In Vitro Comparison of Denture Cleansers Effect on Locator Attachment Retentive Male Inserts (Scanning Electron Microscope Study)

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Abstract

Purpose: The aim of this study was to compare surface changes of the locator attachment nylon retentive male inserts after immersion in three different cleansing solutions by using Scanning Electron Microscope (SEM).

Material and Methods: 18 mandibular overdentures were constructed to fit over a mandibular clear acrylic resin test model. 36 specimens of clear, and blue Locator attachment retentive male inserts (n=36) were soaked into three different denture-cleansing solutions. Group I: water; Group II: Corega cleansing solution, and Group III: Protefix cleansing solution. The retentive male inserts wear evaluated by using Scanning Electron Microscope, after, insertion/removal test for 120, 360, and 720 continuous cycles corresponding to one month, three months, and six months clinical simulation. The surface changes of retentive male inserts was compared between different time periods using repeated measures ANOVA followed by Bonferroni; and, among different groups using One way ANOVA followed by post-hoc tukey.

Results: Denture-cleaning solutions significantly induced surface changes of different retentive male inserts of locator attachments after one month clinical simulation. Water induced wear of blue male insert significantly higher than corega and protefix solutions after six months clinical simulation ($p=0.027$). Blue retentive male insert revealed significant increased surface changes after soaking in water in comparison to clear male inserts after six months clinical simulation.

Conclusions: Locator attachment retentive male inserts in various colors are affected differently by varied cleansing agents. The surface changes of clear retentive male inserts were insignificant with water, corega, and protefix at six months clinical simulation. While; the surface changes of blue attachments increased significantly with water possibly requiring more frequent replacements of these types of attachments.

Key Words: Locator attachment, Surface changes, Denture-cleaning solutions

Introduction

Implant-retained overdentures are a well-recognized treatment modality particularly in the restoration of edentate patients with studies showing superior patient-based outcomes of implant-retained complete overdentures in comparison to conventional complete removable prosthesis [1]. There are a variety of implant retention systems which can be utilized to retain an implant overdenture. These systems are comprised of two parts; one part connected to the implant directly or via a bar and the other within the prosthesis [2]. The retention of the attachment systems is hugely variable [3]. Some studies have shown that there is variation in retention when using the same attachment system. It is also well reported that these attachment systems decrease in retention over time [4].

Denture care is indispensable for general health of not only elderly, fragile and immune-compromised patients but also for healthy patients. Dentures can be cleaned mechanically, chemically or by their combination. Denture cleaning pastes with their active ingredients and/or tooth pastes are commonly used in the mechanical method [5]. The effective and regular cleansing of denture is important for mainly oral health. The chemical method of cleansing is commonly used by elderly patients tend to have compromised ability to manually clean their dentures effectively [6]. Various in vitro and in vivo studies have shown that chemical cleansing of denture are effective enough to remove biofilm, food debris and also tobacco stains from the denture surface [5,7].

One major concern with attachment systems for implant overdentures is that wear changes over time, reflected clinically by loss of retention [8]. Attachments wear is a complex process involving a loss of material from one or two surfaces in relative motion against one another; the mechanisms involved could be adhesive, abrasive, surface fatigue, or corrosive [9]. Wear-induced loss of retention in attachment-retained overdentures poses a major clinical problem; thus, routine maintenance is required to ensure successful long-term outcomes [10]. Various studies [11-14] were evaluated the influences of chemical cleansing solutions of dentures on the retention of the overdentures attachments; these studies have focused on Hader bars and clips, Dio orange O-rings of the DIO system or Locator attachments

There are several denture cleansers available in the market such as ‘corega’ and, ‘protefix’. Few studies have compared the effect of denture cleansing solutions on the surface changes (wear) of different attachments. Only limited data are currently available on the popular locator attachment concerning the effect of these cleansing solutions on its surface changes (wear). Hence, this in vitro study was conducted to evaluate the effect of these common cleansing solutions on the surface changes of locator attachment nylon retentive male inserts by using scanning electron microscope (SEM).

