Dextran and Its Derivatives: Biopolymer Additives for the Modulation of Vaterite CaCO₃ Crystal Morphology and Adhesion to Cells

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Nowadays, a great demand for the development of novel drug delivery systems with high potential for bench-to-market transition attracts scientific attention toward materials that are already approved for biomedical use.

Here, controlled fabrication of hybrid organic inorganic mesoporous crystals is realized in physiologically relevant conditions by co-synthesis of vaterite CaCO₃ in the presence of dextran (DEX) or its functional derivatives. The effects of DEX molecular weight and chemical structure on morphology, porosity, and stability of the hybrids are investigated. Molecular weight of DEX does not affect the crystal growth but leads to the partial blocking of crystal pores. Co-synthesis of DEX functionalized with either carboxymethyl (CM) or diethylaminoethyl (DEAE) groups drastically increased crystal porosity without influencing crystal size. pH-dependent vaterite-to-calcite recrystallization is significantly suppressed by inclusion of carboxymethyl-dextran (CM-DEX), making vaterite crystals stable in acidic medium, whereas the incorporation of diethylaminoethyl-dextran (DEAE-DEX) has no effect. The hybrids prepared with charged DEX derivatives possess stronger adhesion to normal human dermal fibroblasts: three times higher crystal adherence compared to pristine crystals. These results provide fundamental physical–chemical insights into the crystallization of DEX/vaterite hybrids and are discussed in view of the potential of these functional delivery carriers for biomedical and other applications.

1. Introduction

The last decades of biomedical science are accompanied by an exponential growth in the number of new drugs and therapeutic, diagnostic, theranostic and vaccination strategies. Whilst vast drug discoveries revolutionize the biomedical field, their controlled, smart delivery lags behind in its development and remains a challenge—preventing the wide introduction of novel therapeutics into clinical practice. Despite the large number of publications on new drug delivery strategies, the translation of the basic research to clinical application is disappointing. This is due to biocompatibility and safety issues, complicated approval and governmental regulation, as well as the large-scale manufacture challenges prevailing over the challenge proposing new and effective drug delivery approaches. In view of this, researchers draw their attention to drug delivery systems made of materials already approved for biomedical use.

Inorganic crystals of calcium carbonate are among them. CaCO₃ is a non-toxic natural biomineral that is commonly used as a cheap reinforcement material for paper and plastic coatings, as well as a safe buffering agent for food products, hygiene aids and cosmetics.[1–4] Known and used for centuries, CaCO₃ gained its economic importance from the middle of the 19th century;[5] since 1983, the safe use of CaCO₃ has been approved by the Food and Drug Administration Agency as a food and pharmaceutical additive/ingredient. Its modern uses include a source of calcium, coloring (white) additive, acidity regulator, and bulking agent. Recently, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) reevaluated the use of calcium carbonate (E170) taking into account the latest available toxicological data and confirmed that the use of CaCO₃ causes no toxicological concerns.[6]

CaCO₃ can exist in a form of several polymorphs.[7] Up to now, the use of rhombohedral calcite—it’s thermodynamically favorable form—is the most common. However, in recent years another form—the anhydrous metastable vaterite polymorph—attracted interest due to its developed mesoporous structure.[8,9] The capacious vaterite interior allows for the wide utilization
of these inorganic crystals as versatile containers for antimicrobials,[28] functional nanoparticles,[11,12] medical drugs,[13–15] and bioactive molecules.[16,17] Another advantage of vaterite is its large capability to undergo dissolution in body fluids compared to other polymorphs of CaCO₃.[58] In view of this, vaterite crystals have been recognized as promising drug delivery systems. Non-toxic and biodegradable,[4] vaterite can be easily produced in the size range from tens of nanometers to hundreds of microns.[19–23] Vaterite porosity and shape can also be controlled, making vaterite crystals a versatile platform for a diverse range of applications,[22] including use as hard carriers, templates for soft drug delivery vehicles (e.g., polyelectrolyte or alginate capsules), and CaCO₃/alginate hybrid materials.[23] As a drug container, vaterite crystals generally have high loading capacities for various drugs.[24,25] Recently, vaterite therapeutic, diagnostic, and therapeutic uses have been demonstrated in vitro and in vivo.[21,26–29]

One of the main drawbacks of vaterite crystals are their inherent metastability and rather quick degradation/recrystallization in aqueous environments.[30] Degradation/decomposition of vaterite is required for hard templating of polymer, protein, inorganic or hybrid structures[22,31–34] by means of loading the vaterite pores, followed by vaterite solubilisation. Although such properties may be beneficial for some medical applications, for example, pH-triggered release in tumor sites,[35–37] many other applications require the stabilization of vaterite and the suppression of its recrystallization. This is especially important for the use of vaterite in implantable drug delivery systems, tissue engineering platforms, food/cosmetic additives and storage materials, which are designed for prolonged action.

