Effect of Laminin Substrate on Attachment and Morphology of 661W Cells in Culture

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PURPOSE

Cell culture substrates may differ with respect to charge, hydrophilic character, and specific interactions with cell surface receptors, the latter specifically when the substrate is coated with cognate attachment proteins [1]. One distinguishing feature of neurons in culture is elaboration of neurites, which have some similarity to dendrites and axons, the latter requiring extracellular matrix (ECM) components for extension, stabilization, and function [2]. 661W cells (immortalized mouse retinal cone cells) are a surrogate cell culture model for retinal photoreceptors. Although they don't develop outer segments or become morphologically polarized, they can exhibit a neuronal phenotype in response to culture conditions. Here, we examined the effects of different types of cell culture substrate treatments with regard to their effects on 661W cell attachment and neuron-like morphological development *in vitro*.

BACKGROUND

To study the role of photoreceptors in retinal function and disease, a reliable, immortalized, *in vitro* model of photoreceptors (661W cells) has been developed. *In vitro* models such as these adversely have a few limitations: lack of in vivo morphology, function, and may not truly substitute for physiological photoreceptor cells [3]. Studies involving 3D neuronal cultures have shown that using laminin as a substrate assists neuronal differentiation, extension, and dendrite formation [4]. Such extension and dendrite formation can be easily observed under light microscopy and qualitative assessment can be made on neuronal cell cultures. However, use of laminin substrate and its effect on 661W cell culture conditions has not been studied heretofore.

METHODS

661W cells (passage #30-32) were cultured in partially defined DMEM/F12 medium (containing 0.2% bovine calf serum) and plated onto 12-well culture plates (N=5)**.** Tissue culture plastic (TCP) was coated with: **1)** poly-L-ornithine (PORN) and 20% fetal bovine serum (FBS); **2)** laminin-nidogen complex [5] (65 µg/ml in modified Hank's balanced salt solution (MHBSS)); **3)** iMatrix-511 [6] (Nippi laminin-511 fragment, at 2.5 µg/ml in modified Earle's balanced salt solution (MEBSS); **4)** MEBSS alone; or **5)** no additions (TCP). 661W cells were seeded at a density of 15,000 cells/well. Images were obtained with an inverted microscope, 24 h post-incubation. Each cell in a given field was evaluated with respect to five cell body morphology categories: triangular, polygonal, elongated/bipolar, ellipsoid/miscellaneous, or rounded. To quantify extension of neuronal processes, the average number of neurites per field was determined. Length of the five longest neurites/field was measured and compared as a function of substrate condition. Statistical analysis of the data was performed (Student's *t*-test, significance threshold p<0.05).

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RESULTS

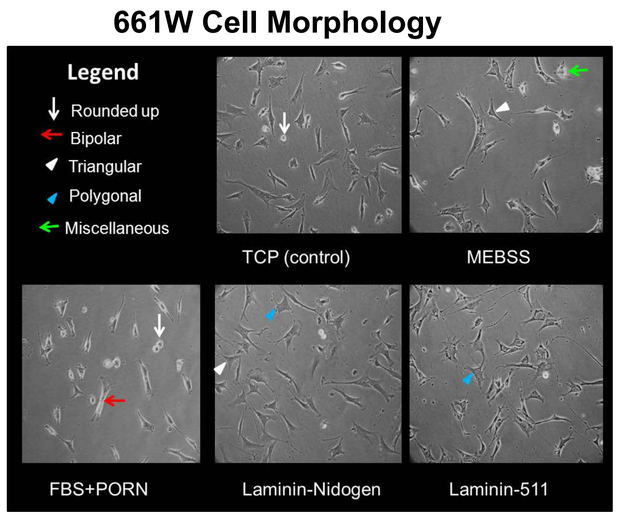

Fig. 1. Classification of 661W cell morphology under various substrate conditions. 661W morphology was determined based on the categories described in upper left panel (*Legend*). All images were taken 24h post-plating using an inverted photomicroscope with a 20x objective. [See *Methods* for definition of abbreviations.]

