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Cytotoxicity of Resin-Based Cleansers: An In Vitro Study

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Abstract

Background: Cell culture has been used to study the cytotoxicity of denture base resins. Indeed, denture cleansers and their effects on the resin cytotoxicity need deep concern. The aim of the present *in vitro* study was to investigate the cytotoxic effect of a heat cured denture base resin treated with two denture cleansers on mouse L-929 fibroblasts.

Methods: Under aseptic conditions, sample disks of a heat treated resin were prepared, following manufacturers' instruction and divided into 12 groups of ten as follows: I) soaking in 1% sodium hypochlorite, II) soaking in alkaline peroxide solution, and III) soaking in water bath for 1, 24, 72 hours and a week. Suspensions of the fibroblasts with acrylic disks were put in 24-well culture plates, and the culture media containing RPMI-1640 environment plus antibiotics and 10% fetal bovine serum were added. After incubation of the plates at considered time intervals, cytotoxicity of the resin was carried out by MTT assay.

Results: A significant difference was noticed for solutions in relation to the biocompatibility of the acrylic resin samples at determined time intervals. The cleansers' soaking samples showed higher cytotoxicity in comparison to those immersed in water at each time interval. The difference between cytotoxic effects of the samples immersed in water or cleansers were significant after 72 hours and one week.

Conclusion: Overnight immersion in alkaline peroxide and 1% hypochlorite solutions increased the cytotoxicity of the heat cured acrylic resin, but water storage improved the biocompatibility of the material tested.

Keywords: Cytotoxicity; Denture base resin; Cleansers; Fibroblasts (L-929)

Introduction

Acrylic resin is still the most frequently used material in denture base fabrication. Despite its satisfactory aesthetic properties, the ease of processing and accurate fit, residual methyl methacrylate (MMA), resulting from incomplete conversion of monomers into polymer, has the potential to cause irritation, inflammation, and an allergic response of oral mucosa.[1-4] Clinical signs and symptoms most frequently reported include erythema, erosion and a burning sensation of the oral mucosa and tongue.[4] The pathologic effects of substances such as MMA, formaldehyde, benzoic acid, methacrylic acid, dibutyl phthalate, phenyl benzoate, phenyl salicylate and dicyclonhexyl phthalate existing in the chemical composition of acrylic resin depend on the way of their entrance to the oral cavity.[1,5]

Residual monomer concentration varies with the methods and the conditions of polymerization.[2,6,7] The variations in the 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 biological and physical properties of the acrylic resins.[7,8]

To ensure the safety of dental materials, *in vitro* cytotoxicity tests have been developed. Testing of dental materials by cell culture methods is relatively

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simple to perform, reproducible and cost effective, and can be carefully controlled.[3] Different parameters such as cell growth inhibition, cytolysis, cytoplasmic markers and changes in metabolic activity are used to evaluate cytotoxicity of dental materials.[9-12] Cytotoxic test systems vary in the way of cells employments. Most cells are transformed or are of tumor origin, like the model for cell response. Normal diploid cells differ from established or transformed cells in many ways and may respond differently to cytotoxicity challenge.[13-15] However, the use of both permanent and primary cells is recommended for a better screening of the cytotoxic effects of denture base resins.[16] Cell culture methods are more suitable than costly controversial animal experiments, which may have uncontrollable variables.[17]

Although denture storage in water could reduce the toxic agents which leach out of denture base[3,8], usage of alkaline peroxide or sodium 1% hypochlorite solutions, as two common cleansers, may change the cytotoxicity of acylic resin material tested.

Today, few data exist about the effect of different denture cleansers on the resultant biocompatibitity of acrylic resins. Therefore, the objective of this study was to determine the cytotoxic effects of denture base resin, following treatment with alkaline peroxide and 1% sodium hypochlorite as denture cleansers.
MTT assay as a common biologic test for evaluating cytotoxicity was used in this study.[3]

Materials and Methods

The material tested was a heat cured acrylic resin (Meliodent; Heraeus Kulzer, Berkshire, Germany). One hundred and twenty disk shaped specimens of acrylic resin material (2 mm thick; 5.5 mm in diameter) were fabricated according to the manufacturers' specifications. The polymerization cycle was 7 hours at 70°c, followed by 1 hour at 100°c. Mimicking heat cured denture base fabrication, finishing and polishing were carried out. The disks were exposed to ultraviolet light for 20 minutes to kill the microorganisms which had contaminated the disk during fabrication.

Sixty acrylic disks (except for 1 hour) were immersed in fresh 1% sodium hypochlorite (Golrang-Pakshoo Co., Iran) or alkalin peroxide (Thramed Dusseldrof, Spain) solutions for 8 hours daily, and for the rest of the day, they were stored in distilled water. Forty disks were stored only in distilled water at 1, 24 and 72 hours and 1 week time intervals.

