Perfused 3D Cell Culture Platform for Long-term Monitoring of Glucose and Lactate Metabolism in Tumor Spheroids

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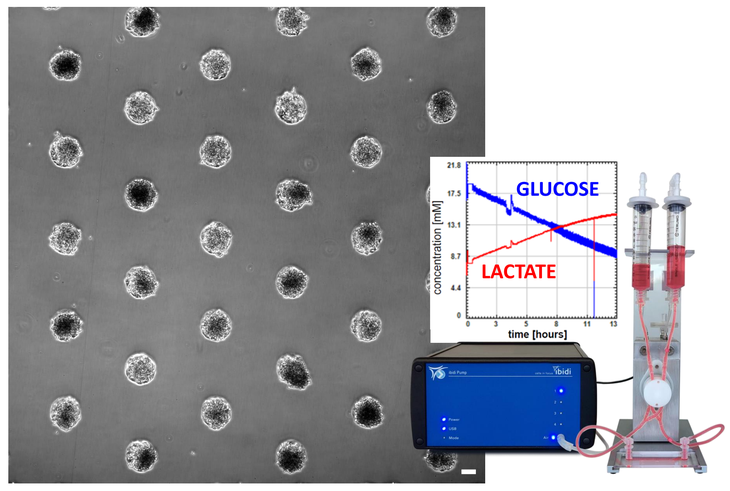
Background

3D cell cultures have emerged as powerful tools in various disciplines, including drug discovery, toxicology, and therapeutic development. Their enhanced physiological relevance makes them ideal for investigating intricate biological processes, particularly in cancer research. Gaining a comprehensive understanding of cellular behaviour within these 3D environments necessitates real-time, multi-parameter monitoring to track metabolic and proliferation rates over extended time periods.

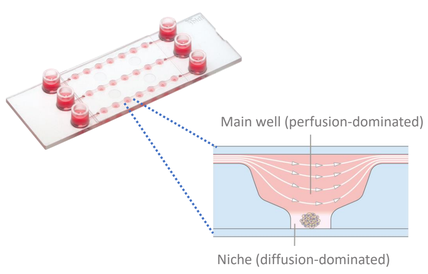
The presented platform integrates microfluidic chips for assembly and cultivation of 3D cell aggregates, a perfusion system to provide nutrients and remove waste products, and electrochemical sensors for targeted monitoring of the metabolic rate of the spheroids. The system's design facilitates the serial connection of multiple cultivation units, enabling simultaneous monitoring of thousands of spheroids. This high-throughput capability significantly improves the sensitivity of the platform for robust and reliable metabolic rate measurements.

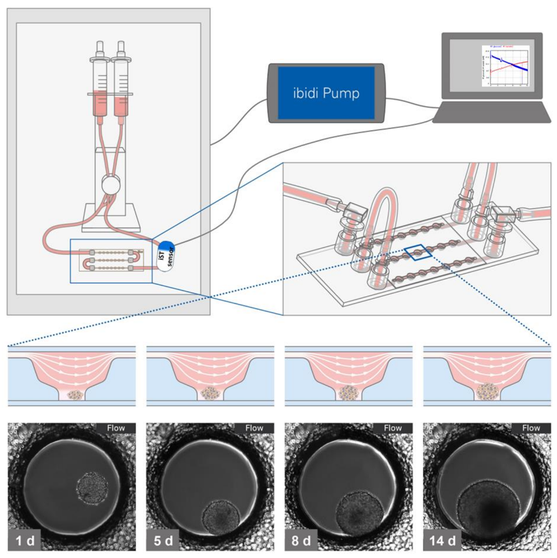


Microfluidic chips for long-term spheroid cultivation under flow

µ-Slide Spheroid Perfusion

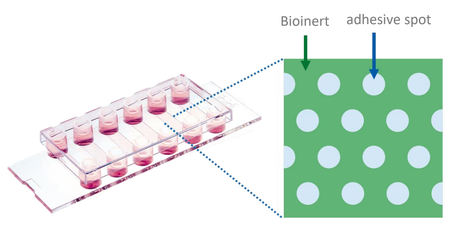
- Self-assembly of cells in microwells with bioinert surface
- Spheroids grown in suspension under flow
- 21 spheroids per chip

