Crystal Phase of Fibrous Calcium Phosphates Prepared with Sodium Alginate

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This study investigated the effects of preparation conditions on the crystal phase of the fired fiber prepared with sodium alginate. Hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, hereafter referred to as HA, was only formed in fiber fired at 900°C under proper conditions. There was no significant difference in the crystal phase of the fired fibers prepared using different sodium alginate concentrations and syringe nozzle diameter, although fiber diameters were enlarged with increasing in either. No effects of phosphate type on the crystal phase of the fired fiber were found, but the aging time and the rinsing time had great effects. Sodium calcium phosphate, NaCaPO_4 , and HA were formed when the aging time was shorter than 5 min. Chlorapatite, $\text{Ca}_5\text{Cl}(\text{PO}_4)_3$, and HA were formed when the rinsing time was shorter than 3 sec, and HA was formed when the rinsing time was 5 min to 1 hour, whereas β -TCP, β - $\text{Ca}_3(\text{PO}_4)_2$, and HA were formed when the rinsing time exceeded 2 weeks.

Key words: Crystal phase, Fiber, Calcium phosphate

INTRODUCTION

HA is regarded as an effective biomaterial for dental applications and orthopaedic prosthesis as a substitute for bony tissue¹⁻⁴⁾. HA has a similar microstructure to biological apatite, with excellent biocompatibility, osteoconductivity and nontoxicity⁵⁻⁷⁾. Clinical success requires the simultaneous achievement of a stable interface with connective tissue and a match of the mechanical behavior of the implant with the tissue to be replaced⁸⁾. The bonding of synthetic HA to bone achieved by a direct chemical bond. It has been reported that tissue ingrowth, stimulation of new bone formation, and the biological fixation of the implanted material depend upon the nature of the microstructure configuration⁹⁾. Furthermore, intimate and stable contact with the host bone is the main prerequisite for osteoconduction. However, powdered HA may not provide this stable interfacial contact when implanted into sites where tissue movement occurs¹⁰⁾. Therefore, it can be expected that fibrous HA has better fixation in biological tissue than powdered HA^{11,12)}. Iwasaki reported that fibrous HA was prepared using a reaction of ion exchange of sodium alginate¹³⁾. However, CaO also formed in the fiber and was removed by chemical etching¹⁴⁾. This study attempted to obtain pure fibrous HA and the effects of various conditions on the crystal phase of fibrous calcium phosphates prepared using sodium alginate were investigated.

MATERIALS AND METHODS

A starting material was prepared by mixing 5 wt% sodium alginate and 6.27 mM phosphate, and a spinning solution was prepared by mixing 500 ml of 0.1 M CaCl_2 and 500 ml of 0.5 M $\text{Ca}(\text{CH}_3\text{COO})_2$ and adjusted to pH 7 by the addition of CH_3COOH . The starting material was extruded from a syringe into the spinning solution, immediately gelled by an ion exchange reaction of sodium for calcium; the gel fiber was then aged in the spinning solution. Subsequently, the gel fiber was gently rinsed with distilled water to remove the spinning solution covering the fiber, and dried at 37°C. The fired fiber was prepared by firing the dried fiber at 200–900°C for 3 hours.

Table 1 shows the preparation conditions employed in this study. Twenty-four kinds of specimens were prepared by changing six factors; firing temperature, concentration of sodium alginate, diameter of syringe nozzle, type of phosphate, aging time, and rising time. To evaluate the crystal phase, the fired fibers were analyzed using X-ray diffractometry* and

Table 1 Experimental conditions

Condition	No.	Firing temp. (°C)	Sodium alginate(wt%)	Nozzle (mm)	Phosphate	Aging time	Rinsing time
Firing temperature	1	*					
	2	200					
	3	400	5.00	1.25	$\text{Na}_4\text{P}_2\text{O}_7$	1 h	5 min
	4	600					
	5	900					
Concentration of sodium alginate	6		5.00				
	7	900	1.25	0.40	$\text{Na}_4\text{P}_2\text{O}_7$	1 h	5 min
	8		0.625				
Diameter of syringe nozzle	9			0.40			
	10	900	5.00	0.56	$\text{Na}_4\text{P}_2\text{O}_7$	1 h	5 min
	11			1.25			
Type of phosphate	12				Na_2HPO_4		
	13	900	1.25	0.40	Na_3PO_4	1 h	5 min
	14				$\text{Na}_4\text{P}_2\text{O}_7$		
Aging time	15					0 min	
	16					1 min	
	17	900	5.00	1.25	$\text{Na}_4\text{P}_2\text{O}_7$	5 min	5 min
	18					10 min	
	19					1 h	
Rinsing time	20						0 s
	21						3 s
	22	900	5.00	1.25	$\text{Na}_4\text{P}_2\text{O}_7$	1 h	5 min
	23						1 h
	24						2 w

* : dry, without firing

* Rotaflex RAD-rC, Rigaku, Tokyo, Japan

Fig. 1 X-ray diffraction patterns of the fibers before and after firing at 200, 400, 600 and 900°C.

