While breast cancer has an overall 5-year survival rate of 89%, the rate for patients with stage 4 metastatic disease is only 26%. Immunotherapies have the potential to improve the prognosis for these patients while also providing better treatment options for all breast cancer patients since they have fewer side effects enabling longer treatment times and the use of combination therapies and reduced chances of developing resistance. Currently these treatments are tested in standard 2D cell cultures that are inaccurate in mimicking in vivo drug response or animal models where the immune system differs from humans in numerous ways including T-cell subsets, cytokine receptors, and costimulatory molecule expression. We have developed 3D models of human breast cancer that span the subtypes, ER+, HER2+, and triple negative, are composed of numerous stromal cell types including fibroblasts and adipocytes, and incorporate immune cell types including macrophages and T-cells under either static or perfusion culture systems.

### Methods

**Microtumor Model Development**

- Seed ASCs into porous scaffold
- Isolate PBMCs from human blood
- Select for CD14+ monocytes and stain with PKH26
- Select for CD3+ lymphocytes and stain with CFDA-SE
- Seed cells into porous scaffold with ECM
- Resected breast tumor
- Fat Differentiation
- Viability Analysis:
  - PrestoBlue® Assay
  - PicoGreen® Quantitation
- Cell Migration Analysis:
  - Multiphoton Microscopy
  - Flow Cytometry
  - Histology
- Immune Cell Characterization:
  - Perfusate Analysis with Cytokine Array
  - Flow Cytometry
  - Histology

**Patient Derived 5x 3D Perfusion Microtumor**

- 5x 3DMT Culture for 1 week
- CD3+ CD14+ Fat
- BRB60/HMF or Fat
- 5x Culture

### Conclusions

- Expansion of primary breast cancer cells was successful across all subtypes with an 80% success rate (16/20) on primary samples; Cells isolated from breast core biopsies have also been successfully expanded (data not shown)
- Expanded primary breast cancer cells demonstrated high EpCAM positivity and stable gene expression throughout expansion culture
- Our 5x 3D perfusion microtumor culture models lymphocyte and monocyte migration and invasion in the breast cancer microenvironment.
- Incorporation of CD3+ lymphocytes and CD14+ monocytes into our 5x 3D perfusion microtumor culture led to a pro-inflammatory phenotype
- The design and use of the 3DKUBE® is covered by patents 8,855,460 and 9,575,055
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