

Localized nucleic acid delivery using magnetic nanoparticles

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INTRODUCTION: We have previously developed Magnetofection™ which is nucleic acid delivery into cells guided and enhanced by magnetic fields using nucleic acids or viral and nonviral gene vectors associated with magnetic nanoparticles. These magnetic vectors can be targeted by magnetic fields in vitro and in vivo and magnetic guidance greatly improves both the efficacy and the kinetics of nucleic acid and gene delivery [1,2]. Magnetofection is suitable for overexpressing genes and for silencing gene expression (antisense oligonucleotides, siRNA). We have provided proof-of-principle for magnetically localized gene and oligonucleotide delivery upon administration in the blood circulation or local application in the gastrointestinal tract. However, magnetic trapping against physiological blood flow rates is difficult to achieve, at least in major blood vessels. Solutions to this problem have to be provided in various manners. Magnetic field technology needs to be optimized for magnetic drug targeting. Most importantly, drug formulations have to be provided that are more susceptible to magnetic fields than standard magnetic nanoparticles and that are optimized for vascular administration. Therefore, we have adapted the technique of using microbubbles as drug carriers for magnetic drug delivery. Microbubbles are gas-filled spheres with a protein, polymer or lipid shell, and are currently used in the clinics as contrast agents for ultrasound imaging. Microbubbles have been used very successfully as carriers for drugs and nucleic acid. Localized delivery can be induced by application of suitable ultrasound to a target area [3,4].

METHODS: Magnetic microbubbles were prepared in glass serum vials from a mixture of surfactant-coated magnetic nanoparticles, soybean oil, a cationic lipid (Metafectene, Biontix, Munich, Germany) and fluorescence labeled nucleic acids (plasmid DNA and antisense oligonucleotides) in aqueous buffer. The gas space above the

suspension was filled with perfluoropropane. The vial was shaken for 1 min at 25.000 rpm using a MiniBeadBeater (Biospec Products Inc., Bartlesville, OK, USA). Magnetic retention at various flow rates was measured using an HPLC pump, an electromagnet and microbubbles prepared with radioactive-labeled DNA. Localized nucleic acid delivery in vivo was examined by intravital microscopy using a mouse skin chamber model.

RESULTS: We have developed magnetic microbubbles in that we incorporated a high load of magnetic nanoparticles in microbubble shells. These bubbles can easily be loaded with nucleic acids or cytostatics. Importantly, the magnetic retention of these bubbles at a given flow rate is tremendously improved compared with the same quantity of magnetic nanoparticles in liquid suspension. Using a skin chamber model in mice, we were able to demonstrate by intravital fluorescence microscopy that nucleic acids can be locally delivered to the vasculature and surrounding tissue within the chamber by applying a suitable magnetic gradient field to the target area in combination with ultrasound of 1 MHz frequency. Without magnetic field or without ultrasound, no localized delivery was feasible.

DISCUSSION & CONCLUSIONS: These results indicate that magnetic microbubbles are highly promising drug carriers that can be remote-controlled within the blood circulation by a combination with two independent physical forces.

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