

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 TGF β 1 and its Receptor 2 (TGFBR2) or against GAPDH as a negative control. Lipofectamine was used at 1.6 μ l/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:** TGF β 1 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 significantly improve the silencing efficacy of the evaluated siRNAs. siTGF β 1- and siTGFBR2-transfected cells showed viability percentages equivalent to those of control untransfected cells. **CONCLUSION:** It is possible to silence *in vitro* TGF β 1 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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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 $25 \pm 3\%$ at a concentration of 3 μ g/well (N/P = 11, $n = 3$ and triplicate replicates). Under the same experimental conditions, DOS reduced EGFP expression to $27 \pm 2\%$ at a concentration of 6 μ g/well (N/P = 22) and to $32 \pm 2\%$ at a concentration of 3 μ g/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 μ g/well LOS cell viability is $83 \pm 4\%$, at 6 μ g/well LOS cell viability is $46 \pm 8\%$, while at 6 μ g/well DOS cell viability is only $32 \pm 9\%$. Transfection of cells with LipofectamineTM2000 resulted in reduction of EGFP expression to $37 \pm 3\%$, with cell viability of $91 \pm 6\%$. 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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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*. 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