FIRST POSTER PRIZE

P-II-9

Chitosan-based chemical platforms for launching antimicrobial peptides against ESKAPE pathogens

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Introduction: The burden of bacterial wound infections has considerably increased due to antibiotic resistance to most of the currently available antimicrobial drugs. The most opportunistic and multidrug-resistant pathogens, which can colonize the wound, are part of the ESKAPE bacterial collection (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Enterobacter* sp.) [1]. Antimicrobial peptides (AMPs) have been recognized as promising alternatives to conventional antibiotics, that could fight antimicrobial resistance through different mechanisms of action [2]. Despite the high number of investigated AMPs, very few reached the clinics. This is mainly due to their low bioavailability, fast degradation, and high cytotoxicity. As such, AMP-chitosan conjugates and different delivery systems have been developed to overcome the side effects of AMPs.

Aims: To evaluate the activity and safety performances of chitosan-based platforms for coupling AMPs with different lengths and ramification degrees, including linear and dendrimeric AMPs. Additionally, to test different delivery strategies of the AMP-chitosan conjugates for topical applications, such as bandages, gels, and nanoparticles [3].

Methods: The chemistry for coupling 4 cationic *N,N,N*-trimethyl, *O*-carboxymethyl, and 2 *N*-aryl (pyridyl and aminocinnamyl) chitosan derivatives to AMPs of different generations (first, second, and third) was performed *via* thioether-haloacetyl reaction, following *in vitro* antimicrobial activity, hemolysis, and cytotoxicity assays. AMP-trimethyl chitosan conjugates were selected for further *in vivo* studies on Gram-negative *P. aeruginosa*-infected mice. AMP-chitosan was incorporated into hyaluronic acid (HA) hydrogel, which upon lyophilization turned into a foam/bandage-like formulation. Nanoparticles were obtained by coacervation with ultra-low molecular weight HA. Mice with bioluminescent *P. aeruginosa*-infected excisional wounds were treated with AMP-chitosan and chitosan ban-dages, sulfadiazine cream, or no treatment, followed by bioluminescence imaging for 7 days.

Results: The new chitosan-AMP conjugates showed high selectivity by killing the ESKAPE pathogens, including *P. aeruginosa,* and very low toxicity toward mammalian cells, as well as extremely low hemolysis to red blood cells. Electron microscopy revealed that the 4 chitosan derivatives coupled to AMP destroyed both the inner and outer membranes of *P. aeruginosa*. Moreover, chitosan-AMP conjugates showed synergetic effects at extremely low concentrations. AMP conjugates showed an *in vivo* antibacterial activity similar to the best antibiotic treatment.

Conclusions: We have successfully designed and coupled a library of at least 4 chitosan derivatives to several AMPs of different lengths and ramifications. The covalent coupling of AMPs to chitosan overcomes the drawbacks of AMPs alone by significantly reducing the cytotoxicity and hemolysis and increasing the bioactivity toward ESKAPE. The chitosan-AMP conjugates can be used as potent antimicrobial therapeutic agents, to eradicate pathogens such as those present in acute and chronic infected wounds.

Keywords: antimicrobial agents, infected wounds, ESKAPE pathogens

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SECOND POSTER PRIZE

P-VI-6

Measuring cytokine-secretion dynamics of single cells to deepen the understanding of systemic autoinflammatory diseases

