Templated CaCO$_3$ Crystallization by Submicrometer and Nanosized Fibers

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Supporting Information

ABSTRACT: Electrospun submicrometer-sized poly(ε-caprolactone) (PCL) meshes and nanosized multiwalled carbon nanotubes (MWCNTs) were used as a template for preparing porous and interconnected inorganic–organic hybrid materials composed of CaCO$_3$. Herein, we describe the proportion and incorporation method of submicrometer-sized plasma-treated PCL meshes over areas $>1$ mm$^2$ with CaCO$_3$ using three crystallization methods including the use of poly(acrylic acid) (PAA). We found that flexible and rigid acid-functionalized MWCNTs showed a clear capacity and effects to penetrate calcite particles. MWCNTs interacted differently with the individual growth planes of CaCO$_3$, indicating that fibers can undergo changes depending on sulfonate or carboxylate groups, adopt different orientations in solution, and thereby elicit changes in CaCO$_3$ morphology. In summary, the use of PCL and acidic MWCNT fibers as an additive for substrate templates and experimental crystallization provides a viable approach for studying various aspects of biomineralization, including the production of controlled particles, control of porosities, and defined morphologies at microscale and nanoscale levels.

INTRODUCTION

Biomineralization is the process by which living organisms ranging from higher vertebrates and plants to unicellular organisms produce hierarchical inorganic–organic hybrid biominerals.$^{1−3}$ Representative examples of biominerals are bones and teeth in vertebrates, shells in molluscs, exoskeletons in crustaceans, coccoliths in marine algae, plant opals in higher plants, and magnetite in bacteria, among others. $^{2,5}$ Biominerals play crucial roles in living organisms such as protection, motion, storage, optical and gravity sensing, defense, and detoxification.$^{5−9}$ They are highly organized from the molecular to the nanoscale, with intricate nanoarchitectures that ultimately make up myriad different functional soft and hard tissues.$^{1,9−11}$ These nanostructured hybrid biominerals present a complex shape, hierarchical organization, uniform particle size, and novel morphologies that often have high strength and remarkable properties. Therefore, chemists are inspired by the underlying molecular processes and aim to mimic biological crystallization to transfer the concepts to modern composite materials and the control of mineral crystallization. The toughness of biominerals depends on the degree of crystallization, soluble and/or insoluble polymer matrices, the shape (platelet, fibers, etc.), and the type of inorganic minerals.$^{12,13}$

It is well known that biominerals are valuable materials not only for understanding the biomineralization concepts but also for novel confined-materials synthesis and design, avoiding undesirable pathological biomineralization.$^{14,15}$ The inspiration from biomineralization and nanocomposite materials that control crystal nucleation and growth is of great interest to materials scientists who seek novel materials syntheses with fibrillar hydrogel structures and/or crystalline matrices and interfaces based on crystalline forms analogous to those produced by nature, such as calcium salts, silica, hydroxyapatite, or iron oxides.$^{16−21}$

The current work discusses the selection and use of calcium carbonate (CaCO$_3$) as a biologically relevant system. CaCO$_3$ has three anhydrous polymorphs: calcite (trigonal), aragonite (orthorhombic), and vaterite (hexagonal). This biomineral often consists of single crystals intimately associated with macro-molecular organic matrices. Calcite is one of the most common biomineral phases.$^{22−25}$ Because of its biocompatible and biodegradable nature, encapsulation, and drug delivery capacity, strong interest for pharmaceutical and biomedical applications...
has also emerged. Recently, hybrid nanoparticles of the CaCO₃—polyaniline biomaterial as a photothermal agent for cancer ablation were demonstrated as a new platform for the next generation of in vivo cancer photothermal therapy agents.²⁶ The aim of this study was to design hybrid composite materials using electrospun submicrometer-sized PCL meshes and nanosized MWCNTs as templates for preparing porous and interconnected organic—inorganic hybrid materials composed of CaCO₃. Template-directed mineralization as strategy has become one of the most popular methods for preparing hybrid composite materials.²⁷ Templated synthesis is an exciting area of research capable of producing highly controlled nanostructures with unique structures, morphologies, and advanced properties.²⁸ Therefore, the understanding of bioinspired directed mineralization mechanisms, charge/polarization matching, epitaxy based on CaCO₃ using different nanomaterial assemblies as a template, and the growth induction of inorganic materials has been extensively investigated.²⁹

