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

Lidia Delgado¹, Gloria Jurado², Gema Galayo², Elena Ogalla², Lourdes Moreno³, Juan C Rodríguez-Aguilera⁴, Ángel Cebolla⁵, Carolina Sousa⁶, Maria Flores² and Sebastián Chávez⁷

¹Department of Genetics, Universidad de Sevilla, Seville, Spain. ²Ingenierías Tecnológicas SL, Spain. ³Department of Microbiology and Parasitology, Universidad de Sevilla, Seville, Spain. ⁴Centro Andaluz de Biología del Desarrollo, Universidad Pablo de Olavide, Seville, Spain. ⁵Biomedical SL, Spain

Abstract
Detection and characterization of microorganisms usually involves culture during more than 20 generations in order to achieve the formation of microcolonies 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 microorganisms 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 microspheres. Using a Cellena® Flow Focusing® microencapsulator, we managed to produce monodisperse alginate microcapsules 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.

2) Detection of gliadins in microencapsulated bacteria

Colonies of bacteria growing in gliadin-containing microparticles.

Bacteria expressing gliadinase activity, isolated from agricultural samples, were detected by its ability to degrade gliadin when growing inside the microcapsules obtained with a Cellena microencapsulator. 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 Trypan 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 KRN 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 (gluatinase 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.