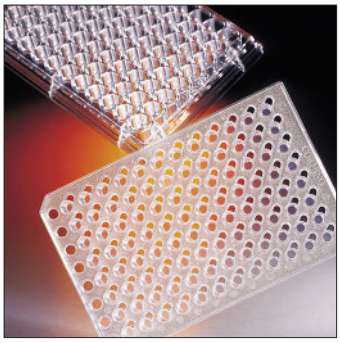
Considerations When Optimizing Coating Protocols for Corning® Transwell® Permeable Supports



SnAPPShots

A brief technical report from the Corning Applications Group

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Introduction

Extracellular matrices (ECM) proteins are necessary for the successful *in vitro* attachment and culture of many cell lines. Three of the most commonly used ECM biological coatings are collagen, laminin, and fibronectin. These coatings are often used to enhance cell attachment, but it can also influence cell proliferation, spreading, invasion, and a variety of other cellular functions. Biological coatings have the ability to impact growth and functionality of cells; based on this knowledge, we evaluated various criteria which we found to be important through previous work by Corning and others, such as coating concentration, length of coating time, and different coating solutions. These are some of the many considerations that should be evaluated when constructing a coating protocol. The following document demonstrates the effects of various biological coating procedures on the attachment and spreading of HT1080, MDCK/MDR1, Neuro-2a and NG108 cells. We realize that there are a variety of reasons for coating with ECMs. This document is to serve as a starting point for optimizing your own biological coating protocols for use with Corning Transwell permeable supports.

Materials and Methods

Cell Culture

HT1080 (ATCC® Cat. No. CCL-121), Neuro-2a (ATCC Cat. No. CCL-131), and NG108 cells (ATCC Cat. No. HB-12317) were detached using HyQTase™ (HyClone® Cat. No. SV30030.01) and MDCK/MDR1 (Dr. Piet Borst, Netherlands Cancer Institute) were detached using Trypsin (Mediatech Cat. No. 25-052-CV). Cells were re-suspended in Iscove's Dulbecco's Medium (IMDM) (Mediatech Cat. No. 10-016-CM) supplemented with 1x ITS solution (Mediatech Cat. No. 25-800-CR) for a serum-free medium (SFM), or 2% fetal bovine serum (FBS). HT1080 cells were cultured in SFM and all other cells were in 2% FBS containing medium. Cells were seeded at a density of 6 x 10^4 cells/cm^2 in Transwell permeable supports and allowed to attach for 30 to 60 minutes in a 37ºC, 5% CO_2 humidified incubator. Following incubation, the permeable supports were fixed and stained with crystal violet (Fisher Scientific® Cat. No. 23-750025) and allowed to dry before image acquisition using an Olympus® inverted microscope with a 10x objective.

Collagen Coating

Stock solutions of Type I Rat Tail Collagen (Sigma-Aldrich® Cat. No. C3867) were diluted to 2 mg/mL in either sterile water, phosphate-buffered saline (PBS) (Mediatech Cat. No. 21-031-CM), 70% ethanol, or 0.02M Acetic Acid. The solutions were stored at 4ºC for short term use (less than 1 month) or -20ºC for long term storage (greater than 1 month). For more details, please refer to the protocol *Collagen Coating Corning Transwell Inserts* (CLS-AN-122) available in the document library at www.corning.com/lifesciences. Working solutions of collagen were prepared using each of the 4 diluents by making four 1:5 serial dilutions between 10 µg/cm^2 and 0.016 µg/cm^2. Twenty-five microliters of working solution were added to each well of a 1.0 µm PET 96 well HTS Transwell (Corning Cat. No. 3392). Transwell permeable supports were also coated with collagen solutions for varying periods of time before cells were added. All Transwell permeable supports were washed once with PBS before seeding cells. Control wells consisted of cells with no collagen coating on the insert.

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Fibronectin and Laminin Coating

