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Mg^{2+} Tunes the Wettability of Liquid Precursors of $CaCO_3$: Toward Controlling Mineralization Sites in Hybrid Materials

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S *Supporting Information*

ABSTRACT: Amorphous and liquid precursors of calcium carbonate are believed to be central species of biomineralization, which serves as an important inspiration for materials chemists in the quest for new and improved organic−inorganic hybrid materials. It has become increasingly clear that magnesium ions exhibit an important function through kinetic stabilization of the metastable precursors. We show that they additionally tune the wettability of liquid precursors of $CaCO_3$, which is a crucial requirement for successful mineralization of proteinaceous organic matrices. Moreover, tunable wettability offers straightforward means to control mineralization sites in organic−inorganic hybrids.

Biomineralization serves as an important inspiration for materials chemists.[1,2] The most abundant biomineral, calcium carbonate, thus is a prominent model system, and it has become increasingly clear that amorphous calcium carbonate (ACC) plays a central role in biomineralization processes.[3,4] An amorphous precursor bears the advantage that it can be molded into any shape and subsequently crystallize and thereby facilitates the formation of complex off-equilibrium morphologies[5] typical for biominerals.[6] Amorphous states are always metastable with respect to crystalline phases, at least at large particle sizes, and it is believed that additives play an important role in the kinetic stabilization of ACC: both (macro-) molecules[7−11] and, interestingly, Mg^{2+}-ions incorporated in ACC.[12−18]

With respect to hydration, Mg^{2+}-ions are remarkably different from Ca^{2+}-ions. The hydration layer of Ca^{2+} is very labile owing to its relatively low charge density, with a mean lifetime of a single water molecule in the first coordination sphere of $\sim 10^{-8}-10^{-9}$ s, which is significantly longer and lies in the μs regime for Mg^{2+}.[19] For Ca^{2+}, it has been proposed that the water coordination increases from 6 to 10 as the concentration decreases,[20] however, this observation has not been confirmed by other studies that are consistent with 8-fold coordination of aqueous Ca^{2+}.[21] In contrast, Mg^{2+} is octahedrally coordinated by six water molecules.[19] The distinctly stronger hydration of Mg^{2+} compared to Ca^{2+} appears to bring about a stronger hydration of precipitated ACC in presence of Mg^{2+}, where the Mg−O bond lengths are comparable to those of hydrated $CaCO_3$.[13] Similarly, it has been found that hydrated ACC and anhydrous ACC relate to stabilized and transient amorphous phases in biomineralization, respectively.[3,22] Additionally, the Mg^{2+} content in ACC can be increased by the introduction of carboxylated molecules,[23] which can also enhance the Mg^{2+} signature in growing calcite.[24] In general, the lattice constants of crystalline calcium carbonate decrease with increasing magnesium content.[15]

Polymer-induced liquid precursors (PILP's)[25] can be utilized in bioinspired syntheses of delicate morphologies of $CaCO_3$.[26] While the understanding of these species is rather limited,[22] it appears that PILP represents a precursor to ACC, which may also form in absence of polymers.[27] Recently, the liquid character of PILP has been directly proven by means of NMR spectroscopy, and its stabilization in absence of polymers has been associated with the abundance of bicarbonate at near-neutral pH values.[28] The transition from PILP to ACC appears to be further based upon dehydration,[29] and continues energetically downhill from ACC to crystalline $CaCO_3$.[30]

Here, the known role of Mg^{2+} in stabilizing ACC, and also PILP,[31] is expanded by considerations of wettability. From the point of view of colloid chemistry, wettability[32] of liquid precursors is a crucial property when it comes to the mineralization of organic matrices and is thus of fundamental importance in biomineralization as well as bioinspired approaches to functional materials.

