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ORIGINAL RESEARCH

An *in vitro* Investigation into the Cytotoxicity of Methyl Methacrylate Monomer

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ABSTRACT

Aim of this study was to assess the cytotoxicity of monomer. An *in vitro* study was designed to study the growth inhibitory effect of monomer (Stellon, Denture Material Improved, Type I, Class I, Dental Products of India Limited) on cells seeded in petri dishes and maintained in an incubator with 5% carbon dioxide at 37°C. The growth of V79 cells (fibroblast cells) maintained in a culture medium to which monomer was added was studied for a period of 5 days. Results of this study pointed out that even at a concentration of 1 µl of monomer, the cell growth was significantly inhibited, when compared to the control group. The number of viable cells decreased dramatically whereas dead cells increased in the culture groups treated with the monomer. The cytotoxic effect was dose dependent. As the concentration increased from 1 to 10 µl there was a marked inhibition of cell growth and a corresponding increase in dead cell count. Results of this study proved beyond doubt that monomer is indeed cytotoxic even in very low concentrations. Thus, it becomes imperative to adopt every possible means to minimize residual monomer content in heat cured resins. Also precautions to minimize tissue contact should be taken while handling monomer by the dentist and dental personnel in the laboratory.

Keywords: Acrylic resin, Methyl methacrylate monomer, Cytotoxicity, *In vitro* study.

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INTRODUCTION

Polymethyl methacrylate is one of the most widely used resins in dentistry[1]. Ninety-eight percent of all denture bases are constructed from methyl methacrylate polymers or copolymers.[2] The tissue compatibility and allergic sensitization of the skin to the components of denture plastics has been a source of considerable contention. The usual component singled out as an irritant is residual monomer which is leachable from these resins into water and saliva.[3] In more recent years, the emphasis has turned to the possible toxic or adverse effects that the material might present to the host. Fisher[4] stated that monomeric methyl methacrylate is a sensitizing agent and can cause an allergic contact type eczematous reaction on the skin and oral mucosa. The mucosa may be further compromised by xerostomia producing a dry, fragile epithelium. Also there is a risk in patients with previous allergic diseases and burning mouth syndrome. In these cases a high incidence of sensitivity to denture allergens has been observed usually to methyl methacrylate.[3] Osteomyelitis, mobility, gingival recession and epithelial downgrowth around the resin implant, increased pocket depth and bone loss have accompanied use of this material and have caused a 50 to 75% failure rate.[5] Also bone resorption under chin implants, bone reaction and possible methyl methacrylate emboli have been reported following implantation of methyl methacrylate.[6] Acute toxic non dermatological reactions were experienced by some prosthodontists following activities such as working with methyl methacrylate or other synthetic resin materials. The reactions were expressed as eyes, respiratory or general symptoms in connection with exposure to volatile liquids and grinding dust. Such reactions were of transient nature while permanent adverse reactions were reported in dental technicians.[7] Dentist and technician should therefore refrain from handling the acrylic resin dough with bare hands. The high concentration of monomer in the dough may produce local irritation and even serious sensitization of the fingers. The monomers used in resins are volatile and nasal olfactory epithelium can be affected. Mucosal degeneration and necrosis have been reported. Repeated inhalation can result in lung irritation. This study emphasized the need for well ventilated working places.

A study by Charnley[8] investigated and identified the cytotoxicity and genotoxicity of methacrylates. Methyl methacrylate exerts its toxic effects by interacting with the

cell membrane and formation of micronuclei indicative of chromosomal damage was noted. In view of the complications and controversies of this widely used material it becomes imperative to investigate thoroughly the biocompatibility of methyl methacrylate liquid monomer. Testing of dental materials by using cell culture methods are suitable as an alternative to costly, controversial animal experiments which may have many uncontrollable variables. In view of the above complications and controversies of this widely used material, it becomes imperative to investigate thoroughly into the biocompatibility of methyl methacrylate. An *in vitro* study was designed to study the growth inhibitory effect of monomer (Stellon, Denture Material Improved, type I, class I, Dental Products of India Limited) on cells seeded in petri dishes and maintained in an incubator with 5% carbon dioxide at 37°C.

