Relationship Between Tumor Cells and Fibroblasts by Coculture of SKOV3 Ovarian Cancer and MRC5 Fibroblasts

Jacob Wipf, Francesca Paradiso

HOUSTON Methodist LEADING MEDICINE

Gifted and Talented Mentorship Program

Background

As a defining characteristic of a tumor, a cell's microenvironment plays an important part in the way a cell grows and migrates. The **microenvironment** can impose barriers for immune reaction by recruitment of other cells primarily by fibroblasts.[1] Fibroblast ability to manufacture collagen allows surrounding tumor tissue to stiffen by a more disorganized crosslinking of collagen fibers compared to normal tissue.[2] This adaptation to the cancer is what makes the fibroblast a primary factor to cancer proliferation. Fibroblast recruitment to the tumor is an important factor to its development of cancer as well. Inactive or quiescent fibroblasts can be turned "cancer-associated fibroblasts" by unknown causes. Not only normal fibroblasts, but other mesenchymal cells are recruited.[4] These are the common aggressive behaviors that give cancer it's known dangerous connotation.

SCAFFOLD MAKING

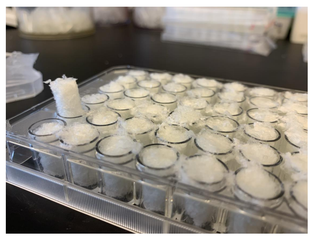
Objectives

Develop a collagen solution that can provide an optimal environment for specified cancer cells. Pack the solution in a compact and uniform manner.

Methods

(1) Weigh out collagen and mix with milliQwater.
(2) Adjust the pH of the new solution to approximately 5-6 pH using NaOH as a base to counter acids already in the collagen.
(3) Sieve the solution three times using a 500-micrometer sieve to rid it of preservatives.
(4) Stir in BDDGE to strengthen the collagen by cross-linkage and leave for 24 hours in 4 degrees Celsius containment.
(5) Fill wells with developed collagen and freeze dry.

Results



SCAFFOLD CHARACTERIZATION

Objectives

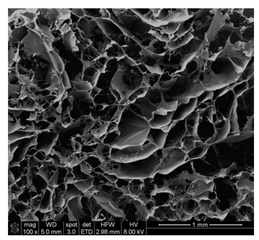
Prepare developed collagen scaffolds for scanning electron microscopy. Identify physical characteristics of models.

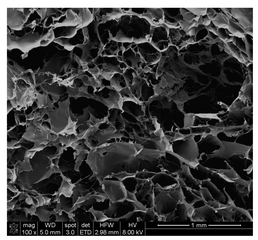
Methods

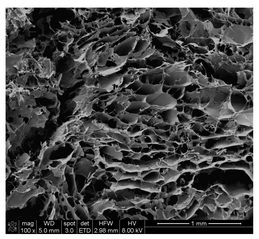
(1) Cover manufactured collagen scaffold with thin layer of platinum using vacuum sputter machine.
(2) View the scaffolds in a scanning electron microscope.

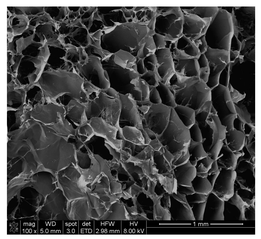
Results

SEM Images









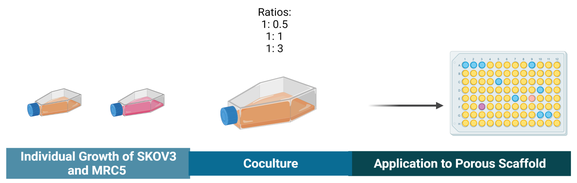
Cell culture & Coculture

Objectives

Provide space for cells to grow by periodically dividing the culture and providing more room to grow. Observe dyed cells on scaffold through immunofluorescence microscopy. Document cell growth among ratios.

Methods

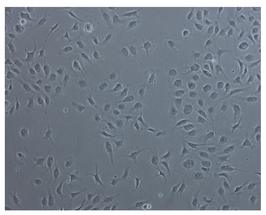
(1) Remove growth medium.
(2) Add PBSH and trypsin. View cells detachment under microscope.
(3) Insert into centrifuge.
(4) Remove liquids to isolate cell pellet.
(5) Dilute pellet with medium.
(6) Add freezing agent and freeze overnight.



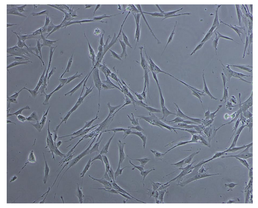
(1) Apply and dye cells to scaffolds.
(2) View under fluorescent microscope.

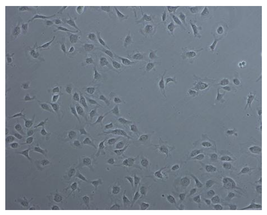
Results

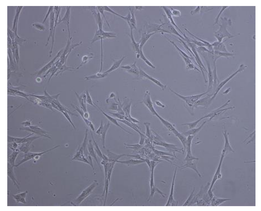
SKOV3



MRC5







Conclusions

- The 3D model used, a porous scaffold, has been an **informative tool** for examining developed **cocultures** of cancer and tumor stroma
- Coculturing cancer cells with high **ratio of stroma** could **increase** their **proliferation** and **metastatic potential**
- 3D models provide the newest technological insights in **preclinical drug evaluation**

References

[1] Jiacheng Huang, Lele Zhang, Dalong Wan, Lin Zhou, Shusen Zheng, Shengzhang Lin, and Yiting Qiao Extracellular matrix and its therapeutic potential for cancertreatment 2020
[2] Boyi Zhang, Fei Chen, Qixia Xu, Liu Han, Jiaqian Xu, Libin Gao, Xiaochen Sun, Yiwen Li, Yan Li, Min Qian, Yu Sun Revisiting ovarian cancer microenvironment: a friend or a foe? 2018
[3] Luke Bu, Hideo Baba, Naoya Yoshida, Keisuke Miyake, Tadahito Yasuda, Tomoyuki Uchihara, Patrick Tan & Takatsugu Ishimoto Biological heterogeneity and versatility of cancer-associated fibroblasts in the tumor microenvironment 2019
[4] João Rodrigues, Marcel A. Heinrich, Liliana Moreira Teixeira,and Jai Prakash 3D In Vitro Model (R)evolution: Unveiling Tumor–Stroma Interactions 2020