VILNIUS UNIVERSITY

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INVESTIGATION OF QUANTUM DOTS MIGRATION IN THE ORGANISM USING OPTICAL METHODS

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Abbreviations

BM – basement membrane

CdSe/ZnS-mPEG – CdSe/ZnS core/shell structured quantum dots coated with amphiphilic polymer and methoxy-polyethylene glycol

CdTe-MPA – CdTe core quantum dots coated with mercaptopropionic acid

HE – hematoxylin and eosin staining

MPA – mercaptopropionic acid

mPEG – methoxy-polyethylene glycol

NP – nanoparticles

PAS – periodic acid-Schiff staining

PL – photoluminescence

QD – quantum dots

RES – reticuloendothelian system

RGB – 3 channel detector (red, green, blue)

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1. INTRODUCTION

Nanotechnologies offer new methods for the diagnostics and therapy to deal with medical problems which are not solved using current techniques. Nanoparticles (NP) possess new properties which are not prominent in the bulk materials of the same chemical composition, e.g. photoluminescence (PL), superparamagnetism, thermal and electrical superconductance, etc. It enables to apply NP as contrast agents for optical, magnetic resonance, X-ray or multimodal imaging for diagnostics. NP are shown to increase the efficiency of drug and gene delivery for therapeutic purposes. At the nanoscale the particles reach high surface to volume ratio which is important for surface-based interactions with biomolecules like cellular receptors, enzymes or genetic material. The physical properties and biological effects of NP can be easily modulated by chemical synthesis and surface modifications. These possibilities expand the field of biomedical NP applications and make them superior over conventional organic drugs.

Quantum dots (QD) are semiconductor NP which exhibit exceptional optical properties like photostability, high photoluminescence quantum yield, size tunable emission spectrum, broad absorption spectra, flexible surface engineering, etc. It enables to use QD for non-invasive optical imaging of tumours, visualization of certain anatomical structures during surgery and studying the processes of angiogenesis. QD can be functionalized to achieve tissue specific accumulation for targeted imaging. More to add, they can be conjugated with drug molecules and serve as drug delivery vehicles. As biodistribution of all inorganic NP mainly depend on size and surface interaction with biomolecules, QD can be used as a model to study the localization of other NP with similar geometrical and superficial properties. However in order to reach optimal beneficial effects of QD and to avoid adverse biological effects, some issues regarding QD biodistribution have to be solved.

It was shown that QD can be used for vascular imaging with high contrast to surrounding tissues. However the accumulation of QD in the vessel walls and the transfer mechanisms of QD to the extracellular space are not fully explored. This issue is important for QD applications related with QD accumulation in specific tissues like brain or foetus, as many drugs are impermeable to the blood-brain or placental barriers. For instance, QD could be used for optical imaging during pregnancy in the absence of

QD effects on embryogenesis. It was shown that QD might be embryotoxic, but the passage of QD to the foetus and mechanisms of QD toxicity remain undiscovered (1).

On the other hand, QD can be used for non-invasive cancer imaging as it was shown that QD might accumulate specifically in the tumour in respect to surrounding healthy tissues. However the biodistribution of QD within the cancerous as well as healthy tissues is not extensively studied. The knowledge about QD localization in the tissues (cells, extracellular matrix, blood vessels, etc) is required to reach the biological target and to maximize the beneficial effects of QD application.

Another issue regarding biomedical QD applications is the estimation of QD permeability through the protective biological barriers of skin, respiratory tract, digestive tract and other. It was shown that QD are impermeable to healthy skin and it‘s main barrier capacity is attributed to the stratum corneum. However the barrier properties of other layers of the skin remain undisclosed. It is important in the case of mechanical epidermal damage. It is also useful to elucidate what are the further QD migration pathways after reaching the dermis and what biological effects can be induced due to QD deposition in skin tissues. On the other hand, QD biodistribution in the skin is important for diagnostic applications which are based on intradermal or subcutaneous QD injection.

QD clearance from the body and the potential risk of long-term accumulation are inevitable issues for any QD application. It was shown that >99% of cadmium which was injected as PEG coated CdSeTe/ZnS QD remained in the tissues of experimental rats up to 4 months (2). It means that these QD were not excreted from the body and some of these QD were degraded releasing cytotoxic compounds. However the reasons of poor QD clearance properties and the mechanisms of QD retention in the body remain unrevealed. Long-term accumulation of QD raises the question about the chemical stability of nanocrystal structure in biological environment.

These premises indicate that safe and efficient biomedical applications of QD require fundamental research on QD penetration through biological barriers, diffusion properties within different tissues, migration pathways in the body, deposition in organs, clearance properties and potential harmful effects.

The aim and objectives

The aim of this dissertation was to investigate the main pathways of CdSe/ZnS-mPEG quantum dots (QD) migration in the tissues of experimental animals using the methods of fluorescence microscopy and spectroscopy.

The objectives:

1) To investigate the penetration of QD through the walls of blood vessels and to evaluate the accumulation of QD in the internal organs.

2) To investigate the penetration of QD through the placental barrier and to evaluate QD accumulation in the embryos.

3) To investigate the biodistribution of QD in skin tissues.

4) To determine the main pathways of QD migration in the organism after different administration routes.

5) To compare the stability and the application possibilities of CdTe-MPA and CdSe/ZnS-mPEG for biomedical imaging *in vivo*.

Scientific novelty and actuality

For the first time, the penetration of QD through the epidermis was investigated in basal-apical direction and the significance of the basement membrane in the barrier properties for the QD penetration through the skin was discussed. These findings reveal biodistribution pattern of QD in the skin tissues and explain their limited passage to the epidermis, hair follicles, sebaceous and sweat glands. These findings are important for the research of NP penetration through the skin barrier. The results can be used for designing NP-based pharmaceuticals which require comprehensive knowledge of NP biodistribution in the skin and their abilities to reach particular tissue targets.

For the first time was shown, that the migration of PEG-coated QD in connective tissues depends on the structure of extracellular derivatives: QD freely migrate in the areolar connective tissues (dermis, epineurium, epimysium, *tunica adventitia*, etc.), but they can not permeate densely organized tissue fibers (basement membranes, perineurium) and don't pass to the epidermis, hair follicles, skin glands, nerves and muscle cells.

For the first time, it was shown that PEG-coated QD do not penetrate to the *tunica media* of healthy blood vessels from the lumen as well as from the exterior of the vessel. It contributes to the general understanding of restrictions for QD diffusion in tissues. This finding can be used for application of NP to visualize the damages of the vessel walls, e.g. atherosclerotic plaques, which are characterised by the disruption of structural integrity. It opens new frontiers for the NP-based diagnostics and therapy.

For the first time, it was shown that PEG-coated QD do not penetrate through the placental barrier and avoid accumulation in the embryos. This finding is important for developing safe by design NP based products, which could be used during pregnancy. These results also contribute to the explanation of QD embryotoxicity mechanisms, as it was not clear whether QD exhibit toxic effects due to their accumulation in embryos or by degradation in maternal tissues and the formation toxic compounds which are transferred through the placenta by different metabolic pathways. Our results support the latter scenario.

Defended statements

1) Intravascularly located CdSe/ZnS-mPEG QD adhere to the endothelium, but they can not permeate it and don‘t accumulate in the *tunica media*.
2) QD can pass to the *tunica adventitia* from the surroundings tissues of the blood vessel, but they are unable to penetrate into the *tunica media*.
3) QD localize in the vascular compartment of most organs and they don‘t extravasate into extracellular space. QD extravasate from sinusoidal capillaries.
4) QD appear in rat placenta but they are not transferred through the placental barrier to the foetuses.
5) The migration of QD in the connective tissues depend on the organisation of the extracellular tissue fibers: QD can freely migrate in the loose connective tissues (dermis, epineurium, epimysium, *tunica adventitia*, etc.), but they can not permeate densely organized tissue fibers (basement membranes, perineurium).
6) The main clearance pathway of QD from the connective tissues is the lymphatic drainage which results in QD accumulation in lymph nodes and pour into blood circulation.
7) Topically applied QD are retained by the stratum corneum and don‘t pass to the deeper layers of the skin.
8) CdSe/ZnS-mPEG QD possess better stability in tissues, lower toxicity and are more suitable for fluorescence imaging *in vivo* than CdTe-MPA QD.

2. MATERIALS AND METHODS

Quantum dots (QD)

Two types of QD were used in the experiments (fig. 1.): a) CdSe/ZnS QD coated with methoxy-polyethylene glycol (mPEG), abbreviated CdSe/ZnS-mPEG (Qtracker-655, λ_{PL}=655±5 nm, non-functionalized, Invitrogen Inc.), b) CdTe QD coated with mercaptopropionic acid, abbreviated CdTe-MPA (λ_{PL}=630±5 nm, PlasmaChem GmbH).

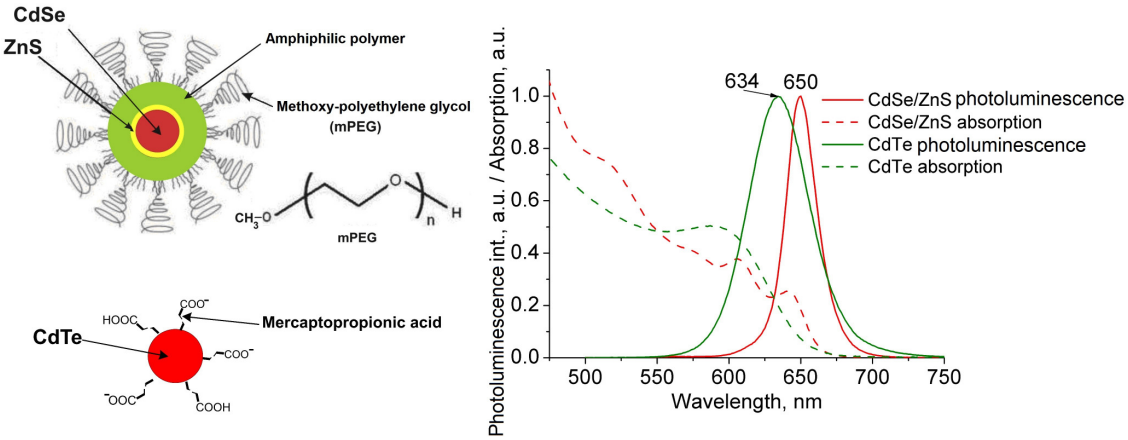

Fig. 1. The model of QD structure and spectroscopic properties of CdSe/ZnS-mPEG and CdTe-MPA QD which were used in the experiments.

Experimental animals

Albino *Wistar* rats and *Balb/c* mice were obtained from the State Scientific Research Institute of Innovative Medical Center (Vilnius, Lithuania). The animal husbandry and experiments on animals were carried out according to the national and European regulations and were approved by the Lithuanian Animal Care and Use Committee (permission no. 0019). Animals were housed under conditions of constant temperature, humidity and standard light /dark cycle. Food and fresh drinking water were available *ad libitum*. Animals were acclimated for at least 7 days before the experiments.

Intravenous injection of QD

In order to estimate QD penetration through the vessel walls and QD biodistribution in the organism 200 µl of CdSe/ZnS-mPEG QD solution at 0,4 µM was injected to the lateral tail vein. Mice were euthanized after 1 h (n=3) and 24 h (n=3). Control group was injected with saline (n=3). Internal organs were removed and blood

samples of 50 µl were collected for fluorescence spectroscopy. Organs were also fixated in 10% formaldehyde solution for 24 h and prepared using standard paraffin embedding technique for microscopical examination. Additionally some organs were frozen at 253 K to prepare slices of ~0,5 mm thickness for fluorescence microscopy.

Subcutaneous injection of QD

In order to determine QD localization in skin tissues 50 µl of CdSe/ZnS-mPEG QD solution at 0,4 µM was injected subcutaneously to the leg (n=5). After 1 h and 24 h mice were euthanized and fluorescence spectra of internal organs were measured and tissues were fixated in the 4% formaldehyde solution for at least 24 h.

QD penetration through placental barrier

For the pregnant rat model female rats were mated overnight with males of the same strain. Vaginal smears from each female rat were collected and subjected to microscopic examination on the following morning in order to determine the oestrous cycle and the presence of sperm. The day of sperm detection in vaginal smears was designated as day 0 of gestation.

3 h after intraperitoneal injection of 500 µl CdSe/ZnS-mPEG QD solution at 0,8 µM the animals (n=4) were sacrificed, internal organs with were excised and analyzed by fluorescence spectroscopy. Afterwards tissues were processed by cryomicrotomy and paraffin embedding techniques for confocal fluorescence microscopy (n=3 rats). Control animals (n=2 rats) were not injected with QD.

Fluorescence spectroscopy

Fluorescence spectroscopy was performed using *Cary Eclipse* spectrometer (*Agilent Technologies*, USA) with fiber optics module ($S_{measured} \approx 20$ mm^2) in order to determine QD accumulation in the embryos and maternal tissues. The organs were washed with saline and dried before measurements. The spectra of internal organs were recorded using the excitation wavelength of 450 nm. The spectra were analyzed and compared with the autofluorescence of the control tissues (uninjected with QD).

Tissue preparation and microscopy

In order to visualize QD localization in the tissues, the samples were prepared by cryomicrotomy (sliced at ~240 K, thickness 20 µm) and standard paraffin embedding techniques (thickness 4 µm). The unstained slices were used for fluorescence microscopy to avoid QD elution and screening effects of the histological dyes. Tissue

slices were imaged using *Nikon Eclipse TE2000* inverted microscope (objectives: x10/0,25, x20/0,5 *Plan Fluor* and x60/1,4 *Plan Apo VC* oil) with confocal scanning system *C1si* which is equipped with RGB detector (channels: 433-467 nm; 500-590 nm; 620-755 nm) and 32-channel spectral detector.

After fluorescence imaging tissue slices were deparaffinised and stained with hematoxylin-eosin (HE) or periodic acid-Schiff (PAS) stains to visualize tissue structure. Brightfield transmission microscopy images were recorded with *Leica DFC-290* RGB CCD camera. Image processing was performed using *ImageJ 1.43* and *Nikon EZ-C1 Bronze version 3.80* software.

Long-term stability of QD in vivo

The solutions of 0,8 μM CdSe/ZnS-mPEG and ~3 μM CdTe-MPA QD of 0,5 ml were injected intraperitoneally (n=3 each) or into the muscle of thigh (n=1 each). 7 days after QD injection animals were euthanized and tissues from the injection site (muscle, omentum) were prepared for fluorescence spectroscopy and tissue histology (paraffin embedding technique).

3. RESULTS

3.1. Distribution of quantum dots (QD) in mice after intravenous injection

Intravenous injection of CdSe/ZnS-mPEG QD resulted in the appearance of QD PL in the spectra of all internal organs after 1 h (kidney, lungs, liver, thymus, spleen, skeletal muscle, heart, etc.). Highest QD PL intensity was observed in the spectra of blood plasma and skin of the injection site (fig. 2). The latter was caused due to the tissue damage and partial extravasation of QD solution during injection procedure. 24 h after injection QD could be observed only in the blood plasma and skin of the injection site. However QD could not be detected in other tissues.

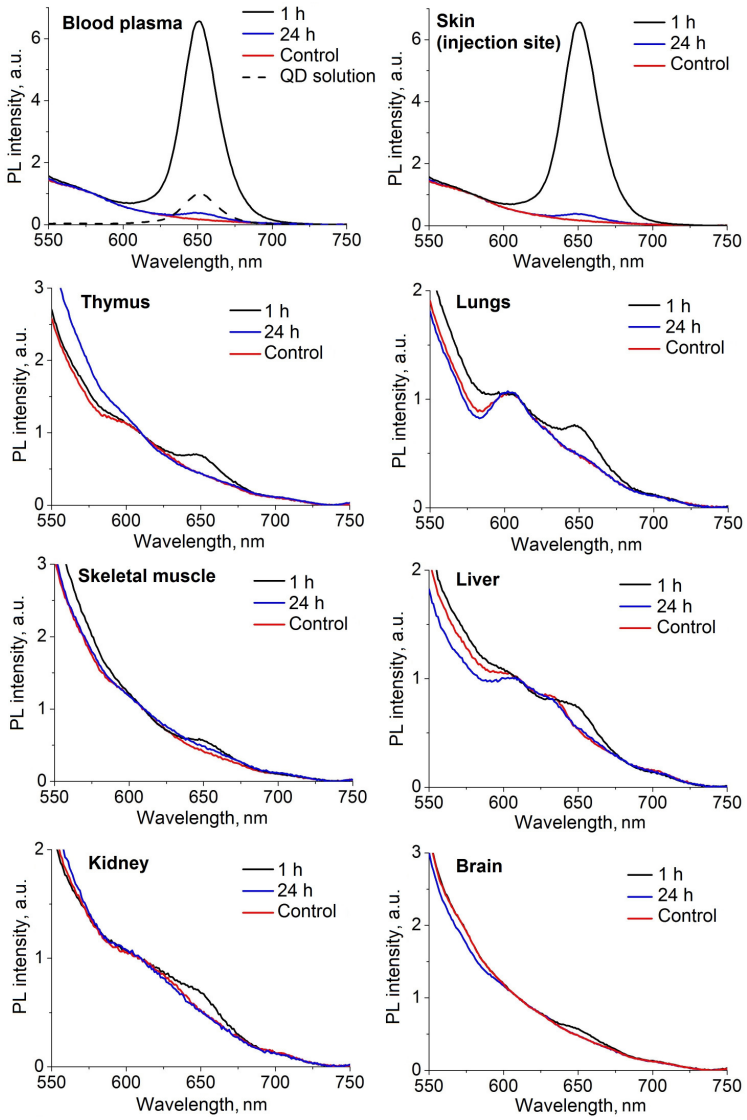

Fig. 2. The averaged and normalized fluorescence spectra of mice tissues 1 h and 24 h after intravenous injection of CdSe/ZnS-mPEG QD solution. Control animals were

injected with saline. The curves represent averaged spectra ($n_{animals/group}$ =3, $n_{spectra/tissue}$=3, $\lambda_{excitation}$=450 nm).

