Cell adhesion profiling for eukaryotic cell culture

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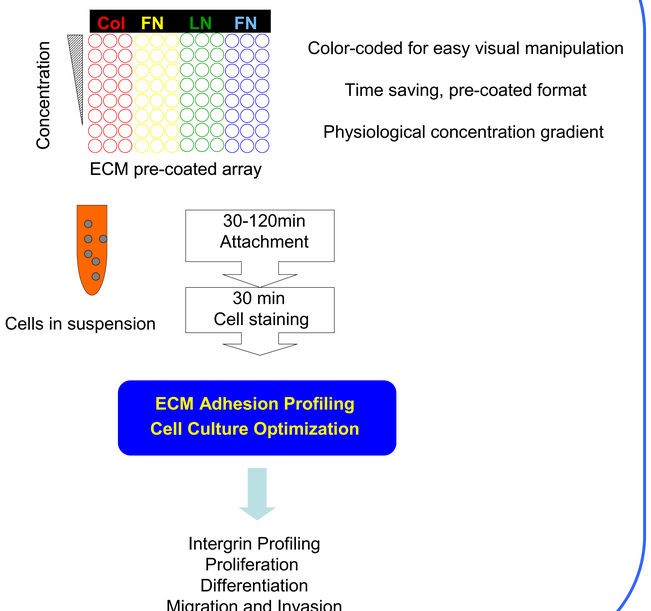
Abstract

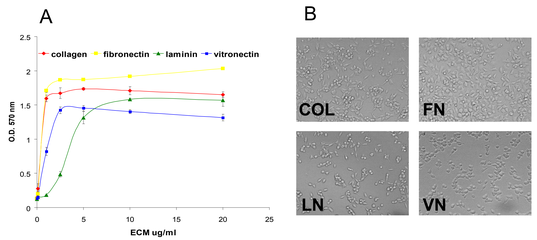
Extracellular matrix (ECM) proteins are produced intracellularly and are subsequently secreted into the surrounding cellular medium, actively regulating a diverse range of cell functions including adhesion, differentiation, proliferation, migration, invasion and survival. ECM proteins are critical for *in vitro* culture of many known cell types including neural stem cells and are key building blocks of the physiologically normal 3-D cellular environment. A primary utility of ECMs in *in vitro* cell culture is to promote cellular adhesion while maintaining cell viability and maximizing cell proliferation for downstream cell-based applications. In cases where optimal cell growth conditions are not well defined and critical adhesion protein requirements are unknown, identifying the ideal ECM protein(s) at functionally relevant concentration(s) is a time-consuming and labor-intensive process. Here, we present a multiparametric assay that allows researchers to: 1) quickly identify ECM(s) that best promote cell-type specific adhesion and 2) pinpoint the optimal ECM concentration required to promote maximum adhesion for a particular cell type. We utilize a polystyrene 96-well plate format to array the most commonly used ECM proteins such as collagen I, laminin, fibronectin and vitronectin, at ranges of functionally relevant concentrations. Applying this assay to several different cell types including human and rat neural stem cells, we show a rapid, sensitive and reliable method for determining and optimizing cell-type and cell-function specific ECM requirements. The assay is amenable to bright field, fluorometric and colorimetric detection methods for microscopy. The assay is also amenable to colorimetric and fluorescent plate readers.

Schematic of ECM Cell Adhesion Optimization Array



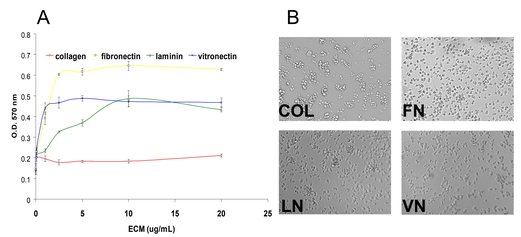
Cell Adhesion Profiling to Various ECM Proteins and Concentrations

