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In Vivo Behavior of Nanoparticles: Impact of Surface Modifications on the Spatiotemporal Microdistribution of Quantum Dots

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"There is plenty of room at the bottom."
Richard Feynman, 1959

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List of Abbreviations

BM	Basement membrane
ECM	Extracellular matrix
MNT	Membrane nanotube
MPS	Mononuclear phagocyte system
MRI	Magnetic resonance imaging
NM	Nanomaterial
NP	Nanoparticle
PEG	Polyethylene glycol
PET	Positron emission tomography
QD	Quantum dot
TEM	Transmission electron microscopy

Summary

In biomedical research, various nanoparticles (NPs) are being developed for clinical applications ranging from diagnostics to therapy, utilizing their unique physicochemical properties as well as their high versatility. For each application it is essential that the NPs efficiently reach their target site in the body, for example, a specific cell type or substructure within an organ. Hence, the aim of this thesis was to study the microdistribution of quantum dots (QDs) in muscle tissue of healthy mice. To investigate the influence of surface modifications on the tissue distribution, QDs with either a polyethylene glycol (PEG) or a carboxyl surface coating were applied.

Chapter 2 [Nekolla *et al.*, 2016] demonstrates by means of *in vivo* real-time fluorescence microscopy, particle tracking, and transmission electron microscopy that the microdistribution of QDs is strongly influenced by their respective surface modification. Locally injected carboxyl QDs preferentially bind to constituents of the extracellular matrix, such as collagen fibers and basement membranes. Furthermore, carboxyl QDs are localized in caveolae of endothelial cells as well as in endothelial junctions, enabling them to translocate into the vessel lumen. In contrast, PEG QDs show little interaction with tissue components, but mainly diffuse in the interstitial space. The data suggest that constituents of the extracellular matrix act as a selective barrier depending on the QD surface modification.

Chapter 3 [Rehberg, Nekolla *et al.*, 2016] shows that immune cells play a part in the microdistribution of NPs in the tissue. By intraarterial injection of carboxyl QDs it was demonstrated that perivascular and tissue-resident macrophages are interconnected by microtubule-containing tubular membranous structures, so-called membrane nanotubes (MNTs). Inside these MNTs, carboxyl QDs are exclusively contained in vesicles, which are transported along the microtubules by molecular motors.

Taken together, this thesis elucidates the extra-, intra-, and intercellular distribution of QDs at the microscopic tissue scale. The choice of surface modification critically

influences the microdistribution, which should be considered for the future design of NPs that are intended for the use in biomedical applications. Furthermore, it is important to keep in mind that the distribution of NPs in the tissue takes place via different routes including the transport via networks of cells interconnected by MNTs.

Zusammenfassung

In der biomedizinischen Forschung werden diverse Nanopartikel (NP) für klinische Anwendungen, die von Diagnostik bis Therapie reichen, entwickelt. Dabei werden die einzigartigen physikalisch-chemischen Eigenschaften sowie die große Vielseitigkeit der NP genutzt. Für jede Anwendung ist es essentiell, dass die NP im Körper ihr Ziel erreichen, z.B. einen bestimmten Zelltyp oder eine spezifische Unterstruktur in einem Organ. Daher war das Ziel dieser Dissertation, die Mikrodistribution von Quantenpunkten (*quantum dots*, QDs) in Muskelgewebe von gesunden Mäusen zu untersuchen. Um den Einfluss der Oberflächenmodifikation auf die Verteilung im Gewebe zu erforschen, wurden QDs mit Polyethylenglycol (PEG)- oder Carboxyl-Oberflächengruppen verwendet.

Kapitel 2 [Nekolla *et al.*, 2016] zeigt mit Hilfe von Echtzeit-Fluoreszenzmikroskopie, Partikel-Tracking und Transmissionselektronenmikroskopie, dass die Mikrodistribution von QDs stark von der Oberflächenmodifikation beeinflusst wird. Lokal injizierte Carboxyl-QDs binden an Elemente der Extrazellulärmatrix wie Kollagenfasern und Basalmembranen. Darüberhinaus befinden sich Carboxyl-QDs in endothelialen Caveolae sowie in Zell-Zell-Kontakten zwischen Endothelzellen, was die Translokation in das Gefäßlumen erlaubt. Im Gegensatz dazu tritt nur wenig Interaktion zwischen PEG-QDs und Gewebekomponenten auf, vielmehr diffundieren PEG-QDs hauptsächlich im Interstitium. Die Daten deuten darauf hin, dass Bestandteile der Extrazellulärmatrix je nach QD-Oberflächenmodifikation als selektive Barriere wirken.

Kapitel 3 [Rehberg, Nekolla *et al.*, 2016] legt dar, dass Immunzellen einen Anteil an der Mikrodistribution von NP im Gewebe haben. Mithilfe von intraarterieller Injektion von Carboxyl-QDs wurde gezeigt, dass perivaskuläre und gewebsständige Makrophagen durch röhrenförmige Membranstrukturen, sog. *membrane nanotubes* (MNTs), die Mikrotubuli enthalten, verbunden sind. Carboxyl-QDs befinden sich in den MNTs ausschließlich in Vesikeln, die mit Hilfe von molekularen Motoren entlang der Mikrotubuli transportiert werden.

Zusammengefasst erläutert diese Dissertation die extra-, intra- und interzelluläre Verteilung von QDs auf der mikroskopischen Gewebeebene. Die Wahl der Oberflächenmodifikation hat einen entscheidenden Einfluss auf die Mikrodistribution. Dies sollte für die zukünftige Entwicklung von NP für biomedizinische Anwendungen bedacht werden. Darüberhinaus ist es wichtig zu berücksichtigen, dass NP im Gewebe auf unterschiedliche Art und Weise verteilt werden. Dazu zählt auch der Transport in Netzwerken von Zellen, die durch MNTs verbunden sind.

Publications and Manuscripts Originating from this Thesis

Chapter 2

K. Nekolla, K. Kick, S. Sellner, K. Mildner, S. Zahler, D. Zeuschner, F. Krombach, M. Rehberg. *Influence of surface modifications on the spatiotemporal microdistribution of quantum dots in vivo.* ***Small***. 2016. 12(19):2641-2651.

Chapter 3

M. Rehberg, K. Nekolla, S. Sellner, M. Praetner, K. Mildner, D. Zeuschner, F. Krombach. *Intercellular transport of nanomaterials is mediated by membrane nanotubes in vivo.* ***Small***. 2016. 12(14):1882-1890.

Publications not included into this thesis:

M. Lasch, K. Nekolla, A. Klemm, J.-I. Pagel, U. Pohl, S. Dietzel, E. Deindl. *Estimating shear stress in murine peripheral collateral arteries by two-photon line scanning.* In preparation.

B. Uhl, Y. Vadlau, G. Zuchtriegel, K. Nekolla, K. Sharaf, F. Gaertner, S. Massberg, F. Krombach, C. A. Reichel. *Aged neutrophils contribute to the first line of defense in the acute inflammatory response.* ***Blood***. 2016. 128(19): 2327-2337

K. Kick, K. Nekolla, M. Rehberg, A. M. Vollmar, S. Zahler. *New view on endothelial cell migration: switching modes of migration based on matrix composition.* ***Arteriosclerosis, Thrombosis, and Vascular Biology***. 36(12): 2346-2357

M. Borowiak, W. Nahaboo, M. Reynders, K. Nekolla, P. Jalinot, J. Hasserodt, M. Rehberg, M. Delattre, S. Zahler, A. Vollmar, D. Trauner, O. Thorn-Seshold. *Photoswitchable inhibitors of microtubule dynamics optically control mitosis and cell death.* ***Cell***. 2015. 162(2):403-411

S. Sellner, S. Kocabey, K. Nekolla, F. Krombach, T. Liedl, M. Rehberg. *DNA nanotubes as intracellular delivery vehicles in vivo.* ***Biomaterials***. 2015. 53: 453-463

S. Dietzel, J. Pircher, A. K. Nekolla, M. Gull, A. W. Brändli, U. Pohl, M. Rehberg. *Label-free determination of hemodynamic parameters in the microcirculaton with third harmonic generation microscopy.* ***PLOS ONE***. 2014. 9(6): e99615

