Viscosity and Wettability of Carboxymethylcellulose(CMC) solutions and Artificial Saliva

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Destruction of oral soft and hard tissues and resulting problems seriously affect the life quality of xerostomic patients. Although artificial saliva is the only regimen for xerostomic patients with totally abolished salivary glands, currently available artificial salivas give restricted satisfaction to patients. The purpose of this study was to contribute to the development of ideal artificial saliva through comparing viscosity and wettability between CMC solutions and human saliva.

Commercially-available CMC is dissolved in simulated salivary buffer (SSB) and distilled deionized water (DDW). Various properties of human whole saliva, human glandular saliva, and a CMC-based saliva substitutes known as Salivart and Moi-Stir were compared with those of CMC solutions. Viscosity was measured with a cone-and-plate digital viscometer at six different shear rates, while wettability on acrylic resin and Co-Cr alloy was determined by the contact angle.

The obtained results were as follows:

1. The viscosity of CMC solutions was proportional to CMC concentration, with 0.5% CMC solution displaying similar viscosity to stimulated whole saliva. Where as a decrease in contact angle was found with increasing CMC concentration.
2. The viscosity of human saliva was found to be inversely proportional to shear rate, a non-Newtonian (pseudoplastic) trait of biological fluids. The mean viscosity values at various shear rates increased as follows: stimulated parotid saliva, stimulated whole saliva, unstimulated whole saliva, stimulated submandibular-sublingual saliva.
3. Contact angles of human saliva on the tested solid phases were inversely correlated with viscosity, namely decreasing in the order stimulated parotid saliva, stimulated whole saliva, unstimulated whole saliva, stimulated submandibular-sublingual saliva.
4. Boiled CMC dissolved in SSB (CMC-SSB) had a lower viscosity than CMC-SSB ($P < 0.01$ at shear rate of 90 s^{-1}).
5. For human saliva, contact angles on acrylic resin were significantly lower than those on Co-Cr alloy ($P < 0.01$).
6. Comparing CMC solutions with human saliva, the contact angles between acrylic resin and human saliva solutions were significantly lower than those between acrylic resin and CMC solutions, including Salivart and Moi-Stir ($P < 0.01$).

The effectiveness of CMC solutions in terms of their rheological properties was objectively confirmed, indicating a vital role for CMC in the development of effective salivary substitutes.

Key words: CMC, Saliva, Viscosity, Wettability, Contact angle

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I. INTRODUCTION

The basic function of the salivary glands has been to supply lubricatory molecules, not only to coat the food, but also the oral soft and hard tissue. The lubricatory film allows food to travel easily through the digestive system, and provides smooth tissue surface with minimal friction. Without appropriate lubrication, food is retained and impacted around teeth, making eating difficult and unpleasant, and increasing plaque formation.[1)]

The development of effective saliva substitutes requires an understanding of both the rheological and biological properties of natural human saliva, which is composed of a complex of macromolecules, primarily proteins and glycoproteins such as proline-rich proteins, α-amylase, mucins, statherins, cystatins, and histatins.[1-4)]

The importance of saliva becomes readily apparent in individuals whose capacity for saliva production is diminished. For example, most xerostomic patients have difficulty in essential functions such as speech, taste, mastication, and swallowing.[1,5,6)] Both intrinsic and extrinsic approaches are used to address the complaints of xerostomic patients.[7)]

The intrinsic approach is to employ sialogogues, such as pilocarpine and cevimeline, in order to maintain or stimulate hypofunctional glands.[6,8,9)] The extrinsic approach, the only regimen for patients with completely abolished salivary glands, is to administer a saliva substitute.[3,7)] Clinically, artificial saliva has served as a replacement modality for individuals exhibiting hyposalivation. For sale as an "over-the-counter" item,artificial saliva has traditionally been function-oriented or formulated to replenish particular function(s) of saliva such as lubrication, viscosity, tissue hydration, surface tension, and/or anti-microbial properties. Currently, extrinsic saliva substitutes are divided into two groups based upon the presence or absence of natural mucins.[10-12)]

Although these saliva substitutes may decrease some symptoms of oral dryness in xerostomic patients, the alleviating effects of today's commercially-available substitutes are short-lived and, therefore, of limited benefit to patients.[7,13)] Despite this, several studies have reported that mucin-based saliva substitutes are more effective than their CMC-based counterparts.[14-16)] But, mucin-based saliva substitutes is not available in United States, so several CMC-based saliva substitutes is used in United States.