Fig. 2 FT-IR spectra of the fibers before and after firing at 200, 400, 600 and 900°C.

FT-IR spectroscopy**. The diameters of dried and fired fibers were determined by optical microscopy, and the average diameter and the standard deviation were calculated for each group of 10 specimens. Furthermore, to determine the lattice parameters of HA in the fired fibers after rinsing, 27 specimens prepared using different rinsing times, 3-sec to 1-hour, were analyzed by X-ray diffractometry. The a -axis and c -axis dimensions were calculated from the positions of the (300) reflection and the (002) reflection, respectively.

RESULTS

Fig. 1 shows X-ray diffraction patterns of the fibers before and after firing at various temperatures. The fibers before and after firing at 200 and 400°C were amorphous. HA and β -TCP were observed at 600°C. HA alone was observed at 900°C. Fig. 2 shows FT-IR spectra of the fibers before and after firing at various temperatures. The spectra of the

** JIR-Diamond-20, JEOL LTD., Tokyo, Japan

Table 2 Diameter of dried fibers and fired fibers at 900°C

Condition	No.	Level	Diameter(μm)	
			Dried fiber	Fired fiber
Concentration of sodium alginate	6	5.00 wt%	108.18(16.21)	53.97(9.92)
	7	1.25 wt%	48.65(19.96)	16.67(2.01)
	8	0.625wt%	18.81(7.27)	10.32(3.01)
Diameter of syringe nozzle	9	0.40mm	108.18(16.21)	53.97(9.92)
	10	0.56mm	133.57(19.40)	67.47(17.73)
	11	1.25mm	287.14(33.40)	161.82(14.52)
Type of phosphate	12	Na_2HPO_4	46.11(16.71)	25.32(6.42)
	13	Na_3PO_4	43.16(16.13)	22.54(6.39)
	14	$\text{Na}_4\text{P}_2\text{O}_7$	48.65(19.96)	24.13(2.60)

The figures shown are averages (standard deviation).

Vertical lines indicate no significant difference at the level of 0.05.

Fig. 3 X-ray diffraction patterns of the fired fibers prepared without and with 1-min, 5-min, 10-min and 1-hour aging.

Fig. 4 FT-IR spectra of the fired fibers prepared without and with 1-min, 5-min, 10-min and 1-hour aging.

Fig. 5 X-ray diffraction patterns of the fired fibers prepared without and with 3-sec, 5-min, 1-hour and 2-week rinsing.

Fig. 6 FT-IR spectra of the fired fibers prepared without and with 3-sec, 5-min, 1-hour and 2-week rinsing.

fiber before and after firing 200°C showed that alginate still remained, because peaks due to alginate were observed around 1620, 1420, 920 and 820 cm^{-1} . At 400°C, the PO_4 bands were observed around 1040 and 580 cm^{-1} , indicating the presence of amorphous calcium phosphate. The peak intensities of the PO_4 groups increased with increasing firing temperatures and the PO_4 bands in the 600–570 cm^{-1} region were more clearly split at 600 and 900°C, indicating higher crystallinity. Furthermore, CO_3 bands around 1430 and 870 cm^{-1} were also observed in the spectra of the fibers fired at 400, 600 and 900°C.

Table 2 shows the average diameter of dried and fired fibers of specimens No. 6–14 listed in Table 1. The diameter of the fiber significantly increased with increasing concentrations of sodium alginate and syringe nozzle diameters. However, there was no significant difference in the X-ray diffraction patterns and FT-IR spectra among these specimens. Furthermore, there was no significant difference in the diameter, X-ray diffraction pattern, or FT-IR spectra of the fired fibers prepared using three kinds of phosphates. Fig. 3 shows X-ray diffraction patterns of the fired fibers prepared using different aging times in the spinning solution. All the diffraction peaks in the X-ray diffraction pattern of the fiber prepared without aging were identified with sodium phosphate, Na_3PO_4 . The fiber prepared with 1-