The most widely used approach to control recrystallization of the carbonates is the use of polymer matrices, which stabilize the vaterite nanocrystallites and suppress the transportation of ions from the crystal surface, as this recrystallization is known to be a solution-mediated mechanism.[38,39] Previously, mucin acted to hamper the recrystallization rate of vaterite to calcite, in which the vaterite-to-calcite transformation acted as the mechanism of doxorubicin release and was attributed to the reduced ion mobility in aqueous solution in the presence of mucin.[13] Whilst poly(N-vinyl-2-pyrrolidone) (PVP) has acted to alter the recrystallization rate of vaterite to calcite via the change in the rate-determining step of the vaterite-to-calcite transition.[40] Besides these, some known examples of polymeric matrices to control vaterite recrystallization include: polycarboxylate-type superplasticizers,[41] poly(aspartate),[42] poly(amidoamine),[43] carboxymethylulinin,[44] and lentiln,[45] for instance.

In this study, the crystallization of vaterite has been controlled by the presence of widely used biodegradable and biocompatible macromolecular modifiers: dextran (DEX) and its functional derivatives. The natural macromolecule, DEX, is already used in clinical practice, for example, as a plasma volume expander[46] and as a coating for nanoparticles used as therapeutic and imaging agents, as well as other bioapplications.[47,48] Both DEX derivatives utilized in this study, CM-DEX and DEAE-DEX, are also important biomaterials with clear application value regarding drug delivery and therapeutic applications.[49–54] This study sought the understanding of the effects of DEX and its derivatives on the crystallization and further possible stabilization of hybrid vaterite-based microcrystals and provides a fundamental platform for their further development as drug delivery systems. Physical-chemical properties of the hybrids are analyzed employing a set of modern methods and techniques such as scanning electron microscopy (SEM), Brunauer, Emmett and Teller (BET) analysis, X-ray diffraction (XRD), thermal gravimetric analysis (TGA), and Fourier-transform infrared spectroscopy (FT-IR). The interaction of the hybrids with biological cells (normal human dermal fibroblasts [NHDFs]) is also assessed.

2. Results and Discussion

2.1. Crystallization of Vaterite in the Presence of Nonionic DEX

2.1.1. Crystal Size and Morphology

Pristine vaterite CaCO₃ crystals (diameter within the range of ≈7–11 µm) were utilized in this study as a control, with their vaterite polymorphism confirmed via both XRD and FT-IR (Figure S1 and S2, Supporting Information, respectively). Vaterite microcrystals were synthesized according to a well-established protocol, and resulted in a spherical shape and mesoporous structure, typical for hexagonal vaterite crystals grown by Ostwald ripening in aqueous media.[18,55] SEM images of representative vaterite CaCO₃ crystals are provided in Figure 1A. For co-synthesis of DEX, nonionic DEXs (average molecular weights from 4 to 2000 kDa) were added to the CaCl₂ solution, which were then mixed with Na₂CO₃ at an initial ratio (DEX:CaCO₃) of 1:10 w/w. Under these conditions, DEXs of different molecular weights were entrapped within the pores of the vaterite during its growth, resulting in the formation of hybrid DEX<sup>FTTC</sup>/vaterite CaCO₃ crystals containing less than 10%<sub>w</sub> of DEX<sup>FTTC</sup>, as was estimated by TGA from the weight loss at 550 °C (typical TGA curves are shown in Figure S3, Supporting Information). The homogeneous distribution of DEX<sup>FTTC</sup> molecules throughout the entire volume of the crystals was confirmed by fluorescence microscopy of the hybrid crystals (Figure 1) and did not depend on DEX molecular weight.

