Glioblastoma migration along constraints with different geometries: how to mimick brain parenchyma invasion?

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GLIOBLASTOMA MIGRATION ALONG CONSTRAINTS WITH DIFFERENT GEOMETRIES: HOW TO MIMICK BRAIN PARENCHYMA INVASION?

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ABSTRACT

A microfluidic device is demonstrated to analyze glioblastoma migration along constraints with precisely designed geometries. This in-vitro model reveals physiologically relevant glioma invasion scenarios: full migration along constraints, suspended motion by extreme constriction, and limited migration associated with the ejection of plasma membrane particles due to the continuing extension.

KEYWORDS: Microfluidics, Glioblastoma, Cell migration, Mechanical constraints

INTRODUCTION

Glioblastoma, one of the most aggressive human cancers, is responsible for a median overall patient survival of only 15 months [1]. To actively migrate within the brain, malignant glioma cells respond to chemical cues [2] and exploit the micro-vascular route network with various physical constraints [3]. A better understanding of glioma brain invasion leading to recurrence and malignancy is crucial for future research and identification of new therapeutic targets. This work proposes a microfluidics approach to monitor glioma migration along various *in vitro* physical constraints specifically designed to understand how pressure during cell contraction and adhesion leads to plasma and nucleus membrane alterations.

EXPERIMENTAL

Two parallel channels handle solutions by controlling the flow separately with pumps (Figure 1a). They are connected by constraints to stress the migrating cells. The stress level on the cells is modulated by the dimensions (width and length of the constraints), geometry (bifurcation (angle)) and the cell/fluid velocity. The device was fabricated as PDMS molded on 10-µm high patterned photoresist (SU8) structures with 4-µm-wide constraints and 3 different bifurcations (Figure 1b).

The glioblastoma cell line U87MG were grown in culture DMEM medium, and incubated at 37°C, 5% CO_2. For the resuspension, cells at 70% confluence were cleaned with warm PBS and detached from flask surface with trypsin, centrifuged and used at the $2.5x10^5$ cells/ml density.

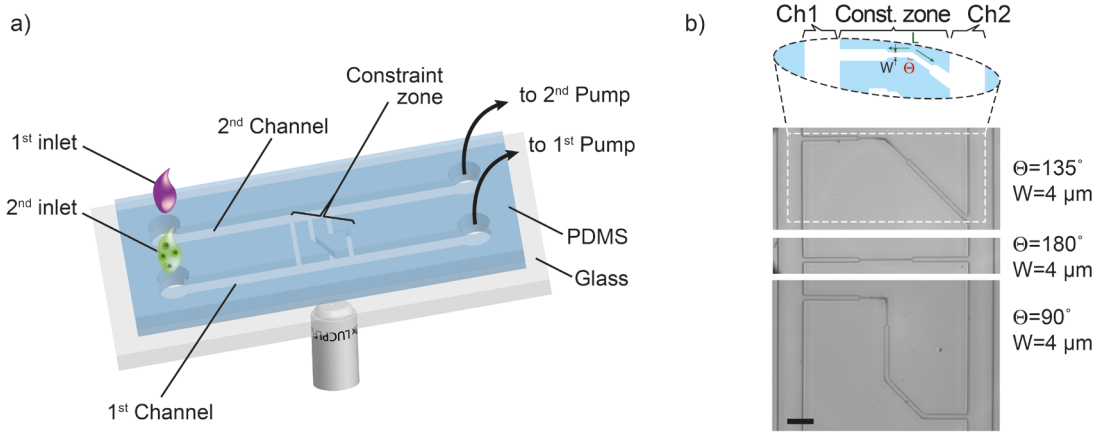

Figure 1: a) Overall view of the microfluidic device with two parallel channels and constraints connecting them. b) Different constraint geometries in a fabricated device to analyze the glioma migration. Scale bar: 100 µm.