Fig. 7 Lattice parameters of the a -axis (left) and c -axis (right) of HA in the fired fibers as a function of rinsing time.

min and 5-min aging showed diffraction peaks of hydroxyapatite and/or sodium calcium phosphate. The intensity ratio of HA to sodium calcium phosphate increased with aging time. After 10-min aging, the fired-fiber showed only diffraction patterns of HA. There was no remarkable difference in the X-ray diffraction patterns from 10-min to 1-hour aging. Fig. 4 shows FT-IR spectra of the fiber prepared with different aging times. The spectra of the fired-fibers prepared with 1-min and 5-min aging had strong PO_4 bands around $1100\text{--}1010\text{ cm}^{-1}$ and $600\text{--}570\text{ cm}^{-1}$, indicating the presence of HA¹⁵⁾. After 10-min aging, these absorption bands became strong, indicating high crystallinity. FT-IR spectrum of the fiber prepared without aging showed quite different PO_4 absorption peaks from those of the other fibers, as described in X-ray diffraction study (Fig. 3).

Fig. 5 shows X-ray diffraction patterns of the fired fibers prepared using different rinsing times. X-ray diffraction patterns of unrinsed fibers without and those rinsed for 3 sec showed the peaks due to HA, CaO and chlorapatite. After 5-min and 1-hour rinsing, the X-ray diffraction patterns indicated the formation of pure HA. In specimens rinsed for 2 weeks, peaks due to β -TCP were observed in the X-ray diffraction patterns. Fig. 6 shows FT-IR spectra of the fired fibers prepared with various rinsing times. After rinsing for 5 min, strongly PO_4 bands were observed around the $1100\text{--}1010\text{ cm}^{-1}$ and $600\text{--}570\text{ cm}^{-1}$ absorption bands, indicating the presence of HA. After rinsing for 2 weeks, absorption bands around 1300 and 540 cm^{-1} were observed. This suggests that β -TCP was formed in the mixture with HA in the fired fiber¹⁶⁾.

Fig. 7 shows lattice parameters of the a -axis and c -axis of HA of the fired fibers prepared with various rinsing times. The lattice parameters of the a -axis were in inverse proportion to the logarithm of rinsing time up to about 10 min, whereas those of the c -axis are in the range from 6.875 to 6.890 Å . After rinsing for 10 min, the parameters of a -axis became a constant 9.43 Å .

From these results, the crystal phases of the fired fibers prepared under various conditions are summarized in Table 3.

Table 3 Crystal phases of fired fibers

Condition	No.	Level	Crystal phase
Firing temperature	1	*	amorphous alginate containing calcium and phosphate
	2	200°C	amorphous alginate containing calcium and phosphate
	3	400°C	amorphous calcium phosphate
	4	600°C	β -TCP, HA
	5	900°C	HA
Concentration of sodium alginate	6	5.00wt%	HA
	7	1.25wt%	HA
	8	0.625wt%	HA
Diameter of syringe nozzle	9	0.40mm	HA
	10	0.56mm	HA
	11	1.25mm	HA
Type of phosphate	12	Na ₂ HPO ₄	HA
	13	Na ₃ PO ₄	HA
	14	Na ₄ P ₂ O ₇	HA
Aging time	15	0min	Na ₃ PO ₄
	16	1min	NaCaPO ₄
	17	5min	NaCaPO ₄ , HA
	18	10min	HA
	19	1h	HA
Rinsing time	20	0s	chlorapatite, HA
	21	3s	chlorapatite, HA, CaO
	22	5min	HA
	23	1h	HA
	24	2w	β -TCP, HA

* : dry, without firing

DISCUSSION

The results of the present study showed no significant difference in the crystal phase of the fired fiber prepared with different concentration of sodium alginate and syringe nozzle sizes, although fiber diameter strongly depended on these factors. It is postulated that the type of phosphate used has no effect on the fiber. One-hour aging was employed in these experiments, Nos. 1–14 and 20–24 listed in Table 1; it seems that 1-hour aging was sufficient for gelation of the starting material by the reaction of ion exchange of sodium for calcium. Our previous study¹⁷⁾ demonstrated that sodium alginate solution had higher viscosity with increased concentrations of sodium alginate, and its rheological behavior was pseudo-plastic. This implies that the flow rate depends more strongly on the concentration of sodium alginate than shear stress, and that the gellation of the fiber is completed immediately upon leaving the syringe. It seems that the cross linking of the alginate-calcium-phosphate is formed immediately and has strong mutual bonding. As a result, the phosphorous ions hardly release from the gel structure. As shown in Figs. 3 and 4, the aging time in the spinning solution has a great effect on the crystal phase of the fired fiber. This seems to be caused by the persistence of Na⁺ ions in the gel fiber due to the insufficient ion exchange of sodium for calcium during a short aging time. These Na⁺ ions were incorporated in the apatite structure by firing. This suggests that the aging time should be extended at least 10

min after apparent gelation to allow completion of the ion exchange.

