A COMPARATIVE EVALUATION OF MICROHARDNESS AND SURFACE TEXTURE OF BIOGLASS PASTE AND TOPICAL FLOURIDE ON ERODED ENAMEL:AN *IN VITRO* STUDY.

DISSERTATION

Submitted to the

BABU BANARASI DAS UNIVERSITY, LUCKNOW, UTTAR PRADESH

In the partial fulfillment of the requirement for the degree of

MASTER OF DENTAL SURGERY

In

CONSERVATIVE DENTISTRY & ENDODONTICS

By

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Under the guidance of
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BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES,

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Batch: 2018-21 **Enrollment No.: 11803220558**

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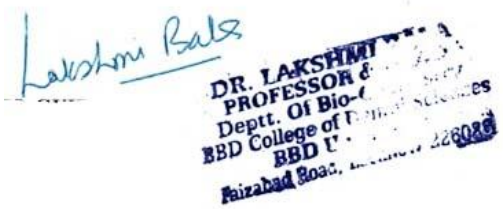

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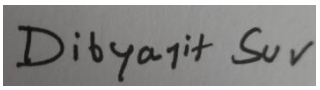

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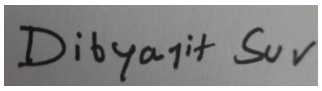

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Dr. Dibyajit Sur

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LIST OF ABBREVIATIONS

S NO	ABBREVIATION	FULL FORM
1	NCCLs	Non Carious Cervical Lesion
2	%	Percentage
3	BAG	Bioactive Glass
4	Mpa	MegaPascal
5	EDX	Energy Dispersive X Ray Spectroscopy
6	Ph	Potential of Hydrogen
7	PLLA/BG	Porous Poly(l-lactide)/Bioactive glass
8	CPP-APP	Casein phosphopeptide–amorphous calcium phosphate
9	CO2	Carbon dioxide
10	mTBS	Microtensile Bond Strength

11	SEM	Scanning Electron Microscopy
12	FE-SEM	Field Emission Scanning Electron Microscopy
13	HAP	Hydroxyapatite
14	ACPF	Amorphous Calcium Phosphate Fluoride
15	RMGIC	Resin-Modified Glass Ionomer Cement
16	PAA	Poly Acrylic Acid
17	Ca	Calcium
18	P	Phosphate
19	VHN	Vickers Hardness Number
20	Mm	Millimeter

ABSTRACT

Dental erosion is caused by a reduced ph level in the oral environment, which causes the teeth to erode. In saliva, both hydroxyl- and fluor-apatite are unsaturated at the same time, resulting in layer-by-layer loss of tooth hard tissue. The current study evaluates the effect of using a 45S5 bioglass paste and topical fluoride application on the cross sectional micro-hardness and the chemical surface changes of eroded enamel.

The buccal surface of forty extracted non-carious molars has been used to obtain enamel discs. The enamel surfaces were ground flat and each disc was covered with two layers of acid resistant nail varnish, with the exception of an exposed treatment window (3 mm x 2 mm) on the buccal surface of the tooth.All specimens were exposed to orange juice with a pH of 3.5 for 60 minutes.The specimens were divided into four groups: 45S5 bioglass paste group (n=10), fluoride gel group (5 min application) (n=10), fluoride gel group (24 h application) (n=10), and control group (n=10). Every group's specimens had their cross-sectional micro-hardness assessed. The top eroded enamel surfaces of five specimens from each group were examined using SEM/EDS.. The cross-sectional micro-hardness of the four groups was compared using a one-way ANOVA.

When compared to fluoride and control specimens, 45S5 bioglass paste considerably improved the sub-surface degraded enamel. ($p < 0.05$)

The 45S5 bioglass paste effectively increases the micro-hardness of the subsurface eroded enamel surface and has greater remineralization efficiency than the other three groups, according to the current in vitro investigation.,but other in vivo studies comparing bioglass to other remineralising agents are required to evaluate its efficacy.

Dental erosion,Orange juice,Bioactive Glass,Flouride,SEM/EDS,Vickers Microhardness.

INTRODUCTION

Loss of tooth structure is a major concern as it may cause hypersensitivity,pain. There are many factors contributing to loss of tooth structure like Dental Caries,Attrition,Abrasion and Erosion.According to National Health and Nutrition Examination Survey around 92% of adults aged 20-64 years have dental caries in their permanent tooth and Dental caries is one of the major reasons for the loss of tooth structure among all other factors[1]

One of the many factors contributing to loss of tooth structure is Non Carious Cervical Lesions.

The etiology of Non Carious Cervical Lesions seems to be related to different factors: hexogen and endogen acids, mechanical abrasive action, tooth flexion under axial and non-axial loads. [2]

According to the study done by hegde et al it has been concluded that Non Carious Cervical Lesions accounted for 46 percent of the tooth decay.[3]

Acording to the study done by Yoshizaki et al,it has been concluded that 67.8 percent patients in their study had Non Carious Cervical Lesions.[4]

According to the study done by Bomfim et al,it has been concluded that 76.8 percent patients in there study had Non Carious Cervical Lesions.[5]

Non Carious Cervical Lesions are characterized by loss of hard dental tissue near the cemento enamel junction.Commonly their shape is like a wedge with the apex pointing inwards.Other times,they appear as regular depressions,like a dome or a cup.Their main characteristics is the presence of hard mineralized tissue.[2]

The Non Carious Cervical Lesions are classified on the basis of the mechanism causing the onset in ;Abrasions,Stress lesions and Erosion

Abrasion- Abrasion is defined as surface loss of tooth structure resulting from direct frictional forces between teeth and external objects or from frictional forces between contacting teeth components in the presence of abrasive medium [6],one of the main causes is improper brushing technique.Their aspect is characterized by dome shape with a very smooth and shiny tissue.According to the study done by Brandini et al prevalence of abrasion is 53 percent. [7]According to the study done by Hegde et al The prevalence of abrasion is 23 percent.[3]

Stress lesions or Abfraction lesions- Abfraction is the pathological loss of tooth substance caused by biomechanical loading forces that result in flexure and failure of enamel and dentin at a location away from the loading.[8] According to the study done by Barttlet et al the prevalence of abfraction is 27 percent.[9]According to the study done by Hegde et al The prevalence of abfraction is 6.3 percent.[3]

Erosion-The cause is the chemical aggression from acids;characteristic is the uniform loss of mineralized tissues beginning from the gingival margin extending in the occlusal direction. [10]According to the study done by Salas et al the prevalence of dental erosion is 30percent.[11]According to the study done by hegde et al Prevalence of dental erosion is 6 percent.[3]

The oldest forms of tooth wear were mostly seen on the occlusal, incisal, and proximal surfaces, but modern erosive tooth wear includes the buccal and palatal/lingual surfaces[12] as well. Tooth wear was formerly only considered problematic if the tooth's function was lost. This could be one reason why dental erosion has been missed in research as a probable cause..[13]

It is generally recognised that lifestyle and behavioural factors have an impact on both oral and general health. Lifestyles evolve over time and are frequently influenced by sociocultural forces. Food and drinking habits, degree of physical activity, stress-related disorders, and/or substance misuse are some of the most common variables.

As previously said, a fundamental change in today's lifestyle is the drastically increasing use of acidic beverages, particularly among children and young adults[14]. Another example is when people adopt a new "healthy lifestyle," with the unintended consequence of having a diet with a higher acidity content. Vegetarians and people who diet or fast to lose weight are examples of this. People consuming a vegetarian diet tend to eat more fruits and vegetables than people following a nonvegetarian diet.Consumption of these acidic foods may lower the pH level in the oral cavity and lead to dental erosion.[15]

Dental erosion is caused by a decreased ph in the oral environment, which causes simultaneous unsaturation of both hydroxyl- and fluor-apatite in saliva, resulting in layer-by-layer loss of dental hard tissue. Early enamel erosion generates little clinical darkening or softening of the tooth surface, making it difficult to detect both visually

and tactilely in the clinical setting.[16] Furthermore, at these early stages, patient complaints are typically absent or limited. When the erosive damage is more extensive, the macromorphology alters more dramatically.[17] Patients' oral health-related quality of life will be affected since the problem will be easier to recognise and more likely to display symptoms..[18]

An eroded surface, it was previously believed, always gave the impression of having a matt surface.[19] Dental erosion, was further said, could only be detected on teeth with no opposing occlusal connections.[20] Today, it is recognised that an erosive lesion's surface appearance is either blank or matt, and that erosion can be detected even when opposing occluding contacts exist between the tooth surfaces. Small concavities can form as the erosive lesion becomes uneven. Most of the time, though, the surface is somewhat rounded or flat, and it sometimes appears to have the impression of having “melted.” [21]

Tooth surface hardness appears to have a role in the development of erosive damage in both primary and permanent teeth, according to studies. Despite the fact that primary teeth are softer than permanent teeth, the erosive process is the same on both. Children's salivary sugar clearances are slower and their salivary flow rates are lower than adults'. Primary teeth are also "smaller" than permanent teeth. Because of the form of the hard tissue, the quality of the saliva, and other salivary circumstances, deciduous teeth are more likely to erode than permanent teeth.[22-26]

One of the most significant defence mechanisms against dental erosion is saliva. The rate of salivary production, as well as the individual's capacity to swallow, affects oral clearance of an acidic product..[27] According to studies, a dry-mouth people has a higher chance of erosion than someone with a normal salivary secretion rate, and children with erosion have saliva with qualities similar to children with high caries activity, although having low caries activity .[28] It's also been claimed that in cases of erosion, salivary buffering capacity is more important than it is in cases of dental caries.[29] The crucial pH is 5.5, and if it falls below that, plaque can re-inhabit, contributing to demineralization and caries onset. In this regard, it should be emphasised that children have a lower rate of salivary production and a lower swallowing capacity than adults.[30]

The thickness of the pellicle that saliva develops on teeth differs not just between people, but also between different parts of the mouth. According to studies, the salivary pellicle provides some protection against acid erosion on enamel, depending on its thickness..[31,32] On the other hand, a quick building of new pellicle on a recently eroded surface will firmly adapt to the degraded surface, preventing remineralization. A pellicle's ability to guard against erosion was found to be restricted in the face of a weak acidic challenge on enamel, and nonexistent in the face of a similar challenge on dentin..[33] It will be obvious that the numerous parameters influencing pellicle and plaque formation can have a significant impact on the location and severity of erosive damage.

Early enamel erosion causes little clinical darkening or softening of the tooth surface, and is therefore a common indicator of dental erosion on a cusp tip, with or without dentinal involvement, and is most commonly found on first molars, especially in the lower jaw.. Cuppings are significantly linked to dental erosion and should be carefully examined, as they have been shown to be a signal of the commencement of erosion. The pulp might be visible through the residual tooth material in advanced cases of erosion[34]. This is most noticeable in the primary dentition's maxillary central incisors, although it can also be visible in the permanent dentition.

Materials like bioactive glasses and topical fluoride are used to prevent erosion or to start Remineralization of the sub-surfaces of enamel that may improve the micro-mechanical properties of these regions and render them more resistant for any further erosive or cariogenic challenges.