Spectroscopic analysis reflects general accumulation of QD in the organ, however it lacks information about spatial distribution of QD in the tissues. Confocal fluorescence microscopy was used to assess QD localization in the tissues. The analysis of the frozen tissue sections (thickness ~0,5 mm) revealed that QD (red) mainly accumulated in the blood vessels of skeletal muscles, cerebral hemispheres, uterus, heart, skin and other tissues (fig. 3). The network of blood vessels could be clearly seen in the background of green tissue autofluorescence. There was no exatravasation of QD to the intercellular space.

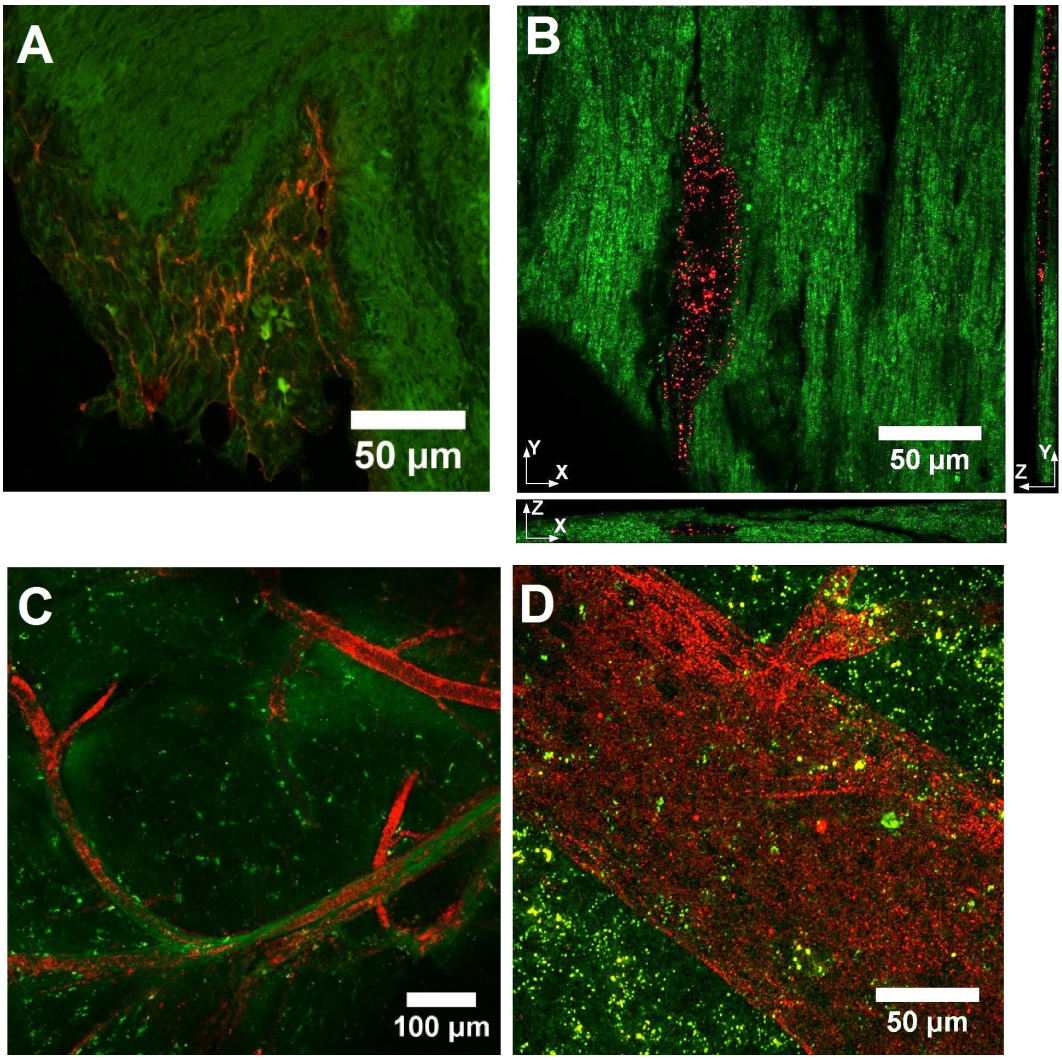

Fig. 3. Confocal fluorescence microscopy images of frozen mice tissue sections: A – uterus wall, B – skeletal muscle, C, D – cerebral hemispheres 1 h after intravenous injection of CdSe/ZnS-mPEG QD (red). Green color corresponds to the tissue autofluorescence (A, B, D: x60/1,4 obj., C: x20/0,5 obj., $\lambda_{excitation}$=488 nm). A–C) single

plane images; B) additional projections of optical sections in perpendicular planes (depth z=23 μm); D) Cumulative projection of 30 optical sections (z=6 μm).

Optical sectioning of single vessels was performed to analyze detailed QD localization in the vessels. Fig. 4 shows the confocal fluorescence images of the single cerebral artery at different focal depths. In the superficial layers of the vessel the QD PL (red) is negligible and the autofluorescence (green) can be clearly recognized (fig. 4 B-D). The autofluorescence forms stripes as it is caused by the elastic lamina of the vessel wall, which is folded due to absence of the internal blood pressure. Going deeper to the specimen the autofluorescence becomes weaker and the PL of QD increases (fig. 4 E). QD PL is strongest in the middle of the vessels. It is caused by QD presence in the lumen (fig. 4 F-H). However QD distribute unevenly: the bright red background is interrupted by circular dark shadows which correspond to the erythrocytes. It means that QD localize in blood plasma, but they don't pass into the erythrocytes. Going even more deeply to the specimen, the QD PL decreases and the autofluorescence of the posterior wall becomes visible (fig. 4 J-K).

Optical sectioning of single blood vessels showed that QD localize in the lumen of the vessels but they are not found in the vessel wall.

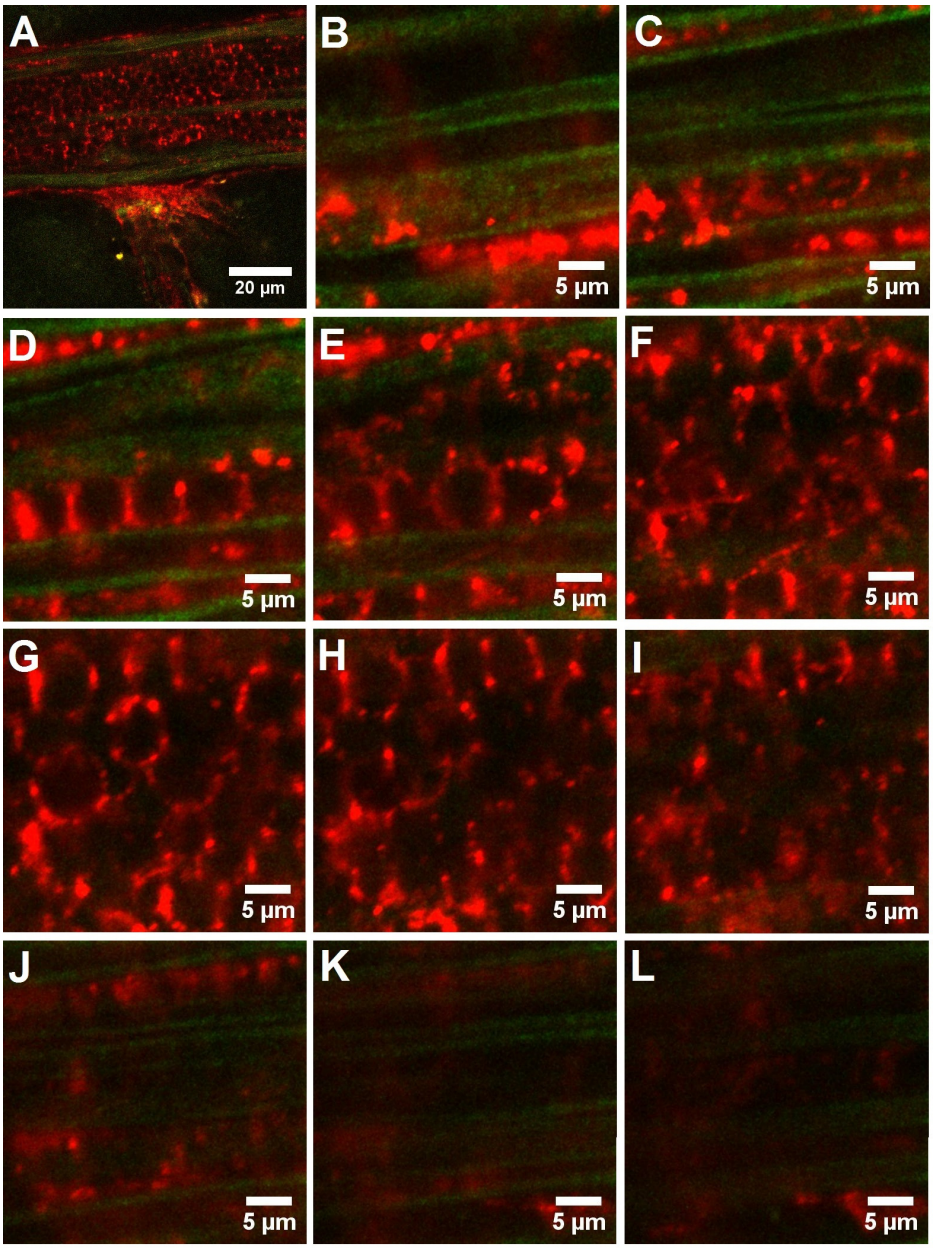

Fig. 4. Optical sectioning of a single cerebral vessel using confocal fluorescence microscopy. Frozen tissue slices were prepared 1 h after intravenous injection of CdSe/ZnS-mPEG QD (red). Green color corresponds to the tissue autofluorescence. (x60/1,4 obj., Δz=0,5 µm, $\lambda_{excitation}$=488 nm).

Additional data about QD localization in the layers of the vessels wall could be retrieved from the paraffin embedded tissue sections of the skin at the injection site (fig. 5). As QD extravasated in the damaged area, QD PL could be seen around the vessels. Confocal microscopy images clearly showed that QD accumulated in the outer layer of the wall *tunica adventitia*, but they could not cross the border of the middle layer of the wall (*tunica media*) and didn't accumulate in this layer. QD could also be detected in the

lumen of the vessels due to presence in the blood. QD adhered to the endothelium, but they couldn't penetrate through it and pass to the *tunica media*.

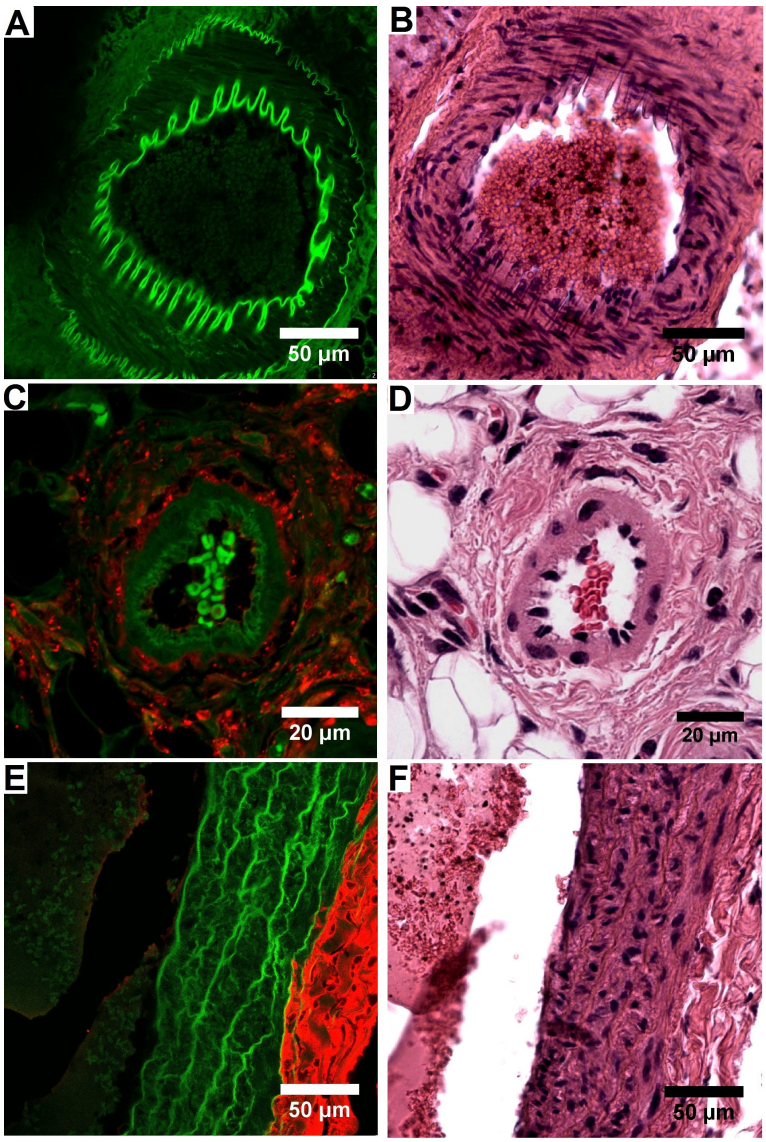

Fig. 5. Confocal fluorescence microscopy images (A, C, E) of paraffin embedded tissue sections of dermis from the injection site 1 h after intravenous injection of saline (A) and of CdSe/ZnS-mPEG QD (red) (C, E). QD accumulation in the vessel walls of an artery (B) and a vein (E) are visible. Green color corresponds to the tissue autofluorescence. (x20/0,5 obj., $\lambda_{excitation}$=488 nm). The transmission microscopy images (B, D, F) of HE stained tissue slices help to recognize tissue structure.

QD outlined even small blood vessels: arterioles, venules and capillaries without accumulation in the wall of the vessels. It shows that the barrier properties of the vessel wall don't depend on the presence of the muscle fibers. The barrier of the capillaries probably is formed by the basement membrane of the endothelium and the neighbouring fibers of the surrounding connective tissue.

Discussion of QD distribution in the organism after intravenous injection

The results of confocal microscopy show that intravenously injected CdSe/ZnS-mPEG QD localize within the blood vessels and the extravasation of QD to the extracellular space in most organs is negligible. It means that QD accumulation in organs 1 h after injection which was assessed by fluorescence spectroscopy is determined by the QD presence in the blood vessels, but not in the extracellular space or tissue cells. 24 h after injection QD PL could be detected only in the spectra of skin at the injection site and blood plasma. However QD PL in other tissues was observed neither by fluorescence spectroscopy nor by confocal microscopy. These temporal changes are mainly attributed to the decrease of QD concentration in the blood. The stability of QD PL quantum yield is also important as it might be altered in vivo due to interaction with biomolecules. QD clearance from the blood is mainly explained by the uptake to the reticuloendothelian system (RES) and accumulation in liver, kidneys, spleen and bone marrows (3-5). However in our study we didn't observed temporal increase of QD PL intensity in RES organs. Confocal microscopy of liver sections showed that QD localized not only in the vascular compartment, but also in the liver cells. It indicates that QD have extravasated through the wall of sinusoidal capillaries and accumulated in the liver tissue. It could partially explain the decrease of QD PL intensity in the spectra of blood plasma.

The results of confocal microscopy show that intravascularly located QD mainly resided in the blood plasma, while there were no QD in the erythrocytes. QD adhered to the endothelium, but there was no QD PL in the deeper layers of the vessel walls. It indicates that QD are not transferred through the endothelium to the middle layer (*tunica media*) of the wall. It was shown that endothelial cells might internalize QD and this process depends on the surface coating of the particle: QD terminated with carboxyl or amine groups were observed in the endosomes, but PEG coated QD could not be detected in the endothelial cells (6, 7). However it remains unclear if QD can be translocated on the basal side of the endothelium. Our results show, that QD do not penetrate tunica *intima* and don't accumulate in the deeper layers of the vessel wall.

The results also show that QD which were located outside the blood vessels penetrated into the external layer of the vessels (*tunica adventitia*) and they outlined the border of the *tunica media*, but didn't cross it. It shows that extracellular tissue

organization impeded QD diffusion into the *tunica media*. It was probably determined due to the proximity of the muscle cells and the basement membrane which forms a continuous layer and may act as a filter for the diffusion of various compounds.

3.2. Penetration of QD through the placental barrier

The embryotoxicity of NP first of all depends on the ability of the compounds to cross the maternal-foetal barrier and to accumulate in the embryonic tissues. We used fluorescence spectroscopy and confocal microscopy to investigate the penetration of CdSe/ZnS-mPEG QD through the placenta in the 13th day of embryogenesis when the placental structure is completely formed and can be clearly identified by histological examination.

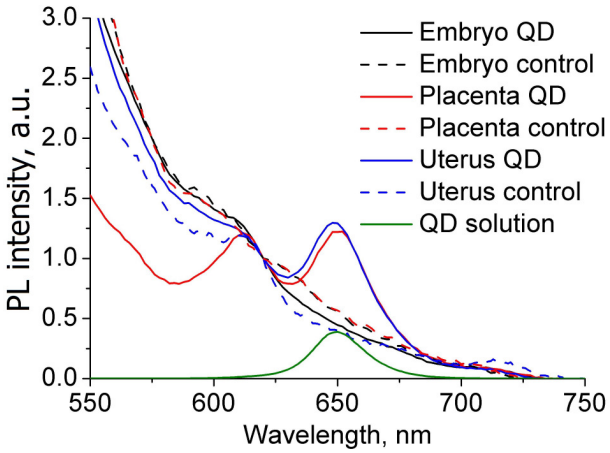

Fig. 6. The averaged and normalized fluorescence spectra of rat tissues indicating the presence of CdSe/ZnS-mPEG QD (PL peak at 650 nm) in the maternal tissues (uterus, placenta) but not in embryonic tissues 3 h after QD injection, 13th day of embryogenesis (n_{rats}=3, $n_{embryos}$=10, $\lambda_{excitation}$=450 nm).

Fluorescence spectroscopy of the tissues excised 3 h after CdSe/ZnS-mPEG QD injection revealed that characteristic PL band of QD at 650 nm could be detected in the tissues of uterus and placenta (Fig. 6). However QD fluorescence was not registered in the embryonic tissues: embryo, yolk sac placenta and umbilical cord.

