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Effect Of Incorporation Of Silica Doped Nanohydroxyapatite, A Novel Nanobioactive Filler, Into Dentin Adhesives On Nanoleakage Of Class V Resin Composite Restorations

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Abstract

Background:- Although composites are widely used for restoring cervical lesions, nanoleakage at the resin-dentin interface is still a major challenge to every clinician.

Aim: Objective of this study is to evaluate the effect of experimental dentin bonding agents incorporated with nanohydroxyapatite and silica-doped nanohydroxyapatite particles with remineralizing potential on nanoleakage at the dentin-adhesive interface.

Settings and design:- An invitro study

Materials and methods:- Forty eight class V cavities were prepared on buccal and palatal surfaces of twenty four maxillary premolars. After acid etching, cavities were randomly allocated into 3 groups of 16 cavities each according to the type of adhesive agent applied**. Group I(Control):-** Conventional nanofilled dentin bonding agent.(CN-DBA) **Group II:-** 0.2weight% nanohydroxyapatite incorporated dentin bonding agent.(NH-DBA) **Group III:** 0.2weight% silica doped nanohydroxyapatite incorporated dentin bonding agent.(Si-NH-DBA)**.** After bonding agent application cavities were restored with composite. After thermocycling, specimens were immersed in 50% ammonical silver nitrate solution for 24 hours and placed in photo developer solution for 8 hours. Samples were sectioned in bucco-lingual direction, polished and observed under Scanning electron microscope. Length of silver nitrate penetration along the cavity preparations was measured as percentage of total cut dentin surface penetrated by silver nitrate.

Statistical analysis used: One-way ANOVA and Tukey's post-hoc analysis at 0.05 significant level.

Results:- All the groups showed some extent of nanoleakage with highest percentage observed in CN-DBA group(58.31%), followed by NH-DBA group(38.93%) and lowest for Si-NH-DBA(30.18%).

Conclusion:- Silica-doped nanohydroxyapatite effectively reduces the nanoleakage at the resin-dentin interface because of its bioactive property and remineralizing ability

Key message:- Nanoleakage at resin-dentin interface, which is jeopardising the longevity of adhesive restorations is effectively reduced by novel nanobioactive fillers

Keywords: Dentin-adhesive interface; Nanohydroxyapatite; Scanning electron microscope; Silica-doped nanohydroxyapatite; Silver nitrate

Introduction

As we enter a new epoch we find an ephemeral increase in the use of adhesive composite restorative materials.[1] The quintessential goal of resin dentin bonding is to seal the dentinal tubules against entry of bacteria and their toxins [2] thereby increase the longevity of composite restorations.[3]

In reality, after acid etching interfibrillar spaces get opened for about 20-25nm.[4] Adhesives with bigger size may not completely encapsulate the exposed collagen matrix. This poorly infiltrated zone is subjected to special type of leakage called "nanoleakage".[5] In nanoleakage, porosities are located between resin infiltrated collagen hybrid layer and the unaltered mineralised dentin.[6] Through these pathways, intrinsic water in dentin and the residual water serve as functional medium for hydrolysis of resin matrices by collagenolytic enzymes and jeopardizing the longevity of resin-dentin bonds.[5]

Therefore it's meritorious to quest for sophisticated dentin adhesive which is efficient in reducing nanoleakage. One of the momentous advances over last few decades is the application of nanotechnology to bonding agents for improving its physical and mechanical properties[7] Furthermore it is desirable to remineralize the partially demineralised dentin to produce mechanically resistant hybrid layer.

Hydroxyapatite (HAP) is biologically and chemically similar to the mineral constituent of human teeth. It is documented that 0.2wt% nanohydroxyapatite **(nano$_{HAP}$)** incorporation into dentin adhesives improves its degree of conversion and polymerization rate.[8,9]

Even though HAP has good biocompatibility, it has limited osteogenic capability, in this way element doping could bring special properties to HA P.[10] It has been demonstrated that silica doped nanohydroxyapatite **(Si-nano$_{HAP}$)** serves as a promising material for hard tissue replacement owing to their virtuosity to form carbonate containing apatite layer.[11]

So far there is no literature available on effect of these nanobioactive fillers on nanoleakage. This paper concerns on nanoleakage evaluation at dentin-adhesive interface using **nano$_{HAP}$** and **Si-nano$_{HAP}$** incorporated dentin adhesives.

