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Statement of Purpose: Colorectal cancer is the third most common cancer and its incidence increases with ageing. Understanding the mechanisms of tumour growth rely in further advances to unveil cancer-causing agents, drug screening and in the development of personalized therapies. Standard 2D *in vitro* models and *in vivo* animal models have undoubtedly contributed to the development of anti-cancer drug candidates. Yet their translation into successful clinical trials is critically low, which reinforces the need of a deeper understanding of tumorigenesis (1). Therefore, 3D models integrating tissue engineering (TE) strategies with microfluidic technology have sparked the expectation on physiologically relevant microfluidic *in vitro* models (2). The aim of this work is to establish a 3D microfluidic model that enables the reconstitution of physiological functions of microvascular tissue that emulates the human colorectal tumor microenvironment.
This model will be established via a microfluidic device with an encapsulating hydrogel compartment comprising a co-culture system of HCT-116 colorectal cancer cells and human intestinal microvascular endothelial cells.

Methods: This microfluidic device functions as a multiphase micro-bioreactor, which was built based on three major compartments; a central chamber seeded with cells embedded in a hydrogel matrix (Matrigel used as model gel), being sided by a lateral pair of channels perfused by culture medium. Human colon cancer HCT-116 cells (ATCC) and intestinal microvascular endothelial cells (Innoprot) were cultured with specific media according to supplier's specifications, at 37°C in a humidified atmosphere containing 5% CO_2. Fabrication quality control and characterization was done by optical 3D profilometry. Particle, molecule and cell tracking assessment were performed by transmitted light and fluorescence microscopy. Permeability of the hydrogels to drug transport was validated with color and fluorescent dyes within the microfluidic platform, assessed by bright-field and fluorescence imaging.

Results: Herein, we describe an "on chip *in vitro* tumor disease model" (Figure 1 a) and b)) that works as a screening tool for the generation and maintenance of stable gradients of Gemcitabine-loaded dendrimer nanoparticles (Figure 1c). The model includes a core region defined by human colorectal cancer cells (HCoMECs) embedded in a soft 3D matrix (Matrigel®) being laterally surrounded by perfused engineered endothelial microvessels (Figure 1d). Viability studies based on automated field of view imaging of the microfluidic 3D model corroborate the fact that cells are exposed to a gradient of Gemcitabine. Our system has proven to be robust, easy to operate and reproducible, opening also the possibility of gene expression analysis when cells are retrieved from the chip. In summary, we're proposing an efficient tumor-on-a-chip toxicity screening 3D platform, providing a more physiological context compared to conventional multi-well assays.

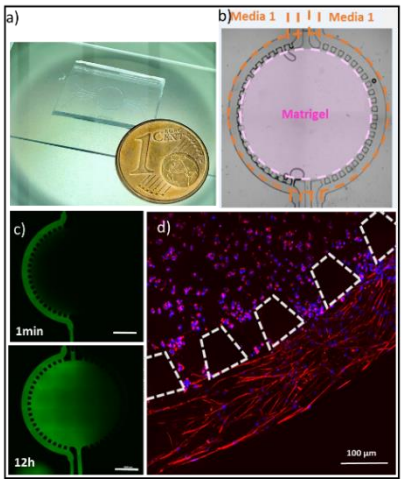

Figure 1- a) Image of microfluidic chip; b) Schematic of chip design: central chamber filled with Matrigel and two independent lateral channels for media perfusion; c) FITC-labeled nanoparticles' gradient at 1min and 12 hours (in green); d) Microvascular 3D microenvironment at day 5: DAPI/Phalloidin staining of HCT-116 cells in Matrigel (central chamber) and HCoMECs (lateral microchannels).

Conclusions: The concept colorectal tumor-on-a-chip model described herein represents a promising tool to better understand cell migration and tumorigenesis processes. The developed on-chip model can allow obtaining molecule gradients, which is foreseen to play an important role in assessing the influence of chemoattractants and drugs. Moreover, the model offers the potential to include complex microenvironments, e.g. by including other cell types, such as immune cells or patient-derived cells, which can be key to bringing us closer to an effective treatment of colon cancer.

References

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