Abschlußcolloquium des SFB 1112

Seminarzentrum der Freien Universität Berlin

Otto-von-Simson-Str. 26, 14195 Berlin

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Zeitplan für das SFB 1112 Abschlusscolloquium (15.06.2018) – Seminarzentrum der FU Berlin, Otto-von-Simson-Str. 26

Beginn	Ende	Vortragende/r	Titel
Sitzung 1: Chair: Burkhard Kleuser			
09:00	9:10	Eckart Rühl	5 Years SFB 1112: Needs and visions
09:10	9:50	Maike Windbergs (U Frankfurt)	Cells and fibers – biological composition of the skin and therapeutic implications
09:50	10:10	Annika Vogt (C04)	Common determinants of nanocarrier penetration and drug release: practical implications for dermatotherapy
10:10	10:30	Alexa Patzelt (C05)	Topical drug delivery in hair follicles
10:30	10:50	Kaffeepause (Foyer)	
Sitzung 2: Chair: Marcelo Calderón			
10:50	11:10	Achim Gruber (C03)	Topical in vivo nanocarrier delivery in mouse models of dermatitis
11:10	11:30	Burkhard Kleuser (Z01)	Interaction of CMS nanocarriers for cutaneous drug delivery and Langerhans cells of the skin
11:30	11:50	Monika Schäfer-Korting (C02)	Beyond skin: Perspectives for 3D in vitro models
11:50	12:05	Christian Hausmann (C02)	Fibroblasts from aged donors...
12:05	12:25	Martina Meinke (B01)	Monitoring and overcoming the skin barrier observed by EPR
12:25	12:40	Pin Dong (B01)	Eudragit pH sensitive nano-particle promoted cutaneous penetration and drug release on skin
12:40	13:40	Mittagspause (Mensa)	
Sitzung 3: Chair: Martina Meinke			
13:40	14:00	Eckart Rühl (B02)	Label-free-spectromicroscopy Approaching the molecular level
14:00	14:20	Ulrike Alexiev (B03)	Innovations from fluorescence microscopy: Cluster-FLIM and single molecule approaches
14:20	14:35	Johannes Stellmacher (B03)	Cluster-FLIM for nanocarrier penetration in oral mucosa and model studies
14:35	14:55	Roland Netz (B04)	Data-based modeling of drug diffusion in healthy and damaged human skin
14:55	15:15	Sven Staufenbiel (A01)	Optimization of nanocrystals, polymeric and lipid nanoparticles for the improved treatment of inflammatory skin diseases

Beginn	Ende	Vortragende/r	Titel
15:15	15:35	Rainer Haag (A02)	Dendritic structures for drug transport in skin and mucus
15:35	15:50	Fatemeh Zabihi (A02)	Synthesis and skin penetration of biodegradable poly (glycerol-co-succinic acid)
15:50	16:10	Kaffeepause (Foyer)	
Sitzung 4: Chair: Ulrike Alexiev			
16:10	16:30	Andreas Lendlein (A03)	Depsipeptides- An innovative nanocarrier system
16:30	16:50	Marcelo Calderòn (A04)	Breaking the barrier - potent anti-inflammatory activity following efficient topical delivery of etanercept or dexamethasone using responsive nanogels
16:50	17:10	Daniel Klinger (A05)	Amphiphilic nanogels as new dermal delivery vehicles
17:10	17:30	Kerstin Danker (C07)	Oral mucosa: Drug delivery by nanocarriers
17:30	18:10	Annette Moter (Charité)	Insights into biofilms: From bench to bedside and back
18:10	18:20	Eckart Rühl	5 Years SFB 1112: Perspectives
18:30	Barbecue, Takustr. 3		

Cells and fibers – biological composition of the skin and therapeutic implications

Maike Windbergs

Institute of Pharmaceutical Technology and Buchmann Institute for Molecular Life Sciences, Goethe University, Frankfurt am Main

The human skin comprises a complex three-dimensional assembly of different cell types, mainly fibroblasts and keratinocytes. The inner structure, mechanical stability and flexibility is based on the extracellular matrix (ECM), an endogenous interconnected fiber network.
The native ECM in the human skin provides a tailor-made environment for cell proliferation and migration as well as for transport of nutrients. From a translational research perspective, two aspects have a high potential:
On the one hand, detailed knowledge about the native ECM can guide development of artificial membranes for advanced in vitro models of the human skin and overcome limitations of generally applied hydrogels for tissue cultivation.
On the other hand, ECM derived data can guide the design of novel skin therapeutics, especially wound dressings. Both aspects can be addressed by polymer fibers generated by electrospinning.
In this context, the talk will present electrospun fibers and their interactions with human skin cells for tissue engineering as well as approaches for using drug-loaded biocompatible wound dressing with controlled drug delivery.