As organic matrix, electrospun fiber meshes (Figure S1) were employed, which are blends composed of poly(ε-caprolactone) and poly(pentafluorophenyl methacrylate) that can be post-functionalized with amino-functional molecules.[33] To mimic biological organic matrices, three different bioinspired oligopeptides have been employed in post-functionalization, the sequences of which are supposed to differ in mineral binding specificity but not distinctly in side-chain chemistry. The first is based on the binding motif of statherin, a protein found in saliva known to prevent calcium phosphate nucleation, (Asp-pSer-pSer-Glu-Glu-Arg-Phe-Leu-Arg-Arg-Ile-Gly-Gly-Lys).[34] The other two consist of 3 and 5 repeats of the (Asp-Asp-Arg-Arg)-unit found in Pif80,[35] a protein important for calcium carbonate binding during the formation of nacre. The peptide-decorated fiber meshes thus emulate the wettability of proteinaceous matrices based upon mineral binding motifs from biomineralization. As a reference, fiber meshes were also functionalized with 2-aminoethanol, where the fiber surfaces carry hydroxy functionalities.

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PILP droplets generally form in solution when poly(aspartic acid) is present at μg/mL concentrations, and range from ~100 nm up to hundreds of micrometers in size.[26] SEM micrographs show compelling indirect evidence of PILP droplets, 100−200 μm in size, that have attached to the surface of the OH-bearing fiber meshes (Figure 1). Without Mg^{2+} (Figure 1 a,b), they have drawn into the matrix, most likely by capillarity, demonstrating that the PILP droplets do wet the fibers. EDX analyses verify that light-gray sections of the micrographs correspond to mineralized parts, while the dark-gray areas contain negligible amounts of calcium carbonate (Figure S2). Fiber mineralization can be achieved under a range of different poly(aspartic acid) and Ca^{2+} concentrations (Figure S3), and attenuated total-reflection-infrared (ATR-IR) spectra (Figure S4) indicate a mixture of calcite and vaterite in all cases, without any trend in the distribution of polymorphs or any clear indications for the presence of ACC under the different conditions. SEM analyses at higher magnification show that indeed, without Mg^{2+}, the fibers are completely covered and enclosed by the mineral (Figure S5).

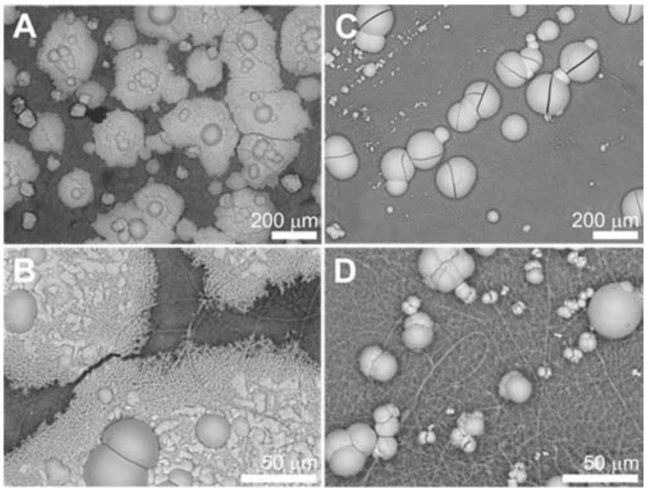

Figure 1. Mineralization of OH-bearing fiber meshes utilizing PILP droplets in absence (a,b) and in presence (c,d) of Mg^{2+}. The results shown were obtained utilizing 10 μg/mL poly(aspartic acid), 5 mM $CaCl_2$ (15 mM $MgCl_2$), after 1 day in the gas diffusion crystallization assay.

In the presence of Mg^{2+}, however, the mineral does not penetrate into the hydroxy functional organic matrix (Figure 1c,d). The Mg-PILP droplets neither wet the individual OH-bearing fibers nor are they drawn into the fiber matrix. Although small particles are attached to the fibers, they do not enclose them (Figure S5) and in principle show the same nonwetting behavior as the larger Mg-PILP droplets (Figure 1c,d). Their state of nonwetting suggests little to no energetic benefit for heterogeneous nucleation.[36] Variations of the poly(aspartic acid) and Ca^{2+} concentrations in presence of Mg^{2+} do not affect the outcome; wetting of the OH-bearing fiber meshes with Mg-PILP cannot be achieved (Figure S6).

