EFFECT OF COLONIZATION OF MICROORGANISMS ON ORTHODONTIC BONDING MATERIALS: AN SEM STUDY

Munjal Puneet,* Ragini, Goyal Sandeep***, Goyal Sonia****, Padmanabhan Prajeesh*******

Demineralization on the tooth surface around orthodontic brackets occurs, with microorganisms being the principal causative factors. This in - vitro study was conducted to evaluate and compare growth of Streptococcus mutans, Staphylococcus aureus and Pseudomonas on three orthodontic bonding materials and the changes in surface characteristics of the bonding materials.

120 standardised pellets were divided into 2 groups as Control group (30 pellets) and Study group (90 pellets), which were subdivided into 3 control subgroups and 9 study subgroups of 10 pellets each. The pellets were weighed and inoculated with respective bacteria, the culture media being artificial saliva and nutrient broth. Control group was not inoculated with any bacteria. The sample was incubated at 37° C for 7 days. Study was carried out under total aseptic conditions. The pellets were re-weighed after the growth of bacteria and weight differential percentage was calculated. Intergroup comparisons were done using t-test. The surface was examined under scanning electron microscope (SEM) to see for the surface changes.

Streptococcus mutans showed maximum growth on all the bonding materials tested, while Pseudomonas showed the least growth. Rely-A-Bond showed the least growth of all the bacteria except for the Staphylococci which showed least growth on Curc-On-Touch. SEM pictures revealed the greatest surface damage was on the Enlight material, mainly caused by Streptococcus mutans.

The orthodontic bonding adhesive should be chosen cautiously to avoid dental caries and in-between – treatment bracket failures.

Key-words: Bbonding materials; orthodontics; microorganism growth;

Introduction

The oral environment provides the proper conditions for the colonization of a complex microbiota. Orthodontic appliances have been found to induce specific changes in the oral environment such as decreased pH, increased plaque accumulation [1-2] and elevated Streptococcus mutans levels [3-5]. Composite adhesive materials provide niche for growth of microorganisms because of their rough surface [6,7] and due to organic and carbon content which is essential for bacterial growth [8]. Bacterial growth may also lead to weakening of the bond between the tooth surface and the bracket due to damage to the material caused by bacterial products [8]. It also produces discoloration of enamel appearing as a white spots [9-12].

Studies have shown a shift in microbial population in the presence of fixed orthodontic appliances, limited information is available as to which bonding material would be less prone to adhesion of bacterial species and plaque accumulation and what surface changes / damage occur on the material [13]. The search of a bonding material that has minimal adherence and growth of microorganisms with subsequent less damage to bonding material may thus prove to be beneficial in orthodontics.

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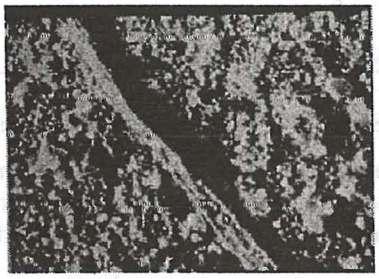


FIG 1 A

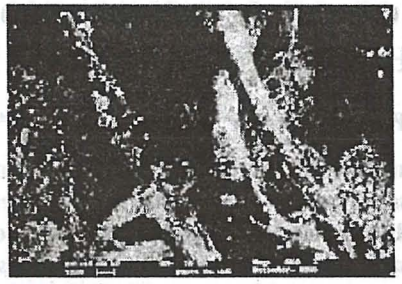


FIG 1 B

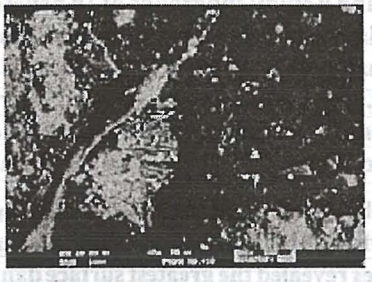


FIG 1 C

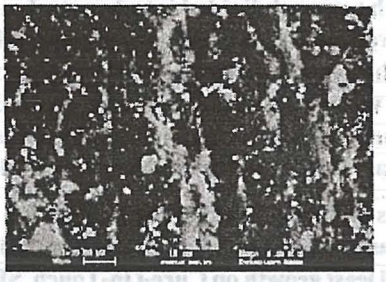


FIG 1 D

Fig 1

A: Surface Characteristics Of Enlight (Control Group, 500 X)
B: Surface Characteristics Of Enlight After Growth Of Strept. mutans (500 X)
C: Surface Characteristics Of Enlight After Growth Of Staph. aureus (500 X)
D: Surface Characteristics Of Enlight After Growth Of pseudomonas (1000 X)

Aims and Objectives:

1. To determine which material ie Enlight, Cure- On -Touch, & Rely-a-bond shows the greatest growth of bacteria over its surface & the material which shows the least growth.
2. To determine the changes in surface topography of the study sample after the inoculation of microorganisms by means of SEM.

