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RESEARCH AND EDUCATION

Wettability of 3 different artificial saliva substitutes on heat-polymerized acrylic resin

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ABSTRACT

Statement of problem. The prosthodontic problems faced by a patient with xerostomia are of great concern. To aid in retention, artificial saliva substitutes should exhibit good wettability on the denture base.

Purpose. The purpose of this in vitro study was to evaluate the wettability of 3 different artificial saliva substitutes on heat-polymerized acrylic resin and to compare these properties with natural saliva and distilled water.

Material and methods. A total of 150 heat-polymerized acrylic resin specimens were prepared with 25×15×2 mm dimensions. The specimens were divided into 5 groups (n=30): human saliva, distilled water, Aqwet, Mouth Kote, and Stoppers 4. The advancing and receding contact angle values were measured by using a goniometer, and the contact angle hysteresis and equilibrium angle were calculated. One-way ANOVA and the Bonferroni multiple comparisons test were performed to determine the difference between contact angle values among the groups (α=.05).

Results. The means of the 5 groups differed significantly (P<.05). The comparison between human saliva and Aqwet showed no significant difference for advancing contact angle, receding contact angle, contact angle hysteresis, or equilibrium contact angle, while comparison between the remaining groups indicated statistically significant (P<.05) results. All 3 saliva substitutes used in this study (Aqwet, Mouth Kote, and Stoppers 4) had significantly better wetting properties than distilled water.

Conclusions. Human saliva had the lowest advancing, receding, and equilibrium contact angle values and the highest angle of hysteresis on heat-polymerized acrylic resin. Aqwet had better wetting ability than the other artificial salivary substitutes tested and was comparable to the human saliva on heat-polymerized acrylic resin. All saliva substitutes have better wetting properties than distilled water. (J Prosthet Dent 2018;■:■-■)

Xerostomia is a condition categorized by qualitatively and/or quantitatively altered saliva secretion and/or increased dehydration of the oral mucosa.[1] This decrease in the flow rate of saliva is the consequence of hypofunction of the salivary glands or interruption of stimuli.[2] The prevalence of xerostomia in the general population has been reported to be between 22% and 26%.[3,4] Dryness of the oral mucosa renders it susceptible to irritation and epithelial atrophy, leading to possible inflammation, fissuring, and ulceration.[5-10] In patients with xerostomia, dry mouth can reduce denture retention and make eating difficult.[1,6,7] Artificial saliva has served as a replacement for individuals exhibiting hyposalivation.[11] However, the quality and quantity of saliva is only one of the factors governing denture retention. In patients with xerostomia, for a denture to exhibit adequate adhesion to the supporting mucosa, the saliva substitutes must flow easily over the denture surfaces to ensure adequate wetting.[1,12] The tendency of adhesives to spread or wet the adherent is referred to as wettability. The tendency of a liquid to spread increases when the contact angle decreases; therefore, the contact angle is a useful indicator of wettability.[12,13]

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Clinical Implications

Appropriate wettability of artificial saliva substitutes, comparable to natural saliva, is essential for adequate denture retention. The wetting properties of different saliva substitutes identified in this study will enable clinicians to select the most appropriate salivary substitute for patients with xerostomia. This will also help improve the prosthetic experience of such patients.

Contact angle can be defined as the angle of contact of a liquid on a rigid surface as measured within the liquid at the contact line where 3 phases (liquid, solid, and gas) meet.[14] Contact angle values have been recorded as advancing contact angle and receding contact angle. The advancing contact angle is defined as the angle that a liquid drop forms on a dry solid surface. The receding contact angle is formed when the liquid recedes on a previously wet solid surface.[12,15] From the standpoint of the denture retentive force measurements, the receding contact angle is more important because the film of saliva between a denture base and the tissues recedes over these surfaces during denture dislodgement.[16] The difference between advancing and receding contact angles is a universal property of most surfaces and is called contact angle hysteresis ($\theta A - \theta R$).[12,15,17,18] The force required to separate 2 surfaces increases with an increase in the hysteresis angle.[15] This analysis of denture retention shows that retention occurs only when hysteresis of the denture-saliva contact angle exists.[15] The average of advancing and receding contact angles is the equilibrium contact angle ($[\theta A + \theta R]/2$).[17-19] The smaller the equilibrium contact angle and the greater the contact angle hysteresis of fluid, the greater will be the wettability.[18] Thus, in the present study, all contact angle values were measured for a detailed understanding of the wettability of saliva substitutes.