The fiber-enhanced composite materials have the pores fully filled by the organic phase that modulates the mechanics of the inorganic crystals by analogy to occluded proteins or micelles.³⁰⁻³² Such composite structures have multiple potential applications in the technical field as well as in the medical field (e.g., 3D scaffolding).³³ Regarding the architecture, the present approach can be considered, to some extent, to be like a steel-reinforced concrete construct. In addition, the first report of bioinspired crystallization of CaCO₃ on electrospun cellulose acetate submicrometer fibers as a scaffold by Yu et al. demonstrated the viability of an organic—inorganic material interface.³⁴ In this context, CNTs represent versatile materials with novel properties, making them potentially useful in many applications such as nanotechnology, electronics, optics, and other fields of materials science. While CNTs could be incorporated into mineral crystals, raw CNTs appear as insoluble bundles of several fibers held together by II–Π interactions. Covalent functionalization could introduce bulky groups, which weaken the interactions, separate the nanotubes, and form interactions with the solvent, allowing the dispersion of the materials. Several methods have been proposed to functionalize CNTs.³⁵,³⁶ Among them, the coupling of highly reactive aromatic radicals, produced by the thermal decomposition of diazonium salts, with CNTs is an easy way to produce water-dispersible CNT systems.³⁷ Here, we have used the method to synthesize different covalently functionalized MWCNTs that show clear effects on calcite particle mineralization.³⁸ Previously, dispersed CNTs have been used to produce composites with hydroxyapatite crystals.³⁹⁻⁴¹

In summary, this article explores a bioinspired approach of templating from the nanoscale to the microscale using electrospun fiber meshes composed of submicrometer and nanometer PCLs and MWCNTs as templates to obtain integrated porous crystal—fiber interface nanomaterials.

■ MATERIALS AND METHODS

Materials. Poly(ε-caprolactone) (PCL, Sigma-Aldrich) was reprecipitated prior to use.³⁸ Chloroform (CHCl₃, 99+%, Merck, Darmstadt, Germany), methanol, tetrahydrofuran (THF), and N,N-dimethylformamide (DMF, 99+, Sigma-Aldrich, Steinheim, Germany), were used as received. For aqueous solutions, bidistilled water was used (0.055 μS/cm, 23.4 °C). All salts used for crystallization were obtained from Sigma-Aldrich (high purity grade, Steinheim, Germany). CaCO₃ powder (99+%), calcium chloride (CaCl₂) dihydrate (96%) from (Sigma-Aldrich), ethanol, and ammonium carbonate were obtained from ACS-Merck and used without further purification. MWCNTs, produced by CVD growth from acetylene, were obtained from Sun Nanotech (China). The material was washed with 6 M HCl for 24 h to clean out metal contamination.

Diazonium Ion Synthesis and Covalent Modification of Carbon Nanotubes. Multiwalled CNTs (CNT, Sun Nanotech) were modified by coupling with aromatic radical cations, produced by the thermal decomposition of diazonium ions.⁴³ Aromatic amines were diazotized with sodium nitrite and concentrated HCl in an ice bath.⁴⁴ Diazotized amines were mixed with MWCNTs in an ultrasonic bath. Then, the temperature was raised to 60 °C and heated for 1 h. The reaction mixture was acidified with concentrated HCl and centrifuged (10 000 rpm) to separate the modified CNT. The precipitate was washed several times with water and dried under vacuum. Raw or functionalized CNT were dispersed in purified water with the aid of ultrasound. Dynamic light scattering (DLS) measurements were performed in a Malvern 4700 DLS. The measurements were made at an angle of 90° at 25 °C. As shown before,⁴³ the CNTs functionalized in this way render a monomodal dispersion (Figure S.1.I) that is stable for months whereas raw CNTs show clusters of different sizes and precipitate spontaneously after a few minutes.

