Mechanisms of Enhanced Functions of Osteoblasts on Undoped and Yttrium-doped Nanocrystalline Hydroxyapatite

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Statement of Purpose

Hydroxyapatite (HA) is a promising material for orthopedic applications because it has been shown to promote bone regeneration when compared to titanium. Recently, it has been demonstrated that functions of osteoblasts (bone forming cells) are enhanced on nanocrystalline compared to microcrystalline HA in bulk compacts [1]. In previous studies [2, 3], it was found that a novel HA coating technique, IonTite™, produced nanocrystalline HA coatings which maintained the original crystallite size (<100 nm) of HA particles. In addition, the undoped and especially yttrium (Y)-doped nanocrystalline HA coatings increased calcium deposition by osteoblasts in vitro when compared to titanium, undoped/Y-doped microcrystalline (UltraCap) HA, and traditional plasma-sprayed HA coatings. The objective of the present in vitro study was to determine mechanisms of why osteoblast functions were enhanced on undoped and Y-doped nanocrystalline HA. Specifically, protein (fibronectin (Fn) and vitronecn (Vn); proteins believed to enhance osteoblast adhesion and differentiation) interactions on the undoped and Y-doped nanocrystalline HA was tested in this study.

Methods

Materials: Undoped and Y-doped UltraCap (microcrystalline) HA and nanocrystalline HA powders were prepared through a wet chemistry process. The resultant precipitates were sintered at 1100 °C for 1h to produce UltraCap HA while some of the precipitates were hydrothermally treated at 200 °C for 20h to produce nanocrystalline HA. Surface areas of the synthesized powders were measured by a BET surface analyzer (Beckman Coulter).

Protein interactions: The relative amounts of Fn and Vn adsorption onto undoped and Y-doped (1.2 and 3.6 mole%) UltraCap and nanocrystalline HA powders were determined using ELISA. For this reason, undoped/Y-doped nanocrystalline HA powders were incubated in fetal bovine serum (FBS; Hyclone) for 4h at 37 ˚C. After rinsing with PBS and blocking with 5% non-fat milk overnight, a primary rabbit anti-bovine Fn (Chemicon) and anti-bovine Vn (Accurate Chemical) antibody were added separately and then were incubated at room temperature for 1h. The powders were rinsed with PBS/0.05% Tween 20 (Sigma), and then a secondary anti-rabbit antibody conjugated with horseradish peroxidase (Biorad) was added. Adsorbed Fn and Vn were quantified separately with an ABTS substrate kit (Vector Laboratories) at an absorbance wavelength of 405 nm.

Results / Discussion

Results of this study showed that Fn and Vn adsorbed onto the 3.6 mole% Y-doped nanocrystalline HA powders in significantly great amounts compared to any other powder tested. The results correlated to the fact that BET surface area of the 3.6 mole% Y-doped nanocrystalline HA was also greatest of all of the powders tested. Therefore, the amount of Fn and Vn adsorption may have simply increased because of greater surface area due to doping HA with Y. Fn adsorption increased on undoped and 1.2 mole% Y-doped nanocrystalline HA powders compared to undoped and Y-doped (1.2 and 3.6 mole%) UltraCap HA powders; however, significant differences for undoped and 3.6 mole% Y-doped UltraCap HA versus undoped and 1.2 mole% Y-doped nanocrystalline HA coatings were not obtained. Interestingly, 1.2 mole% Y-doped UltraCap HA powders exhibited the lowest Fn adsorption of all the powders. Moreover, the amount of Vn adsorbed onto 3.6 mole% Y-doped UltraCap HA powders was significantly greater than 1.2 mole% Y-doped UltraCaP, and undoped and 1.2 mole% Y-doped nanocrystalline HA powders in spite of greater surface areas of nanocrystalline HA powders (by > 200 times). These results suggested that Y dopants influenced the amount of Fn and Vn adsorption onto the UltraCap and nanocrystalline HA not only due to decreasing crystallite size (and, thus, greater surface areas) but also due to changes in surface chemistry/charge.

Conclusions

In conclusion, Y-doped HA increased the amount of Fn and Vn adsorbed on UltraCap and nanocrystalline HA powders. The mechanisms of enhanced osteoblast functions on nanocrystalline HA coatings found in the previous study [2, 3] may be partially explained by modulated Fn and Vn adsorption due to Y dopants and the consequent nanotopography. Thus, undoped and Y-doped nanocrystalline HA should be further studied for orthopedic applications.

References

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