The most frequent remineralizing agent is fluoride. When the acid attacks the enamel surface, the pH rises, and the fluoride in the microenvironment prevents the enamel from dissolving.

Due to the extremely low pH (in the case of titanium tetrafluoride) and the potential for minor discolouration, there are certain negative side effects.a dull feeling on the tooth surface and an astringent sensation (in case of highly concentrated tin-containing fluoride solutions).[35]

Bioactive glasses are silicate-based and can form a strong chemical bond with the tissues. These biomaterials are highly biocompatible and can form a hydroxyapatite layer when implanted in the body or soaked in the simulated body fluid. Due to several disadvantages, conventional glass processing method including melting of glass components, is replaced by sol-gel method with a large number of benefits such as low processing temperature, higher purity and homogeneity and therefore better control of bioactivity.[36]

The greater disadvantages of BAGs are the not so great optimal mechanical property and the meagre break resistance. The out bending-tensile rigidity of the greater part of the BAGs varied between 40 and 60 MPa, and they are not, therefore, usable for loading applications.[37]

As we know that eroded enamel surface undergoes a lot of changes when subject to such conditions.The structural changes on the eroded surfaces of the enamel under scanning electron microscope is visualised.The elements present on the eroded surface of the enamel before ersosion and after erosion are analysed by EDX

In this study The cross sectional microhardness of the eroded enamel surface after the application of the bioglass paste and topical fluoride is measured.To measure the cross sectional microhadness Vickers hardness test is used as it shows very accurate readings

AIM

AND

OBJECTIVES

AIM & OBJECTIVES

Aim of the study:

The aim of this research is to see how a bioglass paste compares to Flouride gel on enamel when it is exposed to erosion.

Objectives of the study:

1. To assess the effect of using a bioglass paste and topical fluoride application on the cross sectional microhardness and surface texture of the eroded enamel.

2. To correlate the effect of using a bioglass paste and topical fluoride application on the surface texture of the eroded enamel.

REVIEW

OF

LITERATURE

Hench LL and Wilson J (1993)[38] provided a comprehensive overview of all types of ceramic and glass materials. They quoted that the enormous growth of the field of bioceramics is due to the importance of bioactive materials which stimulate repair and regeneration of dentinal tissues.

Stoor P etal (1998)[39] studied the effects of bioactive glass S53P4 on the oral microorganisms *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Actinomyces naeslundii, Streptococcus mutans, and Streptococcus sanguis*. They found that, in aqueous solutions the powdered bioactive glass S53P4 appears to have a broad antimicrobial effect on microorganisms of both supra- and subgingival plaque.

Lussi A and Schaffner M. (**2000)**[25]; Studied the progression rate and risk factors of dental erosion and wedge–shaped defects of teeth over a 6–year period,and found that there was a definite statistically significant progression of non-carious dental hard tissue defects in their sample.

D.G. Gilliam et al(2002)[40]Performed a study on the effects of a new dentrifice formulation containing a modified bioglass material replacing part of the abrasive silica component and concluded that the inclusion of bioactive glass particles in a suitably formulated vehicle may be an effective desensitizing agent for the treatment of dentin hypersensitivity.

Sirimaharaj V et al.(**2002)**[41]; studied the consumption patterns of acidic foods and drinks among several sport groups and to examine any relationships between consumption patterns and erosion of teeth. They found that consumption of acidic foods and drinks was frequent among most athletes and the incidence of dental erosion was also more in them.

Arii-pekka forsback et al (2004)[42] Performed an in vitro study on the use of bioactive glass and the biomimetic method on dentin mineralization and found that Bioactive glass S53P4 may develop into a clinical product that could be used in the mineralization of dentin and its tubules in a physiological environment.

K. Zhang et al(2004) [43] stated in their study,that a phase separation technique is used to process porous poly(l-lactide)/bioactive glass (PLLA/BG) composites. The addition

of bioactive glass enhanced the elastic modulus, but decreased the tensile strength and ductility of composites.

Olfat E. Hassanein et al(2006)[44] studied the ability of bioactive glass to remineralize carious enamel and dentin, with and without the use of Zeolite and found that bioactive glass has the potential for remineralizing carious enamel and dentin

Misra et al(2008)[45] compared the effects of introducing micro and nanoscale bioactive glass particles on the various properties (thermal, mechanical and microstructural).They found that the incorporation of nano scale-Bioactive Glass particles had a significant reinforcing effect by enhancing the elastic modulus as function of nano scale-Bioactive Glass content.

Reynolds EC et al. (2008)[46]; investigated the ability of CPP-ACP to increase the incorporation of fluoride into plaque and to promote enamel remineralization *in situ*.They came to a conclusion that CPP-ACP enhanced remineralisation.

Prabhakar et al(2009)[47] evaluated the effect of slow release of bioactive glass on remineralization of carious lesions as compared to fluoride, and found that Bioactive glass can be used as an alternative to fluoride as a remineralizing agent to enhance the remineralization in the absence of fluoride.

H.M. Honório et al (2010)[48] evaluated the interaction between caries and erosion processes and suggested that the combination of erosive and cariogenic challenges showed less subsurface demineralization than a cariogenic challenge alone.

Lata S et al. (2010)[49]; conducted an in vitro study on enamel blocks of human premolars for evaluating the remineralization potential of fluoride and ACP-CPP and the combination of ACP-CPP and fluoride on early enamel lesions. The present study concludes that; ACP-CPP cream is effective, but to a lesser extent than fluoride in remineralizing early enamel caries at surface level

Bakry AS et al. (2011)[50]; Conducted a study,in which 45S5 bioglass was mixed with phosphoric acid and irradiated with CO_2 laser and examined as a possible aid in the treatment of dentin hypersensitivity. They found that that 45S5 bioglass could occlude the dentinal tubule orifices with calcium-phosphate crystals,and improved the mechanical organization of these crystals

Bakry AS et al. (2011)[50] evaluated the biocompatibility of 45S5 bioglass mixed with 50% phosphoric acid which has been suggested to treat dentine hypersensitivity and incipient enamel caries on the rat pulpal cells.They concluded that 45S5 bioglass paste can serve as a biocompatible material that can potentially be used safely on dentine.

Salvatore Sauro et al(2012)[51] evaluated the microtensile bond strength (mTBS) of two ‘‘simplified’’ self-etching adhesives bonded to air-abraded dentine using experimental bioactive glass powders containing polyacrylic acid.They came to a conclusion that air-abrasion procedures performed using pure Bioglass or Bioglass containing 15 wt% Poly Acrylic Acid do not interfere with the immediate bonding performance of self-etching adhesives.

D.H.J Jager et al(2012)[52] : Evaluated erosive potential of beverages, using exposure times from 3 to 30 min, and to analyse the relationship between erosion and several drink parameters. :They found out that Exposure times between 3 and 30 min resulted in very different estimates of erosive potential.

Bakry AS et al. (2013)[53]; conducted an invitro study in which, the efficiency of decreasing the dentin permeability exerted by the interaction layer formed between bioglass and dentin was compared to a resin-containing oxalate desensitizing agent (MS Coat One) and a resin-free oxalate desensitizing agent (Super Seal). The results stated that application of 45S5 bioglass paste to dentin was able to occlude patent dentinal tubule orifices with a layer of calcium-phosphate crystals, while the oxalate containing agents were able to form small crystals which were found in dentinal tubule orifices and scattered along the superficial parts of the dentinal tubule lumen.

Meng Deng et al(2013) [54];investigated whether BioGlass, when used before and after Hydrogen Peroxide bleaching, or mixed with Hydrogen Peroxide for bleaching, can benefit the bleaching therapy as evaluated in terms of color, microhardness and morphology of bovine enamel. They concluded that The alkalinity and accelerated ionic releasing of BioGlass in Hydrogen Peroxide make BioGlass a promising biomimetic adjunct for bleaching therapy to ensure the lifelong integrity of tooth.

Masahiro IIJIMA et al(2013)[55] investigated the effect of a bioactive glass coating on crystal formation on the surface of alumina disk specimens.They came to a conclusion

that Bioactive glass coating influenced crystal formation on the surface of alumina disk specimens in artificial saliva solution.

Asad Mahmood et al(2014) [56] studied the abrasive action of a 45S5 bioactive glass based toothpaste on enamel as a function of the particle size and shape of the glass,and they came to a conclusion,that the particle size, grinding process and particle shape strongly influence the abrasive action of bioactive glass based toothpastes.

Narayana et al(2014)[57] investigated the efficacy of bioactive glass containing product on remineralization of artificial induced carious enamel lesion and to compare its efficiency with other remineralization products using an *in-vitro* pH cycling method.The found that bioactive glass is an effective remineralizing agent.

E. Khan et al(2014) [58] determined the potential of two commercial bioactive glasses as secondary preventive products against enamel erosion and found that all three groups except bioglass showed a general trend of decrease in surface microhardness over time.

A.S. Bakry et al(2014)[59] examined the possibility of forming a Bioglass® 45S5-phosphoric acid paste "interaction layer" on the artificially induced enamel lesions using the field emission scanning electron microscopy (FE-SEM) coupled with energy dispersive X-ray spectroscope (EDS), and X-ray diffraction (XRD). they suggested the possible use of the current technique for treatment of incipient enamel eroded lesions

Z. A. Guclu et al(2016) [60] performed an vitro study to investigate the comparative in vitro enamel remineralisation potential of commercial toothpastes containing bioactive glass (BG) particles, hydroxyapatite (HAP) particles or casein phosphopeptide – amorphous calcium phosphate (CPP-ACP) nanocomplexes.They concluded that BG and CPP-ACP both coated the enamel surface, although their ability to remineralise the body of the lesion was compromised at low pH.

Renita Soares et al(2017)[61] evaluated the ability of Casein Phosphopeptide-Amorphous Calcium Phosphate Fluoride (CPP ACPF), Bioactive Glass (BAG), fluoride enhanced Hydroxyapatite (HA) gel and self-assembling peptide P11-4 to remineralise artificial carious lesions in enamel using a 30 day pH cycling model

through surface microhardness analysis and SEM. They concluded that remineralising potential of self assembling peptide P11-4 was observed to be the highest followed by CPP-ACPF, BAG and fluoride enhanced HA gel

Michelle Alexandra Chinelatti et al(2017)[62] Studied the effect of a bioactive glass ceramic for the control of erosion and carios lesions. They suggested that Bioactive glass ceramics provides superior and continuous remineralizing effect for control of erosion and carious lesions.

Pattaranut Benjasuwantep et al(2017) [63] evaluated the remineralization effect of bioactive glass on enamel carios-like lesions compared with fluoride based products in primary teeth using micro-computed tomography to be an alternative remineralizing agent to avoid risk of fluorosis especially in young children. They concluded that, bioactive glass may be used in place of 500 ppm fluoride or CPP-ACP for remineralization of caries without the risk of fluorosis especially in young children.

