Summary and conclusions

Thanks to worldwide research nanotechnology is one of the key drivers of the current scientific advancements in the 21st century. The field has expanded astonishingly over the last decade, where it is estimated that nearly 10 % of all publications indexed in Web of Science in 2016 involved nanotechnology. Nonetheless, despite this extensive research the translation of these widely investigated nanoparticles (NPs) toward the clinic is rather limited. For organic particles such as liposomes and polymeric particles this is mainly owing to their lack of efficacy evoked by the many intra-and extracellular barriers they encounter. The translation of inorganic particles is primarily restricted by safety concerns, since many of them, including Quantum Dots (QDs), are made up of heavy metals that are known to be toxic. As confirmed in this thesis, it is established that the physicochemical features define how the cell 'sees' the particle and thus dictates the NP's efficacy and toxicity – what you see is what you get!

While this perception is fully acknowledged by the nanotechnology community it remains challenging to connect certain physicochemical properties to specific biological effects, making it hard to predict the therapeutic power or potential toxicity of a NP. This is primarily due to faulty project design, the lack of representative models and the inherent complexities associated to working in the nano-range. The difficulty to link NP physicochemistry to desired or unwanted effects is an issue that affects nearly every research field that aims to take advantage of the unique and powerful properties of NPs, yet in this thesis we aimed to aid in overcoming this issue in context of retinal gene delivery (Part I, Chapter 1-4) and autophagy (Part II, Chapter 5 and 6).

In Chapter 1 we provided an overview of the most prevalent acquired diseases and most well-documented inherited retinal diseases that could be treated with retinal gene therapy. We furthermore described the morphology and functions of the Müller cell, the target cell type for our non-viral approach.

Based on the overview of publications linking physicochemical properties of therapeutics and their carriers presented in Chapter 2, we concluded that the ideal physiochemical characteristics of a therapeutic (carrier) highly depends on the barrier it needs to overcome and therefore also on the preferred administration route. We further found that nearly each barrier undergoes changes in function of age and disease, an important notion when evaluating the potential of carriers to cross delivery barriers. We found many useful in vitro and ex vivo approaches to study

drug delivery barriers in the posterior segment of the eye, though argue that there is still a great need for more straightforward and representative models.

Convinced of the latter argument, we presented the so-called 'vitreoretinal explant' in Chapter 3, an novel ex vivo model especially developed to look into the interaction of nanomedicines with the vitreoretinal (VR) interface. This interface comprises the vitreous and the inner limiting membrane (ILM), both well-recognized drug delivery barriers. We confirmed the viability of this explant model and validated its value by means of polystyrene beads. Since 40 nm beads more efficiently crossed the VR interface than 100 or 200 nm particles we concluded that the entry of NPs into the retina is size-dependent. Moreover, we found that removing the vitreous, as commonly done for culture of conventional explants, led to an overestimation of NP uptake, and concluded that the principal barrier to overcome for retinal entry is

unquestionably the ILM. This VR explant is currently the most representative ex vivo model available that is alive for a sufficiently long time to study NP uptake in the retina.

In Chapter 4 we applied an elementary set-up to examine if Müller cells, which we regard as an ideal target for neuroprotective strategies, are able of efficiently processing lipoplexes and expressing their pDNA or mRNA cargo. Here, we found that mRNA lipoplexes outperformed the DNA lipoplexes in transfection of healthy Müller cells since both the number of transfected cells as well as the level of GFP expression was higher for mRNA lipoplexes. In Müller cells that were exposed to hyperglycemia, oxidative stress or hypoxia no changes in mRNA-induced expression was observed when compared to healthy Müller cells. On the other hand, we did find that Müller cells treated with oxidative stress or hypoxia were more sensitive to lipoplex-induced toxicity while hyperglycemia had a protective effect. Although preliminary, this study indicates that, despite a stressful environment, Müller cells are capable of taking up lipoplexes and expressing their nucleic acid cargo.

Chapter 5 presented an overview of the most relevant reports on NP-mediated autophagy alterations and intended to investigate the interplay between NP physicochemistry and autophagic changes. Hence, several NP properties were put forth as probable influencing factors of NP-induced autophagy disruption or upregulation such as size, charge and chemical composition. However, owing to the shortcomings of some studies and the contradicting claims made in literature it was virtually impossible to draw general conclusions. We thus judged that systematic studies which include sufficient characterization data could greatly support us to truly elucidate the impact of NP physicochemistry on NP-associated autophagy changes.

In view of this understanding we performed a study in Chapter 6 with as goal to carefully define the influence of QD coating on lysosomal health and autophagy. Our study showed that the cellular effects induced by QDS on HeLa cells were strongly dictated by the surface coat (i.e. MPA or PEG) of the otherwise identical particles. MPA-coated QDs proved to be highly compatible as a result of lysosomal activation and ROS reduction, two cellular responses that help the cell to cope with NP-induced stress. In contrast, PEG-coated QDs were substantially more toxic owing to a rise in ROS production and lysosomal impairment. This impairment next resulted in autophagy dysfunction which likely added to their toxic effects. Taken together, our study showed that coating QDs with MPA is a better strategy than PEGylation for imaging applications.

In Chapter 7, we summarized the advances in retinal gene therapy and nanotoxicology and discussed potential challenges that hinder NPs to advance further into clinical stages. We brought forward several guidelines that we believe could aid the nano field to progress and reconstructed them into a general approach that could aid researchers in overcoming the NP delivery barriers after intravitreal injection.