Materials and Method: The study was carried out in the Dept Of Orthodontics, D A V Dental College, Yamuna Nagar, Haryana, India in collaboration with the Dept. Of Microbiology, P G I Chandigarh, under strict aseptic conditions to avoid false results. Following materials were used for the study.

1) Orthodontic Bonding Materials
- Enlight (Ormco Corpn. USA) - Light cure
- Cure- on –Touch (Scientific Pharmaceuticals Inc. USA) - Self cure
- Rely-a-bond (Reliance Ortho Products Inc. USA) - Self cure

2) Templates to prepare round standardized pellets measuring 6 mm x 3 mm size.

3) Pure strains of Streptococcus mutans, Staphylococcus aureus and Pseudomonas.

4) Nutrient Broth: containing Minced buffalo meat 50 gms; Papain 5 gm; and Distil water 200 ml.

5) Artificial saliva: containing NaCl (40 ***mg / 1000 ml.***); KCl (40 ***mg / 1000 ml.***); $CaCl_2$ (80 ***mg / 1000 ml.***), NaH_2Po_4 (70 ***mg / 1000 ml.***), Na_2S (70 ***mg /***

1000 ml.); NH_4No_3 (100 *mg / 1000 ml.*); and $FeCl_3$ (0.02 *mg / 1000 ml.*)

6) 2% Gluteraldehyde for sterilizing the pellets.
7) Normal saline to wash the pellets.

Equipment used for study

1) Electronic Balance (SHIMADZU Co. Korea) least count 0.01 gm.
2) Incubator (York Scientific Industries Ltd. India).
3) Light cure apparatus (Light Cure 2500 – 3M Dental Products, Germany)
4) Ni - Cr wire loop used to carry microorganisms from agar plate to nutrient broth.
5) Sterilized pipettes (100 il Gilsom Inc, USA & 1000 il Accupipet Inc USA) 1000 il for adding saliva & Nutrient broth to test tubes & 10 il for inoculating the microorganisms.
6) Laminar flow chamber (Kartos International India)
7) Scanning electron microscope. (Leo 335 VP)
8) Autoclave (Unitex Surgicals, India).

Method

Sample pellets of bonding materials being studied were prepared by manipulating them according to the manufacturer's recommendations. 120 pellets were prepared and divided into two groups (Table 1)

1. Control Group (N = 30)
2. Study Group (N = 90)

Control group: 30 pellets were randomly placed in control group designated as ø, & subdivided into 3 subgroups of 10 each. [10 Enlight, 10 Cure- On - Touch and 10 Rely- A- Bond] ie ø A, ø B, & ø C respectively.

Study group: 90 pellets were divided into 3 groups of Enlight, Cure- On -Touch and Rely- A- Bond designated as A, B, & C respectively, which in turn were divided in 3 subgroups (a total of 9 subgroups) each containing 10 pellets, to be inoculated with bacterial species ie Streptococcus mutans, Staphylococcus aureus and Pseudomonas designated as 1, 2, & 3 respectively. So 9 subgroups were created as A1, A2, A3, B1, B2, B3, C1, C2, & C3.

