Novel drug formulations –
Impact from the nanoscale

POLYMORFI

2017
Oikeat työkalut
nanopartikkelien karakterisointiin

- Partikkelien koko
- Zetapotentiaali
- Nanopartikkelien lukumäärä
- DLS
- SEC
- NTA (Nanoparticle Tracking Analysis)
- RMM (Resonant Mass Measurement)
- ITC, DSC ja Laserdiffraktio

Yli 30 vuotta asiantuntemustusta asiakkaan hyväksi
The XXVIII Symposium of the Finnish Society of Physical Pharmacy

9:00 Opening of the Symposium  
*Chairman of the Society, Martti Kaasalainen*

9:05 Functional nanomaterials for intracellular interaction to direct cell fate  
*Ciro Chiappini, King's College London, United Kingdom*

9:40 In situ amorphisation of poorly water soluble drugs  
*Petra Priemel, University of Copenhagen, Denmark*

10:15 Nanovaccines for cancer therapy: a multistage approach for personalized medicine  
*Flavia Fontana, University of Helsinki, Finland*

10:35 In-situ monitoring of phase transformation and dissolution of drugs in multifunctional wound healing nanofibrous mats  
*Urve Paaver, University of Tartu, Estonia*

10:55 Coffee break

11:15 Nanosafety and potential health risks of nanomaterials  
*Helene Stockman-Juvala, Finnish Institute of Occupational Health*

11:45 Polymer-based drug delivery systems  
*Tero Jalkanen, Bayer Oy*

12:00 Silica-composite formulations in controlled drug delivery  
*Mika Jokinen, DelSiTech*

12:15 Hosmed Oy.  
*Ismo Lokinoja*

12:30 Lunch break

13:30 Nanoparticles and interfaces for drug delivery: design and analysis  
*Françoise Winnik, University of Montreal, Canada*

14:20 Nanofibrillar cellulose in controlled drug release formulations  
*Timo Laaksonen, Tampere University of Technology, Finland*

14:55 Engineering anti-cancer immunity using polymeric nanoparticles that transform into synthetic cell surface receptors in response to intracellular pH  
*Bruno de Geest, Ghent University, Belgium*

15:30 Posters & sponsor exhibitions & refreshments

18:00 Symposium dinner at restaurant Hus Lindman
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The traditional gathering for the Physical Pharmacy community has now crystallized into its 28th form, again during the cold winter months here in Finland. The focus for our series of symposia has always been to bring together the different scientific aspects that make up the modern pharmaceutical industry. The symposium of 2017 turns our view on the next frontier – formulations in nanoscale. This is reflected in the multiple lecture and poster abstracts contained in this volume of Polymorfi, ranging from pure formulational aspects, to the various materials that can be used with novel nanoscale fabrication techniques, as well as to issues of safety that have to be closely regarded.

Along with the multitude of abstracts, we have again requested a commentary from a fellow scientist to introduce us to a topic that is rapidly gaining notice through its advanced technological merits. Professor Hongbo Zhang from Åbo Akademi University describes us how microfluidics can currently be applied in the fabrication of drug containing smart formulations and how these can ease the challenges presented by, for example, the high cost and limited availabilities of new APIs.

In keeping with modern trend of miniaturization, this edition of Polymorfi builds upon the layout of its predecessors, but trades the old larger paper format to a more compact size, that is familiar to most of us as the current size of Finnish doctoral theses. The new format should also make the reading of the electronic form far easier on modern tablet devices, foregoing the necessity to zoom in for readability.

We wish all our readers a rewarding experience with the 28th volume of Polymorfi!

Ermei Mäkilä and Henrika Wickström

The Polymorfi editorial team for 2016
From the chairman

Research of nanoscale features in drug delivery systems has demonstrated their potential, for example, in controlled drug release, in the drug penetration through biological barriers, and in boosting the immunoresponse. Many of these applications are facilitated by our ability to modify the physicochemical properties of the particles. On the other hand, these properties are in key role if the toxicity of nanomaterials is considered. In order to understand the physics and chemistry in nanoscale, which in many cases differ from our intuitive understanding, the need for academic and industrial collaboration in research is of the utmost importance. New analytical tools are needed together with new innovative ways to apply these tools.

The Finnish Society of Physical Pharmacy has gathered to its 28th symposium to probe these issues. We have excellent line-up of interesting invited speakers, poster presentations, industrial talks and especially good participation activity. As a long term plan to widen the symposium into a more international event, we have this year adapted a practice from scientific conferences and invited two poster abstract submitters to present their work as an oral contribution instead. The experiment was encouraging and we hope that this would in future gather more people abroad to participate.

I wish you all an interesting symposium, and success in collaborative efforts toward new innovations!

Martti Kaasalainen
Chairman of the Finnish Society of Physical Pharmacy 2016

The Finnish Society of Physical Pharmacy

Members of the Board 2016–2017 and the Organizing Committee of the XXVIII Symposium

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Lecture abstracts
Functional nanomaterials for intracellular interaction to direct cell fate

Ciro Chiappini
Department of Craniofacial Development and Stem Cell Biology, King’s College London, United Kingdom

Direct material interaction within the intracellular space has broad enabling potential across biomedicine, with transformative impact in tissue engineering, precision medicine and fundamental cell and molecular biology. Efficiently accessing and sensing the intracellular milieu with minimal cell perturbation are key aspects of direct intracellular interaction in that they enable detection, study and control of biological processes at the molecular level inside living cells. The development of a versatile platform that can efficiently handle delivery, sensing and collection processes to mediate diagnosis and localised treatment in vivo still represents a major bioengineering challenge. High aspect ratio nanostructures (nanowires/nanoneedles) have recently risen to prominence as an effective approach to safe, minimally invasive access to the intracellular space on the large scale with resolution approaching that of a single cell.

This talk will report on the contribution to direct intracellular interaction of the recent development of porous, biodegradable nanoneedles1 for tissue engineering in vivo2 and molecular diagnostics in clinical tissue samples3. These nanoneedles efficiently (>90%) delivery nucleic acids and nanoparticles to the cytosol2,4. Used in vivo they do not induce detectable cell death, alter tissue architecture or elicit immune response. They mediate in vivo delivery of a VEGF expressing plasmid in vivo that yields local VEGF expression and induces neovascularization, in proof or principle for highly local in situ gene therapy2. A nanoneedle-based sensor3 detects single-cell protease activity to discriminate cancer and healthy clinical samples of esophageal mucosa resections.

The ongoing development of this platform technology involves applications for neural differentiation, cardiac reprogramming, and multimodal spectroscopy analysis of molecular tissue replicas, alongside fundamental investigation of the role of nanoneedles as biophysical cues in epigenetic remodeling.

Figure 1. An array of nanoneedles (green) interacting with a human cell (pink).

References


About the presenter

Dr. Ciro Chiappini is Lecturer in Nanomaterials and Biointerfaces at King’s College London, United Kingdom. He earned a PhD in Biomedical Engineering at the University of Texas at Austin (USA) in 2011. He worked as Newton Fellow and Marie Curie Fellow at Imperial College London until 2016. Dr. Chiappini develops functional interfaces for tissue engineering and precision medicine.
Invited Lecture

In situ amorphisation of poorly water soluble drugs

Petra Priemel

Department of Pharmacy,
University of Copenhagen, Denmark

Poor aqueous solubility is an increasingly common issue encountered during the development of modern small molecule drugs. The conversion from the crystalline state into the amorphous form of a drug improves its dissolution rate and results in a higher apparent solubility than its crystalline counterpart. However, due to the intrinsic instability of the amorphous form recrystallisation will occur during storage and/or dissolution.

A new approach to improving aqueous solubility has been developed in which crystalline drugs are combined with a polymer and amorphised in situ, directly before application. The resulting amorphisation process was observed for combinations of a drug, including indomethacin, naproxen or ibuprofen, together with Eudragit E PO in buffer at a neutral pH. Once the in situ amorphised samples were exposed to an acidic medium drug dissolution was increased in comparison to both a glass solution of drug and polymer and crystalline drug polymer mixtures.

A second method to prepare amorphous formulations in situ using a common household microwave has been explored. Compacts of indomethacin and polyvinylpyrrolidone (PVP) were pre-conditioned at different relative humidities and microwaved using different settings. The content of amorphisation was dependent on the energy input from the microwave and the water content of the compact. In intrinsic dissolution testing the microwaved tablet generated results similar to those of quench cooled glass solution of indomethacin and PVP. Furthermore, the amorphisation of indomethacin was tested with PVP of different molecular weights. This study highlighted the importance of the glass transition temperature of the plasticised polymer and the amount of water the polymer can bind in the formulation during microwaving.

In situ amorphisation avoids stability issues during the storage of the amorphous form as the drug polymer mixture is amorphised directly prior to application. The patient could simply place the compact into water and wait for a short period of time to allow amorphisation to occur or microwave a compact before application of the medicine.

About the presenter

Petra Priemel completed her PhD “Solid state conversions on the surface” at the University of Otago, New Zealand, in 2013. She was supervised by Dr. Clare Strachan, Dr. Holger Grohganz and Prof. Dr. Thomas Rades. She then worked as a Postdoc at the University of Lille II on colonic drug delivery of proteins and at the University of Copenhagen on cutaneous and pulmonary formulation development for antibiotics. Currently she is working on understanding key parameters in the dissolution process of amorphous drugs and their formulations.

Dr. Priemel’s research interests include solid state properties of drugs, formulation development, characterization and evaluation as well as antimicrobial treatment of infections.
The production of different types of engineered nanomaterials is rapidly increasing in all industrial sectors. The use of nanotechnologies brings remarkable possibilities for new innovations and precise applications. In addition to the technological progress, significant amounts of resources have been allocated to research on nanosafety during the last ten years.

The unique dimensions and physico-chemical properties of nanosized materials are crucial for their technological applications, but also for their potential hazardous effects on humans upon exposure. Unintentional exposure may occur during all steps of the life cycle of the material, including production of the raw material, handling and using the nanomaterial in the formulation of products, professional or consumer use of nanoproducts, and in the waste or recirculation phase. In many cases, occupational exposure is, however, the only situation where exposure is likely to occur at significant levels.

At this stage there is no epidemiological data on hazardous effects of nanomaterials. Research on the toxicological properties of nanomaterials has therefore mainly been carried out using in vivo or in vitro models, covering oral, inhalational and dermal exposure to various types of nanomaterials. Due to the very small dimensions of nanomaterials they may behave differently in the body than larger particles of the same material. The behavior is however highly dependent also on other physico-chemical properties than size, for example solubility.

Current data show that there are no common ‘nanospecific’ effects that could be related to all nanomaterials. However, it may generally be concluded that inhalation is the exposure route of highest concern. Due to their small size, nanomaterials may accumulate in the alveoli of the lungs, resulting in an overload situation in which macrophages are not able to work efficiently enough. As a result, persistent inflammation may occur, potentially resulting in genotoxic effects followed by tumor development. There is particular concern that fiber-like materials (e.g., certain types of carbon nanotubes) may cause such effects.

Studies on the dermal penetration of nanomaterials through intact skin indicate that, despite their small size, nanoparticles as such are generally not able to reach the systemic circulation via the skin.

Thousands of different nanomaterials have been synthesized, but only a minority of them are already commercially in use. In any case it is clear that it will not be possible to perform toxicological studies on all new materials. Attempts to identify critical properties related to health hazards and to be able to group materials for risk assessment purposes are therefore going on, for example in large EU-funded research projects.

About the presenter
Helene Stockmann-Juvala has been working with toxicological and risk assessment issues at the Finnish Institute of Occupational Health since 1998. She graduated in 1999 and obtained her PhD (Pharm.) degree in 2007 at the University of Helsinki, Faculty of Pharmacy. Currently she holds a position as Senior Specialist at the Finnish Institute of Occupational Health, focusing on hazard and risk assessment of chemicals, including nanomaterials.
Cells have developed various mechanisms to preserve their homeostasis when exposed to “invading” biological or artificial particles. They use the most suitable modes of particle internalization and elimination, depending on the size, surface, and composition of particles. Routing of particles is carefully regulated through dynamic morphological changes of the plasma membrane and activation of signal transduction pathways. Hence, when designing particles or interfaces for therapeutical applications, it is important to evaluate their interactions with the biological environment in vivo in order to gain a realistic description of their “biological identity”.

Some insight into the in-vitro aggregation status of particles can be gained from the analysis of their size in cell culture media in the presence and absence of serum proteins. Asymmetrical flow field-flow fractionation (AF4) is a mild technique to separate hydrodynamically the constituents of a mixture as they migrate in a thin channel within a parabolic laminar flow profile onto which is applied a secondary perpendicular external field, the cross-flow [1, 2]. As a consequence of the balance between forces imposed by the two flows, particles are separated into fractions according to their hydrodynamic size, with smaller particles eluting before larger ones. It is versatile in terms of the analyte size range, from a few nanometers to microns, and readily allows quantitative analysis of nanoparticles in the presence of soluble macromolecules, as shown in the fractogram of a suspension of CdSe/ZnS quantum dots (QDs) in cell culture media (DMEM) containing foetal bovine serum (FBS) (Figure 1) [3].

AF4 is useful also to evaluate the stability of drug formulations, as demonstrated in a study of polymer/antibody fragments complexes [4].

Biomaterials implants also alter their identity upon contact with biological media, such as blood in the case of stents or catheters. Surface analysis techniques, such as atomic force microscopy (AFM) examination, zeta-potential measurements, surface plasmon resonance (SPR) spectroscopy, and quartz crystal microbalance with dissipation (QCM-D) measurements, can be used in order to determine important mechanical cues, such as surface topography, roughness, charge, and rheology, known to influence the adhesion of proteins and cells on a substrate. This approach was described in an assessment of substrates coated with the modified polysaccharide phosphorylcholine-chitosan (CH-PC)
Figure 2. Chemical structure of chitosan-phosphorylcholine (CH-PC) and fibrinogen adsorption on CH-PC substrates.

consisting of bioadhesive chitosan grafted with non-fouling phosphorylcholine groups (Figure 2) [5, 6]

References


About the presenter

Françoise M. Winnik obtained her PhD from the University of Toronto. She worked as a research scientist in the Xerox Research Center of Canada, before joining McMaster U (Hamilton ON) in 1993 as an Associate Professor. Since 2000, she is a professor in the Université de Montréal. She currently holds a FiDIPRO position (Tekes) in the University of Helsinki. She is the Editor in Chief of Langmuir, the ACS journal for colloids and interfaces. Her research interests include self-assembly of amphiphilic polymers, nanoparticles and biointerfaces.
Nanofibrillar cellulose in controlled drug release formulations

Timo Laaksonen

Laboratory of Chemistry and Bioengineering, Tampere University of Technology and Division of Pharmaceutical Biosciences, Centre for Drug Research, University of Helsinki, Finland

Different systems with a variety of rate-controlling mechanisms have been developed for controlled release of drugs. To formulate drug delivery systems with desirable mechanical properties and drug releasing profiles, polymers with a broad variety of physicochemical properties are required and constant improvements of the existing materials and the creation of new polymers are needed. Nanofibrillar cellulose (NFC) has attracted a lot of attention recently as a natural alternative for these purposes.

