Original Research Article

Evaluation of cytotoxicity and cell adhesion property of BioRoot canal sealer with two different type of endodontic sealers

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Abstract

Aim: In vitro cytotoxicity test, represent the first stage of biocompatibility of screening process. The different assays being assessed are cell membrane, enzyme activity, genetic effect and in addition cell adhesion and spread over root end material has been suggested as an evaluation criteria. The present study was conducted with two different root canal sealers to evaluate its cytotoxicity and cell adhesion property in comparison with BioRoot canal sealer. **Material and methods:** A total of 3 sealers were selected AH plus, MTA and Bioroot canal sealer. The human fibroblast was removed during gingivectomy in patients free of periodontal diseases and stored in Dulbecco-modified Eagle medium (DMEM) (Gibco, Grand Island, NY). The cytotoxicity test was assessed by MTT reduction test (Metabolic Assay test). The cell attachment was assessed by Zeiss Compound Microscope. **Results:** The result showed highest fibroblast attachment cell survival by MTT assay with Bioroot canal sealer followed by MTA and the least for AH Plus sealer. Also it was seen that the maximum fibroblast was seen on Day 1 and it considerably reduced from Day 3 to Day7.
Keywords: AH Plus, MTA, Bioroot canal sealer, Cytoxicity , MTT assay, Scanning Electron Microscope.

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INTRODUCTION

The choice of a biocompatible sealing material is crucial to the clinical success of endodontic therapy.[1] Although sealers were developed to be confined within the root canal system, their extrusion over the apical constriction is frequently observed.[2,3] Therefore, these materials should have good biocompatibility and be well tolerated by the peri-apical tissues.[4] Endodontic sealers are supposed to fill the irregularities between the dentinal walls and the gutta-percha core as well as the lateral or accessory canals and bond both to gutta-percha and dentin.[5,6] In addition to defined requirements of mechanical and physical properties[7], the biological compatibility of root canal sealers is important because they come into contact with periapical tissues[8] and the tissue response to the sealers may influence the final outcome of the root canal treatment.[9] Each one of the sealer has its own merits and demerits. Zinc Oxide Eugenol was the most commonly used sealer and was used as the standard in many studies for the comparison with other sealers. Presently, routinely used sealers in Endodontics are based on epoxy resin, calcium hydroxide, Glass Ionomer, polycaprolactone and Bis-GMA. Pre-mixed bioceramic endodontic sealer (Endosequence BC Sealer, Brasseler USA, Savannah, GA, USA) was recently proposed as an alternative root canal filling material. Calcium phosphate was first used as bioceramic restorative dental cement by LeGeros *et al.* [10] However, the first documented use of bioceramic materials

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as a root canal sealer was by Krell and Wefel. [11] The sealers with good biocompatibility are beneficial to aid or stimulate the repair of injured tissues. [1] In-vitro cytotoxicity tests represent the first stage of biocompatibility screening process, with different assays being used to assess, the effects of a biomaterial on cell number, cell growth, cell membrane integrity, enzyme activity, or genetic effects. [12] In addition, cell adhesion and spread over root-end filling materials has been suggested as an evaluation criterion [13]

MATERIALS AND METHODS

MTA Sealer (Brasseler, Savannah, GA, USA, Batch No. 0900458) is a premixed ready-to-use injectable material, based on a calcium silicate composition; Pulp Canal Sealer EWT (Pulp Canal Sealer EWT; SybronEndo, Orange, CA, USA, Batch No. 9-1222) is a Zinc Oxide Eugenol based sealer; AH Plus Jet (Dentsply/Detrey, Konstanz, Germany, Batch No. 1004002041) is an epoxy resin based root canal sealer and consists of a paste system, with paste A containing epoxy resin and iron oxide, and paste B containing amines and silicone oil.

