Culture Platform to Assess the Effect of Hydroxyapatite Properties on Breast Cancer Cells

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Statement of Purpose: Hydroxyapatite (HA), a mineral present in mammographically dense microcalcifications, exhibits varying morphology and composition at different stages of breast cancer. However, it remains unclear if changes in HA properties impact breast cancer malignancy. To test a possible functional relationship between HA physicochemical properties and breast cancer, a screening tool is needed that permits evaluating breast cancer cell behavior as a function of systematically varying HA materials parameters. Here, we have utilized a biomimetic approach to develop such a culture platform and have investigated the malignant potential of breast cancer cells in response to these conditions.

Methods: Poly(lactide-co-glycolide) (PLG)-coated 96-well plates were hydrolyzed and incubated in a set of modified simulated body fluid (mSBF) solutions, which contained twice as much [Ca^{2+}] and [PO_4^{3-}] than conventional SBF solutions, and various [CO_3^{2-}], ranging from 0 to 27 mM. After mineral coating formation, mineral morphology, carbonate content and crystallinity, phase, and surface roughness were characterized by SEM, FT-IR, XRD, and AFM, respectively. MCF7 (poorly metastatic), MDA-MB231 (highly metastatic), MCF10AT1 (pre-malignant), and MCF10DCIS.com (malignant) breast cancer cells were cultured on the different mineral-coated surfaces and their adhesion and secretion of interleukin-8 (IL-8), a marker of malignancy, were measured by fluorimetric DNA assay and ELISA, respectively.

Results: Mineral coatings formed in mSBF solutions consisted of carbonated HA phases (XRD) with carbonate incorporation (FT-IR) that changed as a function of the utilized [CO_3^{2-}] (data not shown), resulting in a change in carbonate content and crystallinity of HA (Fig. a and b). Varying carbonate incorporation not only led to microstructural changes of the HA from plate-like to spherulitic, but in the range of 0 to 4.2 mM [CO_3^{2-}], also affected surface roughness (Fig. c and d). Hence, the degree of carbonate incorporation influences both chemical and physical properties of HA. Previous results indicate that HA carbonate content in breast microcalcifications correlates with breast cancer aggressiveness [1]. Indeed, adhesion of MDA-MB231 was increased on HA coatings formed with 0 and 2 mM [CO_3^{2-}] relative to all other HA coatings suggesting that surface roughness changes may contribute to these differences (Fig. e). The effect of HA properties on IL-8 secretion was assessed representatively on 0, 4.2, and 15 mM [CO_3^{2-}] HA coatings given their distinct chemical and physical characteristics. Highly metastatic MDA-MB231 secreted more IL-8 than poorly metastatic MCF7 and dramatically increased IL-8 secretion on 4.2 mM [CO_3^{2-}] HA coatings relative to all other conditions (Fig. f). Importantly, MCF10A cells with increased malignancy (T1<DCIS) exhibited similar behavior, while culture on PLG control surfaces was unsuitable to reveal these cell line-specific differences. Interestingly, ductal carcinoma in situ (DCIS) is most frequently associated with microcalcifications and both MCF10DCIS and MDA-MB231 upregulated IL-8 most significantly on 4.2 mM [CO_3^{2-}] HA coatings (Fig. g). Collectively, these results suggest that HA materials properties can regulate the malignant potential of breast cancer cells and that the specific materials properties of 4.2 mM [CO_3^{2-}] HA coatings may be particularly relevant. Future studies will be necessary to distinguish between the effects of HA crystallinity and surface roughness, which may exert independent albeit related effects.

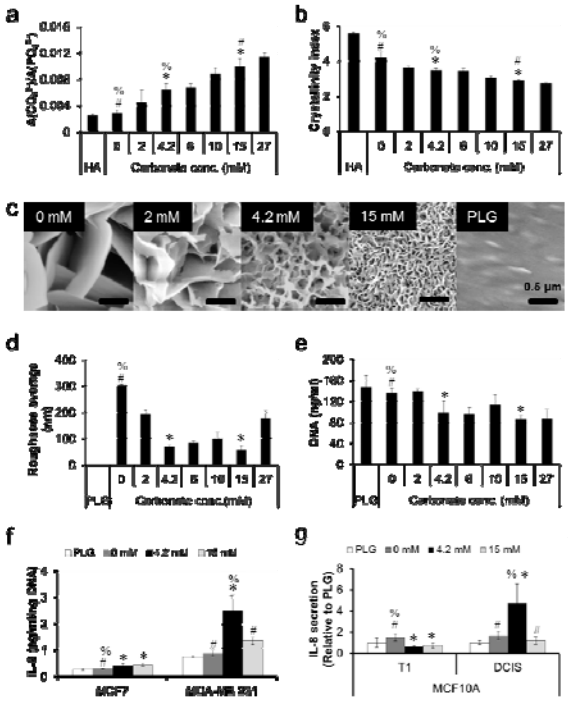

Figure. Characteristics of HA coatings: (a) Relative carbonate composition and (b) Crystallinity index from FT-IR analysis. (c) Representative SEM images. (d) Surface roughness from AFM images. Breast cancer cell behaviors on HA coatings: (e) MDA-MB231 adhesion and IL-8 secretion of (f) MDA-MB231 and MCF7 cells and (g) MCF10A series of varying malignancy. *, #, and % indicate significant differences ($P < 0.05$) between 0 mM, 4.2 mM and 15 mM [CO_3^{2-}], respectively.

Conclusions: We have utilized a biomimetic approach to control HA materials properties on cell culture substrates. Our results suggest that HA physical and chemical properties influence IL-8 secretion by breast cancer cell lines and thus, possibly regulate malignancy in patients. Furthermore, our data suggest that the developed HA-containing culture platform may be applied to study the relevance of HA mineral properties to breast cancer.

Reference: [1] Baker R. Br J Cancer. 2010;103:1034-1039.