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An in-vitro comparison of hardness and SEM-EDS micro analysis after inclusion of 1% Octenidine Dihydrochloride in tissue conditioners for treatment of denture stomatitis

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Introduction

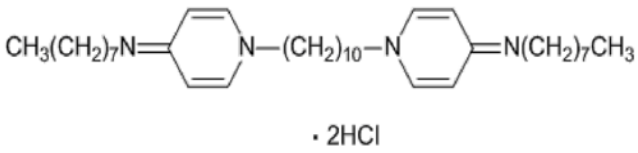
Prolonged use of ill fitting dentures cause irritation of the oral soft tissues resulting in deprived blood supply leading to resorption of the supporting bony foundation. [1]. Because soft tissue mucosa is trapped between denture and underlying bone its form and condition gets altered and consequently ,denture tends to get loosen. [2] Many patients present with diseased tissues of the denture foundation areas secondary to ill-fitting dentures, inaccurate centric relation records and under-extended denture borders. In an edentulous individual with a complete or a partial denture prosthesis the masticatory load and functional stresses are transmitted to the bone through mucoperiosteum. These functional stresses may lead to chronic soreness, pathologic changes of oral tissues, and subsequent bone loss resulting in loss of accurate adaptation of the denture to the underlying tissues [3].Tissue conditioning is an effort to restore the health of the abused tissues of the denture foundation area before final impressions are made. [4,5] These resilient liners either long term or short term are used to replace the fitting surface of the denture when patients cannot tolerate the hard denture base. High resilience of these materials helps in absorbing the impact energies of mastication and

distributes them over a large area of tissues. Hence, they are used to reduce the mechanical abuse of the oral soft tissues and, also to improve the comfort and masticatory function of the patient. They are used to retain over denture bar attachments, extra oral prosthesis, and compensates for the volumetric shrinkage of acrylic resin [6,7].

Ideal requisites of denture lining materials are good biocompatibility, permanent resilience, low water sorption, dimensionally stable, high bond strength to denture base, good color stability and resistance to microbial growth. During their use, these materials are immersed in saliva, foods, water, denture cleansers, thermal cycling, and masticatory force which cause leaching of their components or water absorption. Due to their soft nature these resilient materials are considered more susceptible to microbial adhesion than to the thermo-polymerized acrylic resin. The loss of surface integrity and surface roughness may begin in a matter of 3–4 days. Surface roughness of the resilient liners may differ among materials. Fungi such as Candida albicans first adhere to a lining surface and then penetrate inside the material. [8,9] Due to their soft nature they have higher tendency to interact with microorganisms so, and more susceptible which may cause denture-induced stomatitis [10].

Denture stomatitis is most frequent opportunistic infection amongst the wearers of removable dentures. Various topical agents have been used for relieving the clinical sign and symptoms of denture stomatitis. But the diluent effect of saliva and cleansing action of oral musculature tend to decrease the concentration of these drugs to sub therapeutic level. Also, success of topical application of drugs in the oral cavity may be compromised by lack of patient compliance.[2,4] Furthermore, maintaining the regular and optimal dose of topical antifungal agents is challenging in geriatric denture wearers due to reduced motor skills, cognitive impairment, and memory loss [11] .Systemic antibiotics if used are effective but they do not eradicate microorganisms from denture surface leading to chances of reoccurrence. Therefore, recent trends to prevent this microbial colonization, is by incorporation of antimicrobials agents in tissue conditioners to extend their clinical longevity and prevent microorganism proliferation. The additional advantages of incorporating antimicrobial agents in tissue conditioners or resilient liners is that the drug release in the oral cavity aid in simultaneous recovery of injured tissues, prevention of fungal infections and reduce the need for patient compliance. [11,12]

Octenidine Dihydrochloride, abis-(dihydropyridinyl)-decanederivative, is a cationic surfactant, used in concentration of 0.1-2.0%. It is similar in its action to Quaternary Ammonium. Previous studies with this compound have shown it to be effective in inhibiting the growth of plaque forming bacteria and are of somewhat broader spectrum of antimicrobial activity. Low concentration (0.1%and less) of the substance are found to be bactericidal and fungicidal. Octenidine at 0.1% has shown to reduce plaque, gingivitis and bleeding when used as a mouth rinse. [13,14] Andrew M Slee & John R. O' Connor did a study to check the antibacterial activity of OCT.