1 Introduction

1.1 Nanomaterials

1.1.1 Definition

The word “nano” is derived from the Greek word “nanos”, meaning “dwarf”. The name already indicates that nanomaterials (NMs) are very small objects and, in fact, their sizes are comparable to those of proteins or small viruses.[1] More precisely, NMs are defined as objects with at least one dimension on the nanometer scale, i.e., in the 1 nm to 100 nm size range.[2,3] Thus, not only particles with a sub-100 nm diameter (i.e., nanoparticles (NPs)), but, for example, also long-stretched carbon nanotubes or graphene sheets belong to the category of NMs.[4]

1.1.2 Sources and Applications of Nanomaterials

NMs are generated in many natural processes, for instance, during forest fires or sand storms.[5,6] Further examples for natural NMs are volcanic ash and ocean spray.[5,6,7] Moreover, there are diverse anthropogenic NMs that are, for example, contained in exhausts from combustion engines (contributing to ambient particulate matter air pollution), smoke from combustion (from cooking and heating as well as power plants), or cigarette smoke.[5,7] Furthermore, the field of nanotechnology enables the synthesis of NMs with desired compositions, morphologies, and physicochemical properties. These engineered NMs are utilized in a wide variety of applications, for instance, in (bio)sensor technology,[8] surface coatings,[9] photovoltaic devices,[8] wastewater treatment,[10] cosmetics,[11] sunscreen,[12] and food packaging.[13] In 2015, engineered NMs were contained in over 1600 consumer products.[14]

1.1.3 Biodistribution of Nanomaterials

The described sources and applications indicate that NMs can be incorporated into the human body, for example, by inhalation, oral, or dermal uptake.[7,14] In addition, in biomedical applications, engineered NMs can also be introduced into the body by injection or implants.[14] From these gates, NMs can potentially translocate into the circulation and lymphatic system and thus distribute in the whole body.[7] The distribution of a compound of interest in the body is called biodistribution. It can be determined by dissecting animals and analyzing the amount of compound in different organs. Alternatively, the distribution of the compound – labeled with a suitable contrast agent – can be determined using noninvasive imaging methods, such as magnetic resonance imaging (MRI), molecular imaging (e.g., positron emission tomography (PET)), or optical imaging.[15]

The biodistribution of NPs is significantly influenced by the adsorption of biomolecules on the particle surface occurring upon contact with biological media and leading to the formation of a so-called “corona”.[16] It consists of a stable “hard” corona with strongly adsorbed molecules and a “soft” corona with a dynamic composition of weakly bound molecules.[16,17] The type of corona depends, amongst others, on particle size and characteristics like surface chemistry, hydrophobicity, and charge.[17] By adsorption of opsonins (mostly immunoglobulins and complement proteins, but also other serum proteins, such as C-reactive protein or fibronectin) on the particle surface, NPs can be recognized by the mononuclear phagocyte system (MPS), a part of the immune system consisting of phagocytic cells.[18,19] As a result, in the circulation the majority of NPs underlies rapid clearance and is deposited mainly in liver and spleen.[18,19] If the NPs are small enough (less than 8 nm in hydrodynamic diameter), they may be renally cleared and therefore excreted with the urine.[20] Alternatively, degradation or – if the NPs are not biodegradable – accumulation in the body, mainly in the organs of the MPS, takes place, which may imply toxic effects.[18,20]

On the tissue level, compartments such as the endothelium or the extracellular matrix (ECM) can pose transport barriers for NPs.[21] If these barriers are overcome,

cellular uptake of NPs can take place via different endocytotic pathways including phagocytosis, clathrin- or caveloae-mediated endocytosis, and macropinocytosis, the dominant mode of uptake being influenced by composition, size, shape, and surface characteristics.[22] Inside the cell, NPs can accumulate in the endo-lysosomal system or escape into the cytoplasm and even enter the nucleus, if size allows for it.[22]

1.1.4 Nanotoxicology

Ambient particulate matter air pollution is known to correlate with cardiovascular diseases, respiratory illnesses, and cancer.[23,24] It is suggested that in particular the nanosized component contributes to the adverse health effects of airborne particulate matter – the reason being the increased surface area per mass unit (see section 1.1.5.1).[23,25,26] The scientific field which investigates negative health effects of (engineered) NMs is called nanotoxicology.[7] NM-induced toxic effects in the human organism mainly arise from oxidative stress. It is caused either directly by activating cells (e.g., macrophages) to produce reactive oxygen species or indirectly by introducing chemicals, such as soluble metals or radicals, which are adsorbed on the particle surface.[25,26] Oxidative stress is associated amongst others with changes in the cytoskeleton, unregulated signaling, release of proinflammatory mediators, and DNA damage.[5,23,25] Possible consequences are inflammation, cytotoxicity, and carcinogenesis.[25,27] Diseases associated with the uptake of ambient nanoparticulate matter include asthma, allergic, cardiovascular, neurologic, and autoimmune diseases as well as cancer.[5,25,26] Regarding engineered NMs, particle size, composition, shape, surface functionalization, charge, and concentration are important factors influencing biodistribution and potential toxic effects.[27] Thus, the characteristic “nanoscale” does not automatically imply toxicity. Examples for engineered NMs with positive health effects are antibacterial silver NPs or inorganic NPs, which can be intrinsic antioxidants.[28,29] Moreover, various nontoxic NPs are used in and developed for biomedical applications.[2,30]

1.1.5 Biomedical Applications of Nanoparticles

Nanotechnology does not only play a role in industrial applications, but is also employed in biomedicine. Engineered NMs qualify for a broad spectrum of clinical applications ranging from diagnostics to therapy due to their special physicochemical properties, the possibility of custom-made production, and high versatility.[2,31,32] In the following, the focus is on NPs, as they are the main type of NMs developed for biomedical applications.

1.1.5.1 Nanoparticle Design for Biomedical Applications

Inorganic NPs developed for biomedical applications can, for example, be composed of metals, metal oxides, semiconductors or silica, whereas organic nanoconstructs typically consist of polymers, dendrimers, lipids, or DNA (see Figure 1).[4,33] In addition, different (organic or inorganic) materials can be combined to generate composite NPs, an example being quantum dots (QDs) encapsulated in a gelatin shell.[21] In clinical trials, mostly liposomal and polymeric NPs have been used up to now.[34]
Physical properties of engineered NPs involve aspect ratio and shape (e.g., spherical, cubic, or rod-shaped), as well as features like porosity or rigidity.[4,12] Importantly, also the surface chemistry, which influences surface charge and hydrophobicity, can be engineered. For instance, surface functionalities like amine or carboxyl groups can be attached.[4] Besides, the NPs can be functionalized by attaching various targeting ligands, such as small molecules, peptides, or antibodies, to the particle surface.[4,35]
An essential feature of NPs is the high ratio of surface area to volume, which increases drastically with decreasing particle size.[2,3] To illustrate, when a cube with 1 cm edge length is divided into single cubes with 10 nm edge length, their total surface area is a million times larger than that of their bulk counterpart with 1 cm edge length. The increased surface area can render NPs very reactive, because a large amount of molecules, such as proteins or nucleic acids, can bind to the particle surface.[23]
Overall, properties like increased surface area per unit of mass and effects like quantum confinement (see section 1.1.6) lead to unique chemical, mechanical, optical and electronic features of NPs.[3]