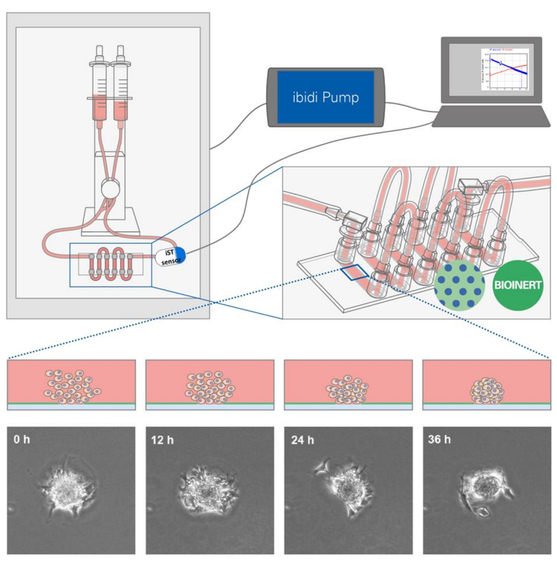




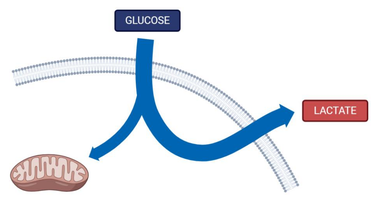
µ-Slide VI 0.4 Multi-Cell µ-Pattern

- Self-assembly of cells on adhesive spots on bioinert surface
- Spheroids grown on micro-pattern under flow
- > 1000 spheroids per chip



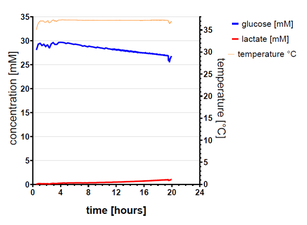


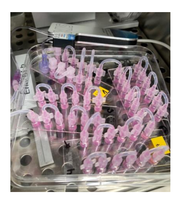
Real-time monitoring of metabolic rate of growing spheroids

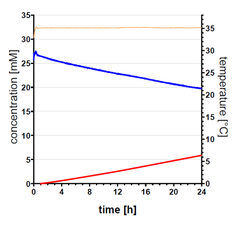


- Warburg effect → glucose breakdown with lactate production
- Biosensor serially connected to chips with spheroids
- Detection of glucose and lactate concentration kinetics in circulating medium
- Serial connection of multiple spheroid cultivation units increases the sensitivity of the system

Glucose/lactate measurement in µ-Slide Spheroid Perfusion and in µ-Slides VI 0.4 with spheroids on micropattern

