succinyl-tetralysine [DPPE-succ-(Lys)4]) was synthesized, which efficiently reduced cytotoxic effects and further augmented the internalization efficiency of the MLs [5]. In conclusion, the results indicate that the use of cationic lipids for transfection purposes should be carefully considered as they can induce severe cytotoxic effects. By carefully controlling the physicochemical properties of the liposomal systems used, many of the cytotoxic effects can immediately be reduced. These data highlight the need for careful optimization of cationic liposome formulations and that great advances can still be made with respect to diminished toxicity and enhanced internalization.

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A6
Intracellular iron oxide nanoparticle coating stability determines nanoparticle usability and cell functionality

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Iron oxide nanoparticles are routinely exploited as T2/T2* contrast agents [1]. One of the most active topics in this biomedical research area is the non-invasive imaging of pre-labelled stem or therapeutic cells upon transplantation *in vivo* in [2]. To this end, commercial particles such as Endorem® are frequently employed, however, the particles display several characteristics which makes them less suitable for *in vivo* labelling [3]. In the present work, the effects on cell physiology of in-house produced cationic magnetoliposomes (MLs), that is, 14-nm diameter iron oxide cores each individually enwrapped by a lipid bilayer containing 3.33% of distearoyltrimethyl ammoniumpropane (DSTAP)[4] – a cationic lipid – are compared with the effects of Resovist (carboxydextran), Endorem (dextran) and VSOP (citrate) iron oxide particles. When the particles are incubated at high dosages, reaching high intracellular iron levels, this results in a transient decrease in cell cycle progression, actin cytoskeleton remodelling and focal adhesion formation and maturation [5]. The extent of these effects is in line with the intracellular iron concentration and appears to be common for all particles. When reaching similar intracellular iron concentrations and when verifying that the different particles are routed along the same way and are therefore exposed to similar intracellular microenvironment at fixed time points, it is shown that intracellular stability of the coating molecules is of high importance. The results *in vitro* show that citrate-coated particles are rapidly degraded, whereas those coated with dextran are more stable, but still less than the MLs. The degradation of the particles can be shown by the increase in free ferric ions, and the distorted *r*1/*r*2 ratio of the particles, hampering their use for long-term imaging. Labelled cells further show increases in reactive oxygen species and transferrin receptor expression in C17.2 neural progenitor cells and impeded functionality of PC12 rat pheochromocytoma cells. The extent of these effects is in line with the degradability of the particles *in vitro*. The MLs appear to be the most stable particles and further show a high persistence of the label in continuously proliferating C17.2 cells. In conclusion, the results indicate that the type of coating material used is highly important with regard to maintaining cell functionality and stability of the label. Further characterization of cell-nanoparticle interactions is both warranted and needed [1].

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A7
Nuclear inclusion of inert and chromatin-targeted polystyrene beads and plasmid DNA containing nanoparticles

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Introduction and aim: The nuclear membrane is currently one of the major cellular barriers to the effective delivery of plasmid DNA (pDNA). Cell division has a positive influence on the transfection efficiency from naked pDNA and nanoparticles containing pDNA. At the end of mitosis, the pDNA near the chromatin is probably randomly included in the nuclei of daughter cells during reassemby of the nuclear envelope around chromatin. However, very little is known on the nuclear inclusion of nanoparticles during cell division. We were interested if inert nanospheres get randomly enclosed in artificial Xenopus nuclei and in nuclei of dividing cells. We investigated nanospheres with a different size and charge, and whether the enclosure could be enhanced by the use of chromatin binding peptides such as AT-hooks. *Material and methods*: Non-targeted positively charged, poly-ethyleneglycol (PEG)-ylated and negatively charged green fluorescent polystyrene nanospheres (Molecular Probes) of 100, 200 or 500 nm were used. The 100 nm nanospheres were also modified with Mel-28 (GPSKPRGRPPKHKAKT), mutated Mel-28 (GPSKPGGGPPGHKAKT) or HMGA2 (SPKRPRGRPKGSKNKS), containing an AT-hook or a mutated AT-hook (targeted nanospheres). Artificial nuclei were obtained with the 'Xenopus egg extract (XEE) nuclear assembly reaction'. The enclosure of the nanospheres in the artificial nuclei and upon microinjection was visualised by confocal fluorescence microscopy. *Results and conclusions*: Periodically the non-targeted nanospheres were able to get enclosed in the artificial nuclei but enclosure was rather limited. The enclosure of the positively charged spheres is higher than that of the negatively charged and the PEG-ylated variants, likely as a result of aspecific interactions with the nett negatively charged chromatin. Size is also important: spheres with a diameter of

200 nm and 100 nm are better enclosed than the 500 nm variants. The enclosure of spheres modified with chromatin binding peptides is indeed higher than the enclosure of the non-targeted spheres and the spheres modified with the mutated AT-hook. When polystyrene spheres were injected in the cytoplasm of HeLa cells, initially, the nanospheres spread homogenously in the cytoplasm. Upon cell division, however, the nanospheres accumulated in a specific perinuclear region and enclosure in the nuclei of divided cells was never observed. Therefore, reaching the nucleoplasm seems to be very difficult and we question whether the chromatin binding peptides are able to target the nanospheres into the daughter nuclei of living cells. It thus seems that nuclear inclusion in the XEE assay does not represent the situation in living cells

