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Contamination of microbes and its disinfection in mouthguards used in sports: An original research

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ABSTRACT

Aim: Purpose of the research was to assess the amount of microbial accumulation and further disinfection of mouthguards usually used in sports.

Methodology: Decontaminating effect of diverse substances on EVA mouthguards hitherto contaminated with saliva and broth culture of Enterococcus faecalis and Candida albicans. Afterwards, the mouthguards were undertaken to the following treatments solutions (A) Untreated; (B) 5 min with sterilized distilled water (C) 5 min with H2O2 (D) 5 min with a physiological solution (E) toothbrush and fluoride toothpaste (F) 5 min with 0.5% NaOCl.

Results: The maximum efficacy against E. faecalis was demonstrated by H2O2 (84.19% bacterial load reduction). H2O2 showed a better reduction of salivary cell load. The maximum efficacy against C. albicans was seen when immersed in 0.5% NaOCl which caused a 92.95% decrease of cell load.

Conclusion: Hydrogen peroxide, 0.5% sodium hypochlorite allowed to obtain an optimum disinfection of the mouthguard.

Keywords Disinfection, mouthguards, sports dentistry, bacterial load

INTRODUCTION

The sports dentistry may be a new area during which the performance of the dentist aims to stop and treat oral diseases and injuries resulting from physical activities. The sports dentistry, and other dental specialties, believes in deterrence. Most of the injuries can be reduced or minimized by the use of mouthguards.[1] Mouth guards are removable intra-oral devices, commonly used in the upper arch, the area most susceptible to trauma. Mouthguards, if used correctly, make the protection of the teeth and soft tissues like gums, lips and cheeks during an impression. The mouthguard should be utilized in all sports events where contact, fall or accident can occur,[2] mainly in case of athletes using orthodontic appliances, due to the

larger likelihood of cuts and lacerations in the mucosa adjacent to brackets and wires.[3]According to the American Academy of Sports Dentistry, the use of mouthguards is responsible for 80% reduction in the risk of dental trauma. Athletes in contact sports have a 10% chance of having an accident in the mouth during sports. Without the use of customized mouthguard, the risk of dental trauma increases more than 60 times.[4]Mouthguards are produced utilizing silicone, EVA or porous polymers. These removable intra-oral devices are often purchased able to use or custom-made for every athlete through the impression of their teeth, construction of the device and adjust for the individual occlusal and orthognathic movements.[5]With the expansion and appreciation of the game in recent years, alongside growing concern about the security of athletes and prevention, the utilization of mouthguards has grown and become increasingly common among some sports. But only limited studies have assessed these devices being contaminated with saliva. It is little known that human saliva from a healthy individual houses more than 100 million bacterial cells per 1 ml of saliva.[6]The characteristics of the microorganisms isolated from the mouthguards enable them to disseminate systemically and/or be aspirated into the respiratory tract. As a result, the immune system would be compromised and athletes would be more susceptible to diseases. The spectrum of microorganisms found in mouthguards raises the question as to whether the risk from wearing mouthguards is worth the tooth protection they provide. At the present time, there are no acceptable decontamination methods available. The major problem is that mouthguards, like dentures, are very porous. With use, microorganisms invade these porosities and thrive in the presence of food and water from their host athlete. Unfortunately, as with dentures, it is very difficult for sanitizing solutions to penetrate these pores. However, the biting actions athletes perform during mouthguard wear end in a systemic showering of microorganisms throughout the mouth, oesophagus, and trachea.[7]A recent systematic review of clinical studies concluded that the clinical evidence was lacking and that, although many chemical agents contained in oral hygiene products have proven in vitro activity against S. aureus, their clinical efficacy remains to be confirmed by further high-quality randomized controlled trials.[8] However, a recent study showed that commercial mouth-rinses are ineffective against oral MRSA biofilm.[9]Good personal hygiene is that the key to prevention and control of community associated MRSA outbreaks. Proper practices amid athletes comprise of frequent hand washing, covering abrasions or seeping wounds, not shared personal items, requiring showers after all gaming events, wearing sandals in showers, quarantining athletes who have infections, and washing protective gear after each use.[10] Therefore, it is important to evaluate the colony forming units (CFU) in relation to mouthguards after usage of different disinfecting agents so that a proper sanitization regimen can be put in use.

AIM OF THE STUDY

Purpose of the research was to assess the amount of microbial accumulation in terms of colony forming units (CFU) and further disinfection of mouthguards usually used in sports.

METHODOLOGY

A vitro study was carried out where For EVA mouthguard contamination, saliva and different Microorganisms were used. The bacterium was subsequently cultured on MacConkey plates without crystal violet incubated at 37 °C for 24 h to confirm the purity of the microorganism. In this study, the antimicrobial effects of the following substances were studied: Sterile distilled water, hydrogen peroxide (H_2O_2), physiological saline solution, fluoride toothpaste (Colgate); 0.5% sodium hypochlorite solution (NaOCl).Before proceeding with the experiment, EVA mouthguards were sterilized with alcoholic solutions. Samples were incubated overnight in nutrient broth at 37 °C to verify absolute sterility. Any sample that

showed turbidity of the nutrient broth was discarded and replaced with a new one. Subsequently, the clouds, in triplicate, were immersed, respectively, in *E.faecalis*and *C. albicans*broth cultures, saliva solution Then, they were incubated at 37 °C (35 °C for *C. albicans*) for 24 h, to facilitate adhesion of microorganisms. After the contamination, the EVA mouthguards were subjected to the following disinfectant treatments:

A. Untreated; non-cleansed specimens were used as positive controls;
B. Immersion for 5 min in sterile distilled water (H2O d);
C. Immersion for 5 min in solution of hydrogen peroxide (H2O2);
D. Immersion for 5 min inside the physiological solution;
E. Brushing with common toothbrush and fluoride toothpaste;
F. Immersion for 5 min into 0.5% sodium hypochlorite (NaOCl);

After the treatment, samples were taken using sterile Swabs. Where they were then streaked onto the respective culture medium (MacConkey without crystal violet for *Enterococcus faecalis*, Sabouraud agar for *Candida albicans*and Blood Agar for saliva samples). Subsequently, they were incubated at 37 °C (35 °C for *C. albicans*) for 24 h. After incubation, the number of CFU/ml was determined. Antimicrobial activity was evaluated by comparing growth on the control and the test plates and expressed as percentage reduction for each disinfectant.To assess the exterior of contaminated and subsequently treated EVA mouthguard, obtained samples were treated with 2% solution of glutaraldehyde in 0.1 M PBS for 2 h at 4 °C and post fixed for 1 h at 4 °C in 1% of osmium tetroxide in the same buffer solution. After thorough washing with PBS, samples were dehydrated in an ascending series of alcohols (50%, 75%, 95%, 100%), allowed to dry on absorbent paper for 48 h, and then observed with a scanning electron microscope. As for the statistical analysis of the results, the $\chi 2$ test, and p values < 0.05 were measured significant statistically.

RESULTS

The antimicrobial effect of different substances towards E. faecalis, C. albicans and salivary micro-organisms grow on the surface of EVA mouthguards were analysed. Antimicrobial activity was evaluated by comparing growth on control and test plates and expressed for each disinfectant, in terms of percentage of cells load reduction over untreated surface. E. faecalis grows easily on EVA mouthguardssurface, the cells appeared quite dispersed with few aggregates. All the tested solution determined a significant reduction of bacterial load. (Table 1) The highest statistically significant efficacy has been demonstrated by hydrogen peroxide (84.19% bacterial load reduction). Treatment with distilled water, toothbrush and fluoride toothpaste showed a lower efficacy with a reduction of 53.67%, 55.2% respectively. (Table 2) It is interesting to highlight that fluoride toothpaste was deposited on mouthguard surface, forming aggregates, within which the salivary bacteria could be trapped. The highest efficacy, statistically significant, has been demonstrated by 0.5% sodium hypochlorite, which caused a 92.95% reduction in cell load (p=0.03). (Table 3) The lowest efficacy has been demonstrated by physiological solution (34.04%)

Table 1- CFU seen in the groups related to various disinfectants

CFU/ ml	Group A	Group B	Group C	Group D	Group E	Group F
	63	39	12	56	37	10

Table 2- Bacterial load reduction observed in case of various groups

Variables	Group A	Group B	Group C	Group D	Group E	Group F
% of	2%	53.67%	84.19%	34.04%	55.2%	92.95%

bacterial load reduction						
Mean ±SD	4.37±3.99	2.38±2.09	1.12±0.97	3.79±3.11	2.15±1.99	1.03±0.23

Table 3- Intergroup variability assessed with chi-square test

Statistical measurement	Group A	Group B	Group C	Group D	Group E	Group F
Chi square value	1.45	1.88	1.05	1.956	1.0442	1.692
p value	2.34	0.78	0.0411	0.59	0.655	0.03

**p<0.05 = significant*

DISCUSSION

In the present study, the EVA mouthguards have been contaminated in vitro with broth culture of Enterococcus faecalis, Candida albicans and saliva. E. faecalis is a gram-positive, non-motile, facultative anaerobic microbe, normal human microflora commensal. E. faecalis can even survive in extreme environmental conditions (acid/alkaline pH, high salts-heavy metal concentrations, low nutrient concentrations). It can dwell between 10 to 45 °C and is resilient to a temperature of 60 °C for 30 min. It can defensive against a variety of antibiotics and intra-canal drugs. It is highly infectious and able to modify the host responses.[11,12]Candida albicans is usually a commensal organism of the oral cavity, but can become pathogenic under varied conditions and it is regularly found on dental prostheses.[13-15]Our data show that there is no ideal method. Undoubtedly, hydrogen peroxide, 0.5% sodium hypochlorite make it possible to achieve optimal disinfection of the device, offering more encouraging results than the literature.[16,17,18] In this study, 0.5% sodium hypochlorite for 5 min determined a significant reduction of *C. albicans*load. Salles et al. showed that 0.5% of NaOCl applied in immersions for 20 min were effective, eliminating all microorganisms evaluated. The difference is due to the separate times of action.[19]Few authors have dealt with the disinfection of the mouthguards. Barton recommends sanitizing daily and soaking between uses in a commercially available antimicrobial denture-cleansing solution.[20] Ogawa showed that washing with sterilized water and a ventilated environment is effective for hygienic storage of dental devices made of EVA.[21]As far as safeguarding is concerned, many athletes leave their mouthguards everywhere, not worrying about likely contamination.[22]The most suitable method remains storage in perforated containers that allow ventilation, after plugging the mouthguard with a napkin to eliminate liquid residues.[23]Further studies are required to find an ideal product to disinfect the mouthguard, which does not have side effects after a long time on the device, compromising its structure and function. The care of the oral protection device is important and must be entrusted to the patient/athlete. He will be able to fulfil this commitment through a few simple steps that must be necessarily associated with the usual oral hygiene rules, as instructed by the sports dentist.

CONCLUSION

Different disinfectants are tested for the custom mouthguard decontamination. Hydrogen peroxide, 0.5% sodium hypochlorite determined a significant reduction of the micro-organisms adherent on the surface and allowed to obtain an optimum disinfection of the mouthguard.

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