MATERIALS AND METHODS

To investigate the cytotoxicity of monomer an *in vitro* study was conducted. The growth of V79 cells maintained in a culture medium to which monomer was added was studied for a period of 5 days. The growth of cells which took place in the presence of monomer was compared to the growth of cells maintained in the culture medium to which no monomer was added.

The cell culture preparations were done inside a working bench provided with laminar flow of sterile air. This working bench Klenzaids working bench (Fig. 1) was placed in a room provided with a facility for ultra violet radiation. V79 cells required for this study was obtained from the National Faculty for Animal Tissue and Cell Culture, Pune. These cells were maintained in a culture flask of culture area 80 cm^2. The culture flask supplemented with Eagles Minimum Essential Medium (Sigma Co. Ltd. USA, Cat No. M 0268) and 10% Fetal calf serum (Sigma product Cat No. F2442) was incubated at 37°C in a humidified atmosphere of 5% carbon dioxide in air to ensure normal growth of cells. Other materials used were 30% polyethylene glycol as solvent for monomer, 0.25% trypsin [Sigma, Cat No. T 4049] for trypsinising the cells and trypan blue dye for staining dead cells (Fig. 2). Methyl methacrylate monomer [Stellon Denture Material Improved, Type I, Class I, Dental Products of India Limited] for heat cured resins was used for this study. As this study was designed to screen very small doses of monomer for cytotoxicity equivalent to the residual monomer that leached out from cured resins, the concentrations of monomer screened for cytotoxicity were 1, 5 and 10 µl of monomer per ml of culture medium respectively. Four groups of petri dishes were prepared.

- *Group I:* No monomer was added (Control group).
- *Group II:* 1µl of monomer was added to each 1ml of culture medium.
- *Group III:* 5µl monomer was added to each 1ml of culture medium.
- *Group IV:* 10µl of monomer was added to each 1ml of culture medium.

From each group V79 cells were sub cultured into 12 smaller petri dishes of 9 cm^2. All these petri dishes were incubated in Steri-Cult 200 incubator at 37°C in a humidified environment of 5% carbon dioxide in air. On the first day the petri dishes were left undisturbed for cell multiplication to take place. On day two, three petri dishes were taken from each group and the mean viable cells, mean dead cells and total cell count were calculated. The same procedure was carried out on day two, three and four. Leitz, Labovert, Binocular Microscope was used. The mean viable and dead cell count of Groups I, II, III and IV were compared with the control group (Figs 3 and 4). Growth curves were plotted to compare the effect of different concentrations of monomer on cell growth in groups II, III and IV with the cell growth

