Acini and Microfluidics

3D epithelial cell culture in beads
A microfluidic approach for high throughput screens

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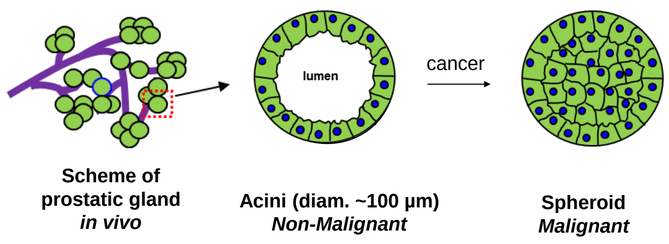
Aims and Perspectives

The challenge facing 3D cell culture today is to adapt current models to a systems biology approach - in particular, to enable RNA interference-based screens to study the effects of the microenvironment on cellular function. We have developed a microfluidic system which delivers highly-controlled conditions for the seeding and growth of polarized cellular spheres, or **acini**, within matrigel beads. We use high-throughput flow handling to change culture conditions and to move acini through flow-analysis devices on an acini-by-acini basis. Using these optimized parameters we can now design RNAi screens that will identify genes important for the regulation of acini formation in a 3D environment.

Cellular model

Traditional 3D cell culture in Matrigel and its limitations

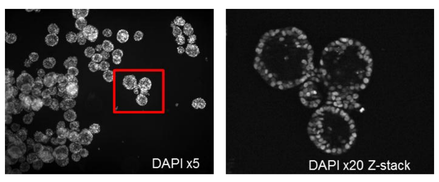


Scheme of prostatic gland *in vivo*

Acini (diam. ~100 µm) *Non-Malignant*

Spheroid *Malignant*

When cultured in 3D on laminin-rich soft substrates (**Matrigel®** rigidity range 500 Pa) epithelial cells form polarized cellular spheres (**acini**), the structure and physiological function of which highly resemble that of glandular tissue *in vivo.*

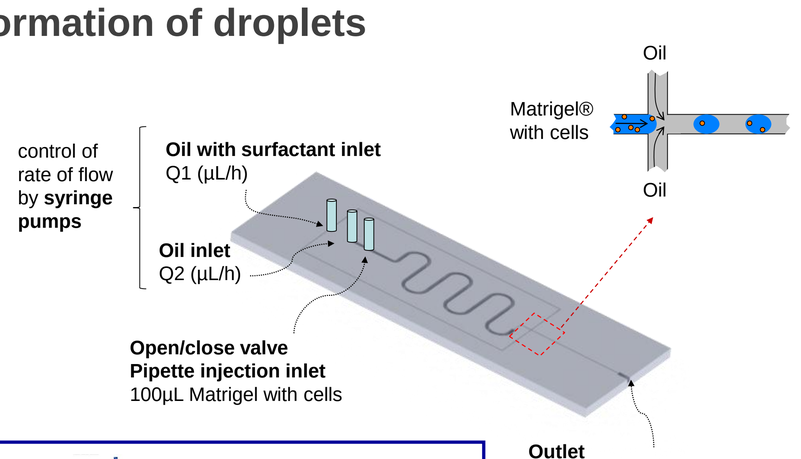


Limitations of standard 3D cell culture:

- Low control over number and distribution of cells in well (structures are overlapping and/or merging)
- Requires multidimensional acquisitions and cannot be easily automated
- No control over environment of acini
- Difficult to recover single living acini from the ECM

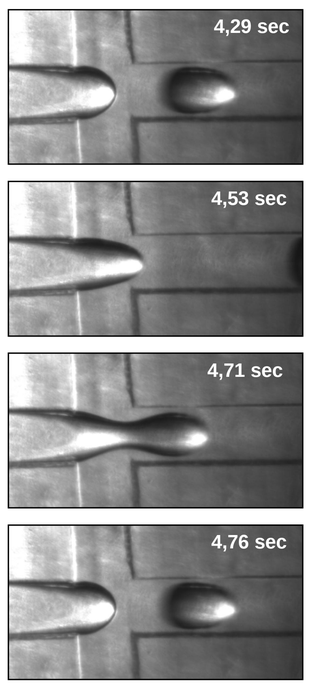
Droplet microfluidics

Formation of droplets



4° C — Formation of droplets → **37° C** — Gel polymerization transfer into culture media

