칼슘 포스페이트 골 시멘트에서의 테트라사이클린 방출 거동

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Release Behavior of Tetracycline from Calcium Phosphate Glass Bone Cement

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ABSTRACT

골 시멘트 자체 기능 외의 특성을 가질 수 있도록 하는 새로운 조성의 비정질 칼슘 포스페이트 분말을 개발하였다. 이 분말로 만든 골 시멘트에서의 테트라사이클린 방출 거동은 두 가지 항생제 담지 방법으로 비교하였고, 이 전 논문에서 이미 입증된 조성의 브러샤이트계 시멘트와도 비교하였다. 첫 번째 담지 방법은 시편의 마이크로 기공에 의해 발생하는 삼투압 현상으로 항생제 약물에 시편을 담그어 담지시키는 방법이고, 다른 방법은 분말과 항생제를 용해시킨 액상을 섞는 방법이다. 비정질 칼슘 포스페이트 분말은 유리 제조의 전형적인 방법인 용해 후 급냉시키는 방법을 이용하여 얻은 비정질 칼슘 포스페이트 유리를 갈아서 얻었다. 시멘트 페이스트를 섞을 때 중합열이 없기 때문에, 우리는 테트라사이클린의 변성없이 골 시멘트 시편을 만들 수 있었다. 골 시멘트로부터의 테트라사이클린 방출 시험은 6일 간 진행되었고, 그 결과 첫 번째 항생제 담지 방법으로 만든 시편보다 두 번째 방법으로 만든 시편으로부터 테트라시이클린 방출이 더욱 지속적으로 나타났다. 또한 항생제 담지에 따른 물리적 성질의 변화를 고려하여 압축강도 시험을 하였다. 그 결과 테트라 사이클린을 담지한 시편과 그렇지 않은 시편과의 통계학적 유의차는 없는 것으로 나타났다. 이런 결과를 토대로 새로운 조성의 비정질 칼슘 포스페이트 시멘트는 다른 재료의 골 이식재처럼 생물학적 인자 혹은 약물 전달 시스템에 있어서 충분한 가능성이 있다고 판단된다.

KEY WORDS: Amorphous calcium phosphate glass, Antibiotics release, Bone cement, Brushite, Tetracycline

INTRODUCTION

Recently, many investigations about the modification of bone grafts with biological molecules and drugs have been performed broadly. These modified bone grafts enable a direct and a well-defined application of drugs to infected or fractured hard tissue[1,2]. Bone grafts with biological substances or drugs are more efficient than systemic administration due to lower doses and reducing side effects[3]. Besides, these modified bone grafts can determine suitable contents of drug for patients who demand actual contents for their disease. There are lots of bone grafts based on various materials. For bone grafts based on polymeric materials, their mechanical and chemical properties are not good as ceramic materials. But most of ceramic bone grafts have upper melting temperature than those of biological molecules and drugs[4,5].

However, some ceramic bone grafts have an advantage which does not need to sinter at high temperature. As the representable ones, those are materials based on calcium phosphate. Calcium phosphate have been proved to be useful in not only in dentistry, but also bone grafts in orthopedics[6]. There are many kinds of calcium phosphate

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materials. Calcium orthophosphate cement do not require sintering to make a specific shape[7]. Therefore, calcium phosphate cement can contain biological molecules and drugs which are not damaged their effects due to the low temperature process, and can release continuously[8,9]. On the other hands, calcium phosphate glass has advantages which can control compositions to react on hard tissue easily and to regenerate hard tissue due to its biodegradable characteristic[10]. But synthesis temperature of calcium phosphate glass is still too high to contain biological molecules and drugs.

Therefore, we investigated brand-new composition bone cement powder and aqueous solution system using the amorphous calcium phosphate (ACP) glass and basic solution. The advantages of this cement are that eliminating needless process like the second heat treatment one for hardening and loading the biological molecules and drugs efficiently. The powder from ACP glass was synthesized like self-setting reaction cement with aqueous solution at body temperature. Due to the temperature of cementation, we could load tetracycline (TTC) on that ACP glass powder and basic solution. Therefore, we experimented on the release of TTC which is known to have diverse characteristics as well as antibacterial characteristics. TTC hastens the attachment of cells on surface of dentin and has inhibition efficacy on collagenase. When it is administered a dose topically, TTC also help new bone formation[11,12,13].

The aim of this study is that new composition ACP cement has potential for containing antibiotics without denaterization and controlling the behaviors of release, that is, release contents of the antibiotics per time could be controlled easily by clinicians or the other users. We chose TTC which is one of the antibiotics, for release, and experimented on release test with two drug loading methods. We also compared with previously studied brushite cements.

