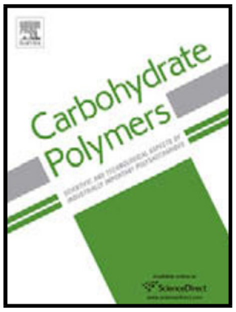


Title: Co-effects of Amines Molecules and Chitosan Films on
In Vitro Calcium Carbonate Mineralization

Author: Jifei Cui John F. Kennedy Jun Nie Guiping Ma

PII: S0144-8617(15)00614-1
DOI: http://dx.doi.org/doi:10.1016/j.carbpol.2015.06.096
Reference: CARP 10087

To appear in:

Received date: 4-4-2015
Revised date: 29-5-2015
Accepted date: 30-6-2015

Please cite this article as: Cui, J., Kennedy, J. F., Nie, J., and Ma, G.,Co-effects of Amines Molecules and Chitosan Films on In Vitro Calcium Carbonate Mineralization, *Carbohydrate Polymers* (2015), http://dx.doi.org/10.1016/j.carbpol.2015.06.096

Highlights

1) Morphologies of $CaCO_3$ were obtained by the co-effect of chitosan and positively charged monomers;
2) The effect of different monomers on mineralization of $CaCO_3$ was investigated and the possible reason was analyzed.

Co-effects of Amines Molecules and Chitosan Films on In Vitro Calcium Carbonate Mineralization

Jifei Cui[1], John F. Kennedy[2],Jun Nie, Guiping Ma[1]*

1. Beijing Laboratory of Biomedical Materials Beijing University of Chemical Technology, Beijing 100029, P R China
2. Chembiotech Laboratories, University of Birmingham Research Park, Vincent Drive, Birmingham B15 2SQ, UK

Corresponding Author
Tel(Fax):+86-01064421310
magp@mail.buct.edu.cn

Abstract

Amines monomers, N,N-dimethylaminoethyl methacrylate (DMAEMA),N,N-dimethylethanolamine (DMEA), 2-dimethylaminoethylamine (DMEDA) and N-methIyldiethanolamine (MDEA) were used to induce the formation of calcium carbonate ($CaCO_3$) crystals on chitosan films, by using $(NH4)_2CO_3$ diffusion method at ambient temperature. The obtained $CaCO_3$ particles were characterized by scanning electron microscope (SEM), X-ray diffraction (XRD) and Energy dispersive spectroscopy (EDS).The influence of reaction variables, such as the additive concentration and their types were also investigated on the products. The morphologies of $CaCO_3$ crystals, inter-grown in cube-shape, were controlled by DMAEMA and DMEA. It was observed that the morphologies of $CaCO_3$ changed from the cube grown arms to massive calcite with a hole on the face by increasing the concentrations of DMEDA and MDEA. While the precipitation grew on chitosan film without any organic additive, only single cube-shaped crystals were obtained. By these results the possible mechanisms can be proposed that electronic movement of the groups on the monomer effected ions configuration and molecules absorbed on the exposed surface, resulted the change of the surface energy, which caused the change in the morphology of $CaCO_3$.

Key words: Calcium carbonate, Mineralization, Amine molecules, Chitosan film

1. Introduction

In nature, biological organisms produce polymer/inorganic hybrid materials such as bone, teeth, diatoms and shells, which inspired physicists, chemists, and materials scientists to mimic such structures and their properties(Nudelman&Sommerdijk (2012); Xinet al.(2014)). At present, calcium carbonate ($CaCO_3$)are the most abundant crystalline biogenic minerals found in nature. Moreover, $CaCO_3$ have been widely studied owing to their significance in biomineralization processes producing unique organic/inorganic material (Gong et al. (2008);Vilela et al., (2010); Chan, Wu, Li, & Cheung, (2002)),biological activities of protein-adhesive properties, cell compatibility, and hard tissue compatibility (Ma et al. (2013))and an attractive combination of properties, including opacity, high hardness and density, and low cost. Scientists inspired by the functions of $CaCO_3$in nature have attempted to produce different morphologies and properties of $CaCO_3$ crystals, and propose the formation mechanisms in vitro by mimicking the biomineralization processes(Dmitrovic et al., (2012); Lee, Park, Kwak& Cho, (2010)). So chemistry at ambient temperature plays with organic molecules to open the door to a much wider range of possible polymorphs and morphology.

Chitosan as an abundant biopolymer influences the mineralization process of $CaCO_3$is always used as templates or together with soluble addictive(Hosoda& Kato (2001)) for the deposition of $CaCO_3$. By the coexistence of PAA and chitosan membranes the thin island crystals of $CaCO_3$ with aragonite and vaterite forms, which develop into continuous films, were generated(Wada, Suda, Kanamura&Umegaki. (2004)). The work of Neira-Carrillo and his co-workers(Neira-Carrillo, Retuert, Martínez, & Arias (2008))dealt with the effect of the constrained volume given by crosslinked chitosan as a sphere on the in vitro $CaCO_3$ crystallization. Until now, most of the soluble additives which together which chitosan to control the mineralization of $CaCO_3$ were polyanionic or had–COOH groups.

