RESEARCH PAPER | Medical Science | Volume : 5 | Issue : 11 | November 2015 | ISSN - 2249-555X

Comparison of Cytotoxicity of Biodentine Retrograde Filling Material Against Mineral Trioxide Aggregate and Glass Ionomer Cement- an Invitro Study.

KEYWORDS: Biocompatibility, Biodentine, cytotoxicity, Sulforhodamine B assay ,Glass ionomer cement , Human Gingival Fibroblast.

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ABSTRACT

AIM OF THE STUDY : *To compare the cytotoxicity of potential retrograde filling materials against on Human Gingival Fibroblasts cell line by means of the Sulforhodamine B assay .*

MATERIALS AND METHODS : *9 samples each of Biodentine , Mineral trioxide aggregate Angelus and Glass ionomer cement Type IX were prepared for the study and set at 37 0C in 100% relative humidity for one day . The set materials were immersed in Dulbecco Modified Eagle culture medium for 24 hrs. Fibroblasts cultured in Dulbecco Modified Eagle Medium were used as a control group. The test materials extracts were then separated and then tested in culture wells in close proximity to growing cell culture and incubated for 24 hrs & 48 hrs. . Cytotoxicity/ Survival fraction was estimated by Sulforhodamine B assay. One way ANOVA test , Bonferroni test and Paired t test were used to evaluate the statistical significance of the results.*

RESULTS : *Cells exposed to extracts from MTA Angelus and Biodentine showed the highest survival fraction percentage after 24 hrs & 48 hrs at all elute concentrations , whereas cells exposed to Glass-ionomer cement type IX extracts displayed the lowest survival fraction percentage.*

CONCLUSION: *On serial dilution of cement extracts , Survival fraction percentage continued to increase on further dilution and showed better results for cell viability and compatibility of all the root filling materials tested. The degree of cytotoxicity in descending order was Glass-ionomer cement type IX, MTA Angelus & Biodentine in the cell line tested for both 24hr and 48 hrs exposure period of the study*

INTRODUCTION

The primary goal to achieve progress in endodontic surgery is to preserve the tooth by removing all possible periradicular pathosis in order to restore health and function of tooth periodontium. This requires root end resection, the complete curettage of the periapical pathosis and the need for the firm seal of the root apex with the root-end filling material[1].

Endodontic surgery has proven to be a success over the years and it relies primarily upon the sealing ability of retrograde filling material. This is made possible by the Osteo-proliferative effect of the retrograde filling material that would promote bone formation in close contact with the tissues. Recently, new and improved retrograde filling materials that are calcium - silicate based have been introduced[1].

Mineral Trioxide Aggregate Angelus , which contained 80% Portland cement and 20% bismuth oxide, was extensively tested and was found to provide distinctly less cytotoxic effects and better results concerning biocompatibility, microleakage protection, bioactivity with innumerous applications in clinical and surgical dentistry. Ever since it has been brought into the limelight in dental literature, it has been utilized in various treatment options ,such as root-end filling material, pulp capping material and a root or furcal perforation repair material[1,29]. In spite of the fact that MTA has undergone rigorous testing with exceptional results , the lookout for the most ideal retrograde filling material is still in question.

In 2011, Biodentine with active biosilicate technology was introduced by Septodont . Since it lacked many flaws of other bioceramic cements ,it was known to yield only promising benefits especially for its excellent Osteogenic and Biomimetic potential. Its main constituents are Calcium carbonate , Tricalcium silicate , Zirconium oxide and mostly essentially a setting accelerator and a water-reducing agent namely Calcium chloride.Its unique property of being a fast-setting dentine substitute has made it an essential commodity for daily use particularly as a suitable repair material for perforations , as a coronal restoration material and in close proximity with the pulp as a pulp capping agent[3].

Biodentine is claimed by the manufacturer to possess benefits such as superior sealing ability, biocompatibility, regenerative capabilities and antibacterial properties [28].

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Periradicular cells can undergo cell death by apoptosis or necrosis , if the toxicity of a root filling material is not taken into account when essentially utilized as a retrograde filling or a perforation repair material. Dental materials should possess either the property of biological neutrality or induce repair when its soul purpose is to allow simultaneous healing , replace lost tissue and restore the functional ability of the tooth. Hence, it is essential that the clinical outcome cannot be compromised over the toxicity of dental materials used on pulpal and periapical tissues[3,4].

Cell cultures studies may offer an excellent tool to augment our knowledge of imminent cytotoxic effects of materials, in order to estimate their efficacy on humans. In-vitro tests are performable ,cost-effective, relevant, repeatable and suitable in majority of conditions as an adjunct to in-vivo experiments[1].