Each disk was placed into each well of a sterile 24-well culture plate containing 0.1ml cell (3×10^5 cells) and 0.9 ml RPMI (Gibco BRL, Scottland) with 10% FBS (Gibco) and then placed at 37°c in Co_2 incubator (5% Co_2). After 1, 24 and 72 hours and one week interval, they were assessed for cytotoxic effects. The medium without disks was also considered as negative control. Another medium in which PRMI-1640 was replaced with distilled water had the role of positive control. Distilled water can cause the lysis of all cultured cells.

Mouse fibroblast cells L-929 (Pasteur Institute, Iran) were cultured in RPMI-164, supplemented with streptomycin and penicillin and 10% fetal bovine serum. They were maintained at 37°c in Co_2 incubator (5% Co_2).

The MTT cytotoxicity assay was first described by Mosmann[18] and the results of this test are directly related to the number of viable culture cells.[19] The test measures the action of the intracellular enzyme on tetrazolium salts which is converted into soluble form of formazan. The cells were subcultured and placed into 24-well culture plates (3×10^5 cell/ 100 microlitre of culture medium). The MTT test was done separately after one hour, 24 and 72 hours and one week of incubation. One hundred microliters of MTT solution (Sigma Chemical, USA) was added to the samples after the proper time and then incubated for 4 hours at 37°c. When the color changed, 200 microliters of HCL-Isopropanol was added to each well and mixed to dissolve the dark blue formazan crystals. Then, the Optical Density (OD) of the plates was tested with ELISA procedure (Anthous 2020, Australia). The absorbance represents the total number of viable cells.

Statistical analysis of the data was performed, using one-way analysis of variance (ANOVA). A p value <0.05 was considered as statistically significant. Repeated measurement was used to rule out the possibility of re-measuring.

Results

The eluates of acrylic resin disks soaked in alkaline peroxide, 1% sodium hypochlorite solutions and water revealed different biocompatibilities at each time duration (Figure 1). The mean and standard deviation (SD) of optical density for control and experimental groups are shown in Table 1. Although after 1 and 24 hours, the samples which were immersed in alkaline

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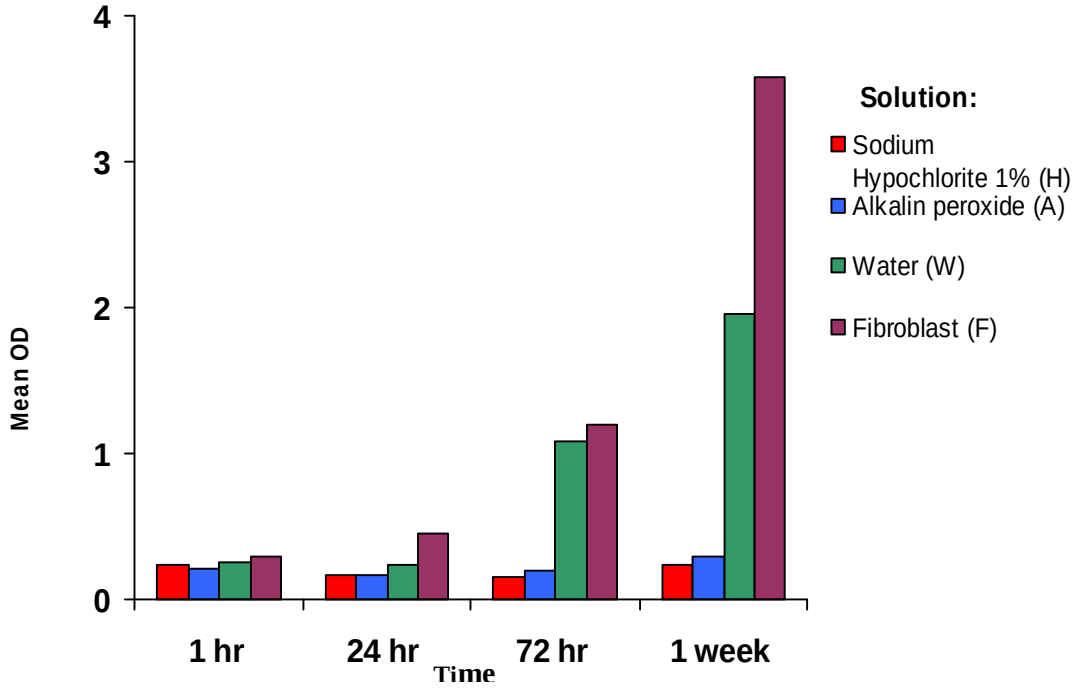

Fig. 1: Mean Optical Density (OD) at each time interval

Table 1: Mean and standard deviation of Optical Density in three solutions and fibroblasts

