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-

ciency. (1). A muscle-specific eukaryotic gene expression plasmid, pSV40E/MCK-HIF1a, was constructed by integrating SV40-enhancer with MCK promoter to regulate HIF-1a gene expression. (2) *In vitro* and *in vivo* studies both indicated that, compared with the natural MCK promoter, the SV40E/MCK hybrid promoter significantly increased HIF-1a gene expression, while retaining a good muscle-cell specificity. Although less efficient than the nonspecific CMV promoter, the hybrid promoter provided more stable gene expression and represented a good compromise between transcriptional activity and muscle specificity. (3) *In vitro* biological effects of increasing HIF-1a gene expression were analyzed in myoblasts to evaluate the function of the muscle-specific gene expression system. Real-time PCR showed up-regulation of several critical angiogenic genes expression, such as VEGF, ANGPT-1, MMP-2 and SDF-1, which were previously demonstrated to facilitate new blood vessel formation and/or maturation. Transwell cell migration assay revealed that pSV40E/MCK-HIF1a transfected L6 cells could recruit progenitor cells derived from bone marrow and muscle tissue. These observations suggested the muscle-specific gene expression system may be useful for stimulating new blood vessel growth and maturation in ischemic limbs while restricting the therapeutic effect to muscle tissue. (4) When reporter gene was transferred into mice limb skeletal muscles, using various nonionic natural polymers, including hyaluronic acid, alginic acid and dextran, the formulated plasmid/polyer resulted in different levels of reporter gene expression, depending upon the type and concentration of the polymers. Some of them showed better performance than naked DNA and these results indicated that the pSV40E/MCK-HIF1a combined with a suitable nonionic polymer may provide a safe and efficient gene therapy system for treatment of limb ischemia.

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A34
Amphipathic CPPs upregulate Ca in cells' cytosol and induce lysosomal exocytosis

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Cell penetrating peptides (CPP) promote the uptake of different bioactive cargo molecules that makes the harnessing of CPPs a promising strategy for drug design and delivery. The translocation mechanism of CPPs into cells, however, has still remained elusive. Direct passage of peptides across the plasma membrane might interfere with its integrity and introduce disturbances. In our study we assessed how cells compensate the disturbances and which processes are induced in response to CPP uptake. Applying fluorometry, flow cytometry and fluorescence microscopy we demonstrate that the uptake of various CPPs enhances the calcium levels in Jurkat and HeLa cells' cytoplasm. The elevated cytoplasmic free calcium concentration evokes downstream effects of membrane repair response and lysosomal exocytosis. Our results indicate that ten of the most commonly used CPPs can be divided into three groups based on their interaction with plasma membrane, the induction of calcium influx, and downstream responses: (1) primary amphipathic CPPs (e.g. MAP, TP) that modulate plasma membrane integrity inducing influx of calcium ions into cells and activate membrane repair and lysosomal exocytosis starting, from low concentrations; (2) arginine-rich, secondary amphipathic, CPPs (e.g. Penetratin, pVEC) that induce changes in the intracellular calcium concentration or subsequent responses at relatively high concentrations and (3) non-amphipathic CPPs (e.g. Tat, Arg9) that do not evoke changes in the intracellular calcium concentration or subsequent responses even at high concentrations. Triggering of the plasma membrane repair response may help cells to recover by replacing the misorganized or membrane active CPPs containing plasma membrane regions, whereas non-amphipathic CPPs could infiltrate without subsequent cellular responses.

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A35
Cellular delivery of oligonucleotides by PepFect

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PepFect (PF) series of peptide based transfection reagents have been developed for the delivery of oligonucleotides (ON) and plasmids into cells. Some PFs are also capable of nuclear delivery of oligonucleotides, for example phosphorothioate 2′-O-methyl RNA oligomers translocate into nucleus and rescue the luciferase expression in the splicing redirection assay after coupling to PF. The optimal ratio of ON with PF for obtaining the functional complexes has been described earlier, but it is not known how such particles interact with the cell surface, enter cells, and reach nucleus. In order to characterize the oligonucleotide delivery by PFs, we labelled 2′-OMe ON with 1.4 nm Nanogold (NG) particles. The membrane interaction, uptake, and intracellular traffic of ON–NG after complexing with PFs were mapped by transmission electron microscopy to unravel their internalization mechanism. PFs pack the Nanogold-labelled ON into small (~200 nm) particles in solution. Smaller particles of ON–NG–PF complexes associate later to form bigger assemblies at the surface of HeLa cells and are taken up by cells in vesicles. The size, electron density and regularity of ON–NG–PF containing structures vary largely depending on the PepFect and its concentration. In cells the majority of the complexes locate in the endosomal/lysosomal vesicles after four hours of incubation. However, the vesicles often have a discontinuous membrane and the Nanogold-labelled oligonucleotides can be found in the cytosol. In addition, with the help of some PFs, the oligonucleotides also reach the cell nucleus. Our results demonstrate that non-covalent complexes of Nanogold-labelled oligonucleotides with PepFects form particles that concentrate at the cell surface and enter cells by endocytotic mechanism. The finding that oligonucleotides have reached nucleus suggests that ON–PF complexes could induce the destabilization of endosomal mem-