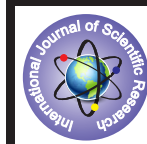


## Cytotoxicity of a Newer Resin Cement Compared to Glass Ionomer and Mineral Trioxide Aggregate an in-Vitro Study



Science

KEYWORDS : MTA, CH, DMEM, GIC

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### ABSTRACT

#### OBJECTIVE:

The aim of the study was to compare and to check the cytotoxicity of a newer resin cement neoprene with and without addition of barium to that of mineral trioxide aggregate and glass ionomer cement.

#### MATERIALS AND METHODS:

Growth and maintenance of human gingival fibroblast was done in Dulbecco's modified eagle's medium (DMEM) the test samples were divided into four groups and the elute of the test materials were made in contact with cells and also the resin cement with and without addition of barium sulphate was serially diluted upto 1:5 and MTT assay was performed for 24 hrs and 48 hrs of contact with cells. cells with DMEM was set as control group

Statistical analysis was done using one way –ANOVA test Tukey's HSD test

#### RESULTS:

Results showed that resin cement with addition of barium sulphate showed the highest viability followed by neoprene, GIC and MTA. No statistical significant result in both serially diluted resin cements.

#### CONCLUSION:

In this study extracts of neoprene with barium sulphate had the highest viability followed by neoprene, GIC and MTA. statistically significant result were seen between the cells exposed to control group and cells exposed to MTA and also between the extract of neoprene with barium sulphate and MTA

### INTRODUCTION

An ideal root end restorative material should fulfill certain properties such as biocompatibility, radiopacity, antibacterial action, should be dimensionally stable, should be easy to handle, and it should be unaffected by blood contamination<sup>1</sup>

Maintaining biocompatibility, prevention of microleakage and stability of the material in the peri-apical tissues are important criteria to be fulfilled by a root end restorative material<sup>2</sup>

Therefore to fulfill the above requirements, a good quality apical root end restorative is mandatory for the success of endodontic surgery.

Many materials have been experimented for the search of an ideal root end restorative, namely amalgam, guttapercha, zinc oxide eugenol cements, glass ionomer cements, gold foil pellets, cavit, composite resin and recently MTA and Biodentine<sup>3</sup>

In the year 1993 a new material was developed in Loma Linda university, initially the material was used as root end filling material<sup>4,5</sup>

Later with advancement MTA was used as an alternative to various clinical uses such as capping of pulp tissue, root end closure and for repairing furcal perforations etc<sup>6,7</sup>

The main reasons for use as a root end restorative material are the unique properties of MTA which include its biocompatibility, its good sealing ability and capability of MTA to promote regeneration of dental pulp and peri-radicular tissues.

However MTA has a few drawbacks such as difficulty in manipulation, longer setting time, limited resistance to washout before setting and possibility of staining the tooth structure<sup>8</sup>

The material has low solubility but low compressive strength and therefore is not recommended for placement in functional sites<sup>8</sup> also because of the high cost of MTA its use has been limited in various countries<sup>9</sup>

Therefore the search for an ideal root end filling material and new root repair materials is an ongoing process to improve the properties.

An ideal orthograde or retrograde filling material should seal the pathway of communication between root canal system and surrounding tissues and all most all endodontic failures occur as a result of leakage of irritant into periapical tissue<sup>10</sup>

Hence the need to check the cytotoxicity of this newer resin cement (neoprene) to human fibroblast cells.

Therefore the purpose of the study was to evaluate the cytotoxicity of this newer resin cement to fibroblast in comparison with MTA and GIC.

### METHODOLOGY:

#### Materials used:

The present study was conducted in the Cell Culture Laboratory, Central Research laboratory, A.B Shetty Institute of Dental Sciences, Deralakatte, Mangalore.

#### Materials used in this study:

##### Test materials:

- 1-Neoprene
- 2-Pro- root MTA
- 3 Glass ionomer cement

### GROWTH AND MAINTAINANCE OF CELL CULTURE:

To determine the biological response of these cements, Human gingival fibroblast cell lines were obtained

The explants were cultured in Dulbecco modified Eagle medium (DMEM) supplemented with 100mg/ml penicillin G, 50mg/ml streptomycin, 0.25 mg/ml fungizone and 10% fetal bovine serum (Gibco) was used as the cell culture medium. Cells were subcultivated using 0.25% trypsin for 2 mins at 37°C. Counting of cells was done after trypsinization by using Nuebaer's Chamber and then they were grown at  $3.6 \times 10^4$  density.

**PREPARATION OF CEMENTAL ELUTES :**

Neoprene, neoprene with addition of barium sulphate, pro-root MTA and fuji II Glass ionomer cement were used in this study and were molded into 3x 3mm using a metallic mold according to manufacture instruction

**Manipulation:**

Three samples of each material were prepared to set under aseptic conditions at 37°C in 100% relative humidity for 2 days.