Electrospinning Setup. PCL solutions were prepared by dissolving the polymer overnight by mixing with a rock and roll shaker using THF/DMF (1:1). The electrospinning setup was composed of a setup described in previous work.⁴⁵ Plastic 1 mL syringes with disposable blunted tips (Howard Electronics, JG20-2, nominal inner diameter 0.584 mm, El Dorado, KS, USA) were positively charged, and an aluminum foil was used as the collector in a vertical setup. The polymer solution was fed at a rate of 1 mL/h by the syringe pump to the blunted needle tip, where a voltage of 15 kV was applied. The spinning distance between the tip and ground collector was 10 cm. The resulting fiber meshes were dried, cut into 0.5 × 0.5 cm² pieces, and sputtered with Pd/Au for SEM observation (LEO 1550-GEMINI, Zeiss, Oberkochen, Germany). To evaluate the fiber diameter, ImageJ software (1.38x, Wayne Rasband, National Institutes of Health, Bethesda, MD, USA) was used. Wide-angle X-ray scattering (WAXS) of the PCL and PCL/composites was conducted on a PDS 120 diffractometer (Nonius GmbH, Solingen, Germany) with Cu Kα radiation.

Microscopy. In the case of CNT fibers and gas diffusion (GD) crystallization, both SEM and light microscopy (LM) measurements of CaCO₃ were applied to all crystal samples. The use of the LM was necessary to prove that the SEM micrographs show real structures instead of drying artifacts resulting from the sample preparation. SEM of CaCO₃ crystals was performed on a LEO 1550-GEMINI (Zeiss, Oberkochen, Germany) instrument. Additionally, LM images were taken with a VHX digital microscope (Keyence VHZ-1100).

Crystallization Methods. CaCO₃ Crystallization with Electrospun Submicrometer PCL Fibers. For crystallization, submicrometer-sized fibers of 0.2–1.2 μm diameter were used.⁴² A self-supported fiber mesh of ~0.8 × 0.8 cm² was used for the different crystallization methods. Air plasma treatment of the mesh was performed for 1 min using an 18 W PDC-32G plasma cleaner (Harrick Plasma, Ithaca, NY, USA). The concentrations of the respective CaCl₂, Na₂CO₃, and PAA solutions can be seen in Table 1, as can the solution volume and mineralization time. After mineralization, the composites were dried in air for corresponding analytics. For SEM, sputtering was performed with crystallization samples. Here, CaCO₃ powder was dissolved in an acidic aqueous solution (saturated at pH 3.6, adjusted with HCl), and then its pH was shifted to neutral conditions (pH 7, NaOH).

CaCO₃ Crystallization with CNT Fibers. The crystallization of CaCO₃ was based on the gas diffusion (GD) method. The diffusion of CO₂ into different concentrations (5, 10, and 25 mM) of an aqueous solution of CaCl₂ in the presence of acidic CNT fibers (10 wt %) as an additive was performed, and then CaCO₃ was slowly precipitated. The crystallization of CaCO₃ was carried out in closed desiccators at room temperature. GD crystallization was performed using raw multiwalled (CNT-1) and functionalized sulfonic (CNT-2, CNT-3) and carboxylate (CNT-5) CNT fibers at 20 °C for 2 and 5 days. The GD method was performed as we described in previous work.⁴⁶⁻⁴⁹ The pH is very important in the biomineralization field. In the gas diffusion method, CaCO₃ crystallization is performed at a defined mineralization time,
Table 1. Different Conditions of CaCO₃ Mineralization

<table>
<thead>
<tr>
<th>methods</th>
<th>solution concentration</th>
<th>time</th>
<th>volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>gas diffusion (GD)</td>
<td>10 mM CC</td>
<td>3 days</td>
<td>~10 mL</td>
</tr>
<tr>
<td>GD, no plasma</td>
<td>10 mM CC</td>
<td>3 days</td>
<td>~10 mL</td>
</tr>
<tr>
<td>GD</td>
<td>25 mM CC, 50 mM PAA</td>
<td>1 day</td>
<td>~10 mL</td>
</tr>
<tr>
<td>GD</td>
<td>25 mM CC, 50 mM PAA</td>
<td>3.5 days</td>
<td>~10 mL</td>
</tr>
<tr>
<td>GD</td>
<td>25 mM CC, 50 mM PAA</td>
<td>1 week</td>
<td>~10 mL</td>
</tr>
<tr>
<td>double diffusion (DD)</td>
<td>10 mM CC and NC</td>
<td>2 days</td>
<td>~30 mL</td>
</tr>
<tr>
<td>DD, no plasma</td>
<td>10 mM CC and NC</td>
<td>6.5 days</td>
<td>~30 mL</td>
</tr>
<tr>
<td>DD</td>
<td>2.3 mM CC and NC</td>
<td>2 weeks</td>
<td>~30 mL</td>
</tr>
<tr>
<td>DD&lt;sup&gt;15&lt;/sup&gt;</td>
<td>0.02 mM CC and NC, cooled at 4 °C</td>
<td>8 days</td>
<td>~30 mL</td>
</tr>
<tr>
<td>vacuum-assisted&lt;sup&gt;15&lt;/sup&gt;</td>
<td>8 mM CC and NC</td>
<td>1 min</td>
<td>~0.5 mL</td>
</tr>
<tr>
<td>vacuum-assisted&lt;sup&gt;15&lt;/sup&gt;</td>
<td>16 mM CC and NC</td>
<td>1 min</td>
<td>~0.5 mL</td>
</tr>
<tr>
<td>vacuum-assisted&lt;sup&gt;15&lt;/sup&gt;</td>
<td>0.1 M CC, NC and PAA</td>
<td>20 min</td>
<td>~0.5 mL</td>
</tr>
<tr>
<td>centrifugation&lt;sup&gt;18&lt;/sup&gt;</td>
<td>0.1 M CC, NC and PAA</td>
<td>60 min</td>
<td>~0.5 mL</td>
</tr>
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</table>
<sup>a</sup>CC, CaCl₂ solution; NC, Na₂CO₃ solution; PAA, poly(acrylic acid) solution; no plasma, PCL fibers were not plasma-treated.

