Biochemical Interactions Between Polymeric Resins Used for Occlusal Splints and Saliva

A pilot study comparing the CAD/CAM technology and the conventional approach

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The CAD/CAM technology has been successfully integrated in clinical and laboratory aspects of dental medicine. The present in vitro study focuses on the biochemical interactions between saliva and three types of polymeric resins for occlusal splints. Dental material samples were produced from 3D printed, milled and self-cured resins and were incubated with saliva samples from 20 healthy volunteers. The results showed that the 3D printed and milled polymeric resins did not produce any significant changes in oxidative stress parameters (uric acid, TAC, GGT, OXSR-1) or inflammatory markers (IL-2, IL-6). On the other hand, the self-cured acrylic resin produced a significant decrease in the salivary TAC and uric acid, the most important antioxidants in saliva, affecting the capacity of saliva to protect the oral environment against oxidative stress.

Keywords: polymeric resins, saliva, oxidative stress, inflammation

Over the last decade, the digital technology (CAD/CAM) has challenged the classical approach and has been rapidly adopted in restorative dentistry, management of temporomandibular and sleep disorders, orthodontics, oral and orthognathic surgery, in both clinical and laboratory aspects. One further step in this direction is the three-dimensional printing, which has been primarily used in engineering but nowadays also in dentistry in producing parts and end products like casts, surgical guides, occlusal splints and teaching aids [1].

The most commonly used 3D printing techniques in dentistry are the following: stereolithography (SLA), digital light processing (DLP), selective laser sintering (SLS), selective laser melting (SLM), electron beam melting (EBM), triple jetting technology (Polyjet Printing) and fused deposition modelling printing (FDM). Compared to other systems, the SLA printing method offers very good accuracy and the smoothest printed surfaces, providing a high quality of printing [2].

The manufacturing of surgical templates and occlusal splints can be done basically in 3 ways: the conventional approach, CAD/CAM milling process and an additive process from a digital model by means of 3D printing [3]. The advantages of the digital techniques over the conventional one include: accuracy due to absence of polymerization shrinkage, reduced chairside and laboratory working time, reproducibility, reduced cost [3]. On the other hand, the traditional method is more technique sensitive, the practitioner may need extra time for passive fit and occlusal adjustments, and, in the direct method, the residual monomer creates discomfort for the patient [4].

Occlusal splints are proven to be efficient in the treatment of temporomandibular disorders (TMD) and the prevention against tooth surface loss as a result of clenching and grinding (bruxism). The most frequently used occlusal splints are: stabilization splints, with the primary goal to reduce muscle pain and the activity of the masticatory muscles and the repositioning splints, which try to change the position of the condyle in the temporomandibular jaw [5].

Controlled clinical trials have shown that the use of an occlusal appliance significantly reduces the symptoms of myofascial pain, prevents the negative effects of parafunctional activity and helps in repositioning the disk-condyle assembly. Moreover, occlusal devices can test whether the patient can tolerate a new occlusal scheme in complex restorative treatment or when an increased occlusal vertical dimension (OVD) is planned [6].

Both above-mentioned types of splints should be worn according to the instructions of the clinician, during the day and/or during the night, which implies that the splints interact with the oral environment at least 7-8 h per day.

To the best of our knowledge, testing of dental materials' pro or antioxidant activity in saliva has not been yet implemented in biocompatibility analysis *in vitro* or *in vivo*. The aim of this pilot *in vitro* study was to test the interactions between polymeric resins for occlusal splints and saliva. The polymeric resin samples were obtained through milling, 3D printing (CAD/CAM) and conventional process (self-cured acrylic resin).

Experimental part

Material preparation

Three types of dental materials were selected for this study: printable resin (*Dental LT Clear/Formlabs/U.S.A.*), milled PMMA/Poly-methyl-methacrylate (*Polident PMMA*

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CAD-CAM Discs/Splint/Transparent/Polident/Slovenia) and self-cured acrylic resin (*Premacryl™ Plus/Clear-O/ SpofaDental/ Czech Republic)*.

Dental LT Clear Resin is a Class II long-term biocompatible resin, recommended for splints, occlusal guards, retainers or printed orthodontic appliances. This material has high optical transparency, good fracture and wear resistance; its' flexural modulus is higher than 1300 MPa.

*Polident PMMA (*Poly-methyl-methacrylate) *CAD-CAM Discs/Splint* are suitable for fabrication of occlusal splints, bite-guards, therapeutic splints or milled templates; it has a flexural strength of at least 114 MPa and Elastic Modulus is at least 2771 MPa.

Premacryl™ Plus/Clear-O is a modified self-curing methacrylate resin usually used in the dental laboratory for traditional manufacturing of occlusal splints and orthodontic appliances.

