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Comparison of cytotoxicity between 3D printable resins and heat-cure PMMA

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ABSTRACT

Aim: The aim of this study was to evaluate and compare the cytotoxicity of polyurethane and polyoxymethylene printable resins with conventional heat cure polymethyl methacrylate denture base resins.

Methods: The study followed ISO-10993-5 guidelines. It comprised of three groups. Fifteen cuboidal samples measuring 10x10 × 10mm dimension were prepared for each group. The polymethylmethacrylate samples were fabricated using conventional denture processing techniques, while the polyoxymethylene samples were printed using fused deposition modeling and the polyurethane samples using stereolithography technique. Post fabrication the samples were evaluated for cytotoxicity using the MTT assay with the VERO cell line. The percentage of cell viability was calculated to determine the cytotoxic effects.

Results: Statistical analysis revealed a significant difference in the cell viability of the experimental groups ($p \leq 0.0001$). The polyoxymethylene group showed the highest % cell viability (62.78 %), followed by the polymethylmethacrylate group (52.43 %), and the least was observed in the polyurethane-based resin group (46.47 %). The findings indicate polyoxymethylene group displayed least cytotoxicity, followed by polymethylmethacrylate, and polyurethane-based resin.

Conclusion: Polyoxymethylene resin exhibited the minimum cytotoxic properties among the tested materials, followed by polymethylmethacrylate and polyurethane resin.

1. Introduction

Denture base resins play a fundamental role in prosthodontics for fabricating removable dentures. Polymethylmethacrylate (PMMA) has been the desired material due to its simplified processing techniques, biocompatibility, and acceptable esthetics.[1] Conversely, with the rapid improvements in 3D printing technology, printable resins like polyurethane (PU) and polyoxymethylene (POM) have emerged as favorable alternatives for fabrication of prosthesis.[2,3] PU, known for its versatility in use offers exceptional mechanical properties and flexibility, making it suitable for prosthodontic applications.[4] However, POM, a thermoplastic resin, exhibits remarkable mechanical characteristics such as high strength, stiffness, hardness, low friction coefficient, and dimensional stability, making it well-suited for varied dental applications.[5]

Though PMMA is broadly accepted and considerably used in denture fabrication it has limitations.[6,7] The low impact strength, decreased fracture toughness, color changes and staining over time are major limitations of PMMA.[8–10] Alternatively, PU offers the advantage of improved toughness and adaptability through 3D printing, but there is inadequate information on its biocompatibility in dental applications. Comparably, POM exhibits suitable mechanical properties for framework fabrication, but less clinical data available for long-term biocompatibility in intraoral use.[11–14]

Considering the potential clinical uses of 3D printable resins in prosthodontics, it is essential to evaluate their cytotoxicity before envisioning their intraoral use. The evaluation of cytotoxicity of these materials is indispensable for safety and biocompatibility.[8,15] Hence, this study aimed to estimate and compare the cytotoxic effects of PU and POM printable resins with conventional PMMA denture base resins.

This study will provide valuable insights on 3D printable resins to be used as viable alternative to PMMA on biocompatibility. By considering the benefits and shortcomings of these materials, informed decisions can be made on material selection for specific clinical requirements that can assist in achieving successful prosthodontic outcomes.

2. Materials and methods

The study was approved by Institutional review board. All

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procedures involving cell lines were conducted in accordance with the ethical standards outlined in the Declaration of Helsinki and its subsequent amendments.

The study design had three distinct experimental groups: Polymethylmethacrylate (PMMA), Polyoxymethylene (POM), and Polyurethane (PU). The sample size was estimated by a pilot study. The pilot study established an effect size of 0.49 with a significance level (alpha error) of 5 % and a study power (1 - beta error) of 80 %. The sample size of 15 specimens per group was obtained based on the pilot study parameters and it ensured adequate statistical rigor and power to detect differences in cytotoxicity.

ISO-10993-5 guidelines were followed in fabrication and testing samples.[16] Cuboidal samples of 10x10 × 10mm dimension were fabricated for each investigational group. The wax samples of the specific dimensions were made and PMMA samples were fabricated following conventional heat cure-denture processing techniques. The POM samples were printed through fused deposition modeling (FDM) with CREALITY ENDER 7 printer and PU samples were printed with ANYCUBIC MONO SLA, through stereolithography (SLA) technique.

VERO cell lines (King's Institute, Guindy), employed for cytotoxicity studies, were cultured and maintained under standard laboratory conditions. These cells were chosen for their high sensitivity in detecting cytotoxic effects. The cells were cultured in a humidified atmosphere containing 5 % CO2 at 37 °C, using minimal essential medium supplemented with 10 % FBS, penicillin (100 U/ml), and streptomycin (100 μg/ml). The reagents for this study were procured from standard sources. MEM (Hi Media Laboratories) Fetal Bovine Serum (Cistron Laboratories), Trypsin, methylthiazolyl diphenyl-tetrazolium bromide (MTT), and Dimethyl sulfoxide (DMSO) (Sisco Research Laboratory Chemicals, Mumbai). The other chemicals and reagents used in the study were sourced from Sigma Aldrich, Mumbai.

