

Monomer Leakage Behavior of Conventional and CAD/CAM Denture Acrylic Materials Under Different pH Values

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ABSTRACT

Computer-aided design and Computer-aided manufacturing (CAD/CAM) has emerged as a new approach for the fabrication of removable prosthesis offering many advantages over the conventional fabrication methods. The pre-polymerized polymethyl Methacrylate (PMMA) pucks used for the fabrication of CAD/CAM removable prosthesis has a significantly enhanced physical and mechanical properties. This study aims to evaluate the effect of different salivary pH values on monomer leakage from heat-cured and CAD/CAM denture acrylic materials. Two groups of 60 discs were fabricated from heat-cured and CAD/CAM acrylic materials. These acrylic samples were subjected to mechanical brushing and thermocycling according to a standardized protocol. The discs of the two acrylic materials were immersed and incubated in three salivary solutions with different pH values (acidic, 5.7; neutral, 7; basic, 8.3) for 30 days, after which the amount of leaked monomer in the saliva solution in the two groups was determined using high-performance liquid chromatography (HPLC). Both the acrylic material type and salivary pH value had a significant effect on monomer leakage. An acidic salivary pH caused the most monomer leakage in both acrylic material groups ($P < 0.05$). The heat-cured acrylic material leaked less monomer than the CAD/CAM acrylic materials. The acidic salivary pH values were associated with higher amounts of monomer leakage in both heat-cured and CAD/CAM denture acrylic materials. In-laboratory immersion of newly fabricated heat-cured and CAD/CAM acrylic dentures in an acidic solution might be recommended to allow most unreacted monomers to leak before delivering the denture to the patient.

KEY WORDS: ACRYLIC, CAD/CAM, DENTURE, MONOMER LEAKAGE, SALIVARY PH.

INTRODUCTION

Despite advances in preventive dentistry, edentulism is still a major public health problem and is considered an important indicator of the oral health of elderly population where the loss of some or all remaining teeth has a negative impact on the health-related quality of life (Cunha-Cruz et al 2007; Emami et al 2013; Batista et al 2014; Silva-Junior et al 2017; Batista et al 2018).

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Accordingly, the use of removable dental prostheses has increased among older patients who are the primary wearers of dentures in the general population (Dye et al 2012; Kassebaum et al 2014; Kassebaum et al 2017). Several materials have been used for the construction of removable prosthesis and dentists have long been searching for ideal materials for the construction of dentures. Nowadays, Polymethyl methacrylate (PMMA) resin is considered the material of choice for the fabrication of removable prostheses. Despite its weak flexural and impact strength and low fatigue resistance, it has many advantageous properties, including good mechanical features, ease of fabrication and repair, aesthetic properties and stability in the oral cavity (Dogan et al 2007; Nakamura et al 2007; Mohamed 2008; Alla et al 2013; Gad et al 2017; Zafar 2020).

Similar to any other dental materials used inside the oral cavity, PMMA resin denture base materials are subjected to changing wet oral environment which is physiologically characterized by natural saliva and its components (Zidan et al 2020). Potential harmful effects may arise from pH changes due to cariogenic biofilms in the oral ecology, diet intake and different enzymes (Turssi et al 2003). These phenomena can lead to the leaching out of plasticizers and soluble components from the acrylic over extended periods (Mohamed 2008; Marsh and Zaura 2017; Du et al 2020). It is widely reported in the literature that substances leaching out from denture base acrylic resins can cause cytotoxic effects (Koutis and Freeman 2001; Gonçalves et al 2006; Mörmann et al 2013; Rashid et al 2015). Unreacted residual monomers are the main substances that leach out from acrylic resins by the process of diffusion, the quantity of which is highly related to the polymerization reaction of acrylic resins (Chaves et al 2012; İça et al 2014; Nik et al 2014). Unreacted monomers may cause toxic effects, adverse allergic reactions and significant damage at the cellular level (Drozd et al 2011; Goiato et al 2015; Çakırbay et al 2018; Degirmenci et al 2020).

