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**An investigation of the effect of cleaning solutions on plaque removal, surface roughness and hardness of oral appliances**

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[redacted]

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19 Dec 2018

# USAF Graduate Prosthodontics

# Master of Science Committee

## Report on Oral Examination

We the undersigned, as participants of the USAF Master of Science Committee, report that on 19-Dec-18 we examined Capt Theresa Ki m regarding (his (her) (protocol / thesis) defense for a research project entitled: An investigation of the effect of cleaning solutions on plaque removal, surface roughness and hardness of occlusal device.

By signing our names below we recommend (approval/disapproval) for the aforementioned resident to proceed with the process of obtaining a Master of Science degree from the Uniformed Services University of the Health Sciences (USUHS).

[redacted]
AF/USUHS Prosthodontist

[redacted]
Primary Mentor

[redacted]
Non-USUHS Prosthodontist

Committee Member

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USUHS Faculty

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USUHS Faculty

Additional Individual

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Director of Research, 59 DTS/SGDTP

Additional Individual

Comments/Recommendations:

- THESIS ONLY
- SIGNIFANT WORK INTO "WITY" INFORMATION APPEARS THAT WAY
- CONSIDER IF ACCOMPLISHING LIMITED SAMPLES
- REVISIT STATISTICS / RAW DATA

# An investigation of the effect of cleaning solutions on plaque removal, surface roughness and hardness of oral appliances.

Original Research

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ABSTRACT:

*Purpose:* The purpose of this *in vitro* study was to compare the effectiveness of cleaning solutions on bacterial plaque removal and changes in surface roughness and hardness of the oral appliances.

*Material and Methods:* After Proform Soft EVA and Essix A+ specimens (n=96 each) were immersed in artificial saliva with *Streptococcus mutans* for eight hours, the following cleaning steps were applied daily at room temperature for 15 days, using Dawn dish soap, Listerine antiseptic mouth rinse, Retainer Brite tablet, or TheraBreath mouth rinse: Treatment (1) Cleaning solutions for 16 hours (as a positive control), (2) Cleaning solutions for 10 min and bench dry for 16 hours, (3) Cleaning solutions for 10 min and immersed in water for 16 hours, and (4) Immersed in water for 16 hours (as a negative control).

*Results:* Type of cleaning solution had significant effect on surface roughness ($p=0.0145$). Dawn caused a significant decrease in roughness when compared to Listerine or TheraBreath, which caused a significant increase ($p < 0.05$). Type of cleaning solution and treatment had a significant effect on hardness ($p=0.001$). Listerine caused the most variation and TheraBreath caused a decrease in hardness regardless of the treatment type. The overall mean for the change in bacterial growth of TheraBreath was greater than other cleaning agents, and the significant interaction effect indicates that Treatment 2 yielded the most bacterial growth with TheraBreath.

*Conclusion:* Dawn caused the least changes in roughness and hardness of the oral appliances, and still had good antibacterial properties. Listerine can be a good antibacterial solution but may affect structural longevity of the prosthesis. TheraBreath may need to be re-considered as an antiseptic solution.

Numerous types of oral appliances are available for multiple purposes in the field of dentistry. Historically, protective guards have been worn during sports to prevent physical trauma from impact and night guards are worn to protect dentitions from parafunctional habits during sleep. They are also widely accepted as the materials for clear orthodontic braces, temporary retainers, bleaching trays, occlusal splints, and useful in treating Temporomandibular disorder (TMD) or Obstructive Sleep Apnea (OSA). The rigidity varies depending on the purpose of the appliances and also types and composition of the ingredients. Mainly, oral appliances can be divided into three types: hard, soft and dual-laminate (hard and soft). Ethylene vinyl acetate co-polymer (EVA) is a soft, "rubber-like" material that is commonly used for soft oral appliances. The higher percentage of EVA in the material, the softer and more flexible it is. The hard component can be fabricated using heat or chemically polymerized poly (methyl methacrylate) (PMMA), polyurethane or other thermoplastic or thermosetting polymers. Combination of hard and soft materials has shown to increase dimensional stability and resistance to forces and distortions[1] from repetitive intraoral movement and two/three-body wear in addition to repeated temperature fluctuation [2,3].

