

Cytotoxicity Evaluation of Four Perforation Repair Materials Using Human Gingival Fibroblasts - An In-Vitro Study

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CERTIFICATE

Certified that the dissertation on **CYTOTOXICITY EVALUATION OF FOUR PERFORATION REPAIR MATERIALS USING HUMAN GINGIVAL FIBROBLASTS - AN IN-VITRO STUDY** done by **Dr. SHALINI CHANDRAN**, Part II Post Graduate student (MDS), Branch III - Conservative Dentistry & Endodontics, Saveetha Dental College and Hospitals, Chennai submitted to The Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment for the M.D.S. degree examination in March 2005, is a bonafide research work done under my guidance and supervision.

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INTRODUCTION

The goal of endodontic therapy is to eliminate bacteria from the root canal system and to subsequently establish an effective barrier to prevent further passage of microorganisms or their products into periapical tissues¹. Endodontic perforations interfere with this goal because of damage of periodontal attachment apparatus and subsequent bacterial proliferation.

An endodontic perforation may be defined as “an artificial opening in a tooth or its root, created by boring, piercing, cutting or pathologic resorption which results in a communication between pulp space and periodontal tissues”².

The prognosis after endodontic perforation not only depends on the size and location of the defect, the length of time that the perforation is open to the oral environment before being sealed and the amount of periodontal ligament irritation³, but also on the choice of repair materials⁴.

The mechanical and physical properties of repair materials such as Amalgam, Cavit, Glass Ionomer, Calcium Hydroxide and Mineral Trioxide Aggregate have been investigated with varying degree of success. The search for an ideal perforation repair material is a challenge that should be based on sound research guided by reason and rationale. The repair materials used are placed in intimate contact with hard and soft tissues of the periodontium. These may pose a threat to health and or endodontic treatment outcome by causing local or systemic adverse effects either through direct contact with or leaching of liberated substances into the periodontal tissues and alveolar bone. Biocompatibility is thus as important as the physical and chemical features when selecting a material for perforation repair⁵. For this purpose ADA and ISO have established guidelines for biological evaluation

of dental materials and have employed initial, secondary and usage tests for the same.

A review of recent literature indicates that repair materials have been subjected to little biological scrutiny. Since the primary treatment objective of endodontic perforation repair is the regeneration of periodontal connective tissue attachment apparatus⁶, the repair material should be able to support periodontal regeneration.

This study attempts to screen four perforation repair materials; Bone Cement, Amalgam, Glass Ionomer and Mineral Trioxide Aggregate for cytotoxicity and also to evaluate whether there is a change in this effect with passage of time using human gingival fibroblasts by MTT assay.

REVIEW OF LITERATURE

Samuel Seltzer et al³ (1970) studied the periodontal effects of root perforation before and during endodontic treatment procedures on six rhesus monkeys. The floor of the pulp chamber were perforated and closed with either Zinc Oxide and Eugenol cement alone or by Zinc Oxide Eugenol cement and a overlying Amalgam. These perforations were closed at intervals ranging from immediately to 10 months afterwards. They concluded that after perforation, damage to the periodontium always occurred. The reaction ranged from mild to severe, depending in part how quickly the perforation was sealed. The most severe reactions occurred when the perforation were not closed immediately. When the perforations were left open, epithelial proliferation was seen and root and bone resorption also was noticed.

Irving H. Sinai et al ⁷ (1977) described in detail the prognosis and treatment of various endodontic perforations. He stated that the main causes of perforation included resorption, caries and operator performance. The prognosis for a tooth with a perforation is related to the location of the perforation, negotiability of the canal, contamination and treatment. Alternative treatment approaches include routine endodontic treatment, correction and stimulation of calcification. In most instances, a perforation can be treated to induce satisfactory healing.

Mahmoud E. Eldeeb et al ⁸ (1982) conducted an animal study to compare the clinical, radiographic and histological changes using Amalgam, Cavit and Calcium hydroxide in repair of furcation perforations. The control teeth were left covered with dry cotton pellets. They concluded that sealing ability amalgam was superior to Cavit and Calcium hydroxide.

Jose and Tamara Oynick ⁹ (1985) described a technique for the treatment of endodontic perforations. When dealing with furcation perforations, they advocated a 30 second application of a small amount of formecresol at the perforation site for disinfection, followed by the placement of a thick mixture of Super EBA cement, packing it carefully with a suitable size plugger bigger than the perforation in order not to overfill it. The thickness of the mixture is very important because therein could lie the difference between success and failure. Their study was based on the fact that the Super EBA cement could be used as an alternative to Amalgam because of its very low solubility and high adhesiveness and adaptation to the dentinal walls.

