

Comparative Evaluation of Bacterial Colonization Color Stability and Halitosis of Oral Appliances Fabricated Using Cold Cure Acrylics, Heat Cure Acrylics and Thermoforming Sheets: An *In Vivo* Study

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ABSTRACT

Aim: Removable oral appliances form an integral part of interceptive orthodontic procedures. Bacterial colonization leading to halitosis and poor color stability are the major disadvantages of the same in spite of patient acceptability. The aim of the present study was to evaluate bacterial colonization, color stability, and halitosis from the oral appliances fabricated using cold cure, cold cure under pressure pot, heat cure acrylics, thermoforming sheet, Erkodur and antibacterial thermoforming sheet, Erkodur–bz.

Materials and methods: A group of 40 children was divided into five groups and the appliances were delivered. Bacterial colonization and halitosis were evaluated before giving the appliance to the patient after 1 and 2 months. Color stability was evaluated before giving the appliance to the patient and then after 2 months. This study was designed as a single-blinded randomized clinical trial.

Results: Results have shown that after 1 and 2 months, bacterial colonization was higher from the appliances fabricated with cold cure and was lower for the Erkodur group, which was statistically significant. Color stability was more from the appliances fabricated with Erkodur and was less for cold cure, which was statistically significant. Halitosis after 1 month was more from the appliances fabricated with cold cure and less from Erkodur group, which was statistically significant. After 2 months, halitosis was more from the cold cure group and less from Erkodur group, which was not statistically significant.

Conclusion: Thermoforming sheet, Erkodur showed better results than other groups in terms of bacterial colonization, color stability, and halitosis.

Clinical significance: When removable appliances are indicated for minor orthodontic tooth movement, Erkodur is preferable due to the advantages of easy fabrication and less bacterial colonization.

Keywords: Color stability, Erkodur, Erkodur–bz, Halitosis, Thermoforming sheets.

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INTRODUCTION

Removable appliances are widely used in pediatric dentistry for the interception and prevention of various malocclusions. Acrylics have been used for the fabrication of appliances since their introduction into dentistry. Apart from advantages of acrylics like their acceptability, esthetics, and desirable characteristics like easy handling, studies are showing some limitations like allergy, cytotoxicity, malodor, surface discoloration, surface roughness, bacterial colonization, and also recent studies have shown the leaching of toxic products from the acrylic resins.¹ So, due to the above limitations, thermoforming materials have acquired existence within the field of dentistry.

Thermoforming is a process during which a thermoplastic sheet is heated, deformed by a shaping force, and allowed to set in the new shape on cooling. Appliances fabricated with this technique have many advantages over acrylics which include esthetics, ease of fabrication, uniform thickness, negligible leachability, consume less time for fabrication, minimal shrinkage, more acceptance from the patient, selection of colors, etc. Antibacterial properties have also been incorporated into thermoforming sheets, which decrease bacterial colonization and surface discoloration. In regard to that, Erkodur–bz thermoforming sheets with antibacterial properties were newly introduced by Erkodent Co, Germany. To the best of our knowledge, no

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studies were reported on Erkodur–bz, and literature is scantily comparing the thermoforming sheets (Erkodur and Erkodur–bz) with cold cure (cold cure and pressure pot) and heat cure acrylics with respect to bacterial colonization, color stability and halitosis from the removable appliances in children. So, these parameters were taken into consideration and evaluated in the present study.

MATERIALS AND METHODS

- Cold cure (auto polymerizing) acrylic resin (rapid repair denture material and Dentsply).
- Heat polymerizing resin (Trevalon and Dentsply).
- Thermoforming sheets viz., Erkodur 2 mm, Erkodur-bz 2 mm (Erkodent).
- Erkopress thermoforming machine.
- Spectrophotometer.
- HC-212SF FitScan breath checker.

This study was conducted on 40 children aged between 6 and 14 years reporting to the Department of Pedodontics and Preventive Dentistry.

