

# In vivo cytotoxicity of injection molded and conventional pressure pack acrylic resin dentures

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## ABSTRACT

**Context:** Acrylic resins are widely used in the fabrication of denture bases and have been shown to be cytotoxic as a result of substances that leach from the resin. **Aim:** The purpose of this study was to compare the cytotoxicity of the injection molded and conventional pressure-pack heat polymerized acrylic base resin systems with evaluation of the micronucleus frequency on the oral mucosa of the participants. **Materials and Methods:** This study was carried out by examining the 20 patients' buccal mucosa who begin to use complete dentures that fabricated by different heat polymerized systems. The micronucleus incidence was scored in the buccal epithelial of each patients. Epithelial cells were collected from oral mucosa by a wooden spatula before applying the prosthesis in the mouth, after 1 week and 1 month and they were smeared on to clean microscope glass slides then examined under light microscope. **Results:** Micronucleus frequency was higher in individuals' buccal mucosa who used conventional press pack dentures than injection molded heat polymerized groups. There was not statistically significant differences between conventional and injection molded heat polymerized groups ( $P>0.05$ ). Number of micronucleus at the end of the 1 week was much more than after 1 month for each groups. **Conclusion:** Complete dentures that fabricated by injection molded denture base materials is less cytotoxic than conventional pressure pack acrylic resin.

## Key words

Acrylic resin, cytotoxicity, original article, polymerization method, residual monomer

## INTRODUCTION

A variety of acrylic resins exist in dental market for the fabrication of dental prosthesis. They may be classified by polymerization mode as heat-polymerized, auto-polymerized, microwave-polymerized, and visible light-polymerized.<sup>[1]</sup> The biocompatibility of these materials are very important factor in their clinical use. Biocompatibility means, acceptance of foreign material by the surrounding and whole body tissues.<sup>[2]</sup> Generally heat-polymerized acrylic resins are mostly preferred in dentistry. Polymethylmethacrylate (PMMA) is heat-polymerized acrylic resin and it has an excellent esthetic properties, enough strength, facility to repair and easy molding technique so it is still most frequently used material for denture bases since its introduction in 1937.<sup>[3]</sup> Despite its satisfactory properties, it has

the potential to elicit irradiation, inflammation and allergic reactions in the oral environment. Acrylic resins contains formaldehyde, methyl methacrylate monomer, methacrylic acid, benzoic acid, plasticers, phenyl benzoate, phenyl salicylate and dicyclohexyl phthalate. These materials can be responsible for hypersensitivity and allergic conditions to dental laboratory persons and denture wearer prior and after the polymerization.<sup>[4]</sup> Leaching to residual monomer methyl metacrylate is one of the main factors effecting the cytotoxicity and biocompatibility of acrylic resins.<sup>[5,6]</sup> The amount of the residual monomer release is dependent on the temperature, polymerization time and processing method.<sup>[7,8]</sup>

Acrylic resins have been modified to improve physical and chemical properties such as injection molding. It allows directional control of the polymerization process through the flask design.<sup>[4]</sup> There are many injectable PMMA systems and they claiming to fabricate more accurate denture bases than conventional PMMA by constant flow of material from the sprue compensates for the polymerization shrinkage.<sup>[9,10]</sup>

The cytotoxicity of microwave, heat-polymerized and auto-polymerized acrylic resin dentures were evaluated by some studies<sup>[11,12]</sup> but there is no study about compare the

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cytotoxicity of heat-polymerized acrylic resins each other with *in vivo* test method. These components cytotoxic potentials are known but cytotoxic potentials in person's target cells should be researched by further studies.

During cellular division a micronucleus (MN) is separated from the main one generated by late chromosome fragments. It has been regarded as indicators for genotoxic exposures since 1937.<sup>[13]</sup> The MN test can be applied to exfoliated cells from the human oral tissues.<sup>[14]</sup> The frequency of micronucleated cells reflects the capacity of target tissues to activate procarcinogens into reactive species, or to inactivate or trap ultimate carcinogens.<sup>[14-16]</sup>

The purpose of this study was to compare the cytotoxicity of the injection-molded and conventional pressure-pack PMMA acrylic base resin systems with evaluation of the micronucleus frequency on the buccal mucosa. Hypothesis was that conventional pressure-pack PMMA acrylic resin would show lower cytotoxic effect than injection molded acrylic resin.

## MATERIALS AND METHODS

This study was carried out by examining 20 patient's buccal mucosa who begin to use complete dentures that fabricated by different heat polymerized systems. Participants who had no systemic disease and did not use medicine since one month and between 45-60 ages were selected from Mustafa Kemal University, Faculty of Dentistry. This research's Ethic Committee number is B. 30. 2. MKU.0.01.01.00/3143/33-34. It was provided from Mustafa Kemal University department of Ethic Committee.

