

***In Vitro* Production of CTCs using 3D Cultures of Human Tumor Tissues and Tumor Cell Lines**

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Chief Technology Office

Management of Open Innovation



Purpose:

To ensure an Innovative Product Pipeline through the Identification, Assessment, and Recommendation of Significant Technology Opportunities, and to Facilitate the Internalization of Technology upon Business Approval.

Managing Open Innovation:

The integrated process of seeking and assessing external technologies, together with building business consensus for internalization and ultimate commercialization.

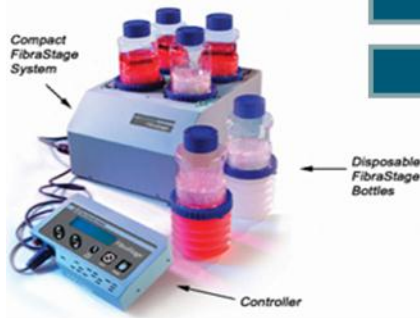
A New In Vitro Model System for Cancer Research



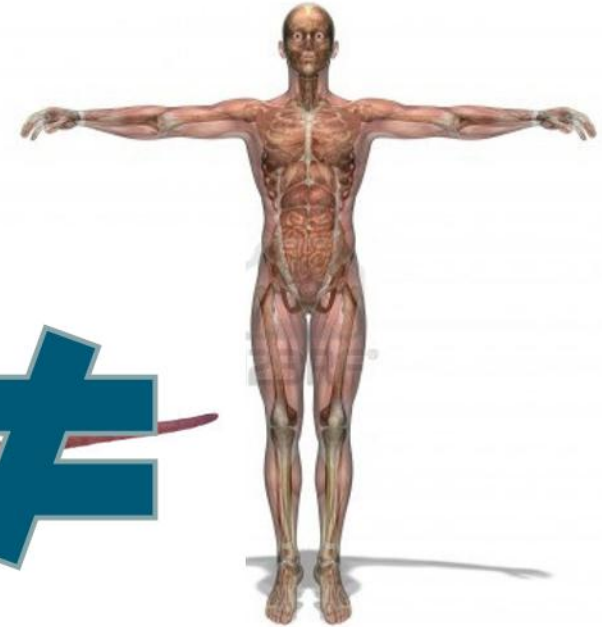
Lab and animal tests are imperfect predictors of clinical outcomes



2D – Flat surface



Simple 3D



A New In Vitro Model System for Cancer Research

Why are new in vitro drug discovery tools needed?

1. **Pharmaceutical companies need improved and reliable methods for predicting a drug candidate's efficacy and toxicity earlier in the process.**
2. **Relevant *in vitro* tumor models could reduce the cost of drug discovery significantly by replacing some animal testing.**
3. **Animal testing is not always an accurate predictor of clinical outcome and drug failures are very expensive.**
4. **Ethical concerns over animals research influences the public's perception, acceptance and support of research.**
5. **These ethical concerns are influencing regulatory agencies to discourage the use of animals in research. Animal testing for cosmetics is banned in Europe and this trend is likely to continue.**

COST, ACCURACY, ETHICS

The ideal *in vitro* model should produce tissue that looks and acts like real tumors

This talk describes the results of a CTO-sponsored feasibility study by RealBio Technology, Inc. to demonstrate the utility of the D⁴ System for the *in vitro* study of cancer biology

