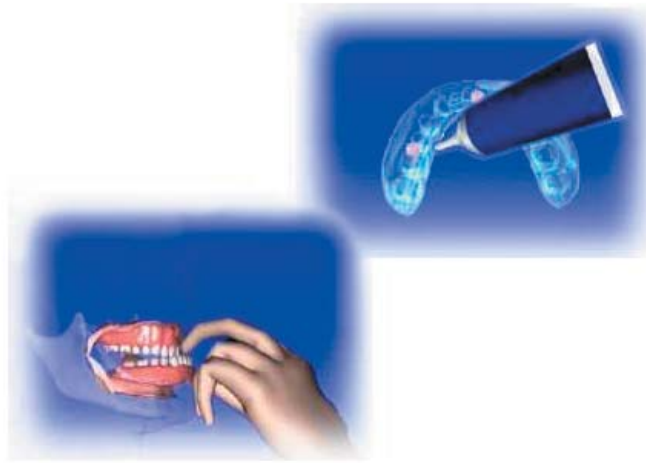


## A Clinical Study to Assess the Breath Protection Efficacy of Denture Adhesive

Graham J. Myatt, PhD; Sheri A. Hunt, PhD;  
Ashley P. Barlow, BSD Pharm Med; J. Leslie Winston, DDS, PhD;  
Alice Bordas, Dent D; Mohammed El Maaytah, BDS, MSc



This randomized and controlled, examiner-blind, 3-period, crossover clinical trial was designed to determine the effect of an experimental denture adhesive, a marketed denture adhesive (European Fixodent<sup>®</sup> Fresh), or no denture adhesive on the breath odor of 37 adults wearing full maxillary and mandibular dentures. Breath quality was measured at baseline and at 3 and 6 hours after the start of each treatment period via monitoring of volatile sulfur compounds (VSCs) using a halimeter and second-person organoleptic grading. A 48-hour washout phase separated treatment periods. There were no statistically significant differences in VSCs between any of the treatment regimens over 6 hours. Both the denture adhesive treatments were superior in breath quality improvement in organoleptic scores compared to no denture adhesive at 3 and 6 hours ( $p=0.0001$ ). This research demonstrates the ability of both an experimental and marketed denture adhesive to deliver superior second-person breath benefits relative to no adhesive. The results indicate that Fixodent<sup>®</sup> denture adhesives provide the denture wearer with a noticeable improvement in breath.

**Keywords:** Denture adhesive, clinical trial, breath malodor

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## Introduction

While bad breath is frequently associated with food related odors such as garlic and onion, the primary source of oral malodor is the oral microflora.<sup>1</sup> Most treatment modalities for bad breath involve targeting microbial plaque, whether it is through conventional oral hygiene practices or incorporation of antimicrobial ingredients into oral care products.

The oral microflora changes with age of the host.<sup>2</sup> An infant begins life with a sterile oral cavity and rapidly acquires its initial microorganisms from its mother. The child then transitions into the acquisition of other species from the environment with the eruption of teeth, onset of puberty, and progression to adulthood. Oral disease processes, including caries and periodontal disease, also influence the makeup of the oral microflora in any given patient. Once the teeth are lost, edentulous adults return to an oral microflora that closely resembles that of the infant prior to the eruption of teeth.<sup>2</sup>

The presence of dentures in edentulous patients creates yet another environment with its own microflora. Several studies have examined denture plaque both on the denture surface itself and the underlying supporting tissues.<sup>3-7</sup> Collectively, these reports have revealed a denture plaque composition similar to plaque on the tooth surface or at the junction of the tooth and the gingiva.

