Modeling Tumor Architecture using the RealBio D^4 ™ Culture System and its Application in the Development of Immunohistochemistry Assays

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Introduction

Standard *in vitro* models of cancer research involve the use of two-dimensional (2D) conditions. While these models allow for the quick growth of homogenous cell populations cost effectively, they are not physiologically relevant and do not model the actual complexity of cancer tumors. Regardless, this is the current method used to produce control slides for cancer screening. Disrupting cell-to-cell interactions impact protein expression which does not translate to *in vivo* tumor biology, a critical aspect when using these systems in Immunohistochemistry for cancer diagnostics. Xenograft animal models better recapitulate the *in vivo* tissue microenvironment and architecture; however, they are expensive, time-consuming and present issues inherent to the nature of the non-human host. Tissue-specific architecture and elements in the surrounding microenvironment are essential components and can be recapitulated using three-dimensional (3D) cell culture models (1,2,3). Tissue-like structures produced using these methods can be used when developing immunoassays for immunohistochemistry. The amount and quality of tissue produced can serve as a high throughput screening for pipeline products. Another advantage includes the reduction of animal subjects. The ideal *in vitro* method should produce a tissue structure that mimics a real tumor. The RealBio D^4 ™ Culture System is an advanced, disposable cell culture platform capable of developing full three-dimensional cell structures *in vitro.* Cell structures mimic the natural composition, configuration and function of normal and diseased tissue. This *in vitro* culture system has been previously used as an *in vitro* model for cancer research capable of recapitulating the human tumor microenvironment and heterogeneity.

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Aim

To develop 3D tissue-like structures from Lung Papillary Adenocarcinoma cell line NCI-H441 to produce control slides for their use in the Ventana automated stainers using the RealBio D^4 ™ Culture System.

Materials and Methods

Lung Papillary Adenocarcinoma cell line NCI-H441 was grown in T150 flasks using conventional tissue culture technique until a density of ~1 x 10^7 was attained. Single cell suspension were seeded into Real Bio D^4 chamber by slowly infusing the cell suspension into the system (Figure 1 and 2). The culture chamber was kept in a 37° C/5% CO_2. Cell culture media was pumped into the chamber at flow rate of 1 mL/1 sec. Glucose consumption, lactate production and viability were monitored weekly in the cell culture chambers for a period of 28 days. Cell culture feeding was dependent on glucose consumption. Cultures were terminated by removing all the culture media followed by Phosphate Buffered Saline (PBS) wash. *In situ* fixation of the culture scaffolds occurred by infusing 10% cold Neutral Buffered Formalin (NBF) followed by overnight incubation at 4° C. Tissue scaffold was processed for a period of 8 hours using different Ethanol concentrations followed by Xylene and Paraffin infiltration. Cut slides from tissue scaffold were stained with Ventana anti-Total c-Met (SP44) and anti-Ki-67 (30-9) antibodies using the BenchMark ULTRA and XT platforms, respectively.

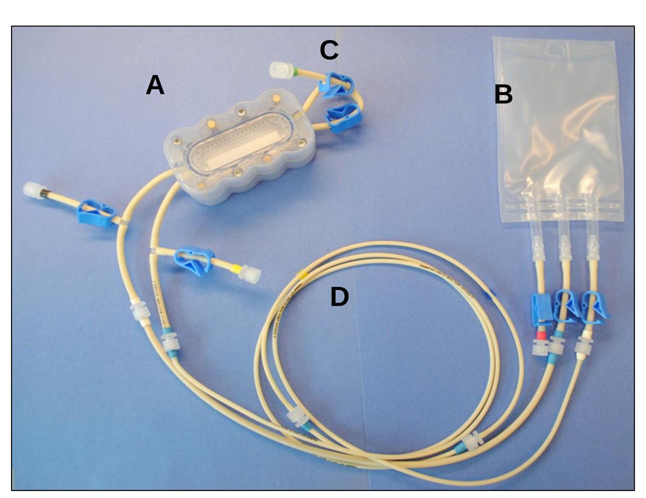

Figure 1. RealBio D^4 ™ Culture System Assembly. A= Cell culture chamber, B= Reservoir bag, C= Recirculating loop and D= System tubing