Several studies have aimed to quantify the amount of diffusing monomer and other leachable components from acrylic-based materials into the saliva. One study found that the maximum concentration of residual monomer leaching into the saliva of patients wearing complete dentures in their post-insertion period peaked one day after the insertion and that despite this amount of released monomer being at a none toxic levels, it could still potentially sensitize complete denture patients and induce an allergic reaction (Singh et al 2013). Another study attempted to quantify the residual monomer elution of conventional and computer-aided design/computer-aided manufacturing (CAD/CAM) dental acrylic-based materials during artificial aging and it found that both CAD/CAM and conventional polymers eluted residual monomer within different aging time (Engler et al 2020). Another important factor to be considered in the diffusion of monomers from acrylic-based materials is the salivary pH value which is known to affect biodegradation of the material and it was found that the amount of monomer released from different denture base acrylic material

processed by different polymerization methods and stored in different storage conditions is higher when stored in an acidic saliva environment in comparison to neutral saliva (Bettencourt et al 2010; Tuna et al 2013; Akay et al 2017; Sá et al 2020).

In recent years, CAD/CAM technology has become an alternative to conventional methods in the fabrication of removable prostheses. In 1994, the first scientific article discussing the use of CAD/CAM in the fabrication of complete dentures was published (Maeda et al 1994). Since then, numerous CAD/CAM denture systems have been introduced into the market (Kattadiyil et al 2013; Steinmassl et al 2017). CAD/CAM-fabricated complete dentures have several advantages over conventionally fabricated complete dentures, such as decreased porosity, enhanced predictability of the desired outcomes and excellent fitting accuracy (Bidra et al 2013; de Mendonça et al 2016). Because the acrylic used for the fabrication of dentures using CAD/CAM technology is pre-polymerized, the prosthesis seems to contain less residual monomer and is more hydrophobic than the conventionally processed one, resulting in a more bio-hygienic prosthesis (Masri and Driscoll 2015).

A recent research that studied CAD/CAM dentures and aimed at evaluating the color stability of it when immersed in different beverages found that milled denture blocks had greater resistance to stain accumulation in comparison to the conventional one (Al-Qarni et al 2020). However, limited data are available on the properties related to the monomer leakage of CAD/CAM processed denture material when the salivary pH values alternate between acidic and basic conditions. This study has aimed to evaluate the effect of different salivary pH values on monomer leakage from conventional and CAD/CAM acrylic denture base materials, with a null hypothesis that there is no difference between the two types of acrylic denture base materials in terms of the effect of the salivary pH values on monomer leakage.

MATERIAL AND METHODS

Two types of acrylic resin materials were used: a CAD/CAM-manufactured resin (IvoBase® CAD; Zenotec, Wieland Dental, Germany) and a heat-cured resin (SR Ivoclar High Impact®; Ivoclar Vivadent AG, Liechtenstein). Two groups of 60 discs were fabricated. The dimensions of the discs were 10 mm (diameter) × 3 mm (thickness). Each of the two groups was divided into three subgroups, with 10 discs each. The CAD/CAM Acrylic discs were designed with predetermined dimensions using Zenotec® CAD software (Wieland Digital Denture; Ivoclar Vivadent, Schaan, Liechtenstein). PMMA blocks were used (Opera system, Principauté de Monaco, French), and the milling procedure was performed using Zenotec® selection (Wieland Digital Denture; Ivoclar Vivadent, Schaan, Liechtenstein). The discs were then finished and polished using a dental laboratory polishing machine with a vacuum cleaner (Aspyclean+ M2V®, Manfredi, Italy), pumice (Interdent, Slovenia) and a rag polishing wheel (Rag muslin wheel; Kerr, USA).

For the fabrication of Heat-Cured acrylic resin discs, a putty molds of the preferred disc dimensions were fabricated using a polyvinyl siloxane putty material (Express STD®; 3 M ESPE, United States). The silicone molds were filled with melted base plate wax. A Bantam flask was filled with a plaster mix with a powder : water ratio of 100 g:47 cc (Lab Plaster Fast Set®; Dentsply, Canada), and then the putty mold was immersed in the plaster mix so that the top of the mold was flushed with the top of the plaster mix. After the plaster was set, a thin layer of petroleum jelly (Vaseline) was applied to the top. The upper half of the flask was then fixed to the bottom half and filled with plaster mix, and then the lid of the flask was placed on the top. After that, the flask was placed in a wax elimination machine (Wapo-Ex®; Wassermann, Germany) for 30 minutes at 90 °F to 100 °F. The flask was then opened, and the melted wax was washed away using boiling water.

A thin layer of separating fluid (Ivoclar Vivadent; Schaan, Liechtenstein) was applied to the plaster surface. The heat-cured acrylic provided as a single capsule containing premeasured polymer and monomer (SR Ivocap High Impact®; Ivoclar Vivadent AG, Liechtenstein) was then mixed for 5 minutes using a cap vibrator (Cap vibrator®; Ivoclar Vivadent, Schaan, Liechtenstein). The mixture was poured into the putty mold and pressed using a pressure apparatus (OL 463, Manfredi, Italy). Next, the flask assembly was placed in a polymerization bath (100 °C water) for 35 minutes (Electronic Denture Curing System; Nevin Labs™; USA). The discs were finished and polished using a dental laboratory polishing machine with pumice (Interdent, Slovenia) and a rag polishing wheel (Rag Muslin wheel®; Kerr, USA).

