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Uptake dynamics of graphene quantum dots into primary human blood cells following in vitro exposure

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Human leukocytes obtained from samples of leukapheresis products of three healthy donors stimulated by granulocyte colony stimulating factor (G-CSF) were exposed to graphene quantum dots. A time- and concentration dependent uptake was observed with a significantly greater uptake into monocytes and granulocytes in comparison to lymphocytes, suggesting a better incorporation ability of cells with phagocytotic properties. We estimate that one cell can incorporate up to 2 billion quantum dots without significant viability changes. The uptake rates also correlate with the cell membrane area. Looking at the different lymphoid subsets a greater uptake was found into CD19+ B-, CD56+ natural killer cells and CD34+ hematopoietic stem cells (HSC) in comparison to CD4+ T- and CD8+ T cells. Independent of the cell type studied, the observed uptake dynamics is consistent with a diffusion-driven process, which allows the determination of cell-specific membrane permeabilities for the graphene quantum dots.

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Self-cleaning materials using the photocatalytic effect of titanium dioxide

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Germs are present in all areas of everyday life and can lead to dangerous infections. Surfaces with antimicrobial properties are used to reduce the risk of infection in sanitary facilities and hospitals. Apart from the addition of biocides or antibiotic agents to synthetic materials, recent studies show that applying the semiconductor titanium dioxide (TiO_2) generates antibacterial surfaces. The photocatalytic active TiO_2 leads to the development of reactive oxygen species (ROS) that are able to kill germs. The aim of the present study is to use TiO_2 to generate antibacterial polymeric bulk materials. AEROXIDE® TiO_2 P25 and KRONOClean® 7000 were incorporated in different polymeric materials to find the best TiO_2/polymer combination concerning photocatalytic and antibacterial activity.
As matrix material TPS and TPU were compounded with different concentrations of TiO_2. Test samples were produced by injection molding. The photocatalytic effect of the test samples was investigated by using contact angle measurements (photo induced superhydrophilicity) and methylene blue trials, before and after irradiation with UV light. In addition specimens were treated with plasma to increase the photocatalytic effect. The antimicrobial effect was examined by detecting the reduction rate of E.coli on photocatalytic active TiO_2/polymer compounds in microbiological tests.
An incorporation of titanium dioxide in the matrix materials up to 20 wt% was possible. After electromagnetic irradiation (UV) and plasma treatment the samples showed different intensities of the photocatalytic effect depending on the concentration of titanium dioxide. Evidence of a biocide effect was determined.
The study indicates that using titanium dioxide as an additive is a promising approach for the development of polymeric materials with antimicrobial properties. Further modifications of titanium dioxide compounds, long-term stability trials, investigation on mechanical properties and benchmarking with biocide disinfectants will be part of future investigations.

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Impedance spectroscopy as a new tool to monitor re-epithelialization in wounded reconstructed human epidermis

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Here we present a new in vitro test method to determine the efficacy of wound healing therapies in a physiological human three-dimensional environment, which could help to reduce the amount of animal testings. For this, we developed procedures to introduce and non-destructively assess wounds in reconstructed human epidermis (RHE) that closely resembles the histological architecture of human epidermis. Following maturation, RHEs with a surface area of 1.1 cm² were injured locally with a dermal punch (3 biological replicates with 8 technical replicates) resulting in defined wound areas of 3.1 – 50.2 mm². Subsequently, epidermal wound healing (EWH) was monitored for 14 days using a highly sensitive and non-invasive method called impedance spectroscopy (ImpSpec). The epidermal barrier was estimated by calculating the transepithelial electrical resistance at 1,000 Hz ($TEER_{1000}$) as well as fitting an equivalent circuit modeling the measured impedance spectra and resulting in the tissue's resistive and capacitive properties. After wounding, ImpSpec analysis showed an instant drop in the $TEER_{1000}$ and in the fitted resistance R_{RHE} by 7.6 kΩcm² (-95.2%) and 20.8 kΩ (-98.5%) respectively. This initial loss in impedance and the efficacy of EWH strongly depends on the wound size (-93.1% $TEER_{1000}$ and -97.6% R_{RHE} for 3.1 mm² to -98.3% $TEER_{1000}$ and -99.9% R_{RHE} for 50.2 mm² wound size). With ImpSpec, the recovery of the epidermal barrier can be monitored on-line and correlated to microscopic imaging of wound closure. Within 14 days, the $TEER_{1000}$ and R_{RHE} recovered and increased significantly. Additionally, EWH was histologically analyzed, confirming re-growth of a basal layer via migrating and proliferating keratinocytes in early stages of EWH. Along with the increasing impedance during EWH, the gradual regeneration of spinous and granular layer in the interim phase and the formation and strengthening of a high-resistive stratum corneum in the late phase of EWH was observed.

