CALCIUM PHOSPHATE NANOPARTICLES FOR GENE TRANSFER AND SILENCING - A 2D AND 3D CELL CULTURE MODEL STUDY

NATANIEL BIALAS[1]*, LEONARDO ROJAS SANCHEZ[1], VIKTORIYA SOKOLOVA[1], MATTHIAS EPPLE[1]

INSTITUTE OF INORGANIC CHEMISTRY AND CENTER FOR NANOINTEGRATION DUISBURG-ESSEN (CENIDE), UNIVERSITY OF DUISBURG-ESSEN, GERMANY
*E-MAIL: NATANIEL.BIALAS@UNI-DUE.DE

[ENGINEERING OF BIOMATERIALS 143 (2017) 34]

Introduction
Gene transfer as a tool to control internal processes in living cells is of great importance in cell biology and biomedicine. Calcium phosphate (CaP) nanoparticles may serve as nucleic acid carriers with high biocompatibility as CaP is a natural component of the human body in bone and teeth [1]. Cell culture models are essential tools to investigate many intracellular processes, including gene transfer and silencing, as well as particle uptake [2]. When compared to a traditional monolayer 2D cell culture model, a spatial 3D cell culture model, due to its higher complexity and more realistic parameters, better reflects the natural cytoarchitecture of tissues, serving as a bridge between *in vitro* and *in vivo* studies [3].

Materials and Methods
CaP nanoparticles were synthesized, stabilized with poly(ethyleneimine), loaded with nucleic acids (DNA/siRNA) and coated with a silica shell. Afterwards they were purified by ultracentrifugation and characterized by dynamic light scattering, nanoparticle tracking analysis and scanning electron microscopy [4]. HeLa cells were transfected with DNA-loaded CaP nanoparticles. For gene silencing in HeLa-EGFP cells, siRNA-loaded CaP nanoparticles were applied. In both cases, the gene transfer efficiency was determined by transmission light microscopy and fluorescence microscopy [2]. The uptake of CaP nanoparticles by HeLa cells was studied by confocal laser scanning microscopy. The cytotoxicity of the nanoparticles was evaluated with a live/dead assay for mammalian cells (Invitrogen™ L3224). All studies on cell cultures were carried out in 2D and 3D (spheroid) models.

Results and Discussion
CaP nanoparticles had spherical morphology with an average size of 150 nm and a positively charged surface with an average zeta potential of +25 mV. Transfection and gene silencing in human cells with the use of nucleic acid-loaded CaP nanoparticles were demonstrated in both 2D and 3D cell culture models, as well as the uptake of CaP nanoparticles by cells. The nanoparticles were not cytotoxic.

Conclusions
CaP nanoparticles can be successfully used as tools for gene delivery and silencing, both in 2D and 3D cell cultures. They have the potential to be widely applied in the treatment of genetic diseases.

References
[1] V. Sokolova, M. Epple, Angew. Chem. Int. Ed. 47 (2008) 1382-1395.
[2] S. Chernousova, M. Epple, Gene Ther. 24 (2017) 282-289.
[3] M. Zanoni, F. Piccinini *et al.,* Sci. Rep. 6 (2016) 19103.
[4] D. Kozlova, S. Chernousova *et al.,* J. Mater. Chem. 22 (2012) 396-404.