6 Summary

The objective of this thesis was the design of new nanostructured diagnostic and therapeutic colloidal systems which offer the possibility of a flexible surface modification. Furthermore, these nanoparticles and the influence of their modification should be characterized regarding their properties in vitro and in vivo. Therefore two different kinds of colloidal systems were developed, both providing a flexible surface modification due to electrostatic interactions:

(I) self assembled systems based on polyelectrolytes

(II) solid polymeric nanoparticles

Ia) Self assembled diagnostic systems

Highly water-soluble cyanine dyes (TSC, TSC-CS) were complexed with oppositely charged polyelectrolytes (PEI, PEG-PEI, cationic starch) and nanoparticulate complexes were obtained. The size of the complexes could be controlled by the charge ratio of the complex partners as well as the molecular weight of the polymer. A self stabilizing ionic shell on the surface of the complexes was provided by a charge excess of the polymer. By means of a combined charge titration with the blockcopolymer Glu(10)-b-PEG(110) and the potential targeting molecule NADP stable surface modified nanoparticles could be prepared. The increase of the complex stability could be shown via incubation in mouse plasma as physiological testing medium. The relevant information was given by typical changes in absorption spectra, possible due to the formation of J-aggregates in the course of complex preparation. Such J-aggregates, underlying a certain packaging order, can be used for displaying physicochemical alterations. Based on these characteristic properties of the complexes, the utilized components for surface modification were investigated regarding their impact on the resulting complex stability. Charge strength, size of the molecules and sterically stabilizing effects were identified as key factors for a successful modification.

These completely new systems offer the advantage to efficiently encapsulate hydrophilic compounds within the matrix of water-insoluble complexes. Decisive arguments for this advantageous formulation are a simple and surfactant free preparation as well as multifunctional surface modification via electrostatic interactions. Especially the utilization of

optical imaging, a radiation free detection method, makes it an interesting testing system for in vitro and in vivo applications.

Ib) Self assembled therapeutic systems

Nanoparticles of the weakly basic drug Vatalanib succinate were formulated by means of complexation with the anionic cyclodextrin derivative βCDPO$_4$. The desired size and final drug load was tailored via the ratio of the two complex partners within the preparation. Stable and narrow distributed colloidal particles were only obtained with an excess amount of the basic drug, although these complexes were electrostatically stabilized by the phosphate groups of the cyclodextrin. By comparison with Vs-SBβCD complexes it was found, that the complex stability depends obviously on the type and the number of charged groups on the CD. The results of different physicochemical investigations (FTIR, DSC and XR-PD) indicated a combination of a charge and inclusion complex for the Vs-βCDPO4 nanoparticles. An incubation of these complexes in the biorelevant media FaSSIF and FeSSIF revealed a sufficient stability, which allows a prediction regarding their subsequent behaviour in vivo. A successful surface modification of this therapeutically applicable system was achieved via flexible electrostatic interactions. In order to assure a sufficient complex stability after titration, a complete surface charge conversion due to an excess of the modifying blockcopolymer was necessary. In case that a complete compensation of surface associated charges was achieved, destabilization was observed.

Such nanoparticulate systems have been described for the first time in this thesis and reveal a new technological strategy to overcome the obstacle of the pH-dependent solubility of weakly basic drugs.

IIa) Solid polymeric nanoparticles

Based on the encapsulation of polyamines in a polymeric matrix, cationically functionalized PBCA nanoparticles were prepared via nanoprecipitation. The utilization of polycations with a sufficient molecular weight was prerequisite for a stable encapsulation. This was demonstrated with two different nanoparticulate systems: PBCA-PEI and PBCA-P(DMAEMA). Low molecular weight dyes could be also encapsulated in the particle core, provided that dye and polycation preformed an ionic complex before a fast precipitation process took place along with the matrix polymer. Additional fluorescent dyes within the

particle core enabled a detection of the particles in the course of cell culture and animal experiments. These particles showed a better colloidal stability compared to self assembled systems. By means of electrostatic interactions different surface modifications were successfully carried out, observing no influence of the encapsulated dye. As key factors for a successful modification were identified: (i) ionic charge strength, (ii) solubility of the titrant and (iii) a sterically stabilizing compound which compensated the minimized electrostatic stabilization. In order to evaluate the suitability of these nanoparticles in biological systems, cell culture investigations were performed.