The conventional PMMA complete dentures were fabricated using conventional flasking and pressure-pack technique. Type II dental stone (Moldano, Bayer, Germany) was used within a dental flask. In accordance with the manufacturer's instructions, a 1:3 monomer to polymer ratio, by weight, was used for the conventional PMMA complete dentures. The polymerization of the resin was performed by immersion in boiling water for 20 min and then prosthesis were finished and polished.

For the injection molded complete prosthesis, the dentures were flased with Ivocap flask according to the manufacturer's instructions. Resin and monomer capsules were triturated for 5 min and injected into the flask. Hydraulic pressure was applied with hydraulic pressing apparatus (Kavo Electrotechnisches, D-7970, Werk Germany) for 5 min and then placed in 100°C boiled water for 35 min. Flasks were placed in cold water for 20 min and flasks were opened to get out prosthesis and then prosthesis were finished and polished. Materials that were used in this study are listed in Table 1.

All the prosthesis were fabricated by the same person and technician laboratory to eliminate the differences of fabrication.

The MN incidence was scored in the buccal epithelial of each patients. Epithelial cells were collected from oral mucosa by a wooden spatula before applying the prosthesis in the mouth and after 1 week and 1 month and they were smeared on to clean microscope glass slides. The cells were fixed with cold 100% methanol (Merck, Darmstadt, Germany). The slides were aged at 37°C one night and then stained with 5% giemsa for 10 min. The slides were screened and 3000 nucleated cells were analyzed for the presence of MN at a final ×100 magnification with light microscope (Olympus, Japan). Countryman and Heddle's micronucleus criteria was used for scoring to micronucleus.<sup>[7]</sup>

## Statistical analysis

Micronucleus incidence differences that dentures caused after the applying in the month were analyzed by Mann Whitney-U test method.

## RESULTS

According to smear samples evaluation that derived from the buccal mucosa of the participants, MN intensity in a week later compared with the control group was increased. It was observed that MN intensity dropped back close to control datas after a month [Table 2]. There is no statistical significance between before and after using of the conventional and injection molded heat polymerized dentures ( $P>0.05$ ). Micronucleus intensity was higher in individuals' buccal mucosa who used

**Table 1: Materials used in this study**

Brand name	Material type	Manufacturer	Processing method
SR-Ivocap	Heat-polymerized (Injection-molding) acrylic resin	Ivoclar Vivadent, Schaan, Lichtenstein	Boiling water/35 min
Meliodent	Heat-polymerized (Conventional-molding) acrylic resin	Dental Bayer Limited Pharmaceuticals, UK	Boiling water/20 min

**Table 2: Incidence of the MN derived from the buccal mucoza before and after applying dentures/1000**

Time	System	Mean	SD
Before	Conventional	10.9	(0.7)
	Injection molded	10.1	(1.0)
After 1 week	Conventional	15.3	(0.8)
	Injection molded	13.3	(1.2)
After 1 month	Conventional	11.9	(0.7)
	Injection molded	10.6	(1.3)

SD - Standard deviation

conventional press pack dentures than injection molded heat polymerized groups. There was not statistically significant differences between conventional and injection molded heat polymerized groups ( $P>0.05$ ).

## DISCUSSION

Genotoxicity is one of the most important side effects of the chemicals. Dental materials, in particular resins used in prosthetic dentistry, are frequently in long-term contact with the oral tissues, and the chronic exposure to these products of a significant proportion of the population means that the release of genotoxic substances by dental resins must be evaluated.<sup>[17]</sup>

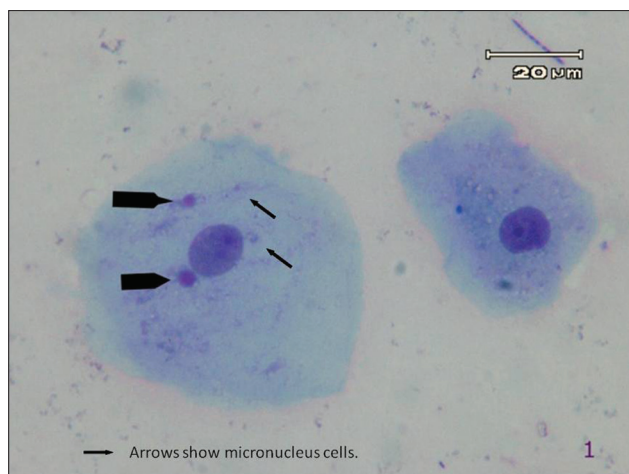
Micronucleus test method was firstly mentioned in 1970's.<sup>[18]</sup> Residual monomer leaching from the dental resins can be in saliva or blood in the oral environment and bring about cell abnormalities.

