Breast cancer: new perspectives for in-vitro studies

Introduction

Breast cancer is considered the most prevalent cancer worldwide, with over 2.3 million women affected in 2020. This disease can occur also for men, with a prevalence of 0.5-1%[1] *(Figure 1)*.

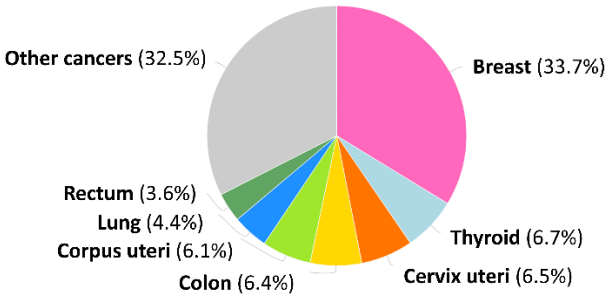
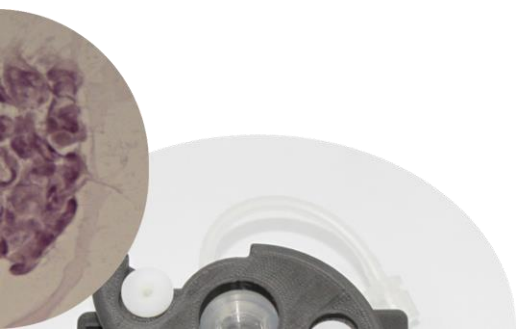


Figure 1: Estimated number of prevalent cases (5-years) in 2020, worldwide, females, all ages[2]

Cancer arises when breast cells start to proliferate without control. According to the specific type of cells which become cancerous, it can be classified as:

- *In situ:* the uncontrolled growth is confined to the epithelial cells of the ducts or to the lobules in the glandular tissue
- *Invasive:* the invasion occurs in the surrounding breast tissue
- *Metastatic:* it's the worst scenario, characterized by the presence of metastasis which spread through the lymph nodes or towards other organs

Breast cancer can be efficiently treated, especially when the disease is early detected. The treatments often consist in a combination of surgical removal and radiation therapy. Certain drugs (i.e. anti-proliferative agents, anti-hormonal treatment, monoclonal antibodies) are used to inhibit cancer growth and metastasis proliferation.
Unfortunately, these approaches often fail, especially when the amount of circulating metastasis is relevant.



Advanced in-vitro models

Understanding the mechanisms involved in the cancer development and spreading to other tissues have always been great challenges for the scientific community. An alternative approach to study breast cancer is represented by advanced in-vitro models, characterized by a 3D cells topology, exposed to a dynamic environment, where medium flows, mimicking the blood action in human circulation.
In the following paragraphs, two different approaches are described.

The in-vitro cells sensitivity to a drug (i.e. Trastuzumab) has been evaluated by *Mazzanti et al.*[3]
In this study, MCF7 and BT474 breast cancer cells have been independently grown in a fluidic chamber (i.e. LiveBox1 by IVTech), both in static and dynamic conditions, investigating the differences *(Figure 2)*. Cells have been cultivated in a sphero like model, embedded in a Matrigel solution.

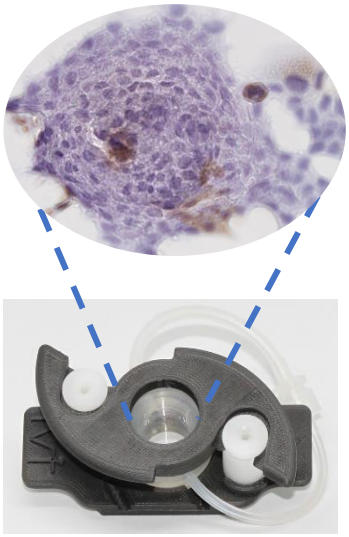


Figure 2: MCF7 cells are seeded in LiveBox1[3]

It has been observed that in dynamic conditions, the hypoxia level is higher than the control (2D static conditions). This feature contributes to better mimic the tumor microenvironment, observed in-vivo *(Figure 3)*.