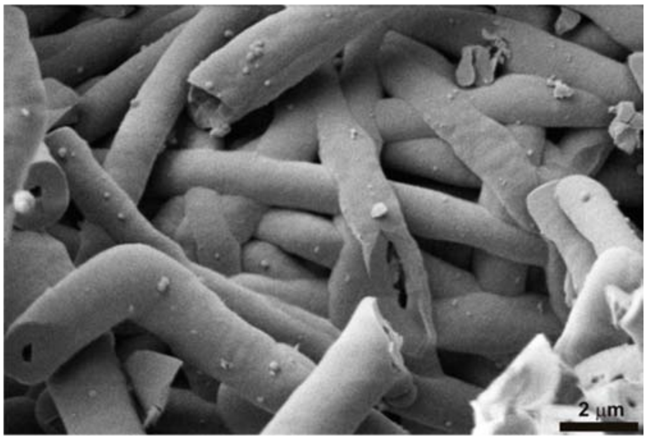

Figure 2. Mineralization of the fiber mesh decorated with the peptide unit from statherin utilizing PILP droplets in presence of Mg^{2+}. The result shown was obtained with 50 μg/mL poly(aspartic acid), 10 mM Ca^{2+}, and 30 mM Mg^{2+} after 4 days in the gas diffusion assay.

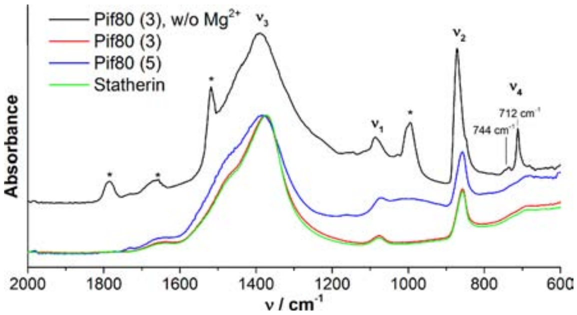

Figure 3. ATR-IR spectra of oligopeptide-decorated fiber meshes, with 3 repeats of the Pif80 unit (Pif80 (3)), 5 repeats thereof (Pif80 (5)), and with the unit of statherin as indicated. The fiber meshes were mineralized utilizing 50 μg/mL poly(aspartic acid), 10 mM Ca^{2+}, and 30 mM Mg^{2+}; in addition, one example is shown for the mineralization of the Pif80 (3) mesh without Mg^{2+}. The mineralization time was 1 day for the sample prepared without magnesium, 4 days for the other specimes. The four vibrational modes of carbonate are indicated, bands from the fiber meshes are labeled with asterisks; the spectrum of Pif80 (3) without Mg^{2+} is offset for clarity.

The situation entirely changes for the mineralization of the fiber meshes decorated with oligopeptides. Here, wetting can only be achieved in presence of Mg^{2+} (Figure S7). This is shown for the fiber mesh decorated with the peptide unit from statherin in Figure 2 (mineralized meshes with other oligopeptide functionalizations are shown in Figure S8). At fracture edges, the mineralized fibers appear to be hollow, which is likely due to the fact that the organics degrade over time at elevated pH in the crystallization assay if no layer of $CaCO_3$ protects them (Figure S7). In this case, Mg-PILP droplets are significantly smaller than those seen in absence of Mg^{2+} (cf. Figures 1 and S7 without Mg^{2+} versus Figures S6 and S8 with Mg^{2+}). This potentially reflects the influence of Mg^{2+} on the surface tension of PILP droplets, thereby affecting droplet size and/or aggregation. Figure 3 shows ATR-IR spectra of the peptide-functionalized fiber meshes, which have been wetted by Mg-PILP droplets, in comparison to a nonwetting result obtained without Mg^{2+}. In the nonwetting case, the spectrum shows several vibrational bands that arise from the organic matrix, and the ν_4 carbonate modes are indicative of a mixture of calcite and vaterite, as for OH-bearing meshes (calcite: $\nu_4 = 712\ cm^{-1}$, vaterite: $\nu_4 = 744\ cm^{-1}$). When wetted by Mg-PILP, on the other hand, vibrational bands from the matrix are largely, if not completely, suppressed, and the carbonate vibrational modes dominate the spectra. Considering the limited penetration depth of IR radiation in the ATR technique, these observations are commensurate with the above assessments of wettability based on inspections of SEM micrographs. Furthermore, in line with previous studies, the IR data show that in presence of Mg^{2+}, ACC is stabilized. This is apparent especially from the ν_4 region, where bands indicative of crystalline modifications are absent (Figure 3), while the overall spectral features resemble those expected for ACC.[37]

