

THE INDUCEMENT AND MODULATION OF COLLAGEN FIBER ON CRYSTAL MORPHOLOGY OF CALCIUM CARBONATE**

by

LIHONG FU,^{1*} SHULING CHAI¹ AND JINGQIU CHENG²

¹*School of Light Chemistry and Environmental Engineering,*

Shandong Institute of Light Industry

JINAN 250353, CHINA

²*Division of Transplant Immunology Key Laboratory of Transplant Engineering*

and Immunology Ministry of Health P.R China

CHENGDU, 610065, CHINA

ABSTRACT

Based on the fundamental principles of biomineralization, a CaCO_3 /proteins composite can be synthesized by biomimetic synthesis under dynamic conditions, using collagen fiber as the matrix. The inducement and modulation of collagen fibril on the crystal morphology of calcium carbonate was studied. The crystalline structure and polymorph of calcium carbonate at different mass ratio of [Collagen fiber]/ $[\text{Ca}^{2+}]$ was characterized using X-ray diffraction (XRD) and refined with Rietveld method. The microstructure parameters, such as the lattice strain, bond length, crystallite size (A, B and C), density of calcium carbonate cell, were calculated to characterize the crystalline structure of calcium carbonate at atom level. The polymorph of calcium carbonates can be changed by the [collagen fiber]/ $[\text{Ca}^{2+}]$ ratio. The degree of super-saturation and number of nucleation have greater influence on the lattice strain, the lattice strain positively correlates to crystallite size nonlinearly. The crystals face, being a more perfect face and with minor defects, had greater stability. By increasing [collagen fiber]/ $[\text{Ca}^{2+}]$ ratio and crystallite size rise, the vitality of calcium carbonate enhanced.

RESUMEN

Basado en los principios fundamentales de la biomineralización, un compuesto CaCO_3 /proteína puede ser sintetizado por síntesis biomimética bajo condiciones dinámicas, utilizando la fibra de colágeno como matriz. La inducción y la modulación de la fibrilla de colágeno en la morfología cristalina del carbonato de calcio ha sido estudiada. La estructura cristalina y polimorfa del carbonato de calcio en diferentes relaciones de masa de [Fibra de Colágeno]/ $[\text{Ca}^{2+}]$ fue caracterizada mediante difracción de rayos X (DRX) y refinado con el método de Rietveld. Los parámetros de la microestructura, como la tensión del entramado, la longitud de los puentes, tamaño de los cristallitos (A, B y C), la densidad de las células del carbonato de calcio, se calcularon para caracterizar la estructura cristalina de carbonato de calcio a nivel de los átomos. La polimorfía del carbonato de calcio puede ser cambiada por el coeficiente [Fibra de Colágeno]/ $[\text{Ca}^{2+}]$. El grado de super-saturación y número de nucleación tienen gran influencia en la tensión del entramado, que se correlaciona positivamente con la no linealidad en el tamaño de los cristallitos. Los cristales más perfectos que se enfrentan a la cara y con defectos menores tienen mayor estabilidad. Al aumentar la relación [Fibra de Colágeno]/ $[\text{Ca}^{2+}]$, y aumentando el tamaño del cristallito, la vitalidad de carbonato de calcio es mayor.

* Corresponding author – E-mail: fulh12@yahoo.com.cn

A Technical Note based on a presentation at the XXIX IULTCS Congress and 103rd annual meeting of the American Leather Chemists Association at the J W Marriott Hotel, Washington, DC, June 20-24, 2007.

Manuscript received August 28, 2008, accepted for publication December 11, 2008

INTRODUCTION

As one of the most important process in the beamhouse, liming has many unique functions related to subsequent leather quality and character. But lime blast, which may often be formed in the liming process, severely affect leather quality. It is very important to understand the forming and stabilization of lime blast to prevent and eliminate lime blasts.

