Initial studies of mechanical compression on neurogenesis with neonatal neural stem cells

Leyla Esfandiari, MS[a], Michelle Paff, MS[b], William C. Tang, PhD[c,d,*]

[a]Biomedical Engineering Interdepartmental Program, University of California, Los Angeles, California, USA
[b]Department of Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, California, USA
[c]Department of Biomedical Engineering, University of California, Irvine, California, USA
[d]Department of Electrical Engineering and Computer Science, University of California, Irvine, California, USA

Abstract

In this article we demonstrate the effect of mechanical compression on the behavior of cultured neural stem cells using a microelectromechanical system platform. Polydimethylsiloxane (PDMS)-based stretchable substrates were used on a neurosphere (NS) assay to investigate the role of mechanical forces on the formation of radial glial processes and neuronal migration. To induce mechanical compression on NS, the PDMS culturing substrate was patterned with micron-sized wells. NS were cultured on the prestretched device. After 48 hours, when the NS had grown to the size of the well's width, the stretched substrate was released. The experimental results showed that applied mechanical compression on neural stem cells could be a factor accelerating the radial glial formation, which is associated with neurogenesis and neuronal migration.

From the Clinical Editor: This study demonstrates that mechanical compression on neural stem cells could be a factor accelerating the radial glial formation, which is associated with neurogenesis and neuronal migration.
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Abnormal cortical development during embryogenesis can lead to severe neurological disorders. Investigating the mechanisms of brain development may lead to new avenues for developing more effective therapies to treat brain disorders due to abnormal cortical development.[1,2] Current research has focused primarily on the biochemical and genetic cues that trigger neurogenesis signaling cascades during brain development, whereas fewer studies have investigated the influence of mechanical forces, which may also play an important role in neurogenesis.[3] Several studies have targeted the effect of mechanotransduction on neuronal differentiation,[4,5] but the

effects of mechanical force as a trigger of complex signaling cascades on neurogenesis and neural migration during brain development has not been investigated. The aim of the work is to implement microelectromechanical system technology to study the effects of mechanical forces on neurogenesis and neural migration during brain development. Our ultimate goal is to establish a set of automated microtools for elucidating the role of microbiomechanical cues in neurogenesis.

Methods

Animals

Animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, Irvine. The mice were purchased from Jackson Labs (Bar Harbor, Maine) and Charles River (Wilmington, Massachusetts). All mice were housed in the animal facility, and animal experiments that were conducted followed the guidelines by the IACUC. Animals were kept in the facility with free access to food and water.

*Corresponding author: Henry Samueli School of Engineering, 5200 Engineering Hall, University of California, Irvine, California 92697-2700, USA.
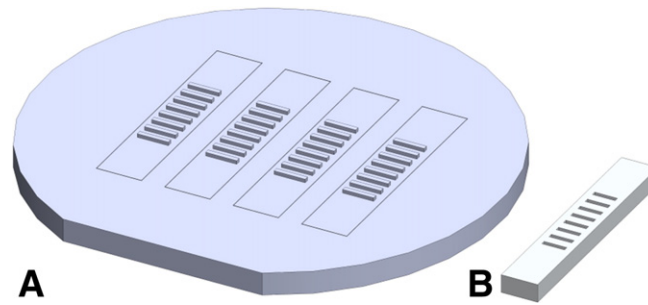*E-mail address:* wctang@uci.edu (W.C. Tang).

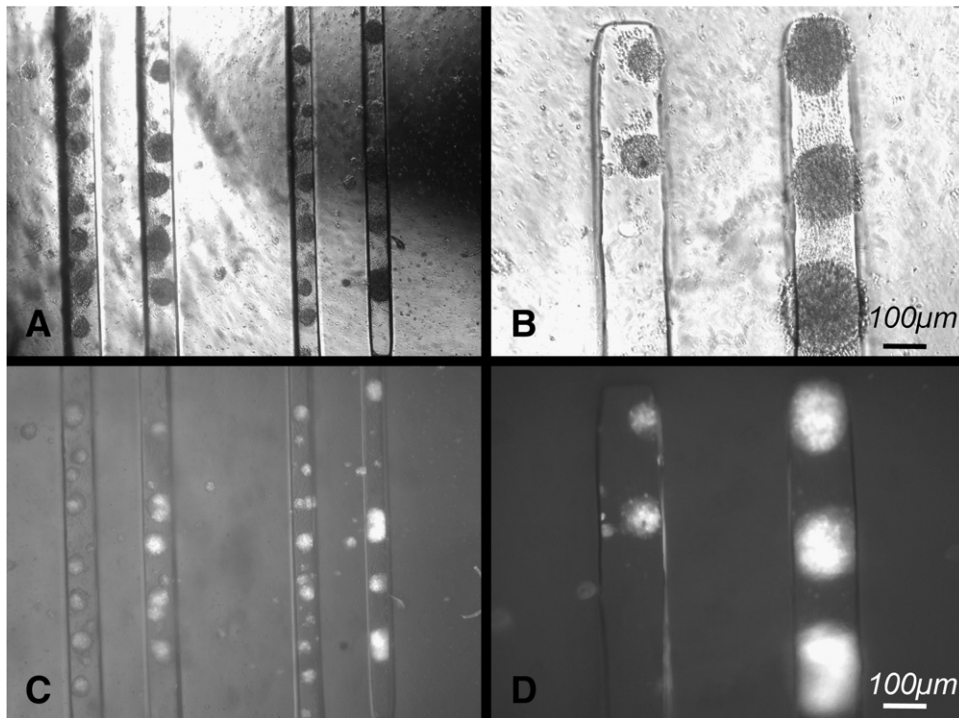Figure 1. **(A)** Drawing of SU-8 mold on silicon. **(B)** Resulting PDMS substrate with embedded wells.