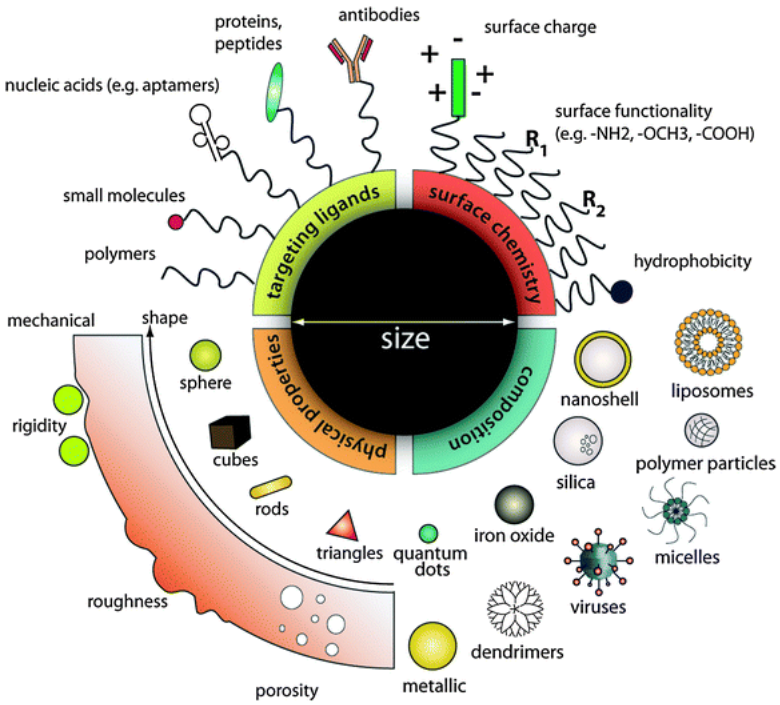

Figure 1: Characteristics of NPs engineered for biomedical applications. Various NPs can be designed by manipulating size, composition, and physical properties. Moreover, diverse chemical surface groups and/or targeting ligands can be attached for functionalization. Reproduced from Ref. 4 with permission from The Royal Society of Chemistry.

1.1.5.2 Nanoparticle-based Diagnostics

In biomedical diagnostics, magnetic NPs qualify for the use in MRI, with particle size and composition influencing the contrast.[36] For example, superparamagnetic iron oxide NPs, so-called SPION, which can be equipped with targeting ligands and drugs, have been utilized as MRI contrast agents.[37,38] Gold NPs can serve as contrast agents in x-ray imaging and computed tomography, as they feature high x-ray attenuation, the potential to attach targeting ligands, and nontoxicity.[39] Moreover, radiolabeled NPs can be utilized in molecular imaging.[40] Besides, fluorescent NPs, such as QDs or NPs with a fluorescent label, can be used in optical imaging.[41] In addition, NPs are developed for multimodal imaging, including hybrid PET/MRI or MRI/ultrasound imaging.[42,43]

1.1.5.3 Nanoparticle-based Therapy

In nanotechnology-based therapy, NPs are designed to serve as nanosized drug carriers.[32] Most clinical trials involving drug nanocarriers focus on cancer therapy, i.e., they are loaded with a chemotherapeutic agent.[30,44,45] However, nanomedicine also tackles diseases like myocardial infarction, Alzheimer's disease, acute lung injury, rheumatoid arthritis, and diabetes.[34,46]

NPs acting as drug nanocarriers provide advantages such as reduced systemic toxicity and protection from enzymatic degradation of the encapsulated drug.[47] Moreover, a prolonged drug circulation time and reduced renal clearance can be achieved.[34,45] Furthermore, the delivery of drugs with low solubility in water can be facilitated by encapsulating them in NPs.[34] The accumulation of NPs at the site of disease can be achieved by different targeting strategies. Tumor tissue can be passively targeted by utilizing the enhanced permeability and retention effect, which is marked by an enlarged accumulation of NPs within tumor tissues because of a leaky vasculature as well as augmented retention due to poor lymphatic drainage.[30] Active targeting can be accomplished by attaching targeting ligands to the NP surface. For instance, folic acid can be used to specifically target folate receptors that are overexpressed in many tumors and transferrin can be utilized to deliver drugs across the blood-brain barrier.[30,45] The attached ligands can also enhance intracellular drug delivery, for example, by receptor-mediated endocytosis, and thus even overcome multidrug resistance.[35]

In addition to the possibility of targeting, NPs offer another important advantage: they can be rendered "smart" in order to respond to internal or external stimuli. Internal stimuli utilize the fact that pathological sites are often marked by an increased redox potential, an elevated expression of specific enzymes (e.g., matrix-metalloproteinases), and a more acidic pH.[30,35] On the other hand, smart NPs can be designed to response to external stimuli, such as light irradiation (e.g., for light-triggered drug delivery) or ultrasound, in which the NPs act as ultrasound-responsive nanocarriers (e.g., lipid nanobubbles).[37,48] In addition, a magnetic field can be used

to accumulate magnetically sensitive NPs in the target area.[35] Temperature can act as internal stimulus, utilizing hyperthermia in inflamed or tumor tissues, or an external heat source can be applied to activate thermo-responsive NPs, e.g., for drug and/or gene delivery.[48]

Due to their versatility, NPs can be designed to simultaneously or sequentially act as imaging agents and drug delivery vehicles. Thus, they can be employed in theranostics to serve as combined diagnostic and therapeutic tools.[47,49]

1.1.5.4 Drawbacks and Future Perspectives

The vast extent and variety of nanomedical research shows that NPs hold great promise for the use in biomedicine. Nevertheless, a major drawback of NPs is that opsonization (see section 1.1.3) allows the cells of the MPS to remove the majority of NPs from the bloodstream before they can act as diagnostic or therapeutic tool.[18] However, opsonization and thus recognition by the cells of the MPS can be reduced by coating the particle surface with hydrophilic polymers. The most widely used polymer is polyethylene glycol (PEG), which prevents binding interactions by exerting steric hindrance.[18,50] In this regard, already the first nanomedical product on the market – liposomal doxorubicin – was PEGylated.[51]

Apart from potential clearance, intravascularly applied NPs have to overcome further biological barriers in order to reach their target cells. They have to cross the endothelium, permeate the interstitial space, and enter the cell as well as (if necessary) the cell nucleus.[21,22] For other routes of administration, additional transport barriers, such as the skin or the mucosa of the lung or intestine, have to be penetrated first.[21] However, several strategies have been developed to overcome these hurdles.[21] Moreover, the situation can be different under pathophysiological conditions. For example, in tumor or inflamed tissue endothelial gaps widen and thus facilitate extravasation of NPs from the vascular system.[21]

Regarding nanotoxicological aspects, future developments should focus on biocompatible and biodegradable nanoconstructs based on natural (e.g., gelatin or hyaluronic

acid) or synthetic (e.g., polylactic acid) polymers.[45,48]

1.1.6 Quantum Dots

QDs are highly fluorescent nanocrystals made of semiconducting materials.[31] With sizes in the range of a few nanometers, they provide optical and electrical properties that are not found in their respective bulk materials. The charge carriers are strongly confined in all three spatial dimensions (quantum confinement effect) so that energy levels are no longer continuous, but discrete and directly depend on QD size (see Figure 2).[52] An incoming photon with an energy higher than the band gap excites an electron from the valence band into the conduction band, creating an electron-hole-pair or exciton, which produces a photon upon electron-hole recombination.[53] The wavelength of the emitted photon depends on the size of the QD, as for smaller QDs the stronger charge carrier confinement leads to a larger band gap.[53] The optically active core (e.g., CdS, PbSe, or InAs) is typically passivated with a shell (typically ZnS) to protect the core from oxidation, enhance quantum yield, and decrease leakage of heavy metals from the core.[54,55]

QDs are utilized as fluorescent imaging tools, as they feature high brightness and photostability making them superior to traditional fluorophores.[54] Wide absorption spectra and narrow emission spectra (full-width at half-maximum ca. 25–40 nm) allow for multiplexing, in which QDs with different colors can be simultaneously excited and detected.[53,55] As explained above, the emission wavelengths of QDs are size-tunable, ranging from the ultraviolet to the infrared.[53] Near infrared-emitting QDs are especially suited for imaging living tissues.[55]

Biomedical applications of QDs involve super-resolution microscopy, single-particle tracking/ single-molecule tracking (e.g., QD-tagged molecular motors), drug delivery (e.g., conjugation of a drug to the QD surface), gene delivery, and multimodal imaging (magnetic QDs for fluorescence detection and MRI).[53,57] Moreover, QD-based immunoassays (utilizing QD-labeled antibodies) and QD-based fluorescence resonance energy transfer (FRET) have been reported.[52] Apart from biomedical

