Pulp response to high fluoride releasing glass ionomer, silver diamine fluoride, and calcium hydroxide used for indirect pulp treatment: An *in-vivo* comparative study

Atish Korwar, Sidhartha Sharma, Ajay Logani, Naseem Shah

Abstract

Aims and Objectives: The study aims at determining pulp response of two high fluoride releasing materials silver diamine fluoride (SDF) and Type VII glass ionomer cement (GIC) when used as indirect pulp treatment (IPT) materials. **Materials and Methods:** Deep Class V cavities were made on four first premolars indicated for extraction for orthodontic reasons. SDF, Type VII GIC, and calcium hydroxide base are given in three premolars, and one is kept control. Premolars were extracted 6 weeks after the procedure and subjected to histopathological examination to determine the pulp response. The results were analyzed using Chi-square test. **Results:** No inflammatory changes were observed in any of the groups. Significantly more number of specimens in SDF and Type VII GIC groups showed tertiary dentin deposition (TDD) when compared to control group. No significant difference was seen in TDD when intergroup comparison was made. Odontoblasts were seen as short cuboidal cells with dense basophilic nucleus in SDF and Type VII GIC group. **Conclusion:** The study demonstrated TDD inducing ability of SDF and Type VII GIC and also established the biocompatibility when used as IPT materials.

Keywords: Calcium hydroxide, high fluoride releasing glass ionomer, indirect pulp treatment, pulpal response, silver diamine fluoride

Introduction

The management of deep carious lesions approaching pulp poses a significant challenge to the practitioner. The ideal treatment of such lesions involves the removal of all infected dentin to prevent further cariogenic activity and provides appropriate liner for pulp protection, but the potential risk of pulp exposure is high. In such situations, indirect pulp treatment (IPT) by placing of a liner, which is biocompatible, with antimicrobial and remineralizing abilities, is highly desirable.[1] The success of IPT is determined clinically by asymptomatic, vital teeth, and histologically by tertiary dentin deposition (TDD) adjacent to the site of injury. The overall response of tooth to injury represents complex interplay between injury, defense, and regenerative process.[2,3]

High fluoride releasing materials silver diamine fluoride (SDF) and Type VII Glass ionomer cement (GIC) are proposed newer IPT materials. Their remineralizing and antimicrobial properties have been established.[4,5] SDF has been termed to be a "silver-fluoride bullet" because of rapidity of its action.[6] Fluoride and silver interact synergistically to form fluorapatite and silver phosphate. Silver phosphate releases silver ions that have a high polarizing effect. Silver interacts with sulfhydryl groups of proteins and with DNA, altering hydrogen bonding and inhibiting respiratory processes, DNA unwinding, cell-wall synthesis, and cell division leading to bacterial cell death. Adverse effects of SDF are stated to be pulpal irritation, tooth staining, tissue irritation, and fluorosis though these have not yet been well-studied.

Type VII GIC that is GC Fuji VII is claimed to release 6 times more fluoride as compared to conventional GIC. Till date, its use is restricted to fissure sealing, root surface protection and to control hypersensitivity.[7]

The present histopathological study was planned to evaluate the pulpal response and TDD potential of these two high fluoride releasing materials and to compare them to Calcium hydroxide when used as IPT materials in deep cavities.

Materials and Methods

Nine subjects; six females and three males (age range: 14–21 years, with the mean age of 17.5 years) that

Centre for Dental Education and Research, All India Institute of Medical Sciences, New Delhi, India

Correspondence: Prof. Naseem Shah,
Department of Conservative Dentistry and Endodontics, Centre for Dental Education and Research, All India Institute of Medical Sciences, New Delhi - 110 029, India.
E-mail: naseeemys@gmail.com

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required extraction of bilateral maxillary and mandibular first premolars as per the orthodontic treatment plan, were included in the study. Subjects were explained about the study verbally and through patient information sheet and written informed consent was obtained. This study was conducted in the Department of Conservative Dentistry and Endodontics, Centre for Dental Education and Research, All India Institute of Medical Sciences, New Delhi after institutional ethical clearance.

After administration of local anesthesia, Class V cavities were made on buccal surface of premolar 1 mm coronal to cementoenamel junction with TF-11 flat ended tapered diamond bur (Mani Inc., Tochigi, Japan) in airotor handpiece under copious water coolant. Preparation of axial wall was continued until pink discoloration was observed due to pulp proximity without pulp exposure. This was done to achieve a remaining dentine thickness of 0.25–0.5 mm to simulate the condition in IPT procedure. Cavity walls and floor were conditioned with a 17% disodium ethylenediaminetetraacetate (Largal Ultra, Septodont, France) solution for 15 s. Then cavities were rinsed with sterile water and dried with sterile cotton pallets for 5 s.