The cryosectioned tissue slices were used to investigate QD localization using confocal microscope coupled with a spectral detector which enables the discrimination of the QD PL from the tissue autofluorescence with spectral resolution. This technique revealed that QD are accumulated in the labyrinthine zone of the placenta (Fig. 7.). QD appeared distributed in the samples not homogenously, but patterned, indicating QD

accumulation in the areas with lower autofluorescence (green) background. In the labyrinthine zone the maternal blood sinuses have strong absorbance and lack of endogenous fluorophores which are more abundant in the connective foetal tissue. Therefore maternal blood results in lower autofluorescence intensity and darker green areas in the image when compared with foetal tissue. In this way, QD are mostly distributed in the maternal blood sinuses.

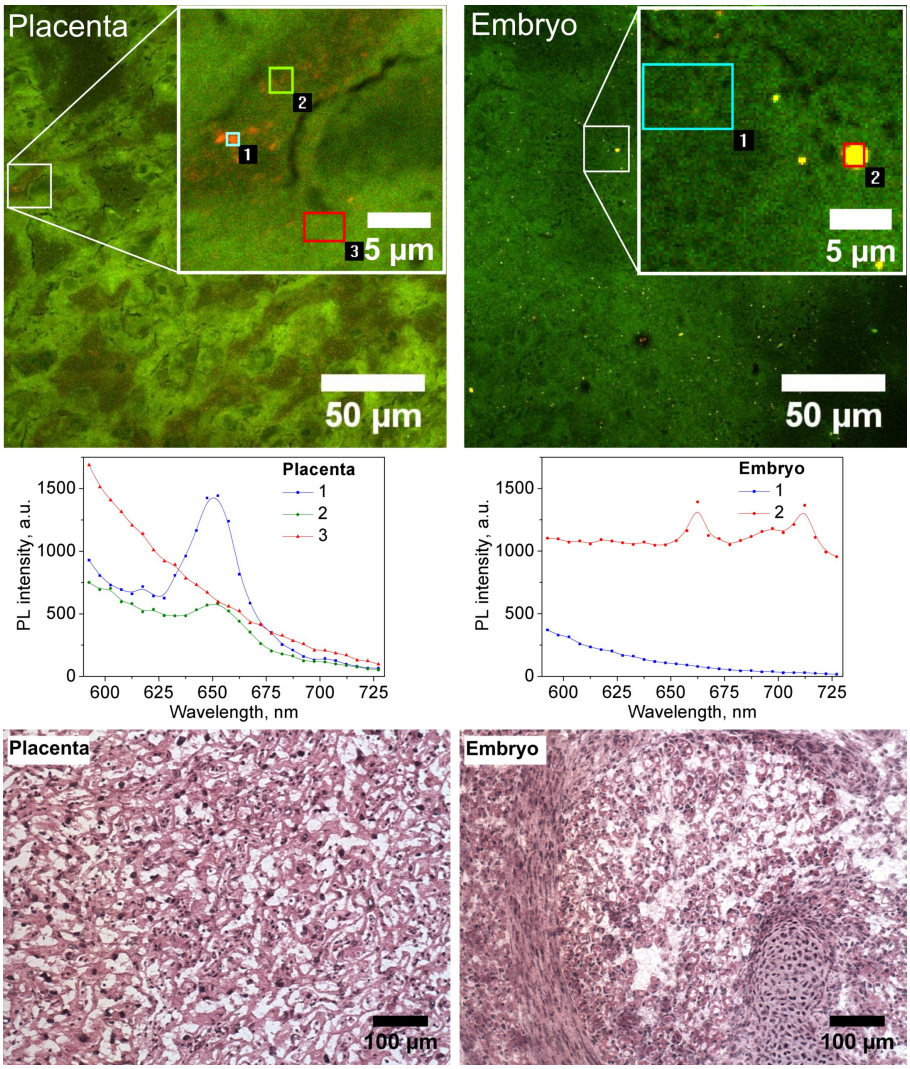

Fig. 7. Confocal microscopy images (top) of rat placenta (labyrinthine zone) and embryo tissues (cryosectioned slices) 3 h after intraperitoneal injection of CdSe/ZnS-mPEG QD (x60/1,4 obj., $\lambda_{excitation}$=488 nm). QD PL (peak at 650 nm) was identified by spectral imaging (middle) in placenta but not in the embryonic tissues. The patterned red QD distribution in placental tissue resembles the network of maternal blood sinuses which can be seen in HE stained histological slices (bottom, x10/0,25 obj.).

The examination of the embryo tissues showed no appearance of QD in the samples. There were seen some yellowish endogenous objects, but they exhibited fluorescence spectra which wasn't characteristic to QD. The results of confocal

microscopy and fluorescence spectroscopy show that QD are found in the labyrinthine zone of the placenta, but there is no QD accumulation in the embryos. This finding suggests that QD do not penetrate through the placental barrier.

Discussion of QD penetration through the placental barrier

After intraperitoneal injection of CdSe/ZnS-mPEG QD the concentration increases up to 2,5 h post injection and then starts decreasing exponentially (data not presented). According to the human perfusion models, the equilibrium between maternal and foetal compartments is reached after 2-3 h (8). Therefore the incubation time of 3 h should be sufficient for QD to reach the foetus in the case of the QD passage through the placental barrier.

However the results show that CdSe/ZnS-mPEG QD do not pass to the embryonic tissues. It is explained due to maternal-foetal barrier which is constituted of three continuous trofoblast layers. The trofoblast cells are joined by the tight, adhesive and gap junctions which limit the intercellular passage of macromolecules (9). Alternatively the molecules can be transported transcellularly involving the internalization of the compounds and excretion on the foetal side of the barrier.

Yamashita K. *et al.* have shown that some NP (silica and TiO_2 NP with diameters of 70 nm and 35 nm) can cross the placental barrier in pregnant mice and cause neurotoxicity in their offspring (10). They showed that the NP were found in the placenta, foetal liver and foetal brain. Moreover, they found that mice treated with these NP had smaller uteri and smaller foetuses than untreated controls. The authors concluded that these detrimental effects are linked to structural and functional abnormalities in the placenta on the maternal side, and are abolished when the surfaces of the silica NP are modified with carboxyl and amine groups.

It is also shown that the penetration of NP depends on the size of the particles. Polyamidoamine (PAMAM) dendrimers of the 5-6 nm size can penetrate through the intercellular junctions in human placenta perfusion model (11). The NP accumulated in the connective tissue of the chorion and there was almost no significant accumulation in the trofoblast. Meanwhile the polystyrene NP of 50-240 nm size accumulated mainly in the trofoblast via the transcellular pathway (8). It shows that the penetration pathway depends on the physical properties of the NP.

The hydrodynamic diameter of CdSe/ZnS-mPEG QD used in our study is greater than 23 nm (12). It was shown that QD of this size can not cross the glomerular barrier and therefore can not be filtrated in kidneys (13, 14). The placental barrier is physiologically designed to restrain the diffusion of various compounds even more. Therefore CdSe/ZnS-mPEG QD are too big to pass between the trofoblast cells.

The transcellular transfer is too complexed to be explained only by the physical properties of QD. First of all, it should include the internalization of QD which depends on the opsonization and charge of the QD surface. It is known that PEG coated QD possess neutral charge and they can avoid biomolecule interaction and endocytosis much better then QD with polar surface (terminated with carboxyl or amino groups) charge (7). It was also observed that QD localize in endosomes, multivesicular bodies and other organelles, but the secretion of QD is highly unlikely process (15, 16). It means that under theoretical QD uptake to the trofoblast cells, they can remain in this layer without the excretion to the foetal side of the barrier. These facts and our results lead to an assumption that QD may accumulate in the placenta but they are not transferred to the embryonic tissues.

Other authors showed that cadmium which is found in the core of QD can be observed in mice embryos after maternal exposure of QD by the means of mass spectrometry (1). However, fluorescence microscopy analysis didn‘t confirm QD presence in the embryos. These results can be explained that QD were degraded in maternal tissues which resulted in the release of cytotoxic cadmium compounds (2). These compounds exhibit other metabolic pathways than QD and they can be transported through the placental barrier (17). The stability of QD in the pregnant organism is of crucial importance as it was reported earlier that QD toxicity *in vivo* is mainly attributed to the destabilization of QD, degradation of inorganic core and the release of cytotoxic Cd^{2+} ions into the tissues (3).

3.3. Distribution of QD in the skin

QD distribution in the skin after subcutaneous injection

After subcutaneous injection of CdSe/ZnS-mPEG QD the non-homogeneously distributed red PL of QD was detected in the dermis, hypodermis and between the

underlying muscle fibers (Fig. 8). No red fluorescence of the QD was found in the epidermis layer and hair follicles. QD accumulation pattern after 24 h didn't significantly differ from the samples taken after 1 h.

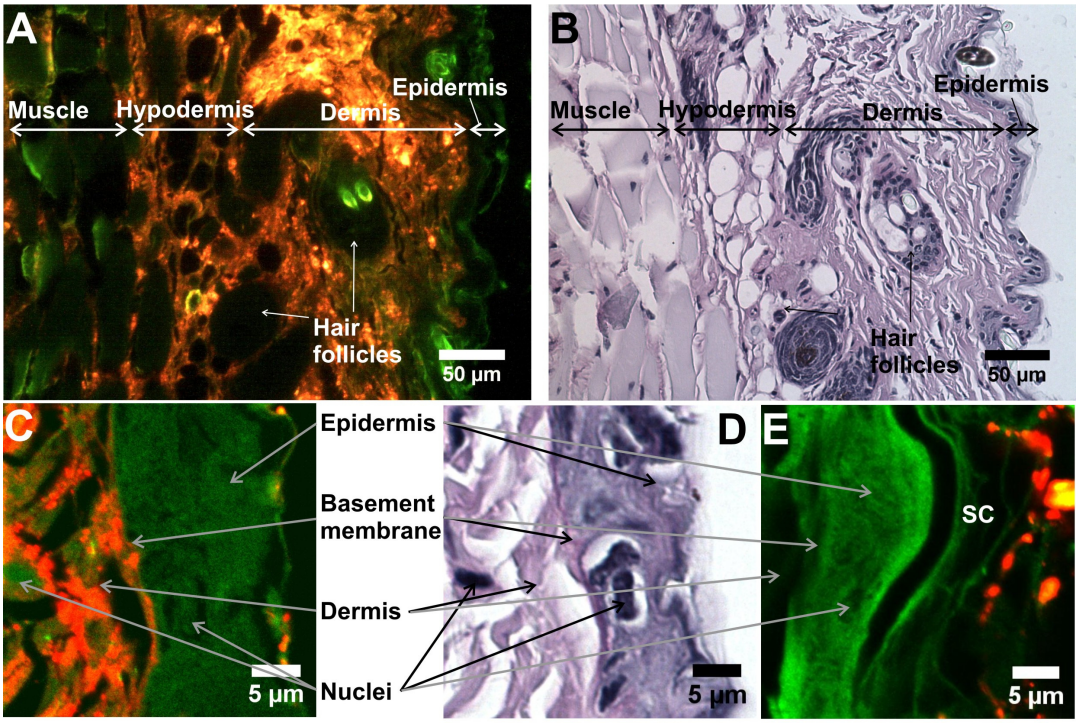

Fig. 8. Confocal fluorescence (A, C - unstained) and brightfield (B, D - PAS stain) microscopy images of mice skin 24 h after subcutaneous injection of CdSe/ZnS-mPEG QD solution showing QD (red) distribution in the skin layers and the underlying muscle tissue (A). Higher magnification (C) reveals detailed accumulation of QD along the basement membrane (D) indicating that QD are prevented from passing through the epidermal-dermal junction. Topically applied QD (E) are found only in the stratum corneum (SC), but not in the deeper layers of the skin (paraffin embedding).

After subcutaneous injection QD are homogenously distributed in the dermis indicating that the loose network of dermal fibers allows NP diffusion in this tissue. It can be seen that QD pattern resembles the arrangement of collagen fibers indicating that they are continuously covered with QD. It manifests that QD interact with the biomolecules of extracellular matrix. The detailed analysis of the samples using confocal microscopy revealed that QD were abundantly accumulated along the epidermal-dermal junction, without crossing this border. The QD were prevented from the uptake to epidermis (fig. 8 C).

Additional experiments were performed to investigate QD penetration through the skin after topical application. The results show that QD accumulate in the superficial layers of stratum corneum (fig. 8 E) but they were not observed in the deeper layers of

epidermis. These results show, that QD are retained from passage to the viable epidermis due to the barrier properties of stratum corneum.

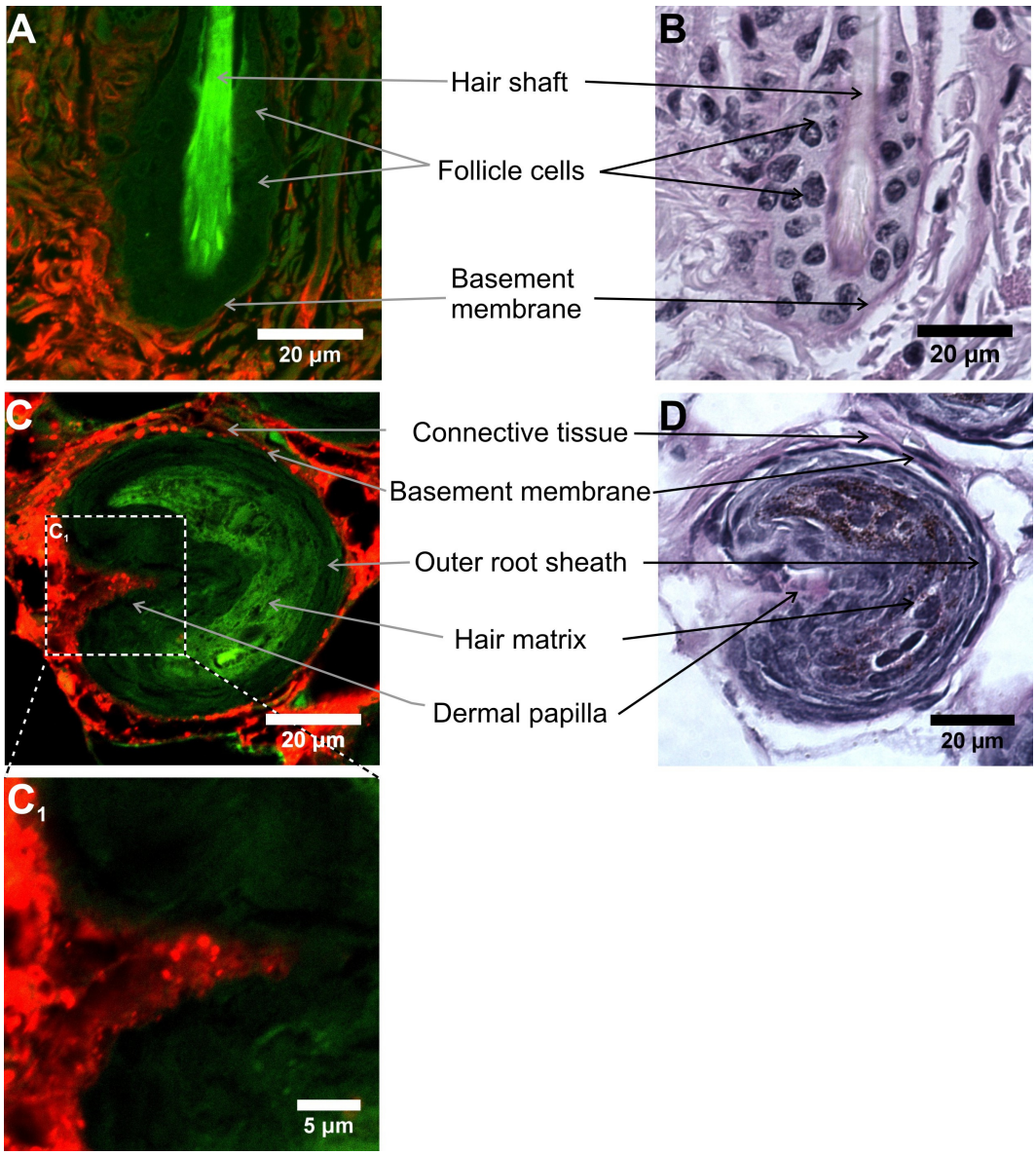

Fig. 9. Confocal fluorescence (A, C, C_1 - unstained) and brightfield (B, D - PAS stained) microscopy images of the longitudinal section of the hair follicle (A, B) and the hair bulb (C, D) after subcutaneous injection of CdSe/ZnS-mPEG QD solution (paraffin embedding). The QD (red) are distributed in the connective tissue of dermis, but they are sequestered by the BM from passing to the hair follicle. The enlarged image (C_1) shows high QD fluorescence contrast at the dermal-epidermal junction.

Figure 9 represents the longitudinal section of a hair follicle after subcutaneous injection of QD. QD are found in the dermis around the hair follicle, but they are retained from passing to the outer root sheath cells or deeper hair follicle layers (fig. 9 A). There was also no QD accumulation in the sebaceous glands. We observed high QD PL contrast in the dermal papilla compared to the hair bulb cells (fig. 9 C), it means that

QD transport to the hair matrix is prevented. PAS staining, which is used for the imaging of the basement membrane (BM) (18), resolved that QD don't cross the BM indicating that this structure is highly impermeable to PEG coated QD (fig. 9 D).

Intracellular localization of QD

CdSe/ZnS-mPEG QD in the dermis are mainly found adhered to the extracellular fibers. However detailed confocal microscopy analysis revealed that there are some cells which contain internalized QD (fig. 10 A). These cells accumulate QD in the cytoplasm, particularly, in the vesicular structures instead of being homogenously distributed (fig. 10 A_1). QD are abundantly assembled in the perinuclear area, but they are not found in the nuclei. These cells don't show any morphological signs of toxicity or imparity. The observed QD PL pattern resembles localization in the endosomes and/or endoplasmic reticulum. The spectroscopic examination revealed that the fluorescence spectra of the intracellularly located QD matches the spectra in saline solution. It means that spectroscopic properties of QD are retained *in vivo* and QD are prevented from the degradation in cellular compartments.