The null hypothesis was that addition of **nano$_{HAP}$** and **Si-nano$_{HAP}$** into dentin adhesives will not have any effect on nanoleakage of class V resin composite restorations.

Materials and Methods:

Preparation of Class V cavities:

A study was designed and Sample size calculated based on the findings of a preliminary study with standard deviation value of nanoleakage of 10% and the difference in nanoleakage between the groups was 10% with 80% power and 95% confidence interval. The final sample size was found to be 16 in each group. Based on this twenty four caries free maxillary premolars indicated for orthodontic extractions were included in the study. Exclusion criteria were teeth with defects and cracks. On these twenty four premolars forty eight class V cavities were prepared on both buccal and palatal surfaces [figure 1a] with the following dimensions: mesio-distal width: 3 mm, cavity depth: 1.5 mm and occluso-gingival height: 2mm with the occlusal margin in enamel and gingival margin in dentin with butt joint as the cavosurface using a straight fissure diamond burs of 1mm diameter with 3.8mm length (Mani Inc, SF-31, ISO 109/013 FG). The burs were renewed after every fifth cavity preparation. Cavity dimensions were controlled using periodontal probe.

After acid etching for 15 sec with 37% phosphoric acid gel (D-tech, Ivoclar Vivadent**)**, and rinsing specimens were randomly allocated into 3 groups of 16 cavities each according to the type of adhesive agent applied, as follows:

Group I (Control): Conventional nanofilled dentin bonding agent (CN-DBA).

Group II:- 0.2wt% nanohydroxyapatite incorporated dentin bonding agent (NH-DBA).

Group III:- 0.2wt% silica doped nanohydroxyapatite incorporated dentin bonding agent (Si-NH-DBA)**.**

Preparation of experimental dentin adhesives:

Two experimental dentin bonding agents were prepared by mixing **0.2wt% nano$_{HAP}$**[9] (Sigma Aldrich, India), **0.2wt% Si-nano$_{HAP}$** (Sigma Aldrich, India) into 1gm of conventional nanofilled dentin bonding agent (CN-DBA) (Tetric n bond, Ivoclar vivadent, USA) individually. The adhesive solutions were homogenized by ultra-sonication using probe

Volume 5, Issue 1; January-February 2022; Page No 481-488

sonicator apparatus (Sonopuls UW2200, Bandelin, Germany) for 1 minute. The adhesive solution containers were arranged in cooling water bath to prevent heat generation during sonication which affects the organic solvent ingredients.

Procedure for application of dentin bonding agent and composite restoration:-

Dentin bonding agents were applied using a fully saturated micro brush with slight agitation to cover the entire surface of the cavity and were gently air dried 0.5-1 mm away from the prepared surface for 2-3 seconds to allow the solvent to evaporate. The adhesive agent was light cured with Blue phase LED Light curing unit (Ivoclar vivadent, India) for 20 seconds according to the manufacturer's instructions.

Subsequently all the cavities were restored obliquely in two increments with nanohybrid resin composite (Tetric N-Ceram, Ivoclar Vivadent) and was cured for 40 seconds with blue phase LED curing unit (Ivoclar vivadent, India). All the restorations were polished with polishing disks (Soflex, 3M ESPE, India) in series of decreasing abrasiveness. All cavity preparations, restorations and finishing procedures were undertaken by the same operator and were stored in artificial saliva at 37°C for 24 hours.

To simulate oral conditions the samples were subjected to thermocycling procedure comprising of 1000 cycles at 5 ± 2°C and 55 ± 2°C with a dwell time of 30 seconds and 10 seconds for specimen transfer.

Preparation of Ammonical silver nitrate:- Ammonical silver nitrate was prepared by dissolution of 25g of silver nitrate crystals (Sigma Aldrich, Bangalore, India) in 25 ml of distilled water. Ammonium hydroxide (Sigma) with 28% concentration was used to titrate the black solution until it became clear. This solution was diluted to 50 ml with distilled water, yielding a 50 wt% solution (pH = 9.5).[12]

The root apices of all the teeth were sealed with sticky wax. The entire surface of tooth received two layers of fast setting nail varnish, except 1 mm away from the restorative margins and immersed in ammonical silver nitrate tracer solution for 24 hours. The silver stained teeth were rinsed with distilled water and placed in photo developer solution for 8 hours under a fluorescent light to allow reduction of the silver ions to metallic silver particles within the voids along the bonded interfaces.

