European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 7, Issue 11, 2020

Determination of cytotoxicity level of various elastomers using indirect method- An invitro study

Ashna Godhwani[1], Akshar Bajaj[2], Krithi Ventaraman[3], Rohaan Billimoria[4]
Debapriya Biswas[5], Mishri Parikh[6]

[1] BDS, Nair Dental Hospital College, Mumbai, Maharashtra, India (2016)
[2] BDS, Sharad Pawar Dental College, Wardha, Maharashtra, India (2018); MDS Prosthodontics, Manipal College of Dental Sciences, Mangalore, India, (2021)
[3]BDS - M. S. Ramaiah Dental College & Hospital, Bangalore, India (2011); MDS Prosthodontics - M. S. Ramaiah Dental College & Hospital, Bangalore, India (2016)
[4]Manipal College of dental sciences, Mangalore, India (Oct 2020)
[5]BDS - Government Dental College & Hospital, Mumbai, India (2019)
[6]III yr BDS - AMC Dental College, Ahmedabad, India (2022)
Email: ashnagodhwani@gmail.com

Abstract: Background:Elastomers are routinely used as final impression materials. This study was an attempt to determine cytotoxicity level of various elastomers using indirect method.
Materials & Methods: In this studyPoly vinyl ether silicone (PVES) (EXA'lence light body), poly vinyl siloxane (PVS) (Flexceed light body and polyether (PE) impression material (Impregum) were classified Ito group I, II and III respectively. A total of 12 specimens were prepared. Dulbecco's modified Eagle's medium was used for growing mouse cell line NIH/3T3. Cytotoxicity level of allelastomers were measured with the test 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide assay.
Results: The mean ± SD cell viability survival of cells at day 1 in group I was 25.4 ± 8.23, 106.2 ± 31.5 in group II and 121.4 ± 16.2 in group III. On day 3, it was 23.7 ± 7.21, 68.2 ± 32.7 and 99.4 ± 14.2 in group I, II and III respectively. On day 7, it was 24.8 ± 6.45, 19.4 ± 31.5 and 73.6 ± 15.8 in group I, II and III respectively. The difference was significant (P< 0.05). There wasdecline in the survival rate with Poly vinyl ether silicone as found on day 1,poly vinyl siloxane and polyether showed on 3rd and 7th day.
Conclusion:Authors found that cell viability for poly vinyl siloxane(PVS) was highest as compared to other elastomeric impression material.

Key words: Cytotoxicity, elastomeric impression, Poly vinyl siloxane

1. Introduction

Elastomers are routinely used as final impression materials in prosthodontics. Essential requirement for the best impression material is superior flexibility, integrity, dimensional stability,elastic recovery of maximum intensity and sufficient mechanical properties to tackle stresses in oral cavity.[1] It should have high mechanical, tensile and tear strength. It should be non- tearable. Vinylpolysiloxanes (VPSs) are addition silicones having silane groups are newly introduced elastomers in market. Similarly other useful material that possesses

properties of both polyether (PE) and poly vinyl siloxane (PVS) is poly vinyl ether silicone (PVES) elastomers.[2]

PE has properties of dimensional stability and wettability.However, it is quite tedious to remove PE impression material from mouth and subsequently chances of die breakage are always exist. It is found that PE has various toxic effects inside the oral cavity. These reactions occur after 1 day of insertion that can lasts upto 2-3 days.[3] Usually patient manifests irritation and in some cases delayed hypersensitivity reaction occur which require immediate dental assistance. Pain, xerostomia, stomatopyrosis, cheilitis granulomatosis, dermatitis and dysphagia are characteristics findings. Lui et al[4] reported high tensile strength of 3M ESPE and Impregum, two different Vinylpolysiloxanes.

Reddy et al[5] and Chandar et al[6] reported various cytotoxicity reactions. Direct and indirect tests are available which can be employed either by inserting cells into materials or by using eluted extract of impression materials. This study was an attempt to determine cytotoxicity level of various elastomers using indirect method.

2. Methodology

This invitro study comprised of three elastomers materials such as Poly vinyl ether silicone (PVES), poly vinyl siloxane(PVS) and polyether(PE) impression material. Approval of the study was obtained from ethical committee. We divided following impression materials in various groups. Group I had Poly vinyl ether silicone (PVES), group II had poly vinyl siloxane(PVS) and group III had polyether(PE) impression material.

We prepared 12 specimens from all elastomeric materials. They were prepared as per manufacturer's instructions. All these specimens were inserted in sterilized brass mold (2 cm×1.8 cm). After polymerization, all were stored in a glass container.