Analysing the brightfield images of the stained tissue slice, the cells containing QD can be differentiated. Highest QD accumulation was observed in the cells, which exhibit granulated cytoplasm (fig. 10 B, square contour). According to skin physiology such granules might be characteristic to mast cells and macrophages. However, QD were not observed inside the muscle fibers and adipocytes despite of abundant accumulation around these cells. It means that QD uptake *in vivo* strongly depends on the cell type.

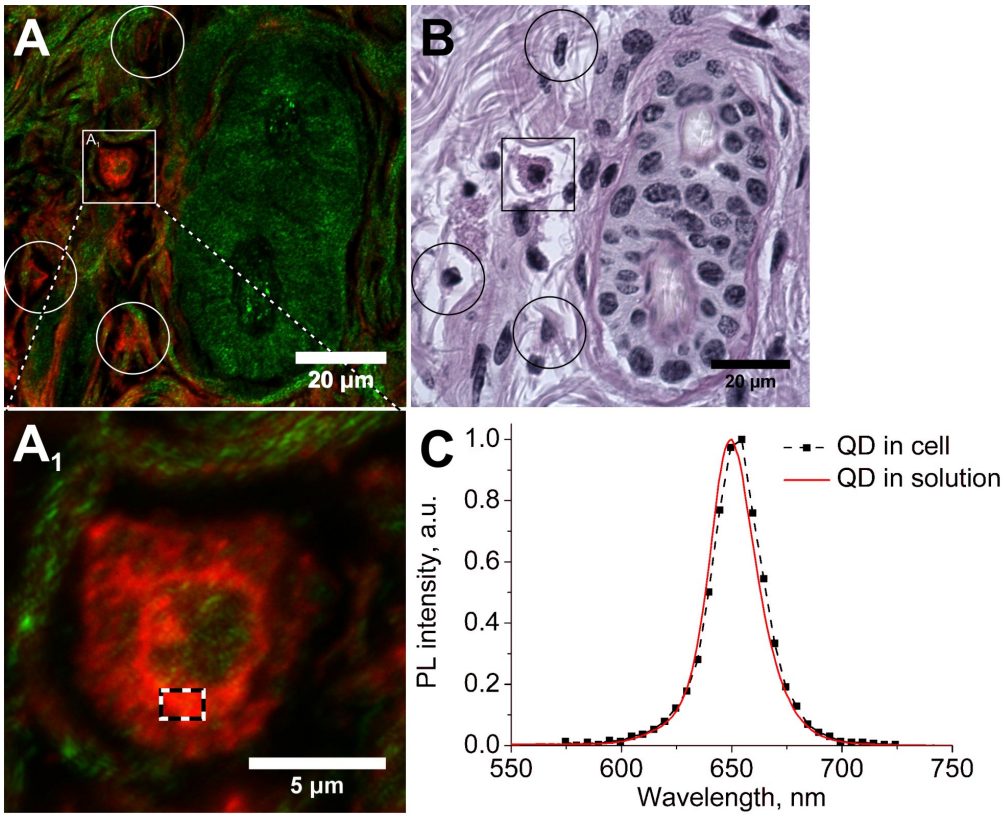

Fig. 10. Confocal fluorescence (A, A_1- unstained) and brightfield (B - PAS stained) microscopy images of mice dermis showing intracellular QD (red) uptake in vivo 1 h after injection of CdSe/ZnS-mPEG QD (paraffin embedding). The cells marked with white contour show QD presence in the cytoplasm. Enlarged image (A_1) of a single cell reveals heterogeneous QD pattern and high accumulation in the perinuclear region. QD PL spectrum (C) in the cell (from rectangle in A_1) matches the QD spectrum in the saline indicating no appearance of spectroscopic changes *in vivo*.

QD localization in other structures

As abundant QD retention in the dermis was observed up to 24 h, we hypothesized if QD could be excreted via sweat glands. Therefore QD were injected into the palm of mice where the highest density of the sweat glands is reported (19). The microscopy images show that QD are found accumulated around the sweat glands, but there is no NP permeation into the epithelial cells of the sweat glands (fig. 11 A, B).

QD were also observed in the epineurium of the peripheral nerves (fig. 11 C). However, QD PL outlines the borders of the nerve without deeper penetration into the densely packed tissue of the perineurium. Similarly QD accumulated in the *tunica adventitia* of the large blood vessels, but they were prevented from passing to the *tunica media*. These observations show that QD diffuse in the loose connective tissue but they are unable to permeate the barriers of tightly organized collagen fibrils.

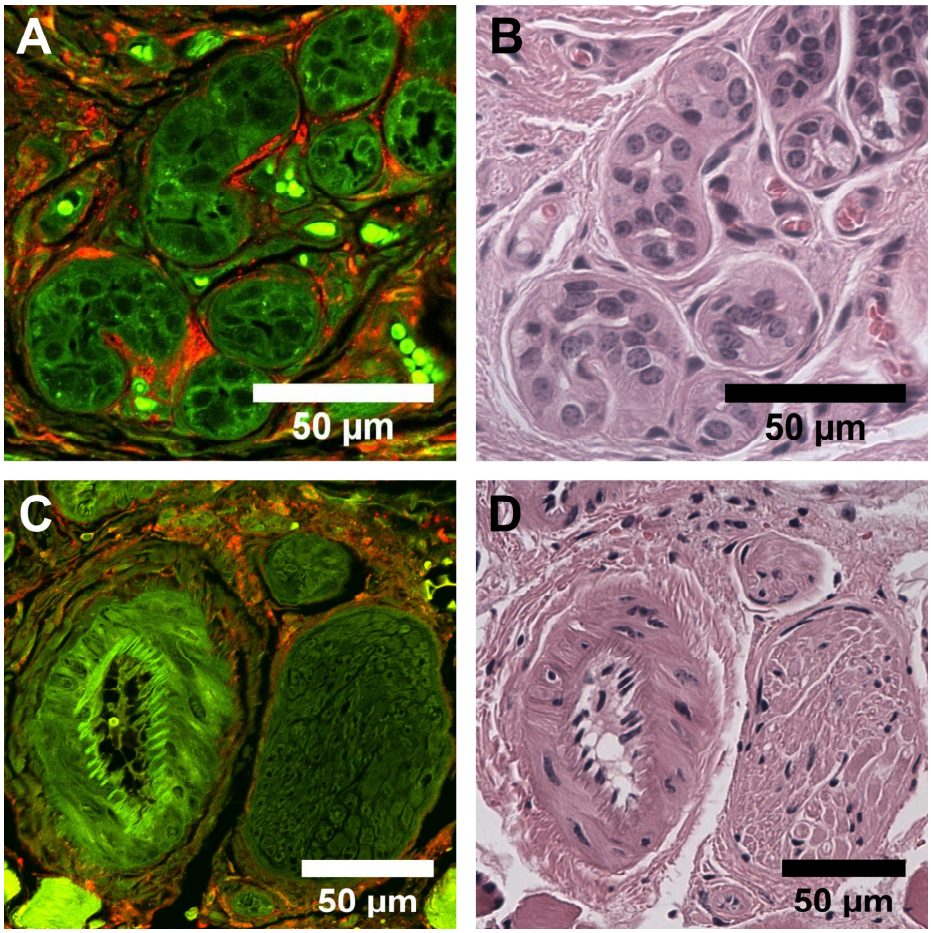

Fig. 11. Confocal fluorescence (A, C - unstained) and brightfield (B, D - HE stained) microscopy images of mice dermis showing QD (red) accumulation in the connective tissue around the sweat gland ducts (A) and outlining artery and peripheral nerve borders (C) in vivo 1 h after subcutaneous injection of CdSe/ZnS-mPEG QD (paraffin embedding). There is no QD accumulation in the sweat glands, *tunica media* of the artery and inside the nerve.

The presented results show that subcutaneously injected NP distribute in the dermis, hypodermis and the muscle tissue. QD migrate in the connective tissue, penetrate between the muscle fibers and adipocytes. However, QD diffusion in the tissue is limited by specific dermal structures and, therefore, QD fluorescence is absent in the epidermis, hair follicle, sebaceous and sweat glands. All these structures are outlined by the BM which organizes the epithelial cells and predominantly plays the major role as the limiting factor for NP migration in the skin. QD are prevented from passing through the densely organized fibrils of the connective tissue coating in the peripheral nerves, blood vessel walls and muscle fascicles.

Penetration of QD through the skin after topical application

After topical application of CdSe/ZnS-mPEG QD solution on shaved mice skin, QD PL could be detected in the skin samples by confocal fluorescence microscopy (fig. 12). QD localized mainly in the superficial layers of stratum corneum. QD could also be detected in the bends and wrinkles as well as superficial part of the hair channel.

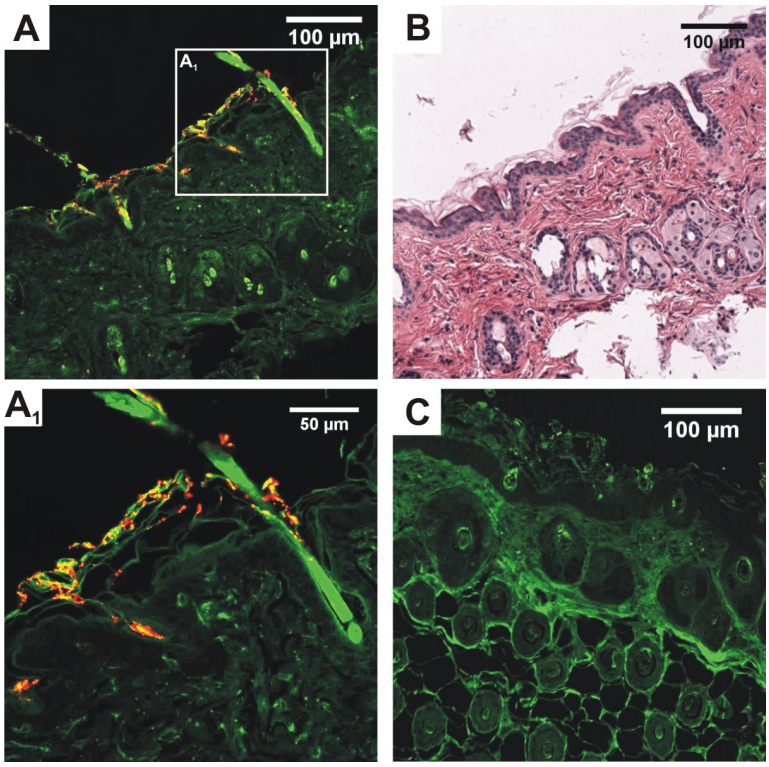

Fig. 12. Confocal fluorescence microscopy images (A, A_1, C) of mice skin 2 h after topical application of CdSe/ZnS-mPEG QD solution on shaved mice skin. Transmission microscopy of HE stained tissue slice (B) visualizes skin structure and helps to recover QD localization in skin layer. C – Control sample without QD application.

Discussion of QD distribution in the skin

Our results show that subcutaneously injected QD distribute widely in the dermis, hypodermis and between the underlying muscle fibers. QD migrate within the loose connective tissue of the dermis. However QD diffusion in the tissues is limited by certain extracellular structures which prevent QD from passing to the epidermis, hair follicles, nerves, blood vessel walls, sebaceous and sweat glands.

Topically applied QD are found in the stratum corneum of the skin, however no penetration to deeper layers of epidermis was detected. The QD retention in the superficial layer of epidermis is primarily caused by dense cellular network which is tightly interconnected with lipids and proteins, which all together create a highly impermeable barrier for various exogenous compounds (20).

We demonstrate high QD accumulation along the dermal-epidermal junction without passing to the epidermis after subcutaneous injection. It is an interesting observation because in most studies the barrier properties of the skin are attributed to the stratum corneum (21, 22).

The role of the BM in the overall skin permeability to NP is not widely discussed in the literature. Baroli B. *et al*. investigated metallic NP penetration through human skin ex vivo and showed that NP mainly accumulated in the stratum corneum with little penetration to the viable epidermal layers, particularly stratum granulosum (22). However, the authors didn't observe NP permeation to the dermis (22). Kuchler S. *et al.* investigated the penetration of fluorescently labelled solid lipid NP and dendrimeric core-multishell NP and observed the concentration decrease from stratum corneum towards the deeper skin layers for both types of NP (23). Fullerenes were applied to human skin biopsies and they permeated into the epidermis but could not penetrate the BM (24). These studies and the current review by Prow T.W. *et al.* highlight limited or no NP permeability from epidermis to dermis (25). Our results contribute strongly to this opinion as we show QD retention in the stratum corneum without any penetration to the deeper layers after topical QD administration.

The main proposed mechanisms of drug delivery through the skin are the intracellular diffusion of hydrophobic particles through the cellular membranes and the transcellular penetration of hydrophilic NP (25, 26). Therefore the main hindrance comes from the alternation of the moiety going through the epidermis. Our results show that QD are impermeable to the dermal-epidermal junction after subcutaneous injection. Unlike cellular epidermis, the BM barrier arises from the densely packed protein layers (18) and there are no specific carriers, lipid membranes or transcellular spaces which are characteristic to the epidermal cells and can contribute to QD transfer. In this way, BM acts as a passive filter for QD and we assume that it determines high BM resistance for the transport of in both directions: epidermal-dermal as indirectly shown by others (22, 24-26) and the dermal-epidermal direction as indicates this study. The QD transport across the BM should depend on their physicochemical properties as the penetration through other biological barriers was shown to be sensitive to the size, surface charge, hydrophylicity and biofunctionalization of NP (27, 28).

QD retention by the BM might be used for evaluating the integrity of the epidermal-dermal junction. Bennett K.M. *et al.* applied ferittin NP for MRI imaging of the BM in the kidney (14). The cationic NP were observed accumulated in the BM of the glomerules and in the filtration slits between the podocytes. The NP penetrated only through the fenestrations which are characteristic for glomerular capillaries. The BM of the epidermis lacks of these fenestrations and it is integral, but the mechanical or radiation damage might disintegrate BM structure and alter barrier capacity for the QD. Therefore QD could be used to assess the integrity and physiological state of the BM.

Hair follicle

As many compounds are impermeable to the stratum corneum much attention is paid to the pilosebaceous unit as an alternative drug delivery pathway (29, 30). The hair follicles extend deep into the skin and the thickness of the stratum corneum layer is progressively reduced going deeper. The lower part of the hair infundibulum has a weak barrier as the corneocytes are smaller and crumbly, which increases the permeability for drugs (31). More to add, there is a rich capillary blood supply available to distribute the substances systemically. Our results show that QD accumulate abundantly along the dermal-follicle junction but they don't penetrate across this barrier. QD are prevented from passing to the hair bulb even in the anagen phase of hair life cycle (characterised by the presence of dermal papilla), when the cells grow most rapidly and the cells internalize the extracellularly located substances most efficiently. The fact that even highly proliferating cells of the hair bulb do not internalize QD indicates that the transport of QD from dermis to the hair matrix is strongly limited or even completely prevented.

It confirms the significance of the BM as the preventive structure for QD migration and may explain low efficiency of transfollicular transport of other reported NP reported. Lademan J. *et al.* (2007) investigated the follicular penetration of polymeric NP (size 320 nm) and showed that NP penetrate deeply into the hair follicles providing long-term deposition in the epidermis (29). NP accumulated along the epidermal-dermal junction. However, the NP penetration from the follicle to the dermis was not discussed (29). Similar results were presented in a recent study, where drug loaded polymer NP (size 30-96 nm) penetrated deeply in the hair follicle, but they were sequestered from

passing to the dermis (32). It was shown that transcutaneously applied 40 nm NP penetrated the skin predominantly via follicular route and possibly passed into the perifollicular dermis, by entering the meshwork of Langerhans cells, which are particularly concentrated in the upper parts of human hair follicles (33). However intercellular migration to the dermis was not observed suggesting that NP were transferred within the Langerhans cells. These reports show that NP are impermeable to the follicular-dermal junction after topical application. Our results complement these studies as we show QD retention by dermal-follicular junction from dermal compartment. The limited transfollicular transfer of QD may be explained by assigning the QD interceptive properties to BM.

Limited transfollicular penetration of NP can be advantageous for drug delivery as they provide rapidly sourcing carriers that can accumulate in the hair canal and create a drug depot without passing to other tissues of the body.

Intracellular QD uptake in vivo

We observed intracellular QD uptake by dermal cells. According to morphological analysis they were internalized by different cellular types. The macrophages, mast cells and eosinophils could be identified from other dermal cells. As QD distributed heterogeneously in the cytoplasm and mainly accumulated in the perinuclear region it seems that they passed by endocytotic pathways. Kuchler S. *et al.* showed intracellular uptake of solid lipid NP to the keratinocytes *in vitro* with the same distribution pattern (23). There are several types of dermis cells which endocytotic activity for various NP was shown earlier *in vitro*. Fullerenes were found assembled in the lysosomes, mitochondria, and endoplasmic reticulum of the human mast cells (34). Gold NP were reported to accumulate in fibroblasts (35). QD were found to be rapidly endocytized by dendritic cells (36) and macrophages (37) and to localize in the lysosomes. However uptake of QD or other NP by dermal cells *in vivo* is not extensively described.

Cellular uptake of drug-loaded NP can become clinically relevant when applied to the damaged skin. In the case of dendritic cells or macrophages QD could be, later on, transported to the regional lymph nodes. On the other hand, the intracellular localization increases the risk of toxic effects because it was shown that QD modulate the expression of genes which are associated with inflammatory, immune and apoptotic responses in

human keratinocytes and dermal fibroblasts (38). The degradation of QD and the release of toxic compounds *in vivo* after oral (25) and intravenous administration (2) was reported. As we showed active cellular uptake and accumulation of QD in dermal cells, including mast cells it is highly possible that NP induce the inflammatory and immune responses in vivo as well.

QD migration pathways in dermis

The long-term QD accumulation in the dermis suggest that they might be excreted to the skin surface as it was shown for organic substances including drugs (39). However, we observed that QD don't penetrate into epithelial cells of the sweat glands. It minimizes the possibility that QD could be excreted from the organism with the sweat as it requires QD uptake to the sweat gland cells.

