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EFFECTS OF A TEST AGENT ON ORAL BIOFILM, PH BUFFERING, DRY MOUTH AND GINGIVAL HEALTH

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OUTLINE

Our goal is to identify in patients with xerostomia the effects of a novel test agent (Oral Essentials™ Hydrating Formula mouthwash, Beverly Hills, CA) vs a control agent (Biotene™ Dry Mouth Oral Rinse) and no treatment on dry mouth, oral biofilm, salivary pH and buffering capacity, gingival health, and tooth sensitivity.

Our specific aims in this project are to:

(1) Use 2-Tone disclosing solution combined with digital photography and image analysis to quantify biofilm presence at baseline and after 7-day use of a test and a control agent each vs no xerostomia treatment product with a 1-week washout between the 3 legs of the study.

(2) Use conventional clinical indices (PI, mSBI, GI) to quantify gingival health at baseline and after 7-day use of a test and a control agent vs no xerostomia treatment product with a 1-week washout between each of the 3 legs of the study.

(3) Quantify salivary volume, pH and pH-buffering performance at baseline and after each 7-day study leg.

(4) Document subject self-evaluation of dentinal sensitivity at baseline and after 7-day use of a test agent and a control agen vs no xerostomia treatment product with a 1-week washout between each of the 3 legs of the study.

(5) Document subject self-evaluation for dry mouth using a standardized questionnaire at baseline and after 7-day use of a test agent and a control agent vs no xerostomia treatment product with a 1-week washout between the 3 legs of the study.

Ten subjects with xerostomia (please see inclusion/exclusion criteria below) participated in this study. The study had 3 legs, whereby in two legs subjects used a test or control xerostomia intervention; in the third leg they used no intervention for xerostomia. Subjects were randomized with regard to sequence of the 3 legs of the study. Subjects also underwent plaque staining and standardized photography at baseline and at the end of each study leg. Digital image analysis was performed to identify plaque age (color-based) and surface coverage in the oral cavity.

Plaque Index (PI), Gingival Index (GI) and Sulcus Bleeding Index (mSBI) were recorded at baseline and at the end of each study leg to provide a quantitative measure of gingival health. Salivary volume, pH and buffering capacity were determined by asking the subject to pool saliva in the floor of their mouth for a 5 minute duration, then expectorate the saliva into a sterile collecting cup. During the leg incorporating test and control agent use, subjects rinsed with the test/control agent. Saliva pooling/expectoration were collected twice, at t=10 and t=40 mins. During the "no test agent" leg, subjects rinsed with water instead. Additionally, subjects completed a standardized self-evaluation questionnaire for dry mouth and dentinal sensitivity at each visit.

PROTOCOL

Subjects: a total of 10 subjects with xerostomia as defined below were recruited from University of California, Irvine. They were recruited by word-of-mouth, flyers and e-mail recruitment in full compliance with and approved UCI IRB protocol. All subjects signed an informed consent at study begin, as well as statement of patient rights and photographic release forms. Subjects received an incentive payment at study completion.

Subject inclusion/exclusion criteria:

Inclusion Criteria

- Males and Female subjects of all races and ethnicities, aged between 18-75 years old.
- Sign and date IRB approved informed consent and HIPAA authorization forms.
- Participate in all scheduled exam visits and procedures.
- Be available for follow up on the telephone
- Demonstrate an unstimulated whole saliva flow rate below 0.2 ml per minute and a stimulated saliva flow rate less than 0.5 ml in 5 minutes
- At least 5 natural teeth in each quadrant (excluding third molars)

Exclusion Criteria

- Unable or unwilling to sign the informed consent form.
- Participation in any other clinical study involving the mouth or xerostomia within the last 30 days prior to enrolment into this study.
- Pregnant or nursing women (self reported)
- Subjects who cannot defer dental treatment during the study dates.
- History of significant adverse effects following use of oral hygiene products such as toothpastes and mouth rinses or allergy to personal care/consumer products or their ingredients.
- Significant past unresolved or current medical problem history
- Cannot participate in the scheduled oral exam visit(s)
- Presence of other major pathologies, such as herpetic infection, major recurrent apthous ulcer, or other ulcer forming diseases, abscesses, granulomas, or severe gingivitis, which may compromise the ability to perform measurements
- Other significant disease or disorders that, in the investigator's opinion, would exclude the subject from the study including systemic conditions that would influence the course of periodontal disease.
- Active acquired immunodeficiency syndrome (AIDS) or Hepatitis B/C (self reported)
- Smokers
- Self-reported GERD condition
- Presence of any condition, abnormality or situation at Baseline that in the opinion of the Principal Investigator may preclude the volunteer's ability to comply with study requirements, including completion of the study or the quality of the data.

