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ORIGINAL ARTICLE

Analysis of the Cytotoxic Effects in Removable Dentures Fabricated Using Two Different Processing Techniques: An Observational Comparative Study

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ABSTRACT

Objective: To compare the cytotoxicity of polymethyl methacrylate dentures fabricated through compression molded and injection molded processing techniques. **Material and Methods:** The injection-molded group utilized IVOCAP resin, while the compression-molded group used DPI heat-cure resin. Twenty patients were selected and divided into two groups of ten. Buccal epithelial cells were collected from each patient pre-insertion and three months post-insertion of the dentures. The micronucleus assay was used to determine cytotoxicity. Intergroup comparisons were conducted using the Mann-Whitney U test, while intragroup comparisons were carried out using the Wilcoxon signed-rank test. **Results:** The intergroup comparison demonstrated a statistically significant difference in the dimensions of micronuclei measured post-smear ($p=0.027$). The group using the compression molding technique had higher mean values of micronuclei (24.2 ± 7.6) than the injection-molded technique. Intragroup comparisons conducted with the Wilcoxon signed-rank test revealed a highly significant difference between pre-smear and post-smear values in both groups ($p<0.001$). **Conclusion:** Injection-molded dentures demonstrated significantly lower cytotoxicity than those fabricated using the compression-molding technique.

Keywords: Cytotoxicity Tests, Immunologic; Acrylic Resins; Polymethyl Methacrylate.

Introduction

Polymethyl methacrylate (PMMA) is one of the most commonly used materials in the fabrication of removable dentures due to its favorable aesthetic qualities, durability, and ease of handling. PMMA offers excellent translucency and can be easily manipulated and repaired during denture-making process, making it particularly suited to prosthetic applications. PMMA is valued for its biocompatibility, meaning it generally interacts well with oral tissues without causing harmful or immune reactions when it comes into contact with bodily tissues or fluids. This compatibility is particularly important in denture fabrication, as dentures remain in close and prolonged contact with the oral mucosa, requiring materials that do not provoke adverse responses [1,2].

Despite its biocompatibility, PMMA has been associated with occasional tissue inflammation and allergic responses, particularly affecting the oral mucosa. These reactions are primarily attributed to the residual components within acrylic resins, such as formaldehyde, methyl methacrylate monomer (MMA), methacrylic acid, plasticizers, and other stabilizing agents. In particular, the leaching of residual monomer MMA plays a significant role in the cytotoxicity of PMMA resins. This release of monomer occurs based on factors like polymerization time, temperature, and the specific technique used during fabrication. Typically, the less polymerized the material, the higher the concentration of residual monomer, which can lead to increased cytotoxicity. Thus, the processing methods used in denture fabrication are critical in determining the final product's safety and biocompatibility [3-6].

Among the methods available for processing PMMA dentures, common techniques include auto-polymerized, heat-polymerized, microwave-polymerized, and light-polymerized methods [4]. Traditional heat-cure methods have been widely used in compression molding techniques for many years, but advances in injection molding technology have introduced an alternative that controls monomer flow and reduces polymerization shrinkage more effectively. Injection molding uses a specialized flask system to optimize resin flow, minimize porosities, and reduce the release of residual monomers, which are the main contributors to cytotoxicity [5]. The potential advantage of injection molding over traditional pressure-pack methods lies in its ability to deliver a higher degree of polymerization, resulting in lower monomer release and, consequently, a more biocompatible final product [6-8].

In assessing the cytotoxicity of these materials, researchers utilize the presence of micronuclei in buccal epithelial cells as a biomarker. Micronuclei are small, extranuclear bodies that form due to chromosome fragments or whole chromosomes not being incorporated into the main nuclei during cell division. These structures serve as genotoxic indicators, helping to identify early cellular damage and potential carcinogenic risk. The presence of micronuclei is often used as a predictive marker for the genotoxic impact of various materials on the epithelium, including acrylic resins, which are recognized for their potential cytotoxicity due to leaching agents [9].

This study aimed to compare the cytotoxic effects of PMMA dentures fabricated through injection molding and traditional compression molding techniques. Buccal mucosa samples were collected from participants fitted with either type of denture, and the presence of micronuclei was analyzed preoperative and post-operative denture insertion. Our hypothesis posits that injection-molded dentures will demonstrate a lower cytotoxic profile, indicated by a reduced occurrence of micronuclei in buccal epithelial cells. Through this comparison, we seek to establish a foundation for safer material selection and enhanced denture fabrication practices in prosthetic dentistry.

Material and Methods

Study Design and Ethical Clearance

The study design was an observational comparative study and was approved by the Scientific Review Board of Sri Ramachandra University, Faculty of Dental Sciences (CSP/18/May/70/161). All participants provided informed consent before their involvement.

