3D Cell Culture of Lung Cancer, A549, Cell Line, Models *in vivo* Xenograft Tumors

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Introduction

Lung cancer is one of the most common and deadliest types of cancer. There are 200,000 new patients diagnosed with lung cancer every year in the United States and 160,000 patients pass away each year because of the disease.. Only 5% of these tumors are able to be treated with surgery. There are no early signs or symptoms of the disease. When symptoms such as fatigue, weight loss, persistent cough, pain associated with breathing do present, 60% of the cases are diagnosed too late for surgery to be a viable option. (Crofton & Douglas,1981).

Treatments include surgery, radiation therapy and chemotherapy and are dependent on the type and stage of the tumor. The latest drug therapies for patients suffering from a specific type of non-small cell lung carcinoma show such promise that the importance of identifying these specific patients has never been greater.

The A549 adenocarcinoma cell line has been used in cell biology as a model for Alveolar epithelial type II (ATII) cells. The cell line was produced from a type II pneumocyte lung tumor in 1972 and expresses some key characteristic features of ATII cells.

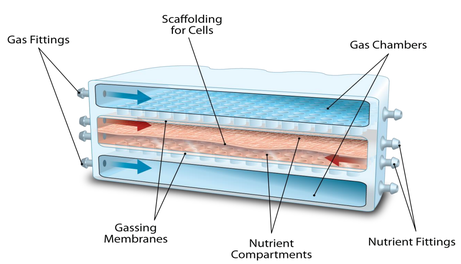
Aim

To develop 3D tissue-like structures from A549, a lung adenocarcinoma cell line, utilizing RealBio D4 ™ Culture System to produce control slides for use on Ventana automated staining platforms.

Methods

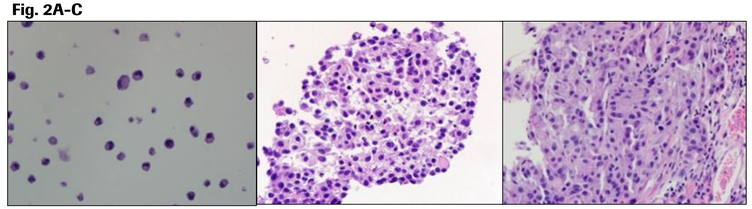
- A549 adenocarcinoma cell line, A549, were thawed and grown in T150 flasks in a 37C/ 5% CO_2 incubator.
- After reaching appropriate cell density (variable for each process), cells were either made into agarose pellets according to OP2100-280, injected into severe combined immunodeficient (SCID) mouse and harvested according to OP2100-222 , or cultured in RealBio D4 ™ Culture System
- Each tissue model (agarose pellet, xenograft tissue, or scaffold from RealBio D4 ™ Culture System) was fixed in 10% NBF, paraffin embedded, and sectioned with microtome
- Slides from each tissue model were stained H+E on the Symphony using N6C3 protocol, anti-ALK and anti-Ki-67 on the Benchmark XT, and anti c-MET on the Benchmark Ultra using standard protocols taken from individual product inserts.
- Slides were visualized on the Olympus BX51 scope and evaluated by qualified reader

Figure1 Culture chamber for RealBio D4 ™ Culture System model:
Cells were injected into nutrient fittings (Day 1), and chamber was placed in 37° C/5% CO2 incubator for ~4weeks. Cell s were fed as needed according to measured glucose levels. Scaffold is removed, fixed, and paraffin embedded for tissue sectioning



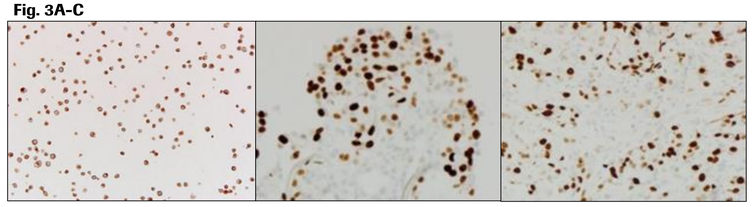
Results

Fig. 2A-C



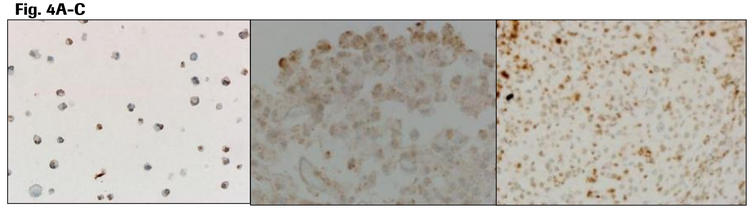
2A) A549 2D culture, 40x, H&E | 2B) A549 3D culture, 40x, H&E. | 2C) A549 xenograft, 40x, H&E.