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A8
mRNA delivery to cervical carcinoma and mesenchymal stem cells mediated by cationic carriers

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We complexed mRNA encoding luciferase (mLUC) to either one of the cationic lipids Lipofectamine (LF) or DOTAP/DOPE, or to linear poly(ethyleneimine), a cationic polymer (linPEI). After incubating the resulting lipo- or polyplexes with HeLa cells for different periods of time, we determined luciferase expression by a bioluminescence assay. Both extent and duration of luciferase expression were dependent on the type of complex used. With LF, mRNA expression lasted for about 9 days maximally, which is not significantly shorter than what can be achieved with pDNA polyplexes. When electroporation was used to transfer mLUC into the cells, luciferase expression lasted for 12 h only. An important characteristic of mRNA-mediated transfection by means of all three complexes is that it could already be detected 30 min after adding the complexes to the cells. In order to estimate the number of positive cells, we transfected the cells with an mRNA encoding Green Fluorescent Protein (GFP) and compared the results with transfection by means of pDNA. With transfection by means of mRNA complexed to LF or DOTAP/DOPE a substantially larger fraction of cells (>80%) was transfected than with pDNA (40%). After establishing the characteristics of mRNA-mediated transfection by means of expression of reporter proteins, we tested the carriers for their ability to mediate expression of a functional protein in mesenchymal stem cells. For that purpose we complexed an mRNA encoding CXCR4, a receptor binding stromal derived factor 1, to the cationic lipids and the polymer. The resulting complexes were incubated with mesenchymal stem cells and CXCR4 expression was assayed. The fraction of CXCR4-positive cells was approximately 80% and 40% for mRNA-cationic lipoplexes and lin-PEI polyplexes respectively. The results of these experiments indicate that mRNA, under certain conditions, may be preferable to pDNA to achieve transfection, particularly in cases requiring transient protein expression.

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A9
Cellular uptake of long-circulating pH-sensitive liposomes: evaluation of the liposome and its encapsulated material penetration in cancer cells

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Print 3G, a peptidic antagonist of oncoprotein involved in breast cancer, could reduce the angiogenic development of breast tumors, leading to tumor dormancy. The necessity of intravenous administration of Print 3G led to the development of long-circulating liposomes as drug carriers. Pegylated liposomes, too large to be collected by fenestrated organs, accumulate passively in solid tumors thanks to the EPR effect. The strategy was to combine the protective properties of PEG with the transfection properties of pH-sensitive lipids that could promote the uptake of liposomes by cells and avoid lysosomal sequestration and degradation of entrapped materials such as peptides. In this study, we compare two formulations in terms of cellular uptake using confocal microscopy. The first one is composed of SPC:CHOL:mPEG-750-DSPE (47:47:6), used as 'standard' liposomes, and the second one composed of DOPE:CHEMS:CHOL:mPEG750-DSPE (43:21:30:6), used as pH-sensitive liposomes.

Firstly, we evaluated the penetration of an encapsulated model molecule, calcein, in Hs578t human breast cancer epithelial cells. When calcein was encapsulated in standard liposomes, its penetration was effective only in a few cells. On the contrary, the majority of cells were fluorescent when calcein-loaded pH-sensitive liposomes were applied on cells for three hours. Secondly, we studied the penetration of liposomes themselves in Hs578t cells using 25-[(nitrobenzoxadiazolyl)methylamino]norcholesterol (NBD-CHOL) as a fluorescent marker of the phospholipid membrane. The obtained results were comparable to those obtained with calcein: a higher penetration of liposome was observed for pH-sensitive liposomes. Finally, the cellular uptake of liposomes using both NBD-CHOL and rhodamine encapsulated in the inner cavity of vesicles was evaluated with Hs578t cells and compared with WI26 human diploid lung fibroblast cells. This experimental design allowed us to follow simultaneously the cell distribution of the encapsulated material and of the liposome itself. Confocal pictures obtained with pH-sensitive liposomes on both WI26 and Hs578t cells allowed us to visualize co-localized red and green of rhodamine and NBD-CHOL, with a higher degree of colocalization in an area close to the nucleus. In comparison with 'standard' liposomes, we observed a higher penetration of the encapsulated material and of the liposome itself in breast cancer cells. Moreover, we visualized a colocalization near the nucleus of liposomes components. From results obtained with fibroblastic cells, there was no difference in terms of cellular uptake between the two formulations. In perspective, we would like to compare these results, obtained with model molecules, with experiments performed with biotinylated Print 3G to assess its cellular distribution. Moreover, it would be interesting to correlate results obtained with confocal microscopy with a possible increase of the peptide efficacy against cancer cells when it is encapsulated in long-circulating pH-sensitive liposomes.

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