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Product Application Bulletin

MAPTrix®-L

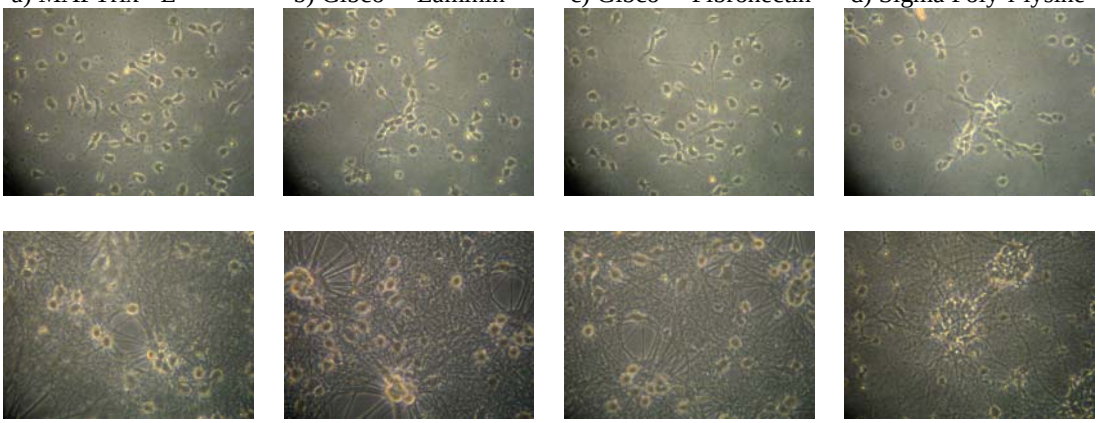
(laminin peptide motif containing mussel adhesive protein)

Abstract:

Adhesion of cells on surface is a basic and important concept in the fields of neural cell culture and morphogenesis of the nervous system. Conditions for culture of neural cells have yet to be optimized, and poly-l-lysine and laminin have been frequently used as a substrate of choice. Morphology of primary rat hippocampal cells grown on mouse laminin, human fibronectin, or poly-l-lysine was studied in a 24 well using Phase Contrast Interference Microscopy. Immunohistochemistry was used to investigate the fluorescence levels of labeled neurons in a culture, adhesion to an ECM coating, presence of actin containing processes as well as dendrites and axons, and expression levels of the glutamate receptor. Morphology, focal adhesion (FA) and focal adhesion kinase (FAK) protein levels were comparable on laminin, human fibronectin, and MAPTrix®-L coated well, but low expression level was observed from cells on poly-l-lysine. Glutamate receptor function also varied in cells cultured on differently coated substrates, correlating with immunological quantification of the glutamate receptor (GluR1). Neural cells grown on MAPTrix®-L, laminin, and fibronectin displayed improved cell morphology and receptor function as compared with poly-l-lysine. These studies indicate that MAPTrix®-L ECM has a similar bioactivity to natural ECM component to consider in obtaining optimal neural cell cultures.

Figure 1. ECM and poly-l-lysine affect the morphology of neural cells

a) MAPTrix®-L b) Gibco™ Laminin c) Gibco™ Fibronectin d) Sigma Poly-l-lysine



Morphology of rat hippocampal cells grown on MAPTrix®-L, mouse laminin, human fibronectin, and poly-l-lysine was studied in a 24-well format using Phase Contrast Interference Microscopy in 2 and 14 days, respectively. Differences in morphology between cultures grown on the various substrates were observed. Pyramidal neurons exhibited long interconnecting processes with growth cones on ECM and MAPTrix® while highly branched with relatively long processes neurons were observed on poly-l-lysine.