A Mechanical brushing was performed according to the recommendations of the International Organization for Standardization (ISO). The specimens were brushed with soft toothbrushes mounted on a toothbrush simulator (ZM-3.12; SD Mechatromik GmbH, Germany) (Figure 1). The specimens were subjected to linear toothbrush abrasion movement at a rate of 356 brush strokes (back and forth) per minute. The machine provides a 200-g vertical load over each specimen and a 5-mm path starting from the center of each specimen and brushes six specimens simultaneously. The total brushing time was 50 minutes, with 17,800 cycles (representing one year). Brushing was performed in distilled water (23±3 °C) and dentifrice (Crest Cavity Protection Regular Paste; P&G, Germany) (Figure 2). Using an SD Mechatronik GmbH thermocycler (SD Mechatronik, Germany), all the specimens were stored in distilled water and subjected to thermocycling between 5 °C and 55 °C, with a dwell time of 30 seconds and a transfer time of 12 seconds for 1,000 cycles (Pusz et al 2010).

For the process of artificial saliva preparation and incubation of the samples, an artificial saliva was prepared at three different pH values (5.7, 7 and 8.3). An electrolyte composition similar to that of human saliva was used in this study, as shown in Table 1 (Kostic et al 2015):

Figure 1: Tooth brushing Simulator Machine

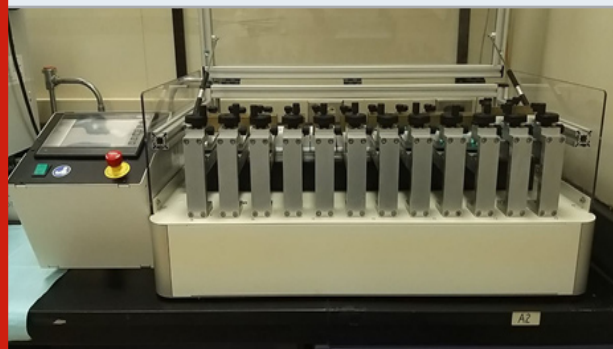


Figure 2: Brushing of the PMMA discs sample.



Table 1. Chemical Composition of Artificial Human Saliva

Na ₂ HPO ₄	0.260 g/l
NaCl	0.700 g/l
KSCN	0.330 g/l
KH ₂ PO ₄	0.200 g/l
NaHCO ₃	1.500 g/l
KCl	1.200 g/l

A buffer solution comprising KH₂PO₄ and Na₂HPO₄ was prepared by dissolving each solution in 1 liter of deionized distilled water. Basic saliva was prepared by taking 500 ml of Na₂HPO₄ and adding KH₂PO₄ gradually until the desired pH was reached. Next, the other salts (NaCl, KSCN, NaHCO₃ and KCl) were added to the saliva, and the volume was completed to 1 liter using deionized distilled water. Neutral and acidic saliva solutions were prepared by taking 500 ml of KH₂PO₄ and adding Na₂HPO₄ gradually until the desired pH was reached. Next, the other salts (NaCl, KSCN, NaHCO₃ and KCl) were added in the same manner as described above. For neutral saliva, a greater amount of Na₂HPO₄ was added to reach the desired pH (Pusz et al 2010). The discs were assorted into 6 groups, with 10 discs in each group, and then were stored in artificial saliva in an incubator (Blanket warming cabinet; Malmel, Australia) at 37 °C for 30 days. High-Performance Liquid Chromatography (HPLC) was used to determine the quantity of residual methyl

methacrylate (MMA) monomer following the immersion of the two types of acrylic materials in artificial saliva at three different pH values.

A UV PerkinElmer Series 200 HPLC system (PerkinElmer, Shelton, USA) equipped with a C18 column was used to perform HPLC analysis. Ten milliliters (ml) of each sample solution was injected and analyzed at 40 °C and a flow rate of 1.0 ml/min (revolutions per minute) with acetonitrile in water (50/50). One reading was obtained from each milliliter of the 10 ml sample. Fifteen minutes after sample injection, the content of MMA was calculated from the area under the peak. The average of 10 readings for each sample was calculated (Mohamed 2008). Statistical Analysis: The data were analyzed using the SPSS statistical software (v16; SPSS Inc., Chicago, IL, USA). The effect of the acrylic material type and pH and their interaction on monomer leakage were analyzed by two-way ANOVA. Tukey's post hoc multiple comparison was used to evaluate the differences in monomer leakage among the three pH values under each type of acrylic material.

