involved in ocular surface inflammation. We also want to determine whether the use of nanoparticulated drug delivery systems, based on cationized gelatine and chondroitin sulfate, as carriers for siRNAs improve the level of gene silencing. *Methods*: HCE cells were transfected with specific siRNAs against TGFβ1 and its Receptor 2 (TGFBR2) or against GAPDH as a negative control. Lipofectamine was used at 1.6 μl/well in 24-well plates and different siRNA concentrations from 20 to 300 nM were assayed. Silencing efficacy was tested, comparing Lipofectamine2000- or Nanoparticle-based transfection, at protein and RNA levels. Potential toxicity was evaluated by means of the XTT test. *Results*: TGFβ1 and TGFBR2 silencing reached 70% at the RNA level (measured by quantitative real-time-PCR) when using Lipofectamine. Lower silencing was detected at the protein level (measured by Western blotting or ELISA). However, the use of nanoparticles did not significantively improve the silencing efficacy of the evaluated siRNAs. siTGFβ1- and siTGFBR2-transfected cells showed viability percentages equivalent to those of control untransfected cells. CONCLUSION: It is possible to silence *in vitro* TGFβ1 and TGFBR2 expression in a corneal epithelial cell line by conventional techniques obtaining acceptable silencing levels while maintaining high cell viability. The use of nanoparticles as siRNA vehicles to improve silencing levels requires further studies

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A29
Efficient siRNA delivery and effective gene silencing by lipoplexes

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siRNA is double-stranded RNA typically 21–24 nucleotide base-pairs long. Gene silencing by siRNA has gained wide acceptance in genomics and is already in different phases of clinical trials as a potential therapeutic. Long chain fatty acid conjugates of spermine have previously been synthesized and evaluated in our research group for both gene and siRNA delivery [1,2]. We report the synthesis of two novel unsymmetrical N4,N9-difatty acid conjugates of the naturally occurring polyamine spermine with the aim of developing structure–activity relationships for their potential as non-viral, self-assembly vectors for siRNA delivery. After transfection with lipoplexes of Alexa Fluor® 647-labelled siRNA (a 24-mer from Qiagen), silencing EGFP expression, both the efficiency of delivery and the effectiveness of knock-down (gene silencing) were evaluated in HeLa cells stably expressing EGFP. Analysis was by FACS 48 hours post transfection. All transfection experiments were carried-out in DMEM containing 10% foetal calf serum. The efficiency of intracellular delivery was measured by the (normalized) fluorescence of Alexa Fluor® 647-labelled siRNA; N4,N9-dioleoylspermine (DOS) showed 150% of the delivery efficiency achieved with N4-linoleoyl-N9-oleoylspermine (LOS). However, knock-down results show that LOS is more effective with a reduction of EGFP expression levels from control (100%) to 25 ± 3% at a concentration of 3 μg/well (N/P = 11, $n = 3$ and triplicate replicates). Under the same experimental conditions, DOS reduced EGFP expression to 27 ± 2% at a concentration of 6 μg/well (N/P = 22) and to 32 ± 2% at a concentration of 3 μg/well (N/P = 11). Cell viability was measured as the percentage of viable cells using the Alamar Blue® assay [3]. The results show that at 3 μg/well LOS cell viability is 83 ± 4%, at 6 μg/well LOS cell viability is 46 ± 8%, while at 6 μg/well DOS cell viability is only 32 ± 9%. Transfection of cells with Lipofectamine™2000 resulted in reduction of EGFP expression to 37 ± 3%, with cell viability of 91 ± 6%. We conclude from these results that the unsymmetrical lipopolyamine LOS is an excellent transfecting agent for the delivery of siRNA producing effective gene silencing in the presence of 10% foetal calf serum.

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A30
Peptide dendrimer based drug delivery system

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In the past decades, dendrimers have been extensively studied for their unique properties such as spherical nanostructure, monodistributed size and numerous peripheral functional groups. Peptide dendrimers, which were synthesized from amino acids, have been reported as biomaterials for disease diagnosis and treatment due to their excellent biocompatibility and degradability. Herein, we reported the synthesis of peptide dendrimers and their biomedical applications as molecular probes for magnetic resonance imaging (MRI) and carriers for drug/gene delivery. The synthesis of peptide dendrimers was according to a previously reported method [1]. The dendrimers with different generations were synthesized and functionalized. Targeting moieties, mPEG, Ga-DTPA complexes and anti-tumor drugs were immobilized on the peripheral groups of the dendrimers. The dendrimers immobilized Ga-DTPA complexes were used as MRI molecular probes and the relaxivity of contrast was tested on 1.5 T MRI both *in vitro* and *in vivo*in. The generations of dendrimers were 2, 3, 4, and galactosyl moiety was used as targeting ligand for liver imaging. The relaxivity of the contrasts were measured and for G4 dendrimer was 100.8 mM-1•S-1, which was much higher than that of the commercial Ga-DTPA product. The signal intensities were determined by choosing an appropriate region of interest in mouse liver tissue. After 10 minutes injection, the SI increase in liver tissue was observed with an averaged enhancement of 43% for G3T and 37% for G4T, respectively. The non-specific dendritic agents G2, G3 and G4 showed low SI increases. The dendritic probes of G2T, G3T and G4T showed 25%, 35% and 34% relative enhanced SI after 1 hour injection. The peptide dendrimers were fabricated gene vectors and gene transfections of generation 3, 4 and 5 of peptide dendrimers were compared, the

results demonstrated that G5 showed the highest gene transfection efficiency both in the medium with or without serum. Peptide dendrimer based drug delivery system was with dual targeting and pH-sensitive functions. Dendrimer–doxorubicin conjugates were synthesized via a pH sensitive bond. The drug release at pH 5.0 was much faster that that at pH 7.4. The sustained release time was as long as 20 hours and more than 90% of the immobilized drugs were released at pH 5.0. The *in vitro* anti-tumor effects of the dendrimer drug delivery system were investigated and it showed that the peptide dendrimer was a promising carrier for drug delivery.