We have evaluated the potential of NFC as a material for controlled drug delivery. For that purpose, drug loaded microparticles and matrix systems (“nanopaper”) with NFC as the matrix former were produced (1,2). Drug loaded films with a matrix structure were produced by a filtration method using nanofibrillar cellulose as a matrix former and poorly water soluble model drugs. Entrapment efficacy of this simple 3-step method was >90% and the final drug loading was in a range of 20-40%. The matrices had excellent mechanical properties and could be easily handled after preparation. Drug release studies showed sustained drug release over periods of up to three months with the drug release kinetics being dependent on the drug used (1).

Another challenge in drug delivery is poor solubility of new drug candidates. Formulating them into nanoparticles has been seen as a way to enhance their poor dissolution rates. Stable storage without losing the benefits of small size is a further challenge in addition to their synthesis. To overcome this, we bound protein coated drug nanoparticles to a NFC network. The matrix protected the particles during storage and formulation processes. Bioavailability of a model drug bound to NFC was found to be excellent (3). Several different types of nanofibrillar cellulose were also tested in order to get better insight on the effect of the cellulose structure on protein binding, aerogel morphology, drug release & stability, and suitability for freeze-drying (4).

Further, NFC was used to stabilize drug-loaded emulsions and to control their drug release rate. Amphiphilic proteins were used to create an emulsion, whose stability was further enhanced by the use of either native or oxidized nanofibrillar cellulose (5). Figure 1 shows NFC stabilized drug-loaded emulsions. (Below) Cryo-TEM and light-microscopy (inset) images.
dized NFC. Both cellulose types were suitable for emulsion formulation and stabilization, but only the oxidized form was suitable for sustained drug release applications. This was due to more favorable protein binding and viscoelastic properties.

In the latest study, NFC hydrogels and freeze-dried aerogels were used as drug releasing matrices. These were evaluated as release matrices for a range of molecules. As expected, the release of large molecules was hindered more than small ones. For example, nadolol was released within 60 h, whereas only 40% of the loaded BSA was released within one week.

References


About the presenter

Timo Laaksonen is acting as an Associate professor at the Chemistry and Advanced Materials Group at Tampere University of Technology. He defended his PhD thesis on physical chemistry of gold nanoparticles from Aalto University in 2007 and completed 2 post-doctoral periods at University of Helsinki, Faculty of Pharmacy, before entering the tenure track at TUT in 2016. His main areas of expertise are physical chemistry, bio- and nano-materials and their use in functional materials and pharmaceutical technology. At TUT he is currently forming a team focusing on the use biomaterials but also on photonic materials and ultrafast spectroscopy. Timo Laaksonen is also a team leader at the Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, where he studies how to create functional (bio)materials for efficient drug release and cell adhesion. He has co-authored 64 publications, with ca. 2300 citations (h-index of 29) an (co-)supervised 6 completed doctoral theses.
Cancer is a leading cause of death and traditional treatment options for cancer patients are prone to causing severe side effects. Recently, immunotherapy – i.e. harnessing the patients’ own immune system at combating cancer - has received major attention. In particular targeting tumours with antibody-based strategies has become increasingly popular. However, this class of therapeutics suffers from exuberant production costs and is prone to inflammatory immune reaction from the host patient. In this project we will develop synthetic polymer-based nanoparticles that recruit endogenous antibodies (i.e. antibodies present in the bloodstream of every individual) to solid tumours to exert subsequent cancer cell killing effects. Utilizing well-defined polymers, self-assembly and efficient ligation chemistry, we will engineer these antibody-recruiting nanoparticles (ARNPs) in such a way that they disassemble after internalization by cancer cells into soluble polymers that contain multiple motifs of a compound that binds to endogenous antibodies through multivalent interaction. Moreover, the polymers also contain a motif that promotes prolonged cell surface expression of these polymers. In other words, upon intracellular unfolding the ARNPs transform into synthetic cell surface receptors that mark cancer cells for recognition and elimination by the immune system.

About the presenter
Prof.dr. Bruno De Geest (°1980) graduated as Chemical Engineer in 2003 from Ghent University where he obtained his PhD in pharmaceutical sciences in 2006 on ‘Polyelectrolyte Multilayer Capsules for Pharmaceutical Applications’. For his PhD work he was awarded the graduate student award for pharmaceutical technology from the AAPS and the Andreas Deleenheer award from Ghent University. After 2 years of postdoctoral research at Utrecht University (The Netherlands) he returned to Ghent University at the Department of Pharmaceutics. From October 2012 onwards he is appointed as professor in Biopharmaceutical Technology. His lab is currently composed of 10 researchers working at the interface between materials chemistry and life sciences with a particular interest in polymer chemistry and immunology. The main focus of his research group is the design of nanoparticulate vaccines and anticancer therapy. Prof.dr. De Geest has published over 120 A1 papers and has an H-index of 33.
The DelSiTech™ Technology – The Natural Solution For Drug Delivery
Nanosized particles have been investigated as drug delivery systems for chemotherapeutics in the treatment of cancer [1]. Recently, the role of the immune system in the fight against cancer has been exploited with the administration of particles as vaccines [2]. The use of nanosized vaccines brings along several advantages: delivery of the antigens to the lymphoid organs thanks to the natural passive targeting, depot formulation of the antigen, possibility of a repetitive display of antigens and adjuvant on the surface [3]. Moreover, nanoparticles can possess intrinsic adjuvant properties [4]. Biologically derived elements like vesicles derived from the cells’ plasma membranes, are currently being investigated as biomimetic camouflages, as drug delivery systems and as innovative source of neoantigens [5].

In this study, adjuvant nanoparticles made of thermally oxidized porous silicon (TOPSi) and acetalated dextran (AcDEX) were prepared by nanoprecipitation in a glass capillary microfluidics device. The particles were then co-extruded with vesicles derived from the membrane of cancer cells (CCM), used as antigenic source, as shown in Figure 1. The nanovaccines (TOPSi@AcDEX@CCM) were highly cytocompatible on established human cell lines of the immune system up to 72 h.

Moreover, we investigated the efficacy of the developed system in increasing the expression of activation markers on both immortalized cells and peripheral blood monocytes (PBMC) isolated from the blood of healthy donors. The nanovaccines showed excellent adjuvant properties, inducing the stimulation of the antigen presenting cells. In addition, after stimulation with the systems, PBMC secreted high amounts of interferon gamma, a key cytokine required in the priming of cytotoxic lymphocytes.

Finally, PBMC activated by incubation with the nanovaccines inhibited the proliferation of the cell line used for the isolation of the cell membrane as antigenic component, demonstrating the efficacy of the nanovaccines in priming a response of the immune system against cancer.

In conclusion, an innovative multistage nanovaccine formulation was developed and evaluated in vitro, showing good cytocompatibility, excellent properties as adjuvant and efficacy against tumor cells.

References

Electrospun (ES) polymeric nanofibers (NF) loaded with drugs can be used as novel drug delivery platforms (DDP) for wound healing therapy or oromucosal drug delivery. The solid state transformation of drugs on the course of delivery and/or during storage can result in significant changes in the performance and bioavailability of a drug [1]. The aim of the present study was to evaluate the chemical and physical solid-state changes of an anti-microbial agent, chloramphenicol (CAM), and anti-inflammatory agent, piroxicam (PRX), loaded in the ES NFs intended for wound healing therapy.

CAM (Sigma-Aldrich C.C., China) was used as an antimicrobial agent in two concentrations 20% and 10%. PRX (Letco Medical, Inc. USA) was used as an anti-inflammatory agent at ratios 1:1 (50%), 2:1 (67%) and 4:1 (80%). With CAM, polyvinylpyrrolidone PVP (Kollidon K90, BASF, Germany) and ethanol (96% w/V) were used as a carrier polymer and solvent, respectively. With PRX, hydroxypropyl methylcellulose HPMC (Methocel K100M premium CR) and 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) (≥ 99.0%) (Sigma-Aldrich C.C.) were used accordingly.

NFs were prepared using an ESR200RD robotized ES system (NanoNC, Korea). X-ray powder diffraction (XRPD) and Raman spectroscopy were used for the physical solid-state analysis. The surface topography of NFs mats was analyzed by scanning electron microscopy (SEM). High-performance liquid chromatography (HPLC) was used to determine the chemical stability of CAM and PRX. Wetting and dissolution of the drug-loaded NFs were monitored by high-resolution optical microscopy (CETI Mag-Text, U.K.) and with Raman spectroscopy. Dissolution rate of CAM and PRX was determined with Sotax AT (Germany) USP basket method. The NFs were analyzed immediately after fabrication (0 day) and after a 3-month storage at low temperature 6±2°C and 0% relative humidity (RH).

The representative SEM images of ES NFs are shown in Fig. 1. All types of ES NFs exhibited very uniform thickness and homogeneity. The XRPD results (Fig. 2) suggest that CAM or PRX incorporated in the ES NFs were in amorphous state and physically stable during the storage of 3 months at low temperature and 0% RH.

The NFs did not show any crystallinity (Fig. 2). The results also suggest that a short-term storage of NF DDPs (up to 3 months) at low temperature and 0% RH did not lead to any decrease in drug concentration in the NFs. Wetting and dissolution tests showed that CAM-PVP NFs dissolve faster than PRX-HPMC NFs.

The change in CAM solid form during NFs dissolution was faster than the transformation of anhydrous amorphous PRX (PRX AM) to PRX monohydrate (PRXMH). The initial phase of wetting (30 sec – 1 min) involves the precipitation of the substance (Fig. 3). It is followed by the rapid dissolution of CAM within 5 min. After that it was not possible to detect any characteristic Raman spectrum (Fig. 4A). The change of the PRX solid-state form occurred within 5 min after wetting.
Crystalline PRXMH was dissolved after the addition of a small amount of purified water (Fig. 4B). As seen in Fig. 3, the wetting, dissolution and crystallization of PRX and the dimensional changes of NFs, can be clearly detected real time by means of high-resolution optical microscopy (Fig. 3).

The present results suggest that poorly water-soluble drugs, CAM and PRX, undergo the solid-state transformation during the release from the polymeric NFs and prior to dissolution. This research work enables us to observe the changes in the solid-state form of poorly water-soluble drugs in NFs when in contact with water and to help us to predict, how these drugs actually are released from NF DDPs when in contact with body fluids.

References


Acknowledgements

This work is financed from the PUT1088 and IUT-34-18 projects. MSc J. Aruväli is thanked for XRPD measurements.
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Poster presentations
Thermosensitivity of electrospun polymer-lipid core-shell fibers

O. Alanen, M. Murtomaa and J. Salonen

Department of Physics and Astronomy, University of Turku, Finland

Controlling the drug release is one of the most interesting issues in the field of biomedicine. Controllable structure is possible to obtain in many different ways [1]. Lately, electrospinning has been used to produce nanoscale materials that can act as drug carriers [2,3]. There have also been several studies of fibers as scaffolds for wound dressings [4,5]. With convenient choices of fiber properties, fibers can be used for controlled drug release. For example, with co- or triaxial fiber, the drug can be packed into the fiber and then release the drug with rate-controlled release. Also zero-order release can be obtained with triaxial fiber.

Electrospinning is a versatile method of producing ultrafine fibers. The diameter of these fibers is usually in nano- and micrometer scale. In electrospinning process, the polymer solution or melt is forced through metallic needle connected to a high voltage. Because of the high electrostatic field present, the solution is charged by induction. When the electrostatic forces toward the collector are greater than the surface tension of the solution, the solution will travel down to the collector.

In this work we introduce thermosensitive coaxial electrospun fibers. The fibers were electrospun by using polyethylene glycol-hydroxypropyl methylcellulose (PEG-HPMC) polymer blend as a shell material and tricaprin as a core material. Ethanol was used as a solvent for both the core and shell materials. For the shell material, some water was added to help HPMC to dissolve. The electrospinning process was noticed to be possible only with certain values of voltage and flow rates. With fluorescein in the core, the coaxial structure of fibers was seen by using fluorescence microscopy. The flow rate ratio was found to affect the diameter ratio of fibers. Furthermore, fibers were heated under microscope and their behavior was observed. The shell of the fibers melted and the core material spread on the microscopic slide. Melting temperature was determined also with differential scanning calorimeter.

References


Biomimetic engineering: designing biocompartmentalized nanoreactors as artificial organelles

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In nature, biological cell is regarded as an intricate microenvironment, in which myriad of enzymes work together to catalyze concurrently multiple chemical reactions concurrently that are crucial for metabolism and cell functions [1]. Inspired by nature, scientists manifest an increasing interest to mimic these compartmentalized enzyme regulatory mechanisms, not only to understand the biological process of cellular metabolism and functions, but most importantly to design customized biomimetic nanomachines with a tremendous potential in a range of applications from high-value biochemical, pharmaceutics, diagnostics to, biomedicine, smart materials, and synthetic organelles [2]. Therefore, mimicking the chemical transformations exhibited by enzymes in cellular compartments has triggered intensive research interest for creating new dynamic materials with tunable enzyme reactivity.

In this work, we aim to develop a compartmentalized cellular nanoreactor consisting of porous silicon nanoparticles (PSi NPs) entrapped with an enzyme, horseradish peroxidase (HRP), and surface coated with a cancer cell membranes to demonstrate the
design of biomimetic cellular nanoreactors and their impact on improving cellular functions for biomedical applications (Figure 1). The enzyme activities and kinetics analyses showed enhanced substrate affinities and reaction rates compared to free enzymes, suggesting a high catalytic activity of the nanoreactors. The in-vitro cell experiments demonstrated that the nanoreactors are cytocompatible, readily integrated with cells, while maintaining the intactness of the structure and being intracellularly stable. The intracellular activity of the nanoreactors in the stimulated oxidative stress conditions demonstrated the fact that the nanoreactors could significantly reduce the intracellular reactive oxygen species levels and supplement the subcellular organelles functions. Therefore, the developed biomimetic nanoreactors can function as artificial organelles inside cells to counteract the oxidative stress that is involved in an array of human diseases. Overall, the cellular nanoreactor featuring a biocompartment enclosed by a cellular membrane closely resembles nature’s biocompartmentalization opens a new avenue for biomimetic nanoreactor design for the further development of customized biomimetic nanomachines using different cell types.

