Establishment of 3D tumor model of Head and Neck Squamous Cell Carcinomas and monocytes cells

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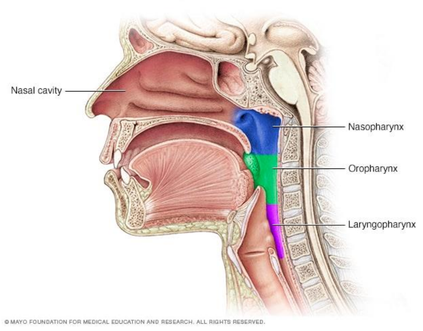
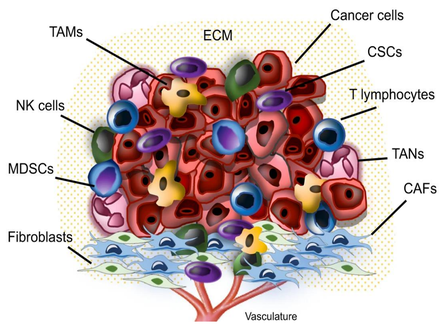
Head and neck cancer

Head and neck carcinomas (HNC) are highly aggressive and frequent cancers in Europe, particularly in Belgium where they come in fourth position in men. HNSCC is composed of a heterogeneous group of tumors developing from the mucosa of the nasal and oral cavity, oropharynx, hypopharynx or larynx. The risk factors of HNCs are mostly related to alcohol and tobacco consumption. In addition, human papillomavirus was recognized as a risk factor for oropharyngeal squamous cell carcinoma.



Tumor microenvironment

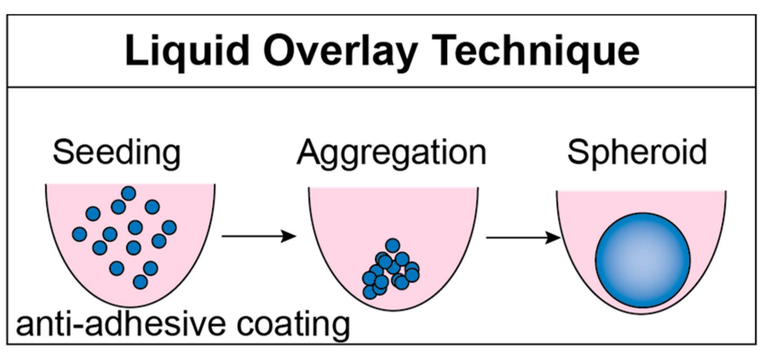
The tumor microenvironment is the ecosystem that surrounds a tumor inside the body. Tumor associated macrophages (TAM) are a key component of the TME, they play an important role in the progression of cancer. Depending on the tumor environment stimuli, macrophages have two different phenotypes: M1 macrophages have anti-tumor effects and M2 macrophages have pro-tumor actions that create a favorable environment for tumor progression. In response to regulation by HNSCC, M2 TAMs dominate the TME and are associated with poor prognosis.

