Microbial encapsulation in monodisperse hydrogel microspheres enables fast and sensitive phenotypic analyses using flow cytometers

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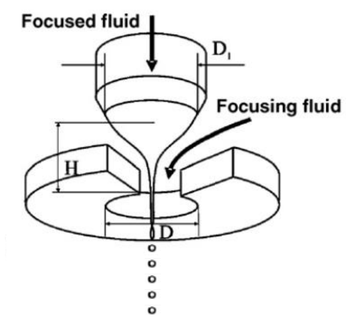
Abstract

Detection and characterization of microorganisms usually involves culture during more than 20 generations in order to achieve the formation of macrocolonies on solid media. Alternatively, microencapsulation allows the detection of microbial growth by monitoring the development of microcolonies from encapsulated individual cells. Microbial proliferation inside the microcapsules can be detected using flow cytometry, provided that the population of microparticles exhibits appropriate optical and mechanical properties and is monodisperse in size and shape.

Here we show the successful application of the Flow Focusing® technology to the microencapsulation of different types of cells in monodisperse hydrogel micropheres. Using a CellENA® Flow Focusing® microencapsulator, we managed to produce monodisperse alginate microparticles containing individual bacteria, yeast and human stem cells. Alginate particle sizes were reproducibly selected from less than 100 µm to over 600 µm, by just replacing the disposable nozzle. Sterility was preserved during the microencapsulation procedure, preventing undesired contaminations.

Microencapsulated microorganisms were utilized for a variety of applications: from characterizing secreted enzymes to detection of thermosensitive mutants. Proliferation inside the particles was monitored by flow cytometry without requiring fluorescent labelling.

1) Cell microencapsulation by Flow Focusing



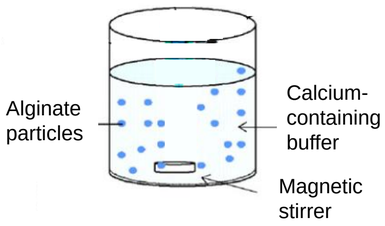
Cellena microencapsulator

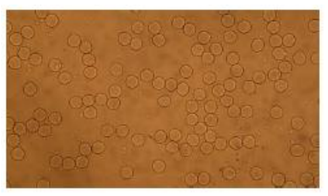
Procedure:

1) Focused fluid: alginic acid solution containing sample cells

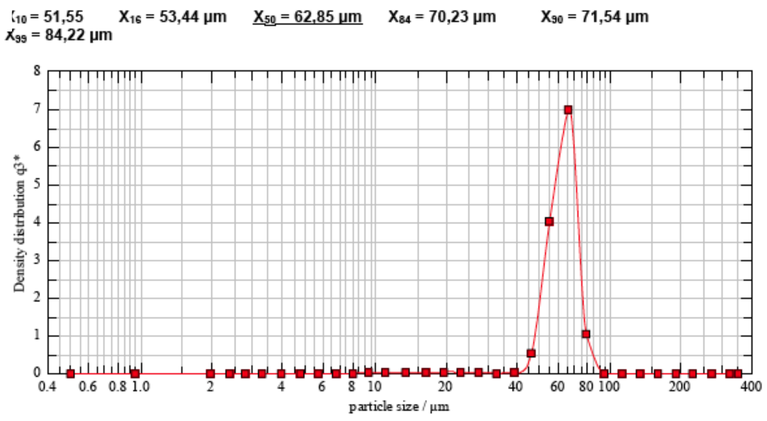
2) The sample is injected through a capillary feed tube.

3) The stationary jet breaks up by capillary instability into homogeneous droplets which gelify in a continuously stirred calcium chloride solution at room temperature.