In the case of calcium carbonate, research has principally concentrated on the influence of anionicadditives as crystal habit modifiers(Ren, Feng &Bourrat, (2011)). However, some studies also focused on the potential role of cationic additives in controlling $CaCO_3$ precipitation(Schenk et al., (2014))and amines were widespread in the biosphere with a very important physiological role. Poly(allylamine hydrochloride) (PAH) was extremely effective in directing the formation of $CaCO_3$ thin films and fibers, where the extent of fiber formation depended on the reaction conditions(Cantaert et al., (2013)). Other polyamines such as, PVAm, PAMA, and PEI were further studied the effect on $CaCO_3$ precipitation(Schenk et al. (2014)).$CaCO_3$ crystals with different morphologies were synthesized under the regulation of DDAB, $[C_{12}mim]Br$and the mixed DDAB/ [C_{12}mim]Br micelles(Zhao et al. (2011)). Various amines such as ethylenediamine, diethylenetriamine(Sugihara et al. (1996)), and other polyamines(Gao, Yu, &Guo, (2006))resulted in the formation of disk-like vaterite crystals that have been reported. It was proposed that the major factor for directing the crystal morphology was the adsorption of the amino groups on the $CaCO_3$ crystal surfaces. Moreover, the designed polymer with charged was synthesized to construct LbL systems: negatively charged star-PAA with positively charged chitosan (CHI), and positively charged star-PDMAEMA with negatively charged poly(styrene sulfonic acid) sodium salt (PSS), as two different models of confined space for mineralization, where different crystal forms of calcium carbonate were obtained(Yanget al. (2013)).The current reports therefore presented no agreement on the effects of positively charged additives on $CaCO_3$precipitation.While a variety of positively charged organic additives have been reported, the co-effect of positively charged soluble organic additives and polycation insoluble substrates was limited.

In our work, calcium carbonate crystals were mineralized in the present of cation molecules and chitosan with controlled size and morphology. A study of the co-effect of a range of amine molecules and CS films on $CaCO_3$ precipitation was presented. And the effect of electrostaticfunction on amine monomers on the mineralization of $CaCO_3$ was also investigated. N,N-dimethylaminoethyl methacrylate (DMAEMA) ,N,N-Dimethylethanolamine (DMEA), 2-Dimethylaminoethylamine (DMEDA) and N-MethIyldiethanolamine (MDEA) were used as crystal modifiers respectively because the polymer was harmless to the human body or monomers could be used as drug intermediates. The $CaCO_3$ crystals were obtained on CS films and under the control of DMAEMA, DMEA, DMEDA and MDEA, respectively. While the precipitations without any organic additive, only the single cube-shaped crystals were obtained on CS films. At ambient temperature, cube pile shaped $CaCO_3$ crystals could be only produced by changing the concentration of DMAEMA or DMEA. And the morphology of $CaCO_3$ was divers because of complicated function of DMEDA and CS films. And with the increase of DMEDA concentrations, the change of $CaCO_3$morphologies was regular and controllable. The transformation law of the co-effect of MDEA and CS was similar to that of DMEDA and CS, except the morphology. Our results clearly demonstrated that molecules comprising strong nucleophilicity groups together with CS were capable of exerting potent effects on morphology. In contrast, when only tertiary combined with CS, the effect was little.

Positively charged additives are generally considered to be much less active, these results may be useful for further investigation of the cationic mineralization.

2. Experimental Part

2.1 Materials

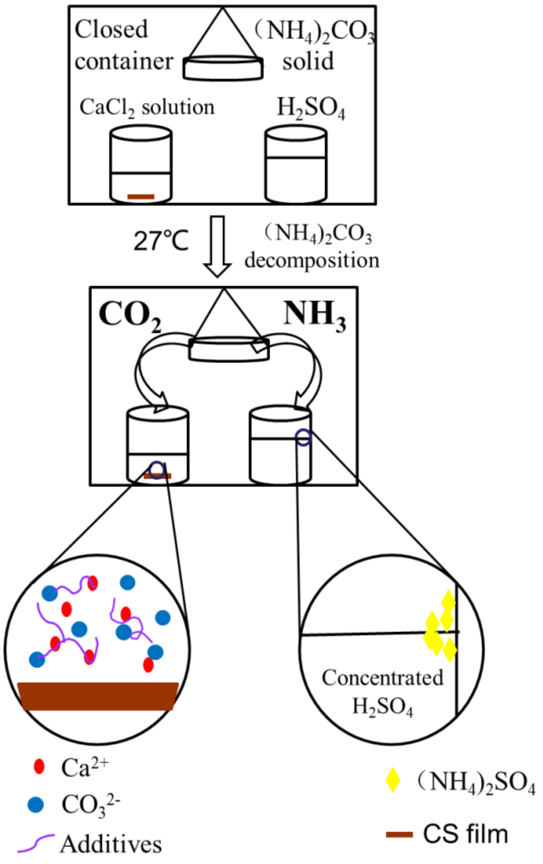
Chitosan (CS; kM_w=100 kD, DA 90%) used in the present work was purchased from Zhejiang Golden-shell Pharmaceutical Co. Ltd (Yuhuan, Zhejiang, China). N,N-dimethylaminoethyl methacrylate (DMAEMA; >99%) ,N,N-Dimethylethanolamine (DMEA; >99%), 2-Dimethylaminoethylamine (DMEDA; >99%) and N-MethIyldiethanolamine (MDEA; >99%) were purchased from Aladdin(Shanghai, China). Other chemical reagents such as calcium chloride dihydrate ($CaCl_2.2H_2O$), ammonium carbonate (NH_4HCO_3) (Shantou Xilong Chemical Factory, Guangdong, China), Acetic acid, Concentrated sulfuric acid (H_2SO_4) (Beijing Chemical Works, China), ethanol, were analytical grade, and used without any further purification.

