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Artikel: Nanoparticles for laser tissue soldering in the brain - chances and risks : an interdisciplinary research project

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3. Relevanz dieses Projektes

Die Identifizierung und die funktionelle Charakterisierung der CYP Enzyme erlaubt, diejenigen CYP Enzyme zu identifizieren, die am Ab- und Umbau eines Wirkstoffes beteiligt sind. Weiterhin können mögliche Arzneimittelinteraktionen untersucht werden, die zum Beispiel zu unerwünschten Wirkungen führen können. Dies wäre der Fall, wenn zwei Arzneimittel-Wirkstoffe über dasselbe CYP Enzym abgebaut werden; infolge des kompetitiven Um- oder Abbaus beider Substanzen durch ein Enzym entstehen höhere Wirkstoffspiegel im Blut. Basierend auf den Daten der Studien können solche Arzneimittelinteraktionen vorhergesagt werden. Bei Nutztieren können die erhobenen Daten auch zur Abschätzung von Arzneimittelrückständen dienen. Dies kann bei der Einhaltung von Dopingbestimmungen bei Sportpferden ebenfalls wichtig sein.

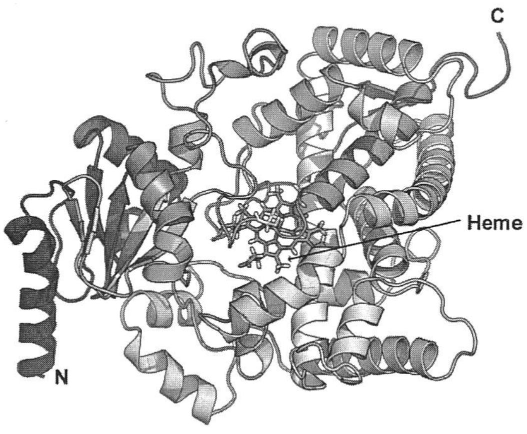

Abbildung 1 *(Farbe siehe Webversion). 3D Strukturmodell des equinen Cytochrom P450 Enzyms CYP3A96 (von Dr. A Pandey)*

Einen weiteren wichtigen Aspekt stellen sogenannte genetische Polymorphismen der CYP Enzyme dar. Bei einem funktionell relevanten Polymorphismus, also dem Auftreten mehrerer Varianten eines CYP Gens, das in die Arzneimittelmetabolisierung involviert ist, wird der Wirkstoff nicht oder wesentlich langsamer abgebaut. Daher können unvorhersehbar hohe Wirkstoffspiegel entstehen, die ihrerseits zu unerwünschten Wirkungen im Organismus führen. Beim Menschen sind solche Polymorphismen zum Beispiel im CYP2D6 bekannt.

Nach Identifizierung der CYPs des Pferdes haben wir nun die Möglichkeit, auch individuelle Polymorphismen in CYPs zu finden. Dies ist ein Schritt in die Richtung, mögliche «poor metabolizers», also Tiere, die bestimmte Substanzen langsamer metabolisieren als andere Individuen, zu identifizieren. Somit sind durch die gewonnenen Erkenntnisse Dosisanpassungen bei solchen Pferden möglich.

4. Schlussfolgerung

CYPs sind für den Arzneimittelmetabolismus von Mensch und Tier von entscheidender Bedeutung, da Medikamenteninteraktionen auch beim Pferd durch eine Induktion oder Inhibition der CYP Enzyme lebensbedrohliche Folgen haben können. Aufgrund von gravierenden Speziesunterschieden ist eine Übertragung von Daten aus der Human- auf die Veterinärmedizin nicht uneingeschränkt möglich. In der Pferdemedizin werden vielfach Arzneimittel eingesetzt, welche in der Humanmedizin keine Anwendung finden. Aus diesem Grund und ebenfalls wegen der geringen Verfügbarkeit von Studien über den Arzneimittelmetabolismus beim Pferd, ist die Erforschung der verschiedenen equinen CYP Enzyme und Kenntnisse über ihre Substratspezifität äusserst relevant. ■

Nanoparticles for laser tissue soldering in the brain – chances and risks. An interdisciplinary research project

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1. Introduction

Nanotechnology involves the engineering and manipulation of particles at a nano scale (< 100 nm = nanometer). One nanometer is one million times smaller than a millimeter or a human hair is about 50 thousand nanometer thick.