Efficient plaque removal is one of the crucial factors in maintaining the integrity of the oral appliances. Once a layer of biofilm accumulates, it creates a rough interface that leads to more bacterial adhesion. If not cleaned efficiently, remaining plaque would harden and become more difficult to remove unless it is scraped or brushed, leading to irreversible surface damage. Numerous *in vitro* and *in vivo* studies have aimed to establish evidence-based guidelines for cleaning acrylic resin prostheses, and utilization of both chemical and mechanical debridement were found to be most effective in biofilm removal[4-14]. Studies have investigated brushing, ultrasonic debridement, using chemical antibacterial and antifungal agents to reduce and/or inhibit biofilm accumulation. *Streptococcus mutans, Candida albicans, Staphylococcus aureus* are some of the commonly investigated bacteria for these studies since they are proven to affect the oral and systemic health, especially for immunocompromised patients.

In addition to commercial denture cleansers or dental-related disinfectants, research on organic acids and essential oils (EO) have increased during last two decades[15-19]. Chlorine dioxide ($ClO_2$)[20] and chlorhexidine (CHX)[21] containing mouth rinses are also available and are also studied as irrigants for endodontic procedures. When studies have compared the antibacterial properties of chlorhexidine (CHX) with EO- containing mouth rinses, inhibited biofilm growth was noted with 0.12% CHX and EO. Van Leeuwen's systematic review[18] concluded that CHX had better plaque control, but long-term control of gingivitis was similar for both EO and CHX as EO effectively reduces supra and subgingival bacteria[19]. EO could be an adequate alternative to CHX since CHX is proven to have some side effects, such as stain or abrasiveness to acrylic resin dentures [16,17]. $ClO_2$ also has shown to have comparable efficacy to CHX in plaque reductions[20]. $ClO_2$ works as an oxidizing agent and disrupts bacterial cell membrane, and has proven to decrease sulfur-producing bacteria, therefore helping with treating halitosis[21].

Despite the years of research comparing the cleaning solutions and methods of the prostheses, a review of interventions for denture cleaning[7] and a survey of dental health professional's recommendations for home care[8] have revealed no consensus on the most appropriate cleaning methods. Also, compared to acrylic dentures, oral appliances have higher risks of distortion and plaque accumulation since they are composed of a series of wells, with thinner and less rigid material. Studies on orthodontic retainers have addressed that the differences in interface dynamics would potentially influence the way biofilm forms[22]. Few studies are available on orthodontic appliances[23-26] and they compared the effects of denture cleaners and other commercially available products with mechanical debridement on thin Essix samples or Hawley-retainer designs.

The purpose of this study was to investigate the effect of various over-the-counter cleaning solutions on the *surface roughness* and the *hardness* of oral appliances and to measure the *efficacy* on plaque removal. The effects of cleansers can easily be studied outside the mouth by simulating the

environment for overnight bacterial growth and biofilm accumulation, and then observing the antibacterial and altered properties due to the cleansers and storage conditions. Our main focus was to standardize the occlusal device homecare to maximize the longevity and maintain the optimal condition of the material. Mechanical regimen would enhance the effect of plaque removal [8,16]; however, this study will examine the use of over-the-counter cleaning solutions alone, on plaque control and surface integrity of oral appliances.

***Materials and Methods***

Four cleaning agents are: Retainer Brite® (Dentsply, Saratoga, FL) mainly composed of potassium monopersulfate and sodium perborate, Dawn® (Proctor and Gample, Cincinnati, OH) dish soap, Listerine® (Johnson & Johnson, New Brunswick, NJ)- antiseptic mouth rinse with alcohol, TheraBreath® (Dr. Harold Katz, LLC, Los Angeles, CA)- mouth rinse with stabilized chloride oxide ($ClO_2$).