2.2 Preparation of CS films

0.3g chitosan and 10mL acetic acid aqueous solution (0.3M) were mixed together at room temperature. The mixture was stirred for 8h to obtain uniform solution,then the solution was poured into Teflon moulds(50mm*50mm*2mm). The moulds with solution were dried and dehydrated at 80℃ for 10h to obtain CS films. The films were cut into 3*3mm^2 slices, washed by water and ethanol twice and dried at 50℃.

2.3 Growth of $CaCO_3$ on Chitosan Films

Mineralization of calcium carbonate was carried out by using the ammonium carbonate diffusion method(Xu, Antonietti, Cölfen, & Fang, (2006))as shown in scheme 1. Mineralization solutions were prepared as follows: firstly,calcium chloride solid was added slowly into an 80 mL aqueous solution of DMAEMA (0.1-2 mg/mL) under continuous stirring at room temperature. The concentration of $CaCl_2$ in the mixed solution was 10mM. CS films were put into the solution as matrix. Then the bottles were covered with Para film which had four 5mm diameter holes and placed in a sealed desiccator which containing 8g crushed ammonium carbonate and concentrated H_2SO_4. Mineralization was then allowed to proceed for 3 days. The obtained precipitates were washed with distilled water and ethyl alcohol three times then dried at 80℃ for 3h. Finally, the same experiments were performed using identical procedures as described above, but in the presence of DMEA, DMEDA, and MDEA, respectively.



Scheme 1. Schematic diagram for preparing calcium carbonate crystals by a gas diffusion method.

2.4 Characterization

$CaCO_3$ crystals were characterized by using a Hitachi S-4700 field emission scanning electron microscope (FE-SEM; Japan), fitted with a field emission source and operated at an accelerating voltage of 20 kV. The power X-ray diffraction (XRD) patterns were recorded on a Bruker D8 Advance diffract meter (Germany) with graphite-monochromatized high-intensity CuKα radiation (40 kV, 40 mA); 0.1 sec/steps scan speed; scan type was locked coupled and 2θ range from 10° to 70° were selected. Energy dispersive spectroscopy (EDS) was performed by using a Hitachi S-4700 scanning electron microscope (SEM-EDS; Japan)at 20 kV.

3. Results and discussion

3.1 The co-effects of additives and chitosan on synthesis of $CaCO_3$

The dissociation constant pKa is 6.3 for chitosan(Wada, Suda, Kanamura, &Umegaki, (2004)), the NH_2 groups of the chitosan membrane surface tend to ionize at pH values below about 9.5 existing in the form of polycations, Chitosan can be regarded as a kind of copolymer in which glucosamine units with NH_2 groups are active units and can be considered as a weak polybase due to the NH_2 groups on its chains. The degree of dissociation of tertiary amine groups of DMAEM, DMEA, DMEDA, and MDEA in solution depends on the pH of the solution. So chitosan has the possibility of forming electrostatic repulsion with the four monomers through electrostatic forces.

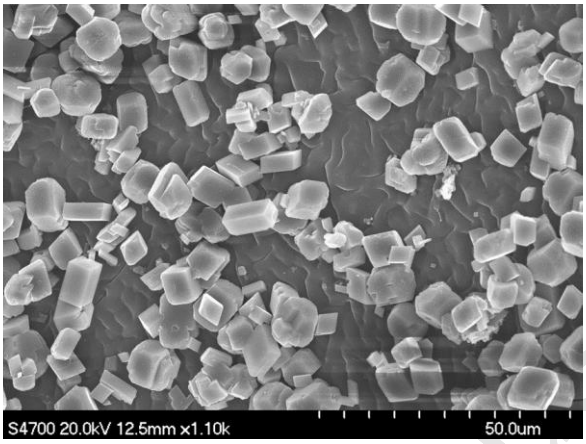


Figure 1.SEM images of $CaCO_3$ crystals grown on CS films with Ca^{2+}=10mM

In our experiment, the results showed that, without additive in the solution, a large number of cube-shapedcalcite crystals precipitated on the chitosan films (Figure 1).

Figure 2 showed that in the presence of a small amount of additives, e.g., 0.1mg/mL, the morphologies of $CaCO_3$ crystals had an obvious change. With the addition of DMAEMA, the SEM image (Figure2a) revealed$CaCO_3$ crystals agglomerated together to form stake-shape with steps on the edge and the average size was 6.5μm.The precipitates in the presence of the DMEA were cube in shape which looked like the shape in present of DMAEMA, with an average size of 5.5μm(Figure 2b). DMAEMA and DMEA caused relatively minor changes in calcite morphology. However, irregular $CaCO_3$ morphologies (Figure2c-d) were grown if changed the group beside tertiary amine. Crisscross and I-shape (approximately 5μm in arm length from center) precipitates were prepared from DMEDA(Figure 2c). In common with DMEDA, MDEA caused dramatic morphological effects (Figure 2d), which were special triangle. As additive to solution, DMAEM, DMEA, DMEDA, and MDEA may affect the morphology and polymorphism of $CaCO_3$crystals through its assembling behavior; for example, “rice-like” particle with a polymorph of calcite was synthesized at lower pH value while spherical particle with a blending polymorph of calcite and vaterite was obtained athigher pH value(Cheng, Shao, &Vollrath, (2008)).The presence of -NH_2 and two -OH groups allowed for interaction with $CaCO_3$ surfaces and thus the transition from a rhombohedral crystal modified by face selective additive adsorption to an irregular crystal. Many biomineralization studies have shown that thermodynamically stable, rhombohedral shaped calcite crystals were formed in the absence of organic additives. Changing preparation conditions (the effect of pH, temperature, ions and different additives) can affect the crystallization behavior, polymorph and morphology.