According to our results, QD were unable to penetrate inside the nerves and accumulated along the perineurium, but they were dispersed in the surrounding connective tissue - epineurium. The epineurium consists of areolar connective tissue with loose bundles of collagen fibrils (40), meanwhile, perineurium is usually represented as a sheet of collagen fibrils forming a lacework structure. Therefore, we assume that the differences in extracellular collagen organization determine limited QD diffusion into perineurium and result the observed QD distribution pattern in the tissue.

Similarly QD were not detected in the muscle fibers, but distributed in the connective tissue between the muscle fascicles. QD were also detected in the *tunica adventitia* of the blood vessels, but they were retained from permeation into the *tunica media*. These observations indicate that QD diffusion through the tissues is limited and depends on the structure and physical organization of the connective tissue fibers. QD can migrate in the loosely organized tissue, but they are not able to penetrate between the densely organized connective tissue fibers.

The limited QD diffusion through the tissue contribute to the slow NP clearance from the injection site. Restricted transfer across the BM leads to inefficient uptake to the blood vessels as the capillaries are outlined by BM and the uptake firstly depends on the QD ability to penetrate through the vessel wall. We assume that it determines that QD are mainly drained from the tissues by the lymphatic system which was observed during fluorescence imaging of mice (data not presented).

3.4. Long-term stability of QD in vivo

Long-term fluorescence imaging applications of QD require stable optical properties to maintain sufficient signal to noise ratio. It is also important to estimate biological effects of prolonged QD accumulation in the tissues as it was shown that QD might degrade and release cytotoxic compounds (1, 38, 41). Therefore we compared the stability of spectroscopic properties *in vivo* of CdSe/ZnS-mPEG and CdTe-MPA QD 7 days in rat tissues.

7 days after intraperitoneal injection of CdTe-MPA QD dark spots in the peritoneal muscle or omentum were observed which were identified by histological examination as necroses (fig. 13). Fluorescence spectroscopy of these tissues showed QD accumulation. However QD PL spectra were red-shifted up to 18 nm in respect to CdTe-MPA spectrum in distilled water (fig. 13). The position of the band peak varied among different places of the same specimen in the range of 745-753 nm. It shows that spectroscopic properties of CdTe-MPA are changing in biological environment and the structure of the QD might be altered. QD PL was localized in the range of few milimeters in the tissues and QD PL could be registered in the surrounding tissues or other rat organs. It shows that QD diffusion and dissemination in the tissues is inefficient.

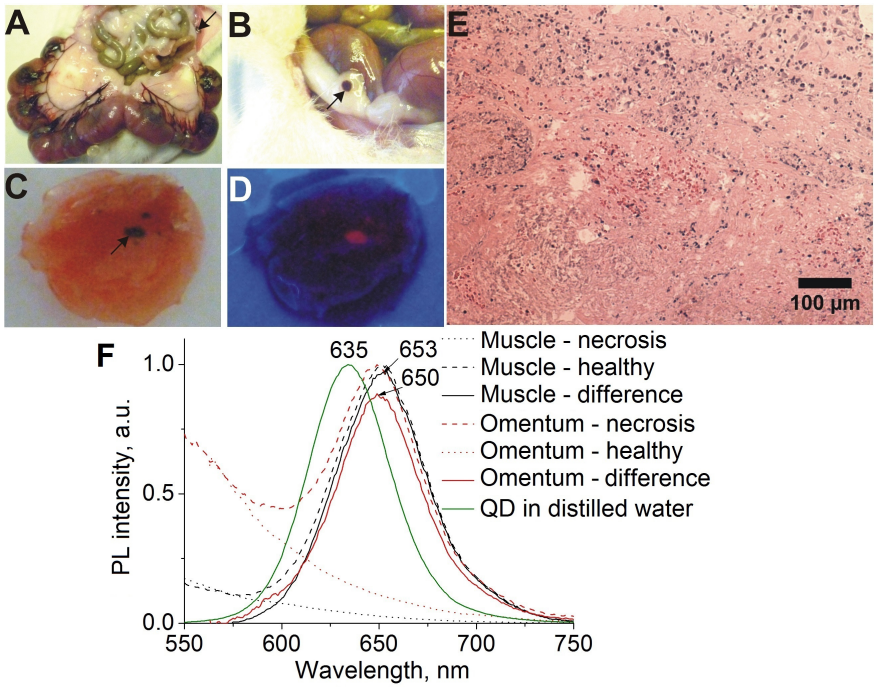

Fig. 13. A-D) The localization of necroses (arrows) in rat abdominal muscle (A, C) and omentum (B) in the injection site 7 days after injection of CdTe-MPA QD solution. QD PL in the necrosis can be seen under UV illumination (D). E) Transmission microscopy image of HE stained necrotic muscle tissue (x10/0,25 obj.). F) Normalized and averaged fluorescence spectra of necrotic and healthy tissues, differential spectra and CdTe-MPA QD spectrum in distilled water ($n_{measurements/tissue}$=3, $\lambda_{excitation}$=450 nm).

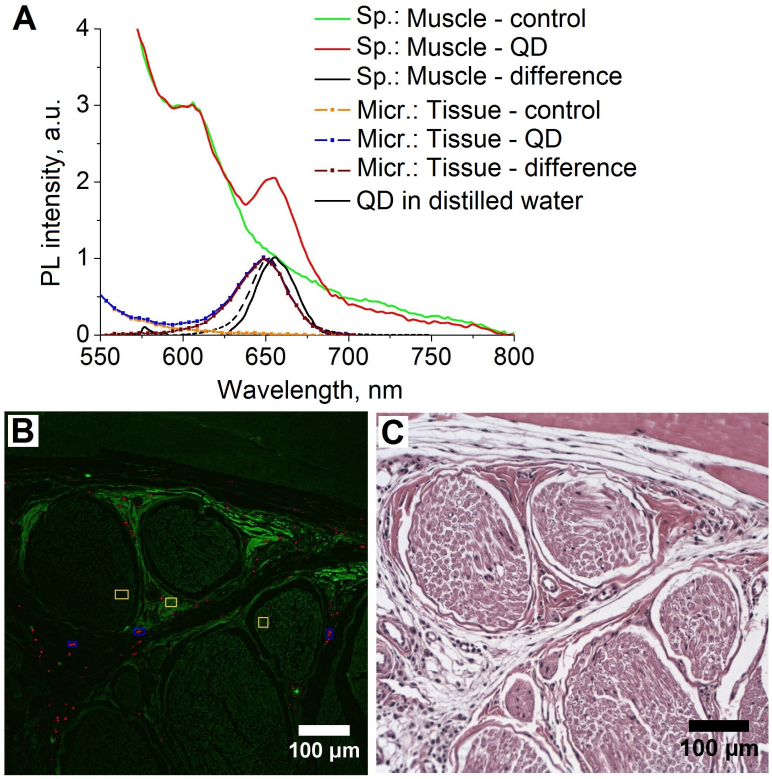

Fig. 14. A) Normalized and averaged fluorescence spectra of rat thigh muscle 7 days after CdSe/ZnS-mPEG injection. "Muscle – QD" represents the spectra in the QD injection site, while control was measured in the opposite thigh of the same animal. "Sp" – spectra were measured using spectrometer ($n_{measurements/tissue}$=3, $\lambda_{excitation}$=450 nm), "Micr." – using

spectral detector of the microscope (averaged spectra from the squared regions in B: yellow – control, blue – QD). B) Confocal fluorescence image of injected tissue (paraffin embedding, x20/0,5 obj., $\lambda_{excitation}$=488 nm); C) Transmission microscopy of the HE stained specimen.

7 days after injection of CdSe/ZnS-mPEG QD solution there were seen no macroscopical tissue damages. QD PL could not be visualized by naked eye under UV illumination. Characteristic PL band of CdSe/ZnS-mPEG QD was registered in the fluorescence spectra of thigh muscle in the region of injection site (fig. 14). QD PL spectra in the tissues were nearly overlapped the QD spectra measured in distilled water. QD Pl spectra in the muscle were measured and using confocal microscope. These spectra had additional component on the shortwave side of the spectra, while longwave side of the spectra overlapped with QD spectra in distilled water. These measurements show that CdSe/ZnS-mPEG maintain relatively stable spectroscopic properties *in vivo*.

QD PL could be distinguished from the autofluorescence background in the tissue region of 2-3 cm, but QD were not detected in other rat tissues. It means that CdSe/ZnS-mPEG distribute more evenly than CdTe-MPA QD in the tissues of a muscle and possess better diffusion properties *in vivo*.

Fluorescence microscopy revealed that CdSe/ZnS-mPEG QD accumulate in the connective tissue, but they were not detected in the muscle fibers, inside the nerves and *tunica media* of blood vessels. Similar QD distribution was observed 1 h after subcutaneous QD injection (chapter 3.3.). It means QD remain their biodistribution properties up to 7 days and during this time can not reach muscle cells, nerves and other discussed targets.

The spectroscopic changes of CdTe-MPA QD could be related with reorganization of the superficial ligands, their desorption and QD aggregation (42, 43). In this case QD loose the passivating organic ligands and are exposed to surrounding biomolecules. It can lead to the oxidation of the CdTe core and the release of cytotoxic compounds. It was shown that CdTe possess cytotoxicity and it is justified by the effects of Cd and Te compounds which impede normal metabolic activity of the cells (44, 45). However MPA is toxic as well (46). It shows that detachment of MPA molecules from CdTe nanocrystal could result tissue necrosis in few ways. Exact mechanisms of QD are still not determined.

The core of CdSe/ZnS-mPEG is stabilized with the ZnS shell, covered with amphiphilic polymer and terminated with mPEG groups. These coatings stabilize the structure of QD and protect nanocrystal from biological interaction. It results in higher structural stability and higher biocompatibility when compared with CdTe-MPA QD. The neutrally charged mPEG were shown to reduce interaction with proteins and intracellular accumulation (12, 47). These properties make CdSe/ZnS-mPEG more favourable for QD applications in the fluorescence imaging of biological systems.

4. CONCLUSIONS

1) Intravascularly located CdSe/ZnS-mPEG QD adhere to the endothelium and extravascularly located QD accumulate in the *tunica adventitia*, but they are unable to penetrate into *tunica media* from any direction.

2) Intravenously injected CdSe/ZnS-mPEG QD localize in the blood vessels of most organs, but they don‘t extravasate or accumulate in the tissues. QD extravasate to the extracellular space through the sinusoidal capillaries and accumulate in the liver cells.

3) CdSe/ZnS-mPEG QD accumulate in rat placenta but they are not transferred through the placental barrier to the foetuses.

4) The migration of CdSe/ZnS-mPEG QD in the connective tissues depends on the structure of extracellular tissue fibers: QD freely migrate in the loose connective tissues (dermis, epineurium, epimysium, *tunica adventitia*, etc.), but they can not permeate densely organized tissue fibers (basement membranes, perineurium) and don’t pass to the epidermis, hair follicles, skin glands, nerves and muscle cells.

5) The main migration pathways of CdSe/ZnS-mPEG QD depend on the administration route: QD injected into connective tissues are drained via the lymphatic system and pour into blood circulation; QD located in the blood are transfered only within it; QD applied on the skin are retained by the stratum corneum and don‘t reach other tissues.

6) Higher stability of optical properties, lower toxicity and more efficient migration in the tissues of CdSe/ZnS-mPEG QD determine that they are more suitable for fluorescence imaging *in vivo* than CdTe-MPA QD.

5. SANTRAUKA (Summary in Lithuanian)

Įvadas

Nanotechnologijos vis plačiau taikomos maisto pramonėje, optoelektronikoje, buitinėje chemijoje, kosmetologijoje ir kitose žmogaus veiklos srityse. Nanodalelės (ND) diegiamos į naujai kuriamus medicinos diagnostikos ir gydymo metodus kaip kontrastinės medžiagos optiniame, magnetinio rezonanso, rentgeno ar daugiamodaliniame vaizdinime. Taip pat naudojamos kaip biosensoriai, vaistų ar DNR nešikliai. ND pasižymi naujomis savybėmis, kurios nebūdingos tos pačios cheminės sudėties medžiagoms, kai jos yra mikrometrų eilės ar didesnių matmenų, pvz., pasireiškia superparamagnetizmas, superlaidumas, fotoliuminescencija (FL) ir kt. Nanoskalėje ženkliai išauga dalelių paviršiaus ir tūrio santykis. Tai vienas esminių veiksnių nulemiančių išskirtinį ND dėmesį biomedicinos tyrimuose, nes jų susikaupimas audiniuose ir sukeliamas biologinis poveikis visų pirma priklauso nuo jų paviršiaus sąveikos su organizmo molekulėmis – ląstelių receptoriais, fermentais, genetine medžiaga ir kt.

Kvantiniai taškai (KT) yra puslaidininkinės ND, pasižyminčios išskirtinėmis fizikocheminėmis savybėmis, tokiomis kaip aukštas FL kvantinis našumas, fotostabilumas, nuo cheminės sudėties ir dydžio priklausomas FL spektras, plati sugerties juosta ir kt. KT paviršius gali būti modifikuojamas įvairiais ligandais, siekiant juos funkcionalizuoti ir pritaikyti specializuotiems biologiniams taikymams. Šios savybės sudaro sąlygas panaudoti KT neinvaziniam fluorescenciniam biologinių objektų vaizdinimui, pvz., piktybinių auglių diagnostikoje, angiogenezės ar kraujotakos sutrikimų tyrimuose. Prie KT paviršiaus prijungus vaisto molekules, galima sukurti daugiafunkcinius nanodarinius, kurie vienu metu suteiktų vaizdinimo galimybę ir pasižymėtų terapinėmis savybėmis. KT gali būti panaudojami kaip neorganinės ND modelis tiriant kitų ND lokalizaciją organizme, nes jų pasiskirstymas labiausiai priklauso nuo jų geometrinių ir paviršinių savybių, o vidinės sandaros įtaka dažniausiai nereikšminga.

Yra parodyta, kad KT gali būti pritaikomi sveikų audinių ir navikų kraujotakos sistemos vaizdinimui (1, 2). Visgi KT lokalizacija kraujagyslių sienelėse, poveikis

endotelio ląstelių gyvybingumui ir su tuo susijęs pašalinis KT poveikis nėra plačiai ištirtas. KT pernaša per kraujagyslių sienelę itin aktuali siekiant KT susikaupimo specifinėse organizmo vietose, pvz., galvos smegenyse ar navikuose, siekiant selektyviai vaizdinti šiuos audinius diagnostikos tikslais ar paveikti jų ląsteles ligų gydymui. Daugelio vaistų vartojimas nėštumo metu yra ribotas dėl galimo šalutinio poveikio embriogenezei. Todėl domimasi KT ir kitų ND medicininiu panaudojimu nėštumo metu tikintis, kad jos kaupsis motinos audiniuose, nepateks į embrioną ir nesutrikdys jo vystymosi. Tačiau kol kas KT prasiskverbimas per placentos barjerą ir jų patekimo į embrioną būdai nėra ištirti. Eksperimentinių gyvūnų tyrimuose yra parodyta, kad KT gali būti embriotoksiški, tačiau šio proceso mechanizmas nėra išaiškintas (3).

Kitas svarbus klausimas susijęs su saugiu KT taikymu biomedicinoje yra jų prasiskverbimas per apsauginius biologinius barjerus, pvz., odos, kvėpavimo takų, virškinamojo trakto ir kt. Yra parodyta, kad ant sveikos odos užtepti KT yra sulaikomi raginio epidermio sluoksnio ir į gilesnius audinius nepatenka (4). Visgi pažeidus paviršinius odos sluoksnius pastebėta, kad KT aptinkami ir dermoje. Konkretūs KT pernašos mechanizmai per epidermį nėra ištirti. Kol kas nėra išaiškinta, kokiuose audiniuose lokalizuojasi į odą patekę KT, kokiose ląstelėse jie kaupiasi, ar jie yra išnešiojami po visą organizmą ir kaip jie gali paveikti audinių funkcijas. Šie klausimai aktualūs tiek vertinant potencialiai žalingą KT poveikį, tiek ir optimizuojant biomedicininius taikymus, kurie susiję su KT įvedimu per odą ar į poodį.

Svarbu paminėti, kad ND kaupimosi, pasišalinimo ir poveikio organizmui tyrimai itin svarbūs toksikologiniu požiūriu. Šiuo metu didžiausi nanotaršos šaltiniai yra pramonė ir autotransportas. Tačiau nanoinžinerijos kuriamų komercinių produktų gamybos apimtys sparčiai auga. Todėl ND pagrįsti produktai tampa potencialiu taršos šaltiniu, kurio rizika žmogaus sveikatai bei aplinkosaugai nėra įvertinta.

Optimaliam KT taikymui biomedicinoje būtinos žinios apie jų pasišalinimą iš organizmo ir ilgalaikį stabilumą audiniuose. Yra parodyta, kad žiurkėms suleidus CdSeTe/ZnS KT, dengtų PEG, praėjus 4 mėnesiams >99% suleistos kadmio dozės išliko organizme (5). Tai rodo, kad KT per šį laikotarpį praktiškai nebuvo pašalinti iš organizmo. Kepenyse ir inkstuose buvo aptikta toksiškų kadmio junginių. Vadinasi, dalis KT suiro ir apnuodijo organizmą. Manoma, kad KT šalinimui iš kūno didžiausią įtaką

turi jų paviršiaus padengimas bei dydis. Tačiau tikslios priežastys, lemiančios ilgalaikį KT susikaupimą ir lokalizaciją organuose, kol kas nėra nustatytos.

Aptartos problemos, susijusios su KT taikymu biomedicinoje, sukelia fundamentinių tyrimų poreikį, kurie suteiktų išsamių žinių apie KT prasiskverbimą per biologinius barjerus, jų farmakokinetiką, lokalizaciją audiniuose, ląstelėse bei jų kompartmentuose, KT stabilumą bei poveikį organizmo funkcijoms, ilgalaikį susikaupimą ir pasišalinimą iš organizmo.

Darbo tikslas

Ištirti CdSe/ZnS-mPEG kvantinių taškų migracijos kelius eksperimentinių gyvūnų audiniuose naudojant fluorescencinės mikroskopijos ir spektroskopijos metodus.