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Engineering of organoid blood vessel patterns with regulated hemodynamics by exosomal functional somatic noncoding RNA angio-morphogens [angiotropins]

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Objective: Different vessel phenotypes result from vasculo-, angio-, arterio- and lymph-angiogenesis by complex interplay of various cells and factors [proteins, RNA, metal ions, metabolites, hormones, environment]. Beyond genetics, diversity, complexity and tolerance of vascular phenotyes are vice versa requisites for function and survival of networked tissue cells with interfaces upon intrinsic and extrinsic [environmental] needs. It may be manifested beneficial or maladaptive entangled with e.g. implant- or cancer-angiogenesis-tolerance reactions. This investigation aimed at whether angio-morphogen RNA sequences for organoid capillary patterns are from inherited germline [Mendelian] or non-Mendelian somatic origin which retranslation does not anymore fit to inherited genes.
Methods: Angiotropin-RNA [ARNA] and MIR126 genes of microRNA-126 [miR-126] structures were used.
Results: miR-126 are small Mendelian split products of MIR126 genes. ARNA are exosomal RNP angiomorphogens from stressed mononuclear cells [shear, exercise, hypoxia, etc.], sequenced after isolation from extracellular fluids where it is active in vitro and tissue to form organoid capillary patterns: By metal ions [Cu, Ca, Na, K], a Mendelian-coded angiotropin-related protein [S100-A12] folds to a stable complex with a somatic non-Mendelian functional $^{5'}$end-phosphorylated, edited, modified, redox- and metallo-regulated non-coding hairpin ARNA [75n] with $^{5'}$CUG$^{3'}$-hairpin loop and modified bases isoG / adenosine-N1-oxide from Fenton-type redox-OH*/NO*-radical modification of adenosine. It is shown that ARNA are formed by stressed mononuclear cells by rearrangement, recombination, mutation, editing, modification, redox- and metalloregulation of inherited MIR-126 gene segments.
Conclusions: The results suggest novel targets for vascular therapy. Some aspects of non-Mendelian ncRNA resemble somatic generation of immune diversity. In this special case, all is achieved on the DNA level under antigen attack, namely for coding new proteins. Here, a general principle is disclosed operated by cells on all nucleic acid, protein and carbohydrate levels to manage any diversity and complexity problems with limited sets of inherited DNA in response to environmental chance reactions.

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Differentiating PPIX from its precursors as a strategy for drug-light interval assessment in photodynamic therapy

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Photodynamic Therapy (PDT) is based on the generation of Reactive Oxygen Species (ROS) inside tumours that destroy cancer cells. The procedure involves three main components: injection of a photosensitizer (PS), presence of oxygen, and light to start the process. Certain variables involved make the process difficult to monitor and optimise. The PDT process starts with the transport of PS inside the tumour, either exogenously or by inducing the synthesis of the PS endogenously to increase selectivity. The drug used to induce the endogenous production is Aminolevulinic Acid (ALA), which is gradually converted to a PS inside the tumour cells. The PS mainly involved is Protoporphyrin IX (PPIX). The interval of time between inoculation and light delivery is known as “Drug-Light Interval” and is one of the main variables influencing outcome of PDT. Based on fluorescence, the sequence of the intensity values of the fluorescence provides information about this interval by assuming the biggest peak as the optimal irradiation time. Too early or too late means that a sufficient concentration of PPIX cannot be ensured in strategic locations inside the cell jeopardising the therapy efficiency. Fluorescence gives no additional information about the actual position and amount of porphyrins inside the tissue. The best starting time for light delivery would be high concentration of PPIX inside the mitochondria. A way to determine this is to monitor the concentration of two precursors of PPIX, Uroporphyrin III and Coproporphyrin III, which are formed outside the mitochondria. We present a novel detection method for differentiating PPIX from these precursors, by monitoring the porphyrins at the same pH and focused on the main emission peaks between 600 – 650 nm, after excitation at 405 nm. The two precursors were distinguishable from PPIX at various concentrations. With this method, localized changes in porphyrin concentration can be identified.