Common determinants of nanocarrier penetration and drug release: practical implications for dermatotherapy

Annika Vogt, Fiorenza Rancan, Janna Frombach, Nadine Döge, Sabrina Hadam, Ulrike Blume-Peytavi

A surplus value of certain nanocarrier types for cutaneous drug delivery has repetitively been suggested, but the field lacks comprehensive studies, which dissect common determinants of penetration and drug release.

Herein, comparison of core-multi-shell-nanocarrier, thermoresponsive nanogels, ethyl cellulose carriers and nanocrystals revealed improved drug penetration in deeper layers of ex vivo human skin. Drug release largely occurred during the stratum corneum (SC) passage. Changes in particle stiffness affected penetration depth, but whether the observed disturbance of SC hydration and lipid organization was a general penetration enhancement effect or specifically attributed to the nanocarriers remains unclear. Interestingly, a surplus value became especially apparent when concentrations lower than clinical standard doses were applied as observed for depsipeptides, but the successful exposure of the skin to higher local drug concentrations occasionally came at the price of irritiation, as demonstrated for dexamethasone nanocrystals and for tacrolimus when applied on barrier-disrupted skin.

High resolution microscopy techniques (STXM, Raman spectromicroscopy, TEM, FLIM, TIRF) and 2PM helped monitor penetration pathways of drugs and nanocarriers across SC and confirmed translocation of low amounts into the viable epidermis predominantly at skin folds and hair follicles. Cellular uptake was increased in impaired skin barrier function and pro-inflammatory environment studied in full-thickness skin and keratinocyte-dendritic cell co-cultures.

Based on those results, we conclude that the investigated nanocarriers enabled modified penetration kinetics and may have dose-sparing effects. Practically, they provide an option for hydrophilic formulations of lipophilic drugs. Physical stratum corneum damage had greater impact on penetration than (bio-)chemical stimuli, but given that chronic skin diseases are typically associated with barrier abnormalities, cytokine increase and immune cell infiltrates, nanocarriers are likely to reach viable cells. This indicates potential for cell targeting, but future chemical compositions also need to consider toxicological and allergological implications.

Topical Drug Delivery in Hair follicles

A. Patzelt, M. Radtke, F. Knorr, S. Jung, R. Netz, J. Lademann

The application of nanocarriers (NC) for transdermal drug transport represents a challenge. NC cannot overcome the intact skin barrier via intercellular penetration but penetrate deeply into the hair follicles (HF). Up to now, the mechanism of the follicular transport of NC has been relatively unknown. First investigations revealed that the follicular penetration pathway seems to be mainly mechanically driven as massage application is mandatory for a deep follicular penetration in ex vivo skin models. Also a size dependency of the NC with regard to the follicular penetration depth has been confirmed experimentally ex vivo and in silico in a two-dimensional stochastic model. The in silico model revealed a ratchet effect for the transport of NC into the HF. In the present investigation we wanted to confirm experimentally the in silico data which indicated that also the massage frequency among other physical parameters influence the follicular penetration depth. The results revealed that the massage frequency has a significant influence on the follicular penetration depth of NC. For the lower massage frequency of 5 Hz, significant deeper follicular penetration depths were confirmed. The knowledge on the mechanism of the follicular penetration process will help to develop NC systems customized for optimized drug delivery via the HF.