Two commercially available thermoplastic materials are used in this study: Pro-form Soft EVA (Keystone, Myerstown, PA) is a clear and flexible EVA material, and Essix A+ (Dentsply, Sarasota, FL) is a clear and hard material that is composed of polypropylene/ethylene copolymer. A PMMA mold was printed using 3D printing machine (Viper si2 SLA System, 3D systems, Rock Hill, SC). Specimens (n=96 hard and 96 soft) had two 8mm x 8mm square wells in 12mm x 8mm x 36mm rectangular bars, fabricated using a Biostar® Vacuum Forming instrument (Biostar® VI 115V, Scheu, Germany). Each specimen was polished, sprayed with Envirocide (Metrex Research, Romulus, MI) disinfectant spray, gently rinsed with deionized water, air dried, and then stored in air-tight container. (Figure 1)

The experimental specimens were immersed in 10ml of artificial saliva, containing oral bacteria, *Strep mutans*, at $10^6$ CFU/ml concentration. The artificial saliva was prepared as described by Lata et al (2010): $Na_3PO_4$ - 3.90 mM $NaCl_2$ - 4.29 mM KCl - 17.98 mM $CaCl_2$ - 1.10 mM $MgCl_2$ - 0.08 mM

$H_2SO_4$ - 0.50 mM $NaHCO_3$ - 3.27 mM, distilled water (DW), with a pH set to a level of 7.2. A pH meter (Accumet XL50, Fisher Scientific) was used to measure pH.

Bacterial isolates *Strep mutans* (ATCC 25175) were cultured on Trypticase Soy Agar with 5% Sheep blood (TSAII) and incubated at 35 $\pm$ 2 °C in ambient air for 48 hours. An inoculation suspension of *Strep mutans* was prepared by harvesting growth of the organism form TSA II and suspending it in sterile saline to a turbidity equal to 0.5 McFarland turbidity standard (approximately 1.5 x $10^8$ CFU/ml), then a 1:100 dilution of the suspension was made resulting in an inoculum suspension of approximately 1.5 x $10^6$ CFU/ml.

Each specimen was covered with 4 mL of inoculation suspension in a 5 mL tube and incubated at 35 $\pm$ 2 °C in ambient air for 8 hours. After incubation the specimens were removed, and specified cleaning steps were as follows for each treatment group at room temperature (21$\pm$2°C): Treatment group 1 (T1) was immersed in cleaning solutions for 16 hours after a gentle rinse with DW. This group served as a positive control. Treatment group 2 (T2) was rinsed with DW, immersed in cleaning solution for 15 min, rinsed with DW, and then bench-dried at room temperature for 16 hours. Treatment group 3 (T3) was rinsed with DW, immersed in cleaning solution for 15 min, rinsed with DW, and then immersed in DW for 16 hours. Lastly, Treatment group 4 (T4) was immersed in DW for 16 hours after a rinse. This group served as a negative control. Specimens were treated every day for 15 days.

*Microbial counts*

On Day 0 and Day15, after 16 hours of immersion in cleaning agent, the specimens were removed and washed with sterile saline to remove unattached bacteria. They were transferred to a conical tube containing 10mls of sterile saline, vortexed, and mixed for two minutes to remove organisms from the mouth guard. The saline was serially diluted and plated on TSA II. All plates were incubated at 35 $\pm$ 2

°C in ambient air for 48 hours. After incubation CFU's on the plates were counted and CFU/ml recovered were calculated to evaluate the effectiveness of the cleaning solutions.

*Surface Roughness and hardness test*

The surface roughness of specimens was measured at the designated corner of the well, at the interval of 2µm on Day 0 and 15, using a non-contact 3D Laser Scanning Confocal Microscope (VK-X250, Keyence, Itasca, IL) at 10x magnification. Figure 2 shows the topography images captured by the laser microscope. The measurements were compared and analyzed using software (VK Analyzer, Keyence). Durometer PTCR 307L ASTM (PTC Instrument, Los Angeles, CA) was used to measure the hardness at Day 0 and 15 on a flat end of the specimen.