The type of the protein backbone/side groups is known to have a large influence not only on the fold of the protein but also on the crystallographic structure of mineral it nucleates. The biological fidelity of polymorph selection is a consequence of the dynamic interaction at the protein-mineral interface. Although the nature of interfacial recognition is not known, it is clear that severing the communication by altering the reaction parameters results in an altered crystal structure.¹ Protein can modulate calcium carbonates not only on morphology but also on polymorph, investigations show that only calcite of calcium carbonate in collagen solutions at different concentrations are formed, and the calcite growth is more and more inhibited as collagen is increasing in concentration.² We studied that the CaCO_3 / gelatin composite was synthesized by the biomimetic synthesis, using gelatin as the matrix, we found that the morphology and orientation of crystal are different for various content of the gelatin.³ Structure determination or characterization is the first and vital step in most materials research, however, In spite of the above studies, variation of lime blasts structure and stability at atom level based on crystal structure has not been reported.

In this paper, the polymorph of calcium carbonate and morphological control of crystals were studied, using the above studies and biological mechanisms as inspiration. The detail information about Ca_2CO_3 crystalline structure were supplied by Rietveld method⁴ which is a method for analyzing variation and distortion of crystalline structure, it is useful tool used in biomaterial science for quantitative phase analysis and structure refinement. This paper mainly discusses the inducement and modulation of collagen fiber on calcium carbonate crystals, as well as stability of crystals. The aim is to further elucidate and understand that effect of collagen fiber on the polymorph and stabilization of calcium carbonate, Furthermore, the results will both provide a starting point for the determination of liming reaction conditions, and an new explaining for the liming phenomena at atom level based on crystal structure.

EXPERIMENTAL

Materials

Materials used in this research include collagen fiber, which was obtained from bovine wet blue shaving dechromed by hydrogen peroxide and treated by ball-milling method.⁵ Calcium chloride was analytically pure grade, and was purchased from Tianjin Guangcheng Chemical Reagent Company. Calcium carbonate was analytically pure grade and was supplied by Shandong Province Laiyang Industry of Fine Chemical Plant. The water was deionized and distilled, the conductivity of which was below $1\mu\text{S}/\text{cm}$.

Method of synthesizing crystals

In this experiment, the method of synthesizing crystals was according to our previous report.³ Na_2CO_3 and CaCl_2 powers at some molar weight were put on respectively the bottoms of two different flasks. The concentrations of Na_2CO_3 , CaCl_2 and collagen fiber are listed in Table I. Collagen fiber solution was added slowly into different flasks on the internal surface of flasks, respectively. Then flask was covered by plastic film, crystallization took place at 4°C for 10 days. After precipitation, the slurry were filtered through a $0.45\mu\text{m}$ cellulose acetate filter, washed with ddH_2O and filtered three times each to avoid the Ca^{2+} , Cl^- and Na^+ residue. Afterwards, the samples were dried under vacuum conditions at 4°C for 1hr.

Characterization of synthesized crystals

X-ray diffraction (XRD), using a X' Per Pro MPD X-ray diffractometer (Philips, Netherlands) with 40 kV, $\text{Cu K}\alpha$ radiation, was employed to analyze the crystalline structure and polymorph of calcium carbonate precipitation at different mass ratio of $[\text{collagen fiber}]/[\text{Ca}^{2+}]$, and then refined with the Rietveld method. The microstructure parameters, such as the lattice strain, bond length, crystallite size, density of calcium carbonate cell, were calculated to characterize the crystalline structure of calcium carbonate at atom level.