Fred W. Benenati et al ¹⁰ (1986) conducted a recall evaluation of iatrogenic root perforations repaired with amalgam and guttapercha in 57 molar cases. The teeth were evaluated using a radiographic and clinical

examination at periods ranging from 3 to 72 months. Success outnumbered failures with both materials even when the repair was delayed upto 60 days. The overall success rate for the non - surgical internal repairs was 54.4%. Guttapercha failed more than Amalgam repairs. Approximately 69% of failures occurred when repair materials extruded beyond the root surface. Surgical intervention in failing teeth resulted in healing of those teeth. Delay of repair had no significant effect on the prognosis of the cases considered and finally Amalgam was found to be a more acceptable repair material than vertically condensed warm Gutta-percha.

Ramon Aguirre et al¹¹ (1986) conducted clinical, radiographic and histological studies on mongrel dogs creating furcation perforations in 48 maxillary posterior teeth and evaluated the furcal region using Amalgam, Guttapercha or Indium foil. The purpose of the study was to determine whether the use of Indium foil matrix for Amalgam would improve the healing response of the periodontal tissues in the furcation when compared with the use of Guttapercha or Amalgam for the repair of mechanical perforations. The results showed Amalgam and Guttapercha to be significantly better than Indium foil though no significant difference was seen between Amalgam and Guttapercha.

R.A.Beavers et al¹² (1986) conducted a study to 1) establish a monkey model system for studying the healing of root perforation, 2) follow and define the normal healing process of periodontal tissues after most root perforations and attempted repair and 3) assess the nature of periodontal tissue response to a hard set Calcium Hydroxide treatment of root perforations under aseptic conditions. The study demonstrated that wounds created into the periodontal ligament below the level of the epithelial attachment from the pulp chamber may heal without migration of epithelium

components to the wound site. Teeth treated with Calcium Hydroxide did not show a different healing pattern but prevented the in-growth of granulation tissue into the instrumented root canal.

James B. Roane and Fred W. Benenati¹³ (1987) in a case report described the successful management of a perforated mandibular molar using amalgam and hydroxyapatite. The technique involved an internal amalgam repair of the mesial root with a strip perforation that was secondarily isolated with a wet mix of Polycarboxylate cement, external amalgam repair of the distal root that also had a perforation, a surgical curettage and reconstruction of the furcation architecture with hydroxyapatite graft. A 21-month follow up examination verified retention of the graft and tissue reattachment. The method presented offers hope for teeth with defects presently considered irreparable.

A. S. High and J. L. Russell¹⁴ (1989) investigated the bacteriocidal properties, tissue compatibility in cell culture and ability to seal cavities as evidenced by dye diffusion of three commercially available gentamycin containing bone cement and amalgam. Amalgam was found to have poor bactericidal properties and poor tissue compatibility but slightly better apical sealing abilities than bone cements.

Robert Balla et al¹⁵ (1991) histologically evaluated the repair of furcation perforation with Tricalcium phosphate, hydroxyapatite, amalgam and Life at intervals of 2, 4 and 6 months after the experiment. The six rhesus monkeys were sacrificed. The histological evaluation revealed lack of complete healing of furcation perforation with any material used. Epithelium was present in the furcation perforation with both experimental and positive control groups. Amalgam, Life, and hydroxyapatite served to cause a lesser degree of inflammation than Tricalcium phosphate. No hard tissue formation

was observed in this study. Inflammation in the furcation area was attributed to inadequate healing ability of the repair material.

Peltola M et al¹⁶ (1992) conducted an in vitro study to evaluate the effect of various retrograde filling materials on gingival fibroblasts and rat sarcoma cells. Inhibition of cell growth around the test particles was most prominent around amalgam and glass ionomer, followed by composite and titanium. The toxic effects of all the materials decreased with time.

G.R. Bruce et al¹⁷ (1993) evaluated the cytotoxicity of amalgam Super EBA and various dentin bonding systems using the agarose overlay test. All of the adhesives tested, except Tenure were cytotoxic. Super EBA displayed minimal cytotoxicity by the initial samples and no cytotoxicity by the long term samples. Amalgam displayed no apparent cytotoxicity in the initial samples, but increased cytotoxicity over time.

Seung Tong Lee et al¹⁸ (1993) in an in- vitro study evaluated the sealing ability of mineral trioxide aggregate for the repair of lateral root perforations. The sealing ability of MT A was compared to that of Amalgam and Intermediate' Restorative Material (IRM). Dye penetration with Methylene blue was performed and the teeth were evaluated under a dissecting microscope. They concluded that MTA had significantly less leakage than IRM and Amalgam. MTA showed the least overfilling tendency whereas IRM showed the least underfilling tendency.

