

A review of the methods used to study biocompatibility of Portland cement-derived materials used in dentistry

Josette Camilleri

Abstract

Advanced restorative dentistry may necessitate the need for surgical intervention to the infected root apex. Once access to the root end is achieved, the root apex is resected and filled with a dental restorative material.

The materials currently in use are not satisfactory due to inadequate biocompatibility and failure to achieve desirable properties in an aqueous environment. With the introduction of a new material, essentially Portland cement used in the building industry, these desirable properties have been achieved. This paper reviews the methods of testing biocompatibility of Portland cement used in dentistry.

Introduction

In restorative dentistry materials are mainly utilized to replace dental tissue lost through dental caries and tooth preparation procedures. When a significant amount of tooth tissue is lost the dental pulp may be adversely affected. This may necessitate advanced conservative procedures involving extirpation of the dental pulp and obliteration of the space with gutta-percha and root canal sealers. Access for these procedures is through the tooth crown. Should this be unsuccessful, surgical endodontics is necessary to allow cleaning and sealing of the root end to prevent further infection.

The main pre-requisites of a material to be used as a root end filling material are biocompatibility and the ability to set in an aqueous environment. Materials of choice for filling the root ends prior to flap repositioning include dental amalgam, glass ionomer cement (GIC), and intermediate restorative material (IRM). None of these materials is the ideal restorative material for the root end of a tooth. Dental amalgam is mercury-based, glass ionomer cements are dimensionally unstable and do not encourage cell growth and IRM is based on eugenol which can be allergenic. The maintenance of a dry field can be problematic during surgery and all these dental cements have to be kept dry until they set.

In the 1990s a new material, mineral trioxide aggregate (MTA) was developed at Loma Linda University as a root-end filling material. The first publication on the use of the material to seal root perforations was published in 1993.¹ It is

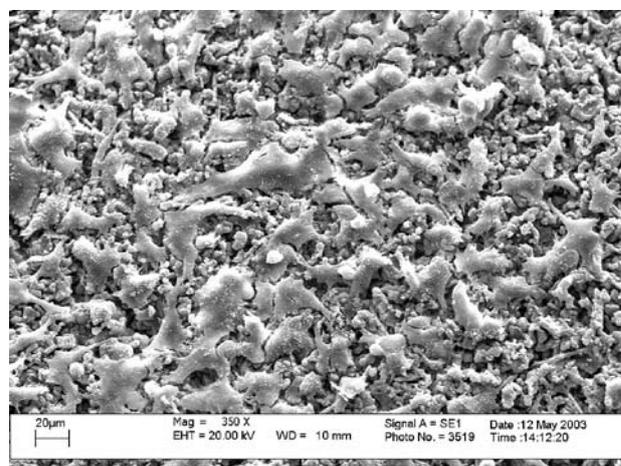


Figure 1: Surface morphology of mineral trioxide aggregate showing cell monolayer over the material (x350) (Camilleri et al. 2004).

Josette Camilleri BChD, MPhil
 Department of Building and Civil Engineering
 Faculty of Architecture and Civil Engineering
 University of Malta, Msida
 Email: joz@global.net.mt

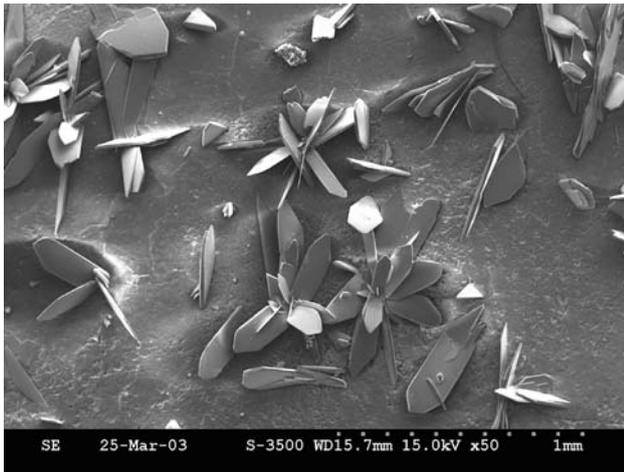


Figure 2: Surface morphology of cement showing calcium phosphate crystals deposited over the surface of the material (x50 mag.) (Camilleri et al. 2005a)