The teeth were removed from the developing solution, rinsed with distilled water and then dried with a paper towel. The root surfaces apical to the cemento–enamel junction were removed. Samples were sectioned in bucco-lingual direction through the centre of the restoration using a diamond disk of thickness 0.3mm under water coolant.

Assessment of nanoleakage under scanning electron microscope (SEM) :-

Sections were polished with 600 grit silicon carbide paper and deproteinised with 2% sodium hypochlorite solution for 2 minutes. They were air dried, mounted on aluminium stubs, sputter coated with gold and observed under SEM(ZEISS, EVO18) using backscattered mode at 200x and 5000x since in low magnification sometimes it is difficult to observe loosely distributed silver nitrate particles.[6]

The length of penetration of silver nitrate along the cavity preparations was measured quantitatively on the SEM monitor with a multi-point measuring device. All the methodology was given in a flowchart. [Figure 1]

Nanoleakage scores were given according to Awliya et al. as the percentage length dentin-adhesive interface that was penetrated by the silver nitrate i.e

P/L x 100,

Where

P= penetration length of silver nitrate along the cut dentin surface.

L= Total length of the prepared dentinal wall.[6]

Tracer penetration was evaluated by a single observer. Statistical analysis

Statistical analysis was performed using the statistical package for the social sciences (SPSS), version 17.0 (SPSS Inc., Chicago III) to compare the nanoleakage among the three groups using One way ANOVA and tukeys post hoc analysis at a 0.05 significant level.

Results

Control group i.e CN-DBA group recorded higher nanoleakage mean percentage (58.31%), followed by NH-DBA group (38.93%) and lowest for Si-NH-DBA group (30.18%). [Table1]. Si-NH-DBA showed

Volume 5, Issue 1; January-February 2022; Page No 481-488

significantly less nanoleakage compared to other groups. [Table1]

Figure 2 shows SEM photomicrograph of nanoleakage at resin dentin interface of CN-DBA group. Irregular silver deposition observed with no gap formation (arrow), within and below the hybrid layer.

Figure 3 shows SEM photomicrograph of nanoleakage at resin dentin interface of NH-DBA group. Mild Irregular silver deposition occurred below the hybrid layer within the tubules with no gap formation.

Figure 4 shows SEM photomicrograph of nanoleakage at resin dentin interface of Si-NH-DBA group. Thick hybrid layer was observed with very minute amount of deposition of silver particles.

Discussion

Adhesion between dentin and adhesive mainly depends on the quality of the formed polymer.[13] Over the last few decades, the search for a polymeric material with a potential to give good adhesion thereby reducing the nanoleakage has become one of the main study objective. In this era application of nanotechnology to resin composites along with bioactive filler addition is gaining importance because of their promising results.[13] So the objective of this study was to evaluate the nanoleakage by incorporating novel nanobioactive fillers into dentin adhesives.

The phenomenon of nanoleakage was first described by Sano et al.[14] The advantage of nanoleakage test is that failure of an optimal seal can be detected without the necessity of gap formation.[15] Ammonical silver nitrate solution was selected for this study, as it has been accepted as a suitable material for measuring both microleakage and nanoleakage.[6] The size of the silver ion is very small (0.059 nm-diameter) when compared to a typical bacterium (0.5-1.0 μm). Moreover, silver nitrate provides a sharp picture of penetration depth with electron microscopic measurable contrast.[16] SEM analysis was carried out using back scattered mode due to its ability to present distinct images with sensitive and accurate analysis.[2]

In the present study highest percentage of nanoleakage was observed with CN-DBA (58.31%) group [Table 1]. Upon SEM evaluation, CN-DBA samples demonstrated irregular silver nitrate penetration with no gap formation, within and below the hybrid layer [figure 2]. Although the presence of nanofillers in the bonding agent has the ability to penetrate the exposed collagen fibrils, more nanoleakage presented with this bonding agent. The presence of minute blebs of retained water that was not evaporated during polymerization can create water rich zones and permits silver uptake.[2]