After setting of the cements, the ultraviolet light was exposed to the disk for 20 minutes on each surface to make certain a sterilized cement and later was placed into 24-well tissue culture plates containing 1 ml DMEM per well and was kept for 24 hrs .

**These cemental elutes were categorized into four groups**

- Group 1- Control Group
- Group 2- Neoprene with addition of barium sulphate
- Group 3- Neoprene
- Group 3- GIC
- Group 4- MTA

DMEM without the material incubated for 24 hrs were used as control group. One milli-litre of extract was taken from each elute after incubation at 37 degree C and 95% relative humidity for 24 hrs and were transferred in to 96 well culture plate containing cells.

A total of five concentrations of the extracts of the resin cement and resin cement with the addition of barium sulphate were achieved by diluting with 1:1 of DMEM. Mean time extract of the material with addition of DMEM kept for 24 hrs and 48 hrs were transferred into a fresh plate containing fibroblast cells and MTT assay was performed for both 24 hrs plate and 48hrs plate.

**MTT assay**

Cell viability using MTT (3,4,5 dimethyl thiazol -2-yl) 2,5 diphenyltetrazolium bromide) assay was performed.

MTT enters the cells and reaches the mitochondria where it is reduced to a formazan crystals which is a dark purple coloured and insoluble.

The storage of these crystals are which are water insoluble are stored in cytoplasm of the surviving test cells. The amount of formazan product formed is directly proportional to level of the number of living cells .

Plates were then incubated in CO2 incubator for 4 hrs and then solubilised in organic solvent in dimethyl sulfoxide (6.25% v/v 0.1 N NaOH in dimethyl sulphoxide (DMSO) and the percentage of light absorbance measured at 570nm using a spectrophotometer

Cell viability is presented as the percentage of the absorbance at 570nm to that detected in the control wells and viability of cells were read using a spectrophotometer.

% of cell viability = Absorbance of sample x100

Absorbance of control

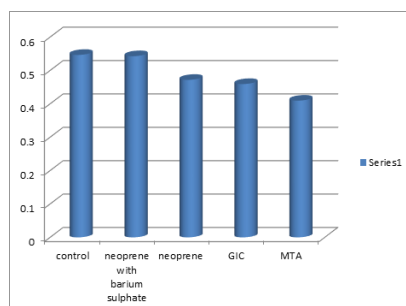
**RESULTS:**

**Cytotoxicity of the set cements after 24 hours.**

Results from MTT assay on the viability of fibroblast cells of the extracts derived from neoprene with barium sulphate, neoprene, GIC and MTA are seen in table 1

**TABLE 1: ANOVA between four groups with control**

24HRS	mean	n	Std deviation	F	Sig.
CONTROL	0.54718	3	0.016901	5.932	.010
NEOPRENE WITH BARIUM SULPHATE	0.54286	3	0.037183		
MTA	0.4096	3	0.00976		



**GRAPH 1: COMPARISON BETWEEN GROUPS AFTER 24 HRS**

After culturing for 24 hours, the extracts from neoprene with barium sulphate showed the highest viabilities followed by neoprene, GIC and MTA respectively .

However post hoc test showed statistical significant result between the control group and MTA and also between MTA and neoprene with barium sulphate.

**TABLE 2: Post hoc test between groups after 24 hours**

(I) GROUPS	(J) GROUPS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.0157000	.0312674	1.000	-.127681	.096281
	3	.0533000	.0312674	1.000	-.056681	.167281
	4	.0677667	.0312674	.554	-.044215	.179748
	5	.1175667*	.0312674	.037	.005585	.229548
2	1	.0157000	.0312674	1.000	-.096281	.127681
	3	.0710000	.0312674	.465	-.040981	.182981
	4	.0834667	.0312674	.235	-.028515	.195448
	5	.1332667*	.0312674	.017	.021285	.245248
3	1	-.0553000	.0312674	1.000	-.167281	.056681
	2	-.0710000	.0312674	.465	-.182981	.040981
	4	.0124667	.0312674	1.000	-.099515	.124448
	5	.0622667	.0312674	.744	-.049715	.174248
4	1	-.0677667	.0312674	.554	-.179748	.044215
	2	-.0834667	.0312674	.235	-.195448	.028515
	3	-.0124667	.0312674	1.000	-.124448	.099515
	5	.0498000	.0312674	1.000	-.062181	.161781
5	1	-.1175667*	.0312674	.037	-.229548	-.005585
	2	-.1332667*	.0312674	.017	-.245248	-.021285
	3	-.0622667	.0312674	.744	-.174248	.049715
	4	-.0498000	.0312674	1.000	-.161781	.062181

\*. The mean difference is significant at the 0.05 level.