Co-synthesis of vaterite and nonionic DEXs of different molecular weights did not influence the polymorphism of the crystals, preserving the predominant formation of vaterite, and had negligible effect on the secondary crystal size, as confirmed by FT-IR spectroscopy (Figure S2, Supporting Information) and SEM (Figure 1B–G), respectively. Of note, there is a slight shift of the ν₄ band (in-plane O–C–O bending[56,57]) of vaterite to lower wavenumbers, from 745 to 741 cm<sup>−1</sup> in vaterite/DEX hybrids: this is likely due to the interaction of the carbonate ions with DEX molecules during the crystal growth phase, such as the hydrogen bonding to amorphous calcium carbonate.[58] Moreover, cracked crystals of the vaterite and vaterite/DEX<sup>FTTC</sup> hybrids bare a similar structure of interconnected cylindrical radial pores, as typically seen throughout the literature.[56,59]

Calculation of the size of the nanocrystallites visible at the external surface of the crystals suggests a ≈2 times reduction of their size in the presence of nonionic DEXs, wherein the molecular weight of DEX had no significant influence on this effect (Figure 2A,B).
Interestingly, the effects of nonionic DEX addition on the crystallization of calcium carbonate established in this study for the mixing method at constant pH, drastically differ from those found for the mixing of H₂CO₃ and Ca(OH)₂. In this case, the addition of DEX significantly affected the pH values during the crystallization process, which resulted in the inhibition of vaterite nucleation and the predominant formation of the calcite.⁶⁰ Of note, typically, the co-precipitation of both small drugs and macromolecules does not affect the crystallinity of the CaCO₃ crystals,⁶¹⁶² even with loading capacities of up to 588 mg g⁻¹ mucin.⁶¹ This is evidenced by the typical vaterite morphologies observed via microscopy and typical FTIR wavenumbers we obtain for such DEX/vaterite hybrids. The suppression of the nanocrystallite growth by nonionic DEXs can be attributed to the adsorption of DEX molecules on the nanocrystallite surface, and consequent suppression of CaCO₃ dissolution, which is a known step in crystal ripening.⁶³ BET analysis of the crystals was in good agreement with SEM and provided additional insights into the crystal structure, as discussed below.

### 2.1.2. Crystal Porosity

Figure 3A,B illustrates typical N₂ adsorption–desorption isotherms obtained for bare vaterite crystals and 2000 kDa-DEXFITC/vaterite hybrids. Analysis of the surface area and typical pore distributions (Table 1), estimated in accordance with the BJH model, indicates the elevation of the total surface area of the hybrid crystals compared to pristine vaterite, whilst preserving the pore size distribution; this correlates with the reduced nanocrystallite size, schematically shown in Figure 2B.
Figure 2. A) The effect of DEX<sup>FITC</sup> molecular weight upon the average size of DEX<sup>FITC</sup>/vaterite hybrid crystal nanocrystallites. Error bars are SD (n = 100), and B) schematic of the effect the presence of DEX<sup>FITC</sup> upon the crystal properties of vaterite CaCO<sub>3</sub> (i.e., nanocrystallite size and pore occupation).

Figure 3. Nitrogen adsorption–desorption isotherms of A) pristine vaterite crystals and B) 2000 kDa DEX<sup>FITC</sup>/vaterite crystals, as well as C) the effect of DEX<sup>FITC</sup> molecular weight upon the hysteresis loop area (as determined by numerical integration) of DEX<sup>FITC</sup>/vaterite hybrid crystals.
Typical pore distributions for pristine vaterite and 2000 kDa DEXFITC/vaterite hybrids are shown in Figure S4, Supporting Information. The molecular weight of DEX held no influence on these effects, despite the range in hydrodynamic diameter of DEXs investigated in this study (13.2–53.8 nm). Of note, the surface area of pure vaterite correlated with that which the literature reported,[64] hence the control crystals synthesized in this study have typical total surface area.

A significant adsorption–desorption hysteresis loop, denoted type H2a, was observed for DEXFITC/vaterite hybrids (Figure 3B). Its width was estimated as the numerically integrated area enclosed within the adsorption–desorption isotherms (Figure 3C). The widening of the hysteresis loop for DEXFITC/vaterite hybrids indicates that a significant volume fraction of the pores remains filled until a lower vapor pressure—which is typical if the narrowing of the pore openings takes place, that is, cylindrical pores are converted into ink-bottle-like pores.[67] In other words, the incorporation of DEX leads to the partial blocking of the vaterite crystal pores. This is in contrast to pristine vaterite crystals in which a type H1 isotherm is observed, suggesting uniform cylindrical pores with minimal pore blocking.[68] The filling of crystal pores with DEX occurs regardless of DEX size, however, there is an increase in hysteresis loop area when DEX molecular weight increases above 40 kDa; this may be due to the larger hydrodynamic radii of higher molecular weight DEXs, and perhaps a greater interaction with the vaterite crystal surface, and hence increased DEX uptake and pore blockage. This may have important consequences for future drug release kinetics.

