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ORIGINAL RESEARCH

Comparative Analysis of the Water Sorption and Cytotoxicity of Two different Denture Base Systems: An *in vitro* Study

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ABSTRACT

Aim: Different biomaterials and techniques have been introduced in the field of prosthetic dentistry with the purpose of replacement and rehabilitation of the edentulous areas. Due to their shorter setting time, the light-activated restorative and prosthetic materials have the capability of releasing few amount of cytotoxic materials in the oral cavity. Polymer materials [urethane dimethacrylate (UDMA) and bis-acryl] are assumed to have high mechanical properties. Polymethyl methacrylate (PMMA) offers numerous advantages of being highly esthetic in nature and at the same time being cost-effective. Hence, this study aimed to assess and compare the water sorption and cytotoxicity of light-activated UDMA denture base resin and conventional heat-activated PMMA resin.

Materials and methods: This study included assessment and comparison of water sorption and cytotoxicity of heat-activated PMMA resin and light-activated UDMA denture base system. Fabrication of heat-activated PMMA resin and UDMA specimens was done by investing the wax patterns in stone molds using manufacturer's instructions. Contraction of the specimens was done for assessment of cytotoxicity and water resorption of the UDMA and PMMA resin samples. All the results were analyzed by Statistical Package for the Social Sciences software version 18.0. Chi-square test and one-way analysis of variance tests were used for the assessment of the level of significance; $p < 0.05$ was taken as significant.

Results: Mean lysis score observed in the PMMA and UDMA groups was 0.4 and 0.3 respectively. While observing at the 3 months time, the mean water resorption in the PMMA and UDMA groups was found to be 37.9 and 40.2 respectively. Significant difference in relation to water resorption was observed between the two study groups only at 3 months time.

Conclusion: Both materials used in this study are nontoxic. Furthermore, UDMA resin materials exhibited lower water resorption after more than 1 month of time of storage.

Clinical significance: Water resorption is similar for different denture base resin systems till 1 months time.

Keywords: Cytotoxicity, Denture base, Water sorption.

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INTRODUCTION

With the purpose of replacement and rehabilitation of the edentulous areas, various new biomaterials and techniques have been introduced in the field of prosthetic dentistry. These technologies also fulfill the purpose of promotion of dental tissue regeneration. Due to its esthetic considerations and various other factors, periodontal soft tissue, among all other oral tissues, has been specifically highlighted.[1,2]

Management of these soft tissues of the periodontal areas is critically important during and after the prosthetic rehabilitation, for the purpose of contouring the soft tissues along the lines of dental restorations. These hold true particularly in the area of anterior tooth regions.[3]

Due to their shorter setting time, the light-activated restorative and prosthetic materials have the capability of releasing few amounts of cytotoxic materials in the oral cavity. However, these appear to be more biocompatible

in comparison to the chemically activated materials which have longer setting time. Due to the natural characteristic of the polymer materials [urethane dimethacrylate (UDMA) and bis-acryl], it is assumed that they have high mechanical properties.[4,5] Polymethyl methacrylate (PMMA) was invented in 1937. They offer numerous advantages of being highly esthetic in nature and at the same time being cost-effective.[6,7]

Literature quotes paucity in the data in relation to the water sorption and toxicity of these newly available light-activated dental base resin materials. Hence, we planned this study to assess and compare the water sorption and cytotoxicity of light-activated UDMA denture base resin and conventional heat-activated PMMA resin.

