Insight into the material properties of a rosette of hiPSCs (human Induced Pluripotent Stem Cells).

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Engineering in-vitro cell culture systems that recapitulate a physio-mimetic environment and allow human pluripotent stem cells (hPSCs) to self-organize and grow in structures that are reminiscent of stages of the embryo development has been of great interest to unlock the potential of hPSC-based applications. The hydrogel-based cell-encapsulation technology pioneered by the "BioImaging and Optofluidics" team and implemented by Treefrog Therapeutics for cell therapy applications has been proven to mimic the epiblast rosette conformation, promoting high cell proliferation and survival while preserving the pluripotency. It is now well accepted that cells perceive mechanical signals provided by their environment or neighboring cells. The robust epiblast-like model, derived from encapsulated hPSCs provides a well-controlled in-vitro model to investigate the mechanobiology of this 3D multicellular system.

A phenomenon of interest has been observed in capsules with high cell-seeding. Multiple cysts arise in these cases and exhibit different coalescence tendencies. Coalescence has been observed in the case of smaller unconfined cysts. However, the systems mostly display an aversion to coalescence and continue growing, in contact with each other, till they are mutually compressed in the confinement of the hydrogel shell. Why and when the systems favour compression over coalescence is hypothesized to be determined by the intrinsic material properties of the cyst and the mechanical constraints at play.

Here, we discuss the initial phases of the study which aims at characterizing the material and mechanical properties of individual lumenized multicellular assemblies. Extensive imaging coupled with precise micro-pipetting mounted on a micromanipulator, has revealed that (1) the pseudo-monolayer of cells does not collapse when it is poked with a sharp glass capillary, (2) if the lumen is emptied by deflating with a capillary, the cyst structure is recovered rapidly. If the lumen is inflated at a constant pressure beyond a threshold, the system transcends from an elastic to a permanently deformed plastic state. We performed a detailed rheological study by applying sinusoidal pressures and measuring the response in strain. Surprisingly, volume conservation of the cellular material seems to break down, which suggests the formation of "fissures" between the cells of the cyst layer voids. This change in material properties probably dominates the behaviour of the system over the plasticity of the individual cells and matrix, hence making this cellular assembly a unique living and mechanical system to probe.

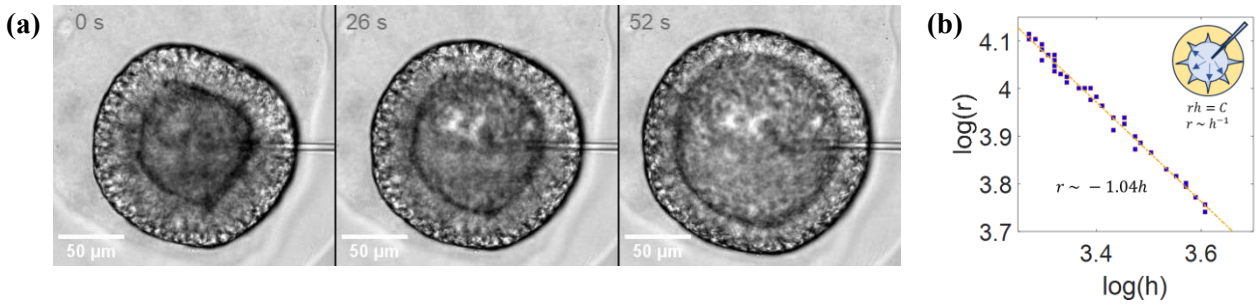

Fig1. (a) Time-lapse of an inflation of an iPSC-cyst under a constant pressure of 50 kPa. (b) Variation of equivalent radius (r) and thickness (h) as the cysts is inflated.