Biocompatibility of Three Root End Filling Materials

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Dental materials should be biocompatible, in order to prevent any adverse effects on the surrounding tissue caused by direct contact. The ideal root end filling material must have certain characteristics, including biocompatibility, satisfying marginal sealing quality, ability to permit or induce repair of alveolar bone, promote periapical healing and antimicrobial activity. In this study, the cytotoxicity of different materials (amalgam, MTA and Biodentine) was evaluated on a permanent fibroblast cell lines (MRC-5 and L929). The cytotoxicity of all three types of materials was investigated using standard biocompatibility tests: DET, MTT and agar diffusion test. MTT test showed that after 24 and 48 hours in both cell cultures, Biodentine had the largest percentage of citotoxicity. The lowest percentage of cytotoxicity showed MTA in both groups. After 72 h in both cell lines, the highest percentage of cytotoxicity had amalgam. The lowest percentage of cytotoxicity showed MTA in both groups. Observing the results of Agar diffusion test, there was no any discoloration detected, neither lysis of cells under the disc. Biocompatibility tests showed high level of cell compatibility of all three tested materials.

Keywords: Biocompatibility, Root End, MTA, Biodentine, Amalgam.

1. INTRODUCTION

Biocompatibility is defined as the ability of a material to function in a specific application in the presence of a corresponding host response.¹ Biocompatible materials must be free of any risks, in order to prevent any adverse effects on the surrounding tissue caused by direct contact. The ideal root end filling material should possess certain characteristics including biocompatibility, satisfying marginal sealing quality, ability to permit or induce alveolar bone repair, periapical healing promotion and antimicrobial activity.²⁻⁵

Commonly used root-end filling materials are mineral trioxide aggregate (MTA), amalgam, glass-ionomer cements, Super EBA and intermediate restorative material (IRM).⁶⁻⁸ Recently, tricalcium silicate-based cement (Biodentine⁹) has been introduced as a new root-end filling material.

Dental amalgam has been widely used root-end filling material for a long time. Amalgam consists of metallic mixtures whose major component is mercury. Contemporary high copper amalgams alloys comprise higher concentrations of copper, silver, tin, and zinc.⁹

Biodentine is a new root-end filling material, setting by mixing the powder and liquid components. The powder consists of dicalcium and tricalcium silicate, the components of Portland cement and calcium carbonate. Zirconium-dioxide is added as contrast medium. The liquid consists of aqueous solution of calcium chloride with a mixture of polycarboxylate.¹⁰

MTA was developed by Torabinejad at Loma Linda University in 1993, and it was originally designed for root end filling and pulp chamber perforations repair. The major ingredients are dicalcium and tricalcium silicates, tricalcium oxide, tricalcium aluminate, and silicate oxide. Bismuth has been added as a radiopacifier.¹¹

In vitro tests are mainly used to assess the biocompatibility of dental materials using determined cell lines as well as primary cells. Assays are providing information about different cellular functions. Commonly used tests are: test with tetrazolium salt (MTT), dye exclusion test (DET), agar diffusion test.

In this study, the cytotoxicity of different materials (amalgam, MTA and Biodentine) was evaluated on a permanent fibroblast cell lines (MRC-5 and L929).

The purpose of this study was to evaluate the biocompatibility of three root end filling materials: amalgam.
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2. EXPERIMENTAL PROCEDURE

Three types of materials were used in this study (amalgam, MTA, Biodentine). The materials were prepared according to the manufacturer’s instructions, and after that they were placed in the disk shaped molds, 10 mm in diameter, 2 mm height and left to set. Three discs of each material were prepared and a total of nine specimens was analyzed. After setting the specimens were removed from the molds and sterilized. Sterilization was performed with cobalt-60 (60Co) radiation source and the dynamics of 9 kGy/1 h. The total dose of gamma radiation was 25 kGy.

