Evaluation of Macrophages Plasticity and Function in Head and Neck Tumor Microenvironment using 3D Multicellular Spheroid Model

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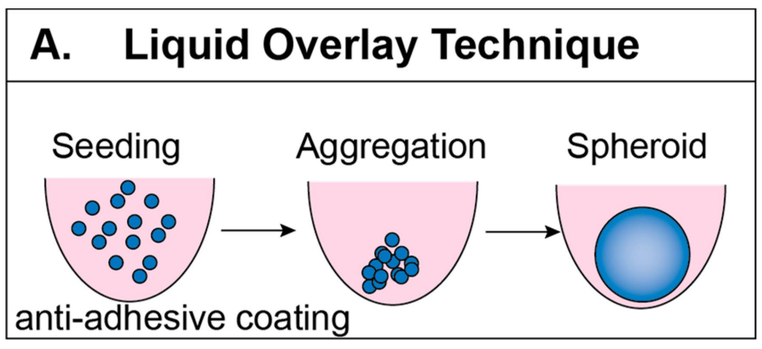
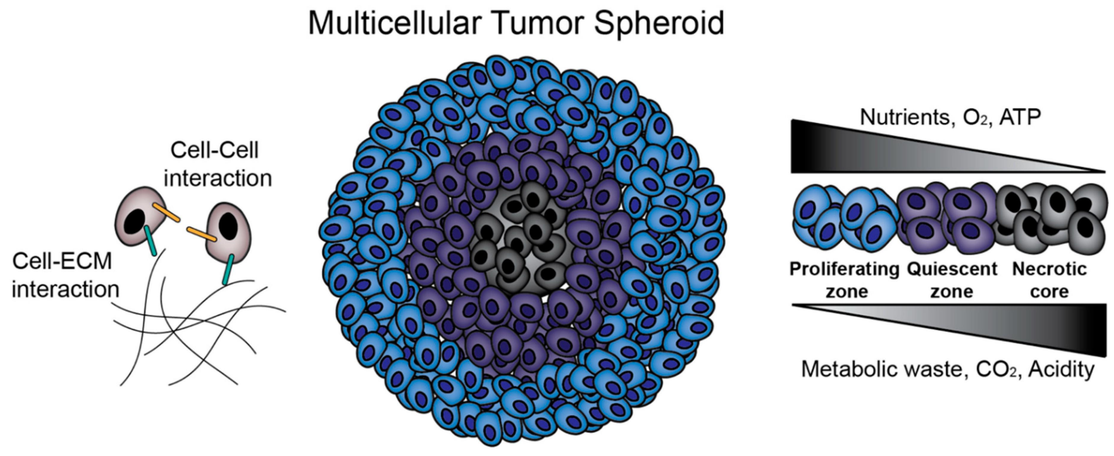
Tumors-associated macrophages (TAMs) are the key components of the tumor microenvironment (TME) and they have been shown to play important roles in the progression of head and neck cancers (HNC). Macrophages can undergo differentiation into two main functional phenotypes depending on the local tissue environment. Based on these functional phenotypes, TAMs can promote tumor progression (M2 phenotype) or inhibit it (M1 phenotype). The presence of M2 macrophages and a high ratio of M2/M1 macrophages in the TME are clinically associated with poor prognosis for HNC patients. Analysis of TAM plasticity in HNC are largely explored and targeting M2 macrophages to repolarize them through M1 phenotype would be a promising cancer treatment strategy.

The aim of the present study is to establish a 3D coculture model in order to characterize the mechanisms underlying the recruitment of TAMs by tumor cells and to repolarize M2 pro-tumoral macrophages into M1 anti-tumoral phenotype.