commercially available as ProRoot MTA (Tulsa Dental Products, Tulsa, OK, USA). The use of MTA as a root-end filling material was identified due to the fact that the material is a hydraulic cement i.e. it sets in the presence of water. A dental cement that sets and develops its properties in the presence of moisture is highly desirable. Mineral trioxide aggregate (MTA) is now used extensively in endodontics. Two commercial forms of MTA are available (ProRoot MTA); namely the grey and the white MTA both with similar chemical and physical properties. MTA is essentially Portland cement (used in the building industry as a binder in concrete) with 4:1 proportions of bismuth oxide added for radiopacity.² The material was originally reported to be composed of calcium and phosphate and its biocompatibility was attributed to its similarity to dental hard tissues.³ However Camilleri and co-workers⁴ have shown that MTA is composed primarily of tricalcium and dicalcium silicate, the main constituent elements of Portland cement, which on hydration produce a silicate hydrate gel and calcium hydroxide, not calcium phosphate as claimed by Torabinejad.^{2,3}

As the evidence presented by Torabinejad and co-workers^{2,3} for the biocompatibility of MTA have been shown to be invalid⁴, further studies were necessary to investigate the biocompatibility of MTA.

Biocompatibility testing

Biocompatibility testing is performed on materials that are placed in the human body. Human tissue reacts to the material in a variety of ways depending on the material type. These special materials which are able to function in intimate contact with biological fluids or living tissue with minimal adverse reaction or rejection by the body are called biomaterials. The mechanism of tissue attachment (if any) depends on the tissue response to the material surface. Materials can generally be categorised into three classes representing the type of tissue response they elicit: chemically inert, bioresorbable, or bioactive.

In vitro biocompatibility of a material is usually assessed

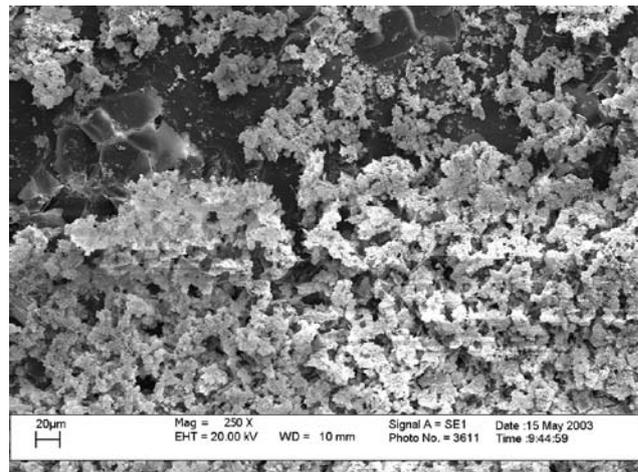


Figure 3: Diffuse calcium carbonate crystals (calcite) deposited over the material after critical point drying x 250 magnification (Camilleri et al. 2004)

against two controls: a negative control material, which does not produce a cytotoxic response and a positive control material, which provides a reproducible cytotoxic response. The purpose of the negative control is to demonstrate background response while that of the positive control is to demonstrate appropriate test system response. Tests are performed either on the material itself or on material extracts. Extraction vehicles include culture medium with and without serum, or physiological saline. Materials tested should have at least one flat surface and should be sterilized prior to subjecting it for biocompatibility testing. Biocompatibility of MTA has been tested using the following methods:

1. cell expression and growth⁵⁻²⁷
2. animal studies²⁸⁻³⁵

Cell expression and growth

The use of cell cultures in the study of endodontic materials was introduced by Rappaport and co-workers.³⁶ Cell culture techniques use either primary cells or immortal cell lines, which