RealBio D⁴™ Culture Chamber



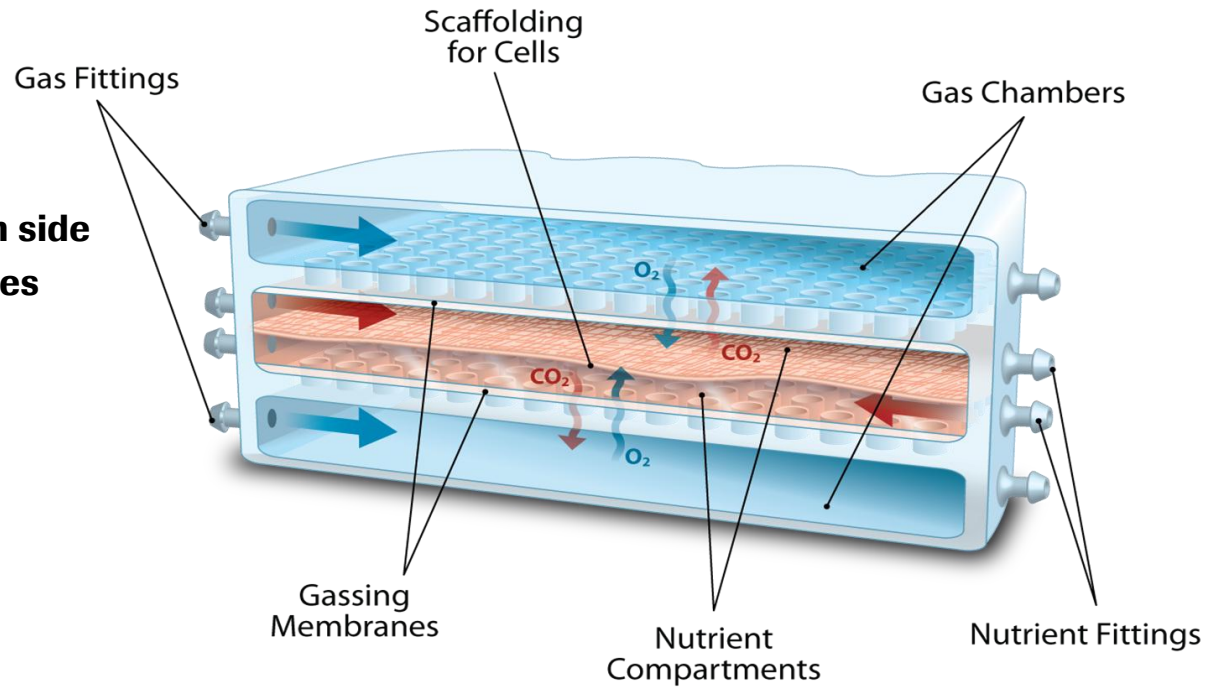
Flexible de-coupling of nutrient and gas supply

🍯 Nutrient Supply:

- **Single Channel**
- **Dual Channel**
 - **Different medium on each side**
 - **Same or different flow rates**
- **Recirculating or single-pass**

🍯 Gas Supply:

