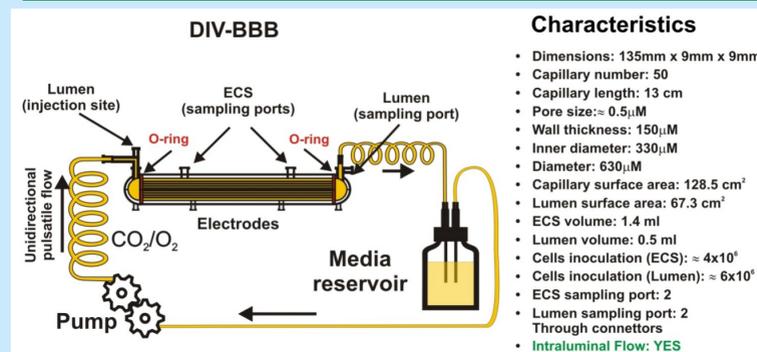


# Glioblastoma Cell Line Metabolism Under Dynamic and Static Conditions: Effects on tumor growth and BBB integrity

## Abstract

Glioblastomas are the most common type of gliomas, with patients surviving 12 months on average, and rarely more than three years. Despite the effort put in the development of effective pharmacological treatments, the results are at most marginal. We planned to: 1) Expand our understanding of the functional and metabolic characteristics of these tumor cell lines; and 2) To characterize their interaction with the brain microvasculature. This is a very important issue since the brain microvessels through the blood-brain barrier (BBB) provide the nutrients and other important factors that facilitate metastatic tumor proliferation and drug resistance. In addition, the BBB may be an essential player in determine the amount of drug delivered to the tumor. In this study we evaluated and compared the levels of glucose consumption and lactate production in three different glioblastoma cell lines (HS683, T98, LN229) grown under dynamic (flow exposure) and static (no flow) conditions. Under static culture conditions, glucose consumption reached steady-state in approximately 6 days regardless of the cell line studied. Lactate production also reached steady-state at around 6 days. Under static conditions, glioblastoma cell lines demonstrated significantly higher glucose consumption in comparison to parallel cultures exposed to flow. When grown under static conditions, all cell lines demonstrated a predominant aerobic metabolic behavior as demonstrated by the amount of lactate produced versus glucose consumed. By contrast, under dynamic condition, glucose metabolism was differently affected depending on the cell line studied. Specifically, exposure to flow promoted an anaerobic shift in T98 cells while the opposite was observed in LN229 and HS683 cells.

## Methodology



**Figure 1: DIV-BBB setup.** The dynamic *in vitro* blood-brain barrier (DIV-BBB) model used in this experiment mimics several BBB characteristics *in situ*. Namely, the DIV-BBB emulates dynamic conditions by enabling intraluminal flow.

### Cell Culture

- Three glioblastoma cell lines (HS683, T98, and LN229) were obtained and initially expanded in T-75 cell culture flasks.
- Cells were stored at 37°C with 5% CO<sub>2</sub> in a humidified incubator.
- Cell growth and viability was assessed daily using inverted light microscopy.

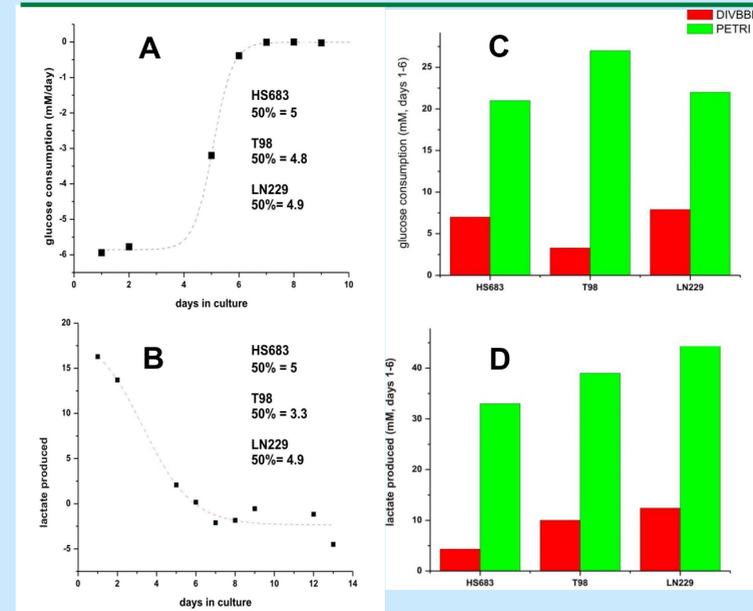
### Static vs. Dynamic Culture Conditions