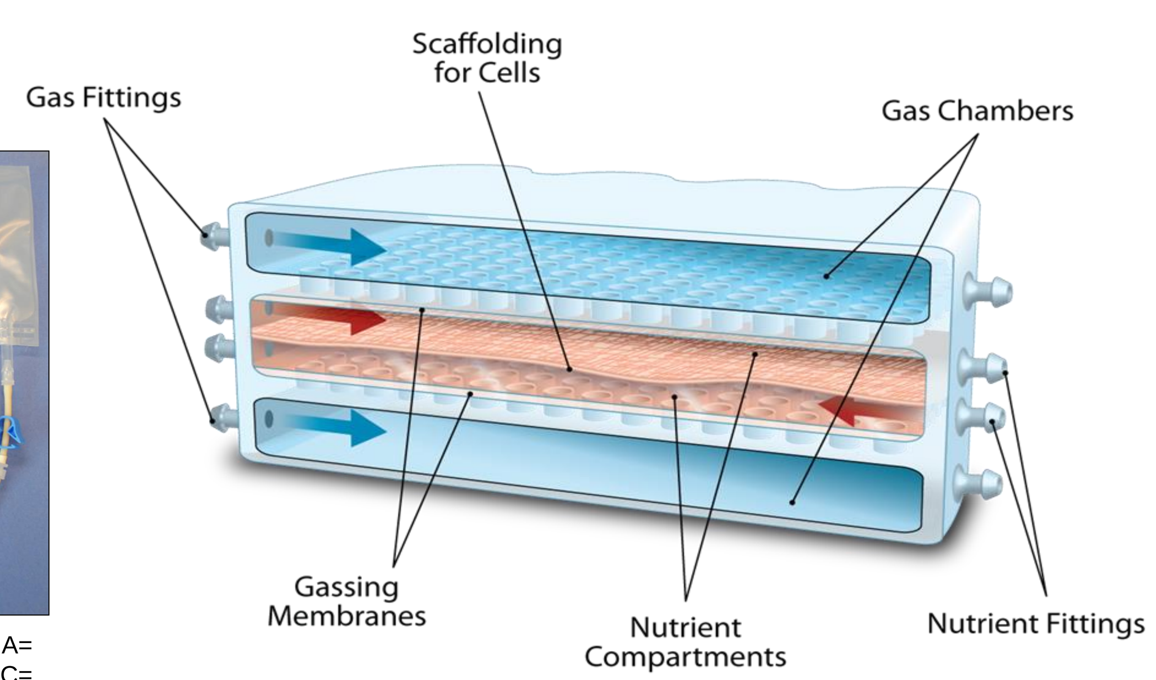

Figure 2. Graphic representation of the RealBio D^4 ™ Culture Chamber. Main components are depicted by black lines. Blue and red arrows represent gas and media flow, respectively.