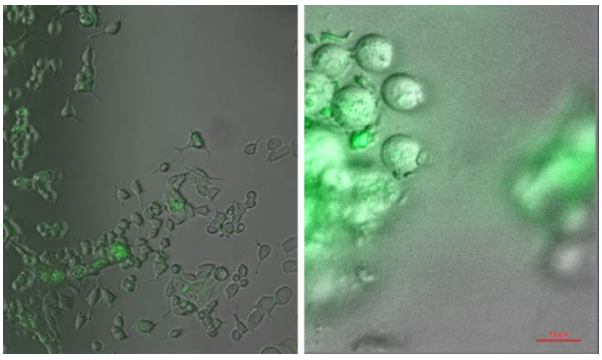


Figure 3: Hypoxia Probe MCF7 treated TRZ for control (left) and 3D dynamic (right) [3]

Moreover, using a fluidic chamber to develop an advanced in-vitro model, it is possible to study the cell sensitivity if exposed to drugs. Evaluating for instance the cytotoxicity of Trastuzumab, cells in a 3D dynamic model are more sensitive than the control (3D static model), as shown in *Figure 4*.

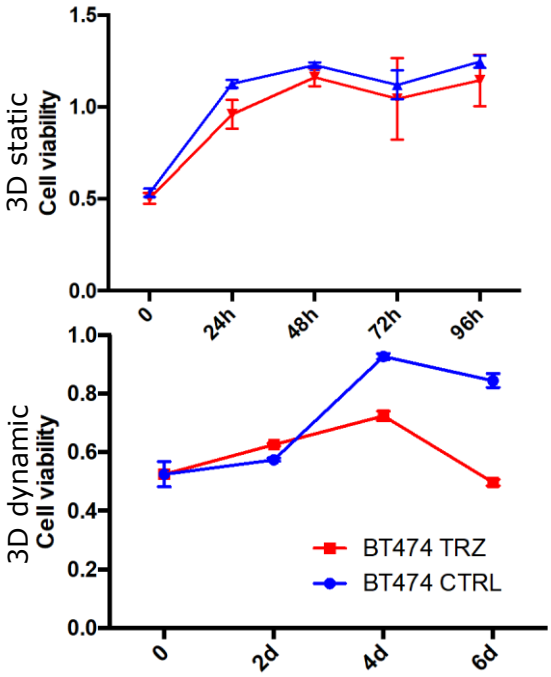


Figure 4: Cytotoxicity tests[3]

Another relevant aspect of breast cancer is represented by the metastasis interaction with healthy tissues. This complex scenario needs a fluidic environment, where metastasis can circulate. The interaction between surrounding tissues and breast cancer metastasis has been evaluated by *Paolillo et al.*[4][5] The target tissue has been obtained culturing human dermal fibroblasts on a polystyrene scaffold (by Sigma-Merck), seeded in a LiveBox1 (IVTech) *(Figure 5)*.

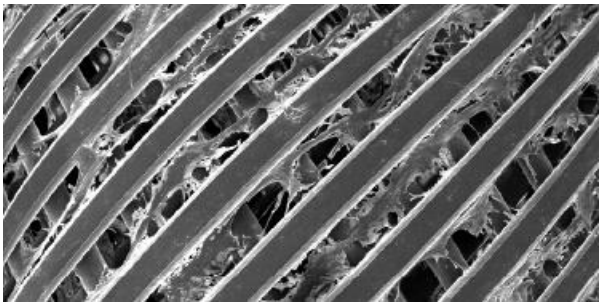


Figure 5: 3D culture of fibroblasts[5]

MCF7 and A549 cells have been de-differentiated in cells that grow in suspension to obtain a metastasis model *(Figure 6)*.

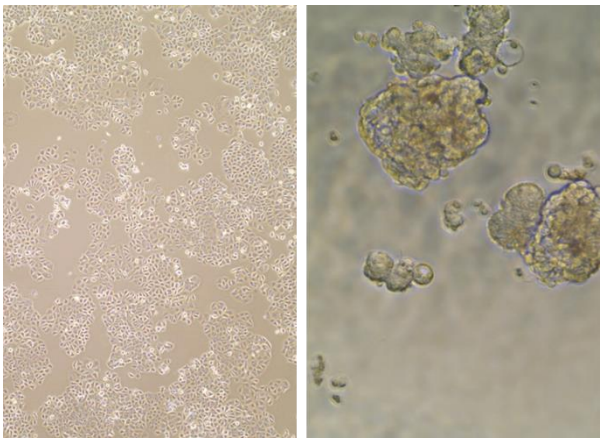


Figure 6: Metastasis model[5]

After 2 days of dynamic stimulation, metastasis have been injected into the LiveBox1 fluidic circuit, where the medium circulated thanks to the action of a peristaltic pump (i.e. LiveFlow by IVTech). The interaction between metastasis and the tissue has been evaluated by fluorescent and SEM analysis. These tests have confirmed that metastasis attached to the target tissue and their interaction was not mediated by the scaffold *(Figure 7)*.