Selection Criteria

The subjects selected for the study were cooperative children requiring interceptive orthodontic therapy and preventive oral appliance therapy for 2 months with good general health, without any dental pain or abscess. Patients under antibiotic therapy, allergic to acrylics, with systemic diseases, and differently abled/special children were excluded from the study.² A total of 40 children were randomly divided into five groups; group I, appliances fabricated with cold cure acrylic material; group II, appliances fabricated with cold cure acrylic material cured by pressure pot technique; group III, appliances fabricated with heat cure acrylic material; group IV, appliances fabricated with a thermoforming sheet containing PETG (polyethylene terephthalate-glycol), Erkodur of 2 mm thickness; group V, appliances fabricated with a thermoforming sheet containing PETG and antibacterial additive pigment, Erkodur-bz of 2 mm thickness. When the thermoforming sheet of 2 mm thickness is used, it gets reduced to an appliance of 1.75 mm thickness. For standardization, the appliances in all the groups were maintained at a thickness of 1.75 mm. Later, the appliances were inserted into the patient, and the three parameters were evaluated.

Evaluation of Bacterial Colonization

After the insertion of the appliances, bacterial colonization was evaluated after 1-month and 2 months. Baseline bacterial colonization was taken as 0. A 1 × 1 cm² was marked on the palatal tissue surface of the appliance. A sterile cotton swab was taken and swabbing was done on that area. Then, the swabs were transferred

into a transport medium (peptone water) and so on to the blood agar culture media and incubated at 37°C for 24 hours, and eventually, the number of colonies forming units (CFU) was counted.

Evaluation of Color Stability

For all the groups, 1 × 1 cm × 1.75 mm blocks were prepared according to the respective fabrication techniques. And baseline color of each material was determined. Based on this, all the appliances were prepared by the identical fabrication technique and at the same thickness (1.75 mm) and delivered to the patients. After 2 months, the patient appliances were collected and placed in artificial saliva and taken for the evaluation of color by using a spectrophotometer in Commission International de L'Eclairage (CIE) L*a*b color scale.

Evaluation of Halitosis

Halitosis was evaluated by employing a portable breath checking instrument, FitScan breath checker. It gives the readings from 0 to 5. The child was made to sit in an upright position and asked to exhale through the mouth. The breath checker was placed 1 cm away from the mouth such that the exhaled air passes into the sensor and the reading is displayed. As halitosis differs from one child to the other, baseline halitosis was taken for each patient just before the insertion of the appliance. Later, the halitosis was evaluated with the appliance in the patient's mouth after 1 and 2 months.

RESULTS

The results were statistically analyzed by one-way analysis of variance (ANOVA) test. After 1 and 2 months bacterial colonization was higher for the cold cure group, followed by cold cure under pot pressure, Erkodur-bz, heat cure, and Erkodur, which was statistically significant (p -value < 0.05) (Table 1). Color stability was more for the Erkodur group, followed by heat cure, Erkodur-bz, cold cure under pot pressure, and cold cure, which was statistically significant (p -value < 0.05) (Table 2). Baseline halitosis was more for heat cure followed by cold cure under pot pressure = cold cure > Erkodur-bz = Erkodur, which wasn't statistically significant (p -value > 0.05). Halitosis after 1 month was more for the cold cure group, followed by cold cure under pot pressure = heat cure > Erkodur-bz > Erkodur, which was statistically significant (p -value < 0.05). After 2 months, halitosis was more for the cold cure group, followed by cold cure under pot pressure, Erkodur-bz, heat cure, and Erkodur which was not statistically significant (p -value < 0.05) (Table 3).

DISCUSSION

Removable appliances are widely used in pediatric dentistry as space maintainers, habit-breaking appliances, for tipping teeth, overbite reduction, etc. Since their introduction, acrylic-based resins have been frequently used for their fabrication. The literature revealed that acrylics cause bacterial colonization,

Table 1: Comparison of bacterial colonization after 1-month and 2-month follow-up between five groups by one-way ANOVA test

Groups	After 1-month follow-up	After 2 months of follow-up
	Mean ± standard deviation (SD)	Mean ± SD
Cold cure	4000 ± 1195.2	5750.0 ± 1982.0
Cold cure under the pot pressure	2937.5 ± 1613.2	3562.5 ± 1801.5
Heat cure	1462.5 ± 663.1	2100.0 ± 960.6
Erkodur	900.0 ± 256.3	1250.0 ± 558.0
Erkodur-bz	2437.5 ± 495.5	2887.5 ± 322.6
f-value	12.474	13.7
p-value	0.000	0.000
	Significant	Significant

