The Effect of Temperature and Concentration of Setting Solution on the Rheological Properties of Injectable Calcium Phosphate

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Injectable calcium phosphate cement (CPC) has been used as bone substitute successfully due to good biocompatibility and osteoconductivity. One of the important mechanical characteristics of CPC is flowablility, which can be evaluated by measuring rheological parameters. However, there have been few studies that measured rheological properties of CPC. The purpose of this study was to evaluate the effects of temperature and concentrations of 2 kinds of setting solutions, hydroxyprophyl methylcellulose (HPMC) and polyacrylic acid (PAA), on rheological properties of CPC.

The CPC used was dicalcium phosphate dihydrate (DCPD). Rheological properties of CPC paste were measured using rheometer. The effect of concentrations of each solution (2% and 1% HPMC and 35% and 17.5% PAA) was evaluated. The effect of temperature (25℃ and 37℃) on the rheological properties of CPC was also investigated. The statistical analysis was carried out with Mann-whitney test with Bonferronis collection. CPC with both setting solutions showed shear thinning behavior. Higher concentrations of setting solution (2% HPMC and 35% PAA) produced significantly higher viscosity than lower concentrations of setting solution (1% HPMC and 17.5% PAA). CPC with HPMC showed significantly higher viscosity at 37℃ that at 25℃. CPC with PAA showed lower viscosity at 37℃ than at 25℃, although the difference was not statistically significant. The results showed that CPC with HPMC or PAA solutions are pseudoplastic and the concentrations of setting solutions and temperature may have an effect on the rheological properties of CPC paste. These results showed that the flowability of injectable CPC could be improved by use of increasing frequency of oscillation. In clinical practice, the use of ultrasonic vibration would be helpful in application of injectable CPC. CPC with HPMC could be more easily applicated at 25℃ than 37℃. The use of lower concentrations of HPMC and PAA solution would be beneficial in terms of flowability.

Key words : calcium phosphate cement, hydroxyprophyl methylcellulose, polyacrylic acid, rheological characteristics

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INTRODUCTION

Calcium phosphate cement (CPC) has been developed as a bone substitute material with many advantages, such as good biocompatibility, self-setting ability, and good moldability for any required shape[1]. Due to these advantages, CPC has been used successfully in osseous surgery in recent years[2]. In dentistry, many successful cases have been reported in which calcium phosphate cement had been used to fill the gap between dental implants and the alveolar socket wall,[3,4] augment the alveolar ridge height[5], preserve and repair alveolar bone[4,6], seal a root canal[7,8]. A CPC powder mixed with a setting solution forms a paste where water acts as a vehicle to dissolve the CPC powder. This dissolution supplies calcium and phosphate ions that precipitate in the form of hydroxyapatite,[9] which is the main mineral in human hard tissues. The good biocompatibility of CPC has been attributed to the formation of hydroxyapatite. When a CPC is applied to a bone defect area using an injection syringe, the flowability and injectability of the CPC are important determinants of its clinical performance. This is particularly true in operations involving hardly accessible defects or narrow cavities when precise placement of a paste to a defect area is required. This is also true when minimally invasive surgical techniques are used.[10] However, until now, no index except for the rheological parameters can describe the flowability of CPC. A rheological study of CPC can provide information on the flow behavior and setting process of CPC, and there are some reports on the rheological properties of CPC.[11-16]

The main shortcomings of CPC are its relatively long setting time and tendency to wash-out. In this study, hydroxyprophyl methylcellulose (HPMC) and polyacrylic acid (PAA) were used to enhance the cohesiveness and anti-washout properties of CPC.[10,17] Some studies reported that the particle size and liquid to powder ratio affect the rheological properties of CPC.[18] In one study, many types of additives, such as chitosan, sodium alginate and starch, were used to improve the rheological properties of CPC.[19] However, there are few reports on the effect of temperature and the concentration of additives on the rheological properties of CPC, which is practically important because a wide variety of additives with different concentrations are used with CPC, and CPC is generally prepared at room temperature and placed in the tissues at human body temperature.