Anti-proliferative tests being a direct measure of the proliferation of the growing cells in direct contact with the root filling material , they are more reliable and productive in estimating the cytotoxicity of the root filling material [8].

Mineral trioxide aggregate ,Biodentine and Glass ionomer cement have been tested for their biocompatibility as a retrograde filling material, both in vitro and in in-vivo study evaluation. Based on an in-vitro study performed by Bonson et al, MTA displayed an Osteogenic phenotype by stimulating periodontal ligament fibroblasts to produce osteonectin, osteopontin, and osteonidogen[7] .

Although, till date,the biocompatibility of Biodentine on fibroblasts cells has not been assessed by determining its anti-proliferative effect on cellular attachment and cytotoxicity .

MATERIALS AND METHODS

a) Source of Data:

After obtaining consent form from patient, Human Gingival Fibroblasts were cultured from freshly prepared cell line prepared from tissue samples of healthy patients who underwent orthodontic extraction in the Department of Oral and Maxillofacial Surgery, A. B. Shetty memorial institute of dental sciences, Mangalore.

b)Material used:

List of Armamentarium and Materials used in the study:

A) **Materials used for testing were :**

3 test materials used in the study were :

1) Biodentine (Septodont, Saint-Maur-des-Fosses, France)

2) Mineral trioxide aggregate Angelus White (*Angelus Indústria de Produtos Odontológicos S/A, Brazil)*

3) Glass ionomer cement Type IX (GC Corporation , Tokyo , Japan)

c) Methodology

The study was done in the Central Research Laboratory of A.B. Shetty Memorial Institute of Dental Sciences.

Cell Culture

Cells were grown in T- 75 flasks as monolayer cultures with culture medium constituting Dulbecco's Modified Eagle Medium added with 10 % Fetal Bovine Serum , 100 mg/ml of streptomycin and 100IU / ml of penicillin. The culture was maintained in Incubator at 37°C , 5% CO_2 in air and 100% humidity (passage no - 12) , they were sub cultured twice / week .

The adherent cells were detached with 2-3ml of 0.05% trypsin (Gibco, 1: 250) by trypsinization and then incubated for 2 to 5 min at 37° C . The cells were then plated in a 96 well plate(10,0000 cells /well) after initial cell count estimation using haemocytometer , which was then followed by incubation for 24hrs for growth .

Preparation of Cement Elutes

9 samples each of Biodentine, Mineral Trioxide Aggregate Angelus and Glass ionomer cement shaped with 3-mm thick sterile teflon moulds with a diameter of 3 mm, were set at 37^0 C in 100% relative humidity according to the manufacturer's instructions under aseptic condition for one day . After setting, the disks were exposed to Ultraviolet light for 20 minutes on each surface to ensure sterility and transferred into 24-well tissue culture plates (3 samples/ well) immersed in 2 ml DMEM per well for 24 hrs. DMEM without the materials incubated for 24 hours was used as control.

After 24hrs, the test material elutes were fully leached out and 1ml of each of the test materials extracts were then aspirated with micropipettes and tested with close proximity in a 96 well tissue culture plate to the growing cell culture for a time frame of 24 hrs & 48 hrs .

Sample Preparation:

Samples of each test material were divided into 3 test groups seeded with Human Gingival Fibroblasts for each Test groups were included:-:

Group I – Control Group

Human Gingival Fibroblasts

Group II -Test Group 1

Biodentine extracts(9 samples)+ Human Gingival Fibroblasts

Group III -Test Group 2

Mineral trioxide aggregate Angelus extracts(9 samples) + Human Gingival Fibroblasts

Group IV -Test Group 3

Glass ionomer cement (type IX) extracts(9 samples)+ Human Gingival Fibroblasts

The extracts were serially diluted 1:1 and 1:2 with DMEM to achieve a total of 9 samples per concentration of each extract for the complete 24 hr and 48 hrs study .Cell number was evaluated by Sulforhodamine B assay for both the time intervals.

Sulforhodamine B assay

300 ml of 10% cold tri chloroacetic acid was added to the 96 well culture plate after culture medium was aspirated before fixation . After microplates were left for 30 min at 4^0 C, they were washed 5 times in deionized water and dried at room temperature for 30 mins. To the above ,300 ml 0.4% (w/v) Sulforhodamine B in 1% acetic acid solution were added to each well (left at room temp for 20 min) . Sulforhodamine B was removed and plates were washed five times with 1% acetic acid before air-drying. Bound Sulforhodamine B was solubilised with 600 ml 10 mm Unbuffered Tris-base solution, plates were left on plate shaker for at least 10 min. Absorbance was read at 492 to 540 nm range in the *ELISA* Plate *Reader.* Experiments were performed in triplicates. Optical density was determined

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by Spectrophotometric analysis and expressed as survival fraction (sf), in which **sf = ODx/ODc**(ODx -optical density of the test wells and ODc - optical density of the control where only fibroblasts cell were placed).

d) Statistical Analysis
One way ANOVA test ,Bonferroni test and Paired t test were used to evaluate the statistical significance of the results using software SPSS (Statistical Package for Social Sciences) Version 15.0. The level of significance was set at $p < 0.05$.

