Original Article

Evaluation of cytotoxicity levels of poly vinyl ether silicone, polyether, and poly vinyl siloxane impression materials: An *in vitro* study

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Abstract

Aim: To assess the cytotoxicity level of newly introduced poly vinyl ether silicone (PVES) compared to poly vinyl siloxane (PVS) and polyether (PE) elastomeric impression materials.

Settings and Design: Comparative -Invitro study design.

Materials and Methods: Mouse cell line NIH/3T3 was grown in Dulbecco's modified Eagle's medium. Samples of three elastomers were dissolved in dimethyl sulfoxide and were tested at various concentrations. Twenty-four well plates with NIH/3T3 cells with different concentrations of elastomeric solutions were incubated at 37°C. 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide assay was performed on day 1, 3, and 7, with a time interval of 15 min, 30 min, 60 min, and 24th h to estimate the cytotoxicity for all three elastomers.

Statistical Analysis Used: Kruskal–Wallis ANOVA test and the period effect within the subjects, repeated-measure ANOVA was done using the Greenhouse–Geisser correction method.

Results: The mean cell viability (survival rate) of NIH 3T3 cells at the concentrations tested was measured. A repeated-measure Kruskal–Wallis ANOVA determined the mean survival concentration on day 1, 3, and 7. PVES showed significant decrease in the survival rate on day 1 than PVS and PE, while PVS and PE had significant decrease in the survival rates of cells on day 3 and 7 which were statistically significant ($P < 0.001$).

Conclusion: PVES shows early cytotoxic signs as compared to PVS and PE, and cell viability for PVS was the highest among all. When making impression with PVES and PE, it is always better to evaluate the impression and gingival sulcus carefully with magnification to prevent adverse reaction, if any material is left inadvertently for longer period of time.

Keywords: 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide assay, biocompatibility, cell viability, cytotoxicity, mouse cell lines

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INTRODUCTION

Final impression materials used in fixed, removable, and implant prosthodontics are elastomers.[1] 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).[2-5] 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 h to 1–3 days.[6,7] The clinical manifestation includes severe pain, dry mouth, burning mouth, swelling of lips, nonspecific cheilitis, dermatitis, and dysphagia.[8,9]

The elastomers can tear and can be trapped in the gingival sulcus under implants during impression making and cause adverse and toxic reactions when remained in contact for longer periods of time.[10-25] There are many reports on cytotoxicity, tissue reactions, and hypersensitivity of various other dental materials.[26-28] The potential cytotoxicity of these materials can be tested either by introducing the cells into the material (direct test) or to the eluted extracts of the impression materials (indirect tests).[2] The assessment of cytotoxicity of these materials is a fundamental step in the evaluation of their biocompatibility. PVES combines the advantages of PVS and PE. No independent *in vitro* study has been reported in the literature on the cytotoxic levels of PVES compared to PVS and PE at the time of start of the study. The aim of this study is to evaluate cytotoxicity of PVES impression material compared to PVS and PE impression materials on NIH/3T3 cells (mouse cell line) cultured *in vitro*. The objective is to evaluate the cytotoxicity level of PVES, PE, and PVS on NIH/3T3 cell for cell viability on day 1, 3, and 7 with a time interval of 15 min, 30 min, 60 min, and 24th h. Null hypothesis means no difference between the PVES, PVS, and PE material in cytotoxicity levels over the three time points on day 1, 3, and 7.

MATERIALS AND METHODS

The study was approved by the Institutional Review Board (IRB Approval No.: IGIDSIRB2014 NRP14PGVRPCI). Elastomeric impression materials used were light body, soft consistency supplied in paste forms: Group A – PVES (EXA'lence light body, GC America, LOT No.: 1306121, expiry: 2.December 2015); Group B – PVS (Flexceed light body, GC America, LOT No.: 1401031, expiry: January 2017); and Group C – PE impression material (Impregum Soft Consistency, hand mix, hydrophilic, 3M ESPE, Germany, LOT No.: 31710, expiry: March 2017).