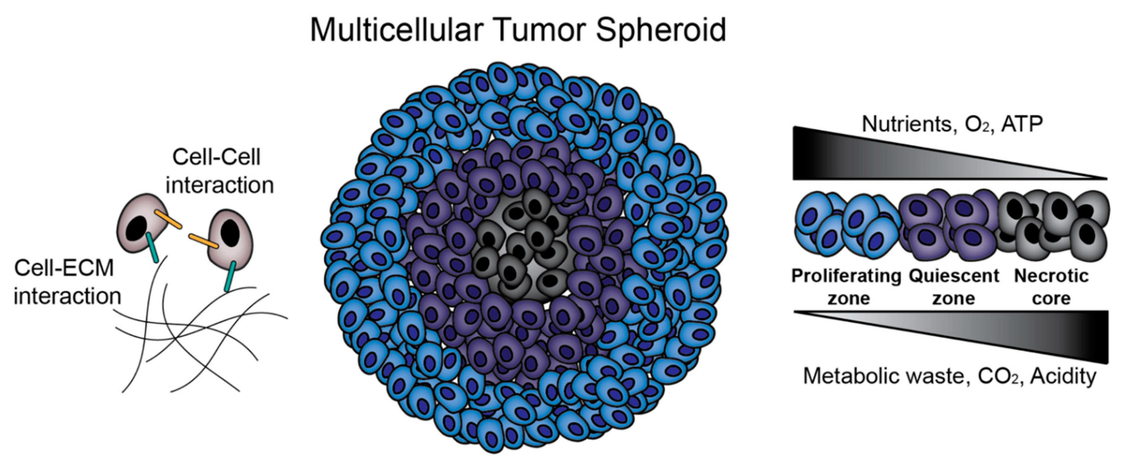


The aim of the present study is to establish a 3D co-culture model in order to characterize the mechanisms underlying the recruitment of TAMs by tumor cells and to evaluate myeloid cell migration and chemokine secretions.

Liquid overlay technique

The liquid overlay technique (LOT), referred to the ultra-low attachment (ULA) technique, is based on the self-aggregation of cells in low adhesion plates with round-bottomed. It favorises cell–cell adhesion that promotes cell-cell interactions and therefore the formation of spheroids.



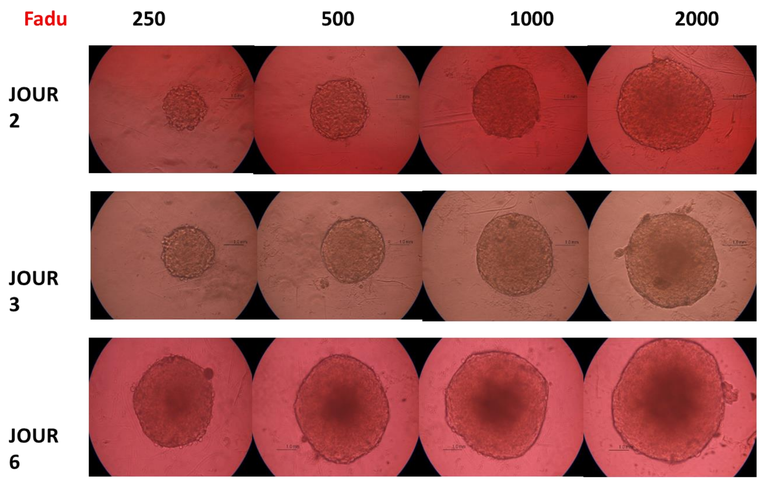


Spheroid produced through LOT can successfully represent the properties of human solid tumors. It simulates cell-to-cell and cell-to-ECM interactions, tissue-specific stiffness, oxygen, nutrient and metabolic waste gradients. This combination of tissue-specific scaffolding cells can mimic the *in vivo* environment (kamatar et al. 2020).

Preliminary results

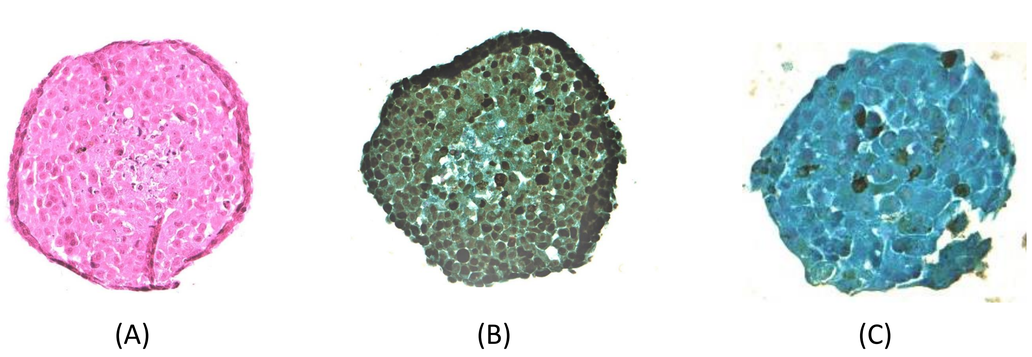
1 Culture conditions of spheroid

❖ The cells capacity to form spheroids by LOT is not similar for all types of cells. Spheroids size can be optimized by modulating the initial number of seeded cells in the non-adherent plates.