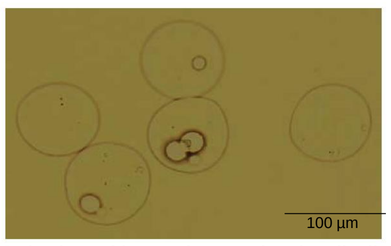


Resulting microcapsules are spherical and monodisperse in size



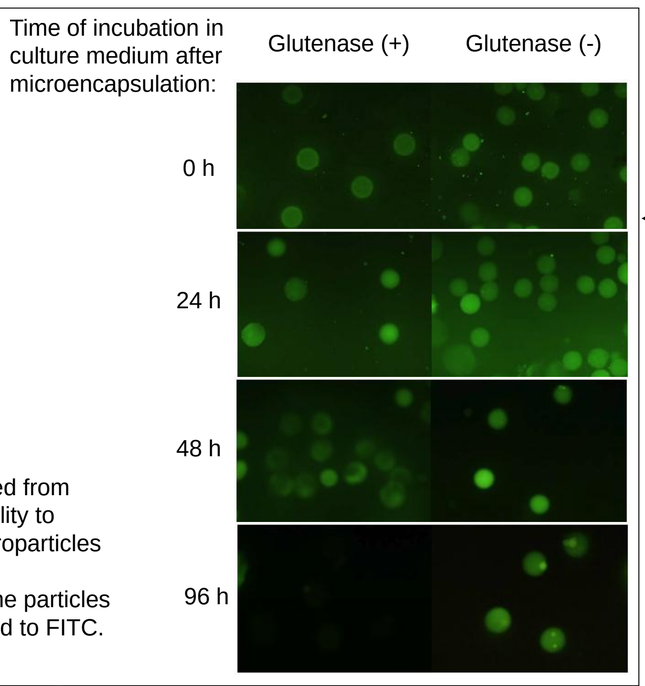
L. Martín-Banderas, M. Flores-Mosquera, P. Riesco-Chueca, A. Rodríguez-Gil, A. Cebolla, S. Chávez and A. Gañán-Clavo
Flow Focusing: A versatile technology to produce size-controlled and specific-morphology microparticles. Small 1: 688-692, 2005

2) Detection of glutenases in microencapsulated bacteria



Colonies of bacteria growing in gliadin-containing microparticles.

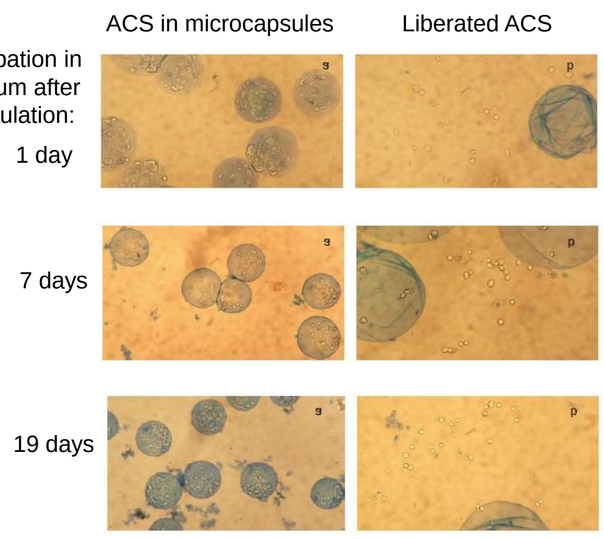
Bacteria expressing glutenase activity, isolated from agricultural samples, were detected by its ability to degrade gliadin when growing inside the microparticles obteined with a Cellena microencapulator.
Gliadin content was detected by incubating the particles with the monoclonal antibody G12, conjugated to FITC.



3) Preserving human stem cells by microencapsulation

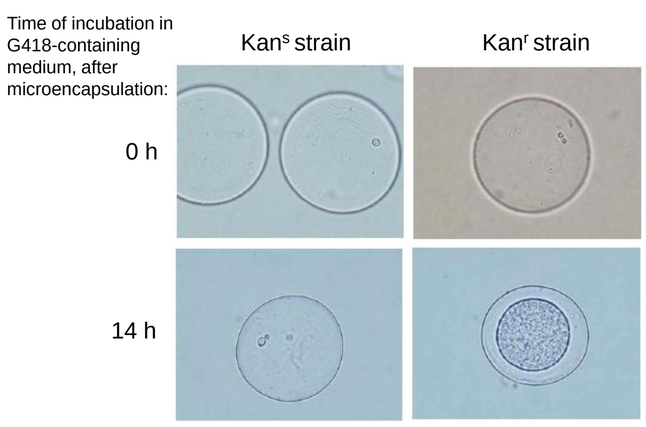
Adipocyte stem cells (ASC) aggregate shortly after being resuspended from monolayer cell culturing:

In contrast, ASC keep separated and alive for more than two weeks after being microencapsulated.
Viability was detected by treatment with Tripan blue.
After the indicated time, cells were liberated by chelating calcium from the particles with citrate buffer.



