide glucose synthesised by several organisms, is a potential nontoxic CPA that could replace DMSO in the preservation of different cell types. The main functions of trehalose include the provision of energy and the regulation of certain metabolic pathways that affect growth. The aim of the current study was to develop and optimise an applicable method for biological research based on the use of trehalose. To improve the loading of trehalose into mammalian cells, the trehalose concentration and the osmotic pressure between the inside and outside of the cells were manipulated using phosphate buffered saline (PBS). The study also aimed to examine the efficacy of a cell-penetrating peptide (CPP) as a trehalose delivery system. The utilised CPP, termed PLR (P21LK158R), has previously been shown to enhance delivery. Trehalose delivery was measured using the anthrone assay, and metabolic activity was evaluated using the presto-blue assay. The data indicate that manipulating osmolarity and the concentration gradient increases membrane permeability; however, they also affect cell stability, possibly due to osmotic shock.

MICROFLUIDIC DEVELOPMENT OF MICROPARTICLES TO ENHANCE CELL ENGRAFTMENT

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Background: Intrahepatic engraftment of islets or hepatocytes can eliminate the need for pancreas or liver transplantation. But, poor revascularisation and inflammatory reactions results in approximately 60-70% of cells failing to engraft. The delivery of immunomodulatory molecules such cytokine inhibitors, and growth factors including cytokines, has been shown to increase cell engraftment. However, delivering and maintaining these molecules locally at therapeutic doses remains a significant challenge. We propose a new paradigm of PLGA-based microparticles fabricated using microfluidic droplet methods as a replicable, automated, and scalable process to produce highly monodispersed microparticles that can provide a tuneable and controlled release. The inclusion of galactose will enable binding to hepatocytes in the liver to ensure localised delivery of immunomodulatory molecules.

Methods: Poly(D,L-Lactide-co-Glycolide) (PLGA) 50:50, Mw 61kDa, and galactosylated PLGA (Gal-PLGA), synthesised using D-(+)-Galactose Mw 180Da chemically attached to PLGA, microparticles were fabricated using a 100µm flow focusing hydrophilic microfluidic chip. PLGA and Gal-PLGA were dissolved in Dichloromethane (DCM) Mw 85Da, to form the dispersed phase. A surfactant solution of Poly(vinyl alcohol-co-vinyl acetate) (PVA) 88% hydrolysed, Mw 25kDa, at different concentrations with or without Tween 20, molecular weight 1227g/mol sourced from Acros Organics, USA, in deionised water made the continuous phase. A syringe pump was used to push both the dispersed and continuous phases through the chip at a rate of 0.15ml/hr and 4ml/hr respectively. At the junction where the dispersed and continuous phase met, droplets pinched off and were collected in deionised water. Microparticles were imaged using scanning electron microscopy and the size distribution of particles was quantified using Fiji (ImageJ), an image analysing software.

Results: 10% PLGA with 2% PVA in water was used with microfluidics to produce microparticles with low polydispersity. Varying the concentration of PVA directly influenced the average particle size and played a role in maintaining the stability of the microfluidic system. 2% and 1% PVA enabled a stable microfluidic system, however, low system stability was observed when 0.5% PVA was used which resulted in higher polydispersity index (PDI) and coefficient of variance (CoV) values for these microparticles. Lastly, 7% Gal-PLGA was used with 2% PVA which successfully produced microparticles. However, notable pores were observed therefore the concentration of PVA was decreased to 1% PVA which reduced the extent of porosity, but pores were still visible. The addition of Tween 20 to the 1% PVA in the continuous phase, resulted in no visible pores on the surface of the Gal-PLGA microparticles.

Conclusions: Microfluidics has the potential to produce monodispersed microparticles. To achieve this, optimisations of materials, surfactants, and operations are needed. Gal-PLGA can also be used as a material to form microparticles. However, it is also evident that further material characterisation and analysis is needed to ensure porosity has been eliminated.

COMPLEX AND PERSONALISED DOSAGE FORMS: A MULTI-MATERIAL HOT-MELT INKJET 3D PRINTED SOLUTION.

