JOURNAL OF BIOLOGICAL REGULATORS & HOMEOSTATIC AGENTS Vol. 32, no. 4, 1045-1049 (2018)

LETTER TO THE EDITOR

THE USE OF CASEIN IN SPORT MOUTHGUARDS: MICROBIOLOGICAL AND ECOLOGICAL VARIATIONS IN ORAL CAVITY

D. TRIPODI, D. MARTINELLI, C. CIARAVINO, D. FULCO, M. TIERI and S. D'ERCOLE

Department of Medical, Oral and Biotechnological Sciences, University "G. D'Annunzio" of Chieti-Pescara, Chieti, Italy

Received January 25, 2018 – Accepted June 8, 2018

Sport mouthguards have the potential to become a microbial reservoir, produce oral and systemic diseases and cause negative changes in the oral cavity. The aim of this study was to monitor oral environmental changes caused by casein and sport-mouthguard *in vivo*, through clinical, salivary and bacterial markers of young athletes. Forty-eight active young athletes in different disciplines were selected and analysed at different times: baseline (T0); after three months of casein application on the mouthguard (T1); and after six months of application (T2). The product used was GC Tooth Mousse®. At T0, clinical monitoring was performed and the following parameters were recorded: Decay-Missing-Filled Teeth (DMFT) index, Plaque index (PL+) and Gingival Bleeding (BOP+). Saliva-Check Buffer GC® and Saliva-CheckMutans GC® salivary tests were then performed. At T0 the athletes demonstrated DMFT 0.03±0.01. PL value was positive in 100% of subjects at T0, T1, and T2. The BI value was always negative. At the three time-points, a significant change in baseline hydration values was observed; baseline viscosity was normal in 50% of cases while it increased in the remaining 50% at T0; it was normal and constant at T1 and T2. The value of the baseline pH underwent an not statistically significant increase at T1 (7.6±0.08) while remaining constant at T2. The amount of saliva produced after 5-min stimulation ranged significantly and gradually from T0 to T1 and T2, with a statistically significant difference. Plaque indicator tests highlighted that at T0 a plaque with a pH of 6.0±0.5 prevailed; at T1 it was 6.25±0.75 while at T2, pH was equal to 6. Tests for the detection of *S. mutans* resulted constant in all subjects at the various observation times, resulting in 67% of patients in whom *S. mutans* was present. The application of casein, within custom-made ethylene-vinyl acetate (EVA) mouthguards, positively influences salivary flow, the increase of pH values, the amount of stimulated saliva and the buffering capacity of the athlete, improving their state of oral health, which is negatively affected by the use of common mouthguards.

To the Editor,

The individual mouthguard is a soft intra-oral device made of plastic and covers the whole palate and occlusal surfaces of the teeth. It is manufactured in ethylene vinyl acetate (EVA) (1).

A factor of poor compliance by the patient is the difficult storage and cleaning of the mouthguard, which tends to accumulate plaque and debris, giving it a bad smell. Glass et al. (2) reported that "boil and bite" mouthguards harboured a range of pathogenic, opportunistic bacteria, yeasts, moulds, etc., and had the propensity to become a microbial reservoir,

Key words: mouthguards, saliva, casein, EVA material, biofilm

Mailing address:
Dr Simonetta D'Ercole,
Department of Medical, Oral
and Biotechnological Sciences
University "G. d'Annunzio" Chieti- Pescara,
Via dei Vestini, 31, 66100 Chieti, Italy
Tel.: +39 0871 3554809 - Fax: +39 0871 3554392
e-mail: simonetta.dercole@virgilio.it

0393-974X (2018)
Copyright © by BIOLIFE, s.a.s.
This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.
Unauthorized reproduction may result in financial and other penalties
DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

and some commercially available devices were contaminated before use. Sport-mouthguards have the potential to produce oral and systemic diseases as the jagged and sharp areas of the posterior regions of typical mouthguards are in close proximity to the pterygoid plexus of veins and therefore near the entire circulatory system. D'Ercole et al. reported that custom-made mouthguards cause changes in environmental oral factors, because they increase Full Mouth Plaque Score (FMPS) and Full Mouth Bleeding Score (FMBS) and reduce the salivary buffering capacity and pH, thus inhibiting the protective effect of saliva (3). Microbiological control of saliva is easy nowadays, even in dental practice with the use of the salivary "chair-side" test (4, 5). It has been demonstrated that a mouthguard is not only a means of trauma prevention but also a vehicle for preventive substances in order to maintain oral health. Different strategies to prevent ecological changes in the oral cavity caused by the use of mouthguards are possible, as demonstrated in a study conducted with the use of chlorhexidine (6).