Nowadays, nanoparticles (NPs) have become part and parcel of everyday life. For example, they are used for aesthetic purposes in food e.g. to make powdered toppings whiter and frostings shinier. While they are often intentionally added, they are also created inadvertently as a byproduct of the process of grinding ingredients. Besides food products including candy like M&Ms, ketchup etc., sun tan lotions and toothpaste often include NPs.

On the other hand, nanotechnology also has an enormous potential for a variety of emerging medical applications including diagnosis, therapy, and

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prevention of human diseases and disorders. Despite the expanding application of nanotechnology in the area of medicine and biology, the effects of engineered nanoparticles on human health have not been adequately evaluated even though there is a large amount of literature on toxic effects of NPs available. Most studies have focused on respiratory exposure, but the long-term health effects associated with NP inhalation and their internalization by cells is not at all fully understood. Besides inhalation, uptake via oral intake, penetration through the skin, uptake via the vascular system, for example when NPs are used as container for molecular drug delivery or as contrast agent in biomedical imaging or direct implantation into the body by medical implants are possible pathways NPs can take to enter the body.

As the range of nanoparticle types and applications increases, the potential toxicities of these novel materials and the properties driving such responses must be understood. A detailed assessment of the physicochemical characteristics of nanoparticles is therefore crucial for the safe and sustainable development of these technologies. Cells phenotypically adapt to alterations in their intra- and extracellular environment via organized alterations to gene and protein expression. Many chemical and physical stimuli are known to drive such responses, including oxidative stress and heat shock. The small size of nanoparticles greatly increases their surface area per unit mass and may facilitate their uptake into cells and across the cells into the blood and lymph stream to reach various target sites. The amount of particles trapped in cells strongly depends on the surface chemistry of the NPs. Important parameters for NP uptake and/or cytotoxic effects include the cell type as well as physico-chemical characteristics of the NPs such as size, shape, or surface charge.

2. Swiss National Research (NRP64) Programme Interdisciplinary project on nanoparticles aimed for laser tissue soldering in the brain

The Swiss Science National Foundation launched a large national program named «Opportunities and Risks of Nanomaterials» (NRP64; *http://www.nfp64.ch*) on nanomaterial research.

Our interdisciplinary research program is part of this NRP64 program. The project partners include researchers from different disciplines including physics (*http://www.iap.unibe.ch*), biotechnology (*http://www.fhnw.ch/lifesciences/*), neurosurgery (*http://www.neurochirurgie.insel.ch*), and veterinary medicine and biology (*http://www.vpi.vetsuisse.unibe.ch*) which unites complementary expertise and establishes a close interdisciplinary research cooperation. Our study aims at investigating possible effects of NPs that are proposed for use in laser tissue soldering in the brain, e.g. cerebral bypass surgery. Laser tissue soldering is a procedure to obtain tissue fusion, which allows to tightly sealing surgical wounds and in particular vascular lesions. It is based on a heat induced denaturation process of proteins like bovine serum albumin BSA, providing the necessary acute tissue strength. This novel tissue fusion technique is perceived as a minimally invasive alternative to the classical use of suture or stitches to close lacerations, which is a powerful perspective in many open and endoscopic surgical applications. This technique provides essential advantages over traditional suturing including speed, immediate water tightness, reduced tissue trauma and faster healing thus reducing the exposure of the patients. This novel laser assisted anastomoses technique will open doors for new avenues of surgical applications especially in neurosurgery where operation time is one crucial parameter. As one major application tissue soldering will be used for cerebral bypass surgery as well as cranial closure techniques. Laser tissue soldering involves the combination of near-infrared radiation, which deeply penetrates tissue, with a biodegradable scaffold in which an exogenous chromophore as heat transducer is embedded e.g. gold nanoparticles (10–80 nm in diameter) or core shell silica nanoparticles (30 -100 nm) containing an encapsulated, indocyanine green (ICG) dye. ICG is a tricarbocyanine fluorescent dye, non-toxic and FDA approved. In the laser-induced tissue soldering process locally confined ICG concentrations are required. The drawbacks of ICG are (i) poor aqueous stability, (ii) strong photo degradation, and (iii) fluorescent intensity decrease at higher concentration.