- 3 million glioblastoma cells from each cell line were plated in T-75 flasks.
- Cell growth was monitored daily using inverted light microscopy.
- 5 million+ glioblastoma cells from each cell line were plated on the abluminal surface of the DIV-BBB.

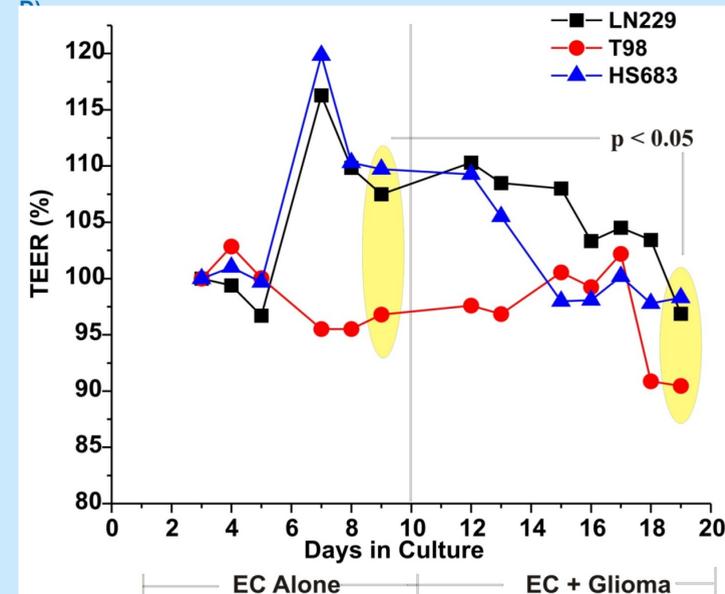
### Glucose-Lactate Sampling

- Cellular medium was sampled daily from each flask.
- Cellular medium was sampled every other day from the DIV-BBB cartridges.
- Glucose/lactate levels were determined using a dual channel immobilized oxidase enzyme analyzer and turn table.
- Flasks were photographed daily using inverted light microscopy in order to assess viability.

## Results



**Figure 2: Glucose consumption vs. lactate production in glioblastoma cells.** The rate of glucose consumption and lactate production significantly decreases after six days in a static culture (Panels A and B). The rate of glucose consumption/lactate production varies between dynamic and static conditions and also between different glioblastoma cell lines (Panels C and D).



**Figure 3: Glioma cells induce permeability changes in endothelial cells grown under dynamic conditions/**

## Conclusions

- We observed that culturing conditions (dynamic vs. static) influence metabolism in glioblastoma cell lines. In addition, metabolic behavior varies from cell line to cell line
- All three cell lines showed sigmoidal growth patterns, and the median growth point was identified as 5 days in HS683, T98, and LN229
- Under static culture conditions, glucose consumption increased by 50% in approximately 5 days. A parallel increase in lactate production was observed in HS683 and LN229. By contrast, T98 cells reached 50% increase in lactate production in 3.3 days.
- Under static conditions, glioblastoma cells demonstrated a significantly higher glucose consumption in comparison to parallel cultures exposed to flow.
- All cell types demonstrated a predominant anaerobic metabolic behavior demonstrated by the amount of lactate produced versus the glucose consumed. A value  $\approx 2$  indicates the full conversion of glucose into lactate without the involvement of the Krebs cycle.
- By contrast, under dynamic condition, the metabolic behavior was affected differently depending on the cell line. Specifically, the exposure to flow determined the shift of T98 cells toward a more pronounced anaerobic metabolism while we observed the opposite effect on LN229 and HS683 cell line.
- Glioma cells impair BBB integrity as *in vivo* when cultured under dynamic conditions