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A31
Pyridylhydrazone-based PEG for pH-reversible lipopolyplex shielding

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PEGylation that is reversed after the therapeutic agent reaches the target cell presents an attractive feature for drug, protein or nucleic acid delivery. Amine-reactive, endosomal pH cleavable ω-2-pyridyldithio poly(ethylene) glycol α-(butyraldehyde)-carboxypridylhydrazone N-hydroxysuccinimide ester (OPSS-PEG-HZN-NHS) was synthesized and applied for bioreversible surface shielding of DNA lipopolyplexes. N1-cholesteryloxycarbonyl-1,2-diaminoethane was reacted with pH-sensitive (OPSS-PEG-HZN-NHS) or the corresponding stable (OPSS-PEG-NHS) reagent. Both types of micelles remained shielded at pH 7.4 as demonstrated by size exclusion column separation after 4 hours of incubation at 37 °C. But only disruption of OPSS-PEG-HZN-Chol micelles was observed at endosomal pH 5 in 30 min, while OPSS-PEG-Chol was almost stable for 8 h in the same conditions. Lipopolyplexes composed of DNA condensed with polyethylenimine (PEI), dioleoyl phosphatidylethanolamine (DOPE) and hydrazone linked pH labile lipid Chol-HZN-PEG were prepared by the ethanol injection technique, with particle size of 160 nm and zeta potential of 8 mV. Pyridylhydrazone-based PEGylated lipopolyplexes was as stable as their non-pH sensitive counterparts at physiological conditions, and had smaller size compared with non-PEGylated variants. At pH 5.4, increasing size was only detectable in pH-reversible lipopolyplexes. Both luciferase and EGFP gene transfections of pH-reversible lipopolyplexes showed an up to 40-fold enhancement in gene expression with reversibly shielded polyplexes compared to stably shielded lipopolyplexes. Investigation of cellular association and uptake by flow cytometry, together with intracellular tracking by CLSM reveal the probability of intracelluar deshielding of PEG. Incorporation of a ligand for transferrin receptor targeting further improved the transfection.

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A32
The 5th generation of poly(L-lysine) dendrimer is a potential carrier for *in vivo* in gene delivery

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Poly(L-lysine) dendrimers have been widely used as reagents for *in vitro* gene transfecion. Here, different generations of dendritic poly(L-lysine)s were synthesized, including G3, onium salt G3 (OG3), G4 and G5, and their characteristics for *in vitro* gene transfection and potentials as *in vivo* gene delivery carriers were evaluated. Gel retardation assays proved that the dendrimers could form complexes with plasmid DNA, and dendrimer G3 could inhibit the migration of pDNA at an N/P ratio of 0.5, G4 and G5 at N/P ratio of 1.0 and onium salt G3 at N/P ratio of 2.0. A DNase I protection assay with G5 showed acquired resistance from combining pDNA with dendrimer; this can resist the nuclease-catalyzed degradation, and the protection capacity of G5 was even stronger than that of PEI. Atomic force microscopy demonstrated that all the 4 generations of dendrimer/DNA complexes showed similar particle size within 100–200 nm. At N/P ratios from 1 to 25, zeta potentials of the 4 dendrimer/pDNA complexes gradually changed from negative to positive with a tendency that the higher generation and higher potential value variants gave a stronger combination potency of the complex with negatively charged cell membranes. *In vitro* cytotoxicity evaluation showed good biocompatibility of each dendrimer within N/P ratios of 1–25. Body weight evaluation of BABL/c mice, together with tissue section observation, blood routine detection and blood biochemistry analysis (liver and kidney function, myocardial enzymes and electrolytes, etc.) of dendrimer G5 also showed good *in vivo* biocompatibility 2 and 7 days after tail vein injection. *In vitro* gene transfection comparison revealed that G5 had an obvious higher efficiency than other dendrimers. Transfection efficiencies of each dendrimer were not influenced by the presence of serum, which is a very important merit for *in vivo*in gene delivery. Quantitative analysis in mRNA and protein level showed that the transfection efficiency of dendrimer G5 was ~60% of PEI's, but PEI had obvious toxicity to cultured cells and its transfection efficiency would be greatly reduced by the presence of serum. Considering that dendrimer G5 had almost the same *in vitro* gene transfection efficiency as G6, we concluded that the fifth generation of poly(L-lysine) dendrimer should be a suitable carrier for *in vitro* gene transfection and, more importantly, a potential carrier to construct *in vivo* gene delivery system.

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A33
Muscle-targeted HIF-1a gene expression system for therapeutic angiogenesis in ischemic limbs

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Therapeutic angiogenesis is expected to be a promising treatment for patients with ischemic disorders such as cardiac and limb ischemia. However, recent clinical trials failed to show much expectant benefits, largely due to suboptimal therapeutic genes and delivery strategies. Herein, we focused on the development of a hypoxia inducible factor-1a (HIF-1a) gene induced muscle-specific angiogenesis strategy that would improve safety and effi-