OCTENIDINE DIHYDROCHLORIDE

Intact preformed in-vitro plaques of four indigenous oral plaque forming microorganisms, Streptococcus mutans, Streptococcus sanguis, Actinomyces viscosis, and Actinomyces naeslundii, were studied. OCT was proved

to be better antimicrobial agent in comparison to Chlorhexidine digluconate in the in vitro plaque bactericidal model. [15] Richard N. Smith, Roxanna N. Andersen and Paui E. Koienbranderin the year 1991 compared the inhibition of intergeneric coaggregation among oral bacteria by Cetylpyridinium chioride, Chlorhexidine digluconate and OCT.

Even at 0.25%, the highest concentration tested with Chlorhexidine digluconate and Cetylpyridinium Chloride, only partial inhibition was observed. OCT at 0.05%, the highest concentration tested, partially inhibited some interactions and completely inhibited the interaction between the coaggregation [16]

Despite these therapeutic advantages it is also essential to observe the effect of drug incorporation on structural properties of Tissue conditioners. This change should be in limits that the soft liner does not loosen its basic properties.One of this basic and important property is hardness.

Hardness is determinant for the evaluation of elastic modulus which in turns defines the functions of dental materials. This statement becomes more relevant in case of Tissue conditioners which are not rigid materials by nature. [17] The addition of 1%Octenidine Dihydrochloride improves the antimicrobial activity but however it is necessary to assess their effect on hardness of Tissue conditioners. Hardness was measured with the help of durometer which is typically used to measure hardness in polymers, elastomers, and rubbers. Besides the effects of drug incorporation on the physical and mechanical properties of Tissue conditioners further information regarding drug dispersion into these soft materials is also required. This was studied with the help of **SEM- EDS** (scanning electron microscope -energy dispersive x-ray spectroscopy). The weight percentage of various elements present and the surface distribution of antimicrobial agent in Tissue conditioners were analyzed to confirm any changes in morphological features of tissue conditioner surfaces.

Materials & Method

The study was conducted in two parts.

Part A: To evaluate and compare the hardness of two tissue conditioners after incorporating antimicrobial agent i.e. 1% Octenidine dihydrochloride (Farblos).

Table 1: Tissue conditioners used in the study

S. No	Tissue Conditioner	Composition
1.	Viscogel(V) (Dentsply)	Powder: Polyethyl methacrylate Liquid: Phthalyl butyl glycolate, ethanol
2.	Coe-soft (C) (GC America)	Powder:polyethyl methacrylate. Liquid: mixture of dibutyl phthalate, benzyl salicylate and ethanol.

Part B: Surface distribution of 1% Octenidine Dihydrochloride and percentage of different components in two tissue conditioners in both control and modified specimens were studied with help of scanning electron microscope -Energy dispersive X-ray spectroscopy (SEM-EDS) ANALYSIS

Part A – Preparation of Test Specimens

Rectangular stainless-steel die of dimensions 36 mm length, 7 mm width & 6 mm thickness according to American Society of Testing and Materials D were fabricated. Varsity dental flasks were used for investing the 4 metal dies in Type III gypsum (Dental Stone) using two pour technique.

Once Gypsum was set, the two halves were separated and the metal dies were retrieved from the flask and moulds were obtained.

A total of 120 samples were divided into two groups of 60 samples each. Each group was then subdivided into 2 subgroups. Different separators for each tissue conditioners were applied. 1% Octenidine Dihydrochloride was incorporated in the liquid of Tissue conditioners and then powder and liquid was mixed in recommended proportion accordingly:

1. Visco-gel 10 gm of powder in 6.6ml of liquid (including 1ml of 1% Octenidine Dihydrochloride in liquid of tissue conditioner).

2. Coe-soft 9.4 gm of powder in 6ml of liquid (1ml of 1% Octenidine dihydrochloride in liquid of tissue conditioner).

Tissue conditioners were mixed for about 30 seconds in a mixing vessel and filled carefully in mould cavity avoiding air entrapment.

The samples were separated from the moulds after the duration of 30 minutes and finished with the help of B P blade.(figure 1)

Group A - VISCO-GEL (V) (60 samples)

Subgroup 1 - VC 30 control samples of Visco-gel.

Subgroup2 - VO30 samples of Visco-gel incorporated with 1% Octenidine Dihydrochloride.

GROUP B: -COE-SOFT (C) (60 samples).

Subgroup 1 - CC 30 control samples Coe-soft.

Subgroup 2 - CO 30 samples of Coe-soft incorporated with 1% Octenidine Dihydrochloride.