RESULTS
MTA Angelus and Biodentine exhibited lesser antiproliferative effect, whereas Glass ionomer cement type IX was more toxic. No statistical significant difference was found between Mineral trioxide aggregate Angelus and Biodentine in the cell line at 24hrs and 48hrs experimental period. Glass ionomer cement type IX cytotoxicity was highest after 24hrs indicating a short and time-independent effect. The effect of Biodentine was more noticeable with highest survival fraction percentage in 24hrs and 48hrs experimental period (Graph I, Graph V).

Viability of the remaining cells was significantly impaired because cell numbers continued to decrease throughout the duration of the experiment from 24hr to 48hrs experimental period.

A) Survival Fraction Percentage Estimation :
For 24 hr experimental period:
Biodentine showed a survival fraction percentage of 89.1 % and MTA Angelus showed a survival fraction percentage of 87.3% whereas GIC type IX showed 83.3 %.(Graph I).Glass ionomer cement type IX inhibited cell numbers by 6% when compared to MTA Angelus and Biodentine after 24hrs of exposure of the material (graph I). For 1:1 dilution , Biodentine showed a survival fraction percentage of 96.4 % and MTA Angelus showed a survival fraction percentage of 90.3% whereas GIC type IX showed 90.4 % (Graph II).For 1:2 dilution , Biodentine showed a survival fraction percentage of 99.9 % and MTA Angelus showed 95.2% whereas GIC type IX showed 94.2 %(graph III).

On serial dilution of the cement extracts , survival fraction percentage continued to increase with further dilution (GraphIV). Biodentine and its extracts on dilution showed the highest survival fraction percentage in the 24 hrs experimental period whereas GIC type IX showed the least survival fraction percentage.(Graph IV)

Based on One way ANOVA test (Table 1), there was a statistically significant difference(p value <0.05) between the first, second and the third test groups without dilution. Multiple comparisons were made using Bonferroni test(Table 2) and there was a significant difference(p value <0.05) between the MTA(Test group 2) and the GIC(Test group 3) ,as well as the Biodentine (Test group 1) and the GIC(Test group 3) but there was no statistical significance between MTA Angelus and Biodentine

Although , with 1:1 and 1:2 dilution of the test groups , there was no significant difference seen in the dilution test groups during the 24hr experimental period (Table 1).

For 48 hr experimental period:

Biodentine showed a survival fraction percentage of 82.8 % and MTA Angelus showed a survival fraction percentage of 80.73% whereas GIC type IX showed 63.4 %(Graph V).Glass ionomer cement type IX inhibited cell numbers by 19% when compared to MTA Angelus and Biodentine after 48hrs experimental period (Graph V).For 1:1 dilution , Biodentine showed a survival fraction percentage of 91.3 % and MTA Angelus showed a survival fraction percentage of 82.8% whereas GIC type IX showed 70.6 %. (Graph VI).For 1:2 dilution ,Biodentine showed a survival fraction percentage of 94.1 % and MTA Angelus showed a survival fraction percentage of 91.9% whereas GIC type IX showed 78.2 %. (Graph VII)

On serial dilution of the cement extracts , survival fraction percentage continued to increase with further dilution (Graph VIII). Biodentine and its extracts on dilution showed the highest survival fraction percentage in the 48 hrs experimental period whereas GIC type IX showed the least survival fraction percentage.(Graph VIII).

Based on One way ANOVA test (Table 3), there was a highly statistically significant difference(p value<0.01) between the first, second and the third test groups. Multiple comparisons were made using Bonferroni test(Table 4) and there was a highly significant difference (p value <0.01) between the MTA(Test group 2) and the GIC(Test group 3) ,as well as the Biodentine (Test group 1) and the GIC(Test group 3) but there was no statistical significance between MTA angelus and Biodentine.

Also , 1:1 amd 1:2 dilution of the test groups proved to be highly significant (p value<0.01)ie; between the MTA(Test group 2) and the GIC(Test group 3) ,as well as the biodentine (Test group 1) and the GIC(Test group 3) but there was no statistical significance between MTA angelus and biodentine during the 48hr experimental period (Table 3, Table 4).