Excluded Medication:

Subjects embarking on a course of medication during the study should inform the Study Coordinator so that a decision can be made as to whether they continue in the study. A five minute unstimulated saliva test may be taken to verify no change in saliva flow has resulted

from use of the new medication. Compliance with the protocol will be checked at each test visit and recorded on the appropriate CRF.

Subject Restrictions

- Subjects must not use other forms of oral hygiene (e.g. non study products, toothbrushes, floss and mouthwashes) while the test retainer is in place
- Subjects will not be allowed to have dental treatment (except emergency treatment) during the study
- Subjects will be asked to refrain from all non-study oral hygiene procedures other than their usual brushing, flossing, and mouth washing routine during the study

RESULTS

Itemized Assessments

1. Biofilm

Surface plaque was quantified three times: at baseline, and after each 7-day study leg respectively. Plaque on oral surfaces was stained using a plaque disclosing solution (2-Tone Disclosing Agent, Young Dental, Earth City, MO) and standardized intra-oral photographs were recorded to document the extent of the plaque staining on all natural teeth. Using image J, software, two assessments were made:

A. Plaque age based on stain color (blue vs red)

B. Percent of total tooth surface covered by plaque.

Using Image J software, % of surface coverage was quantified for old (>24h) and new (<24h) plaque for each study leg (Figure 1).

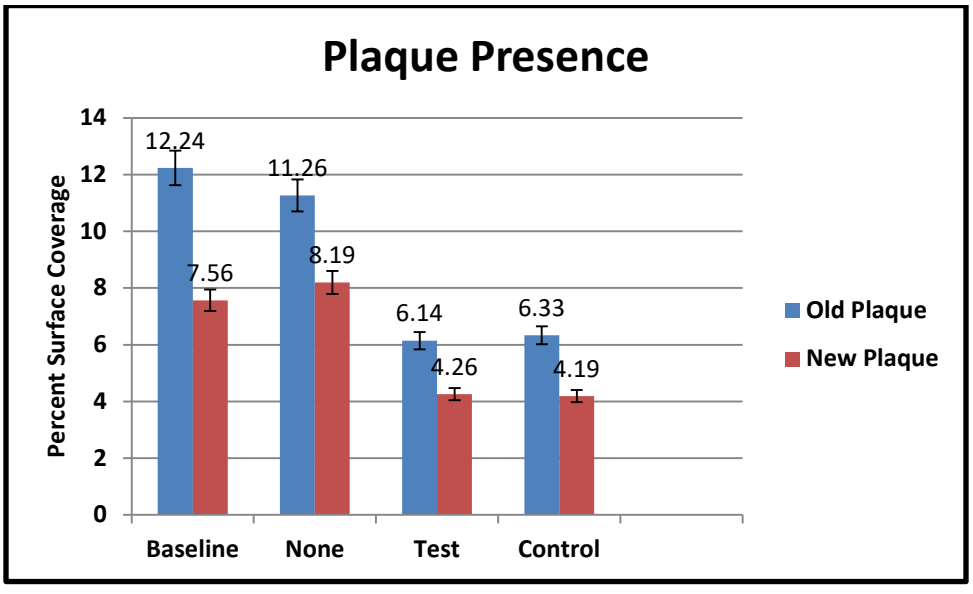

Figure 1: Mean (S.D.) plaque presence at baseline, and after each 7-day leg of the study, expressed as % of image surface area covered by old (>24h) and new (<24h) plaque respectively.