TABLE I
Experiment Parameters

No.	a	b	c	d
$\text{Na}_2\text{CO}_3(\text{mM})$	50	50	10	5
$\text{CaCl}_2(\text{mM})$	50	50	10	5
Collagen fiber (g)	0	0.05	0.05	0.05
$\text{ddH}_2\text{O}(\text{mL})$	100	100	100	100
$[\text{collagen fiber}] / [\text{Ca}^{2+}]$	0	0.02515	0.1335	0.2670

RESULTS AND DISCUSSION

Effect of Collagen Fiber on the Polymorph of Calcium Carbonates

Figure 1 demonstrates the XRD pattern of calcium carbonate crystals modulated by collagen fiber at 4°C for 10 days, collagen fiber concentration was 0.534g/l. a is the XRD pattern of calcium carbonate crystals forming in water, b shows the XRD pattern of calcium carbonate crystals when mass ratio of $[\text{Collagen fiber}]/[\text{Ca}^{2+}]$ is 0.02515, c is 0.1335, d is 0.2670. From XRD it can be seen that calcite and vaterite are formed in collagen fiber at different mass ratio of $[\text{collagen fiber}]/[\text{Ca}^{2+}]$, that collagen fiber can change the polymorph of calcium carbonates. The influence of $[\text{collagen fiber}]/[\text{Ca}^{2+}]$ ratios on the polymorph of calcium carbonates modulated by collagen fiber are listed in Table II. The effect of the different ratios on the morphology is obvious, incremental as collagen fiber is at increasing mass ratio. Only calcite crystal are formed in absence of collagen fiber (control samples). As the mass ratio of $[\text{collagen fiber}]/[\text{Ca}^{2+}]$ increasing from 0.0252 to 0.2670, the calcite increases from 93% to 100%, but vaterite decreases from 7% to 0%. Although, J. Roque et al.⁶ found that vaterite polymorph appeared in all induced crystallization events by soluble matrix extracts from *Mytilus edulis* and polyaspartic acid, but not in blank aqueous solution. F. H. Shen et al.² also indicated that collagen does not change the polymorph of calcium carbonates, only calcite crystals are formed in collagen solutions at different concentration. However, our experimental results show that only calcite crystals are also formed at $[\text{Collagen fiber}]/[\text{Ca}^{2+}]$ is 0.2670.

The Stability of Calcium Carbonate Crystal

Figure 2-5 showed the profiles fitting of Rietveld refinement, that is without collagen fiber, the $[\text{Collagen fiber}]/[\text{Ca}^{2+}]$ ratio is 0.02515, 0.1335 and 0.2670, respectively. With the observed data points overlaid with the calculated pattern, which was drawn as a solid line. The bottom horizontal line showed the difference of observed intensity and calculated intensity based on refined Ca_2CO_3 model by Rietveld refinement. This graph depicted the extent of disagreement between the refined structure model and raw XRD data of tested powders. The fitting indexes of Rietveld refinement RwP , was between 14.78 and 19.68 for all Ca_2CO_3 samples (Table III), the lower RwP is, the better degree of fitting is.

Table III listed results of Rietveld refinement related with CaCO_3 crystalline structure at atom level, such as the lattice strain, crystallite size (a, b and c), bond length and density etc. The Ca_1 was the activist part in CaCO_3 structure, because they were on $[001]$ plane of crystalline structure and easy to replace or exchange with other ions or groups. The distance