Cells were cultured using Dulbecco's modified Eagle's medium (DMEM), 4.5 g glucose/l, L-glutamine and sodium pyruvate, fetal bovine serum (FBS), and 1X phosphate-buffered saline (PBS) (HiMedia Labs, Mumbai, India). Thiazolyl blue tetrazolium bromide 98% (Sigma, Biocorporals, Chennai, India, Cat No.: M2128100MG) and trypsin 0.25% ethylenediaminetetraacetic acid, phenol red (Gibco, Biocorporals, Chennai, India, Cat No.: 25200056 – 100 ml). The cytotoxic tests were carried out on day 1, 3, and 7, with the time interval of 15 min, 30 min, 60 min, and 24th h.

Specimen preparation was done following the International Organization for Standardization standards for cell cytotoxic study.[29,30] The impression materials [Figure 1] were dispensed and mixed individually according to the manufacturer's instructions and placed in a sterilized brass mold of size 1 cm × 1 cm; after polymerization, the specimen was stored in a glass container. A total of eight specimens were made for each impression material. All the prepared samples [Figure 2] were handled in aseptic conditions to avoid biologic contamination during cell culture tests.

The mouse fibroblast cell line NIH/3T3 was obtained from the National Centre for Cell Sciences, Pune. Cells were grown in DMEM, supplemented with 10% FBS, 2 mM-glutamine, 100 μg/ml streptomycin, and 100 U/ml of penicillin. The cells were maintained at 37°C in the incubator with 5% carbon dioxide (CO_2). After few passages, on reaching 85% confluence, cells were washed with 1X PBS and seeded for further cytotoxicity assay.

Indirect cytotoxic testing methods were carried out in this study[2,12] [Figure 3]. NIH/3T3 cells were incubated with polymerized impression materials in 60-mm Petri dishes in 5 ml of culture medium without serum for 15 min, 30 min, 60 min, and 24th h at 37°C under sterile conditions [Figure 3]. The use of culture medium without serum was adopted to avoid the possible interaction or inactivation of substances released by testing materials with serum components.[10] After incubation period, the culture medium containing any soluble extracts of polymerized impression materials was collected in sterile tubes and used for further testing.

3-(4,5-dimethylthiazol-2-yl)-2- 5-diphenyltetrazolium bromide assay

3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide (MTT) assay is one of the most commonly used calorimetric assays to assess cytotoxicity or cell viability.[31] This assay determines principally cell viability through determination of mitochondrial function of the cells by

measuring activity of mitochondrial enzymes, such as succinate dehydrogenase.[32] This method is far superior to the previously mentioned dye exclusion methods because it is easy to use and safe, has a high reproducibility, and is widely used to determine both cell viability and cytotoxicity tests.[32,33]

NIH/3T3 cells were seeded in a 96-well plate at a density of 5×10^3 cells/well with complete DMEM supplemented with 10% FBS [Figure 4]. After 24 h, the used medium was removed and the cells were washed with 1X PBS. Cells were exposed to 100 µl of extracts for day 1, 3, and 7, with the

Figure 1: Impression materials and standardized brass mold

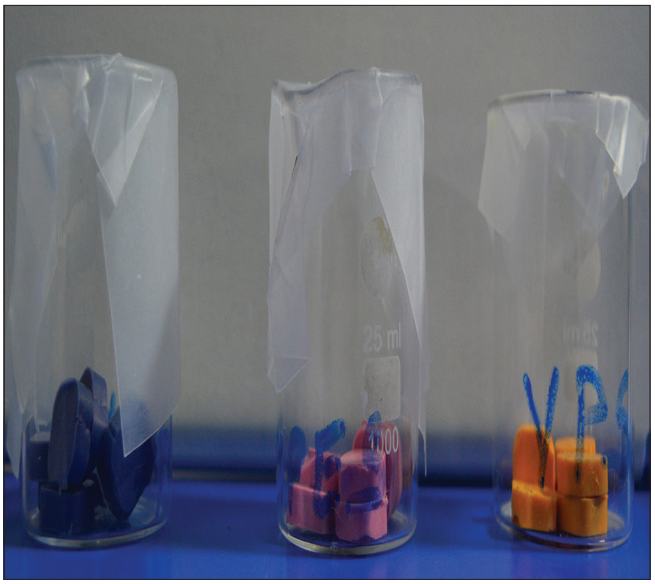

Figure 2: Prepared samples purple polyether, pink poly vinyl ether silicone, yellow poly vinyl siloxane

