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Artificial saliva effect on toxic substances release from acrylic resins

Uticaj veštačke pljuvačke na oslobađanje toksičnih supstanci iz akrilata za bazu zubne proteze

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Abstract

Background/Aim. Acrylic-based resins are intensively used in dentistry practice as restorative or denture-base materials. The purpose of this study was to analyze the surface structure of denture base resins and the amount of released potentially toxic substances (PTS) immediately upon polymerization and incubation in different types of artificial saliva. **Methods.** Storage of acrylic samples in two models of artificial saliva were performed in a water bath at the temperature of 37 ± 1°C. Analysis of the surface structure of samples was carried out using scanning electronic microscopy analysis immediately after polymerization and after the 30-day incubation. The amounts of PTS *per* day, week and month extracts were measured using high-pressure liquid chromatography. **Results.** Surface design and amount of PTS in acrylic materials were different and depended on the types and duration of polymerization. The surfaces of tested acrylates became flatter after immersing in solutions of artificial saliva. The degree of acrylic materials release was not dependent on the applied model of artificial saliva. **Conclusion.** In order to improve biological features of acrylic resin materials, it was recommended that dentures lined with soft or hard cold-polymerized acrylates should be kept at least 1 to 7 days in water before being given to a patient. So, as to reach high degree of biocompatibility preparation of prosthetic restorations from heat-polymerized acrylate was unnecessary.

Key words:
acrylic resins; saliva, artificial; hazardous substances.

Apstrakt

Uvod/Cilj. Akrilati se u stomatologiji često koriste kao restaurativni materijali ili materijali za izradu baza zubnih proteza. Cilj istraživanja bio je analiza količine oslobođenih potencijalno toksičnih supstanci (PTS) iz akrilatnih materijala neposredno nakon njihove polimerizacije i inkubacije u različitim tipovima veštačke pljuvačke. **Metode.** Uzorci akrilatnih materijala potapani su u dva modela veštačke pljuvačke u vodenom kupatilu temperature 37 ± 1°C. Analiza površinske strukture uzoraka vršena je skenirajućom elektronskom mikroskopijom odmah nakon polimerizacije i posle tridesetodnevne inkubacije. Količina PTS u jednodnevnim, jednonedeljnim i jednomesečnim ekstraktima merena je tečnom hromatografijom pod visokim pritiskom. **Rezultati.** Površinski dizajn i količina PTS bili su različiti kod različitih akrilatnih materijala i zavisili su od vrste i trajanja polimerizacionog postupka. Nakon potapanja u rastvore veštačke pljuvačke površine testiranih akrilata postale su ravnije. Oslobađanje PTS nije zavisilo od primenjenog modela veštačke pljuvačke. **Zaključak.** U cilju poboljšanja bioloških svojstava akrilatnih materijala, preporučuje se da zubne proteze podložene mekim ili čvrstim hladno polimerizovanim akrilatima budu potopljene u vodi 1 do 7 dana pre predaje pacijentu. S obzirom na visok nivo biokompatibilnosti, naknadna obrada proteza od toplo polimerizovanog akrilata nije potrebna.

Ključne reči:
akrilati; pljuvačka, veštačka; toksične supstance.

Introduction

Acrylic-based resins are intensively used in dentistry practice as restorative or denture-base materials [1]. These acrylates are made by polymerization of acrylate related monomers and can be classified depending on the factor that initiates the polymerization reaction (as cold, heat or light polymerization) [2]. These materials are considered as biologi-

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cally acceptable, which verifies their wide-spread use in medicine and dentistry. However, a great deal of the available reports referring to adverse effect of particular components of acrylic prosthetic materials on human organism both at local (immunological and inflammatory reactions of oral tissues) and systemic level (changes on respiratory and gastrointestinal tract) [3, 4]. Particular components of acrylic materials could diffuse in saliva from prosthetic restorations, and cause the damage of oral tissues [5, 6]. The potential cytotoxicity is influenced by residual monomers as well as other additives such as initiators and stabilizers, mixing liquids, bonded materials, benzoyl peroxide, tertiary amine etc. [7, 8]. The conversion of monomer into polymer is not complete during the polymerization process, and varying amounts of potentially toxic substances (PTS) remain in the polymerized resin. The values of residual monomers remaining in resins is determined by standards [9], considered only as the amount of monomers in the resin but not taking into account their elution characteristics. Many reports of allergic reactions which were associated with acrylic based resins have been attributed to monomer and additives as benzoyl peroxide [10, 11].

