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Effect of calcium hydroxide and mineral trioxide aggregate on human PDL fibroblast: an in vitro study

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ABSTRACT

Background and Aim

The aim of this in vitro study was to investigate the effect of Calcium hydroxide and MTA (Mineral Trioxide Aggregate) on human periodontal ligament fibroblast (PDL).

Method

The cements were divided into three groups Group 1: Calcium hydroxide, Group 2: MTA and Group 3: Calcium hydroxide + MTA. PDL fibroblasts were cultured in above groups. The proliferations of cells were recorded under microscope.

Results

Group 2 showed no lysis of PDL fibroblasts, whereas Group 1 and Group 3 were found slight toxic in nature as they inhibited the PDL cell proliferation.

Conclusion

MTA provided a more favourable environment for PDL fibroblasts adhesion and proliferation.

Keywords: Calcium Hydroxide Cement, MTA, Periodontal Ligament Fibroblasts, Periodontal Regeneration

INTRODUCTION

In 1920, a new era in the treatment of exposed pulp began when Hermann introduced a calcium hydroxide mixture that induced bridging of the exposed pulp with reparative dentin.The use of calcium hydroxide as a root canal–filling material was proposed by Rhoner in 1940 [1]. The primary reason for using calcium hydroxide is stimulation of the periapical tissues to maintain health or promote healing and secondarily for its antimicrobial effects. Recent studies have demonstrated that calcium hydroxide applied to the root surface in conjunction with surgical periodontal therapy, may promote periodontal regeneration (Stratul et al. 2006) [2]. Sigrun Eick et al stated that oily calcium hydroxide suspension may promote adhesion of osteoblasts and mineralization but does not affect to PDL fibroblasts [3].

MTA was first described in the dental science literature in 1993 by Lee and colleagues and was approved for endodontic use by the U. S. Food and

Drug Administration in 1998 [1]. In 1993, Mohammad Torabinejad centered his research in the development of MTA at the Loma Linda University, California. MTA is endodontic cement that seals pathways of communications between root canal system and periodontium. MTA has high sealing ability, prevention of bacterial leakage, high biocompatibility and no disintegration over time. It has primary pH of 12.5, easy for application and induces cementogenesis, dentinogenesis and osteogenesis [4]. According to the literature, when used as root end seal material, MTA shows greater interaction with PDL compared to calcium hydroxide [5]. In vitro toxicity of MTA was assessed on animal cell lines; however, only few studies investigated its toxicity on cultured human PDL fibroblasts.

Therefore the study was designed to investigate the effect of calcium hydroxide and MTA on human periodontal ligament fibroblast in vitro.

METHOD

Sample preparation

In this *in vitro* study, MTA and calcium hydroxide were separately mixed under sterile conditions on a glass slab exactly according to manufacturer's instructions and transferred into wells with 2 cm diameter and 3 mm height in a 96-well plate. The plate was stored in an incubator at 37°C and 95% humidity for 24hrs to allow complete setting of the materials. The plates were then removed from the incubator and serum and antibiotic free Dulbecco's modified Eagle's medium (DMEM) was added to the wells and incubated for 24 hrs.

Cell cultures

The human periodontal ligament cells (PDL) were obtained from healthy human periodontal tissues isolated from a maxillary first premolar extracted for orthodontic reasons from a 20 year-old female patient without any known medical disorders. Under sterile conditions, the PDL tissue fragments were mechanically removed by scraping the middle third of the root surface with a sharp blade. Tissue explants were maintained in DMEM containing 10 % Fetal Bovine Serum (FBS), 1 % penicillin/streptomycin and 1 % fungizone (Invitrogen). Cultures were incubated in a humidified atmosphere (95 %) of 5 % CO2. Then 3000 cells/200 µL culture medium were transferred to each well of 96-well plate. Peripheral wells of the plates only contained plain culture medium and were considered blank. Plates were transferred to an incubator (in a humidified atmosphere of air and 5% CO_2, at 37°C). After 24 hrs, the culture media were replaced with 200 µL of the prepared cements or control media, and the cells were stored in an incubator for 1hr, 24hrs and 48hrs. Cell lysis was observed by using the Neubauer chamber. This in vitro study was done in the Molecular and Cell Biology Laboratory of Maratha Mandal Dental College, Belguam, India.

STATISTICAL ANALYSIS

Descriptive statistics, such as mean and SD were used. P-values in comparison with controls were determined by Post Hoc Dunnett Multiple Comparisons Test after ANOVA. A p-value less than 0.05 were considered as significant.

RESULTS

Table1: Results for cell viability test (Cements) - O.D values.

S. No.	Sample	Concentration	Absorbance (nm)
1.	Calcium Hydroxide cement	100ug	2.398
2.		100ug	2.102
3.		100ug	1.989
4.	MTA	100ug	0.556
5.		100ug	0.570
6.		100ug	0.604
7.	Calcium OH+MTA	100ug	1.286
8.		100ug	1.182

9.		100ug	1.564
10.	control		0.417
11.	control		0.413
12.	control		0.395

Table 2: Effect of Calcium hydroxide and MTA on cultured human fibroblasts

Cements	
Calcium hydroxide	Slight reduction in cell number
MTA	No change in viability
Calcium hydroxide + MTA	Significant reduction in cell number

Table 3: Comparison of absorbance between groups

	Calcium Hydroxide cement	**MTA**	**Calcium OH+MTA**	**Control**	**F-value**	**p-value**
Absorbance (nm)	2.16 ± 0.21	0.58 ± 0.02	1.34 ± 0.19	0.41 ± 0.012	91.97	P<0.0001

Table 4: Multiple comparison using Dunnett Multiple Comparisons Test with control group for Absorbance between groups

I	**II**	**Mean difference (I-J)**	**p-value**	**Remarks**
Control group	Calcium Hydroxide cement	1.75	P<0.01	Significant
	MTA	0.17	p>0.05	Not significant
	Calcium OH+MTA	0.93	P<0.01	Significant

Group 2 showed no significant results whereas Group 1 and 2 showed significant results.