The specimens were stored in distilled water at room temperature and hardness was evaluated after 24 hours,7 days & 14 days with Shore A Durometer. The obtained results were tabulated and subjected to statistical analysis using three way ANOVA test. (Table: 2,3,4)

Table 2: Hardness in Shore A after 24 Hours

S. No	Group 1 (Visco-gel)	Group 2 (Visco-gel +Oct)	Group 3 (Coe-soft)	Group 4 (Coe- Soft + Oct)
1	24	25	10	14
2	25	27	12	13
3	21	24	11	13
4	24	26	12	15
5	23	27	10	13
6	22	26	12	15
7	24	25	11	14
8	20	27	11	14
9	22	25	13	15
10	24	26	12	14

Table 3: Hardness in Shore A after 7 days

S. No	Group 1 (Visco-gel)	Group 2 (Visco-gel+Oct)	Group 3 (Coe-soft)	Group 4 (Coe- soft + Oct)
1	28	27	12	17
2	27	26	12	14
3	24	29	14	13
4	28	28	12	15
\5	26	27	13	14
6	24	28	12	15
7	28	29	11	13
8	24	25	13	14
9	24	30	12	15
10	26	29	13	14

Table 4: Hardness in Shore A After 14 days

S.No.	Group 1 (Visco-gel)	Group2 (Visco-gel+Oct)	Group 3 (Coe-soft)	Group 4 (Coe -Soft+Oct)
1	26	30	15	18
2	25	34	17	16
3	28	32	16	16
4	27	32	15	17
5	25	30	15	19
6	26	29	14	16
7	25	30	16	19
8	28	34	14	16
9	26	30	17	16
10	25	32	13	19

Part B

In this part of study surface absorption of 1 % of Octenidine Dihydrochloride was evaluated using scanning electron microscopy (SEM). One sample from each subgroup was selected for the study.

Samples were cut with sterile BP Blade in accordance with the size of sputter coater tub. They were dried coated with gold and palladium in sputter coater for about 45 minutes.

The samples were then examined under Scanning electron microscope (SEM) with ZEISS EVOMA10 at 20 KV/EHT and 9.22 Pascal. The selected regions were

submitted to energy-dispersed X-ray analysis attached to SEM to determine and map the main elements into the tissue conditioner matrix (figure 2).

Results

The results (Table 2, 3, and 4) were subjected for analysis and as there were three independent variables, three-way ANOVA test was performed to check for interaction between these variables. The results of 3-way ANOVA showed statistically significant difference for the factors "material" ($p<0.0001$), "antimicrobial agent" ($p<0.0001$), "time" ($p<0.0001$), and the interaction between the factors "material" and "antimicrobial agent" ($p=0.021$), "material," and "time," ($p=0.012$) and "antimicrobial agent," and "time" ($p=0.024$), as well the interaction between all the three factors ($p=0.012$).

Statistical analysis is tabulated in table 5, 6 and 7.

Table 5: Comparison of hardness at different points of time (24 hrs, 7 days & 14 days) among each Subgroup (With or without drug) of each Group.

Material	With or without 1% OCT	Time point	Mean	Std. Deviation	N	Post hoc Tukey's test
Visco- gel	VC	24 hr	22.9	1.6	10	1*2-0.001
		7 days	25.9	1.79	10	1*3-<0.0001
		14 days	26.1	1.2	10	2*3- 0.995
	VO	24 hr	25.8	1.03	10	1*2-0.015
		7 days	27.8	1.55	10	1*3-<0.0001
		14 days	31.3	1.77	10	2*3-<0.0001
Coe-soft	CC	24 hr	11.4	0.97	10	1*2-0.107
		7 days	12.4	0.84	10	1*3-<0.0001
		14 days	15.2	1.32	10	2*3-<0.0001
	CO	24 hr	14	0.82	10	1*2-0.702
		7 days	14.4	1.17	10	1*3-<0.0001
		14 days	17.2	1.4	10	2*3-<0.0001

Table 6: Comparison of hardness among Subgroups (With or without drug) of each Group at different points of time (24 hrs, 7 days & 14 days)

