Cell Culture: Maintaining a Cell Line

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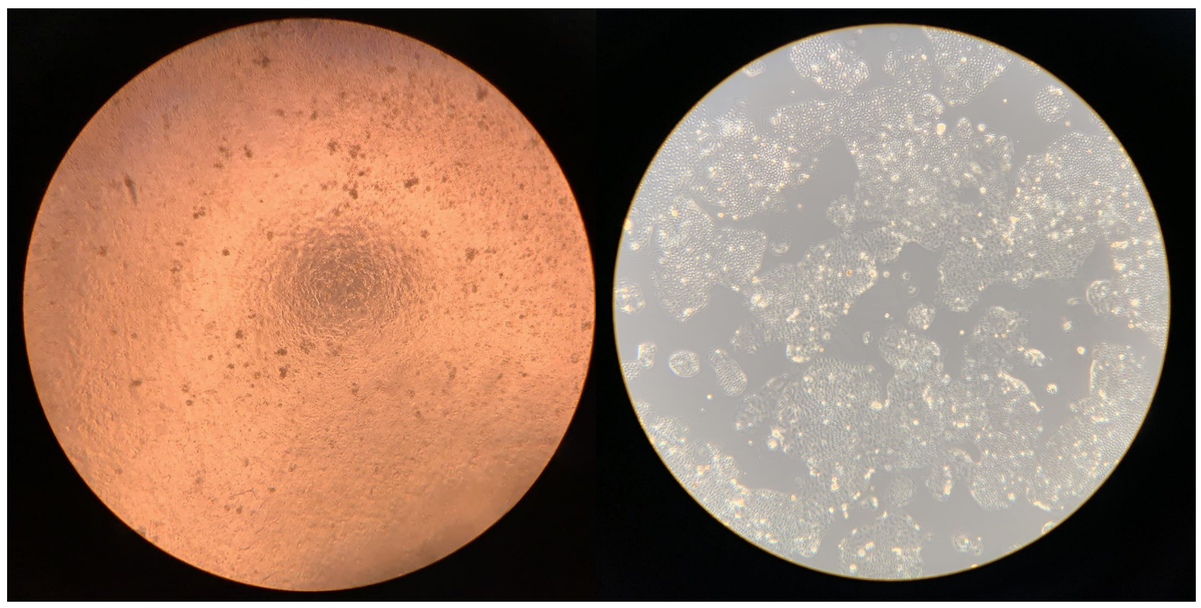
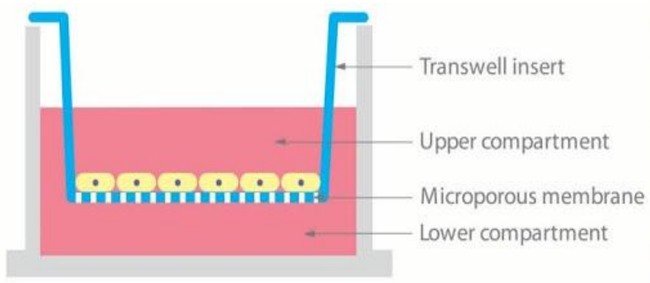
Introduction

I am a research assistant intern at Dr. Younggeon Jin's Gastrointestinal Health and Physiology Lab at the University of Maryland Animal Science Department. My responsibilities involve the processes around maintaining a cell line and assisting with current studies. Cells are grown in a controlled environment and handled with aseptic technique to prevent contamination. I am currently maintaining a Caco-2 cell line, which is in the human colon.



These pictures are of the Caco-2 cell line. The microscope image on the left is in a transwell plate. Based on this picture it is determined to have ~60% of growth. The media color is normally red. Because the color is a light pink/orange, this indicated the media's nutrients have been used and needs to be changed.

The image on the right is clear because this is in the process of being passaged into a new flask. The cells had been washed of the media, thus lacking color.



Transwell plates are shown above. The wells have cups that rest inside them and contain the cells.
To the left is a flask, this is the container cells are primarily grown in.

Aseptic Technique

Aseptic technique is an essential component to cell culturing. It involves the practices to minimize contamination. When performing any work on cells, we use a biosafety cabinet (BSC, shown below) to create a sterile environment with constant air flow. Additionally, a lab coat and gloves are worn at all times when handling flasks or plates. Ethanol is sprayed on the flask/plate and surface it is places on when it comes in/out of the incubator or BSC. Ethanol is also sprayed after the flask/plate touches any surface. Any instruments (e.g. glass pipettes) are autoclaved, opened, and kept in the BSC to maintain its sterility.

Changing Media and Sub-culturing/Passaging

The two main tasks needed to maintain a cell line is changing the media and sub culturing. Cells have requirements to grow such as having nutrients and a warm environment (incubator). Media contains the nutrient the cells use to survive and grow. Once the nutrients are all used by the cells the media is replaced with care to not disturb the cells. Sub-culturing/passaging is done when the cells have no more room to grow in the flask. It is typically done when the flask is 80-90% full. To start sub-culturing the cells, they are washed with a serum to get rid of all the media. Then the cells are detached from the flask, centrifuged down to a compact pebble, and mixed with media. After thus, a fraction is taken and transferred to a new flask to grow. This allows the cells to continuously grow.

Current Status

The lab is shut down and all studies are on pause. There is no current plan for reopening. The cell lines were centrifuged and put into liquid nitrogen.

I would like to thank Dr. Younggeon Jin for giving me this opportunity and being my mentor, and Kristi Manuel for training me.