The difference between plaque isolated from the denture acrylic versus the supporting tissues is negligible and in fact appears to be a continuous heterogeneous intermicrobial matrix.<sup>3,6</sup> Intersubject variability is quite high regardless of the sample collection method used, so it is less important to focus on the strict quantitation of microorganisms as it is to focus on the general trend for predominance of one species over another. Ultrastructural studies performed using the transmission electron microscope have demonstrated an electron-dense layer on the surface of the denture acrylic which resembles acquired dental pellicle and appears to mediate the adherence of the denture plaque mass to the denture itself.<sup>3</sup>

## Oral Microflora at Major Life Stages



### Infant

- Oral cavity is essentially sterile at birth
- Initial microorganisms acquired from mother
- *S. salivarius* (on tongue), *S. mutans*, *Nocardia*, *Actinomyces*, *Veillonella* and others are found with increasing frequency as infant grows



### Child/Adolescent

- Bacterial populations increase
- Eruption of teeth provides different surfaces for bacterial attachment
- Dental caries alters environment; new sites for bacterial attachment; new substrates



### Dentate Adults

- Microbiota becomes more complex
- Oral disease processes (caries, periodontal disease) influence microflora



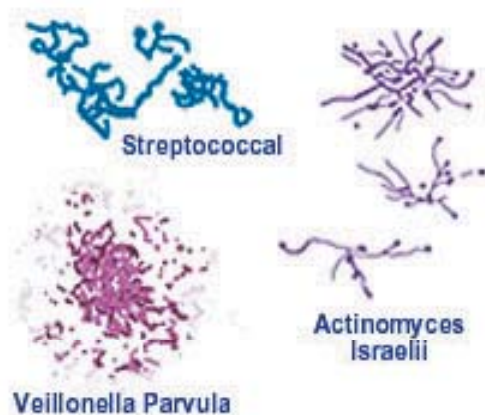
### Edentulous Adults

- Loss of teeth dramatically alters microbiota
- Microflora closely resembles that of infant prior to tooth eruption

Gram-positive cocci were the predominant bacteria isolated from both the palatal mucosa and the surface of the denture, with a mean prevalence ranging from 60-70% of the total plaque composition.<sup>6</sup> The next most frequently isolated group were the Gram-positive rods, with a prevalence of no more than 23%. Speciation of the isolated microorganisms reveals Streptococcal species including: *S. milleri*, *S. mutans*, *S. salivarius*, along with *Staphylococcus*

*aureus* constituting the Gram-positive facultative cocci. This is followed by the Gram-positive rods: *Actinomyces israelii*, *A. viscosus*, and, subsequently, *Veillonella parvula*, a Gram-negative cocci. Gram-negative rods such as *Bacteroides* species and *Fusobacterium* are only rarely isolated.<sup>5</sup> This is important because these Gram-negative rods are often associated with oral diseases/conditions in adults, including periodontal disease and oral malodor.

There is little information in the literature available on oral malodor in denture patients. One study that examined the presence of odor-producing bacterial species in the denture wearer focused on the *Enterobacteriaceae*.<sup>8</sup> Bacterial samples isolated from saliva, tongue, and periodontal pockets revealed a higher prevalence of Enterobacteria in denture wearers when compared with a population of patients complaining of oral malodor, a healthy adult population, and a group of orthodontic patients, with a prevalence of 48%, 27%, 16%, and 13%, respectively.



Members of the *Enterobacteriaceae* are not typically thought of as part of the oral microflora given their high prevalence in the gut; however, they have been isolated from patients with oral malodor and are capable of producing volatile sulfur compounds (VSCs) *in vitro*. VSCs are the primary offensive molecules in bad breath in dentate patients. The most predominant VSCs are hydrogen sulfide (rotten eggs) and methyl mercaptan (rotten cabbage).<sup>9</sup> It is not clear whether this relationship between volatile sulfur production and oral malodor in the denture wearer is comparable to patients with

teeth. A denture wearer's oral malodor has been described as sweet but offensive.<sup>8</sup> Currently the method most frequently used to assess the overall bouquet of breath is via the use of a trained odor judge who utilizes a categorical scale to rate the breath. If VSCs are the focus of the breath assessment, then instrumental techniques such as gas chromatography or a portable sulfide meter are used.

While denture patients frequently complain of bad breath, it is difficult to propose treatment regimens above and beyond frequent cleaning of the dentures, the supporting tissues, and the posterior dorsum of the tongue - where odor-causing bacteria have been found in dentate patients - due to insufficient data. Anecdotal information has provided insight that denture wearers who use a denture adhesive report improvements in oral malodor. The current trial was undertaken to better understand the impact of two denture adhesive formulations versus no adhesive on oral malodor in subjects with full maxillary and mandibular dentures.