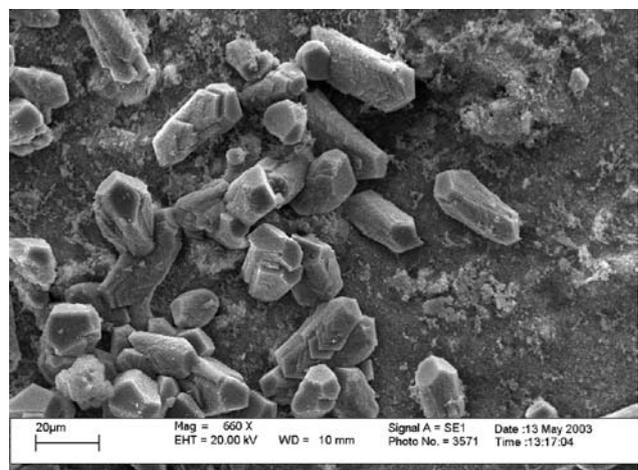


Figure 4: Large calcium carbonate crystals (aragonite) deposited over the material after critical point drying x 660 magnification (Camilleri et al. 2004)

Table 1: Cell type, contact time and method of assessment of the various studies conducted on MTA using cells

Author and date	Cell type	Contact time/days	Method of assessment
Torabinejad <i>et al.</i> 1995	Mouse L929	1	Agar overlay
Torabinejad <i>et al.</i> 1995	Mouse L929	1	Radiochromium release
Koh <i>et al.</i> 1997	MG 63	6	SEM
Koh <i>et al.</i> 1998	MG 63	1-7	SEM
Osorio <i>et al.</i> 1998	Gingival fibroblasts, L929	/	MTT, CV assay
Mitchell <i>et al.</i> 1999	MG 63	2, 4, 7	SEM
Keiser <i>et al.</i> 2000	PDL fibroblasts	1	MTT assay
Zhu <i>et al.</i> 2000	HOBs	1	SEM
Abdullah <i>et al.</i> 2002	SaOS-2	1, 2, 3	SEM
Saidon <i>et al.</i> 2003	Mouse L929	3	SEM
Haglund <i>et al.</i> 2003	Mouse L929, macrophages	3	SEM
Huang <i>et al.</i> 2003	U2OS		MTT assay
Perez <i>et al.</i> 2003	Osteoblasts, MG 63	6, 9, 13	SEM
Pistorius <i>et al.</i> 2003	PDL, Gingival fibroblasts	4	Cytosolic esterase activity
Camp <i>et al.</i> 2003	Gingival fibroblasts	1, 2, 3	fluorescence
Asrari and Lobner 2003	Neurons	12-14	enzyme assay
Balto 2004	PDL fibroblasts	1	SEM
Bonson <i>et al.</i> 2004	PDL, gingival fibroblasts	15	fluorescence
Pelliccioni <i>et al.</i> 2004	SaOS	1, 3	Assay
Camilleri <i>et al.</i> 2004	SaOS	1, 5, 7	SEM
Camilleri <i>et al.</i> 2005	HOS	1-7, 1-21	alamarBlue assay
Koulaouzidou <i>et al.</i> 2005	L929, BHK21/C13 fibroblasts	1, 2	Sulforhodamine-B assay
Nakayama <i>et al.</i> 2005	Rat bone marrow cells	3	"SEM, TEM"
Moghaddame-Jafari <i>et al.</i> 2005	Mouse odontoblastic cells	1	Flow cytometry

are cells derived clonally from normal or malignant cell cultures, usually sarcomas.³⁷ These cells are easily cultured and they exhibit increased phenotypic stability with serial passages which results in increased reproducibility of results in independently conducted experiments.³⁷

Established cell lines have an aneuploid chromosome pattern and thus tend to multiply rapidly with an unlimited life span if appropriately sub-cultured. They are easier to culture and therefore the quality of the cultured cells is more predictable. Aneuploid cells frequently used are L929 mouse fibroblasts³⁸, BHK 21 hamster fibroblasts³⁹, HeLa human cervical carcinoma epithelial cells⁴⁰ and NCTC 2544 human skin epithelial cells.⁴¹

The SaOS-2 cells represent a highly differentiated cell line capable of inducing bone formation and are thus a model for osteoblastic behaviour. They are more highly differentiated and their growth maintains a consistent cell phenotype.^{42,43} These osteosarcoma cells closely resemble the human osteoblast in its ability to express high levels of bone markers. MG 63 cells are derived from human osteosarcoma cells, however have fewer characteristics of mature osteoblasts.