CMC is a common ingredient used to impart lubrication and viscosity. Sorbitol or xylitol is added to provide surface activity and act as a sweetener. However, the combination of carboxymethylcellulose and sorbitol results in a highly viscous mixture having a surface tension significantly higher than that of natural saliva.[7)]

Previous clinical studies on the effectiveness of saliva substitutes have largely depended on subjective reports of xerostomic patients.[13-15)] Few objective data exist regarding the rheological (viscosity) and film-forming (wettability) properties essential to proper function of any saliva substitute. Although an ideal saliva substitute mimics the rheological and biochemical properties of natural human saliva,[10)] the addition of antimicrobials to a solution otherwise having similar rheological properties to human saliva may be an even better solution, and this approach is presently feasible.
We investigated the viscosity and film-forming property of solutions of CMC, human saliva, and commercially-available CMC-based saliva substitutes. This study furthers our understanding of CMC's role in saliva substitutes and may assist in the development of effective saliva substitutes.

II. MATERIALS AND METHODS

1. Saliva collection

Human saliva was collected from 20 healthy donors, aged 25 to 35 years, between 9:00 a.m. and 11:00 a.m. All subjects had refrained from eating or drinking for 2 h prior to collection. Unstimulated whole saliva (UWS) was collected for 10 min by the

spitting method (after swallowing, saliva is collected with closed lips and then expectorated into a vessel one or two times per min). Stimulated whole saliva (SWS) was collected for 5 min, and chewing of paraffin wax (1.0 g) was employed as a mechanical stimulus. Stimulated parotid saliva (SPS) was collected with the aid of a plastic suction cup (modified Lashley cup) placed directly over Stensen's duct orifice. Stimulated submandibular sublingual saliva (SSMSLS) was collected with a custom-made Block and Brottman collector.[17] For the collection of glandular saliva, secretion was stimulated by applying 2% citric acid solution every 30 s to the lateral border of the tongue. All stimulated human saliva collected during the first 2 min was discarded. All human saliva samples were centrifuged at 3,500 *g* for 10 min at 4℃; the resulting clarified supernatant fluid was used immediately for experiments.

2. CMC solution and artificial salivas

Commercially-available CMC were dissolved in simulated salivary buffer (SSB, 0.021 M Na_2HPO_4/NaH_2PO_4, pH 7.0, containing 36 mM NaCl and 0.96 mM $CaCl_2$)[18] and distilled deionized water (DDW) at various concentrations (0.5 and 1.0 %). To investigate the effect of denaturation, animal mucins dissolved in SSB were boiled in a water bath for 10 min. Salivart (Gebauer Co., Cleveland, OH, USA) and Moi-Stir (Kingswood Laboratories, Inc., Indianapolis, IN, USA), a commercially-available CMC-based saliva substitute, was used for comparison with human saliva.

3. Measurement of viscosity

Viscosity measurement was conducted with a model LVT Wells-Brookfield cone-and-plate digital viscometer (Brookfield Engineering Laboratories, Stoughton, MA, USA). Shear rates were varied incrementally from 11.3 to 450.0 s^{-1} at six different speeds. All measurements were carried out at 37℃, and 0.5 mL volume of fluid was used in each test. The viscosity of each sample was measured five times.

4. Preparation of test specimens for contact angle measurement

Heat-cured acrylic resin, Paladent 20 (Herareus Kulzer, Wehrheim, Germany), and cobalt-chromium alloy, Biosil f (DeguDent, Hanau, Germany) were used as surface phases. Ten specimens of each material (30×30×1.5 mm) were prepared to have highly flat surfaces. For acrylic resin specimens, a sheet of wax 1.5 mm thick was pattern-adapted between two plates of glass. The sandwiches of glass and wax were inserted into dental flasks, boiled for 5 min to soften and eliminate the wax, and heat cured. The samples were ground with 600 and 800 grit silicon carbide sandpapers, followed by a felt disc with pumice. Cobalt-chromium alloy specimens (composition in mass %; Co 64.8, Cr 28.5, Mo 5.3, Si 0.5, Mn 0.5, and C 0.4) were cast and finished in the same manner as would be the tissue surface of a removable partial denture framework, according to the manufacturer's instructions.