### Materials and Methods

Edentulous males and females with full maxillary and mandibular dentures and at least 18 years of age provided their written informed consent and were screened for study eligibility; those with oral malodor intensity ranked at least "faint" via organoleptic assessment (score  $\geq 2$ ) and meeting all other entrance criteria were enrolled in this 3-period, examiner-blinded, crossover clinical trial. Volunteers exhibiting poor dental or general health that could potentially interfere with compliance or evaluation measurements, use of medications with xerostomic or taste alteration adverse effects, recent use of antibiotics, or regular use of antimicrobial oral products were excluded from enrollment. In addition, subjects who were unwilling or unable to agree to study restrictions involving use of scented personal products, smoking, and food/drink consumption were not eligible for participation.

Each subject's dentures were thoroughly cleaned by a trained technician using an ultrasound bath and/or brushing to remove all plaque and debris. For the next week, subjects cleaned their dentures at home as needed using a commercial

denture cleanser provided by the clinical site (Corega Denture Cleanser, Stafford-Miller Ltd, Hertfordshire, UK) and supplemented by mechanical cleansing with water and a toothbrush. At the end of this week long acclimation period (treatment day 1) subjects returned to the clinical site having abstained from use of the supplied denture cleanser during the preceding 36 hours. Oral soft tissue evaluations were conducted. Organoleptic assessments were performed, and those participants continuing to have scores of at least 2 were eligible to continue in the clinical trial. For those subjects who qualified, a baseline halimeter measurement was also performed.

Subjects were stratified into “low” and “high” groups according to their baseline organoleptic score and then randomly assigned to one of the six pre-determined treatment sequences. The following specifies the order of use of each of the three 6-hour test regimens:

1. An experimental adhesive containing Calcium/Zinc (Ca/Zn) salt of Poly Vinyl Methyl Ether Maleic Acid (PVM/MA) and Carboxymethylcellulose (CMC)(Procter & Gamble Technical Centres Ltd., Egham, Surrey, UK);
2. A commercially available adhesive containing Ca/Zn salt of PVM/MA and CMC (Fixodent® (Kukident) Fresh, Procter & Gamble Technical Centres Ltd., Egham, Surrey, UK); or
3. No denture adhesive (negative control).

Both the experimental and marketed denture adhesive treatments were supplied in identical white tubes and overlabeled, such that they were indistinguishable. Study site personnel applied 0.75g (+/- 0.05g) of the first assigned denture adhesive to each of the maxillary and mandibular dentures (or no adhesive if indicated by the subject's randomization schedule) and returned the dentures to the subject for insertion. Subjects then wore their dentures continuously for six hours while remaining at the clinical site. Post-treatment organoleptic malodor and halimeter evaluations were conducted three hours after denture insertion and again at six hours post-insertion.

At the conclusion of the treatment period, an oral soft tissue examination was conducted. Each subject's dentures were again thoroughly cleaned

by investigative site personnel in the same manner as at study inception. Subjects were provided with a single tablet of denture cleanser and dismissed, and the following day entered a 48-hour washout period. At-home standardized denture cleansing directions dictated that the supplied denture cleanser should be used within the first 12 hours of the wash out period; subjects were only permitted to use a toothbrush and water thereafter, i.e., within 36 hours of the next treatment day. The schedule of procedures for treatment days 2 and 3 was similar to those followed on treatment day 1.

Subjects were given several instructions concerning pre-visit (screening and treatment) restrictions that were essential for continuing study eligibility. They were instructed to eat breakfast no earlier than 1.5 hours prior to visit time on the morning of each visit day, to avoid smoking within 3 hours of visits, and to discontinue consuming alcoholic beverages and eating highly seasoned/spicy foods or those otherwise commonly associated with oral malodor for the preceding 24 hours. A standardized lunch was provided at the clinical site after the hour 3 post-treatment breath assessments and at least 1-1/2 hours prior to the hour 6 evaluations on treatment days; again bereft of all foods potentially biasing oral malodor assessments. With the exception of water, all other food, drink, and tobacco use was prohibited during the 6-hour treatment periods. Participants were not allowed to use scented personal products (including soap and antiperspirant) on screening and treatment days.