A. Tezvergil-Mutluay et al(2017)[64] evaluated in this invitro study that the degradation of completely demineralized dentin specimens in contact with a filler-free or two ion-releasing resins containing micrometer-sized particles of Bioglass 45S5 (BAG) or fluoride-containing phosphate-rich bioactive glass (BAG-F). They came to a conclusion that the fluoride-containing phosphate-rich bioactive micro-filler incorporated into resin-based composites is more bioactive than BAG 45S5.

Elasser et al(2018)[65] evaluated the remineralizing potential of nano bioactive glass (BG) in comparison with nano hydroxy appetite (HA) incorporated into adhesive on demineralized dentine as affected by PH cycling.They concluded that HA nanorods and NaF have higher efficacy in more acidic condition, while BG nanoparticles was most effective under neutral condition.

Salvatore sauro et al(2018)[66] evaluated the effect of load-cycle aging and/or 6 months artificial saliva (AS) storage on bond durability and interfacial ultramorphology of resin-modified glass ionomer cement (RMGIC) applied onto dentine air-abraded using Bioglass 45S5 (BAG) with/without polyacrylic acid (PAA) conditioning. Finding suggested that the synergic combination of the therapeutic properties of RMGIC to induce fluoride release and the bioactivity of BAG to induce

mineral growth may represent an alternative restoration approach to achieve a long-lasting restoration.

A.A. Taha et al (2018)[67] assessed the ability of this novel fluoride-containing bioactive glass (QMAT3) propelled via an air abrasion handpiece to induce WSL remineralization, in comparison with a commercially available 45S5 glass (Sylc) and artificial saliva (AS). They found that, the novel fluoride-containing QMAT3 glass was capable of enhancing enamel remineralization more effectively than a commercially available bioactive glass (Sylc).

.Luiza Pereira Dias da Cruz et al(2018)[68] compared different bioactive glass formulations to investigate their effectiveness in an in vitro environment. They found that formation of a hydroxyapatite layer occluding the dentine tubules following artificial saliva immersion may be considered an important stepping stone for further evaluation of these bioactive glass compositions.

Mona Ali Abbassy et al(2019)[69] evaluated the effect of using a 45S5 bioglass paste and a topical fluoride(Acidulated Phosphate Gel) as protective agents against acidic erosion for enamel. They found out that 45S5 bioglass paste can efficiently protect the enamel surfaces from acidic erosion challenges.

Saffarpour et al(2019)[70] evaluated the effects of modified 45S5 Bioglass before and during bleaching procedure with 35 percent hydrogen peroxide on tooth color change and physicochemical properties and morphological properties of human enamel.They suggested that using bioglass before hydrogen peroxide has a greater protective effect since it increased microhardness more effectively and retained the integrity of the enamel surface

Qiong et al(2020)[71] studied the remineralisation effect of bioactive glass on artificially induced dentine caries and concluded that Bioactive glass possessed a promising remineralisation effect on artificial dentine caries and could be a therapeutic choice for caries management.

Wenyan Huang et al(2020)[72] conducted a study aimed to fabricate mesoporous BGio lass nanoparticles by well-established sol-gel method and analyze its acidic pH neutralizing and odontogenic potential. They found that the potential application of

BioGlass nanoparticles alone or in combination with SHEDs for pulp-dentin regeneration in pathophysiological acidic environment.

Chenmin Yao et al(2020)[73] In their invitro study, an experimental two-step universal adhesive, ,and self-etch (SE) mode combining a primer, with a bioglass-containing hydrophobic adhesive resin, was multifactorially investigated. It was concluded in the present study that the experimentally developed adhesive is a promising candidate to combine high bonding potential and bond-degradation resistance through reduced water uptake, with anti-enzymatic and anti-bacterial therapeutic effects through gradual ion release from the contained bioactive zinccalcium-fluoride bioglass.

Dimitrios et al(2020)[74] evaluated the influence of air abrasion surface pretreated with bioactive glass 45S5 on bovine enamel erosion induced by a common soft drink.They suggested in their study that air abrasion pretreated with bioglass 45S5 may prevent enamel erosion induced by excessive consumption of soft drinks

MATERIAL

AND

METHODOLOGY

BIOGLASS

Bioactive materials are durable materials that can bind chemically with the surrounding bones and in some cases even with soft tissue. When bioactive materials are implanted in the body, a porous biologically active layer is formed that is a very favorable substrate for the regrowth of bone tissue. The material is ideal as bone cement filler and coating because of its biological activity. A bioactive ceramic is a ceramic that generate a positive reaction in the biological environment of the implants and/or chemical reaction that modify the material in a certain thickness under the surface.[75]

The bioactive ceramic are divided into two classes:

• Ceramics that induct bioactivity due to their chemical composition

• Ceramics in which the bioactivity is induced or by superficial treatment or by filling of the porosity by pharmacological active substance. [76]

The bioactive ceramics are:

• Bioactive glass (BAG) (Bioglass®,)

• BAG-ceramics (Apatite-Wollastonite glass-ceramic, dense and porous hydroxyapatite)..

The most studied is the Bioglass® 45S5. The abbreviation indicates that it contains 45% in weight of SiO2 (oxide creator) and the molar rate between Ca/P is of 5:1. Glasses with significantly lower molar rate (in the form of CaO and P2 O5) do not generate connections with the bone.[77]

The base components are usually SiO2 , Na2 O, CaO, and P2 O5 and given below are percentages in weight of the most common Bioactive glasses. Bioglass composition in wt%

• SiO2 -45 wt%

• Na2 O - 24.5 wt%

• CaO - 24.5 wt%

• P2 O5 -6 wt%.

Bioactive Glasses, as opposed to most technical glasses, are characterized by the materials' reactivity in water and in aqueous liquids. The bioactivity of Bioactive Glasses is derived from their reactions with tissue fluids, resulting in the formation of a hydroxycarbonate apatite (HCA) layer on the glass. The essential chemical property of Bioactive Glasses to release Si+, Ca,2+ and PO4 3− in the tissue fluid, resulting in the initiation of apatite formation on the glass surface has led us to believe that it might also be quite possible to use the materials as vehicles for ectopic mineralization of the surrounding tissue. In this case, the Bioactive Glasses may have therapeutic value as mineralizing agents in caries prophylactics, and also as a desensitizing agent in clinical situations where opened dentinal tubules lead to hypersensitive teeth.[78]

The main advantage of the Bioactive glasses is the high superficial speed reaction that brings to rapid connections to the tissues. Bioglasses are embedded in a biomaterial support to form prosthetics for hard tissues. Such prosthetics are biocompatible, show excellent mechanical properties and are useful for orthopedic and dental prosthetics.[79]

The greater disadvantages are the not optimal mechanical property and the meagre break resistance. The out bending-tensile rigidity of the greater part of the Bioactive glasses varied between 40 and 60 MPa, and they are not, therefore, usable for loading applications.[79]

FLOURIDE GEL

.Although it has been claimed that the concentrated fluoride ions in fluoride varnish induce globules of a calcium fluoride-like substance to develop on the tooth surface, the mechanism of fluoride action in dental caries is still being explored. Protein phosphate in the mouth stabilises these globules, which operate as an insoluble reserve of fluoride at neutral pH.. When a cariogenic challenge is present, such as sugar ingestion, the pH drops and the rate of disintegration of these globules increases. As a result, the solubility constant of calcium and phosphate ions decreases, releasing fluoride and increasing calcium and phosphate ion saturation in plaque fluid.[81] This reaction helps to prevent calcium and phosphate from dissolving from the tooth mineral and/or speeds up the remineralization or reprecipitation of lost minerals.

Sodium fluoride varnish is advocated for moderate and high-risk children, particularly children younger than , as well as for children who are receiving orthodontic treatment.It also is used to prevent and arrest caries in children.It is used for preventing caries, promoting remineralization of caries, and treating tooth hypersensitivity.[82]

Topical application of a sodium fluoride varnish is carried out using a small brush and a very small amount of varnish (especially for young children). The teeth do not have to be very dry and the patient should not eat for approximately two hours after application.Since toothbrushing can remove fluoride varnish, the varnish should be left on the teeth and brushing on the day of application should be avoided whenever possible.[82]

Conventional fluorides with a known anti-cariogenic potential offer some, but limited, protection against erosion as the CaF2 precipitates formed on the surface are readily soluble in acids. Metal containing fluoride compounds showed promising results in prevention of erosion, but might involve some adverse side effects due to the very low pH and the potential to cause slight discoloration, a dull feeling on the tooth surface and an astringent sensation.[83]

There is convincing evidence that fluoride, in general, can strengthen enamel against erosive acid damage; high-concentration fluoride agents and/or frequent applications are considered potentially effective approaches to prevent dental erosion. However, fluorides might be more effective in enamel than in dentin, as the organic matrix influencing the efficacy of fluorides might to some extent be affected by enzymatical and chemical degradation as well as by mechanical abrasion. The use of tin-containing fluoride products might provide the best approach for effective prevention of dental erosion.[83]

METHODOLOGY

The present study was conducted in the Department of Conservative Dentistry and Endodontics and Department of Biochemistry,Babu Banarasi Das College of Dental Sciences,Lucknow in collaboration with Central Institute of Plastics Engineering and Technology Lucknow and Spectral Lab Analysis,Greater Noida

Sample Selection

40 multi rooted mandibular and maxillary molar teeth extracted for orthodontic and periodontal reasons,were collected from Department of Oral and Maxillofacial Surgery,BBD College of Dental Sciences,Lucknow according to selection criteria.Extracted teeth were handled as per safety guidelines of ADA.Collected teeth were cleaned of visible blood and gross debris by washing them in sterile water.Root surface were debrided with hand scaler and stored in 10%Formalin solution until further experiment.

Eligibility Criteria

Inclusion Criteria

Fully developed permanent maxillary or mandibular molars with no caries on the buccal surface.

Exclusion Criteria

- Tooth with Root Fracture.
- Teeth which are earlier restored or endodontically treated.
- Teeth with root resorption.
- Teeth with any developemental anomaly.
- Teeth with any non carious cervical lesion.

