Establishment of 3D co-culture Model of Head and Neck Squamous Cell Carcinomas and monocytes cells

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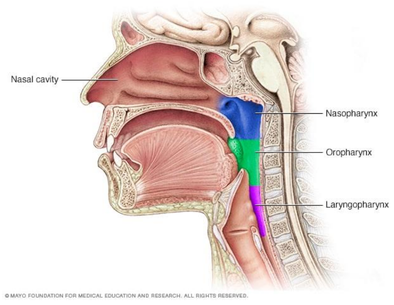
Head and neck cancer

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 vast majority of these (90%) are squamous cell carcinomas, qualified as highly aggressive solid tumors.

Due to late diagnosis, these tumors are often associated with an unfavorable prognosis despite a constant and significant evolution of therapeutic strategies.

The risk factors of HNCs is mostly related to alcohol and tobacco consumption.

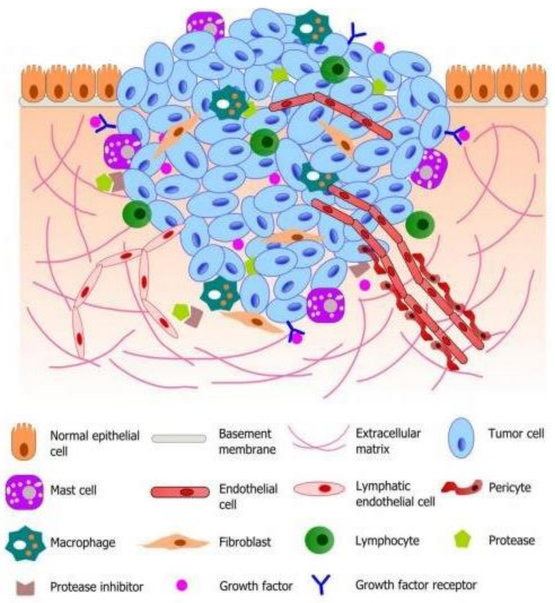
In addition, human papillomavirus was recognized as a risk factor for oropharyngeal squamous cell carcinoma



Tumor microenvironment

The tumor microenvironment is the ecosystem that surrounds a tumor inside the body. It includes immune cells, the extracellular matrix, blood vessels and other cells, like fibroblasts. The tumour microenvironment (TME) shapes disease progression and influences therapeutic response. HNSCC regulate and recruit other immune populations capable of modulating T and NK cell responses, including Tregs, myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), and cancer-associated fibroblasts (CAFs).

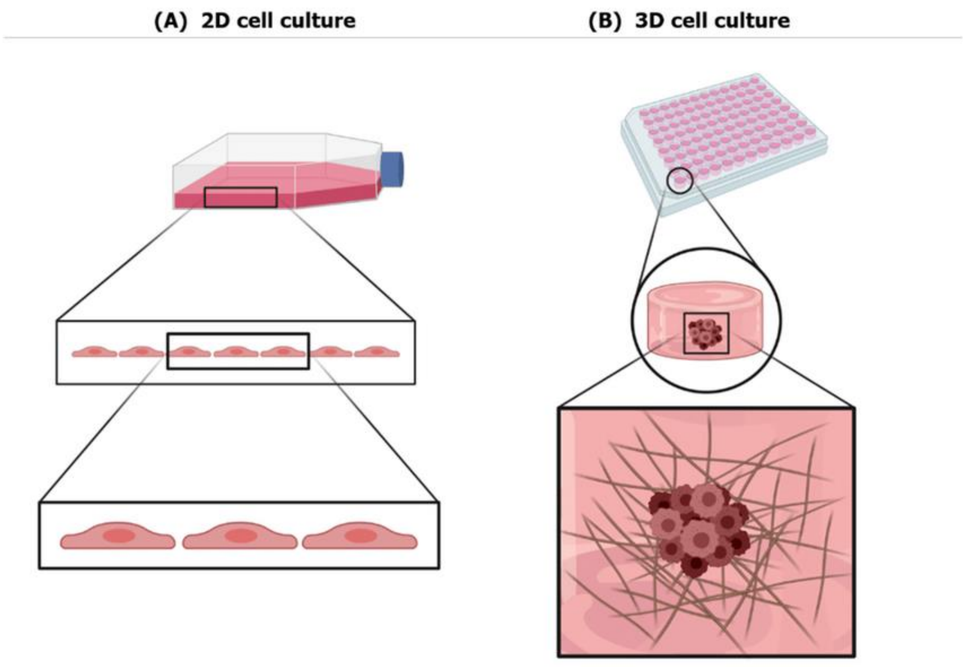
TAM are a key component of the TME and have been shown to play an important role in the progression of cancer. Depending on the tumor environment stimuli, macrophages have two different phenotypes: M1 macrophages have anti-tumor effects and M2 macrophages have pro-tumor actions to create a favorable environment for tumor progression. In response to regulation by HNSCC, M2 TAMs dominate the TME and are associated with poor prognosis.