Organoleptic (second-person) oral malodor assessments were performed by an experienced judge. All assessments were conducted with subjects' maxillary and mandibular dentures in place. Subjects were asked to keep their mouths closed for one minute while breathing through the nose, then each subject placed his/her mouth over one end of a clean cylinder, approximately 1.75 inches long by 1.06 inches in diameter. The other end of the tube was positioned through an opening in the wood screen to where the grader resided on the other side. The grader quantified the extent (intensity) of the oral malodor according to a 6 point scoring system of:



- (0) no odor
- (1) questionable odor
- (2) faint odor
- (3) moderate odor
- (4) strong odor
- (5) very strong odor



Portable sulfide monitor: Halimeter® (Interscan Co., Chatsworth, CA)

All evaluations were made with the grader having no knowledge of prior scoring or subject treatment assignment.

VSC levels were quantified after completion of organoleptic assessments using a portable industrial sulfide monitor (Halimeter®, Model RH17K, Interscan Corp., Chatsworth, CA) housed in an odor-free, plastic laminate booth separating the Halimeter from subjects. To validate instrument performance, VSC measurements were confirmed against primary standards generated by a H<sub>2</sub>S gas permeation tube and a dynacalibrator flowmeter (VICI Metronics Inc., Santa Clara, CA). These comparisons to standards were performed prior to study initiation. After keeping the mouth closed for a timed 30-second interval, each subject placed his/her mouth over one end of a clean cylinder, approximately 1.75 inches long by 1.06 inches in diameter, attached to a Halimeter monitor inlet. While the subject continued to hold his/her breath, the instrument withdrew air from the mouth and measured total VSCs in parts per billion (ppb). These results were transformed using the natural logarithm for analysis.

For both the organoleptic scores and VSC levels, efficacy was evaluated via analysis of covariance (ANCOVA) for crossover studies and included subject, group, period, and treatment as factors. Carryover was not significant and was removed from the statistical model. The baseline score served as the covariate. All statistical testing was at a two-sided 0.10 level of significance, without adjusting for multiple comparisons.

Any clinician-observed oral soft tissue abnormalities and/or negative health effects reported by subjects during a treatment period that were (1) not present at study inception or the end of the previous treatment period or (2) which worsened during that treatment period were classified as adverse events and monitored to resolution. Adverse events were summarized by treatment and type.

## Results

Forty-two subjects entered the pre-treatment 7-day acclimation phase. A total of 37 subjects met continuing eligibility criteria at baseline and were randomized to treatment. Subject baseline characteristics were generally well-balanced among treatment sequence groups. The randomized study population ranged in age from 33 to 80 years, with a mean age of 60.3 years. (Table 1) Subjects were predominantly Caucasian (92%), while the study population was roughly evenly split in gender (51% females, 49% males) and smoking history (54% non-smokers, 46% smokers). Baseline organoleptic scores averaged 2.8, approaching “moderate” breath odor, while baseline VSC levels averaged 59.1 ppb. All randomized subjects (100%) completed all three study periods and were evaluable for all statistical analyses.

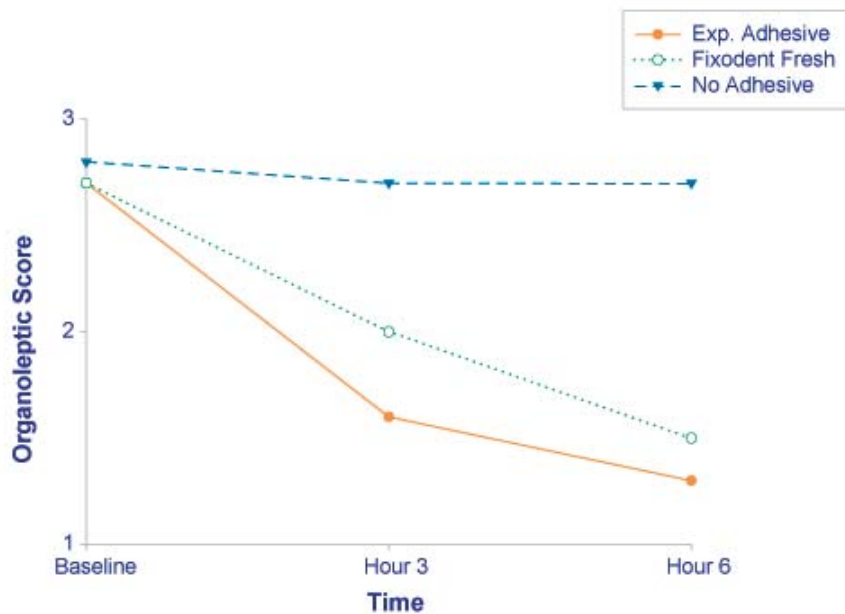
At all evaluation time-points following denture adhesive application (3 and 6 hours), subjects who utilized either the experimental denture