Although the stabilization of PILP and ACC by Mg^{2+} may also influence successful mineralization in terms of kinetics, especially if PILP solidification proceeds rapidly, the results suggest that kinetic stabilization effects do not change the underlying wettability of the PILP phases. First, Mg^{2+} facilitates mineralization of fibermeshes functionalized with different oligopeptides, but this does not apply at all in the case of OH-bearing fibers. Second, when no Mg^{2+} is present and the $CaCO_3$ precursors crystallize, no differences between OH-bearing and oligopeptide functionalized fiber meshes with respect to polymorphism are found. They only differ in principal wetting behavior, underpinning the role of Mg^{2+} in this context.

Strikingly, only either complete wetting or nonwetting conditions are observed (contact angles of 0° and ≥150°, respectively). At first this result may seem strange, as one may suppose that contact angles between those extremes should also be possible. However, the complete wetting may reflect a strong interaction of Mg-PILP with the bioinspired oligopeptides. Moreover, the observed wetting behaviors of the mineral phases are the result of the wettability of their respective precursor PILP phase. In case of Mg-PILP on the oligopeptide-functionalized fibers, the interaction between the droplets and the fiber favors complete wetting of the mineral phase. Whether the precursor phases wet completely or only partially, with the subsequent addition of PILP droplets, the resulting mineral phase will have the same condition of complete fiber wetting.

Conversely, in the case of Mg-PILP on the OH-functionalized fibers, the interaction between the PILP and the fibers is not favorable, and the individual PILP droplets do not wet. Any addition of nonwetting PILP has no effect on the resulting mineral phase, which also does not wet the fibers. As of yet, it is unknown how the Mg^{2+} content alters the interactions and subsequent wetting of the fibers, although the increased hydration of the PILP phase with increasing Mg^{2+} content is thought to play a considerable role. The exact bases of enhanced wetting with Mg^{2+} content, as well as any possible kinetic effects of Mg^{2+} on the PILP to mineral transition on the fiber surface, are the foci of ongoing investigations.

In conclusion, Mg^{2+} distinctly influences the wetting behavior of liquid precursors of $CaCO_3$. In the case of proteinaceous matrices, the presence of Mg^{2+} is required for successful mineralization; without Mg^{2+}, the fibers are not wetted. Hydroxy functional organic surfaces are only wetted by PILP in absence of Mg^{2+}. Thus, wettability can be tuned via distinct types of functionalization of organic surfaces for a given PILP precursor. For a given organic surface functionalization, the tuning of the wettability of PILP's is an additional important function of Mg^{2+}, besides kinetic stabilization of metastable precursors. Experiments at Mg^{2+}/Ca^{2+} ratios of 1/1 and 5/1 (in addition to 0/1 and 3/1 discussed above, see Figure S9 for an overview) show that at a ratio of 1/1 neither hydroxy- nor peptide-functionalized fibers are wetted, while results obtained at a ratio of 5/1 are largely consistent with the findings at 3/1. According to the composition of sea waters,[38] ratios of 3/1 and 5/1 pose an appropriate representation for biomineralization. After all, the results indicate that fine-adjustments of the Mg^{2+} content of the PILP phases may even allow for gradually tunable wetting behavior of (Mg-)PILP on distinct organic surfaces. In any case, this allows for directed mineralization in organic–inorganic hybrid materials, for example, the rendering of tailor-made patterns.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and additional data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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