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Introduction: Disruptions in the regulation of the immune response induce various disorders and clinical manifestations that can be potentially life-threatening. Manifesting by inflammatory flares, systemic autoinflammatory diseases (SAIDs) are a striking example of immune response dysregulation. SAIDs are rare heterogeneous conditions characterized by periodic serous and synovial membrane inflammation that spontaneously resolves. Due to their genetic nature, diagnostic currently relies mainly on genetic testing. However, disease-causing mutations cannot be identified for nearly half of the patients with clinical manifestations of the disease, SAIDs are most frequently of unknown pathophysiology. Therefore, dynamic and highly sensitive studies are needed to understand better the underlying mechanisms of SAIDs and evaluate the consequences of the immune response disruption.
Aims: To address this, we aim to directly measure the dysregulation and activation of the immune cells in response to stimulation on the single-cell level with our platform for functional single-cell measurements.
Methods and Results: We successfully integrated novel bioassays to quantify cytokines secretions dynamically on the single-cell level. Our methods can identify for each individual cell the secreted cytokines quantities, secretion rates, dynamics, co-secretion and different cellular subpopulation according to the secretion pattern.
Conclusions: These developed assays are currently employed in a proof-of-concept study of healthy donors and SAIDs patients of various genetic backgrounds with known or unknown mutations which enables us to measure the immune response with unprecedented resolution and precision. We are hoping that the cytokine profiling of SAIDs patients will deepen the understanding of the pathophysiology of the disease, identify the different disease pathways and could potentially help to define new objective diagnostic criteria based on the measured dysregulation.

Keywords: immunology, single-cell, immune dysregulation, cytokine, microfluidic

THIRD POSTER PRIZE

P-II-7

Temperature-triggered in situ forming lipid mesophase gel for local treatment of ulcerative colitis

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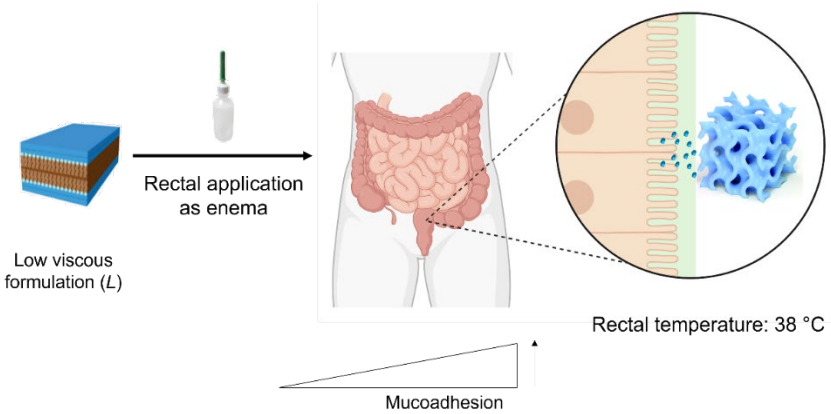
Introduction: Ulcerative colitis (UC) is a chronic inflammatory disorder affecting the colonic mucosa. There is no cure for UC and its chronic relapsing/remitting nature strongly affects patients' quality of life. Current treatments struggle to achieve desired remission rates, prompting the exploration of novel therapeutic approaches that enhance drug delivery to the inflamed region while minimizing systemic effects.
Aim: Leveraging the potential of biocompatible and biodegradable lipid mesophases, we designed a temperature-triggered in situ-forming lipid gel (TIF-Gel) as a platform for localized drug delivery in UC treatment.
Methods: We screened lipid mesophase compositions responsive to the colonic environment using Small Angle X Ray and rheology techniques. The chosen TIF-Gel formulation underwent *in vitro* and *ex vivo* drug release analysis via vertical Franz cells. Mucoadhesion and further validation, including pharmacokinetics, occurred via investigations in murine models of dextran sodium sulphate-induced colitis and T-cell transfer colitis.
Results: This versatile gel effectively accommodates and gradually releases drugs with varying polarities, such as tacrolimus and tofacitinib, over time. Notably, the gel's robust adherence to the colonic wall for a minimum of 6 h prevents leakage and improves drug bioavailability. In 2 established models of inflammatory bowel disease, the TIF-Gel demonstrates enhanced efficacy in reducing inflammation compared to conventional drug delivery methods.
Conclusions: TIF-Gel offers advantages over existing systems, being cost-effective, easy to manufacture, improved colonic retention time and capable of delivering high drug concentrations while minimizing systemic absorption.

