International Journal of Research in Health and Allied Sciences
Journal home page: www.ijrhas.com
ISSN: 2455-7803
Official Publication of "Society for Scientific Research and Studies" [Regd.]

# Original Research

## Assessment of cytotoxicity level of various elastomers using indirect method

Pratik P. Bumb[1], Paaras Kotwal[2], Saniya J Kasat[3], Kurakalva Soundarya[4], Shilpa Sharma[5], PSHL Parvathi[6]

[1]PG 2nd year, Department of Prosthodontics, Teerthanker Mahaveer Dental College and Research Centre, Moradabad, Uttar Pradesh;
[2]PG 2nd year, Department of prosthodontics; NIMS Dental College and hospital NIMS university, Jaipur, Rajasthan;
[3]Pg 3rd year, Department of Prosthodontics; Bharati Vidyapeeth Dental College and Hospital, Pune, Maharashtra;
[4]PG 3rd year, Department of Prosthodontics, Government dental college, Kadapa, Andhra Pradesh;
[5]PG 3rd year; Department of Prosthodontics; NIMS Dental College and Hospital, NIMS University, Jaipur, Rajasthan;
[6]PG 3rd year, Department of Prosthodontics, Lenora Institute of Dental Sciences, Rajanagar, Rajahmundry, Andhra Pradesh

**ABSTRACT:**
**Background:** Final impression materials used in fixed, removable, and implant prosthodontics are elastomers. The present study was conducted to assess cytotoxicity level of various elastomers using indirect method. **Materials & Methods:** Poly vinyl siloxane (PVS), Poly vinyl ether silicone (PVES) and polyether (PE) impression material (Impregum) were classified as group I, II and III respectively. A total of 15 specimens were prepared. Dulbecco's modified Eagle's medium was used for growing mouse cell line NIH/3T3. Cytotoxicity level of all elastomers were measured with the test 3- (4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide assay. **Results:** The mean cell viablility was 130, 78 and 134 at 30 minutes, 102, 62 and 112 at 1 hour and 82, 24 and 110 at 24 hours on day 1, 78, 30 and 108 at 30 minutes, 82, 24 and 106 at 1 hour and 80, 21 and 102 at 24 hours on day 3, 20, 20 and 78 at 30 minutes, 16, 18 and 80 at 1 hour and 12, 14 and 64 at 24 hours on day 7 in group I, II and III respectively ($P< 0.05$). The mean survival rate of cells was 104.2, 126.2 and 120.4 in group I, II and III respectively on day 1, 68.2, 24.2 and 94.6 on day 3 and 18.2, 26.2 and 62.4 on day 7 respectively. The difference was significant ($P< 0.05$). **Conclusion:** Poly vinyl siloxane (PVS) exhibited highest cell viability as compared to other elastomeric impression material.
**Key words:** Cell viability, Elastomeric impression, Poly vinyl siloxane.

Received: 12 December, 2020 Accepted: 16 January, 2021

**Corresponding author:** Dr. Pratik P. Bumb, PG 2nd year, Department of Prosthodontics, Teerthanker Mahaveer Dental College and Research Centre, Moradabad, Uttar Pradesh, India

**This article may be cited as:** Bumb PP, Kotwal P, Kasat SJ, Soundarya K, Sharma S, Parvathi PSHL. Assessment of cytotoxicity level of various elastomers using indirect method. Int J Res Health Allied Sci 2021; 7(2):33-36.

## INTRODUCTION

Final impression materials used in fixed, removable, and implant prosthodontics are elastomers. The newly introduced poly vinyl ether silicone (PVES) elastomer claims combined advantage of dimensional accuracy of poly vinyl siloxane (PVS) and hydrophilic nature of polyether (PE).[1] Multiple adverse reactions have been reported on PE than additional silicone, and it can range from mild irritation to delayed hypersensitivity reaction happening after 24 hours to 1–3 days.[2] The clinical manifestation includes severe pain, dry mouth, burning mouth, swelling of lips, nonspecific cheilitis, dermatitis, and dysphagia. The elastomers can tear and can be trapped in the gingival sulcus under implants during impression making and cause adverse and[7] toxic reactions when remained in contact for longer periods of time.[3]

Several studies have been carried out concerning the cytotoxicity of VPS, whereas results have indicated a high degree of toxicity toward cell cultures compared

to the negative control.[4] Evaluation of biocompatibility is essential when any medical device is to be used on a patient and cytotoxicity testing using the cell culture technique is the simplest and the easiest form of biocompatibility evaluation that can be used to screen a large number of dental materials.[5]
Vinyl polysiloxane possess highest accuracy since they are elastic in nature and show increase in dimensional stability.[6] The recently invented polyvinyl ether silicone (PVES) elastomer has unique features as seen with polyvinyl siloxane (PVS) and PE, such as dimensional stability and hydrophilic behaviour. The present study was conducted to assess cytotoxicity level of various elastomers using indirect method.