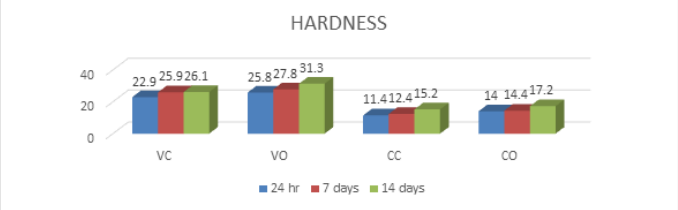
		Subgroup	Mean	Std. Deviation	P value	Significance
Visco-gel	24 hr	VC	22.9	1.6	<0.0001	S
		VO	25.8	1.03		
	7 days	VC	25.9	1.79	0.021	S
		VO	27.8	1.55		
	14 days	VC	26.1	1.2	<0.0001	S
		VO	31.3	1.77		
Coe-soft	24 hr	CC	11.4	0.97	<0.0001	S
		CO	14	0.82		
	7 days	CC	12.4	0.84	<0.0001	S
		CO	14.4	1.17		
	14 days	CC	15.2	1.32	0.004	S
		CO	17.2	1.4		

Table 7: Comparison of hardness among each Group (Visco-gel & Coe-soft) of each Subgroup (With or without drug) at different points of time (24 hrs, 7 days & 14 days)

		Group	Mean	Std. Deviation	P value	Significance
Without 1% OCT	24 hr	Visco-gel	22.9	1.6	<0.0001	S
		Coe-soft	11.4	0.97		
	7 days	Visco-gel	25.9	1.79	<0.0001	S
		Coe-soft	12.4	0.84		
	14 days	Visco-gel	26.1	1.2	<0.0001	S
		Coe-soft	15.2	1.32		
With OCT	24 hr	Visco-gel	25.8	1.03	<0.0001	S
		Coe-soft	14	0.82		
	7 days	Visco-gel	27.8	1.55	<0.0001	S
		Coe-soft	14.4	1.17		
	14 days	Visco-gel	26.1	1.2	<0.0001	S
		Coe-soft	17.2	1.4	<0.0001	S

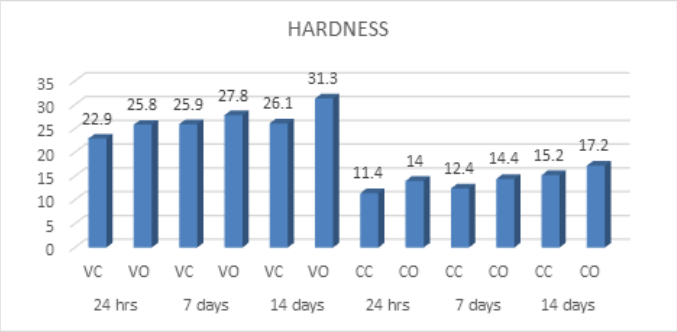
comparison of hardness at different points of time (24 hrs, 7 days & 14 days) among each Subgroup (With or without 1% OCT) of each Group (Visco-gel & Coe-soft) is depicted in the graphs.

Graph 1



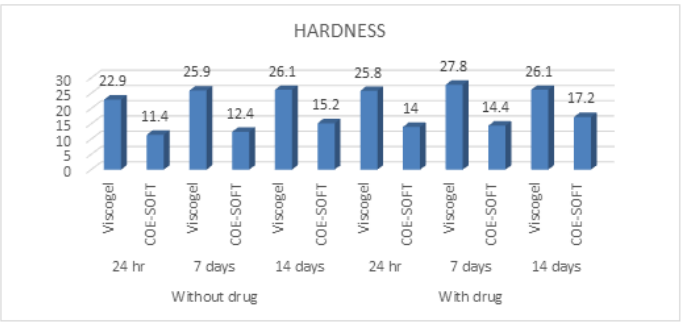
Comparison of hardness among each Subgroup (With or without 1% OCT) of each Group (Visco- gel & Coe-soft) at different points of time (24 hrs, 7 days & 14 days)

Graph 2



Comparison of hardness among each Group (visco-gel & Coe-soft) of each Subgroup (With or without 1% OCT) at different points of time (24 hrs, 7 days & 14 days)

Graph 3



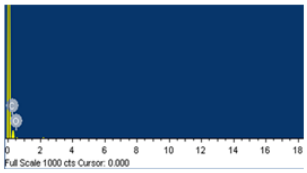
The graphic representation of EDS mapping of control and modified specimen show

Part B

Results of EDS mapping show peaks of chloride present in tissue conditioner matrix of modified samples(along with carbon and oxygen) but not in control depicting that Octenidine Dihydrochloride is well incorporated and can be used as sustained drug delivery system for the treatment of denture stomatitis.

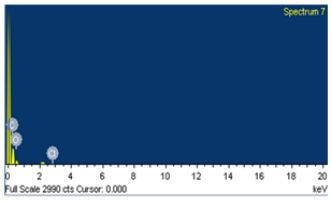
Graphical representation is depicted in graph 4,5,6,7.