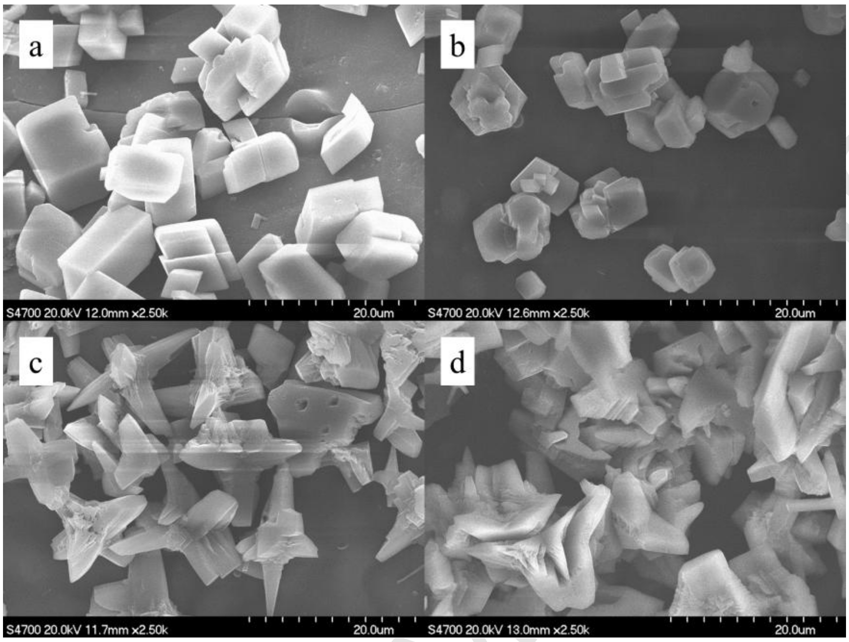


Figure 2.SEM images of $CaCO_3$ crystals grown on CS films using gas diffusion crystallization: (a) DMAEMA; (b) DMEA; (c) DMEDA; (d) MDEA. The concentration of the additives was 0.1 mg/mL

Although the morphologies of the crystals with different additives were distinct, the results of XRD revealed that the mineral phase was consisted of calcite. Figure 3a showed the XRD pattern of CS films. When 0.1 mg/mL DMAEMA was added as the additive, the XRD pattern of the sample was indexed to a single phase of well-crystallized calcite with the hexagonal structure (JCPDS72-1973) No other phases such as aragonite or vaterite were observed. When the crystal modifiers was 0.1 mg/mL DMED, XRD patterns (Figure 3c) of the samples had no (119) characteristic peak, compared with Figure 3b, indicating that the same phase of calcite was obtained. It was clearly observed that the peak intensity dramatically decreased in the present of DMED, implying that the increasing nucleophilicity of groups ($-N(CH_3)_2 < -NH_2$) changed the growth of $CaCO_3$ faces in CS/$CaCO_3$composites. Figure3 suggested that all diffraction peaks except characteristic peaks of the chitosan substrate can be indexed to pure calcite, suggesting that the precipitations of $CaCO_3$ had a high phase purity and were totally composed of calcite crystals. There were a few reports about the mineralization of$CaCO_3$ inducing by molecules with electrostaticfunction(Lu et al., (2005)). This phenomenon was a very interesting result, indicating that a strong interaction existed among calcite particles, cationic molecule and the chitosan matrix, which provided an experimental example for better understanding of the interaction mechanism between $CaCO_3$ crystals and cationic additives.

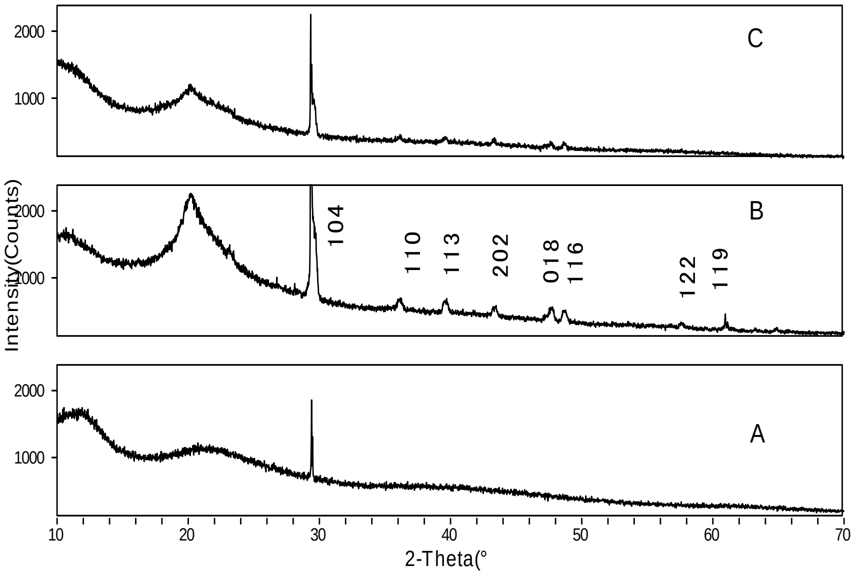


Figure 3.XRD patterns of the CS/$CaCO_3$compositesprepared in the presence of differentadditives. (A)the control ;(B) DMAEMA0.1 mg/mL; (C) DMEDA 0.1 mg/mL