Liquid overlay technique

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





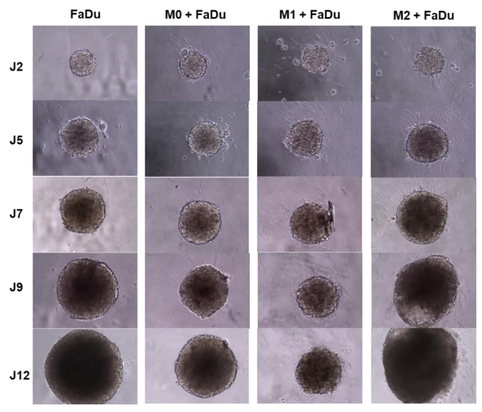
Spheroid produced through LOT can successfully represent the properties of human solid tumors. It simulates cell-to-cell and cell-to-ECM interactions, tissue-specific stiffness, oxygen, nutrient and metabolic waste gradients. This combination of tissue-specific scaffolding cells can mimic the *in vivo*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

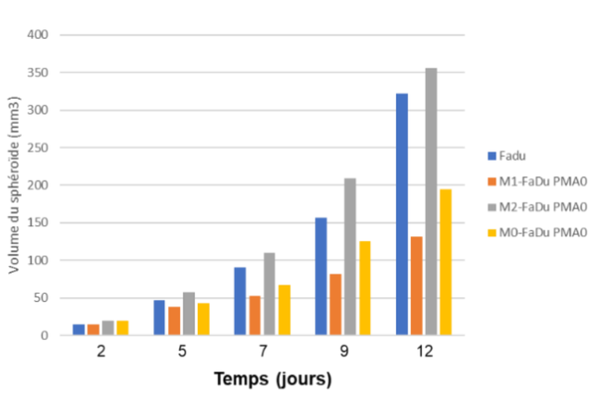
Preliminary results

Functionality of the different subpopulations of macrophage co-cultured with cancer cells

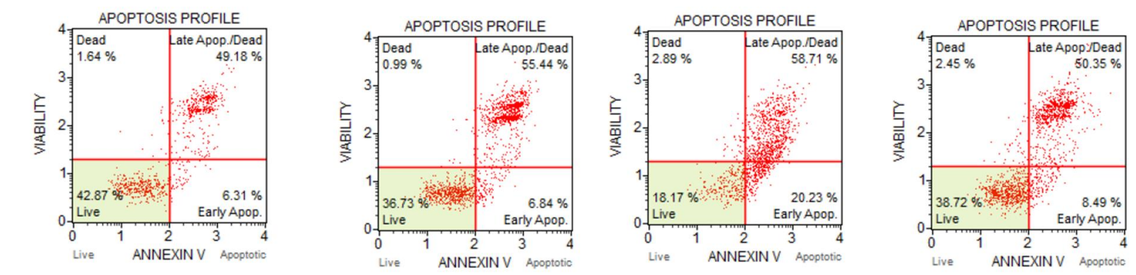
❖ Quantification of the spheroid volume over time

Spheroids are composed of cancer cells and monocytes isolated from peripheral blood mononuclear cells (PBMC) or monocytes differentiated into macrophages M1 or M2 by adding respectively LPS and IFNγ, or IL-4 and IL-13. On days 2, 5, 7, 9 and 12 every spheroid was photographed by digital photography and the volume was quantified using Image J Software.