MATERIALS AND METHODS

1. Synthesis of Amorphous Calcium Phosphate Glass Powder

Glass batch in the system $CaO-P_2O_5-MgO-ZnO-CaF_2$ was prepared with Ca/P ratio 0.6 using $CaCO_3$ (Samchun Pure Chemical Co., Ltd., Korea), H_3PO_4, MgO, ZnO (Duksan Pure Chemical CP., Ltd., Korea), CaF_2 (Junsei Chemical Co., Ltd., Japan) as raw materials and the molar ratio of CaO/CaF_2 was fixed to 9. MgO and ZnO were added at 1mol%, respectively[14,15]. Mixed batches were dried at 100℃ and melted in a platinum crucible at 1250℃. It was subsequently quenched onto a graphite plate conventionally at room temperature. The ACP glass was reduced powder using an alumina mortar and was attrition milled using two different sizes of zirconia balls. To confirm non-crystalline phase, X-ray diffraction analysis was performed (D/MAX 2000, Rigaku, Japan). The powder distribution analysis was performed using a particle size analyzer (Saturn DigiSizer®5200, Micrometritics, USA).

2. Preparation of bone cement specimen and loading tetracycline

The ACP glass powder and 1.5 M NaOH solution were main materials for bone cement. And Na_2CO_3 were added for forming micro-pores and improving strength after immersing in phosphate buffer saline (PBS pH 7.4, Gibco, Invitrogen, USA). The amount of Na_2CO_3 was 7.5 wt% of whole powder and the ratio of powder / liquid was 4 g/ml.

1) Experimental group (EG) : The mixed powder was added to TTC solution at a time and mixed with spatula for 20 seconds. TTC solution is that TTC was dissolved in 1.5 M NaOH solution with using stirrer bar for 3 minutes. This mixed paste was poured into a cylindrical Teflon mold (6 mm D × 12 mm H)[16]. The upper

side and bottom of the mold were covered with glass plates and were fixed with C-clamp. Then the mold was placed in a 37℃, 100% relative humidity box. The mold was removed after 2 hours and was placed in same condition as before again for 22 hours.

2) Control group (CG) : The mixed powder was added to the NaOH solution at a time and mixed with spatula for 30 seconds. After cementation of the paste, specimens were immersed for 10 minutes, 30 minutes, and 60minutes (CG-I : difference caused by loading methods) in prepared TTC solution which was made of 1.5 M NaOH solution and TTC. Afterward process were same with EG. After immersing, the specimen was dried at 37℃ for a day. This group was prepared to compare release behaviors with EG. As control group II, III (CG-II, CG-III : difference caused by components of cements) in TTC release test, two brushite cement formulations were prepared[17,18]. These two groups were prepared to compare release behaviors with EG-c. The components of cements and the contents of loaded TTC are presented in Table 1.

3. Release Behavior of Tetracycline

The specimens of each group were immersed in PBS and stored in 37℃ chamber for 6 days with a change of the effluent after measurement absorbance (1 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days). The volume of PBS was 3 mℓ for each specimen and they were completely surrounded by the solution. The absorbance measurement of effluent was performed at 362 nm using a UV/vis spectrometer (UVD-3200, Labomed, Inc., USA). Quantity of tetracycline was acquired through drawing the calibration curve.

4. Compressive strength

There was a compressive strength test to compare TTC loaded bone cements and unloaded bone cement. The specimens were cylindrical shape those were made in the same process as above. Some specimens were immersed in 37℃ PBS for 1 day. The volume of PBS was twice as much as that of specimens. The specimens were immediately measured by a universal testing machine (3366, Instron, USA). The cross-head speed was 1 mm/min and picked the maximum value of strength out[16]. Results were compared using one-way ANOVA analysis variance followed by a Tukey post hoc test (SPSS, v12.0).

RESULTS

1. Properties of amorphous calcium phosphate glass powder

As a result of XRD analysis, there was none crys-

Table 1. The components of cements and the contents of loaded TTC on each groups per specimen are presented. The contents were calculated by bulk specimen reducing powder and dissolve in PBS. Then we acquired the amount of loaded TTC using absorbance of TTC from UV/vis spectrometer

Cement codes		Component			Content of TTC (mg)		
		Powder phase	Liquid phase	P/L ratio (g/ml)	a	b	c
EG		92.5 wt% ACP glass 7.5 wt% Na_2Co_3	1.5 M NaOH	4.0	412.5	825	1650
CG	I				764.5	974	959.5
	II a	β-TCP	0.2 M H_3PO_4 0.05 M sodium citrate	1.75	1650		
	II b	51 wt% β-TCP 49 wt% MCPM	0.5 M sodium citrate	3.3			