3.2 The effects of additives concentration on the morphology of $CaCO_3$

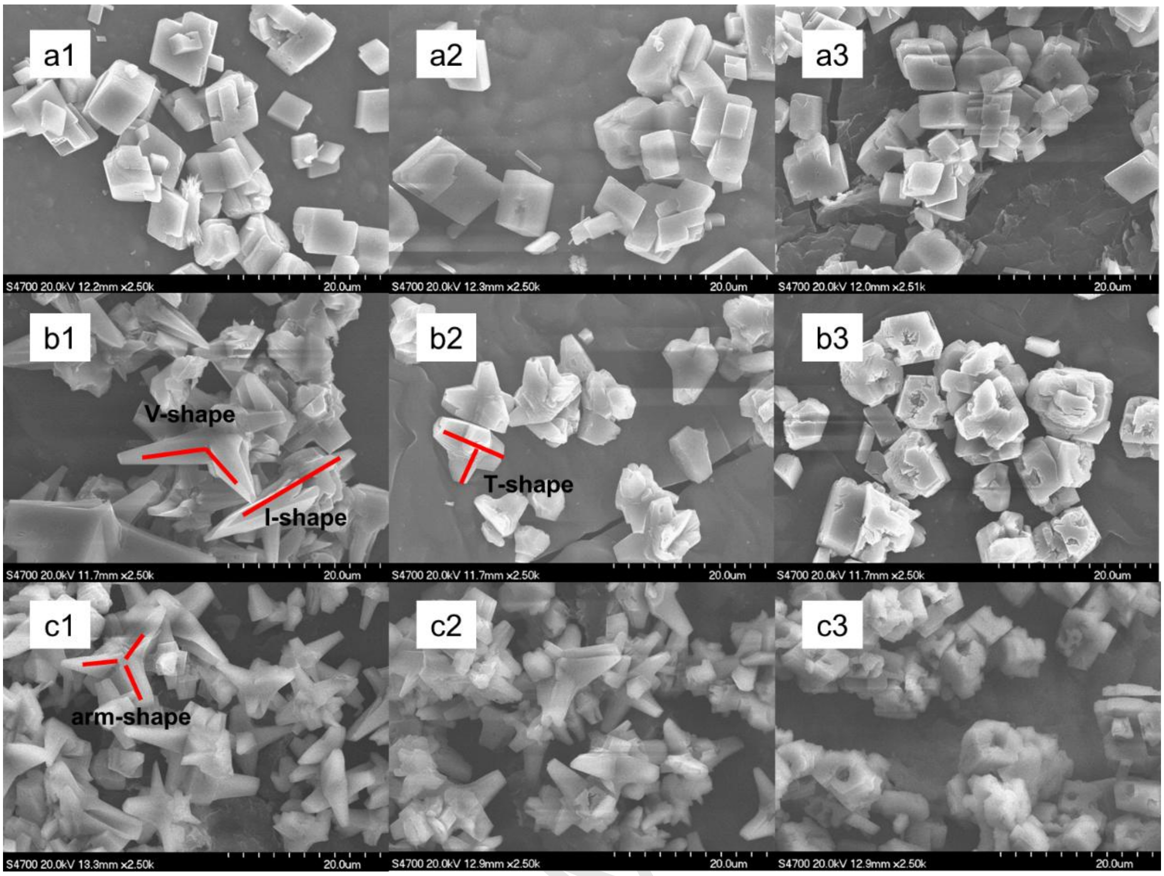


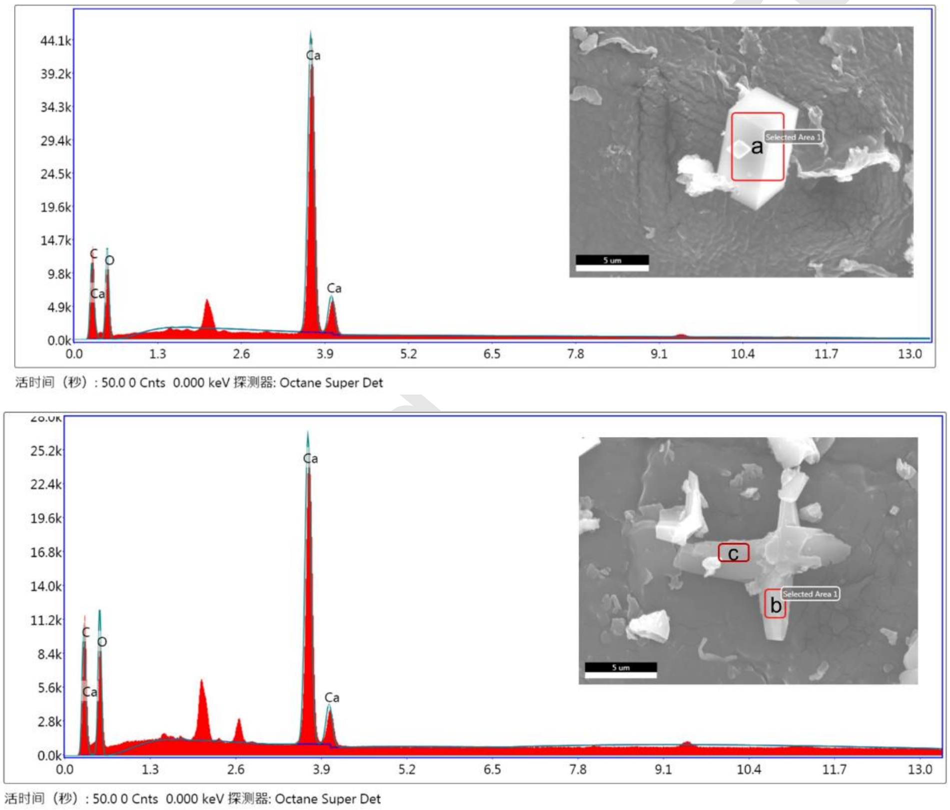
Figure 4.SEM images of $CaCO_3$ crystals grown at different additive concentrations on CS films. (a) DMAEMA: (a1) 0.5mg/mL; (a2) 1mg/mL; (a3) 2mg/mL. (b) DMEDA: (b1) 0.5mg/mL; (b2) 1mg/mL; (b3) 2mg/mL. (c)MDEA: (c1) 0.5mg/mL; (c2) 1mg/mL; (c3) 2mg/mL.

Table 1 pH values of mineralization solution with different concentrations before and after 3 days mineralization

Concentration	pH(0 day→3 days)		
	DMAEMA	DMEDA	MDEA
0.1 mg/mL	8.56→6.91	9.62→7.01	8.97→6.92
0.5 mg/mL	8.96→6.66	10.03→6.96	9.40→6.91
1 mg/mL	8.98→6.88	10.14→7.10	9.69→7.08
2 mg/mL	9.05→6.97	10.31→7.00	9.82→7.28

Three concentrations of additives of 0.5, 1 and 2 mg/mL were usedfor further study. Table 1 shows the pH values of these additives solution, although the initial pH values were different, the pH values were all between 6.5 and 7.5 after 3 days of mineralization. Therefore, both chitosan and additive were positively charged during the mineralization process and the obtained $CaCO_3$ crystals were stable in neutral solution. The concentration of additives was crucial to control the $CaCO_3$ crystal morphology (Figure 4). On the basis of experimental results, with the increase of DMAEMA (Figure 4a) and DMEA concentration, the change of morphologies of $CaCO_3$ crystals was the same trends, while in present of DMEDA (Figure 4b) and MDEA(Figure 4c) the change tendency of morphologies of $CaCO_3$ particles was similar. Figure 4a shows the change trend of $CaCO_3$with the concentration of DMEAMA. At these three concentrations of DMAEMA, the morphology of $CaCO_3$ crystals still calcite rhombohedra. Nevertheless, with the increase of DMAEMA concentration, it can be seen that more $CaCO_3$ crystals were collected to intergrown with several rhombohedra.Figure4b depicts the change of the morphologies of $CaCO_3$ crystals from crisscross and I-shape to the irregular cube with the increase of DMEDA concentration. When the concentration of DMEDAwas0.5mg/mL(Figure 4b1), arm-shaped with different angle crystals were formed and the average length of arm was12μm. With the increasing of the concentration to 1mg/mL (Figure 4b2), novel T-shape crystals were formed with arm length of 4μm. As