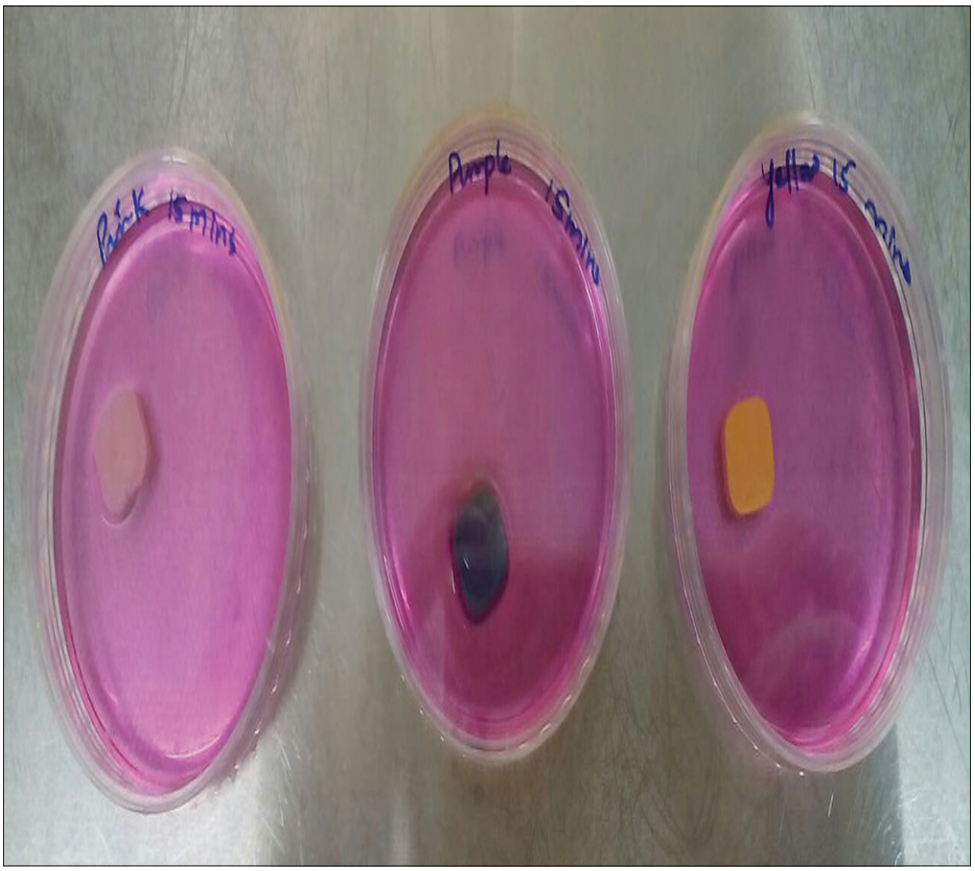

Figure 3: Indirect test

time interval of 15 min, 30 min, 60 min, and 24th h; the time interval is used to differentiate the level of survival percentage of cells and cytotoxic virulence between each time interval. The same time interval is followed for day 3 and 7. 50 µl of MTT (5 mg/ml in PBS) was added to each well. Subsequently, the plates were incubated at 37°C for 3 h at 5% CO_2.[31] At end of the incubation, excess MTT solution was removed and the formazan crystals were dissolved with 100 µl of dimethyl sulfoxide.[16] Finally, the color intensity was measured at 570 nm in SpectoMax M5, Molecular Devices, CA, USA.