Sample

The investigation involved a sample of 20 individuals aged between 30 and 60 years, divided into two groups of ten. Each group was instructed to wear complete dentures fabricated using different techniques. Individuals with poor oral hygiene, diabetes mellitus, bronchial asthma, chronic obstructive pulmonary disease (COPD), oral stomatitis, squamous cell carcinoma, oral ulcers, and developmental disorders were excluded.

Data Collection

In Group A, participants wore removable acrylic complete dentures manufactured by the DPI brand (Bombay Bumrah Trading Corporation Ltd, Mumbai, India), created using the compression molding process. These dentures were made from Polymethylmethacrylate (PMMA), adhering to a monomer-to-polymer ratio of 1:3, as per the manufacturer's guidelines. The traditional flasking method utilized Type II dental stone - Kalabhai Kalstone (Kalabhai Karson Private Limited, Mumbai, India), and excess flash was removed with a hydraulic pressing device D-7970 (KaVo Elektrotechnisches Werk GmbH, Biberach, Germany) for five minutes. The curing process comprised two hours at 74°C, followed by one hour at 100°C, after which the dentures were bench-cooled for 20 minutes. The following day, the dentures were deflasked, trimmed, polished, and prepared for insertion into the patient's mouth.

Group B participants were instructed to wear complete dentures manufactured by the SR Ivocap brand (Ivoclar Vivadent Marketing Pvt. Ltd, Haryana, India), which were created using an injection-molded technique. The process adhered to the manufacturer's guidelines with an Ivocap flask. Type II dental stone (Moldano, Bayer AG, Leverkusen, Germany) was used to fill the dental flasks. The preparation of the SR Ivocap resin (Ivoclar Vivadent Marketing Pvt. Ltd, Haryana, India) involved grinding the capsules containing resin and monomer into a fine powder for five minutes before adding it to the flask. The flasks were then immersed in boiling water at 100 degrees Celsius for 35 minutes and cooled in cold water for 20 minutes before deflasking. After completion, the dentures were polished and prepared for insertion.

Micronuclei Analysis

To assess the incidence of micronuclei (MN) in the buccal epithelium, epithelial cells were collected from the oral mucosa using a wooden spatula before the dentures were inserted. These cells were applied to clean microscope glass slides after one week and one month of denture use and fixed with cold methanol - 100% purity (Merck KGaA, Darmstadt, Germany). The slides were incubated overnight at 37 °C and treated with a 5% Giemsa solution for ten minutes. A total of 3,000 nucleated cells were examined under an Olympus microscope at a final magnification of ×100 to identify the presence of MN, evaluated according to the criteria established by Countryman and Heddle [10]. To ensure consistency and avoid bias in denture production, all prostheses were fabricated by a single technician in a controlled laboratory environment.

The scoring criteria for assessing the micronuclei in the collected epithelial cells included several key variables to guarantee accurate evaluation. The cytoplasm of the cell was required to remain intact, with the cell positioned flat on the microscope slide. There was to be minimal or no overlap with adjacent cells, allowing for

clear observation of individual cells. Additionally, the presence of debris was to be minimal or absent to facilitate accurate analysis. Lastly, the nucleus was evaluated for typical structural characteristics, including a well-defined and smooth nuclear border. These criteria were critical for ensuring that the observed micronuclei were accurately quantified and assessed for their relevance in the study.

Data Analysis

Statistical analysis was performed using Stata statistical software, version 17.1 (StataCorp LLC, College Station, TX, USA). Intergroup comparisons were conducted using the Mann-Whitney U test, while intragroup comparisons were carried out using the Wilcoxon signed-rank test. A p-value of less than 0.05 was considered statistically significant for all analyses.

Results

Pre-smear samples from both the injection-molded (Figure 1A) and compression-molded dentures (Figure 1B) revealed no significant changes in the frequency of micronuclei. However, significant differences emerged in the post-smear samples. The compression-molded dentures exhibited a notable increase in micronuclei frequency (Figure 1C), suggesting a potential negative impact on cellular integrity following their use. In contrast, post-smear samples from the injection-molded dentures showed a significant decrease in micronuclei frequency (Figure 1D), indicating an improvement in cellular conditions after wearing these dentures. These findings underscore the varying effects of different denture fabrication techniques on oral mucosal cells throughout the study period.