Results

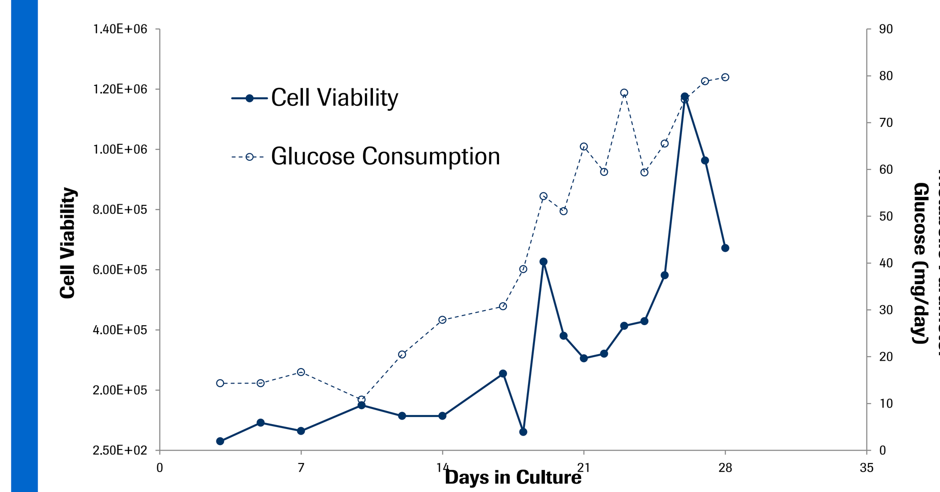

Figure 3. Metabolic and Cellular Viability Profile of NCI-H441 Culture Maintained in the RealBio D^4 ™ Culture System. Estimates of glucose consumption rates between sampling intervals were calculated from glucose concentrations measured in samples taken from the lower flow compartment of culture chambers and from the medium reservoir bags. Mean glucose consumption rate (mg/day) of the two calculation methods is shown. Courtesy of RealBio®.

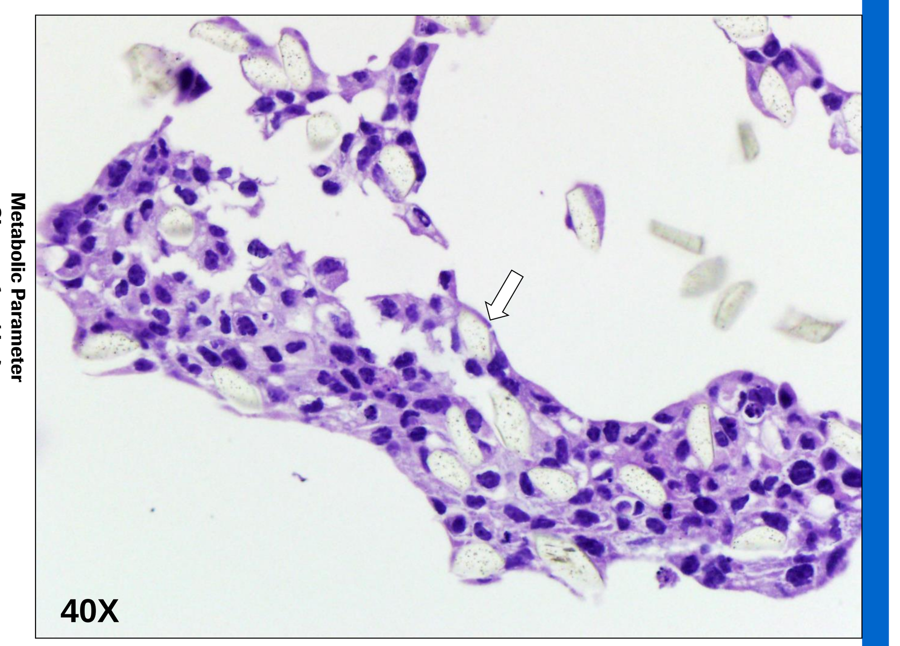

Figure 4. RealBio D^4 ™ NCI-H441 tissue scaffold section stained with Hematoxylin and Eosin (H&E, 40X). Arrow indicates scaffold.

