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Evaluation of Cytotoxic Effect of Different Denture Base Material- Heat Polymerized Resin, Flexible Dental Resin and Microwave Cured Dental Resin

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Abstract

Purpose: The objective of this study was to determine the cytotoxic effect of three different extracts of material used for denture base fabrication and to compare the cytotoxic effect of these materials in mccoy mouse cell line which have fibroblast.

Materials and Methods: Set specimens from heat polymerized resin, flexible dental resin and microwave cured dental resin denture base material were eluted in distil water for 1, 7 and 30 days then eluates were placed in 96 well culture plates with cell line. Cytotoxicity was determined after 24-hour incubation of the cells and eluates. Cytotoxicity was judged using [3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide] (MTT) cell viability assay.

Results: The eluates from heat polymerized resin, flexible dental resin and microwave cured dental resin were cytotoxic to mccoy mouse cell line. Heat polymerized resin was the most toxic denture base material among the denture base resin tested in all cultures. The cytotoxic effect decreased in the order of heat polymerized resin > microwave cure dental resin>flexible dental resin for mccoy mouse cell line at 1st day, 7th day heat polymerized resin > flexible dental resin > microwave cure dental resin and 30th day heat polymerized resin > flexible dental resin > microwave cure dental resin.

Conclusion: The cytotoxic effect depended on the materials tested and the duration for which eluates were taken out.

Keywords: Cytotoxic effect, mccoy mouse cell line, microwave cured resin,flexible dental resin.

Introduction

Denture base resins are vastly used in dentistry for a variety of purposes. Depending upon the factor which starts the polymerization reaction denture base resins can be chemical, heat, light, and microwave polymerization materials. That can be used during denture base construction, relining previous dentures, and for fabrication of removable orthodontic appliances. It has been increasingly questioned for the safety of this material's clinical application as their biodegradation in the oral environment leads to harmful effects. These materials have local side effects and occupational hazards as well.[1]

Biocompatibility has been defined as the ability of a material to perform with an appropriate host response in a specific application. Appropriate host response means no (or a tolerable) adverse reaction of a living system to the presence of such a material. Due to the toxicity of a dental material there may be an adverse reaction. Therefore toxicity may be considered as one reason for non-biocompatibility of a dental material.

Biocompatibility testing can be divided largely into two categories, screening and specific toxicity assays. Screening assays try to determine biological effects under the severe testing conditions. Specific toxicity assays are usually more quantitative than screening assays and may be designed to measure the effects of acute, subchronic or lifetime exposure to the test substance. The use of animals for screening and specific toxicity assays are not performed much for ethical and economic reasons. Therefore, cell culture assays for materials testing have been evolved in order to complement in vivo procedures.

The most widely used biological systems for in vitro toxicity testing of dental materials are cell culture; only a few experiments have been performed on organ cultures; e.g. tooth germs. [2]

During the denture base resin polymerization reaction varying amount of residual monomer, resulting from incomplete conversion of monomers into polymer, has the potential to cause local irritation, inflammation, and hypersensitivity reaction of the oral mucosa. Clinical signs and symptoms most frequently reported include erythema, erosion of oral mucosa, and a burning sensation on the mucosa and tongue.[3]

As residual monomer concentration varies with the methods and the conditions of polymerization it depends upon the variations in chemical composition and purity of the commercially available resin systems, the degree of conversion of their constituent monomers, and manipulative variables may all affect the biologic and physical properties of the acrylic resins.

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. The primary eluate is residual monomer. Numerous reports suggest that residual monomer may be responsible for mucosal irritation and sensitization of tissues. This information is important, not only to assess the biologic effects of such materials, but also to enable a comparison among the different polymerization methods, thus assisting the clinician in selecting a material with minimal cytotoxicity[4].

Till today, few data compare the cytotoxicity of different cell lines with denture base materials. The objective of this study was to determine the cytotoxicity of different eluates of denture base material (heat polymerised resin, flexible dental resin, and microwave cured resin) and to compare the cytotoxic response of these materials on a MCCOY mouse cell line which have fibroblast.[5]

These results obtained from the study may provide valuable data so that we can compare different denture

base material at one time. Which in turn can help us determine treatment option more bio compatible.