Undesirable reaction of oral tissue may occur as the result of toxicity of applied material as well as superficial accumulation of infectious content [12]. In order to be biocompatible, dental restorative material should have such surface design to react with tissue and surrounding agents at the least possible degree [13]. Rough surface of various acrylic materials represents a predilection site for accumulation of plaque, pigments and residual oral tissue [14, 15]. Analysis of the possibility of preparation of acrylic material surface in order to reduce fungal adhesion and microbial plaque in general represents a very significant contribution to the improvement of their biocompatibility [16, 17].

The purpose of the study was to analyze the amount of released PTS and the surface structure of acrylic denture base resins immediately upon polymerization and incubation in two different types of artificial saliva.

Methods

Examined material

To prepare samples two hard and three soft acrylic denture base resins used in prosthetic dentistry for construction and relining of removable dentures were used. The manufacturers and types of cold- and heat-polymerized acrylates used in the study were summarized in Table 1.

The examined material was polymerized according to the manufacturer's instructions. To analyze the influence of artificial saliva on the surface design samples of each acrylic material were prepared in parallelepiped shape, 1 × 2 × 3 mm. In order to determine the dynamics of release of PTS, samples of each test material were made as parallelepiped 10 × 10 × 1 mm (*per* 5 samples in each of the test groups). Preparation of cold-polymerized acrylates was performed at room temperature (18–20°C) for 10–15 minutes without pressure using a condensation silicone mould. Heat polymerization was performed in a water bath (GFS, Germany) within specialized metal flasks for 45 min in boiling water. They were kept in a sterile Petri dish at room temperature, without standard procedure polishing.

Model 1 of artificial saliva was designed according to Preetha and Banarjee [18] (Table 2). Model 2 of artificial saliva represented a modification of model 1 in which 0.20 g α amylase/L deionized water was added.

Table 1

The manufacturers and the used acrylic types of materials

Tested material	Manufacturer	Acrylic type	Content	
			powder	liquid
Bosworth Trusoft	HG Bosworth Company USA	Soft cold polymerized acrylate	Poly (ethyl methacrylate)	Ethyl alcohol, butyl benzyl phthalate
Lang Flexacryl	Lang Dental MFG.Co. USA	Soft cold polymerized acrylate	Poly (ethyl methacrylate)	N-buthyl methacrylate
Lang Immediate	Lang Dental MFG.Co. USA	Soft cold polymerized acrylate	Poly (ethyl methacrylate)	Methyl methacrylate
Triplex Cold	Ivoclar Vivadent, Lichtenstein	Hard cold polymerized acrylate	Poly(methyl methacrylate)	Methyl methacrylate, Ethylene glycol dimethacrylate
Triplex Hot	Ivoclar Vivadent, Lichtenstein	Heat polymerized acrylate	Poly(methyl methacrylate)	Methyl methacrylate, Ethylene glycol dimethacrylate

Table 2

Components of the Model 1 artificial saliva [18]

Components	G components / l deionised water
Xantan gum	0.18
Potassium chloride	1.20
Sodium chloride	0.85
Magnesium chloride	0.05
Calcium chloride	0.13
Di-potassium hydrogen orthophosphate	0.13
Methyl p-hydroxybenzoate	0.35

Incubation of acrylic samples in artificial saliva was performed in a water bath in closed plastic tubes at human body temperature (t = 37 ± 1°C). The ratio of material and artificial saliva was 0.1 g of tested material/1 mL of extraction solution, according to ISO 10993-5: 1992 standard [19].

Scanning electronic microscopy analysis (SEM)

To analyze the influence of artificial saliva on the sample SEM was used. Analysis was used for comparison of surface structure of acrylic materials samples immediately after polymerization cycle (marked as control samples) and after thirty days of immersing in artificial saliva (models 1 and 2), whereby all changes relating to surface appearance, homogeneity and adherence were recorded. The samples were dried and coated with gold layer in ion spray by spattering and analyzed under microscope JSM-5300, JOEL, Japan.