Comparing 24 hrs and 48hr experimental periods:
Survival fraction percentage and cell viability of the fibroblasts was significantly impaired because the cell numbers continued to decrease throughout the duration of the experiment from 24hr to 48hrs experimental period(Graph IX).

There was a highly significant difference (p value<0.01) of the survival fraction percentage of GIC type IX and its extract dilution(1:1, 1:2) between the 24hr and the 48 hr experimental period (Table 5, Table 6, Table 7), thus proving that even on further dilution of the GIC extracts , the survival fraction percentage continued to slowly decrease due to the cytotoxic effect of GIC type IX on prolonged exposure to the material (up to 48 hrs) indicating a time-independent effect.

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TABLES

TABLE 1: ONE WAY ANOVA TEST: COMPARING THE THREE GROUPS (FOR 24HRS)

Percentage
Time: At 24hrs

	Main	N	Mean	Std. Deviation	95% Confidence Interval for Mean		ANOVA F	p value
					Lower Bound	Upper Bound		
Basic	Biodentine	9	89.11	4.99	85.27	92.94	3.423	.045
	Glass Ionomer Cement	9	83.33	3.67	80.51	86.15		sig
	Mineral trioxide aggregate	9	87.36	5.56	83.09	91.63		
Dilution 1:1	Biodentine	9	96.41	7.06	90.98	101.84	1.922	.168
	Glass Ionomer Cement	9	90.41	9.91	82.80	98.03		NS
	Mineral trioxide aggregate	9	90.31	4.84	86.59	94.02		
Dilution 1:2	Biodentine	9	99.94	10.80	91.64	108.24	.926	.410
	Glass Ionomer Cement	9	94.23	9.82	86.68	101.78		NS
	Mineral trioxide aggregate	9	95.21	7.71	89.28	101.13		

Multiple Comparisons

Dependent Variable: Percentage
Bonferroni
Time: At 24hrs

Dilution			Mean Difference	Std. Error	p	
Basic	Biodentine	Glass Ionomer Cement	5.77	2.26	.035	sig
		Mineral trioxide aggregate	1.74	2.26	1.000	
	Glass Ionomer Cement	Mineral trioxide aggregate	-4.03	2.26	.043	sig

TABLE 2:POST HOC ANALYSIS BY BONFERRONI TEST : SUB GROUP ANALYSIS FOR MULTIPLE COMPARISON (FOR 24HRS)

Percentage
Time: At 48hrs

	Main	N	Mean	Std. Deviation	95% Confidence Interval for Mean		ANOVA F	p value
					Lower Bound	Upper Bound		
Basic	Biodentine	9	82.82	8.31	76.43	89.21	21.644	.000
	Glass Ionomer Cement	9	63.42	7.22	57.87	68.97		HS
	Mineral trioxide aggregate	9	80.76	4.52	77.29	84.23		
Dilution 1:1	Biodentine	9	91.32	8.90	84.48	98.16	17.141	.000
	Glass Ionomer Cement	9	70.68	5.49	66.46	74.90		HS
	Mineral trioxide aggregate	9	82.89	7.77	76.92	88.86		
Dilution 1:2	Biodentine	9	94.19	9.97	86.53	101.86	14.841	.000
	Glass Ionomer Cement	9	78.24	5.27	74.19	82.29		HS
	Mineral trioxide aggregate	9	91.98	2.95	89.72	94.25		

TABLE 3:ONE WAY ANOVA TEST: COMPARING THE THREE GROUPS (FOR 48HRS)

Multiple Comparisons

Dependent Variable: At48hrs
Bonferroni

			Mean Difference	Std. Error	p	
Basic	Biodentine	Glass Ionomer Cement	19.39830	3.23900	.000	HS
		Mineral trioxide aggregate	2.05854	3.23900	1.000	
	Glass Ionomer Cement	Mineral trioxide aggregate	-17.33975	3.23900	.000	HS
Dilution 1:1	Biodentine	Glass Ionomer Cement	20.64186	3.54513	.000	HS
		Mineral trioxide aggregate	8.42871	3.54513	.077	
	Glass Ionomer Cement	Mineral trioxide aggregate	-12.21315	3.54513	.006	HS
Dilution 1:2	Biodentine	Glass Ionomer Cement	15.95259	3.17285	.000	HS
		Mineral trioxide aggregate	2.21059	3.17285	1.000	
	Glass Ionomer Cement	Mineral trioxide aggregate	-13.74200	3.17285	.001	HS

TABLE 4: POST HOC ANALYSIS BY BONFERRONI TEST : SUB GROUP ANALYSIS FOR MULTIPLE COMPARISON (FOR 48HRS)