the concentration of DMEDA further increased to 2mg/mL (Figure 4b3), irregular cube crystals (about 8μm) were no arm and with a hole in the middle. It can be seen that the samples in the present of DMEDA and MDEA had similar changing trend, which was that the arm length gradually disappeared with the increase of concentrations. However, the morphology and size of crystals were different, further implying the diversity from the cube to irregular shapes. It was found that both the concentration of additives and the nucleophilicity of groups of molecules had a strong influence on the crystallization behavior and morphology of $CaCO_3$ crystals.

According to the results, DMAEMA and DMEA together with CS showed a weak effect on the morphology of $CaCO_3$, due to the inferiorelectronic effect of groups. However, CS films as insoluble matrix, DMEDA molecules with $–NH_2$ groups and MDEA with two –OHchanged$CaCO_3$ morphology completely. With the increase of additives concentration, the morphology of $CaCO_3$ deviates from the default rhombohedra with six {104} faces and new faces become exposed. This indicated a face selective additives adsorption. The presence of tertiary amine groups allowed for interaction with $CaCO_3$ surfaces and thus the transition from a single crystal modified by face selective additive adsorption to a polycrystal(Naka &Chujo, (2001); Mann et al., (1993)).We supposed that with the increase of nucleophilicity of molecules, the ability of attracting CO_3^{2-} ions became strong, which led to the growth or termination of crystal planes. Andro´ nicoNeira-Carrillo (Neira-Carrillo et al. (2008))and co-workers investigated that different c-PANIs interacted differently with the individual growth planes of $CaCO_3$crystals on controlling the crystallization. Some groups demonstrated that phase separation was driven by the interaction between the positively charged amine groups and the anionic carbonate ions(Cantaert et al., (2012)).The co-effect of the soluble substrate and insoluble matrix on calcium carbonate mineralization at ambient temperature was seldom reported. This result proved the distinct role played by aminemolecules in the process of biomineralization. Clearly, further work was required to investigate the mechanism how to operate in the present of cationic additives.