Darbo uždaviniai:

1) Ištirti kvantinių taškų (KT) prasiskverbimą per kraujagyslių sieneles ir įvertinti jų kaupimąsi vidaus organuose.

2) Ištirti KT prasiskverbimą per žiurkės placentos barjerą ir įvertinti jų kaupimąsi embrionuose.

3) Ištirti po oda suleistų KT pasiskirstymą audiniuose.

4) Nustatyti pagrindinius KT migracijos kelius organizme įvedant juos skirtingais būdais.

5) Palyginti CdSe/ZnS-mPEG ir CdTe-MPR KT stabilumą žiurkės audiniuose ir įvertinti jų pritaikomumą fluorescenciniame vaizdinime *in vivo*.

Mokslinis naujumas ir aktualumas

Pirmą kartą ištirtos odos barjerinės savybės KT prasiskverbimui bazaline – apikaline kryptimi ir įvertinta bazinės membranos reikšmė KT migracijai per epidermį ir kitas odos dalis: riebalų bei prakaito liaukas, plaukų folikulus. Šie rezultatai gali būti panaudoti aiškinant KT prasiskverbimo per odos barjero veikimo principus. Duomenys taip pat svarbūs, kuriant KT ir kitomis ND pagrįstus vaistų pernašos metodus, kurių optimalus taikymas reikalauja išsamių žinių apie jų pasiskirstymą audiniuose, gebėjimą pasiekti bei paveikti tikslinius taikinius odoje.

Pirmą kartą parodyta, kad KT difuzija jungiamuosiuose audiniuose priklauso nuo ekstraląstelinių darinių struktūros: KT laisvai migruoja puriuosiuose jungiamuosiuose audiniuose (dermoje, epineuriume, epimyziume, adventicijoje ir kt.), tačiau neprasiskverbia per audinių struktūras, sudarytas iš tankių skaidulinių sluoksnių (bazinę membraną, perineuriumą).

Pirmą kartą parodyta, kad CdSe/ZnS-mPEG KT neįsiskverbia į sveikų kraujagyslių raumeninį kraujagyslių sluoksnį *(tunica media)* tiek iš kraujagyslės vidaus, tiek iš jos išorės. Šie rezultatai naudingi aiškinantis KT pernašos per kraujagyslių sieneles mechanizmus. Tai sudaro sąlygas taikyti KT ir kitas ND selektyviam kraujagyslių pažaidų, kurioms būdingas sienelių struktūrinio vientisumo pažeidimas (pvz., aterosklerozės židinių), vaizdinimui, diagnostikai ir gydymui.

Pirmą kartą parodyta, kad CdSe/ZnS-mPEG KT yra sulaikomi placentos barjero ir nepatenka į embrionus. Šis faktas svarbus nagrinėjant KT embriotoksiškumo ir teratogeniškumo mechanizmus. Rezultatai taip pat naudingi vertinant KT bei kitų ND patekimo į organizmą riziką nėštumo metu. KT neprasiskverbimas per placentos barjerą aktualus jų taikymams tikslinėje vaistų pernašoje siekiant sukurti tokius junginius, kurie veiktų tik motinos organizmą, bet nepatektų į embrioną ir nepaveiktų jo vystymosi.

Pirmą kartą palygintas nefunkcionalizuotų CdSe/ZnS-mPEG KT kaupimasis skirtingose ląstelėse *in vivo* sąlygomis. Šie rezultatai aktualūs aiškinantis KT kaupimosi audiniuose ir jų biologinio poveikio mechanizmus.

Ginamieji teiginiai

1) Kraujagyslės viduje lokalizuoti KT sąveikauja su endoteliu (*tunica intima*), tačiau pro jį neprasiskverbia ir nepatenka į vidurinį kraujagyslės sienelės sluoksnį (*tunica media*).
2) Kraujagyslių išorėje esantys KT kaupiasi išoriniame sienelės sluoksnyje (*tunica adventitia*), tačiau nepatenka giliau į vidurinį kraujagyslės sienelės sluoksnį (*tunica media*).
3) KT lokalizuojasi daugumos organų kraujagyslėse, tačiau į jų tarpląstelinę terpę nepatenka. KT ekstravazacija į tarpląstelinę terpę gali vykti per sinusinius kapiliarus.
4) KT kaupiasi nėščių žiurkių placentoje, tačiau yra sulaikomi placentos barjero ir nepatenka į embrionų audinius.
5) KT difuziją jungiamuosiuose audiniuose apsprendžia ekstraląstelinių skaidulų organizacija: KT laisvai migruoja puriuosiuose jungiamuosiuose audiniuose (dermoje, epineuriume, epimyziume, adventicijoje ir kt.), tačiau neprasiskverbia per tankius skaidulinius sluoksnius (bazines membranas, perineuriumą) ir nepatenka į epidermį, plaukų folikulus, odos liaukas, nervų vidų ir raumenų skaidulas.
6) Į jungiamuosius audinius patekę CdSe/ZnS-mPEG KT rezorbuojasi į limfos kapiliarus, migruoja limfine sistema ir su limfa įsilieja į kraujotaką.
7) Ant odos užtepti CdSe/ZnS-mPEG KT neprasiskverbia per sveiką ir UVB spinduliuotės pažeistą epidermio barjerą.
8) CdSe/ZnS-mPEG KT pasižymi stabilesnėmis optinėmis savybėmis efektyvesne migracija audiniuose ir mažesniu toksiškumu negu CdTe-MPR KT.

Rezultatų apibendrinimas

Šiame darbe nagrinėjamas CdSe/ZnS-mPEG KT prasiskverbimas per skirtingus audinius ir pasiskirstymas organizme įvedant juos į organizmą skirtingais būdais. KT lokalizacija audinių ir ląstelių lygmenyse suteikia duomenų aiškinant pagrindinius KT migracijos kelius organizme. KT lokalizacija audiniuose analizuojama konfokalinę fluorescencinę mikroskopiją derinant su klasikinės histologijos metodais. Iki šiol toks

būdas buvo pritaikytas tik KT lokalizacijos limfmazgiuose tyrimui (51). Mikroskopijos metodai leidžia įvertinti KT kaupimąsi ląsteliniame lygyje ir tiksliau nusakyti jų lokalizaciją negu plačiai paplitę elementinės analizės (pvz., masės spektrometrija, radioaktyvių izotopų scintigrafija) ar fluorescencinio vaizdinimo metodai.

Eksperimentinių gyvūnų audinių mikroskopijos tyrimai rodo, kad CdSe/ZnS-mPEG KT neprasiskverbia per kraujagyslių sieneles ir nėra pernešami iš tarpląstelinės terpės į kraujagyslių vidų. Tai nulemia, kad KT nėra tiesiogiai rezorbuojami į kraujotakos sistemą ir išnešiojami po visą organizmą. Eksperimentiniai rezultatai rodo, kad į tarpląstelinę audinių terpę suleisti KT migruoja iš suleidimo vietos limfine sistema. Manoma, kad tai apsprendžia dalelių geometriniai matmenys, nes CdSe/ZnS-mPEG KT yra panašaus dydžio (HD ~23 nm (12)) kaip kai kurie tarpląstelinės terpės baltymai ir patogenai, kuriuos pernešti su audinių skysčiu yra prisitaikę limfos kapiliarai. Taigi KT migraciją iš audinių per limfinę sistemą apsprendžia KT dydis ir didesnis limfos kapiliarų sienelių laidumas kraujo kapiliarų atžvilgiu. Tai sudaro palankias sąlygas naudoti KT limfinės sistemos anatominiams ir funkciniams tyrimams bei taikyti KT fluorescenciniame limfmazgių vaizdinime operacijų metu.

KT pernašos ribojimas iš kraujagyslių vidaus į tarpląstelinę terpę sąlygoja tai, kad jie nepatenka į daugumos organų audinius. Tai paaiškina, kodėl mūsų ir daugumos kitų autorių tyrimais nenustatomas KT susikaupimas ne RES audiniuose net ir ilgalaikėje perspektyvoje. Tuo tarpu KT aptikimas RES organuose dalinai paaiškinamas jų kapiliarų sienelės struktūriniais skirtumais, t.y., didesnėmis endotelio poromis, plonesne ir nevientisa bazine membrana, kitų audinių atžvilgiu (4, 52). Kita vertus mikroskopijos rezultatai rodo, kad audiniuose esantys KT patenka į makrofagus endocitozės būdu. Šis procesas gali papildomai prisidėti prie KT kaupimosi RES organuose, kuriuose telkiasi makrofagai ir kitos fagocituojančios ląstelės.

Pažymėtina, kad KT nepatenka į sveikų kraujagyslių vidurinį sluoksnį (*tunica media*) ir tai siejama su KT sulaikymu ties endoteliu (iš vidaus į išorę) ir ties lygiųjų raumenų skaidulų tarpusavio jungtimis bei bazine membrana (iš išorės į vidų). Tai sudaro sąlygas taikyti KT kraujagyslių pažaidų, kurioms būdingi struktūrinio vientisumo pokyčiai (pvz., aterosklerozės židinių), vaizdinime ir diagnostikoje. Todėl tikimasi, kad gauti rezultatai praplės KT taikymo perspektyvas biomedicinoje ir suteiks pagrindą panaudoti KT aterosklerotinių pakitimų tyrimams bei naujų medicinos metodų kūrimui.

Odos audinių mikroskopinė analizė rodo, kad KT neprasiskverbia iš dermos jungiamojo audinio į plaukų folikulus, prakaito bei riebalų liaukas, griaučių skersaruožių raumenų skaidulas ir kitus audinius. Visiems šiems dariniams būdinga bazinė membrana, kuri juos skiria nuo aplinkinės tarpląstelinės medžiagos ir kurioje yra KT. Todėl ji yra pirminis barjeras ribojantis KT difuziją į minėtus audinius. KT taip pat yra sulaikomi tankiai išdėstytų jungiamojo audinių skaidulų, kurios sudaro perineuriumą ir riboja KT patekimą į periferinių nervų vidų. Apibendrinant šiuos faktus galima teigti, jog KT migraciją audiniuose riboja specializuoti tankiai organizuotų ekstraląstelinių skaidulų sluoksniai. Šis apibendrinimas svarbus tuo, jog bazinės membranos juosia visus epitelinius audinius, kurie sudaro pagrindinius organizmo apsauginius barjerus (odos, kvėpavimo ir lytinių takų, virškinamojo trakto ir kt.), tačiau literatūroje KT pernaša per bazines membranas yra praktiškai nenagrinėta ir jos reikšmė KT lokalizacijai audiniuose yra neįvertinta.

KT taip pat yra sulaikomi placentos barjero ir iš motinos į embriono audinius nepatenka. Šį fiziologinį barjerą visų pirma apsprendžia trofoblasto ląstelės, kurios yra glaudžiai susietos tarpląstelinėmis jungtimis. Šie rezultatai papildo KT prasiskverbimo per kraujagysles tyrimus ir rodo, kad KT yra sulaikomi ne tik endotelio bet ir kitų vientisą sluoksnį formuojančių ląstelių. Tarpląstelinės pernašos ribojamas per šiuos sluoksnius grindžiamas tuo, kad tarpai tarp ląstelių yra per maži, kad per juos vyktų KT prasiskverbimas. Tuo tarpu transląstelinė pernaša yra sudėtingesnis procesas, kuris sąlygotas apikalinės membranos receptorių raiškos, KT endocitozės, viduląstelinės pernašos ir sekrecijos bazalinėje membranoje. Manoma, kad svarbiausias vaidmuo šioje grandinėje yra KT sąveikos su transląstelinę pernašą užtikrinančiais endotelio ir trofoblasto receptoriais nebuvimas. KT pernašos mechanizmai per vidinius organizmo barjerus nėra plačiai ištirti. Todėl tikimasi, kad šie darbo rezultatai bus naudingi tiriant KT ir kitų panašaus dydžio bei paviršinių savybių ND prasiskverbimo per biologinius barjerus mechanizmus. Rezultatai taip pat naudingi kuriant ND pagrįstus farmakologinius preparatus, nes efektyviam jų taikymui būtina žinoti, kur organizme lokalizuojasi šios ND ir kokius audinius jos gali paveikti.

Audinių mikroskopijos tyrimai parodė, kad CdSe/ZnS-mPEG KT kaupiasi kai kurių ląstelių viduje *in vivo* sąlygomis ir į jas patenka endocitozės būdu. Viduląstelinis KT susikaupimas priklauso nuo ląstelių tipo: intensyviausia KT FL aptikta

makrofaguose, eozinofiluose, mažesnio intensyvumo – dermos fibroblastuose, tuo tarpu raumeninėse skaidulose, limfocituose bei eritrocituose KT neaptikti. Šie skirtumai grindžiami ląstelių specializacija ir jų skirtingu endocitotiniu aktyvumu. Pvz., makrofagai pasižymi aktyvia nespecifine endocitoze, kuri reikalinga neutralizuojant patogenus. Tuo tarpu eritrocitams endocitozė nebūdinga (53), todėl tai greičiausiai sąlygojo KT nepatekimą į jų vidų. Tai taip pat parodo, kad KT pasyvi difuzija per plazminę eritrocitų membraną nevyksta. Limfocitai sudaro didelės įvairovės ląstelių grupę ir jų endocitotinis aktyvumas priklauso nuo diferenciacijos ir paviršinių receptorių raiškos. Šios ląstelės dalyvauja specifinio imuninio atsako formavime, todėl tikėtina, kad KT patekimui į ląsteles reikalinga specifinė sąveika su atitinkamais ląstelės paviršiaus receptoriais, kurie inicijuoja endocitozę, pvz., DEC-205, CD36, DC-SIGN ar kt. (54). Kadangi tyrime naudoti nefunkcionalizuoti KT, jų sąveika su šiais receptoriais greičiausiai buvo per silpna. KT kaupimasis eozinofiluose bei putliosiose ląstelėse iki šiol nebuvo stebėtas ir tai yra įdomus faktas, nes šios ląstelės dalyvauja alerginėse reakcijose. Pranešama, kad TiO_2 ir fulerenų ND sukelia alerginį atsaką organizme, tačiau šio vyksmo mechanizmai nėra ištirti (55). Taigi ND kaupimasis eozinofiluose gali būti susijęs jų sukeliamomis alerginėmis reakcijomis ir mūsų tyrimo rezultatai gali būti naudingi aiškinantis ND sukeliamo biologinio atsako mechanizmą. Pažymėtina, kad dauguma KT kaupimosi ląstelėse tyrimų atliekami *in vitro* sąlygomis, tačiau KT viduląstelinis susikaupimas *in vivo* nėra plačiai ištirtas. Yra atlikta tyrimų, rodančių, kad specifiniais ligandais funkcionalizuoti KT gali būti selektyviai kaupiami atitinkamus receptorius turinčiose ląstelėse (48, 56, 57). Visgi palyginamųjų tyrimų apie nefunkcionalizuotų KT kaupimąsi skirtingose ląstelėse *in vivo* nėra atlikta. KT kaupimasis ląstelėse yra svarbus prognozuojant jų lokalizaciją bei poveikį ląstelėms klinikiniuose taikymuose ir vertinant iš KT sudarytų elektronikos ar kitų produktų vartojimo saugumą.

Efektyviam KT taikymui taip pat svarbus jų optinių savybių stabilumas ir struktūrinis vientisumas *in vivo* sąlygomis. Darbo rezultatai rodo, kad CdTe-MPR KT patekus į žiurkės audinius kinta jų spektroskopinės savybės, rodančios, kad vyksta KT sąveika su organizmo molekulėmis ir (arba) vyksta paviršinių KT ligandų destabilizacija ir KT agregacija. Šie vyksmai galutiniame rezultate sukelia audinių nekrozę, t.y. ląstelių žūtį. Tuo tarpu CdSe/ZnS-mPEG KT FL savybės išlieka pakankamai stabilios, kad šie

KT galėtų būti pritaikyti ilgalaikiam audinių vaizdinimui. Šie KT nesukelia audinių nekrozės, todėl jie yra pranašesni fluoroforai ir biosuderinamumo atžvilgiu.

Darbo išvados:

1) CdSe/ZnS-mPEG KT prasiskverbimo per kraujagyslių sieneles tyrimai parodė, kad kraujagyslių viduje esantys KT sąveikauja su endoteliu (*tunica intima*), o išorinėje kraujagyslių aplinkoje esantys KT patenka į išorinį sienelės sluoksnį (*tunica adventitia*), tačiau KT neįsiskverbia į vidurinį kraujagyslės sienelės sluoksnį (*tunica media*).
2) Į kraujotaką suleistų KT pasiskirstymo audiniuose tyrimai parodė, kad CdSe/ZnS-mPEG KT lokalizuojasi daugumos organų kraujagyslėse, tačiau į tarpląstelinę terpę ar audinių ląsteles nepatenka. KT prasiskverbia per sinusinius kapiliarus ir kaupiasi kepenų ląstelėse.
3) CdSe/ZnS-mPEG KT pasiskirstymo nėščių žiurkių organizme tyrimai atskleidė, kad KT patenka į placentą, tačiau yra sulaikomi placentos barjero ir nepatenka į embriono audinius.
4) CdSe/ZnS-mPEG KT lokalizacijos odoje tyrimai parodė, kad KT migraciją jungiamuosiuose audiniuose sąlygoja ekstraląstelinių skaidulų organizacija: KT laisvai migruoja puriuosiuose jungiamuosiuose audiniuose (dermoje, epineuriume, epimyziume, adventicijoje ir kt.), tačiau neprasiskverbia per tankius skaidulinius sluoksnius (bazines membranas, perineuriumą) ir nepatenka į epidermį, plaukų folikulus, odos liaukas, nervų vidų ir raumenų skaidulas.
5) CdSe/ZnS-mPEG KT migracija organizme priklauso nuo jų suleidimo būdo: į jungiamuosius audinius suleisti KT juda puriaisiais jungiamaisiais audiniais, rezorbuojasi į limfinius kapiliarus ir kartu su limfa įsilieja į kraujotaką; į kraują patekę KT pernešami tik jo sudėtyje; ant sveikos odos užtepti KT į kitus audinius nepatenka.
6) Didesnis CdSe/ZnS-mPEG KT optinių savybių stabilumas, efektyvesnė migracija audiniuose ir mažesnis toksiškumas negu CdTe-MPR KT sąlygoja tai, kad šie KT yra tinkamesni fluorescenciniui vaizdinimui *in vivo*.

