Factors affecting calcium phosphate nanoparticle mediated gene transfection

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**Introduction**. Calcium phosphate precipitation has been used for over 30 years to deliver different genes to a wide variety of mammalian cells [1]. In spite of widespread use, little attention has been given to the composition and physical properties of the precipitate. Given the current interest and concern regarding nanoparticle use and safety we sought to examine the effects of inorganic nanoparticle morphology, size, composition and surface charge on mediating plasmid transfection. Analysis of the nanoparticles is made possible by incorporating the DNA after the synthesis of the calcium phosphate nanoparticles (CaP-np) in contrast to conventional methods, where it is co-precipitated. Furthermore, transfection of mammalian cells was enhanced when the particles were incubated with plasmid DNA (pDNA) in the presence of $CaCl_2$ and when their diameter was less than 200 nm.

**Methods**. CaP-np were prepared by mixing an aqueous solution of calcium nitrate (18 mM, pH 9.5) and an aqueous solution of diammonium hydrogen phosphate (10.8 mM, pH 9.5). Particles were characterized by transmission electron microscopy (TEM) using a JEOL JEM-2000FX and dynamic light scattering (DLS) (Zeta Sizer Nano ZS Malvern Instruments). pDNA coding for green fluorescent protein (GFP) was obtained from GST (Gene Therapy System, San Diego, CA). PolyFect, a commercial transfection reagent from Qiagen (GmbH, Germany) was used to monitor and compare efficiency of transfection. CaP-np transfection efficiency was monitored after incubation of the pDNA with varying concentrations of $CaCl_2$ added to cultures of NIH/3T3 fibroblasts and MC3T3-E1 preosteoblast cells. pDNA binding to CaP-np was analyzed by agarose gel electrophoresis, $OD_{260}$ nm and Pico Green fluorescence of DNA in the presence of a variety of $Ca^{2+}$ and $Cl^-$ salts. Fluorescence microscopy was performed with a Leica DM IL microscope. GFP expression was quantified in cell lysates using a fluorometer from BMG Labtech (Germany). Cell lines were purchased from ATCC (American Type Culture Collection, Manassas, VA).

**Results and Discussion.** TEM revealed that CaP-np had a spherical morphology with an average diameter of 100 nm. The calcium/phosphate ratio was 1.5, typical of a calcium deficient apatite prepared by precipitation as confirmed by X-ray diffraction. Particle size and morphology did not appear to be modified after addition of pDNA to the particles. Zeta potential measurements showed naked CaP-np had a positive charge of 10mV and after incubation with pDNA this was reduced to -30 mV. pDNA incubated in 10 mM $CaCl_2$ had a zeta potential of -10 mV. pDNA showed higher binding affinity ($p<0.01$) to CaP-np in the presence of $CaCl_2$, than without. Incubation of pDNA with 10 mM $CaCl_2$ (final concentration) enhanced GFP expression compared with other conditions (Figure 1). These results suggest that the interaction of $CaCl_2$ with pDNA and CaP-np is specific in enhancing GFP expression. Similar results were obtained with MC3T3-E1 preosteoblasts. Since particle size has been shown to effect transfection efficiency [2], we examined this parameter and showed that particles smaller than 200 nm enhance the transfection potency of CaP-np with an associated increase in GFP expression ($p<0.05$). Cytotoxicity measurements indicated a minimal deleterious effect of CaP-np and PolyFect on cell metabolism and plasma membrane integrity 48 h post-transfection.

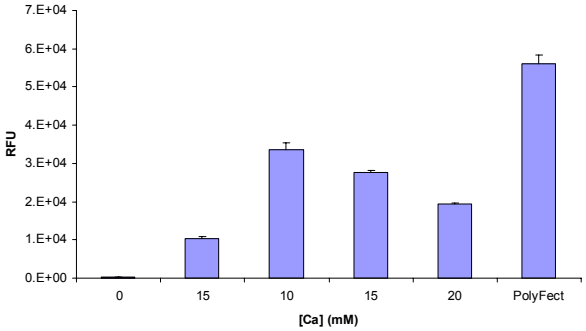


***Figure 1.*** *Effect of calcium concentration on mean GFP expression levels(±sd, n=3) in NIH/3T3 cells transfected with CaP-np-pDNA.*

**Conclusion.** The method used here differs from traditional CaP transfection in that particles were coated with plasmid post-precipitation enabling their full characterisation. We demonstrated that $CaCl_2$ concentration, particle size and charge are important factors influencing plasmid transfection mediated by CaP-np. Preincubation of pDNA with $CaCl_2$ appears to be necessary to achieve significant levels of transfection. Significantly, our modified method achieved reproducible transfections, thereby overcoming a major limitation of this technique. Expression levels between 50 and 100% of PolyFect control levels were routinely obtained without manipulating cell cycle. Additional modifications to the formulation of the particles are in progress to achieve higher levels of transfection that will justify *in vivo* studies. The physical and chemical properties of the particles together with their low cytotoxicity make them a suitable agent to deliver drugs, growth factors and other molecules relevant to biomedical applications.

**References.**

1. (Graham FL & van der EB AJ. Virology 1973; 52:456.)
2. (Prabha S *et al.* Int J Pharm 2002; 244:105-115.)

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