

Evaluation of Biocompatibility of Bioactive Glass, Bioactive Glass-Hydroxyapatite and Mineral Trioxide Aggregate-An in Vitro Study

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Abstract: The aim of this study is to evaluate the biocompatibility of Bioactive glass (BG) and Bioactive glass-Hydroxyapatite (BG-HA) in comparison to Mineral Trioxide Aggregate (MTA). The biocompatibility was assessed by estimation of cell count and alkaline phosphatase activity on Osteoblastic cell line (Saos-2). The biocompatibility was statistically analyzed using one way ANOVA followed by Tukey HSD Post hoc test. The study showed that Bioactive glass-Hydroxyapatite was less cytotoxic than BG and MTA and not significantly different from each other. Bioactive glass-hydroxyapatite showed better biocompatibility compared to bioactive glass and Mineral Trioxide Aggregate.

Key words: Biocompatibility • Bioactive Glass • Bioactive Glass-Hydroxyapatite • Mineral Trioxide Aggregate

INTRODUCTION

The ideal root end filling material should have biocompatible characteristics, dimensional stability, adhesiveness, low solubility and the capacity to create a seal at the apical third of the canal to isolate the root canal system from the periapical region.

Amalgam, Super-EBA, IRM, Diaket, GIC and recently Mineral Trioxide Aggregate are being used as root-end filling materials, but each has its own drawbacks.

Mineral Trioxide Aggregate (MTA) is an accepted retro filling material with good physical, chemical and biologic properties but with the disadvantages of difficult manipulation and prolonged setting time.

Bioactive glass is a type of a bioceramic. It is used as a type of alloplastic synthetic bone graft, which has the property to promote adsorption and concentration of proteins utilized by osteoblasts to form a mineralized extra cellular matrix and, thus, promote osteogenesis by allowing rapid formation of bone [1].

Bioactive glass has been used to reduce the permeability of dentin by providing permanent occlusion of dentinal tubules [2] and also as a pulp capping agent [3].

Bioactive glass-Hydroxyapatite is a new generation composite bioactive material, made up of 50% bioactive glass (BG) and 50% hydroxyapatite mixture.

The purpose of this study was to evaluate the biocompatibility of bioactive glass-Hydroxyapatite on Saos-2 cell line in comparison to MTA and bioactive glass.

MATERIALS AND METHODS

Biocompatibility Test:

Preparation of Material Extract : One gram of Bioactive glass (BG) granules (Perioglas), bioactive glass-Hydroxyapatite (BG-HA) granules (Grabio glascera) and Mineral Trioxide Aggregate (MTA- Angelus) were mixed with McCoy's medium in a centrifuge tube and incubated at 37°C for 24 hours. The supernatants were collected and added to the cell culture dish in triplicates. Then Saos-2 cells were seeded in each plate. A culture dish containing Saos-2 cells without material extracts was used as control group.

The plates were then incubated at 37° C with 5% Carbon dioxide for 72 hours. The test groups were then tested for cell number and alkaline phosphatase activity.

Cell Number: The supernatant was removed from each of the test groups. Saos-2 cells were detached from the surface of the plate using 0.2 % Trypsin. The cells were then washed with Phosphate buffered saline and cells were stained with 4% Trypan blue and cell viability was assessed using hemocytometer.

Alkaline Phosphatase Release: Alkaline phosphatase released in the medium at 72 hours after addition of material extract in Saos-2 cells was measured to check the maintenance of the osteoblastic phenotype. Alkaline phosphatase was assayed by using the conversion of 5mol/L colorless p-nitro phenyl phosphate to colored p-nitro phenol. The colour change was measured spectrophotometrically at 405 nm wavelength.

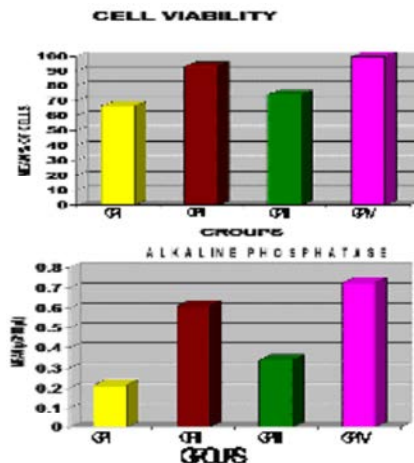
The Study Groups Were:

- Group I - Bioactive glass (BG)
- Group II - Bioactive glass-Hydroxyapatite (BG-HA)
- Group III - Mineral Trioxide aggregate (MTA)
- Group IV- Negative control

Statistical Analysis: Cell viability and alkaline phosphatase activity were analyzed statistically using one-way ANOVA followed by Tukey HSD Post hoc test.

RESULTS

The biocompatibility tests evaluated by cell count and alkaline phosphatase activity showed that group II was not significantly different from group IV; groups I and III were highly significant from group IV and groups I and III were significantly different from group II ($p < 0.01$).