The ICG chromophore or gold nanoparticles selectively and locally convert the laser radiation into heat (Fig. 1). Over time during the healing process, the biodegradable scaffold will break down releasing the embedded NPs into the surrounding tissue and probably also into the blood stream.

The main goal of the project therefore is to determine the NP biodistribution and their toxicokinetic properties, their uptake mechanisms and possible exocytosis of NPs in neuronal cell cultures, and organotypic brain slices. Hereto, we first characterized the newly designed and synthesized NPs and studied the influence of laser irradiation on the stability and on the physicochemical properties since they determine the amount and the pathway of the cellular uptake of the nanoparticles.

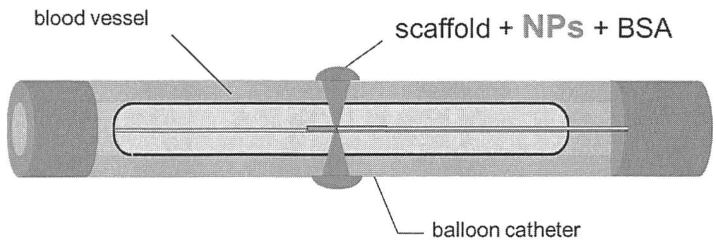

Figure 1 (coulours see web version). Schematic overview of the principle of laser tissue soldering. A biodegradable scaffold containing light-absorbing nanoparticles is placed around the region of the lesion. A laser that was introduced into a blood vessel emits energy, resulting in tissue fusion.

In order to track the nanoparticles two approaches were followed:

1. Use of highly sophisticated methods e.g. two photon microscopy, fluorescence correlation spectroscopy, confocal microscopy, AFM and transmission electron microscopy (TEM) to fully characterize the NPs prior and after laser irradiation and to analyze histological sections.

2. Core shell nanoparticles containing a sensitive fluorochrome or a spin label, but exhibiting the same surface properties and size distribution like the ICG doped particles are monitored by scintillation counting, fluorescence or optical techniques. We also designed, synthesized and investigated silica NPs, incorporating rhodamine dye in the silica core but having otherwise the same structure and properties as the NPs mentioned above. The aim of these experiments was to better follow and track the distribution of the particles inside the cells and organotypic brain slices by multi-photon microscopy.

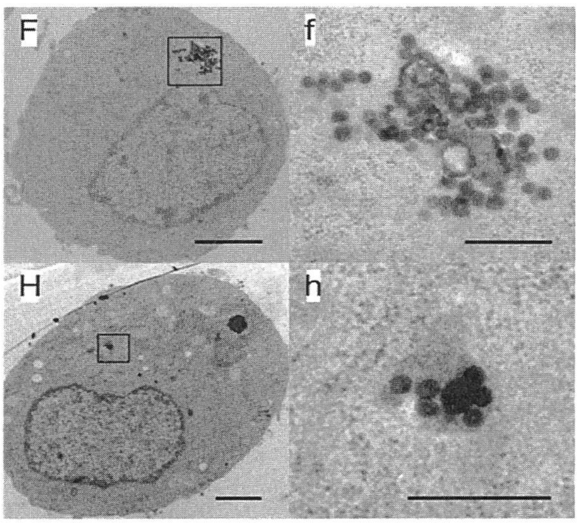

***Figure 2.** Uptake of NPs into N9 cells observed by transmission electron microscopy (TEM). Cells were exposed to nanoparticles for 24 hrs. A higher magnification of the boxed areas in images F and H (scalebar 2 µm) was performed to provide subcellular details (f and h; scalebar 0.5 µm).*

