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Original Article

COMPARISON OF CALCIUM ION RELEASING CAPACITY OF DIFFERENT PULP CAPPING AGENTS BY INDUCTIVELY COUPLED PLASMA-OPTICAL EMISSION SPECTROMETRY TEST"- AN IN VITRO STUDY

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ABSTRACT

Background-The purpose of the present study is to evaluate most suitable calcium Ion releasing material in four different pulp capping materials.

Aim- The aim of this study is to evaluate and compare the calcium ion releasing capacity of different pulp capping agents.

Materials and Method: Eighty specimens (3 mm in diameter and 1.5 mm height) were prepared using a fiber mold (ring), with the desired dimensions (20 for each group) by Four different brands of Ca(OH)2 cements and they were grouped into four groups which are as follows: Group I: Dycal, Group II: Biodentine, Group III: Cal LC, Group IV: Activa Bioactive. All specimen (n=80) were prepared by mixing and curing the cements as per manufacturer's instructions. Each sample was placed on the Bottom of a 4cm high test tube in 10ml deionized water at 37°C. This stored water was collected for Ca analysis and replaced after 7,14 and 21 days. In this manner, ion release was measured after 7, 14 and 21 days by inductively coupled plasma-optical emission spectroscopy test.

Result: Group II demonstrated highest Calcium Ion release followed by Group III ,Group I and Group IV at various time durations was measured and mean was calculated along with the standard deviation. These values were compared using two-ways ANOVA and Tukey's post hoc test which showed highly significant result with $P<0.001$.

Conclusion: Within the limitations of the study Biodentine(180.0008 ppm) released high amount of Ca ion compared to all the other pulp capping materials.

Keywords: ActivaBioactive, biocompatible, Biodentine , biointeractive, reparative dentine,secondary dentine.

INTRODUCTION

Pulp capping materials are placed or coated as a protective layer on the exposed dentine or vital pulp on the floor of deep cavities after removing deep caries or after exposure to trauma. These protective biomaterials should have specific properties such as biocompatibility ,biointeractivity (biologically relevant ions releasing),and bioactivity(apatite-forming ability)to activate the pulp cells and the formation of reparative dentine.[1]

It is known that calcium ions are necessary in cell migration,differentiation and mineralization.Their role in the stimulation of cell proliferation was established much earlier (Swierenga et al. 1976, Das 1981).Torneck et al. (1983) pointed out that the presence of large quantities of calcium ions in vivo could activate ATP, Which played a significant role in the mineralization process. The same authors stated that calcium ions were mitogenic to pulp fibroblasts. Differences of opinion exist about the origin of calcium ions incorporated in tertiary dentine. It has been shown that calcium ions from medicaments do not become a part of mineralized repair tissue (Pisanti&Sciaky 1964) but a resent investigation by Holand et al. (1982) showed that, at the start of mineralization, calcium came from the applied calcium hydroxide and not from the pulp tissue.[2]

The "tunnel defects" in the reparative dentine is the critics if $Ca(OH)_2$ and it is formed under the $Ca(OH)_2$ pulp capping[13]. A tunnel defect is described as the patency from the area of the exposure through the reparative dentine to the pulp, sometimes which involves fibroblasts and capillaries situated within this defect.[3]

MATERIAL AND METHODS

Four different brands of Ca(OH)2 cements were taken and they were divided into four groups.Group I: Self-cured Ca(OH)2 –Dycal (Dentsply caulk, USA), Group II: Self-cure $Ca(OH)_2$–Biodentin (Septodont, FRANCE), Group III: Light-cured Ca(OH)2- Cal LC (Prevest Denpro),Group IV :Light-cured Ca(OH)2-Activa- bioactive(Pulpdent,USA). Total 80 specimen preapared and 20 specimen prepared from each group. All specimen (n=80) were prepared by mixing and curing the cements as per manufacturer's instructions.

The different cement pastes were placed into the plastic molds (3 mm in diameter and 1.5 mm height).The pellet

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with he1.5mm height and 3mm diameter was prepared. The exposed surface area of each sample was approximately 14 mm^2.

