Viscoelastic Properties and Antimicrobial Effects of Soft Liners with Silver Zeolite in Complete Dental Prosthesis Wearers: An In Vivo Study

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Purpose: This study aimed to compare the viscoelastic properties and antimicrobial activity of a soft liner with and without silver zeolite for a period of 4 weeks. ***Materials and Methods:*** Thirty edentulous patients wearing complete dental prostheses were selected. A uniform space was created in the intaglio surface of their maxillary prosthesis, and a soft liner without silver zeolite (control material: S) was applied. After 28 days, the soft liner was replaced with new soft liner containing silver zeolite (test material: SZ) and worn for another period of 28 days. Viscoelastic analysis was conducted on the prostheses using S and SZ materials for newly formed samples (T0) and on samples collected after the 28-day period (T28). Culture tests were performed on both materials at T28. Statistical analysis was done using Student independent *t* test. ***Results:*** The decrease in elasticity from T0 to T28 was found to be 76.49% and 79.11% and the decrease in viscosity was 76.49% and 80.3% for the S and SZ materials, respectively. Hence, the difference was not significant. The mean colony-forming units (CFUs) of *Candida albicans* and gram-negative bacteria in the S material at T28 days was 3,150 ± 1,251, whereas that of the SZ material was 1,084 ± 662. There was a statistically significant difference in the mean CFUs between the two groups ($P < .05$). ***Conclusions:*** The addition of silver zeolite to the soft liner improved the antimicrobial activity while not affecting significantly its viscoelastic properties. *Int J Prosthodont 2015;28:265–269. doi: 10.11607/ijp.3740*

Soft liners are used in the management of abused tissues underlying ill-fitting dentures, functional impressions, and tissue conditioning during implant healing and the relining of maxillofacial prostheses. The viscosity of these materials allows them to be molded over an extended period of time. Soft liners can be acrylic- or silicone-based and form a gel without undergoing chemical reaction. This viscous gel flows under a steady load to serve as a resilient cushion under the dentures. However, the oral environment affects the properties of the soft liner. Indeed, the materials (phthalate and alcohol) leach out, and, thus, the material hardens within a short time and gradually loses its prolonged cushioning effect. This in turn affects its viscoelastic properties because it hardens and also creates porosities replaced in part by water and in part colonized by bacteria and fungi, especially the *Candida* species.[1–6]

In order to minimize the problem of colonization, different products have been tested. Studies have shown antifungal agents such as Clotrimazole incorporated into the soft liner materials to be effective against the *Candida* species only.[7] Limited in vitro evidence suggests that silver zeolite is a potentially effective antimicrobial agent.[8,9] Silver zeolite has been introduced as a crystalline aluminosilicate material with silver ions possessing antimicrobial effect against all microbes. Continuous release of small amounts of silver ions into water results in long-term antimicrobial activity that is not harmful to tissue cells.[10] Moreover, in vitro studies have shown favorable long-term antimicrobial effects of soft liners containing silver zeolite on *Candida albicans* and the bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[11] However, to the authors' knowledge, no in vivo studies have been published

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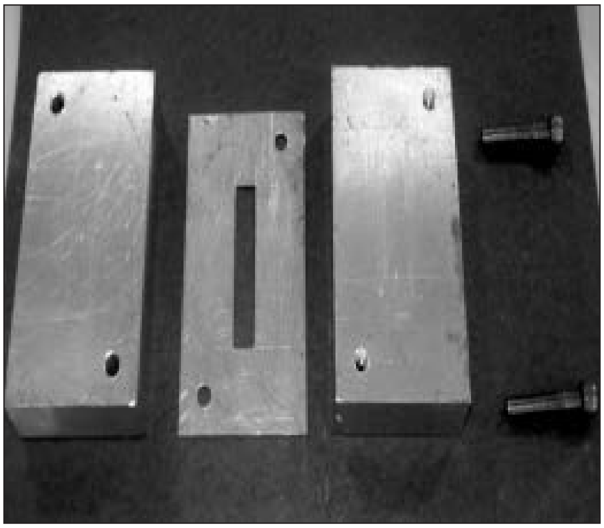

Fig 1 Metal die.