HEK 293



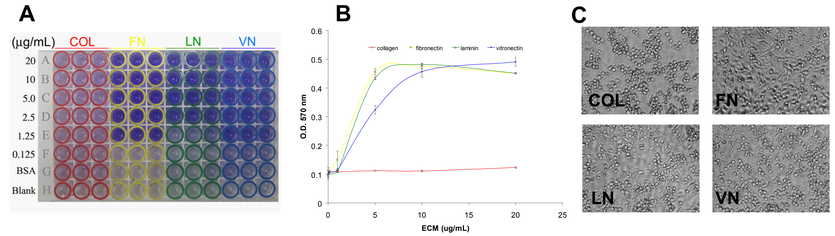
HEK293 cells (1X10^5 cells/well) seeded on the ECM cell adhesion optimization array (Millipore Cat No. ECM541) using serum free medium. Cells were incubated for 120 min at 37°C/5%CO_2. Adherent cells were detected by colorimetric assay using crystal violet. A representative graph showing the adhesion properties of HEK293 cells to various ECMs (A). Both collagen and fibronectin are preferred substrates for HEK293 with optimal concentration at 2.5 μg/mL. Cell morphology can be observed prior to staining as shown in the bright field image (B) 10x objective.

Rat neural stem cell

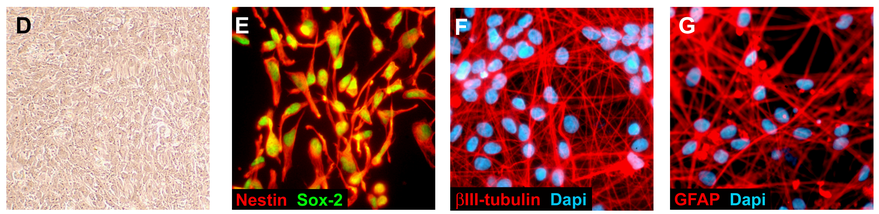


Rat neural stem (RNS) cells (0.5X10^5 cells/well) seeded on the ECM cell adhesion optimization array (Millipore Cat No. ECM541) using serum free medium. Cells were incubated for 120 min at 37°C/5%CO_2 Adherent cells were detected by colorimetric assay using crystal violet. A representative graph showing the adhesion properties of RNS cells to various ECMs (A). Fibronectin is the preferred substrate for RNS cells at 5 μg/mL. Cell morphology can be observed prior to staining as shown in the bright field image (B) 10x objective.

Application of ECM Cell Adhesion Profiling on ReNcell Human Neural Stem Cells



ReNcell ventral mesencephalon (VM) human neural progenitor cells (Millipore Cat No. SCC008) (1x10^5 cells/well) seeded on the ECM cell adhesion optimization array (Millipore Cat No. ECM541) using serum free medium. Cells were incubated for 120 min at 37°C/5%CO_2. Adherent cells were detected by colorimetric assay using crystal violet. A representative view showing the adherent properties of ReNcell VM stem cells to different ECMs on the 96-well plate (A). The optimal concentration for attachment can be identified by the graphic chart (B). For ReNcell VM cells, fibronectin and laminin are the preferred substrates with optimal concentration around 10 μg/mL. Cell morphology can be observed prior to staining as shown in bright field image (C).



ReNcell VM cells were cultured on laminin coated tissue culture plates using ReNcell NSC Maintenance Medium (Millipore Cat. No. SCM005). Cells are grown as a monolayer (D) and express neural stem cell marker Nestin (E, in red), and Sox-2 (E, in green). ReNcell VM cells are able to differentiate into neurons (βIII-tubulin maker, F) and glial cells (GFAP marker, G)

Conclusion

ECM proteins play a critical role in cell adhesion and downstream processes in cell culture. Our data suggests that the optimal ECM requirement for adhesion can be quickly determined by the ECM Cell Adhesion Optimization Array using various cell types such as HEK293, Rat NSC cells and immortalized ReNcell VM cells. The optimized ECM provides essential cell culture condition for cell growth and differentiation.

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