Hatem et al¹⁹ (1994) described about root perforations discussing the causes, diagnosis, prognosis and management of the perforations both surgically and non-surgically.

Thomas R. Pitt Ford et al²⁰ (1995) in an animal study using 7 dogs analyzed the ability of Mineral Trioxide Aggregate (MTA) to repair perforations and was compared with Amalgam. The furcations were either

repaired immediately or under delayed conditions. All repaired specimens were left for 4 months before histological examination. The final results showed that MTA is far more suitable than Amalgam for perforation repair, particularly when used immediately. MTA set within four hours providing a hard barrier against which tissue could organize. MTA also had a high pH thus probably providing the basis for hard tissue formation near the material.

Mahmoud Tarabinejad et al²¹ (1995) evaluated the Bacterial Leakage of MTA as a root end filling material. Statistical analysis of data showed no significant difference between the leakage of amalgam, super EBA and IRM. However MTA leaked significantly less than other root end filling materials indicating its superior sealing ability.

M. Torabinejad et al²²(1995) evaluated the cytotoxicity of four root end filling materials amalgam, Super EBA cement , IRM and mineral trioxide aggregate (MTA) using the agar overlay and radiochromium method on mouse L929 cells. Statistical analysis of the data from the agar overlay technique showed that freshly mixed and set amalgam were significantly less toxic than the rest of the tested materials. With radiochromium method the degree of cytotoxicity of fresh and set material was MTA least toxic followed by amalgam, super EBA, and IRM.

Z. Fuss and M. Trope²³ (1996) discussed the factors which affect the prognosis of root perforations, and formulated a classification based on these predictors. Treatment protocols which would result in the highest possible success rate were outlined.

Donald E. Arens et al²⁴(1996) described in two case reports, the use of Mineral Trioxide Aggregate (MTA) to repair the furcation perforations. The two cases substantiate that MTA is a suitable material for furcal perforation repair in patients probably because it is biocompatible and more

important, sets in the presence of moisture and therefore blood does not affect the sealing ability.

John N. Dean et al²⁵ (1997) in a clinical study in 6 dogs evaluated a combined surgical repair and guided tissue regeneration technique to treat recent root canal perforations. The mandibular 4th premolar and 1st molars were perforated on the distal root. The surgical treatment that followed normal root canal treatment was divided into 3 groups. In the 1st group, the perforations were filled with reinforced Zinc Oxide Eugenol and Intermediate Restorative Material and the osteotomy site near the furcation was then filled with freeze - dried demineralized human cancellous bone chips, a xenograft in this case (FDDDB). In the 2nd group, the osteotomy area was covered with Gortex Augmentation Material (GTAM) without any bone graft. In the third group no regenerative material was used. The controls included unfilled perforations without bone grafts and/or GTAM. Histomorphometry revealed a statistically significant decrease in inflammation and more bone regeneration when root perforations were filled and GTAM was used respectively.

W. Randy Snyder et al²⁶ (1997) studied the effects of various dental restorative materials used in perforation repair on osteoblastic cells, thus assessing the activation of bone forming osteoblastic activity. The materials evaluated were GC dentin cement, Super-EBA, Roth Root canal cement, Amalgam, Ketac Endo, IRM, Cavit-G and Ketac Bond. The effects of these materials on osteoblastic responses in bone were measured using biosynthesis of matrix proteins, osteopontin (OPN) and osteocalcin (OCN) as indices. The mRNA levels were quantified in the samples and compared with controls. Significant differences were detected in several materials. Cavit-G was the only material that showed a statistically significant increase

in the measured mRNA levels of OCN indicating that it contains an ingredient exerting a positive osteoblastic effect. Ketac Endo was the only material that showed a statistically significant increase in mRNA levels for OPN.

Eng Tiong Koh et al ²⁷ (1998) in an in vitro study evaluated the cytomorphology of osteoblasts in the presence of mineral trioxide aggregate (MTA) and Intermediate Restorative Material (IRM). Cytokine production was analysed by ELISA assay. Scanning electron microscopy revealed healthy cells in contact with MTA at 1 and 3 days in contrast to IRM. The ELISA assay revealed raised levels of all interleukins at all periods when cells were grown in the presence of MTA. It was concluded that MTA provided a biologically active substrate for bone cells and stimulated interleukin production.

