

# Evaluation of Effect in the Mouth with New Spray-type Oral Moisturizer: A Preliminary Study

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## Abstract

**Background:** This study was to evaluate the effect of new spray-type oral moisturizer containing Poly- $\gamma$ -glutamic acid ( $\gamma$ -PGA).  $\gamma$ -PGA can be classified as a pseudopolyamino acid, which contains only repeated glutamate units. Bacillus subtilis was isolated from natto, fermented soybean health food containing abundant  $\gamma$ -PGA. It reported that  $\gamma$ -PGA could absorb thousands of times more water than its own weight. **Methods:** 79 volunteers were randomly allocated to two groups. The experimental group used this new moisturizer, and the control group used nothing. The effect of moisturizer was investigated by measuring amylase activity by salivary amylase monitor, oral moisture by Mucus<sup>®</sup>, stimulated salivary flow rates at predetermine times. **Results and Conclusion:** In the amylase activity, there was not any significant difference in the control group. In the experimental group, a decline was admitted in baseline and after 30 minutes ( $p < 0.01$ ). In the oral moisture, there was not a significant difference in both groups. In the stimulated salivary flow rates, there was not a significant difference in the control group. In the experiment group, an increase was admitted at baseline and after 10, 20, and 30 minutes ( $p < 0.01$ ). These results suggest that new moisturizer was able to improve the environment in the mouth.

**Key Words:** Xerostomia, Spray-type oral moisturizer,  $\gamma$ -PGA, Oral health

## Introduction

Recently, the percentage of elderly person has been increasing. Accordingly, there has been a focus on patients with aspiration pneumonia, xerostomia, and glossodynia. Xerostomia causes changes in tastes as well as the difficulty in swallowing, talking, and maintenance of dentures. There is also dysgeusia, trauma, and pain of the oral mucosa.

In 2013, Aquabalance<sup>®</sup> medical mouthspray was commercialized as the spray-type oral moisturizer containing  $\gamma$ -PGA by LION Dental Products Company in Japan. The aim of this study is how the oral environmental changes with the spray.

## Materials and Methods

### Study population

The subjects were 79 healthy Japanese students ranging from 20-28 years old (40 males and 39 females) at Osaka Dental University. The experimental group was 47 subjects (26 males and 21 females) who had Aquabalance medical mouthspray<sup>®</sup> sprayed on the back of the tongue. The control group was 32 subjects (14 males and 21 females) who did not receive spray application.

### Oral moisturizer

Aquabalance medical mouthspray<sup>®</sup> (AB) used in this study, is a spray-type oral moisturizer containing l-menthol,  $\gamma$ -poly glutamic acid (Bacillus natto gum), glycerin, propylene glycol, polyoxyethylene hydrogenated castor oil (60E.O.), xylitol, buffering agent, flavor, and cetylpyridinium chloride.

### Study design

**Examination 1**(Measurement of amylase activity by salivary amylase monitor): Amylase was measured with a salivary

amylase monitor (Nipro Co, Japan) and test strip (Nipro Co, Japan) [7-9]. We used this measurement method because it was easy and less invasive, since the test strip was placed under the tongue for only 30 seconds and also because it only required about one minute from saliva sampling until obtaining the result (Table 1).

**Table 1.** Measuring of the stress value by salivary amylase monitor<sup>®</sup>

Measurement	Experimental	Control	p
Preinitiation	46.04	43.1	
After 10 min.	43.36	40.94	0.123
After 20 min.	43.09	41.34	0.305
After 30 min.	40.94	40.88	< 0.01
There was a significant difference.			

**Examination 2**(Measurement of oral moisture by Mucus<sup>®</sup>): Oral moisture was measured at the lingual mucosa (10 mm from the apex linguae on the surface of the tongue) and at the right and left buccal mucosa (10 mm from the angle of the mouth) [10] (Table 2).

**Table 2.** Measuring of the oral moisture by Mucus<sup>®</sup>

Measurement	Experimental	Control	p
Preinitiation	28.42	27.99	
After 10 min.	28.55	28.11	0.679
After 20 min.	28.18	27.73	0.355
After 30 min.	28.33	27.93	0.717
There was no significant difference.			