2.1. Cell Lines

Cell Lines Tests were carried out using human fibroblasts (MRC-5) and mouse fibroblasts (L929) which grow affixed to the surface of the court (Costar, 25 cm²) in the Dulbecco’s modified Eagle’s medium (DMEM, Gibco BRL, UK) supplemented with 4.5 g/l of glucose and 10% FCS (fetal calf serum, NIVNS). The medium contained antibiotics: penicillin 100 IU/ml and streptomycin 100 µg/ml. The cell line was maintained under standard conditions: at 37 °C in an atmosphere of saturated humidity and 5% CO₂ in incubator (Heraeus). Cells were cultured for two weeks with a medium changes twice a week. Experiment cells were used in the logarithmic phase of growth between the third and tenth transplantation. The experiment involved the products of formazan and it was used for the estimation of cell attachment and proliferation. Cells were collected in quadruplicates in a microtiter plate with 96 holes. The fraction of surviving cells was expressed as a percentage of control values according to the formula:

\[ CI = \left(1 - \frac{N_i}{N_s}\right) \times 100 \]

where \(N_s\) is the number of cells in the samples and the \(N_i\) is the number of cells with tested substance.

Cytopotoxicity was expressed in percents according to formula:

\[ \%K = \left(\frac{N_i}{N_s}\right) \times 100 \]

where \(N_s\) is the number of cells in the samples and the \(N_i\) is the number of cells with tested substance.

2.2. Colorimetric Test with Tetrazolium Salt (MTT)

MTT test is based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to the products of formazan and it was used for the estimation of cell attachment and proliferation. Cells were collected in the logarithmic growth phase, trypsinized, counted, and resuspended in 0.1% trypan blue. The viability of cells used in the experiment was higher than 90%.

2.3. Dye Exclusion Test (DET)

The trypan blue dye exclusion test was applied for the separation of viable and nonviable cells. Cells were harvested in the logarithmic growth phase, trypsinized, counted, and resuspended in 0.1% trypan blue. Viable cells were seeded in Petri dishes (Center well, Falcon) into the tested substance/disks in the concentration of 2 \(\cdot\) 10^5/1 ml. The control samples did not contain tested substance. Petri dishes with seeded cells were left in thermostat at 37 °C and 5% CO₂ for 24 hours (h). At the end of incubation after 24 hours, cell counting was performed using the inverted microscope chambers for counting. Neubauer’s chamber was used, and the cells were counted in four squares. Each square was divided into 16 smaller squares making a total of 64 squares. Then, 100 µl were taken and 100 µl of 0.1% trypan blue color was added. After being shaken intensively, several drops were applied to both field chambers for counting. The dead cells were dyed in trypan-blue while the viable cells were unstained, according to previously described protocol.

Number of cells in 1 ml of the suspension was calculated according to formula:

\[ X = x \cdot 10^2 \cdot 1000 \]

10 — Depth of Commerce
2 — dilution factor
1000 — volumetric coefficient

\[ x \] — number of cells in 16 squares (the average number of cells in 4 · 16 squares).
2.4. Agar Diffusion Test

Eagle’s Basal Medium which contained 2.2 g/l sodium bicarbonate, 3.0 g/l HEPES and 50 ml/l of serum was used in this test. Double concentration of medium was prepared without HEPES, and Na₂CO₃ 1 g/l was reduced. Agar medium was sterilized by autoclaving and filtration. Neural-red solution was kept and protected from light. Petri dishes were used in diameter 100 mm suitable for cell culture. Cells were harvested in the logarithmic growth phase.

2.4.1. Calculation

Discoloration zone around the test material and the control was assessed using inverted microscope with a calibrated partition index discoloration (Decolorization index) and the index of lysis (Lysis Index) was determined for each sample.

Cell response = Decolorization Index/Lysis Index

The statistical significance of the differences in cell viability between tested materials was analyzed by two-tailed Student’s t-test and a p value of less than 0.05 was considered significant.