Figure 2. Neurospheres inside the prestretched PDMS wells. **(A**, **B)** Bright-field images with different magnifications. **(C**, **D)** Fluorescent images of **A** and **B**, respectively.

Stretchable substrate

Flexible polydimethylsiloxane (PDMS) substrate with embedded wells was fabricated to apply mechanical compression. Each well was 5 mm long, 60 µm wide, and 400 µm deep. To form the wells on the PDMS substrate, a photolithographic technique was used to pattern SU-8 photosensitive resist on a silicon substrate to form the mold.[6] The PDMS precursor was cast over the mold and cured overnight. After demolding, the surface topology with an imprint of the mold was formed on the PDMS substrate (Figure 1).

Experiment

Neural stem cells were collected from the cerebral cortices of E12.5 embryos of timed pregnant τ-GFP transgenic mice. The τ-GFP transgenic mouse has green fluorescent protein (GFP)

in the exon of the τ gene. Since the τ protein is expressed only in neurons, visual identification of live migratory neurons is possible in real time without the use of immunocytochemistry.

It has been shown that radial glial cells can develop between adjacent neurospheres (NS) and can support neuronal migration.[7] The three-dimensional structure of NS may be more physiologically relevant than two-dimensional culture systems and allow application of mechanical compression to the cells.[8] We have previously reported 7-day viability of NS on poly-L-ornithine–coated PDMS,[9] which was also used as the substrate in this work. τ-GFP$^+$ E12.5 mouse embryos were identified with fluorescent microscopy. The cerebral cortices were carefully dissected, and the cells were dissociated with gentle pipetting and then plated at 200,000 cells/mL into 10-cm culture dishes with culture medium containing Dulbecco's Modified Eagle Medium–Nutrient Mixture F-12 (Life Technologies, New York) including B27
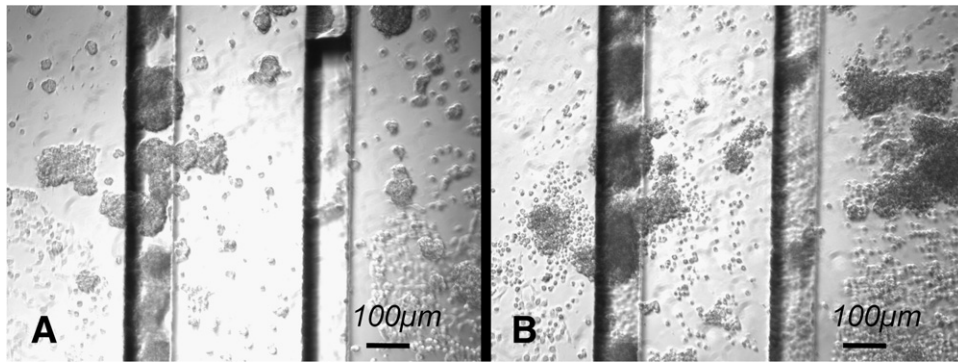
Figure 3. Control experiment without mechanical compression. **(A**, **B)** Neurospheres in prestretched PDMS wells **(A)** after 48 hours and **(B)** after 72 hours.
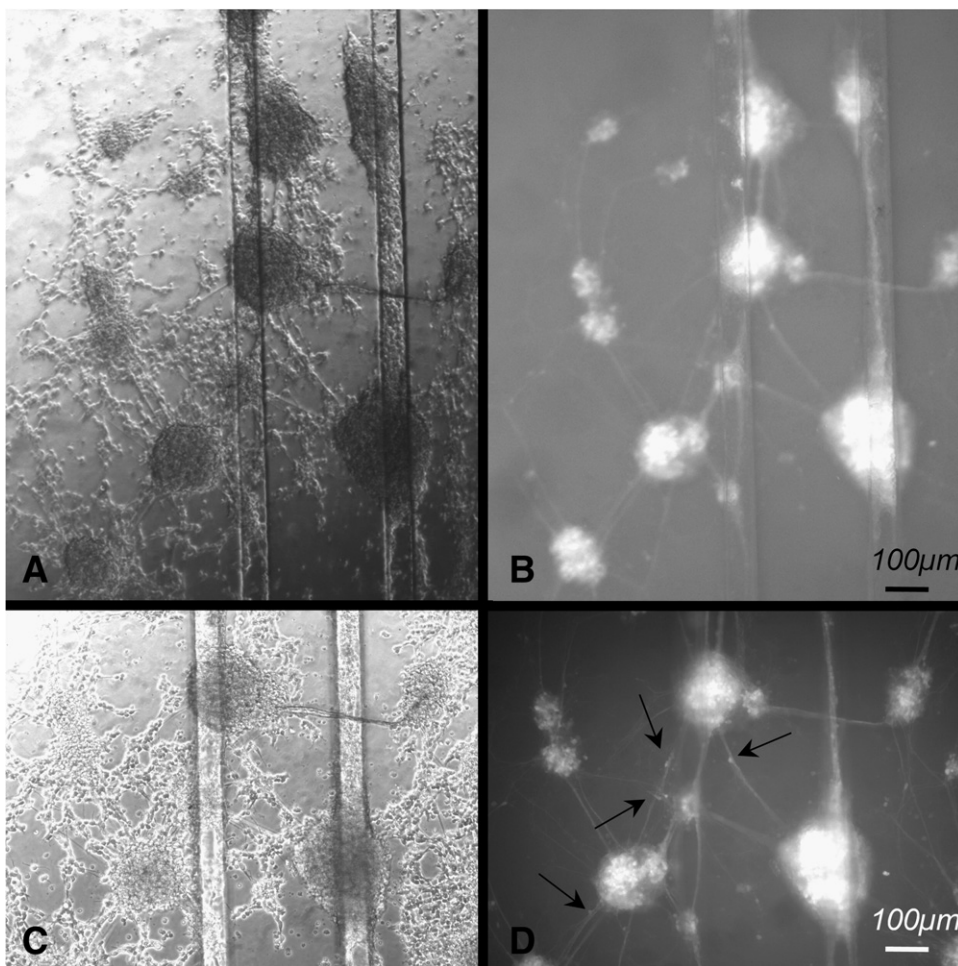