5. Measurement of contact angle

Measurement of contact angle and surface tension was done with a Phoenix 300 (Surface Electro Optics Co., Ansan, Korea). Contact angles were measured on the photographs as follows: 10 L droplets of each liquid were positioned on the test specimens by means of a 1 mL syringe with a blunt point. After 30 s, a tangent to the droplet was drawn from the point of air-fluid-solid phase intersection. Contact angles between this tangent line and the dental material surface were calculated from enlarged photonegatives of the droplets. Measurements of contact angle were performed ten times for each test solution.

6. Statistics

The Student's *t*-test, ANOVA, and Duncan's

multiple range test were used to compare the mean values of viscosity and contact angle.

III. RESULTS

1. Viscosity

The viscosity of human saliva was found to be inversely proportional to shear rate, a non-Newtonian trait of biological fluids. The mean viscosity values at various shear rates increased as follows: SPS, SWS, UWS, and SSMSLS (Fig. 1). Representative values are those at a shear rate of 90 s^{-1}: 1.33 ± 0.29, 1.91 ± 0.54, 2.52 ± 0.59, and 3.88 ± 1.12, respectively.

The viscosity values for 0.5% CMC solutions dissolved in SSB were also dependent on shear rate, but the viscosity values for 1.0% CMC solutions dissolved in SSB were constant regardless of shear rate (Fig. 2). An increase in viscosity was found

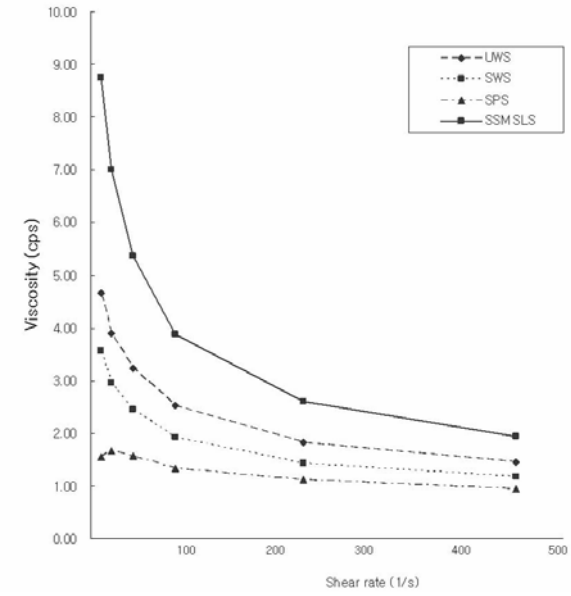

Fig. 1. Viscosity values of human saliva at various shear rates.
UWS: unstimulated whole saliva
SWS: stimulated whole saliva
SPS: stimulated parotid saliva
SSMSLS: stimulated submandibular- sublingual saliva

with increasing CMC concentration, as one would expect. Boiled CMC dissolved in SSB (CMC-SSB) had a lower viscosity than CMC-SSB ($P < 0.01$ at shear rate of 90 s^{-1}). CMC-SSB had much lower viscosity than CMC dissolved in DDW (CMC-DDW) regardless of shear rate ($P < 0.01$).

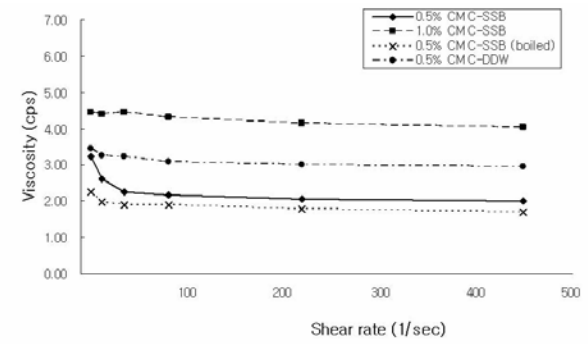

Fig. 2. Viscosity values of CMC in different conditions.
CMC-SSB: carboxymethylcellulose in simulated salivary buffer
CMC-DDW: carboxymethylcellulose in distilled deionized water