MATERIAL AND ARMAMENTARIUM

For Sample Preparation

- Hand Scaler(API,India)
- 10% Formalin Solution(Polyformalin pvt limited)
- Saline water

For Sectioning

- Micromotor,Straight Handpiece(NSK,Japan)
- Mandrel(Dentsply Maillefer,Switzerland)
- Silicon Carbide Disc
- Excavator(API,India)

For Erosion

- Pure Orange Juice(Tropicana)

For countering Erosion

- Flouride Gel 1000 PPM(Septodont)
- Bioglass Paste(MO-SI,USA)

Methodology

Specimen Preparation

To obtain the enamel specimens the crown were sectioned longitudinally in mesiodistal direction.(Fig no 7).Sectioned surfaces were embedded In acrylic blocks.(Fig no 8)Water-cooled silicon carbide discs were used to flatten the sectioned surfaces of the samples.;alluminium oxide sandpaper for decreasing granulations. Except for the window(5 x 5mm)., Two layers of acid-resistant nail varnish were applied to the flat enamel surfaces. The window dimensions were confirmed in all the samples, with a Williams periodontal probe to ensure uniformity of the enamel window

Inducing Erosion

By immersing all specimens in pure orange juice for one hour(Fig no 26), early enamel erosion was induced in all of them. The erosion test was performed at room temperature (about 25 degrees Celsius) with continuous magnetic stirring at a low speed (120 rpm).(Fig no 9)

Specimens were randomly divided into four groups(n=10)

Group A(Flouride gel application for 5 minutes)

Group B(Flouride gel Application for 24 hours)

Group C(Bioglass paste application for 2 hours)

Group D(Control Group)

Application of Flouride Gel and Bioglass paste

Group A

- Flouride gel(Septodont) was applied to the treatment window of 10 sectioned enamel surfaces with the help of a microbrush at room temperature and then kept for 5 minutes and then washed away with sterile water.(Fig No 15-16)

Group B

- Flouride gel(Septodont) was applied to the treatment window of 10 sectioned enamel surfaces with the help of a microbrush at room temperature and then kept for 24 hours and then washed away with sterile water.(Fig No 17-18)

Group C

- • To make a bioglass gel, one-tenth of a gram of 45S5 bioglass powder (MO-SI, USA) was mixed for one minute on a glass slab with 0.2 mL of 50 wt percent phosphoric acid made by diluting 85 wt percent phosphoric acid (Halix Chemicals, India) in distilled water (pH 2).(Fig No 28) The acidic gel was applied with a microbrush to the treatment window of 10 sectioned enamel surfaces at room temperature and left for 2 hours before being rinsed away with sterile water.

Group D

- Remaining ten specimens were not applied with the bioglass paste or fluoride gel and was kept intact.(control group)

Bonding Agent Application

- A layer of bonding agent was immediately applied over the sectioned enamel surfaces where bioglass phosphoric acid gel was applied.(Fig No 19)
- A layer of boding agent was applied over the sectioned enamel surfaces where fluoride gel was applied for 24 Hours.(Fig No 24)

At room temperature, all specimens were kept in a remineralizing solution of 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, 0.13 M KCl, and 5 mM NaN_3 adjusted to pH 7.0 with HEPES buffer for 24 hours.(Fig No 26-27)

The layer of bonding agent was carefully removed with an excavator after 24 hours of storage in the remineralisation solution from Group B((Flouride Gel application for 24 hours) and Group C (Bioglass Paste application for 2 hours), and then rinsed with water spray.

Checking for Microhardness

The cross-sectional micro-hardness of five specimens from each group was tested using a Vicker micro-hardness tester(Spectro Analytical Labs Limited,Greater Noida).(Fig No 30) after they were implanted and longitudinally sectioned through the centre having a 136 degree angle between opposing faces on a diamond pyramid micro-indenter. At room temperature, a 25-gram load was employed for 5 seconds (23 degree Celsius). The following equation was used to calculate the Vicker's hardness number (VHN):

(VHN = 1854.4P/d2, where P is the applied force in grammes and d is the indentation's average length in mm.)

Three rows of eight indentations were created, one in the middle region of the exposed dental enamel and the other two 100 mm on either side of it. At 30, 40, 50, and 100 mm from the outer enamel surface, indentations were produced. To avoid indenting close to the surface of the lesion, the locations chosen were 20–40 mm away.

SEM/EDS Top Surface Examination

The SEM/EDS was used to study the top enamel degraded surface of five specimens from each group. All specimens were dehydrated in an escalating ethanol series (50–100%), gold coated, and SEM/EDS was used to evaluate the surface chemical characterisation and morphological aspects of the specimens.(Fig No 29)