Cytotoxic testing of elastomeric impression materials were carried out with indirect testing method. We used mouse cell line NIH/3T3, Dulbecco's modified Eagle's medium for growth assessment. Thirty six plates containing NIH/3T3 cells with Poly vinyl ether silicone, poly vinyl siloxaneand polyetherimpression material 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 was measured at 15 minutes, 30 minutes, 1 hour and 24th hour on day 1, day 3 and day 7.

Data obtained after doing all steps were tabulated andmentioned as mean and standard deviation. SPSS version (20.0) was used for analyzing results with ANOVA test andChi-square test. 0.05 value was designated as significance.

3. Results

Table IDistribution of elastomers materials I various groups

Groups	**Group I**	**Group II**	**Group III**
Material	Poly vinyl ether silicone (PVES)(EXA'lence light body)	Poly vinyl siloxane(PVS)(Flexceed light body)	Polyether(PE) (Impregum)

Table I shows that materials used was EXA'lence light body, Flexceed light body and Impregum which was classified in group I, II and III respectively.

Table II Measurement of cell viability on day 1

Time period	Group I	Group II	Group III
15 minutes	56	154	156
30 minutes	77	126	149
1 hour	67	135	137
24 hour	28	81	137

Table II, graph I shows in group I on day 1, cell viability at 15 minutes was 56, at 30 minutes was 77, at 1 hour was 67 and at 24 hours was 28. In group II, it was 154, 126, 135 and 81 at 15 minutes, 30 minutes, 1 hour and 24 hours. In group III, it was 156, 149, 137 and 137 at 15 minutes, 30 minutes, 1 hour and 24 hours.

Graph IMeasurement of cell viability on day 1

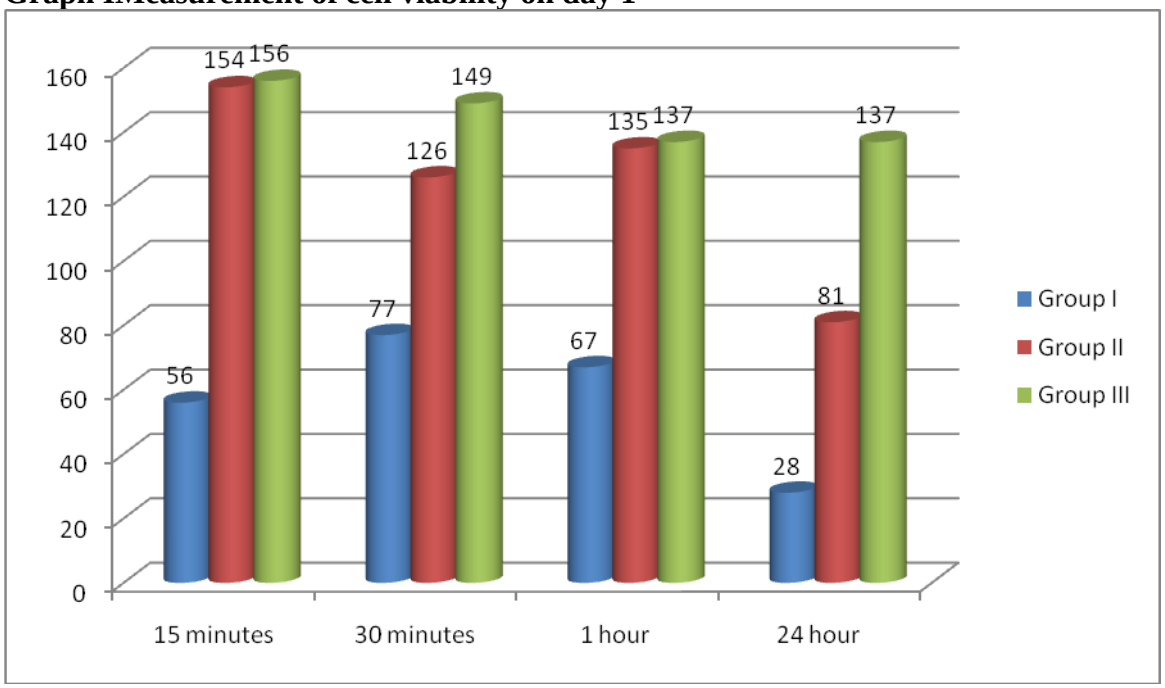

Table III Measurement of cell viability on day 3

Time period	Group I	Group II	Group III
15 minutes	37	83	105
30 minutes	29	71	115
1 hour	23	83	113
24 hour	29	82	115

Table III, graph II shows in group I on day 3, cell viability at 15 minutes was 37, at 30 minutes was 29, at 1 hour was 23 and at 24 hours was 29. In group II, it was 83, 71, 83 and 82 at 15 minutes, 30 minutes, 1 hour and 24 hours. In group III, it was 105, 115, 113 and 115 at 15 minutes, 30 minutes, 1 hour and 24 hours.