References


Drug loaded composite scaffold for bone regeneration

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Tissue engineering coupled with drug delivery plays a key novel role in the treatment of various diseases through the combination of materials science, biomedical engineering and pharmaceutical sciences. This can create a useful ground in which the new tissue can be regenerated in the shorter period of time with higher efficiency.

Bone tissue engineering is considered as a novel science, which tries to treat the bone defects [1, 2]. In this study, we have developed composite bone scaffold by joining hydroxyapatite, gelatin and silica, the promising materials in bone tissue engineering with appropriate biocompatibility, osteoconductivity and drug delivery potential. These materials were coupled with conductive polypyrrole polymers, which makes a composite bone scaffold for regeneration medicine. Conductive and non-conductive scaffolds were made by slurry casting method and they were loaded with vancomycin antibiotic (Figure 1). Their characteristics were compared in different experiments in which scaffolds containing polypyrrole illustrates good mechanical properties, higher protein adsorption, promoted biomineralization and higher vancomycin release over long time compare to non-conductive scaffolds. Long time antibiotic release are desirable after implantation surgery, in which preventing infection and probable second surgeries is necessary. Moreover, Osteoblast cells could be perfectly immersed into the gelatin matrix and stay viable during 14 days.

Overall, new conductive composite bone scaffolds were innovated and the attained results strongly verifies the applicability of this conductive scaffolds, which encourages its further development in tissue engineering applications.
Reference


Development and evaluation of nanobiotics in vitro and in vivo

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Aim: This study focuses on the development and investigation of the antibacterial activity of so-called “nanobiotics”, composed of inorganic & organic constructs. For this purpose, the extent of in vitro bacterial growth inhibition caused by the produced nanobiotics is investigated. In addition, Drosophila melanogaster (D.melanogaster) is used as an in vivo animal model. The antibacterial activity of the nanobiotics in the gastro-intestinal (GI) tract of D.melanogaster is explored. The antibacterial activity of the developed nanobiotics was determined from the analysis of the in vitro and in vivo studies.

Methods: The synthesized nanobiotics are composed of cerium oxide (CeO2) nanoparticles as antibacterial and anti-oxidant core, with porous silica (PSiO2) shells and antibacterial polymer chitosan coating, to create CeO2@PSiO2@Chitosan nanocomposites. The designed nanocomposite aims to eliminate the challenges of unstable cerium oxide cores by coating them with porous silica shells. In addition, the porous structure of the shell in the nanocomposite design enables it to be utilized as a drug carrier. By taking advantage of the porous structure of the shell, the anti-bacterial herbal extract, capsaicin, was loaded into the nanocomposites; which was then further coated with a well-known anti-bacterial polymer, chitosan, as capping agent [1]. In the in vitro studies, the extent of time-dependent bacterial growth inhibition of Escherichia coli-top 10 caused by the produced nanobiotics was investigated. Transmission electron microscopy (TEM) images of the nanobiotics treated with bacteria were studied. In the in vivo study, fluorescent dye incorporated nanocomposites were visualized in the intestines of D.melanogaster by a wide-field fluorescence microscope, after oral dosing of D.melanogaster with the fly food that contained varying concentrations of nanocomposites. The flies were fed with this food and incubated at 25°C for 2 hours, 4 hours and 24 hours. Additional test was conducted to understand the antibacterial activity of the nanobiotics in the in vivo animal model. The antibacterial activity of the nanobiotics was studied by using Polymerase chain reaction (PCR). For this technique, 16S ribosomal RNA gene amplicon analysis was used as the primer.

Results: The proposed nanocomposite-based nanobiotics were successfully prepared and characterized. Bacterial growth inhibition results from in vitro tests showed significant growth inhibition by the improved composite design of nanobiotics, especially compared with the pure cerium oxide cores. The rupturing of the bacterial cell membrane and bacterial growth inhibition were observed under TEM after the bacteria were treated with the nanobiotics. The efficient localization of the nanobiotics in the intestines of D.melanogaster was visualized through wide-field fluorescence imaging after feeding the flies with nanocomposite homogenized fly food.

Discussion: The observed growth inhibition results from the in vitro experiments revealed that multiple antibacterial constructs in the designed nanobiotics system helps to improve its antibacterial activity. The visualization of the nanocomposites in the in vivo experiment results suggested the operability of the nanobiotics in the intestines of the in vivo animal model.
Electrospraying and electrospinning are important top-down electrohydrodynamic (EHD) processes in fabricating polymeric nanoscale (or microscale) platforms for pharmaceutical and biomedical applications. It is well-known that various process parameters in these EHD processes, such as electric field strength, flow rate, needle diameter and distance to collector, can significantly affect the formation of final nano- and microparticles/fibers [1,2]. To date, only a limited number of techniques are available to monitor the spraying/spinning step of these processes. Furthermore, only little is known on the behavior and properties of the electro-sprayed/spun droplets/jet and the EHD process performance.

The aim of the present study was to investigate the applicability of diode laser illumination as a novel technique for the high-speed imaging and monitoring of polymer solution droplets/jet produced in an EHD process. Special attention was paid to the effects of spraying/spinning flow rate, and the type and amount of polymer on the velocity and size distribution of droplets/jet.

For EHD nanofabrication, two solutions of polymers were prepared (Table 1). The solution I, was composed of polyvinyl-caprolactam (PCL, Sigma Aldrich, MW 80 000) and poly-vinylpyrrolidone (PVP90, BASF) dissolved in the binary mixture of chloroform and methanol at a volume ratio of 3:1. The solution II was prepared by dissolving PVP90 in methanol. Electrospaying/spinning experiments were carried out using an ESR200RD robotized electrospinning system (NanoNC, Korea). The experiments were carried out at different process conditions (flow rate, voltage) in order to see the effect of these parameters on the processing and the morphology of the final nanomaterials. The distance between the syringe tip and the collector was 10 cm. The droplet velocity and size distribution were measured by a diode laser stroboscope and CCD camera system designed for high speed imaging measurements (HiWatch, Oseir Ltd., Tampere, Finland) (Figure 1). The morphology of the nanostructures was studied by scanning electron microscopy, SEM (NanoSEM 450, FEI Corp., USA).

High-speed imaging of the droplet/jet of the EHD process was successfully carried out with a diode laser illumination technique. Different “operating modes” for the electronozzle was observed with HiWatch high-speed imaging. With the organic solutions of polymers (PCL, PVP), a tiny drop hanging from the

**Table 1. Composition of the polymeric solutions used for the electrohydrodynamic (EHD) nanofabrication experiments.**

<table>
<thead>
<tr>
<th>ID</th>
<th>PCL</th>
<th>PVP90</th>
<th>Chloroform</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>80 mg</td>
<td>24 mg</td>
<td>6 ml</td>
<td>2 ml</td>
</tr>
<tr>
<td>II</td>
<td>0 mg</td>
<td>240 mg</td>
<td>0 ml</td>
<td>5 ml</td>
</tr>
</tbody>
</table>

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**References:**


**High-speed imaging of electrospinning liquid jet and nanofibers by using a diode laser stroboscopy**

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Electrospraying and electrospinning are important top-down electrohydrodynamic (EHD) processes in fabricating polymeric nanoscale (or microscale) platforms for pharmaceutical and biomedical applications. It is well-known that various process parameters in these EHD processes, such as electric field strength, flow rate, needle diameter and distance to collector, can significantly affect the formation of final nano- and microparticles/fibers [1,2]. To date, only a limited number of techniques are available to monitor the spraying/spinning step of these processes. Furthermore, only little is known on the behavior and properties of the electro-sprayed/spun droplets/jet and the EHD process performance.

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High-speed imaging of the droplet/jet of the EHD process was successfully carried out with a diode laser illumination technique. Different “operating modes” for the electronozzle was observed with HiWatch high-speed imaging. With the organic solutions of polymers (PCL, PVP), a tiny drop hanging from the
A nozzle was observed, and the drop ejected relatively stable liquid streams from its surface. The drop form and diameter remained nearly constant, thus indicating that a jet formation was at least nearly continuous.

The jets were of different forms depending on the spraying parameters (Figure 2): (A) continuous, i.e. fibre-like structure; (B) droplet stream; (C) breaking stream, where a continuous jet spontaneously breaks into a scattered droplet spray at almost fixed distance. Continuous streams were found to be either stationary or oscillating in direction.

The two EHD process variables studied (voltage and flow rate) were shown to have a significant influence on the final properties of the nanostructures. As shown in the SEM images (Figure 3), the application of Solution I resulted in nanoparticles. Higher utilized voltage levels raised the nanoparticle density on the collector. Increasing the flow rate in the EHD process obviously magnified the single droplet size. As expected, solution II gave the nanofibers, but with bead-like structured defects. Here, increasing the voltage resulted in a smaller fiber diameter.

In conclusion, diode laser illumination technique can be used in monitoring and diagnostics of the EHD nanofabrication processes. The analysis of the velocity and size of individual droplets is possible but the measurements of continuous jets is more challenging. Different modes of operation in the electrospray/jet formation can be identified. Voltage and flow rate applied in electrospinning have a significant influence on the final properties of the nanostructures.

References

[\textsuperscript{159}Dy] \text{THCPSi} produced at CERN ISOLDE for radiation theranostics

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The study of porous nanoparticles as drug carriers is a growing field in cancer-therapy research. The porous properties of the particle enable anti-cancer drugs to be loaded in the particles and the surface of the particle can be modified with targeting moieties. As the particles are injected into the bloodstream the penetrative capabilities of the particle enable the carried drugs to be targeted precisely to the tumor. This could enable a high treatment efficiency together with a low strain on the surrounding tissue. Our project uses mesoporous silicon nanoparticles (PSi) which are biodegradable, and thus suitable for use in a living body [1]. The PSi structure is produced through electrochemical etching of a multilayer film on a silicon wafer and passivated by thermal hydrocarbonization (THCPSi) [2]. The porous region is then loaded with radioactive nuclei. The nuclide can be chosen not only for imaging purposes but so that its decay properties allow for radiotherapy as well [3]. To load the radioactivity we ion-implanted THCPSi wafers with a radioactive ion beam at ISOLDE at CERN. The chosen radionuclide \textsuperscript{159}Dy with a half-life suitable for long in-vivo tests, was produced through proton-induced spallation of a tantalum target. The irradiated wafers were then post-processed into a particle distribution at University of Helsinki. Prostate xenografts (5x10\textsuperscript{6} PC-3MM2) were injected subcutaneously into the hip of 15 NMRI-Foxn1 nude mice. The [\textsuperscript{159}Dy]THCPSi particles were then injected directly into the tumor. In-vivo stability tests of the particles were performed over three weeks on the tumor-bearing nude mice. The activities of the tumors were measured at even time points and the biodistribution of [\textsuperscript{159}Dy]THCPSi was obtained based on the harvested organs. Autoradiographic studies of histological sections of the tumors were performed with the novel autoradiography unit Beaver\textsuperscript{TM} [4]. Promising results were obtained on the stability of the [\textsuperscript{159}Dy]THCPSi particles inside the tumour and we wish to present these.

References


Acknowledgements

This study was supported by the Financing projects PUT1088P and IUT34-18.

Antibody-nanoparticle conjugation: are they there, or are they not there?

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Nanoparticles have been increasingly used in nanomedicine, especially for targeted drug delivery. The process of coupling inorganic nanoparticles to targeting moieties such as antibodies may be a straightforward process as such, however, to determine the coupling efficiency and the long-term stability of the antibody-nanoparticle construct in different media require laborious and often semi-quantitative approaches. To measure the coupling efficiency, the nanoparticles can be tested with ELISA, but finding the matching secondary antibody can be time-consuming. Another approach includes electron microscopy, where the antibodies are labelled with a small...
gold nanoparticle (1). This approach is easier, but lacks quantitative information.

Fluorescence correlation spectroscopy (FCS) can determine diffusion coefficients, hydrodynamic radii, average concentrations, kinetic chemical reaction rates, and singlet-triplet dynamics. With the appropriate settings, it is possible to study the binding of a fluorescently labelled antibody to a nanoparticle(2). The diffusion time of the free dye, the dye-labelled antibodies, and nanoparticle-coupled antibody with dye can be measured. This approach allows calculating the number of antibodies per nanoparticle. However, the size difference between the nanoparticle and the antibody is a limiting factor for this method. The diffusion signals from smaller nanoparticles (i.e. 60 nm) and from the antibodies can be difficult to separate. However, dual-color fluorescence cross-correlation spectroscopy (FCCS) can circumvent this problem by labelling both, the nanoparticle and the antibody. In this study, mesoporous silica nanoparticles were labelled with fluorescein isothiocyanate (FITC) and the antibody was labelled with Alexa 647 to assure proper discrimination between the two channels. With FCCS it is possible to study both, coupling efficiency and also long-term stability.

References


Preparation of core-shell fibers using coaxial electrospinning and phase separation method

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Introduction: Electrospinning (ES) is a method widely used for producing core-shell structured microfibers and nanofibers. The unique structure can be applied in biomedical applications as well as drug delivery systems (DDS) (1). It is possible to incorporate biologically active substances (APls), growth factors, and living cells within the fibers. To achieve porosity within fibers either porogenic excipients with post-ES modification or phase separation methods are used.

Aim: The aim of this study was to produce porous core-shell nanofibers using coaxial ES method.

Methods: Monoaxial and coaxial ES were performed using automatic ES system ESR200RD. Polycaprolactone (PCL) and polyethylene oxide (PEO) were used as polymers in different solvents and solvent systems (Table 1). Different process and environmental parameters were varied to achieve porosity. Drug release studies were performed with model antibacterial drug chloramphenicol (CAM) loaded fibers using modified dissolution tests. Fiber morphology was evaluated with scanning electron microscopy (SEM). X-ray powder diffractometry (XRDP), Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy were used for the solid state characterization.