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Background: Polypharmacy is the most commonly used type of treatment used for chronic or elderly patients and can involve the regular consumption of anywhere from 8 and up to 60 medicines. While this approach can be effective, it can lead to low compliance (32.6%) among older patients and misconsumption, with consequential increased risk of adverse reactions and drug interactions. Thanks to its versatility, 3D printing offers a potential elegant manufacturing solution to this problem by enabling the production of personalised, multi-drug dosage forms with complex delivery profiles. Of particular interest would be the possibility to utilise lipidic materials as the main excipients for the solid dosage forms, especially for enhancing the delivery of poorly soluble drugs. Hence, this work aimed to develop a novel thermal jetting apparatus for multi-material printing for pharmaceutical application.

Methods: We developed a new approach for a solvent free 3D printing system capable of fabricating multi-material solid dosage forms via the jetting of melted materials. For this purpose, we modified a commercially available printer (PiXDRO LP50) to include a dual reservoir unit capable of dispensing two different materials during the same additive process. This included the design and development of complementary reservoir, support structures alongside with support temperature control unit and power supply. A commonly used pharmaceutical lipid, Compritol HD5 ATO (matrix material) and Fenofibrate (model drug) were used to prepare both drug-free and drug-loaded inks with drug concentrations varying between 5% and 30% (w/w). Using our bespoke system, we produced several proof-of-concept multi-material solid dosage forms with tailored dosing and release. This included single and multi-material complex 3D patterns with defined localised drug loading whereby drug-free ink is used as a release-retarding material. Various characterizations were performed to optimize the 3D printed tablet formulation.

Results: DSC, TGA, Shear viscosity analysis were used to ensure the ink printability. SEM, EDX, ATR-FTIR, Raman Spectroscopy and XRPD showed that the printlets demonstrated the required physical properties regardless of geometry and composition with the drug being found mostly in its amorphous state. *In vitro* release studies showed tailored release patterns and displayed immediate, extended, delayed and pulsatile drug release depending on drug localisation within the printed formulations and tablet geometry.

Conclusions: Using our novel system, we were able to produce several proof-of-concept multi-material solid dosage forms, with complex geometries and drug distributions able to demonstrate ‘programmable’ drug release patterns in *in vitro* studies.

NASAL DELIVERY OF PEPTIDES NANOFIBERS FOR ISCHAEMIC STROKE

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Background: Stroke is the second highest cause of death and the third leading cause of disability worldwide, with ischaemic stroke subtype accounting for 85% of cases. Recombinant tissue plasminogen activator (r-tPA) is the only FDA-approved thrombolytic agent, effective when administered intravenously within 4.5 hours after stroke onset, thus benefiting only 1-5% of patients. Angiotensin-(1-7) [Ang-(1-7), H_2N-DRVTIHP-COOH] has been shown to be neuroprotective by binding the Mas receptor and exerting anti-inflammatory, angiogenic, vasodilatory and anti-oxidative activities. Ang-(1-7) do not cross the blood-brain barrier in significant amounts and is cleared from the blood in less than 30 minutes. Our working hypothesis lies in engineering novel lipidized Ang-(1-7) analogs by the palmitoylation of the free hydroxyl group of Tyrosyl[4] residue to trigger the self-assembly of the peptide amphiphile (PA) in stable nanofibers that will be both enzymatically stable and brain permeable, while conserving binding to the Mas receptor. Formulating nano-in-microparticles based on mucoadhesive polymers of intermediate molecular weight, such as quaternised glycol chitosan (QGC) and quaternised pullulan (QPUL), we will direct and maximise the uptake of the new PAs via a direct nose-to-brain non-invasive delivery strategy for the early treatment of stroke patients.

Methods: Peptides were synthesized using solid phase peptide synthesis and characterized (ESI-MS, HPLC, FTIR, NMR). Self-assembly studies were undertaken using pyrene and thioflavin T (ThT) assays and complemented with transmission electron microscopy studies (TEM), while conformational studies using circular dichroism (CD) under different physiological pH, different temperatures and upon dilution were also carried out. The enzymatic stability in mouse plasma (50%v/v) and liver and brain homogenates (50%w/v) was assessed. An oxidative stress model was developed using organotypic brain slices treated with hydrogen peroxide and dihydroethidium (DHE) to measure superoxide in live cells. Novel QGC and QPUL polymers were synthesized and characterized (NMR, FTIR, GPC-LALLS), while nano-in-microparticles were prepared using spray-drying.