4) Phenotypic analyses of yeast mutants

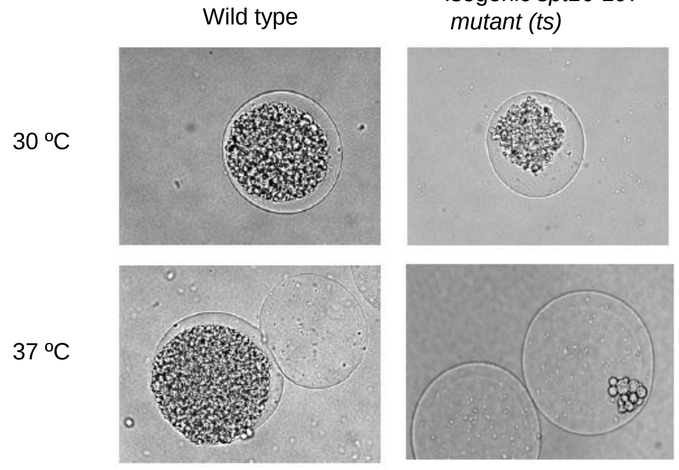
Detection of Kan^r *Saccharomyces cerevisiae* cells:



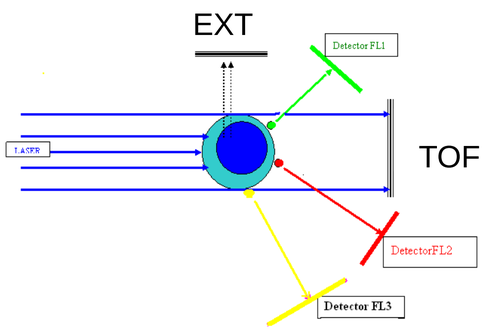
In spite of cell proliferation, microcapsules remain intact and do not increase in size:

Detection of thermosensitive *Saccharomyces cerevisiae* cells:

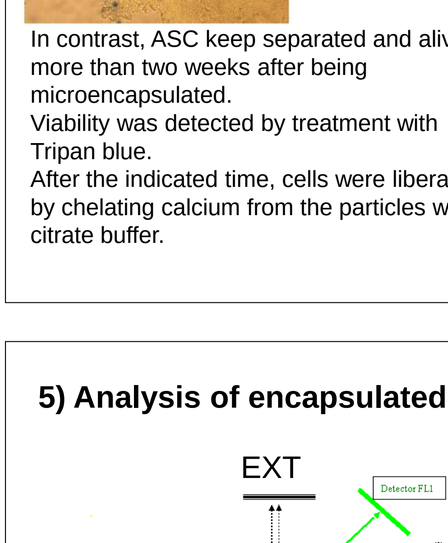
After microencapsulation, cells were incubated in culture medium for 16 hours at the indicated temperature.

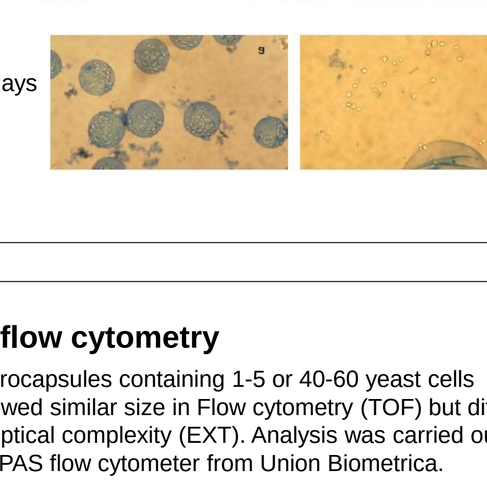


5) Analysis of encapsulated yeast by flow cytometry



Microcapsules containing 1-5 or 40-60 yeast cells showed similar size in Flow cytometry (TOF) but differed in optical complexity (EXT). Analysis was carried out in a COPAS flow cytometer from Union Biometrica.





6) Conclusions

- The Flow Focusing technology, utilized in Cellena microencapsulators, allows efficient encapsulation of bacteria, yeast and human cells.
- Microencapsulation can be used for phenotypic microbial analyses, including the expression of specific characters (glutenase in bacteria) or growth-related phenotypes (antibiotic resistance and ts in yeast).
- Encapsulation in alginate capsules is reversible, as cells can be liberated by treatment with citrate.
- Microcapsules are spherical and monodisperse in size, allowing analysis in flow cytometers.
- Proliferation inside the particles can be detected by monitoring optical complexity, without requiring fluorescent labelling.