Fig. 1: Klenzaids working bench with laminar flow of sterile air

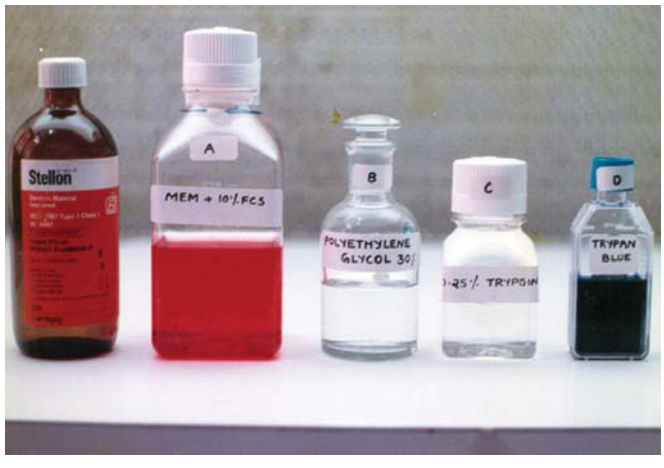

Fig. 2: Chemicals used for the study

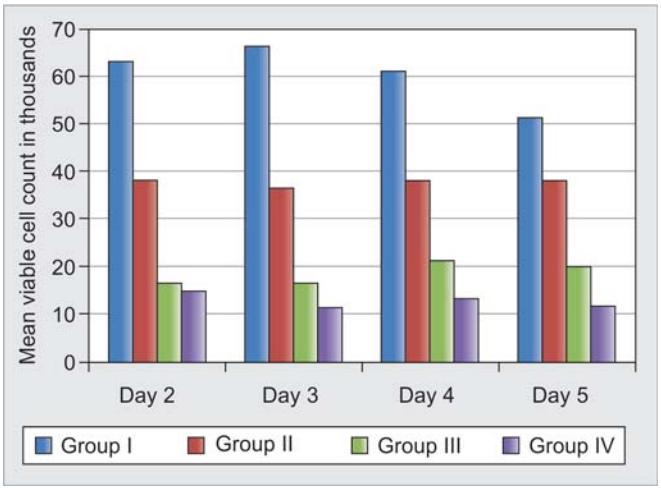

Fig. 3: Histogram showing comparison of mean viable cell count in groups I, II, III and IV on days 2, 3, 4 and 5

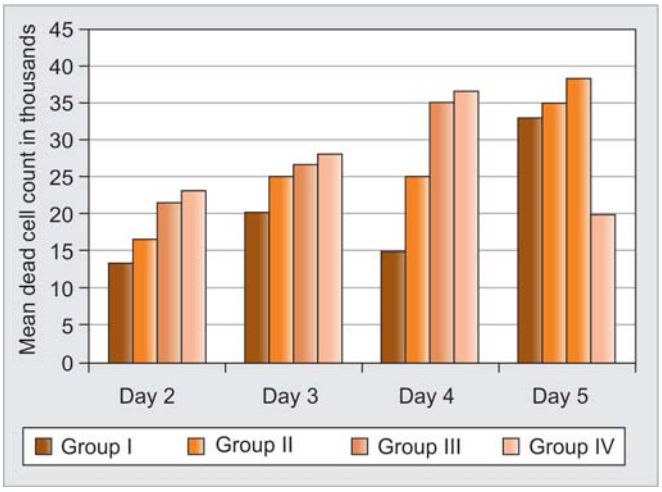

Fig. 4: Histogram showing comparison of mean dead cell count in groups I, II, III and IV on days 2, 3, 4 and 5

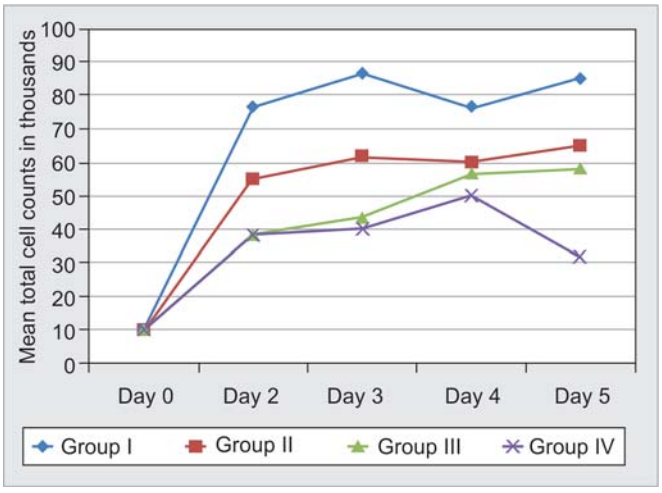

Fig. 5: Comparison of the growth curves of groups I, II, III and IV on days 0, 2, 3, 4 and 5

in the control group (Fig. 5). Results were statistically evaluated using Kruskal Wallis one-way ANOVA test (Table 1).

Table 1: The values of significance for viable cell count, dead cell count and total cell count on days 2, 3, 4 and 5

Day	*Viable cell count*	*Dead cell count*	*Total cell count*
2	0.0009	0.0100	0.0076
3	0.0000	0.1504	0.0000
4	0.0011	0.0004	0.0536
5	0.0030	0.0658	0.0060

Observation and Results

This study was designed to study the cytotoxicity, if any of 3 selected concentrations of monomer.

Figure 3 is a histogram showing the comparison of mean viable cell counts between the four groups on each day. It was seen that on day two, group I showed the maximum count and group IV showed the minimum count. On all the other days the same pattern was observed, i.e. maximum count being in group IV, with groups II and III in between.

