

# Determining the Stiffness Threshold of a PEG Hydrogel for Motor Neuron Axon Extension

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Motor neuron degenerative diseases such as amyotrophic lateral sclerosis (ALS) currently have no cures and mostly unknown causes. For motor neurons to function properly, axon extension is necessary. The ability of motor neurons to extend axons is strongly influenced by their microenvironment, which is the extracellular matrix (ECM), and stiffness is one of the most important characteristics of the ECM. The purpose of this research was to (i) explore the association between the stiffness of the matrix microenvironment of motor neurons and their axon extension; and (ii) determine whether there exists a stiffness threshold at which motor neurons can no longer extend axons. Mouse embryonic stem cells were differentiated into motor neurons and encapsulated in photoinitiated thiol-ene poly(ethylene glycol) (PEG) hydrogels formed by crosslinking 4-arm PEG norbornene with matrix metalloproteinase degradable peptides. These hydrogels provide a three-dimensional in vitro environment mimicking the structure of the ECM. Stiffness was manipulated by varying the PEG weight percentages of the gels. Axon extension was quantified with average growth rate and axon density for each of the different stiffness levels. The research results showed a significant negative relationship between stiffness and overall axon extension. Furthermore, it was determined that a stiffness threshold exists and that it is between 3500 and 6500 Pa. The findings demonstrate that matrix stiffening can inhibit axon extension and thus hinder the growth of newly introduced motor neurons after injury or disease. This research provides insight on the design of transplantable matrices capable of directing axonal outgrowth, which could lead to a possible future cure for motor neuron diseases.