Cell type

MTA and Portland cement show good induction of cell proliferation with the formation of a cell monolayer (Figure 1). Assessment of biocompatibility *in vitro* using

cell culture techniques has been widely used to study the biocompatibility of MTA as a biomaterial. (Table 1). Cell types used varied from immortal cell lines to animal cells and fibroblasts. Cell lines are usually the preferred cell types used for cytological investigations. Primary osteoblasts have however been shown to be more appropriate for testing endodontic materials in cell culture as they are more sensitive and form mineralized nodules when exposed to differentiation media. As cell lines do not behave osteogenically, primary osteoblasts are preferred.¹⁶

Evaluation of cell proliferation

a. Scanning electron microscopy

The method preferred for evaluation of cell proliferation was scanning electron microscopy (SEM) followed by enzyme assay. The main problem with the use of the scanning electron microscope in cell culture studies with MTA is the material reaction with the preparation media. Calcium hydroxide, a by-product of calcium silicate hydration, reacts with phosphate buffered solutions producing calcium phosphate crystals over the material surface (Figure 2).⁴ Camilleri and co-workers showed that this artifact was responsible for Torabinejad's conclusion that MTA resembled dental hard tissue. In addition

critical point drying, an essential step for material preparation prior to viewing under the scanning electron microscope causes cement carbonation (Figures 3, 4).²³

b. Enzyme assay

Enzyme assay, the next most preferred method to study cell proliferation on mineral trioxide aggregate, would seem to be more reliable as it avoids material preparation. Enzyme assay measures the metabolic activity of cells grown over the materials under study. This can be done by using alamarBlue™ and methyltetrazolium (MTT) assay in a method outlined in ISO 10993-5.⁴⁴ Cell proliferation is determined using a redox indicator that can be used to quantitatively measure proliferation of cells.⁴⁵ As the cells grow in culture, their metabolic activity maintains a reducing environment in the surrounding culture medium, whilst growth inhibition produces an oxidized environment. Reduction causes colour change of the alamarBlue™ indicator from non-fluorescent (blue) to fluorescent (red). In addition the cell activity on material elution can also be measured. This ensures that no toxic substances are leached from the material. The MTT assay⁴⁶ is dependent on the intact activity of the mitochondrial enzyme, succinate dehydrogenase, which is impaired after exposure of cells to toxic surroundings. The test involves the conversion of a tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide an insoluble formazan product, which can be quantified by spectrophotometry. Due to the poor knowledge of the chemical constitution of MTA very few studies were published on the material extracts of the material.

c. Cytokine expression

Biocompatibility can also be determined by quantifying the effect of the material on the normal bone physiology. Bone remodeling takes place in alternating phases of resorption and deposition. The first phase involves the recruitment and differentiation of the osteoblast precursors. The second phase is the production and mineralization of the bone matrix by the mature osteoblasts. The cytokines involved in bone formation are divided into cytokines that stimulate bone cell proliferation and those that have an inhibitory effect on the mature osteoblasts. The former include cytokines such as IL-1, tumour necrosis factor and macrophage-colony stimulating factor. For bone resorption to take place, bone formation must be halted. At this phase, stimulation of osteoblast precursors occurs in preparation for renewed bone formation. Here cytokines stimulate both precursor proliferation and mature osteoblast activity. MTA induced expression of cytokines from bone cells and exhibited good cell attachment. MTA caused an increase in IL-4 and IL-10 levels.¹⁵ Another study showed an increase in Interleukin 6, and 8 with no increase in levels of Interleukin 1 α and 1 β .⁹ Conversely other researchers^{6,7} showed a rise of both IL-1 α and 1 β together with IL-6 after the cells were in contact with the material for 144 hours. MTA increases osteocalcin levels⁴⁷ and also preferentially induces alkaline phosphatase

expression and activity in both periodontal ligament and gingival fibroblasts²¹ demonstrating an increase in osteoblast mineralizing activity. In one study where no cytokine production was demonstrated, cell lysis and protein denaturing around the MTA was observed.¹⁴

d. Other methods

The agar overlay method and radiochromium release methods⁵ were the least preferred methods to study cell proliferation. The main disadvantage of radiochromium release method is the use of radioactive isotopes.