Multiple Comparisons

Dependent Variable: At48hrs
Bonferroni

			Mean Difference	Std. Error	p	
Basic	Biodentine	Glass Ionomer Cement	19.39830	3.23900	.000	HS
		Mineral trioxide aggregate	2.05854	3.23900	1.000	
	Glass Ionomer Cement	Mineral trioxide aggregate	-17.33975	3.23900	.000	HS
Dilution 1:1	Biodentine	Glass Ionomer Cement	20.64186	3.54513	.000	HS
		Mineral trioxide aggregate	8.42871	3.54513	.077	
	Glass Ionomer Cement	Mineral trioxide aggregate	-12.21315	3.54513	.006	HS
Dilution 1:2	Biodentine	Glass Ionomer Cement	15.95259	3.17285	.000	HS
		Mineral trioxide aggregate	2.21059	3.17285	1.000	
	Glass Ionomer Cement	Mineral trioxide aggregate	-13.74200	3.17285	.001	HS

TABLE 5 :COMPARISON OF 24HRS AND 48 HRS SURVIVAL FRACTION PEERCENTAGE USING PAIRED T TEST

Basic

Main		N	Mean	Std. Deviation	95% Confidence Interval for Mean		t value	p
					Lower Bound	Upper Bound		
Biodentine	At24hrs	9	89.11	4.99	85.27	92.94	3.102	.015
	At48hrs	9	82.82	8.31	76.43	89.21		sig
Glass Ionomer Cement	At24hrs	9	83.33	3.67	80.51	86.15	10.068	.000
	At48hrs	9	63.42	7.22	57.87	68.97		HS
Mineral trioxide aggregate	At24hrs	9	87.36	5.56	83.09	91.63	2.084	.071
	At48hrs	9	80.76	4.52	77.29	84.23		NS

TABLE 6 :COMPARISON OF 24HRS AND 48 HRS SURVIVAL FRACTION PERCENTAGEUSING PAIRED T TEST-WITH 1:1 DILUTION

Dilution 1:1

Main		N	Mean	Std. Deviation	95% Confidence Interval for Mean		t value	p
					Lower Bound	Upper Bound		
Biodentine	At24hrs	9	96.41	7.06	90.98	101.84	1.430	.190
	At48hrs	9	91.32	8.90	84.48	98.16		NS
Glass Ionomer Cement	At24hrs	9	90.41	9.91	82.80	98.03	5.105	.001
	At48hrs	9	70.68	5.49	66.46	74.90		HS
Mineral trioxide aggregate	At24hrs	9	90.31	4.84	86.59	94.02	2.506	.037
	At48hrs	9	82.89	7.77	76.92	88.86		sig

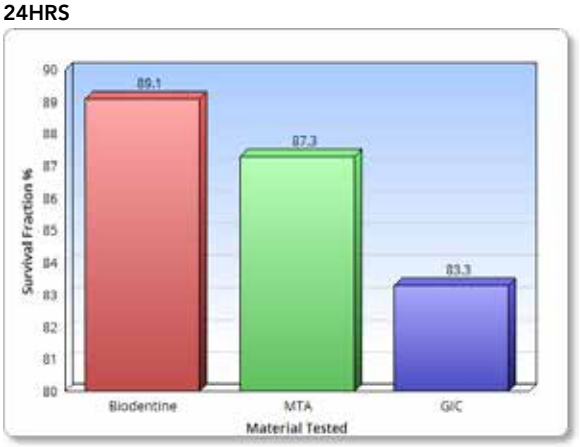
TABLE 7:COMPARISON OF 24HRS AND 48 HRS SURVIVAL FRACTION PERCENTAGE USING PAIRED T TEST-WITH 1:2 DILUTION

Dilution 1:2

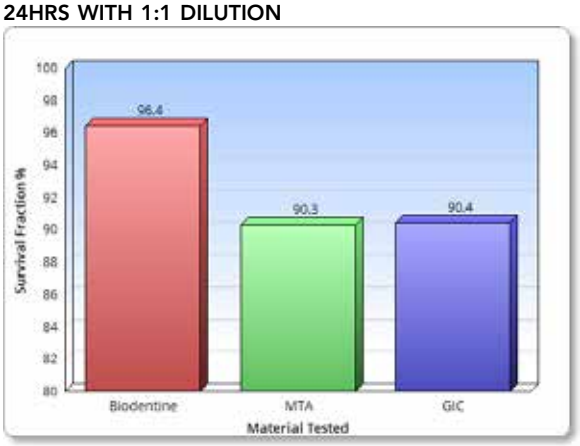
Main		N	Mean	Std. Deviation	95% Confidence Interval for Mean		t value	p
					Lower Bound	Upper Bound		
Biodentine	At24hrs	9	99.94	10.80	91.64	108.24	1.084	.310
	At48hrs	9	94.19	9.97	86.53	101.86		NS
Glass Ionomer Cement	At24hrs	9	94.23	9.82	86.68	101.78	3.960	.004
	At48hrs	9	78.24	5.27	74.19	82.29		HS
Mineral trioxide aggregate	At24hrs	9	95.21	7.71	89.28	101.13	.979	.356
	At48hrs	9	91.98	2.95	89.72	94.25		NS