- **Passive ambient**
- **Active**
 - **Same gas on both sides**
 - **Gradient**



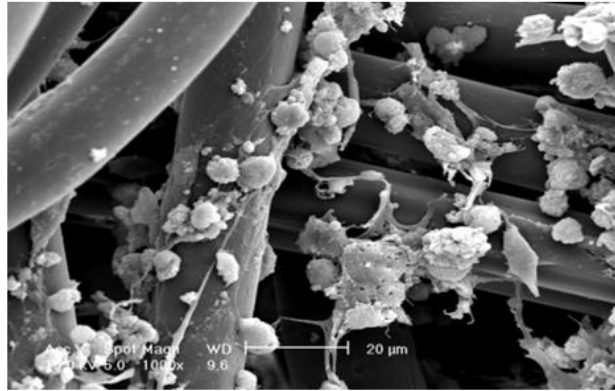
RealBio D⁴™ Culture Chamber



An artificial immune system developed by \$4.5 MM DARPA grant

Human bone marrow mononuclear cells in 3D culture

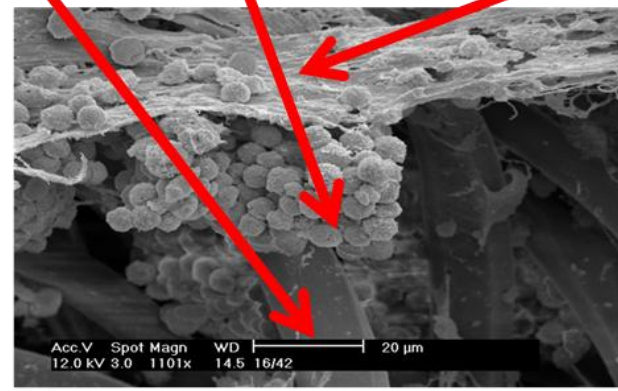
2 Days in Culture



Scaffold Fiber

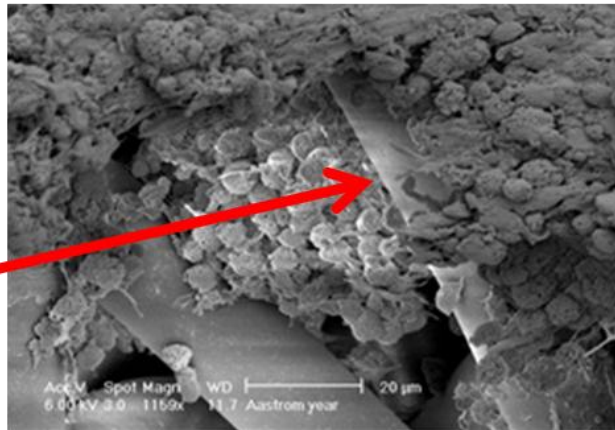
Cell Clustering

Extracellular Matrix

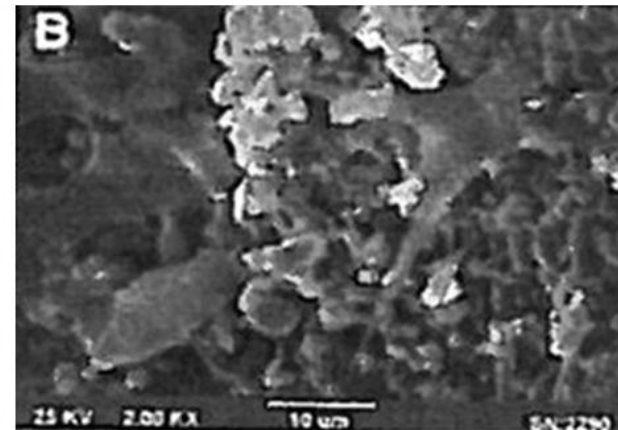


DARPA Data

Cells cluster around fibers like a spicula



420 Days in Culture



Real Bone

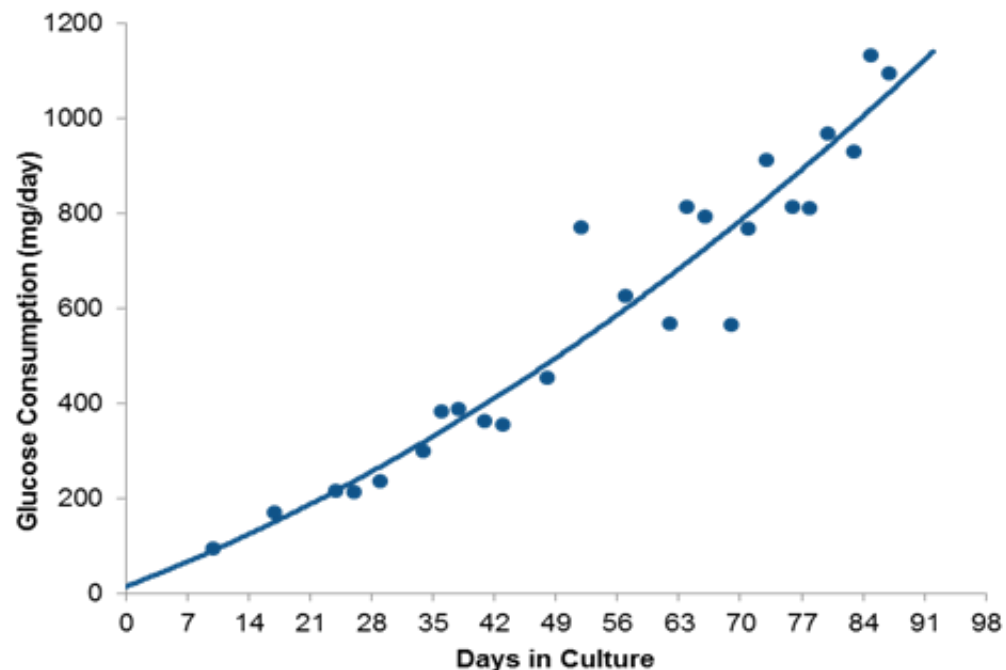
RealBio D⁴™ Culture Chamber



Tumor tissue expands for extended periods without sub-culturing

Glucose Consumption for a Primary Pancreatic Tumor Culture in the 3D Perfusion Cultures System.

Primary tumor tissue cultured in the RealBio D⁴™ Culture System exhibits increased glucose consumption over extended culture periods reflecting continued tissue expansion without the need for subculturing.



RealBio D⁴™ Culture Chamber



Tumor tissue grown in vitro is similar to in vivo tumor

P0 Tumor *in vivo*

3D Culture of P1 Tumor

3D Culture of P1 Tumor

Heterogeneous Primary Pancreatic Tumor Cell Populations in Long-Term 3D Perfusion Culture Show Arrangements Reminiscent of *In Vivo* Organization.