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Automatic algorithm to generate customized microporous membranes by additive manufacturing

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Membranes have a wide spectrum of applications in medical devices. They are crucial elements e. g. in technologies for dialysis, fractionation of blood and clean rooms. Furthermore, in in-vitro diagnostics and bioreactors customized membranes are required to realize compartments. Additive Manufacturing (AM) allows to generate microporous membranes. Therefore, multiple layers with pores are printed with an offset to one another to overcome the restricted resolution of AM methods. Compared to conventional technologies AM can generate customized membranes regarding both filter geometry and pore diameter.
In this study we developed an algorithm in Blender 2.78a for customized membranes. The user can specify the desired pore size, pore form (triangular, rectangular or circular), dimensions of the membrane and whether or not a fixation frame is needed. The minimum pore size, layer height, and need for support structures were stored as pre-settings according to the selected AM technology. Additionally, a method to derive technology-dependent compensation values to improve the accuracy and reproducibility of the pores size, was established. So far two different additive manufacturing technologies were evaluated: Digital Light Processing and Multi Jet Modelling.
To validate if the desired pore size complied to the actual pore size, the membranes were tested with glass beads in following ranges: 0-50, 40-70, 70-110 and 90-150µm. The amount and diameter of glass beads before and after a filtering process, conducted for 2 min in a shaker unit, were analysed by automatic image analysis (Image J). The results showed an efficiency comparable to commercially available wire mesh membranes. So far the reproducible pore size was reduced to a minimum of 40 µm. Further reduction was limited, since the variance of the printing accuracy exceeded the pore size. Combined with improved printing technologies we expect our introduced approach to overcome these limitations in pore size.

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Additive manufactured multimicrophasic (MMP) systems for biomedical applications – perspectives and concepts

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Additive manufacturing is one of the most trendsetting technologies. So far its main advantage is seen in the freedom of design which allows the single-step fabrication of complex parts with undercuts and lightweight construction. However, further progress in 3D printing technology regarding machines and materials such as improved printing resolution, multicomponent printing, and printability of challenging materials including high performance polymers (e.g. PEEK) and elastomers (e.g. TPE, silicone rubber) offers options for further innovations.
In this study we demonstrate the 3-D printing of multicomponent materials with oriented inner structures on a microscale level, which we name multimicrophasic (MMP) systems. An important feature of MMP systems for biomedical applications is the possibility of realizing defined anisotropic properties. Combination and controlled micro arrangement of various materials with deviating hardnesses (e.g. thermoplasts + elastomers) via 3-D printing creates parts with smooth hard-soft-transitions, as well as orientation dependent tensile and compression properties. Thus the MMP approach allows the fabrication of biomedical parts imitating the biomechanics of the human body.
A further promising perspective of MMP parts for biomedical applications is the possibility to realize a new quality of biodegradable systems. By combining materials in the printing process with different rates of degradation the disintegration of parts can be adjusted in a time and location dependent way. This approach also allows changes of part properties over time, like growing pores within scaffolds or a stepwise reduction of part stiffness for healing stage adapted implants.
In our presentation we will demonstrate various examples of MMP parts featuring the above mentioned properties. Furthermore, we will address future technological challenges to promote the MMP approach. We will show that additive manufacturing offers not only new options for geometric part design but also possibilities to realize so far impossible material properties.

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Current developments of an in vitro wound healing experimental system for photobiomodulation therapy research

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Wounds remaining unhealed after three months of standard care require advanced wound healing therapy. Photobiomodulation (PBM) applies light as treatment and can influence chronic wound healing processes. The dose-response of PBM-treated in vitro and animal tissues is observed as biphasic, where cellular activity is activated or inhibited at a threshold light dosage. Light is fundamental to the therapeutic processes, however ‘the medicine’ (light properties) and ‘the dose’ (irradiation parameters) delivered to the system are often unverified or not reported in the literature. More understanding of the relationship between PBM treatment and biological response is necessary to optimize PBM as a clinical therapy to modulate chronic wound healing. Our overall aim is to develop a multi-modal advanced wound healing device that applies a combination of mechanical pressure, electrical fields, and light as treatment for chronic wound healing therapy. This work represents the current developments of our PBM device, study system, and analysis techniques towards a pilot study. Our wound system is an in vitro 3D organotypic tissue consisting of keratinocytes on top of a collagen dermal equivalent embedded with fibroblasts, macrophages, and neutrophils. We developed an image acquisition and planimetry system to measure the wound surface area and used these systems to develop a reproducible wounding technique. Our developed PBM device can operate in an incubator and is flexible to be configured to apply a range of therapeutic ‘medicines’ at a prescribed ‘dose’ in specification. We will apply a specified and verified light treatment to wounded tissue samples over 21 days. The dose-response will be evaluated by the wound surface area reduction and rate of wound closure as compared to untreated wound controls. This pilot study will demonstrate the first treatment application of our developed PBM device.