NH-DBA group showed significantly less nanoleakage (38.93%) compared to CN-DBA group [Table 1]. Upon SEM evaluation NH-DBA samples demonstrated silver nitrate penetration with no gap formation below the hybrid layer within the dentinal tubules [figure 3]. In NH-DBA group, bioactive $nano_{HAP}$ particles were used. Because of their very small size, nanoparticles efficiently penetrate into the acid etched dentinal tubules along with the adhesive resin [9] and because of their bioactive properties they are able to remineralize the demineralized dentin.[4] Some studies reported that addition of 0.2wt% $nano_{HAP}$ to bonding agents increases the depth of cure, modulus of elasticity, diametral tensile strength and microshear bond strength.[9] The reduced nanoleakage in this study might be due to the addition of $nano_{HAP}$ to the adhesive resin which improved the inorganic filler content. Degradation of inorganic fillers is less compared to polymer matrix and lead to a more mechanically resistant material.[17]

Si-NH-DBA group showed less nanoleakage (30.18%) which was statistically significant when compared with CN-DBA group and NH-DBA groups. SEM evaluation of Si-NH-DBA group samples showed thick hybrid layer with minute/no deposition of silver particles [figure 4]. Si-$nano_{HAP}$ has many advantages compared to pure HAP. *Invitro* studies showed good adhesion and proliferation of cells on Si-$nano_{HAP}$ materials, including osteoblast[18] and fibroblasts[19]. It was also reported that Si-$nano_{HAP}$ with specific Si contents exhibited better biocompatibility than the pure HAP through total DNA quantification or growth activity evaluation of osteoblastic cells.[20] In addition, Si-$nano_{HAP}$ coatings have the ability to form carbonate containing apatite layer on implants in presence of simulated body fluids. These coated surfaces provide higher number of nucleation sites for the formation of apatite crystallites.[11]

Volume 5, Issue 1; January-February 2022; Page No 481-488

Botelho et al. stated that the surface charge of Si–HAP was much lower than the pure HAP. Therefore, Si–HAP was in favour of Ca2+ adsorption and easier to form amorphous apatite layer on the surface, thus showing a better bioactivity.[21]

The less nanoleakage in Si-NH-DBA could be better explained by a principle i.e re-incorporation of mineral into the partially demineralized dentin matrix. The mineral precipitated may act as a site for further nucleation and the remineralized tissue may be more resistant to degradation.[22] In this study Si-NHAP is not only a mineral which is in nano-form but also bioactive which has considerable osteogenic ability.

The null hypothesis of the study was rejected as the incorporation of $nano_{HAP}$ and Si-$nano_{HAP}$ into conventional dentin bonding agents significantly reduced the nanoleakage at the dentin-adhesive interface.

To the best of our knowledge, this is the first study on nanoleakage evaluation by incorporating these novel nanobioactive fillers into dentin bonding agents. There is very limited literature available to support or contradict the results of the present study. Limitation of the study is that cyclic loading of the specimens were not done. Within the limitations of the present study, it is stated that **Si-$nano_{HAP}$** can be incorporated in dentin adhesives because of its nanoparticles size, carbonate containing apatite forming ability and bioactivity. However, other properties like effect on collagenolytic activity, colloidal stability need to be assessed along with simulated invivo, clinical and randomised control trials.

Conclusion

HAP containing experimental bonding agents decreased the nanoleakage. Addition of silica doped nanohydroxyapatite owing to its carbonate containing apatite forming ability demonstrated significantly lesser nanoleakage. Bioactive silica-doped nanohydroxyapatite particles could be used to reduce the nanoleakage and enhance the longevity of functional restoration.

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Volume 5, Issue 1; January-February 2022; Page No 481-488

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Figure legends:-

Figure 1:-Methodology flow chart. (1a) Class V cavity preparation on buccal and lingual sides of premolar; (1b) Dentin bonding agents incorporated with nano bioactive fillers application; (1c) Composite restoration; (1d) Varnish application; (1e) Immersion in Ammonical silver nitrate solution; (1f)Buccolingual sectioning of tooth; (1g) Observation under scanning electron microscope.

Figure 2:- Backscattered SEM images of the resin-dentin interface of CN-DBA group (5000x). Irregular silver deposition observed with no gap formation, within and below the hybrid layer (arrows).

(D) Dentin; (C) Composite resin; (H) Hybrid layer;

Figure3:- Backscattered SEM images of the resin-dentin interface of NH-DBA group (5000x). Silver deposition without any gap formation below the hybrid layer within the dentinal tubules was observed (arrows).