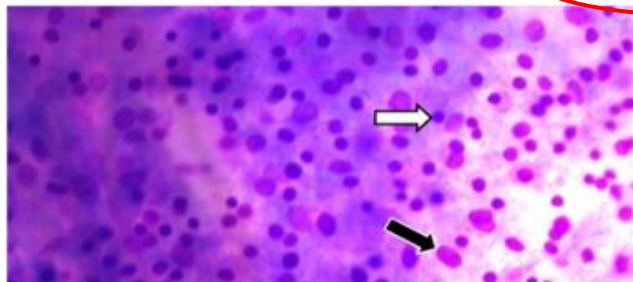
Direct staining and imaging (without sectioning) of primary metastatic pancreatic tumor tissue grown in the RealBio D⁴™ Culture System for 3 months (right panels) clearly reveals multiple cell types including the predominant fibrous and spherical/globular morphologies observed in tumors grown *in vivo* (left panel) in addition to cells with very large vacuoles represented in lesser numbers as *in vivo*.

RealBio D⁴™ Culture Chamber

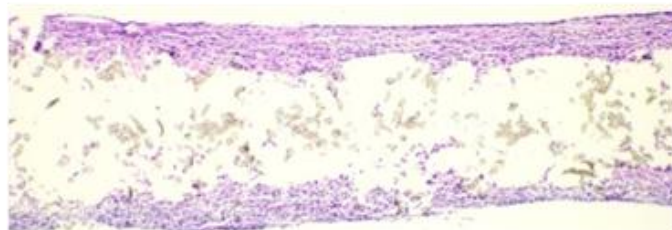


Tumor tissue grown in vitro is similar to in vivo tumor

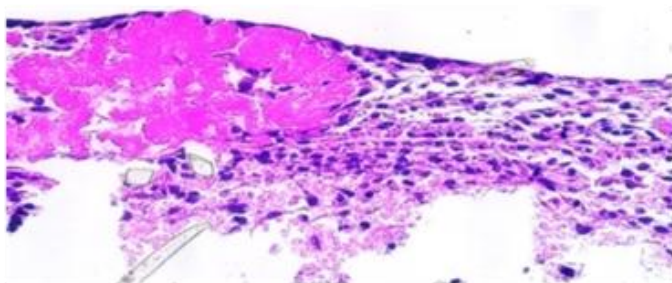
Long-term 3D Perfusion Cultures of Metastatic Sarcoma Tissue.



Metastatic sarcoma tissue rapidly expanded in the RealBio D⁴™ Culture System. Direct staining and imaging (without sectioning) of the culture scaffold just 17-days after seeding (Panel A) revealed multiple cell types (white and black arrows) completely covering the culture scaffold.



Histological cross-sections taken from metastatic sarcoma cultures after 166 days (5 ½ months) revealed dense tissue masses above and below the culture scaffold (tissue depth ~200-500 microns).



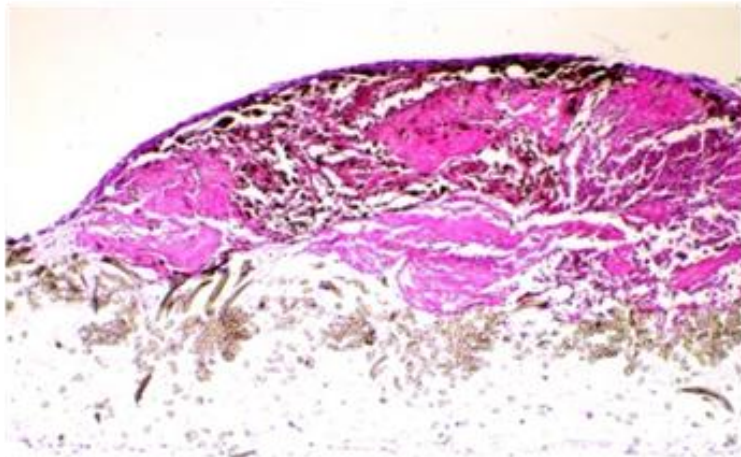
Higher magnification of metastatic sarcoma tissue after 166 days in the RealBio D⁴™ Culture System reveals natural, *in vivo*-like cell organization and tissue structure.