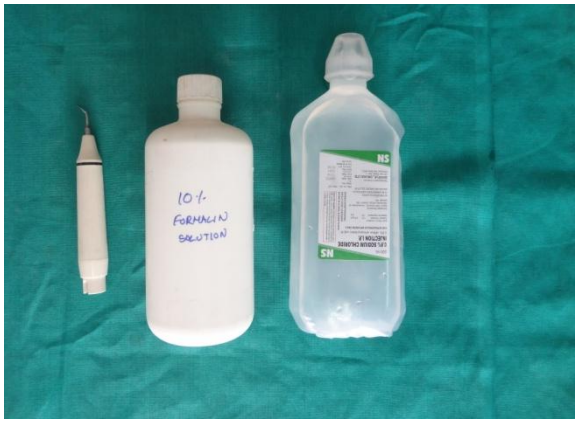


FIG NO 1

Scaler,10%Formaline solution,Saline

FIG NO 2

Micromotor straight handpiece,mandrel ,silicon carbide disc,Excavator

FIG NO 3

Pure orange juice

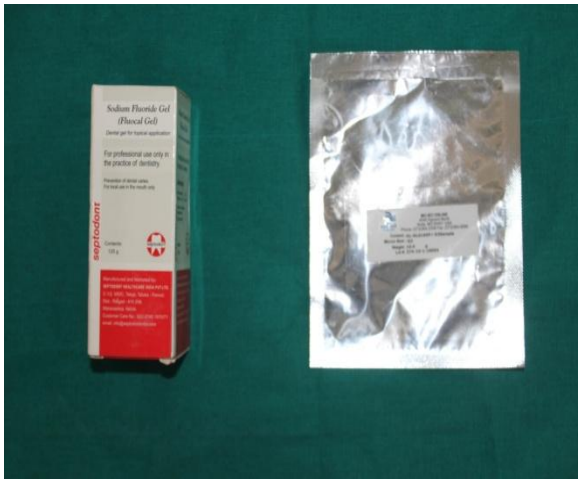


FIG NO 4

Flouride gel and Bioglass Paste

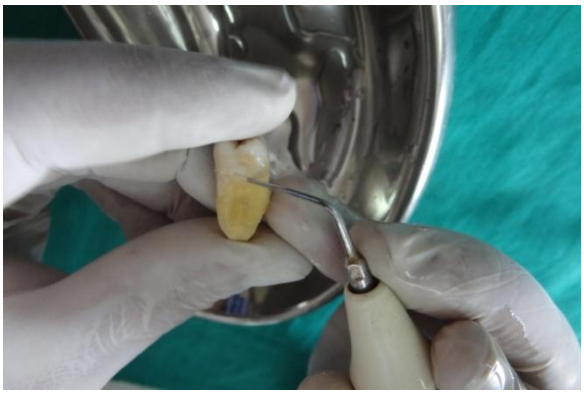


FIG NO 5

Scaling with Ultrasonic

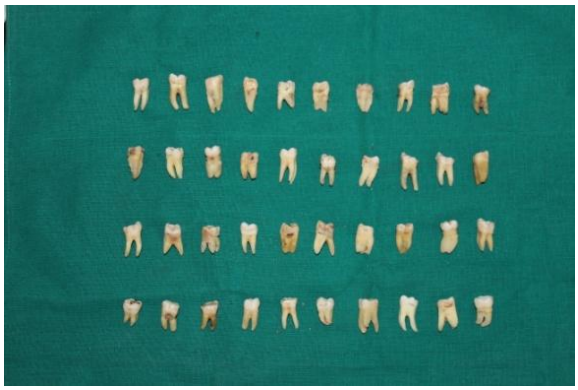


FIG NO 6

TOTAL COLLECTED SAMPLE

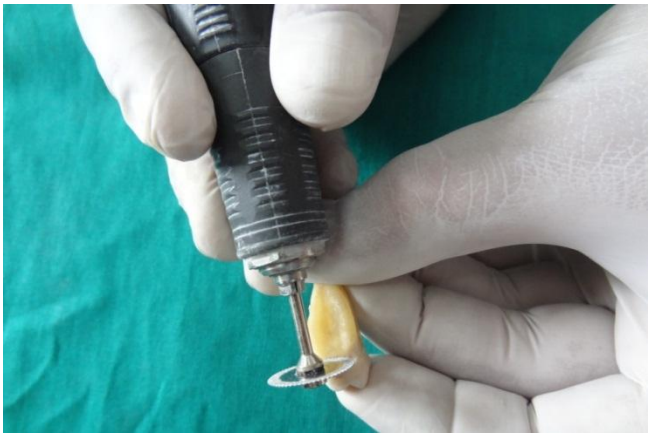


FIG NO 7

ENAMEL SPECIMEN SECTIONED LOGITUDINALLY IN MESIO DISTAL DIRECTION

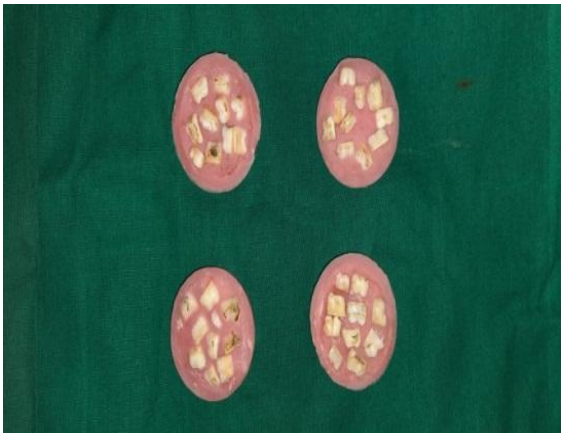


FIG NO 8

ENAMEL SPECIMENS WERE DIVIDED INTO 4 GROUPS .EACH GROUP HAVING 10 ENAMEL SPECIMENS AND WAS EMBEDDED IN ACRYLIC BLOCKS

FIG NO 9

MAGNETIC STIRRER

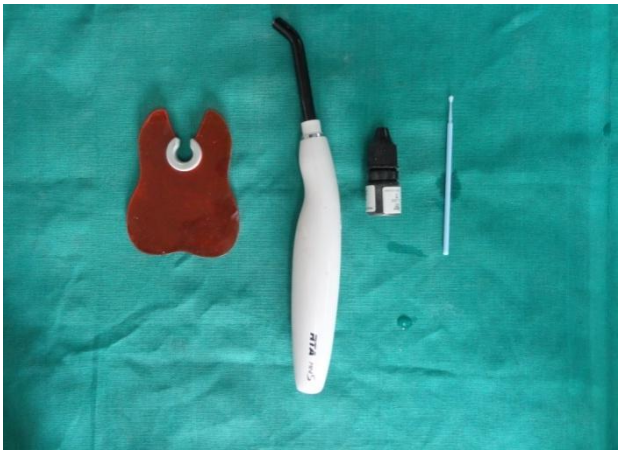


FIG NO 10

CURING LIGHT,BONDING AGENT,MICROBRUSH

FIG NO 11

ORANGE JUICE PLACED IN THE MAGNETIC STIRRER

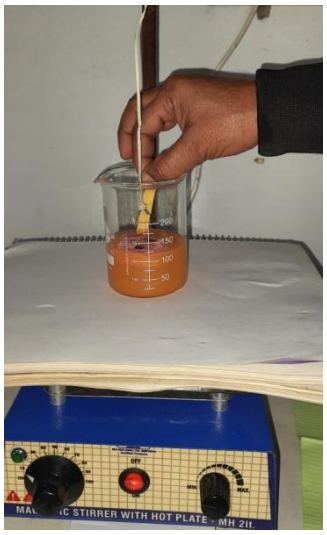


FIG NO 12

Ph IS MEASURED OF THE ORANGE JUICE

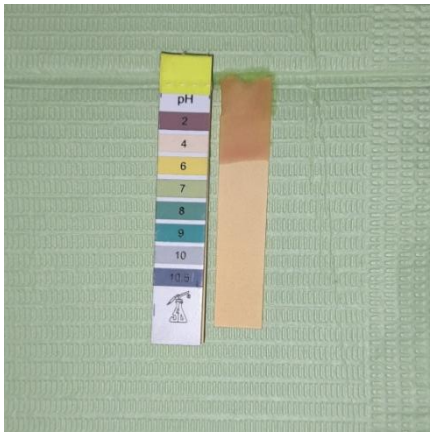


FIG NO 13

Ph MEASURED WITH LITMUS PAPER

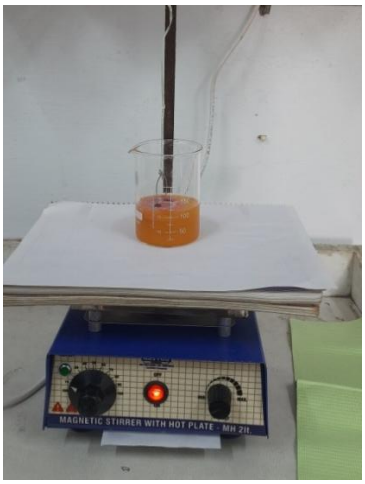


FIG NO 14

SPECIMENS IMMERSED IN ORANGE JUICE AND KEPT FOR 4

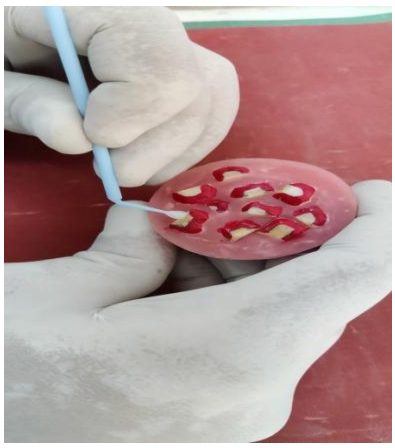


FIG NO 15

FLOURIDE GEL APPLIED IN GROUP 2 AND KEPT FOR 5 MINS

FIG NO 16

FLOURIDE GEL WASHED AWAY WITH STERILE WATER AFTER 5 MINS

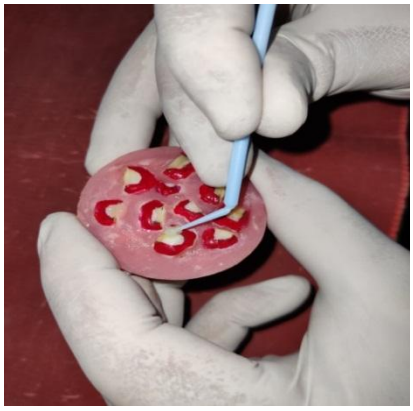


FIG NO 17

FLOURIDE GEL WAS APPLIED IN GROUP 3 AND KEPT FOR 24

FIG NO 18

FLOURIDE GEL WAS WASHED AWAY AFTER 24 HOURS WITH STERILE WATER

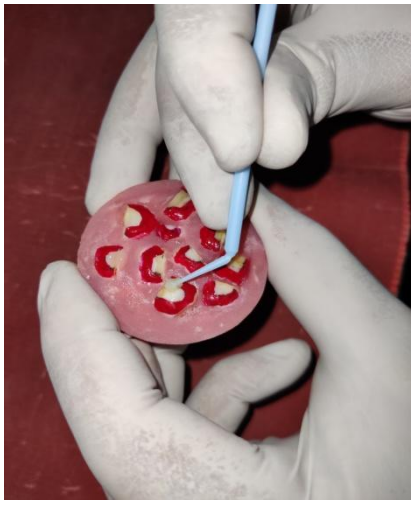


FIGURE NO 19

BONDING AGENT WAS APPLIED ON THE SPECIMENS IN GROUP 3

FIGURE NO 20

ALL SPECIMENS IN GROUP 3 WERE LIGHT CURED

FIG NO 21

MIXING OF BIOGLASS WITH PHOSPHORIC ACID

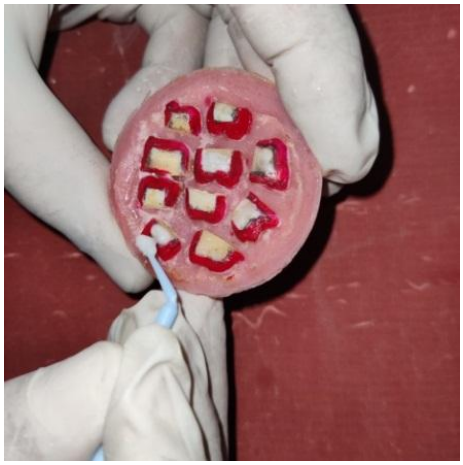


FIG NO 22

BIOGLASS WAS APPLIED ON ALL SPECIMENS IN GROUP 4 AND KEPT FOR 2 HOURS

FIG NO 23

BIOGLASS PASTE WAS WASHED AWAY AFTER 2 HOURS WITH STERILE WATER

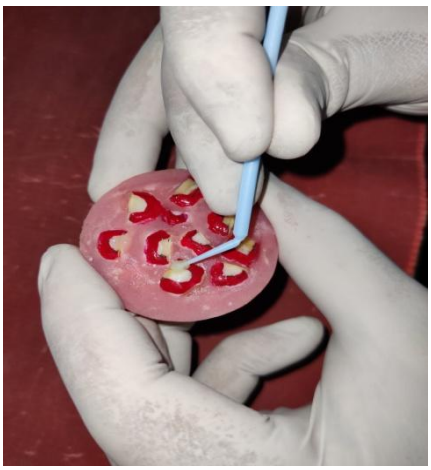


FIG NO 24

BONDING AGENT WAS APPLIED ON ALL SPECIMENS IN GROUP 4

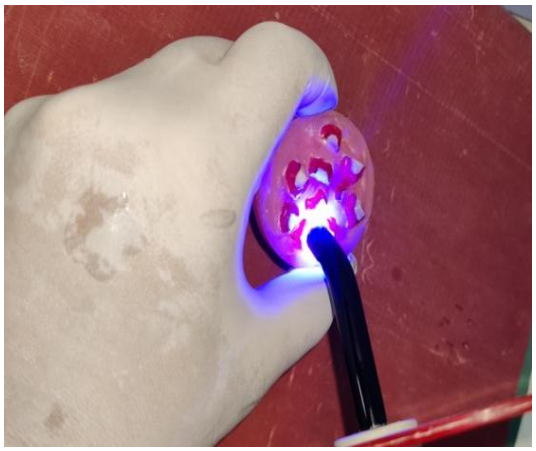


FIG NO 25

ALL SPECIMENS IN GROUP 4 WERE LIGHT CURED

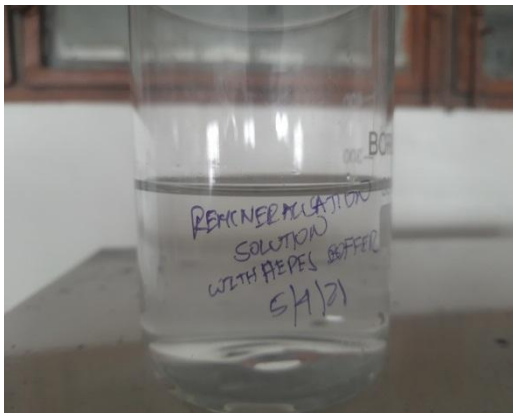


FIG NO 26

REMINERALISATION SOLUTION

FIG NO 27 & 28

ALL SPECIMENS WERE STORED IN THE REMINERALISATION SOLUTION FOR 24 HOURS

FIG NO 29

SCANNING ELECTRON MICROSCOPE(QUANTA FEG 450,FEI,THE NETHERLANDS)

FIG NO 30

VICKERS MICROHARDNESS TESTING MACHINE

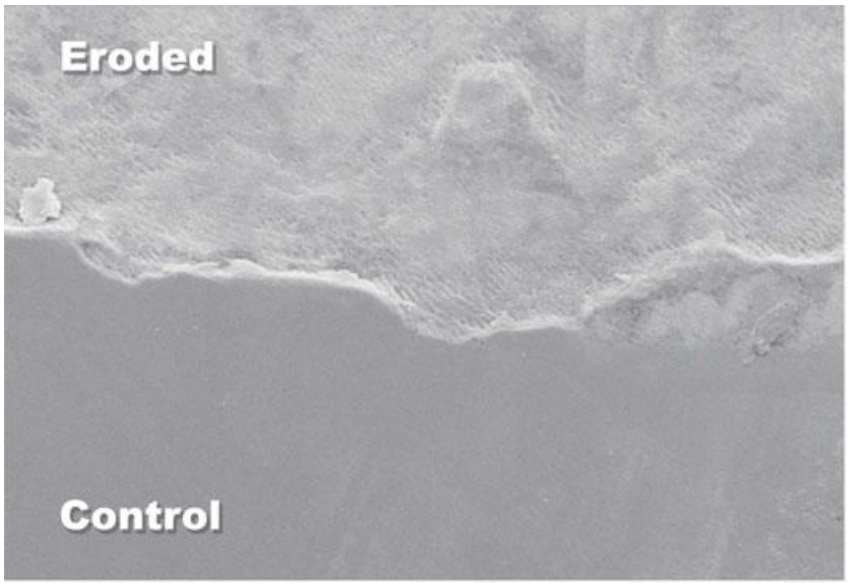


FIG NO 31

SCANNING ELECTRON MICROSCOPY(800X MAGNIFICATION) SHOWING THE ERODED AREA VS THE UNTREATED AREA

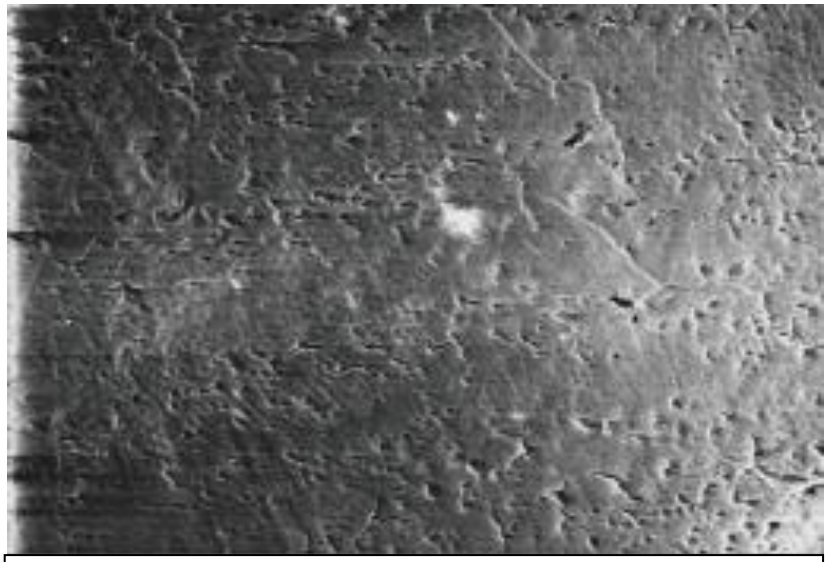


FIG 32 SCANNING ELECTRON MICROSCOPY OF ENAMEL SPECIMENS IN GROUP 1 (NO REMINERALISING AGENT WAS APPLIED) AT 40,000X MAGNIFICATION

