Encapsulation in monodispersed hydrogel microspheres enables fast and sensitive phenotypic analyses using COPAS large particle flow cytometry.

Work performed in 2010 by:
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Objective

Detection and characterization of microorganisms usually involves culture during more than 20 generations in order to achieve the formation of visible macro colonies on solid media. Similarly, handling and analysis of other cell types individually is difficult. Few methods exist for establishing and maintaining clonality. Here we show the successful application of microencapsulation to address these challenges. Different types of cells can be encapsulated and these particles can be analyzed and dispensed as monodispersed hydrogel microspheres.

Introduction

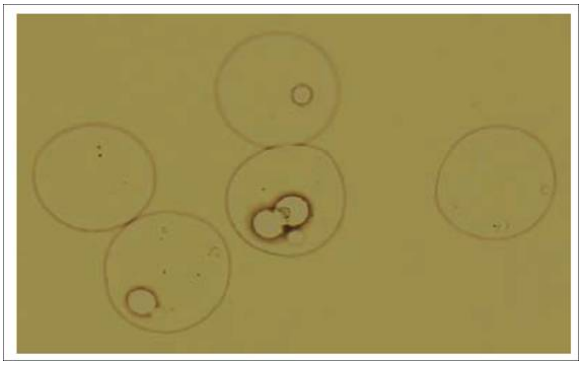
Three dimensional scaffolds provide significant innovative advances in the handling and use of live cells. Scaffolds can be used in a number of ways, such as for the isolation of individual cells to form clonal populations, for establishing partial barrier for cells from their environment, and for creating a matrix allowing the formation of 3D cell cultures. These methods have important implications for the biomedical engineering field and the areas of biomanufacturing and bioprocessing. However, much is left to be learned about 3D-matrix fabrication and use. For example, the chemical composition and engineered functionalities affect the biology of the encapsulated cells. One particular system that has achieved wide usage has been the alginate polymer. A variety of cell types can be enclosed in alginate, maintaining their morphology and function; they proliferate, form cell clusters, and even lay down extracellular matrix components. Furthermore, the cells can be encapsulated in small particles that can then be handled, characterized and analyzed. These features make alginate a widely acceptable material for three dimensional scaffolds of live cells.

Materials and Method

Using a Cellena® Flow Focusing® microencapsulator, we managed to produce monodispersed 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 COPAS large particle flow cytometry without requiring fluorescent labeling.

Sample 1: Detection of glutenases in microencapsulated bacteria

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



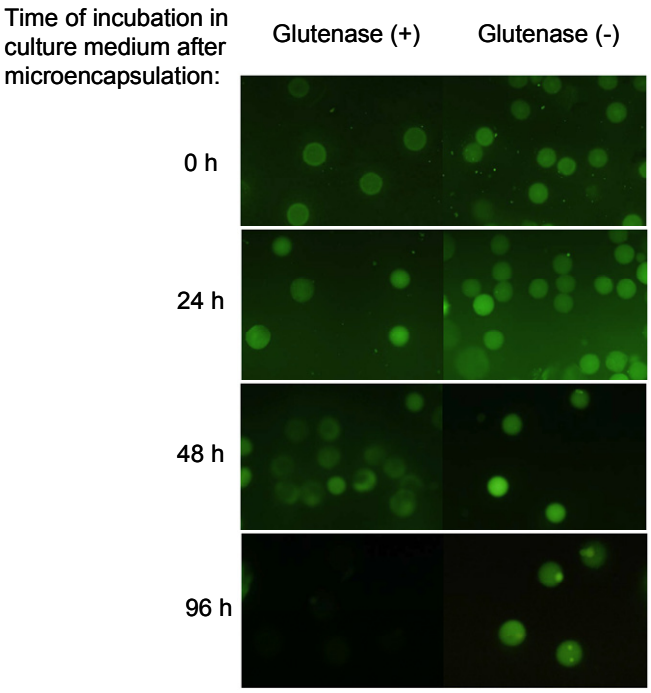


Figure 1 and 2: Colonies of bacteria growing in gliadin-containing microparticles.
Fluorescence intensity of each individual alginate particle can be detected and quantified by the COPAS large particle flow cytometer.