(Xu et al., 2014)

In vitro Models

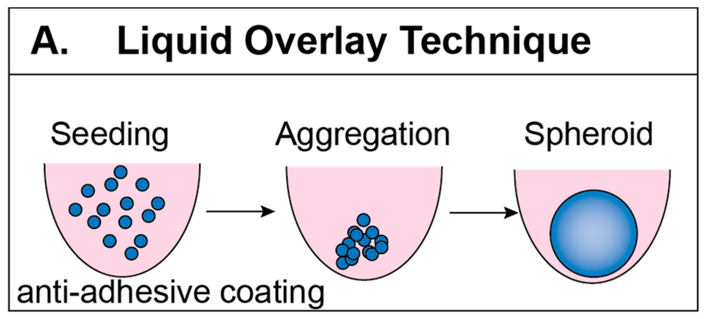
To identify the key cellular mechanisms induce an immunosuppressive tumor microenvironment, we established 3D co-culture model with head and neck cancer cells and monocytes. Using this model, we analyzed the influence of tumor cells on monocytes and their immune suppressive phenotype

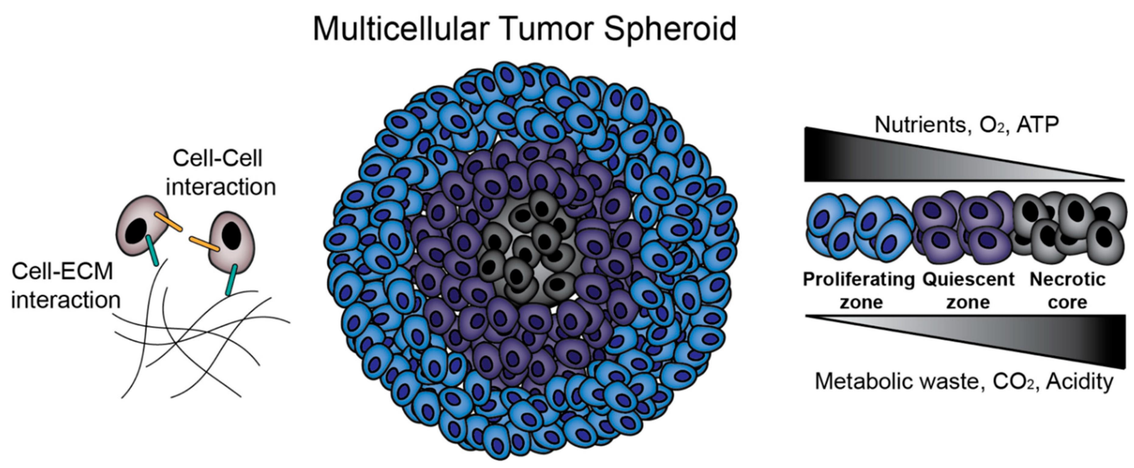


2D culture	3D culture
• Cells are grown as monolayers on flat solid surface	• Cells are grown in low adhesion plates
• Lacking cell-cell and cell-matrix interactions that are present in native tumors	• Enhanced cell-to-cell and Cell-extracellular matrix (ECM) interactions and signaling
• Not representative of in-vivo environment	• Better stimulation of in-vivo environment
• Need for animal testing for validation	• Reduction in animal usage
• Well established	• Not as widely explored

