

Development of Bioactive Paste with Improved Handling

Chikara Ohtsuki, Toshiki Miyazaki*, Hirofumi Inada,
Masanobu Kamitakahara and Masao Tanihara

Graduate School of Materials Science, Nara Institute of Science and Technology,
8916-5 Takayama-cho, Ikoma-shi, Nara 630-0192, Japan

Fax: +81-743-72-6129, E-mail: ohtsuki@ms.naist.jp

*Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology,
2-4, Hibikino, Wakamatsu-ku, Kitakyushu-shi, Fukuoka 808-0196, Japan

Fax: +81-93-695-6032, E-mail: tmiya@life.kyutech.ac.jp

Bioactive paste consisting of calcium phosphates shows bone-bonding property, i.e. bioactivity, and is extensively used in orthopedic fields to fill bone defects. This type of paste is made by mixing powder of calcium phosphates and liquid. The paste is then injected into the bone defect to be hardened *in situ*. Operation of shaping this paste must be completed within as short time as several minutes in clinical usage. In this study, we attempted to develop bioactive paste that sets after exposure to body fluid. Powder was prepared by mixing tetracalcium phosphate and dicalcium phosphate, while liquid was prepared by dissolving cellulose acetate in ethyl lactate. Putty-like paste was obtained after mixing the powder and the liquid, and it was able to be formed into desirable shapes before exposure to aqueous environment. The paste set through precipitation of hydroxyapatite and cellulose acetate after exposure to aqueous solution. This type of self-setting bioactive composite is attractive as novel bone substitutes with easy handling.

Key words: Bioactive paste, Hydroxyapatite, Cellulose acetate, Self-setting, Handling

1. INTRODUCTION

Several kinds of paste consisting of calcium phosphate ceramics show bone-bonding property, i.e. bioactivity, and are extensively used in orthopedic fields to fill bony defects with complex shapes [1-4]. This type of pastes is made by mixing powder of calcium phosphates and liquid. The paste is then injected into the bony defect to be hardened *in situ* through precipitation of hydroxyapatite. Operation of mixing of the powder and the liquid, however, must be completed within as short time as several minutes in clinical usage. As a result, mechanical properties of the paste are highly dependent on technique of mixing. Generally easy handling is an important factor that largely governs quality of biomaterials. It has been desired to develop novel bioactive pastes, which can be formed into desirable shapes without mixing, and sets after exposure to body fluid in the bony defect. It is also a problem that previous bioactive pastes consisting of calcium phosphates have much higher young's modulus than natural bone. Mechanical properties analogous to those of natural bone are required on design of a novel bioactive paste.

In this study, we design novel bioactive paste that consists of powder of calcium phosphates and liquid containing cellulose acetate. Cellulose acetate hardly dissolves in water, while it dissolves in specific kinds of organic solvent, including ethyl lactate. Cellulose acetate dissolved in ethyl lactate precipitates when it is exposed to water. Powder mixture of calcium phosphates consisting of tetracalcium phosphate (TTCP, $\text{Ca}_4(\text{PO}_4)_2\text{O}$) and dicalcium phosphate anhydrate (DCPA,

CaHPO_4) is also set after they are reacted with water to precipitate hydroxyapatite [1]. Therefore, mixed paste consisting powders of TTCP and DCPA, and ethyl lactate solution containing cellulose acetate, is expected to set through precipitation of both hydroxyapatite and cellulose acetate, when it is exposed to aqueous solution such as body fluid. We attempted to fabricate pastes with several compositions, in order to examine setting behaviors and structural changes of the paste after exposure to an aqueous solution.

2. MATERIALS AND METHODS

2.1 Preparation of paste

TTCP was synthesized by heat treatment of powder mixture with equimolar of β -tricalcium phosphate (β -TCP, $\beta\text{-Ca}_3(\text{PO}_4)_2$) and calcium carbonate (CaCO_3) in an electronic furnace up to 1500°C at a rate of 5°C/min, kept at the temperature for 10 h (=hours) and cooled to room temperature. This heat treatment was repeated twice. Dicalcium phosphate anhydrate (DCPA, CaHPO_4) was synthesized by heat treatment of dicalcium phosphate dihydrate (DCPD, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) up to 250°C at a rate of 5°C/min, followed by keeping at the temperature for 10 h and cooled to room temperature. The calcium phosphates were pulverized to be particles less than 45 μm in size. The examined powder was prepared by mixing equimolar of the synthesized TTCP and DCPA. The obtained calcium phosphates powder was hereafter denoted as CP.