RealBio D⁴™ Culture Chamber

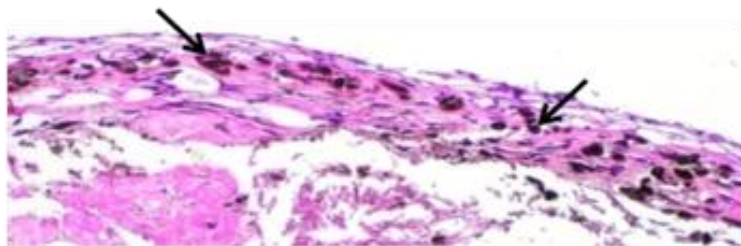


Tumor tissue grown in vitro is similar to in vivo tumor

Long-term 3D Perfusion Cultures of a **Metastatic Melanoma** Tumor.



Metastatic melanoma tumor tissue expanded slowly during a 3-month culture period in the RealBio D⁴™ Culture System using non-optimized conditions. The cultured tumor retained a high degree of cell diversity but minimal cell migration was observed resulting in the highest cell densities on the upper surface of the culture scaffold (where the cells were seeded) and only sparse cell populations in the lower regions of the scaffold.



Higher magnification of the metastatic melanoma tissue after 3-months in 3D perfusion culture clearly shows melanin-containing cells (arrows) organizing near the surface of the cultured tumor just below cells arranged in a manner reminiscent of natural stratum corneum.

RealBio D⁴™ Culture Chamber



Workflow for establishing tumor tissue in vitro

Cultures

- **Human metastatic pancreatic tumor from mouse xenograft ~ 1.2 g tissue**
- **Mechanical/enzymatic dissociation**
- **RealBio D⁴™ Culture Systems (6) for up to 6 weeks**
- **Non-optimized conditions**
 - **Ambient gassing**
 - **Simple medium (DMEM + 5% FBS)**

Sampling

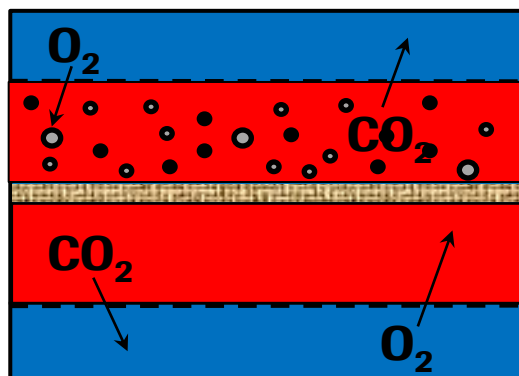
- **5 mL sample from lower compartment 3x per week**
- **Evaluate culture activity (glucose, lactate, cell migration)**
- **Cells shipped to Vitatex for CTC analysis**

RealBio D⁴™ Culture Chamber



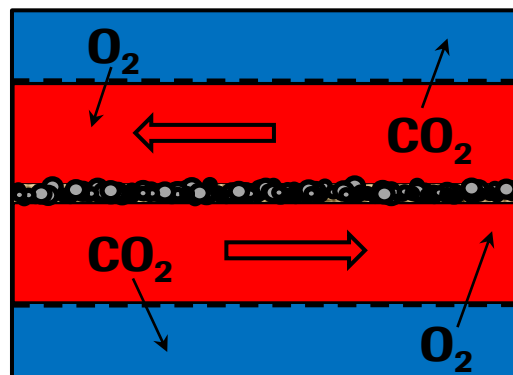
Workflow for establishing tumor tissue *in vitro*

Seed



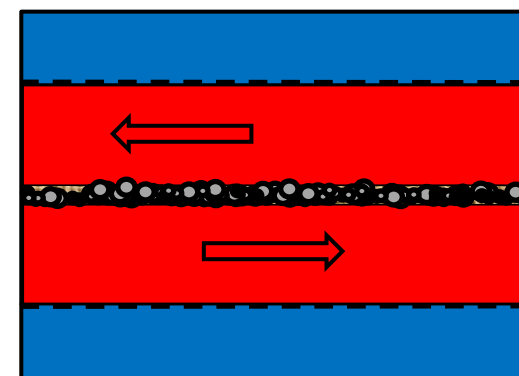
- Infuse tumor cells into upper compartment
- No flow for 24 hours
- Cells settle into and migrate throughout scaffold

Growth



- Re-establish nutrient flow
- Tissue density achieved in a couple→several weeks
- Cell turnover slows as tissue density is achieved
- Cells continue to migrate throughout scaffold