Three groups of samples were obtained for the present study, corresponding to the selected materials, as follows: group 1 -printed resin samples; group 2 -milled resin samples; group 3 - self-cured resin samples (fig. 1). Six printed resin samples were produced, respectively three from each of the two other materials, resulting in 12 samples in total. The obtained disk-samples were designed to be circular in cross section, 2 mm thick, with a 30 mm diameter and a 2 mm edge.

All studied materials were obtained according to the producers' recommendations. The printed and milled samples were manufactured by using the CAD-CAM technology. Based on SolidWorks software, a STL file (fig. 2) with the required dimensions was generated and exported to a 3D printer (Form 2/FormLabs Inc./U.S.A.), in order to produce the printed resin samples (Stereo-lithography/SLA). The same STL file was also exported to a CAM machine for obtaining the milled PMMA samples. In addition, the self-cured acrylic resin samples were prepared by a certified dental technician, according to a settled protocol, as specified by the manufacturer: mixing ratio/by weight - 2 g of powder to 1 g of liquid; mixing time -1.5 min; working time - 5-6 min. at a temperature of 25°C (from the start of mixing to the start of setting); setting time - 15 min at a pressure of 0.2 -0.3 MPa, in water at 50-60°C.

Saliva collection

1.0 - 2.0 mL of unstimulated saliva samples were obtained from 20 healthy volunteers, in the morning, between 9 and 10 AM. All subjects were asked not to eat, brush their teeth or use mouth rinse for at least 2 h prior to sample collection. All subjects included in our study agreed to participate voluntarily and signed an informed consent.

All saliva samples were collected in sterile test tubes. After collection, saliva samples were immediately incubated for 12 h, at 37°C with samples of dental materials (one dental material sample / 500µL of saliva). After the incubation period the saliva samples were centrifuged for 10 min at 3000 rpm to remove bacterial and cellular debris. For the control group saliva samples were centrifuged without prior incubation with dental material. All determinations were performed using the supernatant.

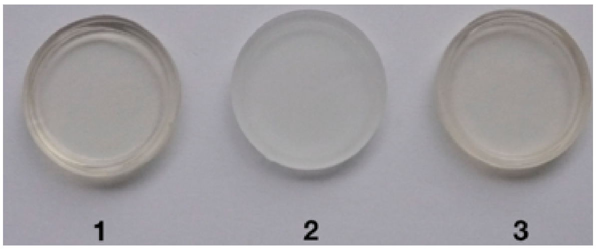

Fig. 1. Types of obtained samples: 1 - printable resin; 2 - milled PMMA; 3 - self-cured acrylic resin

Fig. 2. CAD template for printed and milled samples

Saliva analysis

Salivary uric acid, GGT (gamma glutamyl transferase), albumin, TAC (total antioxidant capacity of saliva), OXSR-1 (oxidative stress responsive kinase 1), IL-2 (Interleukin 2) and IL-6 (Interleukin 6) were assayed in the incubated and control samples, immediately after sample centrifugation.

The concentrations of all salivary parameters were expressed relative to the salivary concentration of albumin in order to avoid the salivary flow influence. Salivary albumin, uric acid and GGT were measured using analysing kits from Biosystems (Barcelona, Spain) on a biochemistry automatic analyser A25, Biosystems (Barcelona, Spain), according to the supplier's instructions. For the salivary TAC and OXSR-1 measurements ELISA analyzing kits from Blue Gene – China and Abbexa -UK, respectively have been used. IL-2 and -6 were measured using an automatic chemiluminescence analyser, IMMULITE 1000 (Siemens, Germany).

All our experimental data were expressed as means ± SD (standard deviation). The data were analyzed statistically using Student's t-test. A p value < 0.05 was considered statistically significant.

Results and discussions

Our experimental results are presented in the following table 1.

Analysing table 1, figure 3 and figure 4 it can be observed that saliva incubation with milled and printed samples did

Sample	**Uric acid** mg/mg alb.	**TAC** nmoli/mg alb.	**GGT** U/mg alb.	**OXSR -1** mg/mg alb.	**IL-2** pg/ml	**IL-6** pg/ml
3D Printed resin	1.8±0.35	1.1±0.14	5.1±0.8	0.58±0.09	<5	<5
Milled resin	1.9±0.29	1.2±0.11	5.8±1.3	0.6±0.04	<5	<5
Self- cured acrylic resin	**1.2 ±0.2 Pi<0.05**	**0.82±0.09 Pi<0.05**	5.5±1.1	0.55± 0,04	<5	<5
Control	1.8±0.25	1.2±0.17	5.6±1.0	0.52±0.07	<5	<5