Results: ESI-MS, FTIR and NMR confirmed the synthesis and lipidisation of Ang-(1-7) lipopeptides as well as their parent analogues. Conformational studies revealed a stable β-sheet secondary structure of the lipopeptides upon different physiological pH, temperatures up to 37°C and upon dilution. Critical aggregation concentrations (CAC) studies indicated the formation of micelles (46.93 ± 6.81 nm) at 5.77 ± 1.62 μM (pyrene assay) and nanofibers (length: 0.2-1μm, width: 29.99 ± 5.65 nm) at concentrations above 54.16 ± 9.06 μM (ThT assay) in PBS (pH 7.4, 10 mM). Stability studies of lipidised analogues showed less than 20% degradation in 24h compared to Ang-(1-7) that was more than 50% degraded within 46, 5, and 11 minutes in plasma, brain and liver homogenates respectively, and was completely degraded after 4h. Coronal 300-μm thick slices treated with 10 mM H_2O_2 had ≥50% of cells producing superoxide compared to control, both in hippocampus and cortex. QGC (MW 60 KDa, DA 6.57%, DQ 11.46%) and QPUL (MW 60 KDa, DQ 32. 22%) polymers were synthesised and employed to prepare nano-in-microparticles with 1% peptide loading. Permeability studies across nasal lamp epithelia mucosa and across RPMI2650 cell layers and an all human blood-brain barrier model are pending.

Conclusions: Novel enzymatic stable Ang-(1-7) analogues were synthesized, able to form stable nanofibers stabilized by β-sheets. Their efficacy is being tested in *ex vivo* models of ischaemic stroke (oxidative stress, oxygen-glucose deprivation). Nano-in-microparticles have been prepared to enable nose-to-brain delivery, while their permeability will be tested using *ex vivo* nasal epithelial mucosa and cell monolayers, as well as an all human blood-brain barrier model.

Image-guided phase change nanodroplets for the treatment of brain tumours

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Background: High-Intensity Focused Ultrasound (HIFU) has attracted notable attention in the last years due to its ability to alter tissue characteristics and enhance the delivery of therapeutic molecules. In preclinical models (including non-human primates), HIFU has proved to intensify the permeability of macromolecules and nanoparticles through the Blood-Brain Tumor Barrier (BBTB). The BBTB acts as an inhibitory factor for the antineoplastic drugs, preventing most of them from entering the tumor and reducing its efficacy.

The phase-change nanodroplets (NDs) have a perfluorocarbon core that starts oscillating upon activation with HIFU energy and potentially causes a reversible permeability of the BBTB for a short period. This study analyses the preparation of lipid-based NDs, labelled with fluorescent probes and drug-loaded to create a targeted drug delivery vehicle.

After the HIFU application, the gas-cored NDs will create a localised BBTB opening due to the cavitation effect, while they will selectively release the encapsulated drug molecules to the tumor site. Moreover, they can be adopted to be MRI (magnetic resonance imaging) traceable.

Methods: The lipid mixture is dried up until there is a thin lipid film. After the hydration of the film and a series of sonications and the addition of the perfluorocarbon the NDs are formulated. The ND stability was assessed measuring their size over time and their their ability to cavitate was measured with high-speed camera and/or passive acoustic methods.

Results: The primary characterisation experiments proved that our formulated blank and drug-loaded NDs start cavitating after the HIFU exposure, while there was a clear correlation of lipid and gas composition to the cavitation effect. Furthermore, the encapsulation efficiency of the SN-38 (a topoisomerase I inhibitor) reached ~100% without disturbing the stability of the NDs.

After further investigation, successful preparation and physicochemical characterisation of the drug-loaded NDs, the preliminary *in vitro* experiments in BBTB cell models showed an increase in membrane penetration after the application of FUS and NDs.

Conclusions: The physicochemical characterization data showed ND stability at 37ºC and for at least a week in fridge storage. Moreover, the cavitation results matched the ones of the approved microbubbles. However, further studies need to be conducted before we move into animal studies.

EVALUATING WOMEN'S PREFERENCES AROUND COLOUR, FRAGRANCE AND SIZE OF DRUG-RELEASING VAGINAL RINGS

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Background: To date, seven vaginal ring products for contraception and hormonal therapy have reached the market and other a large number of new experimental vaginal rings are currently in clinical/preclinical development. The outer diameter of marketed vaginal rings ranges from 54 mm to 56 mm, while cross-sectional diameter varies between 4 and 9 mm, although historically a much more extensive range of dimensions have been evaluated. Marketed vaginal rings are either opaque white or transparent. Here, we assess women's preferences around three key design characteristics of vaginal ring products – colour, fragrance and size – with a view to developing products with increased user acceptability, adherence and efficacy.