Determination of the amount of PTS

Determination of the presence and amount of PTS was performed in solutions of two different models of artificial saliva after removal of acrylic samples. In order to establish the dynamics of PTS release from the examined samples, extraction periods of one, seven and thirty days were adopted. Quantity of released materials was analyzed by using high-pressure liquid chromatography (HPLC), Agilent 1100 Series (USA), with DAD 1200 detector and analytical column SUPELCO Discovery HS C18 250 × 4.6 mm, 5 μm, Sigma-Aldrich, USA. Methanol [M = 32.04 g/moL, Chromasolv HPLC chromatography grade (purity 99.9%) Sigma-Aldrich GmBH, Steinheim, Germany] was used as an eluent. The mobile phase flow was 1 cm^3/min, and sample injection volume was 20 μL. Since all tested compounds have maximal absorbance around 205 nm, this wave length was selected for calibration curve construction and further sample testing. Determined PTS and reagents used for calibration curves were presented in Table 3.

Calibration curves were made from a solution series of each examined substance in methanol. The initial concentration of tested compound was 1 mg/cm^3, from which, afterwards, a solution series of lower concentrations diluted by methanol were made. From the obtained chromatograms retention time of each compound (R_t) and peak surface area (A) were read. Table 4 shows the R_t values, λ_{max}, concentration range (C) at which peak surface dependence is linear and linear correlation coefficient (R).

Two-way ANOVA for repeated measures was used for statistical analysis of differences in the amounts of PTS in relation to the type of materials and artificial saliva. Data were analyzed in SPSS software, version 16.0.

Results

Figure 1 shows the results of SEM analysis of all the analyzed acrylic materials immediately after the polymerization cycle and 30 days after incubation in two different models of artificial saliva.

Surfaces of samples of soft cold polymerized acrylates analyzed immediately after polymerization showed a granular structure and unequal granular size (10–100 μm) that

Table 3

Potentially toxic substances (PTS) pressure determined in acrylic samples and reagents used for high-pressure liquid chromatography (HPLC) calibration curves

PTS	Used agents	Manufacturer
MMA	$CH_2 = C(CH_3)COOCH_3$, M = 100.12 g/moL, 98,5%	Aldrich, Milwaukee, Wisconsin, USA.
BuMA	$CH_2 = C(CH_3)COOC_4H_9$, M = 142.20 g/moL, 99%	Sigma-Aldrich GmbH, Steinheim, Germany.
EGDM	$CH_2 = C(CH_3)COO(CH_2)_2OCOC(CH_3) = CH_2$, M = 198.22 g/moL, 98%	Fluka Chemie GmbH, Steinheim, Germany.
EMA	$CH_2 = C(CH_3)COOC_2H_5$, M = 114.14 g/moL, 99%	Aldrich, Milwaukee, Wisconsin, USA.
BP	$(C6H5CO)_2O_2$, M = 242.23 g/moL, 99%	Fluka Chemie GmbH, Steinheim, Germany.
dBuFt	$C6H4\text{-}1,2\text{-}[CO_2(CH_2)_3CH_3]_2$, M = 278.34 g/moL, 99%	Aldrich, Milwaukee, Wisconsin, USA.

MMA – methyl methacrylate (monomer); BuMA – buthyl methacrylate (monomer); EGDM – ethylene glycol dimethacrylate (comonomer-cross-linker); EMA – ethyl methacrylate (monomer); BP – benzoyl peroxide (initiator); dBuFt – dibuthyl phthalate (plasticiser).

Table 4

Values of R_t, λ_{max}, concentration range for which linear dependence of peak surface exists and concentration and R for compounds determined by high pressure liquid chromotogrophy (HPLC) method

PTS	Rt (min)	λmax (nm)	Linear correlation for compounds C, (mg/cm^3)	R
MMA	2.637	207	0 to 0.14	0.988
BuMA	2.946	208	0 to 0.10	0.999
EGDM	3.049	208	0 to 0.10	0.995
EMA	2.289	207	0 to 0.14	0.991
BP	3.332	204 and 236	0 to 0.12	0.998
dBuFt	3.514	205 and 225	0 to 0.10	0.996

PTS – potential toxic substances; Rt – retention time of each compound; R – linear correlation coefficient; MMA – methyl methacrylate (monomer); BuMA – buthyl methacrylate (monomer); EGDM – ethylene glycol dimethacrylate (comonomer-cross-linker); EMA – ethyl methacrylate (monomer); BP – benzoyl peroxide (initiator); dBuFt – dibuthyl phthalate (plasticiser).

were noticeable on the material surface (Figures 1a, 1b, 1c and 1d). In contrast to all the other material, heat polymerized acrylic material Triplex Hot, immediately after polymerization, had no granular structure at all (Figure 1e).