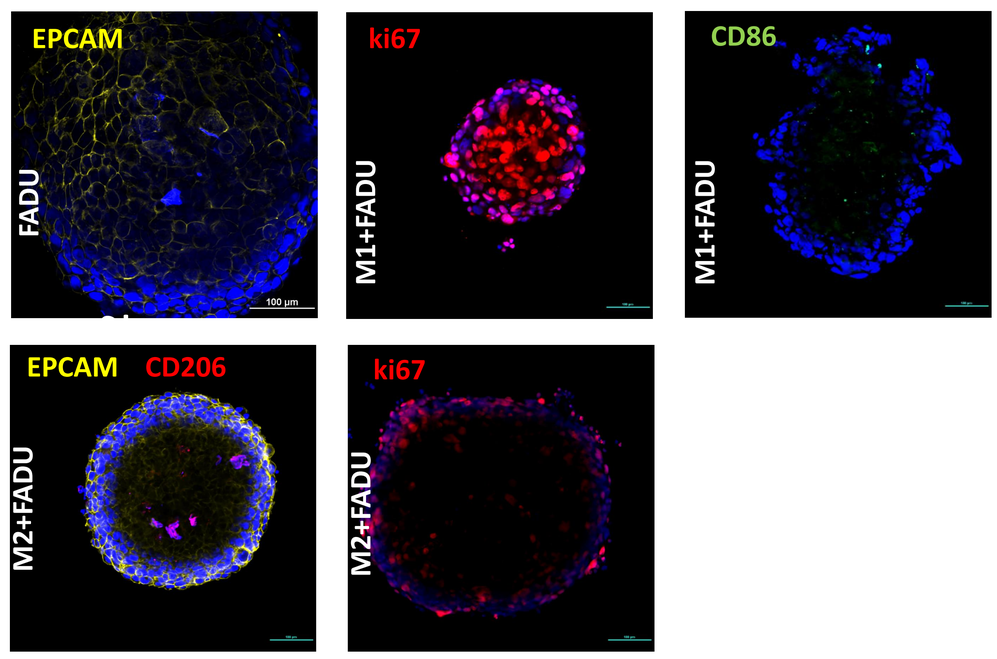
2 Paraffin embedding and immunohistochemical application of spheroid.

❖ Spheroids are fixed and embedded in 2% high melting temperature agarose, then embedded in paraffin wax. Paraffin blocks are sectioned (10µm) for Hematoxylin & Eosin (A), immunohistochemical staining with proliferation marker ki67 (B) and Macrophage marker CD68 (C).

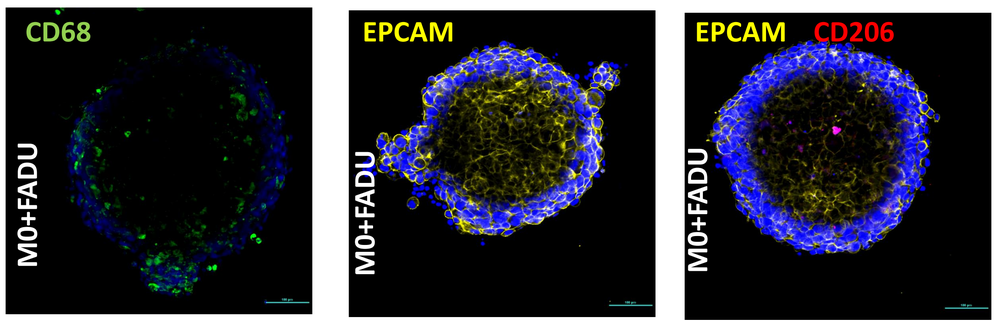


3 Immunofluorescence

❖ Macrophages subtypes (M1 and M2) are generated and co-cultured with Fadu cancer cells spheroids. After co-culture, macrophages phenotypes are analyzed by the identification of some markers by immunofluorescence : M1: CD86, M2 : CD206, Cancer cells : EPCAM and proliferation marker Ki67.



❖ M0 macrophages are cultured with Fadu-cells in order to investigate the influence of these cells on M0 phenotype.



Perspectives

A spheroid based on co-culture model will be a useful tool to assess the ability of M2 macrophages to repolarize into anti-tumor M1 macrophages and evaluate new treatment strategies which could be beneficial for patient survival.

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