This study evaluated the effects of temperature and the concentration of 2 types of setting solution, hydroxyprophyl methylcellulose (HPMC) solution and polyacrylic acid (PAA) solution, on the rheological properties of CPC. The null hypothesis of this study was that the temperature and concentration of the setting solution will have no effect on the rheological properties of a CPC paste.

MATERIALS AND METHODS

1. Preparation of injectable CPC

Dicalcium phosphate dihydrate (DCPD, Riedel de Hann, Germany) was used as the injectable calcium phosphate cement in this study. Two different setting solutions were used to prepare the CPC pastes. One was a sodium phosphate solution (0.05M/L) with hydroxyprophyl methylcellulose (2% and 1 %) (pH7.4) and the other was a polyacrylic acid solution (35 % and 17.5 %)

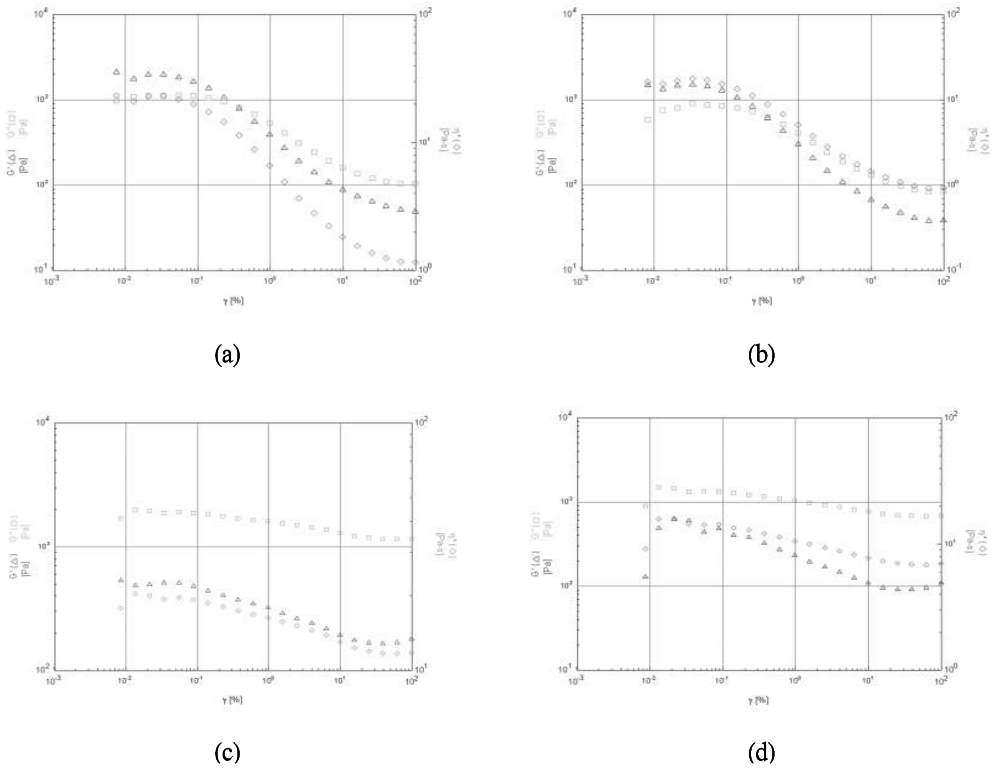
2. Rheological test

The complex viscosity (η^*), shear storage modulus (G'), and shear loss modulus (G'') of the

CPC mixed with the 2 setting solutions was measured using a rheometer with a cone and plate measuring geometry (AR-G2, TA instrument, New Castle, Delaware). The calcium phosphate cement was prepared by mixing DCPD powder and each setting solution immediately before the start of each test. Thereafter, the CPC was transferred to the lower plate of the cone and the plate geometry of the rheometer. The time elapsed between mixing and the start of the measurements was 3 min. The rheological test was carried out at 25℃ and 37℃.

3. Statistical analysis

The difference in the rheological parameters was analyzed statistically using a Mann-Whitney test with a Bonferroni collection. The confidence level was set to 95%.