In this study on dilution of the neoprene and neoprene with barium sulphate after 24 hours, no statistical significant difference was seen up to the concentration of 1:4

**TABLE 3:ANOVA test for serial dilution of neoprene**

HRS	MEAN	n	SD	F	Sig.
NEOPRENE: 1:1	0.463733	3	0.018772	.203	.931
1:2	0.4859	3	0.04894		
1:3	0.461767	3	0.0157		
1:4	0.458533	3	0.017563		

**TABLE 4:ANOVA test of serial dilution of neoprene with barium sulphate**

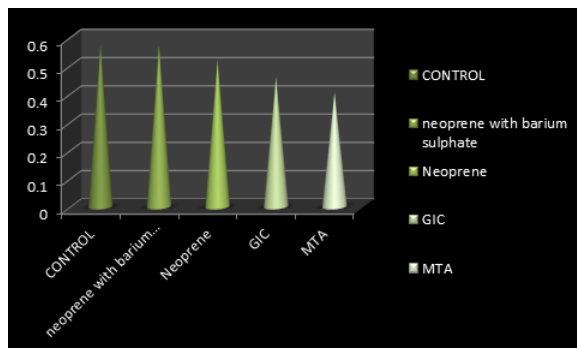
HRS	MEAN	N	SD	F	Sig.
NEOPRENE WITH BARIUM SULPHATE 1:1	0.0187	3	0.054989	.831	.535
1:2	0.04894	3	0.027666		
1:3	0.0157	3	0.046662		
1:4	0.017563	3	0.018796		

**Cytotoxicity of set cement after 48 hours.**

Results of MTT assay on cell viability of fibroblast cell line after 48 hours in different concentration of extracts derived from neoprene cement with and without addition of barium sulphate as shown in table 2(A)

**TABLE 5:ANOVA test between groups after 48 hrs**

	N	Mean	Std. Deviation	ANOVA	
				F	p-value
CONTROL	3	0.573	0.042	6.71	0.007*
neoprene with barium sulphate	3	0.573	0.074		
Neoprene	3	0.520	0.060		
GIC	3	0.459	0.019		
MTA	3	0.405	0.024		



**GRAPH 2: COMPARISON BETWEEN GROUPS AFTER 48 HRS**

Cellular viability usually depends on the type of material ,culture media and incubation time

Cells incubated with extracts of neoprene with barium sulphate had the highest viability followed by neoprene , GIC and MTA

**TABLE 6:POST HOC TEST BETWEEN GROUP AFTER 48 HRS**

(I) Group	(J) Group	Mean Difference (I-J)	95% Confidence Interval		p-value
			Lower Bound	Upper Bound	
Control	MB	0.0003	-0.142	0.143	1.00(NS)
	M	0.0526	-0.090	0.195	1.00(NS)
	G	0.1137	-0.029	0.256	0.17(NS)
	MT	0.1676	0.024	0.310	0.01*
Neoprene with barium sulphate	M	0.0523	-0.090	0.195	1.00(NS)
	G	0.1134	-0.029	0.256	0.17(NS)
	MT	0.1673	0.024	0.310	0.01*
neoprene	G	0.0611	-0.082	0.204	1.00(NS)
	MT	0.1150	-0.028	0.258	0.16(NS)
GIC	MT	0.0538	-0.089	0.197	1.00(NS)

Bonferroni post hoc test \*P<0.05 statistically significant However, our study showed statistically significant difference between the cells exposed to negative group and cells exposed to MTA in our study Neoprene with barium sulphate showed the highest viabilities among all the groups

A significant difference in cell viability between neoprene with barium sulphate and MTA was seen, where in cells exposed to neoprene with barium sulphate showed more viability.

This study has shown that there is no statistical significant result seen between GIC and MTA however there was significant difference in cell viability between neoprene with barium sulphate and MTA Our study has also shown that , no statistically significant result in cell viabilities of the diluted extract concentrations of neoprene and neoprene with barium sulphate at 1:4 and extracts to DMEM contact (p>0.05)

	N	Mean	SD	ANOVA		
				F	p-value	
NEOPRENECEMENT	1:1	3	0.374	0.044	14.45	0.001*
	1:2	3	0.406	0.004		
	1:3	3	0.499	0.039		
	1:4	3	0.512	0.018		
NEOPRENE WITH BARIUM SULPHATE	1:1	3	0.486	0.026	2.21	0.16(NS)
	1:2	3	0.635	0.058		
	1:3	3	0.501	0.136		
	1:4	3	0.580	0.060		

**TABLE 7:ANOVA TEST FOR SERIAL DILUTION OF NEOPRENE AND NEOPRENE WITH BARIUM SULPHATE.**

**DISCUSSION;**

Materials used as root end filling should possess good biocompatibility especially when they are placed in direct contact with the tissues such as for pulp capping, or as a root end restorative<sup>11</sup>, for perforation repair<sup>3</sup>etc and growing need for an ideal retrograde material makes study of cytotoxic effects of great importance.