Material and Methodology

Preparation of Specimens: The materials tested were the heat polymerized resinDpi Heat Cure, the Flexible dental resinLucitone FRS, Dentsply, and the Microwave cured denture base resin Acron^{TM} MC, GC Lab Technologies Inc. For the purpose or standardization of sample, a stainless steel circular metal die with a 10 mm diameter and 1 mm thickness was milled in the center portion of l mm thick stainless steel sheet using milling machine. 90 sample disks of the denture base resins were fabricated under aseptic conditions in moulds. Packing and processing were carried out in accordance with the manufacturer's instruction. One surface of each disc was finished and polished and other surface left unpolished to represent intaglio surface of prosthesis. The sample disks were rinsed thoroughly with sterile deionized water prior to their use in the experiment. Set samples were placed in the air tight container directly after processing. Cytotoxicity was determined after 24 hour incubation of the cells and eluates of days 1, 7 and 30 from the entire specimen. Samples were prepared for each material by placing disc in distil water in autoclaved bottles. Eluates were taken out in vaccutainer sterile test tubes at 1, 7, and 30 days, involving three materials to be tested, so total 90 samples are made.

Cell culture

Media preparation: A complete culture medium, Mccoy 5A Medium L- Glutamine and Sodium Bicarbonate (Himedia Laboratory) 10% fetal bovine serum (FBS, Himedia Laboratory) and antibiotics (100 U/mL gentamycin was prepared. The medium was sterilized by filtration (0. 22µ Millipore filter).) Cultures were maintained at 37 ^{0}C in a humidified atmosphere of 5% CO_2 and 95% air.Cell line procured from National centre for cell science (NCCS PUNE) was subcultured. The used medium is removed aseptically from the Flask and washed with Phosphate buffer saline.

Trypsinization: The cell culture was then treated with TPEG [0. 25% Trypsin, 0.02gm Ethylene diamine tetra acetic acid (EDTA) and 0.05gm glucose in phosphate buffer saline]. The trypsinization was done for 1-2 minutes. The process of trypsinization was then terminated by adding DMEM into the flasks.

Centrifugation: The content of flasks was centrifuged at 1000 rpm for 10 minutes. The supernatant medium was discarded and the pelleted cells were resuspended in fresh medium.

Cell Quantification: Under sterile conditions, volume of trypan blue (dilution factor-2) and mixed gently by pipetting.Both sides of Hemocytometer chamber were filled with cell suspension (approximately 10 µl) and viewed under an Inverted microscope using 20 X magnification. Number of viable (bright cells) and non-viable cells (stained blue) were counted.

Determination of denture material cytotoxicity by [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) cell viability assay: Effects of eluates of denture materials on culture media is assessed by MTT kit obtained by Himedia Laboratory. This assay is based on the cellular conversion of a tetrazolium salt into a blue formazan product that is easily detected by an Elisa reader. To test the effect of eluates obtained from specimen on cell viability, an aliquot (50 µl)of control or denture disk conditioned media was added to the wells seeded with 1×10^5 fibroblasts or epithelial cells in a 96-well plate along with fresh 50µl of growth media and incubated for 24 hours at 37°C. Following the incubation.Every sample was duplicated. 10 µl of MTT added to each well including control and wrapped the plate with aluminium foil to avoid exposure to light. Plate

again placed in incubator for 3 hour then100µl of solubilization solution is added to wells.Gentle stirring done on a gyratory shaker to enhance dissolution. Measured the absorbance on an ELISA reader at 570nm wavelength with a reference wavelength of 650nm.

Results and Discussion

Statistical analysis was done by Statistical Package of Social Science (SPSS Version 20; Chicago Inc., USA). Data comparison was done by applying specific statistical tests to find out the statistical significance of the comparisons. Quantitative variables were compared using mean values and qualitative variables using proportions.

Significance level was fixed at $P \leq 0.05$.

Mean values ± SE were calculated and the data obtained were analyzed by means of a linear model ANOVA followed by Tukey's post hoc tests for comparison among groups.

