Short-term Effect of Mechanical Plaque Control on Salivary Mutans Streptococci in Preschool Children

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Purpose: To determine the effect of mechanical tooth cleaning by toothbrush and dental floss on mutans streptococci in the saliva of preschool children.

Materials and Methods: This blinded, randomised controlled clinical trial included 54 3-year-old preschool children with detectable mutans streptococci in saliva. The children were randomly divided into a test and a control group. Dental college students cleaned the teeth of test group participants with toothbrush and dental floss under the indication of a plaque disclosing agent once a day. The control group received no intervention. Dentocult SM Strip mutans (D-SM) strips were used to test the mutans streptococci in saliva.

Results: The D-SM test scores declined from 1.82 to 0.95 for the test group after the teeth were cleaned 10 times ($P < 0.001$) and the scores increased to 1.62 after tooth cleaning ceased for 2 weeks ($P > 0.05$ compared with baseline). The D-SM level of the control group did not change significantly.

Conclusion: Meticulous and continuous plaque control with toothbrush and dental floss can decrease the mutans streptococci level in preschool children. However, the effect ceased as the intervention ceased.

Key words: dental flossing, Dentocult SM Strip, mutans streptococci, preschool children, toothbrushing

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Mutans streptococci have been identified as important microorganisms responsible for the onset and progression of dental caries (Fitzgerald and Keyes, 1960; Thenisch et al, 2006). A number of studies have been performed with the aim of eliminating mutans streptococci from teeth as a way to reduce the risk of caries. These studies included measures such as professional tooth cleaning, fluoride treatments and the use of various antimicrobial agents (e.g. chlorhexidine) (Kristoffersson et al, 1984; Axelsson et al, 1987; Nomura et al, 2004; Asokan et al, 2008). Collectively, these studies seem to indicate that intensive professional tooth cleaning combined with fluoride treatments or antimicrobial agents are able to reduce the number of mutans streptococci in saliva.

These interventions performed regularly at short intervals have proven to reduce caries incidence (Axelsson and Lindhe, 1975; Axelsson and Lindhe, 1977; Klimek et al, 1985). However, the previously reported application of tooth cleaning, fluoride or antimicrobial agents was previously dependent on the dental professional; therefore, it was expensive and hard to conduct in developing countries with limited resources and manpower. In the studies mentioned, the interventions included not only mechanical tooth cleaning, but also fluoride or antimicrobial agents; therefore, the sole effect of mechanical tooth cleaning to mutans streptococci was confounded and questioned. The participants of these studies were mainly adolescents; preschool children were rarely recruited.

The prevalence of early childhood caries among 5-year-olds in China was reported as about 70% to 80%; moreover, the oral hygiene habits and status

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Table 1 The schedule of participants' screening, group division, intervention and measurements

Date		Period	Content	Participants
9/5/2011	Monday	Noon	D-SM test	All 3-year-old preschool children (n=156))
		Afternoon	Oral examination (dmfs and DI-S)	
Screening and group division (54 children were screened with detectable MS and randomly divided into test and control group)				
9/13/2011	Tuesday	Afternoon	Toothbrushing and flossing	Test group
9/14/2011	Wednesday	Afternoon	Toothbrushing and flossing	Test group
9/15/2011	Thursday	Afternoon	Toothbrushing and flossing	Test group
9/16/2011	Friday	Afternoon	Toothbrushing and flossing	Test group
9/17/2011	Saturday	Weekend		
9/18/2011	Sunday			
9/19/2011	Monday	Morning	Toothbrushing and flossing	Test group
9/20/2011	Tuesday	Afternoon	Toothbrushing and flossing	Test group
9/21/2011	Wednesday	Afternoon	Toothbrushing and flossing	Test group
9/22/2011	Thursday	Afternoon	Toothbrushing and flossing	Test group
9/23/2011	Friday	Noon	D-SM test	Test and control groups
		Afternoon	Toothbrushing and flossing	Test group
9/24/2011	Saturday	Weekend		
9/25/2011	Sunday			
9/26/2011	Monday	Morning	Toothbrushing and flossing	Test group
		Noon	D-SM test	Test group
		Afternoon	Oral examination (DI-S)	Test and control groups
Intervention ceased for 2 weeks				
10/11/2011	Wednesday	Noon	D-SM test	Test and control groups

of preschool children were poor (Wong et al, 2001; Wang et al, 2002; Zhu et al, 2003). Thus, the aim of the present study was to verify whether meticulous mechanical tooth cleaning by toothbrush and dental floss in preschool children decreases the mutans streptococci level.