GRAPHS

GRAPH I : SURVIVIAL FRACTION PERCENTAGE AFTER 24HRS



GRAPH II : SURVIVIAL FRACTION PERCENTAGE AFTER 24HRS WITH 1:1 DILUTION



GRAPH III: SURVIVIAL FRACTION PERCENTAGE AFTER 24HRS WITH 1:2 DILUTION

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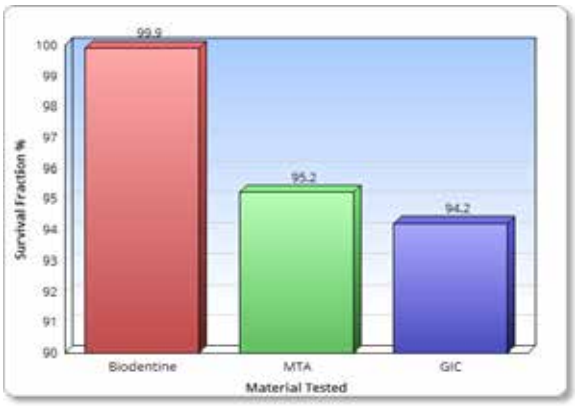
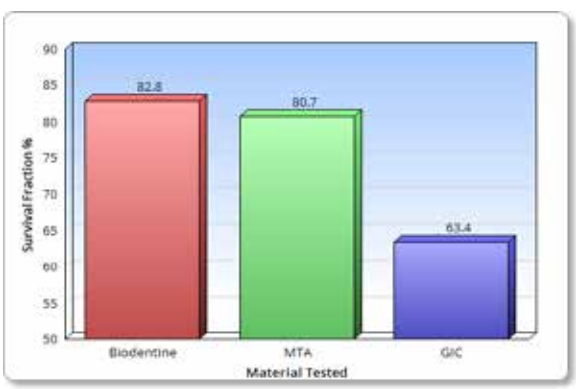
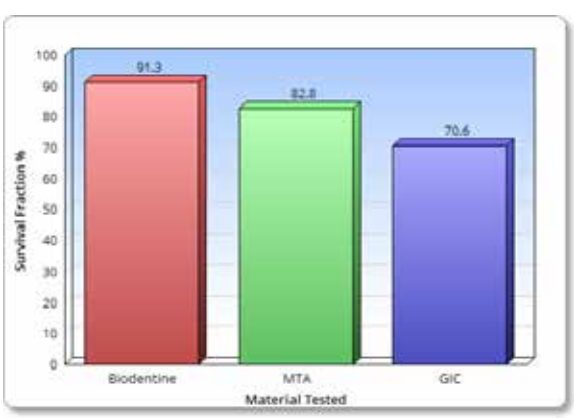