In each subject, cavities in three teeth were given a lining with SDF (Saforide, Toyo Seiyaku Kasei Co. Ltd, Japan) mixed with Zinc Oxide powder (Group A - 9 premolars), Type VII GIC (GC Fuji VII, GC Corporation, Tokyo, Japan) (Group B - 9 premolars), and calcium hydroxide (Dycal, Dentsply International Inc., USA) (Group C - 9 premolars). One tooth in each of the 9 patients was taken as control, where a cotton pellet was placed (Group D). All the cavities were restored with Cavit G (3M ESPE, Germany). Type VII GIC and $Ca(OH)_2$ were used as per the manufacturer's instructions. SDF being a viscous liquid was mixed with zinc oxide powder as done in previous studies.[4,5]

After 6 weeks, the teeth were extracted atraumatically preventing damage to restoration and the tooth. The extracted teeth were fixed in 10% neutral-buffered formalin for 24 h and were then decalcified in 7% nitric acid for 7–14 days. Later, they were embedded in paraffin wax and labiolingual sections of 5–6 μm thickness were prepared using microtome (Microm International GmbH, Walldorf, Germany) and stained with Hematoxylin and Eosin.

Sections were observed for pulpal inflammation and TDD under light microscope at various magnifications (×10, ×40, and ×100). Pulpal inflammatory changes such as dilatation of arterioles and presence of inflammatory cells (polymorphonuclear leukocytes, macrophages, lymphocytes, and plasma cells) were observed. Other histological features of the pulp including calcification, necrosis, and the odontoblast cell layer were also observed.

The results were analyzed using Chi-square test at a 95% level of significance.

Results

Pulp tissue adjacent to cavity preparation showed no inflammatory cells (neutrophils, macrophages, lymphocytes, plasma cells) or dilated arterioles in the subodontoblastic cell-rich zone of Hohl in the pulp, suggestive of absence of inflammation in any of the groups.

TDD was seen in 5 specimens of SDF group, 6 specimens of Type VII GIC group and 3 in calcium hydroxide group [Table 1]. None of the control group specimens showed any TDD. Tertiary dentin of reparative type was observed in SDF and Type VII GIC group with a definite incremental line of Owen. These specimens showed cuboidal odontoblasts with the dense basophilic nucleus, entrapped within the newly formed tertiary dentin, signifying osteodentin formation [Figures 1 and 2].

Three specimens in calcium hydroxide group showed similar osteodentin formation; however, incremental line of Owen was typically absent [Figure 3]. In specimens where TDD was not observed that is in the control group and the remaining specimen of calcium hydroxide group, tall columnar odontoblasts with clearer cytoplasm were observed [Figure 4].

Significantly more number of specimens from SDF and type VII GIC groups showed TDD when compared to control group when Chi-square test was used ($P < 0.05$). No significant difference was observed when intergroup comparison between all the three test materials was made ($P > 0.05$).

Discussion

The search for an ideal material for lining deep cavities is a matter of ongoing research. Need to evaluate different types of materials clinically and compare them with each other cannot be overemphasized.[8]

Table 1: Number of specimens showing tertiary dentin deposition in each group

Test materials*	Tertiary dentin deposition	
	Present	Absent
Group A	5	4
Group B	6	3
Group C	3	6
Group D	0	9

*Group A: Silver diamine fluoride; Group B: Type VII glass ionomer cement; Group C: Calcium hydroxidel; Group D: Control

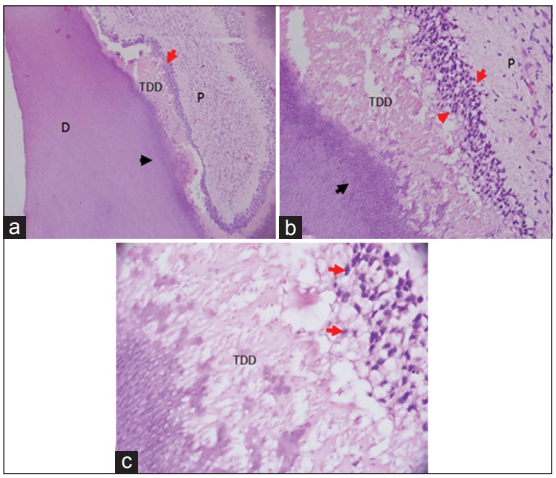

Figure 1: Silver diamine fluoride. (a) ×10 (b) ×40: Histology reveals tertiary dentin deposition between the remaining dentin thickness (D) and pulp tissue (P). No inflammatory changes (black arrow - Incremental line of Owen) (c) ×100: Cuboidal odontoblasts (red arrow) with entrapment – "osteodentin"