MATERIALS AND METHODS

This randomised, controlled clinical trial was conducted in Beijing, China in 2011 and was approved by the Peking University Biomedical Ethics Committee. The study is registered in the Chinese Clinical Trial Register (ID: ChiCTR-TRC-11001553), which is integrated into the WHO International Clinical Trials Registry Platform (ICTRP).

Study population

The study was conducted in a kindergarten, 'Centre for Child Education Peking University'. The mutans streptococci in saliva were measured among all 3-year-old preschool children (n = 156) by Dentocult SM Strip mutans (D-SM, Orion Diagnostica; Espoo, Finland). Subjects who had used antibiotics or antimicrobial agents within the last month before the test or had had professional tooth cleaning or topical fluoride treatment were not included in the study. Finally, 54 preschool children were screened with the D-SM test and scored 1, 2 or 3 (see below). The screened preschool children (N = 54; 21 girls, 33 boys) with detectable mutans streptococci in saliva were randomly divided equally into a test and a control group.

Intervention

The test group of preschool children received mechanical tooth cleaning but no instruction in their own daily oral hygiene care. Tooth cleaning was performed by 39 dental college students. These students had finished theoretical courses and had not received any clinical training; thus, this was their first time cleaning another person's teeth. They received 30 minutes of training on dental models, focussing on plaque disclosing, brushing and flossing, before the intervention. First, the participants' teeth were disclosed with Trace Solution (Young Dental; Earth City, MO, USA). Second, the students tried to brush off all the disclosed plaque with a manual toothbrush and tap water only. Third, after tooth brushing, every approximal tooth surface was flossed with Colgate Total Wax Coated Dental Floss containing no fluoride or antimicrobial agents (Colgate; New York, NY, USA). Brushing and flossing was performed in the kindergarten's bathroom and the time required for each tooth cleaning was recorded. Tooth cleaning was performed once a day for 10 days except weekends, for a total of 10 sessions of tooth cleaning per child (Table 1). The control group of preschool children did not receive any intervention and they followed their own daily oral hygiene care.

Table 2 The daily schedule of preschool children in kindergarten

Time	Activities
7:40	Arrival
7:40-8:10	Breakfast
8:10	Tooth cleaning
9:00	Drinking milk
11:15-11:45	Lunch
11:45	Tooth cleaning
12:10-14:30	Afternoon nap
14:30-15:00	Drinking water and eating fruit
16:30-17:00	Supper
17:00	Leaving

Routine situation: tooth cleaning was performed by preschool children themselves; no toothpaste was used for children younger than 3 years old in case of paste swallowing; children over 3 years used non-fluoridated toothpaste.

D-SM test

According to the daily schedule as shown in Table 2, preschool children took an afternoon nap from 12:10 to 14:30. D-SM tests were conducted according to manufacturer's instructions from 14:00 to 14:30 while the preschool children were awake but still in bed, as also described in other studies (El-Nadeef and Bratthall, 1991; Jeevarathan et al, 2007). The plastic strip with the roughened side was turned against the tongue 10 times; no paraffin wax was used. The plastic spatulas (strips) were immediately transferred to test tubes containing 6 ml of selective broth. The broth had been activated by adding a bacitracin disk 15 min before use. After 48 h of incubation at 37°C, the plastic spatulas were removed from the broth and left to air dry at room temperature. Each strip was compared with a standard chart supplied by the manufacturer, and a score from 0 to 3 was then given, where a score of 0 indicated <10,000 CFU/ml, 1 indicated <100,000 CFU/ml, 2 equalled 100,000–1,000,000 CFU/ml and 3 meant >1,000,000 CFU/ml. The plastic spatulas were serially numbered and group information was not revealed; therefore, the examiner was blinded to the division of groups. The schedule of D-SM testing is shown in Table 1.

