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Functionalized Calcium Phosphate Nanoparticles: Applications for Gene Transfer

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Abstract – Transfection is a widely used method in molecular biology for the introduction of foreign nucleic acids (DNA or RNA) into eukaryotic cells that permits to control intracellular processes, *i.e.* the induction or inhibition of protein expression. Nucleic acids alone cannot penetrate the cell membrane, therefore special carriers like cationic polymers of inorganic nanoparticles are required. Single-shell and multi-shell calcium phosphate nanoparticles were prepared and functionalized with DNA and siRNA. Thereby, the expression of enhanced green fluorescing protein (EGFP) can be induced (by using pcDNA3-EGFP) or silenced (by using siRNA). The single-shell nanoparticles were prepared by rapid mixing of aqueous solutions of calcium nitrate and diammonium hydrogen phosphate. The multi-shell nanoparticles were produced by adding further layers of calcium phosphate and DNA to protect DNA from the intracellular degradation by endonucleases. The size of the nanoparticles according to dynamic light scattering and electron microscopy was up to 100 nm with a zeta potential around -30 mV. The transfection efficiency of the nanoparticles was tested *in vitro* in cell culture.

One of the most intriguing challenges in biotechnology in recent years was the possibility to modify the cell genome, thus obtaining selected cell properties. Therefore there were many attempts to modify cells to influence the synthesis of defined proteins. The transfection of cells with plasmid DNA leads to the subsequent expression of the desirable proteins (stable or transient), whereas the application of different types of RNA may result in silencing the chosen genes. These may be applied for the gene therapy as well as for diagnostics or cell biological research.

Unfortunately, the nucleic acid itself cannot penetrate the cell membrane. Therefore, specific carriers and protectors are needed. In the past years many carriers were explored but none of them showed completely satisfactory results because of either toxicity or low efficiency of the transfection [1]. We chose calcium phosphate nanoparticles as transfection agent because of their high biocompatibility and biodegradability.

The classical calcium phosphate transfection method was discovered by Graham and van der Eb in 1973 [2]. According to this method, the formation of nanoparticles occurs on the DNA backbone. Calcium chloride solution is mixed with DNA. The subsequent addition of phosphate-buffered saline solution results in the formation of calcium phosphate/DNA precipitates. This dispersion can be added to a cell culture, and the agglomerate will be taken up by the cells.

The preparation of functionalized calcium phosphate nanoparticles was described by us in the last years [3,4]. A dispersion of calcium phosphate nanoparticles is prepared by rapid mixing of aqueous solutions of calcium nitrate and diammonium hydrogen phosphate at pH 9 under constant stirring. Immediately after mixing, the nanoparticles are functionalized with the aqueous solution of nucleic acid. They form a stable colloidal dispersion.

Such nanoparticles consist of a calcium phosphate core and an outer shell of nucleic acid, whereas the precipitates of the standard method consist of a poorly defined DNA/calcium phosphate precipitate. The interactions between calcium phosphate and nucleic acid are electrostatic. To enhance the DNA protection against attack of nuclease, the single-shell nanoparticles were modified by addition of additional layers of calcium phosphate (for protection) and nucleic acid (for electrostatic stabilization).

Transfection experiments were carried out with DNA-functionalized calcium phosphate nanoparticles [3] and also with siRNA-functionalized calcium phosphate nanoparticles [5]. In both cases a high efficiency of transfection was observed.

[1] V. Sokolova, M. Epple, Angew. Chem. Int. Ed. 47 (2008) 1382.
[2] F. L. Graham, A. J. van der Eb, Virology 52 (1973) 456.
[3] V. V. Sokolova, I. Radtke, R. Heumann, M. Epple, Biomaterials 27 (2006) 3147.
[4] V. Sokolova, O. Prymak, W. Meyer-Zaika, H. Cölfen, H. Rehage, A. Shukla, M. Epple, Mat.-wiss. u. Werkstofftech. 37 (2006) 441.
[5] V. Sokolova, A. Kovtun, O. Prymak, W. Meyer-Zaika, E. A. Kubareva, E. A. Romanova, T. S. Oretskaya, R. Heumann, M. Epple, J. Mater. Chem. 17 (2007) 721.