and the effect of pH is eliminated by fixing the pH using TRIS buffer at pH 9.0. After mineralization, the CaCO₃ crystals were rinsed with distilled water, dehydrated in a 50 to 100% gradient ethanol solution, dried at room temperature, and observed. CNT fibers in the desired quantity were added to the CaCl₂ solutions under stirring to ensure complete dissolution to obtain a CaCl₂ solution with the CNT additive. Then, equal volume CaCl₂ solutions (10 mL) were added to glass bottles with a volume of 12 mL. The bottles were covered with Parafilm, with three pinholes to allow diffusion of NH₃ and CO₂, and placed in a desiccator. Microscope coverslips (5–8 mm diameter) were placed in the CaCl₂ solutions (at the bottom of the bottles) to simplify the recovery of the crystals for microscopy. Finally, two glass bottles (10 mL) of crushed (NH₄)₂CO₃ were also covered with Parafilm, punched with three needle holes, and put at the bottom of the desiccator. After the mineralization reaction had taken place, the crystals were recovered, briefly washed with water, and allowed to dry. After gold coating, the crystals were examined with a scanning electron microscope. It is important to mention that all glassware (glass bottle and small pieces of glass substrate) was cleaned and sonicated in ethanol for 5 min and rinsed with distilled water and the filter was soaked in a H₂O−HNO₃ (65%)−H₂O₂ (3%) solution. Then the glassware was rinsed with distilled water and finally dried in air with acetone. Additionally, a stock aqueous solution of CaCl₂ at different concentrations was freshly prepared in boiled distilled water and bubbled with N₂ for 2 h before use. Typical rhombohedral calcite crystals were obtained in the absence of fiber additives, whereas CNT additives selectively yield truncated and interpenetrated CaCO₃ crystals.

**RESULTS AND DISCUSSION**

Nonwoven meshes composed of submicrometer-sized PCL fibers and nanizeded MWCNTs are used as templates for crystallization processes to facilitate the generation of fiber-enhanced or-directed CaCO₃ composites as well as porous crystalline materials potentially with bimodal pore systems as shown schematically in Figure 1. The task here was to fully embed nanometer- and submicrometer-sized templates within CaCO₃ crystals. The fibers could then be removed by means of calcination or leaching. Porous crystals would be attractive for electronic, optical, sensory, or drug delivery applications, whereas a knowledge of molecular mechanisms of conformational changes on the microstructure of CaCO₃ composite hybrid materials could be interesting for controlling mechanical, medical, and optical applications. An ultrastructural investigation between organic and inorganic layers of hybrid composite materials is necessary, and surface properties, transport mechanisms of CaCO₃ nanoparticles, and the quantitative conformation change in organic molecules observed by atomic force microscopy (AFM) can be addressed.<sup>50,51</sup>

To effectively fill the pores of the PCL meshes, different crystallization routes have been explored. Direct crystallization methods were conducted in the first attempts, and amorphous calcium carbonate (ACC)-mediated pathways were performed in a second set of experiments. The latter were inspired by biomimeralization in nature where ACC is believed to play an important role as a precursor form. ACC is believed to play an important role as a precursor form, and the meshes were mineralized by means of gas and double ion diffusion, precipitation, centrifugation, and vacuum-assisted infiltration with a transient ACC phase (Table 1).<sup>54,55</sup>