(a) (b) (c) (d)

Fig. 1. Strain sweep curves of the CPC at 3 min after mixing. (a) CPC with 2% HPMC, frequency = 100 s^{-1}, temperature= 25℃ (b) CPC with 2% HPMC, frequency = 100 s^{-1}, temperature= 37℃ (c) CPC with 35% PAA, frequency = 100 s^{-1}, temperature= 25℃ (d) CPC with 35% PAA, frequency = 100 s^{-1}, temperature = 37℃. The storage modulus (G′), loss modulus (G″) and complex viscosity (η^*) decreased nonlinearly within the shear rate range, which exhibits shear thinning. The linear viscoelastic range of the injectable CPC is not obvious. (CPC : calcium phosphate cement, HPMC : hydroxyprophyl methylcellulose, PAA : polyacrylic acid)

RESULTS

1. Preliminary measurement of rheological properties of CPC with HPMC or PAA.

1) Strain sweep

A strain sweep test was carried out to determine the limit of the linear viscoelastic range The result of the strain-sweep test indicated that the storage modulus (G′), loss modulus (G″), and complex viscosity (η*) were non-linear in the strain-sweep range of these tests. (Strain from 0.01% to 100%) (Fig. 1). The data in Fig 1 show that the linear viscoelastic range of the injectable CPC was too small to measure. Therefore, in order to simplify the shear experimental procedure, a low strain of 0.1% was used to examine the frequency test.

2) Time -sweep

Fig. 2 Shows the results of the time sweep test of G′, G″ and η* at a low frequency and low strain

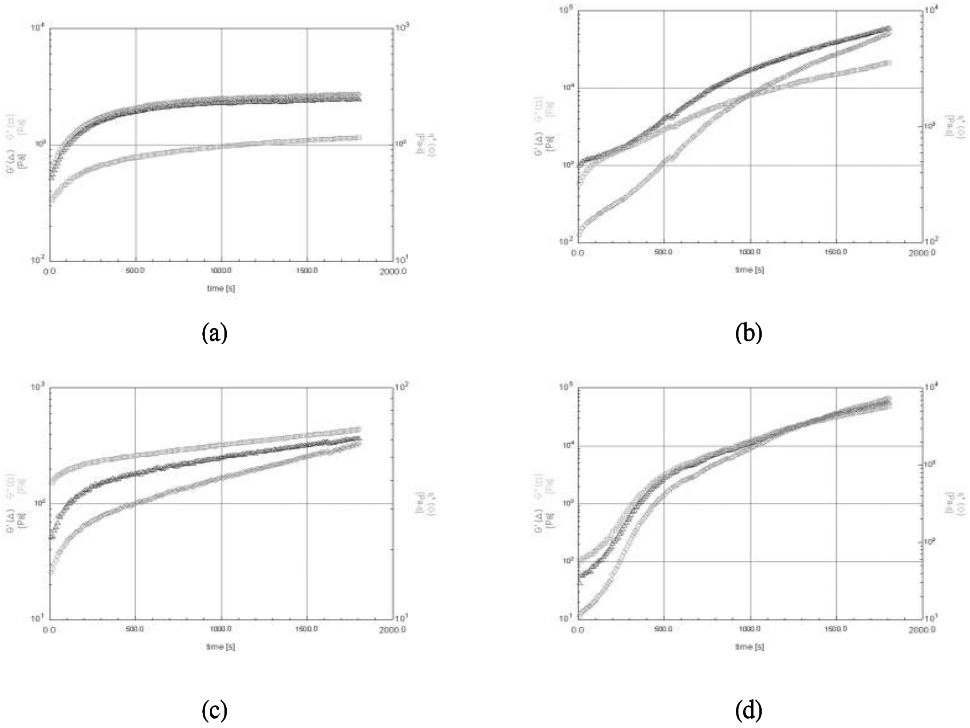
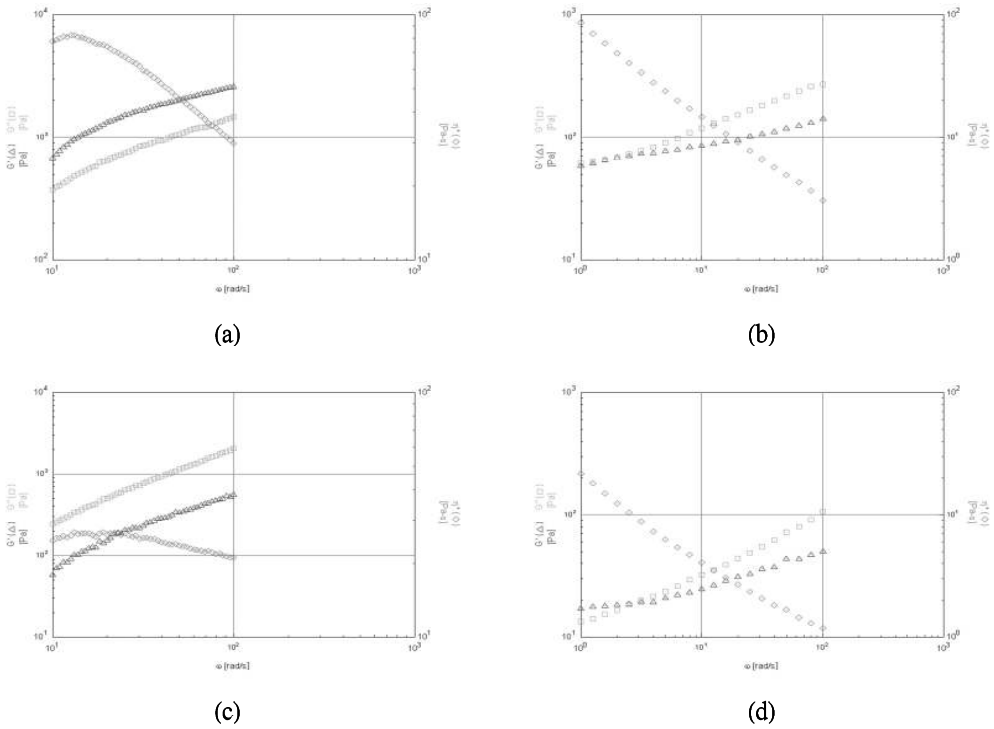