An ideal saliva substitute should mimic the rheological and biochemical properties of natural human saliva.[11] Some clinical trials have reported that the efficacy of carboxymethylcellulose or mucin-based saliva substitutes is greater than that of natural saliva.[1,8,20] However, other studies reported conflicting results regarding the effectiveness of these saliva substitutes,[11,21,22] and studies on biophysical characterization of normal and artificial saliva are lacking. Additionally, data on mucopolysaccharide- and hydroxyethylcellulose-based saliva substitutes are scarce. Considering the importance of the wettability of saliva substitutes on acrylic resin denture bases, the purpose of this study was to evaluate the wetting properties of 3 different commercially available artificial saliva substitutes on heat-polymerized acrylic resin by using contact angle measurements and to compare these properties with human saliva and distilled water. The null hypothesis was that no differences would be found among the contact angle values of human saliva, distilled water, and 3 artificial saliva substitutes.

MATERIAL AND METHODS

The 5 groups tested were an unstimulated whole saliva specimen (human saliva), distilled water, a carboxymethylcellulose-based saliva substitute (Aq-wet; Cipla Ltd), a mucopolysaccharide-based saliva substitute (Mouth Kote; Parnell Pharmaceuticals), and a hydroxyethylcellulose-based saliva substitute (Stoppers 4; Woodridge Labs, Inc). Each group included 30 acrylic resin test specimens. The sample size (n=30) was determined using the expected mean difference and standard deviation values from a previous study[1] using the formula: $n=2\ (Z\alpha + Z\beta)^2\ (s)^2 / d^2$, where, $Z\alpha$ is the z variate of alpha error (a constant with value 1.96) and $Z\beta$ is the z variate of beta error (a constant with value 0.84). The standard deviation (S) of 2 and the mean difference (d) of 1.5 were obtained from the previous study.[1] The sample size was calculated to be n=27.9. Therefore, approximately 28 specimens per group were needed in the present study, and a sample size of 30 per group was chosen.

To prepare the molds for the fabrication of acrylic resin test specimens, rectangular plates with dimensions of 25×15×2 mm were prepared from a preformed acrylic resin sheet of 2 mm in thickness. The dimensions were checked with digital vernier calipers (Digital Caliper; CASON). The plates were invested in utility denture processing flasks (Dental Flask; Sethi Surgimed Devices) to prepare the molds. Heat-polymerized acrylic resin (Trevalon; Dentsply Sirona) was packed into the molds and processed according to the manufacturer's instructions. The specimens were finished in a conventional manner to obtain an even thickness of 2 mm with flat cherry stones and abrasive paper; however, no polishing was done for the surface to be tested (tissue surface) to simulate clinical practice. The opposite sides (polished surface) of the specimens were finished and polished manually.[12]

To remove any contaminants from the test surface, the specimens were first cleaned with a household soap and then cleaned with alcohol (Surgical Spirit; Tkm Pharma) to remove any soap residue, followed by immersion in ultrasonic cleaner (Dental Ultrasonic Cleaner; R.K. Transonic Engineers Pvt Ltd) for 15 minutes.[23] To verify the effectiveness of the finishing and cleaning procedures, the specimens were examined by using a scanning electron microscope (JSM 6380A Analytical Scanning Electron Microscope; JEOL) under ×1000 magnification.[23] After the cleaning procedures, the