Methods: Drug-free silicone elastomer vaginal rings (i) of different dimensions (external diameters and cross-sectional diameters: 57.7 × 5.6 mm; 57.6 × 6.2 mm; 57.6 × 7.9 mm; 55.0 × 9.0 mm), (ii) including different fragrances (lemon, grapefruit, spearmint, camphor and lavender) and (iii) having different colours (white, pastel orange, light pink, pastel green, mellow yellow and mauve) were manufactured via injection molding using medical grade elastomers and colour masterbatches. Women's preferences and opinions were collected and assessed through three focus group discussions (FGDs) conducted in eThekwini District, South Africa in March 2021. Vaginal products of varying colours, sizes and fragrances were distributed to women to visualize, handle and smell. Basic demographic data were collected from participants. Focus group discussions were audio recorded, transcribed and translated. The qualitative data were thematically analyzed based on inductive and deductive coding.

Results: 16 women aged 20–34 years participated in the FGDs. Opinions on ring colour were varied, with some women clearly preferring coloured products while for others this was not an important attribute. Participants also had varied preferences for the different types and intensities of fragrances. Concerns about colour and fragrance were linked to perceptions of vaginal health and safety related to chemical composition. There was more agreement on preferred vaginal ring size; flexibility and width were considered important factors for insertion and comfort with use.

Conclusions: Choice and options in sexual and reproductive health products facilitates increased choice and overall uptake.

A 3D PRINTED REACTOR-IN-A-CENTRIFUGE (RIAC) FOR THE PRODUCTION OF THERAPEUTICALLY RELEVANT LIPOSOMES

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Background: Liposomes have been extensively investigated and clinically employed for the delivery of bio-active compounds, including chemotherapy drugs and vaccines, demonstrating improved pharmacokinetic behaviour and therapeutic efficacy. Traditional bulk production methods often suffer from limited control over liposome size dispersity and lamellarity, and may rely on laborious multi-step procedures. Microfluidic-based methods have been introduced to provide greater control over the end-product characteristics; however, their widespread adoption is often hindered by complexities and costs associated with device manufacturing and operation, as well as the relatively short device lifetime and low production rates. In this study, we demonstrate production of therapeutically relevant liposomal formulations, using a novel reactor-in-a-centrifuge (RIAC) device. RIACs rely on a cost-effective and single-step manufacturing process, and are actuated by conventional laboratory centrifuges to drive reagents through the reactor. The reactor concept developed in this study has the potential to simplify production of organic and inorganic nanomaterials over more complex flow-reactor technologies.

Methods: RIACs were manufactured in polylactic acid (PLA) using a fused deposition modelling (FDM) printer (Ultimaker S5). The device architecture included two reservoirs connected to a spiral-shaped mixing channel. Channels were printed within a cylindrical body, which could be hosted in a standard 50 mL centrifuge tube. Liposomes were produced through a 'solvent exchange' mechanism, which was driven by advective mixing between ethanol (where liposome constituents were solubilized) and deionized water. The formulations investigated in this study contained distearoylphosphatidylcholine (DSPC) and cholesterol, as the core liposome constituents. Liposome average size, size dispersity (or polydispersity index, PDI) and surface charge were measured as a function of different production- and formulation-related parameters. These included temperature, centrifugation time, relative centrifugal force, total lipid concentration, and presence of a PEG-moiety. Formulations containing cationic and biotinylated lipids were also investigated, in order to demonstrate RIAC's ability to synthesize functionalised liposomes with potential application in targetable delivery of genetic materials.

Results: The mean size of the produced DSPC:cholesterol liposomes could be tuned in the range of 140 nm to 200 nm, by varying the RIAC actuation parameters. The optimised actuation method (centrifugal force: 2000 rcf, centrifugation time: 4 min) resulted in the production of liposomes with a therapeutically relevant mean size of 174 nm and a narrow size distribution (PDI = 0.10), at a production rate of ~6 mg/min. Notably, PEGylated liposomes were stable up to one month, at a storage temperature of 4°C. The versatility of the developed production method was also demonstrated by successful synthesis of cationic and biotinylated liposomes, with a mean size of ~120 nm and relatively low size dispersity (PDI < 0.2). Experiments are currently being performed to further evaluate liposome morphology through fluorescence microscopy imaging.