Abstract Abschlusskolloquium SFB 1112 – Projekt C03

Topical in Vivo Nanocarrier Delivery in Mouse Models of Dermatitis

C03 tested three of the best characterized nanocarrier systems of the SFB 1112, the first generation hPG-amid-C18-mPEG-core multishell nanocarriers (CMS), the biodegradable hPG-$PCL_{1.1K}$-$mPEG_{2k}$-CMS, and the thermoresponsive dPG_pNIPAM-nanogels (tNG_dPG_pNIPAM) in mouse models of healthy skin, atopic dermatitis and psoriasis. All three nanocarriers penetrated into the stratum corneum but not further when repeatedly applied topically. hPG-amid-C18-mPEG-CMS slightly increased penetration of the hydrophobic model compound nile red into the viable epidermis compared to a cream-formulation. hPG- $PCL_{1.1K}$-$mPEG_{2k}$-CMS, in a hydrophilic gel-formulation, enabled penetration of the hydrophobic and large molecular weight, immunosuppressive drug tacrolimus through the stratum corneum in the atopic dermatitis model. However, the amounts of tacrolimus subsequently found in viable epidermis, dermis, circulation and liver were slightly lower compared to the amounts delivered by the standard cream formulation. Tacrolimus-loaded CMS seems to show clinical efficacy similar to the standard tacrolimus cream formulation. No measurable amounts of tacrolimus loaded to dPG_pNIPAM-nanogels in a hydrophilic formulation penetrated into the viable skin, circulation or liver in our model of atopic dermatitis. Altogether, these results indicate particle potential that may be fortified by improvements in their design or pharmacological formulation.

Interaction of CMS nanocarriers for cutaneous drug delivery and Langerhans cells of the skin

Authors

Alexander Edlich, Pierre Volz, Robert Brodwolf, Michael Unbehauen, Rainer Haag, Ulrike Alexiev and Burkhard Kleuser

Abstract

Owing their unique chemical and physical properties CMS nanocarriers are thought to underlie their exploitable biomedical use for a topical treatment of skin diseases. This highlights the need to consider not only the efficacy of CMS nanocarriers but also the potentially unpredictable and adverse consequences of their exposure thereto. As CMS nanocarriers are able to penetrate into viable layers of normal and stripped human skin *ex vivo* as well as in *in vitro* skin disease models the understanding of nanoparticle crosstalk with components of the immune system requires thorough investigation. Our studies highlight the biocompatible properties of CMS nanocarriers on Langerhans cells of the skin as they did neither induce cytotoxicity and genotoxicity nor cause reactive oxygen species (ROS) or an immunological response. Nevertheless, CMS nanocarriers were efficiently taken up by Langerhans cells via divergent endocytic pathways. Bioimaging of CMS nanocarriers by fluorescence lifetime imaging microscopy (FLIM) and flow cytometry indicated not only a localization within the lysosomes but also an energy-dependent exocytosis of unmodified CMS nanocarriers into the extracellular environment.

Beyond Skin: Perspectives for 3D in vitro Models

Monika Schäfer-Korting and Sarah Hedtrich

Freie Universität Berlin, Institute of Pharmacy, Pharmacology & Toxicology, Berlin

The penetration of nanomaterials as well as their payload can be studied in reconstructed full-thickness skin (RHS) and advanced techniques such as x-ray microscopy (Yamamoto et al, 2016 and 2017) and fluorescence life-time imaging microscopy (Alnasif et al, 2014) at high spatial resolution. Moreover, an increasing number of RHS-derived disease models closely mimicking the disease patterns, indicating their suitability for the investigation of disease pathways and for the preclinical testing of innovative nanocarriers for drug delivery. In fact, reconstructed human organs may allow to overcome the high attrition rate of drug candidates failing in clinical phase II studies (Arrowsmith & Miller 2013). Finally, a growing political and social pressure to develop alternatives to animal models promotes the search for valid, cost-efficient, and easy-to-handle systems, lacking interspecies-related differences.

This presentation gives an overview on disease models and their use for the testing of nanocarrier-enhanced drug delivery by CRC 1114 members (Hönzke et al, 2016; Giulbudagian et al, 2017; Eckl et al, 2014; Witting et al, 2015; Zoschke et al, 2016).

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Fibroblasts from Aged Donors as a Tool to Introduce Patient Diversity into Reconstructed Human Skin

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Elderly patients are heavily underrepresented in early drug development, which might contribute to the high number of failing drug candidates in clinical trials [1]. Human-based test systems, being able to depict diversity, are currently missing. Reconstructed human skin (RHS), resembling aged skin, has been established by several groups, yet all inducing aging artificially [2]. Herein, we compared gene expression of juvenile normal human dermal fibroblasts (NHDF; foreskin, <9 year-old boys) and *in vivo* aged NHDF (breast skin, 60 to 70 year-old women) in monolayer and RHS culture as well as their respective impact on RHS morphology and barrier functionality, while creating juvenile and aged RHS. Gene array analysis revealed striking differences between NHDF monolayer and RHS culture as well as juvenile and aged RHS. Dermal and viable epidermal thickness, collagen I, fibroblast count, and surface pH decreased in aged RHS, whereas *stratum corneum* thickness and matrixmetalloproteinase-1 expression increased. This is well-in accordance to *in vivo* studies [3-7]. The overall increase in barrier lipids of aged RHS might explain the slight decrease of caffeine and testosterone permeation.
Taken together, fibroblasts regulate more than just dermal homeostasis, directly influencing epidermal differentiation as well as barrier functionality.