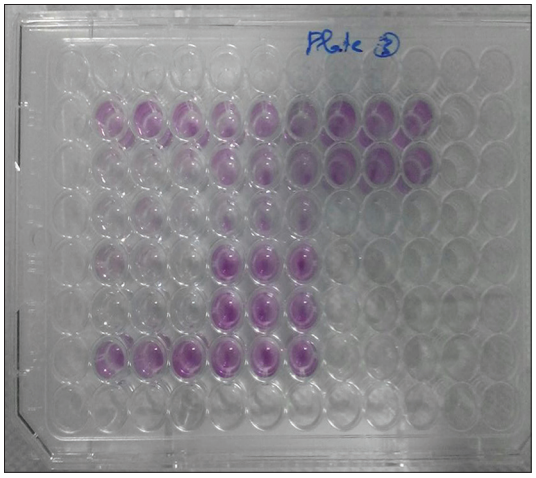

Figure 4: 3-(4, 5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide assay

Table 1: Cell viability (survival of cells at different time point in a single day of measurement)

	Day 1			Day 3			Day 7		
	PVES	PE	PVS	PVES	PE	PVS	PVES	PE	PVS
15 min	57	158	156	34	84	102	19.92	21.03	85
30 min	78	125	147	27	74	115	20.3	12.9	77
60 min	66	135	137	23	84	116	29.43	16.4	84
24 h	25	78	138	25	81	115	28.36	18	66

PVES: Poly vinyl ether silicone, PE: Polyether, PVS: Poly vinyl siloxane

Table 2: Mean survival rate (average cell viability survival of cells on each day)

	Mean (SD)			*P*
	PVES	PE	PVS	
Day 1	25.55 (8.102)	107.5 (33.887)	119.75 (18.719)	0.0001
Day 3	22.13 (5.105)	65.5 (17.682)	92.25 (10.782)	0.0001
Day 7	23.6 (4.316)	17.76 (2.339)	72.04 (15.825)	0.0001

P value is computed using nonparametric method - Kurskal-Wallis test. SD: Standard deviation, PVES: Poly vinyl ether silicone, PE: Polyether, PVS: Poly vinyl siloxane

Continuous data will be reported as mean and standard deviation and dichotomous data as percentages. Data will be checked for normality to use parametric or nonparametric test. To estimate the difference in the primary outcome ANOVA test or Kruskal–Wallis ANOVA test 4 will be used to check , cell viability (survival rate of cells) at various time point on day 1, 3, and 7. To assess the period effect, ANOVA test, using Greenhouse–Geisser method, will be used to test the cell viability changes over time in each group. The alpha error was set to 0.05 to determine the statistical significance.

RESULTS

The amount of cell viability (survival rate) of the NIH 3T3 cells on each day of measurement at various time points (15 min, 30 min, 60 min, 24th h) for each material independently, from the eluates obtained from PVES, PVS, and PE, is presented in Table 1. The nonparametric method Kruskal–Wallis ANOVA test is used to measure mean and standard deviation of cell viability (survival rate of cells) of the NIH 3T3 on day 1, 3, and 7 for the eluates obtained from PVES, PVS, and PE [Table 2]. Statistically significant difference was observed at each day ($P < 0.001$). To assess the period effect within the subjects, repeated-measure ANOVA was done using the Greenhouse–Geisser correction method and the results are presented in Table 3. Pairwise comparison of each day is reported in Tables 4-6.

DISCUSSION

The study rejects the null hypothesis of no difference between the PVES, PVS, and PE material in cytotoxicity levels over the three time points on day 1, 3, and 7. The mean survival rate (cell viability) at day 1 for PVES was very low mean (25.55) compared to PE (107.5) and PVS (119.75) with $P < 0.001$ being statistically significant. At day 3 and 7, PVES (22.13 and 23.6) materials' cell viability unchanged for PE (65.5 and 17.7) and PVS (92.5 and 72.04) with $P < 0.001$. These results inferred that there will be early inflammatory signs with PVES compared PE and PVS; hence, PVS and PE should not be inadvertently left in the gingival sulcus after impression making. To assess the period effect in all three groups, PVES was not statistically significant as there was no change from day 1 to 7, but PE and PVS