*Statistical analysis*

Statistical analysis was carried out using SAS version 9.4 (Statistical Analysis Software, Cary, NC). A three-way analysis of variance (ANOVA) was used to evaluate material, cleaning agent, and treatment factors, and the interaction effects for dependent variables (a change in surface roughness; a change in hardness; a change in bacterial growth). Significant effects amongst main factors or interaction effects were determined by appropriate Tukey's Post Hoc multiple comparison tests ($\alpha = 0.05$) with Bonferroni correction.

***Result***

Change in bacteria growth (Figure 3)

The ANOVA result on the change in bacteria growth found that all the main and interaction effects were significant. The significant three-way interaction among material, cleaning agent, and treatment ($F(9, 160) = 4.39$, $p < 0.0001$) indicates that the main effect of material, cleaning agent or treatment depended on the different combination of those three factors. The overall mean for the change in bacteria growth of TheraBreath was the larger than other cleaning agents, but the significant interaction effect indicates that the bacteria growth of TheraBreath was worst when T2 was used. The material only made a significant difference in the combination of TheraBreath and T2 (851.33 CFU/ml for hard material vs. 1135.83 CFU/ml for soft material).

Change in surface roughness (Figure 4)

The ANOVA result on the change in surface roughness found a significant main effect for the cleaning agent ($F(3, 160) = 3.62$, $p = 0.015$). No other main or interaction effects were significant. The post hoc comparisons among the cleaning agents showed that the change of Dawn (-0.004 ± 0.014mm Ra) was significantly different than the change of Listerine (0.001 ± 0.003mm Ra) and TheraBreath (0.001 ± 0.005 mm Ra).

Change in hardness (Figure 5)

The ANOVA result on the change in hardness found that the main effects were significant for the cleaning agent ($F(3, 160) = 4.19$, $p = 0.007$) and the treatment ($F(3, 160) = 8.99$, $p < 0.0001$). The interaction effect between the cleaning agent and treatment ($F(9, 160) = 3.98$, $p = 0.0001$) was also significant, which indicates the main effect of cleaning agent or treatment depended on the different combination of those two factors. For both materials, Listerine had the largest decreases in hardness

when T4 was used, while it had the largest increases in hardness when T2 or T3 was used. Overall, TheraBreath had decreases in hardness regardless of different treatments, except for T3 with hard material.

***Discussion***

The duration of the study, 15 days, was designed to mimic wearing the appliance overnight (eight hours) and its handling and storage (16 hours) for two weeks. Shore D hardness scale was used to measure the changes in hardness, since Shore 00 or A scales are for softer polymers. During the study, the hardness of soft and hard specimens ranged between 69 and 87, and between 83 and 100, respectively.

When compared, the mean values of hard (n=96) to soft (n=96) material, soft specimens had greater changes for roughness and bacterial growth, possibly due to more porous surfaces than the hard specimens.

When compared the mean values of the cleaning agents (n=48 each), TheraBreath had the largest change for bacterial growth for T2 only and decrease in hardness. Dawn had overall decrease in surface roughness, and Listerine and Retainer Brite had increase in hardness for soft specimens.

When compared the mean values of type of treatments (n=48 each), T2 (clean and then store in water) had the largest value for bacterial growth, most likely due to TheraBreath's ineffectiveness as an antibacterial agent for *Strep mutans*. Interestingly, T4 (negative control with water) showed generalized downward trend of hardness for both type of polymers. This may indicate that water absorption occurs and that it can influence the hardness of the appliance.

Studies done in the 90s have suggested that 0.2microns is the threshold roughness level for initiating bacterial adhesion on a substrate[27,28]. The duration of our experiment has created enough changes in surface roughness past the threshold and promoted bacterial adhesion. Frequent mechanical

debridement, prolonged chemical exposure, and insertion-removal of appliance in clinical setting could increase the magnitude of changes in the physical properties.

