

Microfluidic models of metastasis: in vitro approaches to study the tumor microenvironment

Jelle Sleeboom and Jaap den Toonder¹

¹Microsystems, Mechanical Engineering, Eindhoven University of Technology

Cancer is still one of the deadliest diseases in the world, accounting for nearly 1 in 6 of all deaths worldwide. The majority of these cancer-related deaths are not directly caused by the primary tumor, but by the formation of secondary tumors through metastasis, see Figure 1. However, our understanding of this complex process is still lacking, such that the successful treatment or prevention of metastasis has been difficult to achieve. One of the main reasons for the gaps in our knowledge is the extreme complexity of the metastatic cascade: cancer cells invade into surrounding tissue, then intravasate into the blood circulation, survive in the circulation, extravasate into another tissue, to finally proliferate and develop into a secondary tumor. During all of these steps, the cells are affected and steered by their environment, provided by other cells, extracellular matrix (ECM), soluble factors, and physical forces, see Figure 2. In the primary tumor, the collection of these cues is referred to as the tumor microenvironment (TME) [1].

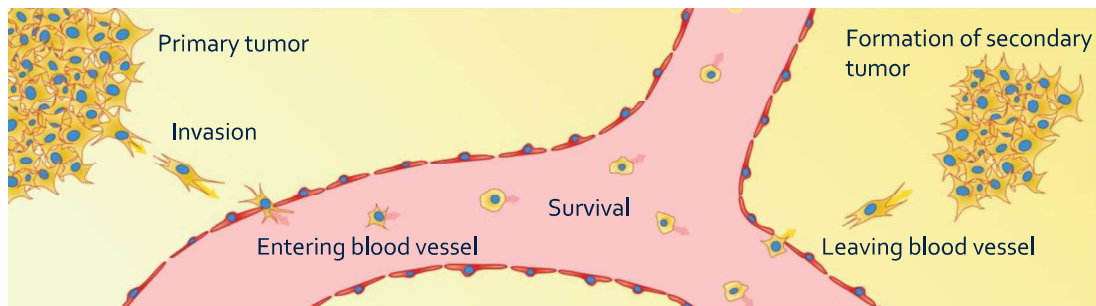


Figure 1: The process of metastasis, spreading of cancer through the body through the blood circulation, encompasses a cascade of events.

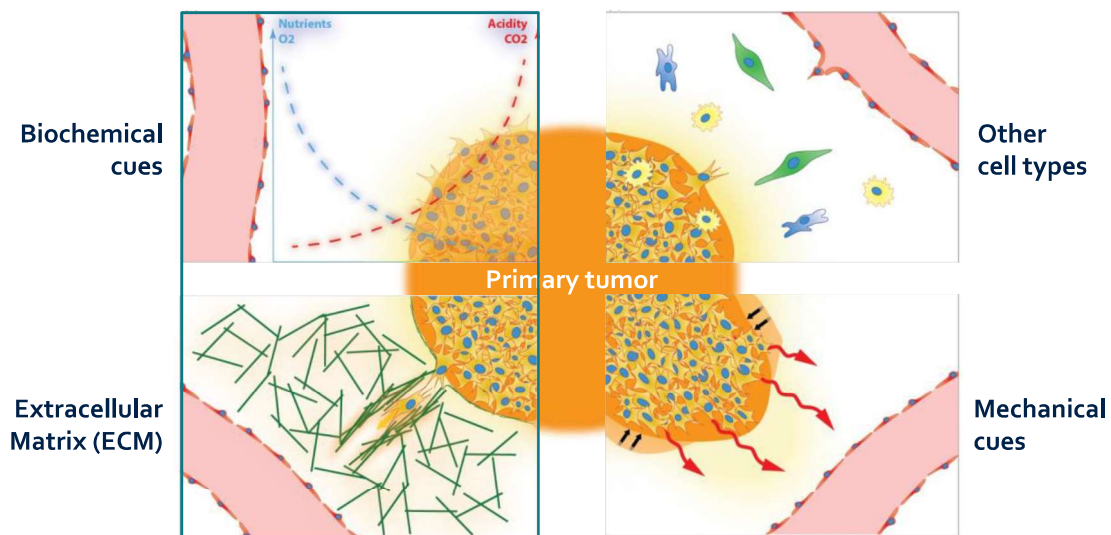


Figure 2: The first step of metastasis is invasion, which is directed by many different cues in the Tumor Microenvironment (TME); in our research, we focus on the influence of chemical cues, in particular oxygen gradient, and the nature of the Extracellular Matrix (ECM).

In our research, we have explored the factors of the TME that are relevant to the onset of metastasis, and developed methods to study them in vitro, using **Cancer-on-a-Chip (CoC) models**. These in vitro models, based on **microfluidic chips**, contain small chambers for cell culture as well as fluid channels, enabling control over local gradients, fluid flow, tissue mechanics, and composition of the local environment. These properties

make CoC devices promising tools to investigate the TME. We have focused on the development of novel in vitro systems to investigate two specific factors from the TME: local oxygen gradients, and the ECM (see Figure 2).