Liquid was prepared by dissolving various amounts of cellulose acetate in ethyl lactate. Compositions of the liquid are given in Table. I. The powder was mixed with

the liquid at various powder to liquid (P/L) ratios under an ambient condition. Protocol of the preparation of the examined paste is illustrated in Fig. 1.

2.2 Measurement of setting behavior

The pastes were soaked in a buffer solution in order to examine setting properties and structural changes in body environment. The solution contained 142 mmol/L of NaCl and was buffered at pH 7.40 by using 50 mmol/L of tris(hydroxyethyl)aminomethane and appropriate amount of HCl. The solution is hereafter denoted as Tris-NaCl solution. Setting time of the mixed paste was measured in Tris-NaCl solution at 36.5°C according to JIS T6602 [5]. Apparatus for measuring setting time was shown in Fig. 2. Weight of 300 g was loaded into the pastes with a Vicat needle apparatus with 1 mm² in cross section. The setting time was defined as a time when a trace of the Vicat needle did not remain on the surfaces of the pastes. Change in crystalline phase of the pastes after soaking in Tris-NaCl solution was examined by powder X-ray diffraction.

3. RESULTS

Putty-like pastes were obtained after mixing the powder and the liquid, and they could be formed into desirable shapes before exposure to aqueous environment. Figure 3 shows setting time of the pastes prepared from CP and liquids with various concentrations of cellulose acetate. The pastes are denoted as "CP+Liquid" on the figure. The pastes were prepared at same powder/liquid (P/L) ratio of 2.0. Paste was set within 120 min. when the ethyl lactate solution contains 30 mass% of cellulose lactate or more, whereas the pastes is not set within 360 min when it contains 10 mass% of cellulose acetate or less. Setting time was reduced by increase in concentration of cellulose acetate in the liquid.

Figure 4 shows setting time of the pastes prepared from CP and CA50 (CP+CA50) with various P/L ratios. Setting time showed tendency to decrease with increasing powder/liquid ratio of the paste. Paste with P/L ratio of 2.0 approximately sets at 60 min.

Figure 5 shows powder X-ray diffraction patterns of the pastes prepared from CP and liquids (P/L ratio = 2.0) with various concentrations of cellulose acetate, which were soaked in Tris-NaCl solution for 7 d (=days). The diffraction pattern of mere powder of CP was also given on the figure. Peaks assigned to DCPA and TTCP were detected for CP powder. The pastes after soaking in Tris-NaCl solution showed broad peaks assigned to hydroxyapatite, as well as those assigned to the trace amounts of TTCP. Figure 6 shows powder X-ray diffraction patterns of the pastes prepared from CP and CA50 with various powder to liquid ratios, which were soaked in Tris-NaCl solution for 7 d. Broad peaks assigned to hydroxyapatite as well as the trace of TTCP were detected for all the pastes after the soaking in Tris-NaCl solution. The calcium phosphates powder almost was reacted to form hydroxyapatite during the soaking irrespective of P/L ratios and concentrations of cellulose acetate.

4. DISCUSSION

The results described above show that the pastes

Table I Compositions of liquid.

Liquid	Cellulose acetate / g	Ethyl lactate / mL
CA0	0	100
CA10	10	100
CA30	30	100
CA50	50	100

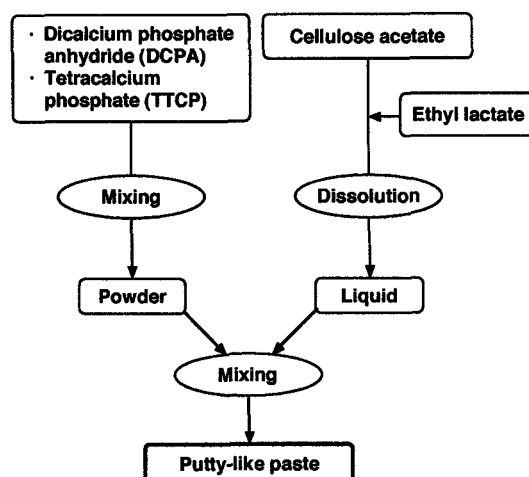


Fig. 1 Preparation of paste.