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Monitoring and overcoming the skin barrier observed by EPR

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Electron paramagnetic resonance (EPR) spectroscopy is a sensitive method for qualitative and quantitative investigations of paramagnetic substances in biological tissue or nanocarriers. EPR can be used for example to identify radicals, which are formed during stress induction in skin tissue, or it can be used to study the fate of spin labels after their application to skin by monitoring their spectral evolution. The line shape gives information about the microenvironment and mobility of the paramagnetic substances. Two applications will be presented here:

1. The spin label TEMPO is amphiphilic and distributes in the lipophilic and hydrophilic parts of the skin after topical application. The partitioning is different for various skin models, diseased and intact skin. This might be related to the thickness of the stratum corneum (SC) but also to the organization and composition of the SC lipids. Therefore, the partitioning differences likely report on the status of the skin's function as a barrier.

2. The drug Dexamethasone (Dx) was covalently labeled with the spin label 3-(Carboxy)-2,2,5,5-tetramethyl-1-pyrrolidinyloxy (PCA) to investigate the penetration efficiency into healthy and barrier disrupted skin for three penetration times of 0.05% DxPCA formulations in aqueous nanocrystal dispersion vs. in base cream. Nanocrystals promote the penetration of DxPCA into the viable epidermis compared to base cream: for intact skin, a 3-fold concentration increase of DxPCA was found, for barrier disrupted skin, a doubling was determined. The analysis of spectral changes shows the dissolution of the nanocrystals and thus confirms the release of DxPCA into the skin.

Abstract for Abschlußkolloquium des SFB 1112

Eudragit® pH sensitive nanoparticle promoted cutaneous penetration and drug release on skin

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Nanoparticles (NPs) have been shown a promising approach to deliver drugs into skin. However, the underlying dynamics of the drug release from NPs, especially, how the physiological changes of diseased skin influence the drug release, remain poorly understood. In this work, we utilized electron paramagnetic resonance (EPR) and confocal laser scanning microscopy (CLSM) to comprehensively investigated the penetration behaviors of probes loaded Eudragit® pH sensitive NPs on both intact and barrier disrupted skin. Using EPR spectroscopy drug release of spin labeled lipophilic dexamethasone (DxPCA) from NPs on *ex vivo* skin could be detected, as well as an improved cutaneous drug penetration was found in comparison to a cream formulation. Compared with intact skin, there was not only twofold higher penetration in deeper skin layers, but also a faster and enhanced drug release on barrier disrupted skin, which was established by partially removing stratum corneum by tape stripping. In accordance, CLSM studies emphasize the enhanced skin penetration of lipophilic probe nile red (NR) loaded to NPs than cream, whose penetration depth into glabrous skin was 160 µm. Moreover, intrafollicular NR related fluorescence indicates the transfollicular penetration of NR from the pH sensitive NPs. In conclusion, the Eudragit® pH sensitive NPs improve the cutaneous penetration and control the drug release of a lipophilic drug, especially on barrier disrupted skin. This may allow targeted delivery to lesional skin, avoiding side effects.

Keywords: pH sensitive nanoparticles, cutaneous penetration and drug release, hair follicle penetration, EPR, CLSM

Label-Free-Spectromicroscopy Approaching the Molecular Level

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Label-free approaches devoted to topical drug delivery of skin by nanocarriers is reported. The aim of these approaches is to increase the spatial resolution below the diffraction limit of optical microscopy and to probe quantitatively the drugs and nanocarriers sensitively by their intrinsic properties without any label in human or murine skin as well as skin models, which were provided by the collaborating partner groups. The methods that have been explored were stimulated Raman microscopy, which is a sensitive label-free technique that was mostly used to probe changes induced by topical drug delivery by nanocarriers in the skin matrix . However, the spatial resolution is diffraction limited. Furthermore, X-ray microscopy has been explored as a label-free exploiting element- and site-selective excitation of *ex vivo* skin samples quantitatively with respect to drug and nanocarrier penetration profiles. The spatial resolution reaches below 50 nm, so that details of the penetration processes become accessible, such as barriers near the stratum corneum, stratum granulosum, and the basement membrane. Finally, photothermal expansion and optical near-field microscopy were employed for probing topically applied drugs in skin samples selectively in the infrared regime. Here, the spatial resolution reaches below 10 nm, so that molecular details of the penetration process become accessible to a significantly higher degree than with the other approaches used in this project. Future work aims to improve these techniques for a quantitative detection allowing for a molecular understanding of drug delivery processes by approaching the molecular level.