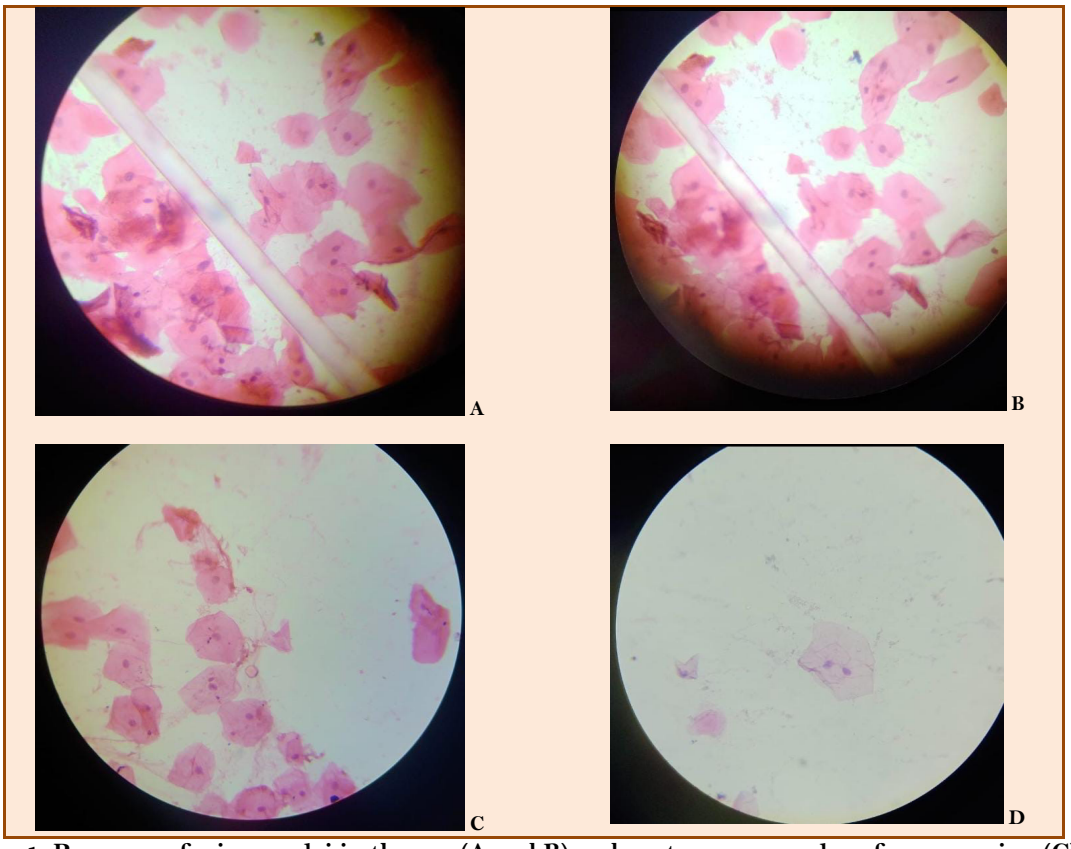

Figure 1. Presence of micronuclei in the pre (A and B) and post smear samples of compression (C) and injection molded dentures (D).

The intergroup comparison (Table 1) demonstrated a statistically significant difference in the dimensions of micronuclei measured post-smear (p=0.027). The group using the compression molding technique had higher mean values of micronuclei (24.2 ± 7.6) than the injection-molded technique. This suggests that the method of denture fabrication may influence the presence of micronuclei in the buccal epithelium.

Table 1. Intergroup comparison.

Period	Group	N	Mean	SD	Std. Error Mean	p-value*
Pre-Smear	Injection Molded	10	8.90	3.542	1.120	1.000
	Compression Molded	10	8.90	2.183	0.690	
Post-Smear	Injection Molded	10	17.70	3.945	1.248	0.027**
	Compression Molded	10	24.20	7.642	2.417	

*Mann-Whitney U test; **Statistically significant.

Further analysis through intragroup comparisons (Table 2) revealed a highly significant difference between pre-smear and post-smear values in both groups (p<0.001). This finding indicates that wearing dentures, irrespective of the fabrication technique, resulted in a substantial increase in the dimensions of micronuclei over the study period. The significant results highlight the potential impact of denture wear on cellular changes in the oral mucosa, emphasizing the need for further investigation into the long-term effects of various denture materials and techniques on oral health.

Table 2. Intragroup comparison.

Period	Group	N	Mean	SD	Std. Error Mean	p-value*
Pre-Smear	Injection Molded	10	8.90	3.542	1.120	<0.001**
	Compression Molded	10	17.70	3.945	1.248	
Post-Smear	Injection Molded	10	8.90	2.183	0.690	<0.001**
	Compression Molded	10	24.20	7.642	2.417	

*Wilcoxon signed rank test; **Statistically significant.