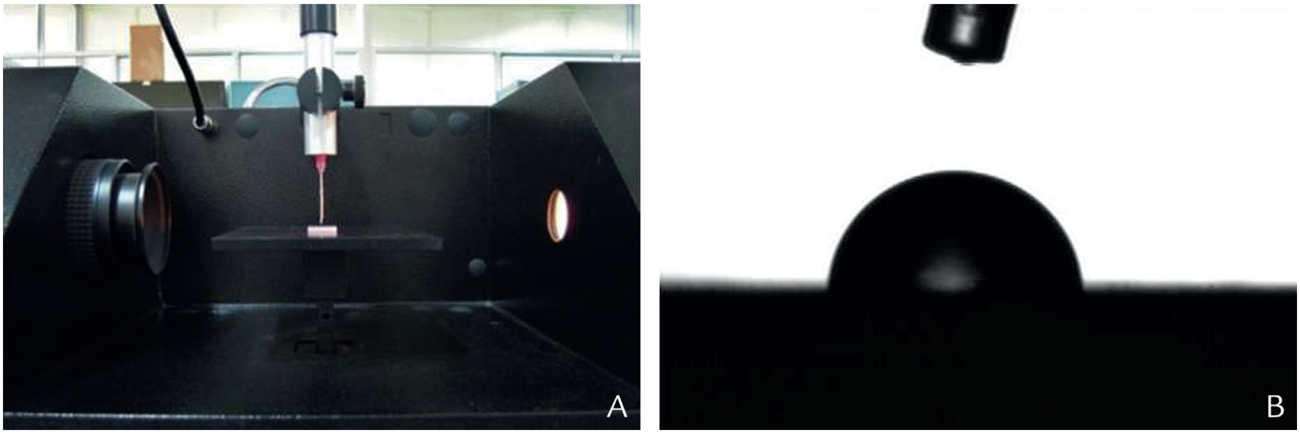

Figure 1. A, Advancing contact angle measurement on horizontal surface. B, Digital image.

specimens were dried in an oven at 44°C for 30 minutes and then cooled to room temperature. All specimens were first sequentially numbered and then randomly allotted to 5 different groups (n=30) using a computer-generated random number generator (http://www.randomnumbergenerator.com).

Under aseptic conditions, human saliva was collected from a healthy male donor aged 27 years between 9:00 AM and 11:00 AM. The donor refrained from eating and drinking for 2 hours before collection of the saliva sample. Unstimulated whole saliva was collected by requesting the donor to relax and minimize all movements. The saliva was collected by allowing the donor to tilt his head forward and drool the saliva from the lower lip into a test tube. The saliva was centrifuged at 3500 rpm for 20 minutes, and the resultant clarified supernatant fluid was used immediately for the experiment.[8,11] Centrifuged saliva exhibits similar lubrication to human whole saliva.[8] A contact angle goniometer (Digidrop; GBX), consisting of a photography area and computer software, was used to measure the contact angles. The photography area consists of an adjustable table or the specimen holder, the source of illumination, and a Nikon camera. It also has a syringe holder with a metal housing for the automatic syringe. The computer software (Windrop++) was used to measure the contact angles.

A clean and dry glass syringe was filled with human saliva up to the 3 mL mark. The syringe was carefully fitted into the metal housing, which had a knob on its superior aspect and was graduated in microliters so that the liquid used for each drop could be standardized. The knob was turned clockwise to expel liquid through the needle. The acrylic resin specimen was placed in the center of the table just below the needle of the syringe (Fig. 1A). The software program Windrop++ was opened on the computer, and the option to measure the contact angle was selected. The page showed the needle tip and the acrylic resin surface on the screen (Fig. 1B).

An option box containing "drop is formed" was clicked. The specimen holder table then moved upward till first contact with the drop was established, and the formation of the drop was shown digitally. After the drop contacted the specimen surface, the measurements could be done for any length of time. The advancing contact angles for liquids are generally independent of time and in the range of 30 seconds to 5 minutes.[1,8,12,24] Therefore, readings were made after 1 minute. This drop was then blotted off the specimen, and a new area on the same specimen was chosen for the receding contact angle measurement. The specimen was placed on an incline of 24° to the horizontal plane (Fig. 2),[12,25] the drop was formed, and the readings were made. After the values were obtained, the specimen was removed, a new specimen was placed, and the same procedure was performed for 30 specimens in the first group. This procedure was repeated for all the specimens in all the 5 groups, and the measurements were recorded. From these advancing and receding contact angles, contact angle hysteresis and the equilibrium contact angle were calculated using the equation[15,18,19]:

Contact angle hysteresis=$\theta A-\theta R$, equilibrium contact angle=$(\theta A+\theta R)/2$, where θR is the receding angle and θA is the advancing angle.

The data were tabulated and analyzed with software (Prism 5 v5.0.1.0 for Windows; GraphPad Software). One-way ANOVA and a multiple comparison test (Bonferroni test) were performed to find the difference in contact angle values of all the groups (α=.05).

RESULTS

The mean difference, standard deviation, and standard error for the contact angle values of human saliva, distilled water, Aqwet, Mouth Kote, and Stoppers 4 are presented in Table 1. The results showed that all the saliva substitutes used in the study had significantly

