Biomineralized 3-D Culture Systems for Studying Metastatic Breast Cancer

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Statement of Purpose: Breast cancer, the leading cause of cancer related deaths among women worldwide, frequently metastasizes to trabecular regions of bone leading to secondary tumor growth, increased bone fragility, and pathological fracture due to osteolysis. Experimental evidence suggests that tumor-derived soluble factors are involved in these processes, but the role of the physicochemical properties of bone in modulating the secretion of these factors remains unclear. We have developed a novel 3-D culture system using porous biomineralized scaffolds and breast cancer cells to study the integrated effects of microenvironmental conditions and bone mineral on metastatic breast cancer cell behavior and circumvent the limitations of conventional 2-D systems in modeling in vivo conditions[1]. We fabricated and characterized poly(lactide-co-glycolide) (PLG)/hydroxyapatite (HA) composites and used them as biomaterial scaffolds to determine if bone mineral promotes osteolytic tumor growth by regulating mammary tumor cell proliferation and the expression of pro-angiogenic and osteolytic morphogens. Our results suggest that bone mineral plays a vital role in influencing breast cancer cell behavior, and show that 3-D biomineralized polymer scaffolds serve as innovative tools for studying the underlying mechanisms and effects of osteolytic bone metastasis.

Methods: *PLG/HA Scaffold Fabrication and Characterization*: Scaffolds were fabricated by gas-forming/particulate leaching (GF/PL) based on a modified version of a protocol by Kim et al.[2] They were characterized by compressive testing, SEM, and EDS-elemental analysis. Compressive testing was performed with an EnduraTEC loading system. Output data was analyzed to give elastic moduli via MATLAB code. SEM was used to determine microarchitecture of scaffolds, and EDS-elemental analysis was used to examine surface composition via the Evex Software Package. PLG scaffolds without HA were used as controls. *3-D Cell Culture*: Scaffolds were statically seeded with MDA-MB231 cells, and seeding efficiency and viability were verified by calcein/propidium iodide staining and fluorescence and confocal imaging. Following dynamic culture for up to 10 days, scaffold lysates and medium samples were collected and analyzed for DNA content and factor secretion through Hoechst Assay and ELISA (R&D), respectively.

Results: Compressive testing revealed that incorporation of HA resulted in a two-fold increase in elastic modulus relative to control scaffolds (data not shown). SEM showed that scaffold microarchitecture resembled trabecular bone, and EDS verified that HA was presented to cells at scaffold surface (Fig. 1).

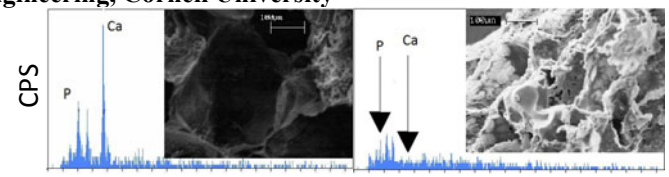

Figure 1 EDS and SEM analysis of PLG/HA (left) and PLG Control Scaffolds (right)

DNA analysis and ELISA showed that scaffolds with HA promoted proliferation of MDA-231 cells and upregulated secretion of interleukin-8 (IL-8) and basic fibroblastic growth factor (bFGF), but produced no effect on secretion of vascular endothelial growth factor (VEGF) when compared with HA-free control scaffolds (Fig. 2). Calcein/propidium iodide stains revealed no cytotoxicity due to experimental or control scaffolds and verified similar seeding efficiency and cell viability for both groups (data not shown).

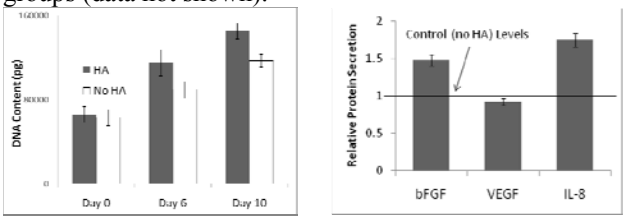

Figure 2 Cell Proliferation (left) and Secretion of Osteolytic and Angiogenic Factors (right)

Conclusions: Biomineralized 3-D culture systems have been developed to study metastatic breast cancer. PLG/HA composite scaffolds were used to recreate physicochemical characteristics of bone that may be implicated in the malignant transformation of breast cancer cells during bone metastasis such as enhanced stiffness and surface-available HA. In contrast to culture in HA-free control scaffolds, mammary tumor cells grown within biomineralized matrices exhibited enhanced proliferative, angiogenic, and osteolytic potential. Specifically, cells upregulated secretion of bFGF and IL-8, while VEGF release was unchanged. Notably, IL-8 not only promotes tumor vascularization, but also modulates osteolytic remodeling by stimulating osteoclastogenesis[3]. Thus, our data suggests that interactions between breast cancer cells and bone mineral may play an important role in increasing osteolytic bone metastasis via upregulation of IL-8. Insights gained through this study along with further investigations using biomineralized 3-D breast cancer models may lead to a better understanding of the signaling pathways that contribute to osteolytic bone metastasis and the identification of novel therapeutic targets for cancer.

References:

1. Fischbach, C, et al. Nat Methods 10: 855-60 (2007)
2. Kim, S, et al. Biomaterials 27: 1399-1409 (2006).
3. Bendre, MS, et al. Bone 33: 28-37 (2003).