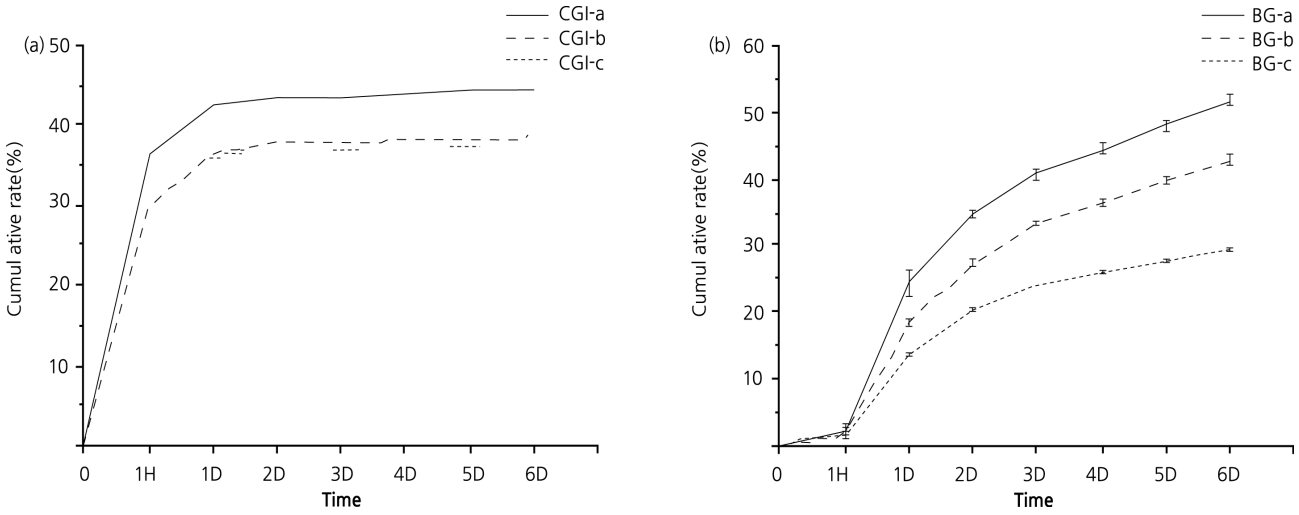

Figure 1. *The release behaviors of TTC from CG-I and EG. (a) CG-I showed initial burst release and after 1day, the release rate of TTC was very slow. (b) EG showed no initial burst and constant release (n=3).*

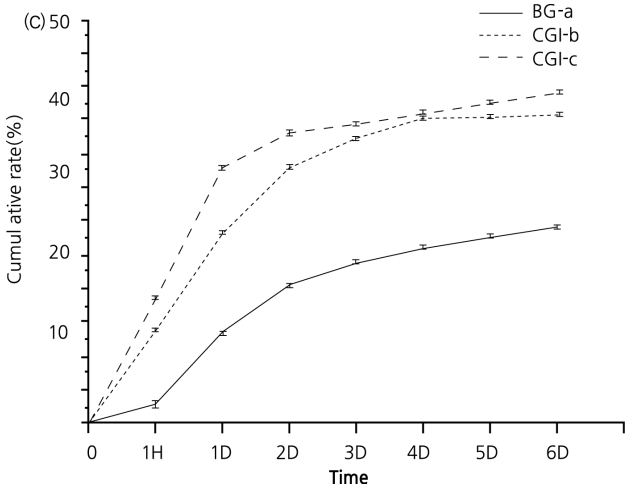

Figure 2. *The release behaviors of TTC from EG-c, CG-II-a, b (n=3). The characteristics of each cement specimen (P/L ratio, component, etc.) let them be different.*

talline peak. Therefore, we could conclude that the calcium phosphate glass powder had amorphous phase. The powder size was distributed at about 5 μm and 1.5 μm broadly, due to the two different size of zirconia balls which used in attrition milling. Therefore, we could expect that the binary particle distribution made the bone cements specimen had many micro-pores[19].

2. Release behavior of tetracycline

The release of TTC under two method conditions showed very different aspects (Fig. 1). In the CG-I, each and every dipping time conditions followed a first order kinetic with an initial burst release, where upto 30% of loaded TTC was released within 1 day. The reason of an initial burst release was that the micro-pores of bone cement specimen were not consisted of open pores but most of those are closed pores. Therefore, it was difficult to absorb the tetracycline solution broadly. Therefore, most of TTC which was burst at the initial stage release was that absorbed only inside the surface nearby of the cylindrical specimen. In contrast, the case of EG indicated that the release of TTC from cylindrical specimens showed no initial burst of TTC and the slopes of EG were regular. These results proved that loaded TTC were spread broadly inside the bone ce-