3. Besides programmed cell death (apoptosis), viability and cytotoxicity of the cells and detection of oxidative stress after NP exposure, we investigate autophagy, a process that cells use to get rid of metabolic and probably other waste products.

As a result of the research in design and synthesis of the NPs, the ICG was (i) stabilized in the scaffold matrix, (ii) protected against the aqueous environment in the tissue, and (iii) the dye revealed optical stable if stored in the dark. The NPs have been characterized by all means of analytical methods e.g. IR spectroscopy, DLS, thermogravimetric analysis, zeta potential measurements and scanning electron microscopy. This work demonstrates a new route for stabilizing ICG at high concentrations. The comparison of naïve and laser-irradiated NPs (heated up to a temperature of around 85°C, the maximum temperature tolerated during the soldering procedure) revealed that the NPs are extremely stable and do not change their properties.

We demonstrated the uptake and internalization of Au NPs in macrophages, N9 microglial cells (Fig. 2) and SH-SY5Y neuroblastoma cells after an incubation time of 24 hours. Our data show that more NPs were taken up by the microglial cells compared to the neuron-like cells.

In addition, an increase in oxidative stress was found after certain times of NP exposure at the highest NP concentration used. Reactive oxygen species (ROS) are involved in many cellular pathways leading to cell death.

The NP uptake was measured using high content analysis to allow quantitative measurements. The uptake was dependent on the cell type, the NP type and the incubation time. NPs were found as single particles, but also as clusters primarily in lysosomes (Fig. 3), but also in the cytoplasm and in the endoplasmatic reticulum, but not in mitochondria or in the Golgi apparatus. It is likely that lysosome enzymes can degrade different coating materials and trigger ROS production. ROS formation can rupture the lysosomal membrane and release the lysosomal enzymes.

Contrary to cell experiments, organotypic brain slices have the advantage of longevity and contain multiple cell types common to a functioning brain making them one of the most useful models for studying uptake of NPs and effects on the brain. As compared to cell lines and primary brain cell cultures, the main feature of organotypic slice cultures is the maintenance of a well preserved, 3-dimensional tissue morphology of the brain with its highly complex microorganization of diverse interconnected cell types such as neurons, astrocytes, oligodendrocytes and microglia, similar to the in vivo situation. Furthermore, organotypic slice cultures can be kept for extended periods of time (up to 26 weeks). Compared to in vivo models, such slice culture systems offer the advantage that specimens can be manipulated in a controlled environment and under defined conditions. A procedure to prepare these organotypic brain slices is shown in Fig. 4. We are currently investigating NP uptake in hippocampal rat brain slices as well as primary cells of that brain region. First results show that NPs were found at the outer border of hippocampal tissue slices after 24 h of incubation. In the hippocampus, different neuronal cell types are organized into layers. Based on the fact that the NPs might not get in touch with the dentate gyrus in our experimental setup, it seems reasonable that NPs were only found in the outer layers of the hippocampal slices 24 h after administration. This might be due to the administration of the NPs by dripping the NP in solvent on the surface of the organotypic brain slices.

We are currently working on intracellular trafficking of NPs and time-lapse uptake and possible exocytosis. The uptake mechanisms will be studied in detail to get insight in how cells engulf the NPs synthesized for laser tissue soldering in the brain. Uptake of the NPs will be investigated in primary cells of the hippocampus.