MATERIALS AND METHODS

This study was conducted in the Department of Prosthodontics of the dental institution and included assessment and comparison of water sorption and cytotoxicity of heat-activated PMMA resin and light-activated UDMA denture base system. Ethical approval was taken from the Institutional Ethical Committee, and written permission was obtained after explaining in detail the entire research protocol. Fabrication of heat-activated PMMA resin specimens was done by investing the wax patterns in stone molds. Whole of this was done within a dental flask unit and the same procedure which is used for the construction of conventional denture was used for curing the heat-activated PMMA resin specimens as per instructions given by the manufacturer. Finishing of all the acrylic specimens was done after removing them from the unit. Digital caliper was used for arranging the desired sizes of the specimens (5 mm diameter and 1 mm thickness for each specimen). Preheating of the UDMA baseplate resin was done for 2 minutes followed by preheating of a prefabricated 5 × 1 mm thick wax pattern. The preheating was done at 55°C. This enabled easy adaptation of the material. Application of the separating agents was done on the stone molds followed by warming the resin and adapting them to the mold using figure pressure. Removal of the UDMA specimens was done after cooling. Processing of the specimens was done in light-processing unit for 10 minutes. Similar procedure was used for obtaining the UDMA cytotoxicity test specimens. Culturing of the mouse connective tissue fibroblasts cell lines L929 was done in Dulbecco's Modified Eagle's medium which was supplemented with 10% fetal calf serum and 2 mm/mL L-glutamine. Antibiotics were not added to the culture medium. Cultivation of the cultures was done in an incubator at 37°C till the point of achievement of confluence by cell monolayer approximately after 7 days. Harvesting of the cultures was done using 0.25% trypsin solution. Seeding of the stock cultures was done in 35 mm diameter culture dishes followed by subculturing once a week. After the formation of the confluent cell layer, removal of the material was done, followed by replacement with a complete medium which contained 1.5% agarose. Preparation of four additional dishes was done for positive and negative control materials. Dulbecco's Modified Eagle's medium and absolute phenol were used as negative and positive controls respectively. Inverted microscopic observation was used for evaluating the cell response after exposition period of 1 day at 37°C. The following criteria were used for scoring of the cell lysis[8]:

- 0: No detectable cellular lysis
- 1: Cell lysis detected in <20%
- 2: Cell lysis detected in 20 to 40%
- 3: Cell lysis detected in 40 to 60%
- 4: Cell lysis detected in 60 to 80%
- 5: Cell lysis detected in >80%

Classification of the cytotoxicity was done into following classes[8]:

- 0 to 0.5: Nontoxic
- 0.6 to 1.9: Mildly toxic
- 2.0 to 3.9: Moderately toxic
- 4.0 to 5.0: Markedly toxic

After fulfilling the International Organization for Standardization requirements, a water resorption test was performed according to the criteria previously described in the literature.[8] For obtaining the wax patterns, a stainless steel mold was used, which measured 1 mm^3 in dimension. For packing of the PMMA and UDMA resins, placements of the wax patterns of the test specimens in the dental stone were done. Manufacturer's instructions were followed for packing of resins and for carrying out their polymerization process. Drying of all the specimens was done in a desiccator for a time period of 24 hours followed by removal at room temperature for 60 minutes until unless a constant mass was reached (M1). Immersion of the specimens was done in distilled water for 24 hours, 7 days, 1, and 3 months time. Drying and cleaning of all the specimens was done after removing from water after each time period. The specimens were waved in the air for approximately 15 seconds and weighted again (M2). Calculation of water sorption was done using formulae as described previously[8]:

$$\text{Water sorption } (\mu g/mm^3) = M2 - M1\ (\mu g)/\text{Volume of disk } (mm^3)$$

All the results were analyzed by Statistical Package for the Social Sciences software version 18.0. Chi-square test and one-way analysis of variance (ANOVA) tests were used for the assessment of the level of significance; $p < 0.05$ was taken as significant.

RESULTS

Cytotoxic assay and their lysis score is highlighted in Table 1. Lysis score observed in the PMMA and UDMA

Table 1: Cytotoxic assay and their lysis score

Groups	*Number*	*Lysis score*
PMMA group	15	0.4
UDMA group	15	0.3
Positive control	15	3
Negative control	15	0

Table 2: Statistical difference of mean water resorption in each group base resin

Denture base resin material	*24 hours*	*7 days*	*1 month*	*3 months*
PMMA group	12.1^{1}	26.1^{2}	37.9^{3}	63.8^{4}
UDMA group	12.5^{1}	27.9^{2}	40.2^{3}	57.1^{5}

Same superscript letter denotes nonsignificant difference between groups

groups was 0.4 and 0.3 respectively. Lysis score of positive control and negative control specimens was 3 and 0 respectively. Table 2 shows the statistical difference of mean water resorption in each group base resins. At the 24 hours time period, the lysis score was found to be 12.1 and 12.5 in the PMMA and UDMA groups respectively. At the 7 days time, the mean water resorption was 26.1 and 27.9 in the PMMA and UDMA groups respectively. While observing at the 3 months time, the mean water resorption in the PMMA and UDMA groups was found to be 37.9 and 40.2 respectively. Significant difference in relation to water resorption was observed in between the two study groups only at 3 months time ($p < 0.05$).