Fig. 2. Time sweep curve of the CPC paste 3 min after mixing. Strain = 0.1 %, frequency = 10 s^{-1} (a) CPC with 2% HPMC at 25℃ (b) CPC with 2% HPMC at 37℃ (c) CPC with 35% PAA at 25 ℃ (d) CPC with 35% PAA at 37℃. Note that the storage modulus (G′), loss modulus (G″), and complex viscosity (η*) increased with increasing hydration time, showing the setting of the CPC paste. (CPC : calcium phosphate cement, HPMC : hydroxyprophyl methylcellulose, PAA : polyacrylic acid)

region. Obviously, G′, G″ and η* increased with a prolonged time sweep, which showed that the CPC paste had set gradually. The complex viscosity of CPC with HPMC was higher than that of CPC with PAA.

2. The effect of the concentration of each additive

The effect of the concentration of each additive was evaluated using a frequency sweep test (Fig. 3). Two concentrations of PAA (17.5 and 35 %) and HPMC (2% and 1%) were used for the time sweep test. The complex viscosity of the CPC paste (CPC with 2% HPMC [n=10] versus CPC with 1% HPMC [n=5], CPC with 35% PAA [n=8] versus CPC with 17.5% PAA [n=5]) was analyzed statistically (frequency : $10s^{-1}$). The CPC with 2% HPMC showed a significantly higher complex viscosity. (p= 0.0027) than that with 1% HPMC. Similarly, the CPC with 35% PAA showed a significantly higher complex viscosity (p=0.0043) than that with 17.5% PAA.

