Amorphous magnesium phosphate nanoparticles as nonviral DNA carriers

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Statement of Purpose: Nonviral gene delivery has gained much attention due to its potential in addressing the viral gene delivery limitations like unintentional mutagenesis. Different natural and synthetic materials such as polymers, lipids, and calcium phosphate particles have been developed as DNA carriers. However, there is still a need for development of highly efficient gene vehicles. Amorphous magnesium phosphate nanoparticles are potential candidates for biomedical applications as they are biocompatible, highly biodegradable, and lack cytotoxicity. Present study investigates the application of amorphous magnesium phosphate nanoparticles as nonviral DNA vehicles. Positively charged Mg^{+2} ions are hypothesized to significantly enhance the uptake of amorphous magnesium phosphate attached plasmid DNA by the osteoblasts. Here, different plasmid DNA to amorphous magnesium phosphate nanoparticle (1:1, 1:100, and 1:200) ratios was examined. pCMV6-AC-GFP vectors were attached to the amorphous magnesium phosphate and were used as the cargo. The optimal concentration of cargo to carrier was found to be 1:200.

Methods: The AMP nanospheres were synthesized using our previously developed microwave technique [1]. Plasmid proliferation was completed via the electroporation technique. Plasmid production, purification, and isolation were executed following the previously published procedure by Wagner [2]. Plasmid vectors were mixed with AMP nanoparticles at different ratios (Table 1).

Table 1. Content composition of Plasmid:AMP (w/w)

Group	Plasmid:AMP (w/w)	Plasmid	Polyfect	Fluor.
1	1:1	Y	N	None
2	1:100	Y	N	Low
3	1:200	Y	N	Medium
4	-	Y	Y	High
5	-	Y	N	None
6	-	N	N	None

XRD, SEM, TEM, and zeta potential analyzing methods were used for physical characterizations. Fluorescent microscopy was used to monitor expression level of GFPs.

Results: SEM image and particle size distribution of the synthesized nanoparticles are illustrated in Figure 1. Based on the SEM images and ImageJ program average diameter of spherical nanoparticles was 226 nm. Mean surface charge of nanoparticles was -19.3 mV. Fluorescent images of groups 2, 3, and 4 are shown in Figure 2. AMP-DNA uptake mechanism is illustrated in Figure 3.

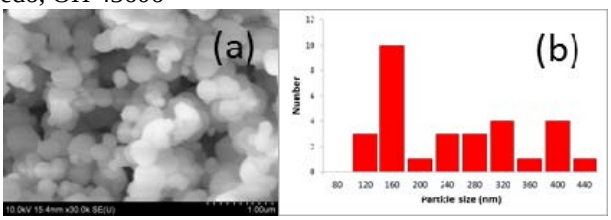

Figure 1. (a) SEM at (30K), (b) Particle size distribution of AMP nanoparticles

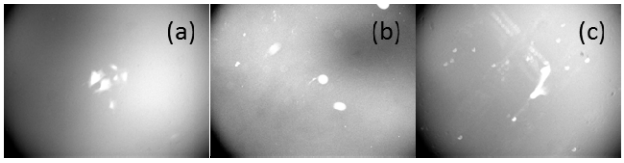

Figure 2. Fluorescence microscopy images of groups (a) 2, (b) 3, (c) 4

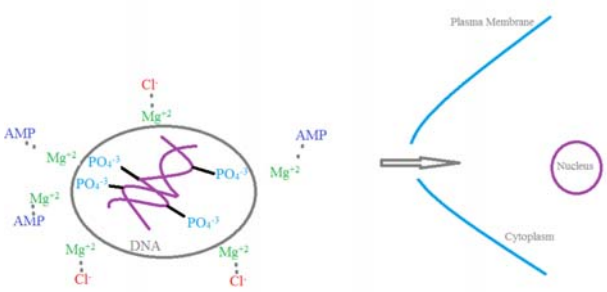

Figure 3. Schematic presentation of the AMP-DNA uptake in osteoblastic cells

Conclusions: The overall results show the ability of magnesium phosphate nanoparticles as gene delivery vehicles. The exact uptake mechanism of nanoparticle attached DNA molecules is unclear, however we suggest that despite the slightly negative surface charge of the AMP nanoparticles, the positively charged magnesium ions (Mg^{+2}) ionically bond to the negatively charged phosphate (PO_4^{-3}) groups of plasmid DNA. Moreover, Mg^{+2} ions shield and protect the plasmid DNA from cytoplasmic nuclease activities. This process justifies the fluorescent signals observed in AMP-DNA complexes verses the naked DNA. Figure 3 provides a schematic presentation of the AMP-DNA uptake.

References:

[1] Zhou H, Luchini TF, Bhaduri S. Microwave assisted synthesis of amorphous magnesium phosphate nanospheres. J Mat Sci: Mat Med 2012;23:2831-7.

[2] Wagner D. calcium phosphate nanoparticles synthesis and manufacture using microwave processing for biomedical applications. Toledo, Ohio: University of Toledo; 2011.