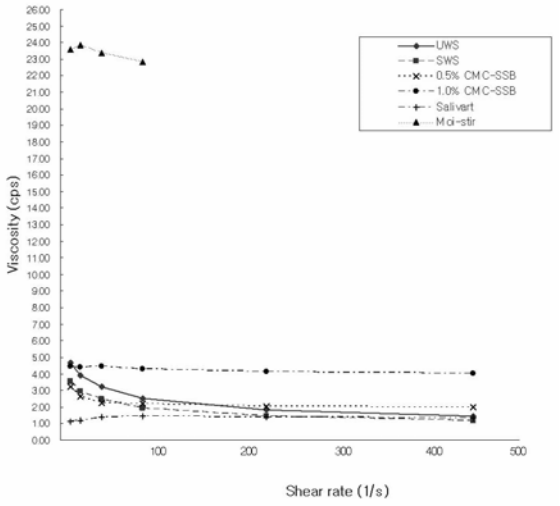

Fig. 3. Viscosity values of human saliva, CMC solutions, and CMC-based saliva substitutes.
UWS: unstimulated whole saliva
SWS: stimulated whole saliva
CMC-SSB: carboxymethylcellulose in simulated salivary buffer

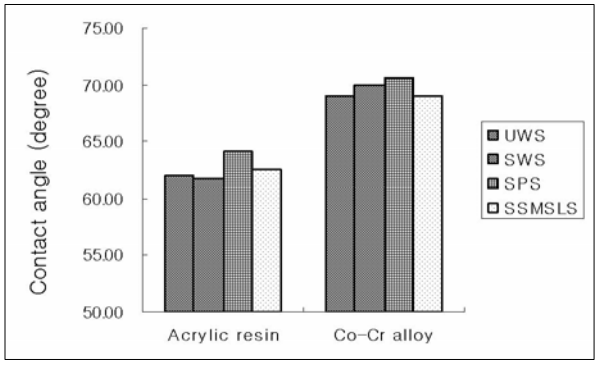

Fig. 4. Contact angles of human saliva.
UWS: unstimulated whole saliva
SWS: stimulated whole saliva
SPS: stimulated parotid saliva
SSMSLS: stimulated submandibular-sublingual saliva

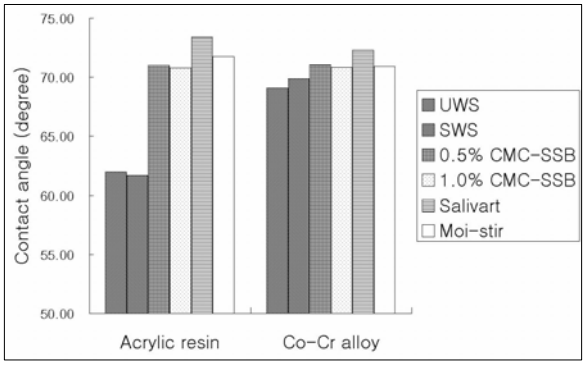

Fig. 5. Contact angles of human saliva, CMC solutions, and CMC-based saliva substitutes.
UWS: unstimulated whole saliva
SWS: stimulated whole saliva
CMC-SSB: carboxymethylcellulose in simulated salivary buffer

Comparing CMC solutions with human saliva, 0.5% CMC dissolved in SSB displayed viscosity similar to UWS at shear rates of 90 s^{-1} and 225 s^{-1}. Viscosity for CMC-based saliva substitutes was constant regardless of shear rate. Viscosity for Salivart was significantly lower than that of human whole saliva at shear rates of 90 s^{-1} and 225 s^{-1}, whereas Viscosity for Moi-Stir was significantly higher than that of human whole saliva at tested shear rate. ($P < 0.01$) (Fig. 3).

2. Contact angle

In human saliva, the mean contact angles on Co-Cr alloy fit a pattern exactly opposite that of the viscosities, namely decreasing in the order SPS, SWS, UWS, and SSMSLS. Though the contact angles of human saliva on acrylic resin displayed a slightly different pattern from that on Co-Cr alloy, SPS had the highest contact angle on both substrates. For human saliva, contact angles on acrylic resin were significantly lower than those on Co-Cr alloy ($P < 0.01$) (Fig. 4).

A decrease in contact angle was found with increasing CMC concentration. The contact angle of boiled CMC-SSB was much higher than that of CMC-SSB. There was also no significant difference in contact angle between CMC-SSB and CMC-DDW (data not shown).

Comparing CMC solutions with human saliva, the contact angles between acrylic resin and human saliva solutions were significantly lower than those between acrylic resin and CMC solutions, including CMC-based saliva substitutes ($P < 0.01$) (Fig. 5).