	After one hour		After 24 hours		After 72 hours		After one week	
solution	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
hypochlorite sodium 1%	0.244	0.050	0.173	0.036	0.158	0.056	0.235	0.096
alkaline peroxide	0.215	0.018	0.170	0.040	0.192	0.013	0.289	0.117
water	0.256	0.015	0.224	0.044	1.087	0.141	1.959	0.523
fibroblast	0.292	0.024	0.454	0.092	1.203	0.165	3.582	0.951

peroxide (A), 1% sodium hypochlorite (H) and water (W) revealed cytotoxic effects, the resultant cytotoxicity by both cleansers was higher than that caused by water. At both time intervals, alkaline peroxide was in priority, followed by 1% sodium hypochlorite. After 72 hours, the samples stored in both cleansers showed almost the same effect on fibroblasts; however, water had a lower level of cytotoxic effect in comparison to both cleansers; the effect was statistically significant ($p<0.001$).

After one week, the specimens immersed in both cleansers showed approximately the same level of biocompatibility, which was significantly lower than that of the specimens stored in water ($p<0.001$).

After 72 hours and 1 week, the resultant cytotoxic effect of acrylic resin samples was significantly lower than that after 1 hour and 24 hours soaking in water ($p<0.05$).

Discussion

On the basis of the previous studies, it can be concluded that the cytotoxic effect of denture base resin may be related to storage time, powder to liquid ratio, polymerization method and cycle.[17,20,21] To the best of our knowledge, no study has been carried out on cytotoxic effect of acrylic resins after immersion in denture cleansers. Our data revealed that cytotoxicity of denture base resin could be affected by the type of cleansers and duration of storage.

The cytotoxic effect of autopolymerized resins is

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greater than that of heat-activated ones. This effect is attributed to leaching of more cytotoxic components such as MMA, formaldehyde, metacrylic acid and benzoic acid.[20-22] Therefore, to decrease the bias, only one common type of heat-cured acrylic resin (Meliodent) was used for all groups in our study. Since previous studies showed that long curing cycles followed by boiling at 100°c provides the maximum conversion of residual monomer[3,7], we used a similar polymerization cycle for fabrication of samples. Moreover, the denture cleansers used in this study were alkaloid type and their pH was more than 7.0. It was demonstrated that leaching of cytotoxic component can occur at a range of pH from 4.0 to 6.8.[23]

Based on Baker et al's study, the cytotoxic effect may occur for several days after polymerization. But it can be minimized if the denture is immersed in water for 24 hours.[22] According to the authors, it is recommended that dentists soak the prostheses in water for at least 72 hours before inserting them in the patient's mouth, especially in patients with infected, inflamed or lacerated mucosa as a result of concurrent medications or nutritional problems.

Among the cleansers, the samples immersed in water showed the least cytotoxicity. It is possible that oxygen has been expelled during the immersion of resin in water and has probably reduced the oxygen effects and resulted in a higher degree of polymerization.[3] But alkaline peroxide when mixed with water and sodium perborate decomposes and releases peroxide which in turn decomposes and releases O_2. The cleansing effect of this product depends on the O_2 release. On the other hand, 1% sodium hypochlorite affects the organic matrix by its OH^- (hydroxyl) group and resists against stain.[24] Perhaps, the O_2 radical disturbs the methyl methacrlyate polymerization and so acrylic resin samples immersed in alkaline peroxide were more cytotoxic than those immersed in sodium hypochlorite solution.

The data of this study are relevant but cannot be directly transferred to clinical scenarios. However, the *in vitro* methods could play an important role in analyzing the cytotoxicity of denture base resins.[3,17]

Although this study showed that cytotoxicity of the resin might vary by alkaloid cleansers, future research is recommended to identify the cytotoxic effect of other cleansers and the individual components of eluates that are responsible for the cytotoxic effect. Moreover, *in vivo* and human clinical studies should be done to clarify the biocompatibility of acrylic resins after soaking in denture cleansers.

Within the limitation of this in vitro study, the following conclusion may be drawn. 1. Both 1% alkaline peroxide and hypochlorite solutions increase the cytotoxicity of the heat cured resin in comparison to those immersed in water and 2. The longer a denture is soaked in water, the less cytotoxic effect of acylic resin will be.

