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Superparamagnetic iron oxide nanoparticles (SPIONs) as non-viral vectors for gene delivery in *vitro*

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INTRODUCTION: The treatment and control of acute and chronic inflammatory processes of the bone and cartilage remain a major goal in orthopedic research. Gene therapy would be an attractive alternative to chemotherapy which is accompanied often by various side effects that are detrimental to the patient. The true benefits of these approaches can only be realized when delivery methods are perfected or new ones developed.

METHODS: Superparamagnetic nanoparticles (SPIONs) were synthesized according to Chastellain et al.[1] SPIONs were coated either with polyvinyl alcohol (PVA) (Mowiol® 3-83, Clariant) and further functionalized with amino-groups and fluorochromes Cy3.5 or Texas red, or alternatively coated with 25 kDa polyethylenimine (PEI) (Aldrich). Intracellular uptake of PVA-SPIONs into cells was evaluated by confocal microscopy and flow cytometry. The feasibility of using SPIONs coated with PVA (PVA-SPIONs), for the delivery of a DNA expression plasmid encoding the green fluorescent protein (GFP) gene (pEGFP-C1 plasmid, Clontech), into different cell lines within a magnetic field, was subsequently explored, and evaluated using fluorescent microscopy and flow cytometry. The gene expression after delivery with PVA-SPIONs was further compared with that of particles coated with polyethylenimine (PEI), PEI-SPIONs.

RESULTS: Flow cytometric analysis showed that the PVA coated particles were taken up, resulting in more than 80% of the synovial cells being labelled with Cy3.5. Gene delivery was also achieved using PVA-SPIONs, and 17.7% of 293T cells expressed GFP. In contrast, in cells transfected with PEI-SPIONs, 43.5 % of 293T cells expressed GFP. The green fluorescence in cells transfected with PVA-SPIONs was faint and seen clearly after 48 hrs of incubation, while in cells transfected with PEI-SPIONs the fluorescence intensity was high and seen clearly after 24 hrs of incubation. This indicated a difference in either particle uptake by the cells or gene release from these polymers. Although PVA-SPIONs were less toxic to the cells, these results showed that PEI-SPIONs were more efficient gene vectors. This was confirmed in synovial cells where up to 96.2% of the cells expressed GFP after transfection with PEI-SPIONs. Gene transfection using of PEI-SPIONs in presence of magnet for 5 min resulted in significantly higher proportion of cells expressing GFP when compared with conventional transfection system with lipofectamine, calcium phosphate and PEI alone.

DISCUSSION & CONCLUSIONS: Our results show that PEI-coated SPIONs are very efficient for non-viral gene delivery, resulting in high transfection efficiency *in vitro*. High transfection efficiency was achieved within minutes and the transfection rates achieved were significantly higher than those achieved with conventional transfection methods. The PVA-coated particles were less efficient in gene delivery, however their efficient uptake and low toxicity is to be explored for protein delivery. Preliminary studies by our group have shown that the use of PEI-coated SPIONs is also feasible in gene delivery *in vivo*. Further studies will evaluate the toxicology and *in vivo* efficiency of these transfection systems. This study will serve as a basis for further studies with plasmids, peptides and proteins targeted at inhibiting joint inflammation and cartilage matrix degradation.

REFERENCES: [1] M. Chastellain, A. Petri, H. Hofmann: Particle size investigations on a multi-step synthesis of PVA coated superparamagnetic nanoparticles, *J. Colloid and Interface Science*, 278 (**2004**) 353-360.

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