| DEX molecular weight [kDa] | DEX hydrodynamic radii [nm] | Crystal size [µm] | Surface area [m² g⁻¹] | Pore distribution [nm] |
|----------------------------|-----------------------------|--------------------|------------------------|------------------------|
| No DEX                     | –                           | 11.4 ± 1.0         | 3.95                   | 5–30                   |
| 40                         | 6.6                         | 6.6 ± 1.1          | 19.0                   | 3–28                   |
| 150                        | 9.0                         | 8.2 ± 1.3          | 19.6                   | 2–30                   |
| 500                        | 15.9                        | 7.3 ± 0.8          | 28.7                   | 3–28                   |
| 2000                       | 26.9                        | 9.3 ± 0.8          | 20.1                   | 5–30                   |

Figure 4. Imaging of ionic DEXFITC/vaterite hybrid crystals. A) CM-DEXFITC/vaterite hybrids and B) DEAE-DEXFITC/vaterite hybrids are shown, with i) transmittance and ii) fluorescent images of crystals, iii) SEM overview of spherical vaterite crystals, iv) cross-section of a single crystal, and v) the typical surface morphology is shown. Scale bar is 10 µm for (i), (ii) and (iii), 1 µm for (iv), and 100 nm for (v). The DEX molecular weight used is 150 kDa.
as evidenced via fluorescence microscopy. CM-DEX holds an apparent slight accumulation within the center of the vaterite crystals, which may be due to the formation of CM aggregates during co-synthesis, as reported with certain proteins in the presence of the same ions.\cite{17,71} DEAE-DEX has a higher fluorescent signal on the crystal surface; in either case it is unclear why, but this is likely due to the difference in encapsulation capacities between the two DEXs, which is related to the electrostatics of the DEX molecule and CaCO$_3$ crystal.

2.2.2. Crystal Porosity

From the analysis of N$_2$ adsorption–desorption isotherms (Figure 6), notably, the surface area of CM-DEXFITC/vaterite hybrids is dramatically increased to $\approx 41$ m$^2$ g$^{-1}$, despite no significant alteration in the crystal size. This can be explained by the reduction of the nanocrystallite size upon CM-DEX co-synthesis with vaterite as well as the possible effect from the presence of precipitated DEX itself; such effect of nanocrystallite size on the surface area is also reported.\cite{22} The large extent of vaterite crystal filling with CM-DEX is evident from the sizeable increase in hysteresis loop width compared to pristine vaterite and nonionic DEXFITC/vaterite hybrids, indicating partial blocking of internal pores. Comparatively, DEAE-DEX slightly increases the surface area of these hybrids to $\approx 6$ m$^2$ g$^{-1}$ compared to the bare crystals, which correlates to the small decrease in average nanocrystallite size (Figure 5) and the little uptake of DEX as evidenced by the small change in mass during TGA curve analysis (Figure S3, Supporting Information). Despite these differences in the extent of pore filling, both CM- and DEAE-DEXFITC/vaterite hybrids hold similar pore distributions, which are akin to that of pristine vaterite and nonionic DEXFITC/vaterite hybrids as determined via BET analysis; such parameters are summarized in Table 2. The ability to produce DEXFITC/vaterite hybrids of varying charge with negligible effect on the crystal size and pore distribution may be invaluable for future drug delivery applications; especially for the selective encapsulation of charged low molecular weight species, for which the large pore sizes of pristine vaterite are typically a hindrance.\cite{76}

2.3. Vaterite-to-Calcite Recrystallization at Different pH

The controlled recrystallization of vaterite to calcite at varying pH is of great importance regarding their use as drug delivery vehicles, as the encapsulated drug should not be released before reaching its target site. The pH-dependent recrystallization of

Table 2. The effect of charged 150 kDa DEXFITC upon the average crystal size, specific surface area, and the pore distribution of the DEXFITC/vaterite hybrids.

| DEX charge | Crystal size [µm] | Surface area [m$^2$ g$^{-1}$] | Pore distribution [nm] |
|------------|------------------|-------------------------------|------------------------|
| No DEX     | 11.4 ± 1.0       | 3.95                          | 5–30                   |
| (-)        | 7.8 ± 0.6        | 40.5                          | 5–28                   |
| (+)        | 6.2 ± 1.0        | 5.89                          | 3–30                   |

Figure 5. The effect of DEXFITC charge upon the average size of charged 150 kDa DEXFITC/vaterite hybrid crystal nanocrystallites. Error bars are SD ($n = 100$).