Table 1. Used polymers and solvents

<table>
<thead>
<tr>
<th>Polymer (API)</th>
<th>Concentration</th>
<th>Solvent / ratio</th>
<th>Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoaxial electrospinning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEO</td>
<td>3%</td>
<td>H2O</td>
<td>25.2°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18 % RH</td>
</tr>
<tr>
<td>PCL</td>
<td>12.5%</td>
<td>THF:DMSO</td>
<td>9:1</td>
</tr>
<tr>
<td></td>
<td>25.2°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCL+CAM</td>
<td>15% + 4%</td>
<td>THF:DMSO</td>
<td>9:1</td>
</tr>
<tr>
<td></td>
<td>25.2°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>60 % RH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coaxial electrospinning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEO</td>
<td>3% +</td>
<td>H2O</td>
<td>25.2°C</td>
</tr>
<tr>
<td>PEO + CAM</td>
<td>4.096%</td>
<td>THF:DMSO</td>
<td>9:1</td>
</tr>
<tr>
<td>PCL</td>
<td>12.5%</td>
<td>THF:DMSO</td>
<td>9:1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25.2°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>60 % RH</td>
</tr>
</tbody>
</table>

Keys: PEO—Polyethylene oxide; PCL—polycaprolactone; CF—chloroform; DMSO—dimethylsulfoxide; THF—tetrahydrofuran; CAM—chloramphenicol.
Results: Porous core-shell fibers were obtained with co-axial ES (Figure 1). Porosity was achieved with a phase separation method only at high humidity (60% RH). The composition of PEO 3% H2O and PCL 12.5% THF:DMSO was used and drug-loaded nanoporous fibers were successfully prepared at high humidity. Fibers produced by monaxial and coaxial ES showed different drug release profiles. It was confirmed that CAM was in an amorphous state within the fibers using both ES techniques (Figure 2).

Conclusions: Coaxial ES method with a phase separation can be used to produce core-shell fibers at high humidity. Drug incorporation changed the morphology of the fibers but porosity remained.

References:


Acknowledgements

This project is financed from PUT1088P and IUT-34-18 projects. MSc J. Aruväli is thanked for XRPD measurements.

Quantitative analysis of pharmaceutical solid-state forms using low-frequency Raman spectroscopy

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Introduction: Low-frequency Raman spectroscopy is a technique that has recently been applied for solid state studies [1-4]. For pharmaceuticals, it is very important to be able to control and monitor crystallinity, because the solid-state structure can determine the bioavailability, stability, and manufacturing aspects of a drug. The advantage of low-frequency Raman spectroscopy is that it gives additional information on the lattice vibrations of a crystal which cannot be
collected by the conventional, mid-frequency Raman spectroscopy. The resulting distinct spectral features between different solid state forms have been used for characterizing polymorphs of several pharmaceuticals, however, the technique is still not widely used in the field of pharmaceutical research. The purpose of this study is to further evaluate low-frequency Raman spectroscopy for quantitative analysis of complex ternary mixtures, and to compare the results with those obtained using the more conventional mid-frequency analysis.

Methods: Mixtures of three solid state forms of piroxicam (form I, II and monohydrate) were used to calibrate and test quantitative models. The samples were measured with two low-frequency Raman systems, one capable of simultaneous low- and mid-frequency analysis, and a conventional FT-Raman spectrometer.

The first low-frequency Raman spectrometer (LF-785), capable of collecting both low and mid-frequency spectra, was a homebuilt system with 785 nm laser module (Ondax, Inc.) followed by BragGrate bandpass filters (OptiGrate Corp.). Backscattered light from the sample was collected and filtered through a set of volume Bragg gratings (OptiGrate Corp.), focused into an LS 785 spectrograph (Princeton instruments) and dispersed onto a CCD detector (Princeton instruments PIXIS 100 BR CCD). Spectra were collected with 5-7 cm$^{-1}$ resolution, each spectrum consisted of an average of 60 scans each with an integration time of 0.01 s.

The second low-frequency Raman system (LF-830) was a prebuilt 830 nm SureBlockTM XLF-CLM THz-Raman system (Ondax Inc.) with a CCD detector. The spectra were acquired with 2 cm$^{-1}$ resolution, and 60 scans with 1 s integration time were averaged.

The FT-Raman instrument consisted of a multi-Ram FT-Raman spectrometer (Bruker Optik), 1064 nm Nd:YAG laser and D 418 Ge detector. Spectra were collected using 2 cm$^{-1}$ resolution and 32 scans.

Quantitative partial least squares (PLS) models were built using different spectral regions after standard normal variate transformation of the data (SIMCA v13.0.3, Umetrics, Sweden).

Results: Both the low and mid-frequency spectral regions showed clear differences between the different solid-state forms of piroxicam. When combined with multivariate analysis, the amount of each form could be predicted with the models based on all studied spectral regions and Raman setups. When evaluating the data from the LF-785 setup, where all experimental sources of error had been accounted for, it was observed that the signal intensities were higher at the low-frequency range and the quantitative models performed better compared to the mid-frequency fingerprint region. This implies that there is an advantage in using low-frequency over mid-frequency Raman data.

Conclusions: Low-frequency Raman spectroscopy is well-suited for quantitative analysis of multiple solid state forms of piroxicam. It offers the potential advantage of stronger Raman scattering and larger spectral differences compared to mid-frequency Raman spectroscopy. Low-frequency Raman spectroscopy could be a very valuable analytical tool when multiple solid state forms need to be detected, identified and quantified.

References

pH-Degradable Imidazoquinoline-Ligated Nanogels for Lymph Node-Focused Immune Activation

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Efficient delivery of antigens and immune-stimulatory molecules to antigen-presenting cells in the draining lymph nodes is a key requirement for developing the next generation of therapeutic anti-cancer vaccines.[1] Due to their strong cytokine induction towards Th1 and cytotoxic T cell responses, imidazoquinoline-based Toll-like receptor TLR 7/8 agonists (e.g. IMDQ) are of primary interest as adjuvants in cancer immunotherapy.[2] However, in soluble form, they readily diffuse after administration and evoke systemic inflammatory responses that cause dose-limiting toxicity.

For this study 50-nm-sized, pH-degradable nanogels conjugated with the TLR 7/8 agonists IMDQ were designed by precise crosslinking of amphiphilic reactive ester block copolymers based on pentafluorophenyl methacrylate (PFPMA) and tri(ethylene glycol) methyl ether methacrylate (MEO3MA). While the hydrophilic MEO3MA block guarantees nanoparticles stability and tissue mobility, the PFPMA block allows for self-assembly into nanoparticles in polar aprotic solvents (e.g. DMSO) followed by IMDQ ligation and crosslinking with 2,2-bis(aminooxy)propane. The latter installs pH-sensitive ketal moieties, which render the nanogel crosslinks susceptible to acid hydrolysis.[3]

In experiments with in vitro cultured dendritic cells the potency of the TLR7/8 agonist ligated to the nanogels was largely retained. Importantly, in vivo these nanogels were able to focus subsequent immune activation only on the draining lymph nodes whilst dramatically reducing systemic inflammation. Thereby, total draining lymph node cellularity was increased by vast recruitment of antigen presenting cells. Mechanistic studies revealed a prevalent passive diffusion of the nanogels to the draining lymph node. In addition, immunization in mice demonstrated that relative to soluble TLR7/8 agonist, imidazoquinoline-ligated nanogels induce superior antibody and T-cell responses against a tuberculosis antigen.[3] The results show that nano-sized carriers are able to localize immune activation of imidazoquinoline-based Toll-like receptor TLR 7/8 agonists to the draining lymph nodes and, thus, enable their applicability as vaccine adjuvant for immunization.

References


Figure 1. Acid-degradable nanogel particles promote uptake into antigen-presenting cells and localize the immune activation of a covalently linked TLR7/8 ligand to the draining lymph nodes.
Local anesthetic-lidocaine encapsulated chitosan nanoparticles for dermal patch formulations

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Nanoparticles are widely used as drug carriers in pharmaceutical applications. They offer possible solutions to many current problems by means of smaller, lighter, faster and better performing materials, components and systems [1]. Chitosan (CHT) is one of the most commonly used natural biomaterials and it is made by treating the chitin shells of shrimp and other crustaceans. Chitosan nanoparticles as drug carriers have the advantage of providing controlled drug release, they can also aid to improve drug solubility and stability, enhance efficacy, and reduce toxicity. In addition, CHT is highly biocompatible [2]. The aim of this study is to develop a polymer film patch containing lidocaine-loaded chitosan nanoparticles as a local anesthetic formulation.

In this study, the lidocaine nanoparticles were prepared by ionic gelation between positively charged chitosan and negatively charged tripolyphosphate (TPP). The ratio between chitosan and TPP was 1:2. In the preparation process of lidocaine encapsulated chitosan nanoparticles, lidocaine was dissolved in DCM (dichloromethane) and chitosan (1mg/ml) was dissolved in acetate buffer solution (10 mM @ pH 5) separately. Afterwards, the chitosan solution was added to the DCM-lidocaine solution. After homogenization of the obtained emulsion, TPP (3mg/ml) was added under stirring. After 1 hour of stirring, the content of DCM in the lidocaine encapsulated chitosan-TPP particles was evaporated by heating the sample (36°C). The obtained sample was lyophilized. Nanoparticles were characterized by using electron microscopy imaging, dynamic light scattering and X-ray diffraction analysis.

The lidocaine encapsulated chitosan nanoparticles were incorporated into bi-layered polymer film patches. In this study, the first layer of the patch was composed of 15% hydroxypropyl methylcellulose (HPMC) mixed with 5% glycerol as plasticizer. Lidocaine encapsulated nanoparticles were incorporated into the first film layer. Second layer of the bi-layered film was composed of 15% hydroxypropyl cellulose (HPC) and 5% microcrystalline cellulose (MCC) as a filling material. Thickness of HPMC film was 200 µm and HPC film was 500 µm. Franz diffusion cell system was used to investigate the drug release from the obtained patches. Films were characterized with scanning electron microscopy (SEM) and scanning white light interferometry (SWLI).

Nanoparticle-incorporated patches have provided three times more drug incorporation compared to only lidocaine incorporated patch formulation. The nanoparticle-incorporated patch formulation has provided sustained released profile. In addition, flux of lidocaine from films was compared by using Franz cell diffusion, and it shows that the flux is higher when the formulation contains nanoparticles.

References

3D printed biofunctional scaffolds integrated with smart drug delivery for muscle tissue engineering

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The traditional treatments of dysfunctional tissue or organs have been surgical treatments with organ transplantations or artificial organs. Even though the techniques have been advancing in the past decades, the challenges still remain in terms of biocompatibility, biofunctionality, immune rejection and shortage of the donated organs. Stem cell-based therapies has been rapidly advancing in the last 15 years, and they are among the most promising approaches for treatment of diseases and for replacing damaged or lost tissue for successful regeneration such as heart, neuronal, vascular and muscle tissues [1,2]. However, stem cell therapies still face challenges in low survival, migration and control of cell differentiation.

Stem cells are precursor cells with the ability of self-renewal and differentiation features, and they participate in tissue and organ development and maintenance. Stem cell renewal/differentiation decisions must be controlled, and the Notch signaling pathway plays a crucial role in stem cell decision making. Notch signaling should be active for stem cell renewal, and inactive for differentiation [3].

The project aims to develop novel biocompatible 3D printed scaffolds integrated with Notch signaling modulator nanocarriers [4,5,6,7] for achieving controlled stem cell differentiation in a muscle tissue model. Collagen coated PLA (poly-lactic acid) and nanofibrillar cellulose were used as scaffold materials and mesoporous silica nanoparticles (MSNs) were used as nanocarriers for Notch modulators. Hybrid scaffolds (nanoparticles and biopolymers) were prepared with a) pre-formed 3D scaffold printed with MakerBot© with post-printing incorporated nanoparticles; b) nanoparticles incorporated in the polymer filaments prior to 3D printing with BioBot©. Biocompatibility and functionality of scaffolds and nanoparticles were investigated for promotion of directed growth, cell attachment and alignment.

Cellular uptake and toxicity of different size and surface functionalized of MSNs; softness of nanocarriers incorporated hydrogel; adherence, viability and differentiation of stem cells with hybrid scaffolds prepared with the first method were evaluated to date. Early results show promise for tissue formation in the hybrid scaffold with collagen and PLA. Nevertheless, biocompatibility of nanofibrillar cellulose for muscle tissue and proper design and porosity of hybrid scaffold for cell growth and control of differentiation with Notch signaling still required to be investigated prior to in vivo studies. The prospective results will provide the basis for a new generation of biomaterials for stem cell-based regenerative therapies based on rational design.

References

The effect of co-amorphization on the ability of amino acids to stabilize the supersaturation of indomethacin.

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Majority of the newly discovered drug candidates are considered as poorly soluble, which has raised an interest towards formulation strategies that enhance the dissolution properties of those drugs. One such strategy is to formulate the drug in an amorphous form, which, due to the higher internal energy, often possesses increased apparent solubility and dissolution rate, but also poor physical stability. To overcome the stability issues, co-amorphous mixtures of drugs and other small molecular weight compounds have been introduced [1].

In the present study, we investigated the ability of arginine (ARG), phenylalanine (PHE) and tryptophan (TRP) to stabilize the supersaturation of indomethacin (IND) either when the amino acids and IND were formulated as cryo-milled binary co-amorphous mixtures or when the amino acids were freely in solution. To study these properties, dissolution and precipitation tests were performed in buffer solutions (FeSSIF blank (pH 5.0) and FaSSIF blank (6.5)), and in fasted and fed state simulated intestinal fluids (FaSSIF (pH 6.5) and FeSSIF (pH 5.0), respectively). The influence of amino acids on IND precipitation was evaluated with excipient gain factor (EGF).

The EGFs obtained from the dissolution studies with amorphous IND and co-amorphous mixtures (Figure 1) provide that ARG had the most significant effect on the dissolution of IND, which can be mainly attributed to salt formation between acidic drug and basic amino acid. In some media, statistically significant differences between co-amorphous mixtures and amorphous IND were observed also in the presence of TRP and PHE, but their effect was much weaker when compared to ARG, which may result from the lack of strong intermolecular interactions. However, in precipitation studies, these amino acids seemed to have no effect on the IND precipitation, and also ARG delayed precipitation only in FaSSIF blank and FaSSIF.