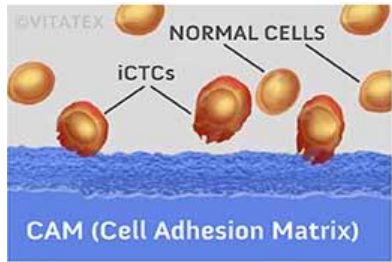
Culture & Harvest



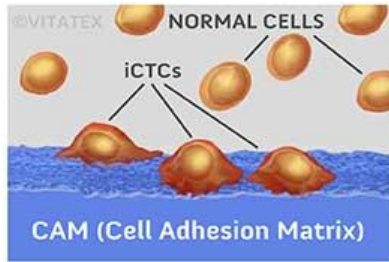
- Viable cells can migrate out of scaffold
- Periodic harvest from lower compartment
- Apoptotic cells migrate from tissue

Vitatest iCTC Detection

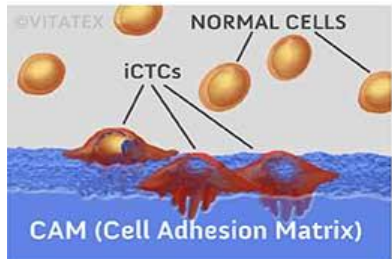
Infiltrating CTCs are isolated by their ability to bind and digest collagen



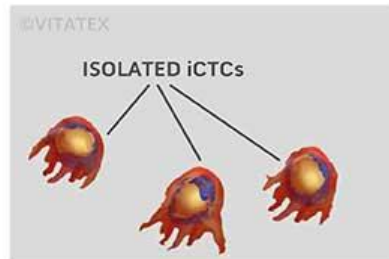
1 Patient samples are added to CAM-coated plates or in tubes.



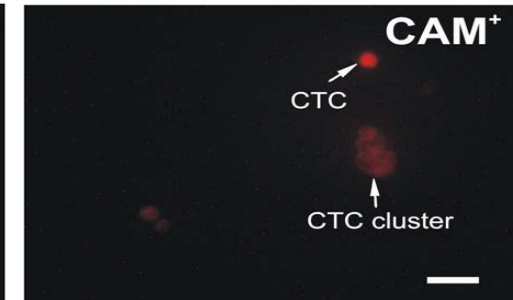
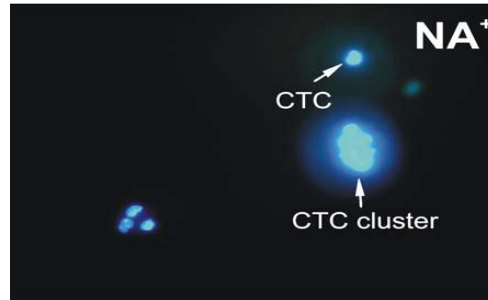
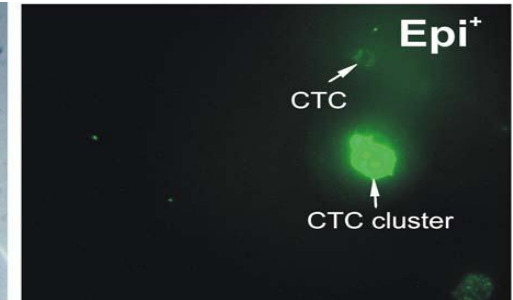
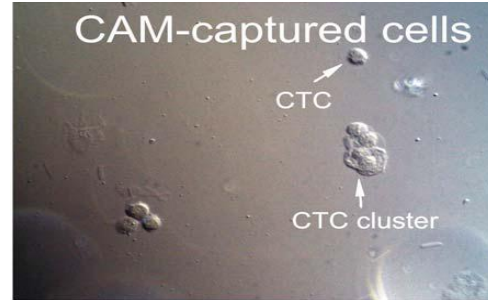
2 iCTCs are capture based on their preferential adhesion to CAM.