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List of publications

Publications listed in the *Thomson Reuters Web of Science* database:

1. Kulvietis V, Zurauskas E, Rotomskis R. Distribution of polyethylene glycol coated quantum dots in mice skin. Experimental Dermatology 2013, 22(2):157-159.
2. Žalgevičienė V, Kulvietis V, Bulotienė D, Didžiapetrienė J, Rotomskis R. The effects of nanoparticles during critical periods of pregnancy in rats. Medicina (Kaunas) 2012, 48(5): 256-264.
3. Kulvietis V, Zalgeviciene V, Didziapetriene J, Rotomskis R. Transport of Nanoparticles through the Placental Barrier. Tohoku Journal of Experimental Medicine 2011;225(4): 225-234.
4. Kulvietis V, Streckytė G, Rotomskis R. Spectroscopic investigations of CdTe quantum dot stability in different aqueous media. Lithuanian Journal of Physics 2011, 51(2): 163–171.
5. Jazdauskaitė E, Kulvietis V, Rotomskis R. Combined risk effects of quantum dot nanoparticles exposure and ultraviolet radiation to skin. Proceedings of the 10th international conference on medical physics (Medical Physics in the Baltic States); Kaunas, Lithuania. Technologija, 2012, p. 67-71.
6. Kulvietis V, Rotomskis R. Accumulation of quantum dots in Ehrlich ascites tumour in mice. Proceedings of the 8th international conference on medical physics (Medical Physics in the Baltic States); 2010 October 14-16; Kaunas, Lithuania. Technologija, 2010, p. 50-53.
7. Kišonas J, Kulvietis V. Spectroscopic study of quantum dots distribution in vivo. Proceedings of the 8th international conference on medical physics (Medical Physics in the Baltic States); 2010 October 14-16; Kaunas, Lithuania. Technologija, 2010, p. 54-58.

Other peer-reviewed publications:

1. Kulvietis V, Žalgevičienė V, Didžiapetrienė J, Bulotienė D, Rotomskis R. Distribution of nanoparticles in the pregnant rat: the morphologic and spectroscopic study. Papers on Anthropology 2011, 20: 218-228.
2. Kulvietis V, Žurauskas E, Ričardas R. Investigation of quantum dots distribution pathways in mice. Conference proceedings of the 4th European conference for clinical nanomedicine; 2011 May 23-25, Basel, Switzerland. 2011, p. 125.
3. Karabanovas V, Kulvietis V, Rotomskis R, Valius M. The pathway of carboxyl-coated quantum dots accumulation in the embryonic fibroblast NIH3T3 cells. Conference proceedings of the 4th European conference for clinical nanomedicine; 2011 May 23-25; Basel, Switzerland. 2011, p. 119.
4. Kulvietis V, Karabanovas V, Rotomskis R, Valius M. Investigation of quantum dots cellular uptake and distribution in vitro and in vivo. Conference proceedings of the 3rd European conference for clinical nanomedicine; 2010 may 10-12; Basel, Switzerland. 2010, p. 85-6.
5. Kulvietis V, Janutytė I, Bagdonas S, Karabanovas V, Rotomskis R. Effect of medium pH on stability of quantum dots: spectroscopic study and biological implications. Proceedings of the 6th international conference on medical physics

(Medical Physics in the Baltic States); 2008 October 10-11; Kaunas, Lithuania. Technologija, 2008, p.15-8.

Presentations in national and international conferences:

1. Kulvietis V, Zalgeviciene V, Bulotiene D, Rotomskis R. Penetration through the placental barrier and embryotoxicity of semiconductor nanoparticles. European society for photobiology 2013 congress; 2-6 September; Liege, Belgium. Abstract Book, p.111-2.
2. Jazdauskaitė E, Kulvietis V, Rotomskis R. Photostability of quantum dots and its implication in biosafety of nanomaterials. Open Readings 2013 March 20-23, Vilnius, Lithuania. Abstract book, p. 45.
3. Jazdauskaitė E, Kulvietis V, Rotomskis R. Nanodalelių prasiskverbimo per apsauginį odos barjerą spektroskopiniai tyrimai. 40-oji Lietuvos nacionalinė fizikos konferencija; 2013 June 10-12; Vilnius, Lithuania. Vilniaus universitetas, p.119.
4. Niciūtė I, Kulvietis V, Rotomskis R. Kvantinių taškų ir infraraudonojo dažo pasiskirstymo eksperimentiniuose gyvūnuose tyrimas fluorescenciniais metodais. (Investigation of biodistribution of quantum dots and infrared dye in experimenatl animals using fluorescence methods). 40-oji Lietuvos nacionalinė fizikos konferencija. (40th National Physics Conference of Lithuania); 2013 June 10-12; Vilnius, Lithuania. Vilnius Universitety, p.136.
5. Jazdauskaitė E, Kulvietis V, Rotomskis R. Combined risk effects of quantum dot nanoparticles exposure and ultraviolet radiation to skin. Medical Physics in the Baltic States: Proceedings of the 10th international conference on medical physics; 2012 November 8-10; Kaunas, Lithuania. Technologija, 2012, p. 67-71.
6. Kulvietis V, Karabanovas V, Jagminas A, Rotomskis R. Application of magnetic nanoparticles in cancer imaging and therapy. Modern radiation oncology: economical aspects and innovations in the treatment. 2012 September 14-15, Palanga, , Lithuania.
7. Kulvietis V, Kišonas J, Rotomskis R. Quantum dot migration in the lymphatic system of mouse after subcutaneous injection. Nanomedicine:Visions, risks, potential. 2012 April 19-20; Berlin, Germany, p. 27.
8. Bajerčius H, Kulvietis V. Kvantinių taškų stabilumo vandens ir kraujo tirpaluose tyrimai (The stability of quantum dots in aqueous solutions and blood). VU MF Studentų mokslinės draugijos LXIV konferencija (The students‘ conference of Medical Faculty of Vilnius University), p. 222-3.
9. Kulvietis V, Rotomskis R. Fluorescencinis odos vaizdinimas naudojant kvantinius taškus (Fluorescence imaging of skin using quantumk dots). 39-oji Lietuvos nacionalinė fizikos konferencija (39th National Physics Conference of Lithuania). 2011 October 6-8; Vilnius, Lithuania. Vilnius University, p. 161.
10. Kulvietis V, Žalgevičienė V, Didžiapetrienė J, Bulotienė D, Rotomskis R. Distribution of nanoparticles in the pregnant rat: the morphologic and spectroscopic study. Baltic Morphology VI. Abstracts of presentations; 2011 September 22-23; Tartu, Estija, p. 36.
11. Kulvietis V, Žurauskas E, Ričardas R. Investigation of quantum dots distribution pathways in mice. 4th European conference for clinical nanomedicine. Conference proceedings; 2011 May 23-25; Basel, Switzerland, p. 125.

12. Karabanovas V, Kulvietis V, Rotomskis R, Valius M. The pathway of carboxyl-coated quantum dots accumulation in the embryonic fibroblast NIH3T3 cells. 4th European conference for clinical nanomedicine. Conference proceedings; 2011 May 23-25; Basel, Switzerland, p. 119.
13. Kulvietis V, Rotomskis R. Accumulation of quantum dots in Ehrlich ascites tumour in mice. Medical Physics in the Baltic States: proceedings of the 8th international conference on medical physics; 2010 October 14-16; Kaunas, Lithuania. Technologija, 2010, p. 50-53.
14. Kišonas J, Kulvietis V. Spectroscopic study of quantum dots distribution in vivo. Medical Physics in the Baltic States: proceedings of the 8th international conference on medical physics; 2010 October 14-16; Kaunas, Lithuania. Technologija, 2010, p. 54-58.
15. Kulvietis V, Rotomskis R. Distribution of quantum dots in Ehrlich ascites tumour. 5th Baltic congress of oncology; 2010 May 14-15; Riga, Latvia, p. 74-75.
16. Kišonas J, Kulvietis V, Žalgevičienė V, Rotomskis R. Spectroscopic investigations of quantum dots biodistribution after sucutaneous injection in mouse model. 5th Baltic sea region conference in medical sciences; 2010 May 14-16; Vilnius, Lithuania, p. 214-5.
17. Kulvietis V, Karabanovas V, Rotomskis R, Valius M. Investigation of quantum dots cellular uptake and distribution in vitro and in vivo. 3rd European conference for clinical nanomedicine. Conference proceedings; 2010 May 10-12; Basel, Switzerland, 2010, p. 85-6.
18. Kišonas J, Kulvietis V, Žalgevičienė V, Rotomskis R. Po oda suleistų kvantinių taškų pasiskirstymo pelės organizme tyrimas panaudojant spektroskopinės analizės metodą (The distribution of subcutaneously injected quantum dots in mice). 62-oji Vilniaus universiteto medicinos fakulteto studentų mokslinės draugijos konferencija (62th student conference of medical students of Vilnius University); 2010 April 26 – May 3; Vilnius, Lithuania.
19. Kulvietis V, Janutytė I, Streckytė G, Bagdonas S, Rotomskis R. Vandenyje tirpių CdTe kvantinių taškų stabilumo spektroskopiniai tyrimai (Spectroscopic study of CdTe quantum dots stability in aqueous solutions). 38-oji Lietuvos nacionalinė fizikos konferencija (39th National Physics Conference of Lithuania); 2009 June 8-10; Vilnius, Lithuania. Vilnius University, 2009, p.152
20. Kulvietis V, Janutytė I, Bagdonas S, Karabanovas V, Rotomskis R. Effect of medium pH on stability of quantum dots: spectroscopic study and biological implications. Medical Physics in the Baltic States: proceedings of the 6th international conference on medical physics; 2008 October 10-11; Kaunas, Lithuania. Technologija, 2008, p.15-18

References

1. Chu M, Wu Q, Yang H, Yuan R, Hou S, Yang Y, et al. Transfer of quantum dots from pregnant mice to pups across the placental barrier. Small. 2010 Mar 8;6(5):670-8.
2. Lin CH, Chang LW, Chang H, Yang MH, Yang CS, Lai WH, et al. The chemical fate of the Cd/Se/Te-based quantum dot 705 in the biological system: Toxicity implications. Nanotechnology. 2009 May 27;20(21):215101,4484/20/21/215101. Epub 2009 May 5.
3. Fitzpatrick JA, Andreko SK, Ernst LA, Waggoner AS, Ballou B, Bruchez MP. Long-term persistence and spectral blue shifting of quantum dots in vivo. Nano Lett. 2009 Jul;9(7):2736-41.
4. Ballou B, Lagerholm BC, Ernst LA, Bruchez MP, Waggoner AS. Noninvasive imaging of quantum dots in mice. Bioconjug Chem. 2004 Jan-Feb;15(1):79-86.
5. Yang RS, Chang LW, Wu JP, Tsai MH, Wang HJ, Kuo YC, et al. Persistent tissue kinetics and redistribution of nanoparticles, quantum dot 705, in mice: ICP-MS quantitative assessment. Environ Health Perspect. 2007 Sep;115(9):1339-43.
6. Rehberg M, Leite CF, Mildner K, Horstkotte J, Zeuschner D, Krombach F. Surface chemistry of quantum dots determines their behavior in postischemic tissue. ACS Nano. 2012 Feb 28;6(2):1370-9.
7. Praetner M, Rehberg M, Bihari P, Lerchenberger M, Uhl B, Holzer M, et al. The contribution of the capillary endothelium to blood clearance and tissue deposition of anionic quantum dots in vivo. Biomaterials. 2010 Sep;31(26):6692-700.
8. Wick P, Malek A, Manser P, Meili D, Maeder-Althaus X, Diener L, et al. Barrier capacity of human placenta for nanosized materials. Environ Health Perspect. 2010 Mar;118(3):432-6.
9. Steed E, Balda MS, Matter K. Dynamics and functions of tight junctions. Trends Cell Biol. 2010 Mar;20(3):142-9.
10. Yamashita K, Yoshioka Y, Higashisaka K, Mimura K, Morishita Y, Nozaki M, et al. Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. Nat Nanotechnol. 2011 May;6(5):321-8.
11. Menjoge AR, Rinderknecht AL, Navath RS, Faridnia M, Kim CJ, Romero R, et al. Transfer of PAMAM dendrimers across human placenta: Prospects of its use as drug carrier during pregnancy. J Control Release. 2011 Mar 30;150(3):326-38.
12. Ballou B, Ernst LA, Andreko S, Harper T, Fitzpatrick JA, Waggoner AS, et al. Sentinel lymph node imaging using quantum dots in mouse tumor models. Bioconjug Chem. 2007 Mar-Apr;18(2):389-96.
13. Choi HS, Liu W, Misra P, Tanaka E, Zimmer JP, Itty Ipe B, et al. Renal clearance of quantum dots. Nat Biotechnol. 2007 Oct;25(10):1165-70.
14. Bennett KM, Zhou H, Sumner JP, Dodd SJ, Bouraoud N, Doi K, et al. MRI of the basement membrane using charged nanoparticles as contrast agents. Magnetic Resonance in Medicine. 2008 Sep;60(3):564-74.
15. Cleal K, He L, Watson PD, Jones AT. Endocytosis, intracellular traffic and fate of cell penetrating peptide based conjugates and nanoparticles. Curr Pharm Des. 2013;19(16):2878-94.

16. Damalakiene L, Karabanovas V, Bagdonas S, Valius M, Rotomskis R. Intracellular distribution of nontargeted quantum dots after natural uptake and microinjection. Int J Nanomedicine. 2013;8:555-68.
17. Eisenmann CJ, Miller RK. The placental transfer and toxicity of selenite relative to cadmium in the human term perfused placenta. Placenta. 1994 Dec;15(8):883-95.
18. Hashmi S, Marinkovich MP. Molecular organization of the basement membrane zone. Clin Dermatol. 2011 Jul-Aug;29(4):398-411.
19. Kennedy WR, Sakuta M, Quick DC. Rodent eccrine sweat glands: A case of multiple efferent innervation. Neuroscience. 1984;11:741-9.
20. Elias PM. Stratum corneum defensive functions: An integrated view. J Invest Dermatol. 2005 Aug;125(2):183-200.
21. Monteiro-Riviere NA, Wiench K, Landsiedel R, Schulte S, Inman AO, Riviere JE. Safety evaluation of sunscreen formulations containing titanium dioxide and zinc oxide nanoparticles in UVB sunburned skin: An in vitro and in vivo study. Toxicological Sciences. 2011 Sep;123(1):264-80.
22. Baroli B, Ennas MG, Loffredo F, Isola M, Pinna R, Lopez-Quintela MA. Penetration of metallic nanoparticles in human full-thickness skin. J Invest Dermatol. 2007 Jul;127(7):1701-12.
23. Kuchler S, Radowski M, Haag R, Schafer-Korting M. Nanoparticles for skin penetration enhancement - a comparison of dendritic core-multishell nanotransporters and solid lipid nanoparticles. Eur J Pharm Biopharm. 2009 Feb;71(2):243-50.
24. Kato S, Aoshima H, Saitoh Y, Miwa N. Biological safety of LipoFullerene composed of squalane and fullerene-C60 upon mutagenesis, photocytotoxicity, and permeability into the human skin tissue. Basic & Clinical Pharmacology & Toxicology. 2009 Jun;104(6):483-7.
25. Prow TW, Grice JE, Lin LL, Faye R, Butler M, Becker W, et al. Nanoparticles and microparticles for skin drug delivery. Adv Drug Deliv Rev. 2011 May;63(6):470-91.
26. Seto JE, Polat BE, Lopez RFV, Blankschtein D, Langer R. Effects of ultrasound and sodium lauryl sulfate on the transdermal delivery of hydrophilic permeants: Comparative in vitro studies with full-thickness and split-thickness pig and human skin. J Controlled Release. 2010 Jul;145(1):26-32.
27. Shimada A, Kawamura N, Okajima M, Kaewamatawong T, Inoue H, Morita T. Translocation pathway of the intratracheally instilled ultrafine particles from the lung into the blood circulation in the mouse. Toxicol Pathol. 2006;34(7):949-57.
28. Gao JH, Chen K, Xie RG, Xie J, Lee S, Cheng Z, et al. Ultrasmall near-infrared non-cadmium quantum dots for in vivo tumor imaging. Small. 2010 Jan;6(2):256-61.
29. Lademann J, Richter H, Teichmann A, Otberg N, Blume-Peytavi U, Luengo J, et al. Nanoparticles - an efficient carrier for drug delivery into the hair follicles. European Journal of Pharmaceutics and Biopharmaceutics. 2007 May;66(2):159-64.
30. Lauer AC, Lieb LM, Ramachandran C, Flynn GL, Weiner ND. Transfollicular drug-delivery. Pharm Res. 1995 Feb;12(2):179-86.
31. Lademann J, Richter H, Schaefer UF, Blume-Peytavi U, Teichmann A, Otberg N, et al. Hair follicles - A long-term reservoir for drug delivery. Skin Pharmacology and Physiology. 2006;19(4):232-6.
32. Morgen M, Lu GW, Du D, Stehle R, Lembke F, Cervantes J, et al. Targeted delivery of a poorly water-soluble compound to hair follicles using polymeric nanoparticle suspensions. Int J Pharm. 2011 Sep;416(1):314-22.

