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COMPARATIVE EVALUATION OF THE CYTOTOXICITY OF BIODENTINE CEMENT MIXED WITH A NEW POTENTIAL REGENERATIVE SCAFFOLD HYDROGEL TO PERIODONTAL LIGAMENT FIBROBLAST CELLS.

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ABSTRACT

Aim of the study: To investigate the cytotoxicity of Biodentine (Septodont, France) cement mixed with a new potential regenerative scaffold hydrogel (Placentrex) to periodontal ligament (PDL) fibroblast cells. **Methodology:** Periodontal ligament fibroblast cells were seeded into 24 well plates containing Biodentine (Group1), placentrex (Group 2), Biodentine coated with placentrex (Group 3) and Biodentine mixed with Placentrex gel (Group 4) respectively, in 6 wells each for 7 days by changing the growth medium every other day. On the 3rd day, and 7th day, the cell viability and proliferation of the cells was assayed using the MTT assay test and the absorbance reading at 490nm was recorded in an ELISA plate reader. **Results and Conclusion:** Within the limitations of the present study, Both Biodentine and Placentrex are highly biocompatible materials Biodentine coated with Placentrex gel was least cytotoxic and showed increased proliferation of cells. The use of Placentrex gel as an injectable scaffold has immense potential in regenerative procedures.

KEYWORDS: Biodentine cement, Placentrex gel, cytotoxicity.

1. INTRODUCTION

Fundamental research has envisioned the development of newer dental materials. Till date, no material has been developed that is considered ideal. Materials are made to perform more sophisticated functions in the body for longer period. Thus it is necessary to evaluate the use of material based on the risk-benefit analysis. Biocompatibility of a restorative material is a fundamental requirement for any new restorative material. Thus, evaluation of biocompatibility for any new restorative material and weighing the degree of possible benefits against the possible risks is a must.

Biodentine ™ (Septodont, France) is a bioactive cement manufactured using active Biosilicate technology. It is biocompatible according to ISO standards, has a short setting time and also a high compressive strength.

Placentrex is a drug containing peptides (FNP-III, CRF), nucleotides (PDRN, NADPH) & glutamate and is derived from an extract of fresh term, healthy, human placenta. This is the most available but least investigated organ and contains a wide range of biologically active substances and materials, therefore rightly calling it the biological brilliance.

Placentrex has so far been used in the fields of obstretics, gynaecology, surgery and orthopaedics mainly for its wound healing, tissue regeneration and antimicrobial properties.

Several major areas of research have been identified that might have application in the development of regenerative endodontic techniques.

These techniques are Root canal revascularization via blood clotting, postnatal stem cell therapy, pulp implantation, scaffold implantation, injectable scaffold delivery, three-dimensional cell printing and gene delivery which are still in nascent stages of development Of these methods, scaffold implantaton may be of two types depending on the nature of the scaffold -rigid scaffold and injectible scaffold. Injectable scaffolds are materials with the bioactive growth factors can be injected into the narrow root canals.

Furthermore if the root canal filling material can offer additional properties that decrease the bacterial survival and promote bioactive mechanisms necessary for regeneration and healing then some of the ideal requirements of the filling material might be viewed less important when the distinct advantages are considered.

There is no information on the cytotoxicity of Biodentine with Placentrex gel Also, there is no comparative study evaluating the cytotoxicity of Biodentine with Placentrex to periodontal ligament fibroblast cells and their effect on proliferation of cells. Therefore the aim of the study was to evaluate and compare cytotoxicity and their effect on proliferation of periodontal ligament fibroblast cells with *Biodentine, Placentrex, Biodentine mixed with Placentrex and Biodentine coated with Placentrex* at 24 hour, 48 hour and 72 hour interval.

The null hypothesis formulated for the study was there will be no difference in the cytotoxicity and effects on proliferation of cells with Biodentine, Placentrex, Biodentine mixed with Placentrex and Biodentine coated with Placentrex at 24 hour, 48 hour and 72 hour interval.