RESULTS AND DISCUSSION

At $\alpha = 0.05$ and a sample size equal to 10 under each pH value used (acidic, neutral, basic), the power of the study was estimated to be 88%. Two-way ANOVA was performed to evaluate the effect of salivary pH on monomer leakage into the saliva. Both the material type and pH of the saliva significantly affected monomer leakage ($P = 0.03$ and $P = 0.00$, respectively) (Table 2). The mean and standard deviation (SD) of monomer leakage when the two acrylic material types were soaked in salivary solutions with different pH values are presented in table 3. The highest amount of monomer leaked from the CAD/CAM material when the material was soaked

in an acidic salivary solution, while the least amount of monomer leakage occurred in the basic solution (table 3). Post-hoc multiple-comparison analysis revealed that the monomer leakage of the CAD/CAM material soaked in an acidic solution was significantly higher than that of the neutral and basic pH solutions ($P = 0.01$ and $P = .00$, respectively);(table 4). Similarly, heat-cured acrylic exhibited most of the monomer leakage when the material was soaked in an acidic salivary solution; however, the lowest monomer leakage was observed when the material was soaked in neutral pH solution (table 3). Post-hoc multiple-comparison analysis revealed that the monomer leakage of the heat-cured acrylic material soaked in an acidic solution was significantly higher than that of the two other salivary solutions. ($P = 0.00$; table 4).

Acrylic-based resins are frequently used in daily dental practice. These acrylic resins are used to replace lost tissue and transfer masticatory forces from the denture to the residual ridges because they can provide essential properties and have the necessary characteristics for use in diverse functions. Although acrylic resins have many desirable properties, one of their main drawbacks is that they contain residual monomers that may leach out and trigger undesirable side effects (Oliveira et al 2010; Ivkovic et al 2013; Kostis et al 2015). Diffusion is the mechanism that underlies residual monomer leakage from acrylic resins in which the constant contact of saliva with the material causes expansion of the openings present between the polymer chains, causing the unreacted monomer to diffuse out. Thus, the substances that are leached out from the denture bases into the saliva are transferred to the oral structures, causing adverse allergic reactions (Urban et al 2009; Kopperud et al 2011;Chaves et al 2012; Gautam et al 2012; Nik et al 2014; Choudhary et al 2020).

Table 2. Two-way ANOVA of the effect of two independent variables material type and pH value on monomer leakage (uV.sec).

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Disc Material	426398.26	1	426398.26	4.86	0.03
pH	2635911.05	2	1317955.52	15.03	0.00
Disc Material * pH	440815.82	2	220407.91	2.51	0.09
Error	4735157.61	54	87688.10		
Total	49121084.98	60			
Corrected Total	8238282.73	59			

Based on the results obtained from this study, the null hypothesis was rejected indicating that the variation in salivary pH values had a significant effect on the monomer leakage from the acrylic materials used in the study. The results demonstrated that, when different acrylic resin materials were soaked in saliva with different pH values, the greatest amount of monomer leakage occurred in the acidic salivary solution, a finding that was in agreement with other studies (Faltermeier et al 2007; Bettencourt et al 2010; Akay et al 2017; Sá

et al 2020). One study evaluated the residual monomer using high performance liquid chromatography (HPLC) for microwave-cured, conventional heat and injection-technique acrylic materials that were stored in neutral and acidic artificial saliva for 24 hours and it was found that all three materials exhibited higher monomer release into the acidic saliva (Tuna et al 2013). The chemical structure of the monomers used to prepare the resins could directly affect the amount of eluted monomer. Lefebvre et al (1995) studied the pattern of release of

cytotoxic substances from four light-polymerized denture base resins and suggested that different components may leach out at different rates and that the release of cytotoxic resin components may continue for several days.

Table 3. Monomer leakage (uV.sec) from the three different acrylic materials (CAD/CAM and heat-cured) when soaked in three solutions with different pH values (acidic, neutral, basic).

Acrylic material	pH	Mean	Std. Deviation
CAD/CAM	Acid	1320.52	159.31
	Neutral	750.54	146.11
	Basic	658.21	122.36
Heat-Cured	Acid	922.57	65.43
	Neutral	628.57	138.56
	Basic	672.34	73.43

Table 4. Post hoc multiple-comparisons analysis to compare the effect of salivary pH value on monomer leakage for each acrylic material type.