DISCUSSION

The use of cell culture to test the biocompatibility of dental materials is gaining importance because it offers a significant cost effective and repeatable tool to advance knowledge of possible toxic effects of materials. Study reports used animal cell lines such as L929 or 3T3 mouse fibroblasts to measure toxicity of dental materials (Al-Hiyasat et al. 2005; De Menezes et al. 2009) [6,7]. PDL fibroblasts are easier to culture and they have better morbidity rate. According to Melcher's concept, PDL fibroblasts have ability to form new connective tissue attachment as they play important role in regeneration. The protein and collagen content production is significantly greater in PDL fibroblasts. PDL fibroblast have higher alkaline phosphatase level, higher rate of proliferation and rapid turnover of extra cellular matrix [8].

A huge number of scientific reports describe the properties of MTA, specially the biocompatibility of this cement in comparison with any other developed endodontic cement [9]. MTA is a bioactive material which is hard tissue inductive, conductive and biocompatible. MTA was designed for surgical and repair purposes because it can seal off the internal and external tooth surfaces. Studies showed that MTA has the capacity to induce alkaline phosphatase expression and activity over the PDL and gingival fibroblasts. MTA enhance cementoblast attachment and growth, and has a role in the production of mineralized matrix gene and protein expression [10]. De Deus et al evaluated cytotoxicity of MTA on PDL fibroblasts at 24 hrs, 48 hrs and 72 hrs and they discovered no significant difference on PDL fibroblasts at different concentrations [11].

P. Yan et al discovered MTA as nontoxic over human PDL and MTA appear to induce the differentiation of human PDL fibroblasts [12].Camp et al did in vivo study and found MTA

biocompatible with PDL fibroblasts, and have positive influence on cellular behaviour of PDL fibroblast [13]. Rhythm Bains et al did study in vivo on management of furcation with MTA and found MTA biocompatible and have excellent regenerative property [14]. In our study MTA proved non toxic over PDL fibroblasts as it showed no lysis of PDL fibroblasts at interval of 24hrs and 48hrs.Absorbance rate over time period of 1 hrs,24 hrs and 48 hrs is 0.556nm ,0.570nm and 0.604nm respectively.

The important reasons for using calcium hydroxide as a root-filling material are stimulation of the periapical tissues to endorse healing and secondly for its antimicrobial effects.[1] The exact mechanism of calcium hydroxide is unknown, but the following mechanisms of actions have been proposed as, Calcium hydroxide has antibacterial activity. It has a very high pH that promotes repair and active calcification. The alkaline pH of calcium hydroxide neutralizes lactic acid from osteoclasts and prevents dissolution of mineralized components of teeth. It denatures proteins and makes them less toxic. It diffuses through dentinal tubules and may communicate with the periodontal ligament space to prevent external root resorption and promote healing [15].

Stratul et al (2006) noticed an excellent healing of soft tissue at the surgical site without any sign of inflammation, necrosis, abcess formation or allergic reactions [2]. Sculean et al did 6 month case report study on intrabony defect with oily calcium hydroxide. No significant difference of PDL cell proliferation between the oily calcium hydroxide suspension and the pure calcium hydroxide is reported by A. Kasaj et al [16]. Oily calcium hydroxide demonstrated efficacious results in adjunct to periodontal regenerative therapy but very little knowledge available about molecular mechanism of oily calcium hydroxide on PDL fibroblast. We used calcium hydroxide powder mixed with saline. In our study calcium hydroxide showed slight lysis of PDL fibroblast compared to MTA. Absorbance rate over time period of 1hr, 24hrs and 48 hrs is 2.398nm, 2.102nm and 1.989nm respectively. Third group of our study is combination of MTA and Calcium hydroxide. We could not find any study reporting use of MTA in combination with calcium hydroxide. In our study mixture of MTA and calcium hydroxide showed significant lysis of PDL fibroblasts. Absorbance rate over time period of 1hr, 24hrs and 48hrs is 1.286nm, 1.182nm and 1.564nm respectively. Control group showed absorbance rate as 0.417nm, 0.413nm and 0.395nm over time period of 1hr, 24hrs and 48hrs.

Overall, freshly mixed materials have higher cytotoxicity due to the release of some materials during their setting. After completion of setting, the material becomes structurally more stable and its primary cytotoxicity decreases. Therefore, in the present study, cells were exposed to elute of set materials, like many other similar studies. Our findings revealed that neat concentration of MTA had no significant cytotoxicity at all three time intervals ($P<0.05$) Calcium hydroxide and combination of calcium hydroxide and MTA provided less favourable environment for PDL cell adhesion and proliferation, whereas MTA appears to be safe material.

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