In order to study the effect of **oxygen gradients** on cancer cell migration, we have developed a microfluidic platform that is capable of generating a controlled and stable gradient between hypoxic (<1%) and normoxic (~7%) oxygen saturation, shown in Figure 3(a) [2]. Cells are cultured in a microfluidic chamber in which the oxygen gradient is generated using a neighbouring channel, separated from the cell chamber by a thin polydimethylsiloxane (PDMS) barrier, in which a continuous flow is maintained of leaching fluid that consumes oxygen. The design of the chip was guided using numerical simulations (Figure 3(b)) and the oxygen gradient was confirmed by experiments (Figure 3(c)). Using the developed system, we have studied the migration of MDA-MB-231 breast cancer cells in this defined oxygen gradient. Figure 3(d) shows the tracks of three cells within the cell chamber, and Figure 3(e) shows many cell tracks superposed and shifted to the origin. The migration of the cancer cells was found to have a bias towards low oxygen levels, as shown in Figure 3(f). In addition, we have investigated the migration of another breast cancer cell line (MCF-7), and two glioblastoma cell lines (U87, and U251). Here we found that the U251 cells exhibit behaviour similar to the MDA-MB-231 cells. In contrast, the MCF-7 and U87 cells did not show the bias towards low oxygen, but migrated in random patterns. We also demonstrate that the platform enables staining for relevant markers, such as E-cadherin, N cadherin, and Vimentin.

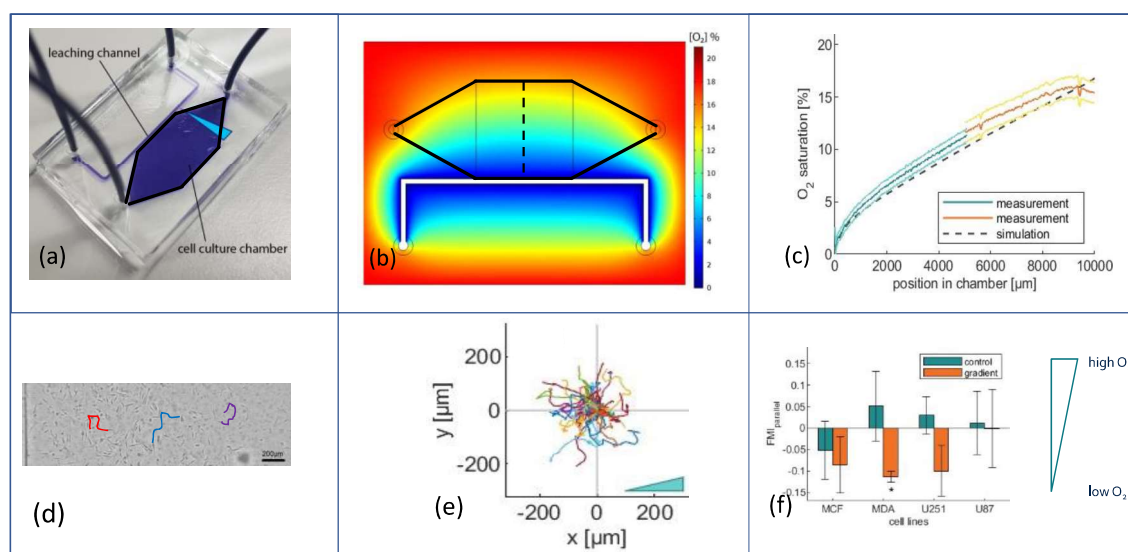


Figure 3: Oxygen gradient microfluidic device used to study cancer cell migration. (a) A picture of the microfluidic device filled with Brilliant Black BN dye. The chip is 25x30 mm. The gradient direction in the chamber is indicated with a blue triangle; (b) Simulation of the steady-state oxygen saturation in the chip. The chamber outline is shown in black; (c) The average oxygen concentration across the entire chamber, based on 10 measurements done within 1 hour; (d) Traces of three MDA-MB-231 breast cancer cells in the device; (e) Representative migration tracks for MDA-MB-231 breast cancer cells, the direction of the oxygen gradient is indicated by the blue triangle: Low oxygen at the tip, and high oxygen at the base. The starting points of the migration tracks of each cell are offset to (0,0). The graph contains the data of 50 tracked cells in a single experiment. (f) The average forward migration index parallel to the oxygen gradient, extracted from the tracks for four cell lines: MCF-7, MDA-MB-231, U251, and U87. Here, a negative value indicates a migration bias towards lower oxygen concentration.

Next to the oxygen gradient chip, we have developed devices that enable us to study the effect of the ECM on the first step in metastasis: invasion. In vivo, cancer cells initially reside in a soft basement membrane before invading the fibrous and stiffer stromal ECM [3]. To recapitulate this heterogeneous pre-invasive ECM composition, we have developed a microfluidic cell encapsulation method that creates a similar tissue morphology. In our model, MCF-7 breast cancer cells are encapsulated in Matrigel beads that mimic the basement membrane, which are then embedded in a fibrous collagen I hydrogel, mimicking the stromal ECM, as shown schematically in Figure 4(a). Figure 4(b) shows the actual cell encapsulation device, which has a flow focusing region in which droplets of liquid Matrigel are formed encapsulating cancer cells; this is region is cold so that Matrigel is liquid. In a subsequent meandering channel kept at higher temperature, the Matrigel gels. After harvesting these Matrigel beads containing cancer cells and embedding them in collagen I matrix, we were able to make live observations of the early stages of invasion of MCF-7 breast cancer cells, Figure. 4(c), and we

demonstrated the possibility to quantify individual cell positions after invasion using image analysis, Figure 4(d).