Figure 4 is a histogram showing comparison of the mean dead cell counts in the four groups on each day. On day two the mean count was minimum in group I and maximum in group IV. Mean count in group II was higher than in group I and that in group III was higher than groups I and II.

Figure 5 is a line graph showing comparison of the growth curves of the four groups. The mean total cell count in groups I, II, III and IV were plotted as a line graph for comparison of the growth curves. It can be interpreted that cell growth in group II was less than that of group I, that of group III was less than groups I and II, that of group III was less than groups I and II and that of group IV was less than groups I, II and III.

DISCUSSION

In recent years there has been a lot of controversy regarding the complications and untoward side effects from the use of acrylic resins. Emphasis is now placed on the possible toxic effects this material presents to the host.

In the present study three concentrations of monomer were screened for cytotoxicity and it was seen that there was a significant dose dependent inhibition of cell growth. To evaluate the cytotoxic potential of methyl methacrylate the number of viable cells in group I [control group] was considered to be the optimum for that particular day, and was considered as 100% growth. As the concentration of monomer increased from 1 to 10 µl/ml the number of viable cells showed a corresponding decrease (Fig. 3).

On day 0, all the four groups were seeded with equal number of cells, and were left undisturbed in the incubator for one day for cell multiplication to take place. When the cells were counted on day two it was seen that the total cell count was maximum in group I and showed a minimum

value in groups III and IV. The same pattern was observed on days three, four and five. The growth curve of group I was thus at a higher level than the other groups on all days and that of group IV was at the lowest level with groups II and III being in between (Fig. 5). This proved that the effect of monomer on inhibition of cell growth was dose dependent, i.e. as the concentration of monomer increased from 1 to 10 µl/ml there was a marked inhibition of cell growth and a corresponding increase in dead cell count. The results of this study agree with that of Tsuchiya et al[9] and that of Pamush and Petty.[10]

Though *in vitro* cell culture methods can be used to assess biocompatibility of denture materials it would seem that the part played by natural defense mechanisms is being overlooked.[11] Intraorally, various protective mechanisms play a major role in combating harmful stimuli. That monomer is cytotoxic has been proved beyond doubt by this *in vitro* cell culture experiment. However, further research can be directed to evaluate the tissue response to acrylic and its reaction products *in vivo*, in order to consolidate the results of this present study.

CONCLUSION

With the implication of methyl methacrylate monomer as the culprit behind the various adverse reactions to acrylic resins the current study was undertaken to evaluate the cytotoxic potential of the same. The growth inhibitory effect of 3 concentrations of monomer which were introduced to four groups of petri dishes seeded with V79 cells were compared with a control group. Results of the study pointed out that even at a concentration of 1 µl of monomer, the cell growth was significantly inhibited (Table 1). The number of viable cells decreased dramatically whereas dead cells increased in the culture groups treated with the monomer. The cytotoxic effect was dose dependent. As the concentration increased from 1 to 10 µl there was a marked inhibition of cell growth and a corresponding increase in dead cell count (Fig. 5). Further studies could be directed to evaluate the *in vivo* response of tissue to acrylic and its reaction products and if possible to device a fool proof method to eliminate the prime culprit the residual monomer, from acrylic appliances and prosthesis.

In view of the complications and side effects associated with its use every possible means should be adopted to minimize tissue contact and subsequent irritation with monomer. It is therefore the obligation of the dentist to ensure that monomer content in appliances should be kept to the minimum possible. Precautions in handling monomer has to be emphasized and its wide spread use in compromised situations of tissue health is to be curtailed. In the laboratory, strict precautions should be observed while handling monomer. Kneading of the dough with unprotected fingers should be avoided.[12] Monomer bottle should be kept tightly closed to minimize evaporation and subsequent irritation with monomer. Laboratory should be well ventilated. Those who develop contact dermatitis should get a patch test done immediately, also use p/l ratio as suggested by the manufacturer.[13,14] Adhere to manufacturer's instructions regarding curing time. Use heat cured resins instead of self cured ones. Further research to single out the irritant and a search for an alternative compound to replace the irritant material is of utmost importance.

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