All the pellets were weighed separately 3 times and the mean was taken. The pellets were sterilized in 2% Gluteraldehyde in separate glass vials which

Table 1: Distribution Of Sample In Control And Study Groups

	CONTROL ø (N = 30)	A (N = 30) Enlight	B (N = 30) Cure-on-touch	C (N = 30) Rely-a-Bond
1	**Group A** N = 10 Enlight	**Group ø A1** N = 10 Streptococcus mutans	**Group B1** N = 10 Streptococcus mutans	**Group C1** N = 10 Streptococcus mutans
2	**Group ø B** N = 10 Cure-on-touch	**Group A2** N = 10 Staph. aureus	**Group B2** N = 10 Staph. Aureus	**Group C2** N = 10 Staph. aureus
3	**Group ø C** N = 10 Rely-a-Bond	**Group A3** N = 10 Pseudomonas	**Group B3** N = 10 Pseudomonas	**Group C3** N = 10 Pseudomonas

A - Enlight
B - Cure – on - Touch
C - Rely – a - Bond

1. Streptococcus mutans
2. Staphylococcus aureus.
3. Pseudomonas

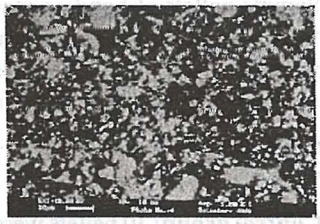


FIG 2 A

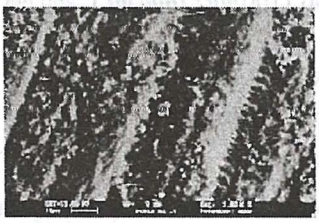


FIG 2 B

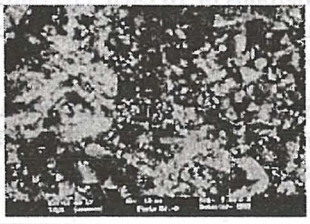


FIG 2 C



FIG 2 D

Fig 2

A: Surface Characteristics Of Cure – On – Touch (Control Group, 1000 X)

B: Surface Characteristics Of Cure – On – Touch After Growth Of Strept. mutans (1000 X)

C: Surface Characteristics Of Cure – On – Touch After Growth Of Staph. aureus (1000 X)

D: Surface Characteristics Of Cure – On – Touch After Growth Of Pseudomonas (1000 X)

were pre-marked for further reference. The pellets were washed in sterile distilled water & dried in incubator and then individually placed in previously marked & sterilized empty test tubes. Each pellet was picked up separately with the help of sterilized straight forceps and placed at the bottom of the test tube. Then, artificial saliva and nutrient broth was poured into the test tube in the ratio of 1 : 2 (1000 il of saliva and 2000 il of nutrient broth). The mouth of the test tube was heated both prior and after pouring the two solutions. The entire process was carried out in laminar flow chamber. Marked test tubes were then inoculated with 1000 CFU of Streptococcus mutans, Staphylococcus aureus & Pseudomonas separately with the help of sterile micropipettes (100 il). All the tubes were kept in incubator at 37° C for 7 days. The test tubes were removed from the incubator on day 7 and the pellets were again dried in the incubator,

Table – 2: Weight Gain Of Control Group Sample

	Enlight N = 10 (ø A)	Cure - on -Touch N = 10 (ø B)	Rely - A- Bond N = 10 (ø C)
Mean weight before inoculation (gm.)	0.2360	0.1655	0.2145
Mean weight after growth (gm.)	0.2379	0.1674	0.2165
Mean weight gain (gm.)	0.0017	0.0019	0.0020
%age weight gain	0.7180	1.148	0.9282

reweighed thrice; the mean final weight & weight gain percentage were recorded.

The test pellets were viewed under SEM to see the surface changes occurring after growth of bacteria. The samples were coated with gold – palladium (the thickness of coat was about 30 nm) in a polaron sputter (E-5000) under vacuum pressure (0.1 – 0.001 Torr) for 10 minutes, the samples were then transferred to a SEM (LEO 335 VP) operating at 15 KV accelerating voltage for visual analysis and photography. The sample was scanned as a whole initially and representative photomicrographs of desired area were obtained at variable magnification (500 x to 1000 x).

The data thus obtained was subjected to statistical evaluation. Student's t – test was applied for inter-group comparison.