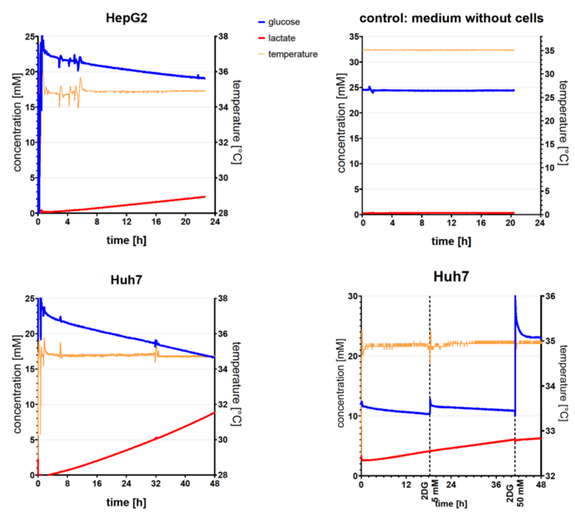




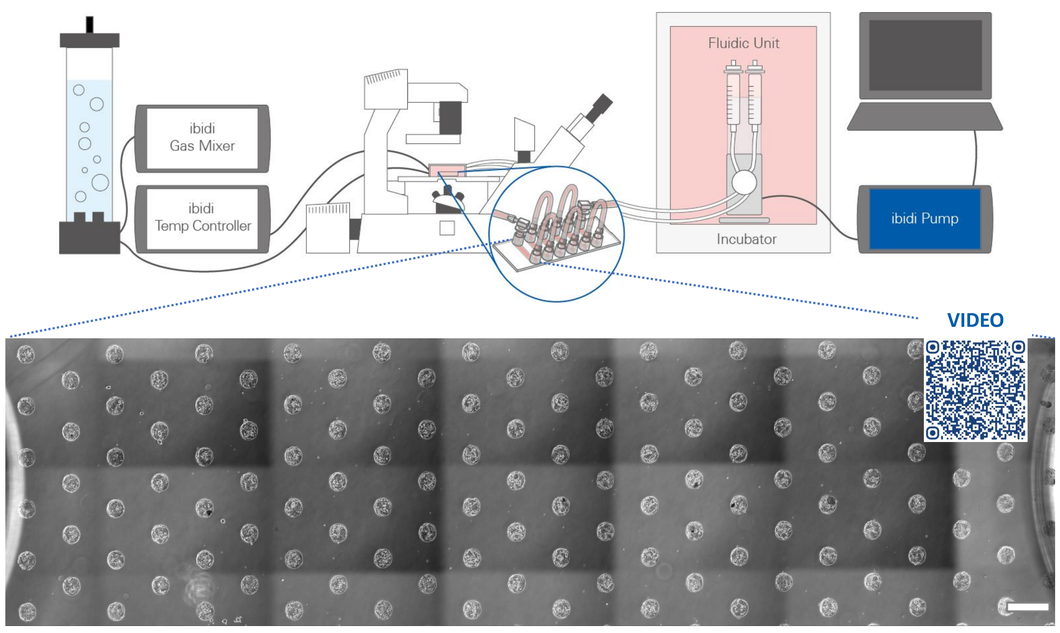


Glucose/lactate metabolism in hepatic cell lines

- Metabolic rate was monitored in perfused 3D cell cultures of hepatic cell lines HepG2 and Huh7 for 1-2 days
- Glucose uptake and lactate secretion was detected in real-time
- In control experiment with cell culture medium without cells, glucose and lactate concentrations remain constant
- Perturbation of glucose metabolism by the glycolysis inhibitor 2-deoxy-glucose led to a decrease in lactate secretion rate



Time-lapse imaging of spheroids under flow



Stitched image of a channel of µ-Slide VI 0.4 with HepG2 spheroids on micro-pattern with 200 µm adhesive spots; scale bar = 500 µm.

Outlook: 3D spheroid invasion assay in collagen matrix

3D cell culture on micro-pattern under flow

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Monitoring of metabolic rate

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Embedding spheroids on patterns with 3D collagen matrix

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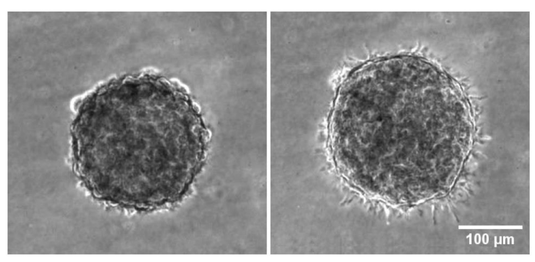
Time-lapse imaging and evaluation of cell invasion



L929 (fibroblasts) spheroids grown on micropattern in µ-Slide VI 0.4 after 2 weeks of cultivation under flow were embedded in 3D collagen matrix. Within several hours, cells from the spheroid start to invade the surrounding matrix.

Summary and Outlook

This 3D cell culture platform facilitates measurement of glucose uptake and lactate production in spheroids. It enables real-time monitoring of their metabolic rate, making it suitable for drug screening and toxicological studies. Additionally, the platform allows for time-lapse microscopy evaluation of further cellular characteristics, such as tumour invasive potential.