This study had aimed to establish a simple cleaning guideline for oral appliances. Based on the results, Dawn dish soap is effective in biofilm removal without altering the physical properties. Cleansing tablet and mouth rinses can affect the physical properties of the appliance, therefore, careful selection of the products is recommended.

## *Conclusion*

Compared to acrylic resin removable prostheses, thermoplastic appliances do not have established guidelines for dental health professionals to recommend to their patients. Evidence-based guidelines for dentures[4] would be a good reference for the providers, and it is crucial to review the active ingredients of the cleaning agents and their biocompatibility prior to providing recommendations. Routine maintenance and/or replacement of the appliance are critical in improving long-term prognoses of patients' dentitions, existing restorations, systemic health and patient-provider relationships.

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***Figures***

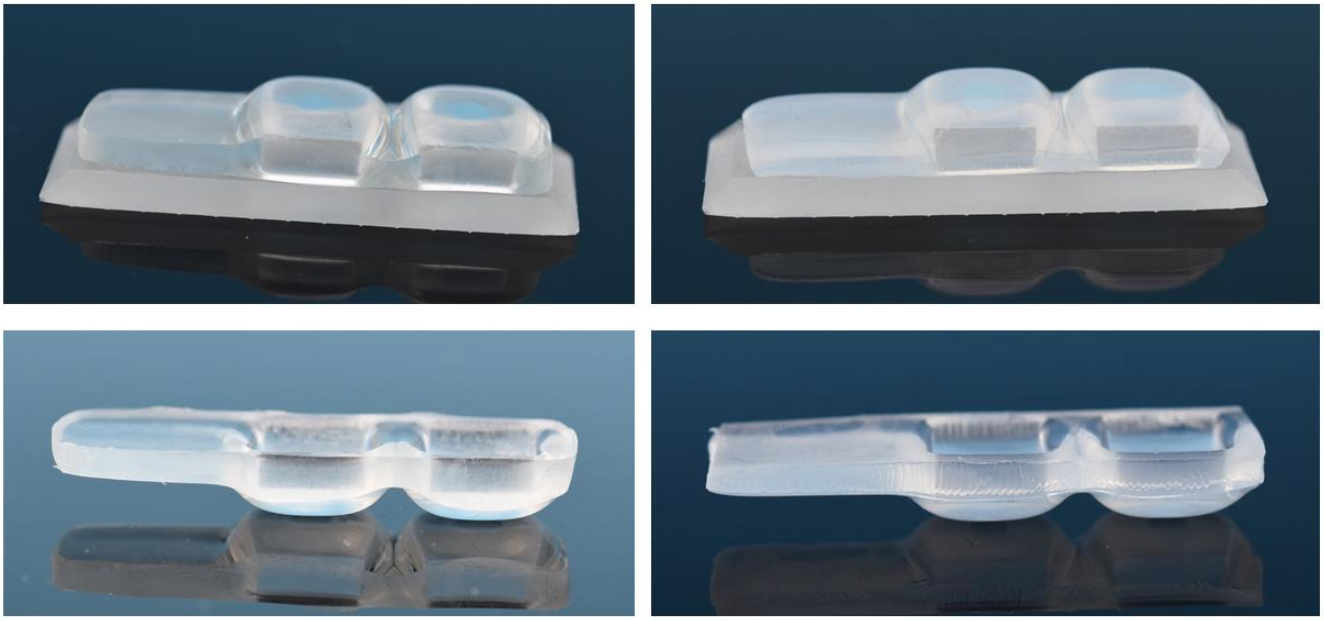


Figure 1: Fabricated specimens: hard (left column) and soft (right column) with/without PMMA mold