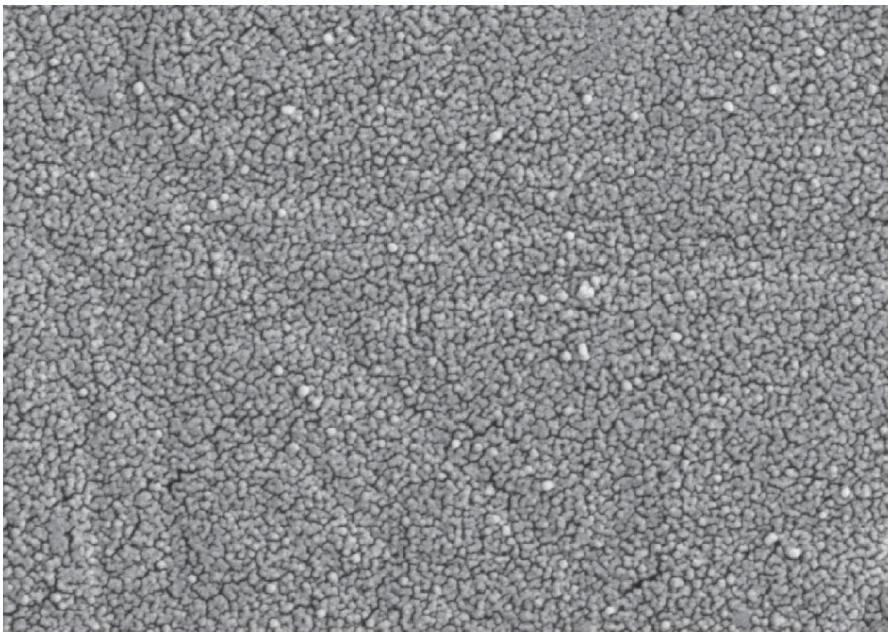


FIG 33 SCANNING ELECTRON MICROSCOPY OF ENAMEL SPECIMENS IN GROUP 2(FLOURIDE WAS APPLIED FOR 5 MINS) AT 40,000X MAGNIFICATION).

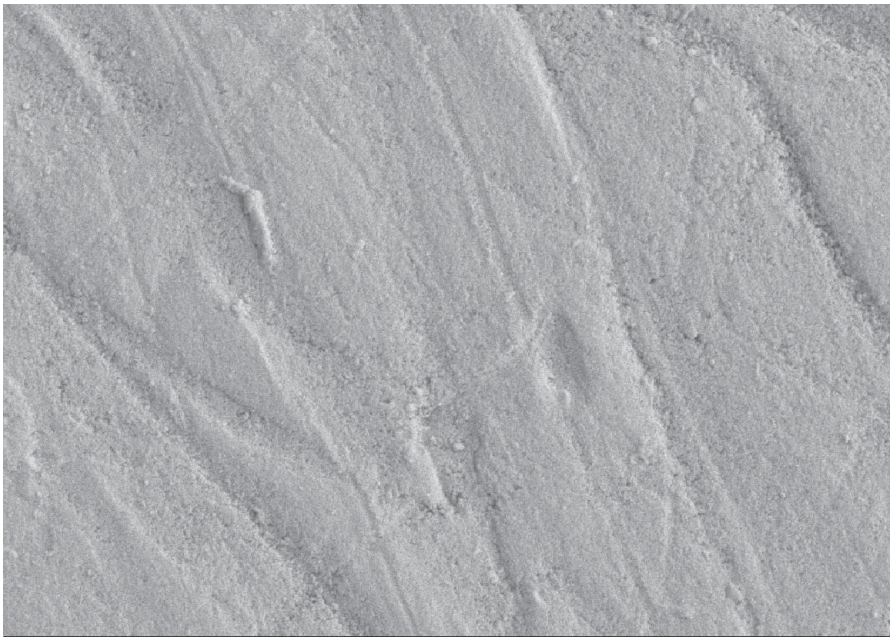


FIG 34 SCANNING ELECTRON MICROSCOPY OF ENAMEL SPECIMENS IN GROUP 3 (FLOURIDE WAS KEPT FOR 24 HOURS) AT 40,000X MAGNIFICATION.

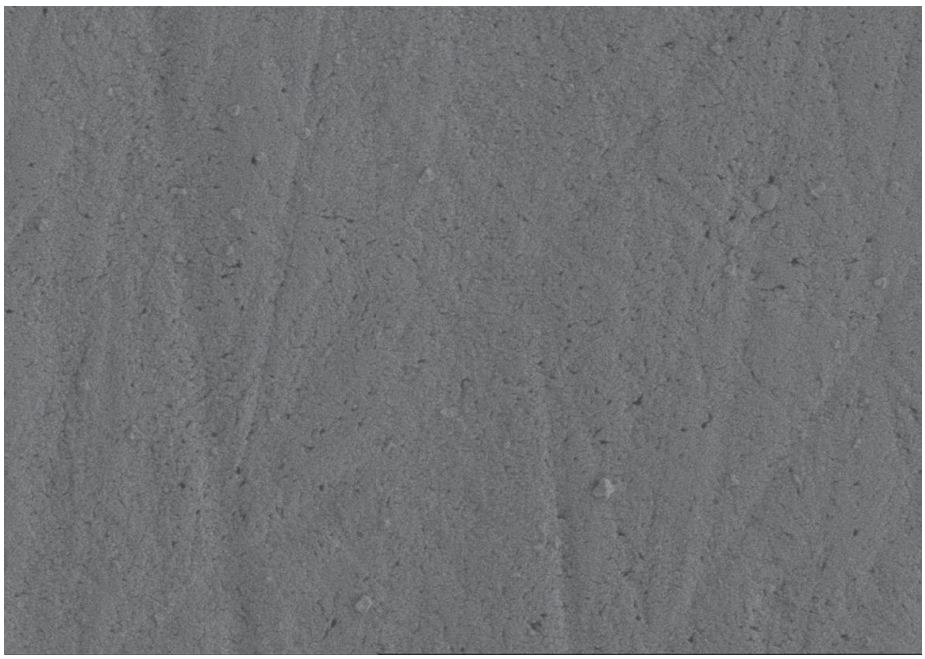
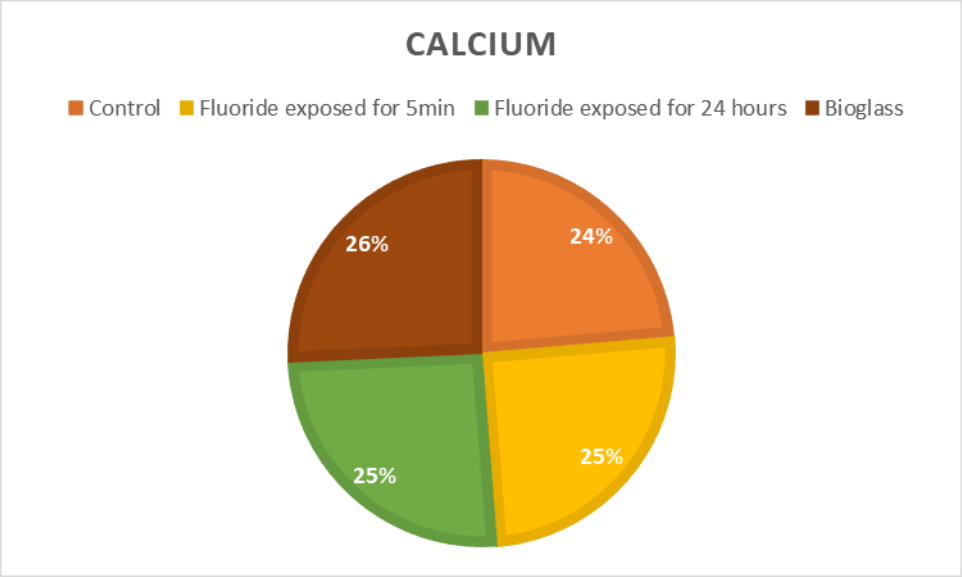


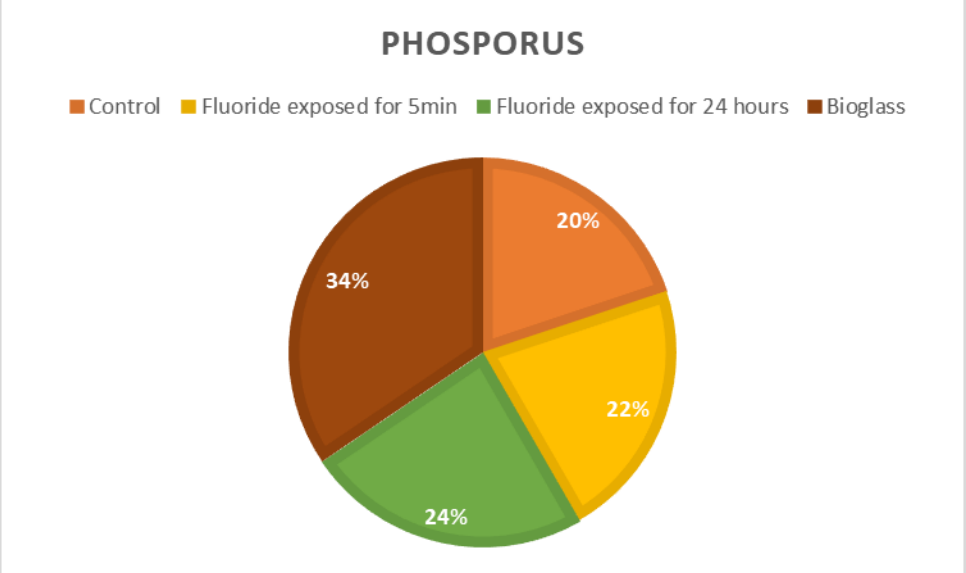
FIG 35 SCANNING ELECTRON MICROSCOPY OF ENAMEL SPECIMENS IN GROUP 4(BIOGLASS WAS APPLIED)AT 40,000X MAGNIFICATION.