Conclusions: The flow-through production method proposed in this study has the potential to be an effective and versatile approach to simplify the synthesis of therapeutically relevant liposomal formulations, due to the single-step and pump-free nature of the process. Future studies could investigate scaling-up strategies to achieve greater production rates and improve continuity of device operation.

IMPROVED ANTIFUNGAL ACTIVITY OF ITRACONAZOLE USING MULTIMPLE COMBINATION OF BIOADHESIVE-THERMOSENSITIVE IN SITU VAGINAL GEL-GEL FLAKES- SOLID DISPERSION IN CANDIDIASIS RAT MODEL

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Background: The treatment of vaginal candidiasis using conventional dosage form resulted in ineffective therapy. Furthermore, as one of antifungal agents, the effectiveness of itraconazole (ITZ) is hampered by its poor aqueous solubility. Here, we developed mucoadhe-thermosensitive *in situ* vaginal gel containing gel flake- solid dispersion of ITZ to overcome the problems

Methods: The optimization of solid dispersion and gel-flake formulations of ITZ was performed using a composite central design. The gel flakes- solid dispersions were further incorporated into *in situ* vaginal gel using PF-127 and PF-68, as the gelling agents, with the addition of hydroxypropyl methylcellulose (HPMC) as the mucoadhesive polymer.

Results: The results exhibited that the optimized formulation of solid dispersion was able to significantly improve the solubility of ITZ in water and simulated vaginal fluid to reach the values of 4.211 ± 0.23 and 4.291 ± 0.21 mg/mL, respectively. In addition, the optimized formulation of gel flakes had optimum entrapment efficiency and drug-loading capacity. The obtained *in situ* vaginal gel provided desirable physicochemical characteristics and could retain more than 4 mg of ITZ in the vaginal tissue after 8 h. Importantly, as per the *in vivo* antifungal activity using infection animal models, the incorporation of the solid dispersion technique and gel-flake system in the formulation of the bioadhesive-thermosensitive in situ vaginal gel led to the most significant decrease of the growth of Candida albicans reaching <1 log colony-forming units (CFU)/mL or equivalent to <10% of the total colony after 14 days, indicating the improvement of ITZ antifungal activity compared to other treated groups.

Conclusions: The incorporation of ITZ into bioadhesive-thermosensitive *in situ* vaginal gel and gel flakes-solid dispersions could significantly enhance the solubility of ITZ and improve *in vivo* antifungal activity in candidiasis rat model.

Control over the α polymorph of indomethacin using a supercritical carbon dioxide-based method

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Background: The polymorphic control of active pharmaceutical ingredients (APIs) is vital in the manufacture of medicines within the pharmaceutical industry. Crystallisation methods based on supercritical CO_2 are capable of producing unique solid forms of APIs, nevertheless, the control over the polymorphic form remains a challenge. The work that I present shows that the gas antisolvent (GAS) method enables control of the polymorphic form of indomethacin.

Methods: The GAS method based on supercritical carbon dioxide was explored to control the polymorphism of indomethacin. The effect of the pressure, stirring rate, temperature, solvent, and additive was studied.

Results: The temperature parameter defined the predominance of the formation of the α and γ forms of indomethacin. Nevertheless, absolute control over the production of the α metastable form of indomethacin was achieved only when the poloxamer 407 was used in the experiments regardless of the other parameters. Therefore, a detailed molecular modelling study was also conducted and gave insight into the role of poloxamer 407 in the formation of the α polymorph.

Conclusions: Control over the polymorphic form of indomethacin was achieved with the GAS process using poloxamer 407 as an additive. The two experimental conditions that had an effect on the polymorphism of indomethacin were the temperature and the presence of an additive. The α polymorph was consistently obtained from the experiments using this additive independent of all other conditions. The extensive molecular modelling conducted revealed that the α polymorph of indomethacin was favoured in the presence of poloxamer. Therefore, the GAS method together with the use of additives shows potential for controlling the polymorphism of APIs.

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CHITOSAN COATED POLYMERIC NANOCAPSULES FOR ENHANCING THE PERMEATION OF CELECOXIB IN VAGINAL TISSUE

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Background: Vaginal administration of drugs suffers from many limitations, e.g., the short contact time of the drug with the mucosa or continuous carrier wash-out. Thus, the development of new carriers for gynecological use is necessary. Celecoxib, a selective cyclo-oxygenase-2 inhibitor, has been administered orally as an anti-inflammatory drug. It is a poorly water-soluble drug with oral bioavailability of around 40%. Besides, long-term oral administration of celecoxib produces gastrointestinal side effects. The hypothesis of the present work was to augment the permeation of celecoxib through the vaginal mucosa via its incorporation within chitosan-coated polymeric nanocapsules formulation.