In the present study, it was decided to use casein inside the mouthguard, due to its many useful properties, as confirmed by the widespread use in dental practice (7, 8).

The purpose of this study was to monitor the possible ecological changes of the oral cavity by the determination of salivary and bacterial clinical markers at baseline, 3 months and 6 months after treatment, where casein was applied in the mouthguard, making use of salivary chair-side tests.

MATERIALS AND METHODS

Patient selection

Forty-eight young male athletes aged between 10 and 14 years, were selected. Twenty-four soccer players, 12 rugby players and 12 athletes were referred to the Department of Paediatric Dentistry, Department of Medical, Oral and Biotechnological Sciences, University "G. D'Annunzio" Chieti-Pescara in the period 2015-17.

Inclusion criteria were absence of any active caries and mouthguard requirement. Exclusion criteria were periodontitis, partial or total removable prosthesis, poor medical conditions (diabetes, asthma), use of systemic antibiotics or local antimicrobials during the 3 months preceding the sport treatment placement and patients under medication that could affect the saliva flow rate.

The selected subjects participated voluntarily in the study. Patients and their parents were first given oral and written information on the study's purpose. Informed consent was given by signing a protocol. (Privacy Law DL 196/2003). In this study, the approval from the Ethics Committee is not reported, as it is not required for works that are based on research protocol on medical devices already used in the clinical protocols approved by the Department.

Clinical monitoring

The study was divided into three periods of screening: baseline, before the application of the mouthguard-baseline (T0); after three months of casein application on mouthguard during training (T1) and after six months of application (T2). The mouthguard used was custom made with EVA and multilayered. The product used was GC Tooth Mousse (GC Dental Co. Ltd. Suzhou, China). Training was carried out for the selected subjects for the application of casein according to the guidelines of the manufacturer, i.e., cleaning their teeth before applying and distributing the casein, both on the teeth and the mouthguard. Patients avoided eating and drinking and brushing the teeth at least 2 h prior to taking the samples at all stages.

During the first visit the cleaning procedure of the sport mouthguard was explained to each patient, i.e., washing for 5 min with hydrogen peroxide, and storing in perforated containers after blotting the mouthguard with a napkin to eliminate liquid residues.

A dental examination was performed at T0 and the data obtained were later collected in the appropriate Medical Records along with personal data and complete medical history. In addition, an examination of intraoral mucosal was performed to assess the presence/absence of bad habits and/or parafunctional habits and to evaluate the level of oral hygiene of the individual patients. Information (according to WHO criteria) was later collected for indices in regards to eating habits and decayed missing filled teeth (DMF-T) to assess prevalence of caries, plaque (Plaque Index) and gingival bleeding (Bleeding Index) to evaluate the oral hygiene and periodontal status. Patients avoided eating or drinking and stopped tooth

brushing at least 2 hours before taking the samples at all stages. All procedures were carried out by a calibrated researcher, and commercial kits were used according to the manufacturer's instructions.

Prior to treatment, extra and intraoral photos of the athletes followed by dental impressions were taken to create the gypsum study models.

At all three observation times, the "Saliva-Check Buffer GC" and "Saliva-Check Mutans GC" salivary tests were performed on each athlete in order to obtain the following information: visual inspection of the hydration level (test 1); consistency of saliva (test 2); salivary pH (not stimulated; test 3); amount of saliva (stimulated; test 4); salivary pH (stimulated; test 5); saliva check mutans (GC; test 6); GC plaque indicator kit (test 7).

Test 1 was used to visually evaluate the secretion of the labial gland on the lower lip.

Test 2 was used to visually assess the consistency of basal saliva in the oral cavity. If the saliva was dense and foamy the viscosity was considered increased, if the saliva was watery and transparent then viscosity was considered normal.

In Test 3, the patients were asked to deposit their saliva in a container. A pH test strip was then inserted into the collected saliva sample for 10 seconds. Then the colour of the strip was verified and statically compared with the reference graph included in the package: - red: very acidic (pH 5.0 to 5.8); yellow: moderately acidic (pH 6.0 to 6.6); - green: normal (pH 6.8 to 7.8).

