# Mechanical stimuli activation of calpain is required for myoblast differentiation and occurs via an ERK/MAP kinase signaling pathway.

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# Mechanical stimuli activation of calpain is required for myoblast differentiation and occurs via an ERK/MAP kinase signaling pathway.

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**Key Words:** muscle development, calpains, signal transduction, mechanical stimulation

## Abstract

Myogenesis is a complex sequence of events, including the irreversible transition from the proliferation-competent myoblast stage into fused, multinucleated myotubes. During embryonic development, myogenic differentiation is regulated by positive and negative signals from surrounding tissues. Stimulation due to stretch- or load-induced signaling is now beginning to be understood as a factor which affects various signal transduction pathways, gene sequences and protein synthesis. Evidence of the involvement of mitogen-activated protein kinase (MAPK) cascade activation in myoblast fusion, cell membrane and cytoskeleton component reorganization due to the activity of ubiquitous proteolytic enzymes known as calpains has been reported. Whether there is a link between stretch- or load induced signaling and calpain expression and activation is not known.

## Introduction

Using a magnetic bead stimulation assay and C2C12 mouse myoblasts cell population, we have shown that mechanical signals transmitted through the C2C12 cells interaction with laminin cause an increase in cellular differentiation. This signaling results in an increase in the number of myotubes formed in the cultures, with each individual myotube containing fewer nuclei. Mechanical stimulation increases not only the expression of m-calpain but also the overall activity of calpain in the cells through the MAPK signaling cascade.

## Materials and Methods

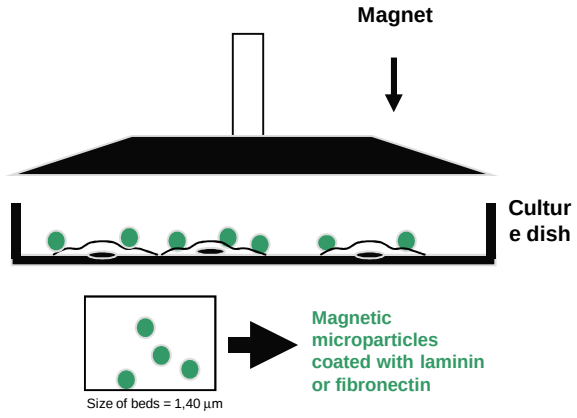


**Fig.1** A magnetic field of 0.5 mT was generated by an electromagnet (Power Generator 0-30 Volts, 0.1-100 Hz; Elcanic A/S, Denmark). The magnet produced alternating MF at frequency of 1 Hz. Magnet was placed 10 mm over the monolayer of cells during the stimulation period. Cells lacking beads but placed under the magnetic field were used as an additional control.

## Results

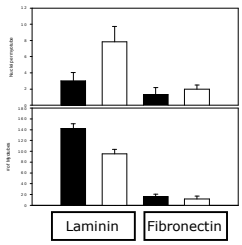


**Figure 2**. Frequency and size of myotubes formed by C2C12 cell populations in the presence and absence of mechanical stimulation. The number of nuclei per myotube (a) and the total number of myotubes (b) in cultures of cells stimulated with micro spheres (black) as compared to un-stimulated controls. Stimulation with laminin coated microspheres causes an increase in the number of myotubes formed, but the resultant myotubes are significantly smaller. Few if any myotubes are seen when cultures are stimulated with fibronectin coated micro spheres. Error bars - ± SE.

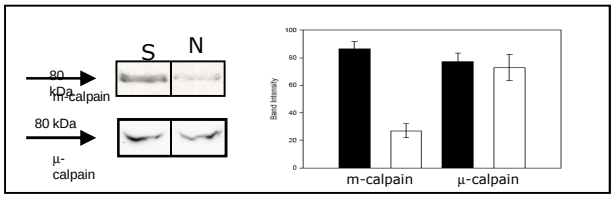


**Figure 3.** Protein extracts from control C2C12 cells (N) and stimulated C2C12 cells (S) were resolved in 8% polyacrylamide gel, transferred onto membrane and detected using antibodies against m- and µ-calpain. Mechanical stimulation increases m-calpain but not µ-calpain expression

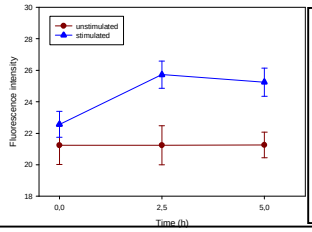


**Figure 4.** Quantisation of calpain activity using a fluorescent substrate probe. The control cells did not show an increase in calpain activity over the course of the experiment, while mechanically stimulated cells showed a marked increase in calpain activity by 2.5 h after which time no further increases were observed.

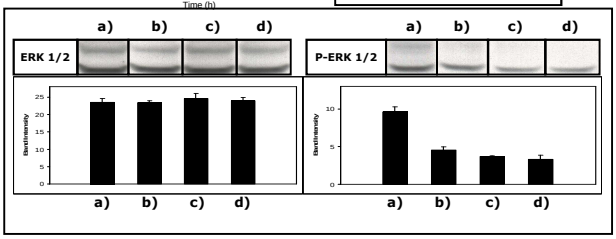


**Figure 5.** Mechanical stimulation with laminin coated beads shows a significant increase in the level of phosphorilation of the ERK 1/2 pathway compared to the other stimulations. **a)** mechanical stimulation laminin **b)** chemical stimulation laminin **c)** mechanical stimulation fibronectin **d)** chemical stimulation fibronectin.

## Conclusions

**We have shown that mechanical signals transmitted through the C2C12 cells interaction with laminin cause an increase in cellular differentiation.**

**This signaling results in an increase in the number of myotubes formed in the cultures, with each individual myotube containing fewer nuclei.**

**Mechanical stimulation increases not only the expression of m-calpain but also the overall activity of calpain in the cells.**

**Mechanical stimulation through the laminin receptor induces signaling through the MAP-K signaling cascade.**