Materials and Methods

The test group consisted 18 mandibular overdentures were constructed with 36 specimens of clear, and blue Locator attachment nylon retentive male inserts (n=36) were soaked into three different denture-cleansing solutions to evaluate the effect of these common cleansing solutions on their surface changes (wear). To simulate the clinical use of dental attachments, a normative edentulous mandibular heat cure

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clear acrylic resin model was fabricated for an edentulous mandibular stone cast [15]. Two implants fixtures 3.7 mm in diameter and 12 mm in length (TioLogic, Dentaurum, Ispringen, Germany) were inserted at the canine sites bilaterally in the edentulous mandibular clear heat cure acrylic resin model; perpendicular to the occlusal plane with the aid of a surveyor parallelometer by utilizing an acrylic resin stent for determining the accurate positions of implants. The mucosa was simulated by using a 3-mm thick autopolymerized resilient silicone layer (Softliner®, Promedica, GmbH, Neumünster, Germany) to cover the clear heat cure acrylic resin model to simulate resilient edentulous ridge mucosa [16].

On the normative edentulous mandibular heat cure clear acrylic resin model, six mandibular overdenture were constructed for each denture-cleansing solution and used throughout the study. For every experimental overdenture 2-female metal housing of Locator attachments were picked up to the intaglio surface of each experimental overdenture. After the finish of pick-up procedure, black processing retentive male inserts were removed from the Locator attachments female housings and the different studied retentive male inserts of the locator attachments were inserted

The cleansing solutions used in this study are represented in *Table 1*. According to cleansing solution; overdentures were divided into three groups:

Group I: six overdentures included different retentive male inserts were cleaned by tap water as a control group. Three overdentures included clear retentive male inserts (medium retention; 2.270 g), ultimately, three overdentures included blue retentive male insert (very low retention; 680 g).

Group II: six overdentures included different retentive male inserts were cleaned by corega tabs. The 6-experimental overdentures locator attachments include retentive nylon male inserts as that of group I.

Group III: six overdentues included different retentive male inserts were cleaned by protefix active cleanser. The 6-experimental overdentures locator attachments include retentive nylon male inserts as that of group I.

Table 1. *Cleansing solution composition and, manufacturer.*

Cleanser	Composition	Manufacturer
Corega TAB	Potassium Monopersulfate; Sodium Bicarbonate; Sodium Lauryl Sulfoacetate; Sodium Perborate Monohydrate; Sodium Polyphosphate	Block Drug Company, Inc., Jersey City, New Jersey-NJ, USA.
Protefix active cleanser(PAC) TAB	Sodium bicarbonate, Potassium caroate, Sodium perborate, Citric acid, Sodium laurly sulphate, Aroma	Queisser pharma Flensburg, Germany
Water	-	-

Patient simulation

Overdentures were undergo an insertion/removal test Four times, then soaked into the corresponding denture cleansing solution, according to the manufacture instruction as shown in *Table 2*. Overdentures were placed in glass cups contain the corresponding cleansing solution of each group and ensuring the immersion of retentive male inserts during the soaking period. Repeated insertion, removal, and soacking into the cleansing solution was done for 120, 360, and 720 continuous cycles corresponding to one month, three months, and six months. Nylon retentive male inserts were removed from its female metal housings by a core locator instrument. Then; surface changes (wear) of nylon retentive male inserts as a result of insertion, removal and soaking into the cleansing solution were evaluated by scanning electron microscope.

Table 2. *Study groups and their Time of overdenture immersion according to the manufacture instruction.*

Groups	Solution	Time of immersion (per day)
Group I	Water	8 hours
Group II	Corega Solution	10 minutes
Group III	Protefix Solution	5 minutes

Samples preparation

The samples were holed using sample holder, coated with gold/palladium using Hummer VI deposition system, about 1.0-1.5 minutes of sputtering. Samples were studied using electron microscope (JOEL-JSM-6510LV) by using X25, X 150 magnification.