Disc material	(I) pH	(J) pH	Mean Difference (I-J)	Sig.
CAD/CAM	Acidic	Neutral	569.98	0.01*
		Basic	662.31	0.00*
	Neutral	Acid	-569.98	0.01*
		Basic	92.33	0.87
	Basic	Acid	-662.31	0.00*
		Neutral	-92.33	0.87
Heat-Cured	Acidic	Neutral	294.00	0.00*
		Basic	250.23	0.00*
	Neutral	Acid	-294.00	0.00*
		Basic	-43.77	0.59
	Basic	Acid	-250.23	0.00*
		Neutral	43.77	0.59

* The mean difference is significant at the .05 level.

Heat-cured acrylic resin showed the least monomer leakage in both acidic and neutral solutions compared with the CAD/CAM material. Many studies were conducted to evaluate the amount of monomer leakage from heat-cured acrylic compared with that of other materials and all presented similar findings in which the heat-cured acrylic material showed less monomer leakage. This finding might be related to the high polymerization temperature needed to cure the acrylic material (Vallittu et al 1998; Shim and Watts 1999; Sideridou and Achilias 2005; Mohamed et al 2008; Chaves et al 2012; Nik et al 2014). In a recent study conducted to compare the residual monomer concentration and cytotoxic effect of three acrylic materials that were hot-cured or

polymerized under pressure and at lower temperatures, the authors reported that the acrylic material polymerized at high temperatures has a lower residual monomer concentration, while self-curing materials polymerized at lower temperatures have a higher concentration of residual monomer, leading to a lower number of living cells that might trigger allergic reactions shortly after the new denture is delivered (Raszewski 2020).

CAD/CAM denture base acrylic resin is supplied as pre-polymerized blocks which are produced in industrially controlled conditions with standardized pressure and temperature and are known to have enhanced material-specific properties (McCabe and Walls 2013). As a result of the polymerization of PMMA blocks used for the milling of denture under high temperature and pressure, long polymer chains are formed leading to a higher degree of monomer conversion and lower values of residual monomer as well as minimal porosity (Kattadiyil et al 2013; Mörmann et al 2013; Murakami et al 2013; Nguyen et al 2014; Akin et al 2015; Kattadiyil et al 2015). In a recent study that aimed to evaluate the amount of monomer released from a CAD/CAM acrylic material when soaked in water, the results demonstrated that the CAD/CAM acrylic material released very little monomer. However, the amount released was not different from that released from conventionally heat-cured acrylic material (Steinmassl et al 2017). This finding agreed with ours when the two acrylic materials were soaked in neutral and basic salivary solutions. On the contrary, one study that instigated the mechanical properties including monomer leakage between heat cured and CAD/CAM denture base material found that CAD/CAM material leached lower amount of monomer compared to heat cure denture acrylic material and this variation was attributed to the method of polymerization under high pressure (Ayman 2017).

The presence of unreacted residual monomers in denture base acrylic resins is inevitable, and every effort should be applied in laboratory and clinical settings to reduce the exposure as much as possible (Rashid et al 2015). Generally, and regardless of the acrylic material type, lower pH values were associated with more monomer leakage. Because lower amounts of monomer leakage occurred from the heat-cured acrylic material in the acidic solution, this material might be the material of choice when treating patients who report a high intake of an acidic diet. Similarly, using acidic solutions as storage media for dentures before denture insertion might be warranted to eliminate larger amounts of monomer release.

The salivary pH value in the oral cavity changes continuously between acidic and basic based on the dietary intake of the patient. Consequently, it might be necessary to subject the same acrylic material to alter salivary pH values and study the effect of this parameter on monomer leakage. Similarly, acrylic materials are subjected to many other factors that might affect monomer leakage. These factors include enzymes in the oral cavity, cleanser agents, different

brushing techniques, polymerization techniques, surface treatments and chewing forces. Further investigation is needed to study the effects of the combination of these factors on acrylic materials, particularly the newly introduced CAD/CAM materials.

CONCLUSION

Within the limitations of this study, acidic salivary pH values were associated with higher amounts of monomer leakage in both heat-cured and CAD/CAM denture acrylic materials. It might be recommended to immerse newly fabricated heat-cured and CAD/CAM acrylic dentures in an acidic solution to allow most unreacted monomers to leak before delivering the denture to the patient.

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