There was a significant reduction in old and new plaque presence after use of test or control products for 1 week vs no product ($p<0.05$). Old and new plaque levels were similar for the test

and the control products (p>0.1). Old plaque presence fell to approximately 50% of the baseline value, while new plaque presence was reduced to approximately 60% of the baseline level.

2. Gingival Health

Clinical Indices were quantified three times, at baseline, and after each 7-day study leg respectively using 3 standard numerical scales: Plaque Index (Quigley-Hein, Turesky Modification Plaque Index) (P.I.), Gingival Inflammation (Loe and Silness Gingival Index) (G.I.) and Gingival Bleeding (mSBI).

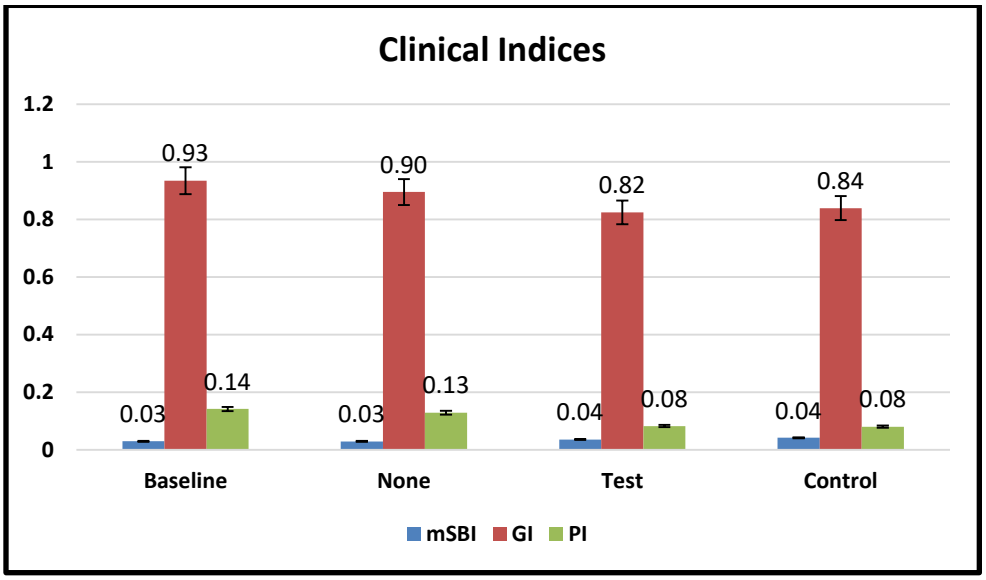

Figure 2: Mean clinical indices (S.D.) at baseline, and after each 7-day leg of the study

There was a significant reduction in clinical Plaque Index by approx. 60% after use of test or control products for 1 week (p<0.05). Generally clinical indices were similar for the test and the control products (p>0.1). The reduction in Gingival Index after 7 Days of test or control product use almost reached a significant level (p=0.0563 for test product, p=0.0643 for control product). All other values did not change significantly during the course of this study.

3. Saliva

Salivary volume, pH and buffering capacity were recorded at the beginning and end of each study leg. Buffering measurements were made using GC Saliva-Check Buffer Kit .

3.1. Saliva Volume

Baseline	None	Test	Control
2.25 ml (0.35)	2.64 ml (0.27)	5.45 ml (0.48)	5.02 ml (0.42)

Table 1: Mean saliva volume (S.D.) collected over 5 minutes in subjects at the beginning and end of each study leg.

The volume of saliva collected over 5 minutes in subjects with xerostomia was approximately doubled at the end of the test and control study legs, representing a statistically significant increase in each case ($p<0.05$). In the study leg with no intervention, saliva production did not change significantly ($p>0.1$).

3.2. Salivary pH

Baseline	None	Test	Control
7.25 (0.75)	7.13 (0.67)	7.20 (0.68)	7.25 (0.62)

Table 2: Mean salivary pH at baseline and at the end of each study leg.

PH values did not differ significantly between study legs and time-points ($p>0.05$).