## MATERIALS & METHODS

The present study comprised of 15 specimens prepared from 3 elastomers materials such as poly vinyl siloxane (PVS), Poly vinyl ether silicone (PVES) and polyether (PE) impression material.
These materials were divided into 3 groups. Group I comprised of poly vinyl siloxane (PVS), group II comprised of Poly vinyl ether silicone (PVES) and group III had polyether (PE) impression material. All these specimens were inserted in sterilized brass mold of dimension 3 cm×2.4 cm. Indirect testing method of cytotoxic testing of elastomeric impression materials was used. Mouse cell line NIH/3T3, Dulbecco's modified Eagle's medium was selected for growth assessment. 45 plates containing NIH/3T3 cells with different materials were obtained. These plates were incubated at the temperature of 37°C. Cell viability or cytotoxicity level was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide assay. Cytotoxicity level was calculated at 30 minutes, 1 hour and 24th hour on day 1, day 3 and day 7. Results were assessed statistically. P value less than 0.05 was considered significant.

## RESULTS

**Table I Assessment of cell viability at different interval**

| Duration | Time | Group I | Group II | Group III | P value |
|---|---|---|---|---|---|
| Day 1 | 30 minutes | 130 | 78 | 134 | 0.02 |
| | 1 hour | 102 | 62 | 112 | |
| | 24 hours | 82 | 24 | 110 | |
| Day 3 | 30 minutes | 78 | 30 | 108 | 0.05 |
| | 1 hour | 82 | 24 | 106 | |
| | 24 hours | 80 | 21 | 102 | |
| Day 7 | 30 minutes | 20 | 20 | 78 | 0.04 |
| | 1 hour | 16 | 18 | 80 | |
| | 24 hours | 12 | 14 | 64 | |

Table I, graph I shows that mean cell viablility was 130, 78 and 134 at 30 minutes, 102, 62 and 112 at 1 hour and 82, 24 and 110 at 24 hours on day 1, 78, 30 and 108 at 30 minutes, 82, 24 and 106 at 1 hour and 80, 21 and 102 at 24 hours on day 3, 20, 20 and 78 at 30 minutes, 16, 18 and 80 at 1 hour and 12, 14 and 64 at 24 hours on day 7 in group I, II and III respectively (P< 0.05).

