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Effect of toothpaste containing arginine on dental plaque—A randomized controlled *in situ* study

Yiyuan Xue[a,b], Qian Lu[a,b], Yuan Tian[a,b], Xuedong Zhou[a,b], Lei Cheng[a,b,**], Biao Ren[a,*]

[a] State Key Laboratory of Oral Diseases, National Clinical Research Center for Oral diseases, West China Hospital of Stomatology, Sichuan University, No. 14, Section 3, Renmin South Road, Chengdu 610041, China

[b] Department of Operative Dentistry and Endodontics, West China Hospital of Stomatology, Sichuan University, Chengdu, China

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ABSTRACT

Objectives: To evaluate the effects of 8% arginine-containing toothpaste on the dental plaque of no caries (NC) and high caries (HC) individuals *in situ*.

Methods: 6 no caries (DMFT = 0) and 6 high caries (DMFT ≥ 6) individuals wearing a self-developed *in situ* dental plaque acquisition device were involved in a randomized double-blinded crossover study for 6 weeks: including lead-in (1 week), arginine-free (2 weeks), washout (1 week) and arginine-active (2 weeks) stages. The *in situ* plaque samples were collected at the endpoint of arginine-free and arginine-active stages and subjected to lactic acid production, metabolic activity, live/dead bacteria ratio and total biofilm biomass detections.

Results: The arginine-containing dentifrice reduced lactic acid production significantly in both the NC and HC groups, while the inhibitory abilities in the HC group were stronger than that in the NC group. In addition, the arginine-containing dentifrice didn't significantly decrease the metabolic activity, live/dead bacteria ratio and total biofilm biomass in either the NC or the HC group.

Conclusions: Arginine-containing toothpaste can significantly reduce the lactic acid production from the *in situ* plaques to a low level without changing the metabolic activity, live/dead bacteria ratio and total biofilm biomass through a critical clinical randomized double-blinded crossover study.

Clinical significance: Arginine is a potential ecological prevention and control agent for dental caries. Meanwhile, the *in situ* model is an easy and pragmatic way to evaluate oral hygiene products (clinical trial registration: ChiCTR-INR-16010226).

1. Introduction

Dental caries is one of the most common infectious and chronic diseases in human oral cavity. The loss of the oral microbial balance caused the increase of glycolytic acid production such as lactic acid from fermentable carbohydrates is considered as the major cariogenic agent. The increase of aciogenic/aciduric oral bacteria can not only cause the demineralization of the tooth by leading to a drop of pH value, but also provides a competitive acidic growth environment over other commensal species [1–3]. Arginine, an alkali-generating substrate, can inhibit tooth demineralization by neutralizing glycolytic acid, which is a promising strategy for dental caries prevention through an exogenous source from oral care products [4–7].

Recently, our group demonstrated that arginine may augment ecological benefits by enriching alkali-generating *S. sanguinis* and prevent the overgrowth of the periodontal pathogen *P. gingivalis* in multi-species biofilms *in vitro* [8]. Meanwhile, accumulating conventional clinical trial data have shown that arginine-containing toothpaste significantly increased ecological benefits, rendering it less susceptible to cariogenic challenges [9–13]. Those studies mostly focused on the control of multispecies biofilms cultured in saliva *in vitro*, which may not be representative of dental plaque under real clinical conditions. However, according to previous studies *in vivo*, the 3-dimensional structure of the dental plaque was crashed, when bacterial samples were scaled from the tooth surface or dorsum of the tongue [10,14–16]. To get whole clinical dental plaques from oral cavity and access the *in situ* dental plaque characters, a self-developed *in situ* dental plaque acquisition device was developed in our lab and was designed to clinically reproduce the oral environment of individuals with different caries statuses and to obtain the integrated biofilm without the distorting of the

* Corresponding author.
** Corresponding author at: State Key Laboratory of Oral Diseases, National Clinical Research Center for Oral diseases, West China Hospital of Stomatology, Sichuan University, No. 14, Section 3, Renmin South Road, Chengdu 610041, China.
E-mail addresses: chenglei@scu.edu.cn (L. Cheng), renbiao@scu.edu.cn (B. Ren).

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3-dimensional structure during formation, collection or analysis processes.

The aim of the current study was to clinically evaluate the effects of arginine-containing toothpaste on plaque samples collected from the self-developed *in situ* dental plaque acquisition device by the analysis of acid production, metabolic activity and biomass change.

2. Materials and methods

2.1. Registration and ethical aspects

This *in situ*, double-blinded, single-center, randomized controlled crossover study was authorized by the Institutional Review Board, West China Hospital of Stomatology, Sichuan University, Chengdu, China (WCHSIRB-ST-2014-085) and the Chinese Clinical Trial Registry (registration no. ChiCTR-INR-16010226). The manuscript was prepared in compliance with the CONSORT checklist. A principal investigator was responsible for adherence to the study protocol, two investigators performed all clinical and technical procedures. An external monitor followed the study (initiation, follow-up, and close-out). Written informed consent was obtained from all the participants in the study. Participants had the right to withdraw from the study at any time and for any reason without prejudice.