Furthermore, chlorapatite was formed in the fired-fiber with insufficient rinsing time as shown in Fig. 5. LeGeros⁸⁾ reported that the substitution of Cl for OH in apatites prepared from aqueous and non-aqueous systems is accompanied by expansion in the *a*-axis and contraction in the *c*-axis dimensions. The lattice parameters of the chlorapatite were *a*-axis = 9.647 and *c*-axis = 6.771, and the lattice parameters of HA were *a*-axis = 9.442 and *c*-axis = 6.882, due to the differences in the ionic radii of the substituents. In the present study, the substitution of OH for Cl in apatite was also observed by a contraction in the *a*-axis and a slight expansion in the *c*-axis dimensions. It seems that the surfaces of gel fibers were covered with the spinning solution containing Cl⁻ and Ca²⁺ ions even after complete gelation in the solution, and the needless spinning solution covering the fiber was gradually removed by rinsing. Thus, insufficient rinsing time resulted in the formation of HA and chlorapatite in the fired-fiber. This suggests that the gel fiber should be thoroughly rinsed, although excess rinsing time resulted in the formation of HA and β -TCP. This seems to be caused by a decrease in the Ca/P ratio of the gel fiber due to the release of Ca²⁺ ions from the gel structure during prolonged soaking.

From these results, it is concluded that fibrous HA with a high degree of purity can be obtained by properly controlling of these factors during preparation.

CONCLUSION

Fibrous calcium phosphates were prepared using an ion exchange reaction of sodium for calcium. Although the diameter of the fiber increased with both the sodium alginate concentration and the syringe nozzle diameter, there was no significant difference in the crystal phase of the fibers prepared by varying these factors. No effect of phosphate type on the crystal phase of the fired fiber was observed, but the aging time and the rinsing time had great effects. The fired fiber contained sodium phosphate and/or sodium calcium phosphate when the aging time was insufficient, whereas the fired fiber consisted of HA and chlorapatite when the rinsing time was insufficient. These results indicate that pure fibrous HA can be obtained by properly controlling these factors.

REFERENCES

- 1) Hench, L. L.: Bioceramics: From concept to clinic, *J Am Ceram Soc* **74** (7): 1487-1510, 1991.
- 2) Froum, S. J., Kushner, L., Scopp, I. W. and Stahl, S. S.: Human clinical and histologic responses to durapatite implants in intraosseous lesions, *J Periodontol* **53** (12): 719-725, 1982.
- 3) Moriwaki, Y.: Some application of hydroxyapatite ceramics as a bio-material (1), *The Nippon Dent Rev* **512**: 187-197, 1985. (in Japanese)
- 4) Nery, E. B., Lynch, K. L., Hirthe, W. M. and Mueller, K. H.: Bioceramic implants in surgically produced infrabony defects, *J Periodontol* **46** (6): 328-347, 1975.
- 5) Ducheyne, P.: Bioceramics: Material characteristics versus *in vitro* behavior, *J Biomed Mater Res* **21** (A2): 219-236, 1987.
- 6) Denissen, H. W., de Groot, K., Makkes, P. Ch., van den Hooff, A. and Kloppe, P. J.: Tissue response to dense apatite implants in rats, *J Biomed Mater Res* **14**: 713-721, 1980.
- 7) van der Meulen, J. and Koerten, H. K.: Inflammatory response and degradation of three types of