33. Vogt A, Combadiere B, Hadam S, Stieler KM, Lademann J, Schaefer H, et al. 40 nm, but not 750 or 1,500 nm, nanoparticles enter epidermal CD1a+ cells after transcutaneous application on human skin. J Invest Dermatol. 2006 Jun;126(6):1316-22.
34. Dellinger A, Zhou ZG, Norton SK, Lenk R, Conrad D, Kepley CL. Uptake and distribution of fullerenes in human mast cells. Nanomedicine-Nanotechnology Biology and Medicine. 2010 Aug;6(4):575-82.
35. Pernodet N, Fang XH, Sun Y, Bakhtina A, Ramakrishnan A, Sokolov J, et al. Adverse effects of citrate/gold nanoparticles on human dermal fibroblasts. Small. 2006 Jun;2(6):766-73.
36. Sen D, Deerinck TJ, Ellisman MH, Parker I, Cahalan MD. Quantum dots for tracking dendritic cells and priming an immune response in vitro and in vivo. Plos One. 2008 Sep;3(9).
37. Clift MJD, Brandenberger C, Rothen-Rutishauser B, Brown DM, Stone V. The uptake and intracellular fate of a series of different surface coated quantum dots in vitro. Toxicology. 2011 Aug;286(1-3):58-68.
38. Romoser AA, Chen PL, Berg JM, Seabury C, Ivanov I, Criscitiello MF, et al. Quantum dots trigger immunomodulation of the NF kappa B pathway in human skin cells. Mol Immunol. 2011 Jul;48(12-13):1349-59.
39. Barnes AJ, Brunet BR, Choo RE, Mura P, Johnson RE, Jones HE, et al. Excretion of methadone in sweat of pregnant women throughout gestation after controlled methadone administration. Ther Drug Monit. 2010 Aug;32(4):497-503.
40. Stolinski C. Structure and composition of the outer connective-tissue sheaths of peripheral-nerve. J Anat. 1995 Feb;186:123-30.
41. Karabanovas V, Zakarevicius E, Sukackaite A, Streckyte G, Rotomskis R. Examination of the stability of hydrophobic (CdSe)ZnS quantum dots in the digestive tract of rats. Photochemical & Photobiological Sciences. 2008;7(6):725-9.
42. Boldt K, Bruns OT, Gaponik N, Eychmuller A. Comparative examination of the stability of semiconductor quantum dots in various biochemical buffers. J Phys Chem B. 2006 Feb 9;110(5):1959-63.
43. Gui M, Bao L, Xia Y, Wei C, Zhang S, Zhu C. Indication of intracellular physiological pH changes by L-cysteine-coated CdTe quantum dots with an acute alteration in emission color. Biosens Bioelectron. 2011 Dec 15;30(1):324-7.
44. Wang L, Zhang J, Zheng Y, Yang J, Zhang Q, Zhu X. Bioeffects of CdTe quantum dots on human umbilical vein endothelial cells. J Nanosci Nanotechnol. 2010 Dec;10(12):8591-6.
45. Liu L, Zhang J, Su X, Mason RP. In vitro and in vivo assessment of CdTe and CdHgTe toxicity and clearance. J Biomed Nanotechnol. 2008 Dec 1;4(4):524-8.
46. Hazardous substances data bank. National Institute of Health. Accessed on 2013.10.08. Link: Http://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+hsdb:@rn+107-96-0.
47. Mosqueira VC, Legrand P, Morgat JL, Vert M, Mysiakine E, Gref R, et al. Biodistribution of long-circulating PEG-grafted nanocapsules in mice: Effects of PEG chain length and density. Pharm Res. 2001 Oct;18(10):1411-9.
48. Smith BR, Cheng Z, De A, Rosenberg J, Gambhir SS. Dynamic visualization of RGD-quantum dot binding to tumor neovasculature and extravasation in multiple living mouse models using intravital microscopy. Small. 2010 Oct 18;6(20):2222-9.

49. Smith JD, Fisher GW, Waggoner AS, Campbell PG. The use of quantum dots for analysis of chick CAM vasculature. Microvasc Res. 2007 Mar;73(2):75-83.
50. Gopee NV, Roberts DW, Webb P, Cozart CR, Siitonen PH, Latendresse JR, et al. Quantitative determination of skin penetration of PEG-coated CdSe quantum dots in dermabraded but not intact SKH-1 hairless mouse skin. Toxicol Sci. 2009 Sep;111(1):37-48.
51. Kosaka N, Mitsunaga M, Choyke PL, Kobayashi H. In vivo real-time lymphatic draining using quantum-dot optical imaging in mice. Contrast Media Mol Imaging. 2013 Jan-Feb;8(1):96-100.
52. Sarin H. Physiologic upper limits of pore size of different blood capillary types and another perspective on the dual pore theory of microvascular permeability. J Angiogenes Res. 2010 Aug 11;2:14,2384-2-14.
53. Burns ER, Pollack S. P. falciparum infected erythrocytes are capable of endocytosis. In Vitro Cell Dev Biol. 1988 May;24(5):481-6.
54. Garcia-Perez BE, De la Cruz-Lopez JJ, Castaneda-Sanchez JI, Munoz-Duarte AR, Hernandez-Perez AD, Villegas-Castrejon H, et al. Macropinocytosis is responsible for the uptake of pathogenic and non-pathogenic mycobacteria by B lymphocytes (raji cells). BMC Microbiol. 2012 Oct 31;12:246,2180-12-246.
55. Ema M, Kobayashi N, Naya M, Hanai S, Nakanishi J. Reproductive and developmental toxicity studies of manufactured nanomaterials. Reprod Toxicol. 2010 Nov;30(3):343-52.
56. Lu RM, Chang YL, Chen MS, Wu HC. Single chain anti-c-met antibody conjugated nanoparticles for in vivo tumor-targeted imaging and drug delivery. Biomaterials. 2011 Apr;32(12):3265-74.
57. Santra S, Yang H, Stanley JT, Holloway PH, Moudgil BM, Walter G, et al. Rapid and effective labeling of brain tissue using TAT-conjugated CdS:Mn/ZnS quantum dots. Chem Commun (Camb). 2005 Jul 7;(25)(25):3144-6.

Curriculum vitae (CV)

PERSONAL INFORMATION

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WORK EXPERIENCE

2010.07 - current

Junior research fellow

Institute of Oncology, Vilnius University, Vilnius, Lithuania

- Planning and performing scientific experiments. Main methods: confocal microscopy, spectroscopy, fluorescence imaging, animal models, cell cultures.
- Data analysis
- Dissemination of scientific results (publications, conferences)
- Supervision of students
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Business or sector Science / Health care

2008.07 – 2009.11

Manager

UAB "Musu prekyba", Vilnius, Lithuania

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Sector E-commerce

2007.07 – 2010.07

Engineer

Institute of Oncology, Vilnius University, Vilnius, Lithuania

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-Dissemination of scientific results (publications, conferences)

Sector Science / Health care

EDUCATION AND TRAINING

2009 - 2013

PhD in Biophysics (Biomedical sciences)

Vilnius University, Vilnius, Lithuania

Doctoral thesis: "Investigation of quantum dots migration in the organism using optical methods "

2007 - 2009

Master of Biophysics

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	Master thesis: “Accumulation of CdTe quantum dots in Ehrlich ascites tumor cells”
2008.02 - 2008.07	Erasmus studies
	Copenhagen University, Copenhagen, Denmark
	30 ECTS: Research Work (15 ECTS), Selfassembling nanosystems (7,5 ECTS), Video microscopy and image analysis (7,5 ECTS)
2004.07 - 2004.08	Summer school studies
	University of Luton, Luton, Great Britain
	Course “Analyzing English and Intercultural Communication”
2003 - 2007	Bachelor of Biophysics
	Vilnius University, Vilnius, Lithuania

PERSONAL SKILLS

Mother tongue(s): Lithuanian

Other language(s):

	UNDERSTANDING		SPEAKING		WRITING
	Listening	Reading	Spoken interaction	Spoken production	
English	C1	C1	B2	B2	B1
German	B1	B1	A2	A2	B1
Russian	B2	B1	B1	A2	A1

Communication skills
- intercultural communication skills gained through international conferences, projects and living abroad.
- Interdisciplinary communication skills gained through discussions with scientists of different research areas
- Team working skills gained through research and educational projects
- Pedagogical skills gained through lectures, working with students and pupils.

Organisational / managerial skills
- Leadership skills gained through supervision of students’ research projects
- Budget management and organisational skills through planning scientific research and working as e-commerce manager.

Computer skills
- Competent with Microsoft Office tools, OriginLab, ImageJ, Corel Draw.

Projects

- Supervisor. „Students‘ research practice“ (Nr. M 2012-01/SMP 12-033) Duration: 2012.07.02-09.02. Supported by Research Council of Lithuania.
- Junior research fellow. „Penetration of nanopartciles through the placental barrier and effect on embryogenesis“ (Nr. MIP-10440). Duration: 2010.07-2011.12. Supported by Research Council of Lithuania.
- Senior specialist. „ Multifunctional nanoparticles for specific non-invasive early diagnostics and treatment of cancer “ (Nr. 2004-LT0036-IP-1NOR). Projekto trukmė: 2008.08-2010.06. Supported by Norway Grants and republic of Lithuania.Lector „Training of the personnel of the Institute of Oncolo, Vilnius University” (Nr. VP1-1.1-SADM-10-V-01-004/D4-116). Supported by European Social fund.
- Supervisor, lector. “School is alive in art” Undergraduate research projects. Together with National Gallery of Art. Duration 2013.03-04. Supported by EU structural assistance.
- Lector. „National academy of pupils“. The training school of talented pupils. 2011.01.02-05

Awards

2012 Scholarship of the Research Council of Lithuania
2011 Scholarship of State Studies Foundation
2000-2002 I-III places in Vilnius city Olympiads of highschool students: Biology, Chemistry, Physics and Mathematics.

Gyvenimo aprašymas (CV)

Asmeninė informacija

Vytautas Kulvietis
Gimimo data: 1984.07.11
Adresas: J.Franko g. 8-7, LT-08431, Vilnius, Lietuva
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El. Paštas: vytautas.kulvietis@vuoi.lt
Pilietybė: Lietuvis

Darbo patirtis

Datos	Nuo 2010.07 mėn iki dabar
Pareigos	Jaunesnysis mokslo darbuotojas
Pagrindinės veiklos	Mokslinių eksperimentų planavimas, vykdymas, rezultatų analizė, pristatymas konferencijose, publikacijų rengimas, vadovavimas studentų tyrimams, darbų saugos organizavimas, naujų metodų diegimas.
Darbovietė	Vilniaus universitetas Onkologijos institutas, Biomedicininės fizikos laboratorija, Vilnius, Lietuva

Datos	Nuo 2008.07 iki 2009.11
Pareigos	Projektų vadovas
Pagrindinės veiklos	Verslo organizavimas, produktų vadyba, e-prekybos portalo www.ciapigiau.lt kūrimas
Darbovietė	UAB „Mūsų prekyba“., Vilnius, Lietuva

Datos	Nuo 2007.06 iki 2010.07
Pareigos	Inžinierius
Pagrindinės veiklos	Mokslinių eksperimentų planavimas, vykdymas, rezultatų analizė, vadovavimas studentų tyrimams.
Darbovietė	Vilniaus universitetas Onkologijos institutas, Biomedicininės fizikos laboratorija, Vilnius, Lietuva

Išsilavinimas

Datos	Nuo 2009.10 – 2013.12
Kvalifikacija	Doktorantūra (Biomedicinos mokslai, biofizika – 02B)
Įstaigos pavadinimas	Vilniaus universitetas, Fizikos fakultetas Disertacijos tema: „Kvantinių taškų migracijos organizme tyrimai optiniais metodais“

Datos	Nuo 2007.09 – 2009.06
Kvalifikacija	Biofizikos magistras
Įstaigos pavadinimas	Vilniaus universitetas, Gamtos mokslų fakultetas Baigiamojo darbo tema: „CdTe kvantinių taškų susikaupimas Erlicho ascito navikinėse ląstelėse“

Datos	2003.09 – 2007.06
Kvalifikacija	Biofizikos bakalauras
Įstaigos, pavadinimas	Vilniaus universitetas, Gamtos mokslų fakultetas Baigiamojo darbo tema: „Fotosensibilizatorių kaupimasis žiurkės galvos smegenyse“

Kvalifikacijos kėlimas

Datos	2008.02 – 2008.07
Kvalifikacija. Įstaiga	Studijos pagal Erasmus programą. Išklausyti kursai: 1) Savitvarkės nanosistemos, 7,5 ECTS („Selfassembling nanosystems“), 2) Video mikroskopija ir vaizdų analizė 7,5 ECTS („Video microscopy and image analysis“). 3) Mokslo tiriamasis darbas 15 ECTS (Research work). Kopenhagos universitetas, Danija.

Datos	2004.07 – 2004.08
Kvalifikacija. Įstaiga.	Stažuotė vasaros mokykloje: “Analyzing English and Intercultural Communication” („Anglų k. analizė ir tarptautinis bendravimas“). Liutono universitetas, Didžioji Britanija

Datos	2001.09 – 2003.06
Kvalifikacija.Įstaiga.	Lietuvos jaunųjų matematikų mokykla, Vilniaus universitetas.

Dalyvavimas projektuose:

1. Lektorius, organizatorius Nacionalinės Dailės Galerijos edukaciniame modulyje „Mokykla gyva mene“. Paskaita, ekskursija ir pažintiniai laboratoriniai darbai 9-12 kl. moksleiviams, vykdant projektą „Muziejus – mokykla – moksleivis. Muziejų ir bendrojo lavinimo mokyklų nacionalinis partnerystės tinklas“, įgyvendinamo pagal 2007 – 2013 m. Žmogiškųjų išteklių plėtros veiksmų programos 2 prioriteto „Mokymasis visą gyvenimą“ VP1-2.2-ŠMM-10-V priemonę „Neformaliojo švietimo paslaugų plėtra“. 2013
2. Vadovas. „Studentų mokslinė praktika“ (Nr. M 2012-01/SMP 12-033) Projekto trukmė: 2012.07.02-09.02. Finansuoja Lietuvos mokslų taryba.
3. Metodinės medžiagos rengimas. „Biotechnologijos kvalifikacijos tobulinimo programa gamtos mokslų mokytojams“ (Nacionalinio lygio programa). (Nr. VP1-2.2-ŠMM-03-V-01-004). Trukmė: 2011-2013. Finansuoja LR Švietimo ir mokslo ministerija.
4. Jaunesnysis mokslo darbuotojas „Nanodalelių pasiskirstymas organizme, prasiskverbimas pro placentos barjerą ir poveikis embrionui“ (Nr. MIP-10440). Projekto trukmė: 2010.07-2011.12. Finansuoja Lietuvos mokslų taryba. Krūvis: 0,25 etato.
5. Vyresnysis specialistas. „Daugiafunkcinės nanodalelės specifinei ir neinvazinei ankstyvajai vėžio diagnostikai ir gydymui“ (Nr. 2004-LT0036-IP-1NOR). Projekto trukmė: 2008.08-2010.06. Finansuoja Lietuvos Respublika ir Norvergijos finansiniai mechanizmai. Krūvis: 0,5 etato.
6. Lektorius „Sveikatos specialistų, prisidedančių prie sergamumo ir mirtingumo nuo onkologinių ligų mažinimo, kvalifikacijos kėlimas Vilniaus universiteto Onkologijos institute“(Nr. VP1-1.1-SADM-10-V-01-004/D4-116). Projekto trukmė: 2009.05-2014.04. Finansuoja Lietuvos Respublika ir Europos socialinis fondas. Krūvis: 4 val.

Kiti moksliniai renginiai:

- Tarptautinė mokykla: “Advanced methods in biophysics”. 2007 lapkričio 26-30; Trakai, Lietuva.
- Konferencija: “Teaching Biophysics: Curruculum, Methods, Problems”. 2006 birželio 8-10; Vilnius, Lietuva,
- Lietuvos mokslo ir studijų fondo finansuojama vasaros praktika: ”Application of databases for biophysical data presentation” Vilniaus Gedimino technikos universitetas; 2005; Vilnius, Lietuva

Pedagoginė veikla

2011-2013 Straipsniai mokslo populiarinimo žurnale "Jaunasis tyrėjas", temos: "Biomimetika ir biologinė nanodalelių sintezė", "Pirštų atspaudų paslaptis", "Kas yra fluorescencija?".

2011-2012 Metodinės medžiagos rengimas. „Biotechnologijos kvalifikacijos tobulinimo programa gamtos mokslų mokytojams“. (Nr. VP1-2.2-ŠMM-03-V-01-004). Trukmė: 2011-2013. Finansuoja LR Švietimo ir mokslo ministerija.

2011.01.02-05 Lektorius "Nacionalinėje moksleivių akademijoje", biochemijos sekcijoje (4 val).

2010.10.25-29 Lektorius mokymuose pagal projektą „Sveikatos specialistų, prisidedančių prie sergamumo ir mirtingumo nuo onkologinių ligų mažinimo, kvalifikacijos kėlimas Vilniaus universiteto Onkologijos institute“ (4 val.)

2010-2013 Vadovavimas studentų mokslo tiriamiesiems darbams: Ieva Niciūtė (VGTU), Edita Jazdauskaitė (VU FF), Aušra Marčiuškaitė (VU GMF), Justina Pupkaitė (VU GMF). Juras Kišonas (VU MF), Paulius Čekanauskas (VU MF), Herkus Bajerčius (VU MF)

Asmeniniai gebėjimai

Gimtoji kalba: Lietuvių

Užsienio kalbos*	Supratimas		Kalbėjimas		Rašymas	
English	C1	Įgudęs vartotojas	B2	Pažengęs vartotojas	B1	Pažengęs vartotojas
German	B1	Pažengęs vartotojas	B1	Pažengęs vartotojas	A2	Pradedantysis vartotojas
Russian	B2	Pažengęs vartotojas	B1	Pažengęs vartotojas	A1	Pradedantysis vartotojas

Socialiniai gebėjimai: Geri bendravimo įgūdžiai tarptautinėje ir tarpdisciplininėje aplinkoje, komandinis darbas, partnerio poreikių suvokimas ir interesų derinimas, pedagoginiai ir reprezentaciniai įgūdžiai.

Techniniai gebėjimai: Fluorescencinė ir sugerties spektroskopija, fluroescencinė konfokalinė mikroskopija, darbas su laboratoriniais gyvūnais, audinių preparavimas, reoencefalografija.

Darbo kompiuteriu gebėjimai: MS Office, Origin Lab, Statistica, Neuron, Maple, ImageJ, Corel Draw. Susipažinęs su: Matlab, HTML, PHP scripting, Turbo Pascal programavimas, duomenų bazės.

Vairuotojo pažymėjimas: B kategorija nuo 2002 m.