Mechanisms of Enhanced Osteoblast Functions in the Presence of Hydroxyapatite Coated Iron Oxide Nanoparticles

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Statement of Purpose: In order to use hydroxyapatite (HA) as injectable nanoparticles that can be directed at will to various locations in the body to treat bone defects, HA was coated onto iron oxide nanoparticles in this study. Nanoparticles were characterized via transmission electron microscopy (TEM), dynamic light scattering, X-ray diffraction, Zeta potential and vibrating sample magnetometry. Nanoparticle uptake by osteoblasts was studied using TEM. Long-term osteoblast experiments demonstrated greater alkaline phosphatase activity, total protein synthesis, collagen synthesis and calcium deposition after 7, 14 and 21 days in the presence of greater concentrations (up to 200 μg/ml) of HA-coated iron oxide nanoparticles. The possible mechanism of enhanced osteoblast functions in the presence of nanoparticles could relate to the amount and type of proteins adsorbed onto nanoparticle surfaces. Albumin and fibronectin adsorption to the nanoparticles was measured to answer this question and provided a plausible mechanism for enhanced osteoblast functions in the presence of HA coated magnetic nanoparticles.

Methods: Magnetite nanoparticles and HA coatings were prepared by a wet chemical method similar to a previously described method [1].

Osteoblasts (human osteoblasts (CRL-11372, ATCC), population number 9) were seeded onto polystyrene substrates at a density of 100,000 cells/cm^2 and were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% FBS, 1% P/S, 50 mg/ml L-ascorbate (Sigma Aldrich) and 10 mM β-glycerophosphate (Sigma Aldrich) under standard cell culture conditions for 7, 14 and 21 days. HA-coated Fe_3O_4 nanoparticles were added to cells at concentrations of 200, 100 and 12.5 μg/ml. At the end of each prescribed time period, the supernatant was removed and osteoblasts on the substrates were lysed using 500 μl of deionized water and three freeze–thaw cycles.

The total intracellular protein content in the cell lysates and intracellular alkaline phosphatase (ALP) activity were determined using commercially available kits and a spectrophotometer, following the manufacturer's instructions.

To determine collagen content, the cell lysates were dried onto a microplate and stained with 1% Sirius Red stain at room temperature for 1 h. After the excessive stain had been removed, the stained collagen was then dissolved with 0.1 M NaOH for spectrophotometric analysis at 540 nm. Intracellular collagen content was normalized by total intracellular protein.

After 21 days of incubation, the extracellular matrix on the substrates was treated with 0.6 M HCl at room temperature for 24 h. Then, the sample was analyzed using the inductively coupled plasma (ICP) technique. To differentiate between the added calcium in HA (in the coatings) and calcium deposited by osteoblasts, the amount of calcium from the HA coatings was calculated from the Ca/Fe ratio for the nanoparticles as described above. The calcium then deposited by osteoblasts was normalized by substrate area.

To determine protein adsorption on nanoparticle surfaces, albumin and fibronectin were added at concentration of 600 μg/ml to nanoparticle solutions and shaken for 2 hours. The samples were then centrifuged and protein amount in the supernatant was determined by a commercially available kit (BCA protein assay-Thermo Scientific) and a spectrophotometer.

Results: Higher ALP activity was observed on samples with 200 μg/ml HA coated nanoparticles compared to control samples at all time periods. After 21 days, osteoblasts cultured in the presence of the 200μg/ml HA coated iron oxide nanoparticles had the highest levels of collagen, which was significantly higher than controls.

One of the most important markers regarding bone formation and osteoblast differentiation is clearly calcium deposition in the extracellular matrix. Osteoblasts cultured with HA coated nanoparticles (all concentrations) deposited more calcium compared to controls. Especially, the calcium deposited by osteoblasts cultured in the presence of the 200 μg/ml HA coated iron oxide nanoparticles increased 10 times compared to controls (Figure 1).

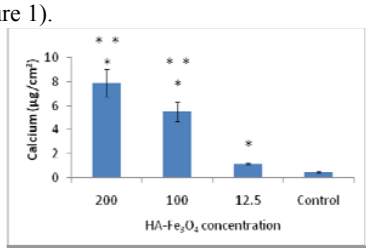

Figure 1. Extracellular calcium deposition in the presence of HA coated Fe_3O_4 nanoparticles after 21 days of culture. Data = mean ± SEM. *p<0.01 compared to control samples. **p < 0.05 compared to samples with smaller amounts of nanoparticles.

Lastly, the measured amount of albumin adsorbed onto nanoparticle surfaces increased with concentration of nanoparticle solutions (Figure 2)

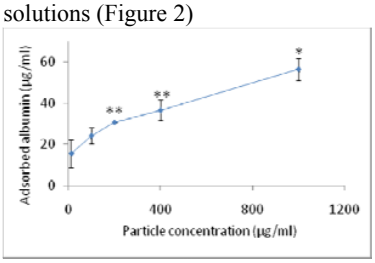

Figure 2. Adsorption of albumin on HA coated nanoparticle surfaces. Data = mean ± SEM. *p<0.05 compared to samples with smaller amounts of nanoparticles. **p<0.05 compared to samples with 100μg/ml and 12.5μg/ml of nanoparticles.

Conclusions: Osteoblast differentiation was studied in the presence of HA coated Fe_3O_4 nanoparticles. Using these nanoparticles at concentrations of 200 μg/ml improved osteoblast differentiation after 21 days of culture, as shown by greater ALP activity, collagen synthesis and calcium deposition compared to control samples without any nanoparticles. Mechanisms of this enhancement could be due to the altered protein adsorption on the nanoparticle surface.

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References:

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