c-MET

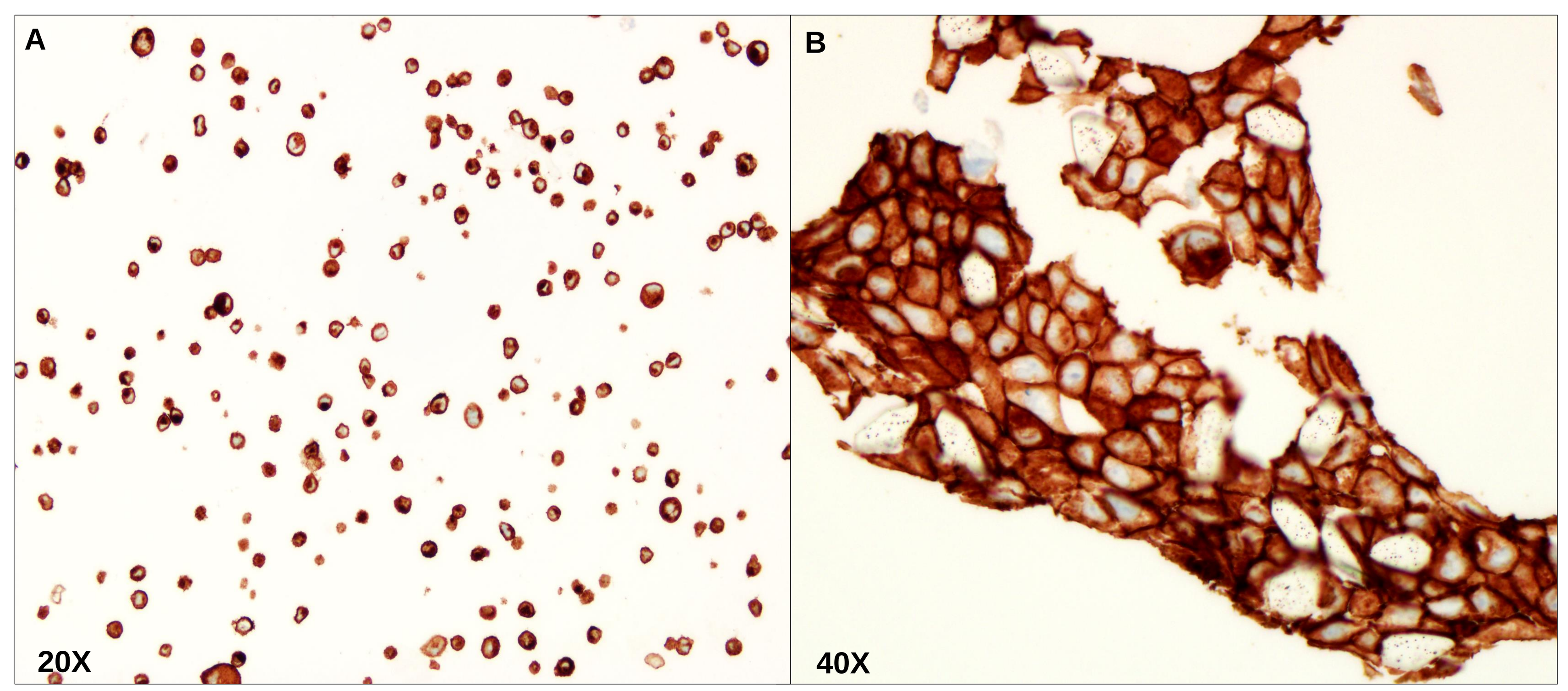

Figure 5. NCI-H441 Stained with Ventana Anti Total c-MET (SP44) Antibody. A= Agarose pellet uniblock (2D) cell line control section stained with Ventana anti c-MET antibody (20X). B= RealBio D^4 ™ tissue scaffold section stained with Ventana anti c-MET antbody (40X).

