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Non-randomized, one way cross-over, open label preliminary clinical trial for silk protein based oral gargling

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Abstract

Oral gargling solution has been used for the control of halitosis. The purpose of this study was to compare the level of hydrogen sulphide concentration between silk oral gargling solution and commercially available oral gargling solution. Total 21 volunteers were included in this study. The relative level of hydrogen sulphide concentration was calculated to the baseline level. In terms of the primary endpoint of the trial, relative level of hydrogen sulphide concentration was 50.84 ± 33.19% with silk group, versus 71.07 ± 21.83% with Listerine group ($P_{non\text{-}inferiority}$ =0.003). In conclusion, the results of oral gargling with a silk protein for healthy individual were non-inferior to oral gargling with Listerine for hydrogen sulphide concentration reduction.

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Introduction

The halitosis is defined as bad breath from mouth and influences on social interaction (Seemann *et al.,* 2016). The halitosis is induced by sulphide based gas which is mainly produced by anaerobic bacteria (Tonzetich *et al.,* 1977). The prevalence of halitosis is reported approximately 30 % in general population (Liu *et al.,* 2006; Porter and Scully, 2006). Reducing halitosis is an interesting issue for dentists. As it is induced by bacterial products, inhibition of bacterial growth has been main target for oral gargling (Park *et al.,* 2012). However, oral bacteria are normal residents in the oral cavity and complete removal is not possible. Accordingly, oral gargling for the treatment of halitosis has anti-microbial agent with sulphide gas neutralizing agent (Thrane *et al.,* 2009; Miyazaki *et al.,* 1995).

Plant-based oils have been used for oral gargling (Charles *et al.,* 2000; Warnke *et al.,* 2013). As these oils do not have strong anti-microbial activity, the purpose of adding these oils into the oral gargling is neutralizing foul odor gases (Charles *et al.,* 2000). For this purpose, spearmint oil, basil oil, and eucalyptus oil can be considered. As these oils are hydrophobic, alcohol is used as a solvent. However, the usage of alcohol in oral gargling has been issued because of its potential carcinogenic activity (Biron, 1999; Reidy *et al.,* 2011). As there has been no direct evidence for its carcinogenic activity, many oral gargling agents have been approved for usage.

Oral anti-septic has been prescribed to the patients having intra-oral wound. The purpose of this agent is to prevent infection. Though oral anti-septic has been used differently to oral gargling,

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its purpose of usage looks alike (Charles *et al.,* 2000; Warnke *et al.,* 2013). If an oral anti-septic has a multifunctional action such as removing halitosis and accelerating wound healing, this will be more appropriate for the patients. Silk sericin has been used for wound dressing to accelerate wound healing (Napavichayanun *et al.,* 2018). As silk sericin is hydrophilic, it can be added into oral gargling without difficulty.

This is a preliminary study to screen the effect of an oral gargling containing silk sericin. First, the effect of reducing halitosis was evaluated after using silk sericin containing oral gargling. Second, comparative study was done with commercially available oral gargling agent.

Subjects and methods

Study population

The study was ethically approved by Institutional Review Board of Gangeung-Wonju National University (GWNUIRB-2018-15). Participants were recruited from dental school students in Korea. Inclusion and exclusion criteria were referenced from previous publication (Seemann *et al.,* 2016). The volunteers having serious dental problems or halitosis were excluded.

Study treatments

Silk based oral gargling contains 0.02% silk sericin, 0.1% 4-hexylresorcinol, aqua, polysorbate 20, menthol 0.02%, and natural oil. Silk based oral gargling did not have alcohol. The control solution was purchased from market (Listerine, McNeil Consumer Healthcare, Fort Washington, PA, USA). Oral gargling protocol was rinsing 10 ml of gargling solution for 30 s.

Study protocol

The study had a non-randomized, one way cross-over, open label preliminary clinical trial design. Subjects were ordered to stop oral hygiene care for 8 h. Boiled eggs were administered to the subjects for halitosis induction. And then oral concentrations of hydrogen sulphide was measured using a Twin Breasor II (iSenLab, Sungnam, Korea). These values were used as baselines. And then treatment was done. All assessments were made at baseline, 1 h after the silk rinse. A week later, the same subjects were recalled and the same measurements were done again using the control rinse. All adverse events were recorded and classified.

Statistical analysis

The primary efficacy measures were change in hydrogen sulphide concentrations from baseline to 1 h after the first rinse with either silk or Listerine. In consideration of the large difference in baseline concentration among subjects, the baseline concentration was set as 100 and relative gas concentration was calculated. Null hypothesis was that silk rinse was inferior to Listerine in reducing hydrogen sulphide concentrations. To evaluate the non-inferiority of the primary outcome, a two-sample t-test for non-inferiority was done using NCSS 12.0.11 (NCSS, Kaysville, UT, USA). The significance level of non-inferiority was set as $P_{non\text{-}inferiority}<0.05$.