3.3. Salivary Buffering capacity

	Baseline	None	Test	Control
pH Buffering	Low (10/10 subjects)	Low (10/10 subjects)	Normal (8/10 subjects) Low (2/10 subjects)	Normal (8/10 subjects) Low (2/10 subjects)

Table 3: PH buffering capacity at (i) baseline, (ii) at the end of each study leg

Salivary pH buffering performance improved significantly in test and control legs of the study ($p<0.05$). No change was observed in the leg when no intervention was used ($p>0.1$).

4. **Subject Questionnaires**

Subjects completed a routine self-evaluation questionnaire (separate attachment) for dentinal sensitivity, dry mouth status and Mouthwash ease of use at every visit.

Oral Health Questionnaire: Clinical criteria showed outstanding and highly significant ($p<0.01$) improvement during the test and control agent use legs of the study except for “oral comfort at waking in the morning” (significant effect only for test intervention) and “do you sip liquids to aid swallowing” (no significant change from baseline for test and control interventions).

Agent Questionnaire: Overall, subject response was very positive. Ease of use, flavor, and mouth feel all scored highly at > 70/100. Almost 80% of subjects stated that they would prefer a longer-lasting product for test and control products.

	Mean score on 100mm VAS (o-best; 100-worst)			
Oral Health Questionnaire	**Baseline**	**None**	**Control**	**Test**
a) dryness/wetness upon waking in the morning (0-wettest; 100-driest)	85	80	45	45
b) mouth comfortable (0)/ uncomfortable (100) upon waking in the morning	90	85	90	35
2. I have trouble maintaining my weight because of swallowing problems (0-no; 100-a lot)	0	0	0	0
3. I have trouble eating certain solid foods (hard to chew, crumbly, sticky)(0-no; 100-a lot)	75	80	40	35
4. I have trouble drinking thin liquids (like water, tea, and Ensure®)(0-no; 100-a lot)	0	0	0	0
5. Food gets stuck in my mouth (0-no; 100-a lot)	65	55	5	10
6. Food gets stuck in my throat (0-no; 100-a lot)	40	20	5	5
7. I choke or strangle on liquids (0-no; 100-a lot)	0	0	0	0
8. I choke or strangle on solid foods (0-no; 100-a lot)	0	0	0	0
9. I have problems with dry mouth (0-no; 100-a lot)	100	100	50	60
10. The amount of saliva in my mouth seems to be too little (0-no; 100-a lot)	100	100	60	50
11. Problems with me dry mouth make chewing and swallowing difficult (0-no; 100-a lot)	70	60	65	40
12. Problems with dry mouth affect my ability to sleep (0-no; 100-a lot)	0	0	0	0
13. Do you sip liquids to aid in swallowing dry foods? (0-no; 100-a lot)	50	50	50	50
14. Problems with dry mouth affect my ability to talk (0-no; 100-a lot)	0	0	0	0
15. Are any of your teeth sensitive to hot, cold, or spicy food or drinks (0-no; 100-a lot)	70	70	60	40
Agent Questionnaire				
16. How long does the effect last while sleeping (in hours)?	N/a	N/a	4	3
17. How long does the flavor last while sleeping (in hours)?	N/a	N/a	4	6
18. How long does the effect last during the day (in minutes)?	N/a	N/a	180	150
19. Please rate the ease of use	N/a	N/a	80	80
20. Please rate the flavor (0-100)	N/a	N/a	80	70
21. Please rate the mouth feel immediately after use (0-100)	N/a	N/a	70	80
22. Please rate the convenience (0-100)	N/a	N/a	80	80
23. What is your overall impression of the product? (0-100)	N/a	N/a	70	70
24. Will you continue to use this product/recommend it to others? Y/N	N/a	N/a	Y (60%)	Y (60%)
Question 1a: 0 = Dry; 10 = Very Wet				
Question 1b: 0 = Uncomfortable; 10 = Comfortable				
Questions 2-4: 0 = None; 10 = A lot				
Questions 5-14: 0 = Never; 10 = Always				
Question 15: 0 = No sensitivity; 10 = Severe				
Question 19: 0 = Very easy; 10 = Very difficult				
Questions 20-22: 0 = Best; 10 = Worst				
Question 23: 0 = Not good; 10 = Very good				
Question 24: 0 = No; 10 = Definitely				

ORAL ESSENTIALS XEROSTOMIA CLINICAL TEST