Ki-67

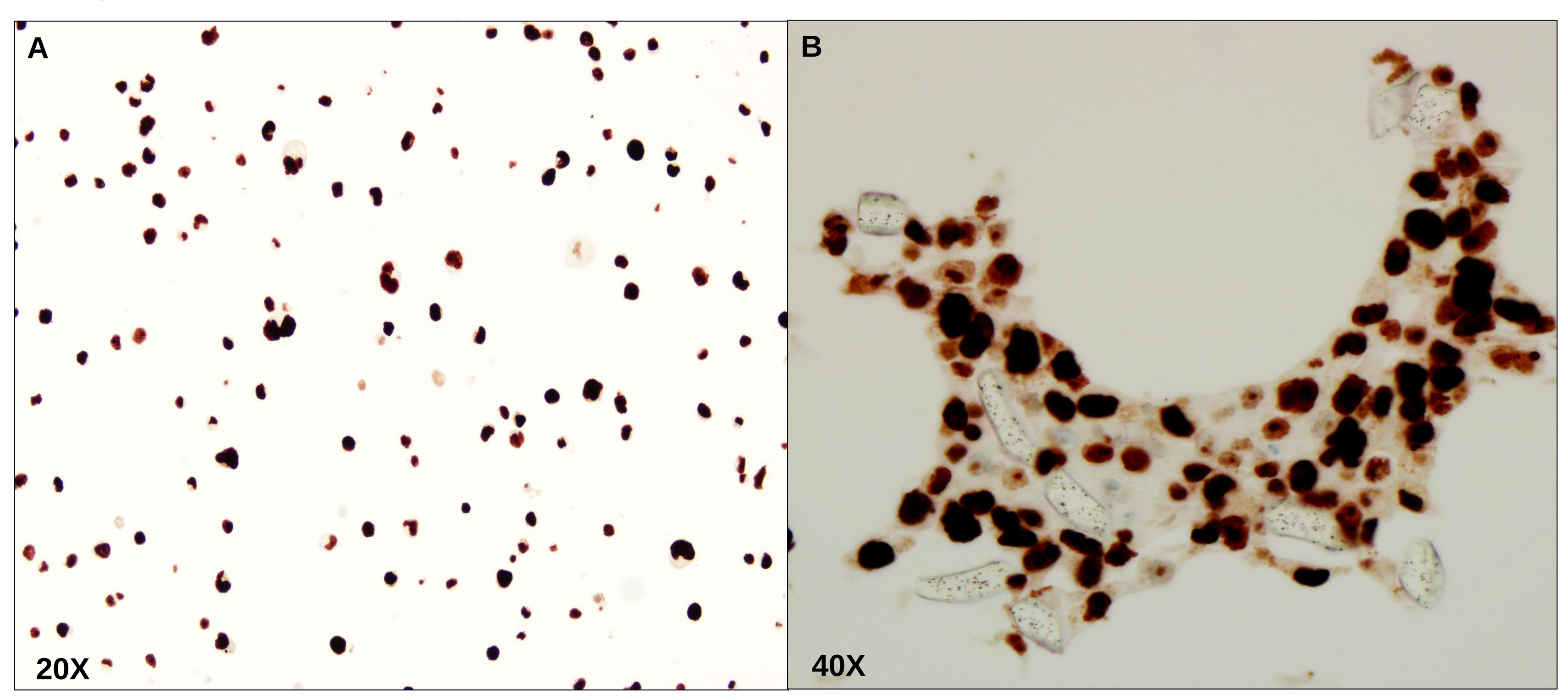

Figure 6. NCI-H441 Stained with Ventana anti-Ki-67 (30-9) Antibody (Amplified). A= Agarose pellet uniblock (2D) cell line control section stained with Ventana anti-Ki67 antibody. (20X). B= RealBio D^4 ™ tissue scaffold sectionstained with Ventana anti-Ki-67 antbody (40X). Mixed cell populations at different proliferating rates can be observed in figure B.