Keywords: ulcerative colitis, lipid mesophase, local delivery, mucosa adherence



Reference:
Carone, M., Spalinger, M.R., Gaultney, R.A. et al. Temperature-triggered in situ forming lipid mesophase gel for local treatment of ulcerative colitis. Nat Commun 2023; 14: 3489

BEST POSTER IN PHARMACEUTICAL TECHNOLOGY

P-II-1

Colonic delivery of aqueous formulations using 3D printed capsules

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Introduction: The human gut microbiota significantly impacts human health, and its imbalance can contribute to various diseases, influencing treatment outcomes. Fecal microbiota transplantation (FMT) has shown promise in treating *C. difficile* infections and is being explored for other conditions. However, its standardization is hindered by invasive administration routes and its effectiveness by high dosing requirements. Oral dosage forms can overcome these obstacles, however delivering live beneficial bacteria to the colon via the oral route is challenging due to harsh gastrointestinal conditions. Therefore, there is a need for new delivery systems to efficiently deliver live bacteria in sufficient amounts.
Aims: The objective of this work is to develop and characterize 3D-printed capsules that can encapsulate an aqueous suspension of live bacteria. These capsules are designed to protect the bacteria from the harsh conditions that are encountered during gastrointestinal transit and selectively release their content at the distal part of the intestine.
Methods: The water-insoluble lid and body were 3D-printed by digital light processing (DLP) using methacrylated poly(Ɛ-caprolactone) and its random copolymers with poly(D,L-lactic acid) and poly(glycolic acid). The locking cap was 3D-printed by fused deposition modeling (FDM) using a water-soluble poly(vinyl alcohol). The locking caps were dip-coated with an enteric polymer, Eudragit S100. The mechanical properties (tensile strength, elongation at break and compression deformation), degradation, and swelling of the capsules in simulated intestinal fluid (SIF) at pH 6.8 were evaluated. Their resistance to proton diffusion was assessed in simulated gastric fluid (SGF) at pH 1.2 by monitoring the pH changes of the inner solution. The release profiles of the capsules loaded with an aqueous solution of Evans blue were determined in SGF pH 1.2, SIF pH 6.8 and SIF pH 7.3. The release was further visualized by surface dissolution equipped with an UV-Vis camera. Finally, the performance of the capsules was evaluated *in vivo* in a beagle dog by monitoring the release of barium sulfate aqueous suspension with X-ray imaging.
Results: Utilizing FDM and DLP 3D printing, we achieved precise fabrication of a soft capsule body and lid, and a locking cap with an enteric coating of 40 µm. The capsules were able to resist compression forces that are expected in the GI tract and restricted proton diffusion. *In vitro* dissolution tests showed that the capsules remained intact for 4.5 h and achieved complete release of Evans blue after 5-6.5 h. X-ray images of an *in vivo* study on a beagle dog showed that the capsules did not leak for 2-2.5 h post administration and after 2.5-4 h they started to deform and release their content. These data suggest that the capsules can release their aqueous content in the distal part of the small intestine or the colon.
Conclusions: In this study, we report a simple design of 3D-printed capsules for the oral delivery of aqueous suspensions to the distal part of the intestine or the colon. These capsules protect their content from gastric fluids and withstand mechanical stress. They open in the late intestine, thus showing promise for the oral administration of bacterial suspensions.

Keywords Capsules, 3D printing, colonic delivery, aqueous suspension.

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BEST POSTER IN PHARMACEUTICAL BIOLOGY AND PHYTOPHARMACOLOGY

P-I-1

Novel inhibitors targeting oncogenic ERK and AKT signaling in melanoma: From compound library screening to target identification