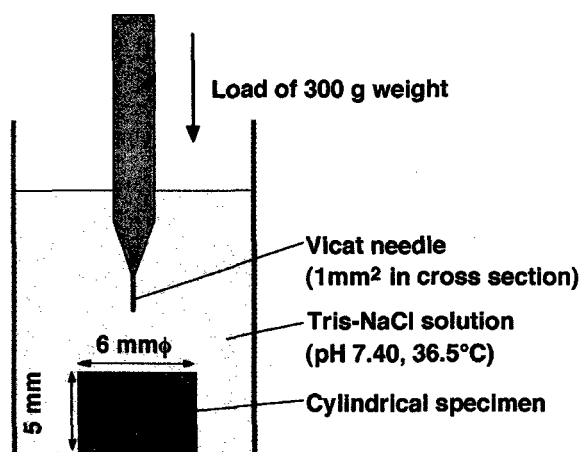


Fig. 2 Measurement of setting time of the pastes (JIS T6602).

solution containing cellulose acetate can set within 120 min. after they are exposed to aqueous solution. Higher content of cellulose acetate in ethyl lactate solution allows to reduce the setting time of the pastes. Setting could be observed within 120 min. even for of the mere CA50 solution ($P/L=0$). Therefore, precipitation of cellulose acetate mainly determines the setting behavior of the paste.

Results of the X-ray diffraction (Figs. 5 and 6) shows that calcium phosphates powder can react with surrounding water to form hydroxyapatite, even when cellulose acetate precipitates on the pastes after exposure to aqueous solution. The precipitated hydroxyapatite has low crystallinity, similar to that of bone apatite. This characteristic brings the possibility for the pastes to make direct bond to living bone in a short implantation period. In addition, the precipitation of hydroxyapatite may form complex structure with cellulose acetate polymer. This type of complex structure gives the set pastes mechanical properties analogous to natural bone, in comparison with conventional calcium phosphate cement.

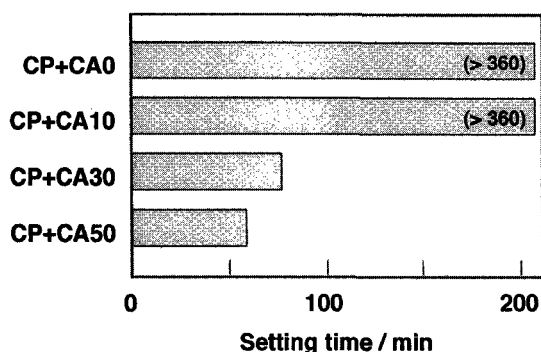


Fig. 3 Setting time of the pastes prepared from CP and liquids with various concentrations of cellulose acetate.

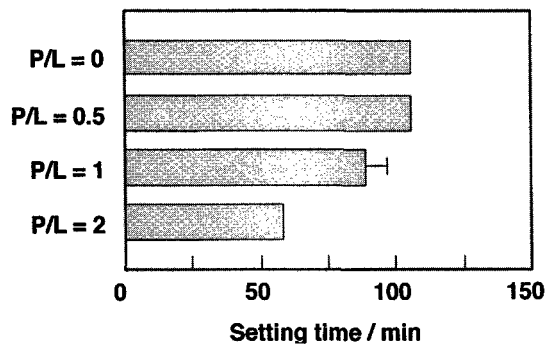


Fig. 4 Setting time of the pastes prepared from CP and CA50 (CP+CA50) with various powder to liquid ratios (P/L).

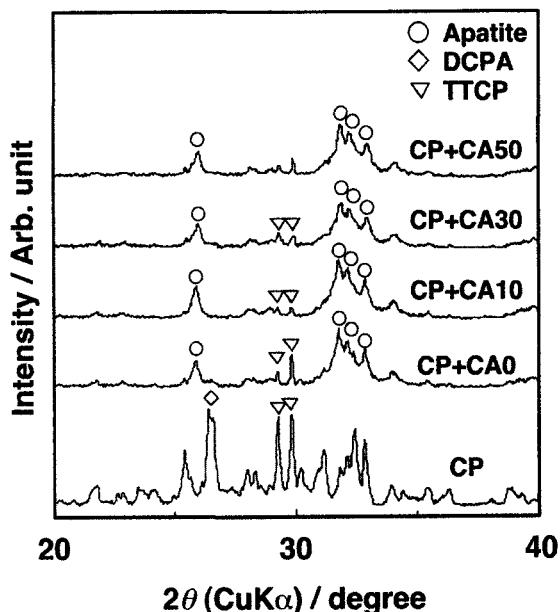


Fig. 5 Powder X-ray diffraction patterns of the pastes prepared from CP and liquids with various concentrations of cellulose acetate, which were soaked in Tris-NaCl solution for 7 d. CP was not soaked in Tris-NaCl solution.