Element	Atom%	CS films (a)	Atom%	DMEDA/CS films (b)	Atom%	DMEDA/CS films (c)
C	12.82		17.40		22.75	
O	**47.89**		**51.46**		**53.10**	
Ca	**39.29**		**31.14**		**24.15**	
total	100	Ca:O =1:3.05	100	Ca:O =1:4.16	100	Ca:O =1:5.5

Figure 5. EDS spectra of $CaCO_3$ crystals obtained on CS filmsin the (a) absence of additives and the (b) presence of DMEDA (0.1 mg/mL). Table showing the calculation of Ca:O ratio.

To further investigate the presence of additives adsorbed on the crystal surface, energy dispersive spectroscopy(EDS) was used as a qualitative spatially resolved detection technique. Although a strong co-effect of DMEDA (and MDEA)and CS films on$CaCO_3$ morphology observed by SEM was obvious, perhaps the low concentration of the molecules used during the gas diffusion method do not allow for the resolution of the presence of nitrogen (atom%) adsorbed on the crystal surface (Figure 5). When there were no additives in the solution, the EDS(Figure 5a) measurement shows the theoretical calcium 39.29% and oxygen 47.89% (atom %), namely calcium/oxygen ratio (Ca/O=1:3) corresponding to pure calcite surfaces. However, when applying DMEDA as the additive, the EDS measurement shows the ratio changes to Ca/O=1:4.16 and 1:5.5, which indicates the adsorbed monomer in varying amount on the faces.

Much of the research stated that some parts of the organic additives provided the initial structural information for the inorganic part to nucleate and grow outward in a favorable way(Ajikumar, Low, &Valiyaveettil, (2005)).As the calcium content is lower than the one expected for $CaCO_3$, the surfaces with adsorbed DMEDA get depleted of calcium ions, indicative of electrostatic interaction between monomer and surface exposed CO_3^{2-} ions. Due to the Ca/O ratio, the amount of adsorbed DMEDA monomer can be roughly estimated supposing that EDS only detected the surface composition and that each of the two monomer amino groups adsorbs and reduces one Ca^{2+} ions. The adsorption of additives on all exposed faces resulted in the minimization of the surface energy(Cölfen, (2007))and the growth of different morphologies. As a result low energy surfaces became exposed and high energy surfaces disappeared.

4. Conclusion

This article proposed the co-influences of the organic cationic amine additives and chitosan films on the morphology of $CaCO_3$ obtained from the mineralization solution with CO_2gas diffusion. It was demonstrated that on CS films different morphologies were synthesized by the addition of DMEDA and MDEA, while the intergrown cube-shape crystals were obtained with DMAEMA and DMEA.I n addition, our results demonstrate that morphological variations in calcium carbonate can be achieved with cationic additives and is not confined to anionic species. Moreover,it seems that the ability of electronic movement of the groups in the monomer is determinant of its influence on the crystallization. Our work also showed that the additive concentration is crucial to control the $CaCO_3$ crystal morphology. At lower concentration of DMEDA and MDEA, the $CaCO_3$ crystal morphology is completely modified and great differences become obvious between the different molecules. These results indicate that the $CaCO_3$/CS composite materials are potential cell culture matrix and filling materials for bone repair. The use of various cationic polymer and monomer in biomimetic materials offers fresh insights into controlling the structure and morphology under mild conditions. Therefore, the contents and order of $CaCO_3$, the mechanism, structure and function need to be further researched and improved.

References

Ajikumar, P. K., Low, B. J. M., Valiyaveettil, S. (2005). "Role of soluble polymers on the preparation of functional thin films of calcium carbonate." Surface and Coatings Technology**198**(1): 227-230.

Cantaert, B., A. Verch, Y.-Y. Kim, H. Ludwig, V. N. Paunov, R. Kröger and F. C. Meldrum (2013). "Formation and Structure of Calcium Carbonate Thin Films and Nanofibers Precipitated in the Presence of Poly (Allylamine Hydrochloride) and Magnesium Ions." Chemistry of Materials, **25**(24): 4994-5003.

Chan, C. M., Wu, J., Li, J. X., & Cheung, Y. K. (2002). "Polypropylene/calcium carbonate nanocomposites." Polymer,**43**(10): 2981-2992.

Cheng, C., Shao, Z., &Vollrath, F. (2008). "Silk Fibroin-Regulated Crystallization of Calcium Carbonate." Advanced Functional Materials, **18**(15): 2172-2179.

Cantaert, B., Kim, Y. Y., Ludwig, H., Nudelman, F., Sommerdijk, N. A., & Meldrum, F. C. (2012). "Think positive: phase separation enables a positively charged additive to induce dramatic changes in calcium carbonate morphology." Advanced Functional Materials, **22**(5): 907-915.

Cölfen, H. (2007). Bio-inspired mineralization using hydrophilic polymers. Biomineralization II, Springer**:** 1-77.