Evaluation of surface changes (wear) of the nylon retentive male inserts in all groups was done by using Computer Assisted digital image analysis (Digital morphometric study. The result images were analyzed on Intel® Core I3® based computer using VideoTest Morphology® software (Russia) with a specific built-in routine for pixel statistics.

Statistical analysis

Data were tabulated, coded then analyzed using the computer program SPSS (SPSS Inc., Chicago IL, USA) version 17.0. In statistical comparison between the different groups, the significance of difference was tested using one way ANOVA (analysis of variance):- Used to compare between more than two groups of numerical (parametric) data followed by post-

hoc tukey. Repeated measures ANOVA (analysis of variance):- Used to compare between more than two groups of numerical (parametric) data followed by post-hoc Bonferroni. P value <0.05 was considered statistically significant.

Results

Scanning electron microscope results

Locator attachment retentive male inserts without denture cleansing solutions immersion shows a smooth, finely grained inner surface as represent in (*Figure 1*).

Figure 2 revealed the surface changes of the clear retentive male inserts when it immersed in the study cleansing solutions at various clinical simulation periods.

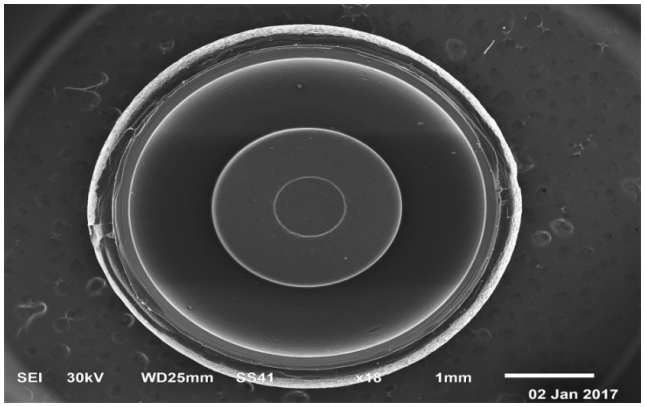

Figure 1. *SEM image (25X) of a dry retentive male inserts shows a smooth, finely grained inner surface.*

