DOI: 10.1017/S1431927603440452
Microsc Microanal 9(Suppl 2), 2003

Crystallization of NaCl and $CaCO_3$ from Solution Using Magnetotactic Bacteria as Laser Beam Energy Absorbers under the Optical Tweezers Microscope

C.N. Keim[1, 2], L.G. Lutterbach[2, 3] and M. Farina[1, 2]

[1] Laboratório de Biomineralização, Instituto de Ciências Biomédicas; [2] Laboratório de Pinças Ópticas, COPEA - UFRJ; [3] Laboratório de Neurobiologia II, Instituto de Biofísica Carlos Chagas Filho; [1, 2, 3] CCS, Universidade Federal do Rio de Janeiro, Cidade Universitária, 21941-590, Rio de Janeiro, RJ, Brazil. E-mail: mfarina@anato.ufrj.br

Magnetotactic bacteria are motile microorganisms presenting intracellular membrane-bounded magnetic crystals, usually composed of magnetite (Fe_3O_4). These structures function as magnetic compass orienting the whole organism along magnetic field lines [1]. When trying to catch marine magnetotactic bacteria with the optical tweezers, we noted that, surprisingly, the laser beam killed the bacteria. Focusing the laser onto magnetotactic bacteria concentrated in the border of a drop of water caused "explosions" as if the water was locally boiling (fig. 1) and/or the formation of salt crystals from seawater. Then, we decided to study the crystallization of NaCl and $CaCO_3$ induced by the laser beam of the optical tweezers directed towards magnetically isolated uncultured marine magnetotactic bacteria.

Samples containing magnetotactic bacteria were collected in Itaipu lagoon, near Rio de Janeiro city, Brazil. The bacteria were magnetically separated and further concentrated at the border of a drop of water put onto coverslips, using a common magnet. The seawater was carefully changed by either NaCl (3.5 to 30%, pH 7.0) or $CaCl_2/NaHCO_3$ (3:1; ionic strength = 0.025 to 0.1M, pH 8.5) solutions. Experiments were performed in an inverted microscope equipped with a Neodymium-YAG (λ = 1064nm, E $\approx$ 100 to 200mWatt) laser set for optical tweezers.

Most of the NaCl crystals formed were cubic (fig. 2). It was observed that the higher the energy of the laser beam and the salt concentration, the easier the crystal formation and growth. Fig. 3 shows a video sequence of the formation of a $CaCO_3$ solid structure when focusing the laser beam onto magnetotactic bacteria. Without the laser beam, the crystals formed in this solution were rombohedric (fig. 4c), as usual for inorganic calcite. However, the structures formed with the laser were rounded and seemed to be composed of many crystals disposed symmetrically regarding to the optical axis of the microscope (fig. 4b). When the laser is on for a long time (many seconds), it is formed also a rim composed of long and thin crystals arranged radially in the border of these structures (fig. 4b). Polarizing light microscopy showed that the crystals are disposed symmetrically in the rounded structures (fig. 5).

The magnetite crystals of the bacteria absorb energy from the laser beam producing heat, which vaporizes water, causing local supersaturation of the solution and crystal growth. As the heat distribution is symmetrical in relation to the optical axis of the microscope, it was grown symmetric $CaCO_3$ structures around the laser beam. The temperature gradient, the time, and the specific chemical species present in solution influence the size and shape of the microcrystals formed.

Acknowledgements: We are thankful to Milton M. Costa and Adriana Fontes for technical help.

Reference:

[1] Lins, U. et al. *Microsc. Microanal.* 6 (2000) 463.
[2] Financial support: COPEA, CNPq, FAPERJ, Instituto do Milênio de Bioengenharia Tecidual.

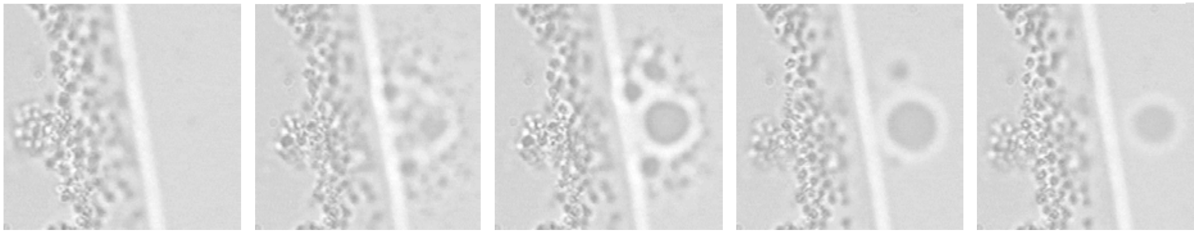

Figure 1: Video sequence showing a “microexplosion” caused by the interaction of the laser beam with magnetite from marine magnetotactic bacteria. Individual figures are 26×26µm.