3. RESULTS

3.1. MTT Test Results

The influence of tested materials on cell growth was measured by MTT assay 24, 48 and 72 hours after incubation. Figure 1 shows the cytotoxicity index (%) of root end filling materials on MRC5 cell line, and Figure 2 shows the cytotoxicity index (%) of root end filling materials on L929 cell line. After 24 and 48 hours MTT test showed that the Biodentine was significantly more toxic than the other materials. MTA showed the lowest percentage of cytotoxicity in both groups. After 72 h in both cell lines, amalgam exhibited the highest percentage of cytotoxicity.

3.2. DET Test Results

The lowest percentage of cytotoxicity showed MTA in both groups, with an average percentage of cytotoxicity of 11.23% on mouse fibroblast cell line L929 and 9.75% on human fibroblast cell line MRC5. The highest cytotoxicity index was 16.35% (Amalgam, mouse fibroblast cell line L929). The highest level of cytotoxicity on human fibroblast cell line MRC5 was demonstrated by Biodentine 10.76% (Fig. 3).

3.3. Agar Diffusion Test Results

Observing the results there was no any discoloration detected, neither lysis of cells under the disc. Cell response is 0/0, which indicates that this test has not established a cytotoxic effect of the tested materials in the cell line L929 and MRC-5.
4. DISCUSSION

Dental materials should have physical performances suitable to create an apical seal, they should be biocompatible and nontoxic, with no effect on cell morphology. In this study, the cytotoxicity of different materials (amalgam, MTA and Biodentine) was evaluated on a permanent fibroblast cell lines (MRC-5 and L929).

Dental amalgam has been commonly used root-end filling material for a long time. Kaga et al. showed that amalgam had little cytotoxicity despite the declared potential toxicity of its mercury components and affinity to discharge toxic elements. The results in the present study agree with previous ones that showed that amalgam has a lower effect on cell growth and morphology.

Recent experiments have shown that MTA is very efficient for filling root ends, restoring impaired roots and repairing root perforations. Under physiological requirements, MTA set progressively (over a period of few hours). The set material has low percentage of cytotoxicity and good biocompatibility without effect on cell morphology and it seems that MTA induces a favorable tissue response. This agrees with our findings that set MTA showed the lowest percentage of cytotoxicity in both cell groups.

Biodentine is a new calcium silicate-based material designed to be placed in permanent and close contact with periradicular tissue. It is important to assess its possible cytotoxic effects. Having in mind that Biodentine is relatively new material in endodontics and periradicular surgery, there are only few reports about its biocompatibility. In the study published by Zhou et al. it has been reported that Biodentine and MTA exhibited similar cytotoxic effects. Results of our study showed that Biodentine was more toxic than MTA and dental Amalgam. However, the results of the cell survival showed high level of cell compatibility of all the tested materials, because none of tested materials had a cytotoxicity index greater than 20%.

Numerous methods are available for cytotoxicity testing of materials. The results of toxicity tests depend on the choice of cell cultures and tests for cytotoxicity. More tests and cell types are definitely useful for more precise prediction of dental materials’ biocompatibility. The methyl-thiazol-tetrazolium (MTT) colorimetric assay is widely used as a standard assay for evaluating the cytotoxicity of new biomaterials and has been routinely used to test dental materials in cell culture systems. Besides MTT, DET and agar diffusion test are also frequently used test for obtaining valuable data regarding dental materials’ biocompatibility.

Experiments with cell cultures are the method of choice for biocompatibility surveys because they are fast, cheap and simple compared to other methods.

5. CONCLUSIONS

- MTT and DET tests showed that MTA had the lowest percentage of cytotoxicity in both groups.
- DET test showed high level of cell compatibility of all the tested materials, because none of tested materials had a cytotoxicity index greater than 20%.
- Agar diffusion test has not established a cytotoxic effect of the tested materials in the cell line L929 and MRC-5.
- The results of the cell survival in MTT, DET and Agar diffusion test, showed high level of cell compatibility of all tested materials.

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References and Notes


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