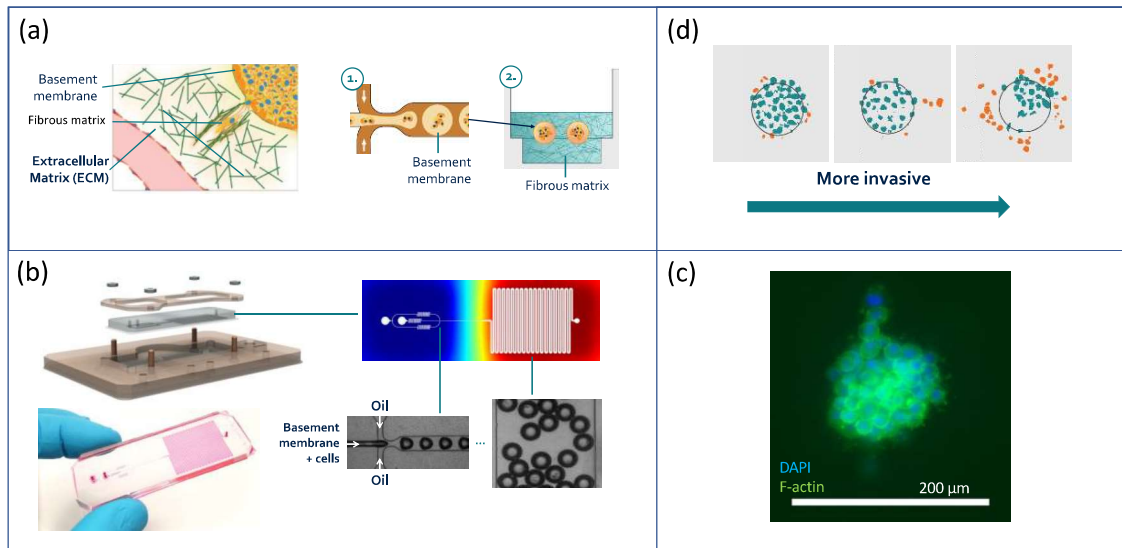


Figure 4: Breast cancer invasion chip. (a) Schematic of cancer cell invasion in which the cell breaks through the basement membrane and enters the fibrous stromal matrix. We recreate this using two steps: encapsulating cells in Matrigel beads using a microfluidic flow focusing device, followed by harvesting the beads and embedding them in fibrous collagen I. (b) Microfluidic cell encapsulation device (75mm x 25 mm); Matrigel beads encapsulating cells are formed in a flow focusing structure at <math><4\text{ }^\circ\text{C}</math> at which Matrigel is liquid; in a subsequent meandering channel at $37\text{ }^\circ\text{C}$, Matrigel gelation happens. (c) We can follow invasion live; the fluorescent image is taken at the moment cells break through the basement membrane (Matrigel) and invade into the stromal matrix (collagen I); (d) Images can be analyzed and invasion can be quantified.

Both systems, for oxygen control and ECM modeling, are designed to fit together, such that the effects of oxygen gradients on cancer invasion can be studied in the future. In a broader perspective, the technologies in this research could be further developed to envelope more of the TME cues, such as additional biochemical cues, cell types, and (fluid-) mechanical cues. Potentially, they could also be applied to study other (patho-) physiological processes in the field of organ-on-a-chip. Many tissues have some form of epithelial tissue and different oxygen levels throughout, such as the lungs, liver, and gut. Summarized, our technologies can aid in deciphering the mechanisms that govern metastasis, and potentially other pathological processes [4].

References

1. Sleebom, J.J.F., Eslami Amirabadi, H., Nair, P., Sahlgren, C.M., & Toonder, J.M.J. den (2018). Metastasis in context: Modeling the tumor microenvironment with cancer-on-a-chip approaches. *Disease Models & Mechanisms* 11. dmm033100. doi:10.1242/dmm.033100.
2. Sleebom, J.J.F., Toonder, J.M.J. den & Sahlgren, C.M. (2018). MDA-MB-231 Breast Cancer Cells and Their CSC Population Migrate Towards Low Oxygen in a Microfluidic Gradient Device. *Int. J. Mol. Sci.* 19, 3047. doi:10.3390/ijms19103047.
3. Sleebom, J.J.F., Sahlgren, C.M., & Toonder, J.M.J. den (2021). A microfluidic model for breast cancer invasion from a heterogeneous ECM. Under review.
4. Sleebom, J.J.F. (2020). Microfluidic models of metastasis: In vitro approaches to study the tumor microenvironment. PhD Thesis, Eindhoven University of Technology.