As a conclusion, it seems that strong intermolecular interactions enhance the dissolution properties of co-amorphous drug-amino acid mixtures. However, because of the previously observed increased physical stability and slight improvement in dissolution properties also with weakly interacting amino acids, the strong interactions may not be an absolute prerequisite for a successful co-amorphous formulation.

References


Figure 1. The excipient gain factors of mixtures of arginine (ARG), phenylalanine (PHE) or tryptophan (TRP) and indomethacin (IND) obtained from dissolution studies with co-amorphous mixtures (DISSO) or from precipitation studies (PRECIP) in different media. Statistical significance is marked with an asterisk.

Physical characterization of electrospun gelatin fiber substrates in the inkjet-printed drug formulations

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1. Institute of Pharmacy, University of Tartu, Estonia
2. Pharmaceutical Sciences Laboratory, Åbo Akademi University, Finland

Solid dosage forms with high dosing accuracy and flexible properties can be fabricated by printing technology [1]. In inkjet-printed drug formulations the drug-containing ink is deposited on a carrier matrix (substrate) and dried. Different biocompatible materials, including electrospun fiber scaffolds, with suitable physical and mechanical properties can be used as substrates for inkjet printing [2,3]. In this study, the aim was to characterize the mechanical properties of electrospun gelatin fiber substrates and evaluate their applicability in the inkjet-printed drug formulations.

The gelatin nano- and microfibers were prepared by electrospinning and thermally crosslinking [2]. Furthermore, piroxicam (PRX) was incorporated into the gelatin microfibers as a model drug compound during electrospinning for producing a drug-loaded fibrous substrate. The printed dosage forms were prepared by depositing lidocaine hydrochloride (LH) on the electrospun fiber mats by piezoelectric inkjet printing (PixDro LP50, Roth&Rau, the Netherlands).

Scanning electron microscopy (SEM) (Zeiss EVO® MA 15, Germany) was exploited to visualize the prepared substrates and printed dosage forms and an ImageJ 1.49V software (National Institute of Health, U.S.) was used to measure the fiber diameters from the SEM images (n = 100). The mechanical properties were studied with a TA.XTplus Texture Analyzer (Stable Micro Systems Ltd., UK) by two methods – puncture and tensile testing. In addition, moisture uptake was studied at relative humidity (RH) of 70% and wetting of substrates was determined by contact angle measurements (Model 100 contact angle goniometer, Ramé-Hart instrument co., US).

The diameter of the gelatin fibers was determined based on the SEM images (Table 1).

The determination of moisture uptake and wetting allowed to evaluate the suitability of the electrospun gelatin substrates for inkjet printing. The moisture uptake of the crosslinked nanofibers and microfibers was 12.8 ± 1.9% and 6.0 ± 1.0%, respectively. Visual inspection showed that the crosslinked nanofiber mats could maintain its original appearance, whereas others were prone to contraction at high humidity. The contact angle of the ink solution on the hydrophilic gelatin substrates was comparable to the reference copy paper. The moisture uptake and wetting of the substrates was dependent on the fiber diameter and the packing density of the fibers within the substrate.

The physical properties, including mechanical strength, elongation and elasticity, of the electrospun gelatin fibers were improved by crosslinking (Figure 1). The addition of PRX into the microfibers decreased the puncture strength and elongation (%) at break; however, it had no remarkable influence on the tensile strength and elastic properties. In addition, the puncture elongation (%) of the crosslinked substrates was noticeably increased after wetting the substrates with water.

Both nano- and microfibrous substrates were suitable for inkjet printing. The LH ink was deposited on the printed area with well-defined edges, suggesting that the wettability and liquid uptake of the substrates was suitable for inkjet printing. The contact angle of water was decreased on the inkjet-printed surfaces, suggesting a high suitability for oral administration.

Table 1. Diameter of the electrospun gelatin fibers after cross-linking (mean ± SD).

<table>
<thead>
<tr>
<th>Fiber diameter (nm)</th>
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<tbody>
<tr>
<td>Nanofibrous substrate</td>
</tr>
<tr>
<td>Microfibrous substrate</td>
</tr>
<tr>
<td>Microfibrous substrate with piroxicam (PRX)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>607.8 ± 79.2</td>
</tr>
<tr>
<td>1186.3 ± 225.7</td>
</tr>
<tr>
<td>1301.1 ± 216.9</td>
</tr>
</tbody>
</table>

Figure 1. Puncture strength (mN/mm2) of gelatin nanofibrous (NF), microfibrous (MF) and piroxicam-loaded (MF-PRX) substrates before and after inkjet printing (n = 3).
Also, the printing of LH on the crosslinked fiber mats increased the puncture elongation (%) of the formulations.

The mechanical properties were highly affected by the randomness of the fiber alignment within the electrospun substrates. Nevertheless, the mechanical properties of the electrospun gelatin substrates seemed to be comparable to many oromucosal film formulations [3].

In conclusion, the wettability and mechanical strength of the substrates was dependent on structure of the fiber matrices. Furthermore, the properties of the inkjet-printed drug formulations were comparable to the initial substrates. Thus, the electrospun gelatin fibers showed a good applicability for inkjet printing after crosslinking.

This work is supported by the NordForsk, the PUT1088 project and IUT-34-18 project. Mirja Palo is thankful for the Finnish Cultural Foundation for financial support. PhD Urve Paaver, MSc Kristian Semjonov (Institute of Pharmacy, University of Tartu) and Prof. Kalle Kirsimäe (Institute of Ecology and Earth Sciences, University of Tartu) are thanked for conducting the SEM imaging.

References


Development of Porous Electrospin Fibers for Tunable Drug Delivery

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Introduction: Electrospinning (ES) is a straightforward method for producing ultrafine fibers with micro- to nanometer range diameters and with desirable surface morphology. Over the past few years drug-loaded nanofibers have been prepared as drug delivery systems where the poorly soluble active pharmaceutical ingredients (APIs) are incorporated. Electrospin fibers with nanopores have been prepared using thermally induced phase separation, selective removal of polymer blends and the breath figure process [1] However, to understand the benefits of tunable drug delivery systems, a deeper insight into the phase behavior of the polymer and solvent systems under different process and environmental conditions and in the presence of an API during ES is needed.

Purpose: The main aim of the study was to develop nanoporous biodegradable polycaprolactone (PCL) fibers for tunable drug delivery. Specific aim was to understand the effect of polymer concentration and voltage, humidity and the presence of model antibacterial drug substance on the pore formation.

Materials and Methods: Biodegradable PCL (MW 80 000; Sigma) was used as a polymer for fiber formation. Different material (polymer concentration, solvent mixtures), process (voltage:11, 13, 15 kV) as well as environmental (relative humidity, RH: 19, 30, 65%) parameters were varied in order to produce porous fibers. ES was conducted at room temperature (21±2°C). Table 1 shows different polymer concentrations and solvent systems tested. Model drug substance was chloramphenicol (CAM; Sigma). The PCL system which gave nanoporous fibers was tested together with

Table 1. Polymer and solvent systems

<table>
<thead>
<tr>
<th>Polymer (PCL)</th>
<th>Solvent system</th>
<th>Solvent ratio, % w/v</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5</td>
<td>CF:DMSO</td>
<td>90:10</td>
</tr>
<tr>
<td>15</td>
<td>CF:DMSO</td>
<td>90:10</td>
</tr>
<tr>
<td>12.5</td>
<td>CF:DMSO</td>
<td>80:20</td>
</tr>
<tr>
<td>15</td>
<td>CF:DMSO</td>
<td>80:20</td>
</tr>
<tr>
<td>2</td>
<td>ACE:DCM</td>
<td>50:50</td>
</tr>
<tr>
<td>5</td>
<td>ACE:DCM</td>
<td>50:50</td>
</tr>
<tr>
<td>12.5</td>
<td>ACE:DCM</td>
<td>50:50</td>
</tr>
<tr>
<td>15</td>
<td>ACE:DCM</td>
<td>50:50</td>
</tr>
<tr>
<td>12.5</td>
<td>THF:DMSO</td>
<td>90:10</td>
</tr>
<tr>
<td>15*</td>
<td>THF:DMSO</td>
<td>90:10</td>
</tr>
</tbody>
</table>

Keys: CF- chloroform; DMSO- dimethyl sulfoxide; ACE- acetone; DCM- dichloromethane; THF- tetrahydrofuran; *- selected solvent system and PCL concentration for further studies with CAM.
an API in different concentrations (4 vs 20%). ES was performed using an ESR200RD robotized ES system. Scanning electron microscopy (SEM) and optical microscopy were used for analysing the morphology and diameter of the prepared fibers. Image J software was used for analysing the fiber diameter and diameter distribution. X-ray powder diffractometry (XRPD), attenuated total reflection Fourier transform infrared (ATR-FTIR) and Raman spectroscopy were used for the solid state characterisation.

Results and discussion: Porous microfibers were obtained with PCL and different binary solvent systems. Largest fiber diameters were obtained with THF:DMSO system (2.0±0.3), CF:DMSO and ACE:DCM systems provided similar diameters (average 1.3±0.5). Higher polymer concentration resulted in higher viscosities and generally increased the diameter of the prepared fibers. Lower PCL concentration resulted in electrospraying instead of ES. No clear correlations were obtained between the fiber diameter and applied voltage. Humidity was the main parameter affecting the pore formation and increasing the humidity enhanced the creation of nanopores (Figure 1). Incorporation of an antibacterial API CAM changed the ES process as well as pore formation. However, the nanopores were still observed on the fibers (Figure 2). Higher concentration of the CAM (20%) produced fewer pores and pores lost their spherical morphology compared to polymeric fibers or 4% CAM drug-loaded fibers. The solid state characterization of the API with XRPD showed that CAM was in an amorphous state within the fibers hence their physical stability needs to be investigated further. The amorphous state was also confirmed by other techniques.

Conclusions: Nanoporous biodegradable PCL microfibers are obtained using THF:DMSO binary solvent system and high relative humidity. The effect of humidity has the largest effect of pore formation on single fibers within the tested systems. Present porous drug-loaded fibers will be further studied for the drug release kinetics and antibacterial efficacy.

<table>
<thead>
<tr>
<th>PCL Concentration (wt%)</th>
<th>Relative Humidity (65%)</th>
<th>Relative Humidity (30%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 kV</td>
<td>1.5±0.3</td>
<td>1.3±0.5</td>
</tr>
</tbody>
</table>

References


Acknowledgements

This project is financed from PUT1088P and IUT-34-18 projects. MSc J. Aruväli is thanked for XRPD measurements.
Solid dispersions from electrospayed enteric polymer micromatrix particles

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The oral bioavailability of a poorly water-soluble drug is often inadequate for the desired therapeutic effect. The bioavailability can be improved by enhancing the physicochemical properties of the drug (e.g., dissolution rate, permeation across the gastrointestinal tract) [1]. Another approach is drug shielding from the gastric metabolism, and targeted drug release to obtain optimal drug absorption [2]. In this study, a poorly water-soluble model drug griseofulvin was encapsulated as disordered solid dispersions into Eudragit® L 100-55 enteric polymer micromatrix particles, which were produced by electrospaying. Similar micromatrix particles were also produced, with griseofulvin-loaded thermally oxidized mesoporous silicon (TOPSi) nanoparticles dispersed to the polymer micromatrices. The in vitro drug dissolution at pH 1.2 and 6.8, and permeation at pH 7.4 across Caco-2/HT29 cell monolayers from the micromatrix particles were investigated [3]. The micromatrix particles were found to be gastro-resistant, while at pH 6.8 the griseofulvin was released very rapidly in a fast dissolving form. Compared to free griseofulvin, the griseofulvin permeability across the intestinal cell monolayers was greatly improved, particularly for the TOPSi doped micromatrix particles. The griseofulvin solid dispersions were stable during a storage for 6 months at accelerated conditions. Overall, the method developed here could prove as a useful oral drug delivery solution for improving the bioavailability of poorly water-soluble or otherwise problematic drugs.

References


Influence of relative humidity on the electrostatic charging of lactose powder mixed with salbutamol sulphate

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Electrostatic charging, or triboelectrification, of powders has a significant effect on pharmaceutical industry. The charging arises from powder–powder and powder–surface contacts. The packing behaviour, flowability, and manufacturing of the powders, among other things, may be hampered as a result. In dry powder inhalers (DPIs), the drug is mixed with an additive powder, for instance with lactose. Frictional contacts with these two unlike powders cause bipolar charging which can lead to different problems. The oppositely charged drug particles may adhere on lactose particles and end up in the throat instead of lungs. Moreover, if electrostatic separation occurs during the transportation, mixing, and handling, the concentrations of the drug–additive mixtures may considerably differ from the planned concentrations. The charging may be reduced, for example, by increasing humidity. However, the powders used in pharmaceutical industry are often sensitive to moisture.

Figure 1. The measured griseofulvin dissolution profiles, with a transition from pH 1.2 to pH 6.8 at 120 minutes.
In this study, electrostatic charging of lactose and its mixtures with salbutamol sulphate (SS) were studied as a function of relative humidity (RH). Powder adhesion on a steel pipe surface was also investigated. The powders were charged by sliding them in a steel pipe. Increase in RH decreased the charging of lactose and mixtures, but the effect on SS was not evident. Furthermore, the charge of the mixtures was reversed from negative to positive as RH was increased and remained positive as the samples were again dried. Humidification also changed the adhesion behavior of the mixtures onto the pipe surface.