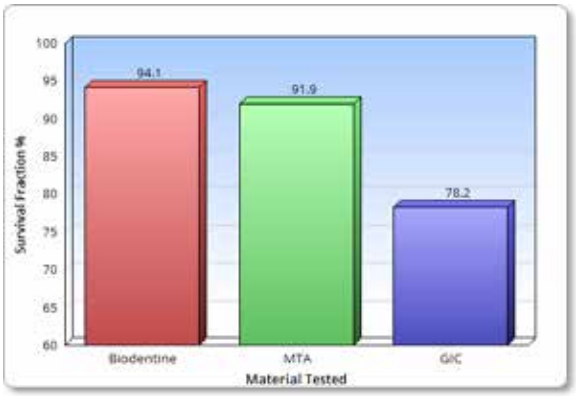
GRAPH V: SURVIVIAL FRACTION PERCENTAGE AFTER 48HRS



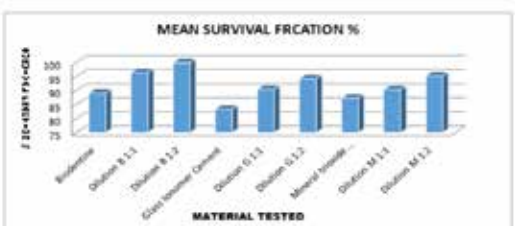
GRAPH VI : SURVIVIAL FRACTION PERCENTAGE AFTER 48HRS WITH 1:1 DILUTION



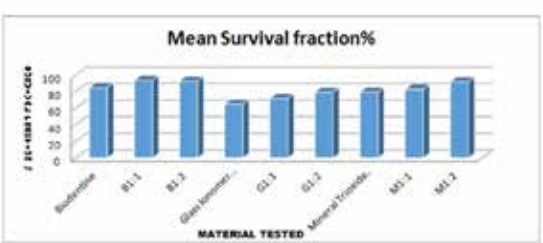
GRAPH VII :SURVIVIAL FRACTION PERCENTAGE AFTER 48HRS WITH 1:2 DILUTION



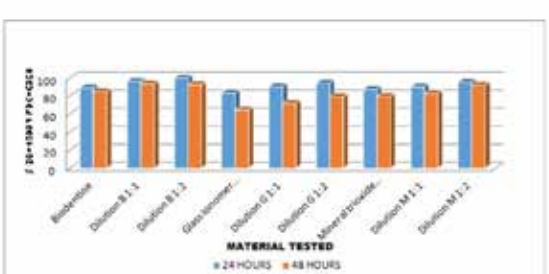
GRAPH IV
COMAPRING MATERIAL AND THEIR DILUTIONS (24HRS)



GRAPH VIII
COMAPRING MATERIAL AND THEIR DILUTIONS (48 HRS)



GRAPH IX: COMPARISON OF THE 24HRS AND 48 HRS SURVIVAL FRACTION % USING PAIRED T TEST



DISCUSSION

A retrograde filling material is ideal when it is biocompat-

Volume : 5 | Issue : 11 | November 2015 | ISSN - 2249-555X

ible, adherent to tooth structure, dimensionally stable, resistant to dissolution, antibacterial, radiopaque, and easy to use .The biocompatibility of the root-end filling materials plays a significant role in the success of endodontic treatment as the main property of such materials since they are placed in direct contact with the periradicular tissues , thus benefiting the long term prognosis of treatment by promoting tissue healing and providing a favourable environment for cellular growth [3].

Survival fraction relies on the culture medium used, the category of material and the amount of time the cells were exposed during incubation [1]. In the present study, Human Gingival Fibroblasts cells were acquired from previously prepared fresh cell lines of Human gingival tissue of healthy patients who had the need for orthodontic extraction in the Department of Oral and Maxillofacial surgery.

Biodentine, as a retrograde filling material has excellent sealing ability due to its Biomimetic mineralization quality and its ability to induce cell proliferation. Therefore it is mandatory to recognise imminent cytotoxic effects on Human Gingival Fibroblasts cells[3]. In apexification procedure, Biodentine eliminates the need for two-step obturation as in MTA by shortening the setting time to 12-16 min and reduces the risk of bacterial contamination. The high specific surface size of particles present in the material matrix and the addition of calcium chloride as accelerator to liquid phase , helps to lower the liquid content , attributing to its short setting time [28].

Mineral trioxide aggregate has been thoroughly examined and recognized as a biocompatible and bioactive material [4] due to its good excellent compatibility with periapical tissues , mechanical stability and regenerative impact on hard tissue and periodontium.

Glass ionomers cements have also gained popularity as the filling material of choice in the treatment of cervical resorptions because of the constant release of fluoride and its essential adhesive property . Fuji IX is comprised of fine grain powder and liquid with polyacrylic acid content , owing to its high viscosity . They are bioactive materials used in the repair of perforations or root resorption cavities and as a temporary restorative material during endodontic therapy[11].

The trait of a short setting time of Fuji IX GIC is comparable to Biodentine and may be of suitable advantage in certain clinical situations such as perforation repair[10,11]. Fuji IX GIC is popularly recognised for its rapid set due to its relative resistant to early moisture and low solubility to oral fluids[9]. In the present study, the biocompatibility of MTA Angelus and GIC type IX was evaluated in comparison with Biodentine.

Several strategic methods that are that are simple, reproducible , rapid and inexpensive are suggested for cytotoxicity testing of materials. The Sulforhodamine B assay was put forward by Skehan and colleagues to determine cell proliferation and drug-induced cytotoxicity for large-scale screening applications[12]. It is used for cell density measurement, based on the determination of cellular protein content. Its principle is primarily based on the potential of the protein dye Sulforhodamine B to bind electrostatically based on suitable acidic pH conditions on protein basic residues of trichloroacetic acid-fixed cells[12]. The method not only allows numerous samples to be tested within a short span of time but also only requires the need of inexpensive reagents and simple equipment to carry out the task .The Sulforhodamine B assay is nondestructive and indefinitely stable but also possesses a colorimetric end point .Hence, the Sulforhodamine B assay is a reliable and highly cost-effective method for screening of cell and material cytotoxic interactions [13] .