Post fabrication the test samples (PMMA, POM, and PU) was exposed to the VERO cells. This exposure was designed to simulate potential contact between the materials and oral tissues.

The invitro assay for Cytotoxic effect (MTT assay) was conducted. The Cells (1 × 105/well) were plated in 24-well plates and incubated under conditions of 37 °C with 5 % CO2. Once the cells reached confluence, the samples were added and incubated for 24 h. Following the incubation period, the samples were carefully removed from the wells and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. Subsequently, 100μl/well of a 0.5 % solution of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) at a concentration of 5 mg/ml was added and then incubated for 4 h. After the incubation with MTT, 1 ml of Dimethyl sulfoxide (DMSO) was added to all the wells. The absorbance at 570 nm was measured using a UV-Spectrophotometer, with DMSO serving as the blank. The measurements were performed, and the concentration required for a 50 % inhibition (IC50) was determined graphically. The percentage of cell viability was calculated using the following formula

$$\% \text{ cell viability} = \text{A570 of treated cells} / \text{A570 of control cells} \times 100.$$

The calculation enabled a direct comparison of cytotoxic effects among the different materials. The data were tabled (Table 1) and statistical analysis was performed using one-way ANOVA to compare the mean percentage of cell viability among the experimental groups.

Table 1
Comparison of mean values, variability, and significance across PM, PO, and PU groups.

Group	N	Mean	Std. Deviation	Std. Error	95 % Confidence Interval for Mean		P value
					Lower Bound	Upper Bound	
PM	15	52.432	.365	.094	52.22	52.63	0.0001
PO	15	62.781	.939	.243	62.26	63.30	
PU	15	46.473	.919	.246	45.94	47.00	

3. Results

The mean cell viability of the PM group was 52.432 %, with a standard deviation of 0.365 and a standard error of 0.094. The 95 % confidence interval for the mean ranged from 52.22 % to 52.63 %. P-value was 0.0001, indicating a high level of statistical significance. PO group exhibited a higher mean cell viability of 62.780 %, with a 95 % confidence interval spanning from 62.26 % to 63.30 %. This group displayed the highest cell viability among all tested materials. In contrast, the PU group had a lower mean cell viability of 46.473 %, accompanied by a standard deviation of 0.919 and a standard error of 0.246. The 95 % confidence interval for the mean was found to be between 45.94 % and 47.00 %. The significance of these findings is further illustrated in Fig. 1, which graphically displays the maximum cell viability percentages for each material. POM resin exhibited the highest cell viability at 62.78 %, followed by PMMA at 52.43 %, and the lowest was observed in the PU group at 46.47 %. Additionally, Figs. 2–4 present results that indicate the POM group as having the least cytotoxicity among the tested materials, followed by PMMA, with PU showing the highest cytotoxicity. Statistical analysis, (Table 2), revealed highly significant differences between the groups ($F = 1614.776$, $p = 0.0001$). The low p-value of 0.0001 demonstrates the strong statistical significance of these group differences.

The post hoc analysis was results (Table 3) confirm the statistical significance of mean differences, with very low p-values (0.001). The results suggest that there are significant variations in means between the PM, PO, and PU groups ($p \leq 0.0001$). The study indicates that the choice of material, whether PM, PO, or PU, has a significant impact on cell viability, with POM resin being the most biocompatible, followed by PMMA, and PU being the least biocompatible material.

4. Discussion

PMMA has been widely accepted denture base resin materials due to its favorable properties, ease of processing and good biocompatibility. The long-standing use of PMMA is credited to its excellent clinical performance and patient acceptance. Conversely, 3D printed resins have acquired significant attention in recent years as potential alternatives to conventional materials in dental applications.[17–19] Fiore AD et al.,[9] and Perea -Lowery et al.,[10] Chhabra et al.[11] have exhibited that PMMA has better flexural and impact strength when compared to newer generation 3D-printing materials. Kraemer Fernandez P et al.,[20] Zeidan AAE et al.,[21] have reported higher surface roughness in 3D printed resins compared to

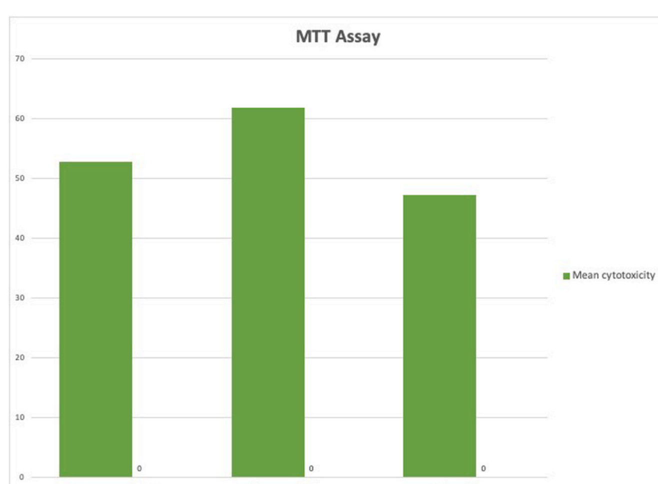

Fig. 1. MTT assay.