**Graph I Assessment of cell viability at different interval**

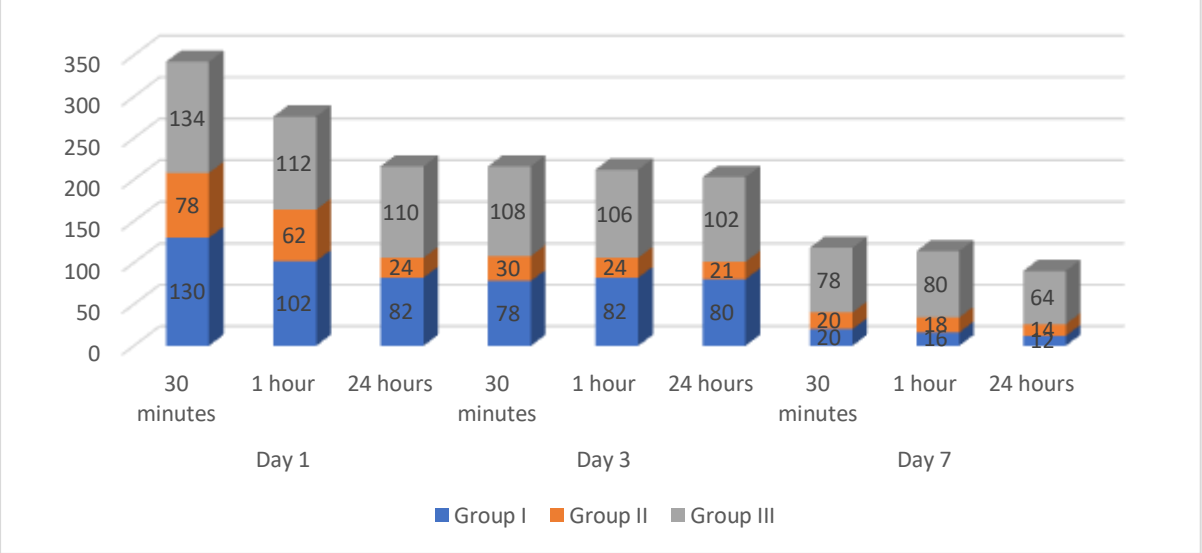
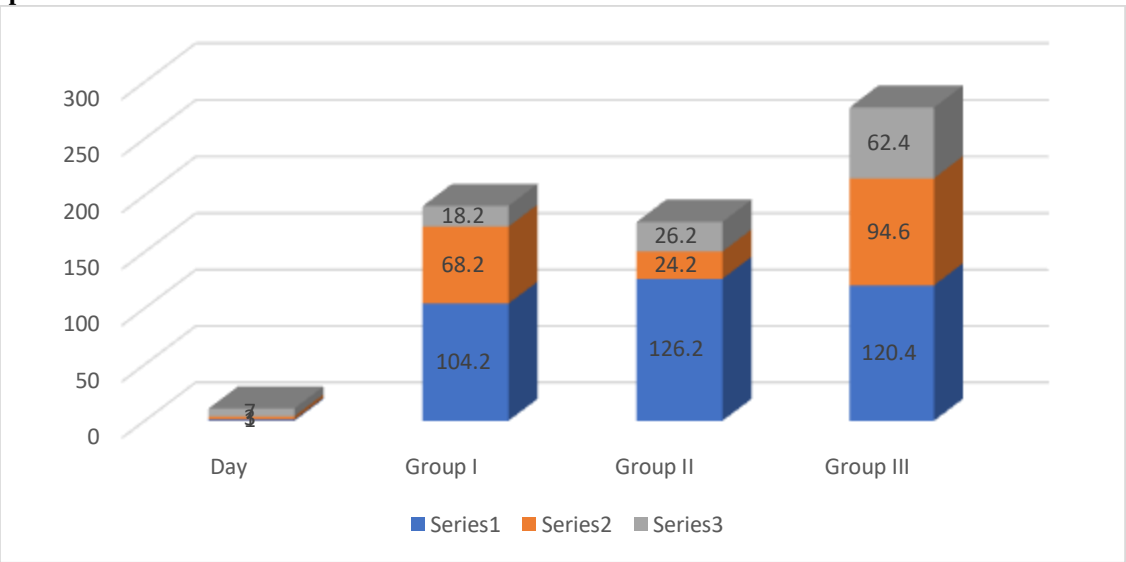


**Table II Assessment of survival rate**

| Day | Group I | Group II | Group III | P value |
|---|---|---|---|---|
| 1 | 104.2 | 126.2 | 120.4 | 0.05 |
| 3 | 68.2 | 24.2 | 94.6 | 0.02 |
| 7 | 18.2 | 26.2 | 62.4 | 0.01 |

Table II, graph II shows that mean survival rate of cells was 104.2, 126.2 and 120.4 in group I, II and III respectively on day 1, 68.2, 24.2 and 94.6 on day 3 and 18.2, 26.2 and 62.4 on day 7 respectively. The difference was significant (P< 0.05).

**Graph II Assessment of survival rate**




## DISCUSSION

Elastomeric dental impression materials are widely used material in prosthodontics for recording exact replica of dental tissues (soft and hard).[8] Additional silicone or vinyl polysiloxane (VPS), polysulfide, polyether (PE), and condensation silicones are among commonly used elastomeric impression materials.[9] They are used in recording the impression of removable and fixed implants.

The biocompatibility of elastomers may be evaluated by determining the cytotoxicity level. The utility of these tests for diagnosing the cytotoxicity of dental materials is well established. The potential cytotoxicity of elastomeric materials may be tested by direct and indirect tests.[10] In direct test, the cells are introduced into the material and in indirect test the cells are inserted to the eluted extracts of the impression materials. Test, such as dye exclusion methods, can be used for measuring the cell viability. However, it has its limitations. 3-(4,5-dimethylthiazol-2-yl)-2-5- diphenyltetrazolium bromide is a calorimetric assay used frequently in evaluating the cytotoxicity or cell viability.[11] The cell viability is assessed principally through the evaluation of mitochondrial function of the cells by calculating the succinate dehydrogenase which is a potent mitochondrial enzyme. This method is safe and easily employed. It has high reproducible capacity which helps in assessing the cytotoxicity and cell viability.[12]

The present study was conducted to assess cytotoxicity level of various elastomers using indirect method.