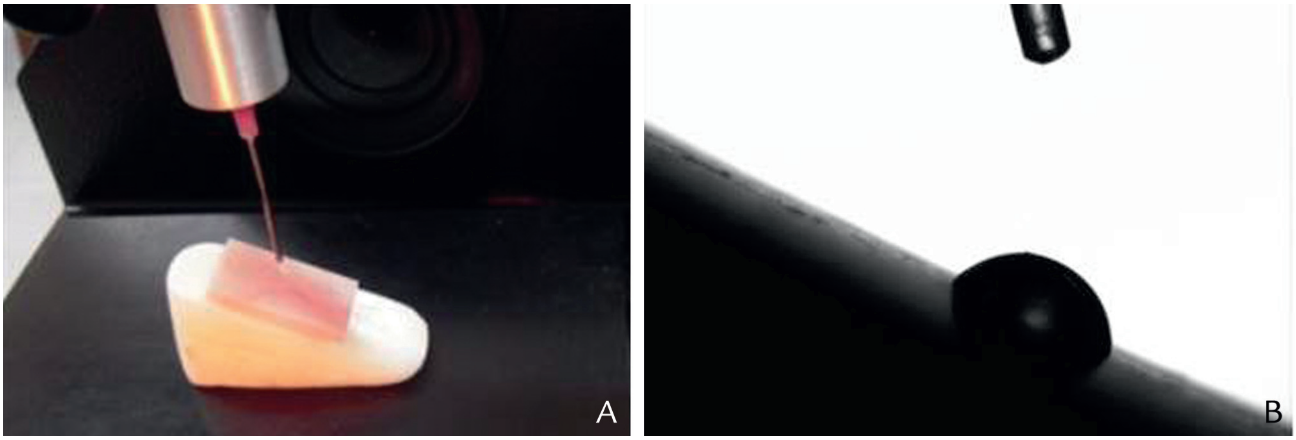

Figure 2. A, Receding contact angle measurement on inclined surface. B, Digital image.

better wetting properties than distilled water ($P<.05$). The carboxymethylcellulose-based saliva substitute had better wetting ability than the other saliva substitutes used in this study and was comparable with human saliva on heat-polymerized acrylic resin. Thus, the null hypothesis for this study was rejected.

DISCUSSION

Treatment of xerostomia is difficult and mainly symptomatic.[26] Water, being readily available, is widely used by individuals with xerostomia to quench their thirst and lubricate the mouth, but relief is usually transient.[6,27] Therefore, saliva substitutes have been developed with thickening agents for longer relief and increased moistening and lubrication of the oral surfaces. Mucin-based saliva substitutes are derived from porcine derivatives, mainly the gastric mucin, and are likely to be objectionable to people who avoid pork.[28] Therefore, this study contained carboxymethylcellulose-, mucopolysaccharide-, and hydroxyethylcellulose-based (nonmucin-based group) saliva substitutes which have been advocated and are available commercially.[1,6,8,29] Wettability of the saliva substitutes on denture base acrylic resin can be measured with contact angle values.[1,11-13,22]

Contact angle measurements have been reported previously, but a consensus on which measurement is the accurate indicator of wettability is lacking. Craig et al[16] measured the advancing and receding contact angles of saliva and water on polystyrene and polymethyl methacrylate materials and concluded that from the standpoint of the denture retentive force measurements, the receding contact angle was the most important. However, Monsenego et al,[15] after deriving an equation to determine the force required to dislodge a denture vertically, concluded that the force required for separating the 2 surfaces increases with an increase in the hysteresis angle ($\theta A-\theta R$). This analysis of denture retention shows that retention occurs only when hysteresis of denture-saliva contact angle exists.[15] Zissis et al[18] concluded that contact angle hysteresis and equilibrium contact angle are reliable measures of wetting properties of materials. Thus, in the present study, all 4 contact angle values were measured to reach an accurate conclusion.

Preparation of the test specimens in this study was carried out meticulously, as contamination of the studied surfaces would be expected to induce an error. The other factor that would affect the magnitude of the contact angle of a fluid on a solid surface is the roughness of the adherent surface, which differs with respect to the solid.[12] In this study, the advancing contact angles of human saliva, water, and saliva substitutes at the left and right boundaries of the drop of fluid were not significantly different for a specific group. These results are supported by Kilani et al.[12] The standardized method for cleaning the test surfaces suggested by previous investigators effectively removed contaminants from the test surfaces.[12,23] The differences between advancing and receding contact angles may have been due to the presence of pores or crevices on the adherent surfaces, which resulted in entrapment of the fluid as it flowed over the solid surfaces.[12,15] Aydin et al[1] showed that the mucin, carboxymethylcellulose, and concentrated ion materials all had better wetting properties than human saliva on the denture base resin, whereas Park et al[11] investigated the viscosity and wettability of solutions of carboxymethylcellulose and human saliva. Aydin et al[1] and Park et al[11] stated that the combination of carboxymethylcellulose (CMC) and sorbitol results in a highly viscous mixture with significantly high surface tension. In the present investigation, this factor might have contributed to better wettability by the CMC substitute than the other liquids.