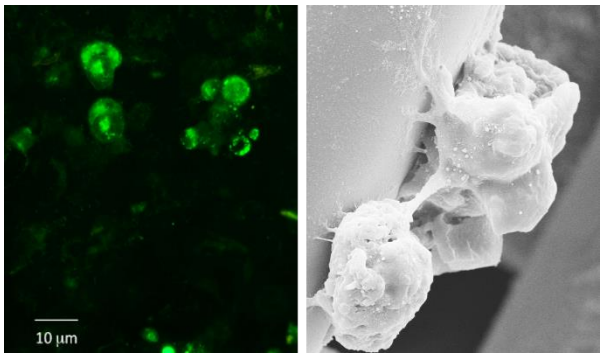


Figure 7: Metastasis adhesion by fluorescent analysis (left) and SEM (right) [5]

The use of a dynamic chamber such as the IVTech products allows to investigate methods to counteract the metastasis adhesion. *Paolillo et al.*[4] analysed 3 different RGD integrin antagonists, injecting them in the fluidic circuit and evaluating their anti-adhesive properties.

Further analysis have demonstrated that none of them had toxic effects on fibroblasts and only cilengitide has significantly reduced metastasis adhesion on the target *(Figure 8)*.

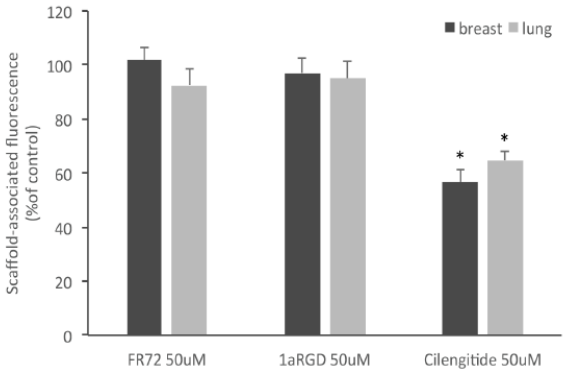


Figure 8: Anti-adhesive properties of RGD integrin antagonists[5]

Future perspectives

An advanced in-vitro model can be considered a powerful tool to evaluate the patho-physiological processes. Indeed, the 3D configuration and the dynamic environment contribute to generate a milieu which better reproduces the in-vivo scenario, allowing to perform complex studies of tissue-tissue interaction. Therefore, the obtained results are more predictive and representative of the human reality. A hybrid approach is suggested, focusing the attention on both the biological as well as the system complexity aspects. Indeed, an advanced in-vitro model could better replicate the human complexity, basing on the use of an in-vivo tissue explant, as a substitution of the primary cells. However, this is not appliable to all the models, considering the biopsies availability. On the other hand, the complexity of the in-vitro model can be increased, interconnecting different tissues and developing a multi-organ approach (MOA). For instance, starting from the previously described models, the implementation of a vascular tissue and its physical connection to the previous model represents a suitable evolution. Using this set-up, the metastasis interaction with the vascular endothelium could be studied. Moreover, this approach is useful to evaluate different ways of drugs administration. Indeed, the breast cancer can be treated using oral drugs. In this case, their bioavailability (e.g. their permeation through the intestinal barrier and their metabolism) is a hot topic that should be investigated.

In conclusion, the refinement of in-vitro models can be achieved increasing both the biological and technological complexities, using a hybrid approach that has to be inspired by the new technologies, currently available on the market.

References:

[1] https://www.who.int
[2] http://gco.iarc.fr/
[3] Maria Chiara Mazzanti, FPS, Pisa, Italy, c.mazzanti@fpscience.it
[4] Mayra Paolillo, University of Pavia, Pavia, Italy, mayra.paolillo@unipv.it
[5] M. Paolillo, et al., *Stem-Like Cancer Cells in a Dynamic 3D Culture System: A Model to Study Metastatic Cell Adhesion and Anti-Cancer Drugs*, Cells 2019, 8, 1431, doi:10.3390/cells8111434www