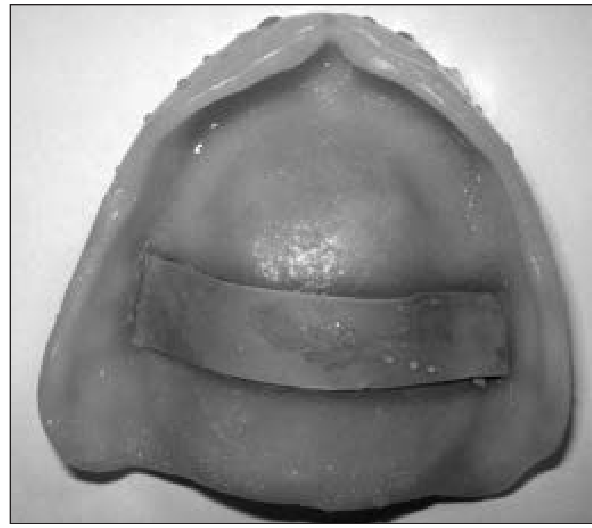

Fig 2 Maxillary denture with putty block.

on this topic because the changes in the viscoelasticity of soft-liner materials in the mouth over time are more rapid when compared to in vitro immersion of the same in water, isotonic saline, artificial saliva, or denture cleansers.[12]

In vitro studies possess limitations in their capacity to simulate the oral environment, and, moreover, the in vivo effect of silver zeolite on the viscoelastic properties of the soft liner and on its antimicrobial activity is unknown. Hence, this study aimed to analyze the efficacy of silver zeolite as an antimicrobial agent and to compare the change in the viscoelastic property of a soft liner with and without silver zeolite in vivo for a period of 28 days.

Materials and Methods

The study was approved by the Publication Oversight Committee, Sri Ramachandra University. The sample size was evaluated by G-star power. Thirty edentulous patients wearing complete dental prostheses with healthy oral mucosa were selected. Patients with systemic disorders such as diabetes, immunocompromised situations, or any form of oral diseases were excluded. Written informed consent was obtained from the patients.

Closed-mouth impressions of the maxilla were made with a medium body impression material (Virtual Monophase) using the prosthesis as a special tray. The impression was disinfected with 5% Glutardehyde for 10 minutes, and the cast was poured using die stone. The prosthesis and the hardened die stone cast were then invested into a dental flask. After opening of the flask, the prosthesis was removed from the die stone and trimmed in order to leave 2 mm of acrylic resin around the teeth. The trimmed denture was placed back into the mold space. A metal die consisting of three parts—upper, middle, and lower—was fabricated. The middle part contained the prototype of the specimen in the following dimensions: 5 cm length × 1 cm width × 1 mm thickness (Fig 1). The three parts were secured tightly using screws. Putty consistency addition silicone was manipulated and placed in the metal die, which was secured tightly using screws, and, after 10 minutes, the set putty blocks were secured to the palatal region of the maxillary cast using cyanoacrylate.

Heat-curing resin was packed into this mold space, and the maxillary prosthesis was retrieved after curing. The intaglio surface of the prosthesis containing the putty block (Fig 2) was replaced by a layer of soft liner (Group S—control; GC Soft-Liner) according to the manufacturer's instructions (2.2 g to 1.8 g powder liquid ratio). The prosthesis was inserted and the soft liner was allowed to reline in the patient's mouth for 10 minutes. The excess material was scraped with a sterile BP blade. The patient was instructed to wear the prosthesis continuously during the day and store it in a container of clean water at night. The patient was also instructed to clean the polished surface of the prosthesis daily with a soft brush and the relined intaglio surface with a wet piece of gauze.[13]

After 28 days, the soft liner without silver zeolite (control material—S) was scraped from the maxillary prosthesis and samples were collected. A new layer of soft liner with silver zeolite (SZ—test material) was

Table 1 Comparative Analysis of Paired Difference Between Elastic Value of Control Group (S) and Test Group (SZ)

	Paired samples statistics using paired *t* test				Difference in value between T0 and T28		
Group	Mean	SD	*t*	*P*	Mean	SD	Percentage decrease
S at T0 S at T28	816.93 192.03	50.593 29.496	59.826	.0001*	624.90	57.21	76.50
SZ at T0 SZ at T28	605.80 126.50	34.183 11.383	69.434	.0001*	479.30	37.81	79.11

S (control group) = soft liner without silver zeolite; SZ (test group) = soft liner with silver zeolite;
T0 = initial properties of the two test materials; T28 = properties analysis after 28 days.
*Significant at $P < .05$.

