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MECHANICAL ACTIONS OF CLEANING AND FEEDING HAVE A MAJOR INFLUENCE ON THE CONTROL OF ORAL MICROBIAL FLORA

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ABSTRACT

The mouth cavity of a human being harbors a mixed population of various microorganisms, bacteria and fungi being the major categories. These microbes can produce harmful effects to the teeth and the inner wall of the oral cavity, producing carries and cavities in teeth, corrosion in the walls, and bad breath. Various cleaning formulations (toothpastes and mouthwashes) are available in the markets which claim for 12-hours protection after brushing with the specific formulation. Here we compare the effectiveness of various formulations with respect to time passed after brushing. We also study the mechanical effects of food and drink intake over the microbial load. The results show that different chemical formulations do not affect the microbial load very distinctly. Rather, the mechanical activities of brushing and intake of food and drink helps to control the load of microbial flora in the oral cavity of humans.

Keywords: Oral flora, toothpaste, mechanical cleaning.

INTRODUCTION

Teeth, the instruments which slice, piece, and grind the solid food materials for us, are always in attack of various microorganisms. The lining of oral cavity is also under such type of attacks. The importance of this lining is basically of two types: it repels microorganisms by the help of saliva,[1] and, some of the underlying cells (i.e. some glands) of the lining produce and provide enzymes required for digestion.[2,3] Mastication by teeth and the enzyme action both are needed for the proper digestion of food materials.[4,5] So any damage to any of these two actions due to oral flora can produce indigestion, malnutrition, and gastrointestinal discomfort. Although the oral flora may contain some species which are considered beneficial for human health, none of them is found obligatory for such beneficial activities as studied yet.[6-8] Therefore it is considered beneficial for oral health to get rid of microorganisms as much as possible.[9]

The sources of oral microbes include air engulfed by the human being, the foodstuffs and water consumed, any infectious disease, water used for cleaning the mouth cavity, any cleaning object, nails of the person, and contact to another person by means of lips, tongue, and teeth.[10-13] These microbes colonize in different regions inside the mouth cavity and eventually distinct microbial species are seen in tongue, lips, hard and soft palates, teeth, etc.[7] Such colonization can produce diseases in these parts of mouth cavity; carries and cavities in teeth, corrosion in the mucus membrane of the inner walls of the oral cavity, and gingivitis, are common of them.[14] Cleaning teeth and mouth cavity regularly and daily is therefore necessary to maintain proper oral hygiene. For this purpose, toothpastes or mouthwashes are used widely as the cleaning formulations to control oral flora, and studies show that those are effective at least up to an acceptable level.[15-17]

Different chemical formulations are available in the markets as toothpastes and mouthwashes which claim to prevent the above mentioned

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diseases and plaque formation. Some of them claim that they provide protection for up to 12 hours after brushing and therefore recommend brushing twice daily, 12 hours apart, to maintain optimal oral hygiene. In the present study, three brands of toothpastes with different chemical formulations are tested for their claims. It has also been studied whether the mechanical brushing activity is enough to maintain oral hygiene properly. The effects of food and drink intake are also tested for any mechanical cleaning of the microbial load of the oral cavity.

MATERIALS AND METHODS

Selection of cleaning formulations and mechanical cleaning devices

Toothpastes of three different brands are chosen at random from various brands which claim for 12 hours protection. Soft toothbrushes of a single brand are used in the study to avoid any discrimination which might arise due to the mechanical effects of a toothbrush used as a cleaning device.

Collection of clinical samples

Saliva and oral swabs are collected as sputum from volunteers of same age group, of which, 50% are males and 50% females. Sputum sample from each volunteer is collected in sterile watch glass and 100µL of this sputum is then mixed well with 900µL of sterile double distilled water. 100µL of this diluted sputum sample is then spread on LB agar plate. For each volunteer, sputum samples are collected just after brushing (labeled as 0 hrs.), six hours after brushing (labeled as 6 hrs.), twelve hours after brushing (labeled as 12 hrs.), and twenty four hours after brushing (labeled as 24 hrs.). For each volunteer, sputum samples are collected after using no toothpaste (labeled as 'control') and after using three brands of toothpastes (labeled as 't1', 't2', and 't3').

Incubation of clinical samples to determine microbial load

LB agar plates spread with diluted sputum samples are incubated at 37^0C for 24 hours.