## Study Design



TABLE 1 BASELINE CHARACTERISTICS: ALL RANDOMIZED SUBJECTS (N=37)	
<b>AGE (YEARS)</b>	
Mean	60.3
Minimum - Maximum	33-80
<b>GENDER (N,%)</b>	
Male	18 (49%)
Female	19 (51%)
<b>RACE (N,%)</b>	
Black	1 (3%)
Caucasian	34 (92%)
Multi-Racial	2 (5%)
<b>SMOKING HISTORY (N,%)</b>	
Yes	17 (46%)
No	20 (54%)
<b>ORAL MALODOR</b>	
Organoleptic Mean	2.8
Mean Volatile Sulfur Compounds (ppb)	59.1

Figure1. Adjusted Mean Organoleptic Scores by Treatment and Time



**TABLE 2  
BREATH ORGANOLEPTIC ANCOVA RESULTS BY TIME:  
MEANS ADJUSTED FOR BASELINE VALUES  
ALL EVALUABLE SUBJECTS (N=37)**

<b>TREATMENT REGIMEN</b>	<b>HOUR 3</b>	<b>HOUR 6</b>
<b>EXPERIMENTAL ADHESIVE (STANDARD ERROR)</b>	1.6 (0.07)	1.3 (0.09)
<b>MARKETED ADHESIVE (STANDARD ERROR)</b>	2.0 (0.07)	1.5 (0.09)
<b>NO ADHESIVE (STANDARD ERROR)</b>	2.7 (0.08)	2.7 (0.09)
<b>BETWEEN-REGIMEN COMPARISONS</b>		
<b>EXPERIMENTAL DENTURE ADHESIVE VS. NO ADHESIVE</b>		
Adjusted Difference <sup>B</sup>	-1.12	-1.41
90% Confidence Interval	(-1.30, -0.94)	(-1.61, -1.21)
Two-sided Statistical Significance	0.0001	0.0001
<b>MARKETED DENTURE ADHESIVE VS. NO ADHESIVE</b>		
Adjusted Difference <sup>B</sup>	-0.75	-1.17
90% Confidence Interval	(-0.93, -0.58)	(-1.37, -0.96)
Two-sided Statistical Significance	0.0001	0.0001
<b>EXPERIMENTAL DENTURE ADHESIVE VS. MARKETED DENTURE ADHESIVE</b>		
Adjusted Difference <sup>B</sup>	-0.36	-0.24
90% Confidence Interval	(-0.54, -0.19)	(-0.45, -0.04)
Two-sided Statistical Significance	0.0010	0.0497
<sup>A</sup> Data adjusted for period and baseline.		
<sup>B</sup> Negative differences represent reductions in organoleptic scores for the first regimen relative to the second.		

adhesive or the marketed denture adhesive exhibited statistically significantly lower mean adjusted organoleptic scores (reduced odor intensity) relative to subjects who did not use a denture adhesive. The largest differences seen were between the experimental denture adhesive and no denture adhesive regimens. (Figure 1 and Table 2) There, the between-group mean difference of 1.12 organoleptic units at hour 3 and 1.41 organoleptic units at hour 6 were both highly significant ( $p=0.0001$ ). The percent reductions in organoleptic scores for the experimental denture

adhesive versus the no denture adhesive regimen were 41% at hour 3 and increased to 52% at hour 6. While smaller in magnitude, the differences in breath malodor seen in comparisons of the marketed denture adhesive and no denture adhesive regimens were also highly statistically significant ( $p=0.0001$ ), with average organoleptic differences of 0.75 and 1.17 at hours 3 and 6, respectively. This is reflected in a 28% breath benefit versus no denture adhesive at hour 3 and an increase to a 43% breath benefit at hour 6.