3 Captured iCTCs are labeled based on their ingestion of FITC- or TRITC-labeled CAM.



4 Captured iCTCs are suspended for analysis by flow or image cytometry, or for gene expression, gene mutations, etc or culture in plates for growth inhibition with drugs or other agents.



iCTC = EpCAM/CK19⁺; CD45⁻; DAPI⁺; CAM⁺

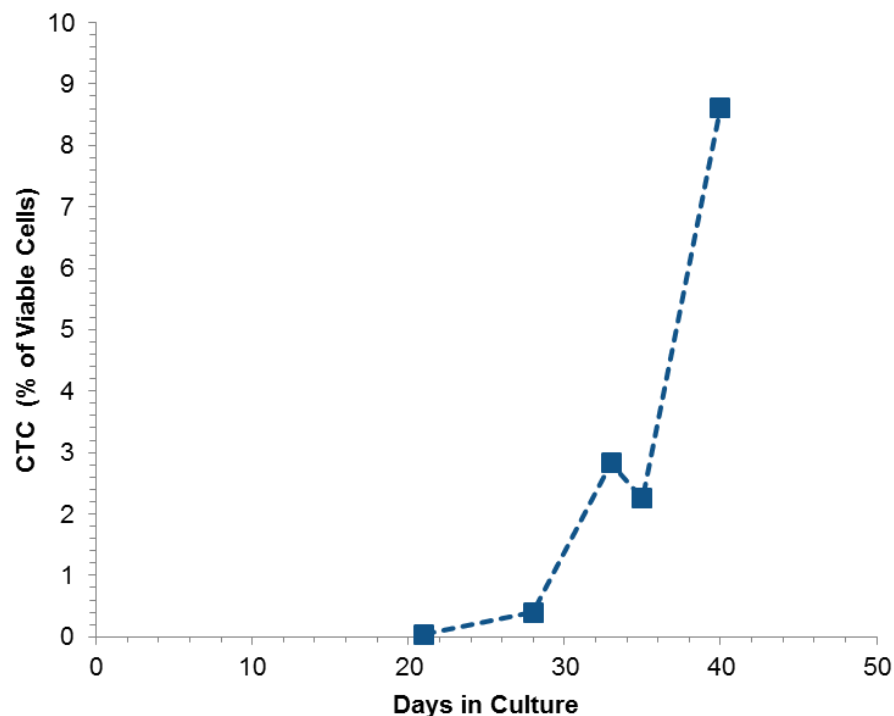
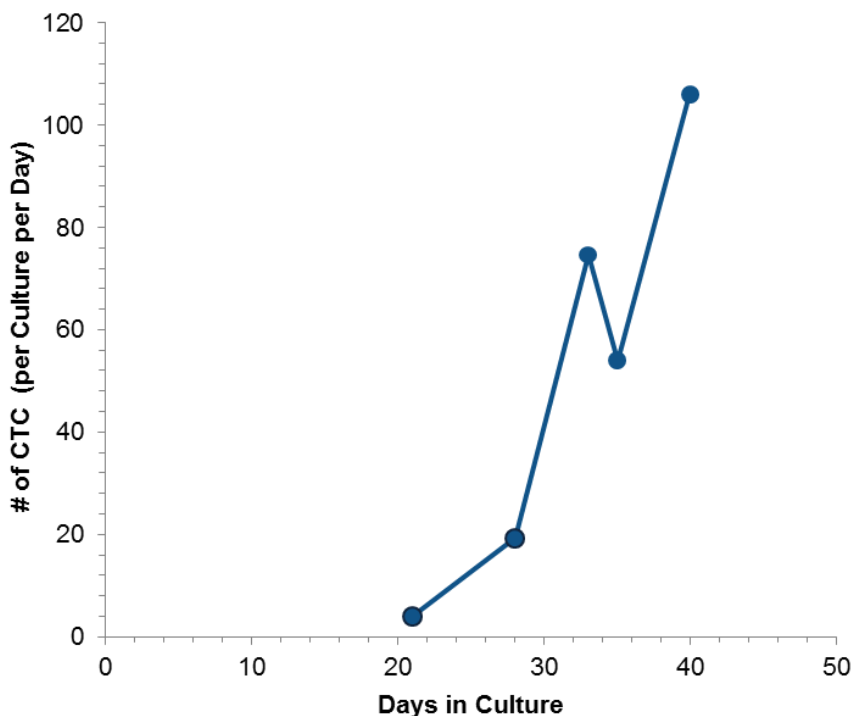
CTPC = CD44/Seprase⁺; CD45⁻; DAPI⁺; CAM⁺