Graph 4: EDS Mapping- Visco-Gel Control



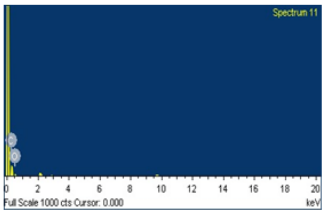
Element	Weight%	Atomic%
C K	69.62	75.33
O K	30.38	24.67
Total	100	

Graph 5: EDS Mapping- Visco-gel with 1% Octenidine Dihydrochloride



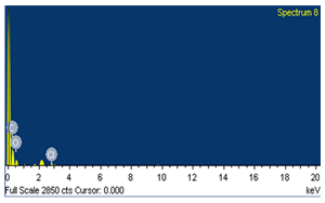
Element	Weight%	Atomic%
C K	64.51	70.88
O K	35.15	28.99
Cl K	0.34	0.13
Total	100	

Graph 6: EDS Mapping- Coe-Soft control (cc)



Element	Weight%	Atomic%
C K	70.69	76.26
O K	29.31	23.74
Total	100	

Graph 7: EDS Mapping- Coe-Soft with 1% Octenidine dihydrochloride



Element	Weight%	Atomic%
C K	58.88	65.78
O K	40.52	33.99
Cl K	0.6	0.23
Total	100	

Discussion

Denture-related stomatitis is the most common form of oral candidiasis with varied treatment modalities including topical and systemic antifungal therapy. Mechanical and chemical oral hygiene care procedures include cleaning and disinfecting the prosthesis along with replacing old dentures, restoration of non-traumatic occlusion and use of overnight denture disinfectants. Mostly the choice and combination of treatment is a personal choice. [17] However, success depends on the methods that reduce microorganisms from the infected mucosa and the intaglio surface of the denture base.

It has been recommended that to reduce the micro-organism it is essential to eliminate the contact between the denture biofilm and infected tissues, thus avoiding a re-infection cycle. This is possible with the use of resilient liners. [18]. But the problem is because of their soft nature and chemical composition they get contaminated with microorganisms.

Tissue conditioners are used to enhance the recovery of denture-bearing tissues from trauma, damage or residual ridge resorption caused by ill-fitting dentures.[19] Microbial growth occurs from the adherence of microbial cells, enhanced by rough surface, and from adhesive interactions between candida species and oral bacteria mostly Candida albicans and oral streptococci.[20,21] Moreover, Staphylococcus aureus, giving rise to pharyngeal and respiratory infections, has been isolated from dentures and the oral cavity in elderly patients with decreased immunological activity.[22,23]Tissue conditioners kept clean by mechanical and chemical method scan get damaged. Hence, without damaging the surface of the tissue conditioner (as this surface roughness will again act as seat for microorganisms) some authors have suggested that the incorporation of antimicrobials in these materials may extend their clinical longevity and help prevent microorganism proliferation.

During clinical use, the soft lining materials are susceptible to hardness changes, which determine the ability of a material to cushion the impacts and are related to its modulus of elasticity.

Tissue conditioners are short term soft liners which are used for the treatment of denture stomatitis and are given for around two weeks. Therefore, in the present study hardness is evaluated after 24 hours,7 days and14 days. Selection of 1% Octenidine Dihydrochloride as antimicrobial agent was based on earlier studies which showed that the compound exhibited broad-spectrum antimicrobial activity as measured by MICs against a variety of gram-positive and gram-negative bacteria. This activity was comparable to that exhibited by Chlorhexidine Gluconate and Biguanide germicidal agents currently used in various commercial skin degerming formulations. There are studies which show good results with antimicrobial agents when used in mouthwashes and root canal medicaments. David M.Sedlock & Denis M. Bailey demonstrated that OCT is a broad spectrum antimicrobial agent which is active biocide at low concentrations. They also mentioned that the compound is bactericidal and candidicidal and is effective against resident skin micro flora. [26].

In the present study hardness has been compared between two tissue conditioners with and without incorporation of drug (OCT) at different intervals i.e. after 24hours ,7days and 14 days .Results confirmed increases in hardness with time in both tissue conditioners i.e. Viscogel & Coe-soft in control and modified specimens. The maximum increase in hardness was seen in first 24 hours then between 24hours and 7days. Change in hardness was found least between 7 days to 14 days.