Table 1
EXPERIMENTAL DATA EXPRESSED AS MEAN ± SD

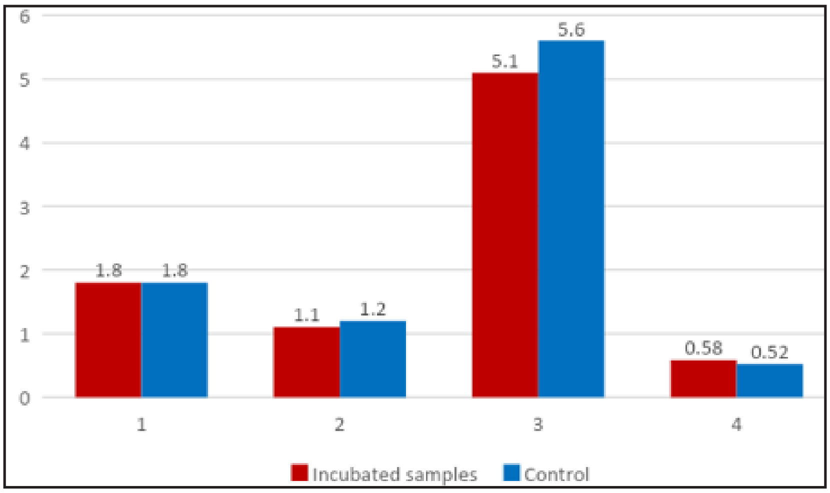

Fig. 3. Experimental data obtained from saliva incubation with printed samples: 1-Uric acid mg/mg albumin; 2- TAC nmoli/mg albumin; 3-GGT U/mg albumin; 4-OXSR-1 mg/mg albumin

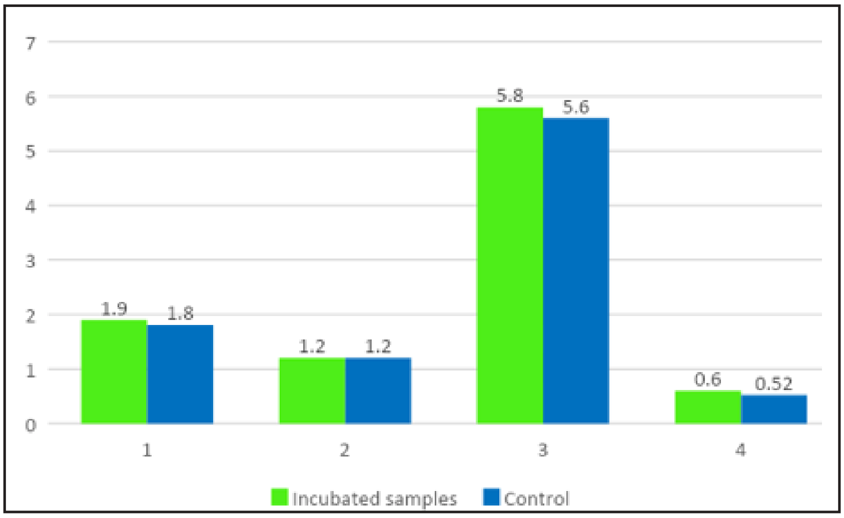

Fig. 4. Experimental data obtained from saliva incubation with milled samples: 1-Uric acid mg/mg albumin; 2- TAC nmoli/mg albumin; 3-GGT U/mg albumin; 4-OXSR-1 mg/mg albumin

not induce any significant changes (p> 0.1) concerning the salivary redox status, indicated by uric acid, TAC, GGT, OXSR-1 levels and the inflammatory status as reflected by the levels of IL-2 and IL-6.

These experimental results show that the samples obtained by 3D printing and milling processes do not modify the redox status of the incubated saliva, which means these materials do not induce oxidative stress in the oral environment. According to various studies, oxidative stress plays an important role in the development and progression of an increased number of oral pathologies, including periodontal disease, oral lichen planus and oral cancer [7,8].

Regarding the self-cured acrylic resin samples incubation of saliva, our results shown in table 1 and figure 5 revealed that salivary uric acid and TAC levels significantly (p< 0.05) decreased compared with control samples (1.2 ±0.2 mg/mg albumin *versus* 1.8±0.25; 0.82±0.09 nmoli/mg albumin *versus* 1.2±0.17 respectively).