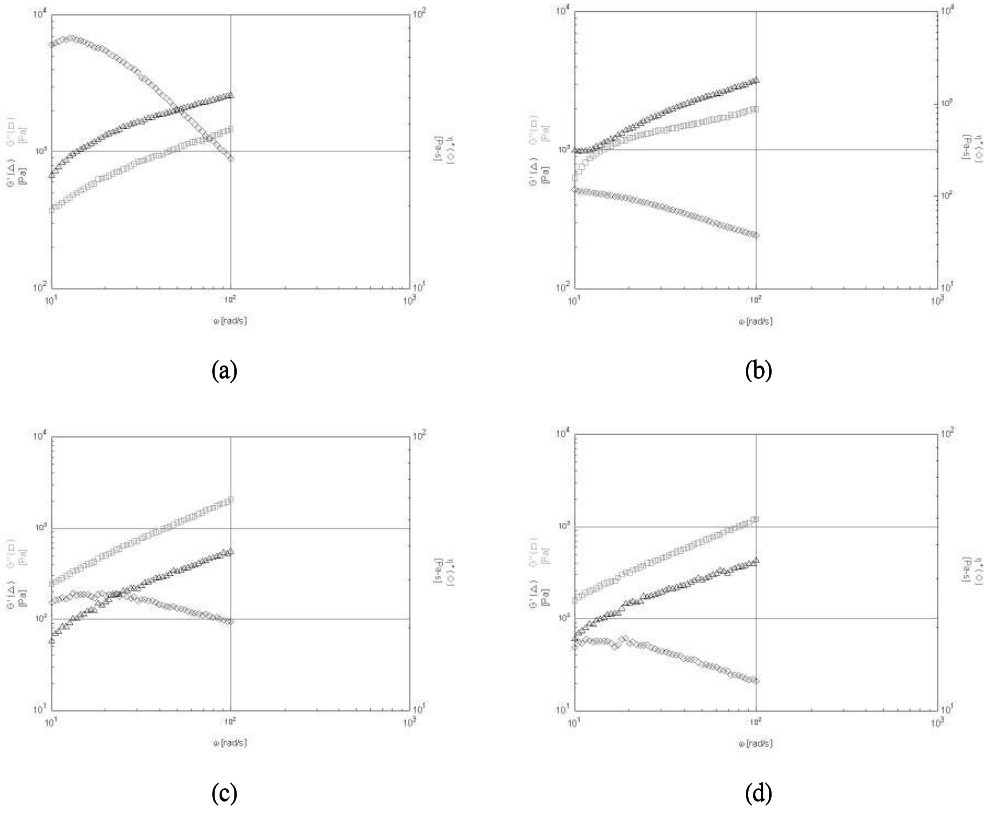


(a) (b) (c) (d)

Fig. 3. Frequency sweep curve of the CPC 3 min after mixing. Temperature = 25°C, P/L ratio =1.0 (a) CPC with 2% HPMC, strain =0.1%, (b) CPC with 1% HPMC strain = 0.1%, (c) CPC with 35% PAA, strain=0.1%, (d) CPC with 17.5% PAA, strain= 1.0%. Note that the storage modulus (G′), loss modulus (G″), and complex viscosity (η*) were higher in the CPC with the higher concentrations. (a) G′, G″, and η* of CPC with PAA (35% by weight) (b) G′, G″, and η* of CPC with PAA (17.5% by weight) (CPC : calcium phosphate cement, HPMC : hydroxyprophyl methylcellulose, PAA : polyacrylic acid)

3. Effect of temperature on the rheological properties of CPC

The effect of temperature on the rheological properties was evaluated suing a frequency sweep test (Fig. 4). The frequency sweep test was carried out on the CPC with 2% HPMC and 35% PAA at 25℃ and 37℃. The results showed that the G′, G″ and η* of CPC with HPMC increased with increasing temperature but those of the CPC with PAA decreased with increasing temperature. The complex viscosity of CPC paste (CPC with 2% HPMC at 25℃ [n=10] versus CPC with 2% HPMC at 37℃ [n=8], CPC with 35% PAA at 25℃ [n=8] versus CPC with 35% PAA at 37℃ [n=5]) was analyzed statistically (frequency = $10s^{-1}$). The CPC



(a) (b) (c) (d)