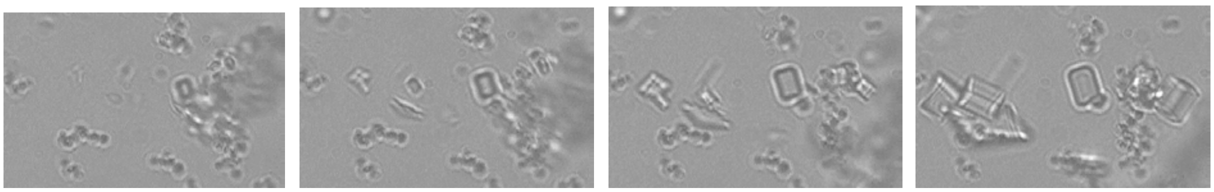

Figure 2: Video sequence showing rapid growth of crystals from a 20% NaCl solution by the action of the laser beam on magnetotactic bacteria. Each micrograph is 22×36µm.

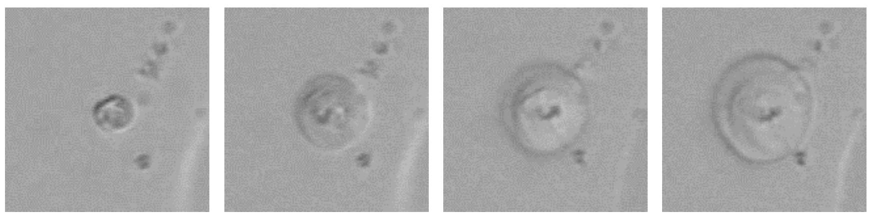

Figure 3: Video sequence of the growth of a $CaCO_3$ rounded particle from a $CaCO_3$ solution (ionic strength = 0.025M). Observe three layers that appear early in the growth. Figures are 14×14µm.

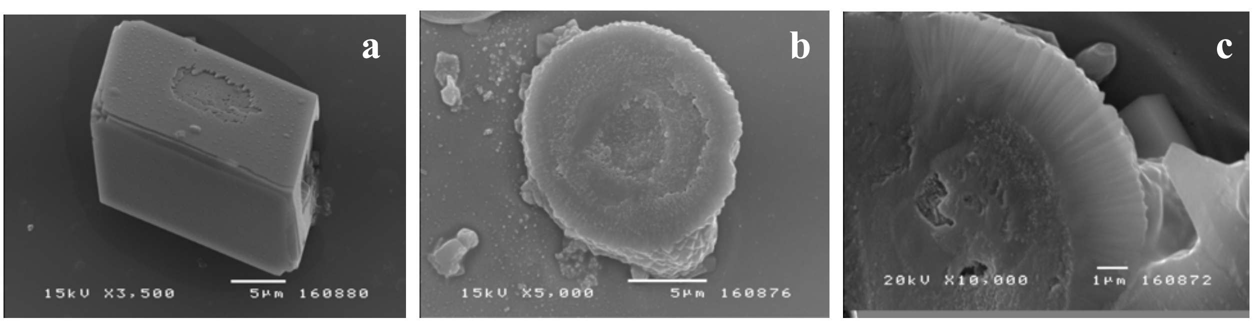

Figure 4: Scanning electron micrographs of (**a**) a rombohedric $CaCO_3$ crystal grown spontaneously from the solution, (**b**) a rounded $CaCO_3$ structure formed by the action of the laser beam on magnetotactic bacteria containing three distinct layers and (**c**) detail of the peripheral region of a rounded structure showing the rim composed of thin crystals disposed radially.

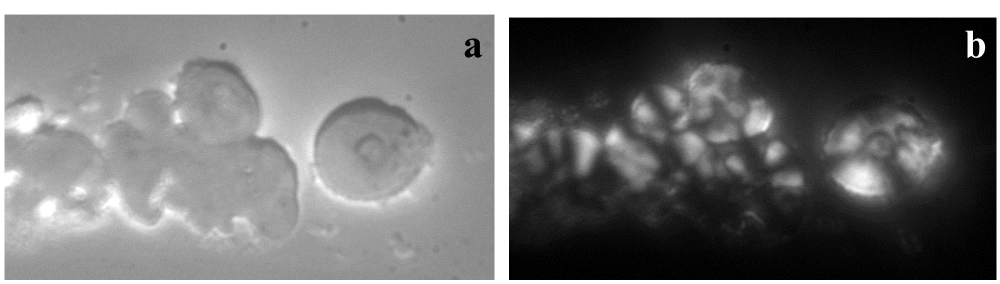

Figure 5: (**a**) Nomarski interference contrast and (**b**) polarized light microscopy of rounded $CaCO_3$ structures formed with the optical tweezers and magnetotactic bacteria. Note the crossed pattern characteristic of a radial symmetry in most of the structures. Each figure is 74×40µm.

https://doi.org/10.1017/S1431927603440452 Published online by Cambridge University Press