Modulating the Design of Mesoporous Silica Nanoparticles to Enhance the Antibacterial Activity

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The bacterial resistance to antibacterial drugs has been attempted to be overcome by discovering new antibiotics and chemically modifying existing antimicrobial drugs. Still, there is no assurance that the new drug developments can catch up to the pathogen’s fast and frequent development of resistance in a timely manner. One of the most promising strategies to address this challenge is utilizing antibacterial nanomaterials to combat the resistance. Among the developing applications of antibacterial nanostructured materials, mesoporous silica particles (MSPs) can be highly feasible. This is due to their flexible and versatile design options, which could in this context be beneficial to employ MSPs themselves as antibacterial agents for combating antibacterial resistance and induce synergetic effects together with existing antibiotics and/or utilize them as vehicles to carry antibiotics for delivery to bacteria. In the literature, very little insight regarding antibacterial properties of silica nanoparticles exist to date. Therefore, we aimed to consider a couple of the decisive properties of mesoporous silica nanoparticles: the shape (morphology) effect together with surface coating. As a special focus, we have investigated the effect of MSPs with different aspect ratios and surface coatings to enhance the antibacterial activity; and also aimed to impart synergistic effects to the already existing antibiotic kanamycin which is difficult to be taken up by bacteria. The produced antibacterial agents samples were targeted against several Gram positive and Gram negative bacteria. Our results revealed that the designed MSPs with the highest aspect ratio had the highest interference on the bacterial growth. In order to investigate the inhibition mechanism of the MSPs, the interaction of the nanoparticles with bacterial membranes and DNA has been assessed using various spectroscopic and imaging techniques. Furthermore, the synergistic antibacterial effect of nanoparticles at LD50 dosage in combination with kanamycin against Vibrio Cholerae was also studied. Our experiments here convincingly showed that the bacterial growth can be inhibited by shape engineering of MSPs and further fine-tuning for reaching synergetic effects together with for kanamycin can be provided with the different aspect ratios, and this phenomenon has good antibacterial potential to be utilized in the future. [1]

References

Heart-homing porous silicon nanoparticles for therapy of heart diseases

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Cardiovascular diseases are the leading causes of high morbidity and mortality worldwide. Current therapies have still limited rate of success, therefore novel/modified nanomaterials are emerging as promising alternatives for the treatment of heart diseases. Our lab has shown that porous silicon-based nanoparticles (PSi NPs) are promising biocompatible nanovectors for drug delivery applications [1−4]. Thus, in this work, we have successfully modified PSi NPs with heart-homing peptides to improve the cytocompatibility of the particles in primary cardiomyocytes, non-myocytes, and H9c2 cardiomyoblasts, enhance their cellular uptake, and improve their accumulation in heart tissue in vivo in a myocardium infarcted (MI) rat model.

The PSi NPs were modified with DOTA (D), heart-homing peptides (ANP, P2 and P3) were covalently conjugated to the NPs and, finally, the NPs were radiolabeled. In vitro studies evaluated the cellular viability and interactions. A MI rat model induced by isoprenaline injection was used to study the ¹¹¹In labelled NPs after i.v. administration. The heart accumulation of the NPs was investigated by SPECT/CT (Figure 1).

The safest concentrations of the NPs found in vitro were up to 50 µg/ml. Un-D-ANP NPs were internalized in higher amounts by primary cardiomyocytes, non-myocytes and H9c2 cardiomyoblasts. A competition assay demonstrated that Un-D-ANP NPs were internalized via the guanylate cyclase A receptor in primary cardiomyocytes. Enhanced accumulation of the NPs was observed for all peptide-modified nano-systems in heart up to 3-fold. Peptide-modified PSi NP-to-control ratio in heart for different peptides at 10 min, 20 min, 4 h, and 24 h. Values are represented as mean ± s.d. (n = 2-5).

References


Figure 1. Representative sagittal SPECT/CT images showing the biodistribution of i.v. administered [¹¹¹In]NPs at 10 min timepoint. H (heart), Lu (lung), Li (liver), S (spleen).
Exploring cellulose discs as carriers in oromucosal drug delivery

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2. Laboratory of Fibre and Cellulose Technology, Åbo Akademi University

Introduction: The dissolution of cellulose has gained a lot of attention recently and environmentally friendly water based solvents have been used to produce cellulose-based materials without toxic by-products and residues. This fulfills the modern environmental, health and safety (EHS) regulations and opens new possibilities e.g. among pharmaceutical applications [1]. Cellulose solutions can be transformed into different shapes e.g by means of coagulation process to be utilized in various fields. For instance, spherical cellulose beads have found application in liquid chromatography, blood purification, etc. and have been investigated for pharmaceutical usage [2]. However, the application of cellulose discs (CDs) made of cellulose solution for oral drug delivery has not been studied so far. Therefore, the objectives of this study were to explore the development of CDs by using cellulose solution which was produced using a novel environmentally friendly procedure and subsequently to employ these discs for oromucosal drug delivery.

Methods: As drug loading solutions; there different concentration of Lidocaine Hydrochloride-LiHCl (80 mg/ml, 20 mg/ml and 5 mg/ml) and Triamcinolone acetonide-TAA (2 mg/ml, 1 mg/ml and 0.5 mg/ml) were dissolved in polymer:ethanol solutions. Freely soluble polymers were chosen for incorporation into CDs; PEG 6000 and PEG 400. 6 different ratios of polymers were prepared to determine the right polymer amount to avoid shrinkage and understand the effect of ratios on drug intake and release.

FTIR (UATR Two, Perkin Elmer) and DSC (Q2000, TA instruments) measurements were performed on pure substances, unloaded & loaded cellulose discs, polymer:ethanol solutions, drug loading solutions and physical mixtures of substances to determine the solid state of the incorporated drug in the CDs. In vitro drug release for loaded CDs (bilayered and without backing layer) was performed according to USP paddle method (USP, 35th ed.). The dissolution medium was pH 6.0 phosphate buffer solution. The released amount of drug was determined by UV/Vis spectrophotometer (PerkinElmer, Lambda 25, Germany).

Results & Discussion: Studies showed that 1:1:2, PEG400:PEG 6000:EtOH ratio was able to keep the size of the CDs up to approx. 90%, also desired drug intake and drug release was achieved with that specific ratio for both model drugs. LiHCl and TAA release from discs without backing layer completed in 10 mins and 2h, respectively. Controlled release of APIs was observed from from bilayered CDs.

Conclusions: Coagulation of dissolved cellulose resulting in small-sized flat cellulose discs (CDs) was successfully performed. The study revealed that it was feasible to load the discs with the local anaesthetic Lidocaine HCl and glucocorticoid Triamcinolone acetonide.

References

Figure 1. 1) wet washed water swollen CD, 2) dry CD, 3) dry PG incorporated CD, 4) dry TEA incorporated CD, 5) dry PEG 6000 incorporated CD, 6) dry PEG 400 incorporated CD, 7) dry PEG 400/PEG 6000 incorporated
Interdisciplinary collaboration, especially the merge of nanotechnology with other fields, including biology, pharmacy and medicine is a trend for future research. My research group focuses on multidisciplinary subjects, which interplay between applied physics, materials science, synthetic organic chemistry, engineering, pharmacy and biology.

To efficiently diagnose and treat diseases, we need to understand the detail mechanism of disease occur by qualitatively and quantitatively analyzing the molecular signals in real time, however this is technically challenging. Herein, we aim to utilize nanotechnology to solve the fascinating problems offered by biology through designing and synthesizing of specific nanomaterials and utilizing specific techniques.

DNA nanotechnology is one important material for bridging biology with nanotechnology. As the central genetic molecule in biological systems, DNA possesses many exceptional properties, including biocompatibility, sequence specificity, molecular recognition ability, and nanoscale controllability, which enables the creation of functional DNA nanostructures for far-ranging of real-world applications. Microfluidic technology is revolutionary for material fabrication, high throughput diagnostics and biological analysis. We will collaborate with Prof. David A. Weitz’s group in Harvard University, and develop the droplet based microfluidic techniques for Single Cell RNA-Sequencing. We will also use microfluidic for nano/micro-fabrications and encapsulation. 3D printing technology is very advanced in producing tailor-made biocompatible and biomimetic scaffolds. Different scaffolds will be printed for stimulating wound healing, bone regeneration and used as cardiac stent.

In summary, we will utilize nanotechnology as tools to investigate the real-world fascinating problems in biology and pharmacy, in order to understand the detail mechanism of cell signaling, gene expression and to develop Nanomachines for diagnostics, and Nanomedicine for disease treatments.

Recent Publications

Microfluidic technology manipulates nanoliter liquid in designed micro-channels with precise control, which has emerged as a promising technique for designing all kinds of advanced drug delivery platforms. [1] Microfluidic templated nanoparticles and microparticles hold great advantages in drug delivery applications and have great potential to be commercialized. [1] Microfluidics include the advantages of monodisperse particle control; robust fabrication and good batch to batch, lab to lab reproducibility; flexible in material choice; very high drug loading efficiency; precise particle size and structure control; energy and material saving etc. [2]

1. Microfluidics for pharmaceutical applications

With the development of pharmaceutical science, novel drugs and strategies for effective and targeted drug delivery have become the research focus and trend for developing new generation of pharmaceutics, in order to enhance the drugs’ bioavailability and specificity, reduce adverse drug effect, improve patients’ quality of life during treatment, and achieve personalized medication. [1] Nanoparticles and microparticles have great impact for advanced drug delivery. To achieve industrial standard and reproducible loading and release profile, the control of size and monodisperse of the particles are highly demanded, while may not be feasible with conventional methods. Moreover, for disease like cancer, co-delivery of several drugs, proteins, nuclear acids with distinct chemical/physical properties may be needed, which is also very challenging with bulk methods. Another challenge is the loading efficiency since many cancer drugs, growth factors, siRNA are very expensive and cannot be wasted. Microfluidic systems allow precise mixing of nano-liter and pico-liter volumes of liquid in designed channels, which can fabricate complex drug carriers with precise control on size and composition. Herein, we present the typical microfluidic systems for pharmaceutical applications and we also highlight the future prospective of microfluidics for pharmaceutical research and industry.

1.1 Droplet Based Microfluidics

Droplet based microfluidics are very powerful for fabricating microparticles, microbubbles, and microgels for drug delivery applications. [3] As a subcategory of microfluidics, the immiscible phases of liquids are manipulated to form droplet in micro-channels. When the two immiscible liquids meet, the dragging force from shear stress and the viscous force compete with each other and the drop pinch-off happened when the viscous force is fully conquered. [3] Reynolds number is a parameter that reflects the characterization of fluid flow, which can be calculated as

\[
\text{Re} = \frac{\rho vl}{n}
\]

where, \( \rho \) is the density of the fluid, \( v \) is the velocity of the fluid, \( l \) is the characteristic length of the channel, and \( n \) is the viscosity of the fluid. [4] By adjusting the flow rates, channel diameter, polymer concentration (viscosity), flow regime, the particles size can be controlled and tailored.
to use. In addition, microfluidics can manipulate single emulsion, double emulsion, multiple emulsion, which can load drug or multiple drugs at high dose.

The most popular geometries in droplet based microfluidics are co-flow, flow-focusing and Y-junction or T-junction; and higher order emulsions are made from combination of those basic geometries. [2]

In single emulsion, the inner flow is emulsified into outer flow and form droplets. Single emulsion can be water in oil (W/O) or oil in water (O/W). W/O emulsion is used for encapsulate hydrophilic drugs, especially in the case of bioactive agents that are sensitive to solvent. For example, the hydrophilic polymers including alginate, chitosan, etc were produced as drug loaded microgel, microbubbles and hydrogel microparticles [1] Since the polymer dissolve in water, the polymerization process is mainly achieved with ionic induced, UV light induced cross-linking or other physically gelation methods. O/W emulsion is to emulsify solvent phase to aqueous phase, which are used for encapsulating hydrophobic drugs into biocompatible polymeric matrix to control the drug release. The polymers and hydrophobic drugs are dissolved in volatile solvent and the droplets are solidified through removal of solvent by evaporation or diffusion. By using environmental responsible polymers, trigger drug release can be designed, for example hydroxypropyl methylcellulose acetate succinate (HPMCAS) is pH responsive and controls the drug delivery along gastrointestinal tract in pH responsive manner. [5] Single emulsion has the advantage of easy handling, but it is limited on co-loading both hydrophilic and hydrophobic drugs in the same formulation. To solve this problem, porous nanoparticles are encapsulated in polymer matrix and nano-in-micro platforms are developed. Using O/W emulsion in microfluidics, we have loaded hydrophobic drug in polymer matrix, and co-loaded hydrophilic drug with the support of porous silicon nanoparticles. [5]

By combining two sets of basic geometries in microfluidic device, double emulsion is formed. Double emulsion has three phases (inner phase, middle phase and outer phase) and two rounds of emulsifying process occur in microfluidics to form droplet in droplet. [6] Generally, double emulsion has W/O/W and O/W/O emulsions. [6] In W/O/W emulsion, the inner water phase with hydrophilic drugs is encapsulated in polymer/liposome shell. After solvent removal, the polymer shell will become solid and form hollow shape microparticles. Similar to W/O single emulsion, O/W/O emulsion is mainly used to produce microgel, but containing oil droplet inside a microgel shell, and the shell is solidified through physically gelation methods. With O/W/O emulsions, open-celled porous poly(N-isopropylacrylamide) (PNIPAM) microgel has been production. [1] In double emulsion, the droplet size and shell thickness is mainly controlled by adjusting the flow rates of inner, middle and outer flows. [6] Since double emulsion has two layers, it is more flexible in drugs co-loading and all kinds of nanoparticles encapsulation. With microfluidic double emulsion, we have developed all in one platform for multiple drugs and particles co-delivery. [7]

Multiple emulsions and Janus particles are two types of advanced use of droplet based microfluidics. [2, 8] While, due to the complicity, those particles are mostly developed as proof of concept in bench.