(D) Dentin; (C) Composite resin; (H) Hybrid layer

Volume 5, Issue 1; January-February 2022; Page No 481-488

Figure 4:- Backscattered SEM images of the resin-dentin interface of Si-NH-DBA group (5000x). Thick hybrid layer with no or mild deposition of silver particles.

(D) Dentin; (C) Composite resin; (H) Hybrid layer;

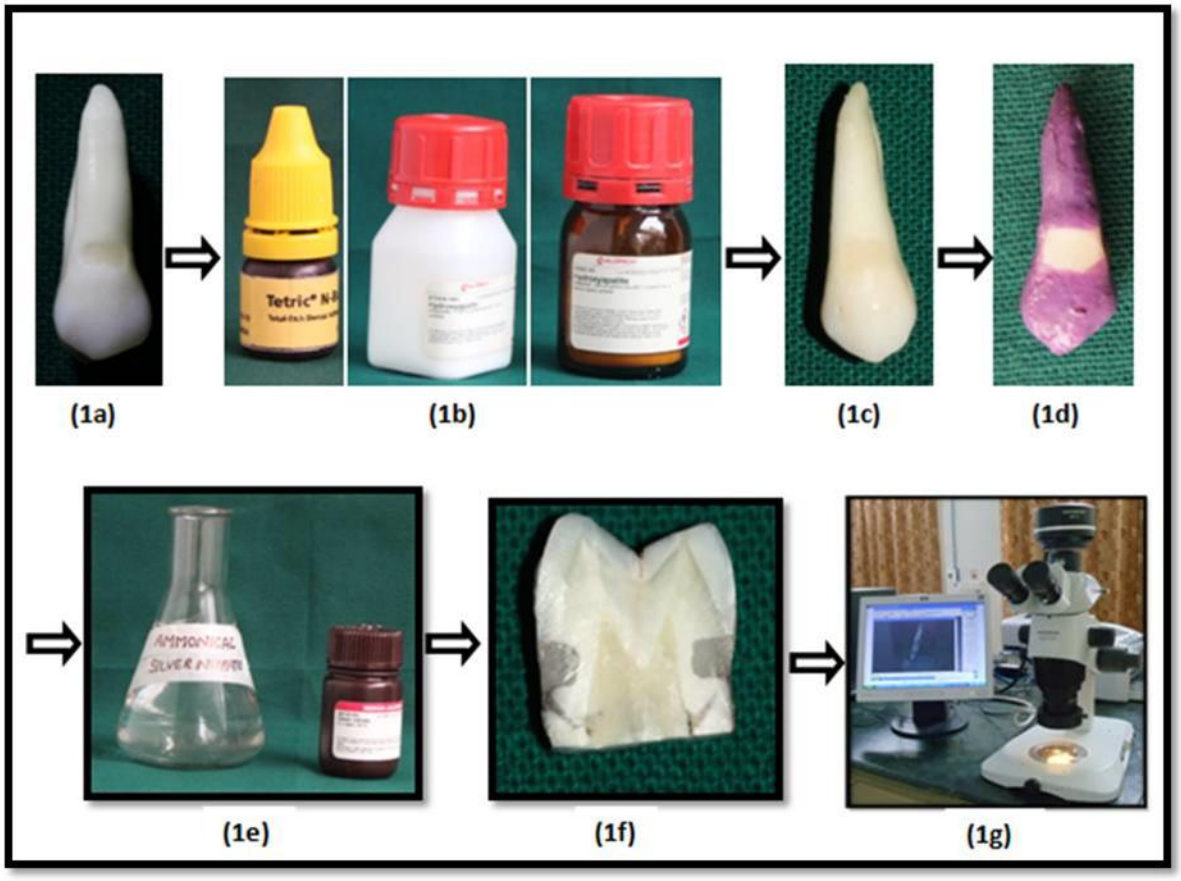
Table 1 Comparison of percentage length of silver nitrate penetration at the resin-dentin interface

GROUPS	N	MEAN (%)	STD. DEVIATION	P VALUE
CN-DBA	16	58.31*,a	12.19	
NH-DBA	16	38.93*,b	10.05	
SI-NH-DBA	16	30.18*,c	6.24	<0.01

Significance level was set at p value 0.05. (Equal alphabetical letters indicate no significant difference).