Specimen preparation

Specimen preparation for Group I:

Specimen in this group were prepared by mixing in equal proportion of base and catalyst (i.e 1:1) to a condensable consistency with plastic spatula on oil impervious paper pad.

Specimen preparation for Group II :

Specimens in this group were prepared b mixing the cement from1 capsule powder: 5 drops of liquid in an amalgamator.

Specimen preparation for Group III and Group IV

Specimens in these groups were prepared by dispensing the cement from the syringe and bulk light cured with LED probe vertically placed as close as possible to the specimen for 20 seconds as recommended by the manufacturer.

Each filled mold was placed on the bottom of standard test tube which was filled with 15 ml of deionized water at 37°C. The stored water was collected for Ca analysis and replaced after 7, 14, and 21 days respectively.

Ca ion analysis testing

After each time interval 5 ml of sample from each specimen was carried for analysis and quantification by Inductively Coupled Plasma Optical Emission Spectrometry (Shimadzu AA-6800 ,JAPAN) was done to get the values in the designated parts per million (ppm) units.

Result :The obtained readings were statically analyzed using One-way analyses of variance(Table 1& Table2). and Tukey multiple comparison tests((Table 3).. The Ca-ion release of experimental groups in the current study in ppm.

Calcium ion release from all groups at various time durations was measured and mean was calculated with the standard deviation. The experiment signifies that the maximum calcium ion released was for Biodentine followed by Cal LC, Dycal and Activa-Bioactive respectively. Overall this deifference in calcium ion release was found to be highly statically significant (P=9.5). Hence most superior material for calcium ion release is Biodentine(Table 1).

Graphs: Grahaps shown experimental groups were demonstrated calcium ion release from different time

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interval (Figure1) and calcium ion release in inter groups in different time interval (Figure 2)

Table 1: One-way analyses of variance test **shows the mean of Ca-ion release for the experimental groups(ppm)**

Groups(Materials)	Means Ca-ion release for exp. Groups)	Standard deviation(SD)	Standard Error(SE)	Median	F	P Values
Group I: Dycal	91.293	3.938686	0.880717	91.87	1528.246	9.5**
Group:II Biodentine	180.0008	6.983333	1.561521	180.95		
Group:III Cal LC	158.8097	4.073333	0.910825	158.5483		
Group:IV Activa Bioactive	88.404667	3.33	0.744611	88.345		

** Highly statically significant

Table 2: ANOVA test for comparison in different groups.

Source of variation	SS	df	MS	F	P-value	F *CRIT*
Between groups	16313.23	5	3262.645	238.9301	1.12E-58	2.293911
Within groups	1556.696	114	13.65523			
Total	17867.92	119				

Table 3: Tukey multiple comparison tests for comparison between individual groups

Comparison Group	Comparison Difference	P Value
BIO DENTINE Vs DYCAL	88.7078	8.2**
BIO DENTINE Vs CAL LC	21.1983	4.9**
BIO DENTINE Vs ACTIVA BIOACTIVE	91.5962	1.3**
CAL LC Vs DYCAL	67.5167	8.1**
CAL LC Vs ACTIVA BIOACTIVE	70.4051	5.5**
DYCAL Vs ACTIVA BIOACTIVE	02.8884	1.12**

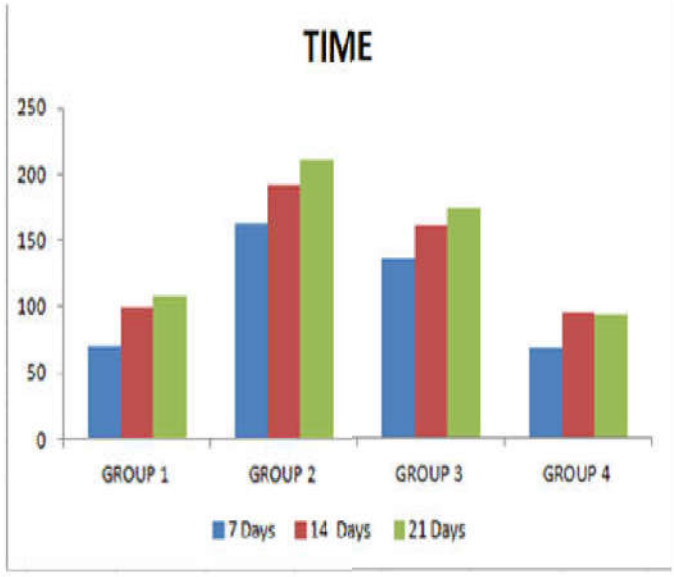

Figure 3 calcium ion release from different time interval

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Figure 4 calcium ion release in inter groups in different time interval