As a starting point, the GD method was employed where a plasma untreated submicrometer fibrous PCL mesh in an aqueous solution of 10 mM CaCl₂ was crystallized in a vessel by the decomposition of (NH₄)₂CO₃ into CO₂, H₂O, and NH₃. Most of the resulting crystals did not show the inclusion of fibers, but they rather expelled and lifted the fibers onto their top surface as visible in the Supporting Information (Figure S.I.2). To improve the interaction between CaCO₃ crystals and PCL fibers during crystallization, the fiber scaffold was plasma-activated (1 min, air, 18 W). During treatment, the ester bonds on the fiber surface are supposed to be cleaved and reacted with hydroxyl, carbonyl, and carboxyl groups.<sup>18</sup> In addition, the fiber mesh would be instantaneously wetted similarly to surface-functionalized fibers in contrast to the superhydrophobic behavior of untreated fibers.<sup>56,57</sup> Plasma-treated fibers were increasingly integrated into the calcite crystals as shown in Figure 2. However, the crystals seemed to grow from the fiber mesh rather than inside the fiber mesh. Attempts to switch the top side of the fiber mesh upside down after 1 day of crystallization in order to promote the ingrowth of crystals into the fiber mat were not successful.

To improve the limited integration of CaCO₃ into the fiber mesh, a double ion diffusion (DD) experiment was conducted.
The mesh was placed between two half U-tube arms, which were simultaneously filled with aqueous solutions of CaCl₂ and Na₂CO₃ (10 mM each). This procedure clearly improved the CaCO₃ incorporation, as can be seen from fully fiber-penetrated calcite crystals shown in Figure 3. Nonetheless, there seems to be a larger number of small crystals compared to those formed during the GD method. This suggests many nucleation points. In addition, there were still some crystals growing from the surface of the meshes. Experiments using plasma-untreated PCL and the variation of both concentrations and reaction times did not change the results dramatically.

Theoretically, the ideal case would be one nucleation point in the middle of the fiber mesh leading to a large single crystal. Practically, this is time-consuming because low supersaturation and hydrophobic surfaces are typically needed to prevent many heterogeneous nucleation sites. However, this might be bypassed using ACC intermediates inspired by nature. By this means, the pores might be filled with a liquidlike ACC, which converts directly into a crystal, or by the introduction of a second crystallization step as shown by Qi et al.58 In addition, Meldrum et al. showed that the ACC stability could be increased by cooling the anion/cation solutions prior to the start of crystallization.59 This was applied in a DD experiment; however, the results did not improve the situation shown in Figure 3. The concept of cooling solutions in advance was also applied to a vacuum-assisted method adapted from Qi et al.58 This procedure was applied to a plasma-treated submicrometer-sized PCL mesh. Cooled aqueous CaCl₂ and Na₂CO₃ solutions were mixed to obtain a translucent phase that then served as an infiltration solution for the fiber mesh upon applying vacuum. Figure 4 summarizes the results using two solutions of different concentrations.

A final solution containing 8 mM [Ca²⁺] and [CO₃²⁻] ions (Figure 4a) resulted in small calcite-like structures after drying. Decreasing the concentration to 4 mM (Figure 4b) led to a large (200 × 500 μm²) incorporation of likely amorphous CaCO₃ into the fiber mat. The WAXS pattern from the latter composite structure indicated no peak for crystalline CaCO₃ (Figure 5). The visible peaks were attributed to the semicrystalline orthorhombic PCL.55 The lack of a crystalline peak might be an indication of ACC, which is in agreement with the liquid-like morphology of the CaCO₃. However, a low mineral content could be an alternative explanation.