IIb) HeLa cell culture investigations with functionalized nanoparticles

Prior characterized PBCA-P(DMAEMA) nanoparticles with a cationically functionalized surface showed a strong adhesion on HeLa cells, presumably due to electrostatic interactions with anionic membrane compounds. According to higher concentrations on the cell surface, more particles were taken up into the cells. The final fluorescence after uptake of unmodified nanoparticles, Glu(10)-b-PEG(110) or with folic acid modified nanoparticles exemplified decreased cell internalization in correlation with a lowered zeta potential. A detailed investigation of the cell uptake mechanism was done with Glu(10)-b-PEG(110) modified nanoparticles. Microscopic data gave clear evidence for receptor-independent endocytosis as major cell uptake mechanism of these nanoparticles. A bright and dot-like fluorescence was obviously caused by internalized endosomes or endolysosomes. In contrast to that, a more plane and less strong fluorescence indicated released particles within the cytoplasm. Reasons for a strong accumulation of the particles close to the nucleus and in the region of mitochondria may be active transport mechanisms via microtubules in direction to the nucleus. Furthermore, it was observed, that longer periods of incubation time or higher particle concentrations lead to an altered distribution closer to the nucleus and an intensified particle uptake. False colour rendering was used to illustrate the particles' distribution depending on the intensity of the fluorescence.

Finally an efficient uptake and release of functionalized and modified PBCA-P(DMAEMA) nanoparticles could be confirmed by the results of the cell culture investigations. These properties make them an appropriate nanoparticulate system both for diagnostic and therapeutic applications.

IIc) In vivo investigation of Glu(10)-b-PEG(110) modified PBCA-P(DMAEMA) nanoparticles via optical imaging

The subsequent animal experiment showed, that under certain conditions Glu(10)-b-PEG(110) modified nanoparticles can be accumulated passively in tumor tissue via the EPR-effect. An ex vivo investigation of the tumors confirmed for three of four mouse an intensified fluorescence contrast in treated tumor tissue. In the case of one mouse the tumor could be also detected in vivo and a delayed, multiple imaging of the pathologic fluorescent tissue was possible. Due to the accumulation in tumor tissue the conclusion can be drawn, that the electrostatically bound blockcopolymer Glu(10)-b-PEG(110) was stable enough on the particle surface for shielding the cationic charges of the core particle and providing a sufficient blood circulation time. According to the imaging data a fast hepatic elimination of particles, which had been cleared from the blood circulation, can be assumed. The faster the complete elimination of these cleared particles, the fewer side effects may be caused. Hence such a nanoparticulate system would provide optimal properties for diagnostic as well as for therapeutic purposes.

With a non-invasive, external imaging technique the passive accumulation of Glu(10)-b-PEG(110) modified, NIR-active PBCA-P(DMAEMA) nanoparticles after i.v. application could be confirmed.

Outlook

Within this theses novel diagnostic and therapeutic nanoparticles were developed in order to provide potential drug delivery systems for an improved drug medication. Based on the modularity of these systems a huge variety of surface modifications for a patient related optimization is possible. Furthermore, the discussed findings of the thesis provide useful information regarding electrostatic interactions and the applicability in terms of colloidal systems and their surface modification. With surface engineered drug delivery systems the key factor for controlling active and passive targeting will be designed more effectively in the future. With different systems, based on ionic self assembly or polymer precipitation, a broad range of transferable data for similar colloidal systems is described in this thesis.