Thomas *et al.* fixed to acrylic discs to hamsters' cheeks, and it caused inflammation in that tissues. They connected this result to unreacted residual monomer and polymerization methods of the acrylic resins.<sup>[19]</sup>

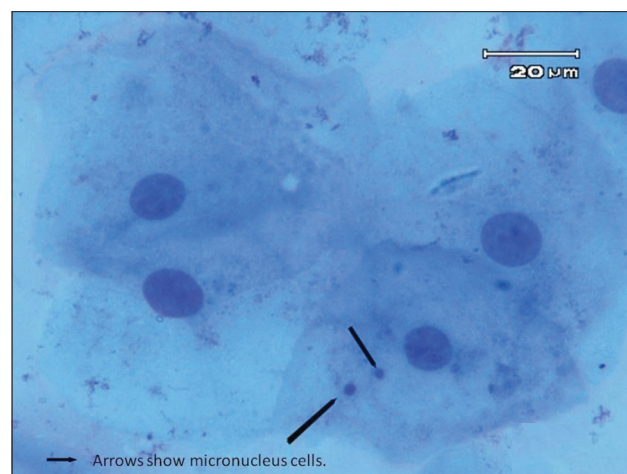
It is important to keep to right proportion of powder and liquid during the preparation of the acrylic dentures.<sup>[20]</sup> Kleinsasser *et al.* found out that the high ratio of the methacrylates HEMA, TEGDMA, Bis-GMA and UDMA induced significant but mild enhancement of DNA migration in the Comet assay as a possible sign for limited genotoxic effects.<sup>[21]</sup> Bis-GMA is composed of the heat polymerized acrylics. After 1 week that the materials applied the participants, increasing of the MN frequency may arise from leaching of this material.

It was reported that the toxic effect of an irritant heat-polymerized acrylic resin dentures can be eliminated by repeating the heating process,<sup>[22]</sup> and the levels of cytotoxicity is different in different systems.<sup>[23]</sup> Different heat polymerized systems were used for this study and after 1 one week [Figure 1] that the dentures used by participant's, cytotoxic effect was much more than at the end of the 1 month [Figure 2] as previous studies.<sup>[1,24]</sup>

Kedjarune *et al.* stated that the amount of residual monomer is dependent on the amount of liquid in the mixture ratio.<sup>[25]</sup> Injection molded acrylic resin monomer/polymer content is carried out originally by manufacturer in capsule. Unreacted residual monomer may be decreased by this original ready ratio. This is one possible explanation for the less cytotoxicity of the injection molded acrylic resins than conventional heat-polymerized systems. Because powder-liquid ratio is carried out by dental staff, it is not an originally prepared capsule form like injection molded system. During preparing of these material's proportion, sometimes they may not be complied with the manufacture's instructions precisely.



**Figure 1:** Microscopic occurrence of MN of the oral mucosa that participants used dentures at the end of the 1 week



**Figure 2:** Microscopic occurrence of MN of the oral mucosa that participants used dentures at the end of the 1 month

Several studies reported that<sup>[25,26]</sup> wearing of newly made dentures may result in oral tissues irritation from these leachable substances, because these studies showed that PMMA released into saliva after incubation for 24 h can cause cell toxicity *in vitro*. On the other hand it may also be possible that minor irregularities of fit in new dentures may also provide a source of irritation that makes the mucosa more susceptible to MMA in first days.

Injection molded system allows directional control of the polymerization process through the flask design. A constant flow of new material from the sprue compensates for the polymerization shrinkage<sup>[27]</sup> and SR ivocap system has less linear dimensional change than conventional system.<sup>[28]</sup> Complete dentures that fabricated by injection molded system were less irritant for oral tissues than conventional system for this study. These prosthesis' tissue harmonies may be better than conventional system due to less polymerization shrinkage and it may also be possible that tissue irritations may lesser for this reason.



According to studies,<sup>[25,28]</sup> long term boiled decreases the residual monomer amount. Dentures which were fabricated by injection molded system was long-term boiled than conventional system. Less cytotoxicity of the injection molded system may arise from long-term boiling than conventional system.

Different methods are presented for reducing the leachable substances from newly made acrylic dentures before applying into patients. Immersion of the prosthesis in water for at least one day or immersion of this dentures in hot water<sup>[29]</sup> or using ultraviolet light<sup>[30]</sup> are some of them.

## CONCLUSION

It was determined that complete dentures that fabricated by injection molded denture base material are less cytotoxic than conventional pressure pack acrylic resin. It is important to choose denture base material to fabricate complete dentures for cytotoxicity. Injection molded denture base materials should be used for dental prosthesis to reduce the frequency of hypersensitivity reactions and help patient's well-being.

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