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Introduction: Malignant melanoma is the deadliest type of skin cancer with unmatched rates of mutations that frequently arise in the MAPK/ERK and PI3K/AKT signaling pathways. Although specific inhibitors of these pathways show spectacular initial results in the clinic, most patients relapse within just a few months [1]. Novel inhibitors targeting aberrant ERK/AKT signaling in melanoma are therefore urgently needed.
Aim: Discovery of novel natural products targeting oncogenic ERK/AKT signaling in melanoma.
Method: Our natural product lead discovery platform of plant extracts was combined with an innovative high-content screening (HCS) assay that quantifies downstream inhibitory activity at the ERK and AKT levels [2]. To further explore the coverage of chemical space for such inhibitors, this HCS was part of an EU-OPENSCREEN program. Transferring from 96- to 384-well format enabled the screening of large pure compound libraries by accessing high-throughput screening (HTS) pipelines.
Results and Conclusion: To this end, we screened our in-house library of 2,576 crude plant extracts as well as additional 25,696 pure natural and synthetic compounds through our scalable screening and discovery pipeline. A total of 46 active compounds with diverse natural scaffolds were confirmed as downstream inhibitors of ERK and/or AKT with IC_{50} values in the low micromolar range. Our approach allows the exploration of the chemical space of natural product libraries consisting of crude extracts and pure compounds while targeting downstream activities of complex signaling pathways. The current challenge aims towards target identification of the most promising hits. Our strategy includes similarity searches and assessment of physical binding as well as enzymatic inhibition with heterologously produced pathway proteins. Ultimately, we envisage to develop these newly discovered inhibitors into lead compounds for future drug development.

Keywords: melanoma, natural products, MAPK/ERK and PI3K/AKT signaling, high-content screening, target identification

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SPECIAL POSTER PRIZE

P-II-8

In vivo evaluation of cyclodextrin microneedles for particulate vaccine delivery

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Introduction: Microneedles have emerged as a promising and innovative approach for vaccine delivery, offering the advantages of painless application, improved stability, and the potential for self-administration. A key highlight is their ability to induce efficient immune activation through skin application. This becomes particularly significant for particulate vaccines, as the dermis houses dendritic cells crucial for the initiation of cellular immune responses. Dissolving microneedles can be fabricated using various matrix materials, including polymers and sugars. The utilization of cyclodextrins is widespread as excipients in pharmaceutical formulations, but largely unexplored as a matrix for microneedles.
Aims: In this study, we evaluate cyclodextrins as a matrix for microneedle fabrication in delivering particulate vaccines through skin. Initial physicochemical assessment was conducted *ex vivo*, and we further seek to assess the safety and immune stimulation in mice using the MHC class-I-binding peptide antigen SIINFEKL incorporated in nanoparticles.
Methods: The antigenic nanosuspension was mixed with cyclodextrins and a series of centrifugation and drying steps led to the formation of the microneedles by solvent casting. To facilitate detachment post-application, a layer of hydroxypropylmethylcellulose was interposed between the needles and the base plate. The histological impact of microneedle application to skin was assessed in mice subjected to a single patch application. The patch was kept for 3 min on the skin. Intravital imaging System (IVIS) was utilized to identify the deposition of labeled particles and subsequent distribution to organs. The immunological CD8 T-cell response stimulated by patch application was evaluated in C57BL/6 mice adoptively transferred T-cell receptor transgenic OT-I cells after microneedle-assisted intradermal model vaccine delivery.
Results: Cyclodextrins were found to be an effective matrix for microneedle fabrication in particulate vaccine delivery, maintaining particle compatibility and structural integrity. IVIS revealed successful particle deposition in mice after treatment. Histological examination displayed transient cellular reactions at the application site compared to non-involved skin. The immunization showed improved antigen-specific CD8 T-cell proliferation and activation towards effector functions, as evidenced by MHCI-SIINFEKL specific pentamer, CD44, and CD62L staining. Importantly, the immune responses were non inferior to intradermal administration of antigen with the poly I:C adjuvant, despite the substantially lower antigen content in microneedles.
Conclusions: This study sheds light on the potential of cyclodextrin-based microneedles for particulate vaccine delivery. Their successful fabrication, structural integrity, penetration efficiency, and immunological response all point to their viability as a promising candidate for further investigation. Future studies could explore the potential to incorporate inclusion complexes for the delivery of hydrophobic extracellular adjuvants.

Keywords: microneedles, vaccine delivery, nanoparticles, cyclodextrins