2. MATERIALS AND METHODS

This *in vitro* study was conducted in the KLE's Dr. Prabhakar Kore Hospital and Research Centre, Belagavi, with the objective of evaluating the cytotoxicity and effects on proliferation of fibroblast cells with Biodentine, Placentrex, Biodentine mixed with Placentrex and Biodentine coated with Placentrex at 24 hour, 48 hour and 72 hour interval.

2.1 Isolation of PDL cell line

Freshly extracted teeth were placed in 5ml of sterile Phosphate buffered saline (PBS) solution and were rinsed with PBS solution in the laboratory.

Following which the PDL cells were scraped with number 21 surgical blade and 1.5 DMEM (Dulbecco's modified Eagle's media) Media was added to the cells and the cell suspension was seeded in 24 well microtitre plate and incubated at 37^0C with a 5% supply of CO_2. Following incubation an inverted microscope (Laomed, India) was used to observe the growth of fibroblast cells.

2.2 Preparation of samples

Materials included Biodentine and Placentrex gel. The materials were mixed on a sterile glass slab and introduced into capillary tubes having a 1-mm diameter and a 4-mm length. The materials at both ends of the tubes were flattened using a spatula and a moist cotton pellet was placed over each end with minimal pressure. All samples were placed in an incubator (37°C, 95% humidity and 5% CO2). The samples of each material were tested after storage time of 7 days after mixing. All groups were then exposed to isolated fibroblast cells for 24, 48 and 72 hours. The experimental groups were prepared in sterile condition as follows:

Biodentine(B)(n=15): Biodentine was manipulated as instructed by the manufacturer, the pipette of liquid was emptied into the capsule of powder. The capsule was then placed in the arms of a mechanical triturator (Vari-MixIII, Dentsply, Caulk) and driven for 30s. The mixed contents of the capsule were emptied into a container using a spatula, both provided by the manufacturer. The cement was creamy in consistency which kept on hardening as time progressed.

Placentrex(P)(n=15): Placentrex gel was loaded into the syringe and filled inside the capillary tubes.

Biodentine coated with Placentrex(Bc) (n=15) : After Biodentine cement was loaded into the capillary tube, placentrex gel was coated with the microbrush.

Biodentine mixed with Placentrex (Bm) (n=15): Placentrex gel was used to mix the Biodentine powder in the ratio of 1:1

Control(C) (n=15): included the untreated cells.

2.3 Cytotoxicity assay

100 microlitre of cell culture was seeded into the wells of four 96-well microplates (RPMI 1640, GIBCO, Carlsbad, CA, USA). The prepared samples of test and control groups were then individually added to wells(n=15) after seeding.

Plates were incubated at 37°C in 5% CO for 24, 48 and 72 hours. Four hours before the end of the incubation time, 20 µL 3-(4, 5-dimethylthiazol-2-yl) 2,5diphenyltetrazolium bromide solution (MTT, Sigma-Aldrich, St. Louis, USA, 5 mg/ml) was added to each well.

Precipitated formazan crystals were dissolved by adding 200 µL solvent (Dimethyl sulfoxide) to each well. The microplates were shaken at room temperature for 10 minutes and prepared for reading by a microplate reader at 630 nm. The percentage of metabolic activity was calculated using the formula: (Test optical density / control optical density)*100. Periodontal ligament cell fibroblasts that were cultured in the empty capillaries were considered as negative controls. The assay was repeated at 24, 48 and 72 hour interval. Statistical analysis of the data was performed by using two-way analysis of variance and Tukey multiple comparison post test, with significance of $p < 0.05$.

3. RESULTS

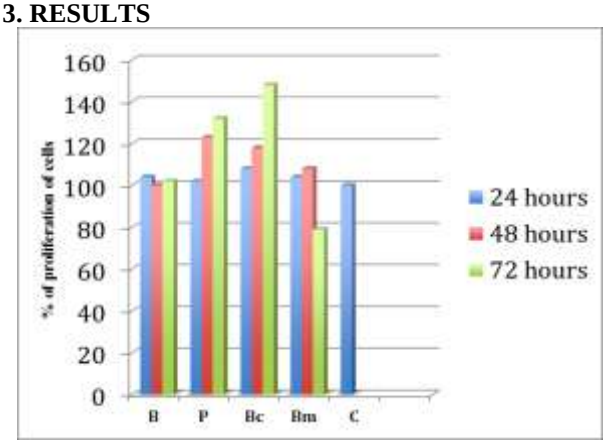

Figure 1: Shows the % of proliferation of Pdl fibroblast cells in each group at 24, 48 and 72 hour period.