Table 2: Comparison of color change after 1-month follow-up between five groups by one-way ANOVA test

Groups	Mean ± SD	f-value	p-value
Cold cure	11.8050 ± 2.3	15.400	0.000
Cold cure under the pot pressure	8.2013 ± 3.1		Significant
Heat cure	7.1625 ± 1.3		
Erkodur	4.3863 ± 0.6		
Erkodur-bz	7.7563 ± 0.3		

Table 3: Comparison of halitosis between baseline, after 1-month, after 2 months follow-up between five groups by one-way ANOVA test

Groups	Baseline	After 1 month	After 2 months
	Mean \pm SD	Mean \pm SD	Mean \pm SD
Cold cure	0.1250 \pm 0.35	0.8750 \pm 0.64087	1.5000 \pm 0.53452
Cold cure under the pot pressure	0.1250 \pm 0.35	0.7500 \pm 0.70711	1.1250 \pm 0.99103
Heat cure	0.2500 \pm 0.46	0.7500 \pm 0.46291	0.6250 \pm 0.51755
Erkodur	0.0000 \pm 0.00	0.1250 \pm 0.35355	0.5000 \pm 0.53452
Erkodur–bz	0.0000 \pm 0.00	0.2500 \pm 0.46291	0.7500 \pm 0.46291
f-value	0.942	3.116	3.285
p-value	0.451	0.027	0.022
	Not significant	Significant	Significant

surface discoloration, and halitosis. In regard to these factors, research is incredibly scanty comparing the removable appliances made with acrylics and the thermoforming sheets.³ So, there is a need for the present study.

Bacterial Colonization

Pathak and Sharma⁴ studied biofilm associated microorganisms on removable orthodontic appliances in 25 children between 10 and 14 years in the mixed dentition. Different studies have suggested that oral bacteria may be risk factors for a variety of prevalent systemic diseases like bacterial endocarditis, aspiration pneumonia, gastrointestinal infection, chronic obstructive pulmonary disease etc., and removable appliances offer as a scaffold for microorganisms related to these infections.⁵ According to Sumi et al., acrylic bases act as a reservoir of respiratory pathogens and can be a risk factor for pharyngeal colonization and aspiration pneumonia.⁶

Bjerklin et al.⁷ reported higher proximal caries progression on canines, premolars, and molars among children treated with removable orthodontic appliances. Batoni et al.⁸ studied the prevalence of *mutans Streptococci* in children with removable orthodontic appliances and found that 83% of children undergoing orthodontic treatment have bacteria in dental plaque.

The palatal unpolished rough surface of acrylic makes a favorable area for bacterial and candidal accumulation due to the hampered natural cleaning mechanism of saliva.⁹ Paranhos et al. showed that the palate has high retention for microorganisms.¹⁰ The fitting area has the greater potential for the collection of debris and microorganisms.¹¹ Eichenauer et al.¹² proved that there were greater densities of microorganisms on the covered area of the palate. Decelis et al.² took swabs from the palatal surface of the tissue surface of the appliances and studied the effect of nitradine on the candida levels of removable maxillary appliances. So, in the present study, the swabs from the palatal unpolished (rough) surface of the appliance were collected to evaluate the CFU.

In the present study, an increase in bacterial colonization after 1 month was cold cure followed by cold cure under pressure pot, Erkodur–bz, heat cure, and Erkodur. After 1 month, bacterial colonization was less for the Erkodur group, followed by the heat cure group, Erkodur–bz, cold cure under pressure pot, and cold cure group. After 2 months, in the Erkodur group, bacterial colonization was less, followed by the heat cure group, Erkodur–bz, cold cure under pressure pot, and cold cure group. In the present study, Erkodur was effective in decreasing bacterial colonization. This can be explained by the fineness and uniform thickness of the polished

surface, which causes decreased food and debris accumulation and decreased bacterial colonization.