OBSERVATION AND RESULTS



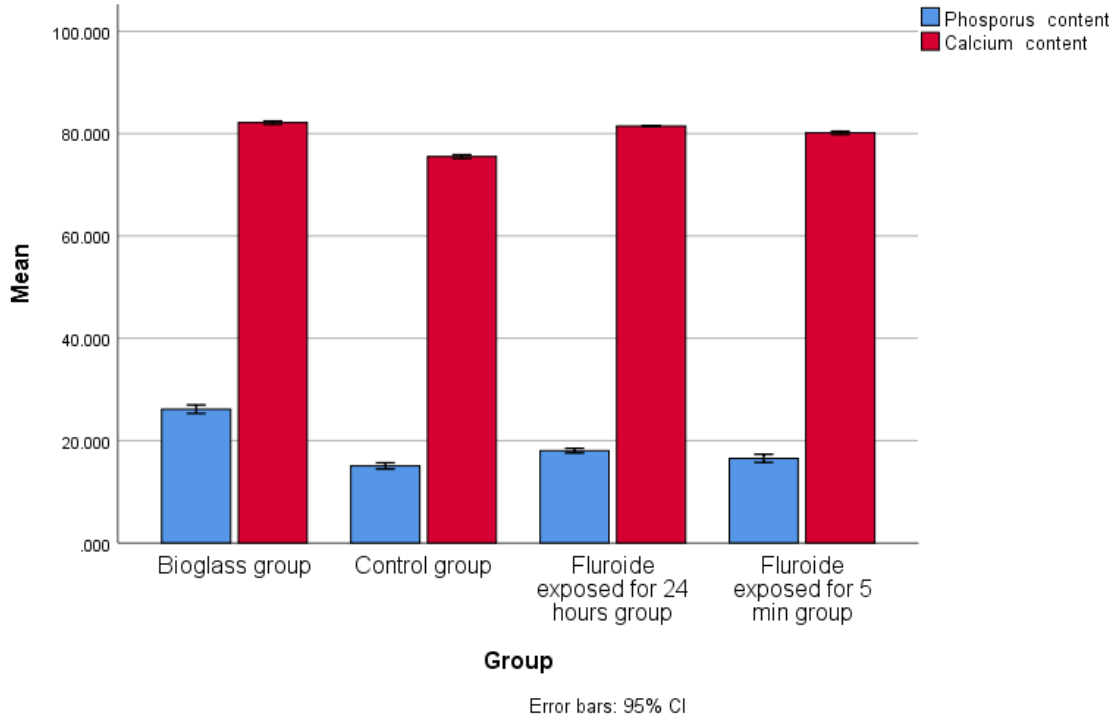
Pie chart 1: - Percentage of calcium present in the tooth after being exposed to different groups

In pie chart 1, calcium content was increased in bioglass group, but were same in fluoride 24hrs,fluoride 5 mins and control group



Pie chart 2: - Percentage of phosphorous present in the tooth after being exposed to different groups

The phosphorous percentage follows a pattern different to the fluoride group i.e., bioglass>Flouride 24 hours>fluoride 5 mins>control



Graph 1: - Mean values of calcium and phosphorous content in all the groups

Graph 1 shows the mean values of calcium and phosphorous in all the groups. The calcium and phosphorous content were obtained by calculating weight/volume of the teeth before and after the intervention. It was observed that the calcium content increased by little margin in the bioglass group whereas the phosphorous content was also increased in the same.

Table 1: - Comparison between groups regarding phosphorous content in tooth

	Sum of Squares	df	Mean Square	F	Sig.	
Phosphorus content	Between Groups	734.034	3	244.678	265.79	.000
	Within Groups	33.140	36	.921		
	Total	767.174	39			

Table 1 values were obtained after the statistical analysis of difference in the phosphorous content of the tooth among all the groups after the exposure. These results were also statistically significant with a p-value of 0.00. But, unlike the fluoride content, the phosphorous content was maximum in the bioglass group and remained almost the same in all other groups. (graph 2)

Graph 2: - Comparison between groups regarding mean value of phosphorous content in tooth

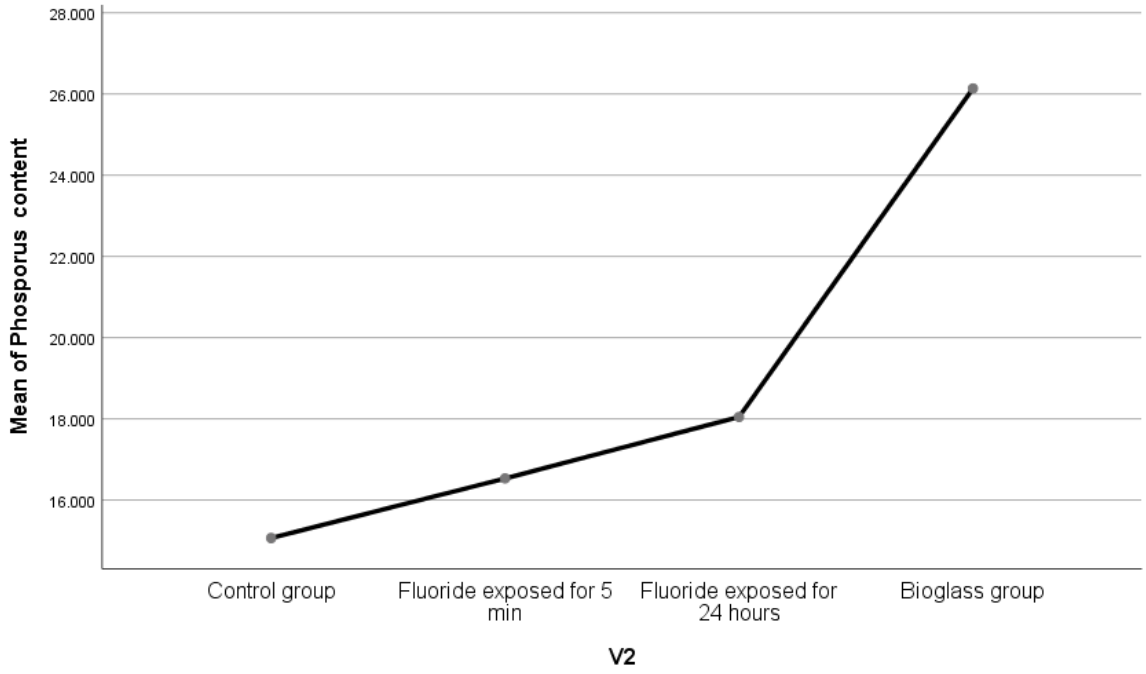


Table 2: - Comparison between groups regarding calcium content in tooth

	Sum of Squares	df	Mean Square	F	Sig.	
Calcium content	Between Groups	268.641	3	89.547	518.031	.000
	Within Groups	6.223	36	.173		
	Total	274.864	39			

Table 2 shows the values after comparing the calcium content among all the groups after the intervention and it was also highly significant similar to the fluoride and phosphorous content (p-value 0.00). the Graph 3 depicts the mean value of the calcium content among all the groups, the bioglass group having slightly more calcium content.

Graph 3: - Comparison between groups regarding mean value of calcium content in tooth

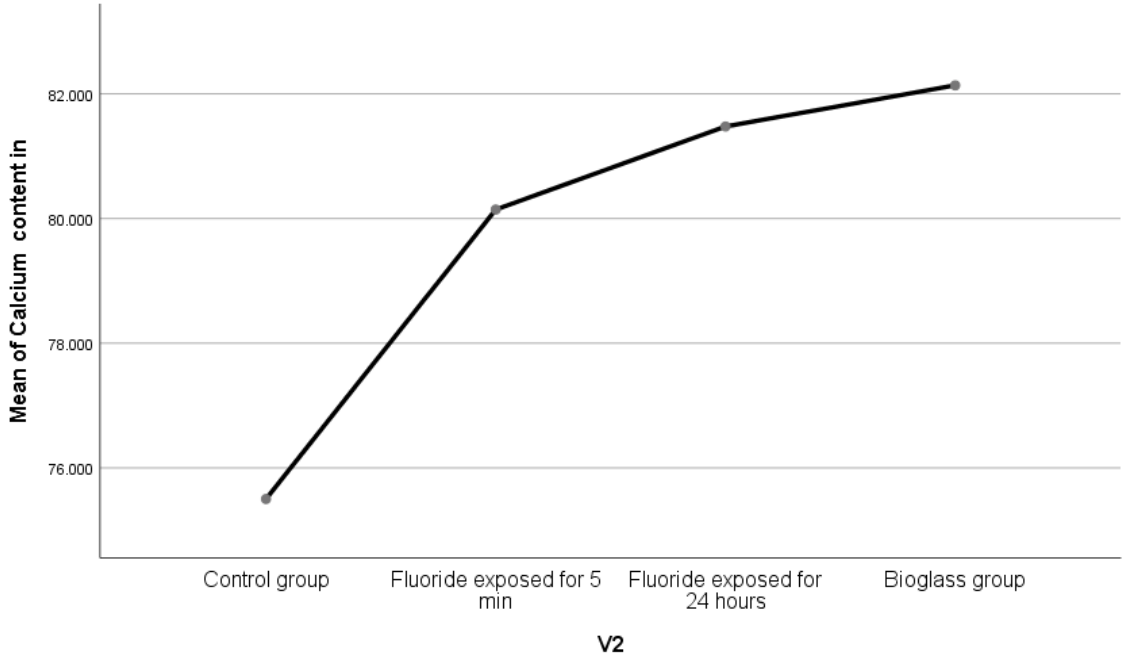


Table 3: - Hardness at 100 microns among different groups

		N	Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		Min	Max
						Lower Bound	Upper Bound		
Hardness at 100 microns	**Control group**	10	214.70	7.804	2.468	209.12	220.28	205	228
	Fluoride exposed for 5 min	10	241.60	9.371	2.963	234.90	248.30	230	258
	Fluoride exposed for 24 hours	10	325.90	8.660	2.738	319.71	332.09	315	338
	Bioglass	10	331.50	9.384	2.967	324.79	338.21	315	342
	Total	40	278.43	52.546	8.308	261.62	295.23	205	342

Table 3 shows the hardness values of the tooth at 100microns in all the groups, control, 5mins, 24hrs and bioglass exposure. Mean values among the groups were

214.7, 241.6, 325.9 and 331.5 respectively and also the total mean value combining all the groups was found to be 278.43.
The minimum and maximum hardness value were 205, 230, 315,315 and 228, 258, 338, 342 respectively among the groups.

Table 4: - Comparison between groups regarding Hardness at 100 microns among different groups

		Sum of Squares	df	Mean Square	F	Sig.
Hardness at 100 microns	Between Groups	104877.875	3	34959.292	448.531	.000
	Within Groups	2805.900	36	77.942		
	Total	107683.775	39			

Table 4 shows the statistical analysis of the hardness value at 100microns when compared among the groups. It showed a significant result with a p-value of 0.00. the hardness increases in all the groups significantly.