During the immersion period in solution of both artificial saliva models, soft cold acrylic materials accumulate salivary components on their surface. After the 30-day immersion in artificial saliva without α amylase, the surface of soft cold-polymerized acrylates became flatter and the appearance of the samples surface remained almost the same after immersion in artificial saliva with enzyme addition (Figures 2a, b, c and d). After immersion of heat polymerized acrylic material in both models of artificial saliva, the surface of this sample was completely flat. The most homogenous surface structure was noticed at heat-polymerized acrylates as compared to all the tested materials (Figure 2e).

The results show that the amount of PTS, regardless of the type of acrylic materials, is mostly released on the first day and continues to decrease during the observation period (Figure 1). However, data clearly suggest that the amount of released PTS is significantly higher immediately after polymerization, compared to the day 7 and 30 after polymerization

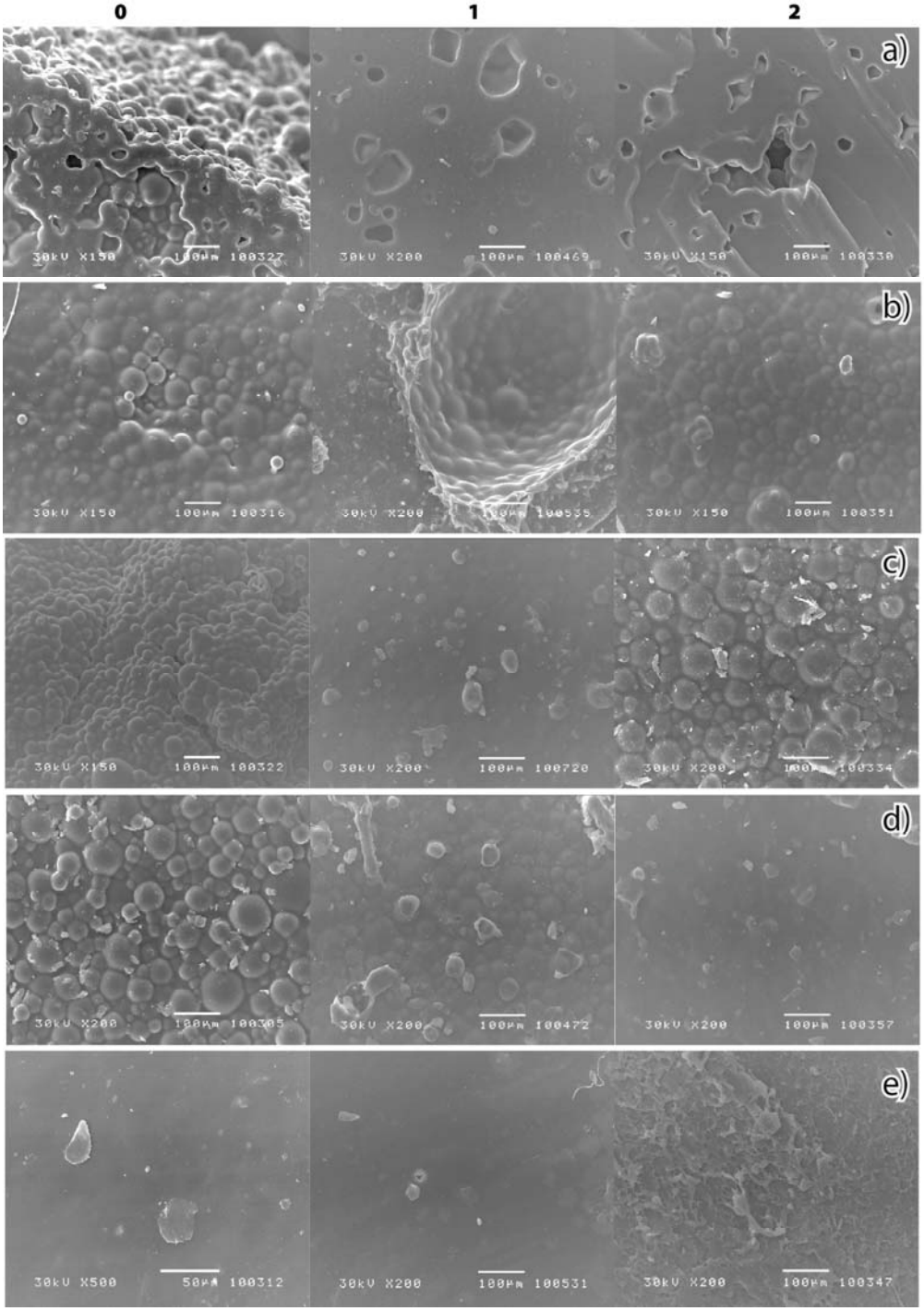

Fig. 1 – Scanning electronic microscopy (SEM) analysis of acrylic materials samples immediately after polymerization cycle (0) and after a thirty-day immersion in two different models of artificial saliva: model 1 (1) and model 2 (2) for different acrylic resins: a) Bosworth Trusoft; b) Lang Flexacryl; c) Lang Immediate; d) Triplex Cold, and e) Triplex Hot.

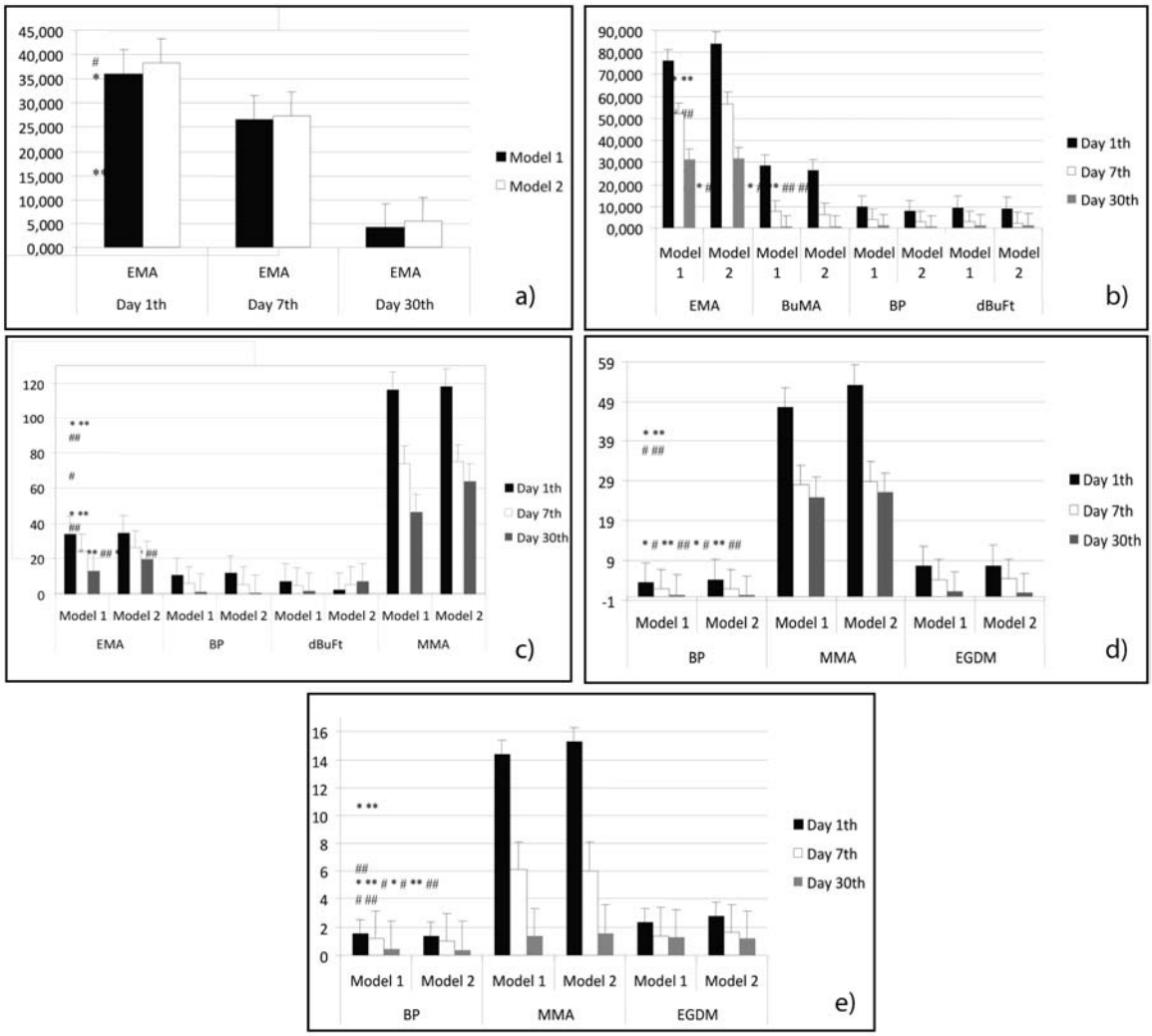

Fig. 2 – Concentrations (mean ± SD, μg/cm^3) of potentially toxic substances given in Table 3 from different acrylic resins: a) Bosworth Trusoft; b) Lang Flexacryl; c) Lang Immediate; d) Triplex Cold; e) Triplex hot at time periods of 1, 7 and 30 days after immersion in two different models of artificial saliva.