Innovations from Fluorescence Microscopy: Cluster-FLIM and Single Molecule Approaches

Ulrike Alexiev (TP B03), Physics Department, Freie Universität Berlin, Berlin, Germany

Fluorescence lifetime imaging microscopy (FLIM) provides new possibilities in the investigation of nanocarrier penetration and drug delivery, as the fluorescence lifetime is highly sensitive to the microenvironment [1,2]. Using Cluster-FLIM, image contrast is enhanced and a clear-cut discrimination between different fluorescent species is feasible [1]. This allows visualization of nanoparticles with an unprecedented precision and sensitivity in skin sections, skin biopsis, living cells and in living tissue [1, 3-5]. The latter is realized in a multiphoton FLIM setup [5]. Moreover, we demonstrate in live-cell experiments fine details of cellular targeting, drug delivery, and endocytosis pathways of a core-multishell nanocarrier.

To further increase resolution and to resolve nanocarrier fine-structure we employed single molecule TIRF microscopy [6]. Using this method we were also able to show the penetration of individual nanoparticles into the viable epidermis, thereby adding new information to the debate of whether nanoparticles can cross the skin barrier [7].

Since nanotoxicity is a major concern in the application of nanoparticles, we recently developed a novel FLIM-based imaging assay (FLIM-ROX) for reactive oxygen species detection. Using FLIM-ROX, we demonstrate the nanotoxicity of subcytotoxic amounts of nanoparticles by linking cellular adverse effects to low-level oxidative stress [5]**.**

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Cluster-FLIM for nanocarrier penetration in oral mucosa and model studies

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The penetration behavior of topically applied drugs in oral mucosa is of great interest for pharmacological purposes. Not only is oral mucosa of immediate importance as target tissue for inflammatory diseases in the mouth, like periodontitis, but since oral veins are drained directly by the jugular vein, oral mucosa additionally gives the opportunity for systemic delivery of drugs while avoiding hepatic metabolism.
Fluorescence lifetime imaging microscopy (FLIM) has been employed to investigate the penetration of a fluorescently tagged core-multishell nanocarrier CMS-ICC and Nanogel-Rhodamine B in intact mucosa as well as mucosa with mechanical impairment of the upper epithelium. The unique fluorescence lifetime signature of the fluorophores were used to identify the conjugates in different tissue layers and discriminate them against the autofluorescent background and even secondary fluorescent staining.

Penetration profiles show an enhanced permeation of the nanocarriers especially in the case of lateral diffusion in barrier disrupted mucosa. Driven by these and previous results on human skin we are now developing a novel approach using a dual-label, consisting of a fluorescent label in conjunction with an EPR probe, for the future use in penetration experiments. With the opportunity to use two independent methods to investigate the same sample we expect to achieve more holistic description and predictions about drug diffusion and biomolecular interactions in tissue penetration.

Data-based modeling of drug diffusion in healthy and damaged human skin

R. Schulz and R. R. Netz

Based on experimental concentration depth profiles of the anti-inflammatory drug dexamethasone in healthy and damaged human skin, we model the time-dependent drug penetration by the 1D general diffusion equation that accounts for spatial variations in the diffusivity and free energy. For this, we numerically invert the diffusion equation and thereby obtain the diffusivity and the free-energy profiles of the drug as a function of skin depth without further model assumptions. As the only input, drug concentration profiles derived from X-ray microscopy at three consecutive times are used. The barrier function of healthy skin relies on the combination of a substantially reduced drug diffusivity in the stratum corneum, dominant at short times, and a pronounced free-energy barrier at the transition from the epidermis to the dermis underneath, which determines the drug distribution in the long-time limit. The resulting free energy profiles for healthy and damaged skin are very similar. In contrast, the diffusivity of damaged skin is 200 times larger in the stratum corneum of damaged skin compared to healthy skin, which explains the mechanism of corrupted skin barrier function.

Optimization of nanocrystals, polymeric and lipid nanoparticles for the improved treatment of inflammatory skin diseases

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Nanocarriers with satisfactory drug loadings within the therapeutic range, with a controlled particle size in the 100 – 800 nm range and with rapid (nanocrystals, amorphous nanoparticles) or extended, pH- or sebum-triggered (nanocapsules, polymer and lipid nanocarriers) release were prepared by physical means.