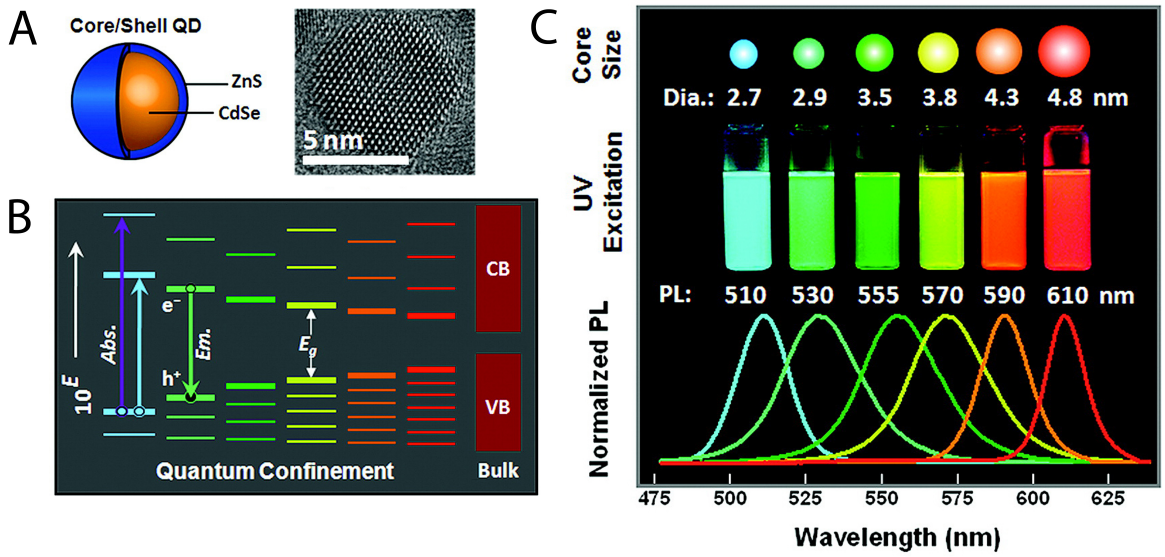

Figure 2: Characteristics of QDs. A) Schematic sketch and transmission electron micrograph of a core-shell QD. B) Semiconductor QDs possess discrete energy levels – in contrast to the bulk semiconductor with conduction band (CB) and valence band (VB). With increasing QD size, the band gap energy (E_g) decreases. Abs.: absorption, Em.: emission. C) With increasing QD size, the smaller band gap leads to longer photoluminescence (PL) wavelengths. Dia.: diameter. Adapted with permission from Ref. 56. Copyright 2011 American Chemical Society.

applications, QDs are, for example, used in photovoltaic cells and light emitting devices.[5]

QD cores often contain heavy metals such as cadmium, raising the question of toxicity. In general, QD toxicity depends on dose, size, composition, charge, and surface chemistry.[31,54] As these factors vary widely, toxicity has to be assessed individually. In any case, low concentration and high stability can reduce toxicity significantly.[53] Thus, potential toxicity should not prevent the use of QDs as they are outstanding fluorescence imaging tools for *in vitro* and *in vivo* biomedical applications. However, QD-based applications probably will not be allowed for medical use in humans. As an alternative to heavy metal-based QDs, biocompatible QDs, for example, based on silicon, are being developed.[58]

1.2 Previous Work

The growing use of engineered NPs as well as increasing air pollution by (nano-sized) particulate matter demands knowledge about interactions between NPs and biological systems. As stated above, it is known that various NPs can translocate into the bloodstream.[7] On the other hand, NPs may be deliberately injected into the circulation in biomedical applications. From this platform, NPs are able to reach various tissues and organs. Apart from that, the (micro)vasculature is essential for numerous regulatory, immunologic, and metabolic functions. Hence, it is crucial to investigate how NPs interact with blood vessel walls, if they are taken up by the endothelium, and if they are able to overcome the blood-tissue barrier. Moreover, in the context of possible adverse health effects of NPs, the potential to elicit an immune response needs to be studied.

Therefore, the fate and effects of NPs *in vivo* were investigated by our group. Commercially available core-shell QDs were used as fluorescent model NPs. To study the influence of surface modifications on the particle behavior, QDs with carboxyl, amino (PEG), or PEG surface groups were applied. These QDs have been designed for the use in *in vivo* imaging applications, e.g., PEG QDs can be used as vascular labels as they exhibit a blood half-life time of several hours. Besides, these QDs have been already employed to assess the influence of surface modifications on cellular uptake mechanisms and cytotoxic effects.[59,60,61,62]

The distribution of QDs was studied in the microvasculature of the mouse cremaster muscle. It was demonstrated that under physiological conditions, surface modification strongly influences the localization of QDs in postcapillary venules, their uptake by perivascular macrophages, and their ability to initiate an inflammatory response.[63] More precisely, carboxyl QDs were found in caveolae of endothelial cells and were rapidly taken up by perivascular macrophages, where they localized in the endo-lysosomal compartment and the cytoplasm.[63] In contrast, PEG QDs were rarely found in the cytoplasm of perivascular macrophages, but were found to be attached to amorphous lipid-containing material in between endothelial cells.

Amino (PEG) QDs were rarely seen both in perivascular cells and endothelial cells. In addition, only carboxyl QD enhanced leukocyte recruitment, which was found to be mediated by mast cell degranulation.[63] Moreover, it was shown that carboxyl QDs do not only accumulate in organs of the MPS (mostly liver and spleen), but also associate with the capillary endothelium of skeletal and heart muscle tissue and thus are cleared from the circulation and deposited in the tissue.[64] In contrast, for PEG QDs an association with the capillary endothelium was absent.[64] In studies under pathophysiological conditions (ischemia-reperfusion), amino QDs, but not carboxyl QDs, were strongly associated with vessel walls of postcapillary venules and increased ischemia-reperfusion-induced leukocyte recruitment.[65] Taken together, these studies provide evidence that the behavior of NPs *in vivo* is strongly influenced both by surface modification and the physiological condition of the tissue.

1.3 Objectives of this Thesis

The studies summarized in the previous section characterized the interactions of QDs with microvessel walls and elucidated the influence of surface modification on particle behavior and the ability to elicit leukocyte recruitment. Moreover, it was observed that shortly after intraarterial administration, carboxyl QDs are not only taken up by perivascular macrophages, but also appear in tissue-resident cells located far away from vessels. However, the mechanism behind this phenomenon remained unclear. In addition, when conducting NP-based diagnosis or therapy, not only interaction with the vessel plays a role, but it is essential that the NPs overcome the blood-tissue border and move through the tissue to efficiently reach their target, for example, a specific cell type. Hence, it is crucial to understand the spatiotemporal dynamics of NPs at the microscopic tissue level and how the behavior may be influenced by surface modifications. Furthermore, not only for nanomedical applications, but also for nanotoxicological studies, information about the microdistribution is required to predict position and concentration of NPs within tissues.

To address this question, in this thesis the local distribution of NPs was investigated at the microscopic tissue level. Core-shell QDs (see sections 1.1.6 and 1.2) were used as fluorescent model NPs. To study the influence of surface modifications on the distribution, QDs with either a carboxyl or a PEG coating were applied. Chapter 2 [Nekolla *et al.*, 2016] focuses on the interaction of QDs with tissue constituents. More precisely, the aim was to characterize the dynamics of QDs in the interstitial space and their interaction with tissue compartments such as the ECM. Moreover, a goal was to investigate the extra- and intracellular distribution of QDs at the blood-tissue interface after interstitial injection. On the other hand, chapter 3 [Rehberg, Nekolla *et al.*, 2016] complements the results from chapter 2 by investigating the tissue distribution of QDs after intraarterial administration and illuminates the appearance of carboxyl QDs in cells far away from the nearest vessel. The aim was to characterize the involved cells and the underlying transport mechanism.