In present study, mean cell viablility was 130, 78 and 134 at 30 minutes, 102, 62 and 112 at 1 hour and 82, 24 and 110 at 24 hours on day 1, 78, 30 and 108 at 30 minutes, 82, 24 and 106 at 1 hour and 80, 21 and 102 at 24 hours on day 3, 20, 20 and 78 at 30 minutes, 16, 18 and 80 at 1 hour and 12, 14 and 64 at 24 hours on day 7 in group I, II and III respectively (P< 0.05). Boraldi et al[13] compared various elastomeric materials with Balb/c 3T3 and human gingival fibroblasts. Result showed clear decline of cellular viability of Balb/c 3T3 tests resulted from express light body. Polyether found to be most cytotoxic material. Primary cell line found to be less sensitive to the toxic effect as compared to permanent cell line.

We found that mean survival rate of cells was 104.2, 126.2 and 120.4 in group I, II and III respectively on day 1, 68.2, 24.2 and 94.6 on day 3 and 18.2, 26.2 and 62.4 on day 7 respectively. Priyaranjan et al[14] in their study elastomeric impression materials which were divided into three groups, group I, II, and III with PVES (EXA'lence light body), PVS (Flexceed light body), and PE impression material (Impregum), respectively. A total of 10 specimens were prepared. Dulbecco's modified Eagle's medium was used for growing mouse cell line NIH/3T3. Cytotoxicity level of all elastomers was measured with the test 3-(4,5-

dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide assay at regular intervals. Results: There was a decline in the survival rate with PVES as found on day 1, PVS and PE showed on 3rd and 7th day. Kruskal–Wallis test showed a significant difference in all groups at various days ($p < 0.05$). Authors found that PVES showed early cytotoxic signs as compared to PVS and PE. Cell viability for PVS was highest as compared to PVES and PE impression materials.

## CONCLUSION

Authors found that poly vinyl siloxane (PVS) exhibited highest cell viability as compared to other elastomeric impression material.

## REFERENCES


1. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65(1-2):55–63.
2. 13. Stone V, Johnston H, Schins RP. Development of in vitro systems for nanotoxicology: methodological considerations. Crit Rev Toxicol 2009;39(7):613–626.
3. Rajasimhan NV, Jayaraman S, Ali DJ, et al. Evaluation of cytotoxicity levels of poly vinyl ether silicone, polyether, and poly vinyl siloxane impression materials: an in vitro study. J Indian Prosthodont Soc 2019;19(4):332–337.
4. Jeng HW, Feigal RJ, Messer HH. Comparison of the cytotoxicity of formocresol, formaldehyde, cresol, and glutaraldehyde using human pulp fibroblast cultures. Pediatr Dent 1987;9(4):295–300.
5. Nassar U, Oko A, Adeeb S, et al. An in vitro study on the dimensional stability of a vinyl polyether silicone impression material over a prolonged storage period. J Prosthet Dent 2013;109(3):172–178.
6. Chen SY, Chen CC, Kuo HW. Cytotoxicity of dental impression materials. Bull Environ Contam Toxicol 2002;69(3):350–355.
7. Aslanturk OS, Askın Celik T, Karabey B, et al. Active phytochemical detecting, antioxidant, cytotoxic, apoptotic activities of ethyl acetate and methanol extracts of Gallium aparine L. Br J Pharma Res 2017;15(6):1–16.
8. Eames WB, Wallace SW, Suway NB, et al. Accuracy and dimensional stability of elastomeric impression materials. J Prosthet Dent 1979;42(2):159–162.
9. Ciesco JN, Malone WF, Sandrik JL, et al. Comparison of elastomeric impression materials used in fixed prosthodontics. J Prosthet Dent 1981;45(1):89–94.
10. Blankenau RJ, Kelsey WP, Cavel WT. A possible allergic response to polyether impression material: a case report. J Am Dent Assoc 1984;108(4):609–610.
11. Rafael CF, Liebermann A. Clinical characteristics of an allergic reaction to a polyether dental impression material. J Prosthet Dent 2017;117(4):470–472.
12. Sydiskis RJ, Gerhardt DE. Cytotoxicity of impression materials. J Prosthet Dent 1993;69(4):431–435.
13. Boraldi F, Coppi C, Bortolini S, et al. Cytotoxic evaluation of elastomeric dental impression materials on a permanent mouse cell line and on a primary human gingival fibroblast culture. Materials 2009;2(3):934–944.
14. Priyaranjan KK, Thota SK, Yaqoob A, Awinashe V, Sayed AJ. An In Vitro Assessment of Cytotoxicity of Polyvinyl Siloxane, Polyether, and Polyvinyl Ether Silicone on NIH/3T3 Cells. The Journal of Contemporary Dental Practice. 2020 Nov;21(11):1263.