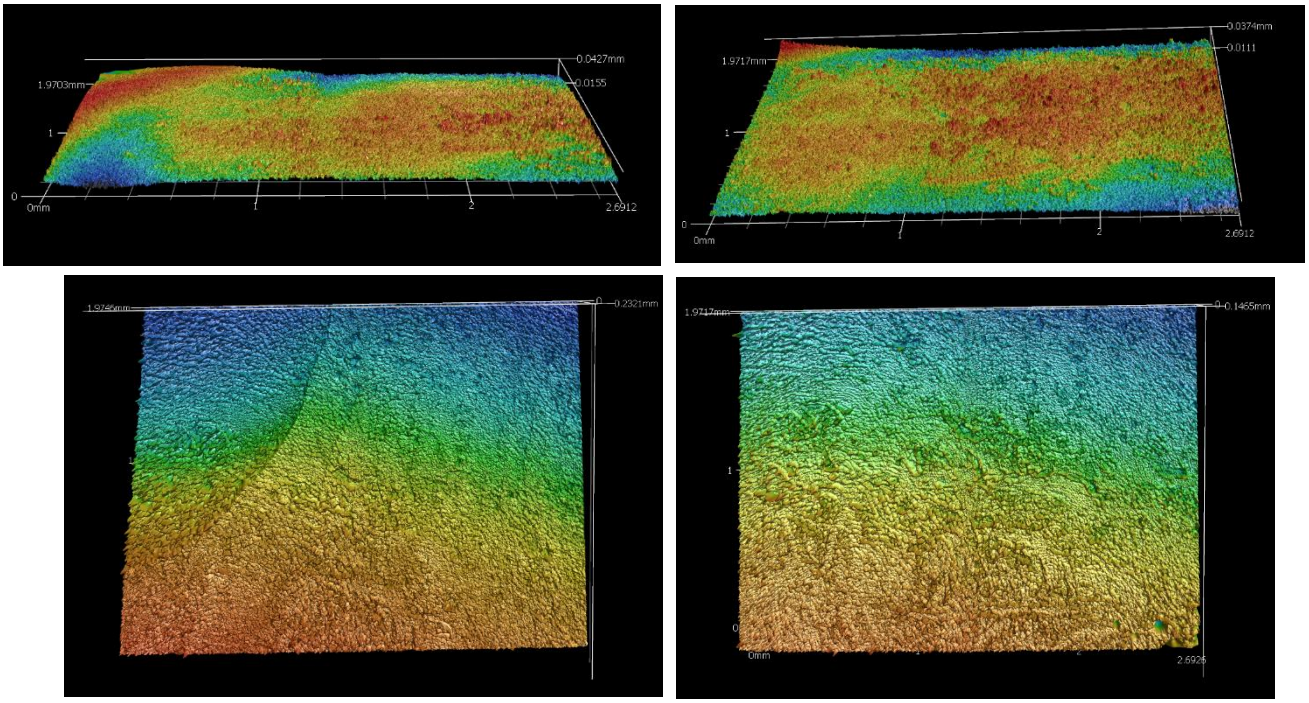
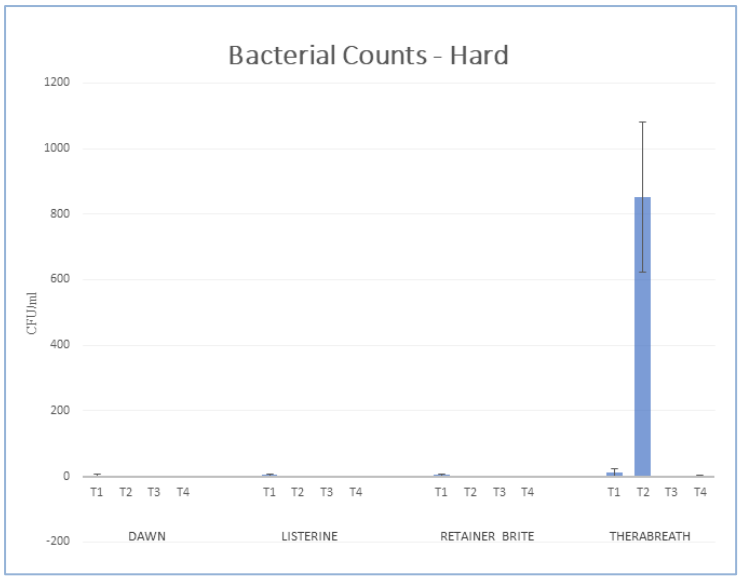