Animal studies

Recommended methods for the evaluation of dental materials include a preliminary test that provides a general toxicity profile for the potential material and a secondary test that evaluates local toxicity, and a usage test in experimental animals. The effect of the test material on animal tissues can be studied by histological evaluation of the tissues involved. The main problem with these studies is the cost and upkeep of the test animals.

Subcutaneous and intra-osseous implantation

Subcutaneous implantation of the materials in test animals showed MTA initially elicited severe reactions with coagulation necrosis and dystrophic calcification.^{30, 34} The reactions however, subsided to mostly moderate with time. Reactions to intraosseous implants of both materials were less intense than with subcutaneous implantation. The main disadvantage with intra-osseous implantation is that the bony cavity created for placement of the material implant is an artificial socket with no resemblance to a tooth suspended in the periodontal ligament. Implantation of MTA in rat connective tissue produced granulations birefringent to polarized light and an irregular structure like a bridge was observed next to the material. In the dentin wall tubules a layer of birefringent granulations was also observed.³¹⁻³³ The tissue reaction to MTA implantation was the most favorable observed in both tibia and mandible of test animals, as in every specimen, it was free of inflammation. In the tibia, MTA was the material most often observed with direct bone apposition.^{29,30}

Histological assessment of peri-radicular tissues

MTA has been used to fill root ends of teeth in experimental animals. The teeth and surrounding bone were then resected and the presence of inflammation around the material used as a marker of material biocompatibility. MTA at the peri-radicular surgical site produced less periradicular inflammation and more fibrous capsules compared with amalgam.⁴⁸ In addition, the presence of cementum on the surface of MTA was a frequent finding.⁴⁹ The most characteristic tissue reaction to MTA was the presence of connective tissue after the first postoperative week. Inflammation was seen occasionally. Early tissue healing

events after MTA root-end filling were characterized by hard tissue formation, activated progressively from the peripheral root walls along the MTA-soft tissue interface.

Conclusions

Both MTA and Portland cement are bioactive materials. The biocompatibility of the materials had originally been attributed to the chemical similarity to tooth hard tissues namely calcium phosphate. However this has been shown not to be the case. MTA produces calcium hydroxide as a by-product of the hydration reaction.⁴ The similarity of action of both MTA and Portland cement to calcium hydroxide had been postulated.^{31,32}

Calcium hydroxide is used extensively in dentistry. When using SEM to study the biocompatibility of dental materials it is imperative to ensure there is no reaction between the material and the reagents used in the experimental procedure. Scanning electron microscopy thus is contraindicated to evaluate cell growth and expression over materials based on Portland cement. Other methods of assessing biocompatibility are thus preferred.

