FLUXION

TECHNICAL NOTE

Cellular Monolayers

Growing mammalian cells in the BioFlux microfluidic channels

Introduction

Many cell adhesion or host-pathogen interaction studies require a monolayer of cells as the adhesion substrate. Tissue culture cells which readily attach to plasma-treated plastic vessels often have difficulty attaching to glass surfaces. To remedy this, glass surfaces can be coated with a variety of natural and artificial growth matrixes, such as collagen, fibronectin or poly-l-lysine. While the matrix required for growth may be cell-line specific, the method of coating the Bioflux channels is extensible to other coatings.

The BioFlux system (Figure 1) is a microfluidic platform designed to run automated shear flow protocols for cell biology and microbiology experiments. This technical note covers a method to coat the glass surface of the BioFlux channels (Figure 2), seed cells and propagate cells in preparation for live-cell adhesion assays.

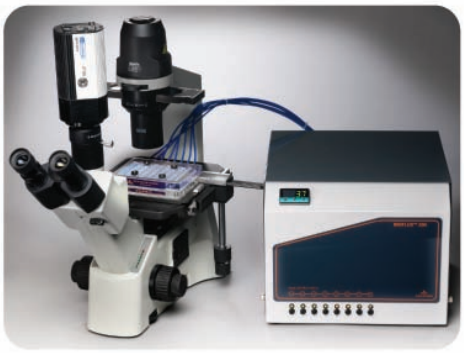

Figure 1: The BioFlux System for live cell assays under controlled shear flow.

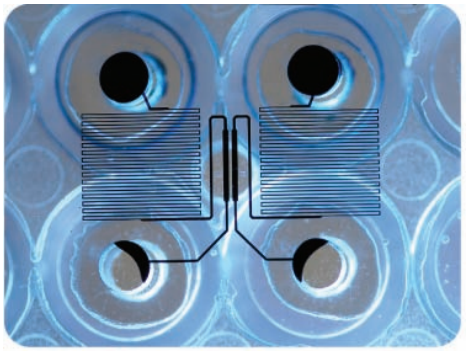

Figure 2: BioFlux Plate channels as viewed from beneath the well plate. Microfluidic flow cells are integrated into the bottom of an SBS-standard well plate. Each fluidic channel runs between pairs of wells and has a central viewing window for observation.

Methods

Materials

All materials for this procedure were sterilized and all open dish steps were performed in a biosafety cabinet under aseptic conditions:
CellTak (BD Biosciences) in 0.1M sodium bicarbonate or collagen I in 0.01M HCl
Sterile, endotoxin free water, PBS with Ca_2+/Mg_2+
Cells – 1-5 x10^6 cells per ml depending on cell type
Trypsin and appropriate growth media for your system

Coating the channels with Cell-Tak™

A solution of Cell-Tak™ (CT) (BD Biosciences, Franklin Lakes, NJ) was prepared in 0.1 M sodium bicarbonate, pH was not adjusted (10ul of CT in 290ul bicarbonate). This yielded a solution with a pH of ~7.4, which according to the manufacturer is within the pH range required for CT adsorption to surfaces. This solution was immediately dispensed into the outlet wells of the channels of interest and perfused at a rate of 30 dyne/cm^2 for 1 minute or until beads of liquid were apparent in the inlet wells and filled the innermost punch hole. The plate was left to incubate at room temperature for 1 hour. The CT solution was removed from the outlet wells to the top of the innermost punch-out. CT coated channels were washed with sterile water from the outlet wells at 10 dyne/cm^2 for 5 minutes. The water was then replaced with PBS and washed for 15 minutes at 10 dyne/cm^2.

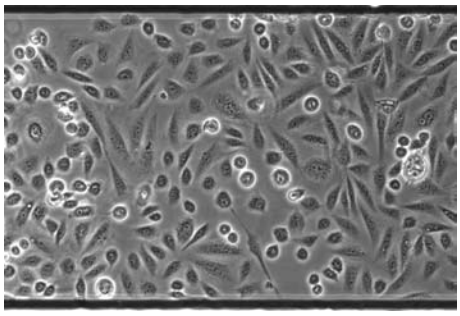

Figure 3. CHO kv 2.1 cells grown in a glass channel coated with Cell-Tak™. Phase-contrast microscopy using 10X objective.