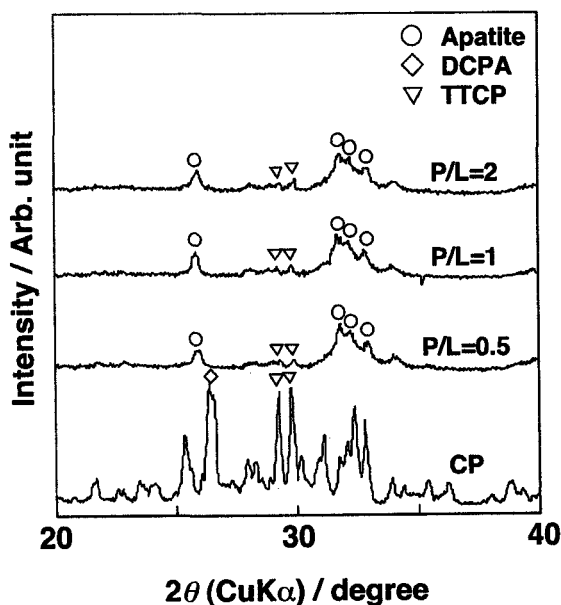


Fig. 6 Powder X-ray diffraction patterns of the pastes prepared from CP and CA50 with various powder to liquid (P/L) ratios. The pastes were soaked in Tris-NaCl solution for 7 d, while CP was not soaked.

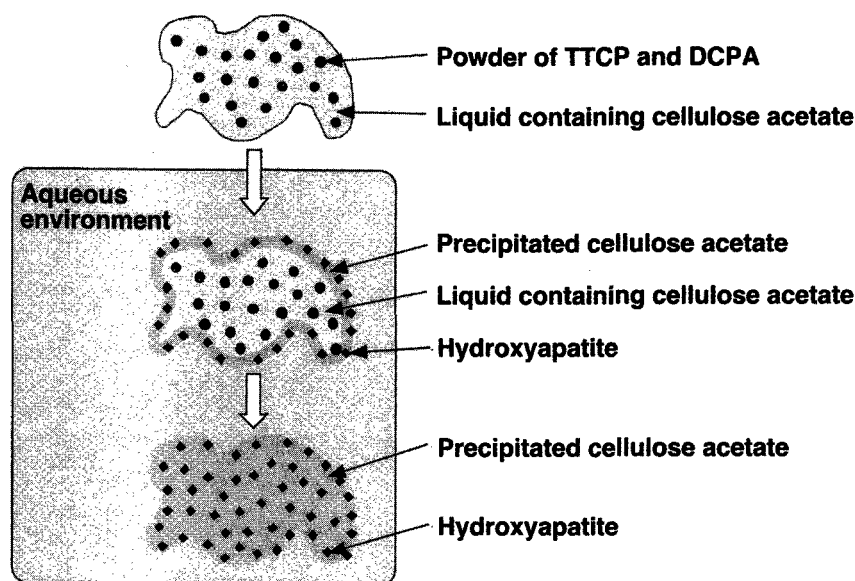


Fig. 7 Schematic representation of the setting mechanism of the pastes consisting of calcium phosphates powder and liquid containing cellulose acetate.

The setting mechanism of the paste developed on the present study is illustrated on Fig. 7. The setting is initiated by release of ethyl lactate into surrounding fluid to precipitate cellulose acetate. Formation of hydroxyapatite after the hydrolysis of calcium phosphates may increase mechanical strength. On the design of this type of paste, we may use the other types of organic polymers that can dissolve in the liquid, ethyl lactate, and may have quite low solubility to water. Appropriate combination of the organic solvent and organic polymer should be clarified in the future researches.

5. CONCLUSIONS

Bioactive paste with easy handling was obtained from calcium phosphate ceramics and ethyl lactate solution containing cellulose acetate. The paste set through precipitation of cellulose acetate as well as low-crystalline hydroxyapatite after exposure to aqueous environment. This type of bioactive composite paste is attractive as novel bone substitutes for repairing complex-shaped bony defects.

ACKNOWLEDGMENTS

This study was supported by Grant-in-Aid for Exploratory Research (14658290), Ministry of Education, Culture, Sports, Science and Technology, Japan.

REFERENCES

- [1] L. C. Chow, *J. Ceram. Soc. Japan*, **99**, 954-64 (1991).
- [2] K. Kurashina, H. Kurita, A. Kotani, H. Takeuchi and M. Hirano, *Biomaterials*, **18**, 147-51 (1997).
- [3] S. Takagi, L. C. Chow and K. Ishikawa, *Biomaterials*, **19**, 1593-99 (1998).
- [4] H.-M. Kim, *J. Ceram. Soc. Japan*, **109**, S49-S57 (2001).
- [5] JIS, Japanese Industrial Standard T6602-1985, Dental Zinc Phosphate Cement, 1985.

(Received November 30, 2003; Accepted February 29, 2004)