TABLE 3 BREATH VSC ANCOVA (NATURAL LOGARITHM SCALE) RESULTS BY TIME ALL EVALUABLE SUBJECTS (N=37)				
TREATMENT REGIMEN	BASELINE MEAN <sup>a</sup>	ADJUSTED MEAN <sup>a</sup>	ONE-SIDED OR TWO-SIDED p-VALUE <sup>b</sup>	
			NO ADHESIVE	EXP. ADHESIVE
<b>Hour 3</b>				
No Adhesive	59.7	65.4		
Exp. Adhesive	63.4	47.9	<b>&lt; 0.001</b>	
Marketed Adhesive	54.6	47.9	<b>&lt; 0.001</b>	0.968
<b>Hour 6</b>				
No Adhesive	59.7	54.1		
Exp. Adhesive	63.4	52.4	<b>0.289</b>	
Marketed Adhesive	54.6	51.4	<b>0.201</b>	0.780

<sup>a</sup> The baseline and adjusted means from the ANCOVA are transformed back to the original scale.

<sup>b</sup> One-sided p-values are boldfaced, all other p-values are two-sided.

In addition, the experimental denture adhesive demonstrated statistically significant reductions in oral malodor versus the marketed denture adhesive. Using the odor judge, the results are shown in Table 2, with a mean difference of 0.36 ( $p=0.0010$ ) at hour 3 and 0.24 at hour 6 ( $p=0.0497$ ). The percent reductions for the experimental denture adhesive versus the marketed denture adhesive were 18% at hour 3 and 16% at hour 6.

There were statistically significant differences in VSC production between both the experimental and marketed denture adhesives when compared with no denture adhesive at hour 3 ( $p<0.001$ ). However, this benefit was not observed at hour 6. (Table 3)

The 3 treatment regimens were well tolerated. Two subjects (5% of the randomized study population) reported a tingling sensation on the palate during the trial. One event occurred while using the experimental denture adhesive and the other event occurred when assigned to the marketed denture adhesive. Both adverse events

resolved and did not occur when the respective subjects were exposed to the other denture adhesive regimen.

### Discussion

This randomized and controlled clinical trial demonstrates the breath protection efficacy of two denture adhesives using a second-person breath measurement method. The reductions in breath odor severity were observed at 3 and 6 hours post-adhesive application, with an increasing magnitude versus no denture adhesive over the 6 hour measurement period. Interestingly, the increased benefit versus patients not wearing a denture adhesive continued to build following the consumption of a meal. The induction of salivary flow through the act of mastication would be expected to reduce oral malodor in all treatment groups; however, the benefit associated with the denture adhesives versus no denture adhesive still increased over the course of the day.

The second measurement method used in this study was a portable sulfide monitor known as a



halimeter. For breath measurements in subjects who are dentate, the halimeter is a very useful tool for following changes in VSCs commonly associated with oral malodor.<sup>10,11</sup> As mentioned previously, the bouquet of bad breath in denture patients appears to differ qualitatively from patients with teeth and the current study provides additional evidence in support of this idea.<sup>8</sup>



*Fusobacterium*, a bacterial species commonly associated with oral malodor in dentate patients, was seen in ~1% of the cultivable dental plaque from maxillary dentures.<sup>6</sup> While statistically significant differences in VSCs were seen between the two denture adhesives and the no denture adhesive regimens at hour 3, the magnitude of the sulfide levels detected with the halimeter were hovering very closely to the lower limit of detection for the instrument and the between regimen differences were very small. Given the specificity of the halimeter for VSCs, it is not surprising to observe small reductions using an instrument designed to measure these compounds if the oral microflora is not comprised of high VSC-producing

microorganisms. The mean baseline VSCs for this denture population (Table 1) are noticeably lower than those seen in dentate populations.<sup>10</sup>

It is important to concede the mere presence of a VSC-producing bacterium is not sufficient to explain denture bad breath. Recall that Enterobacteria were found in high numbers from denture patients and these isolates were capable of VSC production *in vitro*.<sup>8</sup> However, this did not translate into an odor that could be measured using the human nose (organoleptic scores); in the trial described by Goldberg et al.,<sup>8</sup> there was no relationship between the presence of the Enterobacteria and odor judge scores in the denture subjects.