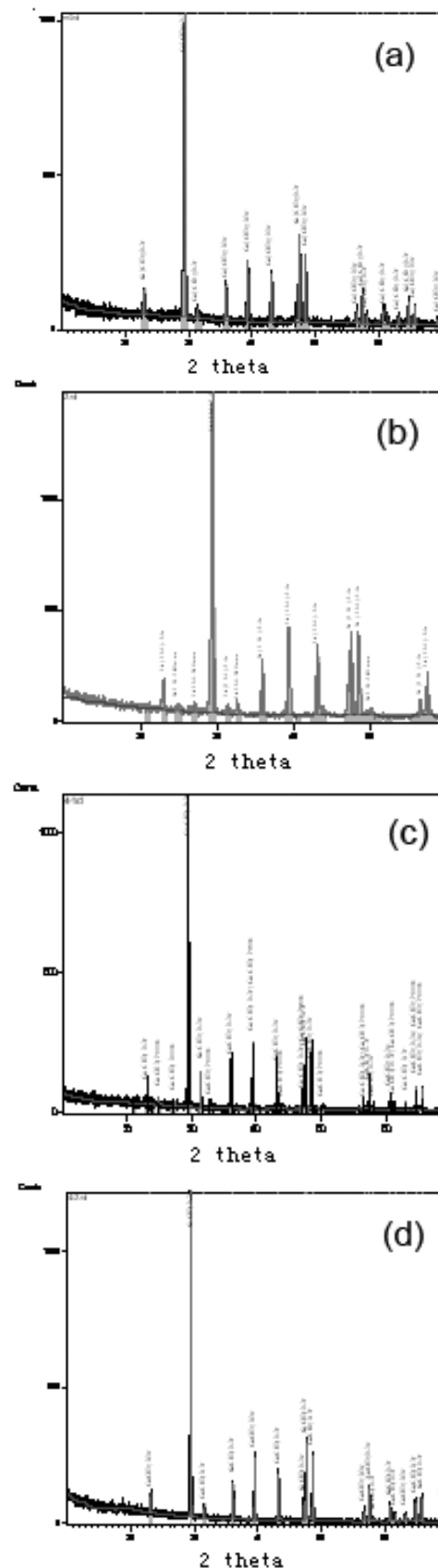


Figure 1: XRD pattern of calcium carbonate crystals modulated by collagen fiber at 4°C for 10 days. Collagen fiber concentration: 0.534g/l. (a) XRD pattern of calcium carbonate crystals forming in water. (b) CaCl_2 : 50mM, Na_2CO_3 : 50mM; The mass ratio of $[\text{Collagen fiber}]/[\text{Ca}^{2+}]$ is 0.02515. (c) CaCl_2 : 10mM, Na_2CO_3 : 10mM; The mass ratio of $[\text{Collagen fiber}]/[\text{Ca}^{2+}]$ is 0.1335. (d) CaCl_2 : 5mM, Na_2CO_3 : 5mM; The mass ratio of $[\text{Collagen fiber}]/[\text{Ca}^{2+}]$ is 0.2670.

TABLE II

**Effect of ratio of [Collagen fiber] / [Ca²⁺]
on the polymorph of calcium
carbonates modulated by collagen
fiber at 4°C for 10 days**

No.	[Collagen fiber] / [Ca ²⁺]	Vaterite/%	Calcite/%
0 [#]	Without collagen fiber	0	100
1 [#]	0.0252	7	93
2 [#]	0.1335	5	95
3 [#]	0.2670	0	100

between Ca₁ and nearest O₃ in the CaCO₃ cell may be a quantitative index to characterize the inter-action of Ca₁ and its nearest atom O₃, which closely related to the activity Ca₂CO₃.⁴ Lattice strain describes the number of perfected crystal faces, the less the lattice strain, the more perfect the crystal face.

From the Table III, it was obvious that the c axis of crystallite size increased significantly from 211 Å to 577 Å, but the density of crystallite decreased from 2.70 to 2.68 g/cm³, as rising of [Collagen fiber]/[Ca²⁺] ratio. The crystallite size is least, but density most in all samples when the ratio is 0.02515. This indicated that the stability of crystal may be enhanced.

The relationship between lattice strain and crystallite size is shown in Table III. It can be seen from Table III that the lattice strain positively correlates to crystallite size nonlinearly. The lattice strain decreases as the crystallite size increase, as listed above 2[#] and 3[#]. So, this implies that the defect occurs at the crystal face or superficial surface, and despite of the increase of the crystallite size, defects in per unit surface area almost keep constant. That is to say that these crystal face with less defect are formed under higher [Collagen fiber]/[Ca²⁺] ratio, it means that the stability of these crystal is very well. On the other hand, Lattice strain without collagen fiber is more than with, exception 2[#] sample. It indicate that stability of crystalline structure change with [Collagen fiber]/[Ca²⁺] ratio. This is because that the [Collagen fiber]/[Ca²⁺] ratio directly affect the value of degree of super-saturation, S, and the number of nucleation. That can be expressed as:⁷

$$S = (\text{Ca}^{2+}) (\text{CO}_3^{2-}) / K_{sp} \quad (1)$$

Where (Ca²⁺) and (CO₃²⁻) is ionic activity of Ca²⁺ and CO₃²⁻, K_{sp} solubility parameter of calcium carbonate. It