It reveals comparative evaluation of Mean cell Viability of McCoy Cell line among Microwave cure, Flexible & Heat cure denture base material at different time interval. Mean McCoy Viable cell was found maximum in number in flexible denture base materials i.e. 0.260±0.023 and it was minimum for microwave and heat cure. i.e. 0.227±0.044 & 0.229±0.046 respectively. It was 0.377±0.127 for controls. It indicates that microwave and heat cure is more toxic as compare to flexible denture base material on 1st Day. Mean Mc Coy Viable cell was found maximum in number in Microwave & flexible denture base materials i.e. 0.288±0.090 & 0.283±0.073 and it was minimum for heat cure. i.e.0.216±0.066. It was 0.392±0.152 for controls. It indicates that heat cure is more toxic as compare to microwave & flexible denture base material on 7th Day. Mean McCoy Viable cell was found maximum in number in Microwave & flexible denture base materials i.e. 0.289±0.056 & 0.277±0.035 and it was minimum for heat cure. i.e.0.265±0.025. It was highest 0.523±0.131 for controls. It indicates that heat cure is more toxic as compare to microwave & flexible denture base material on 30th Day. There was statistically highly significant difference found in % Mean Viable McCoy Cell (Cytotoxicity) among Microwave cure, Flexible & Heat cure denture base material at different time interval**. (p<0.01).**

Comparative evaluation of Mean Viable McCoy Cell from 1st day to 30th day among Microwave cure, Flexible & Heat cure denture base material. Overall it shows that Mc Coy viable cell are continuously increasing with time and toxicity of microwave cure resin base material is decreasing with the time among all material. But there was statistically not significant reduction found in toxicity of all three resin base material from 1st day to 30th Day. **(p>0.05). (Table 1, Figure 1)**

All recent materials must be investigated to confirm their biocompatibility with intraoral tissues. To assess the interaction between materials and host, different cell culture techniques have been proposed. MCCOY MOUSE CELL LINE (FIBROBLAST) are used to determine the cytotoxicity of materials by this we can eliminate the donor biopsy variability, and greater reproducibility is possible.

In the present study, cytotoxicity was represented by the number of viable cells present after exposure to denture base resin eluate when compared to control cultures. The hypothesis behind the study was to compare the cytotoxicity of different material as there was very less study has been done in order to compare cytotoxicity of microwave cure denture base material to heat polymerized denture base material and the introduction of new hypoallergic denture base material such as polyamide which are free of monomer release. Another important aspect considered is storage time, which plays an

important role in the cytotoxicity of acrylic denture base materials.

In the present study three material were tested out of which heat cure shows maximum cytotoxic effect followed by flexible denture base resin then microwave cure denture base material and that was with agreement with earlier studies that shows all of the eluates from denture base resins are cytotoxic to fibroblasts[6,] U-937 human histiocytic lymphoma promonocytic cells[7] and hamster cheek pouch epithelial cells invitro[8]. Our results were also in agreement with **Sheridan et al**[9]who has reported that the cytotoxic effect of acrylic resins was greater in the first 24 hours after polymerization and decreased with time for all the resins evaluated in their study. The authors concluded that the longer the prosthesis is soaked, the less cytotoxic effect is likely to have regardless of the denture base resin it is manufactured from. So study was designed to check the long term effect on cytotoxicity with time, up to 30 days.

Our study shows that the microwave denture base material is least cytotoxic among the three material tested which was in agreement with the **Yunus et al**[10] who determined that lowest residual monomer level recorded with microwave polymerisation. The lowest residual monomer level means that the specimens polymerized by microwave energy achieved the highest degree of conversion, It also appears that the level in the microwave group has already been reduced to approximately half that of the level in bench-cure specimens, indicating that polymerization using microwave energy results in a substantial reduction of residual monomer in a shorter time.

It was found that at baseline day1 the mean cell viability is least when compared to day 30 and as elution time increases the effect of cytotoxicity on cell culture decreases. It can be explained that the toxic substances released into the medium within the first 24 hours are either broken down over time or complexed with other chemicals in the medium that may change their cytotoxic potential. Individual compounds such as dimethacrylate, methyl methacrylate, benzoic acid, and formaldehyde have been shown to act as toxic substances that may release from traditional denture base resins. They may be a cause of the cytotoxic effects observed in this study.