Graph II Measurement of cell viability on day 3

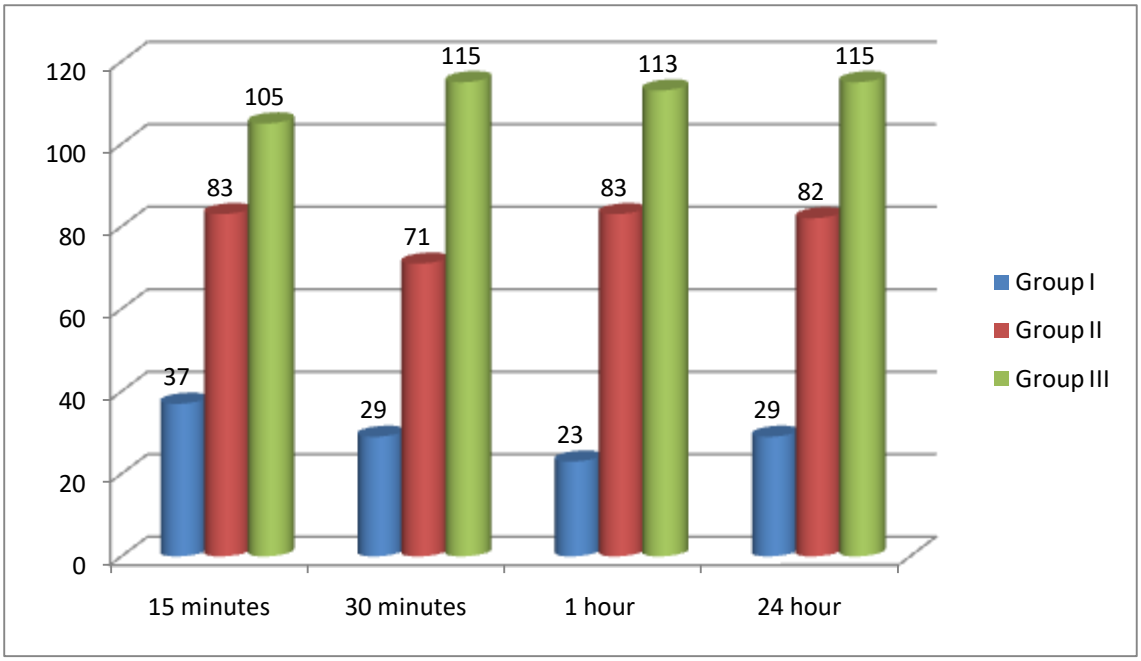

Table IV Measurement of cell viability on day 7

Time period	Group I	Group II	Group III
15 minutes	20	22	86
30 minutes	21	13	76
1 hour	30	17	85
24 hour	29	19	65

Table IV, graph III shows that on day 7, in group I cell viability at 15 minutes was 20, at 30 minutes was 21, at 1 hour was 30 and at 24 hours was 29. In group II, at 15 minutes was 22, at 30 minutes was 13, at 1 hour was 17 and at 24 hours was 19. In group III, at 15 minutes was 86, at 30 minutes was 76, at 1 hour was 85 and at 24 hours was 65.

