3D Printing of Scaffolds with Tunable Modulus and Pore Size for Investigating the Progression of Cancer-Induced Bone Disease In Vitro

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Statement of Purpose: Cancer Induced Bone Disease (CIBD) commonly occurs in patients with advanced metastatic disease from several different primary cancers, including breast, lung, and prostate. Tumor cells establish and alter bone formation leading to extreme pain, bone loss and frequent fractures. Recent studies have begun to investigate the interactions of tumor cells with physical, mechanical and topological facets of CIBD. The majority of these studies have relied on 2D culture, which while valuable, limits our ability to recapitulate complex interactions between multiple cells types and the physical properties of the bone microenvironment. To address this we utilized a 3D perfusion bioreactor (3D Bioteck, LLC, Hillsborough, NJ) as an in vitro model and mammary fat pad implantation of the 3D polyurethane scaffolds (PURs) in vivo and observed the microenvironment's influence on tumor-cell gene expression. Since our previous studies in 2D have indicated that matrix rigidity regulates genes important for CIBD [2], our 3D studies focus on rigidity mediated effects, and allows us to incorporate essential physiological parameters like cellular interactions, shear stress, and mechanical forces that we could not fully capture in 2D. We hypothesize that the rigidity, flow rate, and pore size of the bone microenvironment all contribute to gene expression and bone destruction seen in bone metastatic tumors.

Methods: 3D poly(lactic acid) (PLA) templates were printed with a MakerBot Replicator 3 (MakerBot, Brookyln, NY). A reactive two-component polyurethane (hexamethylene diisocyanate trimer (HDIt), a poly(ε-caprolactone-co-glycolide-co-lactide) triol (polyol, Mn = 300 or 3000 Da), and iron(III) acetylacetonate catalyst) was cast into the mold and cured. Pore sizes were 300 or 500 μm and were 100% interconnected in defined architecture (Fig 1.A). The elastic modulus of the PUR was varied by adjusting the molecular weight of the polyester triol. To investigate the effects of pore size and rigidity on expression of bone metastatic genes by tumor cells, rigid (2600 MPa, representative of cortical bone) and compliant (10 MPa, approximating the basement membrane) PURs were seeded with MDA-MB-231 breast cancer cells (5 x 10^5 cells/scaffold) and mounted in a perfused 3D bioreactor (3D Biotek, LLC, Hillsborough, NJ) for 48 hours (Fig 1.A-E.). In vivo studies were performed by seeding MDA-MB-231-GFP cells on 3D scaffolds by implantation into the mammary fat pads of nude mice. (Fig 1H.)

Results:

To examine the effect of pore size and rigidity on the expression of genes associated with bone destruction, we performed quantitative real-time PCR (qPCR) on tumor cells seeded on 3D PURs. Our data demonstrated that PTHrP expression was 20-fold higher on 300 μm rigid scaffolds compared to 2D compliant films (Fig. 1F) and integrin β_3 ($I\beta_3$) was 50-fold higher (Fig. 1G). In vivo RT-PCR data demonstrated 25-fold higher PTHrP expression between rigid and compliant 300μm PURs (Fig.1.I). Matrix Assisted Laser Desorption/Ionization (MALDI) analysis demonstrated a strong peek in the rigid scaffold and not in the compliant. This peek was recently identified as S100A8, a host (mouse) protein that is reported to be a potent chemoattractant of myeloid cells.

Conclusions: Our data suggest that the rigidity and pore size of the microenvironment can influence the expression of factors associated with bone metastasis and destruction. Furthermore, we have developed a novel approach to studying the interactions between tumor cells and the bone microenvironment that allows us to investigate rigidity and pore size effects in vitro and in vivo and can be used in the future to address cell-cell interactions.

References

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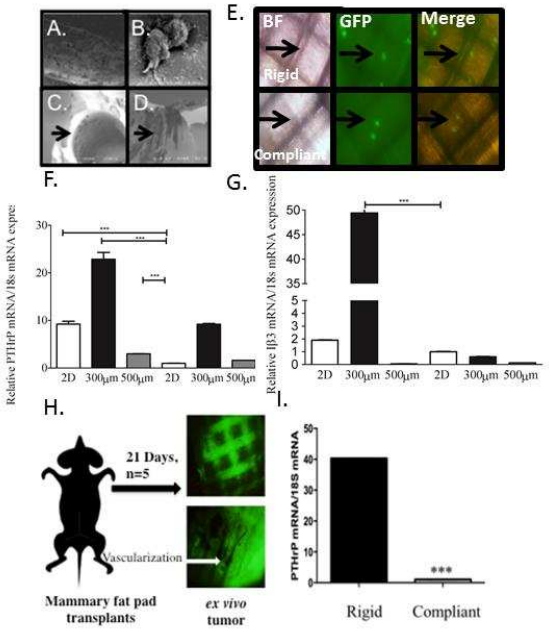

Fig 1. MDA-GFP cells adhere to 3D PURs as shown by SEM (A-D.) and florescence microscopy (E.) *in vitro.* Gene expression for E. PTHrP and F. $I\beta_3$ are affected by pore size and rigidity. G-I. *In vivo* studies (G.) show 40 fold increase in PTHrP gene expression (I)