Figure 2: images captured by laser profilometer
(*Top row*: Dawn T1, hard, Day 0 and 15, *Bottom row*: Retainer Brite T3, soft, Day0 and 15)



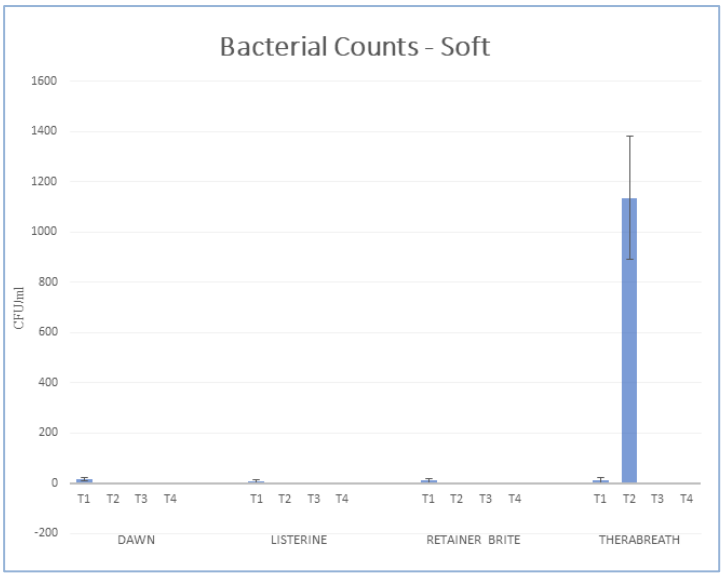
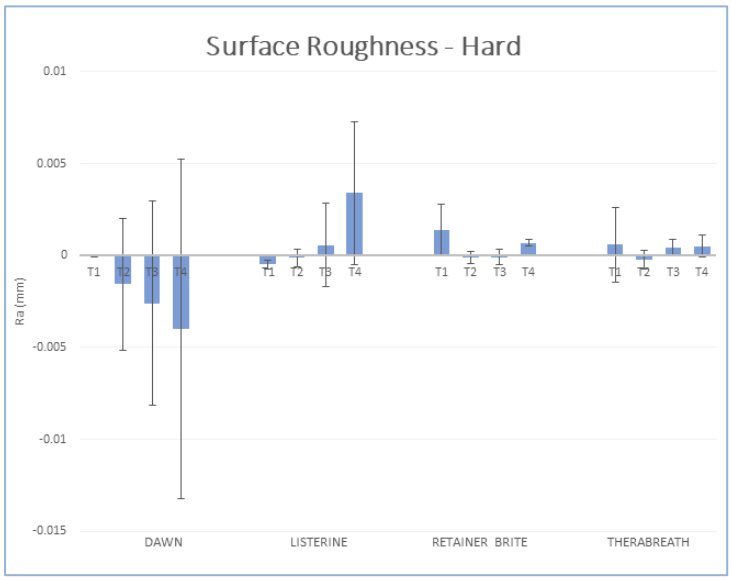




Figure 3: Change in bacterial counts (CFU/ml) for hard and soft specimens for each treatment factor.