DISCUSSION

A root end filling is commonly placed after resection and preparation of the root end during endodontic surgery. The most ideal healing outcome after filling of the resected root end would be formation of a normal attachment apparatus with healthy bone, periodontal ligament and cementum.

Because a retro filling material is in long-term contact with the bone in fibrous tissue of the periapical region, the biocompatibility is a criterion that should be met before widespread clinical use.

Bioactive glass-hydroxyapatite is a less expensive, resorbable synthetic porous ceramic granular graft. The particle size is in the range of 150-500 μm and pore size in the range of 100-200 μm . The material is a composite of amorphous and crystalline phases such as hydroxyapatite, calcium silicate, tricalcium phosphate and amorphous silicon compounds.

The glassy part (BG) is 17% silicon (As SiO_2), 53% calcium (As CaO) and 30% phosphorous (As P_2O_5). The glass is composite with an equal quantity(50%)of synthetic hydroxyapatite(HA).The particles are synthesized as porous granules and mixed to have improved bioactivity.

Bioactive glass- Hydroxyapatite contains calcium-phosphosilicate glass made through low-temperature sol-gel route and composited with hydroxyapatite (A calcium phosphate similar in composition to bone and dental mineral) and tricalcium phosphate phases. These granules act as substrates for osteoblast proliferation and biointegration.

The initial osteoconductive property leading to faster integration into bone is provided by synthetic hydroxyapatite, while the bioactive glass phase promotes rapid bone growth. Previous studies have shown that the composited form has improved mechanical property [3].

Although the biocompatibility can be evaluated by in vivo or invitro systems, we assessed the biocompatibility in invitro systems. The osteosarcoma cell line (Saos-2) was used in this study because these cells represent a model of osteoblastic behavior [4, 5]. They are more highly differentiated and their growth maintains a consistent cell phenotype. These osteosarcoma cells closely resemble the human osteoblast in its ability to express high levels of bone markers.

The use of a permanent cell line allowed easy maintenance in culture. The use of cells in continuous culture permits an accurate evaluation of the changes, independently from factors such as age, metabolic and hormonal states of the donor which may influence the cell in primary culture.

When using an invitro system to assess cytotoxicity, it can either be a direct method where the material physically contacts the cells or an indirect method in which an extract of the material can be used. In this study, the material extract was used to assess the biocompatibility of the materials.

Results of this study showed that the bioactive glass- Hydroxyapatite was found to have better cell viability than bioactive glass or MTA.

The Trypan blue exclusion test was used to indicate cytotoxicity, although it does not give any detailed information other than membrane stability of the cell, it is a standardized and accepted test to monitor possible cytotoxic effects [6].

The calcium-phosphate rich layer of glass granules form a silica gel which induces the primitive mesenchymal cells to differentiate into osteoblasts. Thus, the silica gel is responsible for the osteoconductive properties (7). The phagocytic cells erode the particles internally and expose the Ca-P rich layer to interstitial fluids. Therefore, new bone forms within and external to the particles. Remodeling of the particles is accompanied by replacement with bone tissue.

The BG-HA was found to be more biocompatible than BG, MTA. A high bioactivity of the sol-gel glass may be due to the porous nature of the glass.

In this study, Bioactive glass, bioactive glass-Hydroxyapatite and MTA all showed a good viability but cells on Bioactive glass-Hydroxyapatite showed higher viability values than the other two groups. The control group showed values definitely higher than the other groups.

Alkaline Phosphatase activity is considered as an important marker of osteoblastic activity and hence this was assessed to confirm that the cells did not show any phenotypic change. Alkaline phosphatase was routinely determined by measuring the release of p-nitro phenol from p-nitro phenyl phosphate spectrophotometrically (405 nm at 37°C) as proposed by Lowry *et al* (8).

Alkaline phosphatase (AP) is a hydrolase enzyme and it dephosphorylates nucleotides, proteins and alkaloids under alkaline conditions. The enzyme is present within all tissues of the body but is elevated in cells of the liver, kidney, bone, placenta and embryo and under specific disease states. Under alkaline conditions (pH>10), AP can catalyze the hydrolysis of p-nitrophenylphosphate (p-NPP) into phosphate and p-nitro phenol, a yellow colored by-product of the catalytic reaction. The amount of p-nitro phenol produced is proportional to the amount

of alkaline phosphatase present within the reaction. The amount of AP can thus be reliably quantified by reading the amount of p-nitro phenol formed after the catalytic reaction at 405 nm on a spectrophotometer. Alkaline Phosphatase activity was highest in the control group. Among the study groups, the bioactive glass-Hydroxyapatite showed higher activity than the other two groups.

But further studies should be done before recommending this as a better root-end filling material, when compared to other materials.

CONCLUSION

- Bioactive glass – Hydroxyapatite was less cytotoxic compared to Bioactive glass (Group I) and Mineral Trioxide Aggregate
- The cell viability and alkaline phosphatase activity of Bioactive glass-Hydroxyapatite was not significantly different from the control group.

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