A patient-derived *in vitro* model of colorectal cancer: A perfusion culture system that recapitulates patient tumor composition and structure in 3-dimensions.

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Abstract

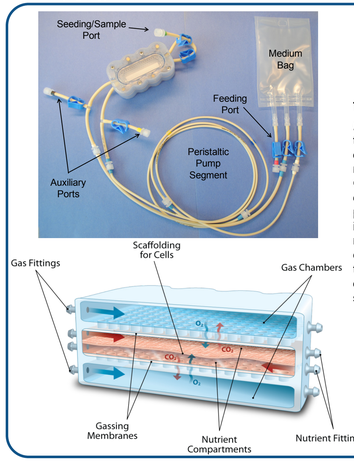
Cancer medicine would benefit greatly from a practical technology that accurately predicts the sensitivity of individual patient tumors to drugs. Previously developed *in vitro* chemosensitivity models have generally failed to do this because they do not incorporate key aspects of the tumor microenvironment (TME), which clearly plays a role in drug resistance. There has been significant interest in patient-derived xenograft (PDX) chemosensitivity testing as an alternative to *in vitro* models, but the applicability of this approach is limited by engraftment percentages and growth rates.

We have developed an *in vitro* technology that is designed to recapitulate the 3D tissue architecture of patient-derived colorectal tumors including accessory (endothelial and immune) cells, starting with freshly excised tumor specimens. The system incorporates: 1) low-sheer, tangential flow of nutrient medium above and below an open, synthetic 3D cell scaffold; and 2) exchange of metabolic gasses via permeable membranes which separate the culture chamber core from gassing chambers located above and below the nutrient flow compartments. This design allows establishment of gas and nutrient gradients across the cell scaffold that closely mimic conditions *in vivo*.

Aliquots of mechanically and enzymatically dissociated, freshly excised, primary human colorectal adenocarcinoma tissue were introduced into the 3D culture system and maintained for up to 3 weeks. Routine maintenance of the cultures involved partial medium exchanges as needed based on glucose concentrations in the circulating medium. The proportions and arrangement of key cell types (e.g., normal muscularis, tumor epithelium, stromal fibroblasts, endothelial cells, and immune cells) in the cultured explants were compared to those of the original tumor by standard histological techniques. Results revealed good agreement between the histology of the original tumor and that of the *in vitro* model. These results suggest that this model technology could be applied to individual patient drug selection as well as studies of colorectal cancer biology that are not feasible or practical *in vivo*.

Introduction

The RealBio D⁴ ™ Culture System was designed to mimic the *in vivo* tumor microenvironment. An open 3D culture scaffold situated between tangential flows of nutrient medium enables cultured cells to maintain natural 3-dimensional morphologies. Gasses and nutrients are delivered in such a way that gradients of each, reminiscent of those in natural tumors, may be formed such that heterogeneous tumor cell populations are supported. Together, these features provide unprecedented potential for modeling key components of tumor structure and behavior such as tumor initiation, expansion and metastasis *in vitro* while manipulating a host of environmental conditions (e.g., hypoxia, acidosis, etc.).



The RealBio D⁴ ™ Culture System.

In vivo-like conditions are promoted within the culture chamber through the use of an open 3D cell scaffold and decoupled nutrient and metabolic gas supplies. Gradients of nutrients and gasses established across the cultured tissue promote varied microenvironments which in turn support long-term maintenance of mixed cell populations that mimic the composition and organization of natural tumors. Also, cell migration out of the open scaffold permits the collection and study of CTCs *in vitro*.

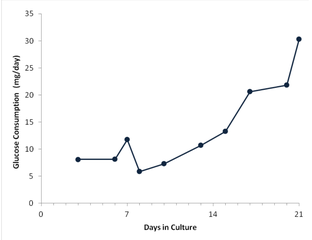
Materials & Methods

3D Perfusion Tumor Cultures: Human colorectal adenocarcinoma tumor tissue was obtained fresh from a consenting patient via the Spectrum Health Universal Biorepository (Grand Rapids, MI). The tumor tissue was minced and then partially digested using Liberase TM (Roche) and DNase I (Roche) before being triturated through successively smaller orifices until all fragments could pass freely through a 16 g needle. Portions of the resulting whole tumor cell suspension were infused into the top compartments of RealBio D⁴ ™ Culture Chambers primed with IMDM supplemented with 10% FBS and a cocktail of antibiotics. The seeded culture systems were placed at 37° C with 5% CO_2 for 4-5 hrs to allow cells to settle into the culture scaffold. After the cell settling period with no medium flow, the culture chambers were placed on a 45° incline and pulsed medium flow was initiated and maintained for the duration of each study (1 mL/min for 1 minute, 45 seconds every 30 minutes). Samples were collected from the bottom compartment of each culture chamber 3 times per week for analyzing routine culture parameters (glucose and lactate concentration, and migrating cells). Partial exchange of the circulating medium in each system was performed 3 times per week in order to replenish nutrients and remove waste products.