1.4 Materials and Methods

Qdot 655 ITK carboxyl quantum dots and Qtracker 655 non-targeted (PEG) quantum dots with a 655 nm fluorescence peak emission as well as Qdot 525 ITK carboxyl quantum dots with a 525 nm fluorescence peak emission were purchased from Life Technologies (Carlsbad, CA, United States). The QDs consist of a CdSe core encapsulated by a ZnS shell and an additional polymer coating with carboxyl or PEG surface groups, respectively. The PEG coating itself consists of short oligomers with a molecular weight of 1–3 kDa. The core-shell dimensions of the elongated 655-QDs are 10 nm $\times$ 12 nm and the spherical 525-QDs have a core-shell diameter of 3–4 nm (measured with TEM; Life Technologies, personal communication). The coated QDs are 18 nm (655-QDs) or 12 nm (525-QDs) in diameter, respectively (determined by size exclusion chromatography; Life Technologies, personal communication). Carboxyl QDs are negatively charged in PBS (with or without serum), whereas PEG QDs exhibit a near neutral surface charge.[64]

The mouse cremaster muscle of healthy mice was employed as a model system for skeletal muscle tissue. Prior to microscopy, the muscle was surgically prepared and mounted on the pedestal of a microscopy stage. QDs were locally microinjected with micrometer precision by using a microinjection system equipped with a micromanipulator or systemically administered via intra-arterial or intrascrotal injection. To visualize the dynamic distribution of QDs and their interaction with tissue structures, *in vivo* real-time reflected light oblique transillumination and epifluorescence microscopy were applied.[66] QD-containing vesicles were imaged with video microscopy and manually tracked to determine vesicle kinetics.

Following *in vivo* experiments, the tissue was (immuno)stained to localize QDs in cells and at tissue structures using confocal microscopy. Additionally, transmission electron microscopy (TEM) was employed to reveal the ultrastructural localization of QDs in the tissue.

For quantitative analysis of extracellular QD dynamics, multiple particle tracking of microinjected QDs was performed. In multiple particle tracking, the microscopic motion of a multitude of particles is imaged using fast video microscopy and subsequently tracked using an automated tracking algorithm. From the resulting trajectories, parameters such as mean squared displacement can be computed to characterize the mode of motion.

In addition to the *in vivo* experiments, the dynamics of QDs were investigated in corresponding *in vitro* studies. QDs were microinjected into two structurally different model hydrogels: porous Matrigel (resembling endothelial basement membranes (BMs))[67] and fibrillar collagen I, the most abundant type of collagens in humans.[68] Subsequently, the distribution was monitored over two hours using time-lapse microscopy. In addition, QD-containing hydrogels were fluorescently stained to analyze colocalization with QDs using confocal microscopy.

1.5 Results

Chapter 2 [Nekolla *et al.*, 2016] focuses on the extra- and intracellular spatiotemporal microdistribution of carboxyl QDs and compares it to the behavior of PEG QDs. As observed by *in vivo* fluorescence microscopy, PEG QDs show little interaction with tissue constituents, but mainly diffuse in the interstitial space. In contrast, carboxyl QDs bind to tissue components quickly after microinjection. More specifically, TEM revealed that carboxyl QDs bind to collagen fibers, fasciae of muscle fibers, as well as BMs, a type of ECM lining the basolateral side of blood vessel walls. In addition, carboxyl QDs are able to translocate into the vessel lumen, as they can be found in caveolae of endothelial cells and in endothelial junctions. Carboxyl QDs even appear in the so-called lateral border recycling compartment, a specialized membrane reservoir of the endothelium.[69] Matched *in vitro* experiments with hydrogels confirmed the *in vivo* QD distribution. While PEG QDs diffuse in the hydrogels, carboxyl QDs immediately bind to collagen I fibers or the Matrigel constituents laminin and collagen IV, respectively. The results indicate that components of the ECM constitute a selective barrier depending on QD surface modification.

Chapter 3 [Rehberg, Nekolla *et al.*, 2016] concentrates on the intercellular distribution of QDs and shows that immune cells play a part in the microdistribution of NPs in the tissue. Previously, our group observed that shortly after intraarterial administration, carboxyl QDs are not only taken up by perivascular macrophages, but also appear in tissue-resident cells located far away from vessels. Expanding this study, we found that perivascular and tissue-resident macrophages are interconnected by a network of so-called membrane nanotubes (MNTs) and that carboxyl QDs are shuttled via these intercellular “bridges”. TEM elucidated that inside MNTs carboxyl QDs are localized in endosomal vesicles that colocalize with microtubules. In addition, video microscopy revealed fast bidirectional vesicle movement arguing for transport along microtubules by molecular motors. Interestingly, this phenomenon cannot only be observed after systemic administration, but also after local interstitial microinjection of carboxyl QDs.

1.6 Discussion and Outlook

Recently, Amin *et al.* predicted that surface modification soon will revolutionize the therapeutic applications of NPs, as their surface properties strongly influence their overall behavior.[70] Accordingly, the knowledge acquired in this thesis suggests guidelines for the future design of smart NPs. When biomedically administered NPs shall take effect in a large region, a surface functionalization with PEG is advisable to ensure a high mobility. In contrast, when it is desired that the locally injected NPs form a depot for slow and continuous drug release, carboxyl surface groups enable the NPs to bind to tissue constituents and remain at the site of injection. In addition, carboxylation may be used to target BMs or the endothelium. As shown in chapter 3 [Rehberg, Nekolla *et al.*, 2016], for intravascularly administered carboxyl QDs, transport over tissue barriers (i.e., blood vessel walls), efficient cellular uptake by macrophages, and intercellular distribution between macrophages takes place. Thus, carboxylated nanosized drug carriers may be used to quickly transport drugs from the blood to cells located deeper in the tissue. Moreover, a combination of carboxyl groups and long, cleavable PEG chains is imaginable: the PEG groups guarantee for a long circulation time and can be cleaved at the target site to expose the carboxyl groups so that the NPs can be efficiently taken up by cells or bind to tissue constituents. In this line, further research is necessary to be able to create smart NPs that meet the requirements of the respective application regarding the distribution in the body and especially in the target tissue.

The PEG and carboxyl QDs used in this thesis are commercially available. Unfortunately, no amino QDs are provided by Life Technologies, but only amino (PEG) QDs. Microinjected into collagen gels, these amino (PEG) QDs rapidly diffuse from the site of injection, thus acting similarly to PEG QDs. Also in the immunofluorescent staining of hydrogels, amino (PEG) QDs do not bind to collagen I or the Matrigel constituents collagen IV and laminin, respectively, but are widely washed out during the staining process. Thus, the amino (PEG) QDs essentially behave like PEG QDs. Unfortunately, no definite conclusion can be drawn, whether this behavior depends on

the PEG coating or on the presence of the amino groups. Nevertheless, as surface modification critically influences the interaction between NPs and biomolecules,[63,71,72] future research studying the microdistribution of NPs with cationic surface groups is encouraged. In this context, it would be especially interesting if cationic QDs are subject to transport in MNTs.

As previously published by our group, the behavior and effects of intravascularly injected QDs are not only critically influenced by surface modification, but also by the underlying tissue condition.[65] Accordingly, it would be interesting to study the local distribution of microinjected QDs under pathophysiological conditions, for example, their binding to constituents of the ECM. In inflamed tissue, the ECM is strongly remodeled by the action of diverse matrix metalloproteinases and certain cytokines, such as tumor necrosis factor or interferon-γ.[73] Similarly, in tumor tissue the amount and composition of the ECM changes drastically (including the overproduction of different types of collagens), leading to disorganization and loss of essential functions of the ECM.[74] Thus, especially in terms of future biomedical applications, the impact of surface chemistry on NP distribution in inflamed or tumor tissues needs to be addressed. In addition, as the physical properties of the ECM strongly influence cell function, it would be interesting to study if the binding of carboxyl QDs to constituents of the ECM influences cellular behavior *in vivo*.[74] Besides, it would be worthwhile investigating potential changes in the assembly of the MNT network of cells under pathophysiological conditions, as this could influence the intercellular transport of NMs.

