

CHANGE IN HYDROXYAPATITE COATING CRYSTALLINITY AFTER CULTURE WITH OSTEOBLASTS

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Introduction

Enhanced bone attachment to orthopaedic implants is obtained with the use of bioactive hydroxyapatite (HA) coatings [1]. High crystalline, non-resorbable HA coatings have the potential problem of introducing an additional interface into the system which could act as a failure site [2]. Therefore, lower crystalline, resorbable HA coatings are being investigated which may provide the short-term benefits of an osteoconductive material without the long-term detrimental effects of coating failure [3]. In this study, the greater amount of chemical resorption taking place on the low crystalline coatings as compared to high crystalline coatings was found to enhance cell proliferation: low crystalline implants had more cells attached and growing at 2, 7 and 14 days. A greater amount of calcium release was found to have a detrimental effect on alkaline phosphatase (AP) production per cell: low crystalline implants had lower AP/cell than high crystallinity coatings for all time periods [4].

The influence of HA crystallinity on coating dissolution and reprecipitation behavior in cell culture was also investigated. Tissue culture experiments were conducted on both high and low crystallinity HA coatings to test the effect of varying coating resorption rates on coating chemistry and morphology. X-ray diffraction was used to determine the extent of the crystallographic changes taking place in the coatings as a result of the exposure to osteoblast cells. In addition, morphological changes in the surfaces of the coatings were analyzed with scanning electron microscopy.

Materials and Methods

Disc shaped samples of surgical grade Ti6Al4V with a thickness of 0.04" were plasma sprayed with a high purity HA powder to achieve a 50-70 micron thick coating. The processing parameters were altered to produce both high (approximately 90%) and low (approximately 70%) crystallinities. Six implants of each type were sterilized with ethylene oxide and placed in 24 well tissue culture plates. Osteoblasts were enzymatically digested from two day post-natal rat calvaria and were cultured to confluence under sterile conditions. Cells were then seeded at a concentration of 5×10^5 cells/ml in a total of 18 wells: 6 wells of each of the two implant types, and 6 wells without implants. An additional six wells without cells served as controls. The cells were cultured for three time periods: 2, 7, and 14 days in DMEM (Eagle) containing nonessential amino acids, 2 mM Glutamine, 10% fetal calf serum, antibiotics and fungicide. The media was changed every two to three days and was saved for chemical analysis.

A Sieman's D500 Diffractometer with Ni-filtered $\text{CuK}\alpha$ radiation was used to analyze the crystalline content of the coatings. A chemical sublimation method was used to fix the cells on the implant surfaces for imaging. An Amray 1400

scanning electron microscope with an accelerating voltage of 30 keV was used to investigate the cell morphology.

Results and Discussion

The micrographs pictured in Figure I show osteoblasts attached to the surfaces of the low and high crystalline coatings. The cells appeared to be well adhered to both surface types, due to the presence of numerous cytoplasmic extensions. The low crystalline surfaces had a more globular appearance compared to the high crystalline surfaces at all time periods. The high crystalline coatings initially had a greater degree of microtexture than the low crystalline, although the average roughness of the two surfaces was indistinguishable.

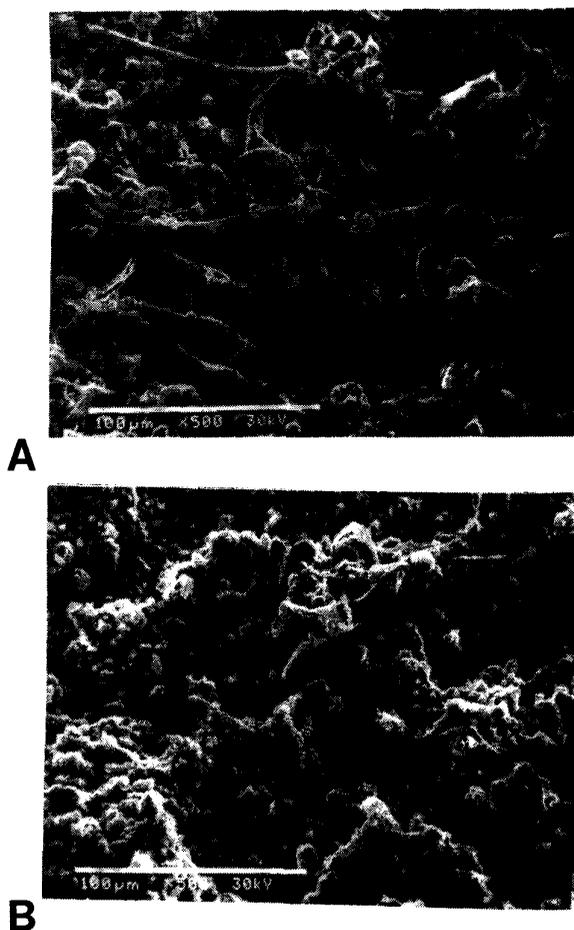


Figure I. Osteoblasts attached to A) low crystalline HA and B) high crystalline HA surfaces at 14 days.