Table 3: Test of with in subject effect over time

Source	Type III sum of squares	Df	Mean square	*F*	*P*
PVES (Greenhouse-Geisser)	23.612	1.951	12.103	0.346	0.716
PE (Greenhouse-Geisser)	16,127.8	1.016	15,874.5	11.821	0.040
PVS (Greenhouse-Geisser)	4588.37	1.286	3567.02	25.726	0.007

PVES: Poly vinyl ether silicone, PE: Polyether, PVS: Poly vinyl siloxane

Table 4: Pair wise comparison: Poly vinyl ether silicone

Time (I)	Time (J)	Mean difference (I-J)	*P*	95% CI for difference	
				Lower bound	Upper bound
Day 1	Day 3	3.425	1.000	−15.655	22.505
	Day 7	1.950	1.000	−17.475	21.375
Day 3	Day 7	−1.475	1.000	−23.055	20.105

CI: Confidence interval

Table 5: Pair wise comparison: Polyether

Time (I)	Time (J)	Mean difference (I-J)	*P*	95% CI for difference	
				Lower bound	Upper bound
Day 1	Day 3	42.000	0.599	−82.462	166.462
	Day 7	89.738*	0.038	8.154	171.321
Day 3	Day 7	47.738*	0.041	3.137	92.338

*$p<0.05$. CI: Confidence interval

Table 6: Pairwise comparison: Poly vinyl siloxane

Time (I)	Time (J)	Mean difference (I-J)	*P*	95% CI for difference	
				Lower bound	Upper bound
Day 1	Day 3	27.500	0.146	−14.007	69.007
	Day 7	47.713*	0.004	28.576	66.849
Day 3	Day 7	20.213	0.172	−12.439	52.864

*$p<0.05$. CI: Confidence interval

were statistically significant ($P < 0.05$). Cell viability for PVS was the highest among all. Pairwise comparison for survival of the impression material confirms that PE and PVS have more survival rate at day 1, 3, and 7 than PVES. Significant differences were found on day 1 and 7 for PE and PVS, whereas no significant differences were found for any of the days in PVES, which denotes that the incidence of cytotoxic effects will be earlier in case of PVES compared to that of PE and PVS.

Boraldi *et al.* and Roberta *et al.* assessed the cell viability after 12 h, 24 h, and maximum of 48 h.[10,12] In the current study, the cell viability is tested after 24 h, 3 days, and 7 days, which will substantiate that both early and delayed cytotoxic signs can be assessed, as in case if impression material is inadvertently left in the gingival sulcus for longer period of 7 days. Studies done by Blankenau *et al.*, Rafael and Liebermann, Batchelor and Todd, Mittermüller *et al.*, Roberta *et al.*, Nally and Storrs, Brunton *et al.*, and Smith and Williams concluded that there is allergic response, contact dermatitis, and gingivitis and may provoke hypersensitive reactions to PE impression material.[6-10,23,34,35] Roberta *et al.* also concluded that PE is more cytotoxic than PVS, which matches with the results of our study.[10] In the current study, along with PE and PVS, PVES is also assessed for cytotoxicity and no study has compared till date. The adverse reactions of these impression materials occur when entrapped material remains within the gingival sulcus, under the suture, within the soft tissues, periosteum, and maxillary sinus; when an impression is made, careful inspection of the gingival sulcus and impression for after removal with magnification is requited to prevent adverse reactions.[36-39]

There are limitations for the current study, as it is an *in vitro* study with mouse cell line and not gingival fibroblast or immortalized human oral fibroblasts. A study done by Sung Kwon and Nam Kim in 2014 stated that selecting the cells lines for the cytotoxic study of impression material is important for interpreting the results for cytotoxic evaluation; using human-based cell lines like hTERT-hNOF (immortalized human oral fibroblasts) would be appropriate for both ease of cytotoxicity test and more accurate clinical relevance.[40] Further, *in vivo* research is needed to confirm the results of this study.

CONCLUSION

PVES shows early cytotoxic signs as compared to PVS and PE. Cell viability for PVS was the highest among all at day 7. When making impression with PE and PVES, it is always better to evaluate the impression and gingival sulcus carefully with magnification to prevent adverse reaction if any material is left inadvertently for longer period of time.

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Conflicts of interest
There are no conflicts of interest.

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