Hany-Anwar M.Makkawy et al ²⁸ (1998) evaluated the cytotoxicity of two resin modified glass Ionomer cement and one dental amalgam on human periodontal ligament cells using the MTS ASSAY. Results of this study demonstrated that both material and time affected cell viability. Amalgam eluate significantly inhibited cell viability at 24hour. At 48 and 78 hour all three materials exhibited an inhibitory effect on cell viability. The authors concluded that resin modified glass Ionomer cements may be viable alternative to dental amalgam as a root perforation material.

Rosa Maria Osoria et al ²⁹ (1998) used an in vitro cell culture model of human gingival fibroblasts and L-929 cells to measure the cytotoxicity of currently used root canal sealers Endomet, CRCS and AH26 and root end filling materials Amalgam, Gallium GF2, Ketac Silver, mineral trioxide aggregate (MTA) and super EBA. Cytotoxic effects were assessed using MTT assay and CV assay. Among root end filling materials, MTA was not

Cytotoxic, Gallium GF2 displayed little cytotoxicity, Ketac Silver, Super EBA and Amalgam showed higher levels of cytotoxicity. CRCS was the least cytotoxic sealer followed by Endomet and AH26.

S.R.Sluyk et al³⁰(1998) in an in-vitro study evaluated the setting properties and retention characteristics of Mineral Trioxide Aggregate (MTA) when used as a furcation perforation repair material. Instron testing was used to measure the force required to displace the material from the perforation. The force measurements showed that MTA resisted displacement of 72 hours to a significantly greater level than 24 hours. When slight displacement occurred at 24 hours, the material demonstrated the ability to re-establish resistance to dislodgment from the dentin wall. The presence of moisture in the perforation during placement seemed to be advantageous in aiding adaptation of MTA to the walls of the perforation.

T.T.Nakata et al³¹(1998) in an in-vitro bacterial leakage study, compared the use of Mineral Trioxide Aggregate (MTA) to Amalgam as a perforation repair material using an anaerobic bacterial leakage model. *Fusobacterium nucleatum* was the bacterium used for evaluating the leakage. 44.4% of Amalgam treated teeth showed leakage probably because the zinc free -Amalgam contracted when set in moist conditions and none of the teeth treated with MTA showed leakage probably because some particles had hydrophilic property like calcium and phosphorous. Therefore they concluded that MTA was significantly better than Amalgam in preventing leakage of *F.nucleatum* past furcation perforation repairs.

Mahmoud Torabinejad et al³² (1999) described the clinical procedures for application of MTA in pulp capping, apexification, root-end filling and repair of root perforations both non-surgically and surgically.

Qiang Zhu et al³³ (1999) evaluated the cytotoxicity of three root end filling materials (amalgam, IRM and Super EBA) using cultures of human periodontal ligament cells and human osteoblast like cells. The results of the study showed that Amalgam had higher relative cell toxicity than super EBA and IRM for both cell types.

Richard S. Schwartz et al³⁴(1999) presented five case reports in which MTA was used to manage vertical root fracture, apexification, perforation repair and repair of a resorptive defect. In each case MTA was shown to allow bone healing and eliminated existing clinical symptoms.

Karl Keiser et al³⁵ (2000) compared the cytotoxicity of mineral trioxide aggregate (MTA) to other commonly used retrofilling materials, super-EBA and amalgam using human periodontal ligament fibroblasts. Cell viability assay was conducted by mitochondrial dehydrogenase activity. In both freshly mixed and 24 hour set states. MTA was less toxic than amalgam and Super-EBA.

Kuo-Wei Tai et al³⁶ (2000) investigated the long term cellular effects of three common perforation repair materials (amalgam, resin and glass ionomer) on cultured human peirodontal ligament cells for the relative kinetics of cell recovery after incubation with the test substance. All tested materials were cytotoxic to human periodontal ligament cells. Amalgam exhibited the least toxicity followed by glass ionomer and resin.

Qiang Zhu et al³⁷ (2000) observed the adhesion of human osteoblasts on commonly used root end filling materials (MTA, IRM, composite and amalgam) with scanning electron microscopy. Results showed that osteoblasts attached and spread on MTA and composite, while amalgam showed no spreading and IRM had neither attachment nor spreading of osteoblasts. The result indicated that osteoblasts had a

favourable response to MTA and composite resin in comparison with IRM and amalgam.

Gary Mathew Holt and Thom C Dumsha³⁸ (2000) compared the root-end sealing ability of Amalgam with varnish, composite with dentin bonding agent and Super Ethoxy Benzoic Acid with a new retrofill material: Bone cement. Single rooted teeth were instrumented and obturated with guttapercha, resected and retroprepared. The teeth were then randomly divided into 4 groups of 20 each, with each group receiving one of the root-end filling material. The Bone cement group was either etched or unetched. The teeth were immersed in silver nitrate and developer for leakage assessment. The teeth were sectioned longitudinally and viewed under measuring microscope. They concluded that there was no significant difference in dye leakage between the composite and Bone cement groups or between Bone cement groups and Super Ethoxy Benzoic Acid.