Methods (cont.)

Coating the channels with collagen I

Collagen I solution (BD Biosciences) was diluted to 50ug/ml in 0.01M HCl. Channels were filled with collagen solution from the outlets at 10 dyne/cm^2 for 3 minutes or until beads of fluid were apparent at inlet wells. The plate was left to incubate for 1 hour at room temperature. The channels were washed from the outlet wells as above except PBS was used for both washes.

Seeding CHO cells into the channels

To seed cells into the prepared channels, the PBS was aspirated to the top of the innermost punch-out. Cells were trypsinized, resuspended in fresh warmed media, and counted prior to addition to the outlet wells. The cell density used was 1 x 10^6 cells/ml. Cells were introduced into the channels at a rate of 1 dyne/cm^2 until all the channels of interest were sufficiently populated with cells. The outlet and inlet wells were washed out with fresh warmed media and a pressure head was established on the inlet side of the channel to feed the cells by gravity flow overnight.

The plate was placed in an incubator for growth overnight. Cells were observed using a Nikon TS100 inverted microscope; images were captured using a CCD camera (QICam). (Figures 3 & 4).

For this experiment using CHO cells, the presence of a collagen or Cell-Tak™ coating significantly enhanced the cellular monolayer coverage (Figures 5 & 6).

Before starting a downstream assay such as cellular adhesion or cell staining, the cells populating the outlet and inlet wells may be removed by trypsinization and manual washing of the well and punch-out area.

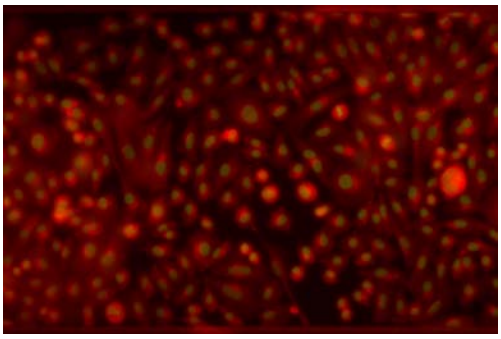

Figure 4. CHO kv 2.1 cells grown in a glass channel coated with Cell-Tak™. Fluorescence microscopy using 10X objective. Cells stained with Hoersht and Wheat Germ agglutinin.

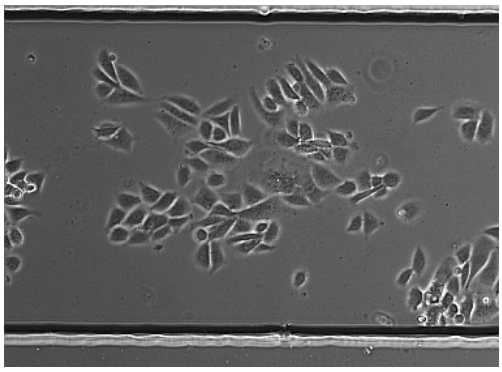

Figure 5. CHO kv 2.1 cells after 45 minutes of attachment in a glass channel coated with Cell-Tak™ (10X objective).

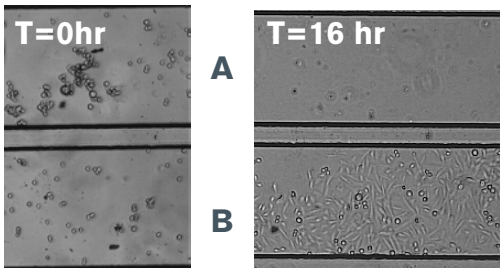

Figure 6. Growth of CHO cells without (A) and with (B) collagen I in the channel (4x objective)

Summary

In order to grow a monolayer of CHO cells in a BioFlux channel, we found that deposition of an extracellular matrix-like substrate was beneficial for cell attachment to the glass surface of the plate. While we specifically tested collagen I and Cell-Tak™, a variety of extracellular matrix proteins are commercially available and could be used to facilitate attachment of a number of cell types. It is important to note that there may only be one coating specific for your cell type.

FLUXION

384 Oyster Point Blvd., #6
South San Francisco, CA 94080

T: 650.241.4777
F: 650.873.3665
TOLL FREE: 866.266.8380

www.fluxionbio.com