Nanocrystals increased the saturation solubility of dexamethasone and tacrolimus with factors ranging between 1.2 and 6.6. The increased solubility and enhanced dissolution rate of nanocrystals significantly improved the rate of drug penetration through the skin in comparison to the commercial cream, as assessed by *ex vivo* skin studies. The modulation of drug release from nanocrystals was obtained by their nanoencapsulation with polymers (*e.g.* Eudragit® RS 100) by the solvent evaporation method to combine both high drug loadings and extended release properties. pH-triggered polymeric nanoparticles which release the drug at the pH-threshold of 7.5, were prepared to target inflamed skin and deep hair follicles. Ethyl cellulose nanoparticles dissolved in sebum, thereby the hair follicles could be targeted for drug release, as demonstrated by *ex vivo* skin studies, where drug release within sebum-containing hair follicles was increased in comparison to sebum-reduced porcine ear skin. Lipid nanoparticles were obtained by high shear homogenization or by a membrane emulsification method by mixing various solid and liquid lipids, which allowed good control over particle size and drug release.

These results underline the potential of the various physically prepared nanocarriers for a better treatment of skin diseases by modulating their properties to meet specific therapeutic needs.

Abstract A02- SFB 1112 Abschlusssymposium

Dendritic Structures for Drug Transport in Skin *and Mucus*

In our cooperation with Prof. Dommisch and PD Dr. Danker in the field of Periodontology and mucosal delivery, the CMS nanocarrier showed an increased delivery of spin-labelled dexamethasone in comparison to conventional cream. While this finding is in agreement with previous projects, it emphasizes the major benefit of CMS nanocarriers in the new application field of mucus delivery: constant exposure of the drug formulation to flushing, e.g., salivation in the buccal mucosa, is a big limitation to creams. Here, CMS formulations renders spatial fixation of the drug possible, thus increasing the efficiency of the applied drug. Our group has also developed new chemical compositions of CMS nanocarriers. A variation of the length of the inner, hydrophobic building block showed an influence on the encapsulation and release properties of dexamethasone and tacrolimus. We also included ester bonds between the building blocks, leading to the degradation of CMS nanocarriers to their parent building blocks. Getting a step closer to fully-degradable nanocarriers, we also synthesized a core building block based on ε-caprolactone and glycidol. The talk will show the potential of the CMS nanocarrier as a platform for suiting all needs but with a demand for tailor-made solutions when it comes to specific demands.

Abstract Karolina Walker- SFB 1112 Abschlusssymposium

CMS nanocarriers for the delivery across redox barriers in the skin

The presentation will focus on the latest results of project A02 on redox-sensitive Core-Multishell nanocarriers.
Using sulfur-based responsive units within the inner shell of the nanocarrier, a complementary set of both reduction- and oxidation-sensitive nanocarriers was synthesized. The aim of this project is to utilize redox-triggered release of a fluorescent model drug for the visualization of redox-barriers within the skin and to subsequently approach a targeted delivery of drugs across redox-barriers. The work is performed in close collaboration with project B02 (Rühl), and C04 (Vogt). Preliminary data suggests conflictive behavior of redox CMS: While skin penetration experiments on excised human skin performed by Fiorenza Rancan (C04-Vogt) have shown penetration of reduction-sensitive CMS into the epidermis, X-ray microscopy measurements by the group of Prof. Rühl on Dexamethasone-containing identical CMS point to absence of any CMS on or in skin. This talk will discuss these contrary findings, suggest reasons and will show why the expertise of each discipline is needed to tackle such puzzling results.

BREAKING THE BARRIER - POTENT ANTI-INFLAMMATORY ACTIVITY FOLLOWING EFFICIENT TOPICAL DELIVERY OF ETANERCEPT OR DEXAMETHASONE USING RESPONSIVE NANOGELS

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Dermal drug transport and release using nanocarriers have been seen to be a powerful research tool for the investigation and development of skin disease therapies. However, the skin barrier is a principal obstacle to achieve drug transport and the current methods that improve penetration produce skin disorders leading to potential infections. The environmentally responsive nanogels (NG) technology is shaping up as a good strategy to transport drugs into the skin, for instance, through the hair follicles.[1] Key features such as nanogel size, lipophilicity, volume phase transition temperature, surface modification, targeting ability, etc., have been correlated with the potential of responsive nanocarriers to topically release bioactive molecules.[2]