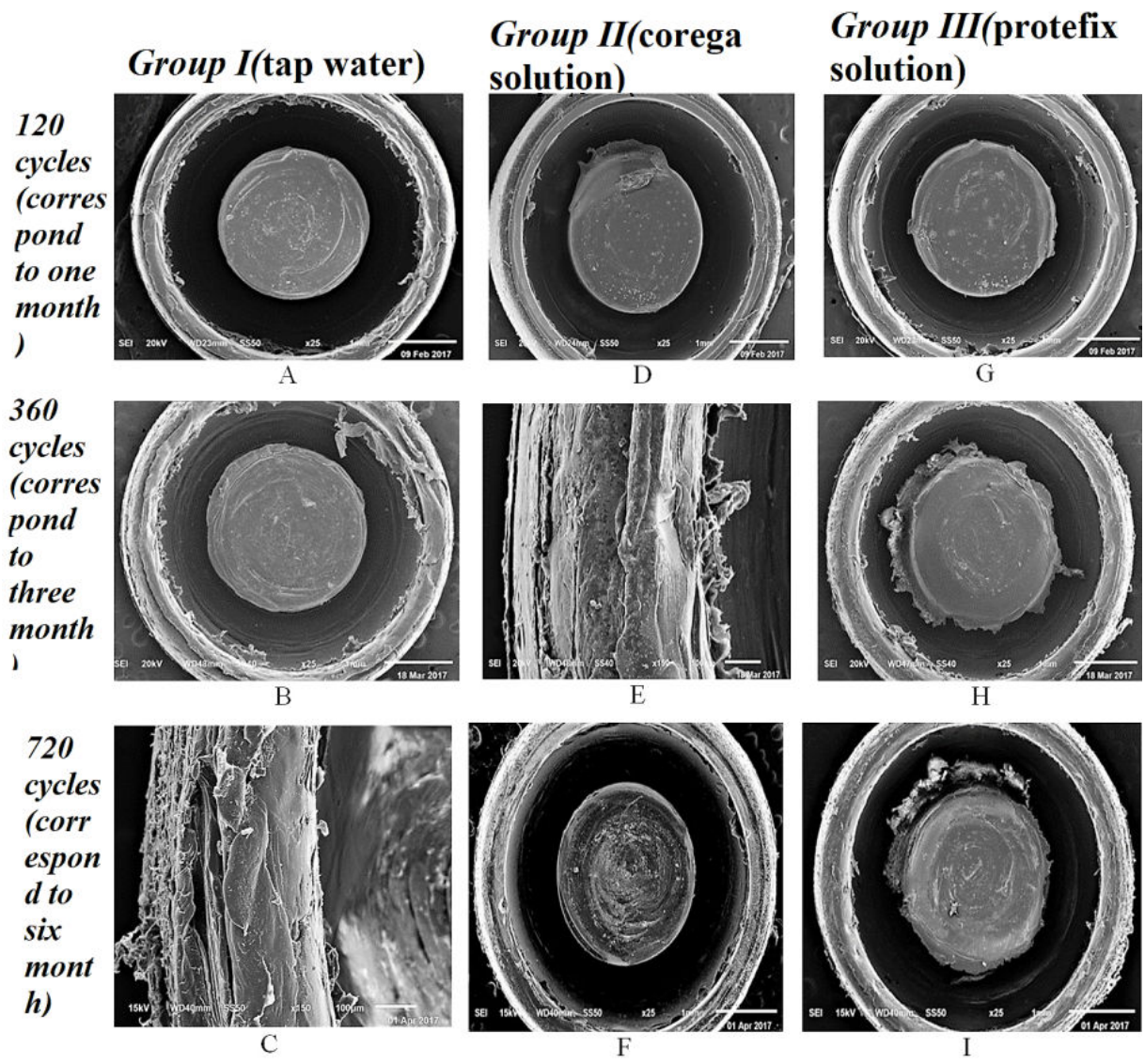

Figure 2. *Surface changes of the clear retentive male insertes when it immersed in the study cleansing solutions at various clinical simulation periods; A. group I (tap water) at 120 cycle, B. group I (tap water) at 360 cycle, C. group I (tap water) at 720 cycle, D. group II (Corega solution) at 120 cycle, E. group II (Corega solution) at 360 cycle, F group II (Corega solution) at 720 cycle, G. group III (Protefix solution) at 120 cycle, H. group III (Protefix solution) at 360 cycle, I. group III (Protefix solution) at 720 cycle.*

In group I; clear retentive male inserts at 120 cycles revealed surface irregularities, scratch lines, and, deformation at the central core; at 360 cycles revealed extensive cracking areas and, crazing randomly propagated in the central core and outer wall; at 720 cycles revealed severe cracking, plastic deformation, surface deterioration, micro-voids, and particle loss. In group II; clear retentive male inserts at 120 cycles divulged minor surface irregularities, scratch lines along the path of insertion/removal, and localized deformation at the central core; at 360 cycles showed imparted areas of surface irregularities, surface roughness, tearing of the walls, and localized deformation at the central core; Whilst, at 720 cycles revealed large area of surface irregularities, voids, deteriorations of the walls, and complete deformation at the central core. In group III; clear retentive male inserts at 120 cycles divulged walls surface irregularities and, areas of deformities, roughness and small voids in the central core; at 360 cycles imparted walls surface irregularities and deformations, surface laceration, and deformation at the central core; at 720 cycles revealed increase walls surface irregularities, increase surface roughness, scratch lines along the path of insertion/removal, and deteriorations at the central core.