CTPC = Circulating Tumor Progenitor Cell

RealBio D⁴™ Cultured Pancreatic Tumor Tissue



CTCs are detectable after 20 days and production increases over time

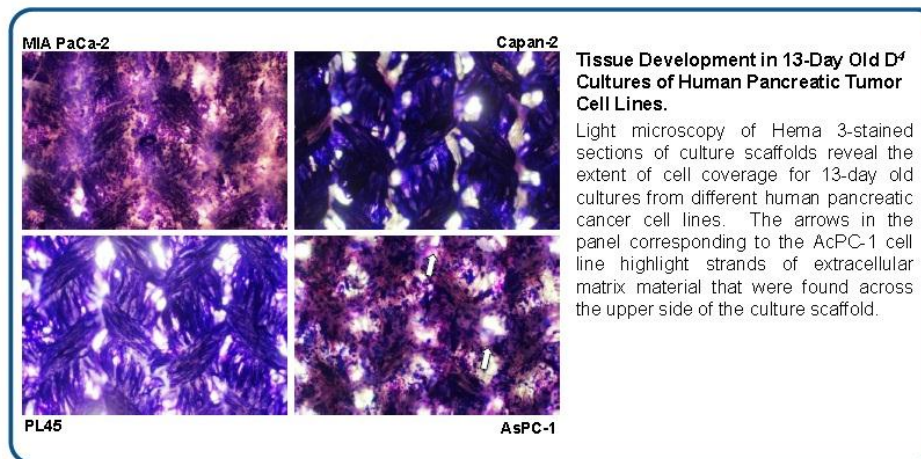
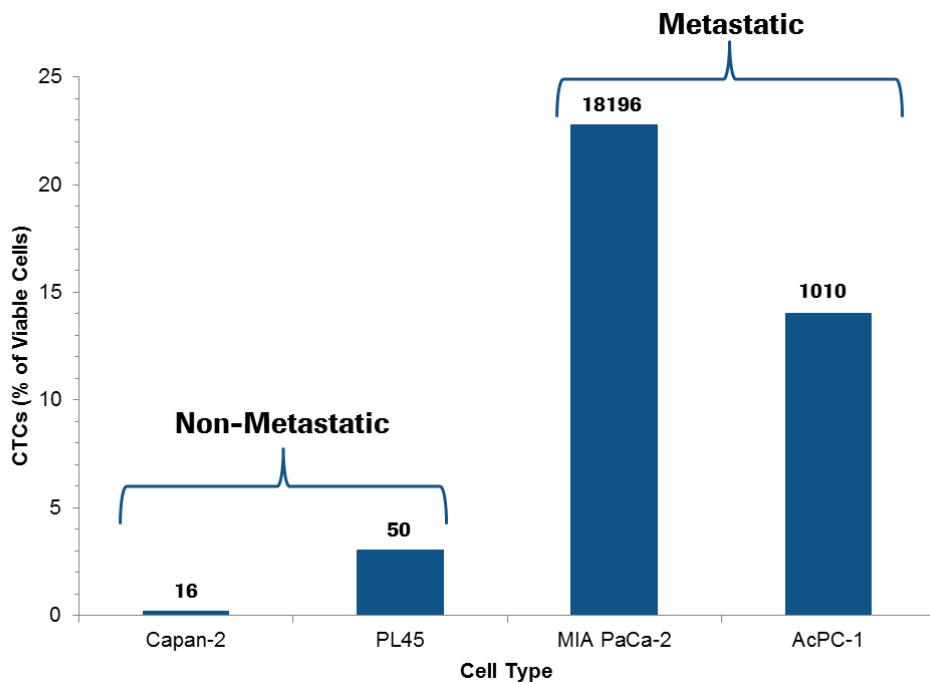


The experiment was terminated after 6 weeks (42 days)

RealBio D⁴™ Cultured Cell Lines



CTC production by cancer cell lines correlates with metastatic potential



Cell Line	Metastatic Potential
Capan-2	Low
PL45	Low
MIA PaCa-2	High
AsPC-1	High

RealBio D⁴™ Cultured Cell Lines

Summary

- 🌸 The 3D perfusion culture system simulates the *in vivo* tissue microenvironment promoting 3D organization on the cellular and tissue level *in vitro*.
- 🌸 CTCs are produced *in vitro* by heterogeneous tumor tissue and cell lines maintained in the RealBio D⁴™ Culture System.

Applications:

- 🌸 *In vitro* modeling of Circulating Tumor Cells (CTCs) including factors that may control CTC formation and migration from the tumor.
- 🌸 *In vitro* study/manipulation of the tissue microenvironment including hypoxia and tumor/stroma interactions
- 🌸 Bio-relevant *in vitro* drug efficacy studies using whole tumor cell populations.

Acknowledgements



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Lee Knoll



Lee Chen

Doing now what patients need next