Though the antibacterial additive was incorporated in Erkodur–bz, it was not effective in decreasing bacterial colonization. This may be explained by the fact that the antibacterial pigment component might not be that effective in decreasing bacterial colonization, and the duration that it is active in the patient's mouth is questionable. For the fabrication of any appliance with thermoforming, near the wire components, acrylic component given by the manufacturer must be added 10 seconds before thermoforming. In the present study, it was observed that the acrylic component wasn't merging so precisely near the wire bending in Erkodur–bz, leading to more bacterial colonization, whereas, for Erkodur, the compatibility is good leaving a finely finished surface near the wire components, thereby less food and debris accumulation and fewer bacterial colonization. If the increase in bacterial colonization from 1 to 2 months is considered, Erkodur–bz was more effective than heat cure but less than Erkodur. This study was done just for 2 months. So, further long-term studies are necessary to judge the long-term effectiveness of this material.

When cold cure, cold cure under pressure pot, and heat cure groups were considered, there was decreased bacterial colonization for heat cure groups. This is in accordance with Domenyuk,¹³ who he found that there was less bacterial colonization for heat cure appliances than cold cure appliances. This was probably because the surface roughness and porosity of the cold cured material were different from that of the heat cured material because of the different processing conditions used. Heat cured dentures typically exhibit lower porosity and less bacterial colonization compared with cold cure dentures.¹⁴ The same reason can be taken to explain the decreased bacterial colonization of thermoforming sheets. Due to the absence of residual monomer and, thereby the porosity, the absence of trimming and polishing causes no surface roughness and thereby, decreased bacterial colonization.

Color Stability

Color stability is the property of any material to retain its color in any specified environment over a period of time. Clinical and *in vitro* studies have implicated dietary compounds as a major etiological factor in the staining of acrylic materials. It is well known that ingredients in Indian food, like turmeric and a few artificial dyes employed in food, may increase the discoloration of denture base polymers. So, the appliances, upon contact with various food materials and beverages in the oral cavity, absorb various contaminants/coloring agents and are subject to sorption (a process of absorption and adsorption of liquids), resulting in

possible discoloration.¹⁵ Discoloration becomes more intense with an increase in exposure time to the food materials.

The CIE Lab uniform color scale has been employed in the current study. In the CIE color system, L^* is defined as the value, or lightness/darkness of the precise color, whereas a^* and b^* specify the particular hue or color. Positive a^* values indicate a predominance of red hue, while negative values indicate a predominance of green. Positive b^* values indicate a bent toward yellow, whereas values within the negative range demonstrate a bluish tone.

In the present study, color measurements were made within the palatal area for every appliance. This area was selected because, for the fabrication of thermoforming appliances, the acrylic component was added near the wire bending. So, the palatal area was freed from the acrylic component and hence was used for the assessment of color. The spectrophotometer was used to evaluate color stability. This can be in accordance with Regis et al.,¹⁶ Subramanya and Muttagi,¹⁷ Singh and Aggarwal,¹⁵ where a spectrophotometer was used to study the color changes. L , a , and b values were obtained before and after giving the appliance to the patients. color variation of specimens ΔE was calculated between the initial measurement and after 2 months using the subsequent equation through the formula $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$. A ΔE value of >3.3 is perceived clinically.

In the present study, Erkodur has more color stability than the opposite groups, followed by the heat cure group, erkodur-bz, cold cure under pressure pot, and cold cure. Erkodur was more color stable due to the absence of residual monomer, uniform thickness, and superior finish. This is in accordance with Bechir et al.,¹⁸ who concluded that appliances fabricated with the vacuum-forming method are more color stable than heat cure material. Erkodur shows more color stability than Erkodur-bz. This could be explained that the pigment component added to decrease the surface discoloration might not be active for a long duration.

Pigment in Erkodur-bz was less compatible with the acrylic component near the wire bending resulting in food and debris accumulation and possible discoloration. Whereas for the Erkodur group, the compatibility is good, leaving a finely finished surface near the wire components, thereby less food, and debris accumulation and thereby less discoloration. Erkodur-bz exhibits the property of superior color stability than the cold cure group under pressure pot and cold cure groups due to the absence of residual monomer. Literature is extremely scanty comparing the color stability of thermoformed sheets with cold cure acrylics. Regarding the color stability of Erkodur-bz and heat cure, there was less difference between the two groups, with a p -value (0.97) not significant. And heat cure material is more color stable than the cold cure group with a p -value of 0.000, which is significant.