Results

Results are shown in Tables 2 – 5. The weight gain for Enlight, Cure-On-Touch and Rely-A-Bond was 0.7180 %, 1.148 % and 0.9282 % respectively (Table 2). Increase in weight of the control samples was mainly due to deposition of salts from the media

Table - 3: Weight Gain Of Different Bacteria On Different Study Materials

	Enlight N = 10 (A 1)	**Cure - on - Touch N = 10 (B 1)**	**Rely - A - Bond N = 10 (C 1)**
Streptococcus mutans			
Mean weight before inoculation (gm.)	0.2406	0.1665	0.2157
Mean weight after growth (gm.)	0.2607	0.1770	0.2281
Mean weight gain (gm.)	0.0201	0.0105	0.0124
%age weight gain	8.348	6.301	5.7400
	Enlight N = 10 (A 2)	**Cure - on - Touch N = 10 (B2)**	**Rely - A - Bond N = 10 (C2)**
Staphylococcus aureus			
Mean weight before inoculation (gm.)	0.2404	0.1650	0.2160
Mean weight after growth (gm.)	0.2576	0.1744	0.2263
Mean weight gain (gm.)	0.0172	0.0094	0.0103
%age weight gain	7.147	5.686	4.7630
	Enlight N = 10 (A 3)	**Cure - on - Touch N = 10 (B 3)**	**Rely - A - Bond N = 10 (C 3)**
Pseudomonas			
Mean weight before inoculation (gm.)	0.2407	0.1676	0.2145
Mean weight after growth (gm.)	0.2538	0.1729	0.2218
Mean weight gain (gm.)	0.0131	0.0053	0.0073
%age weight gain	5.439	3.1570	3.3960

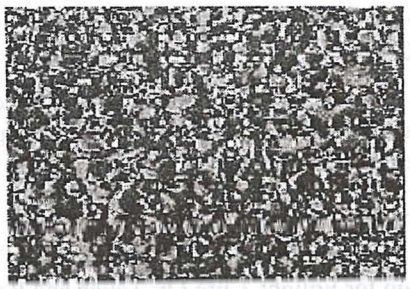


FIG 3 A

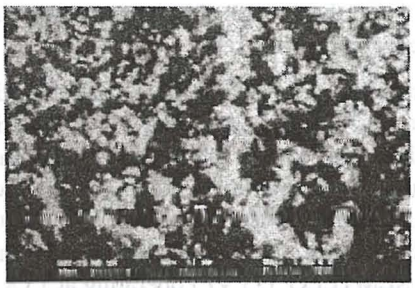


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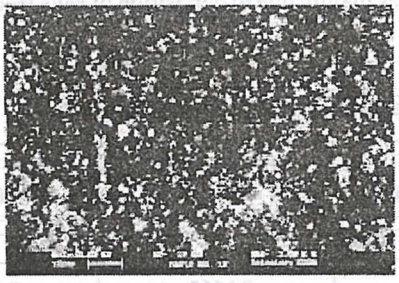


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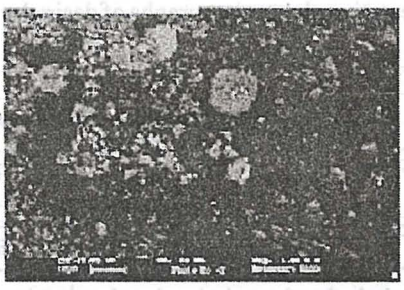


FIG 3 D

Fig 3

A: Surface Characteristics Of Rely – A – Bond (Control Group, 1000 X)
B: Surface Characteristics Of Rely – A – Bond After Growth Of Strept. mutans (1000 X)
C: Surface Characteristics Of Rely – A – Bond After Growth Of Staph. aureus (1000 X)
D: Surface Characteristics Of Rely – A – Bond After Growth Of Pseudomonas (1000 X)

Weight gain for Enlight, Cure-on-Touch and Rely-A-Bond with Streptococcus mutans was 8.348%, 6.301% and 5.7400 % respectively; with Staphylococcus aureus was 7.147%, 5.686% and 4.7630% respectively, and with Pseudomonas was 5.439%, 3.1570% and 3.3960% respectively (Table 3).

Comparison of weight gains of control group with study group and intergroup comparisons showed highly significant differences (Tables 4, 5) showing significant growth. Streptococcus mutans showed the highest growth on all the materials tested. Pseudomonas showed lowest growth on all the materials tested. Streptococcus mutans showed highest growth on Enlight and lowest on Rely-A-Bond. Staphylococcus aureus showed highest growth on Enlight and lowest on Rely-A-Bond. Growth of Pseudomonas on Enlight was highest and on Cure-On-Touch was lowest (Tables 3, 5). Enlight showed the highest weight gain among all the materials tested and Rely-A-Bond showed the least weight gain, except with Pseudomonas with Cure-On-Touch showing the least weight gain.