Figure 6. Nitrogen adsorption–desorption isotherms of 150 kDa A) CM-DEXFITC/vaterite and B) DEAE-DEXFITC/vaterite hybrid crystals.
pristine vaterite to calcite is shown, with enhanced recrystallization at lower pHs. This is likely due to the enhanced solubility of CaCO$_3$ at these conditions$^{[77]}$ wherein the dissolution and subsequent re-precipitation of CaCO$_3$ is the main mechanism responsible for calcite formation.$^{[78]}$ Here, we have demonstrated the enhanced stabilization of CM-DEXFITC/vaterite hybrids, with no vaterite-to-calcite transformation within the range of pH 5.7–9.0 across a 54-day period, as shown in Figure S5C, Supporting Information. Whereas DEAE-DEXFITC/vaterite hybrids appear to transform independent of DEAE-DEX presence, similarly to the pristine vaterite CaCO$_3$ within a range of 5 to 8 h for pHs 6 and 7.6, and ~20 to 30 h for pH 9. The higher uptake of anionic CM-DEX likely inhibits the recrystallization of vaterite via the binding of Ca$^{2+}$ to carboxyl groups along the DEX backbone, and hence limits the ion transport rate in solution.$^{[41]}$ Further to the presence of an internal polymeric matrix, the vaterite crystals may be coated with the layer-by-layer (LbL) coating of polyelectrolytes to suppress the recrystallization, however, such coatings typically only suppress the vaterite-to-calcite transformation for a few days.$^{[12]}$ as opposed to pre-encapsulated matrices as shown in this study, for instance. Here we only investigate one weight percentage of DEX, and further concentrations should be described in future works in order to control the vaterite re-crystallization rate, which holds great implications for tissue culture applications and the encapsulation and subsequent programmed release of small ionic drugs.

2.4. Vaterite-Cell Interaction

Here we present preliminary cell culture in vitro results via the interaction of 1–3 µm pristine and ionic DEXFITC/vaterite hybrid crystals with NHDFs over a 3 h incubation period in

Figure 7. Overlayed transmittance and fluorescence images of NHDFs with A) pristine-vaterite crystals, B) CM-DEXFITC/vaterite hybrids, or C) DEAE-DEXFITC/vaterite hybrids. Images and number of particles are taken after thrice washing with DMEM media supplemented with 10% FBS and 1% penicillin-streptomycin. D) the particle:cell ratio is also plotted as a function of DEX derivative. Error bars represent SD, $n = 3$. 
cell culture medium (Figure 7). Of note, these smaller crystals displayed no significant changes in their morphology when compared to the DEXFITC/vaterite hybrids investigated here. DEAE-DEXFITC/vaterite hybrids showed a weaker fluorescent signal compared to CM-DEXFITC/vaterite hybrids due to the lower amount of DEAE-DEX encapsulated, as aforementioned. After thrice washing the fibroblasts, it appears these vaterite hybrids were able to attach to the cell surface during this rather short incubation period. Previously, >600 nm naftinile-loaded CaCO3 vaterite crystals were uptaken at an average internalization rate of 2.57 crystals per cell (NHDFs), where the LbL deposition of certain biopolyelectrolytes (heparin, poly(arginine), or dextran sulfate) did not have a dramatic effect on this internalization.[28] In our study, the presence of CM- and DEAE-DEX significantly increased cell attachment compared to pristine vaterite, perhaps due to the binding of DEX to certain molecules, such as proteins, on the cell surface. This is similar to those preliminary results reported for the uptake of CM-DEX/vaterite hybrids within HepG2 and SK-Hep-1 human liver cancer cells, with the enhanced uptake of these hybrids.[13] Such attachment bodes well for further tissue culture and local drug delivery applications, such as topical or transdermal treatments involving the encapsulation of antifungal, antibiotic and anti-inflammatory drugs into these hybrids. Future in vitro studies will focus on the internalization of hybrids in these and further cell-types; however, this topic is out of the scope of this study and is our ongoing research.