Graph III Measurement of cell viability on day 7

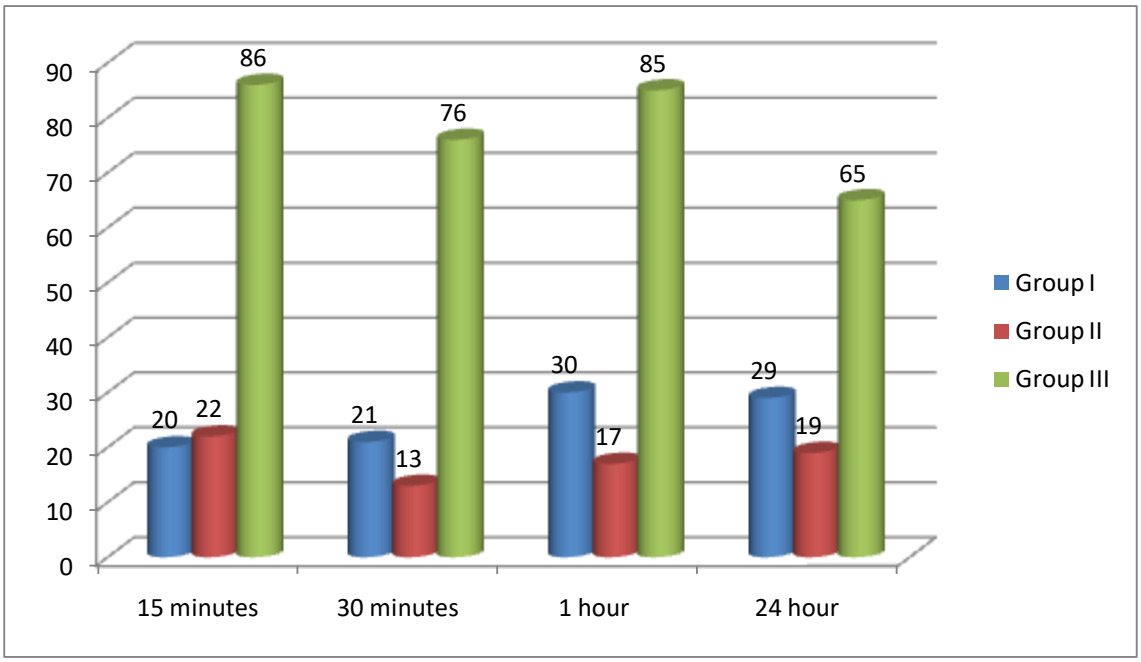

Table VMeasurement of mean survival rate

Time period	Day 1	Day 3	Day 7
Group I	25.4 ± 8.23	23.7 ± 7.21	24.8 ± 6.45
Group II	106.2 ± 31.5	68.2 ± 32.7	19.4 ± 31.5
Group III	121.4 ± 16.2	99.4 ± 14.2	73.6 ± 15.8
P value	0.002	0.001	0.004

Table V shows that mean ± SD cell viability survival of cells at day 1 in group I was 25.4 ± 8.23, 106.2 ± 31.5 in group II and 121.4 ± 16.2 in group III. On day 3, it was 23.7 ± 7.21, 68.2 ± 32.7 and 99.4 ± 14.2 in group I, II and III respectively. On day 7, it was 24.8 ± 6.45, 19.4 ± 31.5 and 73.6 ± 15.8 in group I, II and III respectively. The difference was significant (P< 0.05).

Table VIPeriod effect within the subject

Groups	Sum of squares	Df	Mean square	F	P
Group I	25.28	1.24	12.72	0.31	0.71
Group II	16322.2	1.02	15824.1	12.78	0.01
Group III	4712.4	1.56	3230.2	24.16	0.02

Table VI shows test of within subject effect over time in all groups.

4. Discussion

Dental impression materials should be able to duplicate oral tissues accurately without distortion with maximum details and biocompatibility. Condensation silicones,vinylpolysiloxanes, polyethers and polysulfides are types of elastomers used in prosthodontics for impression making. All possesses advantages and disadvantages.[7] It is very difficult to label one as superior l and other as inferior. They are also grouped as toxic,

non toxic and less toxic. Numerous studies have determined the cytoxicity level of these elastomers either directly or indirectly. Research concerningthe cytotoxicity of VPS have indicateda high degree of toxicity towards cell cultures compared to the negative control.[8]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 easiest form of biocompatibility evaluation that can be used to screen a large number of dental materials.[9]

Inpresent study we used indirect method for determining cell viability or cytotoxicity. We determined cytotoxicity level of Poly vinyl ether silicone (PVES), poly vinyl siloxane(PVS) and polyether(PE) impression material using indirect method.

We observed that in group I on day 1, cell viability at 15 minutes was 56, at 30 minutes was 77, at 1 hour was 67 and at 24 hours was 28. In group II, it was 154, 126, 135 and 81 at 15 minutes, 30 minutes, 1 hour and 24 hours. In group III, it was 156, 149, 137 and 137 at 15 minutes, 30 minutes, 1 hour and 24 hours. Chai et al[10] found that VPS impression materialhas high strain tolerance as comparison to other materials of different categories. VPS impression material can be easily removed from oral cavity accurately with minimal or no distortion.

We found that in group I on day 3, cell viability at 15 minutes was 37, at 30 minutes was 29, at 1 hour was 23 and at 24 hours was 29. In group II, it was 83, 71, 83 and 82 at 15 minutes, 30 minutes, 1 hour and 24 hours. In group III, it was 105, 115, 113 and 115 at 15 minutes, 30 minutes, 1 hour and 24 hours. Roberta et al[11] evaluated the cytotoxicity of polyethers and vinyl polysiloxanes impression material and observed that MTT test, cell counting and light microscopy were effective in determining cellular viabilityof extracts of polyether materials which showed reduction.