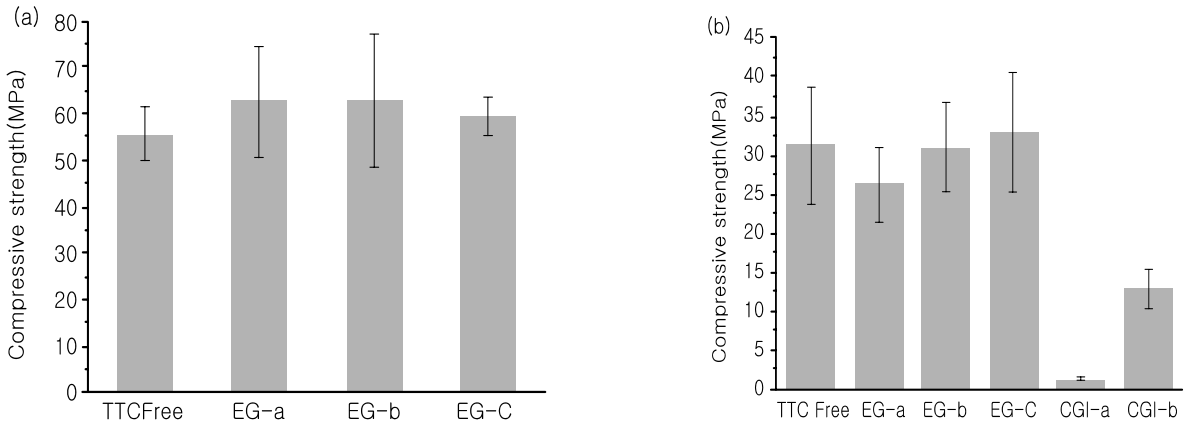

Figure 3. *The results of compressive strength test (n=10). (a) is a graph of dry condition. The results of wet condition (b) showed that each group has different characteristics. The results of CG-II-a, b were quoted from reference 17,18.*

ments specimen. When the specimens were made, TTC was not powder, but was solute of NaOH solution. Therefore TTC could spread broadly into the specimen, and release rate was nearly constant. Fig. 2 showed the release behaviors of TTC from EG-c, CG-II-a, and CG-II-b. They had same contents of TTC but cumulative rate were different. The reason of different cumulative rate is their P/L ratio. P/L ratio has an effect on setting time, porosity, and strength[17]. Actually, the result of compressive strength test proved the theory.

3. Compressive strength

The results of compressive strength test were showed in Fig. 3. There were no statistical difference of compressive strength between TTC unloaded specimens and unloaded specimens, neither hardening at 37℃ nor immersing in PBS at 37℃. There was no significant difference between TTC free specimens and EG ($p<0.05$). As shown Fig. 3, results of wet condition told us that the relationship between P/L ratio and strength. As P/L ratio increased, strength increase, too.

DISCUSSION

Hard tissue of human body consists of various elements. Main element is that we called the hydroxyapatie (HA) which consists of calcium and phosphorus with their ratio is 1.67. ACP glass we are investigating is similar to ingredients of the HA and has many advantages. The way of synthesis of ACP glass is not complex as well as synthesis of HA. Therefore, we could approach easily to acquire the artificial bone through synthesizing ACP glass.

We investigated this ACP glass and the composition what we applied is suitable for applying self-hardening bone cement. Because, there was no heat of polymerization, we could conceive that biological molecules and drugs could be additives of our ACP bone cement easily. We experienced that the control of drug quantity is difficult to mix paste and physical characteristic could be weakened, if amount of drug is added on amorphous calcium phosphate powder. Therefore, we conceived that drug might be as well loaded by liquid phase as powder form. And this way has many advantages that when we mixed paste and the paste was hardened, the loaded drug could be dispersed broadly. Therefore, the drug could release constantly and continuously. The release test of TTC was fine we expected, because of broad dispersion of TTC inside specimen. But the CG-I was not. The reason was that micro-pores inside bone cement specimen were closed pores (not inter-connective). Therefore, it is difficult to pass through and too low pressure to soak the drug solution.

Comparing the release behavior of TTC from ACP

bone cement under mixing powder and antibiotic solution system with simple dipping method showed that drug release behavior can be controlled by the former. The drug release result from this method showed no initial burst release behavior and no de-naturalization of mechanical property (ACP bone ce-ment specimen). Especially, there did not need to devise special and complex device for drug loading due to the ACP cement. And this showed us that the potential which is abilities to load biological mole-cules and various drugs and for other bone grafts to release drugs efficiently through ACP coating. But one thing we mistook in this experiment is that mi-cro-pores inside bone cement specimen were closed pores. Therefore, it was difficult to adapt capillary phenomenon as a control group. In following inves-tigation, we should consider another method to load biological molecules and drugs for control group and another bone grafts. Furthermore, we should find out various factors which can affect when drugs are loaded on bone cement.

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