Fig. 3A-C



3A) A549 2D culture, 20x, anti-Ki67 | 3B) A549 3D culture, 40x, anti-Ki67 | 3C) A549 xenograft, 40x, anti-Ki67

Fig. 4A-C

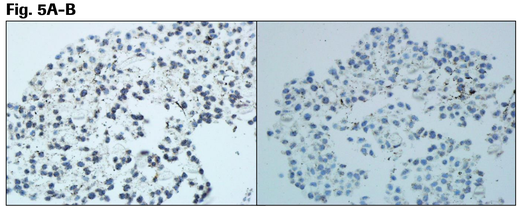


4A) A549 2D culture, 40x, anti-ALK | 4B) A549 3D culture, 40x, anti-ALK | 4C) A549 xenograft, 40x, anti-ALK

The A549 cell line cultured and grown *in vitro* in the RealBio D4 chamber exhibit many morphological and key characteristics of *in vivo* normal tissue. The Ki-67 staining shows the chamber is capable of growing and sustaining mixed cell populations and is comparable to the identically stained A549 xenograft tissue sample. The Ki-67 antibody detects a nuclear antigen present in proliferating cells. Cells not in the proliferation stage, resting, consistently test negative for the nuclear antigen. (Duchrow, 1984) When both the A549 cell line grown in the D4 chamber and the A549 xenograft tissue were stained with the antibody Ki-67 the showed similar results (Figure 3A-C). Proliferating cells and cells still in their resting state were clearly observed. The ability of the D4 chamber to enable growth of cell line cultures resembling tissue like arrangements shows its feasibility in preparing quality morphology relevant control tissue material.
The Anaplastic Lymphoma Kinase (ALK) protein in xenograft and tissue cultured in the RealBio D4 also showed superior staining quality when compared to slides generated via the 2D system (Figure 4A-C).

The RealBio D4 cell culture system produces control culture material suitable for control slide production. The slides of the cell tissue cultured was stained H&E, anti-ALK, anti-Ki-67, and anti-c-MET. A Qualified Reader assessed slides and determined the slides exhibited appropriate staining .

Fig. 5A-B



The A549 cell line cultured and grown *in vitro* in the RealBio D4 chamber stained with MET DNP Probe.

Conclusions

The tissue produced by growing a A549 cell line 3D culture the a RealBio D4 device produces a superior control slide. Once optimized:
- Use of 3D cell culture tissue will decrease costs.
- Use of 3D cell culture tissue will result in the decrease of lot to lot variation.
- Use of 3D cell culture tissue could eliminate of the use of animals and need for xenograft tissue samples.
- Use of 3D tissue has potential to advance the studies and understanding of clinical research.

Discussion

Currently, tissue from cells cultures are processed into agarose cell pellets and embedded to make quality control material. It has been shown that the 2D monolayer layer of cells is unrepresentative physiologically and morphologically of *in vivo* tissue (Lovitt, 2014).

The 3D cell culture grown in the RealBio D4 system has significant advantages over the slides currently produced using 2D cultures and xenograft tissue. The porous material of the scaffold supports the cell line growth of 3D structures. The multicellular tumor structures and spheroids may exhibit *in vivo* tumor specific characteristics, such as nutrient-to-oxygen gradients, increased cell-to-cell interactions, and different rates of cellular proliferation across the 3D structure. These 3D cultures are a superior model to simulate the micro-environmental conditions and growth of *in vivo* tumors. The RealBio D4 culture system creates an intermediate step between the standard 2D monolayer suspension culture and real life *in vivo* tumors

References

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