We found that on day 7, in group I cell viability at 15 minutes was 20, at 30 minutes was 21, at 1 hour was 30 and at 24 hours was 29. In group II, at 15 minutes was 22, at 30 minutes was 13, at 1 hour was 17 and at 24 hours was 19. In group III, at 15 minutes was 86, at 30 minutes was 76, at 1 hour was 85 and at 24 hours was 65. Smith Williams et al[12] in their study suggested that there is allergic response, contact dermatitis, and gingivitis with PE impression materials and this may incite hypersensitive reactions as well.

We found that there was significant difference in mean viability survival of cells in all groups. 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.

3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide (MTT) is a calorimetric assayused for evaluating cytotoxicity through determinng mitochondrial function of the cells by calculating succinate dehydrogenase which is a potent mitochondrial enzyme. This method is safest of all which exhibit high reproducible capacity.[14]

Kwon et al[15] assessed cytotoxicity of vinyl polysiloxane (VPS) is elastomeric dental impression material with positive control with sodium lauryl sulfate (SLS). Results revealed that positive control VPS with more or equal to 2 wt% of SLS was more cytotoxic as compared to commercially available VPS.

The limitation of this study is that indirect method of assessing cytotoxicity of elastomeric impression material was used.

5. Conclusion

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

References

[1] Ciesco JN, Malone WF, Sandrik JL, Mazur B. Comparison of elastomeric impression materials used in fixed prosthodontics. J Prosthet Dent 1981;45:89-94.
[2] Nassar U, Oko A, Adeeb S, El-Rich M, Flores-Mir C. An in vitro study on the dimensional stability of a vinyl polyether silicone impression material over a prolonged storage period. J Prosthet Dent 2013;109:172-8.
[3] Blankenau RJ, Kelsey WP, Cavel WT. A possible allergic response to polyether impression material: A case report. J Am Dent Assoc 1984;108:609-10.
[4] Liu, C.M.; Huang, F.M.; Yang, L.C.; Chou, L.S.; Chou, M.Y.; Chang, Y.C. Cytotoxicity effectsof gingival retraction cords on human gingival fibroblasts in vitro. J. Oral. Rehabil. 2004; 31: 368-372.
[5] Reddy NR, Abraham AP, Murugesan K, Matsa V. An in vitro analysis of elemental release and cytotoxicity of recast nickel-chromium dental casting alloys. J Indian Prosthodont Soc 2011;11:106-12.
[6] Chandar S, Kotian R, Madhyastha P, Kabekkodu SP, Rao P. In vitroevaluation of cytotoxicity and corrosion behavior of commercially puretitanium and ti‑6Al‑4V alloy for dental implants. J Indian ProsthodossntSoc 2017;17:35‑40.
[7] Rafael CF, Liebermann A. Clinical characteristics of an allergic reaction to a polyether dental impression material. J Prosthet Dent 2017;117:470-2.
[8] Batchelor JM, Todd PM. Allergic contact stomatitis caused by a polyether dental impression material. Contact Dermatitis 2010;63:296-7.
[9] Aslanturk OS, Askın Celik T, Karabey B, Karabey F. Activephytochemical detecting, antioxidant, cytotoxic, apoptotic activities ofethyl acetate and methanol extracts of Gallium aparine L. Br J PharmaRes 2017;15:1‑16.
[10] Chai, Y. Takahashi, and E. P. Lautenschlager. Clinically relevant mechanical properties of elastomeric impression materials. International Journal of Prosthodontics 1998; 3: 219–223.
[11] Roberta T, Federico M, Federica B, Antonietta CM, Sergio B, Ugo C.Study of the potential cytotoxicity of dental impression materials.Toxicol In Vitro 2003;17:657‑62.
[12] Smith DC, Williams DF. Composition, characteristics and clinical and tissue reactions of impression materials. Biocompatibility of Dental Materials. Vol. 3. Boca Raton, FL, USA: CRC Press; 1982; 277‑89.
[13] Boraldi F, Coppi C, Bortolini S, Consolo U, Tiozzo R. Cytotoxicevaluation of elastomeric dental impression materials on a permanentmouse cell line and on a primary human gingival fibroblast culture.Materials (Basel) 2009;2:934‑44.
[14] Sydiskis RJ, Gerhardt DE. Cytotoxicity of impression materials. J Prosthet Dent 1993;69:431–5.
[15] Kwon JS, Lee SB, Kim KM, Kim KN. Positive control for cytotoxicity evaluation of dental vinyl polysiloxane impression materials using sodium lauryl sulfate. Acta Odontologica Scandinavica. 2014 Nov 1;72(8):618-22.