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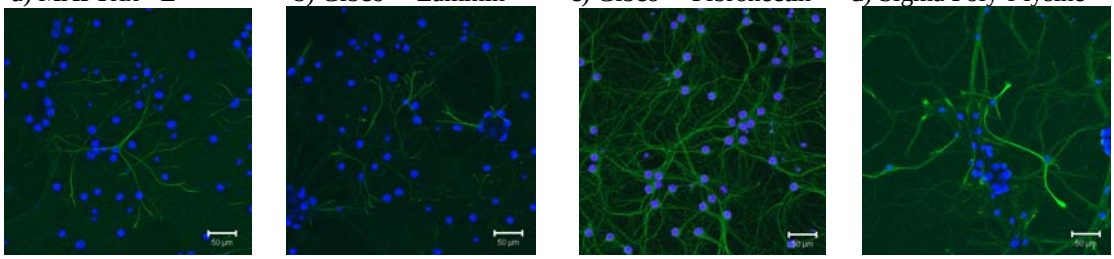
Figure 2. Microtubule-Associated Protein 2 (MAP2) Immunostaining

a) MAPTrix®-L b) Gibco™ Laminin c) Gibco™ Fibronectin d) Sigma Poly-l-lysine

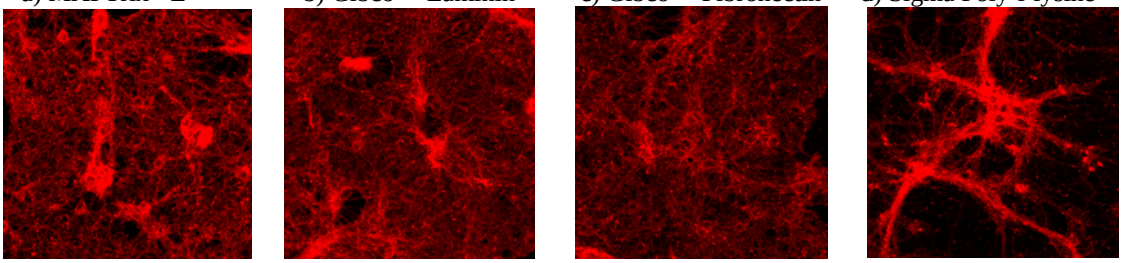


Confocal images showed MAP2 along dendrites and in neuronal cell bodies. MAP2 immunostaining indicated ECMs affects pyramidal dendrite formation of the primary nerve cells. Anti-MAP2 was higher on MAPTrix®-L, laminin, and fibronectin coatings and all of them were comparable, but was significantly less MAP2 fluorescence on poly-l-lysine coatings.

Figure 3. FA and FAK expression in hippocampal neurons cultured on different substrates

3.1 F-actin linked cytoskeleton

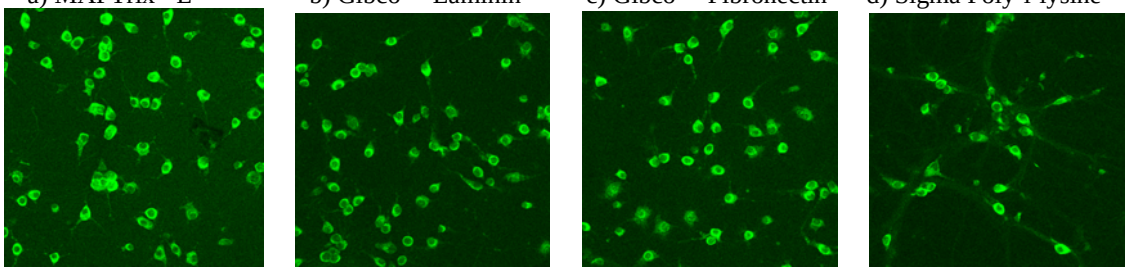
a) MAPTrix®-L b) Gibco™ Laminin c) Gibco™ Fibronectin d) Sigma Poly-l-lysine



F-actin-linked cytoskeleton was particularly enriched on MAPTrix®-L, laminin, and human fibronectin while decreased actin filament polymerization was observed on poly-l-lysine.

3.2 FAK expression level of hippocampal neurons cultured on different substrates

a) MAPTrix®-L b) Gibco™ Laminin c) Gibco™ Fibronectin d) Sigma Poly-l-lysine



Anti-Focal Adhesion Kinase antibodies localized adhesion plaques in 24-well plates coated with different ECMs. Fluorimetry revealed that coatings containing MAPTrix®-L, laminin, fibronectin had greater fluorescence due to FAK while poly-l-lysine was less adhesive.