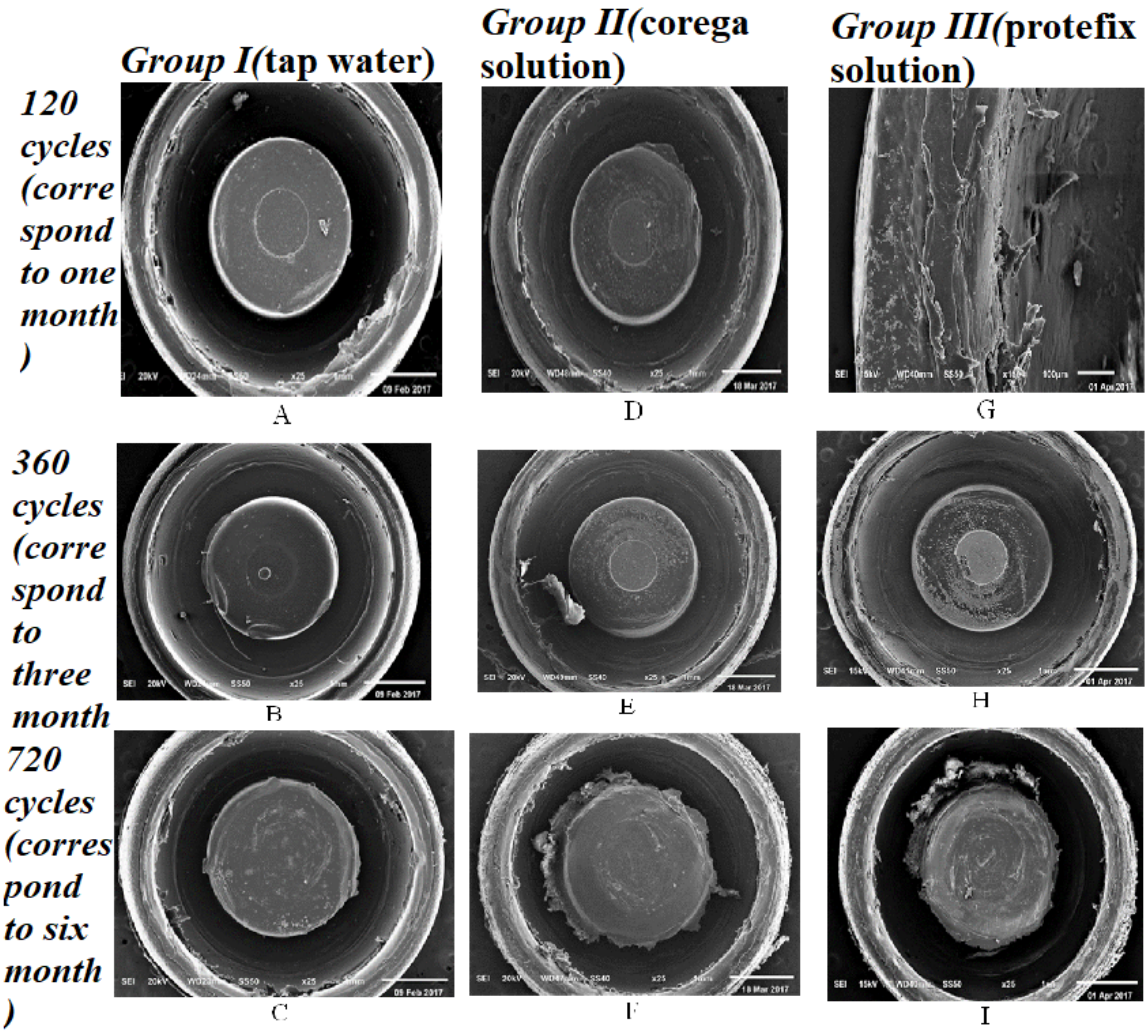

Figure 3. *Surface changes of the Blue retentive male insertes when it immersed in the study cleansing solutions at various clinical simulation periods; A. group I (tap water) at 120 cycle, B. group I (tap water) at 360 cycle, C. group I (tap water) at 720 cycle, D. group II (Corega solution) at 120 cycle, E. group II (Corega solution) at 360 cycle, F group II (Corega solution) at 720 cycle, G. group III (Protefix solution) at 120 cycle, H. group III (Protefix solution) at 360 cycle, I. group III (Protefix solution) at 720 cycle.*