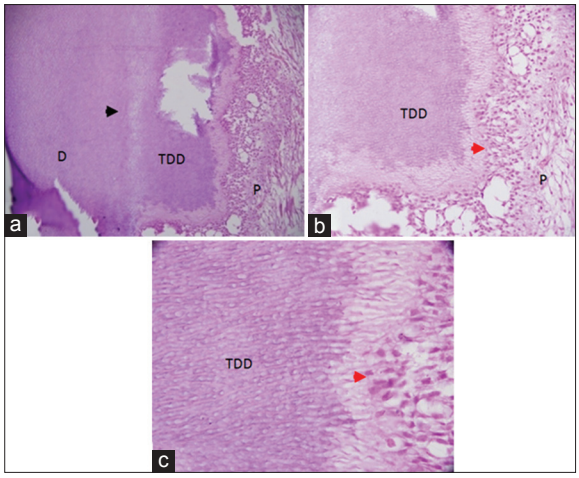

Figure 2: Type VII glass ionomer cement: (a) ×10 (b) ×40: Histology reveals tertiary dentin deposition between the remaining dentin thickness (D) and pulp tissue (P). No inflammatory changes (black arrow - Incremental line of Owen). (c) ×100: Cuboidal odontoblasts (red arrow) with entrapment – "osteodentin"

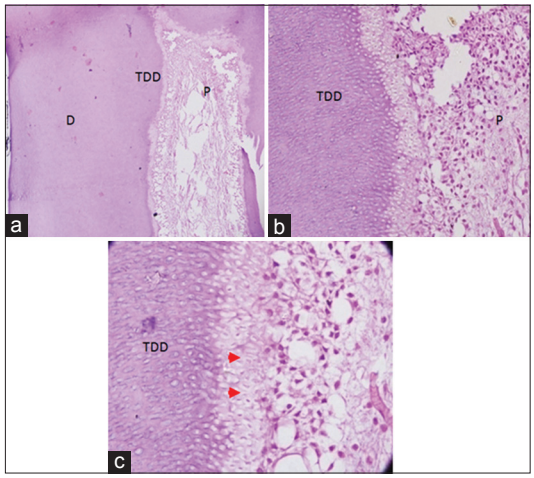

Figure 3: (a) ×10 (b) ×40 (c) ×100: Calcium hydroxide depicts changes similar to silver diamine fluoride and Type VII glass ionomer cement groups

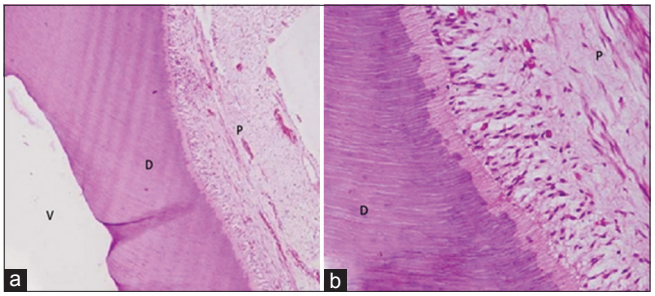

Figure 4: (a) ×10; (b) ×40: Control specimen showing no tertiary dentin deposition or inflammatory cells or dilated arterioles

Tertiary dentin can be classified as being reactionary or reparative in origin. Reactionary dentin is secreted by preexisting odontoblasts, and reparative dentin is secreted by newly differentiated odontoblast-like cells. All the events such as method of preparation, condition of the dentin-restoration wall, presence of bacteria, the type and application method of restorative material used, remaining dentin thickness (RDT), etc., come into play. The importance of these multiple events varies somewhat from one restoration to another and from one patient to another.[9-11]

Calcium hydroxide and other materials like zinc oxide eugenol cement, GIC, dentin bonding agents and composite are utilized for IPT. SDF and type VII GIC are used in "arrest of caries treatment" for the treatment of incipient carious lesions. Both these materials express anti-microbial and caries remineralizing property, desired for IPT.[4,5] However, their pulp irritation and biocompatibility were not known when used in deep cavities, thus this study was planned.