1.2 Self-assembly of nanoparticles in Microfluidics

The development of novel nanoplatform for drug delivery applications are very attractive for pharmaceutical science. The fabrication of monodispersed nanoparticles, especially the fabrication of structured nanocomposites are of great challenge with conventional methods. With microfluidics, two or multiple miscible streams are interfaced and the nanoparticles are formed at the interfacial layer due to nanoprecipitation process. [9] The narrow width of the core flow and fast flow rate of the other flows at small diffusion length scale creates fast mixing and greatly improves the efficiency of nanoprecipitation. The rate of particle formation and monodispersity of the formed nanoparticles are controlled by the diffusive mixing time, which
is determined by geometry of the microfluidic channel, the flow rates, the polymer concentration etc. The fluid flow and mixing patterns are reflected by Re number. The flow behavior can be categorized into laminar flow, vortex and turbulent jet in microfluidics. At low Re number, the flow is mostly laminar flow and with increasing of Re, the flow becomes unstable and changes to turbulent jet. The extremely fast complete mixing (< 0.5 millisecond) can be achieved at very high Re, normally more than 500. [1, 10]

Different types of nanoparticles have been produced with microfluidics. Abhay et al. self-assembled monodisperse liposomes on microfluidic device. [11] Liu et al. had developed a microfluidic platform for which inner flow has higher flow rate than outer flow to create fast vortex (mixing) thus leading to the fabrication of monodispersed nanoparticles. [10]

Microfluidics are also effective in fabricating advanced nanocomposites. The porous silicon nanoparticles were encapsulated with pH responsive polymer to control the drug release. [12] And with continuous two-rounds nanoprecipitation, structured core/shell nanocomposites are developed. [13]

Microfluidic technologies have very high throughput in nanoparticles fabrication. The single nanoprecipitation device and continuous nanoprecipitation device can fabricate 340 and 700 g nanoparticles per day, which can meet the requirement in the pharmaceutical industry. [10, 13]

1.3 Local drug delivery with microfluidic device

Localized drug delivery with long-term and variable dosage control is very attractive for the treatment of many diseases. [1] Under current drug discovery circumstance, a great proportion of drug candidates are very effective through local drug delivery, however they may be ineffective or toxic if delivered systemically. Most of those compounds are discarded from pharmaceutical industry due to the lacking or inconvenience in dosing. If a device can adjustable and precisely delivery those compounds locally, those compounds will become novel drugs, which will greatly reduce the cost of time and money for novel drug discovery process. The precisely control of local drug delivery is very challenging, especially when multiple drugs are dosed. Implantable microfluidic devices can load high quantity of drugs and program the local drug delivery with long-term control. The microfluidic platforms are usually composed of a pump or actuator, a valve, a drug reservoir, and a membrane for controlling the release rate. Those systems can be very simple, for example an ocular drug delivery device was produced by manually compress the drug in the reservoir and the drug release and release rate were controlled by manually pressing the reservoir at different pressure. [14] In advanced case, the reservoir could be sealed by a magnetic responsive iron oxide doped membrane and the drug release was controlled by magnetic field. [15]

2. Future prospectives

Microfluidics have attracted a lot of research interests in pharmaceutical science in recent decades, however the pharmaceutical application of microfluidics is still in its infancy. Microfluidic technology accelerates the development of advanced nano- and micro- platforms for pharmaceutical applications and enables the fabrication of very powerful and complicate platforms for difficult diseases. Microfluidic also opens revenues for industry-led research and commercialization of the nano- and micro-platforms in pharmaceutical industry. [1]

There are some key directions for the future development of microfluidic technologies for industrial applications. First is the amplification of the nano- and micro-platforms to industrial scale in microfluidics. This will involve the development of robust parallel and stackable microfluidic systems. Second, through microfluidics, it is anticipated to construct novel nano- and micro-platforms that are not accessible by conventional methods. Third, to integrate different steps of nanoparticle development into a single system is another attractive application of microfluidic techniques. For example, by assemble several microfluidic devices with different function, a multi-functional microfluidic platform can be
designed to do on chip particles fabrication with real-time feedback control, etc. Finally, the develop of robust and easy carry on microfluidic system can lead to development of commercialized platforms as “nanoparticle synthesis kits” for end users.

Microfluidic technologies have great potential to accelerate the discovery and fabrication of novel nano- and micro-platforms. Among all those techniques, the in vitro screening and fabrication of nanoparticles is supposed to have greatest impact in near future, which may act as new-generation manufacturing technology for US Food and Drug Administration-approved nanoparticles. In addition, due to the flexibility of controlling the nanoparticle size, material of choice, and loading content, microfluidics may also be adopted as a tool for preparing different nanomedicines and theranostic nanoparticles for in vivo applications.

Overall, microfluidics techniques bring exciting opportunities to expand the nanoparticles and microparticles in biomedical applications and advance the clinical translation of nano-and microparticles based therapeutic and theranostic systems.

References

PhD and MSc theses – 2016
Towards Continuous Tablet Manufacturing- Assessment of Some Critical Quality Attributes and Novel Techniques for Quality Monitoring

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Batch processing has been the dominant method for tablet manufacturing, but the first signs of a paradigm shift towards continuous manufacturing, integrated with science-based quality-by-design approach, have appeared so that safe drug products will be able to be produced more efficiently. However, so far, the number of published articles investigating complete continuous manufacturing process is rather low and there is a lack of experience and knowledge about this platform. Before the benefits of continuous manufacturing can be completely achieved, there is a need to improve the current level of process understanding, as well as devising innovative methods for quality monitoring.

This study aimed to provide new insights into continuous tablet manufacturing based on real experimental investigation, and to evaluate the potential of some novel methods for monitoring tablet quality. First, a continuous mixing – direct tablet compression set-up was devised with the goal of producing extended release (ER) tablets. The aim was to improve process understanding with respect to material properties and process variables and to examine how they related to each other during processing. In the second part of the study, an important material property, solid-state behavior, was explored in more detail by investigating an environmentally-induced solid-state transition. The third part of the study evaluated the feasibility of utilizing a multispectral measuring system and terahertz spectroscopy for monitoring tablet quality.

ER matrix tablets were successfully manufactured for the first time with a continuous mixing-direct compression set-up. The properties of these tablets were determined to be strongly affected by both material properties and process variables, and the importance of finding the correct balance between material and process was confirmed. A solid-state transformation from a physical mixture of sample materials (theophylline and nicotinamide) into a cocrystal could be confirmed. The presence of moisture was found to be the pivotal parameter and elevated temperature accelerated the rate of the transformation. Raman spectroscopy combined with principal component analysis was found capable for detecting the transformation as consistently as two reference methods, DSC and XRPD. In the third part, both the methods were confirmed to be capable of monitoring tablet porosity with good accuracy and furthermore, the multispectral measurement system was able to measure the API concentration in the tablet.

In conclusion, good process understanding i.e. the combination of knowledge about both material and process based factors and adequate process monitoring are the guiding lines on the route towards continuous manufacturing. Science-based intelligent processing supervised and controlled by in-line process monitoring equipment will achieve the full potential of continuous manufacturing.

Microscale Freeze-Drying: A Step Toward Rapid Biopharmaceutical Formulation Development

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Biopharmaceuticals such as therapeutic proteins are important drugs that are used clinically in the treatment of severe, potentially life-threatening, medical conditions such as diabetes mellitus, asthma, rheumatoid arthritis, and multiple types of cancer. In order to ensure efficacy, safety, and ultimately the therapeutic success, only pharmaceutical products of the high-
est quality are acceptable. However, biopharmaceuticals are often not stable when prepared as aqueous formulations. Freeze-drying the product is a common strategy in such cases. More specifically, freeze-drying is a gentle downstream processing method to remove the water at a low temperature and increase the stability of the biopharmaceutical formulation. Considering the various biopharmaceutical manufacturing techniques, freeze-drying currently accounts for approx. 17% of the approved biopharmaceutical products.

The development of an optimal biopharmaceutical formulation is a demanding task and often based on the experience of the formulation scientist. In addition, a large scale freeze-drying process may last up to several days and requires protein quantities that cannot be supplied during early-stage development. The developed microscale freeze-drying process has the advantage to be completed within a few hours and requires only minute quantities of a biopharmaceutical. It permits accelerated formulation development and provides scientists with early access to the critical information they need in order to develop a high quality biopharmaceutical product.

I developed a statistical model, PockDrug, which combines three properties (hydrophobicity, geometry and aromaticity) to predict the druggability of protein pockets, with results that are not dependent on the pocket estimation methods. The performance of pockets estimated on apo or holo proteins is better than that previously reported in the literature (Publication I).

PockDrug is made available through a web server, PockDrug-Server (http://pockdrug.rpbs.univ-paris-diderot.fr), which additionally includes many tools for protein pocket analysis and characterization (Publication II).

I developed a customizable computational workflow based on the superimposition of homologous proteins to mine the structural replacements of functional groups in the Protein Data Bank (PDB). Applied to phosphate groups, we identified a surprisingly high number of phosphate non-polar replacements as well as some mechanisms allowing positively charged replacements. In addition, we observed that ligands adopted a U-shape conformation at nucleotide binding pockets across phylogenetically unrelated proteins (Publication III).

I investigated the prevalence of salt bridges at protein-ligand complexes in the PDB for five basic functional groups. The prevalence ranges from around 70% for guanidinium to 16% for tertiary ammonium cations, in this latter case appearing to be connected to a smaller volume available for interacting groups. In the absence of strong carboxylate-mediated salt bridges, the environment around the basic functional groups studied appeared enriched in functional groups with acidic properties such as hydroxyl, phenol groups or water molecules (Publication IV).

I developed a tool that allows the analysis of binding poses obtained by docking. The tool compares a set of docked ligands to a reference bound ligand (may be different molecule) and provides a graphic output that plots the shape overlap and a Jaccard score based on comparison of molecular interaction fingerprints. The tool was applied to analyse the docking poses of active ligands at the orexin-1 and orexin-2 receptors found as a result of a combined virtual and experimental screen (Publication V).

The review of literature focuses on protein-ligand recognition, presenting different concepts and current challenges in drug discovery.

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Development of Computational Methods to Predict Protein Pocket Druggability and Profile Ligands using Structural Data

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This thesis presents the development of computational methods and tools using as input three-dimensional structures data of protein-ligand complexes. The tools are useful to mine, profile and predict data from protein-ligand complexes to improve the modeling and the understanding of the protein-ligand recognition. This thesis is divided into five sub-projects. In addition, unpublished results about positioning water molecules in binding pockets are also presented.
Multi-Approach Design and Fabrication of Hybrid Composites for Drug Delivery and Cancer Therapy

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Drug delivery systems (DDS) have been developed in the last decades to improve the pharmacological properties of free drugs by modifying their pharmacokinetic profile and biodistribution. Major limitations for newly developed drug molecules are the poor water solubility and stability, which can be addressed by DDS. These can protect the drugs from the potentially harsh external conditions found in the biological fluids, and improve their dissolution rate by different strategies, overall increasing the therapeutic activity of the drugs. Additionally, chemotherapeutic agents are nonspecific in nature, leading to deleterious off-target side effects, and poor therapeutic efficacy. Therefore, targeted therapy plays a very important role in cancer treatment, although not without obstacles, since DDS have to overcome a number of biological barriers following their intravenous administration, including renal clearance or opsonization-mediated phagocytosis and efficient extravasation to the tumor.

Mesoporous silicon (PSi) micro- and nanoparticles offer numerous benefits for biomedical applications, in particular for drug delivery. Along with a great biocompatibility and biodegradability, PSi possess mesopores (2-50 nm), where the drugs can be easily loaded and confined in their amorphous state avoiding extensive crystallization, thus, increasing their dissolution rate. However, the release of drugs from this platform is uncontrolled and fast, necessitating the use of strategies to tune the drug release.

In this thesis, multiple approaches were used for the design and fabrication of hybrid composites for drug delivery and cancer therapy, including PSi and polymer-drug conjugate-based DDS produced by different modalities of the microfluidics technology and pH-switch nanoprecipitation. First, the loading and release of drugs with different solubility characteris-

tics from PSi were investigated, and further PSi-lipid and polymer-composites were developed to control the drug release profiles. Overall, it was achieved both a sustained release of hydrophilic and hydrophobic molecules loaded on the PSi and also a reduced initial burst release from the bare PSi particles.

Next, PSi-based nanovectors were envisaged for antitumoral applications. A smart PSi-based hybrid nanocomposite with stealth properties was developed, consisting of a pH-responsive polymeric structure assembled on the surface of drug-loaded PSi nanoparticles. This nanocomposite was extremely efficient avoiding drug release from PSi under physiological conditions, while allowing the release of the drug upon acidification of the medium. Remarkably, the nanocomposites avoided extensive macrophage recognition and phagocytosis.

Thereupon, a tumor targeted theranostic nanoplatform with dual pH- and magnetic-response capacity was designed. The DDS consisted of a polymeric-drug conjugate nanoparticle containing an imaging agent and decorated with a tumor homing peptide for targeted drug delivery, which was successfully applied for intracellular triggered drug release. Overall, the hybrid composites based on PSi and a polymer-drug conjugate represented an advanced contribution to the field of drug delivery and cancer therapy, and in particular to the development of PSi as a platform for advanced drug delivery applications.

Transdermal Iontophoresis Delivery Control by Ion-Exchange Fibers and Nanocarriers

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Biological variation and poor transport efficacy are the major concerns in the development of novel iontophoretic drug delivery systems for the transder-
mal administration of therapeutics. One possibility to overcome these limitations would be to load the drug in interest into a reservoir system such as ion-exchange fibers or nanocarriers prior administration. More precise and homogenous control of drug release and the following transdermal iontophoretic permeation could be obtained as the transdermal device/patch would determine the rate of drug transfer instead of the skin, leading to smaller inter- and intra-subject variability. In addition, the range of molecules delivered by iontophoresis can be expanded as charges could be imparted to neutral drugs by encapsulating them in charged drug carriers. Other benefits raising from such combined systems include enhanced drug transport into or across the skin, improved drug stability and decreased local side effects on skin.

The aim of this thesis was to study in vitro the applicability of systems that combine iontophoresis and either drug-loaded ion-exchange fibers or nanocarriers for the controlled transdermal delivery of therapeutics.

Firstly, drug reservoirs based on cation-exchange fibers were utilized to retard drug release and provide additional control into transdermal transport of a small molecular drug and a peptide. The drug release kinetics could be modified by the choice of the fiber type or the ionic composition of the external solution. The application of pulsed current iontophoresis instead of conventional constant current led to increased transport efficiency of a cationic hydrophobic peptide that has a tendency to adsorb into skin and inhibit electroosmosis as its main transport mechanism. In addition, drug delivery systems combining iontophoresis and nanoencapsulation into polymeric nanoparticles or lipid vesicles for the controlled transdermal delivery of lipophilic or hydrophilic model compound were developed and tested. Although the obtained nanocarriers were considered as suitable for transdermal iontophoretic administration, regarding the colloidal properties, stability and release kinetics, no clear advantage was observed with respect to drug permeation from free drug formulation. Throughout the thesis, the impact of formulation parameters and current type on drug transport efficiency was monitored. Iontophoretic transdermal drug delivery from polymeric nanoparticle-based formulations but not from lipid vesicular nanocarriers was improved by the application of pulsed current.

In conclusion, binding the drug molecules prior iontophoresis into reservoir based on ion-exchange material or nanocarriers is a promising approach to be utilized in controlled transdermal delivery, although the comprehensive evaluation of full potential of such systems tailored for specific drug warrants further investigation in the future.

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**Mesoporous silicon systems for oral protein/peptide-based diabetes mellitus therapy**

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Regardless of the considerable efforts, there have been no major breakthroughs in the development of effective oral protein/peptide delivery. When compared to parenteral administration, oral delivery can significantly improve the patients’ quality of life, especially in chronic conditions, such as diabetes mellitus (DM), which requires multiple injections daily. However, oral absorption of proteins/peptides is severely limited by their physico-chemical properties and various physiological barriers in the gastrointestinal tract.