The results of our study on microwave cure material shows maximum toxicity in first 24 hours which was in agreement with ***Baker et al***, who determined that most of the methyl methacrylate leached into human saliva from resin with in first hour. Progressive degradation of MMA would manifest as a less detrimental effect on cell viability. our experiment report that mean viable cells from 1st day to 7th day keep on decreasing that means MMA release increases with time upto 7th day. After that mean viable cells increase till 30th day which was in disagreement with ***Baker et al***[11].

Our study shows polyamide denture base material has similar toxicity with conventional heat cure in long term like 30 days. McCoy mouse viable cells are increasing with time from 1st day to 7th day and then decrease slightly that was in agreement with **Uzun et al**[12]. (Table 2, Figure 2)

Polyamide is a monomer-free material and does not have a polymerization reaction like PMMA. However, it was significantly cytotoxic compare to control group. At present, we do not have enough knowledge about the reasons of polyamide denture base material cytotoxicity, which mechanism causes the cell death and how it can be minimized. dendritic-shaped particles/surfaces have the extended aging period could alter the surface characteristics of the biomaterials and these alterations are thought to be the cause of the increased cytotoxicity after 7 days aging and it was also indicated by **Yamamoto**[13].

Further research is needed to identify the effect of short- and long-term aging on chemical behaviour, particle releasing characteristics, surface roughness and their relation with the cytotoxicity of polyamide denture base material. On the basis of our results it can be stated that the polyamide denture base material can be used as a denture base material with similar biological safety limits as PMMA.

It has been advocated that the prosthesis should be immersed in water at 50^0C for 60 minutes, to reduce the amount of released monomer and therefore the toxic potential of denture base resins which was also reported by ***Weaver and Goebell.***[2.].The immersion of prosthesis in heated water decreased the hypersensitivity reaction in the examined patient due to further polymerisation in the presence of free radical. By immersing the prosthesis in heated water, monomer molecules diffuse more rapidly, leading to a complementary polymerization reaction.

The recommended limit for exposure to methyl methacrylate monomer in man has been estimated only for the inhaled vapour (100 ppm in air or 410 mg/ m^3/ 8 hour of exposure; Health and Safety Executive, 1986). According to the **BAKER et al**[11], the amount of MMA released from an oral appliance seems comparatively small and is quickly degraded. This MMA release has only been detected from appliances constructed with autopolymerized resin but not with conventionally heat cured acrylic resins and that can be minimized by immersion of appliances in water for 24 hour before insertion for microwave cure denture base material and 7 days for heat cure denture base material.

This in vitro study also demonstrated that poly amide materials represent an alternative to the classic PMMA resins for patients who are allergic to MMA; monomer. But acrylic resin still remain the main stay of denture prosthesis because polyamide denture base material has disadvantages like water sorption, surface roughness, bacterial contamination, warpage, colour deterioration, and difficulty in polishing. In this study, differences in cytotoxicity between different materials used for denture bases is demonstrated with heat cure should be least preferred. To summarize, study indicates heat polymerised denture base material is more cytotoxic than the microwave polymerised resin which was in parallel to **Sheridan et al**[9] .

Study data can not necessarily be extrapolated to clinical scenarios however in vitro study provide a simplified system that minimises confounding variables. From the results of this investigation, it can be suggested that microwave polymerisation could be used to reduce the cytotoxicity but it is the clinician's decision to choose the best material out of the available material on ground of patient requirement such as in condition where there are major undercuts polyamide denture base should be the choice. And where time and biocompatibility is prime concern microwave cure denture base material can be used.

To overcome the limitations of the in vitro tests, denture base materials must be evaluated intraorally. Investigation such as water sorption, and solubility and other properties of these materials is necessary. Future research may be designed to identify the individual components of the eluate that were responsible for the observed cytotoxicity.

Conclusion

1. Comparative evaluation of Mean cell Viability of McCoy Cell line among Microwave cure denture base material at 1^{st} day, 7^{th} day and 30^{th} day was 0.227, 0.288 and 0.289 respectively.
2. There was statistically no significant (p value-0.067) reduction found in cell viability of Microwave cure denture base material from 1st day to 30^{th} day.