Dmitrovic, V., Habraken, G. J., Hendrix, M. M., Habraken, W. J., Heise, A., &Sommerdijk, N. A. (2012). "Random Poly (Amino Acid) s Synthesized by Ring Opening Polymerization as Additives in the Biomimetic Mineralization of CaCO3." Polymers, **4**(2): 1195-1210.

Gao, Y. X., Yu, S. H., Guo, X. H. (2006). "Double hydrophilic block copolymer controlled growth and self-assembly of CaCO3 multilayered structures at the air/water interface." Langmuir, **22**(14): 6125-6129.

Gong, J., Zhou, Z., Hu, X., Wong, M-k., Wong, K-w., & Du, Z. (2008). "Self-assembled chitosan nanotemplates for biomineralization of controlled calcite nanoarchitectures." ACS applied materials & interfaces,**1**(1): 26-29.

Hosoda, N. & Kato, T. (2001). "Thin-film formation of calcium carbonate crystals: effects of functional groups of matrix polymers." Chemistry of materials, **13**(2): 688-693.

Lee, S., Park, J. H., Kwak, D., & Cho, K. (2010). "Coral mineralization inspired CaCO3 deposition via CO2 sequestration from the atmosphere." Crystal Growth & Design, **10**(2): 851-855.

Lu, C., Qi, L., Cong, H., Wang, X., Yang, J., Yang, L., Zhang, D., Ma, J., & Cao, W. (2005). "Synthesis of calcite single crystals with porous surface by templating of polymer latex particles." Chemistry of materials,**17**(20): 5218-5224.

Ma, M. G., Dong, Y. Y., Fu, L. H., Li, S. M., & Sun, R.C. (2013). "Cellulose/CaCO 3 nanocomposites: microwave ionic liquid synthesis, characterization, and biological activity." Carbohydrate polymers, **92**(2): 1669-1676.

Mann, S., Archibald, D. D., Didymus, J. M., Douglas, T., Heywood, B. R., Meldrum, F. C., & Reeves, N. J. (1993). "Crystallization at inorganic-organic interfaces: biominerals and biomimetic synthesis." Science, **261**(5126): 1286-1292.

Naka, K. &Chujo, Y. (2001). "Control of crystal nucleation and growth of calcium carbonate by synthetic substrates." Chemistry of Materials, **13**(10): 3245-3259.

Neira-Carrillo, A., Acevedo, D. F., Miras, M. C., Barbero, C. A., Gebauer, D., Cölfen, H. & Arias, J. L. (2008). "Influence of conducting polymers based on carboxylatedpolyaniline on in vitro CaCO3 crystallization." Langmuir, **24**(21): 12496-12507.

Neira-Carrillo, A., Retuert, J., Martínez, F., & Arias, J. L. (2008). "Effect of crosslinked chitosan as a constrained volume on the in vitro calcium carbonate crystallization." Journal of the Chilean Chemical Society, **53**(1): 1367-1372.

Nudelman, F., &Sommerdijk, N. A. (2012). "Biomineralization as an inspiration for materials chemistry." AngewandteChemie International Edition, **51**(27): 6582-6596.

Ren, D., Feng, Q., &Bourrat, X. (2011). "Effects of additives and templates on calcium carbonate mineralization in vitro." Micron, **42**(3): 228-245.

Schenk, A. S., Cantaert, B., Kim, Y. Y., Li, Y., Read, E. S., Semsarilar, M., Armes, S. P., & Meldrum, F. C., (2014). "Systematic study of the effects of polyamines on calcium carbonate precipitation." Chemistry of Materials, **26**(8): 2703-2711.

Sugihara, H., Ono, K., Adachi, K., Setoguchi, Y., Ishihara, T., &Takita, Y. (1996). "Synthesis of disk-like calcium carbonate. 1. Effect of various organic compounds on the carbonation of the basic calcium carbonate." Journal of the Ceramic Society of Japan, **104**(9): 832-836.

Vilela C., Freire C. S., Marques P. A., Trindade T., Neto C. P., &Fardim P. (2010). "Synthesis and characterization of new CaCO 3/cellulose nanocomposites prepared by controlled hydrolysis of dimethylcarbonate." Carbohydrate Polymers, **79**(4): 1150-1156.

Wada, N., Suda, S., Kanamura, K., &Umegaki, T. (2004). "Formation of thin calcium carbonate films with aragonite and vaterite forms coexisting with polyacrylic acids and chitosan membranes." Journal of colloid and interface science,**279**(1): 167-174.

Xin, J., Chen, T., Lin, Z., Dong, P., Tan, H. & Li, J. (2014). "Phosphorylated dendronized poly (amido amine) s as protein analogues for directing hydroxylapatitebiomineralization." Chem. Commun.**50**(49): 6491-6493.

Xu, A. W.,Antonietti, M., Cölfen, H., & Fang, Y. P. (2006). "Uniform hexagonal plates of vaterite CaCO3 mesocrystals formed by biomimetic mineralization." Advanced Functional Materials, **16**(7): 903-908.

Yang, J., Cao, S., Xin, J., Chen, X., Wu,W. & Li, J. (2013). "Calcium carbonate deposition on layer-by-layer systems assembled from star polymers." Journal of Polymer Research**20**(6): 1-10.

Zhao, Y., Li, S., Yu, L., Liu, Y., Wang, X., Jiao, J. (2011). "The preparation of calcium carbonate crystals regulated by mixed cationic/cationic surfactants." Journal of Crystal Growth, **324**(1): 278-283.