Formation of biomimetic calcite-gelatin/agarose composites: Gel occlusion, aggregate co-orientation and Mg content

/XIAOFEI YIN (1), LURDES FERNÁNDEZ-DÍAZ (2,3), ERIKA GRIESSHABER (1), ANDREAS ZIEGLER (4), PAUL WALTHER (4), FRANCISCO JAVIER GARCÍA-GARCÍA (5), WOLFGANG W. SCHMAHL (1)*

(1) Department für Geo- und Umweltwissenschaften. Ludwig-Maximilians-Universität. 80333 Munich, (Germany)
(2) Departament of Crystallography and Mineralogy. Complutense University of Madrid. 28040, Madrid (Spain)
(3) Institute of Geosciences (UCM-CSIC). 28040 Madrid (España)
(4) Central Facility for Electron Microscopy. University of Ulm. 89069 Ulm. Germany
(5) ICTS-CNME. Complutense University of Madrid. 28040, Madrid (Spain)

INTRODUCTION

Biological hard tissues such as shells, bones and teeth are mineral-organic composite materials with enhanced mechanical properties. In the case of carbonate biological hard tissues, the organic component consists of a mixture of proteins, glycoproteins and polysaccharides, arranged to define a matrix of membranes and of fibrils. The most common mineral component is nanoparticulated calcium carbonate, calcite or aragonite. The characteristics of calcite crystal aggregation and its organization into hierarchical orientation patterns is mediated by the organic matrix, which becomes occluded within the mineral component during the formation of the hard tissue.

Crystallization environments, where biological hard tissues form, share numerous characteristics with hydrogels. This fact has promoted the use of hydrogels to conduct biomimetic crystallization experiments Moreover, the fact that the gel network becomes incorporated into calcite crystals during growth render hydrogel systems especially suitable for studying the role played by extracellular organic matrices in the internal micro-structuring of biological carbonate hard tissues.

In previous studies, we used single gels, e.g. of gelatin, a poly-peptide material derived from natural collagen, and agar, a linear polysaccharide isolated from marine algae, both with and without magnesium in the growth medium, to obtain calcite-gel composites [1,2,3]. The fibrous structure of these gels defined porous networks where local crystallization environments resembled those formed by extracellular, biological matrices. In magnesium-free systems, either single crystals or radial aggregates with very few subunits formed. The occlusion of gel polymeric matrix within calcite during crystal growth had little effect on the internal structuring of the calcite-gel composites. However, it was possible to establish a correlation between an increase in the amount of occluded gel and a decrease in crystal co-orientation [1]. The addition of magnesium to the growth medium had a most marked influence on both, the morphologies of the composites, which became significantly more complex and were bounded by rough surfaces, as well as the internal structuring of the composites, which was characterized by a significant increase in the number of subunits and internal boundaries.

Because the polymeric matrix that is occluded in most biominerals commonly contains covalent interconnections between proteins and oligo- and polysaccharides, in this paper we use intermixed gelatin/agarose hydrogels to mimic better the characteristics of those matrices. Our aim is to study the effect that changes in the gelatin:agarose ratio in the gel, combined with the absence/presence of magnesium in the growth medium, have in the calcite-gel composites features. We pay attention to the amount of gel occluded, and to the distribution of this gel within the composite. Moreover, we look at the number of subunits in the composites and the degree of crystal co-alignment, within and between sub-units. Finally, we also pay attention to the amount magnesium incorporated in the calcite structure of those composite that grow in magnesium-bearing gels.

METHODOLOGY

In our experiments, we apply the double-diffusion method to crystallize calcium carbonate using agarose/gelatine mixed gels, both with and without magnesium in the growth medium. The intermixed hydrogels were prepared with three different gelatin:agarose ratios (1:1, 1:2, and 2:1). High-pressure freezing and cryo scanning electron microscopy (cryo-SEM) is used to investigate features of the polymeric matrix of the different gels. We use field emission scanning electron microscopy (FE-SEM) imaging for the assessment of both, morphologies and surface nanometric features of the obtained calcite-gel composites. We also use FE-SEM to visualize, on microtome polished, etched and critical point dried sample surfaces, the distribution of gels within the composites and the characteristics of the gel-mineral interlinkage. With high-resolution electron backscatter diffraction (EBSD) we determine the microstructures of the composites and their degree of crystal co-aligment.