This thesis investigates the distribution of NPs not only after intravascular administration, but also after local injection into the tissue. With local microinjection, the microdistribution of NPs in the tissue can be directly observed. More precisely, the dynamics of NPs in or their interaction with tissue compartments, such as muscle fibers, microvessels, or connective tissue, can be studied. Also in the context of NP-based therapy, local injection can be considered as a type of administration. That way, tissue barriers, including the vascular endothelium and tissue interstitium, can

be circumvented. As an example, to treat inflamed tissue, a localized and continuous release of an anti-inflammatory drug would be preferential. In this context, micro- and nanosized drug delivery vehicles have been developed to locally treat arthritis. The nanocarriers offer sustained release and reduced side effects in comparison to systemic administration of the drugs.[75] Similarly, Hosseini *et al.* developed nanosized liposomes as carriers for prednisolone, and intramuscular injection resulted in a longer-lasting anti-inflammatory effect of the liposomes compared to free prednisolone in rats.[76]

The results of this thesis reveal that the type of administration partly influences the microdistribution of QDs. Carboxyl QDs are taken up by perivascular and tissue-resident macrophages and are transported between these cells in MNTs, no matter if they are intraarterially or intrascrotally administered or locally microinjected. Additionally, after interstitial microinjection, carboxyl QDs adhere to tissue constituents, such as collagen fibers, muscle fasciae, or BMs. The direction of transcytosis (a mechanism that mediates the bidirectional exchange of macromolecules between blood vessel lumen and interstitial space) is influenced by the type of administration.[77] QDs microinjected into the interstitial space are transported into the vessel lumen, whereas after intraarterial injection, transcytosis of QDs to the abluminal side of the vessel takes place.[63] In this regard, in chapter 3 [Rehberg, Nekolla *et al.*, 2016] it was shown that perivascular macrophages form distinct contact sites at postcapillary venules, however, there was no proof of a direct cellular access to the vessel lumen. Another possibility is that carboxyl QDs are shuttled to the contact sites by transcytosis and subsequently taken up by perivascular macrophages.

In conclusion, this thesis illuminates the extra-, intra-, and intercellular distribution of QDs at the microscopic tissue scale. The microdistribution is critically influenced by the surface modification of the particles, which should be taken into consideration for the future design of NPs that are developed for the use in biomedical applications ranging from diagnostics to therapy. Furthermore, it is important to keep in mind that the tissue distribution of NPs takes place via different routes including the transport via networks of cells interconnected by MNTs.

2 Influence of Surface Modifications on the Spatiotemporal Microdistribution of Quantum Dots In Vivo

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2.1 Author Contributions

K. Nekolla, F. Krombach, and M. Rehberg designed the research.

K. Nekolla, S. Sellner, F. Krombach, and M. Rehberg discussed the results.

K. Nekolla and M. Rehberg wrote the manuscript.

K. Kick, S. Sellner, S. Zahler, D. Zeuschner, and F. Krombach critically evaluated the results and finalized the manuscript.

K. Nekolla

- Performance of the experiments, data collection, data analysis, and interpretation of the data presented in Figures 1-3, Figure 5, Figure S1, and Figure S2.
- Data collection, data analysis, and interpretation of the data presented in Figure 6.
- Preparation of all figures.

K. Kick performed the experiments for Figure 6.

S. Sellner helped with intravital microscopy.

K. Mildner and D. Zeuschner collected and interpreted the data presented in Figure 4.

D. Zeuschner helped with the transmission electron microscopy part of the manuscript.

M. Rehberg performed the experiments for Figure 4 and helped with microscopy.

2.2 Main Manuscript

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3 Intercellular Transport of Nanomaterials is Mediated by Membrane Nanotubes In Vivo

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3.1 Author Contributions

M. Rehberg and F. Krombach designed the study.

K. Nekolla, S. Sellner, F. Krombach, and M. Rehberg discussed the results.

K. Nekolla, S. Sellner, M. Praetner, D. Zeuschner and F. Krombach critically evaluated the results and finalized the manuscript.

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- Performance of the experiments, data collection, data analysis, and interpretation of the data presented in Figures 1, Figure 3, Figure 4A, B, D-F and Figure 5A, B.
- Performance of the experiments of the data presented in Figure 2F, G, Figure 5C, D, and Figure 6.
- Preparation of Figures 1-5 and Figure S1.
- Wrote the manuscript.

K. Nekolla

- Performance of the experiments, data collection, data analysis, and interpretation of the data shown in Figure 4C.
- Analysis and representation of the data shown in Figure 6.
- Preparation of Figure 6.

S. Sellner performed the experiments, data collection, data analysis, and interpretation of the data presented in Figure 2A-E and Figure S1.

M. Praetner performed the initial *in vivo* experiments.

K. Mildner and D. Zeuschner collected and interpreted the data presented in Figure 2F, G and Figure 5C, D.

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3.3 Supplemental Information

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Bibliography

[1] M. Curreli, R. Zhang, F. N. Ishikawa, H.-K. Chang, R. J. Cote, C. Zhou, and M. E. Thompson, "Real-time, label-free detection of biological entities using nanowire-based fets," *IEEE Transactions on Nanotechnology*, vol. 7, no. 6, pp. 651–667, 2008.

[2] J. Conde, J. T. Dias, V. Grazu, M. Moros, P. V. Baptista, and J. M. de la Fuente, "Revisiting 30 years of biofunctionalization and surface chemistry of inorganic nanoparticles for nanomedicine," *Front Chem*, vol. 2, pp. 1–27, 2014.

[3] V. Biju, T. Itoh, A. Anas, A. Sujith, and M. Ishikawa, "Semiconductor quantum dots and metal nanoparticles: syntheses, optical properties, and biological applications," *Analytical and Bioanalytical Chemistry*, vol. 391, no. 7, pp. 2469–2495, 2008.

[4] L. Y. Chou, K. Ming, and W. C. Chan, "Strategies for the intracellular delivery of nanoparticles," *Chem Soc Rev*, vol. 40, no. 1, pp. 233–45, 2011.

[5] C. Chang, "The immune effects of naturally occurring and synthetic nanoparticles," *J Autoimmun*, vol. 34, no. 3, pp. 234–46, 2010.

[6] O. A. Sadik, "Anthropogenic nanoparticles in the environment," *Environ Sci Process Impacts*, vol. 15, no. 1, pp. 19–20, 2013.

[7] G. Oberdorster, E. Oberdorster, and J. Oberdorster, "Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles," *Environ Health Perspect*, vol. 113, no. 7, pp. 823–39, 2005.

[8] D. Jariwala, V. K. Sangwan, L. J. Lauhon, T. J. Marks, and M. C. Hersam, "Carbon nanomaterials for electronics, optoelectronics, photovoltaics, and sensing," *Chem Soc Rev*, vol. 42, no. 7, pp. 2824–60, 2013.

[9] I. Gurrappa and L. Binder, "Electrodeposition of nanostructured coatings and their characterization-a review," *Science and Technology of Advanced Materials*, vol. 9, no. 4, pp. 1–11, 2008.

[10] S. C. Smith and D. F. Rodrigues, "Carbon-based nanomaterials for removal of chemical and biological contaminants from water: A review of mechanisms and applications," *Carbon*, vol. 91, pp. 122–143, 2015.

[11] S. Raj, S. Jose, U. S. Sumod, and M. Sabitha, "Nanotechnology in cosmetics: Opportunities and challenges," *J Pharm Bioallied Sci*, vol. 4, no. 3, pp. 186–93, 2012.

[12] H. Kettiger, A. Schipanski, P. Wick, and J. Huwyler, "Engineered nanomaterial uptake and tissue distribution: from cell to organism," *Int J Nanomedicine*, vol. 8, pp. 3255–69, 2013.

[13] L. Rashidi and K. Khosravi-Darani, "The applications of nanotechnology in food industry," *Crit Rev Food Sci Nutr*, vol. 51, no. 8, pp. 723–30, 2011.

[14] S. M. Hussain, D. B. Warheit, S. P. Ng, K. K. Comfort, C. M. Grabinski, and L. K. Braydich-Stolle, "At the crossroads of nanotoxicology in vitro: Past achievements and current challenges," *Toxicological Sciences*, vol. 147, no. 1, pp. 5–16, 2015.

[15] H. Ding and F. Wu, "Image guided biodistribution and pharmacokinetic studies of theranostics," *Theranostics*, vol. 2, no. 11, pp. 1040–53, 2012.

[16] M. P. Monopoli, C. Aberg, A. Salvati, and K. A. Dawson, "Biomolecular coronas provide the biological identity of nanosized materials," *Nat Nanotechnol*, vol. 7, no. 12, pp. 779–86, 2012.

[17] R. M. Pearson, V. V. Juettner, and S. Hong, "Biomolecular corona on nanoparticles: a survey of recent literature and its implications in targeted drug delivery," *Front Chem*, vol. 2, pp. 1–7, 2014.

