

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 Sousa³, María Flores² and Sebastián Chávez¹

¹Department of Genetics, Universidad de Sevilla, Seville, Spain

²Ingeniatrix 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

⁵Biomedal SL, Spain

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 microspheres. 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.