In contrast with the milled and printed resins, self-cured acrylic resin produces a significant decrease in the salivary TAC and uric acid, the most important antioxidants in saliva. This indicates that the capacity of saliva to protect the oral environment against oxidative stress is affected, thus the oral cavity being at risk. As for the salivary levels of GGT, OXSR-1, IL-2 and IL-6 no significant changes could be observed, which means that did not influence the anti-inflammatory components from saliva. Moreover, the role of saliva as part of the defence mechanisms against oral

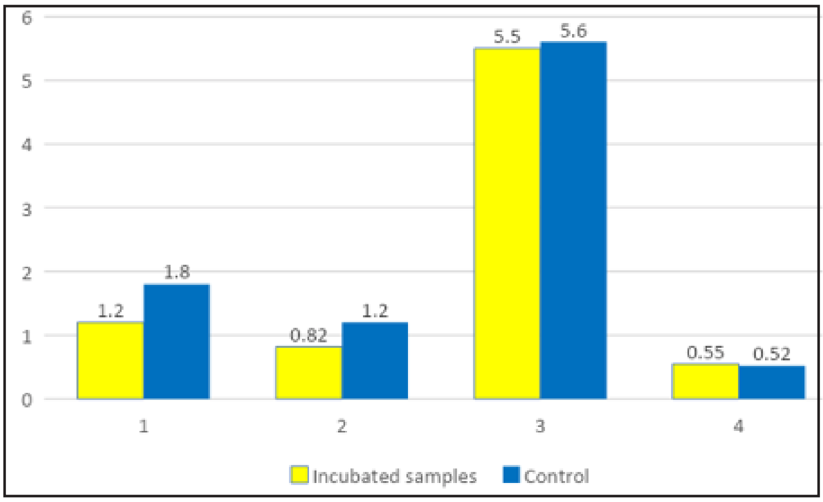

Fig. 5. Experimental data obtained from saliva incubation with self-cured acrylic resin samples: 1-Uric acid mg/mg albumin; 2- TAC nmoli/mg albumin; 3-GGT U/mg albumin; 4-OXSR-1 mg/mg albumin

pathologies (caries or periodontal disease) as well as a useful diagnostic tool has already been proven [9,10].

Various authors have studied whether the digital process and new materials for occlusal splints or surgical guides can become a reliable alternative for the conventional approach. A pilot study in 2013 [3] describes a method of manufacturing an occlusal splint by means of an additive process (stereolithography). Alginate impressions were taken, the casts were scanned with a 3Shape D710 laboratory scanner, the splint was designed using the VISCAM RP Software and produced from a biocompatible liquid photopolymer (Somos WaterShed XC 11122, DSM Functional Materials) in the SLA 350 (3D Systems) machine.

The patient adapted well to the splint and after using it for a period of 6 month, the authors observed a reduced tension in the masticatory muscles and no sign of splint wear [3]. These results show that this material performed well in the oral cavity and proved their therapeutic effect.

Another study published by Berntsen et al in 2018 [11] focuses on the comparison between the conventional approach and the additive approach of manufacturing occlusal stabilization splints for TMD patients, analysing the following parameters: patients´ perception, treatment outcome, time needed for impressions (conventional and scanning) and try-in and the internal fit. The results showed that there were no significant differences in the internal fit of splints (570 ìm for additive and 603 ìm for conventional) and the patients responded well and experienced less TMD symptoms after a period of three months. Although the time for alginate impressions was significantly lower, the patients rated significantly better the digital impression technique.

Shaheen et al studied and validated a protocol for designing and 3D printing of the splints used in orthognathic surgery. The clinical assessment and the comparison with the conventional set-up found clinically acceptable error margins between 0.12-0.88 mm [12].

Regarding the fabrication of occlusal splints, the mechanical properties of the polymers used for the digital manufacturing (milling and 3D printing) are an important issue because they can predict how the materials would perform under clinical conditions (parafunction). Huettig et al [13] found in an in-vitro study that the polishability and wear resistance of the milled and printed materials were comparable with the conventional self-cured acrylic resin for oral appliances, but they pointed out the need for further tests.

Another in-vitro study [14] compared the wear resistance of 4 types of occlusal splint materials (polyamide, conventional, milled and printed resins) with 3 composite materials for direct restorations. The results showed that the polyamide resin and the printed resin wear rates were comparable with those of the composite resins. The authors concluded that the practitioner has the opportunity to choose the material which corresponds to the clinical situation.

Finally, our study, along with other recent ones [15-17], provides valuable results with practical applicability, and further stimulates the interest in developing research involving the study of the interaction between saliva and different dental materials.

Conclusions

Despite the limitations of this *in vitro* study, the reduced number of samples and the short incubation time, we can conclude that the samples produced by milling and 3D printing did not produce oxidative stress and inflammation in saliva. There were no significant changes in the redox status markers (uric acid, TAC, GGT, OXSR-1) or in the inflammatory markers (IL-2, IL-6). On the other hand, our results show that the self-cured acrylic resin reduced the saliva antioxidant defence potential by reducing the levels of TAC and uric acid. Regarding the inflammatory markers, our results show no significant changes in IL-2 and IL-6 induced by the conventional splint material. Our results combined with those from the other studies mentioned in the present article recommend the resins for occlusal splints obtained by CAD/CAM technology for clinical application.

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