In such context, we hereby present further developments on temperature- and enzymatic-responsive nanocarriers. To evaluate the potential of the thermoresponsive NGs for the loading and delivery of therapeutic moieties on inflamed skin models, we effectively adapted the previously developed synthetic methodologies to enable the encapsulation of either a highly hydrophobic drug, i.e. dexamethasone, or a biologically active protein, i.e. Etanercept (ETR). [3-4] The NGs were fabricated by replacing the conventionally used organic solvents by differently tempered aqueous buffer. Etanercept, the anti-TNF fusion protein, could be delivered into reconstructed skin equivalents providing the first evidence for its efficient non-invasive efficacy. Moreover, it could be shown that the delivery of the protein into the viable epidermis occurred explicitly upon its temperature triggered release.

Moreover, thermoresponsive nanocapsules (NCs) and protease-degradable NGs have been explored as novel nanocarriers. The correlation between the high hydration capability and higher flexibility of the NCs and their skin penetration ability has been investigated. The challenging issue of biodegradability has been addressed by incorporation of enzymatically cleavable peptides in the backbone of the NGs.

[1] F.F. Sahle et al., Nanoscale (2017), 9, 172-182.

[2] M. Giulbudagian et al., J Control Release 2016, 243, 323-332.

[3] M. Giulbudagian et al., Theranostics (2018), 8, 450-463.

[4] M. Giulbudagian et al., Nanoscale (2018), 10, 469-479.

Amphiphilic Nanogels as New Dermal Delivery Vehicles

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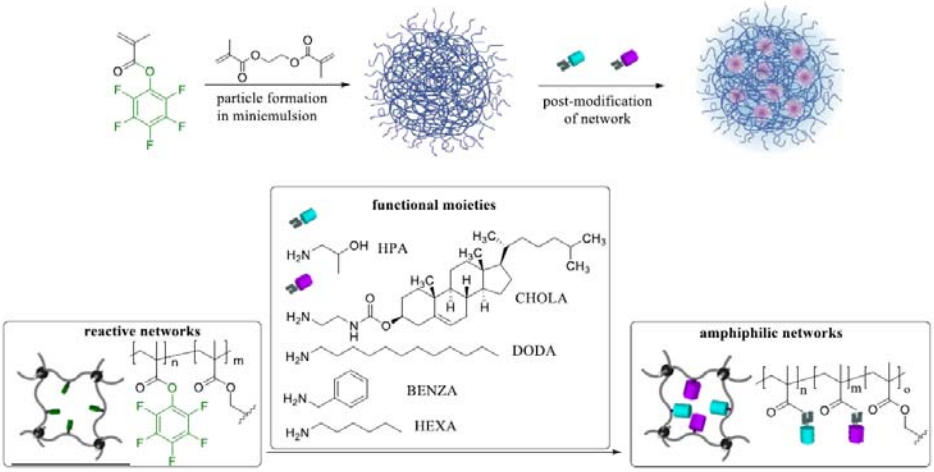
The efficient dermal delivery of drugs requires new concepts in design and function of nanocarriers to address the structural aspects of skin as challenging biobarrier. To address this challenge we have developed a new versatile synthetic platform to control the lipophilicity of nanogels by incorporating hydrophilic and hydrophobic groups into the polymer network (Scheme 1). Depending on the carriers' location in the skin, these adaptive nanogels can present suitable groups at the particle surface, thus matching required amphiphilicity and enhancing skin-carrier interaction. Besides self-adaptability of the exterior, the interior will adapt to the hosted drug entities, as well: the hydrophobic moieties form internal nanodomains for loading with drugs via hydrophobic interactions.

By adjusting the hydrophilic/hydrophobic balance of the polymer network we have prepared a small library of particles to systematically investigate the influence of nanogel amphiphilicity on drug loading capacity and the carrier interaction with biological systems. Incorporation of nile red as hydrophobic model drug has shown that the loading capacity increases with carrier hydrophobicity whereas delivery to the viable epidermis was highest for nanogels with medium hydrophobicity, thus indicating the existence of an optimum carrier amphiphilicity. For these optimized nanogels, dexamethasone leakage was reduced due to its interaction with structurally similar cholesterol domains, as evidenced by EPR spectroscopy. In addition, good biocompatibility of the nanogels was demonstrated by basic toxicological investigations and only minor perturbation of the protein/lipid distribution in excised skin was observed by Stimulated Raman Spectroscopy. Consequently, these results suggest the potential of the amphiphilic nanogels for mild yet efficient dermal delivery.