Roberto Holland et al³⁹ (2001) conducted an animal study to observe the healing process of intentional lateral root perforation repaired with mineral trioxide aggregate (MTA) and Sealapex. Forty – eight root canals of four mongrel dogs were instrumented and filled. After partial removal of filling an intentional lateral root perforation was made with a bur and later repaired. Histological analysis revealed no inflammation and deposition of cementum over MTA in the majority of specimens, while Sealapex exhibited chronic inflammation in all specimens.

Fu-Mei Huang et al⁶ (2002) evaluated the effect of resin modified glass Ionomer cement, compomer and composite resin human gingival fibroblasts. Cytotoxicity was evaluated using MTT assay. The authors noted that all the resinous perforation repair materials evaluated were cytotoxic to gingival fibroblasts by inhibiting cell growth, attachment and proliferation. It

was concluded that these materials might impede periodontal wound healing and regeneration

A. Pistorius et al ⁴⁰ (2003) investigated the several essential metabolic cellular responses of gingival fibroblasts exposed to MTA, amalgam and a chemically inert titanium alloy by means of cell proliferation, protein and lactate synthesis and prostaglandin (PGE₂) release assessment. MTA demonstrated cellular response similar to those of titanium. Amalgam showed as irritation rate higher than that of MTA and titanium.

Hanan Balto et al ⁴¹ (2003) in an vitro study compared the attachment behaviour of human periodontal ligament fibroblasts to root end filling materials, Amalgam, Super EBA and guttapercha by means of scanning electron microscope. Results showed that cold burnished guttapercha provided a better substrate than amalgam and super EBA for cell growth and attachment. Amalgam was the most toxic material, showing early manifestation of cell injury.

Mark A. Camp et al ⁴² (2003) conducted an in vitro study to assess the attachment of human gingival fibroblasts and periodontal ligament fibroblasts to different root end filling materials and also evaluated whether integrins were responsible for any noted attachment. Both cell lines showed superior attachment to resin modified glass ionomer (RMGI). Attachment of gingival fibroblasts to amalgam, mineral trioxide aggregate (MTA) and super-EBA did not significantly differ between these materials. At 24 hour time period the attachment of periodontal ligament fibroblasts to super EBA was significantly greater than the attachment of these cells to amalgam and MTA but significantly less than RMGI. At the 24 hour time for the second experimental series, the attachment of periodontal ligament fibroblasts was

significantly greater than amalgam and super EBA but less than resin-modified glass ionomer.

SUMMARY

In this study four perforation repair materials, Bone cement, Amalgam Glass Ionomer and Mineral Trioxide Aggregate were evaluated for cytotoxicity using human gingival fibroblasts by MTT Assay. The effect of cytotoxicity with passage of time was also evaluated using the time intervals 6hrs, 48hrs and 72hrs.

Under aseptic condition sixteen specimens were prepared as discs of radii 5mm and height 2mm using a custom made mould.

The specimens were divided into 6 groups

- Group I - Bone Cement
- Group II - Silver Amalgam
- Group III - Glass Ionomer Cement
- Group IV - Mineral Trioxide Aggregate
- Group V - Positive control – Methylmethacrylate
- Group VI - Negative control – Medium with serum

The eluates prepared were filtered and added to the culture wells and incubated in a carbon dioxide incubator at 37°C. After 6hrs, 48hrs and 72hrs the MTT Assay was performed and optical density values were obtained. On statistical analysis of the results it was observed that Bone Cement showed the least cytotoxicity. No significant difference was observed on comparing the change in cytotoxicity between different materials with the passage of time.

CONCLUSION

From the present study it can be concluded that:

1. All the materials tested; Bone Cement, Amalgam, Glass Ionomer and Mineral Trioxide Aggregate were cytotoxic with the passage of time
2. No significant difference was observed on comparing the change in cytotoxicity between different materials with the passage of time
3. Bone Cement showed the least cytotoxicity.
4. Amalgam was less toxic than Mineral Trioxide Aggregate.
5. Glass Ionomer was less toxic than Mineral Trioxide Aggregate.
6. On comparing cytotoxicity within the groups Bone Cement, Glass Ionomer and Mineral Trioxide Aggregate did not show an increase in cytotoxicity for the time period 48hours to 72 hours. Amalgam however showed a cumulative effect in cytotoxicity with the passage of time.

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