***p* < 0.001 – means significant differences detected between the day 1 and the day 7 after immersion in model 1 of artificial saliva without α amylase; #*p* < 0.001 – means significant differences detected between the day 1 and the day 30 after immersion in model 1 of artificial saliva without α amylase; ***p* < 0.001 – means significant differences detected between the day 1 and the day 7 after immersion in model 2 artificial saliva with α amylase; ##*p* < 0.001 – means significant differences detected between the day 1 and the day 30 after immersion in model 2 artificial saliva with α amylase.**

($p < 0.001$). Moreover, when the release of PTS in the model of artificial saliva with the addition of α amylase is tested, it is noticed that there is a tendency of PTS to increase but without making statistically significant differences compared to the model of saliva without this enzyme.

Discussion

It has been assumed that the difference in the amount of PTS and adherence degree depend on the type of material and polymerization conditions, as well as that biocompatibility of acrylic dentures may be increased by adequate postpolymerization treatment [20, 21].

Analysis of the possibility of preparation of acrylic material surface in order to reduce fungal adhesion and microbial plaque in general represents a very significant contribution to the improvement of their biocompatibility. Acrylic restorations in the mouth are coated with salivary pellicle, the layer that is formed by an interaction of material and ingredients of saliva. Precipitation of mucin and proteins of saliva plays the most pivotal role in its formation [16, 17]. Salivary pellicle is removed by hygiene procedures, as well as mechanical cleaning of the surfaces, but in contact with saliva (in the mouth), pellicle is produced again at removable denture. The presence of saliva and salivary pellicle formation in a clinical setting may influence PTS release from acrylic materials [22]. Due to the fact that the quality and composition of natural saliva is different in each individual, its chemical composition is impossible to be reproduced originally. Advantages of artificial saliva use under *in vitro* conditions include standardization of experiment conditions and prevention of sample contamination.

Adverse effect of residual monomer has been well established in numerous studies [23, 24]. It was experimentally established that cold-polymerized acrylates, due to their incom-

plete polymerization, had higher amount of residual monomers, and subsequently, higher release in the oral cavity [25]. Higher level of heat-polymerized acrylates' biocompatibility could be explained by their more complete polymerization and more compact internal structure after polymerization cycle. In fact, heat-polymerized acrylates are prepared at the temperature of boiling water that is close to the point of acrylic glass transition temperature, so, the mobility and conversion of monomer units in polymer structure are significantly higher as confirmed by the previous researches [26, 27]. The obtained results are in accordance with those of Baker et al. [28] who examined the release of methyl methacrylate from heat and cold-polymerized acrylates in the patient's oral cavity.

Two models of artificial saliva were prepared in order to evaluate the influence of artificial saliva composition on released of PTS from denture materials and surface design. The first model was without and the other one was with α amylase, which is one of the components of human saliva for digestion of food in mouth. The utilized models of artificial saliva showed values of viscosity and surface tension similar to those of natural saliva [29]. However, none of the used models had active phospholipids and mucin as the most active surface proteins of natural saliva. Adding of α amylase to model of artificial saliva could have compromised the obtained results due to more intensive coating of materials with salivary pellicle in relation to conditions existing in the oral cavity [29]. There was no significant difference in the amount of toxic substances released from all the examined materials over the time in relation to the type of saliva, which indicates minimal effect of α amylase on reduction of acrylic adherence.

Conclusion

The surfaces of the tested acrylates became flat after immersing in both models of artificial saliva. This research proves that the amount of potentially toxic substances from the samples of acrylic material used for making dentures grows over the time and does not depend on the type of used saliva. Slightest changes in the structure show the sample of heat-polymerized acrylates. In order to improve biological features of acrylic resin materials, the authors suggest that dentures lined with soft or hard cold-polymerized acrylates should be kept at least 1 to 7 days in water before being given to a patient.

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