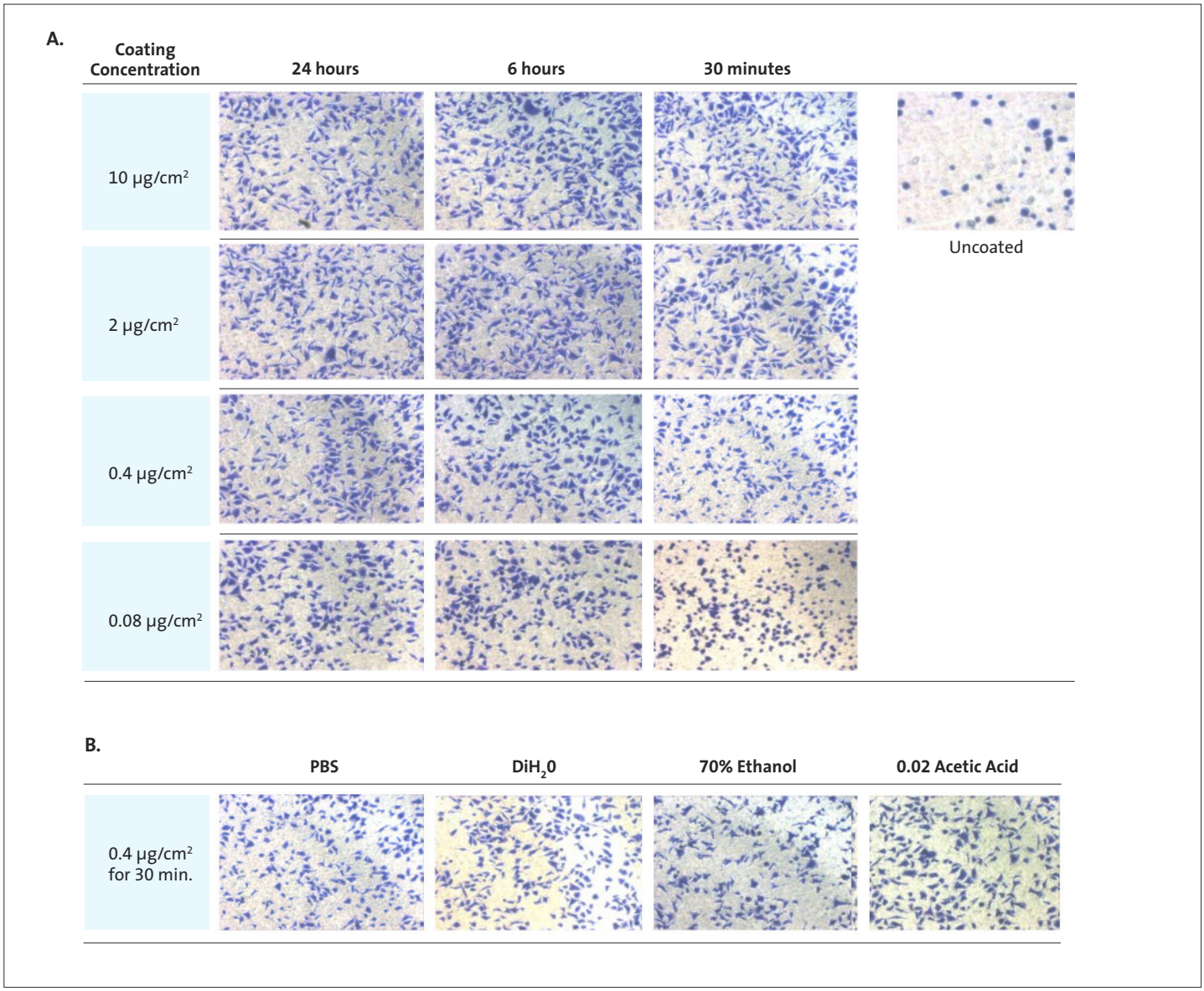
Fibronectin and laminin were purchased from Sigma-Aldrich® (Cat. No. L2020 and F1141, respectively) and aliquoted for storage as described above. For a more detailed protocol on fibronectin or laminin coating of Transwell inserts, please refer to CLS-AN-150 and CLS-AN-149 in the document library at www.corning.com/lifesciences. Multiple concentrations were used to coat the permeable supports for varying periods of time using either PBS or serum-free medium (SFM) as a diluent. As many protocols call for polymerizing laminin at 37°C, this was also examined. All inserts were washed once with PBS before seeding cells, and control wells consisting of uncoated inserts were included.

Results

The following applications were performed to address frequently asked questions about coating ECM proteins on Transwell permeable supports using three commonly used biological coatings: collagen, fibronectin, and laminin. Twenty-five microliters of working solution were added to each well of a 1.0 µm PET 96 well HTS Transwell (Corning Cat. No. 3392) and allowed to coat for varying periods of time before adding cells. Fig. 1A shows HT1080 cells on collagen cultured after 30 minutes for attachment in SFM. MDCK/MDR1 cells cultured in 2% FBS medium were also tested giving similar results (data not shown). The micrographs show that cell attachment and spreading is dependent not only on coating concentration, but also coating time. Similar results were obtained when coated at 10 µg/cm^2 for 30 minutes as compared to a 0.08 µg/cm^2 for 24 hours.

We also examined various solutions for diluting collagen (Fig. 1B). Example micrographs of HT1080 cells attaching to inserts that were coated at 0.4 µg/cm^2 for 30 minutes are shown above. No difference in cell spreading was observed at any time point or coating concentration, regardless of the diluent used (data not shown).