3. Conclusion

The influence of DEX and its derivatives on the crystallization and stability of the vaterite is investigated. DEX is incorporated into the vaterite crystals by means of co-synthesis at physiologically relevant pH 7.4. The presence of DEX, DEAE-DEX, or CM-DEX matrix did not influence the polymorphism of the crystals and had negligible effect on the crystal size. Incorporation of nonionic DEX decreased the nanocrystallite size, with no effect on the pore size distribution of the crystals but lead to the partial blocking the crystal pores. This may influence drug release kinetics. Varying the molecular weight of DEX had no significant influence on these effects.

The use of DEX grafted with either CM or DEAE groups increased the overall crystal porosity without influencing the size of the crystals. In addition, the inclusion of CM-DEX significantly retarded the vaterite-to-calcite recrystallization within the pH range of 5.7–9, whereas DEAE-DEX did not affect the kinetics of recrystallization. This might have important implications for drug delivery, biomineralization, and other biomedical applications.

Moreover, this study shows how the impregnation of biopolymer matrices of different natures can be used as a tool for modulation of cell-drug carrier interactions. Preliminary cell culture studies demonstrate the significant attachment of ionic DEX/vaterite hybrids to NHDFs compared to pristine vaterite. Based on these data, a model of CaCO3 crystallization in the presence of DEX and its charged derivatives as macromolecular additives is proposed. These results open avenues for further use of DEX/vaterite hybrid crystals as additives for food products and packaging, as well as for biomedical applications.

4. Experimental Section

Materials: Calcium chloride dihydrate (Acros Organics, 10158280), sodium carbonate (Acros Organics, 10577182), fluorescein isothiocyanate-labelled dextran at 4 kDa (Sigma, FD4), 40 kDa (Sigma, FD405), 70 kDa (Sigma, FD705), 150 kDa (Sigma, FD150S), 500 kDa (Sigma, 46947) and 2000 kDa (FD2000S), fluorescein isothiocyanate-labelled carboxymethylated xylan 150 kDa (Sigma, 74817), fluorescein isothiocyanate-labelled diethylaminoethyldextran 150 kDa (Sigma, 75005), tris-buffered saline (10X Tris) pH 7.4 (Alfa Aesar, 160764) containing 250 mM Tris, 1.37 mM sodium chloride and 27 mM potassium chloride, 1 mM sodium hydroxide (Fisher, 10528240), and 1 mL hydrochloric acid (Fisher, 10467640) were used to adjust the pH. Dulbecco’s Modified Eagle Medium (DMEM)-low glucose-GlutaMAX supplement (Gibco, 21885108), Trypsin-EDTA solution 10X (Sigma, 59418C), heat-inactivated fetal bovine serum (FBS) (Sigma, F9665), penicillin-streptomycin (Gibco,15140-122). The water used in the experiments was prepared using a Millipore Milli-Q purification system and had a resistivity higher than 18.2 MΩ cm.

Synthesis of Calcium Carbonate Crystals: The fabrication of pure CaCO3 crystals of ~7 μm in diameter used as a control sample in this study is also described elsewhere.[29] Here, 100 mL of 50 mM CaCl2 in 2X TRIS pH 7.4 was added to a 400 mL beaker and agitated at 650 rpm for 60 s. 100 mL of 50 mM Na2CO3 in water was then quickly added, and the mixture was agitated for a further 60 s. After 60 s, the suspension was poured into plastic tubes and left to grow for 20 min at room temperature. The resulting crystals were centrifuged at 1100 g for 5 min and washed twice with water. The sediment was finally suspended in 1 mL of water and dried on a pre-heated glass petri-dish for 1–2 h at 85 °C. For the formation of DEXFITC-loaded crystals, the same protocol was followed adding 1 mL of 50 mg mL-1 DEXFITC aqueous solution to the CaCl2 solution before the agitation and addition of Na2CO3.

Optical and Fluorescence Microscopy: Life Technologies EVOS FL microscope with 40× lens (United States) was used for analysis of the synthesized CaCO3 crystals and DEXFITC/CaCO3 hybrids. Imaging was performed in both transmission and fluorescence modes. The images were processed using ImageJ 1.48 V Software (Wayne Rasband, NIH, USA) to enhance brightness and color, and to take fluorescence profiles. The cells and cell–crystal interaction were imaged using DMi8 Fluorescence Microscope with 20× objective (Leica, Germany).