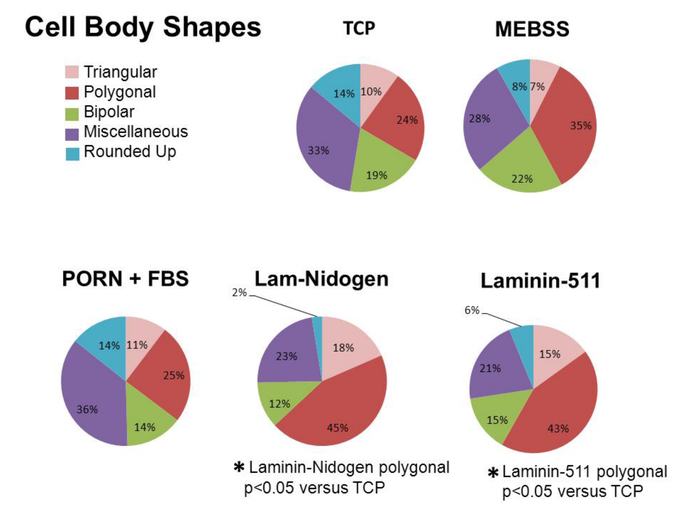

Fig. 2. Analysis of 661W Cell Body Shapes. The number of triangular, polygonal, bipolar, miscellaneous, and rounded up cells were manually counted (presented as percentage of total). Triangular and polygonal shapes are characteristic of well-spread cells, suggesting better attachment. Cells grown on laminin-nidogen and laminin-511 had a significantly larger percentage of polygonal cells compared to TCP alone

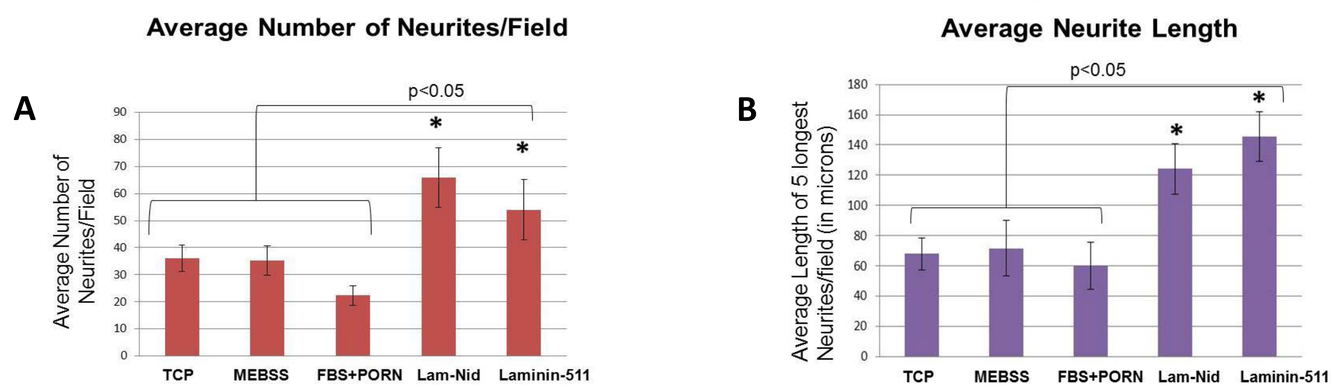

Fig. 3. Quantification of neurite number and length under various substrate conditions. A) The number of neurites per field was counted manually and the average for each substrate condition was calculated (n=5). Values obtained for MEBSS and FBS+PORN conditions were not significantly different, compared to TCP control values. However, laminin-nidogen and laminin-511 coatings significantly increased the number of neurites relative to TCP, MEBSS, and FBS+PORN (*p<0.05, Student's *t*-test). **B)** The five longest neurites per field were measured and the average length for was calculated (n=5). Values obtained for MEBSS and FBS+PORN conditions were not significantly diferent, compared to TCP control. Laminin-nidogen and laminin-511 coatings significantly increased the average neurite length relative to TCP, MEBSS, and FBS+PORN (*p<0.05, Student's *t*-test).

CONCLUSIONS

- Surface treatments containing **laminin** functionalities promote a **more neuronal morphological phenotype** in 661W cells, compared to more conventional cell culture attachment substrates
- Novel application of the defined substrates **laminin-nidogen** complex and **laminin-511 fragment** indicates that they are useful alternatives for multiwell plate assays of neuronal cell lines

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