Results

The sample recruitment and follow-up were done between August 9, 2018, and August 31, 2018 (Fig. 1). For each group, Listerine group was 6 missing cases during follow-up. Two cases of silk group and 3 cases of Listerine group showed too low baseline gas level. Accordingly, these cases were excluded in the statistical analysis. Accordingly, 14 cases were included in silk group and 7 cases were in Listerine group. Complications

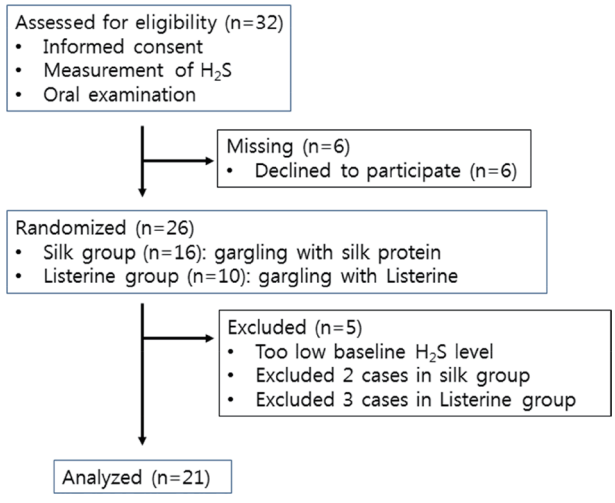

Fig. 1. Subject disposition

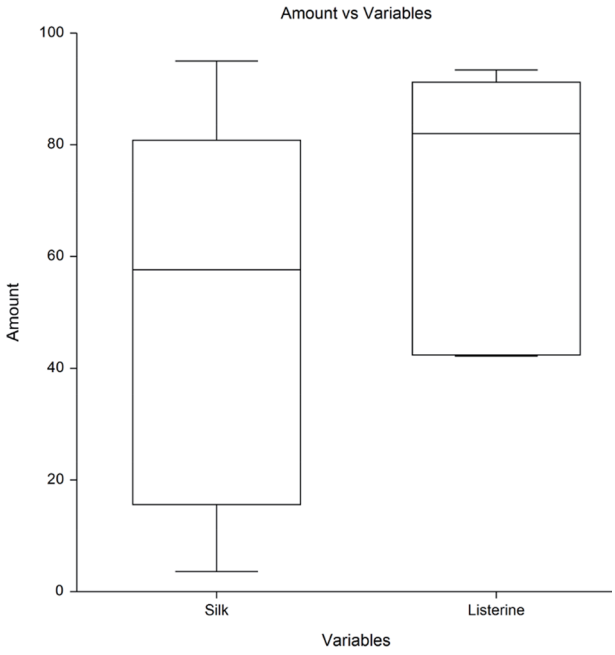

Fig. 2. Mean and confidence interval of hydrogen sulphide concentration reduction for both groups. Amount was a relative value to the baseline as percent. The level of the hydrogen sulphide before administration of gargling solution was set as 100. The hydrogen sulphide reduction in the silk group was not inferior to that of the Listerine group ($P_{non\text{-}inferiority}$ =0.003).

associated with the oral gargling application, such as burning sensation and allergic reaction were not observed. Any complication potentially caused by the silk protein application was not reported.

In terms of the primary endpoint of the trial, relative level of hydrogen sulphide concentration was 50.84 ± 33.19 % (average ± standard deviation) with silk group, versus 71.07 ± 21.83 % with Listerine group (mean difference –20.24, lower limit of one-sided 97.5% confidence limit (CI): –49.38 %, pre-specified margin of non-inferiority –21.83 %, $P_{non\text{-}inferiority}$ =0.003). In undergoing oral gargling with a silk protein for healthy individual, the results were non-inferior to oral gargling with Listerine for hydrogen sulphide concentration reduction (Fig. 2).

Discussion

Silk originated proteins have been used for dressing materials to accelerate wound healing. Oral gargling is frequently subscribed for the patients who receiving oral surgery. In this study, oral gargling containing silk protein had been tested for healthy individual. When the effect of oral gargling was assessed by the change of hydrogen sulphide concentration, undergoing oral gargling with a silk protein for healthy individual, the results were non-inferior to oral gargling with Listerine for hydrogen sulphide concentration reduction ($P_{non\text{-}inferiority}$ =0.003).

An oral rinse containing chlorhexidine has been widely used in patients undergoing oral surgery. Chlorhexidine based oral rinse is effective in inhibiting growth of pathogenic bacteria and halitosis (Young *et al.,* 2003). However, chlorhexidine has an irritation to oral mucosa and some patients don't like its taste (Gurgan *et al.,* 2006; Jones, 1997). Natural oil based oral gargling solution has less irritation compared to synthetic chemicals (Smida *et al.,* 2019). In this study, silk sericin and natural oil were not synthetic chemicals. Complications associated with the oral gargling application, such as burning sensation and allergic reaction were not observed.

Silk protein has been used in various bio-medical fields as scaffold or active ingredient. In this study, silk sericin as degummed product was used for oral gargling. Silk sericin has been used for burn dressing and cosmetics (Ahsan *et al.,* 2018). Silk sericin is known to accelerate wound healing (Napavichayanun *et al.,* 2018; Kim *et al.,* 2018a). Silk sericin can increase tumor necrosis factor-α mildly and this can contribute on bone regeneration (Jo *et al.,* 2017). Silk sericin also can increase the expression of vascular endothelial growth factor via hypoxia inducible factor mediated pathway (Jo *et al.,* 2019). Sericin 1/3 abundant layer of silk mat showed higher level of genes expression associated with inflammation and angiogenesis (Kim *et al.,* 2018b). Angiogenesis is an essential for bone regeneration (Katagiri *et al.,* 2017). In this study, oral gargling containing silk sericin showed non-inferior to oral gargling with Listerine for hydrogen sulphide concentration reduction ($P_{non\text{-}inferiority}$ =0.003).

The limitation of this study was that there was too small number of samples enrolled in this study. Large scale randomized clinical trials are required. The participants for this study were dental school students and supposed to be professional in oral hygiene care compared to general population. When reviewing previous articles, most papers associated with oral gargling test included the subjects having severe level of halitosis. If the subject did not have halitosis, the effect of halitosis inducing gas suppression could not be prominent. This was main pitfall of this study. Applying strict inclusion criteria for the evaluation of halitosis would be required in future study. In addition,

little bacteria formation in the participants by boiled eggs was considered as a limitation, too.

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