To further increase the inorganic mass ratio in the composite, a method adapted from Kato et al. was applied.60 A paste was generated by mixing an aqueous solution containing CaCl₂ and PAA with an aqueous Na₂CO₃ solution, reaching a concentration of 50 mM of each component in the final mixture. By aging and subsequent centrifugation of the resulting precipitate into the fiber mesh, deposition was mainly observed on the mesh surface (see Figure S.I. 3 in the Supporting Information). In contrast, using vacuum-assisted infiltration succeeded in the incorporation of fibers within the dried CaCO₃ crust as evident in a cross section of the film in Figure 6. The CaCO₃ film was probably broken by drying or handling. Nonetheless, the production of the large-scale (>1 mm²) incorporation of PCL fibers was accomplished. The CaCO₃ film itself consisted of small particulates (Figure 6b) that might be composed of ACC as suggested by Kato et al.60

To verify the composite structure, WAXS was performed. No crystalline peak, which could be assigned to CaCO₃, was observed in the diffractogram. This suggests an amorphous form of CaCO₃ and is in agreement with the observations made by Kato et al.60

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Kato et al., who reported the stability of the ACC for at least 200 days under ambient conditions. Overall, this method was feasible for coating a large mesh area within a short period of time. Finally, a novel approach of combining the PAA concept as an ACC stabilizer with the GD method was applied. A solution of 25 mM CaCl₂ and 50 mM PAA was employed so that potentially every Ca²⁺ ion could be coordinated by a CO₃²⁻ group of PAA. The resulting composites can be seen in Figure 7. The inorganic composition could be gradually increased by the crystallization time. Whereas after 1 day only partial coating was achieved, crystallization proceeded in particle-like precipitates, which partially coated the entire fiber and mesh surface after 4.5 days (Figure 7b,d). In Figure 7d, it can be seen that some of the ACC particles transformed into calcite polycrystals. After 1 week, large areas (>1 mm²) were coated as shown in Figure 7c. However, prolonging the mineralization time did not lead to the complete

Figure 4. SEM images of PCL fiber meshes infiltrated by ACC precursor solutions. The incorporation of CaCO₃ was conducted by a vacuum-assisted protocol. (a) 16 mM or (b) 8 mM solutions of CaCl₂ and Na₂CO₃ were mixed, and then ~0.5 mL of the mixture was sucked into the mesh within ~1 min. Scale bars: (a1, a2) 100 μm and (b1, b2) = 2 μm.

Figure 5. Wide-angle X-ray diffraction pattern of CaCO₃ on the fiber mesh indicating no crystalline peak attributed to CaCO₃.

Figure 6. SEM images of CaCO₃ films with incorporated (b) PCL meshes. An ACC paste stabilized by PAA (Mₚ = 2 kg/mol) infiltrated the electrospun PCL nanofiber network by vacuum-assisted means, Aging time, ~ 20 min. Scale bars: (a) 10 μm and (b) 2 μm.
coverage of the 0.8 × 0.8 mm² fiber meshes. In summary, composite fabrication was successful using different approaches ranging from vacuum-assisted methods to precipitation to GD. It seemed to be promising to use ACC intermediates, and rather highly concentrated solutions were applied for efficient fiber embedding and pore filling of the electrospun fiber meshes rather than direct calcite crystallization at low saturation.

To create porous crystals and to reveal the homogeneity of the coating, the organic fibers were removed. For this purpose, leaching with chloroform and calcination at 500 °C (~10⁹/min, 3 h dwell time) in an air atmosphere were performed. Leaching of the sample nicely showed the traces of the initial fibers (Figure 8). To some extent, the porous structure of the mesh was retained because the fiber mesh was not completely infiltrated.

Figure 7. SEM images showed an increased CaCO₃ coating on the PCL fiber meshes with increasing crystallization time using PAA as an ACC stabilizer by means of GD. Conditions: CaCl₂ = 25 mM, ACC = 50 mM for (a) 1, (b, d) 4.5, and (c) 7 days. Scale bars: (a–c) 100 μm and (d) = 2 μm.

Figure 8. SEM images of CaCO₃ demonstrated an intact replica of the PCL fibers upon subsequent leaching (chloroform, 1 day). The sample was crystallized using GD for 4.5 days. Scale bars: (a) 100 μm, (b) = 10 μm, and (c, d) = 2 μm.
morph occurrence, and morphology of the CaCO₃ crystals. The have a strong influence on the crystallization behavior, polymorph occurrence, and morphology of the CaCO₃ crystals. The CONCLUSIONS