Figure 1. HT1080 Cells on Collagen-Coated Transwell® Permeable Supports



The micrographs below show that cell spreading was improved when SFM was used to dilute fibronectin as compared to PBS (Fig. 2). The micrographs also show that similar cell spreading can be achieved when cultured on fibronectin for 30 minutes or 24 hours, depending on the concentration of fibronectin used.

Laminin, another coating used to promote cell attachment and spreading, was also tested under the same conditions as above using Neuro-2a and NG108 cells (NG108 data not shown) (Fig. 3A). It was found that coating times of 24 hours negatively impacted cell spreading (data not shown). No difference was observed in cell spreading regardless of diluent used. Various coating concentrations and coating times were also compared using laminin that was coated at 37ºC and at room temperature (25ºC) (Fig. 3B).

Discussion

In summary, we have found the effectiveness of biological coatings to be directly related to coating concentration and coating time. Our data suggests that similar results can be achieved with either a short coating time with high concentrations or a long coating time with lower concentrations. By monitoring cell spreading of multiple cell lines diluted with various reagents, it was found that the effect on cell attachment can be dependent on the diluent used as well as the temperature at which the coating was applied. These results suggest that each diluent should be examined carefully when setting up experimental conditions. To improve expermental results when coating Transwell permeable supports, we recommend optimizing coating time, concentration and diluent used.