Figure 4. Results of mechanical compression on neurospheres. **(A)** Bright-field image of neurospheres after 12 hours of compression. **(B)** Fluorescent image of **A**. **(C)** Bright-field image after 24 hours of mechanical compression (72 hours in culture). **(D)** Fluorescent image of **C** illustrating fluorescent neurons migrating on processes (*arrows*).

supplement with epidermal growth factor, fibroblast growth factor, and heparin sulfate. Formation of free-floating NS was observed on the second day of culture. The PDMS substrate was stretched and clamped around a glass fixture. Optical measurement indicated that the width of the wells was stretched from the original 60 μm to about 166 μm. The NS were then placed on the prestretched PDMS wells. After 48 hours of culture, most of the NS had grown to a diameter approximately the width of the stretched wells, so that the outer layer of cells on the spheres were in contact with the sidewalls of the wells.

The stretched PDMS was then released after 60 hours in culture, and the wells shrank back to their original width of 60 μm, thus compressing the NS.

Results

After 48 hours of culture, the NS inside the wells grew and aggregated to a diameter about the width of the stretched wells (166 μm), as shown in Figure 2. In the control experiment with identical culture conditions, the PDMS remained stretched. Figure 3 shows the NS in the control experiment after 48 hours (Figure 3, *A*) and 72 hours of culture (Figure 3, *B*). The NS inside the wells were more aggregated and confluent after 72 hours compared with 48 hours. However, there was no sign of meshwork formation between the NS.

In the compression experiment, the PDMS was released after 60 hours in culture and mechanical compression of the NS was initiated. Figure 4 (*A* and *B*) shows the effect of mechanical compression on NS after 12 hours of releasing the stretched PDMS. At this time, numerous processes were observed to have grown between the NS, resulting in the formation of a meshwork. In addition, after 24 hours of mechanical compression, there appeared to be fluorescent neurons present on these processes, probably representing neuronal migration along these processes (Figure 4, *C* and *D*).

Discussion

A previous study by our group showed the formation of radial glial precursors, identified with nestin staining, formed between NS on poly-ʟ-ornithine–coated PDMS after 7 days in culture.[9] Neuronal migration along these radial glial precursors was observed after 8 days. In the current study, similar processes formed after only 3 days in culture with 12 hours of applied mechanical compression, and fluorescent neurons were seen on these processes after 24 hours of mechanical compression. Further experiments are required to confirm our observations made during these preliminary experiments—specifically, staining for radial glial cell markers. Our observations indicate that applied mechanical compression on NSCs cultured as NS could be a factor accelerating the process of neuronal migration. Future studies implementing this method to study the effect of mechanical compression on neuronal migration should include sensor-instrumented platforms to quantify the stresses applied to the NS, as well as gene expression analysis to identify changes associated with mechanical compression.

Acknowledgment

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