For Test 4 the patient chewed a paraffin gum (included in the kit) to stimulate salivary flow. After 5 min, the amount of saliva was measured with the notches on the side of the pan: - <3.5 ml: very little; between 5.0 and 3.5 ml: little; - > 5.0 ml: normal

Test 5 was carried out by placing a buffer strip on an absorbent tissue with the test side facing upwards. Using a pipette, a sufficient amount of saliva was sucked out of the collection pan and a drop was deposited on each of the three test blocks. After 2 min it was possible to calculate the final result by evaluating the colour toning of the strips and summing up the points based on the colour of each block: green: 4 points; green/blue: 3 points; blue: 2 points; red/blue: 1 point; red: 0 points. By evaluating the total sum of the three scores, saliva buffer capacity was evaluated: 0 - 5: very low; 6 - 9: low; 10 - 12: normal/high.

The Saliva-Check Mutans GC test was used for rapid detection of the concentration of *Streptococcus mutans* in the saliva and the evaluation of the patient's susceptibility to caries. To perform this test, the patients were asked not to eat or drink or clean their teeth with toothpaste and a mouthwash within 2 hours prior to the test. Each patient then chewed the paraffin gum contained in the pack for 1 min to stimulate saliva secretion. After this time, the saliva sample was collected in the mixing container, and the analysis was carried out as indicated by the instructions provided, waiting 15 min before checking the results. The result was positive if a thin red line appeared in the test window.

The GC plaque indicator kit test was performed by collecting a sample of plaque and reacting it with the solution included in the kit, or with the plaque indicator solution. The reaction was fermented for 5 min and the colour chromaticity was compared: Green: pH = 7; Yellow: pH = 6.5; Orange: pH = 6.0; Red: pH = 5.5. From red to yellow, the acidity of dental plaque decreases, therefore a yellow sample indicates plaque in which there is an equilibrium of microorganisms and less susceptibility to the onset of dental caries. To the contrary, a red sample demonstrates a major risk of dental caries due to the low level of salivary ph.

Statistical analysis

For statistical analysis, the Matched-Pairs Signed-Wilcoxon-rank test was used. Data are shown as Mean values±Standard Deviation (SD), and for all analyses a P-value of less than 0.05 was considered significant.

RESULTS

From the information obtained, none of the children suffered from food or drug allergies. Data from eating habits showed that most of the subjects regularly took carbohydrates in their diet (pasta, breads, sweets, candies, etc.), although not excessively. In addition, none of the patients had abnormalities, erosions or recessions. Twelve (25%) of the sample had widespread discoloration and 25% showed oral breathing (data not shown).

One patient stopped wearing the mouthguard for more than a month at T2 control and was excluded from the study.

As shown in Table I, concerning clinical indices,

Table I. *Clinical indices of sample.*

	T0	T1	T2
DMFT	0.03±0.01		
Plaque Index (Y/N)	100%	100%	100%
BOP +	0%	0%	0%

T0 = before mouthguard use; T1 = mouthguard plus casein after 3 months; T2 = mouthguard plus casein after 6 months

the young athletes demonstrated the following values: DMFT was 0.03±0.01; PL was positive in 100% of the subjects examined in T0, T1 and T2; BI was negative at all the time-points examined. The results of saliva and bacteria analyses are shown in Table II.

Within the three observation times, there was no significant variation in basal hydration level and salivary flow. The saliva consistency at T0 was normal in 50% of cases and increased in the remaining 50%. It appeared to be normal and constant in T1 and T2.

The pH value was increased significantly at T1 (7.6±0.08) to remain constant at T2. The amount of saliva produced under stimulation varied significantly and gradually from T0 to T1 and T2 (therefore, after application of casein), with a statistically significant difference between observation times. The buffering capacity increased at T1 and T2. This value was statistically significant.

The plaque detector test emphasized that a plaque with pH 6.0±0.5 (orange/red in Plaque Indicator) prevailed at T0; 6.25±0.75 (green) at T1, while at T2 it had a pH equal to 6 (orange), therefore casein had not varied the plaque pH in a statistically significant way.

The tests for the detection of *S. mutans* resulted constant in all subjects at the various observation time-points.

DISCUSSION

Mouthguards represent the best solution to protect teeth in traumatic accidents. A good oral hygiene is essential for the patient and for the good preservation of the mouthguard, as demonstrated by D'Ercole and Tripodi (1).