Cytotoxicity of the cements may show cell death either due to apoptosis or necrosis of the pupal and periradicular cells thus causing delayed wound healing at the time of pulp capping ,perforation repair and during retrograde filling<sup>12</sup>. It is of paramount importance that dental materials should stimulate repair and be biologically inert .<sup>13</sup>

In this study the cell viability was measured qualitatively using MTT assay based on the ability of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble tetrazolium salt MTT into dark blue formazan crystals<sup>14</sup>

MTT assay evaluates the vital cells after made contact with extract of test materials<sup>15</sup>

The merits of this method are its ease,speed,accuracy and its non requirement of radio isotopes<sup>16</sup>

in this study Human gingival fibroblast were used to simulate the clinical environment

An elution (extract) from the cement material were used in this present investigation because it simulates clinical situation in which toxic elements leach into the surrounding tissue<sup>16</sup>

Mineral trioxide aggregate (MTA) is in a powder form which consist of fine hydrophilic particles . Tricalcium silicate, tricalcium aluminate, tricalcium oxide, and silicate oxide are the principle compounds present in this material .<sup>17</sup>

Also these are the principal compounds present in dental hard tissues<sup>18</sup>

A similar result was seen by Osorio et al and reported that MTA was the least toxic to L 929 cells compared to Glass ionomer cement<sup>19</sup>.

In this present study MTT assay exhibited that the cell viability of neoprene with barium sulphate was the highest followed by neoprene ,GIC and MTA in comparison to cells incubated in DMEM alone .

Statistically significant results were seen among the groups on one-way Anova analysis

However in our study statistically significant differences was seen between cells incubated in DMEM ie control group and the extracts from MTA

Significant results were also seen between the extract of neoprene with barium sulphate and MTA extracts.

However in this study no statistically significant result was seen between the control group and neoprene with barium sulphate and between MTA and GIC

Min-Kyung Kang et al conducted a study to understand the biocompatibility of white mineral trioxide aggregate (MTA), and studied comparison with calcium hydroxide liner (Dycal), glass ionomer cement (GIC), and Portland cement , Study showed that GIC, MTA, and Portland cement had no cytotoxic effect compared with the control.<sup>20</sup>

The authors suggested that modified Portland cement or modified GIC can be used as an alternative for perforation repair and as a inexpensive substitute for MTA in root end restoration<sup>20</sup>

According to Min-Kyung Kang et al cytotoxicity of calcium hydroxide was attributed to its high alkalinity<sup>20</sup>

When MTA powder is mixed with sterile water, calcium silicate hydrate and calcium hydroxide is being first formed and then later converts into a poorly porous gel. However the amount of calcium silicate reduces because of the high amount of calcium precipitate being released, this calcium precipitate produces calcium hydroxide which is the reason for MTA high pH value.<sup>20</sup>

The initial pH of MTA after mixing is 10.5 and this pH increases after 3 hrs up to 12.5<sup>21</sup>

A study done by Fridland et al reported that MTA had high sustained pH value in a long term study.<sup>22</sup>

Therefore the cytotoxicity could be attributed to the high alkalinity of MTA

In our study a series of extracts of different concentration of neoprene with barium sulphate and neoprene were made to see the dose response relationship.

However there was no statistically significant difference in the different concentrations of neoprene with barium sulphate and this study showed that cells exposed to the lowest concentrations of neoprene with barium sulphate and neoprene, showed the highest cell viability .

One explanation for the cytotoxicity of glass-ionomer cement could be that there is a sustained fluoride release.

Khalil and Dadara reported that the continuous fluoride showed inhibition of cell division and also caused death of cells of bone marrow.<sup>23</sup>

Neoprene is a commonly used resin cement. It is known for its fluid impervious seal and has a long standing history of use for sealing sea divers suits. it is also easily available and relatively inexpensive.

Barium sulphate was added to neoprene to make it radio-opaque.

The present study showed that the newer resin cement to be more biocompatible than MTA and GIC.

The cost and ease of use warrants more studies to be done on this material, so that this could be used in dentistry as a cheaper and more viable root end restorative.

However the limitation of this study is the disadvantages of these primary cell for its maintenance and difficulty to work with .

Also the cytotoxicity of these materials has been checked for 24 hrs and 48 hrs only.

#### CONCLUSION:

In this study extracts of neoprene with barium sulphate had the highest viability followed by neoprene , GIC and MTA.

statistically significant result were seen between the cells exposed to control group and cells exposed to MTA and also between the extract of neoprene with barium sulphate and MTA

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