Murray *et al*. studied pulpal response to various materials.[12,13] They had reported that RDT in the range of 0.25–0.5 mm elicit maximum TDD. RDT of <0.25 and more than 0.5 mm elicits less TDD response. RDT <0.25 mm leads to death of preexisting odontoblasts and allows the byproducts from restorative material to reach the pulp and show cytotoxic effects. RDT more than 0.5 on the other hand does not cause sufficient response to stimulate tertiary dentinogenesis. Evidence shows that TDD and odontoblast activity is established once 28 days have elapsed,[14] and pulpal inflammatory reactions to cavity preparation subside after about 3 weeks.[15]

In the current study, age of the subjects was ranging from 14 to 21 years with an average age of 17.5 years. This matches with the age at which maximum pulpal response can be achieved. Cavit G was used as an intermediate restoration in all the specimens to minimize microleakage as it is known to provide good marginal seal.

Pulp response was determined after 6 weeks, thus transient inflammatory response to operative procedure was expected to have subsided. All the groups showed absence of inflammatory changes. Other histological features of the pulp tissue, including calcification of the pulp and necrosis were not observed in any of the groups. Thus, the hypothesized adverse pulp reactions to SDF at RDT 0.25–0.5 mm were not observed in the present study. This establishes the safety of SDF and Type VII GIC in deep cavities as IPT agents. A definite incremental line of Owen was observed in both, SDF, and Type VII GIC groups, which indicated disturbance in cellular function of odontoblasts [Figures 1 and 2]. Secondary odontoblasts were seen as cuboidal cells with intensely basophilic nucleus as was seen in these two groups. Tertiary dentin is indicative of reparative response. In these two groups, odontoblasts were seen entrapped within the newly formed tertiary dentin, signifying osteodentin formation. Rapid deposition of tertiary dentin leads to such phenomenon.[16]

In a study by Gotjamanos, silver fluoride showed favorable pulp response, the presence of abundant reparative dentin and a wide odontoblastic layer.[17] Histological assessment of dental pulps of 55 carious primary teeth was carried out at 3–56 months after extraction for orthodontic reasons. No study in literature determines pulp response to SDF.

Biocompatibility of GIC is established by Schmalz *et al*. using *in-vitro* three-dimensional cell culture model.[18] Six *et al*., through animal model found reparative osteodentin formed in response to the GIC.[19] Mousavinasab *et al*. studied pulp response to GIC in human premolars and documented good pulpal *r*esponse and similar TDD potential as $Ca(OH)_2$.[20] The results of the current study are in agreement with the previous studies and showed that SDF and Type VII GIC have good pulp response and TDD potential.

Typically calcium hydroxide group showed only 3 samples with TDD with entrapment of odontoblasts, but there was lack of definite incremental line in these samples, unlike in SDF and GC VII groups. The remaining specimens showed tall columnar odontoblasts with polarized basophilic nucleus without any TDD. This indicates primary odontoblasts with minimum disturbance of the function [Figure 3].

Mechanism of TDD in response to calcium hydroxide is mediated via alkaline phosphatase enzyme, stimulated by hydroxyl ions at pH 10.2 and calcium-dependent pyro-phosphatase. Heithersay suggested that calcium ions reduce the permeability of capillaries so that less interstitial fluid is produced, thus calcium ions concentrate at the mineralization site. The mineral deposition is presumably by calcium derived from the blood supply of the dental pulp. This indicates that calcium hydroxide is an initiator rather than a substrate for TDD.[21,22] Calcium hydroxide showed TDD in lesser number of specimens at 6 weeks, probably indicating that it requires longer time to respond to calcium hydroxide when used as IPT material.

Nakade *et al*. stated that fluoride at micromolar concentrations can stimulate the proliferation and alkaline phosphatase activity of human dental pulp cells.[23] Fluoride at this level stimulated thymidine incorporation into DNA in dental pulp cells, with optimal effects around 50 μMol. It significantly increased the alkaline phosphatase activity in dental pulp cells by 177 ± 12%. Extracellular-matrix synthesis (Type I collagen) was also shown to increase by 150 ± 8.7%. This suggests that fluoride at low concentrations can be a useful therapeutic agent, where increased TDD is desired, such as in IPT procedure. It can be hypothesized that SDF and Type VII GIC release fluoride in amount that can stimulate the TDD when used as IPT materials.

Further studies with larger sample size and longer follow-up period may help to conclusively establish the efficacy of these proposed IPT materials for their biocompatibility and reparative efficacy.

Conclusion

High fluoride releasing materials SDF and Type VII GIC did not induce any inflammation/necrosis in the pulp. They have good tertiary dentin inducing ability. Based on these attributes, they can be recommended as IPT materials for the management of deep cavities.

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