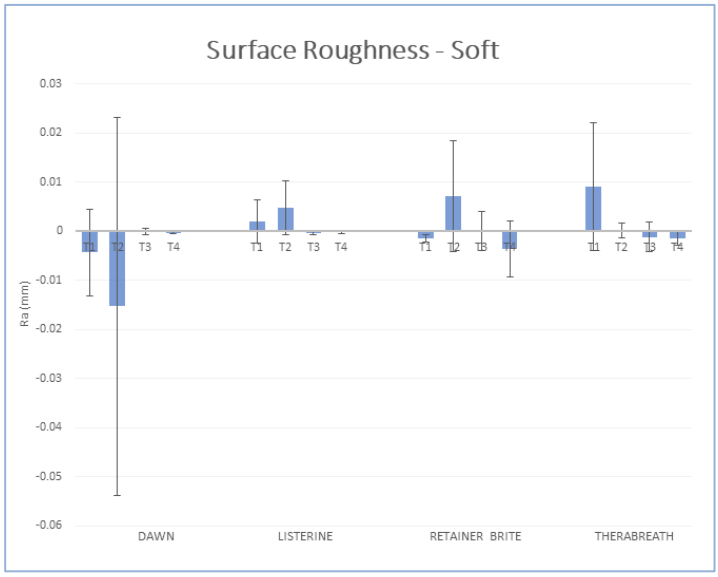




Figure 4: Changes in surface roughness (Ra) for hard and soft specimens for each treatment factor.

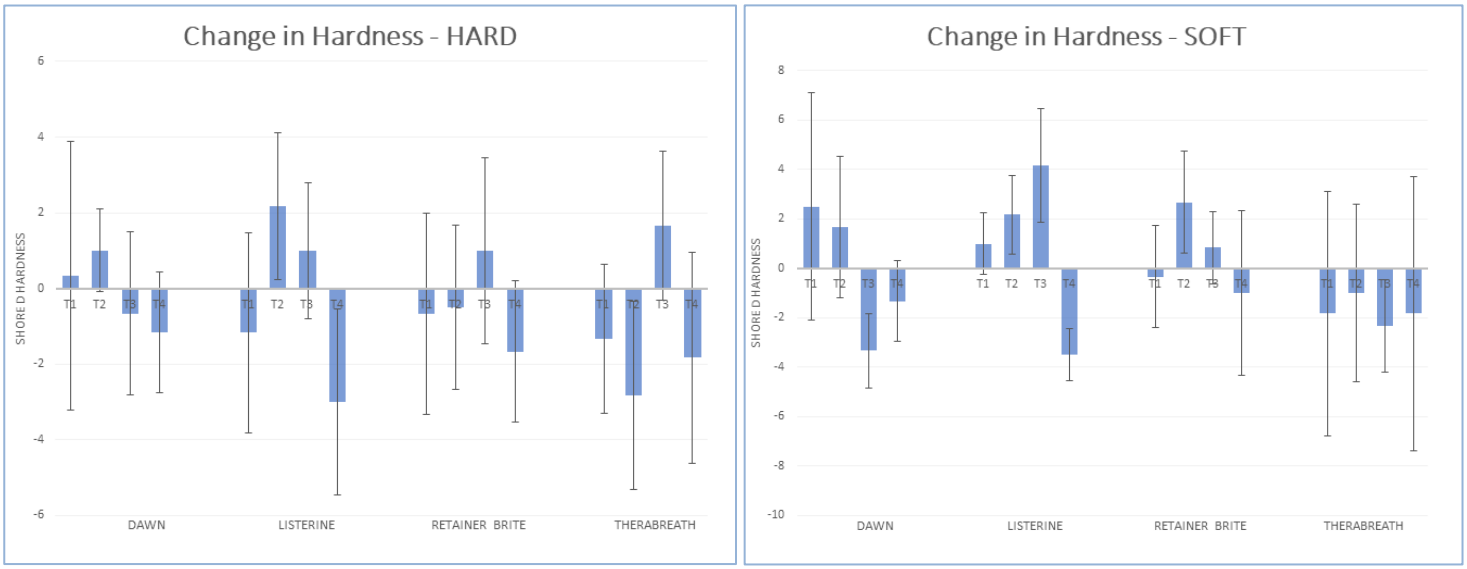



Figure 5: Changes in hardness (shore) for hard and soft specimens for each treatment factor.