Changes in the hardness with time in acrylic soft liners due to degradation gradually increases hardness value as low molecular weight components are leached out from the material after elapsed time, this is in agreement with Terao (1993). These changes could be attributed to either of the following factors: 1. scission of the polymers chains. 2. absorption of water. 3. oxygen cross linking and 4. Leaching of plasticizers.

The first two factors could explain the decrease of hardness value because scission of polymer's chain may increase freedom of molecules movement while absorbed water may act as additional a plasticizer that enhance material resiliency, in the opposite direction the last two

factors indicates reduction in molecules movements and reduces materials elasticity.[11]

It was observed that hardness values of both control and modified specimen were more in case of Viscogel as compared to Coe-soft. This can be explained by the fact that butyl pthyl butyl glycolate which is present in Viscogel in concentration of approximately 86% leached out more rapidly and produced more significant change in hardness. Benzyl salicylate present in coe- soft, being larger molecule would be leached out slower than butyl pthalyl butyl glycolate which would be leached out faster and hence lead to deterioration of the hardness.

According to Craig, Hardness value of 13 to 49 Shore A in 24hours is regarded acceptable for clinical use of these materials. Shore A hardness of 25-30 units without changes during the lifecycle of resilient material is considered clinically suitable. [11]

The highest hardness values as observed after 14 days of Viscogel and coe soft with 1%octinidiene were calculated as 31 and 17 respectively. Both the values are below the mean value as of range of softness desired for even 24 hours; both can be used in the prevention and treatment of denture stomatitis.

Although, the effects of drug incorporation on the physical and mechanical properties of tissue conditioners have been studied, further information regarding drug dispersion into the tissue conditioner is required. In the current study, SEM images demonstrated that all drug containing specimens showed changes at the surface in comparison to drug -free specimens .Control specimens of Viscogel were transparent but modified specimen were milky white in color .Change in color was observed in Coe-soft form of translucent pink to opaque pink.

In modified sample of Coe-soft and Visco-gel , traces of chloride along with carbon and oxygen were present as represented in EDS MAPPING(an analytical technique used for elemental analysis or chemical characterization of sample) where K is the analytic factor. Peaks of chloride can be seen along with carbon and hydrogen which signifies that Octenidine Dihydrochloride is well incorporated in the sample. A similar SEM-EDS Microanalysis study was done by Vanessa Migliorini Urban et al for distribution and identification of Antifungal/Antimicrobial Agents on a modified Tissue Conditioners. They concluded the detection of S2−and Ca2+ ions in the Soft one powder and the presence of only Cl1-ion in the chemical structure of both miconazole and Chlorhexidine were used to confirm the location of these drug particles within the plasticized matrix. Despite the presence of Cl1− ion in the chemical formula of ketoconazole, this element was not found in EDS mapping analysis. Therefore, analysis of the presence and features of nystatin and ketoconazole added to the specimens were only based on comparisons among SEM images from drug-containing and drug-free specimens. The desirable drug activity of a polymeric or plasticized system containing antifungal or antimicrobial agents is strongly related to its ability to release drug particles.[27,28]

In the present study the SEM microscopic view when compared showed homogenous distribution of particle in control specimens. The distribution was non homogenous in case of modified specimens, in acceptable range without altering the roughness microscopically and macroscopically (figure 3,4,5 and 6).

When compared modified specimens of Viscogel and Coe-soft a more uniform distribution of antimicrobial agent was observed in Viscogel than in Coe soft. As drug distribution is related to drug release,[29,30] it may be expected that this pattern of particle distribution within the tissue conditioner could result in constant and effective sustainable release rates for therapies. Large pores can be seen in the modified samples of Coe soft as compared to

tiny homogenously distributed pores in Viscogel attributed that drug leached out faster creating pores and became seat for microorganisms. As the study is an in vitro study, as during function soft liners are exposed to many different fluids and temperatures in oral cavity accurate changes cannot be predicted unless tested in-vivo.

Summary and Conclusion

The role of 1% Octenidine Dihydrochloride as an antimicrobial agent isan effective and viable method for treatment of denture stomatitis.

Within the limitations of the study it is evident that:

1. Drug is well embedded in both tissue conditioners as shown in SEM-EDS analysis.

2. Compared to Coe-soft better solubility and uniform distribution of Octenidine is in Visco-gel.

3. SEM-EDS study proves that Coe-soft has more surface roughness and larger pores size which may lead to increased microbial activity due to greater surface area for adherence.

4. Increase in hardness values of Visco-gel within clinical limits after addition of antimicrobial agent at1st, 7th and 14th day intervals was significantly greater when compared to Coe-soft.

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