DISCUSSION

Commonly used materials for the fabrication of complete and removable dentures are the acrylic resins. Heat-cure acrylic resins are the materials from which majority of the denture bases are made. It is believed that certain toxic materials are released from them, which include formaldehyde, methyl methacrylate (MMA), methacrylate acid, and benzoic acid. These chemicals can cause serious reactions in the surrounding soft tissues.[9] Methyl methacrylate monomer is the major element responsible for majority of these reactions. It can be released from the denture base system into the saliva and the amount secreted into the saliva depends on the type of reason, polymerization reaction, etc.[10,11] The availability of these materials in the environment and the route of insertion of these acrylic resin decides their degree of harmfulness.[12] Literature quotes limited studies that have assessed the cytotoxicity of various denture base systems. Hence, we planned this study to assess and compare the water sorption and cytotoxicity of light-activated UDMA denture base resin and conventional heat-activated PMMA resin.

In this study, we observed that UDMA and PMMA resins exhibited similar levels of cytotoxicity (Table 1). We also observed that significantly lower levels of water resorption were exhibited by UDMA denture base resins in comparison to PMMA resins ($p < 0.05$) (Table 2). Our results were in correlation with the results obtained by Akin et al[8] who also reported similar findings in their study. They assessed the water sorption and cytotoxicity of UDMA and PMMA resins denture base systems. They fabricated cytotoxic and water sorption specimens and observed no cytotoxic effects in either of their two study groups. They also observed significantly lower water resorption in the UDMA study group in comparison to the PMMA study group at both 3 and 6 months storage time.

Akin et al[13] assessed the impact of various surface treatments in relation to the shear bond strength on PMMA and UDMA. They used heat-cured PMMA (Meliodent) and light-activated UDMA (Eclipse) in their study. They divided their study samples into four study groups with 15 samples in each group. The groups were divided based on their surface treatment agents, which included the following groups: Acrylic untreated (group AC), Eclipse untreated (group EC), treated with eclipse bonding agent (group EB), and erbium-doped yttrium aluminum garnet laser-irradiated eclipse (group EL). As per manufacturer's instructions, the preparation of testing specimens was done. Universal testing machine was used for testing the shear bond strength of all the specimens. They compiled the data of all the specimen groups and analyzed them with one-way ANOVA and *post hoc* Tukey–Kramer multiple comparison tests. They observed that in specimens of the group EB, mean bond strength was the highest, whereas in group EC, the observed shear bond strength was the lowest. While comparing bond strength among all the study groups, they observed statistically significant difference except for group EC and EL. From the results they concluded that for fabrication of dentures with Eclipse resin system, Eclipse bonding agent should be used as a component. Pfeiffer and Rosenbauer[14] assessed four different denture base systems and compared their residual monomer amount, water resorption quality, and solubility. The different denture base systems used by them in their study included sinomer, polyan, promysan, and microbase. Gas chromatography was used for the assessment of residual MMA monomer concentration. They observed that sinomer and polyan showed significant lower residual MMA monomer content in comparison to PMMA control group; 0.31% of MMA monomer was contained in sinomer, whereas a MMA monomer content of 0.44% was exhibited by polyan. No detectable amount of residual MMA was present in promysan and microbase. Significantly lower amount of water resorption was seen in promysan in comparison with paladon 65. They observed significantly lower residual monomer in all the tested hypoallergenic denture base materials in comparison with PMMA.

Ebrahimi Saravi et al[15] assessed the cellular toxicity of Futura Gen and GC Reline hard acrylic resins and compared the obtained values with those of conventional heat-cure resin (Meliodent). They placed small discs from each of the acrylic resin in 24 culture plates in the fibroblast culture cell line. They evaluated the amount of light absorption by each plate at the end of 1 hour time, 1 day time, and 1 week time with enzyme-linked immunosorbent assay. They observed significantly lower levels of light absorption in the samples of group Futura Gen in comparison with Meliodent after 1 hour time. A significant lower level of light absorption was observed in cases of Futura Gen in comparison with Meliodent after 1 day time. Approximately similar levels of absorption rates were observed in cases of GC Reline hard, Meliodent, and Futura Gen after 1 week time. Some degree of cytotoxicity was exhibited by all the tested resin materials. Under the lights of their results, the authors recommended immersion of dentures in water for 1 day prior to insertion into the oral cavity of the patient.

CONCLUSION

In terms of cytotoxicity, both materials are nontoxic. Lower water resorption is exhibited by UDMA resin materials after more than 1 month time of storage. However, future studies are recommended.

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