Increased osteoblast density in the presence of calcium phosphate coated magnetic nanoparticles

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Statement of Purpose: Magnetic nanoparticles have been widely used in biomedical research [1]. Our research goal is to treat bone diseases (such as osteoporosis) by using surface modified magnetic nanoparticles. According to previous investigations [2], it is believed that using nanoparticles can create an optimal drug delivery system controlled by an external magnetic field which immediately increases bone density at the porous bone sites. In this study, we reported the increase of osteoblasts (bone forming cells) density in the presence of various coated magnetic nanoparticles.

Methods: The magnetite nanoparticles were prepared by a wet chemical method as previously described [3]. Iron (II) chloride and Iron (III) chloride with a molar ratio of 1:2 were dissolved in deoxygenated water in the presence of HCl. The resulting solution was added drop-wise to a NaOH solution under vigorous stirring and nitrogen flow to obtain magnetite nanoparticles (Fe_3O_4). Maghemite nanoparticles were obtained from magnetite by aeration in boiling water at low pH.

The particles were further coated with calcium phosphate (CaP: the main inorganic component of bone) to tailor them to treat osteoporosis. Briefly, a 1M calcium nitrate solution containing iron oxide nanoparticles was added drop-wise into 0.6M potassium phosphate solution under vigorous stirring. The precipitation was later treated with various thermal conditions to obtain nanocrystalline CaP and amorphous CaP. To reduce nanoparticle agglomeration, these particle solutions were added with surfactants such as citric acid (CA), bovine serum albumin (BSA) and dextrant.

A droplet of nanoparticles was placed on a TEM copper grid and allowed to dry. The imaging was carried out on a Phillips EM420 TEM.

Human osteoblasts (OB: bone-forming cells; CRL-11372 American Type Culture Collection, population number 9) were cultured in Dulbecco's modified Eagle's medium (DMEM; GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; Hyclone) and 1% penicillin/streptomycin (P/S; Hyclone). Cells were grown on a 96 well plate at a volume of 200 µL per well and a seeding density of 3500 cells/cm^2 in the presence of 20 µl of 100 µg/ml iron oxide nanoparticles, CaP coated particles, and a control with no particles. Each solution was replicated three times. Cells were incubated under standard conditions (37°C, humidified, 5% CO_2,/95% air environment) for one, three and five days before the addition of the CellTiter96 (Promega) assay. Cell solutions were incubated and then analyzed using a microplate reader (SpectraMax 300, Molecular Devices) at 490nm for absorbance. A standard curve was also established to correlate absorbance and cell number. The experiment was repeated three times.

Results: TEM images demonstrated that magnetite and maghemite nanoparticles with diameters ~ 20nm were successfully synthesized. Due to their magnetic properties and high surface energy, the nanoparticles formed aggregates.

The synthesized CaP had a rod shape. Nano CaP was crystallized and had average sizes 50 nm long and 20 nm wide. TEM images also showed iron oxide nanoparticles embedded in CaP particles.

Osteoblast proliferation tests conducted at one, three and five days showed that CaP-coated Fe_3O_4 in the presence of CA and BSA increased OB density compare to the controls (no particles) (Figure 1). After one day, OB densities were similar in the presence of CaP-coated nanoparticles with CA and dextrant compared to the control. However, after 5 days, samples with CaP coated Fe_3O_4 added with CA and BSA showed significantly higher OB density compare to the control.

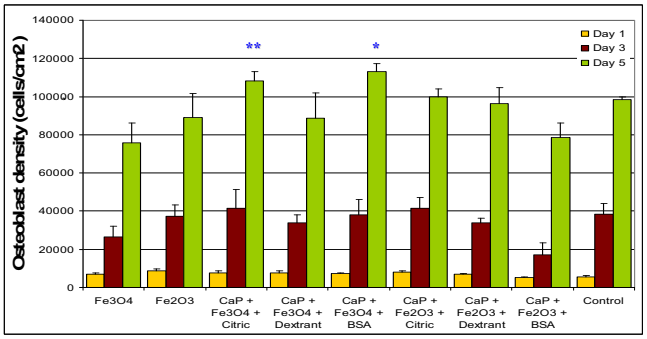

Figure 1. Increased osteoblast density in the presence of CaP coated magnetite (Fe_3O_4) nanoparticles with surfactant CA and BSA after 1, 3 and 5 days of culture. Data = mean +/- SEM; N = 3. * p = 0.013 compared to control, ** p = 0.058 compared to the control (no particles).

Conclusions: Magnetite and maghemite nanoparticles were successfully synthesized and characterized in this study using TEM. Further, these magnetic nanoparticles were coated with CaP in the presence of three different surfactants (CA, BSA and dextrant). After five days, the CaP coated magnetite in the presence of CA and BSA showed significantly higher osteoblast densities compared to controls. For future studies, cell experiments with CaP, iron oxide and surfactants separately should be performed to understand the contribution of each factor to osteoblast proliferation and function process.

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References:

[1] A.K. Gupta, M. Gupta. *Biomaterials*. **26** 2005, pp. 3995–4021.
[2] R.A. Pareta, E. Taylor, T.J Webster. *Nanotechnology* **19** 2008, pp. 265101**.**
[3] Y.S. Kang, S. Risbud, J.F. Rabolt, P. Stroeve. *Chem. Mater.* **8** 1996, pp. 2209-2211.