The present study was parallel to the findings in the studies found in the literature that heat cure acrylics are more color stable than cold cure acrylics. The residual monomer content was accountable for the color changes observed. Austin et al.¹⁹ explained that denture base materials processed by a cold polymerized method have demonstrated up to seven times the level of residual monomer found in conventional heat polymerized materials.

Purnaveja et al.²⁰ showed that there was greater chromatic instability for the cold cure resins since these present a great amount of additional reagents, such as an amine accelerator and benzoyl peroxide^{16,21} which remain after polymerization and alters the color. Thus, cold cure resins are less color stable than heat cure acrylic resins, and the same result was obtained within the present study.

Halitosis

Bad breath (halitosis, *fetor ex ore*) is usually considered to result from the production of malodorous compounds by oral bacteria. Among the oral microorganisms, a variety of gram-negative bacteria are particularly odorogenic, like *Klebsiella* spp., *Porphyromonas* spp., *Prevotella* spp., and *Fusobacterium* spp. etc. According to Goldberg et al., malodor is 13% among orthodontic patients.²² Many oral microbes produce volatile sulfur compounds, which are the prominent elements of oral malodor. Literature is scanty comparing halitosis from orthodontic appliances manufactured from different materials. So, there's a need for this study.

In this study, the FitScan breath checker was used to measure halitosis. When the patient exhales the air from the mouth into the sensor of the instrument, it gives readings from 0 to 5 on the digital display. The 0—no odor, 1—slight odor, 2—moderate odor, 3—heavy odor, 4—strong odor, and 5—intense odor.

Baseline halitosis was more for the heat cure group followed by cold cure and cold cure under pressure pot, and it was least for both Erkodur and Erkodur-bz groups, which is statistically not significant (p -value is 0.451). The measurement of halitosis after 1 month was more for the cold cure group followed by cold cure under pressure pot and heat cure, Erkodur-bz group, and it's least for the Erkodur group, which is statistically significant (p -value is 0.027). Regarding halitosis after 2 months, it's more for the cold cure group followed by cold cure under pressure pot and Erkodur-bz group heat cure, and it is least for both Erkodur which is statistically significant. The change in halitosis after 2 months was highly significant for the cold cure group, significant for cold cure under pressure pot and Erkodur-bz, and for Erkodur, not significant for the heat cure group.

Increased halitosis is observed after the removable appliance therapy in all the groups. The possible reason is that the appliance itself will support the development of denture plaque and calculus but presents different environments to the colonizing species. The fitting surfaces are exposed to high nutrient concentrations and low salivary flow rates, and provide a roughened topography to support and protect the plaque. This is also in accordance with Babacan et al.,²³ who found that the oral malodor increased and reached a critical level during fixed orthodontic treatment. Coulthwaite and Verran²⁴ studied the presence of malodor in orthodontic treatment. Thus, during the orthodontic treatment for children, malodor should not be neglected.

Finally, the current study was done on 40 children for a follow-up of 2-months. These types of studies need long-term follow-up with more sample sizes. Even collection of systemic samples like blood, and urine may additionally help in assessing the reaction of these materials with the systemic circulation. Further research is necessary to conclude the above findings.

CONCLUSION

The following conclusions were drawn from the present study:

- Among all the groups, Erkodur was more effective for all the parameters evaluated within the present study.
- Heat cure acrylic appliances come next to Erkodur in all the parameters.
- The Erkodur-bz group was also equally effective as heat cure showing statistically no significant difference for all the parameters evaluated.
- Cold cure acrylics showed inferior properties for all the parameters evaluated.
- Thus, this study could be helpful in guiding us in selecting an appropriate material for the fabrication of removable appliances.

Clinical Significance

Erkodur is preferable when removable appliances are indicated for children as they have the versatility of easy fabrication, less bacterial colonization, and less halitosis.

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