EPS of living bacteria influence the crystallization of EPS-hydrogel-calcite composite aggregates

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Microorganisms are the most ubiquitous life forms on our planet. As minerals secreted by bacteria nucleate and grow in the extracellular environment, bacterial mineralization is the product of their metabolic activity and chemical reactions associated with the metabolic byproducts [1]. Bacterial extracellular polymeric substances (EPS) surround bacterial colonies and are biofilms that enable mineral formation [2].

Our previous studies [3,4] show that even low concentrations of bacterial EPS in the growth medium induce specific mineral microstructure in the EPS-calcite aggregates. To understand the influence of bacterial metabolic activity at calcite crystallization, we synthesized hydrogel-calcite composite aggregates with *Bacillus subtilis* EPS. The microstructure of the composite aggregates was characterized with scanning electron microscopy and electron backscatter diffraction (EBSD). The bacterial EPS-agarose hydrogel networks were visualized with selective chemical etching and local kernel misorientation derived from EBSD data.

The presence of living bacteria has limited influence on the morphologies of the aggregates, however, it strongly changes mineral organization and polymer distribution within the aggregates. Calcite aggregates that grew with living bacterial communities are radial polycrystals, which show a typical spherulitic organization. Bacterial cells were embedded into the aggregates at crystal growth, as micro-voids (~1 μm), often containing bacterial cells, were observed within the aggregates. Although the aggregates that grew with UV light treated PES have similar morphologies as those grew with living bacterial cells, their subunits are irregular in shape, size and distribution. A dense membrane-like polymeric layer is also present in the obtained composite aggregates.

In a summary, our experiments show that living microorganisms are actively involved in the crystallization process of the aggregates and they strongly influence the microstructure and texture of the mineral. Hence, microorganisms have an important role in the mineralization (biomineralization) process. This characteristic makes it possible and indicates that bacterial EPS can be developed as a biomarker, as a further tool for the recognition and identification of bacterially mediated calcification in present environments as well as in the geological record.

[1] Frankel R. B, Bazylinski D. A, Biologically induced mineralization by bacteria. Biomineralization 54, 95-114 (2003).

[2] Bäuerlein E, Biomineralization of unicellular organisms: an unusual membrane biochemistry for the production of inorganic nano- and microstructures. Angew. Chem. 42, 614-641 (2003).

[3] Yin X, Weitzel F, Griesshaber E, *et al*. Bacterial EPS in agarose hydrogels directs mineral organization in calcite precipitates: species-specific biosignatures of *Bacillus subtilis*, *Mycobacterium phley*, *Mycobacterium smagmatis*, and *Pseudomonas putida* EPS. Cryst. Growth Des. 20, 4402-4417 (2020).

[4] Yin X, Weitzel F, Jiménez-López C, *et al*. Directing effect of bacterial extracellular polymeric substances (EPS) on calcite organization and EPS–carbonate composite aggregate formation. Cryst. Growth Des. 20, 1467-1484 (2020).