The application of casein within the custom-made mouthguard in EVA material influences the

Table II. *Data from salivary test.*

	T0	T1	T2
Basal flow	Normal	Normal	Normal
Consistency	50%Normal 50% Increased	Normal	Normal
Basal pH	7.42±0.27	7.6±0.08*	7.6±0.2*
mL of stimulated saliva	4.64±0.62	7.5±2.12§	10±0.2§**
Buffer capacity	29% normal 57% high 14% low	75% high 25% low	75% high 25% low
Plaque Indicator	75% orange/red	50% green	75% orange
S. mutans	67%positive 33%negative	67%positive 33%negative	67%positive 33%negative

T0 = before mouthguard use; T1 = mouthguard plus casein after 3 months; T2 = mouthguard plus casein after 6 months
** NS, $p>0.05$; § S, $p<0.05$ vs T0; ** S, $p<0.05$ vs T1*

increase of pH values, the amount of stimulated saliva and the buffering capacity of the athlete, improving the state of oral health that was negatively affected by the use of simple mouthguards. These actions are enhanced by the use of casein due to a part of the casein protein called casein phosphopeptide (or CPP), to which calcium and phosphate ions are "tied" in the form of amorphous calcium phosphate (or ACP). CPP-ACFP is superior to fluoride varnish for remineralizing smooth-surface white spot lesions (9). The increase of salivary flow, buffering capacity and pH, due to the use of casein is referred by Hegde and Thakkar (10), through the use of casein-chewing gums.

Contrary to what was expected by the Authors and already demonstrated by several studies (7, 11, 12), in this work, the casein used did not show any efficacy on *S. mutans*. The results of the bacteria tests carried out in this study can be justified in that the test in question presented low sensitivity and low quantitative discrimination.

Several further preventive strategies for the changes that occur in the oral cavity of athletes should be implemented, even if this field is in continuous development.

The application of casein as an oral protector represents a new strategy to fight ecological variations in athletes' mouths, helping the salivary buffering capacity and increasing the salivary pH, avoiding all negative effects on oral health and sport performance. The present study demonstrates for the first time in literature that casein can be useful as a preventive means in the use of sport mouthguards.

Further studies will be carried out to confirm the positive effects of casein application in mouthguards.

REFERENCES

1. D'Ercole S, Tripodi D. I protettori orali nello sport. Sport & Medicina 2017; 1:43-9.
2. Glass RT, Conrad RS, Köhler GA, Warren AJ, Bullard JW. Microbiota found in protective athletic mouthguards. Sports Health 2011; 3(3):244-48.
3. D'Ercole S, Martinelli D, Tripodi D. Influence of sport mouthguards on the ecological factors of the children oral cavity. BMC Oral Health 2014; 5:14-97.
4. D'Ercole S, Tripodi D. The effect of swimming on oral ecological factors. J Biol Regul Homeost Agents 2013; 27(2):551-58.
5. D'Ercole S, Tieri M, Martinelli D, Tripodi D. The effect of swimming on oral health status: competitive versus non-competitive athletes. J Appl Oral Sci 2016; 24(2):107-13.
6. D'Ercole S, Tieri M, Fulco D, Martinelli D, Tripodi D. The use of chlorexidine in mouthguards. J Biol Regul Homeost Agents 2017; 31(2):487-93.
7. Paglia L, Scaglioni S, Torchia V, De Cosmi V, Moretti M, Marzo G, Giuca MR. Familial and dietary risk factors in early childhood caries. Eur J Paediatr Dent. 2016; 17(2):93-99.
8. Esenlik E, Uzer Çelik E, Bolat E. Efficacy of a casein phosphopeptide amorphous calcium phosphate (CPP-ACP) paste in preventing white spot lesions in patients with fixed orthodontic appliances: A prospective clinical trial. Eur J Paediatr Dent 2016; 17(4):274-80.
9. Dashper SG, Catmull DV, Liu SW, et al. Casein phosphopeptide-amorphous calcium phosphate reduces Streptococcus mutans biofilm development on glass ionomer cement and disrupts established biofilms. PLoS One 2016; 11(9):e0162322.
10. Hegde RJ, Thakkar JB. Comparative evaluation of the effects of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and xylitol-containing chewing gum on salivary flow rate, pH and buffering capacity in children: An in vivo study. J Indian Soc Pedod Prev Dent 2017; 35(4):332-37.
11. Mummolo S, Tieri M, Tecco S, Mattei A, Albani F, Giuca MR, Marzo G. Clinical evaluation of salivary indices and levels of Streptococcus mutans and Lactobacillus in patients treated with Occlus-o-Guide. Eur J Paediatr Dent 2014; 15(4):367-70.
12. Pasquantonio G, Condò S, Cerroni L, L.Bikiqu L, Nicoletti M, Prenna M, Ripa S. Antibacterial activity of various antibiotics against oral Streptocci isolated in the oral cavity. Int J Immunopathol Pharmacol 2012; 25(3):805-9.