- calcium phosphate ceramic in a non-osseous environment, *J Biomed Mater Res* **28**: 1455-1463, 1994.
- 8) LeGeros, R. Z.: Apatites in biological systems, *Prog Crystal Growth Charact* **4**: 1-45, 1981.
 - 9) Salyer, K., Holmes, R. and Johns, D.: Replamineform porous hydroxyapatite as bone substitute in craniofacial osseous reconstruction, *J Dent Res* **56B**: 173, 1977.
 - 10) Ylinen, P.: Filling of bone defects with porous hydroxyapatite reinforced with polylactide or polyglycolide fibres, *J Mat Sci Mater Med* **5**: 522-528, 1994.
 - 11) Kasuga, T., Ichino, A. and Abe, Y.: Preparation of calcium phosphate fibers for applications to biomedical fields, *J Ceram Soc Japan* **100** (8): 1088-1089, 1992.
 - 12) Hayashizaki, J., Ban, S., Arimoto, N., Kataoka, H., Miyazawa, K. and Hasegawa, J.: Preparation of hydroxyapatite fiber by using alginate and its application to bioceramics, *Bioceramics* **7** (Proc. 7th Intern. Symp. Ceramics in Medicine): 41-46, 1994.
 - 13) Iwasaki, H.: Fibrous hydroxyapatite, *Ceramics* **24**: 295-299, 1989. (in Japanese)
 - 14) Iwasaki, H. and Kaneko, Y.: Preparation of fibrous hydroxyapatite by use of sodium alginate, *Materials* **37** (412): 60-64, 1988. (in Japanese)
 - 15) Sauer, G. R. and Wuthier, R. E.: Fourier transform infrared characterization of mineral phases formed during induction of mineralization by collagenase-released matrix vesicles *in vitro*, *J Biol Chem* **263** (27): 13718-13724, 1988.
 - 16) Fowler, B. O.: Infra-red spectra of hydroxyapatite, octacalcium phosphate and pyrolysed octacalcium phosphate, *Arch Oral Biol* **11**: 477-492, 1966.
 - 17) Hayashizaki, J., Ban, S. and Hasegawa, J.: Preparation of hydroxyapatite fibers by gelation of sodium alginate. Part 1. Relation between the preparation conditions and the products, *J J Dent Mater*, **12** (Special Issue 22): 46-47, 1993. (in Japanese)

アルギン酸ナトリウムを用いて合成した繊維状リン酸カルシウム塩の結晶相

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リン酸塩を混合したアルギン酸ナトリウム水溶液をノズルを装着したシリンジを用いて, 塩化カルシウムと酢酸カルシウムを混合し酢酸で pH7 に調整した紡糸液中に吐出し, イオン交換させ, ゲル状繊維を合成した。乾燥後, 大気中焼成し繊維状リン酸カルシウム塩を得た。焼成温度の上昇に伴い, 繊維状リン酸カルシウム塩の結晶性は増加し, 900°C焼成時にハイドロキシアパタイト ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, 以下 HA と略す) の単一結晶相を

呈した。ゲル状繊維の紡糸液中での熟成時間が5分以下の時, 焼成繊維は HA 及び NaCaPO_4 の混合結晶相であった。また, 焼成繊維は熟成後の蒸留水による水洗時間が3秒以下の時, HA, クロロアパタイト ($\text{Ca}_5\text{Cl}(\text{PO}_4)_3$) 及び酸化カルシウムの混合結晶相であり, 5分~1時間では HA の単一結晶相であった。しかし, 水洗時間が2週間を越えると, HA と $\beta\text{-Ca}_3(\text{PO}_4)_2$ の混合結晶相を呈した。

臼歯部用光重合型コンポジットレジンの疲労強度

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近年, 歯科材料における疲労特性の重要性が指摘されている。そこで本研究では水中保管した2種類のコンポジットレジンの疲労特性について検討した。試験には Hybrid type と MFR type の2種類のコンポジットレジンをを用い, ダンベル型の試片を作製し, 37°C水中に24時間, 1ヵ月間, 3ヵ月間, 6ヵ月間, 12ヵ月間保管してから疲労試験と引張試験を行った。疲労試験ではステアケース法を用いて 10^5 回における疲労強度を求めた。

24時間後の疲労強度は, Hybrid type で 54.7 MPa, MFR type で 28.1 MPa であり, ともに経時的に減小傾向がみられた。一方, 直接引張強さは Hybrid type で 77.0 MPa, MFR type で 53.5 MPa であり, Hybrid type では水中保管期間にかかわらず変化がみられなかったが, MFR type では経時的に小さくなる傾向が認められた。

機能性タンパク質薄膜によるインプラント用金属表面の化学修飾

(第1報) 修飾表面の分子構造と生体活性

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インプラント用金属に生体活性を付与するために, 金属表面を機能性タンパク質で化学修飾する方法を検討した。今回はシッフ塩基結合法を用い, NiTi 合金表面に接着性タンパク質として知られる血漿フィブロンectin

(pFN) を固定化し, 各処理段階における表面構造を X線光電子分析装置で調べた。さらに, 固定化した pFN をフルオレサミンを用いた蛍光法により定量した。その結果, pFN は合金表面に結合したシランカップリング剤に