The SEM pictures showed a definite difference between the surfaces of pellets before the growth of bacteria (Figs. 1, 2, 3) and after the growth of bacteria, with a definite increase in roughness of the surface of pellets after bacterial growth. Comparing all the groups, the greatest damage was caused to the Enlight, and Streptococcus mutans caused the greatest damage to all the materials tested.

Table 4: Comparison Of % Weight Gain Of Control Group With The Different Study Groups

Groups	Degree of freedom	t – value
ø A – A 1	18	36.558**
ø A – A 2	18	28.758**
ø A – A 3	18	26.102**
ø B – B 1	18	18.226**
ø B – B 2	18	12.940**
ø B – B 3	18	7.488**
ø C – C 1	18	18.154**
ø C – C 2	18	15.628**
ø C – C 3	18	4.465*

Where ** $p < 0.0001$; * $p < 0.001$

Table 5: Inter-group comparison of different materials after bacterial growth

Comparison between Groups	Degree of freedom	t – value	p – value
A 1 – B 1(N= 10) each	18	7.293	$p < 0.0001$**
A 1 – C 1(N= 10) each	18	9.056	$p < 0.0001$**
B 1 – C 1(N= 10) each	18	1.866	$p < 0.01$*
A 2 – B 2(N= 10) each	18	4.080	$p < 0.0001$**
A 2 – C 2(N= 10) each	18	8.465	$p < 0.0001$**
B 2 – C 2(N= 10) each	18	2.628	$p > 0.05$ ***
A 3 – B 3(N= 10) each	18	9.317	$p < 0.0001$**
A 3 – C 3(N= 10) each	18	3.685	$p < 0.001$*
B 3 – C 3(N= 10) each	18	0.424	$p > 0.1$***

A 1 = Enlight + Streptococcus mutans
B 1 = Cure - On - Touch + Streptococcus mutans
C 1 = Rely- A- Bond + Streptococcus mutans
A 2 = Enlight + Staphylococcus aureus
B 2 = Cure - On - Touch + Staphylococcus aureus
C 2 = Rely- A- Bond + Staphylococcus aureus
A 3 = Enlight + Pseudomonas
B 3 = Cure - On - Touch + Pseudomonas
C 3 = Rely- A- Bond + Pseudomonas

** = Very highly significant
* = Highly Significant
*** = Non significant

Discussion

Microbes have been shown to grow and even decompose composites, thus weakening of bond between the tooth and the bracket [8]. These micro-organisms have been known to cause localized demineralization of enamel [9,10,11].

In this study, the comparison of % weight gain of different materials showed a very highly significant increase in weight as compared to their controls (Table 4) indicating a significant growth of microbes on all bonding materials tested. The increase in weight of the control group was mainly due to deposition of salts from the artificial saliva, which was one of the components of the culture media, whereas increase in weight of the study sample was mainly due to the growth of bacteria. So it helped to distinguish the growth of inoculated bacteria in the study group from the other environmental contaminants.

Enlight showed the highest weight gain with all the 3 bacteria when compared to other materials (Tables 3, 5). Rely-A-Bond showed the least weight gain with Streptococcus mutans and Staphylococcus aureus (Tables 3, 5) whereas, its weight gain with Pseudomonas was intermediate between Enlight and Cure-On-Touch, while Cure-On-Touch showing the least weight gain (Tables 3, 5) with Pseudomonas. Thus all the 3 materials inoculated with Streptococcus mutans showed the highest weight gain when compared with groups inoculated with other bacterial cultures. This is of great clinical significance because Streptococcus mutans has been implicated as the major cause of dental caries. Pseudomonas showed least growth on all the bonding materials with the growth of Staphylococcus aureus occurring intermediate between the two bacterial groups (Tables 3, 5).