The limitations of the study include that in vitro investigations cannot duplicate the clinical situation. Clinically, the tissue surface of the denture is irregular,

Table 1. Comparative evaluation of different contact angle values of human saliva, distilled water, Aqwet, Mouth Kote, and Stoppers 4

Contact Angle Values	Groups	N	Mean ±SD	*P* (1-way ANOVA)	Post Hoc Multiple Comparisons (Between Groups)		*P* (Bonferroni Test)
Advancing contact angle	Human saliva	30	58.42 ±1.68	<.001	Human saliva	Distilled water	<.001
	Distilled water	30	78.01 ±1.94		Human saliva	Aqwet	.192
	Aqwet	30	58.92 ±1.17		Human saliva	Mouth Kote	<.001
	Mouth Kote	30	68.85 ±1.18		Human saliva	Stoppers 4	<.001
	Stoppers 4	30	72.13 ±1.49		Distilled water	Aqwet	<.001
					Distilled water	Mouth Kote	<.001
					Distilled water	Stoppers 4	<.001
					Aqwet	Mouth Kote	<.001
					Aqwet	Stoppers 4	<.001
					Mouth Kote	Stoppers 4	<.001
Receding contact angle	Human saliva	30	28.88 ±1.34	<.001	Human saliva	Distilled water	<.001
	Distilled water	30	59.58 ±1.32		Human saliva	Aqwet	.066
	Aqwet	30	29.45 ±0.97		Human saliva	Mouth Kote	<.001
	Mouth Kote	30	47.48 ±1.63		Human saliva	Stoppers 4	<.001
	Stoppers 4	30	51.70 ±1.27		Distilled water	Aqwet	<.001
					Distilled water	Mouth Kote	<.001
					Distilled water	Stoppers 4	<.001
					Aqwet	Mouth Kote	<.001
					Aqwet	Stoppers 4	<.001
					Mouth Kote	Stoppers 4	<.001
Contact angle hysteresis	Human saliva	30	29.51 ±1.99	<.001	Human saliva	Distilled water	<.001
	Distilled water	30	18.43 ±1.64		Human saliva	Aqwet	.920
	Aqwet	30	29.47 ±1.28		Human saliva	Mouth Kote	<.001
	Mouth Kote	30	21.37 ±1.42		Human saliva	Stoppers 4	<.001
	Stoppers 4	30	20.43 ±1.23		Distilled water	Aqwet	<.001
					Distilled water	Mouth Kote	<.001
					Distilled water	Stoppers 4	<.001
					Aqwet	Mouth Kote	<.001
					Aqwet	Stoppers 4	<.001
					Mouth Kote	Stoppers 4	.081
Equilibrium contact angle	Human saliva	30	43.67 ±1.19	<.001	Human saliva	Distilled water	<.001
	Distilled water	30	68.79 ±1.44		Human saliva	Aqwet	.062
	Aqwet	30	44.18 ±0.87		Human saliva	Mouth Kote	<.001
	Mouth Kote	30	58.17 ±1.23		Human saliva	Stoppers 4	<.001
	Stoppers 4	30	61.91 ±1.25		Distilled water	Aqwet	<.001
					Distilled water	Mouth Kote	<.001
					Distilled water	Stoppers 4	<.001
					Aqwet	Mouth Kote	<.001
					Aqwet	Stoppers 4	<.001
					Mouth Kote	Stoppers 4	<.001

Statistically significant at (P<.05)

whereas the surface of the test specimens was flat. Furthermore, the efficacy of salivary substitute in a clinical situation depends upon other factors such as the instructions given and patient expectations and compliance. The human saliva was collected from single healthy young individuals and may not be generalizable to older people as saliva composition changes with age.[30] In the future, recombinant technologies will be used to replenish native macromolecules in artificial saliva and eventually the cloning of salivary glands will be possible.[23] An understanding of the rheological properties of human saliva and salivary proteins may be a realistic approach for the development of more effective artificial saliva.

CONCLUSIONS

Within the limitations of this in vitro study, the following conclusions were drawn:

1. Human saliva had the lowest advancing, receding, and equilibrium contact angle values and the highest angle of hysteresis on heat-polymerized acrylic resin.

2. Aqwet had better wetting ability than the other artificial salivary substitutes tested and was comparable to the human saliva on heat-polymerized acrylic resin.
3. All saliva substitutes tested had significantly better wetting properties than distilled water.

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