Calcium phosphate nanoparticles: Nanocrystalline bone substitution materials and carriers of nucleic acids into cells

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Summary: Calcium phosphate nanoparticles constitute the inorganic component of human hard tissue, *i.e.* bone and teeth. Therefore, they have a high biocompatibility and typically a good biodegradability. This has stimulated their application as bone substitution material, *e.g.* in the form of hydroxyapatite (HAP) and tricalcium phosphate (TCP).
The synthesis of calcium phosphate nanoparticles and their processing into nanocrystalline ceramics permits to fine-tune their biodegradability which is governed by the resorption by osteoclasts. If such nanoparticles are prepared as colloids, they can be used as carriers for drugs and genes, *e.g.* for transfection or gene silencing. It is also possible to stimulate the immune system by appropriate addressing of dendritic cells with calcium phosphate nanoparticles.

Results and Discussion: Calcium phosphate nanoparticles were prepared by precipitation from aqueous solutions, and the water was quickly removed by filtration. Thereby, nanoparticles of calcium phosphate were obtained with a diameter around 50-80 nm (Figure 1). Their internal crystallinity was varied by the synthesis conditions: A rapid precipitation at 5 °C gave fully X-ray amorphous nanoparticles whereas a slower precipitation at 50 °C gave nanocrystalline particles. The addition of carbonate during the synthesis led to nanocrystalline carbonated apatite with high similarity to bone mineral.

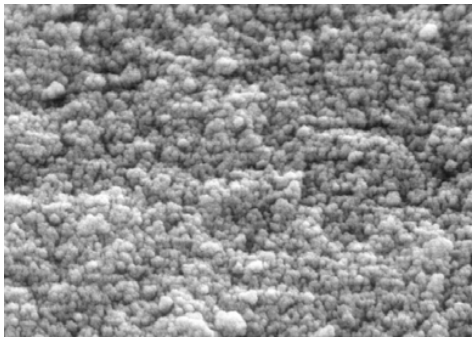

Figure 1: A nanocrystalline calcium phosphate ceramic, consisting of nanoparticles with a diameter around 80 nm

Such nanoparticles can be processed into macroscopic objects by cold-isostatic pressing or uniaxial pressing. The cell-biological response was tested *in vitro* by osteoblast and osteoclast cell culture, showing a good resorption by osteoclasts.
If calcium phosphate nanoparticles are functionalized by suitable polymers, they can be stabilized as a colloidal dispersion by electrostatic or steric stabilization. Typical examples are poly(styrene sulfonate), PSS, poly(allyl amin hydrochloride), PAH, or poly(ethylene imine), PEI. Instead of a polymer, nucleic acids can also be used as stabilizing agents. In this case, calcium phosphate nanoparticles can be used as carriers of nucleic acids into cells. Within the cell, the nucleic acid can either specifically induce the biosynthesis of proteins (DNA; transfection) or specifically inhibit the biosynthesis of proteins (siRNA; gene silencing). This was shown for a number of cell lines
Multi-shell nanoparticles were produced to protect the nucleic acids from intracellular degradation by nucleases. They can also be used to stimulate dendritic cells of the immune system with the TLR ligands CpG and poly(I:C) and induce them to present an antigen of influence (hemagglutinin) to T-cells. This provides a method to stimulate the immune system with such ceramic nanoparticles against persistent chronic infections.
Cells readily take up nanoparticles of calcium phosphate which may therefore be used as highly biocompatible and efficient carriers for drugs and nucleic acids into cells (Figure 2).

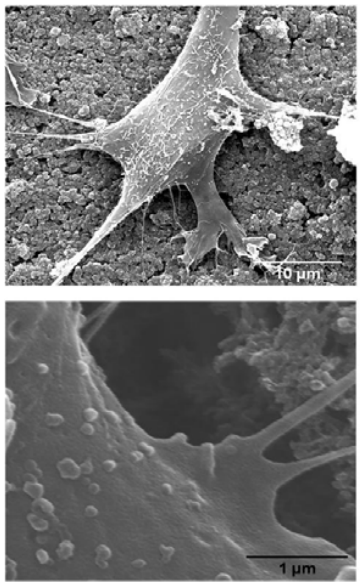

Figure 2: An endothelial cell on the surface of calcium phosphate nanoparticles (top) and magnification of the cell surface with penetrating calcium phosphate nanoparticles (bottom)