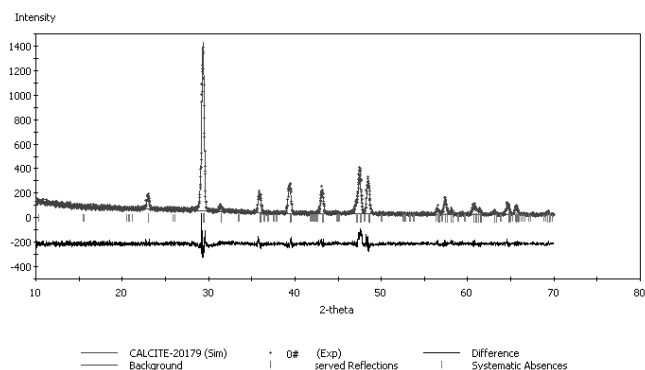


Figure 2: Rietveld refinement of calcium carbonate crystals forming in water

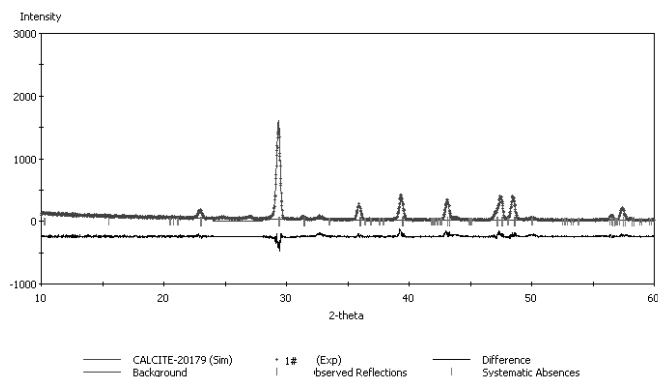


Figure 3: Rietveld refinement of calcium carbonate crystals modulated by collagen fiber (the [Collagen fiber]/[Ca²⁺] ratio is 0.02515)

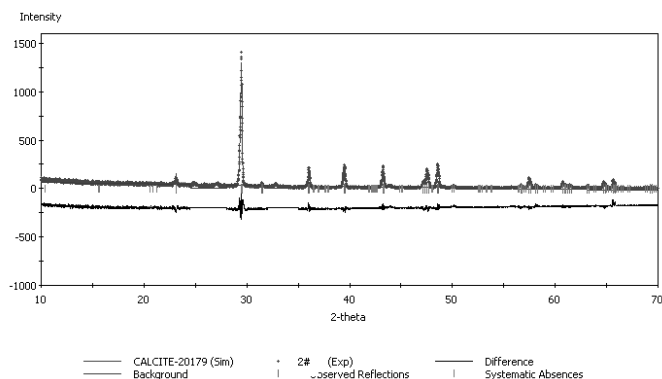


Figure 4: Rietveld refinement of calcium carbonate crystals modulated by collagen fiber (the [Collagen fiber]/[Ca²⁺] ratio is 0.1335)

can be seen from eq. (1) that the ionic activity positively correlates to the S and are linear. While the activation energy of nucleation, G, decreases as S increases.³ So, the nucleation and growth of crystal increase rapidly when the number of nucleation positions is many more and S is larger, it results in increasing the lattice defects. This indicates that abnormally large strain existed in the lattice of sample 2[#] makes crystals easy to degrade. At higher [Collagen fiber]/[Ca²⁺] ratio (0.2670), the distance between Ca₁ and O₃