Figure 3 revealed the surface changes of the blue retentive male inserts when it immersed in the study cleansing solutions at various clinical simulation periods. In group I; blue retentive male inserts at 120 cycles revealed minor irregularities, scratch lines along the path of insertion/ removal, and deformation areas at the central core; at 360 cycles showed surface irregularities, and, surface deformations at the central core; at 720 cycles showed surface irregularities, increase surface roughness and tearing. In group II; blue retentive male inserts at 120 cycles revealed minor wall surface roughness, and small area of deformation at the central core; at 360 cycles showed minor wall surface irregularities and, roughness, areas of deformation at the central core; at 720 cycles' divulged surface irregularities, increase surface roughness, tearing and splitting of the walls. In group III; at 120 cycles revealed surface irregularities, and localized deformation at the walls, scratches at the central core; at 360 cycles showed surface irregularities, scratch of walls surface, roughness, scratches and, voids at the central core; at 720 cycles divulged surface laceration, increase

surface rupture and tearing's, roughness, scratches and, voids at the central core.

Statistical analysis of the results

Percent area for SEM image of clear Locator attachments retentive male inserts analyzed by repeated measure ANOVA followed by post-hoc Bonferroni was presented in *Table 3*.

Table 3. *Comparison among different time interval periods in different denture cleaning solutions groups in clear Locator attachments retentive male inserts.*

Group	Test	-ve control	120 cycles One month	360 cycles Three months	720 cycles Six months	P
Group I (Water cleaning solution)	X	175.07	90.469	129.243	109.927	0.02*
	± SD	± 37.52	± 23.809	± 17.452	± 5.284	
	Post-hoc		P1=0.03*	P1=0.35 P2=0.23	P1=0.04* P2=0.7 P3=0.35	
Group II (Corega cleaning solution)	X	175.07	92.173	110.971	109.995	0.03*
	± SD	± 37.52	± 17.085	±7.062	± 16.204	
	Post-hoc		P1=0.02*	P1=0.06 P2=0.06	P1=0.11 P2=0.4 P3=1.00	
Group III (Protefix cleaning solutions)	X	175.07	108.747	112.307	104.909	0.018*
	± SD	± 37.52	± 8.097	± 6.700	± 18.828	
	Post-hoc		P1=0.03*	P1=0.04* P2=0.6	P1=0.1 P2=1.00 P3=1.00	
SD:standard deviation P:Probability						
*:mild significance <0.05 **:Moderate significance ***:High significance						
Test used: repeated measure ANOVA followed by post-hoc Bonferroni						
P1: significance relative to -ve control						
P2: significance relative to 1M time						
P3: significance relative to 3M time						

For group I; there was significant decrease in smoothness and, finely grained walls surface compared to that of negative control after 120, 720 cycles as P=0.03, P=0.04 respectively. While, after 360 cycles there was insignificance surface changes when compared to negative control. For group II; there was significant decrease in firmness and, regularity of walls surface compared to that in negative control as P=0.02, while after 360, 720 cycles showed insignificance surface changes of clear Locator attachments retentive male inserts. For group III; there was significant decrease in firmness and, regularity of walls surface compared to that in negative control after 120, 360 cycles as P=0.03, P=0.04, respectively.