Scanning Electron Microscopy (SEM): Pure CaCO3 and hybrid DEXFITC/CaCO3 samples were prepared via depositing the dried powder onto carbon tape upon the aluminum sample stub. The crystals were cracked via light mechanical force. The samples were imaged using the JSM-7100F field-emission scanning electron microscope (JEOL, USA) using a probe current of 1 μA and an accelerating voltage of 2 kV. Images of crystal surface topology at high magnification were again processed using ImageJ in order to determine the nanocrystallite diameter.

Brunauer, Emmett, and Teller (BET) Analysis: Nitrogen adsorption–desorption analysis of CaCO3 crystals and DEXFITC/CaCO3 hybrids had been carried out using a QUADRASORB SI (Quantachrome Instruments, USA), 77.3 K. 30–100 mg of the powder was used for each measurement. The samples were degassed at 150 °C for 20 h prior to the measurements. Porosity analysis had been performed using the Barret–Joyner–Halenda (BJH) model.

Thermal Gravimetric Analysis (TGA): TGA was performed for the dried CaCO3 and DEXFITC/CaCO3 hybrids heated from 30 to 800 °C at a rate of 5 °C min−1 in a helium atmosphere using a TGA 4000 thermogravimetric analyzer (PerkinElmer, USA).

X-Ray Diffraction: Samples were deposited and the spectra were taken under the following conditions: copper anode material, Kα1 and Kα2 wavelengths of 1.540598 and 1.54426 Å, respectively, with a generator voltage of 45 kV and a tube current of 40 mA using a PANalytical
Fourier-Transform Infrared Spectroscopy: FTIR spectroscopy was performed on a Cary 630 FTIR Spectrometer (Agilent, USA). 32 scans per sample were performed, with a resolution of 4 cm$^{-1}$.

In Vitro Recrystallization of Vaterite CaCO$_3$: The recrystallization of vaterite into calcite was monitored by pipetting 10 µL of a suspension of bare vaterite crystals, DEAE-DEX$^{14}$TIC/CaCO$_3$, and CM-DEX$^{14}$TIC/CaCO$_3$ (3 mg mL$^{-1}$) between two glass slides sealed together with paraffin to prevent evaporation. The crystal suspensions were prepared with Tris buffer solution at pH 6, 7.4–7.6 and 9, and images were taken over time to study the transformation of vaterite to calcite. All experiments were carried out at room temperature. The percentage of calcite was calculated with the following equation (Equation 1):

$$\frac{V_t - V_i}{V_i} \times 100$$

(1)

where $V_i$ is the initial number of vaterite crystals and $V_t$ is the number of vaterite crystals at different times. The vaterite crystals were distinguished from calcite crystals via their morphology, that is, vaterite crystals were counted as spherical particles, whilst calcite crystals were cubic.

Cell Culture: NHDFs were cultured at 37 °C with 5% CO$_2$ using Dulbecco’s Modified Eagle Medium (DMEM) media supplemented with 10% FBS and 1% penicillin-streptomycin until confluent. The cells were then trypsinized, followed by neutralization with media before counting on a hemocytometer and seeding in a 96 well plate at 10 000 cells per well. These were then incubated for 24 h. For CaCO$_3$ crystal interaction studies, these NHDFs were first incubated in FBS-free media prior to incubation with the CaCO$_3$ crystal suspension (concentration: 0.03 mg mL$^{-1}$ in FBS-free media) for 3 h. The cells were then thrice washed with media to remove any non-attached crystals, followed by subsequent optical and fluorescent imaging.

Keywords

Biomineralization, co-synthesis, drug delivery, mesoporous, recrystallization

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

This work was supported by the Marie Skłodowska-Curie Ph.D. Fellowship programme, EC Grant Agreement No. 801604-DTA3-H2020-MSCA-COFUND-2017. J.C. acknowledges the Ph.D. Bursary from Nottingham Trent University. D.V. and J.C. acknowledge Research Contingency Fund as well as Talent Bridging Fund (Nottingham Trent University). A.V. acknowledges financial support from the Stedler Foundation in the frames of the “Function by Design: Cellular Hybrids” project. The authors thank Kathryn Kroon and Graham Hickman from Nottingham Trent University for the assistance with SEM imaging, and Ava McMullin from Nottingham Trent University for the assistance with the imaging of cells. Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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