Discussion

Acrylic resins, particularly in dental applications, have been shown to exert harmful effects on oral cavity cells, leading to genetic alterations known as genotoxicity. The continuous release of residual monomers from these resins can adversely affect oral health. Prior studies have investigated various methacrylates, including triethylene glycol dimethacrylate (TEGDMA), 2-hydroxyethyl methacrylate (HEMA), and Bisphenol A-glycidyl methacrylate (Bis-GMA), and have demonstrated that these substances can induce harmful changes in the DNA structure of eukaryotic cells, as evidenced by the Comet assay. Hence, it is essential to meticulously blend the correct ratios of polymer and monomer during denture fabrication to minimize these risks [11,12].

The leaching of unreacted monomers is closely tied to the polymer-to-monomer ratio. For example, a polymer-to-monomer ratio of 5:3 has been shown to result in reduced leaching compared to a 4:3 ratio, leading to decreased cytotoxicity. Immersing acrylic prosthetics in water for 24 hours post-curing further diminishes cytotoxicity by allowing residual monomers to leach out, a process that continues to decline over several days following initial polymerization. During the first 24 hours, toxic materials released interact with surrounding molecules, forming new compounds that may subsequently degrade into smaller, less harmful substances. To mitigate the cytotoxic effects of acrylic resins, dentists are advised to soak the prostheses in water for a minimum of one day before patient insertion [13,14].

Conventional denture fabrication methods involve manually mixing materials, which can result in inconsistencies in the powder-to-liquid ratio, leading to increased monomer release. In contrast, injection-molded

dentures use premeasured polymer and monomer ratios from capsules, which helps minimize cytotoxicity after processing. Additionally, dentures produced through injection molding experience less polymerization shrinkage due to the controlled flow of resin through the sprue attached to the flask.

Extending the curing duration in the injection-molding process, followed by a 24-hour immersion in water, can further reduce the cytotoxicity of these dentures. The leaching of residual monomers can alter the mechanical and physical properties of the dentures, which are influenced by the oral environment and contribute to their biodegradation. This biodegradation process involves interactions between saliva, oral microorganisms, and the mechanical forces from chewing [5,15]. Saliva plays a crucial role in biodegradation, as uncured monomers and non-reactive particles become dispersed due to water infiltration, compromising the bond with the polymer matrix.

Water molecules can degrade dental materials and polymers through hydrolysis and enzymatic reactions, with salivary enzymes attacking polymer side chains, leading to detrimental effects. The resulting degradation can increase surface roughness, facilitating plaque and bacterial accumulation.

The biodegradation of acrylic resins can lead to various oral disorders, including sensitization, labial edema, mucosal ulceration or inflammation, pain, local chemical irritation, and conditions such as denture stomatitis and burning mouth syndrome. In severe cases, systemic allergic reactions may occur [16-18]. Previous research indicates that the cytotoxicity of methyl methacrylate (MMA) monomer can disrupt cellular structures, affecting cell membrane integrity, enzymatic activities, and macromolecule production. Direct toxicity from released or residual MMA, along with oxidative stress from free radicals generated during polymerization, contributes to the harmful effects associated with these materials [18-22].

Micronuclei, which are chromosomal fragments or entire chromosomes that fail to reach the spindle poles during mitosis, can serve as biomarkers for genomic damage [23]. The chromosome aberration assay detects specific genetic damage, while the micronucleus assay can identify both chromosome loss and dysfunction of the mitotic spindle due to aneugenic factors. The presence of micronuclei and chromosomal abnormalities may have prognostic implications for cancer development. Although the cytotoxic potentials of these materials are established, further studies are warranted to explore their impacts on target cells, particularly T cells involved in antibody production and innate immune responses. Future research should also investigate the use of micronuclei as a preliminary diagnostic tool for assessing oral cancer risk among denture wearers.

Buccal cells are capable of metabolizing proximate carcinogens into reactive substances, acting as an initial line of defense against inhalation or absorption of harmful agents. A significant proportion of human cancers, approximately 92%, arise in epithelial tissues, including the skin, bronchial epithelium, and the lining of the alimentary canal. Collecting buccal mucosal cells can provide insights into pre-neoplastic changes, with micronuclei observed in these cells potentially indicating the risk of cancer in the upper digestive tract, including early manifestations such as oral leukoplakia [24].

This study's limitations include a small sample size of 20 participants and a short duration of one month, which may restrict the generalizability of the findings. Future research should involve larger cohorts and longer follow-up periods to assess the long-term effects of denture wear on oral health. Additionally, exploring other biomarkers and cell types could provide a more comprehensive understanding of the cytotoxicity associated with different denture materials. Investigating innovative materials or processing techniques that reduce residual monomer release would also enhance the biocompatibility of acrylic resins, improving patient outcomes.

Conclusion

Dentures made from injection-molded denture base materials exhibit less cytotoxicity than those made using traditional molding techniques. Injection molding lowers the risk of hypersensitivity reactions and improves patient health overall because of the decreased monomer leakage, making it safer and more biocompatible.

Authors' Contributions

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All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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