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References

1 Lassila LV, Vallittu PK. Denture base polymer Alldent Sinomer: mechanical properties, water sorption and release of residual compounds. *J Oral Rehabil* 2001;**28**:607-13. [11422690] [doi:10.1046/j.1365-2842.2001.00733.x]

2 Sadamori S, Kotani H, Hamada T. The usage period of dentures and their residual monomer contents. *J Prosthet Dent* 1992;**68**:374-6. [1501194] [doi:10.1016/0022-3913(92)90349-F]

3 Jorge JH, Giampaolo ET, Vergani CE, Machado AL, Pavarina AC, Carlos IZ. Cytotoxicity of denture base resins: effect of water bath and microwave postpolymerization heat treatments. *Int J Prosthodont* 2004;**17**:340-4. [15237883]

4 Weaver RE, Goebel WM. Reactions to acrylic resin dental prostheses. *J Prosthet Dent* 1980;**43**:138-42. [6985962] [doi:10.1016/0022-3913(80)90176-6]

5 Craig RG, Powers JM. Restorative dental materials. 12th ed. St. Louis: Mosby; 2006; pp. 125-162.

6 Tsuchiya H, Hoshino Y, Kato H, Takagi N. Flow injection analysis of formaldehyde leached from denture-base acrylic resins. *J Dent* 1993; **21**:240-3. [8354750] [doi:10.1016/0300-5712(93)90136-E]

7 Vallittu PK, Ruyter IE, Buykuilmaz S. Effect of polymerization temperature and time on the residual monomer content of denture base polymers. *Eur J Oral Sci* 1998; **106**:588-93. [9527360] [doi:10.1046/j.0909-8836.1998. eos106109.x]

8 Lefebvre CA, Knoernschild KL, Schuster GS. Cytotoxicity of eluates from light-polymerized denture base resins. *J Prosthet Dent* 1994; **72**:644-50. [7853263] [doi:10.1016/0022-3913(94)90298-4]

9 Costa CAS. Testes de citotoxcidade em cultura de celulas. In: Estrela Ced. Metodologia cientific: ensino e pesquisa em odontologica. Sao Paulo: Artes Medicas LTDA, 2001; pp. 145-160.

10 Hensten-Pettersen A. Comparison of the methods available for assessing cytotoxicity. *Int Endod J* 1988; **21**:89-99. [3077393]

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11 Wennberg A. Cell culture in the biological evaluation of dental materials: a review. *ATLA* 1986;**13**:4194-202.

12 Barile FA. Inivtro cytotoxicity mechanism and methods. 1st ed. Beca Ratoni: CRC PRESS, 1994; p. 125.

13 Holley RW. Control of growth of mammalian cells in cell culture. *Nature* 1975;**258**:487-90. [1105198] [doi:10.1038/258487a0]

14 Lechner JF, Kaighn ME. Reduction of the calcium requirement of normal human epithelial cells by EGF. *Exp Cell Res* 1979;**121**:432-5. [312735] [doi:10.1016/0014-4827(79)90027-2]

15 Feigal RJ, Yesilsoy C, Messer HH, Nelson J. Differential sensitivity of normal human pulp and transformed mouse fibroblasts to cytotoxic challenge. *Arch Oral Biol* 1985; **30**:609-13. [3863559] [doi:10.1016/0003-9969(85)90081-0]

16 Huang FM, Tai KW, Hu CC, Chang YC. Cytotoxic effects of denture base materials on a permanent human oral epithelial cell line and on primary human oral fibroblasts in vitro. *Int J Prosthodont* 2001;**14**:439-43. [12066639]

17 Jorge JH, Giampaolo ET, Machado AL, Vergani CE. Cytotoxicity of denture base acrylic resins: a literature review. *J Prosthet Dent* 2003; **90**:190-3. [12886213] [doi:10.1016/S0022-3913(03)00349-4]

18 Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983;**65**:55-63. [6606682] [doi:10.1016/0022-1759(83)90303-4]

19 Ciapetti G, Cenni E, Pratelli L, Pizzoferrato A. In vitro evaluation of cell/biomaterial interaction by MTT assay. *Biomaterials* 1993;**14**:359-64. [8507779] [doi:10.1016/0142-9612(93)90055-7]

20 Sheridan PJ, Koka S, Ewoldsen NO, Lefebvre CA, Lavin MT. Cytotoxicity of denture base resins. *Int J Prosthodont* 1997;**10**:73-7. [9484073]

21 Kedjarune U, Charoenworaluk N, Koontongkaew S. Release of methyl methacrylate from heat-cured and autopolymerized resins: cytotoxicity testing related to residual monomer. *Aust Dent J* 1999;**44**:25-30. [10217017] [doi:10.1111/j.1834-7819.1999.tb00532.x]

22 Baker S, Brooks SC, Walker DM. The release of residual monomeric methyl methacrylate from acrylic appliances in the human mouth: an assay for monomer in saliva. *J Dent Res* 1988;**67**:1295-9. [3170884]

23 Lefebvre CA, Schuster GS, Marr JC, Knoernschild KL. The effect of pH on the cytotoxicity of eluates from denture base resins. *Int J Prosthodont* 1995;**8**:122-8. [7575962]

24 Zarb GA, Bolender CL. Prosthodontic treatment for edentulous patient. 12th ed., St. Louis: Mosby, 2004, p: 204.

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