Scheme 1. Synthetic platform for the development of amphiphilic nanogels with tunable amphiphilicity.

Oral Mucosa: Drug Delivery by Nanocarriers

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Background & Aim

Inflammatory diseases of the oral mucosa may be either triggered by oral pathogenic bacteria or developed due to autoimmune responses. None of these inflammatory conditions are easy to treat, since oral biological fluids dilute topically applied drugs and hinder adhesion and penetration of most drug formulations. Therefore, there is a medical need to develop innovative drug formulations that adhere to the oral mucosa and release anti-inflammatory drugs efficiently. Using *ex vivo* and *in vitro* models, we characterised core multishell (CMS) nanocarriers for their potential use as drug delivery systems at oral mucosal tissues.

Methods

For this purpose, we prepared porcine masticatory as well as lining mucosa and performed Franz cell diffusion experiments. Adhesion to and penetration of fluorescently labelled CMS nanocarriers into the mucosal tissues was analysed using confocal laser scanning microscopy (CLSM). The metabolic and proliferative activity of gingival epithelial cells was determined by XTT and sulforhodamine B assays, respectively. The physical barrier integrity was studied by measuring the transepithelial electrical resistance (TEER) in the presence of CMS nanocarriers. Cellular uptake of the nanocarriers by gingival epithelial cells was analysed by CLSM. Potential inflammatory responses were analysed by monitoring the mRNA expression of pro-inflammatory cytokines using real time PCR.

Results

CMS nanocarriers reveal penetration and very fast adherence to mucosal tissues after 5 min. The CMS nanocarriers exhibited no cytotoxic effects in gingival epithelial cells, and were taken up by cells while the TEER is not affected. Only at high nanocarrier concentrations and after long exposure times, the mRNA expression of the pro-inflammatory cytokines IL-6 and IL-8 was increased, while the expression of IL-1β, CCL20, and TNFα remained unaffected by the nanocarriers.

Conclusion

CMS 10-E-15-350 adhered fast and efficiently to the oral mucosa, which is an absolute prerequisite for an efficient drug release. One the basis of our TEER and cellular uptake experiments, we suggest a transcellular route of CMS nanocarriers into the mucosa. Taken together, these findings indicate that CMS nanocarriers are an innovative approach for topical drug delivery at the oral mucosa.

PD Dr. Annette Moter

Insights into Biofilms:
from bench to bedside and back

Medical biofilms are present on a wide range of interfaces and niches in the human body, both as physiological flora or as biofilm-associated infections. While biofilms have been studied extensively *in vitro*, we are just beginning to evaluate these findings for their clinical significance. We know that biofilm-associated infections pose a significant risk for patients, since biofilms tolerate higher concentrations of antibiotics than measured by standard antibiotic susceptibility tests. However, still, we perform routine diagnostic procedures that comprise culture techniques as well as PCR-based detection. These imply disintegration of the biofilm and therefore loss of crucial information about the biofilm itself, such as spatial resolution and distribution of viable cells in the biofilm. We suggest the microscopic technique Fluorescence in situ hybridization (FISH) to complement the information that is lost during routine microbiological testing:

Biofilm architecture: In subgingival plaque of pockets from periodontitis patients, we analysed the composition and architecture of multispecies biofilms comprising periodontal species and yet uncultured bacteria.

Pathogen identification: FISH using species-specific probes in tissue sections of heart valves can prove infection and identify the causative microorganisms in endocarditis patients. This is particularly helpful in culture-negative cases that pose a significant diagnostic problem.

Biofilm activity: To detect the activity and therefore viability of single bacterial cells, we developed FISH-probes for the 16S-23S internal transcribed spacer region that is only present in actively transcribing cells. Using this spacer-FISH we detected positive cells in heart valves of patients who had been given adequate therapy.

Quantification of anti-biofilm activity: We used FISH for measuring the efficacy of antimicrobial agents against biofilms and biofilm susceptibility *in vitro*.

These findings confirm the recalcitrance of bacteria towards antibiotic treatment in biofilms in the clinical setting. They stress the point that our current diagnostic techniques for cultivation and antibiotic resistance testing *in vitro* are not satisfactory. *In situ* analysis of *in vivo* grown biofilms in clinical samples contributes to bridging the gap between bench side and clinical application and vice versa.