TABLE III
Effect of ratio of [Collagen] / [Ca²⁺] on calcite CaCO₃ crystalline structure

Samples		0 [#]	1 [#]	2 [#]	3 [#]
[Collagen fiber]/[Ca ²⁺]		Without collagen fiber	0.02515	0.1335	0.2670
Lattice strain (%)	a	0.07393	0.01748	0.01749	0.01744
	b	/	0.01748	0.01749	0.01744
	c	0.00956	0.00511	0.02300	0.00510
Crystallite Size (Å)	a	305.2725	299.7154	850.2538	610.4866
	b	/	299.7154	850.2538	610.4866
	c	267.9011	211.7928	507.5175	577.3342
RwP (%)		14.78	17.62	16.10	19.68
C-O bond length in CO ₃ group* (Å)		1.284	1.284	1.284	1.287
Ca ₁ -O ₃ bond length in lattice** (Å)		2.360	2.360	2.360	2.370
Density (g/cm ³)		2.7030	2.7076	2.6850	2.6880

*CO₃ group with plane (bond length of all C-O is equal and all bond angle is 120°)

**Ca₁-O₃ is neighbor in lattice and bond length of Ca₁-O₃ is the shortest of Ca-O bond length

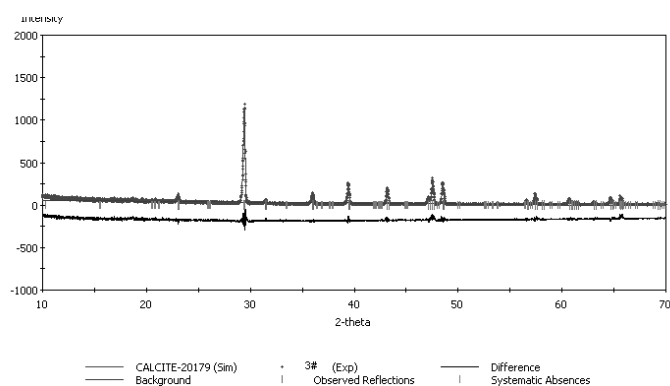


Figure 5: Rietveld refinement of calcium carbonate crystals modulated by collagen fiber (the [Collagen fiber]/[Ca²⁺] ratio is 0.2670)

increased, it showed that Ca₁ was no steady and thus, Ca₁ in the CaCO₃ is easily replaced or exchanged. On the other hand, C-O bond length in CO₃ group also increased as increasing [Collagen fiber]/[Ca²⁺] ratio, it made crystallite size increase.

CONCLUSION

The collagen fiber can change the polymorph of calcium carbonates; the effect of the different [collagen fiber]/[Ca²⁺] ratios on the morphology is evident: with ratio increasing, the calcite increases, and vaterite decreases.

The lattice strain characterized lattice defect is mainly influenced by S and number of nucleation, the crystal face being more perfect face and with minor defects has larger stability. Increasing ionic activity of Ca²⁺ and CO₃²⁻ and collagen fiber content could increase the stability of crystal of calcium carbonate. The lattice strain positively correlates to crystallite size nonlinearly, the crystal face with less defect are formed under higher [Collagen fiber]/[Ca²⁺] ratio, It's stability is larger. The distance between Ca₁ and O₃ in cell increased, as [collagen fiber]/[Ca²⁺] ratio increasing, the vitality of calcium carbonate enhanced.

The detailed information about crystalline structure of calcium carbonate formed in collagen fiber is very attractive as inducement and modulation of collagen fiber on crystal morphology of calcium carbonate are controllable. It is possible to prevent aciculate hides in liming of beamhouse processing. The results from these experiments will provide both a starting point for the determination of liming reaction condition and a new explaining for the liming phenomena at the atom level, based on crystal structure.

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