3. Comparative evaluation of Mean Viability of McCoy Cell at 1st day, 7th day and 30th day among Flexible denture base material was 0.260, 0.283 and 0.277 respectively.
4. There was statistically no significant reduction (p value-0.533) found in cell viability of Flexible cure denture base material.
5. Comparative evaluation of Mean Viability of McCoy Cell at 1st day, 7th day and 30th day among Heat cure denture base material was 0.229, 0.216 and 0.265 respectively. There was statistically no significant (p value - 0.159) reduction found in cell viability of Heat cure denture base material from 1st day to 30th Day.
6. Comparative evaluation of Mean cell Viability of McCoy Cell line at 1st day among Microwave cure denture base material and control was 0.227 and 0.377 respectively. The difference in cytotoxicity was highly significant (p value- 0.001).
7. Comparative evaluation of Mean cell Viability of McCoy Cell line at 7th day among Microwave cure denture base material and control was 0.288 and 0.392 respectively. The difference in cytotoxicity was non-significant (p value- 0.156).
8. Comparative evaluation of Mean cell Viability of McCoy Cell line at 30th day among Microwave cure denture base material and control was 0.289 and 0.523 respectively. The difference in cytotoxicity was highly significant (p value- 0.001).
9. Comparative evaluation of Mean cell Viability of McCoy Cell line at 1st day among flexible denture base material and control was 0.260 and 0.377 respectively. The difference in cytotoxicity was highly significant (p value- 0.005).
10. Comparative evaluation of Mean cell Viability of McCoy Cell line at 7th day among flexible denture base material and control was 0.283 and 0.392 respectively. The difference in cytotoxicity was non-significant (p value- 0.127).
11. Comparative evaluation of Mean cell Viability of McCoy Cell line at 30th day among flexible denture base material and control was 0.277 and 0.523 respectively. The difference in cytotoxicity was non-significant (p value- 0.001).
12. Comparative evaluation of Mean cell Viability of McCoy Cell line at 1st day among heat cure denture base material and control was 0.229 and 0.377 respectively. The difference in cytotoxicity was highly significant (p value- 0.001).
13. Comparative evaluation of Mean cell Viability of McCoy Cell line at 7th day among heat cure denture base material and control was 0.216 and 0.392 respectively. The difference in cytotoxicity was significant (p value- 0.005).
14. Comparative evaluation of Mean cell Viability of McCoy Cell line at 30th day among heat cure denture base material and control was 0.265 and 0.523 respectively. The difference in cytotoxicity was highly significant (p value- 0.001)

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Legends Figure

Table 1 : Comparative evaluation of Mean cell Viability of McCoy Cell line among Microwave cure, Flexible & Heat cure denture base material at different time interval.

Denture Base Material	McCoy Cell Viability					
	1st Day		7th Day		30th Day	
	MEAN	SD	MEAN	SD	MEAN	SD
Microwave cure	0.227	0.044	0.288	0.090	0.289	0.056
Flexible	0.260	0.023	0.283	0.073	0.277	0.035
Heat cure	0.229	0.046	0.216	0.066	0.265	0.025
Controls	0.377	0.127	0.392	0.152	0.523	0.131
ANOVA ‘F’ Value	8.872		4.448		24.738	
p-Value	0.001(HS)		0.010(S)		0.001(HS)	

Table 2: Comparative evaluation of % Mean Viable McCoy Cell from 1st day to 30th day among Microwave cure, Flexible & Heat cure denture base material.

Denture Base Material	McCoy Viable Cell (Toxicity)						Repeated Measure of ANOVA	'p' Value
	1st Day		7th Day		30th Day			
	MEAN	SD	MEAN	SD	MEAN	SD		
Microwave cure	0.227	0.044	0.288	0.090	0.289	0.056	3.666	0.067(NS)
Flexible	0.260	0.023	0.283	0.073	0.277	0.035	0.502	0.533(NS)
Heat cure	0.229	0.046	0.216	0.066	0.265	0.025	2.235	0.159(NS)
Controls	0.377	0.127	0.392	0.152	0.523	0.131	5.757	0.047(NS)

Figure 1 : Comparative evaluation of % Mean Viable Mc Coy Cell among Microwave cure, Flexible & Heat cure denture base material at different time interval.