Ⅳ. DISCUSSION

The importance of saliva in oral health has become increasingly apparent in our expanding aged population. Here, advance in medical procedures and development of medications has been combined in an effort to maintain the "quality of life". Artificial saliva is the only regimen for xerostomic patients with totally abolished salivary glands. Nevertheless, all the currently available artificial salivas have several deficiencies. First and foremost is their lack of substantivity; they have to be replenished on a frequent basis. In general, they lack wettability, have a poor taste, and cannot be selectively targeted to different intraoral sites.

Klestov *et al.* reported that a CMC-containing substitute had offered significant relief of nocturnal oral discomfort in patients with Sjgren's syndrome.[19]

The effect of some saliva stimulants (chewing

gums, pastilles) and substitutes available on the Scandinavian market has been tested on patients with symptoms related to dry mouth. All products relieved the symptoms to some extent but none of them had a sufficiently long-lasting effect, but most patients preferred stimulants to substitutes.[20]

Previous clinical studies have reported relatively weak correlations between subjective mouth dryness and objective sialometric values.[5,21,22] Such a correlation would indicate that the lubricative and hydration functions of saliva are dependent on saliva quality such as viscosity and film-forming property as well as quantity. The practical goal of developing salivary substitutes for xerostomic patients, then, is to achieve a viscoelastic pattern similar to that of human whole saliva.

The efficacy of saliva as a lubricant is at least partially dependent on its viscosity and how this changes with shear rate.[23] According to our results, all animal mucin solutions, as well as human saliva, displayed viscoelastic properties, which is characteristic of macromolecular solutions. 0.5% CMC solutions containing had viscosities similar to that of human UWS at shear rates that would exist during oral functions, such as swallowing or speech (from 60 s^{-1} to 160 s^{-1}).[24] Traditional CMC-based formulations, which have comparatively higher viscosity values,[9,10,12] is not always desirable in terms of the function of the salivary substitute. It is known that an excessively sticky salivary substitute gives rise to unpleasantness and difficulty in masticatory function.[10,25] In our study, while Moi-Stir has displayed higher viscosity than human whole saliva at clinically-important shear rates, Salivart has displayed lower viscosity than human whole saliva among the tested CMC-based saliva substitutes. However Salivart, which has lower viscosity value, also is not desirable because of its insufficient lubrication.

Because the wettability on oral structures and dental materials is indispensable for the maintenance of lubrication and denture retention, the film-forming property seems to have a greater impact on the clinical efficacy of saliva substitutes than does the viscosity alone.[11,26] The present study demonstrated that the contact angle of human saliva was inversely proportional to viscosity, although there was a slight difference according to solid phase. Specifically, human saliva displayed superior wettability on acrylic resin versus other CMC solutions.

Human saliva displayed better wettability on acrylic resin than on Co-Cr alloy, which coincides with a previous report.[27] The good wetting of the acrylic resin by human saliva is of clinical importance because good wettability can improve the retention of removable dentures.[28] Surface treatment of intra-oral removable appliances for enhanced wettability is thus a potentially important consideration for improving their effectiveness and retention in xerostomic patients.

The results of the present study showed that SSMSLS, abundant in salivary mucin, plays a crucial role in effective lubrication and wettability because of its high viscosity and good wettability. This supports previous reports of the important role of salivary mucins in proper oral function.[29-32]

Boiled CMC-SSB displayed lower viscosity values than did CMC-SSB regardless of shear rate. It has been theorized that the boiling step may cause heat degradation of CMC molecules. The difference in viscosity between CMC-DDW and CMC-SSB is attributable to the difference in ionic strength of the two solutions. This was established by a study on the relationship between ionic strength and viscosity wherein an approximately 25% decrease in intrinsic viscosity of canine tracheal mucin was found upon increasing the ionic strength from 50 to 250 mM.[33] It was reported elsewhere that increasing the ionic strength from 35 to 235 mM resulted in an approximately 50% decrease in specific viscosity. [34]

This study provided an objective observation of the effectiveness of CMC solutions in terms of their rheological properties. In the future, recombinant technologies will be used to replenish native macromolecules in artificial salivas and eventually the cloning of salivary gland will be possible. This

complete overcome of xerostomia, however, may need the long-lasting basic salivary research. Therefore the understanding of rheological properties of human saliva and salivary proteins is required first of all, and this may be a realistic approach for the development of more effective artificial saliva.