Figure 2. HT1080 Cells on Fibronectin-Coated Transwell® Permeable Supports

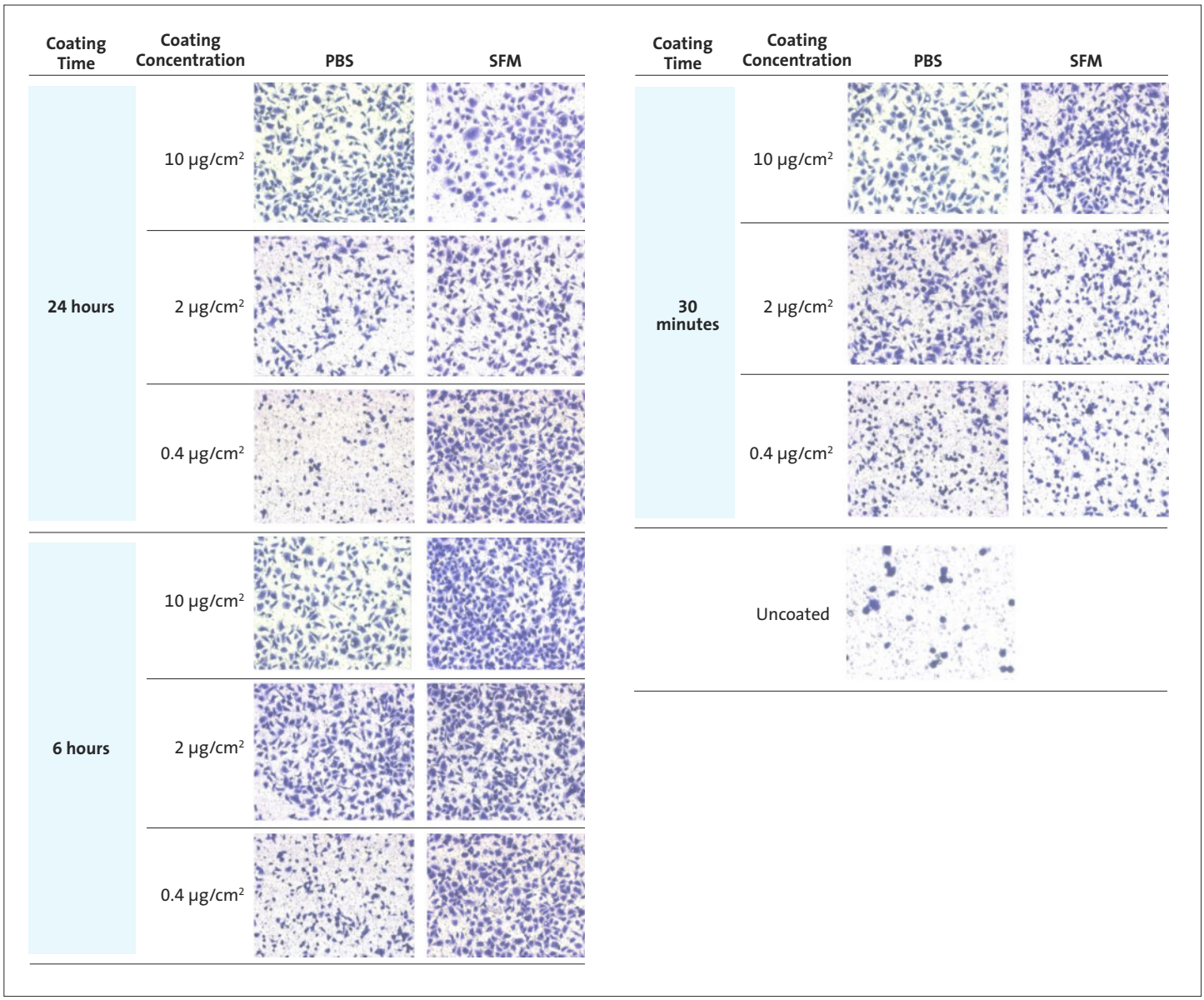
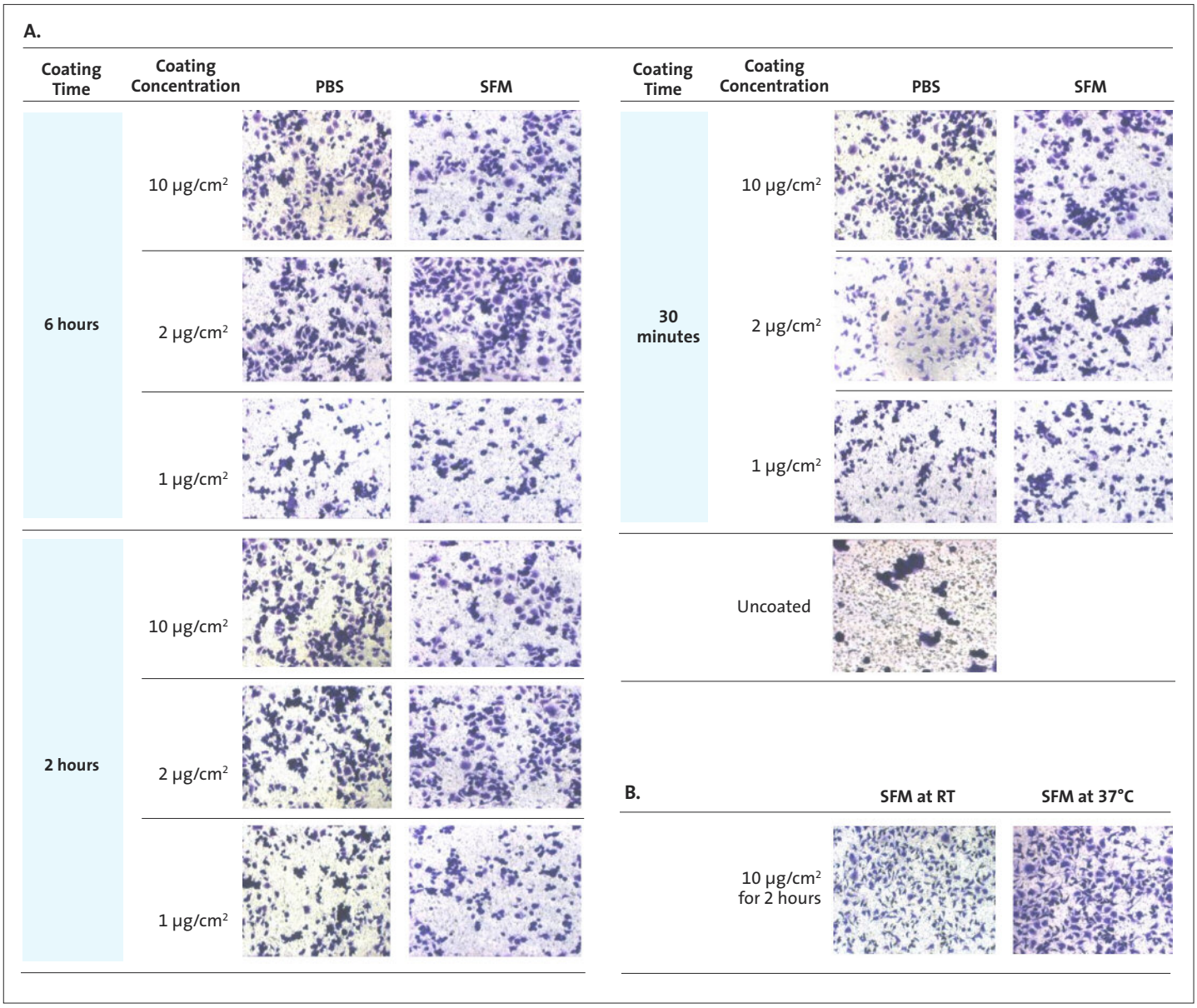


Figure 3. Neuro-2a Cells on Lamininn-Coated Transwell® Permeable Supports



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