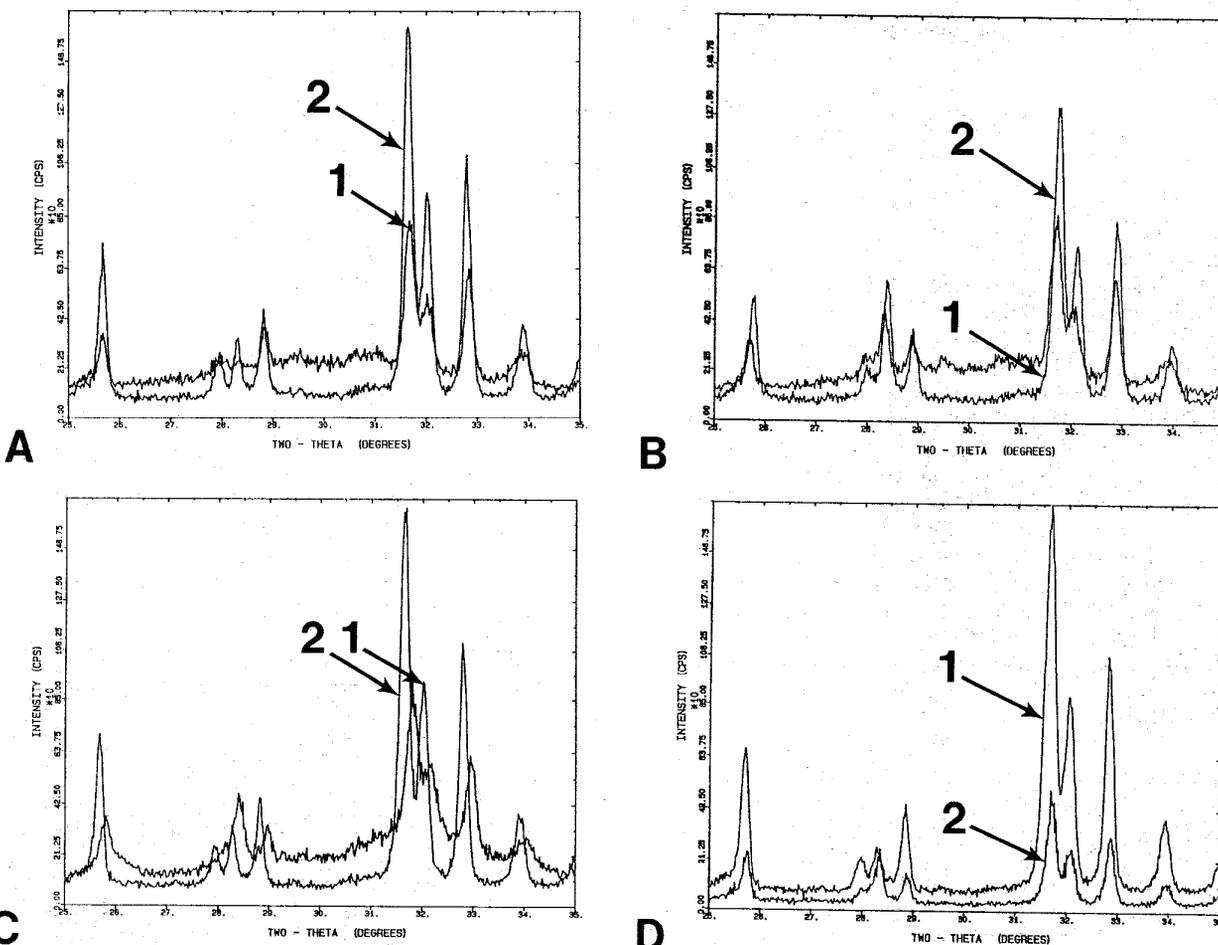


Figure II. X-ray spectra of the low [1] and high [2] crystalline coatings at A) 0 days, B) 2 days, C) 7 days, and D) 14 days.

The x-ray spectra of the two coatings at 0, 2, 7, and 14 days are shown in Figure II. The low crystalline HA coating increased slightly in crystallinity between 0 and 2 days, and 2 and 7 days. More dramatic increases were seen between 7 and 14 days, at which time the amorphous content of the low crystalline HA spectra had decreased. The dissolved amorphous portion of the low crystalline coating may be reprecipitating in an apatitic form and may remain partially in solution.

The high crystalline coating experienced a reversed trend in crystallinity. The crystallinity slightly decreased between 0 and 2 days, and 2 and 7 days. Between 7 and 14 days, however, there is a large decrease in crystallinity along with a loss of amorphous content. Thus, the high crystalline coating has transformed to a poorly crystallized apatite material.

Conclusions and Future Work

Osteoblast cells attached to both low and high crystalline HA coatings, and did not significantly change the morphology of the surfaces.

Significant changes in coating chemistry took place in both low and high crystalline coatings as evidenced by the changes in the x-ray spectra of the two materials. This *in vitro*

study had different influences on the two coating types: the low crystalline HA coating lost amorphous material and increased in crystallinity over the 14 days, while the high crystalline HA coating had a decreasing crystallinity over the same time period.

The crystallographic changes taking place in the HA coatings between 7 and 14 days need to be further investigated.

Acknowledgments

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