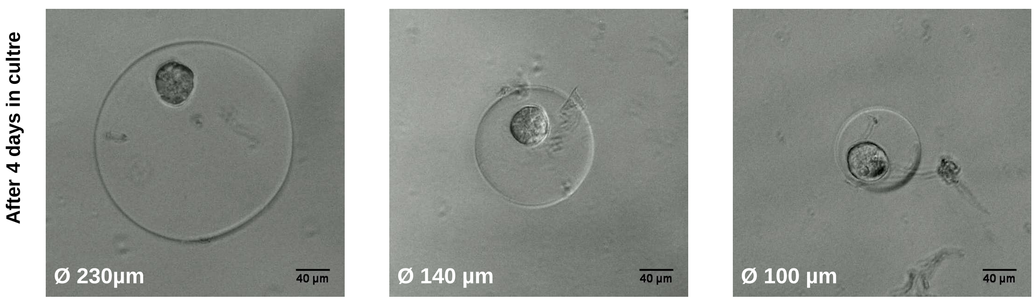
Formation of a droplet in 400 um channel



One bead generates on average a single acini

New possibilities: high throughput screen on living acini

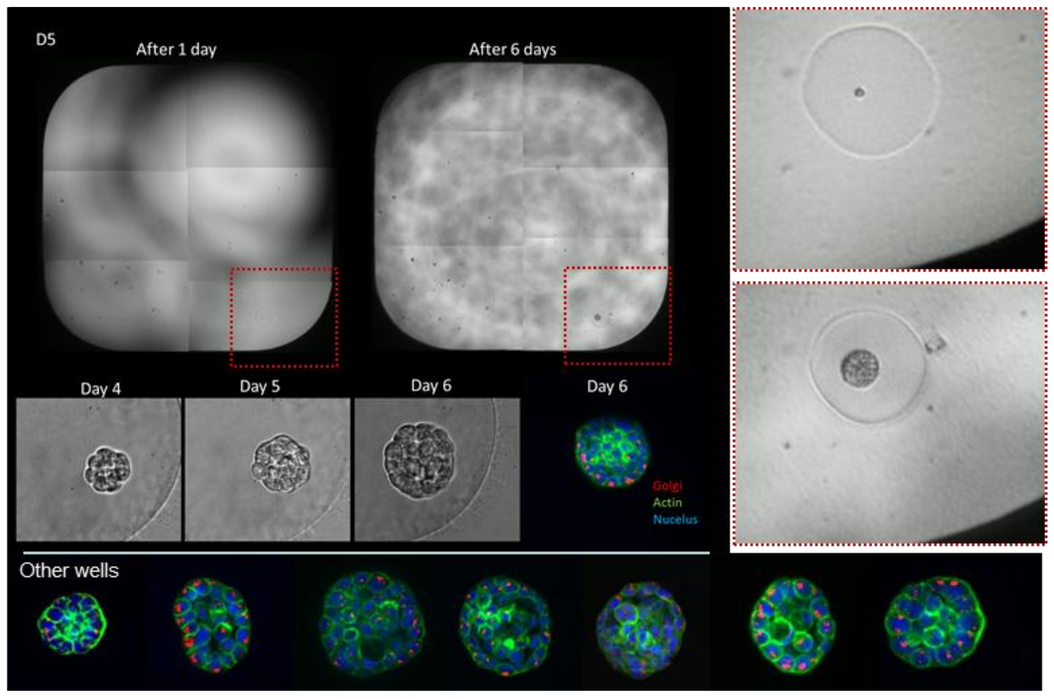
How does the 3D constraint influences acini polarity?