Methods: The chitosan-coated polymeric nanocapsules were prepared by the nanoprecipitation method followed by ultrasonication. Chitosan was used as a coating agent to provide mucoadhesive properties and long residence time on the vaginal mucosa. The developed nanocapsules were characterized in terms of nanocapsules size, surface charge, encapsulation efficiency %, morphology, and mucoadhesive force. In vitro drug release and drug permeability across rabbit vaginal mucosa were also assessed.

Results: The optimized formulation comprised of celecoxib (20 mg), oleic acid (500 mg), lecithin (20 mg), Tween®40 (30 mg), cetyl trimethyl ammonium bromide (CTAB) (7.5 mg) and chitosan (0.3% w/v) displayed a particle size of 574.9 ± 13.06 nm, zeta potential of +34.56 ± 3.65 mV, encapsulation efficiency of 88.54 ± 0.18%, and mucoadhesive force of 2.6 ± 0.56 Pas. The transmission electron microscope images revealed a spherical shape of the optimized chitosan-coated polymeric nanocapsules. In vitro release studies displayed a sustained release pattern of celecoxib from both the non-coated and chitosan-coated polymeric nanocapsules compared to the free drug dispersion. An in vitro vaginal permeation study showed better permeation-enhancing ability of the developed celecoxib laden chitosan-coated polymeric nanocapsules than that of un-coated formulation and free drug dispersion. In addition, the amount of celecoxib uptaken through rabbit vaginal mucosa for the optimized coated formulation was higher than that of the uncoated formula and the free drug dispersion.

Conclusions: The developed chitosan-coated polymeric nanocapsules might provide a promising carrier for vaginal drug delivery and for improved control of inflammation.

Safety Optimisation of a hybrid nanoparticle based on thermo-responsive delivery system for pancreatic cancer treatment.

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Background:

Pancreatic cancer is the 4th most aggressive cancer in the western world. In the United States, it is the third leading cause of cancer-related deaths in the United States. The overall 5-year rate of survival for this type of cancer is 9%; very low in comparison to other cancers [1]. The lack of symptoms results in a delayed diagnosis and therefore, a delay in treatment of the cancer. Current therapies for pancreatic cancer include: fluorouracil, gemcitabine and nab-paclitaxel. Nanotechnology offers the benefit of enhancing drug delivery to the targeted tissue because of increased drug permeability. This also reduces side effects and sustains drug release over a long period of time [2]. Theranostics are a new discovery which offer the added benefit of diagnosis alongside therapy for the cancer which leads to a decrease in requisite time for treatment.

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Methods:

From previous experimentation conducted in the labs, the charge needed to render the system (charged bis-naphthalimide drug molecules on hybrid iron oxide-gold nanoparticles) both stable and reversible upon heat stimulus has been investigated. In order to make headway with this information, it would be vital to develop an optimised formulation which possesses a higher safety profile until triggered heating and drug release. The possibility of addition of specific targeting ligands which will allow for active transport of the nanomedicines to the tumour site will be explored. Hence, the hybrid particles will be surface engineered to protect the drug molecules from metabolism until they are heated and drug release occurs. There will also be addition of targeting peptides onto the hybrid surface in order to enable site specific ability. The most favourable formulations will be tested both in vitro and in vivo.

Results:

The experiment is currently ongoing therefore, no conclusive results yet.

Conclusions:

This is a revolutionary area for nanotechnology therapies to be applied in the treatment of cancers particularly pancreatic cancer.

SMART GEL AND CONTACT LENSES-BASED DRUG DELIVERY SYSTEMS: NOVEL NANOTECHNOLOGY APPROACHES FOR TREATMENT OF COVID-19 ASSOCIATED CONJUNTIVITIS

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Background: The aim of the present work is to develop advanced ocular delivery system of Naphazoline hydrochloride in ophthalmic dosage forms including thermoresponsive hydrogel, contact lenses and ocuserts.