Fig. 4. Frequency sweep curve of the CPC at 3 min after mixing. P/L ratio = 1.0, strain = 0.1% (a) CPC with 2% HPMC at 25℃, (b) CPC with 2% HPMC at 37℃, (c) CPC with 35% PAA at 25℃, (d) CPC with 35% HPMC at 37℃. Note that G′, G″, and η* of the CPC with HPMC increased with increasing temperature but those of CPC with PAA decreased with increasing temperature. (CPC : calcium phosphate cement, HPMC : hydroxyprophyl methylcellulose, PAA : polyacrylic acid)

with 2% HPMC showed a significantly higher complex viscosity at 37℃ than at 25℃. (p= 0.0008). In contrast, the CPC with 35% PAA showed a lower complex viscosity at 37℃ than at 25℃, even though the difference was not statistically significant. (p=0.2486). Therefore, the null hypothesis was rejected from these results.

DISCUSSION

Calcium phosphate cement (CPC) is known to produce hydroxyapatite when it reacts with water after setting.[20] The reported setting time for the original formulation of CPC, which was developed by Brown and Chow, ranged from 15 to 22 minutes. [9] This long setting time is not desirable because the CPC might be washed out before setting when it is applied to human tissues. Various types of additives have been used in an attempt to improve the cohesion tendency of CPC. [17,19,21]. In this experiment, HPMC and PAA were used to impart a gelling effect and anti-wash-out properties to CPC. The strain sweep test is an oscillatory test with variable strain, constant frequency, and constant temperature. Variable strain is produced by increasing the angle of rotation using a step increase. The results of the strain sweep test (Fig. 1) indicated that the linear viscoelastic range of the CPC was too small to measure. For this reason, a strain of 0.1% was chosen because the CPC paste can be treated as a linear viscoelastic body at a strain < 1%. Dynamic oscillatory tests are used to examine the viscoelastic properties of a material under forced oscillation. This method can provide information on the internal structure of the sample. Compared with the steady test, the dynamic oscillatory test is less affected by the heat produced during setting and covers a larger frequency range. Moreover, the dynamic oscillatory test can measure the continuous changes in the internal structure and the network in the same sample without altering the characteristics of the sample. [18] The results obtained by dynamic oscillatory test can provide information on the change in internal microstructure of the CPC. The strain and time, the development of internal structure, e.g. flocculation, agglomeration, or polymerization can be estimated by analyzing the change in the rheological properties as a function of frequency. A frequency sweep is an oscillatory test with a variable frequency, constant amplitude and constant temperature, which provides information on the characteristics and internal structure of a sample. The time sweep provides information on the development or breakdown of a network inside the sample as a function of time. In the rotational tests, only one curve can be obtained, i.e. the time dependent increase in shear viscosity (η). However, additional information, such as G' and G'', on the structural characteristics can be obtained from the dynamic oscillatory tests. The G' to G'' ratio is used to determine the viscoelastic state of a sample?. If $G'/G'' < 1$, the sample displays liquid characteristics and can easily be deformed and flow. As the paste hardens, G'/G'' increases and a great deal of the internal structure inside the sample are formed, which increases the viscosity. In time sweep analysis of the dynamic oscillatory test, the slope of the curve (G', G'', and η^*) represent the hydration process of CPC under a setting process. A higher slope indicates a faster setting process and vice versa. In the frequency sweep test, CPC with both setting solutions showed shear thinning and yield behavior at 25℃. Liu et al also reported shear thinning of the CPC mixed with distilled water. They suggested that under low frequency oscillation, the CPC just deforms and does not flow. In this range, the viscosity increases with increasing frequency. However, the CPC begins to flow when