References

- Lee SJ, Monsef M, Torabinejad M. Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. *J Endod* 1993;19:541-4.
- Torabinejad M, White DJ. Tooth filling material and use. US Patent Number 5,769,638; 1995.
- Torabinejad M, Hong CU, McDonald F, Pitt Ford TR. Physical and chemical properties of a new root-end filling material. *J Endod* 1995;21:349-53.
- Camilleri J, Montesin FE, Brady K, Sweeney R, Curtis RV, Pitt Ford TR. The constitution of mineral trioxide aggregate. *Dent Mater* 2005; 21:297-303.
- Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD. Cytotoxicity of four root-end filling materials. *J Endod* 1995;21:489-492.
- Koh ET, Torabinejad M, Pitt Ford TR, Brady K, McDonald F. Mineral Trioxide Aggregate stimulates a biological response in human osteoblasts. *J Biomed Mater Res* 1997;37:432-9.
- Koh ET, McDonald F, Pitt Ford TR, Torabinejad M. Cellular response to Mineral Trioxide Aggregate. *J Endod* 1998; 24:543-7.
- Osorio RM, Hefti A, Vertucci FJ, Shawley AL. Cytotoxicity of endodontic materials. *J Endod* 1998;24:91-6.
- Mitchell PJC, Pitt Ford TR, Torabinejad M, McDonald F. Osteoblast biocompatibility of mineral trioxide aggregate. *Biomater* 1999;20:167-73.
- Keiser K, Johnson CC, Tipton DA. Cytotoxicity of mineral trioxide aggregate using human periodontal ligament fibroblasts. *J Endod* 2000;26:288-91.
- Zhu Q, Haglund R, Safavi KE, Spangberg LS (2000) Adhesion of human osteoblasts on root-end filling materials. *J Endod* 2000;26:404-6.
- Abdullah D, Pitt Ford TR, Papaioannou S, Nicholson J, McDonald F. An evaluation of accelerated Portland cement as a restorative material. *Biomater* 2002;23:4001-10.
- Saidon J, He J, Zhu Q, Safavi K, Spangberg LS. Cell and tissue reactions to mineral trioxide aggregate and Portland cement. *Oral Med, Oral Surg, Oral Path, Oral Radiol, Endod* 2003;95:483-489.
- Haglund R, He J, Jarvis J, Kamran E, Safavi KE, Spangberg LSW, and Zhu O. Effects of root-end filling materials on fibroblasts and macrophages in vitro. *Oral Med, Oral Surg, Oral Path, Oral Radiol, Endod* 2003;95:739-45.
- Huang TH, Ding SJ, Hsu TC, Kao CT. Effects of mineral trioxide aggregate (MTA) extracts on mitogen-activated protein kinase activity in human osteosarcoma cell line (U2OS). *Biomater* 2003;24:3909-13.
- Perez Al, Spears R, Gutmann JL, Opperman LA. Osteoblasts and MG63 osteosarcoma cells behave differently when in contact with ProRoot™ MTA and white MTA. *Int Endod J* 2003;36:564-70.
- Pistorius A, Willershausen B, Briseno Marroquin B. Effect of apical root-end filling materials on gingival fibroblasts. *Int Endod J* 2003;36:610-5.
- Camp MA, Jeansonne BG, Lallier T. Adhesion of human fibroblasts to root-end-filling materials. *J Endod* 2003;29:602-7.
- Asrari M, Lobner D. In vitro neurotoxic evaluation of root-end-filling materials. *J Endod* 2003;29:743-6.
- Balto HA. Attachment and morphological behavior of human periodontal ligament fibroblasts to mineral trioxide aggregate: a scanning electron microscope study. *J Endod* 2004;30:25-9.
- Bonson S, Jeansonne BG, Lallier TE. Root-end filling materials alter fibroblast differentiation. *J Dent Res* 2004;83:408-13.
- Pelliccioni GA, Ciapetti G, Cenni E, Granchi D, Nanni M, Pagani S, et al. Evaluation of osteoblast-like cell response to Proroot MTA (mineral trioxide aggregate) cement. *J Mater Sci Mater Med* 2004;15:167-73.
- Camilleri J, Montesin FE, Papaioannou S, McDonald F, Pitt Ford TR. Biocompatibility of two commercial forms of mineral trioxide aggregate. *Int Endod J* 2004;37:699-704.
- Camilleri J, Montesin FE, DiSilvio L, Pitt Ford TR. The chemical constitution and biocompatibility of accelerated Portland cement for endodontic use. *Int Endod J* 2005 (accepted for publication).
- Koulaouzidou EA, Papazisis KT, Economides NA, Beltes P, Kortsaris AH. Antiproliferative effect of mineral trioxide aggregate, zinc oxide-eugenol cement, and glass-ionomer cement against three fibroblastic cell lines. *J Endod* 2005;31:44-6.
- Nakayama A, Ogiso B, Tanabe N, Takeichi O, Matsuzaka K, Inoue T. Behaviour of bone marrow osteoblast-like cells on mineral trioxide aggregate: morphology and expression of type I collagen and bone-related protein mRNAs. *Int Endod J* 2005;38:203-10.
- Moghaddame-Jafari S, Mantellini MG, Botero TM, McDonald NJ, Nor JE. Effect of ProRoot MTA on Pulp Cell Apoptosis and Proliferation In Vitro. *J Endod* 2005;31:387-91.
- Torabinejad M, Hong CU, Pitt Ford TR, Kariyawasam SP. Tissue reaction to implanted Super EBA and Mineral Trioxide Aggregate in the mandible of guinea pigs: a preliminary report. *J Endod* 1995;21:569-71.
- Torabinejad M, Ford TR, Abedi HR, Kariyawasam SP, Tang HM. Tissue reaction to implanted root-end filling materials in the tibia and mandible of guinea pigs. *J Endod* 1998;24:468-71.
- Moreton TR, Brown CE Jr, Legan JJ, Kafrawy AH. Tissue reactions after subcutaneous and intraosseous implantation of mineral trioxide aggregate and ethoxybenzoic acid cement. *J Biomed Mater Res* 2000;52:528-33.
- Holland R, de Souza V, Nery MJ, Otoboni Filho JA, Bernabe PF, Dezan Junior E. Reaction of rat connective tissue to implanted dentin tubes filled with mineral trioxide aggregate or calcium hydroxide. *J Endod* 1999;25:161-6.
- Holland R, de Souza V, Nery MJ, Faraco Junior IM, Bernabe PF, Otoboni Filho JA, Dezan Junior E. Reaction of rat connective tissue to implanted dentin tube filled with mineral trioxide aggregate, Portland cement or calcium hydroxide. *Braz Dent J* 2001;12:3-8.
- Holland R, de Souza V, Nery MJ, Faraco Junior IM, Bernabe PF, Otoboni Filho JA, Dezan Junior E. Reaction of rat connective tissue to implanted dentin tubes filled with a white mineral trioxide aggregate. *Braz Dent J* 2002;13:23-6.
- Yaltirik M, Ozbas H, Bilgic B, Issever H (2004). Reactions of connective tissue to mineral trioxide aggregate and amalgam. *J Endod* 2004;30:95-9.
- Sousa CJ, Loyola AM, Versiani MA, Biffi JC, Oliveira RP, Pascon EA. A comparative histological evaluation of the biocompatibility of materials used in apical surgery. *Int Endod J* 2004;37:738-48.