Table 1: Sealers used along with their composition and details of manufacturing company

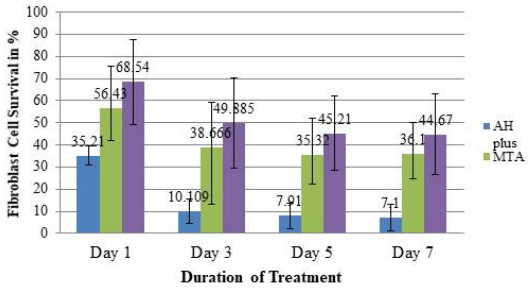
Sealer	Composition	Manufacturer
MTA	Zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, filler and thickening agents	Brasseler USA, Savannah, GA, USA
AH-Plus Jet	Paste A: bisphenol-A epoxy resin, bisphenol-F epoxy resin, calcium tungstate, zirconium oxide, silica, bisphenol-A epoxy resin, bisphenol-F epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide pigments Paste B: dibenzyldiamine, aminoadamantane, tricyclodecane diamine, calcium tungstate, zirconium oxide, silica, silicone oils	Dentsply/Detrey, Konstanz, Germany
BioRoot canal sealer	Powder: tricalcium silicate, zirconium oxide and povidone Liquid: aqueous solution of calcium chloride and polycarboxylate	Septodont, Saint Maur-des-Fosses, France

Cell Culture:

In vitro, cytotoxicity of the endodontic sealers was evaluated using human gingival fibroblast obtained from gingival tissue removed during gingivectomy in patients who were free of periodontal disease.[1] Standard protocols were followed in establishing and maintaining the cultures. Dulbecco-modified Eagle medium (DMEM) (Gibco, Grand Island, NY) supplemented with 100 mg/ml Penicillin G, 50 mg/mL Streptomycin, 0.25 mg/ml Fungizone (Gibco) and 10% fetal bovine serum (Gibco) was used as the cell culture medium. Fibroblasts of the seventh to eighth passage were used for both cytotoxicity and cell adhesion assays.

Metabolic Activity Assay (MTT reduction):

Cytotoxic potential of retrofilling materials was assessed based on norm (ISO 10993-5.2009) (International Standardization Organization) using the 3-(dimethylthiazol-2-il)-2 diphenyl tetrazolium bromide assay (MTT) (Sigma Aldrich St Louis, MO, USA). Briefly, the same cultures that were used to take fibroblast pictures were removed from their conditioned media and 50 µL of dissolution of 1 mg/ mL MTT were added into each well. Cultures were protected from the light and kept at 37 ºC with 5% CO2 for 2 h. After this time, MTT was removed and 100 µL of isopropanol were added (TECSIQUIM, TSQ, Iztacalco, Mexico City). Cultures were incubated for 30 min at room temperature. After this time, dissolution absorbance was measured at (Vermont USA). Absorbance values were normalized considering 100% as the absorbance obtained from untreated cultures 590 nm in a plate reader model Synergy HT, Bio-Tek brand. [14]



Graph 1: Fibroblast cell survival in percentage after Dental Sealer application assessed using MTT assay

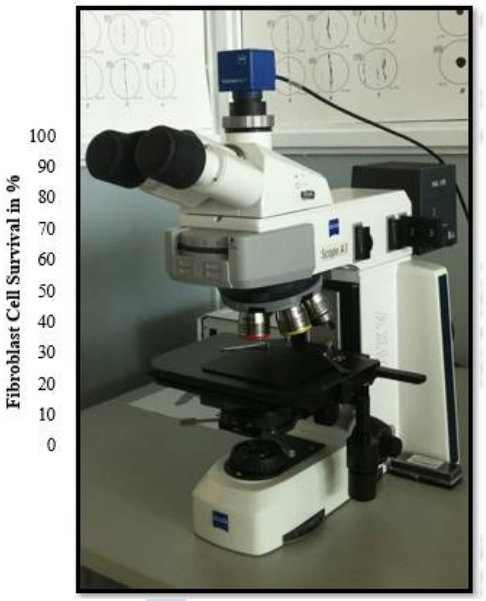
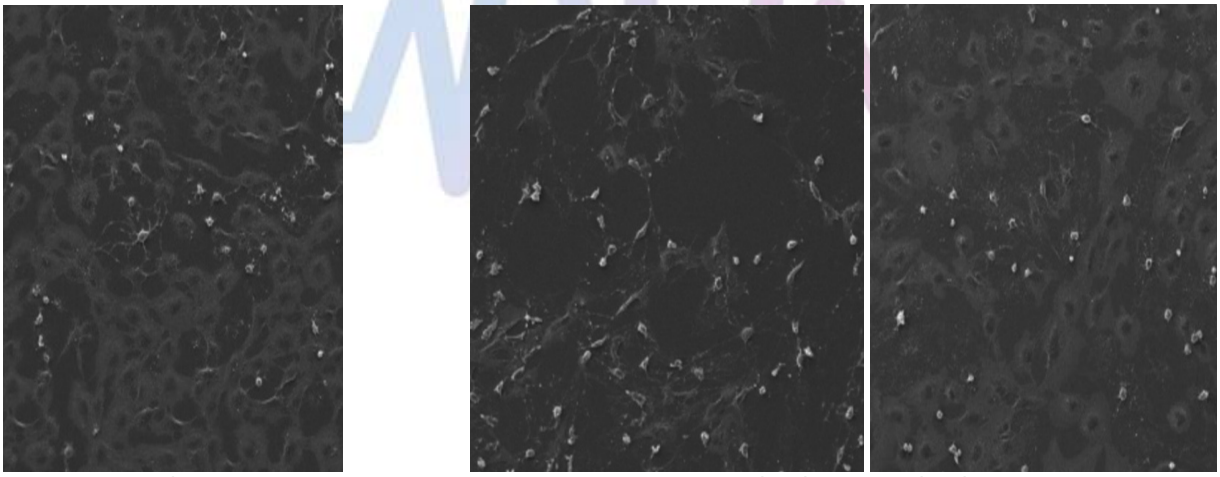