Oral examination

Caries and oral hygiene status were examined on site, using a portable dental chair, mirrors and CPI explorers. Caries diagnosis was based on the criteria suggested by the WHO (1997), recording every tooth surface as sound, carious, filled or missing. The simplified Debris Index (DI-S) (Greene and Vermillion, 1964) was used to evaluate the oral hygiene status, which examined the buccal surfaces of tooth 55, 51, 65, 71 and the lingual surfaces of tooth 75 and 85. One dentist conducted the clinical examination and he was blinded to the division of groups.

Statistical methods

Differences of D-SM scores and DI-S scores between groups and various sections were tested using the non-parametric Mann-Whitney test. $P < 0.05$ was considered statistically significant.

Table 3 Age and caries status of participants			
Group	**Age (mean ± SD, months)**	**dmfs (mean ± SD)**	**No. of dmfs >0 (%)**
Test (n = 22)	47 ± 6.6	5.57 ± 6.86	13 (59%)
Control (n = 27)	49 ± 5.2	4.92 ± 6.23	16 (56%)
Total (n = 49)	48 ± 5.9	5.22 ± 6.47	28 (57%)

Table 4 The D-SM scores for test and control group in different sections				
	Baseline (mean ± SD)	**After tooth cleaning 8 times (mean ± SD)**	**After tooth cleaning 10 times (mean ± SD)**	**2 weeks after intervention ceased (Mean ± SD)**
Test group	1.82 ± 0.80	1.05 ± 1.00*	0.95 ± 0.83*	1.62 ± 0.74
Control group	1.70 ± 0.78	1.48 ± 0.90	NA	1.57 ± 0.99
Non-parametric test (Mann-Whitney Test, compared with baseline separately): *$P < 0.01$. NA: not applicable.				

Table 5 The DI-S scores for test and control group in different sections		
	Baseline (mean ± SD)	**After tooth cleaning 10 times (mean ± SD)**
Test group	1.58 ± 0.70	0.90 ± 0.40*
Control group	1.42 ± 0.56	1.25 ± 0.51
Non-parametric test (Mann-Whitney Test, compared with baseline separately): *$P < 0.01$.		

RESULTS

One child in the test group dropped out due to illness. Four children of the test group completed tooth cleaning less than 4 times because of poor cooperation. These five children were excluded from the analysis. The age and caries status between the test and control groups was balanced (Table 3).

At the beginning, the dental college students were not experienced and the preschool children were not used to having their teeth cleaned by others, so the first brushing and flossing lasted an average of 32 min (SD = 12.18 minutes). As the intervention continued, the cooperation between dental students and preschool children improved. The same intervention took about 11 min the last two times.

The D-SM levels of the test and control groups were equivalent at baseline (Table 3). The D-SM levels of the test group declined after 8 and 10 times of tooth cleaning and it increased to baseline level 2 weeks after the intervention ceased (Table 4). The D-SM level of the control group did not fluctuate significantly.

Similarly, the DI-S level of the test and control groups was also equivalent at baseline (Table 5). The levels of the test group declined after 10 tooth cleanings, while those of the control group did not change statistically significantly.

DISCUSSION

This study was planned to evaluate the effect of mechanical tooth cleaning on reducing mutans streptococci, the initiator of dental caries. The isolation and culture of mutans streptococci colonies is difficult and time consuming; Dentocult SM Strip mutans has gained widespread use due to its ease of handling. There is scientific evidence that the D-SM test scores are clinically comparable to data obtained by elaborate conventional culturing techniques using selective agar media (Jensen and Bratthall, 1989; Pienihakkinen and Jokela, 1995); furthermore, the D-SM test is highly correlated with the presence and progress of caries (Shi et al, 2003). The D-SM test measured bandwidths of bacterial counts rather than exact numbers. Given a steady state, it is based on the observation that the level of salivary mutans streptococci found on the surface of the tongue reflects the level of these microorganisms on the tooth surfaces (Van Houte and Green, 1974; Lindquist et al, 1989).