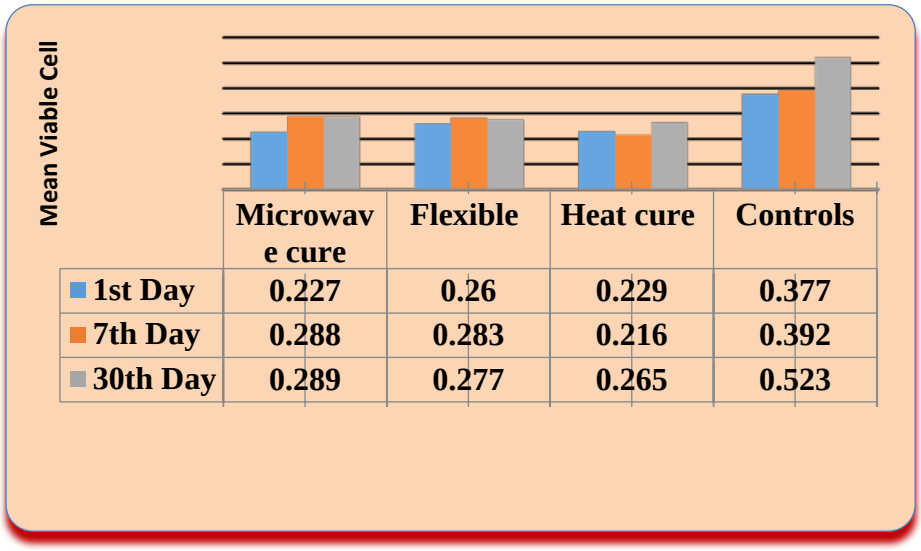
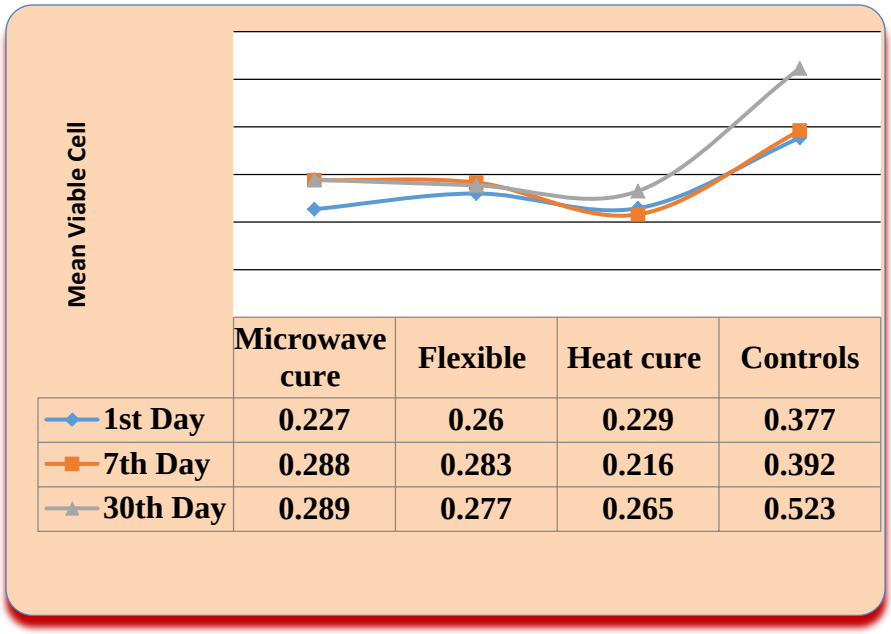


Figure 2: Comparative evaluation of % Mean Viable Mc Coy Cell from 1st day to 30th day among Microwave cure, Flexible & Heat cure denture base material.



	Microwave cure	Flexible	Heat cure	Controls
1st Day	0.227	0.26	0.229	0.377
7th Day	0.288	0.283	0.216	0.392
30th Day	0.289	0.277	0.265	0.523