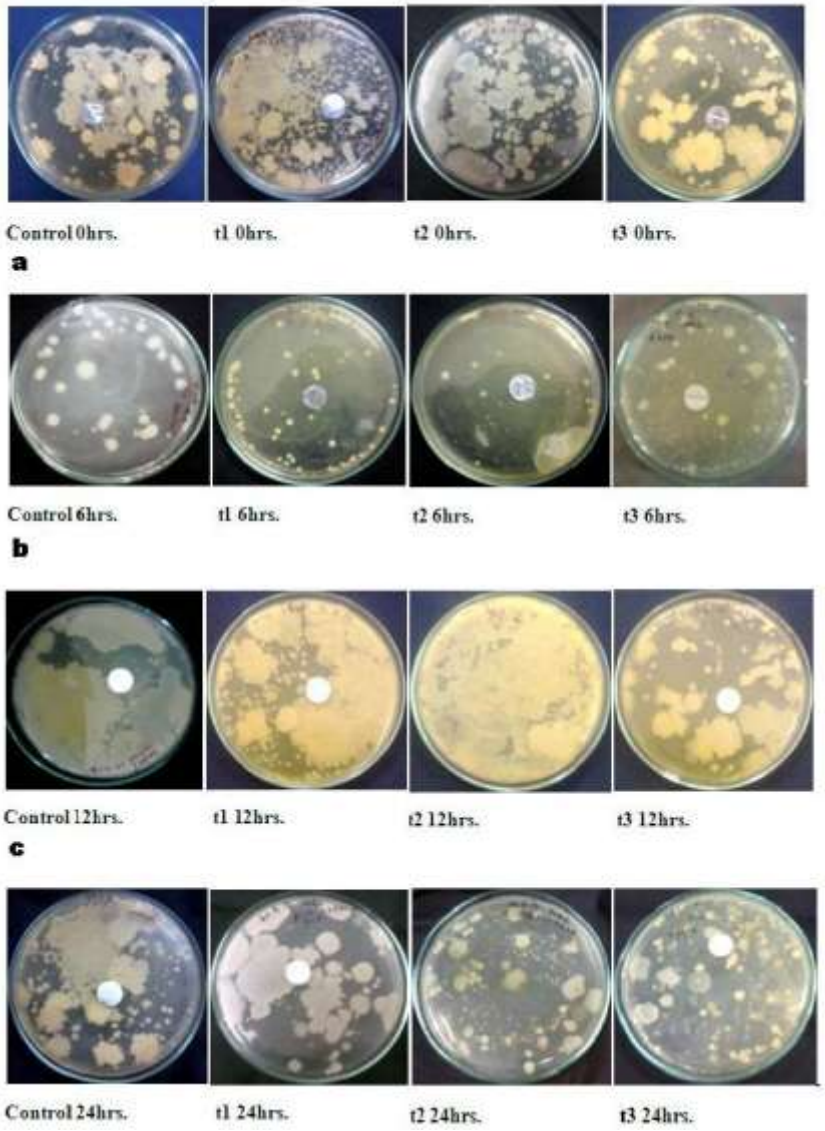
Precautions taken during the study

During the tenure of the experiment, all the volunteers have followed normal diet and have taken no alcoholic drink. All of them have followed same diet and same daily routine of work, eating, and sleep. Smoking was stopped by the volunteers one week ahead of the experiment and is prohibited during the total tenure of the experiment. Brushing is done for 2 minutes with toothbrushes and toothpastes, which are sterilized by ultraviolet radiation in the horizontal laminar air flow hood (STERI CLEAN DM188, Manufactured by DEEPAK MEDITECH PRIVATE LIMITED, DELHI, INDIA) for 30 minutes. Sterile tap water is used for washing the mouth cavity of each volunteer after brushing and before collection of sputum sample each and every time. The results are confirmed by repeated observations.

Table1: Ingredients of the readymade chemical formulations used in the study.