3. Relevance of the project

Mechanism-driven research is important in order to provide a solid scientific basis for safety and risk assessment. Despite the fact that there is a common assumption that small size nanoparticles allows them to easily enter and traverse tissues, cells and organelles since the actual size of engineered nanoparticles is similar to that of many biological molecules (e.g. proteins), our study will gain insight in size-dependent and nano-specific characteristics of cellular uptake of nanoparticles in neuronal cells as well as in brain tissue. The evaluation of potential neurotoxic effects of nanoparticles on neuronal function is required, as specific mechanisms and pathways through which nanoparticles may exert their toxic effect remain largely unknown.

Understanding the unique characteristics of engineered nanomaterials used for laser-assisted soldering of brain vascular lesions (anastomoses) and their interactions with biological systems is key to the safe implementation of these materials in novel biomedical therapy. Moreover, we address the important question of biodistribution of a nanoparticle scaffold used for soldering anastomoses in the brain. This work will open doors for new avenues of research that could eventually lead to safer use of nanomaterials and will enable public health officials in Switzerland and elsewhere to better use current results in making decisions. ■

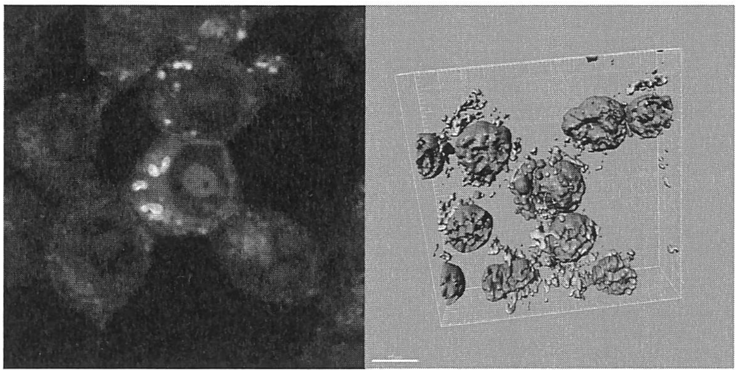

Figure 3 (coulours see web version). Uptake of NPs (core was doped with rhodamine; red) into lysosomes (green) of N9 cells after 24 h of exposure. Cell nuclei were stained with dapi (blue). Left picture: all fluorescence channels are merged to obtain a two dimensional image. Right picture: Imaris software was used in the surpass mode to compute several image slices in order to produce a three dimensional view onto the samples.

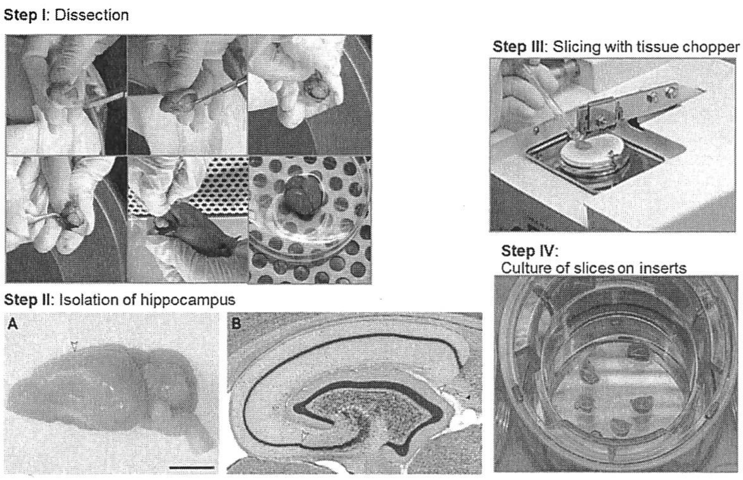

Figure 4 (coulours see web version). Preparation of organotypic hippocampal slice cultures from the brains of rat pubs. After dissection of the whole brain, the hippocampus is prepared. Afterwards, the isolated tissue is sliced with a tissue chopper. Finally, the slices are placed on a semi-permeable membrane in a cell culture dish and exposed to nanoparticles.