[18] D. E. Owens and N. A. Peppas, "Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles," *Int J Pharm*, vol. 307, no. 1, pp. 93–102, 2006.

[19] G. Song, J. S. Petschauer, A. J. Madden, and W. C. Zamboni, "Nanoparticles and the mononuclear phagocyte system: pharmacokinetics and applications for inflammatory diseases," *Curr Rheumatol Rev*, vol. 10, no. 1, pp. 22–34, 2014.

[20] J. P. Almeida, A. L. Chen, A. Foster, and R. Drezek, "In vivo biodistribution of nanoparticles," *Nanomedicine (Lond)*, vol. 6, no. 5, pp. 815–35, 2011.

[21] S. Barua and S. Mitragotri, "Challenges associated with penetration of nanoparticles across cell and tissue barriers: A review of current status and future prospects," *Nano Today*, vol. 9, no. 2, pp. 223–243, 2014.

[22] M. H. Kafshgari, F. J. Harding, and N. H. Voelcker, "Insights into cellular uptake of nanoparticles," *Current Drug Delivery*, vol. 12, no. 1, pp. 63–77, 2015.

[23] E. V. Brauner, L. Forchhammer, P. Moller, J. Simonsen, M. Glasius, P. Wahlin, O. Raaschou-Nielsen, and S. Loft, "Exposure to ultrafine particles from ambient air and oxidative stress-induced dna damage," *Environ Health Perspect*, vol. 115, no. 8, pp. 1177–82, 2007.

[24] R. D. Brook, S. Rajagopalan, r. Pope, C. A., J. R. Brook, A. Bhatnagar, A. V. Diez-Roux, F. Holguin, Y. Hong, R. V. Luepker, M. A. Mittleman, A. Peters, D. Siscovick, J. Smith, S. C., L. Whitsel, J. D. Kaufman, E. American Heart Association Council on, C. o. t. K. i. C. D. Prevention, P. A. Council on Nutrition, and Metabolism, "Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the american heart association," *Circulation*, vol. 121, no. 21, pp. 2331–78, 2010.

[25] S. Kumar, M. K. Verma, and A. K. Srivastava, "Ultrafine particles in urban ambient air and their health perspectives," *Rev Environ Health*, vol. 28, no. 2-3, pp. 117–28, 2013.

[26] P. S. Vinzents, P. Moller, M. Sorensen, L. E. Knudsen, O. Hertel, F. P. Jensen, B. Schibye, and S. Loft, "Personal exposure to ultrafine particles and oxidative dna damage," *Environ Health Perspect*, vol. 113, no. 11, pp. 1485–90, 2005.

[27] X. M. Li, W. Liu, L. W. Sun, K. E. Aifantis, B. Yu, Y. B. Fan, Q. L. Feng, F. Z. Cui, and F. Watari, "Effects of physicochemical properties of nanomaterials on their toxicity," *Journal of Biomedical Materials Research Part A*, vol. 103, no. 7, pp. 2499–2507, 2015.

[28] R. Sandhir, A. Yadav, A. Sunkaria, and N. Singhal, "Nano-antioxidants: An emerging strategy for intervention against neurodegenerative conditions," *Neurochem Int*, vol. 89, pp. 209–26, 2015.

[29] E. Weir, A. Lawlor, A. Whelan, and F. Regan, "The use of nanoparticles in anti-microbial materials and their characterization," *Analyst*, vol. 133, no. 7, pp. 835–845, 2008.

[30] R. Lehner, X. Wang, S. Marsch, and P. Hunziker, "Intelligent nanomaterials for medicine: carrier platforms and targeting strategies in the context of clinical application," *Nanomedicine*, vol. 9, no. 6, pp. 742–57, 2013.

[31] M. Massey, M. Wu, E. M. Conroy, and W. R. Algar, "Mind your p's and q's: the coming of age of semiconducting polymer dots and semiconductor quantum dots in biological applications," *Curr Opin Biotechnol*, vol. 34, pp. 30–40, 2015.

[32] G. Y. Tonga, K. Saha, and V. M. Rotello, "25th anniversary article: interfacing nanoparticles and biology: new strategies for biomedicine," *Adv Mater*, vol. 26, no. 3, pp. 359–70, 2014.

[33] Y. C. Cao, "Nanomaterials for biomedical applications," *Nanomedicine (Lond)*, vol. 3, no. 4, pp. 467–9, 2008.

[34] B. L. Chung, M. J. Toth, N. Kamaly, Y. J. Sei, J. Becraft, W. J. Mulder, Z. A. Fayad, O. C. Farokhzad, Y. Kim, and R. Langer, "Nanomedicines for endothelial disorders," *Nano Today*, vol. 10, no. 6, pp. 759–776, 2015.

[35] V. P. Torchilin, "Multifunctional, stimuli-sensitive nanoparticulate systems for drug delivery," *Nat Rev Drug Discov*, vol. 13, no. 11, pp. 813–27, 2014.

[36] T. H. Shin, Y. Choi, S. Kim, and J. Cheon, "Recent advances in magnetic nanoparticle-based multi-modal imaging," *Chem Soc Rev*, vol. 44, no. 14, pp. 4501–16, 2015.

[37] L. Zhu and V. P. Torchilin, "Stimulus-responsive nanopreparations for tumor targeting," *Integr Biol (Camb)*, vol. 5, no. 1, pp. 96–107, 2013.

[38] R. Jin, B. Lin, D. Li, and H. Ai, "Superparamagnetic iron oxide nanoparticles for mr imaging and therapy: design considerations and clinical applications," *Curr Opin Pharmacol*, vol. 18, pp. 18–27, 2014.

[39] L. E. Cole, R. D. Ross, J. M. R. Tilley, T. Vargo-Gogola, and R. K. Roeder, "Gold nanoparticles as contrast agents in x-ray imaging and computed tomography," *Nanomedicine*, vol. 10, no. 2, pp. 321–341, 2015.

[40] D. S. Abou, J. E. Pickett, and D. L. Thorek, "Nuclear molecular imaging with nanoparticles: radiochemistry, applications and translation," *Br J Radiol*, vol. 88, no. 1054, p. 20150185, 2015.

[41] O. S. Wolfbeis, "An overview of nanoparticles commonly used in fluorescent bioimaging," *Chemical Society Reviews*, vol. 44, no. 14, pp. 4743–4768, 2015.

[42] O. Perlman, I. S. Weitz, and H. Azhari, "Copper oxide nanoparticles as contrast agents for mri and ultrasound dual-modality imaging," *Physics in Medicine and Biology*, vol. 60, no. 15, pp. 5767–5783, 2015.

[43] J. Garcia, T. Tang, and A. Y. Louie, "Nanoparticle-based multimodal pet/mri probes," *Nanomedicine (Lond)*, vol. 10, no. 8, pp. 1343–59, 2015.

[44] M. L. Etheridge, S. A. Campbell, A. G. Erdman, C. L. Haynes, S. M. Wolf, and J. McCullough, "The big picture on nanomedicine: the state of investigational and approved nanomedicine products," *Nanomedicine*, vol. 9, no. 1, pp. 1–14, 2013.

[45] E. Perez-Herrero and A. Fernandez-Medarde, "Advanced targeted therapies in cancer: Drug nanocarriers, the future of chemotherapy," *Eur J Pharm Biopharm*, vol. 93, pp. 52–79, 2015.

[46] M. Rahman, G. Sharma, K. Thakur, V. G. Goni, O. P. Katare, F. Anwar, and V. Kumar, "Emerging advances in nanomedicine as a nanoscale pharmacotherapy in rheumatoid arthritis: State of the art," *Curr Top Med Chem*, 2016.

[47] J. H. Ryu, S. Lee, S. Son, S. H. Kim, J. F. Leary, K. Choi, and I. C. Kwon, "Theranostic nanoparticles for future personalized medicine," *J Control Release*, vol. 190, pp. 477–84, 2014.