Percent area for SEM image of blue Locator attachments retentive male inserts analyzed by repeated measure ANOVA followed by post-hoc Bonferroni was revealed in *Table 4*. For group I; there was significant decrease smoothness, increase irregularities of blue Locator attachments retentive male inserts compared to that in negative control after 120, 360, and 720 cycles as P=0.003, P=0.001and, P=0.001, respectively. For group II there was significant decrease smoothness, increase regularities of blue Locator attachments retentive male inserts compared to that in negative control after 120, 360,and 720 cycles as P=<0.001, P=0.001and, P=<0.001, respectively. For group III there was significant decrease smoothness, regularities of blue Locator attachments retentive male inserts compared to that in negative control after 120, 360 and, 720 cycles as P=0.005, P=0.002 and P=0.004 respectively.

Table 4. *Comparison among different time interval periods in different denture cleaning solutions groups in blue Locator attachments retentive male inserts.*

Group	Test	Negative control	120 cycles One month	360 cycles Three months	720 cycles Six months	P
Group I (Water cleaning solution)	X	179.89	114.801	99.672	83.054	<0.001***
	± SD	± 19.65	± 13.461	± 5.327	± 5.986	
	Post-hoc		P1=0.003**	P1=0.001** P2=0.15	P1=0.001** P2=0.04* P3=0.046*	

Group II (Corega cleaning solution)	X	179.89	95.716	107.175	112.209	<0.001***
	± SD	± 19.65	± 13.982	± 6.838	± 28.184	
	Post-hoc		P1=<0.001***	P1=0.001** P2=0.5	P1=<0.001*** P2=0.76 P3=1.00	
Group III (Protefix cleaning solutions)	X	179.89	107.888	101.865	86.665	<0.001***
	± SD	± 19.65	± 11.497	± 6.916	± 11.986	
	Post-hoc		P1=0.005**	P1=0.002** P2=1.00	P1=0.004** P2=0.1 P3=0.13	
SD:standard deviation P:Probability						
*:mild significance <0.05 **:Moderate significance ***:High significance						
Test used: repeated measure ANOVA followed by post-hoc Bonferroni						
P1: significance relative to -ve control						
P2: significance relative to 1M time						
P3: significance relative to 3M time						

Discussion

This study addressed and tested quantitatively the effect of different denture cleansers compared to water on inducing surface wear of locator attachment retentive male inserts after simulated clinical use. The choice of cleaning solution and attachment type essentially depends on which cleaning solution and which design provides the least wear.

In this study; two types of attachments retentive male inserts were evaluated after immersion in three types of denture cleansing. Although the two types of attachments were made from the same material, the composition of the material may differ to achieve different elasticity and retention force. As the composition changes, the effect of cleansing solution and the attachment may also changes [14].

The cleaning solutions used in this study, are powerful disinfectant that is active against microorganisms and is nontoxic to humans in low concentrations. In this study the effect of denture cleansers was performed where periods of soaking have followed four times removal and insertion. This is similar to that in clinical situations, where periods of soaking are interrupted with periods of use, as the patients wear the dentures during the day and then soak them in denture cleansing solutions during the night [17].

In the current study, the scanning electron microscopy was used to assess the surface changes of the attachments retentive male inserts after immersion in three different cleansing solutions, as the surface changes of nylon morphology including porosities and cracks were observed at the scanning electron microscopy level [18].

The findings of the current study suggest that the immersion of all tested overdenture in various denture cleansers can significantly affect the hardness of retentive male inserts. This is affected by the chemical nature of the denture cleansers, the immersion time as well as the retentive male inserts type [19].

Distinct surface wear patterns were characteristic for the different types of attachments retentive male inserts. As for Locator attachments retentive male inserts surface initially had smooth character with obscure lines orientated perpendicularly to the longitudinal axis which most probably were formed during manufacturing process. As a result of wear Locator attachments produced surface with more irregularities and particle loss could be explained by different types of nylons used to fabricate plastic retentive male inserts [20].

Possible explanations for the behavior of the Locator attachments retentive male inserts in this study may be attributed to the nylon manufacturer; this is an unreinforced polyamide 66 resin for injection molding. The most influential factor on unreinforced polyamide 66 properties and performance is moisture. This type of nylon absorbs moisture from the atmosphere. Low moisture adsorption lowers its strength and stiffness but increases its toughness and elongation. This may provide an explanation regarding the reduction in retention values of Locator attachments after exposure to water compared to the dry situation. As unreinforced polyamide 66 has great affinity for water molecules, this process allows water molecules to diffuse into the polyamide chains, forcing them apart and weakening the attraction between the chains [21].