Is it possible to control size of polarized structures by constraining acini in beads of different sizes?

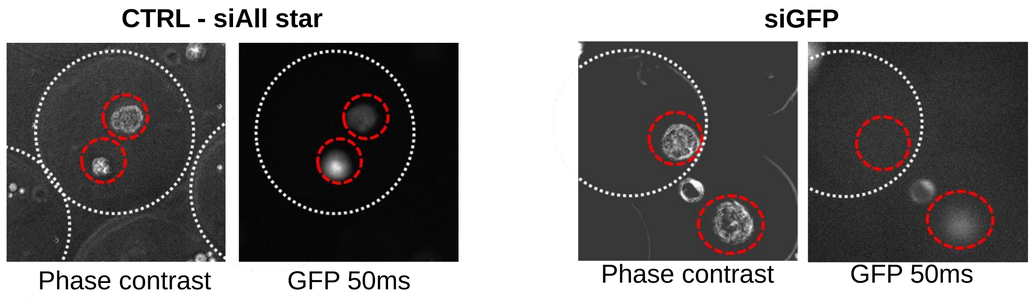
In standard 3D culture acini are growing heterogeneously in size ranging from ~80 µm to even 230 µm. It remains unknown how and if the size of the acini influences its function and response to treatment (f.e. siRNA, drugs).

Is a single cell able to form acinus?

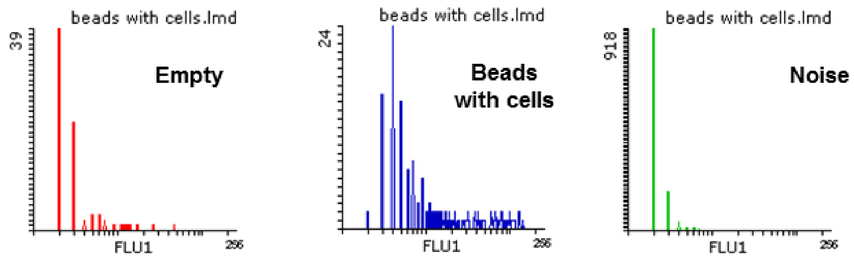


A single cell encapsulated in a matrigel bead and isolated in 384 well plate begins to form acinus only after 6 days of culture. We proof that the autocrine signals are sufficient for development of acini.

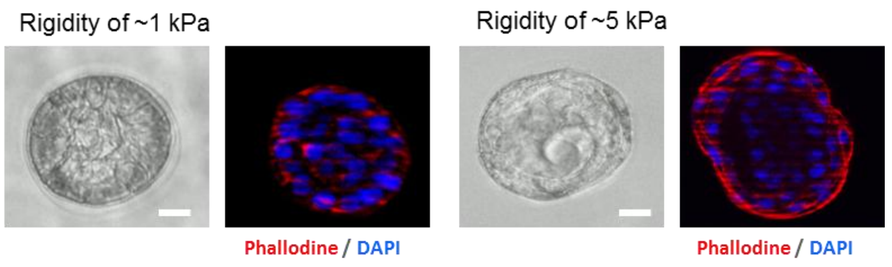
siRNA treatment and flow-based analysis



MCF10A-GFP cells encapsulated in matrigel beads were treated with siGFP (pictures above) and fluorescent signal has been analyzed with large particle FACS (graphs below).



Control over rigidity in 3D



Matrigel as a natural ECM promotes cellular growth and differentiation, however, its rigidity falls in range of 400Pa. Therefore, studies on the effect of stiffness of ECM on morphogenesis of the acini are limited. We propose a system where matrigel beads are immobilized in other hydrogel (in here agarose) of various mechanical properties. At certain moment acini fill up the volume of the bead and start to sense a rigidity from external hydrogel.

Acknowledgments

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