Modelling stem cell differentiation in response to physiological stresses using 3D organoids

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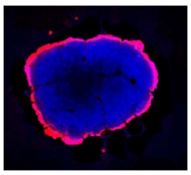
Research Themes: Cellular & molecular regulation of stem cells ; control of differentiation ; disease modelling

Background: Stem cells are a key cell population for tissue formation during development, tissue maintenance and repair throughout life. Stem cell populations are also linked to a range of pathologies, ranging from developmental abnormalities to tumours. In cancer for instance, a small population of stem cells present within the tumour and able to evade oncotherapies can lead to recurrence. Being able to model the behaviour and differentiation of stem cells in vitro is therefore a critical step to (i) characterise their specific biological properties; (ii) analyse their role in disease; (iii) develop new regenerative medicine approaches to harness their therapeutic potential.

→ The aim of this project is to model in vitro, in 3D, the way healthy and disease stem cells respond to specific physiological stresses, and how their differentiation potential is affected.

The differentiation of healthy & disease stem cells will be analysed in response to biomechanical and metabolic cues mimicking the in vivo environment through the use of 3D organoid models.

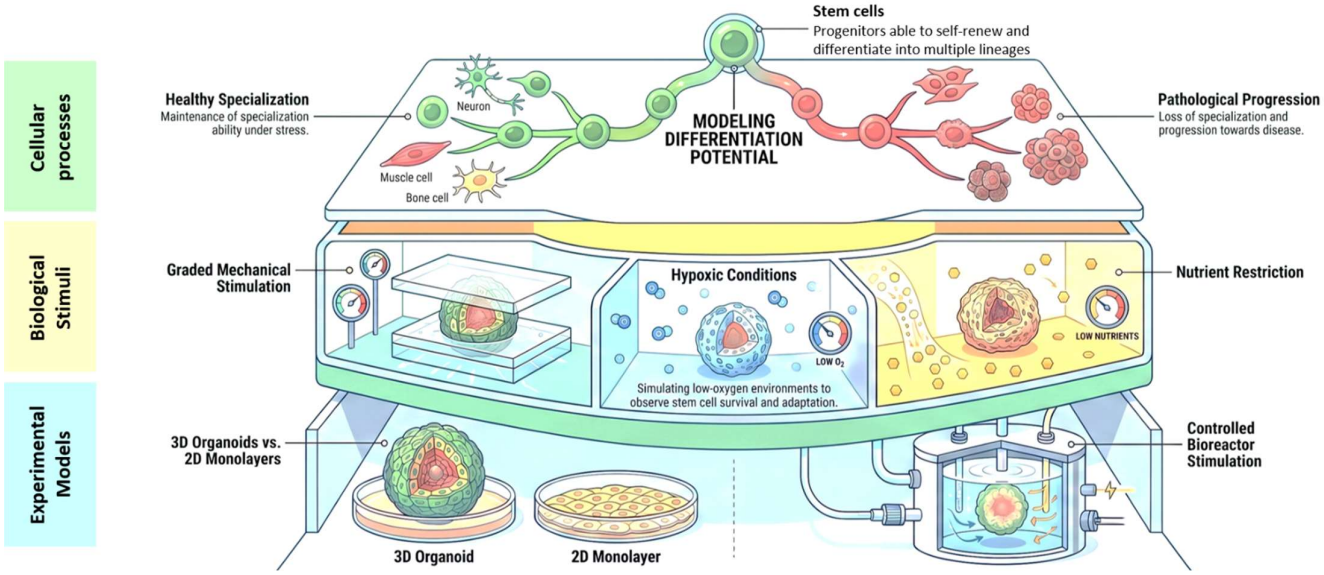


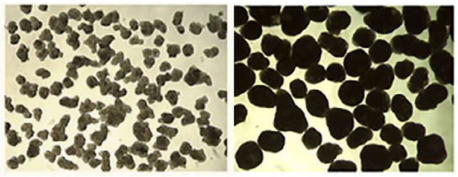
Working with 3D cell structures enables a more physiological analysis of cell behaviour compared to 2D monolayers, and the use of controlled bioreactors allows the stimulation of relevant physical and biochemical pathways to challenge them in a tissue-like environment. Using mesenchymal progenitors as primary example, the way healthy and disease stem cells respond to external stresses will be analysed in three experimental models:

- response to graded **biomechanical stimulation**, which is an important parameter regulating stem cell differentiation during tissue development, and also during tissue repair
- response to **low oxygen** conditions (comparing normoxia and hypoxia), known to influence stem cell behaviour, in both healthy tissue and in tumour masses
- response to **nutrient restriction** (comparing rich vs deprived medium), to model one of the key physiological stresses impacting on tissue dynamics, both in normal and disease models.

Key experimental steps for the project:

1. generating 3D cultures and inducing differentiated cell types, monitored using confocal microscopy
2. using a bioreactor to expose organoids to controlled culture environments (collaboration with the group of M. Conti)
3. analysing the induced phenotypical changes through marker expression analysis at protein and transcript level
4. characterising the differentiated cell populations in the 3D samples using flow cytometry and stemness assays
5. performing metabolic measurements to monitor biological changes in the samples as a result of the applied stimuli





Techniques: Stem cell culture & differentiation, 3D organoid formation, bioreactor culture, histology, immunomarker detection, confocal imaging & 3D reconstruction, metabolic assays, qPCR analysis, flow cytometry.