Methods: Naphazoline hydrochloride loaded niosomes coated with chitosan (N1) were prepared via thin film hydration method, in addition, thermoresponsive gel was synthesized by cold method using poloxamer 407: poloxamer 188 in 1:3, 1:1 and 3:1 mass ratio , (G1), (G2) and (G3), respectively. The prepared niosomes (N1) were incorporated in (G3). Ocuserts were prepared from alginate and calcium chloride solutions using ionic cross-linking containing Naphazoline hydrochloride loaded niosomes coated with chitosan (F1) . Moreover, alginate based contact lens containing free drug (F2), and also readymade contact lens soaked in drug solution (F3) were prepared. Gamma radiation was adopted for the sterilization for the different formulae prior their evaluation. Niosomes were evaluated for particle size, zeta potential, entrapment efficiency and morphology using zeta sizer and SEM. Thermoresponsive gels were evaluated for viscosity, PH and gelation temperature. Thickness, elasticity and transparency of ocuserts were also evaluated. In vitro release and kinetic analysis were studied for all formulae.

Results: The obtained results revealed that niosomes' particles size increased from a value 458 nm to 680 nm upon chitosan coating, polydispersity index (PDI) was 0.257 increased to a value of 0.656, negative zeta potential value of -38.3 mV was transformed to a positive 20 mV indicating successful coating. Entrapment efficiency of Naphazoline hydrochloride was 18% due to its hydrophilicity. The prepared hydrogels showed a pseudoplastic behavior due decline in viscosity upon increased shear stress from 6 rpm to 30 rpm, at which (G1) decreased from 200 to 60 cP, (G2) decreased from 200 to 80 cP and (G3) decreased from 500 to 120 cP, noting that maximum viscosity was exhibited by (G3) due to the higher proportion of poloxamer 407 relative to poloxamer 188. A pH value of 7.4 was detected in all prepared hydrogels, however, gelation temperature varied among them, (G3) showed the desired gelation temperature at 36.5 ± 0.5 °C, while (G1) did not gel at all upon increasing temperature and (G2) gelled at 29 ± 0.5 °C. Ocuserts (F1) and contact lens (F2) showed thickness of 0.3 mm and 0.25 mm respectively. They also showed elasticity due to absence of breakage upon 200 times of folding, however regarding the transparency, (F1) was opaque while (F2) was transparent. In vitro release studies and release kinetic models showed a satisfactory controlled release pattern of the drug from the aforementioned systems as the release of formulae N1, N2, N3 was 30.34%, 29.38%, 98.73% respectively, and the release of F1, F2, F3 was 28.68%, 18.28%, 0.05% respectively.

Conclusions: These diverse systems provide enhanced targeting drug delivery and offer a promising therapeutic strategy for future ophthalmological treatments.

PRAMIPEXOLE-CONTAINING DISSOLVING MICROARRAY PATCHES FOR THE TREATMENT OF PARKINSON'S DISEASE

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Background: Parkinson's disease is a debilitating neurodegenerative disease that predominantly affects dopamine-producing neurones within the brain [1]. Pramipexole (PRA) is a non-ergot dopamine agonist which increases dopamine neurotransmission, restoring balance and alleviating symptoms, such as rigidity and tremor. PRA is currently available in tablet form with a maximum daily dose of 3.3 mg (expressed in terms of PRA base) and a bioavailability of 90%. However, due to the disease, patients usually experience dysphagia and gut motility issues, rendering oral preparations undesirable. Dissolving microneedle array patches (MAPs) can alleviate these problems with an additional benefit of tailoring the drug release kinetics from the MAP by altering the polymer and drug form. The aim of this research was to develop a PRA salt containing-MAP composed of rapidly dissolving, biocompatible, polymers for immediate release of PRA. A second MAP containing PRA base, a more hydrophobic form, was formulated with a biodegradable polymer, poly(lactic-co-glycolic acid) (PLGA), to form implantable tips with the aim of sustaining PRA release.

Methods: A bilayer-casting technique was employed to formulate PRA salt dissolving MAPs. The first layer was composed of PRA salt, poly(vinylpyrrolidone) (PVP) and poly(vinyl alcohol) (PVA). The baseplate layer contained PVP, PVA and glycerol. A similar approach was used to formulate PRA-PLGA MAPs, with the first layer comprising PRA base and PLGA (LA:GA 75:25, viscosity 0.8-1.0 dL/g) dissolved in DMSO. Nile red was added to the solution for staining purposes. Following drying, a second PRA-PLGA layer was cast on several MAPs, again, the baseplate layer contained PVP, PVA and glycerol. This enabled comparison between MAPs with one or two PRA-PLGA casting layers. For both MAP formulations, an excess of the gel/solution was cast on the microneedle mould which consisted of 600 pyramidal needles per 0.75 cm^2, with each needle having a height of 750 µM. The MAPs were characterised in terms of their mechanical strength and insertion efficiency into eight layers of Parafilm M®, which replicated the thickness of skin [2].