Sample 2: Preserving human stem cells by microencapsulation

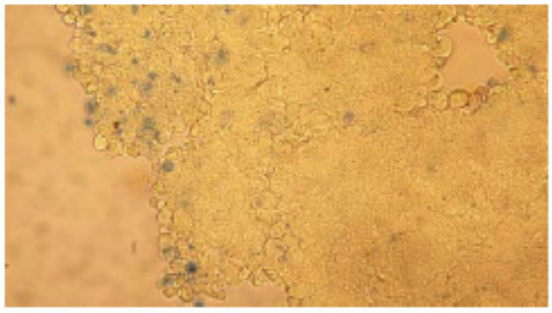
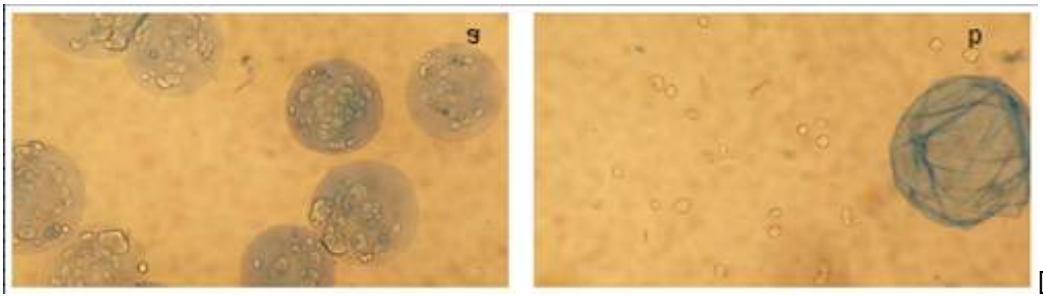
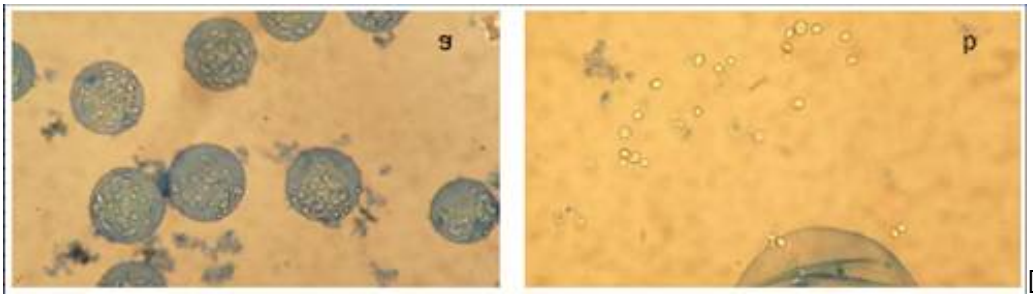


Figure 3: Adipocyte stem cells (ASC) aggregate shortly after being resuspended from monolayer cell culturing.



Day 1

Figure 4a and 4b



Day 19

Figure 5a and 5b

Figure 4a and 5a: ASC aggregates kept separated and alive for more than two weeks after being microencapsulated. Viability was detected by treatment with Trypan blue.
Figure 4b and 5b: After the indicated time, cells were liberated by chelating calcium from the alginate particles with citrate buffer releasing the cells.

Alginate encapsulated ASC can be analysed and sorted using COPAS large particle flow cytometry.

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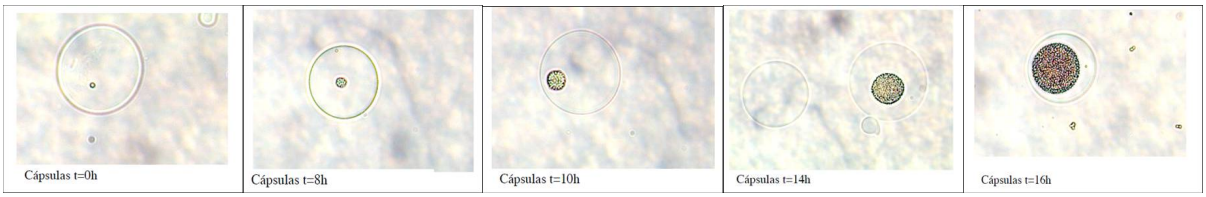
Sample 3: Analysis of encapsulated yeast by COPAS flow cytometry

Microcapsules containing yeast colonies incubated for different times showed similar size (TOF) but differed in optical complexity (EXT). Analysis was carried out in a COPAS SELECT flow cytometer.



T=0h T=8h T=10h T=14h T=16h

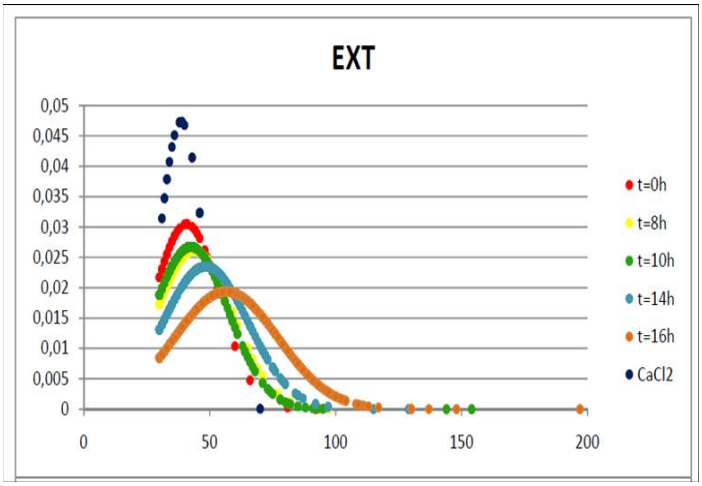


Figure and table 6: Optical density (EXT = x-axis) measurements of encapsulated yeast following the time points indicated (below the images of the encapsulated yeast).

Conclusion

Microencapsulation, using Flow Focussing technology, can be used for phenotypic analyses of the encapsulated cells, such as the expression of specific characteristics of microbial colonies (glutenase expression by bacteria) or growth-related phenotypes (antibiotic resistance and proliferation in thermosensitive yeast). Mammalian cells are delicate and difficult to handle and manipulate individually. However, these cells can be encapsulated within alginate, and these particles remain intact through the steps of analysis and dispensing. Furthermore, fragile cells like adipocyte stem cells remain viable for extended periods of time (in this test > 2 weeks) and can be released and recovered from the alginate particles, allowing for further analysis and use.
All alginate encapsulated cells of various sizes can be analysed and sorted using the COPAS large particle flow cytometers.

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