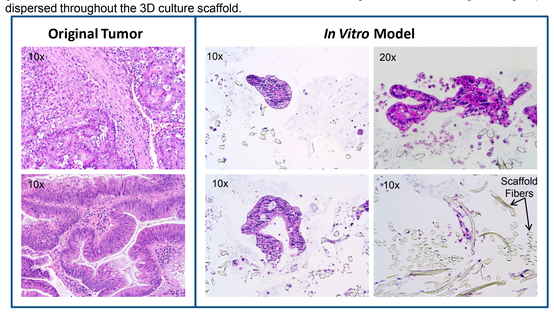
Histology: Tumor cultures were terminated and fixed *in situ* by infusing 10% Neutral Buffered Formalin (NBF) into the culture chambers. After fixation (5 hr), the culture scaffold was removed from the culture chambers and embedded in paraffin for subsequent sectioning and staining using traditional histological methods. A range of IHC probes was used to provide a qualitative comparison of the cellular composition and structure of the patient-derived tumor with that of the *in vitro* model derived from the tumor.

Results

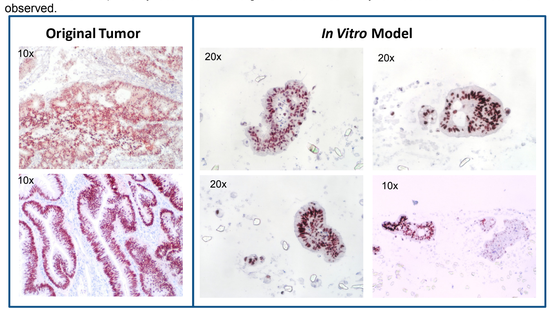
Primary colorectal adenocarcinoma tumor tissue cultured in the RealBio D⁴ ™ Culture System exhibited increasing glucose consumption over extended culture periods reflecting continued tissue expansion without the need for subculturing.



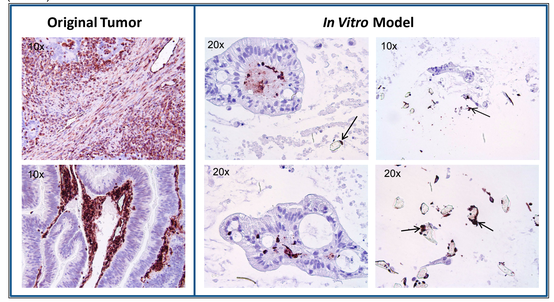
H&E staining revealed structural heterogeneity across different areas of the original tumor. Similarly, glandular tumor structures are maintained in the *in vitro* model along with cells in less organized groups dispersed throughout the 3D culture scaffold.



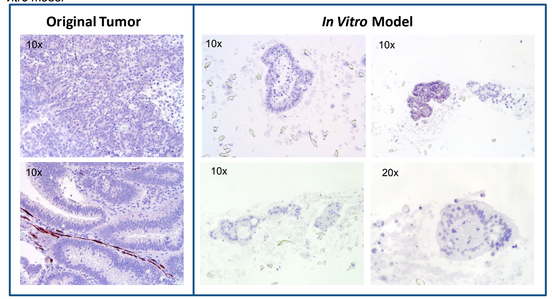
CDX-2 staining confirmed the existence of colorectal tumor epithelial cells in the *in vitro* model where the CDX-2+ cells were primarily localized within organoid structures. Very few isolated CDX-2+ cells were observed.



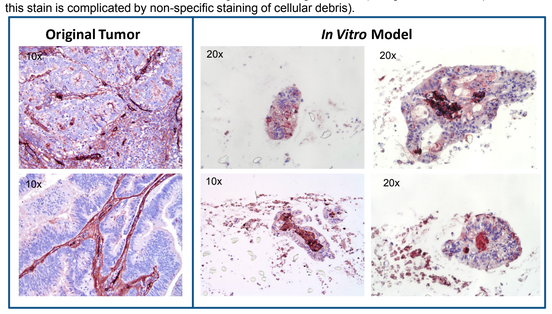
Vimentin+ stromal cells are were found localized within cultured tumor organoid structures and scattered throughout the culture scaffold where they were found directly adhered to the synthetic scaffold fibers (arrows).



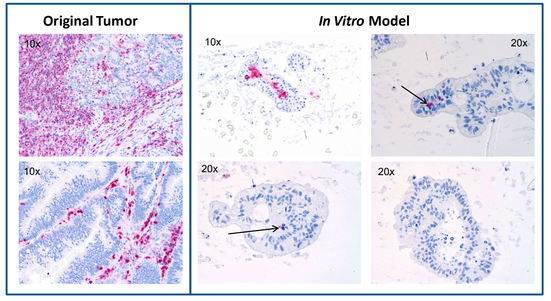
Muscularis cells staining positive for **desmin** were found in adenomatous regions of the original tumor but but not in carcinomatous regions. No desmin+ cells were observed in the sections reviewed for the *in vitro* model



Clusters of endothelial cells staining positive for **Factor VIII** within the cultured tumor structures resemble endothelial structures in carcinomatous regions of the original tumor (though definitive interpretation of this stain is complicated by non-specific staining of cellular debris).



The **pan-leukocyte marker CD45** confirms that tumor-infiltrating immune cells persist in varying numbers within tumor organoid structures maintained in the *in vitro* model for up to 3 weeks.



Conclusions

- **The patient-derived *in vitro* colorectal cancer model described here maintains key cell types associated with natural tumors including tumor epithilium, stromal fibroblasts, endothelial, and immune cells.**
- **The heterogeneous cell populations organize into physiologically-relevant 3D structures within the model system.**
- **The RealBio D⁴ Culture System supports maintenance and expansion of the heterogeneous cell populations for at least 3 weeks.**
- **Next Steps:**
  - **Replication of results using additional patient-derived tumor specimens.**
  - **Further optimization for increased cell densities.**
  - **Development of chemosensitivy assay endpoints.**

Acknowledgements

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