SEM pictures revealed a definite increase in surface roughness of these materials after growth of bacteria as compared to control. Enlight showed the greatest increase in surface roughness as compared to its control. SEM figures indicated a greater roughness of Enlight and Cure- On -Touch when compared to Rely- A- Bond, (Figs. 1 - 3). The greatest surface roughness for the Enlight group occurred with Streptococcus mutans (Fig.1 B), with exposure and the degradation of fibrous matrix. No appreciable difference was noticed in the surface roughness of Enlight group when Staphylococcus aureus and Pseudomonas was inoculated (Fig.1 C, 1 D). The greatest surface roughness for the Cure- On -Touch group also occurred with Streptococcus mutans (Fig. 2 B), with the exposure of fibrous meshwork of the matrix. Surface was degraded comparatively less by Staphylococci and Pseudomonas (Fig. 2 C, 2 D). The greatest surface degradation of the Rely-A-Bond occurred with Streptococcus mutans growth (Fig. 3 B) followed by Cure-On-Touch and Rely-A-Bond (Fig. 3 C, 3 D)

The growth of streptococci appeared to have caused greatest damage to all the bonding materials when compared to other bacteria and the maximum damage occurred on Enlight. The least damage occurred on Rely-A-Bond. The damage appeared to be less on the material group inoculated with Pseudomonas.

Conclusions

Following conclusions were drawn from the study:

- Streptococcus mutans is the most commonly growing microorganisms on all the bonding materials.
- Enlight material showed the highest bacterial growth when compared to other materials.
- Rely-A-Bond showed least growth of all bacteria except with Pseudomonas which showed least growth on Cure-On-Touch.
- S E M study revealed maximum surface damage to the Enlight, mainly caused by Streptococcus mutans as compared to other bonding materials.
- Rely-A-Bond was found to be the superior material over the other bonding materials studied.

Clinical significance

In view of the above findings, the orthodontist should be cautious in choosing the bonding adhe-

sive for his patients to minimize the bond failures during treatment. Also, a proper oral hygiene protocol should be advised to minimize the growth of microorganisms during the treatment to prevent enamel damage.

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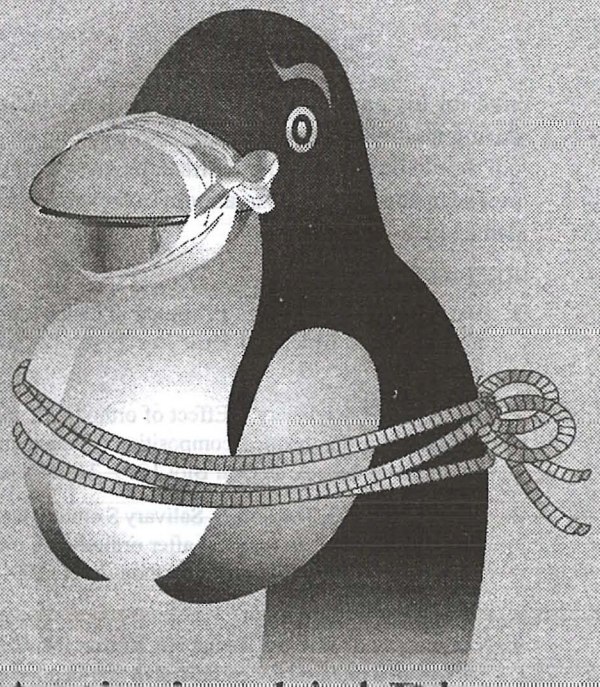
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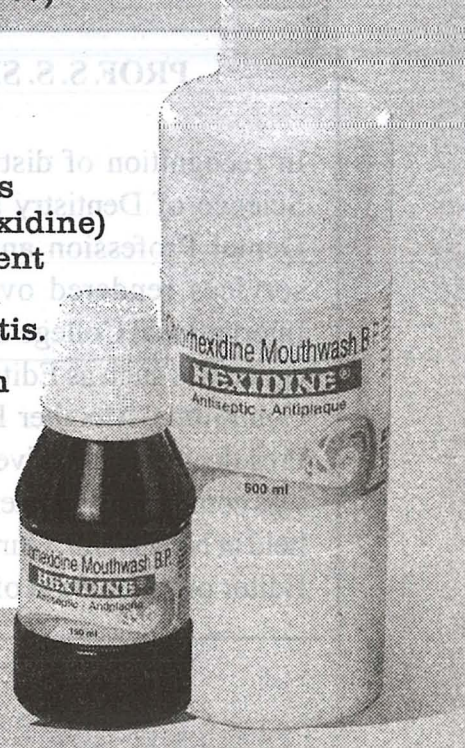


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- Carranza's Clinical Periodontology, Pg. 666, 9th edition, 2004



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