Liquid overlay technique

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





Spheroids produced through LOT can successfully represent the properties of human solid tumors include cell-to-cell and cell-to-ECM interactions, tissue-specific stiffness, oxygen, nutrient and metabolic waste gradients, and a combination of tissue-specific scaffolding cells better simulation of the *in vivo* environment in a living organism.(kamatar et al. 2020)
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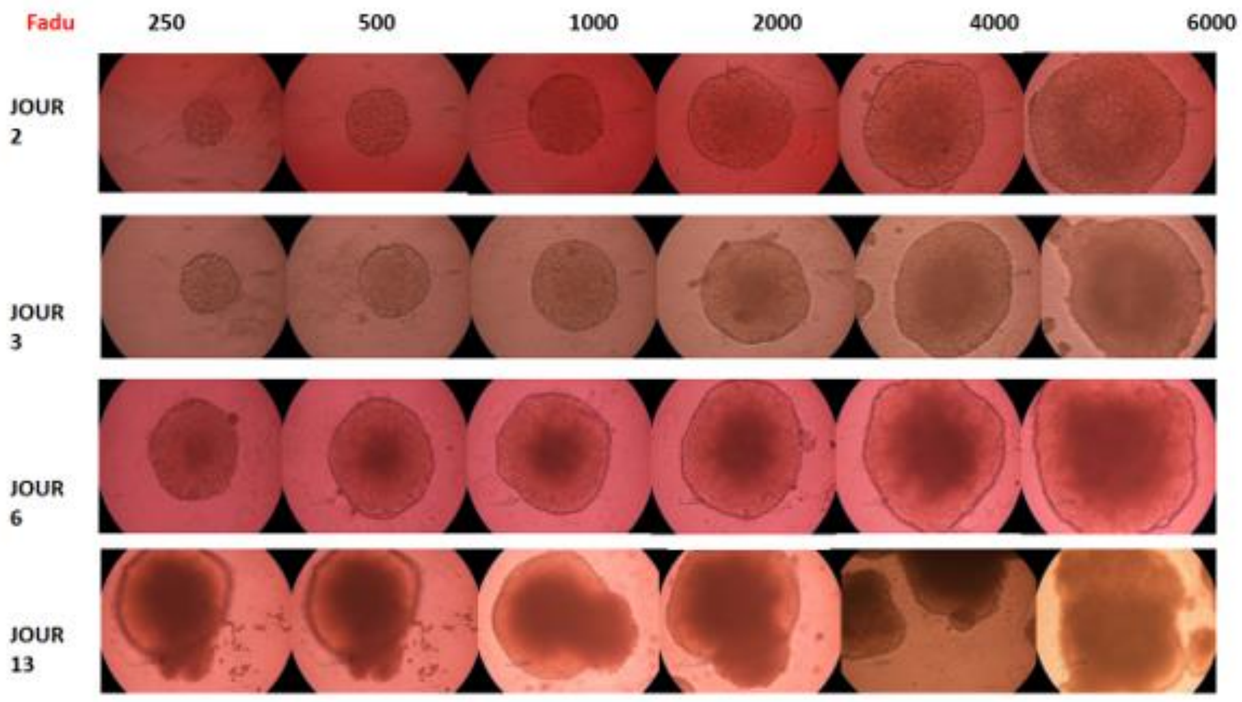
Preliminary results

1 Culture conditions of spheroid

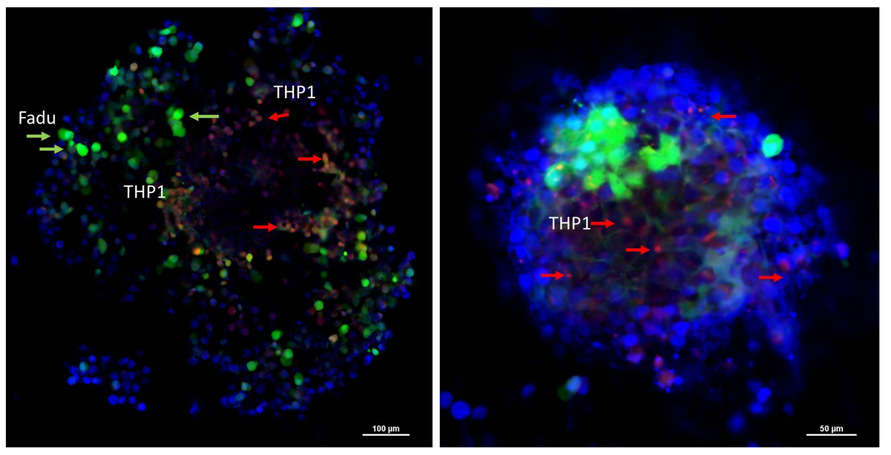
The cells capacity to form spheroids by LOT is not similar for all types of cells. Spheroids size can be optimized by modulating the initial number of cells seeded on the non-adherent .They are verified after 3 days of culture, the diameter of all spheroids increased overtime in a rate that was proportional to the initial number of cells seeded.



2 Paraffin embedding and immunohistochemical application of spheroid.

On day 7, spheroids were fixed and embedded in 2% high melting temperature agarose, then embedded in paraffin wax. (A)Paraffin blocks were sectioned (10mm) for Hematoxylin & Eosin and immunohistochemical staining with proliferation marker ki67(B).

3 Immunofluorescence of co-culture between Fadu spheroid and THP1 cells



Infiltration of transfected THP1-RFP cells in transfected Fadu-GFP spheroid identified by immunofluorescence was observed in co-culture spheroids

4 Perspectives :

A spheroid-based 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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