**Examination 3**(Measurement of stimulated salivary flow rates by Saxon test): Saliva production was measured by

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weighing a folded sterile gauze pad before and after chewing. The low-normal value is 2g/2min [11] (Table 3).

**Table 3.** Measuring of the stimulated salivary flow rates by Saxon test.

Measurement	Experimental	Control	p
Preinitiation	3.22	4.54	
After 10 min.	3.76	4.97	< 0.001
After 20 min.	3.69	4.85	< 0.001
After 30 min.	3.76	4.85	< 0.001
There was a significant difference.			

The subjects were randomly allocated to one of two groups:

Experimental group: 47 persons who used AB.

Control group: 32 persons who did not use AB.

We performed all examinations in baseline after 10, 20, and 30 minutes for experimental and control group. All subjects gave written informed consent to participation in this study after receiving explanation of its purpose.

We carried out this study between noon and 3pm and measured after more than 1 hour of eating and/or drinking. During the experiment, eating, drinking, and conversation were forbidden. The subjects were told to relax in a sitting position.

### Statistical analysis

We compared the experimental group with the control group with T-test. We compared baseline after 10, 20, and 30 minutes for each group.

## Results

### Examination 1

We measured amylase activity by salivary amylase monitor<sup>®</sup>. There was not any significant difference in the control group. In the experimental group, there was a significant difference between baseline and after 30 minutes ( $p < 0.01$ ).

### Examination 2

We measured oral moisture by Mucus<sup>®</sup>. There was not a significant difference in both groups.

### Examination 3

We measured stimulated salivary flow rates by Saxon test. There was not a significant difference in the control group. In the experiment group, there was a significant difference between baseline and after 10, 20, and 30 minutes ( $p < 0.01$ ).

## Discussion

There are a lot of methods to measure mental and physical health. Especially, it is well known that glucocorticoid, catecholamine, amylase, cortisol and chromogranin A are stress markers [12,13]. Glucocorticoid and catecholamine

require blood sample, and could cause stress. On the other hand, Amylase and chromogranin A, which need saliva for inspection, react sensitively to an acute stress load. However, cortisol needs more than 10 minutes to react. There is a small quantity of Chromogranin A in the saliva and there is individual variation. So we selected amylase as a stress marker in this study. We considered that AB was valid for the improvement of the oral environment from the result of Examination 1.

Saliva measuring sheet and mucus<sup>®</sup> are available to check oral moisture. Within the normal range of the secreting saliva, the saliva measuring sheet does not react sometimes because the saliva viscosity and the food residue affect result of the saliva measuring sheet. So, we used mucus<sup>®</sup> in this study.

The result of Examination 2 did not detect significant difference. We considered that there was no change of the moisture in an orally healthy person.

Saxon test and gum test were popular methods for measurement of saliva secretion quantity for outpatients. However, gum test requires more than 10 minutes. And so, Saxon test was conducted in this study. In examination 3, there was a significant difference of stimulated whole salivary flow rate after application of AB.

Poly- $\gamma$ -glutamic acid ( $\gamma$ -PGA) can be classified as a pseudopolyamino acid, which contains only repeated glutamate units. The glutamate units in  $\gamma$ -PGA are linked between the  $\alpha$ -amino and  $\gamma$ -carboxylic acid functional groups, whereas the peptide linkages in proteins are formed between the  $\alpha$ -amino and  $\gamma$ -carboxylic acid groups. *Bacillus subtilis* (natto) was isolated from the traditional Japanese food, natto, fermented soybean health food containing abundant  $\gamma$ -PGA. The hygroscopic and moisturizing effects of  $\gamma$ -PGA were compared with HA. Cross-linked  $\gamma$ -PGA could absorb thousands of times more water than its own weight

## Conclusions

We found that the use of this new spray-type oral moisturizer effectively admitted reducing stress and saliva secretion. It might play an important role in the maintenance of a healthy oral environment.

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