Porous silicon (PSi) has emerged as a promising drug delivery system, owing to its beneficial properties, such as top-down production, customizable particle and pore morphology, easy surface modification, simple drug loading, biodegradability and biocompatibility. Thus, the aim of this dissertation was to develop a multifunctional PSi based platforms that would be able to overcome the physiological barriers and efficiently deliver insulin and glucagon-like peptide-1 (GLP-1) orally.

First, the influence of different PSi surface chemistries was evaluated on the intestinal transport of insulin. Due to the negatively charged surface of PSi, there was minimal interactions with the intestinal cells. Thus, chitosan, a polycationic mucoadhesive biopolymer with permeation enhancing effect, was used to modify the surface of the PSi microparticles. When comparing different surface modification techniques, chemical conjugation of chitosan to PSi exhibited strongest cellular interaction, and the highest in-
sulin permeation and uptake across the intestinal cell monolayers. Secondly, three different nanoparticles (NPs) were developed based on lipids, polymers and PSi, with and without chitosan coating, and evaluated as potential oral GLP-1 delivery system. The results showed that the chitosan-modified PSi NPs were the most efficient nanosystem with the best loading degree and the highest GLP-1 permeation across the cellular monolayer.

To overcome several physiological barriers, the next step was to develop a multistage nanocomposite comprising of chitosan-conjugated PSi NPs that were coated with a pH responsive polymer, in order to deliver GLP-1 and dipeptidylpeptidase-4 (DPP4) inhibitor simultaneously via the oral route. This multistage nanosystem showed enhanced GLP-1 transport across the intestinal cell monolayers and across the rat intestinal tissue. Furthermore, the nanosystem also demonstrated hypoglycemic effect in vivo after the oral administration in diabetic rats. The efficacy of the nanosystem could be attributed to the combined effect of the permeation enhancing chitosan-modified PSi NPs, the presence of DPP4 inhibitor that prevented GLP-1 degradation, and the pH responsive coating that helped in avoiding premature GLP-1 release/depgradation in the stomach.

Moreover, it was shown that the mucoadhesivity and permeation enhancing ability of chitosan-modified PSi NPs could be significantly increased by further surface modification of NPs with either L-cysteine or cell-penetrating peptide (CPP). It was disclosed that electrostatic interactions between the NPs and the glycocalyx were the most prominent pathway for the transport and uptake of insulin from the NPs, together with the contribution of active transport, adsorptive endocytosis and clathrin-mediated endocytosis.

Overall, advanced PSi-based systems were developed which successfully overcame several limitations associated with the oral delivery of biomacromolecules, and thus, showed high clinical potential as oral protein/peptide delivery systems for DM therapy.

**Toward accurate high-throughput physicochemical profiling using image-based single-particle analysis**

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Key physicochemical properties determining the developability of a drug include solubility, dissolution rate, lipophilicity and pKa. Not only do these properties affect synthesis and solid form optimization, choice of administration route, processability and formulation strategies; they also greatly influence, directly or indirectly, the adsorption, distribution, metabolism, excretion, toxicity and efficacy of drugs. However, miniaturized methods that would enable small-scale determination of these fundamental properties in an accurate and rapid way, are lacking. Image-based microscopy could provide an opportune method for non-specific, rapid and miniaturized applications. First, the applicability of image-based microscopy and single-particle analysis in drug dissolution rate measurement was evaluated. This was done by comparing image analysis data with traditional UV spectrophotometric data of individual dissolving drug pellets. It was found that dissolution rates obtained by image analysis and UV spectrophotometry were practically identical. Next, a single-particle trap flow-through device was developed, wherein it is possible to continuously monitor individual drug particles under constant flow conditions. Based on the promising results of image-based dissolution rate analysis, the possibility of acquiring the intrinsic dissolution rate from individual freely rotating particles, trapped inside the flow through device, was evaluated. It was found that image analysis can be used for rapid real-time determination of intrinsic dissolution rates from continuously changing effective surface areas of dissolving individual micro-particles. The method was then further extended to determine the equilibrium solubility of drugs. Based on the diffusion layer dissolution rate model, solubility is the rate limiting factor of dissolution and can therefore be determined. While
solubility is generally determined from bulk solutions after long incubation times, it was shown that the equilibrium solubility can be rapidly determined from individual pure-substance particles by means of the diffusion layer theory and image analysis. Finally, the single-particle method was further miniaturized and a second device developed, in order to allow imaging of individual powder crystals. It was shown that dissolution rate and solubility can be acquired from individual nanogram crystals. The single-particle method was further extended to acquire pKa, logP and logD of the studied substances, using aqueous buffers, simulated physiological solutions and organic solvents. Using this method and device, it is possible to acquire a complete pH-solubility profile for an unknown material of unknown composition, with individual measurements of less than 30 seconds. In summary, these results strongly suggest that image-based analysis of materials could be applied in high-throughput experimentation (HTE) applications. The possibility of acquiring solubility, dissolution rate, lipophilicity and pKa using a single analytical method, could significantly simplify and speed up accurate data acquisition. This in turn, could lead to faster and more informed decision-making and, ultimately, better and more affordable drugs.

Launching New Products in the Finnish Pharmaceutical Industry: A Relationship Approach

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The pharmaceutical industry has a vital reliance on successful new product launches (NPL), which are a critical driver of a company’s performance. The prevailing literature on NPLs is fragmented and has mainly concentrated on a product’s superiority as well as the strategic and tactical launch activities largely omitting the importance of customer relationships. The aim of this thesis is to provide a comprehensive overview on the key determinants of a successful NPL in the Finnish pharmaceutical industry. In practice, this study considers the extent to which a NPL and getting physicians to prescribe a new drug is relational activity.

The role and relative impact of a company’s strategic orientations and their mediating mechanisms were studied with survey data collected from the pharmaceutical companies operating in Finland. Partial least squares (PLS) path modeling revealed that the relationship orientation had the strongest positive impact on both customer acceptance and financial launch success. The company’s accumulated market-based assets represented an alternative mediator in addition to product advantage. Sales force management and relationship marketing activities transformed a relationship-oriented organizational culture into launch performance. PLS regression modeling combined with target projection identified the diversity of determinants affecting launch performance at different stages of NPL. Product advantage and relationship marketing activities contributed to gaining the acceptance of key opinion leaders in the early phase of launch, while market-based assets and a company’s relationship orientation largely determined the acceptance of the majority of target customers in the later phase.

The buyer’s perspective focused on the physician-pharmaceutical industry relationship and was studied by means of theme-interviews among a randomized sample of physicians. The positive relationship orientation of the physicians towards the pharmaceutical industry and whether they actively interacted with pharmaceutical companies were reflected in their early adoption of new drugs, especially when a product had a unique advantage and the physician’s own personal interest accelerated the adoption of a new drug. In comparison, physicians who were negatively oriented towards the pharmaceutical industry and interacted passively adopted a new drug later based on evidence- and experience-based reasoning and the opinions of colleagues.

In conclusion, this thesis calls for a relationship approach in order to complement the traditional sales and marketing approach regarding the launch of new pharmaceutical products. A successful pharmaceutical product launch should focus on appropriate relationship marketing activities that are conducted in a timely manner to achieve customer acceptance and financial launch performance.
Mesoporous silica nanoparticles as versatile intracellular drug delivery platform

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Mesoporous silica nanoparticles (MSNs) have attracted substantial attention for their application in drug delivery and biomedicine. MSNs have been established as a promising and novel drug delivery vehicle due to their unique structural properties, such as high surface area, large pore volume, tunable pore diameter, and narrow pore size distribution. Furthermore, they provide the possibility to include various surface functions and are biocompatible.

For efficient drug delivery using mesoporous silica nanocarriers, their physicochemical characteristics should be controlled to predict their behavior under physiological conditions. The surface function on the particles determines their fate in the physiological environment. Further, the surface functionalization needs to be tailored according to the cargo molecule to be delivered. In this thesis, various surface functionalization strategies of MSNs employing different polymers and lipids were utilized to fabricate novel drug delivery nanocarriers for hydrophobic and hydrophilic drugs, in order to improve the efficacy of poorly aqueous soluble drugs and to achieve sustained or triggered drug release. Adequate surface functionalizations provide colloidal stability and reduce protein adsorption on the particle surface. By the application of zwitterionic coating on the MSN surface, protein adsorption on the particle surface can be diminished.

For intravenous delivery, first passive targeting (extravasation) of nanoparticles at the tumor site is required and then active targeting to cancer cells using small molecular targeting ligands can be achieved, which provides the advantage of lowering the dose and reducing the side effects imparted on healthy cells. In this thesis, MSNs were designed for active cellular targeting using glucose and folic acid as targeting ligands, and further loaded with anticancer drug molecules. Therapeutic efficacy of the drug molecules were significantly improved using MSNs compared to free drug in vitro and in vivo.

For oral drug delivery, the drug molecule should be protected from degradation in the gastrointestinal (GI) tract and permeability through the mucus layer needed to be improved. In this thesis, MSNs were functionalized by polymeric surface grafts, which has facilitated drug transport through the mucosal barrier and enhanced intestinal cellular internalization. Drug targeting in different parts of the intestine could be tuned by surface modifications, and polyethylene glycosylation (PEGylation) of nanoparticles in combination with polyethylene imine (PEI) as particle surface coating enhanced the internalization of MSNs into intestinal epithelial cells.

For the delivery of hydrophilic anticancer molecules after intravenous administration requires protection from non-specific uptake in healthy cells. In this thesis, hydrophilic molecules were loaded in MSNs, which were further coated with lipid bilayer for intracellular drug delivery. MSNs provided delivery to cancer cells without any observed toxicity to normal cells in vivo.

The thesis reports the importance of a) surface modification needed with respect to the properties of the cargo molecules, and b) appropriate evaluation of biophysicochemical interactions of nanocarriers for their future drug delivery applications. This knowledge can facilitate the development of nanomedicines with desired properties for cancer therapy with reduced side effects.

Physicochemical characteristics of silica nanoparticles tailored for nanomedicine

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ISBN: 978-952-12-3406-4
Silica nanoparticles have broad applications as multifunctional nanoparticle systems in nanomedicine. Silica nanoparticles possess a great potential as drug delivery systems: their small size, unique surface properties and loading capacities make them attractive for drug delivery. Moreover, they can serve simultaneously as diagnostic tools. The attractive combination of drug delivery and simultaneous tracking of nanoparticles is known under the term “theranostics.” Hereby, diagnostic and therapeutic features can be united in the same particle with the controlled synthesis of silica nanoparticles. For successful use of nanoparticles in nanomedicine, their physicochemical characteristics need to be well-controlled to predict their behavior in biological matrices. The size, shape, and surface characteristics of silica nanoparticles define their behavior at the nano-bio interface. In this study, silica nanoparticles were prepared with diverse size, shape, surface, and composition with the focus of endowing the nanoparticles with multifunctionality and facilitating their use in biomedical applications. The dispersion stability of nanoparticles is an important aspect for both diagnosis and therapy applications. In this study, the dispersion stability of silica nanoparticles was evaluated after altering the surface physicochemical characteristics by a surface coating process. The process was mainly achieved by physical adsorption of copolymers composed of polyethylene glycol grafted polyethyleneimine (PEG-PEI) prepared with two different grafting ratios. Then, the emphasis was put on evaluating the colloidal stability and redispersibility of particles in the biologically relevant medium. Differences in redispersibility and dispersion stability of particles were observed by tuning of PEG-PEI composition on the particle surfaces. Furthermore, physiological responses (i.e. protein corona formation) to surface modified silica nanoparticles were investigated. In therapeutic applications, when the nanoparticles are designed as drug carriers, internalization of particles by target cells is aimed in order to exert intracellular therapeutic effects. Enhancement in the cellular internalization of nanoparticles can be facilitated by altering the size, shape and surface properties of the particles. In this thesis, silica nanoparticles with spherical and rod-like shapes, porous, non-porous and hollow structures were prepared on the submicron scale for biomedical purposes. Among these approaches, the extent of nanoparticle internalization was altered by modifying the shape and surface modification by preparing similarly sized spherical and rod-like particles with various net surface charges. The obtained results revealed that the particle shape-induced uptake play a predominant role as compared to surface charge dependent uptake. We could show that particles with a higher aspect ratio were internalized more than their spherical counterparts. The surface charge of the particles remained a secondary regulator to control the internalization of particles. In therapeutic applications, targeted drug delivery is a promising approach which benefits from lower doses and avoiding side effects to healthy cells. By the targeted drug deliver strategy, drugs can be protected in a drug delivery system (DDS), e.g. particulate drug delivery system and cannot freely diffuse, and the uptake of the DDS via specific ligand-receptor interaction, where the targeted receptors are mainly expressed at lesion sites, can be provided. Therefore, specific uptake of the DDS mainly by the injured cells reduces the side effects. In this thesis, mesoporous silica nanoparticles were designed as drug carrier with active cellular targeting capability. Additionally, the particles were loaded with a potential anticancer compound. The effect of the free potential anticancer compound and the silica nanoparticle incorporated compound was tested in vitro. The apoptotic effect of the potential anticancer compound was significantly enhanced compared to free compound with the employed targeted mesoporous silica nanoparticle based DDS. Tracking of silica nanoparticles in the biological environment via different imaging modalities during the delivery of drug is an important feature for multifunctional nanoparticles. This is usually achieved by the incorporation of an imaging probe into the silica network. The detectability of particles can be altered by the structural and morphology of silica nanoparticles, as well as the incorporation strategy of imaging probe into silica network. Furthermore, surface coating of the nanoparticles, leaching of the probe from the silica matrix, and the surrounding pH can affect the detectability significantly. In the present thesis, these phenomena were evaluated in order to clarify their influence on detectability of particles via fluorescent and magnetic resonance imaging methodologies. The thesis addresses the critical steps in the synthesis of silica nanoparticles to be used in theranostic applications. Various methods were explored to obtain tailor-made silica nanoparticles. The work provides deep insight into how the physicochemical properties of silica nanoparticles influence their fate in biological environment and can serve as a guideline to design safe and efficient theranostic systems.
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Empyrean Nano edition

Goniometer based X-ray scattering platform

Nanomaterial analysis on multiple lengths scales

• USAXS
• 1D & 2D SAXS / WAXS
• Bio-SAXS
• Total scattering (PDF analysis)

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