Results: MAPs formed from casting aqueous gels containing 10% and 20% PRA salt, had a drug content of 0.70 ± 0.05 mg and 1.40 ± 0.19 mg, respectively, expressed in terms of PRA base, demonstrating uniform drug distribution within the needle tips. After the needles were pierced through Parafilm M® it was found that the 10% salt MAPs were stronger than the 20% MAPs, reflected by a percentage needle height reduction of 2.34 ± 2.91% and 16.49 ± 8.80%, respectively. Interestingly, 75% of needles in both formulations were capable of penetrating through the 2nd layer of Parafilm M® equating to a skin depth of 252 µM. Uniform PRA-PLGA MAPs were formulated with PLGA concentrated in the needle tips. The height of the PLGA tips for one and two layer castings was 239.92 ± 15.62 µM and 298.53 ± 17.79 µM, respectively. This represents 32.0% and 39.8% of the total needle height, respectively.

Conclusions: Uniform PRA base and salt MAPs have been successfully formulated. Future work will focus on *in vitro* permeation of PRA salt as well as permeation and skin deposition of PRA base using Franz cells.

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Development of *Zingiber cassumunar* oil-Loaded Microneedle for Musculoskeletal Disorder

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Background: Musculoskeletal conditions are injuries or disorders of the muscles, nerves, tendons, joints, and cartilage. Globally, musculoskeletal disorders were found to be the most prevalent disease in 2019. Approximately 1.71 billion people with musculoskeletal disorders have been reported recently [1]. *Zingiber cassumunar* Roxb. (Plai) is a local medicinal herb in Asian countries. It has a strong benefit characteristic. For Thai traditional medicine, volatile oil from *Z. cassumunar* has been used to directly apply and penetrate the skin to remedy muscle stress and joint pain. In addition, this volatile oil has been used for a long time for the treatment of muscle inflammation according to its oil contains (*E*)-1-(3,4-dimethoxyphenyl) butadiene (DMPBD) as an active ingredient which proven to be an anti-inflammatory agent [2]. This volatile oil has been developed in various dosage forms such as cream or gel. However, these formulations require a long time for pain relief due to the slow penetration of drugs into the skin as a cause of the protective epithelial barrier. Therefore, a microneedle patch with micro-scale needles bypasses the stratum corneum to deliver molecules. Thus, providing painless skin insertion and effective muscle pain relief.

Methods: The essential oil of Z. *cassumunar* rhizomes is obtained by hydrodistillation using a Clevenger' s type apparatus with water at 130-150°C to obtain light yellowish oil. Dissolving microneedles (DMNs) were fabricated in a casting technique. The formulation consisted of volatile oil with poly (vinyl alcohol) (PVA) and poly (vinyl pyrrolidone) K32. DMNs were evaluated in terms of mechanical properties depending on the compression force test using a texture analyser. Further, insertion properties of DMNs were investigated using the parafilm M ® insertion test.

Results: All cone-shaped needles were formed entirely, sharp needles with a strong base plate and elegant appearance. A compression test was conducted to evaluate the mechanical strength that DMNs can withstand before they deform; after a 32-N compression, the average height reduction rates of the formulated displayed the height reduction with a value of 5.31 ± 0.32%. The force of 32-N was applied to assess the effects of insertion on needle height, using Parafilm M® as an artificial membrane to mimic the skin. It was found that volatile oil-loaded bilayer DMNs penetrated to the third layer of Parafilm M®. MN patches possessed the capability to be inserted into neonatal porcine skin, reaching insertion depths of approximately 250–300 μm. In the case of Parafilm M®, it was penetrated down to the third layer (approximately 370 μm. These results are very similar to previous insertion studies of polymeric MN into Parafilm M®.

Conclusions: The work presented here reports the successful formulation and mechanical characterization of dissolving microneedle arrays containing *Z. cassumunar* oil. Future work will focus on *in vitro* permeation of *Z. cassumunar* oil permeation using Franz cells.

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