Electrospun submicrometer fiber meshes and nanosized CNTs were further used for the hybrid fabrication of PCL with CaCO₃, a biorelevant mineral. It was possible to incorporate submicrometer-sized plasma-treated PCL meshes with areas >1 mm² into CaCO₃ using three crystallization methods including the use of PAA. The additive seemed to be useful in stabilizing ACC to effectively fill the space between the electrospun fibers resulting in composite structures. The organic part could be removed by leaching, and the resulting inorganic pore system did not collapse upon a subsequent calcination step. On the other hand, CNT fibers offer a great range for controlled crystallization applications. It was found that differently functionalized CNTs with acidic groups interacted differently with the individual growth planes of CaCO₃ crystals using the GD method. The GD method showed that the concentration of functionalized CNT fibers and the experimental crystallization conditions are crucial to controlling the CaCO₃ crystal morphology. SEM and EDX analysis confirmed that the presence of acidic groups of the

Figure S.I.5 (a–d) shows CaCO₃ crystals with raw MWCNT (CNT-1) fibers obtained with different CaCl₂ concentrations from 5 to 25 mM. In general, these crystals showed rhombohedral calcite with selective CNT fiber deposition on the CaCO₃ crystals. However, Figure S.I.5 (b–d) shows different morphologies (truncated, round, and aggregated) of CaCO₃ crystals obtained in the presence of functionalized sulfonic acid (CNT-2, CNT-3) and carboxylic acid (CNT-5) CNT fibers as templates. We observed that these CaCO₃ crystals lost their well-developed edges and show elongation on the atomic steps of the {104} face. A similar morphology of calcite with stairstep dendritic structures using synthetic peptides and a synthetic organic polymer such as poly(vinyl alcohol) has been reported. It is important to mention that in all the cases where CNTs were dispersed in aqueous solution the obtained crystals are black as a result of the presence of CNTs inside the calcite crystal. The incorporation of CNTs inside the CaCO₃ particles is an interesting way to introduce new properties (e.g., fluorescent, optical, and electrical) into inorganic crystals.

The incorporation of CNTs inside the biorelevant mineral. It was possible to incorporate submicrometer-sized plasma-treated PCL meshes with areas >1 mm² into CaCO₃ using three crystallization methods including the use of PAA. The additive seemed to be useful in stabilizing ACC to effectively fill the space between the electrospun fibers resulting in composite structures. The organic part could be removed by leaching, and the resulting inorganic pore system did not collapse upon a subsequent calcination step. On the other hand, CNT fibers offer a great range for controlled crystallization applications. It was found that differently functionalized CNTs with acidic groups interacted differently with the individual growth planes of CaCO₃ crystals using the GD method. The GD method showed that the concentration of functionalized CNT fibers and the experimental crystallization conditions are crucial to controlling the CaCO₃ crystal morphology. SEM and EDX analysis confirmed that the presence of acidic groups of the

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MWCNTs effectively controls the morphogenesis and the crystallographic polymorphism of CaCO₃ crystals. Thus, the concentration of soft and/or hard organic templates such as PCL and CNT fibers, the concentration of CaCl₂ reactants, and the time were found to be crucial during CaCO₃ nucleation and growth. This result confirms that these organic nanofibers based on CNT have a great potential to induce porosity into CaCO₃ by direct crystallization processes and to obtain advanced hybrid composite materials with a great potential improvement in properties such as stiffness and bioactivity, as was already shown by SEM analysis. Our suggestion is that at different pH values, functionalized CNT fibers can undergo changes in the charge of the sulfonate or carboxylic acid groups and adopt different orientations in solution and thereby elicit changes in CaCO₃ morphology. Therefore, the presence of acidic moieties along the functionalized MWCNT is crucial for interaction with the growing CaCO₃ on the nanoscale level, as can also be expected from numerous literature reports on the microscale. In summary, we demonstrated that the use of PCL and CNT fibers as an additive or substrate template provides a viable approach for studying various aspects of biomineralization, including the production of controlled particles, soft/hard combined reinforcement, control of porosity, and defined morphologies at microscale and nanoscale levels.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.6b02536.

Additional data from SEM of CaCO₃ crystals and CaCO₃ films obtained with plasma and PCL meshes as well as light microscopy pictures from the CaCO₃/MWCNT and dynamic light scattering (DLS) (PDF)

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Notes

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ABBREVIATIONS

PCL, poly(ε-caprolactone); MWCNT, multiwalled carbon nanotubes; CaCO₃, calcium carbonate; PAA, poly(acrylic acid) solution; GD, gas diffusion; DD, double diffusion; CNT, carbon nanotubes; ACC, amorphous calcium carbonate; WAXS, wide-angle X-ray scattering

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