Statistical test:- One way ANOVA and Tukeys post hoc analysis

Figure 1:-



Volume 5, Issue 1; January-February 2022; Page No 481-488

Figure 2:-

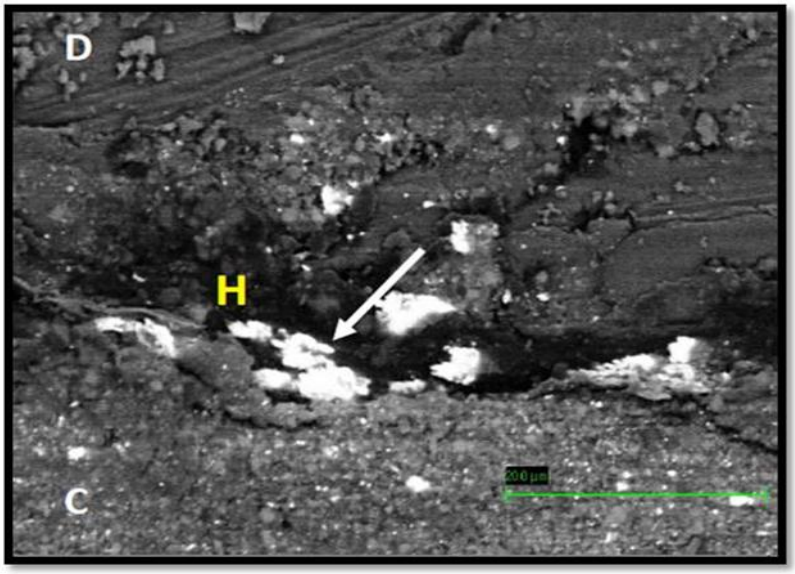


Figure 3:-

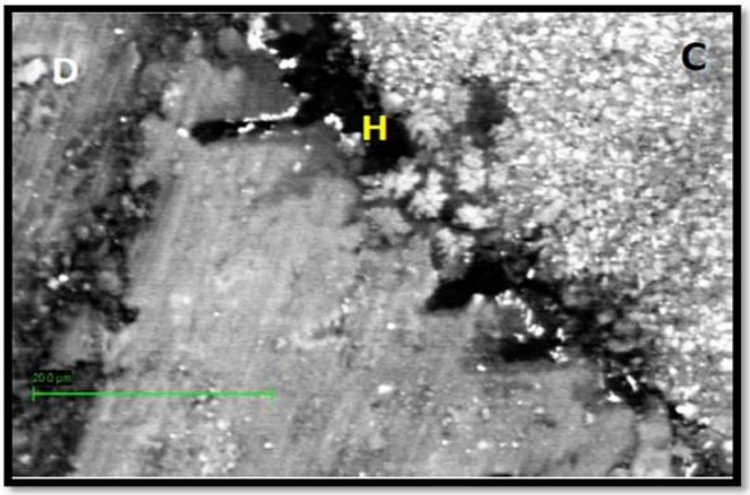
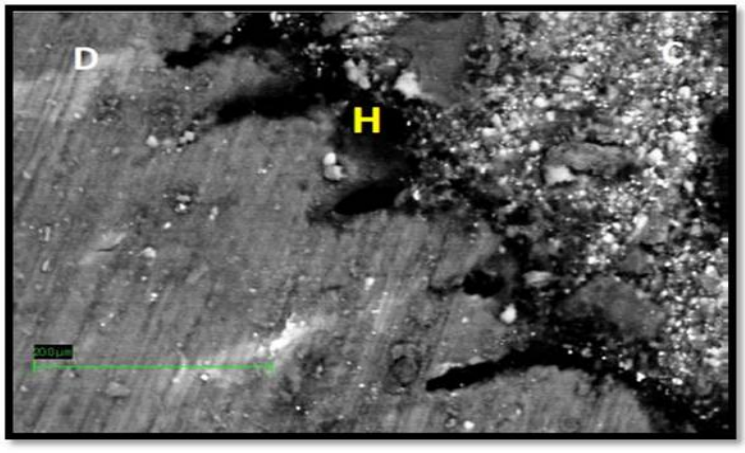


Figure 4:-



Volume 5, Issue 1; January-February 2022; Page No 481-488