Fig. 2. Cell viability heat cure resin.

Fig. 3. Cell viability Polyoxymethylene.

Fig. 4. Cell viability Polyurethane.

conventional materials. The higher surface roughness may lead to increased bacterial adhesion, which affects the oral health of the patients. Additionally, 3D printed resins displayed poor hardness values

Table 2
ANOVA statistics for the variables.

Variable	Sum of Squares	df	Mean Square	F
Between Groups	1986.41	2	993.206	1614.776
Within Groups	25.22	41	.615	
Total	2011.63	43		

Table 3
Post hoc analysis of mean differences and statistical significance between groups.

(I) Groups	(J) Groups	Mean Difference (I-J)	P value	95 % Confidence Interval	
				Lower Bound	Upper Bound
PM	PO	−10.34867*	0.001	−11.0635	−9.6338
	PU	5.95914*	0.001	5.2316	6.6866
PO	PM	10.34867*	0.001	9.6338	11.0635
	PU	16.30781*	0.001	15.5803	17.0353
PU	PM	−5.95914*	0.001	−6.6866	−5.2316
	PO	−16.30781*	0.001	−17.0353	−15.5803

than conventional denture base materials, that affects the durability and resistance to wear.[12,13] The wear resistance has been demonstrated to be comparable to milled or conventionally fabricated resin materials.[20–23]

Despite the challenges, advancements in technology and materials are expected to significantly benefit 3-D printing resin compared to conventional resins. In recent times, a wide range of materials has been employed for various denture applications. The primary criterion for denture base materials is to have low cytotoxicity. Few studies have assessed the cytotoxic potential of commonly used 3D printing materials such as Polyoxymethylene and Polyurethane (PU). Therefore, this study was conducted to assess the cytotoxicity of these materials.

The study suggested that POM demonstrated the least cytotoxicity among the tested materials. The higher cell viability in the POM group can be due to its mechanical properties and biocompatibility. POM's increased strength, impact strength, stiffness, low friction coefficient and hardness may contribute to increasing its biocompatibility.[3,24] Conversely, the mean percentage of cell viability in the PMMA group was 52.43 %, demonstrating a lower cell viability compared to POM. Though PMMA has been broadly used and established denture base resin material, it still exhibited a higher cytotoxicity in this study and it can be considered as limitation of PMMA.[21,23]

The PU group showed the highest cytotoxicity (lowest cell viability), with a mean value of 46.47 %. Lourinho et al.,[3] Della Bona et al.[24] attributed it to 3D printing process, that produces resins with higher surface roughness compared to conventional materials. The increased surface roughness could enhance bacterial adhesion and contribute to cytotoxic effects. Besides, the limited long-term clinical data on PU's biocompatibility and limited testing in various dental applications, may also contribute to its higher cytotoxicity data in the literature.

The results of this study are in consensus with the literature, where POM has been is considered for its favorable mechanical properties and biocompatibility.[3-6] POM's superior mechanical properties, comprising high strength, stiffness, hardness, and impact strength, make it a favorable material for various prosthodontic applications. The high wear resistance and dimensional stability augment its use in removable partial denture frameworks. Furthermore, the low coefficient of friction of POM is beneficial for reducing wear on opposing dentition. POM additionally displayed improved chemical resistance, low water absorption, and superior biocompatibility. These properties are vital in ensuring the long-term biocompatibility, safety of dental materials, reduces the risk of tissue reactions, improves patient comfort and satisfaction.[24]

However, this study has limitations that includes it is invitro nature and the need for further research to validate the biocompatibility and long-term clinical performance of POM and other 3D printable resins in dental applications. Furthermore, conducting analyses on other material properties, such as surface roughness and bonding to denture teeth, will provide a more comprehensive understanding of POM's performance in dental prosthetics that addresses the major limitations of the material.

Ideally the PMMA remains a reliable and well-established material for denture bases, while 3D printable resins, particularly POM display favorable properties for future dental applications. The results of the study emphasized the cytotoxic differences among the tested materials. POM exhibited the least cytotoxicity, followed by PMMA, and then PU. It is essential to consider the specific requirements of each patient when selecting the appropriate material to accomplish successful and robust prosthodontic outcomes. Continued research and developments in material science will play a significant role in improving the performance and increasing the applications of dental materials in prosthodontics.

5. Conclusion

Polyoxymethylene (POM) demonstrated minimal cytotoxicity among the tested denture base resins. It has the potential to enhance patient comfort, reduce complications, increase durability, and stimulate further innovation in dental material science. As the dental field continues to advance, the use of POM and similar materials may contribute to a higher standard of care and improved quality of life for denture wearers.

Declaration of competing interest

Nil.

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