❖ Evaluation of the apoptotic profile of dissociated M1 and M2 spheroids

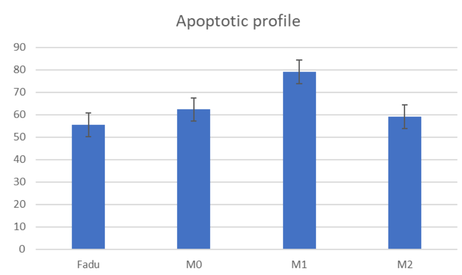


Fadu
Total Apoptotic : 55.49 %

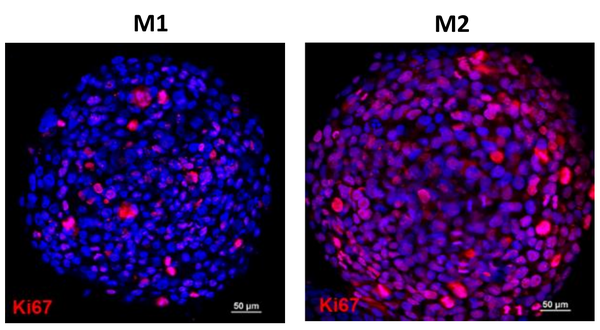
M0
Total Apoptotic : 62.27 %

M1
Total Apoptotic : 79 %

M2
Total Apoptotic : 59 %



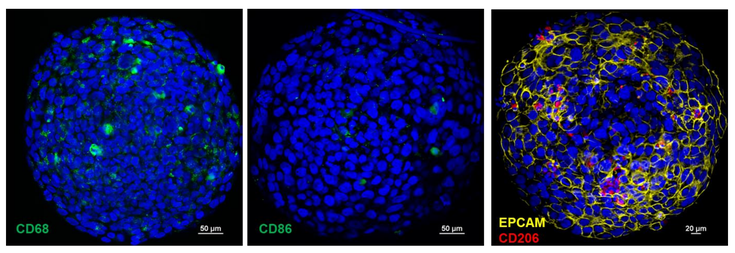
❖ Evaluation of the proliferation profile of M1 and M2 spheroids by the Ki-67 immunofluorescence

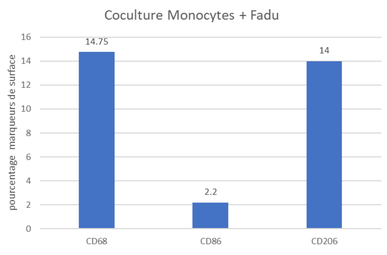


Analyze the influence of tumor cells on monocyte phenotypes

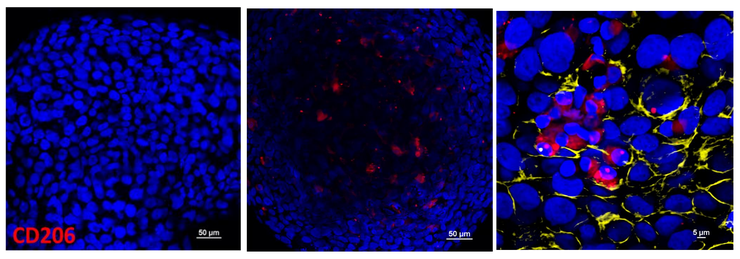
M0 macrophages are co-cultured with FaDu cancer cells in spheroids. In order to prove the infiltration of macrophages inside the spheroid, CD68 is visualized by immunofluorescence and immunohistochemistry

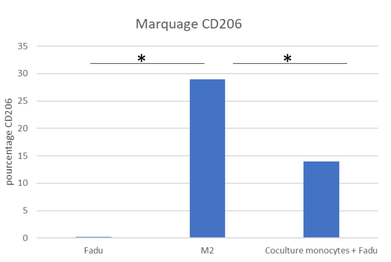
In order to investigate the influence of cancer cells on the polarization of macrophage, their phenotypes are analyzed by immunofluorescence through the identification of some markers: M1= CD86, M2 = CD206, Cancer cells = EpCAM





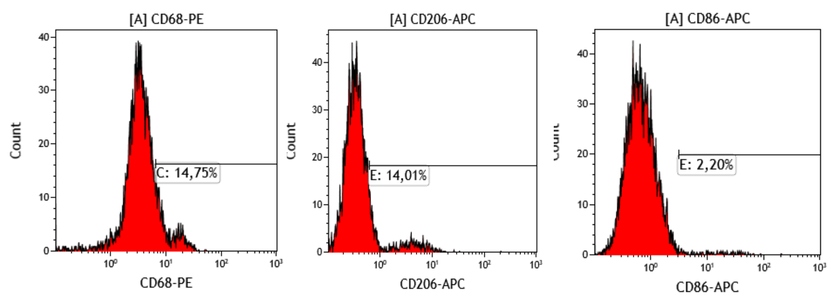
Comparison of M2 marker (CD206) in cancer cell spheroid (A), M2 spheroid (B) and cocultured M0 spheroid with cancer cells (C)





(A) (B) (C)

After coculture, spheroids composed from M0 and cancer cells are dissociated and the markers of M0, M1 and M2 are quantified by flow cytometry



We demonstrated that HNC cells in 3D culture recreates an invasive and immunosuppressive TME and induced polarization of macrophages into M2-like phenotype with high CD206 expression and low CD86 expression

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