After simulated period of one month, a significant increase on wear of all types of attachment was observed with insignificant difference among overdentures groups. This may be attributed to, a mechanical action mainly through friction between the matrix and patrix alone or a combination of chemical action, through the action of cleaning solutions, and mechanical actions induced loss of material from the surface [14].

After simulated period of three months, it was found that in clear and, blue retentive male inserts; there was insignificant change in wear with all cleansing solutions. Also, after simulated period of six months, it was found insignificant change in surface wear of clear retentive male inserts relative to one and three months with insignificance difference among different cleansing solutions. These finding indicates relatively stable wear pattern at three and six months relative to one month clinical simulation. This may be in accordance with Evtimovska et al. [l2] who observed that a significant

loss of retention occurs after the first removal of the locator attachments from the abutments. Furthermore, their study showed that each additional time the Locator attachments retentive male inserts were removed from the abutments, an additional decrease in retention occurred until retention plateaued after the sixteenth pulls [22].

Comparison between different cleansing solution groups of this study and their effect on attachment wear, it was observed that the blue color retentive male insert after simulated period of six months, the effect of water was significant more than corega and protefix cleansing solutions in inducing wear of the retentive male insert. One possible explanation to this finding could be attributed to long soaking time (8 hrs) compared with corega (10 minutes) and protefix (5 minutes). Another possible explanation might be due to presence of chlorine in tab water; which may be responsible for change of the surface morphology of nylon as shown by Gonçalves et al. [23]. Also, porosities and cracks were observed at the scanning electron microscopy level; in addition, a nylon manufacturer (Kelco) claims a deteriorating effect of NaOCl and no effect of sodium bicarbonate on their nylon product [21].

On the other hand, there was insignificant difference among denture cleansers observed in clear color retentive male insert after simulated period of six months. This finding may agree with You et al. [11] who investigated the effect of denture cleansers on the retention of pink Locator attachments for a time equivalent to six months use and observed insignificant difference in retention between groups soaked in different cleaning solutions and water.

When comparing between different retentive male inserts, it was found that the water induced wear of blue retentive male inserts at three months significantly more than clear retentive male insert color. Also, at six months, it was found that water induced wear on blue retentive male insert more than clear retentive male inserts. This may be attributed to hydrolytic degradation is a function of time and, the tensile strength decreased by 80%, and all of the tensile modulus diminished the difference of blue locator retentive male insert material composition and design characteristics compared to clear retentive male inserts to achieve different elasticity and retention force. As the composition changes, the effect of cleansing solutions on the attachments may change [23].

Evtimovska et al. [12] showed that retentive values of attachments tested in their study were significantly reduced over time after multiple pulls; however, it did not considered this situation in this study. Also, did not consider the thermal and chemical conditions of the oral cavity which might have deleterious effects on the locator attachments retentive male inserts. Therefore, further researches considering thermocycling and multiple pulls with longer testing times in this context are indicated.

This study had several limitations. First, the locator attachments retentive male inserts were continuously soaked in the cleansers for a simulated period of six months; however more changes might appear after a longer period of time. The continuous soaking of locator attachments retentive male inserts is actually different from the clinical situation where periods of soaking are interrupted with periods of use, as patients wear their dentures during the day and soak them in denture cleansing solutions at night.

Conclusion

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

1. Retentive male inserts of different colors are affected differently by different cleansing agents

2. The wear of blue attachments increased significantly with water possibly requiring more frequent replacements of these types of attachments. Therefore, water should not be routinely recommended for use as a denture cleanser for blue locator attachment

3. The wear of clear retentive male insert not affected significantly with water, corega, and protefix at six months clinical simulation test

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