The cytotoxicity of the degradation products and elution substances from endodontic cements has to be taken into account , since it might gain access to periodontal tissues in numerous conditions, affecting the healing process [14]. Therefore, extracts of various concentrations derived from Biodentine, MTA Angelus and Glass ionomer cement type IX were examined for cytotoxicity. Here, toxic elements of the retrograde filling material leach into the surrounding fluids in the bony crypt , hence the extracts simulate the postsurgical root-end environment when placed in the culture medium [15].

To observe a possible dose-response relationship between the material and the cells, a series of extracts of various concentrations were made . In the present study, extracts derived from all the materials were examined on fibroblasts cells for their viability and its was clearly brought to notice that cell motility and survival were highly dependent on extract concentration. At a low extract concentration (1:2) the extracts from Biodentine and MTA Angelus showed comparable viabilities of cells and showed no significant difference between each other when compared with the DMEM control.

After preparation and filling of the resected root end , the most ideal healing outcome would be reformation of a normal attachment apparatus with healthy bone, periodontal ligament and cementum. As the wound site advances to the connective tissue phase of healing, the fibroblast becomes the principal cell type present .

In order to determine the cell interactions with the materials, the assessment of surface morphology of the test materials is an important criteria [20].

Both Biodentine and MTA Angleus showed crystalline and uneven surface topography, whereas GIC Type IX surfaces appeared smooth [3].

In this study, the best cell viability , survival and cellular interaction occurred to the surfaces of Biodentine and MTA Angelus.

Based on the findings of Biodentine and MTA , Balto in 2004 [21] , reported good spread and a high density of attached Human periodontal ligament Fibroblasts to the surface of set specimens of MTA with morphology close to that of the controls, in comparison to GIC Fuji IX .Recently it was reported that one of the major leached components of MTA and Biodentine were calcium ions . Since calcium plays a potential role in the process of fibroblast adhesion , the constant release of calcium ions is essential regarding the attachment of cells to the surface of this material [24]. When exposed to physiologic solutions the hydration of tricalcium silicate produces a calcium silicate gel and crystalline calcium hydroxide which then later precipitates to form a hydroxyapatite layer . The formation of calcium induced uneven crystalline surface matrix on the test samples , allows a suitable platform for cell adhesion to take place , which strongly suggests the enhanced biological performance of the bioactive Biodentine material [25,33] .

Volume : 5 | Issue : 11 | November 2015 | ISSN - 2249-555X

Cements extracts from GIC Fuji IX caused significantly more cell death than extracts from Biodentine & MTA Angelus after culture for 24hrs and 48hrs exposure. In agreement with the results, Human Gingival Fibroblasts attached poorly to GIC Fuji IX and displayed poor attachment characteristics, with cells exhibiting features of toxicity.

In general, a relatively smooth surface topography favours cell adhesion and growth. Even though there was increased cellular adhesion on the surface of GIC there was simultaneous cell death taking place . This could be likely due to the poor initial spreading of fibroblasts in a both the exposure periods on the GIC type IX surface compared with Biodentine & MTA Angelus caused by leaching of toxic degradation substances such as aluminum and/or iron ions present in GIC extracts .This adversely affected the cell interactions with the material by causing cytotoxic effect on the cells [16,17].

The results of this study are also supported by those of Al-Sabek et al in 2005 [27], who found that human fibroblasts attached poorly to Ketac Fil.

Based on a study conducted by Tatjana kanjevac et al, among the 8 tested GIC products , GIC Fuji type IX & Fuji plus showed the maximum release of fluoride and simultaneous inhibiting of cell growth, proliferation, mitochondrial activity and protein synthesis as a result , causing increased necrosis and cell death [18,19] .

Therefore ,fluoride release was in direct correlation with cytotoxic activity of glass ionomer cements on Human Gingival Fibroblasts cells . Along with other toxic degradation products , excess fluoride release on prolonged exposure is also considered toxic , which could in turn affect the morphology ,attachment behavior and the survival of the predominant cells present in the periapical tissue.

CONCULSION

Based on the experimental method used in the present study, Biodentine proved to be the most biocompatible materials in a 24hr & 48 hrs experimental period. Cells exposed to extracts from MTA Angelus and Biodentine showed the highest survival fraction percentage after 24 hrs & 48 hrs, whereas cells exposed to Glass-ionomer cement type IX extracts displayed the lowest survival fraction percentage. Biodentine caused gingival fibroblast reaction similar to that by MTA. In contrast, GIC Type IX showed significantly higher cytotoxicity levels than Biodentine and MTA angelus after 48hrs thus affecting cell proliferation and the healing process of periapical tissues close to them over prolonged exposure to the retrofilling material.

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