While VSCs are the predominant components present in oral malodor from dentate patients, there are other compounds such as amines, acid, and indole that may be more important in denture bad breath. Additional investigation into these breath components is necessary to develop targeted treatment modalities for the denture patient. In the meantime, this trial demonstrates that in addition to improving denture hold and function,<sup>12,13</sup> use of denture adhesives will improve bad breath for denture patients. The mechanism whereby the adhesives inhibit denture bad breath is not clear. The antimicrobial activity of the adhesives tested in the trial, both of which contain zinc, has been demonstrated previously *in vitro* and *in vivo*.<sup>14</sup> The presence of the denture adhesive in the mouth long-term may serve as a reservoir for antimicrobial activity.

This research demonstrates the ability of both an experimental and marketed denture adhesive to deliver superior second-person breath benefits relative to no denture adhesive. The results indicate that Fixodent<sup>®</sup> denture adhesives provide the denture wearer with a noticeable improvement in breath.

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## About the Authors

**Graham John Myatt, PhD**



Dr. Myatt is currently a Principal Scientist working in Health Care at Procter & Gamble's Rusham Park Technical Centre, Surrey, UK. Graham received his BSc (Hons) in Chemistry from the University of Manchester, UK in 1989 and his PhD in Applied Physical and Inorganic Chemistry also from the University of Manchester in 1992.

**Sheri Hunt, PhD**



Dr. Hunt is a Senior Scientist at the Procter & Gamble Health Care Research Center in Mason, OH in Oral Care. Dr. Hunt received her BS from the University of Florida in Chemistry then received an MS degree in physical chemistry and a PhD in biophysical inorganic chemistry from the University of California, San Diego, CA.

**Ashley P. Barlow, BSD Pharm Med**

Ashley Barlow is a Senior Clinical Scientist at the Procter & Gamble Company, Rusham Park Technical Centre in Egham, Surrey, UK. After receiving a Bachelors degree in Biochemistry from the University of St. Andrews, Scotland he went on to earn a post-graduate degree in Pharmaceutical Medicine at the University of Surrey, England. He joined P&G in 1995 and has worked in the fields of skin care, laundry, paper and respiratory medicines clinical research. His current responsibilities include the design and conduct of clinical studies to evaluate the safety and efficacy of novel oral care products worldwide. At present Mr. Barlow's research interests include vital tooth bleaching, bio-films, oral digital imaging and clinical methods. He is the author of numerous clinical study reports and co-author on 6 publications in peer reviewed journals.



J. Leslie Winston, DDS, PhD



Dr. Winston is a Section Head at the Procter & Gamble Company Health Care Research Center in Mason, Ohio, USA. She is responsible for the design and conduct of clinical studies to evaluate the safety and efficacy of oral care products worldwide. Her current research interests include oral malodor, oral health of denture patients, and clinical methods for measuring gingivitis. Dr. Winston is a periodontist who received her Doctorate of Dental Surgery degree from the University of Iowa. She received her PhD in Oral Biology from State University of New York at Buffalo as well as her Certification in Periodontics. Her work experience includes both private practice and an academic teaching appointment. Dr. Winston has published her research extensively including over 40 peer-reviewed publications (manuscripts and abstracts).

Address Correspondence to:  
J. Leslie Winston, DDS, PhD  
The Procter & Gamble Company  
PO Box 8006  
Mason, OH 45040-8006  
e-mail: [winston.jl@pg.com](mailto:winston.jl@pg.com)

Alice Bordas, Dent D



Dr. Bordas graduated from the University of Paris VII, Faculty of Dental Surgery. She is currently working at the Aster-Cephac Institute in Paris as Director of the Oral Care Department and Director of Clinical Studies, in charge of performing clinical tests and analysis.

Email : [abordas@aster-cephac.fr](mailto:abordas@aster-cephac.fr)

Mohammed El Maaytah, BDS, MSc

Dr. El Maaytah is an Assistant Professor in the Oral and Maxillofacial Surgery Department at the University of Jordan School of Dentistry in Amman, Jordan.