36. Rappaport HM, Lilly GE, Kapsimlis P. Toxicity of endodontic filling materials. *Oral Surg, Oral Med, Oral Path* 1964;18:785-802.
37. Hughes FJ, Aubin JE. Culture of cells of the osteoblast lineage. In *Methods in Bone Biology*. Arnett TR, Henderson B. Chapman and Hall UK;1998, 1-39.
38. Wennberg A, Hasselgren G, Tronstad L. A method for toxicity screening of biomaterials using cell cultured on Millipore filters. *J Biomed Res* 1979;13:109-20.
39. Tyas MJ. A method for the in vitro toxicity testing of dental restorative materials. *J Dent Res* 1977;56:1285-90.
40. Spangberg L. Kinetic and quantitative evaluation of material cytotoxicity in vitro. *Oral Med, Oral Surg, Oral Path* 1973;35:389-401.
41. Leirskar J, and Helgeland K. A methodologic study of the effect of dental materials on growth and adhesion of animal cells in vitro. *Scand J Dent Res* 1972;80:120-33.
42. Rifas L, Fausto A, Scott MJ, Avioli LV, Welgus HG. Expression of metalloproteinases and tissue inhibitors of metalloproteinases in human osteoblast-like cells: differentiating is associated repression of metalloproteinase biosynthesis. *Endocrinol* 1994;134:213-21.
43. McQuillan DJ, Richardson MD, Bateman JF. Matrix deposition by a calcifying human osteogenic osteosarcoma cell line. *Bone* 1995;16:415-26.
44. International Standard: Biologic evaluation of medical devices Part 5. Tests for cytotoxicity: in vitro methods. ISO 10993-5 1992.
45. O'Brien J, Wilson I, Orton T, Pognan F. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *Eur J Biochem* 2000;267:5421-6.
46. Mosmann T. Rapid calorimetric assay for cellular growth and survival; application to proliferation and cytotoxicity assays. *J Immunol Meth* 1983;65:55-63.
47. Thomson TS, Berry JE, Somerman MJ, Kirkwood KL. Cementoblasts maintain expression of osteocalcin in the presence of mineral trioxide aggregate. *J Endod* 2003;29:407-12.
48. Torabinejad M, Hong CU, Lee SJ, Monsef M, Pitt Ford TR. Investigation of mineral trioxide aggregate for root-end filling in dogs. *J Endod* 1995;21:603-8.
49. Torabinejad M, Pitt Ford TR, McKendry DJ, Abedi HR, Miller DA, Kariyawasam SP. Histologic assessment of Mineral Trioxide Aggregate as root end filling material in monkeys. *J Endod* 1997;23:225-8.