[48] M. Karimi, A. Ghasemi, P. Sahandi Zangabad, R. Rahighi, S. M. Moosavi Basri, H. Mirshekari, M. Amiri, Z. Shafaei Pishabad, A. Aslani, M. Bozorgomid, D. Ghosh, A. Beyzavi, A. Vaseghi, A. R. Aref, L. Haghani, S. Bahrami, and M. R. Hamblin, "Smart micro/nanoparticles in stimulus-responsive drug/gene delivery systems," *Chem Soc Rev*, vol. 45, no. 5, pp. 1457–1501, 2016.

[49] Y. Cohen and S. Y. Shoushan, "Magnetic nanoparticles-based diagnostics and theranostics," *Curr Opin Biotechnol*, vol. 24, no. 4, pp. 672–81, 2013.

[50] R. Gref, Y. Minamitake, M. T. Peracchia, V. Trubetskoy, V. Torchilin, and R. Langer, "Biodegradable long-circulating polymeric nanospheres," *Science*, vol. 263, no. 5153, pp. 1600–3, 1994.

[51] P. P. Wibroe, D. Ahmadvand, M. A. Oghabian, A. Yaghmur, and S. M. Moghimi, "An integrated assessment of morphology, size, and complement activation of the pegylated liposomal doxorubicin products doxil(r), caelyx(r), doxorubicin, and sinadoxosome," *J Control Release*, vol. 221, pp. 1–8, 2016.

[52] T. R. Pisanic, Y. Zhang, and T. H. Wang, "Quantum dots in diagnostics and detection: principles and paradigms," *Analyst*, vol. 139, no. 12, pp. 2968–81, 2014.

[53] K. D. Wegner and N. Hildebrandt, "Quantum dots: bright and versatile in vitro and in vivo fluorescence imaging biosensors," *Chem Soc Rev*, vol. 44, no. 14, pp. 4792–834, 2015.

[54] R. Bilan, F. Fleury, I. Nabiey, and A. Sukhanova, "Quantum dot surface chemistry and functionalization for cell targeting and imaging," *Bioconjugate Chemistry*, vol. 26, no. 4, pp. 609–624, 2015.

[55] I. L. Medintz, H. T. Uyeda, E. R. Goldman, and H. Mattoussi, "Quantum dot bioconjugates for imaging, labelling and sensing," *Nat Mater*, vol. 4, no. 6, pp. 435–46, 2005.

[56] W. R. Algar, K. Susumu, J. B. Delehanty, and I. L. Medintz, "Semiconductor quantum dots in bioanalysis: crossing the valley of death," *Anal Chem*, vol. 83, no. 23, pp. 8826–37, 2011.

[57] C. E. Probst, P. Zrazhevskiy, V. Bagalkot, and X. H. Gao, "Quantum dots as a platform for nanoparticle drug delivery vehicle design," *Advanced Drug Delivery Reviews*, vol. 65, no. 5, pp. 703–718, 2013.

[58] A. Das and P. T. Snee, "Synthetic developments of nontoxic quantum dots," *Chemphyschem*, vol. 17, no. 5, pp. 598–617, 2016.

[59] L. W. Zhang and N. A. Monteiro-Riviere, "Mechanisms of quantum dot nanoparticle cellular uptake," *Toxicol Sci*, vol. 110, no. 1, pp. 138–55, 2009.

[60] L. W. Zhang, W. W. Yu, V. L. Colvin, and N. A. Monteiro-Riviere, "Biological interactions of quantum dot nanoparticles in skin and in human epidermal keratinocytes," *Toxicol Appl Pharmacol*, vol. 228, no. 2, pp. 200–11, 2008.

[61] J. P. Ryman-Rasmussen, J. E. Riviere, and N. A. Monteiro-Riviere, "Surface coatings determine cytotoxicity and irritation potential of quantum dot nanoparticles in epidermal keratinocytes," *J Invest Dermatol*, vol. 127, no. 1, pp. 143–53, 2007.

[62] J. P. Ryman-Rasmussen, J. E. Riviere, and N. A. Monteiro-Riviere, "Penetration of intact skin by quantum dots with diverse physicochemical properties," *Toxicol Sci*, vol. 91, no. 1, pp. 159–65, 2006.

[63] M. Rehberg, M. Praetner, C. F. Leite, C. A. Reichel, P. Bihari, K. Mildner, S. Duhr, D. Zeuschner, and F. Krombach, "Quantum dots modulate leukocyte adhesion and transmigration depending on their surface modification," *Nano Lett*, vol. 10, no. 9, pp. 3656–64, 2010.

[64] M. Praetner, M. Rehberg, P. Bihari, M. Lerchenberger, B. Uhl, M. Holzer, M. E. Eichhorn, R. Furst, T. Perisic, C. A. Reichel, U. Welsch, and F. Krombach, "The contribution of the capillary endothelium to blood clearance and tissue deposition

of anionic quantum dots in vivo," *Biomaterials*, vol. 31, no. 26, pp. 6692–700, 2010.

[65] M. Rehberg, C. F. Leite, K. Mildner, J. Horstkotte, D. Zeuschner, and F. Krombach, "Surface chemistry of quantum dots determines their behavior in postischemic tissue," *ACS Nano*, vol. 6, no. 2, pp. 1370–9, 2012.

[66] T. R. Mempel, C. Moser, J. Hutter, W. M. Kuebler, and F. Krombach, "Visualization of leukocyte transendothelial and interstitial migration using reflected light oblique transillumination in intravital video microscopy," *Journal of Vascular Research*, vol. 40, no. 5, pp. 435–441, 2003.

[67] H. K. Kleinman and G. R. Martin, "Matrigel: basement membrane matrix with biological activity," *Semin Cancer Biol*, vol. 15, no. 5, pp. 378–86, 2005.

[68] G. A. Di Lullo, S. M. Sweeney, J. Korkko, L. Ala-Kokko, and J. D. San Antonio, "Mapping the ligand-binding sites and disease-associated mutations on the most abundant protein in the human, type i collagen," *Journal of Biological Chemistry*, vol. 277, no. 6, pp. 4223–4231, 2002.

[69] W. A. Muller, "How endothelial cells regulate transmigration of leukocytes in the inflammatory response," *American Journal of Pathology*, vol. 184, no. 4, pp. 886–896, 2014.

[70] M. L. Amin, J. Y. Joo, D. K. Yi, and S. S. An, "Surface modification and local orientations of surface molecules in nanotherapeutics," *J Control Release*, vol. 207, pp. 131–42, 2015.

[71] S. Tenzer, D. Docter, J. Kuharev, A. Musyanovych, V. Fetz, R. Hecht, F. Schlenk, D. Fischer, K. Kiouptsi, C. Reinhardt, K. Landfester, H. Schild, M. Maskos, S. K. Knauer, and R. H. Stauber, "Rapid formation of plasma protein corona critically affects nanoparticle pathophysiology," *Nat Nanotechnol*, vol. 8, no. 10, pp. 772–81, 2013.

[72] M. Lundqvist, J. Stigler, G. Elia, I. Lynch, T. Cedervall, and K. A. Dawson, "Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts," *Proc Natl Acad Sci U S A*, vol. 105, no. 38, pp. 14265–70, 2008.

[73] L. Sorokin, "The impact of the extracellular matrix on inflammation," *Nat Rev Immunol*, vol. 10, no. 10, pp. 712–23, 2010.

[74] P. Lu, V. M. Weaver, and Z. Werb, "The extracellular matrix: a dynamic niche in cancer progression," *J Cell Biol*, vol. 196, no. 4, pp. 395–406, 2012.

[75] Z. Zhang and G. Huang, “Micro- and nano-carrier mediated intra-articular drug delivery systems for the treatment of osteoarthritis,” *Journal of Nanotechnology*, vol. 2012, pp. 1–11, 2012.

[76] S. H. Hosseini, A. Maleki, H. R. Eshraghi, and M. Hamidi, “Preparation and in vitro/pharmacokinetic/pharmacodynamic evaluation of a slow-release nano-liposomal form of prednisolone,” *Drug Deliv*, pp. 1–9, 2016.

[77] M. Simionescu, D. Popov, and A. Sima, “Endothelial transcytosis in health and disease,” *Cell Tissue Res*, vol. 335, no. 1, pp. 27–40, 2009.

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Hiermit erkläre ich, Anna Katharina Nekolla, an Eides statt, dass ich die vorliegende Dissertation mit dem Thema:

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