The results showed that Biodentine and placentrex gel were biocompatible and not cytotoxic to the cells. However, the cell viability reduced in the group in which Biodentine was mixed with placentrex gel at 72 hour period.

The cell viability of the Biodentine group was similar to the control and did not show proliferation of cells whereas Placentrex gel showed significant increase in proliferation of cells with the maximum proliferation noted in the Biodentine coated with Placentrex gel group.

4. DISCUSSION

Placement of a material in the body creates a dynamic interface. Therefore a new material must be evaluated with respect to its mechanism of action, safety, its effects on dental personnel and its potential side effects.

Various methods such as Tryptan blue solution, MTT assay and MTS assay are available to assay cytotoxicity. Tryptan blue solution can only distinguish between viable or dead cells.[1]

In this present study MTT cytotoxicity assay was chosen to evaluate cell viability because of its simplicity, precision and accessibility. MTT is a colorimetric assay based on the ability of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble tetrazolium salt into dark blue formazan crystals.[2,3] The amount of formazan produced is directly proportional to the viable cell number.[3,4] Methylthiazol sulfophenyl assay (MTS) is composed of 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium. Its advantage is that the formazan crystals are soluble in tissue culture media and therefore the solving procedure is omitted.[5]

In the present study, capillary tubes containing the test materials (Biodentine and Placentrex) with moist cotton pellets at either ends were used.

They served as the barrier thereby enabling the separation of the cells from the material and also allowing sufficient release of test materials to assess cell viability.

Biodentine is a relatively new calcium silicate-based material with high compressive strength and short setting time, which is suggested as an appropriate substitute for dentin.[6] Biodentine formed a significantly thicker Ca- and Si-rich layer compared to ProRoot MTA as concluded by Han et al.[7] Zhou et al. reported that Biodentine showed similar results to MTA in terms of gingival fibroblast reaction.[8] In a recent study, it was found that Biodentine and ProRoot MTA had acceptable biologic effects.[9] The result of this study revealed no cytotoxic effects on fibroblast cells which is in accordance with previous studies.[8,9] No significant difference between the ProRoot MTA specimens and Biodentine regarding cytotoxicity on monocyte cells was reported by Khedmat et al.[10]

The results of the present study showed that Biodentine is a highly biocompatible material concurring with the results reported by Corral Nunez CM.[11]

The results of the study showed that both Biodentine and placentrex gel individually were biocompatible and the combination of Biodentine coated with placentrex gel was least cytotoxic and showed a statistically significant increase in the proliferation of cells.

The proliferation of cells can be attributed to PDRN and Fibronectin III contained in placentrex gel. PDRN is known to suppress the proinflammatory chemical mediators[11] and interfere with microbial replication thereby exerting a bacteriostatic effect. Fibronectin has high efficacy in stimulating cell migration and wound repair. The rationale is that fibronectin will expose the collagen fibres from within the root surface and facilitate interaction of the gingival fibroblasts and tooth.

The sustained proliferation of the Biodentine coated with placentrex group over 72 hours could be attributed to the presence of several types of cytokines and chemokines which are essential for maintenance and differentiation of stem cells and thus the combination may be beneficial for Direct pulp capping, apexogenesis and in regenerative endodontics.

Placentrex is known to express fibronectin like activity[13], produces cross linkage along with fibrin to form a plug and can thus serve as a scaffold in regenerative therapy.

5. CONCLUSION

With the current research being focussed on attempts at successful regeneration in endodontics, the use of placentrex as an injectible scaffold has immense potential.

However, all aspects of the material need to be investigated, its physicomechanical properties, and various interactions need further evaluation.

Within the limitations of the present study, both Biodentine and Placentrex are highly biocompatible materials. Biodentine coated with Placentrex gel was least cytotoxic showing increased proliferation of cells.

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