Figure 1: Zeiss Compound Microscope

Criteria for Image Analysis:

The image analysis was performed by one operator who was not blind to the study. Cell attachment was assessed by the presence of filopodia (cylindrical/conical processes, often 10-20 µm long with a small diameter); microvilli (the processes of smallest diameter, 0.1-0.2 µm); lamellipodia (flat extensions, with their thickness reaching 0.1-0.5 µm); Blebs (round extensions, their diameters ranging between 1-2 µm).[13]



AH Pluse MTA Sealer BioRoot Canal Sealer

Figure 2: Scanning Electron Microscopy Images (500x) of Gingival fibroblasts adhered to Endodontic sealers

RESULTS

MTT Assay result showed the highest survival of fibroblast for Bioroot canal sealer followed by MTA and least survival for AH Plus on Day 1. On day 3, Day 5 and Day 7 it has reduced gradually in similar manner. (Graph-1)SEM study shows images at (500X) gingival fibroblast adhered to endodontic sealer ,fibroblast adhesiveness is seen maximum to Bioroot sealer followed by MTA and least for AH Plus. Image analysis done on SEM.(Fig:2)

DISCUSSION

The cytotoxicity of endodontic sealers may cause cellular degeneration and delayed wound healing because of the direct contact of sealers with periapical tissues. This study aimed at evaluating the essential property of root canal sealer, such as cytotoxicity and cell adhesion. Cytotoxicity was evaluated following the international standard ISO 10993-5 (ISO 10993. 2009), which describes the tests for assessing cytotoxicity in vitro. Cytotoxicity was assessed

using human gingival fibroblasts, Fibroblast attachment is an essential requirement for the formation of a new attachment apparatus to root surfaces following endodontic surgery22 and thus may be an important predictor of the success of surgical endodontic treatment. In the present study, attachment of cells was assessed qualitatively by SEM, which allows a close observation of cellular morphology and reaction to the filling material and this method has been used by several investigators.[2,4,5] MTA Fillapex was developed in an attempt to take advantage of excellent biological properties of MTA for root canal sealers. According to the present results, MTA Fillapex showed cytotoxicity for all tested incubation periods. Interestingly, Vitti *et al*.[15] showed that MTA Fill apex solubility increases over time, from -9.31% weight variation at day one to -25.55% weight variation after 28 days. Another possible explanation for the cytotoxicity of MTA Fillapex is a highly alkaline pH environment, which is associated with setting products (calcium hydroxide) that releases hydroxyl ions .[15]

CONCLUSION

MTT Assay and Quantitative analysis done by SEM showed Bioroot canal sealer to be least cytotoxic thus showing the highest compatibility followed by MTA while AH-Plus sealer showed maximum cytoxicity in both the analysis.

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