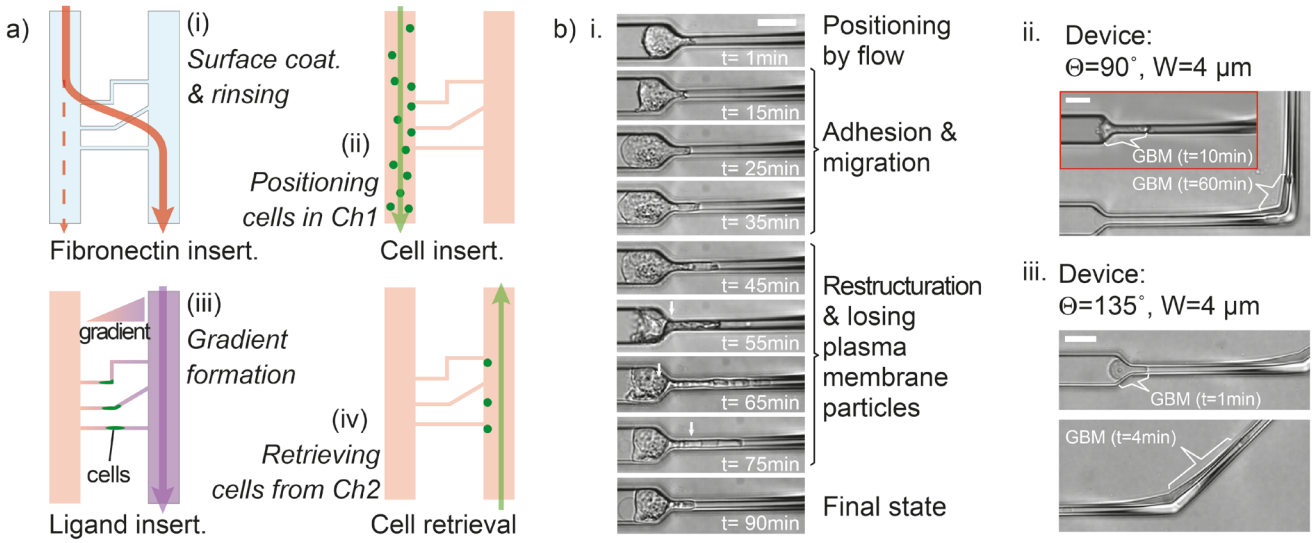

Figure 2: a) An overall view of the proposed protocol. b) Demonstration of different invasion scenarios along the fabricated constraints: i) limited migration with plasma membrane particle extraction, ii) migration until suspending the motion at extreme constraint and iii) full migration. Scale bars correspond to 20 µm.

Experiments were performed on an inverted microscope stage with an integrated incubation unit (37°C, 5% CO_2). First, the device was coated with fibronectin (20 µg/ml, 30 minutes) and rinsed with PBS (Figure 2a-i). Then, glioblastoma cells were injected in the first channel while gently directed towards constraints by controlling the pressure in the second channel (Figure 2a-ii). Although the introduction of chemoattractant in the second channel (Figure 2a-iii) was not demonstrated here, the device is capable of the forming chemoattractant gradient along the constraint zone and cell retrieval after passing through the constraints (Figure 2a-iv).

RESULTS AND DISCUSSION

After positioning by the constraint, cells took several minutes (up to 20 minutes) to adhere on the glass surface and start migrating along the constraint. Then, cells started experiencing re-structuration which ended up with different scenarios. Some cells tried migrating along the constraint but failed (Figure 2b-i, t=55 min). After returning to their earlier shape, several plasma membrane particles were extracted (Figure 2b-i). Some other cells successfully migrated along the constriction, however, with different time-scales (>10x; Figure 2b-ii&iii). A glioblastoma smoothly reaching to a 90° constraint after an initial "restructuration" stopped until losing many plasma membrane particles (Figure 2b-ii) which was not experienced by another glioblastoma moving along a 135° constraint (Figure 2b-iii).

CONCLUSION

Obtained time-lapses show the dynamic behavior of single glioblastomas migrating along narrow channels similar to the pathways on tumor vasculature in the brain. Analyzing the migration behavior along in-vitro channels allows investigating several phenomena, *e.g.* how confined migration leads to the loss of nuclear envelop integrity which would be responsible for genetic instability, tumor recurrence and resistance.

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