This study showed that solely mechanical tooth cleaning with toothbrush and dental floss could re-

duce the D-SM scores in the saliva of preschool children. In principle, the reduction of the D-SM scores observed may be attributed to three possible causes: 1. the bias of the method, for example bias in adherence to the strip, bias in bacitracin content, etc.; 2. the temporarily disturbed steady-state relationship between the high level on the tooth surfaces and the low level found on the tongue; 3. the true reduction of microorganisms on the tooth surfaces. Because this was a random, controlled and blinded clinical trial, bias was excluded. A previous study found that eating and swallowing can decrease the number of mutans streptococci adhering to the surface of tongue to an extent pronounced enough to significantly shift the frequency distribution of the actual D-SM scores (Schlagenhauf et al, 1995). Hence, the D-SM tests in this study were all conducted at the end of the afternoon nap, which ensured that the preschool children had not been exposed to such influences (e.g. eating, drinking, tooth cleaning) for at least 2 hours. In earlier studies, the mutans streptococci level in saliva was measured immediately (usually less than 30 min) after performing the intervention (Wikner, 1986; El-Nadeef and Brathall, 1991; Schlagenhauf et al, 1995). The intervals between the D-SM test and tooth cleaning for the second and third D-SM tests were 4 and 20 h, respectively. The D-SM test scores of the tongue surface was thought to accurately reflect the steady-state level of microorganisms on tooth surfaces.

A study by Schlagenhauf et al (1995) found that the D-SM scores were not significantly influenced by tooth brushing. The present study's positive result might be due to more meticulous and effective tooth cleaning, i.e. the participants' teeth were cleaned with the indication of plaque disclosing agent and approximal surfaces were flossed. Neither of these interventions was included in Schlagenhauf et al's study. In contrast, Wikner (1986) demonstrated that professional elimination of visible dental plaque dramatically temporarily reduced the salivary concentration of mutans streptococci in heavily infected children, which indicated that effective mechanical tooth cleaning alone could decrease the number of mutans streptococci. As opposed to other studies, the current investigation presented the cumulative effect of 8 and 10 interventions instead of just one (El-Nadeef and Brathall, 1991; Schlagenhauf et al, 1995). Thus, the reduction of mutans streptococci in this study cannot be compared with that of previous studies due to different study designs and measurement methods.

A previous study indicated that the reduction effect was transient and mutans streptococci return to baseline level within 30 days after cessation of the interventions (Kristoffersson et al, 1984). In agreement with this, the current study found that the D-SM scores of the test group increased back to baseline level within 2 weeks after the intervention was discontinued. Nevertheless, the present study showed that common oral self-care products are sufficient to maintain good oral hygiene status and decrease the mutans streptococci level in preschool children.

As opposed to previous studies in which the results depended on intervention performed by dental professionals and their instruments (Kristoffersson et al, 1984; Axelsson et al, 1987; Nomura et al, 2004; Asokan et al, 2008), the present intervention might be more easily accepted and conducted by members of the general populace. Unlike young dental college students, preschool children's parents usually do not possess dental knowledge; however, they might be more motivated to clean their own children's teeth since no special instrumentation or skills were needed to do so. Prior to the study, the dental college students had no experience in cleaning children's teeth; the parents were assumed to have more (or at least the same) experience than did the students. Therefore, it is hypothesised that well-motivated parents supported with adequate knowledge are able to clean their children' teeth as effectively as the dental students. Further studies need to be conducted.

CONCLUSION

Meticulous and continuous mechanical plaque control with a toothbrush and dental floss can decrease the mutans streptococci level of preschool children. However, the effect ceases as the intervention ceases.

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