MET DNP Probe

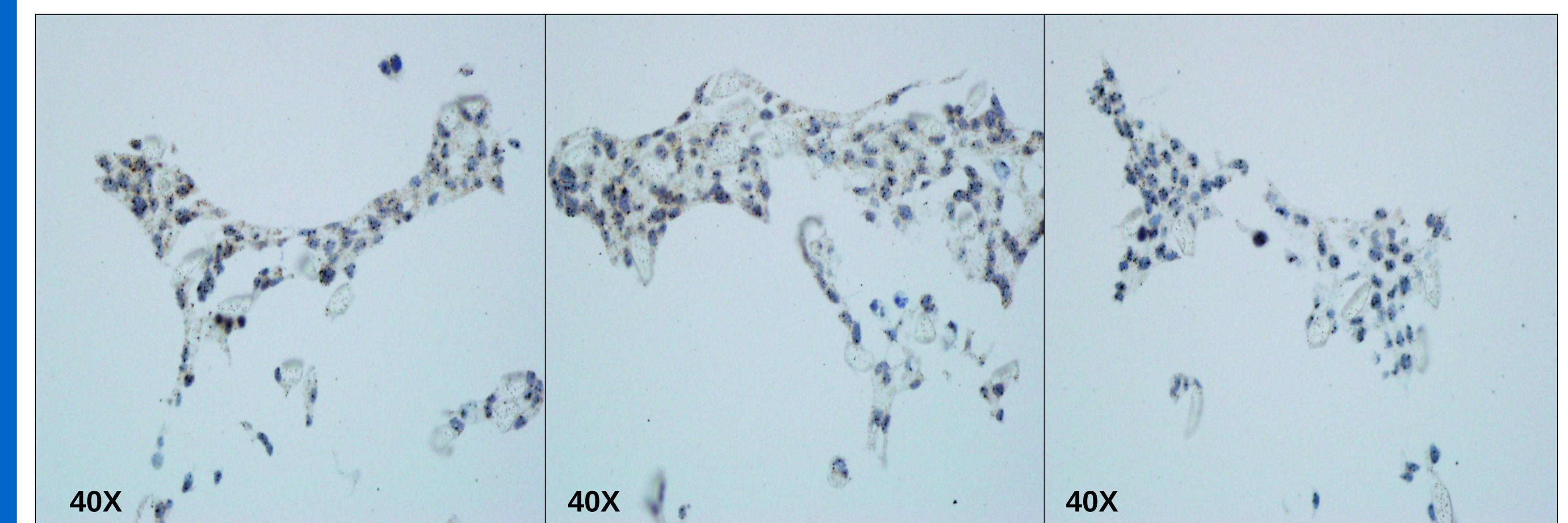

Figure 7. RealBio D^4 ™ tissue scaffold sections stained with MET DNP Probe (40X).

Summary of Results

- **The RealBio D^4 ™ Culture System mimics normal tissue architecture.**
- **Better resembles cell biology as indicated by proliferative cell stages when compared to 2D system.**
- **Superior staining quality with minimal background staining**
- **Improved or equal stability when stored at different ambient conditions as compared to 2D system.**
- **Cost effective and consistent method to produce control slides for the Ventana automated stainers.**

Conclusions and Discussion

- Human tissue is precious and often rare. Because it is obtained directly from patient's biopsies, tissue is only available in small quantities.
- Xenograft production is expensive and requires the use of animal subjects which do not comply with Roche ethics. Regulatory agencies discourage the use of animals in research.
- The RealBio D^4 ™ Culture System is biologically relevant.
- Innovative and reliable method to produce tissue-like structures.
- Tissue-specific architecture and elements in the surrounding microenvironment are essential components that can be recapitulated using the RealBio D^4 ™ Culture System.

The RealBio D^4 ™ Culture System demonstrated to be superior and more cost effective to current 2D models using to develop Immunohistochemistry assays at Ventana Medical Systems/Roche Tissue Diagnostics.

Acknowledgements

We would like to thank RealBio® for providing the Metabolic and Cellular Viability Profile of NCI-H441 Culture data from the Phase 1 trial conducted at their headquarters. Special thanks to Companion Diagnostics. Other individuals that contributed to the development of this project include: Sandor Papp, John Palting, Martin Asimis, Ben Heitz, Traci Degeer and Anna Menzl.

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