2.2. Participants

A total of 12 adult volunteers (mean age, 22.5 ± 2.6 yrs; 6 females and 6 males) were recruited from West China Hospital of Stomatology at Sichuan University (Table S1). The following exclusion criteria were employed: smoker or former smoker, presence of any systemic disease that could alter the production or composition of the saliva, treatment with antibiotics, steroids or any medication known to cause dry mouth in the last 3 months, having any known allergy to previously used oral hygiene products or dental materials, and presence of dental prostheses or orthodontic devices that might affect the oral environment. Participants were categorized into 2 groups. The no caries (NC) group consisted of 6 individuals with no clinical evidence of caries experience [decayed, missing and filled teeth (DMFT = 0)]. The high caries (HC) group consisted of 6 individuals with decayed, missing and filled teeth (DMFT ≥ 6). In this study, 10 participants (5 NC and 5 HC) completed all the study visits. 2 participants were excluded from the study due to self-reported lack of compliance.

2.3. Experimental design

This *in situ*, double-blinded, randomized controlled crossover study was carried out for 6 weeks, including a 1-week lead-in period, 2-week arginine-free phase, 1-week washout period and 2-week arginine-active phase. In each phase, participants were randomly assigned to use either arginine-free (arginine-free phase) or arginine-containing (arginine-active phase) toothpaste. The arginine-free toothpaste was Colgate® Total® Advanced Toothpaste (containing 1450 ppm F^- as sodium fluoride). The arginine-containing toothpaste was Colgate® Sensitive Pro-Relief™ Toothpaste (containing 8% arginine and 1450 ppm F^- as sodium fluoride). Dental plaque samples were collected at the endpoint of both phase 1 (first 2-week treatment) and phase 2 (second 2-week treatment). Participants who used arginine-free toothpaste in phase 1 used arginine-containing toothpaste in phase 2 and vice versa (Fig. 1). During the lead-in and washout period, participants brushed their teeth using Colgate® Total® Advanced Toothpaste (containing 1450 ppm F^- as sodium fluoride).

2.4. Preparation of the palatal device and specimen

The independently designed *in situ* model [17], was made with six hydroxyapatite (HA) slabs recessed into a palatal appliance. A

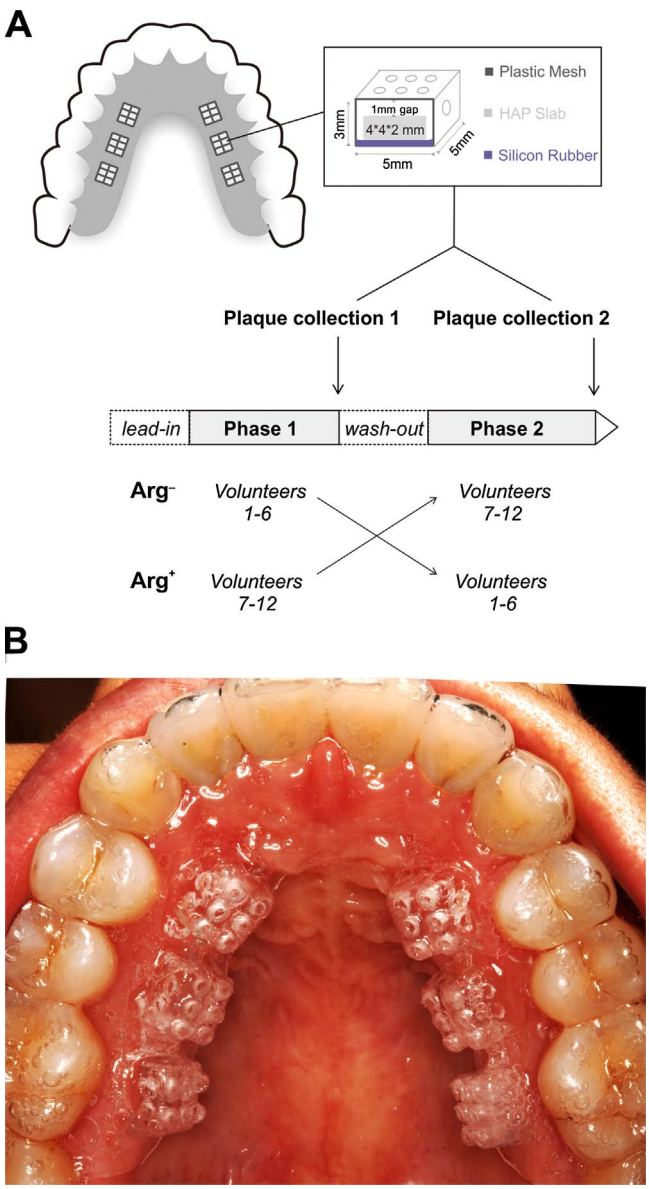

Fig. 1. The self-developed *in situ* dental plaque acquisition device. (A) Schematic illustration of the *in situ* model and the experimental design. (B) Intraoral view of the *in situ* model.

schematic illustration and an intraoral view of the *in situ* model is presented in Fig. 1. Every site had a 1-mm uniform gap covered by plastic mesh to allow for free contact of the saliva with the specimen's surface to form the plaque biofilms and to protect it from a mechanical disturbance. HA slabs (4 mm × 4 mm × 2 mm, BAM, National Engineering Research Center for Biomaterials, Chengdu, China) were stored in 0.1% thymol solution (pH = 7.0) at 4 °C, and randomly assigned to each site, fixed with silicone rubber. New slabs were inserted into the appliance before each stage. To minimize the contact between the tongue and the specimens, the sites were positioned posterior to the incisive papillae. Participants had an appliance try-in appointment where the necessary adjustments were made before each phase.

2.5. Intervention and sample collection

Participants performed oral hygiene with the provided toothbrush and toothpaste habitually. The frequency of tooth brushing was twice a day for 3 min. The recommended amount of dentifrice was approximately 1 g or 2 cm in length. Participants should wear the appliances