the frequency reached the point at which the external strain exceeds the yield strain. In this range, the viscosity decreases with increasing frequency. [18] In this experiment, G′ and G″ increased with increasing frequency. This was different from the results reported by Liu et al., who demonstrated a decrease in CPC with increasing frequency. This difference may be caused by the difference in the setting solution. The CPC with PAA showed similar changes in the rheological parameters to those of CPC with HPMC, and exhibited a yield behavior and shear thinning. The result of the time sweep test showed a gradual increase in G′. This represents the increasing structural strength with time, e.g. due to the additional mechanical entanglements of polymer molecules, or due to particles blocking each other's movement.[22] In the process of paste hardening, the interval where G″>G′ is referred to as the sol state (liquid state), and the interval where G′>G″ is referred to as the gel state (solid state). The time at which G′ = G″ marks the sol/gel transition point. A strong initial hydration reaction occurs after the CPC powder comes in contact with the setting solution containing the additives. During this process, hydrated particles and unhydrated particles co-exist. In this phase, the rheological parameters (such as G′, G″, and η*) of the CPC paste are relatively low. The CPC paste is not resistant to flow and can be injected easily into the target point. As the hydration process progresses, many colloidal particles are produced, which agglomerate to form a flocculent structure through Van der Waals forces. In this phase, G′, G″, and η* increased quite rapidly, and the strength of the network was enhanced quickly.[22] An increase in temperature had an opposite effect on the complex viscosity of CPC with PAA compared with that with HPMC. The complex viscosity of the CPC with HPMC increased with increasing temperature but the complex viscosity of CPC with PAA decreased with increasing temperature. This may be due to the different characteristics of the setting solutions. The PAA is a type of polymer, which has monomers that are rendered more movable at higher temperatures. In contrast, in the CPC with HPMC, the setting reactions may be enhanced by increased temperature, which increases the resistance of the CPC paste to flow.

CONCLUSION

This study examined the effect of temperature and concentration of the setting solutions on the dynamic rheological properties of CPC. The viscosity of the CPC with HPMC increased with increasing temperature (p= 0.0008) but the viscosity of the CPC with PAA decreased with increasing temperature (p=0.2486). The viscosity of CPC increased with increasing concentration of HPMC (p= 0.0027) and PAA (p=0.0043). The CPC paste with both setting solutions showed viscoelasticity, yield behavior and shear thinning. The time sweep test showed that G′, G″ and η* increased with time in the CPC with both setting solutions.

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경화액의 농도와 온도가 인산칼슘시멘트의 유변학적 성질에 미치는 영향에 관한 연구

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유현미 · 장석우 · 박동성

인산칼슘시멘트(calcium phosphate cement)는 우수한 생체친화성 및 골전도성을 가지고 있어 골이식재로 많이 사용되어 왔다. 인산칼슘시멘트의 중요한 물성 중 하나인 흐름성은 유변학적 성질을 측정하여 확인할 수 있지만, 인산칼슘시멘트의 유변학적 성질에 관한 연구는 많이 진행되어 있지 않다. 이 실험의 목적은 인산칼슘시멘트를 hydroxyprophyl methylcellulose (HPMC) 수용액 및 polyacrylic acid (PAA) 수용액과 각각 혼합하여 주사용 인산칼슘시멘트를 만들고, 각각의 용액의 농도(35%와 17.5%의 HPMC, 2%와 1%의 PAA)와 온도(25℃ and 37℃)가 유변학적 성질에 미치는 영향을 연구하기 위한 것이다. 실험에 사용된 인산칼슘시멘트는 dicalcium phosphate dihydrate (DCPD)이며 유변학적 성질은 자동화된 rheometer를 사용하여 측정하였다. 통계분석은 Mann-whitney test를 사용하였다. 높은 농도의 경화액과 혼합된 인산칼슘시멘트는 (35% HPMC와 2% PAA)는 낮은 농도의 경화액과 혼합된 인산칼슘시멘트 (17.5% HPMC와 1% PAA)보다 각각 유의성 있게 높은 점도를 보였다. HPMC 수용액과 혼합된 인산칼슘시멘트는 37℃에서 25℃보다 유의성 있게 높은 점도를 보였다. PAA 수용액과 혼합된 인산칼슘시멘트는 37℃에서 25℃보다 낮은 점도를 보였으나 통계적 유의성은 없었다. 또한 실험에 사용된 모든 종류의 인산칼슘시멘트는 의사가소성(pseudoplastic)을 보였다.

주요어: 인산칼슘시멘트, 하이드록시프로필메틸셀룰로오스, 폴리아크릴산, 유변학적 성질

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