Table 2 Comparative Analysis of Paired Difference Between Viscous Value of Control Group (S) and Test Group (SZ)

	Paired samples statistics using paired *t* test				Difference in value between T0 and T28		
Group	Mean	SD	*t*	*P*	Mean	SD	Percentage decrease
S at T0 S at T28	1.1763 –5.97	0.78160 0.999	36.502	.0001*	7.14	1.07	76.49
SZ at T0 SZ at T28	–2.97 –12.63	0.850 1.497	36.018	.0001*	9.67	1.47	80.3

S (control group) = soft liner without silver zeolite; SZ (test group) = soft liner with silver zeolite;
T0 = initial properties of the two test materials; T28 = properties analysis after 28 days.
*Significant at $P < .05$.

then incorporated into the trough at day T28. The SZ was incorporated into the soft liner by adding 5% dry weight of SZ (approximately 0.1 g) into the soft liner powder.[12,8] The patient was asked to wear the prosthesis for another 28 days and to report for another sample collection.

The samples, which were stored in a sterile container, were sent for culture to identify the presence of *C albicans* and gram-negative bacteria. The specimens were first vortexed for 20 minutes in 10 mL of normal saline. Following a serial dilution up to 10:6, 100 µmL of each dilution was plated in blood agar and plain Saborauds dextrose agar for quantification of microorganisms. Later, the plates were incubated at 5% carbon dioxide. The colony was kept in the carbon dioxide incubator for 48 hours, and the count was done manually. The count was multiplied with dilution factor to quantify the number of colony-forming units (CFUs).

Viscoelasticity is the property of materials that exhibit both viscous and elastic characteristics when undergoing deformation. Viscoelastic materials have elements of both of these properties and exhibit time-dependent strain. Elasticity is usually the result of a bond stretching along crystallographic planes in an ordered solid.[14] Viscosity is the result of the diffusion of atoms or molecules inside an amorphous material. A dynamic mechanical analyzer (DMA 242c) at Indian Institute of Technology, Chennai, India, was used to quantify the viscoelastic properties of the soft liners. Because soft liners exhibit both viscous and elastic characteristics when undergoing deformation, the properties of elasticity and viscosity were analyzed. Samples of the S and SZ materials were made with the metal die to simulate the initial (T0) elastic and viscous properties of the two materials. The specimens were then set in a compression jig and tested at a frequency of 1 Hz and a temperature of 37°C to mimic the oral environment.[8] With the sinusoidal oscillating force applied, the values for Storage and Loss modulus (elastic and viscous values) could be determined and indicated the storage of the energy still remaining in the sample (elastic value) and the amount of the loss of energy in the given sample (viscous value). The same analysis was performed with the samples collected on the maxillary prosthesis of both S and SZ materials after the 28-day period (T28).

All subjects were standardized using inclusion and exclusion criteria, and the microbial evaluation was done by a single evaluator. The evaluator was blinded to the type of specimen given while the viscoelastic properties were analyzed by the DMA.

The results were analyzed using a Student independent *t* test. Final analysis was done to find the significant difference in the growth of microorganisms and changes in viscoelastic property in the control (S) and study (SZ) groups. A *P* value < .05 was considered to be significant.

Results

The analysis of the S material samples (Tables 1 and 2) showed that, between T0 and T28, the mean elastic value decreased by 76.49% or 624.90 ± 57.21 MPa, while the mean viscous value decreased by 76.49% or the equivalent of 7.14 ± 1.07 MPa. Both of these decreases were statistically significant ($P < .05$).

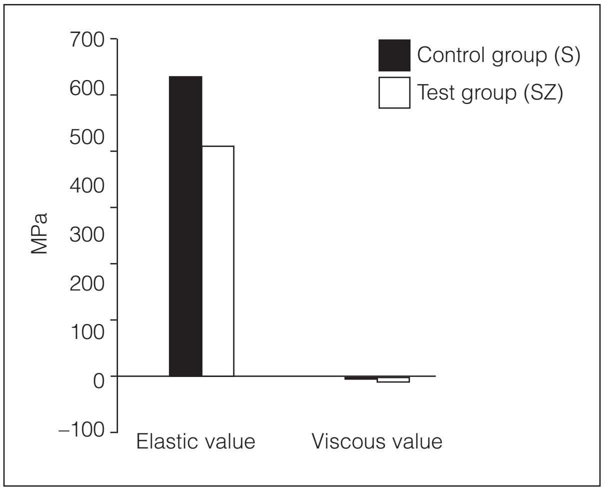

Fig 3 Comparative analysis of paired difference between viscoelastic value of control group (S) and test group (SZ) between T0 and T28 days.