DISCUSSION:

The mechanism of action of calcium hydroxide material used for vital pulp therapy is through the release of calcium and hydroxide ions from the material which permits the participation of these ions in the repair process, stimulating mineralization. Calcium reacts with tissue carbonic gas, forming calcium carbonate, which favors dental pulp cell proliferation and differentiation and thus contributes to the onset of mineralization.[4, 5] Hydroxide ion acts by stimulating the release of alkaline phosphatase which participates in the mineralization process.[6] In addition to their biologic action, calcium and hydroxide ions exert an antimicrobial action, with the former reacting with carbonic gas to remove the source of respiration of anaerobic bacteria and the latter inhibiting the bacterial enzymatic system.[10,6]

Mineralized tissue formation due to contact of Ca(OH)2 and connective tissue has been observed from 7th to 10th day after application.[1] The complete antibacterial activity takes place in 7 days by Ca(OH)2, and the slight inflammation induced by Ca(OH)2 is resolved in 14 days.[7] Even though the recommended application period for the Ca(OH)2 is 4–5 weeks, it is reported that 4–5 weeks of Ca(OH)2 application causes necrosis of the normal cells. Hence, it has been chosen as 7, 14, and 21 days for Ca ion release measurement in this study.[1]There is a lack of international standards and test methods for both conventional and light-cured Ca(OH)2 cements, the deficiency is evident from the scarcity of articles available for the same.

Conventional calcium hydroxide liners have high solubility, lack of chemical or mechanical adhesion to dentin and restorative materials, and in addition presents difficulty in manipulation and application.[8,9] With the purpose of improving the properties of conventional calcium hydroxide cavity liners, resin-based cavity liners containing calcium

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hydroxide were developed. These materials are light-cured, highly resistant to etchants, present superior physical properties, and handling characteristics.[7] So, two such materials namely Cal LC (Prevest denpro) and Activa Bioactive (Pulpdent) were the experimental groups in this study respectively where Cal LC is a light cured radiopaque Calcium Hydroxide paste which is composed of Urethane Dimethacrylate, Triethylene Glycol Dimethacrylate, Silanated Barium Glass, Amorphous Fumed Silica, Barium Sulphate and Calcium Hydroxide. Ca ion release from Dycal occurred during the 21‑day period is in agreement with other studies,[11]

In this study the Mean calcium ion release Value for Biodentine is 180.0008ppm, CAL LC it is 158.8097ppm, Dycal it is 91.293ppm, and Activa Bioactive it is 88.4046ppm with P=9.5.

Based on the calcium and hydroxide ion release from material, it may be concluded that tricalcium silicate material such as Biodentine may be preferable for IPC.

After Biodentine, Cal LC significantly released more ca ion than Dycal and Activa Bioactive due to its high content of calcium hydroxide.

The present study proved that Ca and OH ion release from the pulp capping materials supposed to be continue over time and the action of these on vital tissue can induce the deposition of hard tissue.[12] The chemical dissociation occurs in the presence of fluids and the Ca and OH ions dissociated

from Ca(OH)2 can penetrate the surrounding dentinal tubules.[12] Clinically, it is possible to evaluate that a wet condition may maintain the dissociation constant because of the presence of fluid.[12]

Within the limitation of the present study, it can be concluded that Biodentine released higher amount of Ca ions compared to other pulp capping agents. Among Biodentine and other pulp capping agents, biodentine was found to be the highest Ca ion-releasing material.

CONCLUSIONS:

The present findings confirms that Biodentine is a reliable material in the matter of inducing dentin bridge formation while keeping the vital pulp in both direct and indirect pulp capping procedure. This study also reports Biodentine superiority in relatively easier manipulation, lower cost

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and outstanding clinical outcomes. High biocompatibility and excellent bioactivity further go in favour of this material.

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