Toothpaste label / Ingredients	t 1	t 2	t 3
1	Sorbitol	Sorbitol	Sorbitol
2	Water	Water	Water
3	Hydrated silica	Hydrated silica	Hydrated silica
4	Sodium lauryl sulphate	Sodium lauryl sulphate	Sodium lauryl sulphate
5	PEG-32	PEG-32	PVM/MA Copolymer
6	Cellulose gum	Cellulose gum	Cellulose gum
7	Sodium saccharin	Sodium saccharin	Sodium saccharin
8	Sodium fluoride	Sodium fluoride	Sodium fluoride
9	Zinc sulphate	Zinc citrate	Glycerin
10	Sodium hydroxide	Trisodium phosphate	Sodium hydroxide
11	CI 77019	Methylparaben	Carrageenan
12	CI 16255	Propylparaben	Triclosan
13	CI77491	Triclosan	Limonene
14	CI 77891	CI 77891	CI 77891
15	Flavor (Not specified)	Flavor (Not specified)	Flavor (Not specified)
16	Cocamidopropylbetain		

Figure 1



Effects of chemical formulations on mouth flora load compared to mechanical cleaning. a. Different cleaning formulations show similar effects on oral microbial load. This indicates a minor role of the chemicals used in various chemical formulations. The major role is therefore imparted on the mechanical cleaning activities, as is also evident from the plates shown in the following panels. **b.** Microbial load is minimal in plates containing samples taken at 6 hours after brushing, indicating the mechanical effects of intake of food and drink as well as the antimicrobial effects of the chemical formulations. **c.** Microbial load is increased in samples taken at 12 hours after brushing. This indicates the loss of effectiveness of the antimicrobial activities of the chemicals with time. **d.** Plates having samples taken at 24 hours after brushing show microbial load higher than 6 hrs. plates but lower than 12 hrs. plates, indicating an effect of less food available to the microorganisms during night time.

RESULTS AND DISCUSSIONS

Incubated plates are compared to each other qualitatively and it was found that although the chemical formulations are different in different toothpastes (Table), all of them are equally effective for removing microbes from oral cavity. They do not differ for much as their cleaning activity is considered for oral microbial load even just after brushing. (Figure, panel a).

It is also found that the patterns of microbial loads in control and test plates are similar. This reveals that the major role for cleaning microbes is played by the mechanical action of brushing, not by the specific chemicals used in a cleaning formulation. (Figure, all panels). Although various chemicals used in toothpastes are tested for their antimicrobial activities,[15-17,18] our study reveals that they play a minor role for cleaning the microbes. Probably they are more important for the maintenance of a low-microbial-load environment inside the mouth cavity for a long time after brushing, but the mechanical cleaning actions of toothbrushes actually do wash out the microbes from the oral cavity and teeth.

It has also been found that the 6-hrs. plates show the minimal microbial load. (Figure, panel b) This reveals that the mechanical actions during intake of food and drink help to clean the oral cavity and to maintain low microbial load. The chemicals present in the toothpastes may help for the maintenance of oral hygiene by keeping the microbes controlled, but the pattern is same as of control plates. After brushing, the load should increase as time passes by, and this is reflected in 12-hrs. and 24-hrs. plates. (Figure, panel c and panel d respectively) Therefore it can be said that both the antimicrobial activity of the chemicals and the mechanical cleaning by food and drink intake supplement each other to maintain an optimal oral hygiene for at least 6 hours, but not up to 12 hours.

It has been seen that the microbial load in 24 hrs. plates seems lower than that in the 12 hrs. plates. (Figure, panels c and d) This can be justified by two logics. Firstly, intake of food and drink is lowered during night time and is actually absent during sleeping time. Mechanical action for cleaning microbes is therefore almost absent in the period from 12 hrs. to 24 hrs. But, on the other hand, supply of food particles are also minimized in this period, which particles can serve as the food for the oral microbes. Such food is actually present throughout the day: from 0 hrs. to 24 hrs., but the supply is much high during the period of 0 hrs. to 12 hrs. The second logic is the circadian rhythm, which may play a role in the variations seen in the results.[19,20] To infer for any definite reason(s), extensive studies are required involving more volunteers and quantitative analyses, such as colony count for a particular bacterial species.

CONCLUSION

The present study therefore concludes that a cleaning formulation with some specific chemicals is not as effective as it claims, and the use of such formulations is not mandatory for maintaining proper oral hygiene. Instead, a proper mechanical cleaning device, regular intake of food and drink, and washing mouth cavity with clean water is enough to maintain optimal oral hygiene. For a more specific inference about the control of microbial load of oral cavity, experiments should be designed taking various other parameters, including fasting conditions and genetic make-up of the persons.

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