Fabrication of Living Yeast Cellosomes by Polyelectrolyte Mediated Assembly and Magnetically Responsive Templates

Rawil F. Fakhrullin[1] **Marie-Laure Brandy[4], Olivier Cayre[2], Orlin D. Velev[3] and Vesselin N. Paunov[4]**

(1) Department of Biochemistry, Kazan State University, Kreml uramı 18, Kazan, Tatarstan, Russian Federation 420008. (2) School of Process, Environmental and Materials Engineering, University of Leeds, Leeds, LS2 9JT, UK; (3) Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC 27695, USA; (4) Department of Chemistry, University of Hull, Hull, UK HU6 7RX.

We have developed a new method for fabrication of living multicellular structures that could show how colonial organisms evolved in nature and be used in tissue engineering. These structures, termed cellosomes, were made by layering yeast cells on to templates of aragonite (rod-shaped) and calcite (rhombohedral) microcrystals. We coated yeast cells with a layer of an anionic polyelectrolyte and the templates with a layer of cationic polyelectrolyte to promote their irreversible binding and adhesion. The templates were pre-coated with magnetite nanoparticles so they could be manipulated with an external magnetic field. We used this to extract the cell-coated templates by magnetic separation which is very useful for selective sorting and separation of the cellosomes from the excess of single cells. The templates were dissolved with ethylene diamine tetra acetic acid to give rod- and rhombohedral-shaped, hollow 3D cellosomes. We also pioneered a similar technique for templating of air micro-bubbles with yeast cells to produce spherical cellosomes. We analyzed the obtained hollow structures with Scanning Electron Microscopy to study their arrangement in the cellosome “membrane” and also used fluorescence microscopy after treating them with fluorescein di-acetate to find that the yeast cells were still active in these assemblies and remained viable for more than two weeks. These cellosomes resemble primitive multicellular organisms like *Volvox* to a certain degree, so we could speculate that nature has used a similar assembly mechanism in evolution. The technique, works not only with yeast cells but also with many other kinds of cells. It could be applied with stem cells and opens new possibilities for novel ways of engineering tissues where their shape can be directed by the shape of the micro-template. Our method also works for fabrication of living cellosomes of various shapes and from different types of cells, to produce symbiotic colonies of cells which is the next step in the design of an "artificial" living multicellular organism.
We also report a related technique for polyelectrolyte mediated magnetization of living cells which leads to deposition of an integrated layer of magnetite nanoparticles on the surfaces of yeast cells. We show that these “magnetic yeast” cells preserve their viability and can be manipulated by external magnetic field. We demonstrate the usefulness of these materials by fabricating magnetic yeast cells that have been genetically modified to express their Green Fluorescent Protein (GFP) gene whenever the cells repair damaged DNA. We report a simple microfluidic device in which these cells were directed by external magnetic field and used to simultaneously detect genotoxicity and cytotoxicity.