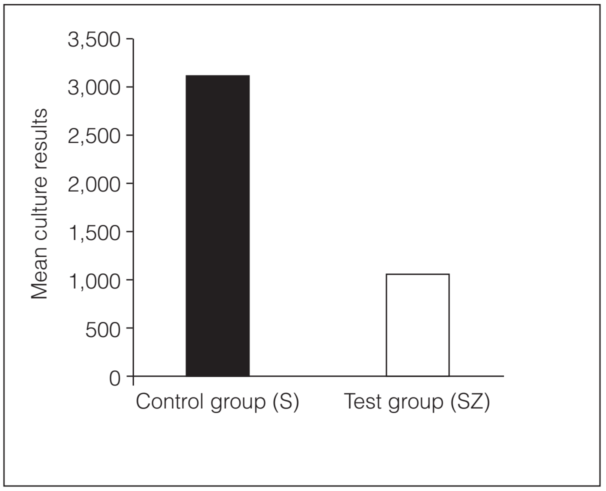

Fig 4 Mean culture results (microbial activity) of control group (S) and test group (SZ) at T28 days.

The analysis of the SZ material samples (Tables 1 and 2) showed that, between T0 and T28, the mean elastic value decreased by 79.11% or 479.30 ± 37.81 MPa, while that of the viscous value by 80.3% or the equivalent of 9.67 ± 1.47 MPa. Both of these decreases were statistically significant ($P < .05$). The difference in the decrease in viscoelastic properties between the two materials was 3% and found to be not significant (Fig 3).

The mean culture results (Fig 4) of S material was 3,150 ± 1,251 after T28 days, whereas that of SZ material was 1,084 ± 662. There was a mean decrease of 65% in the mean CFU between the two materials, which was a statistically significant difference ($P < .05$).

Discussion

The main problem with the use of soft liners has been the colonization of the surface by *Candida* species and gram-positive and gram-negative bacteria.[15] A number of in vitro studies[16] have been done to find out the adherence of microorganisms on various resilient liners. Addition of antifungal agents such as clotrimazole and nystatin in soft liners has decreased the adherence of microorganisms.[17] However, the amount of antifungal agents used in these specimens may be harmful to older people.[14] SZ is a crystalline aluminosilicate material with silver ions possessing antimicrobial effect against all microbes.

Hotta et al[18] proposed that zeolite be used as a carrier for silver because this material has the ability to exchange the ions loaded in its molecular structure for the ions in the ambient environment.

A previous in vitro study done by Matsuura et al[19] concluded that the soft liners containing SZ have shown the antimicrobial effects for 4 weeks on *C albicans* and gram-negative bacteria. An in vivo study by Malmström et al[13] also utilized a 4-week period to evaluate the surface integrity and softness of a soft liner. Hence, in the present study, a 4-week period was chosen.

The viscoelastic property was evaluated using DMA 242c. This was chosen because sinusoidal deflection or deformation contains information about storage modulus for the elastic property and loss modulus for the viscous property of the material.[8] With DMA, it is possible to make a quantitative determination of the mechanical properties of the given sample under an oscillating load and its other functions, ie, temperature and frequency.

The viscoelasticity was decreased by 76% in the control soft liner after 28 days of use in an oral cavity, which was statistically significant ($P < .05$). These results coincided with the in vitro study of Murata et al.[20] There was also a statistically significant difference ($P < .05$) in the viscoelastic results of soft liner with SZ after 28 days of use in the oral cavity (decreased by 79%). On comparing the difference of 3% in the decreases in viscoelasticity between the S and SZ materials after the 28-day period, it was found to be not significant. This study showed that the percentage of the decrease in viscoelasticity properties of soft liners after a certain period of aging inside the oral environment is unaffected by the incorporation of SZ.

The growth of microorganisms decreased by two-thirds (65%) when adding SZ, which was statistically significant. The results of this study demonstrated that soft liners containing SZ have a prolonged antimicrobial effect against all microbes. The results were in accordance with the in vitro studies by Nikawa et al[12] and Matsuura et al,[19] who proposed that SZ combined with soft liners exhibited a greater inhibitory effect on *Candida* growth and gram-negative bacteria.

From a clinical point of view, the present study showed only a 3% decrease in viscoelasticity between the two samples, which is not significant. But, the growth of microorganisms was reduced to one-third by adding SZ to soft liner. This study revealed that SZ incorporated in soft liner is an effective way to prevent microbial contamination. The results of the present study were limited to one soft liner. Further studies need to be done on other soft liners to obtain the differences among the various soft liners used in clinical practice.

Conclusions

This study concluded that the addition of SZ has a significant effect on antimicrobial activity when used with a soft liner. A soft liner with SZ was found to be effective in preventing microbial contamination without altering the viscoelastic property.

Acknowledgments

The authors reported no conflicts of interest related to this study.

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