RESULTS AND DISCUSSION

In the absence of magnesium in the growth media, calcite-gel composites grow as either single crystals or aggregates with few subunits, which are formed by rombohedral, highly co-oriented sub-blocks. The size of these sub-blocks is < 1 μm in all the cases. However, the average size of these sub-blocks is smaller in those composites grown in gels with a gelatin:agarose ratios of 1:2 and 2:1 than in those formed in gels with ratios equal to 1:1. This is again difficult to understand. The number of subunits increases from composites formed in gelatin:agarose 1:1 to 1:2 and 2.1. Moreover, this

palabras clave: Cristalización Biomimética, Composites Calcita-Gel, Difracción de Electrones Retrodispersados (EBSD).

key words: Biomimetic Crystallization, Calcite-Gel Composites, Electron Backscatter Diffraction (EBSD).

* corresponding author: lfdiaz@geo.ucm.es

increase in the number of subunits correlates with a decrease in the degree of co-aligment of calcite crystals. The occluded gel network is homogenously distributed within the composites, although this is significantly less so in those composites formed in gelatin:agarose 1:2 and 2:1, where thin membranes can be distinguished in the occluded network.

The addition of magnesium to the growth medium results in morphologically complex composites, which are bounded by rough and curved surfaces and curved edges. These composites consist of numerous subunits, which appear misoriented in fan-like arrangements. Detailed inspection of these subunits show that they consist of aggregated, spherical nanoparticles, whose size < 70 nm and varies depending on the gelatin: agarose ratio in the gel following the same pattern as observed in composites formed in the absence of magnesium. The mineral component of the composites grown in the presence of magnesium is high-magnesian calcite, with $MgCO_3$ contents that can be as high as 31 mol%.

Differences in the characteristics of the occluded gel network depending on the gelatin:agarose ratio used in the preparation of the hydrogel and the presence/absence of magnesium in the growth medium concern (i) the degree of disruption of the original gel matrix, with changes in the distribution of pore sizes and the absence/presence of broken pore walls and (ii) the development of gel membranes that separate subunits within the composite. We interpret these differences as the consequence of the differences in the mechanical and diffusivity properties of the different hydrogels. The solid content and the gelatin:agarose ratio in each gel as well as the presence/absence of magnesium in the growth medium affect the complex balance between crystallization pressure and hydrogel strength, which in turn determines changes in the characteristics of the gel occlusion. These changes are reflected in microstructural features of the calcite-gel composites.

ACKNOWLEDGMENTS

This research is part of the German-Spanish Joint Acciones Integradas Program (AIB2010 DE-0008), DAAD-50749739. This research was partially funded by projects CGL2013-47988-C2-01-P and CGL2016-77138-C2-1-P (MECC-Spain). We sincerely thank the staff of the National Microscopy Centre (ICTS) of the Complutense University (UCM) for technical support and assistance.

REFERENCES

Nindiyasari, F., Fernández-Díaz, L. Griesshaber, E., Astilleros, J.M., Sánchez-Pastor, N., Schmahl, W. W. (2014): The influence of gelatin hydrogel porosity on the crystallization of $CaCO_3$. Crystal Growth and Design. 14, 1531-1542.

Nindiyasari, F., Griesshaber, E., Fernández-Díaz, Astilleros, J.M., Sánchez-Pastor, N., Schmahl, W. W. (2014): Effects of Mg and Hydrogel Solid Content on the Crystallization of Calcium Carbonate in Biomimetic Counter-diffusion. Systems Crystal Growth and Design, 14, 4790-4802.

Nindiyasari, F., Ziegler, A., Griesshaber, E., Fernández-Díaz, Walther, P., Schmahl, W. W. (2015): Effect of hydrogel matrices on calcite crystal morphology, aggregate formation, and co-orientation in biomimetic experiments and biomineralization environments Crystal Growth and Design, 15, 2667-2685.