V. CONCLUSIONS

The present study was performed to contribute to the development of ideal artificial saliva through comparing viscosity and wettability between human whole and glandular saliva, CMC and CMC-based saliva substitutes

The obtained results were as follows:

1. The viscosity of CMC solutions was proportional to CMC cncentration, with 0.5% CMC solution displaying similar viscosity to unimulated whole saliva. Whereas a decrease in contact angle was found with increasing CMC concentration.
2. The viscosity of human saliva was found to be inversely proportional to shear rate, a non-Newtonian (pseudoplastic) trait of biological fluids. The mean viscosity values at various shear rates increased as follows: stimulated parotid saliva, stimulated whole saliva, unstimulated whole saliva, stimulated submandibular-sublingual saliva.
3. Contact angles of human saliva on the tested solid phases were inversely correlated with viscosity, namely decreasing in the order stimulated parotid saliva, stimulated whole saliva, unstimulated whole saliva, stimulated submandibular-sublingual saliva.
4. Boiled CMC dissolved in SSB (CMC-SSB) had a lower viscosity than CMC-SSB ($P < 0.01$ at shear rate of 90 s^{-1}).
5. For human saliva, contact angles on acrylic resin were significantly lower than those on Co-Cr alloy ($P < 0.01$).

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국문요약

Carboxymethylcellulose(CMC) 용액과 인공 타액의 점도와 습윤성

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박문수·김영준

구강건조증 환자에서 자주 발생하는 구강 연조직과 경조직의 손상은 구강건조증 환자의 삶의 질에 심각한 문제를 일으킨다. 타액선의 기능을 완전히 상실한 구강건조증 환자의 경우 인공타액은 유일한 처치법임에도 불구하고, 현재 통용되고 있는 인공타액은 환자들의 기대치에 비해 많이 부족한 실정이다. 본 연구는 CMC 용액과 인공타액의 점도와 습윤성을 비교함으로써 향후 이상적인 인공타액의 개발에 필요한 정보를 얻고자 시행되었다.

CMC를 타액모방완충용액(simulated salivary buffer, SSB)과 증류수에 용해시켜 동물 mucin 용액을 완성한 후, 이를 인체 전타액, 인체 개별 타액선 타액, 그리고 CMC를 주성분으로 하는 인공타액인 Salivart 및 Moi-Stir와 비교 분석하였다. 점도는 cone-and-plate digital viscometer로 검체 당 6개의 전단율에서 측정하였고, 습윤성은 아크릴릭 레진과 Co-Cr alloy 표면 위에서의 접촉각 측정을 통해 평가하여 다음과 같은 결론을 얻었다.

1. CMC 용액의 점도는 CMC 농도에 비례하여 증가하였으며, 0.5% CMC 용액의 점도는 비자극성 전타액의 점도와 유사하였다. 반면에 CMC 용액의 접촉각은 점도와는 반대로 CMC 농도를 증가시킴에 따라 감소하였다.
2. 인체 타액의 점도는 전단율 증가에 따라 감소하는 non-Newtonian fluid의 특성을 나타내었다. 다양한 전단율에서의 평균 점도는 자극성 이하선 타액, 자극성 전타액, 비자극성 전타액, 자극성 악하선-설하선 타액의 순으로 증가하였다.
3. 인체 타액의 접촉각은 점도와는 반대로 자극성 이하선 타액, 자극성 전타액, 비자극성 전타액, 자극성 악하선-설하선 타액의 순으로 감소하였다.
4. 타액모방완충용액에 용해시킨 CMC를 가열하여 변성시킨 경우 점도가 감소하였다 ($P < 0.01$, 전단율 90 s^{-1}).
5. 인체 타액의 아크릴릭 레진 표면에서의 접촉각은 인체 타액의 Co-Cr alloy 표면에서의 접촉각보다 유의하게 작은 것으로 나타났다 ($P < 0.01$).
6. 인체 타액의 아크릴릭 레진 표면에서의 접촉각은 CMC 용액의 아크릴릭 레진 표면에서의 접촉각보다 유의하게 작은 것으로 나타났다 ($P < 0.01$).

이번 연구에서 CMC 용액의 유동학적 성질을 객관적으로 확인할 수 있었으며, 이상의 결과를 종합하여 보면 CMC는 향후 효과적인 인공타액의 개발에 있어서도 중요한 역할을 수행 할 것으로 기대된다.

주제어: CMC, 타액, 점도, 습윤성, 접촉각