Effect of materials properties of hydroxyapatite nanocrystals on fibronectin conformation

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Statement of Purpose: Breast cancer preferentially metastasizes to bone and induces pathological remodeling. Although the exact mechanism of this process remains unclear, there has been evidence that the nanoscale materials properties of the bone mineral are implicated in breast cancer metastasis. [1] Of particular interest is hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$), a calcium phosphate mineral closely related to the inorganic component of bone used for conferring unique structural and mechanical properties to bone. In this study we seek to understand whether HA materials properties alter the mineral/organic interface in bone, in particular, the quantity and conformation of adsorbed fibronectin (FN), a major extracellular matrix (ECM) protein. More specifically, we have (i) developed 2-dimensional platforms containing HA and FN to mimic the interface between bone and the ECM and (ii) analyzed the changes in FN conformation while varying specific properties of HA, such as size, shape, and crystallinity, as well as HA concentration.

Methods: HA nanocrystals with various crystallinities, and narrow size distributions were synthesized through a two-step method in which a typical wet precipitation reaction of a calcium salt with a phosphate salt was followed by hydrothermal aging for 0 and 3 days. Particles were dialyzed in phosphate buffered saline (PBS), or washed with 0.15 M NH_4OH, rinsed with acetone and dried at 20° C for characterization. X-ray diffraction (XRD) was used to determine particle phase (HA). Fourier Transform Infrared Spectroscopy (FTIR) was used to assess the crystallinity of the nanocrystals. The size and shape of the HA nanocrystals were determined by Transmission Electron Microscopy (TEM). Finally, Förster Resonance Energy Transfer (FRET) was used to assess FN conformation by mixing trace amounts of FRET labeled FN with HA nanocrystals dialyzed in PBS in the 2-dimensional platforms. [2]

Results: The materials properties of the HA nanocrystals are summarized in Table 1. A1 and A3 were both proved to be pure HA by XRD. A3 had larger size and higher crystallinity (splitting factor) than A1 nanocrystals. TEM images showed that A1 and A3 dialyzed in PBS were both elongated along their c crystallographic axis.

ID	Hydrothermal aging time /hr	Length along c axis /nm (XRD)	Splitting factor (FTIR)
A1	0	25	3.95
A3	72	74	6.97

Table 1

FRET intensity ratios (IR) of FN incubated with HA nanocrystals at various concentrations for 24 hrs are shown in Figure 1. At 0.1 mg/ml, FRET IR values for A1 and A3 were not significantly different. At 0.7 mg/ml and 5 mg/ml, A3 had higher FRET IR than A1 particles. With increasing concentration of nanocrystals, FRET IR values decreased for both A1 and A3. One-way ANOVA was used to determine statistical significance between conditions. Tukey's post test was used for pairwise comparisons. Our results indicate that at 0.1 mg/ml, FRET IR for A1 and A3 were not significantly different, with $p>0.05$; at 0.7 mg/ml and 5 mg/ml, FRET IR for A1 and A3 were significantly different, with $p<0.01$.

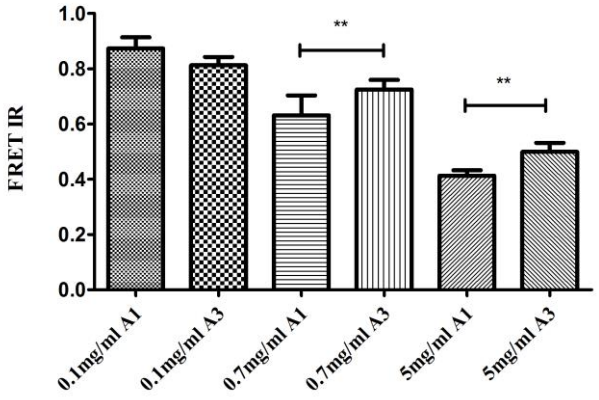

Figure 1

Conclusions: High FRET IR values indicate compact conformation of FN, while low FRET IR values denote extended/unfolded FN conformation. According to Figure 1, FN conformation is not sensitive to materials properties of HA nanocrystals at very low concentrations. However, at higher concentrations, for example, 0.7 mg/ml and 5 mg/ml of nanocrystals, FN adopts more compact conformation when adsorbed on larger and more crystalline A3 particles than on A1 particles, as indicated by higher FRET IR values of A3. Moreover, as the concentration of nanocrystals increases, FN tends to adopt more extended conformation, as indicated by lower FRET IR values, which holds for both A1 and A3. Our results indicate that FN conformation is affected by HA materials properties only at relatively high concentration of HA nanocrystals. This is probably due to enhanced protein-protein interaction, which stabilizes FN compact conformation when more FN becomes adsorbed per unit area (at very low concentrations of HA nanocrystals). Similarly, FN likely adopts more extended/unfolded conformation at higher concentrations as a result of more FN-HA interaction with less FN adsorbed per unit area. Overall these results show that HA materials properties control ECM deposition and conformation, and suggest that HA-induced dysregulated ECM at the mineral/protein interface in bone may play a role in cancer cell recruitment and metastasis.

References: [1] Pathi SP, Lin DDW, Dorvee JR, Estroff LA, Fischbach C. Biomaterials 2011;32: 5112-5122. [2] Smith ML, Gourdon D, Little WC, Kubow KE, Eguiluz RA, et al PLoS Biol. 2007;5(10): 2243-2254.