Table 5: - Hardness at 50 microns among different groups

		N	Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		Min	Max
						Lower Bound	Upper Bound		
Hardness at 100 microns	Control group	10	214.70	7.804	2.468	209.12	220.28	205	228
	Fluoride exposed for 5 min	10	241.60	9.371	2.963	234.90	248.30	230	258
	Fluoride exposed for 24 hours	10	325.90	8.660	2.738	319.71	332.09	315	338
	Bioglass	10	331.50	9.384	2.967	324.79	338.21	315	342
	Total	40	278.43	52.546	8.308	261.62	295.23	205	342

Table 5 depicts the hardness values of the tooth at 50microns in all the different groups. a standard deviation of 210.5, 191.4, 297 and 311.1 was obtained among the control, 5min, 24hrs and bioglass exposure groups with a standard error of 12.81, 63,55, 8.96 and 6.1 respectively.

Table 6: - Comparison between groups regarding Hardness at 50 microns among different groups

	Sum of Squares	df	Mean Square	F	Sig.	
Hardness at 50 microns	Between Groups	109114.200	3	36371.400	33.667	.000
	Within Groups	38891.800	36	1080.328		
	Total	148006.000	39			

Table 6 is the statistical analysis of the hardness values at 50microns among all the groups. it was found to be highly significant (p-value 0.00). the hardness increased in all the groups simultaneously.

Table 7: - Hardness at 40 microns among different groups

		N	Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		Min	Max
						Lower Bound	Upper Bound		
Hardness at 40 microns	Control group	10	184.40	7.863	2.486	178.78	190.02	171	194
	Flouride exposed for 5 min	10	197.10	16.285	5.150	185.45	208.75	176	223
	Fluoride exposed for 24 hours	10	280.40	13.574	4.293	270.69	290.11	265	302
	Bioglass	10	293.10	6.208	1.963	288.66	297.54	285	302
	Total	40	238.75	50.312	7.955	222.66	254.84	171	302

Table 7 shows the hardness values of the tooth at 40microns among all groups. the control group, 5mins, 24hrs and bioglass exposure groups has a minimum value of 171, 176, 265 and 285 respectively and maximum value of 194, 223, 302 and 302.

Table 8: - Comparison between groups regarding Hardness at 40 microns among different groups

	Sum of Squares	Df	Mean Square	F	Sig.	
Hardness at 40 microns	Between Groups	93772.900	3	31257.633	227.393	.000
	Within Groups	4948.600	36	137.461		
	Total	98721.500	39			

Table 9: - Hardness at 30 microns among different groups

		N	Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		Min	Max
						Lower Bound	Upper Bound		
Hardness at 30 microns	Control group	10	173.30	12.490	3.950	164.36	182.24	152	186
	Fluoride exposed for 5 min	10	181.10	13.034	4.122	171.78	190.42	165	201
	Fluoride exposed for 24 hours	10	193.80	12.054	3.812	185.18	202.42	176	210
	Bioglass	10	235.80	20.751	6.562	220.96	250.64	210	276
	Total	40	196.00	28.365	4.485	186.93	205.07	152	276

Table 9 shows us the values of hardness of the tooth at 30microns obtained after each exposure. Standard deviation was found to be 12.49, 13.03, 12.05 and 6.5 in control, 5mins, 24hrs and bioglass exposure groups.

Table 10: - Comparison between groups regarding Hardness at 30 microns among different groups

Hardness at 30 microns	**Between Groups**	23261.800	3	7753.933	34.393	.000
	Within Groups	8116.200	36	225.450		
	Total	31378.000	39			

Table 10 shows a significant result when hardness at 30microns was compared among all the groups (p-value 0.00). the hardness increased significantly in all the groups.

Table 11: - Comparison of Hardness at 100 microns among different groups

Dependent Variable		**(I) Group**	**(J) Group**	**Mean Diff (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
							Lower Bound	**Upper Bound**
Hardness at 100 microns	**Tukey HSD**	**Control group**	**Fluoride exposed for 5 min**	-26.900*	3.948	.000	-37.53	-16.27
			Fluoride exposed for 24 hours	-111.200*	3.948	.000	-121.83	-100.57
			Bioglass	-116.800*	3.948	.000	-127.43	-106.17
		Fluoride exposed for 5 min	**Control group**	26.900*	3.948	.000	16.27	37.53
			Fluoride exposed for 24 hours	-84.300*	3.948	.000	-94.93	-73.67
			Bioglass	-89.900*	3.948	.000	-100.53	-79.27
		Fluoride exposed for 24 hours	**Control group**	111.200*	3.948	.000	100.57	121.83
			Fluoride exposed for 5 min	84.300*	3.948	.000	73.67	94.93
			Bioglass	-5.600	3.948	.496	-16.23	5.03
		Bioglass	**Control group**	116.800*	3.948	.000	106.17	127.43
			Fluoride exposed for 5 min	89.900*	3.948	.000	79.27	100.53
			Fluoride exposed for 24 hours	5.600	3.948	.496	-5.03	16.23

Table 11 shows the significance of hardness of the tooth at 100microns when all the groups were compared individually with all other groups one by one. It was observed that the results were significant in the following comparisons i.e., control vs 5min; control vs 24hrs; control vsbioglass; 5min vs 24hrs; and 5mins vsbioglass. the results were not significant in the following i.e., 24hrs vsbioglass.

Table 12: - Comparison of Hardness at 50 microns among different groups

Dependent Variable		(I) Group	(J) Group	Mean Diff (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Hardness at 50 microns	Tukey HSD	Control group	Fluoride exposed for 5 min	19.100	14.699	.569	-20.49	58.69
			Fluoride exposed for 24 hours	-86.500*	14.699	.000	-126.09	-46.91
			Bioglass	-100.600*	14.699	.000	-140.19	-61.01
		Fluoride exposed for 5 min	Control group	-19.100	14.699	.569	-58.69	20.49
			Fluoride exposed for 24 hours	-105.600*	14.699	.000	-145.19	-66.01
			Bioglass	-119.700*	14.699	.000	-159.29	-80.11
		Fluoride exposed for 24 hours	Control group	86.500*	14.699	.000	46.91	126.09
			Fluoride exposed for 5 min	105.600*	14.699	.000	66.01	145.19
			Bioglass	-14.100	14.699	.773	-53.69	25.49
		Bioglass	Control group	100.600*	14.699	.000	61.01	140.19
			Fluoride exposed for 5 min	119.700*	14.699	.000	80.11	159.29
			Fluoride exposed for 24 hours	14.100	14.699	.773	-25.49	53.69

Table 12 shows the significance of hardness of the tooth at 50microns when all the groups were compared individually with all other groups one by one. It was observed that the results were significant in the following comparisons i.e., control vs 24hrs;

control vsbioglass; 5min vs 24hrs; and 5mins vsbioglass. the results were not significant in the following i.e., 24hrs vsbioglass and 5mins vs control.

Table 13: - Comparison of Hardness at 40 microns among different groups

Dependent Variable		(I) Group	(J) Group	Mean Diff (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Hardness at 40 microns	Tukey HSD	Control group	Fluoride exposed for 5 min	-12.700	5.243	.091	-26.82	1.42
			Fluoride exposed for 24 hours	-96.000*	5.243	.000	-110.12	-81.88
			Bioglass	-108.700*	5.243	.000	-122.82	-94.58
		Fluoride exposed for 5 min	Control group	12.700	5.243	.091	-1.42	26.82
			Fluoride exposed for 24 hours	-83.300*	5.243	.000	-97.42	-69.18
			Bioglass	-96.000*	5.243	.000	-110.12	-81.88
		Fluoride exposed for 24 hours	Control group	96.000*	5.243	.000	81.88	110.12
			Fluoride exposed for 5 min	83.300*	5.243	.000	69.18	97.42
			Bioglass	-12.700	5.243	.091	-26.82	1.42
		Bioglass	Control group	108.700*	5.243	.000	94.58	122.82
			Fluoride exposed for 5 min	96.000*	5.243	.000	81.88	110.12
			Fluoride exposed for 24 hours	12.700	5.243	.091	-1.42	26.82

Table 13 shows the significance of hardness of the tooth at 40microns when all the groups were compared individually with all other groups one by one. It was observed that the results were significant in the following comparisons i.e., control vs 24hrs;

control vsbioglass; 5min vs 24hrs; and 5mins vsbioglass. the results were not significant in the following i.e., 24hrs vsbioglass and 5mins vs control.

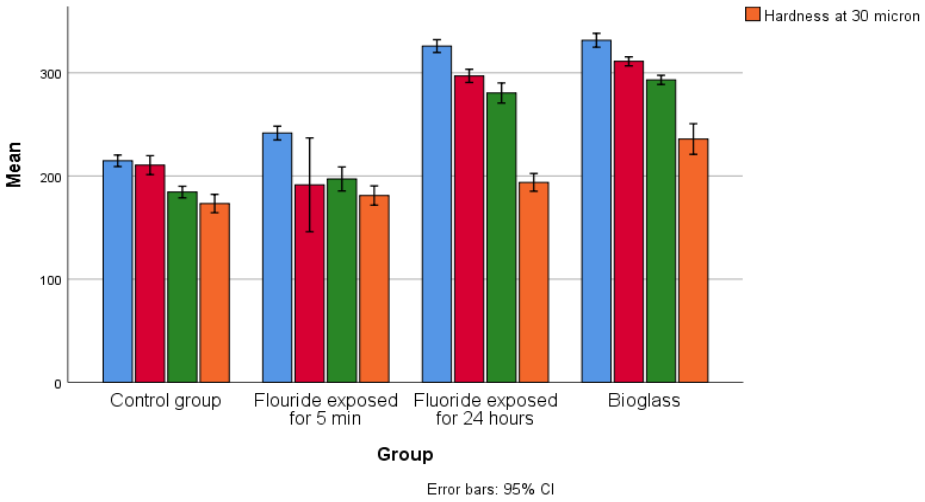
Table 14: - Comparison of Hardness at 30 microns among different groups

Dependent Variable		(I) Group	(J) Group	Mean Diff (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Hardness at 30 microns	Tukey HSD	Control group	Fluoride exposed for 5 min	-7.800	6.715	.654	-25.88	10.28
			Fluoride exposed for 24 hours	-20.500*	6.715	.021	-38.58	-2.42
			Bioglass	-62.500*	6.715	.000	-80.58	-44.42
		Fluoride exposed for 5 min	Control group	7.800	6.715	.654	-10.28	25.88
			Fluoride exposed for 24 hours	-12.700	6.715	.250	-30.78	5.38
			Bioglass	-54.700*	6.715	.000	-72.78	-36.62
		Fluoride exposed for 24 hours	Control group	20.500*	6.715	.021	2.42	38.58
			Fluoride exposed for 5 min	12.700	6.715	.250	-5.38	30.78
			Bioglass	-42.000*	6.715	.000	-60.08	-23.92
		Bioglass	Control group	62.500*	6.715	.000	44.42	80.58
			Fluoride exposed for 5 min	54.700*	6.715	.000	36.62	72.78
			Fluoride exposed for 24 hours	42.000*	6.715	.000	23.92	60.08

Table 14 shows the significance of hardness of the tooth at 50microns when all the groups were compared individually with all other groups one by one. It was observed that the results were significant in the following comparisons i.e., control vs 24hrs;

controlvsbioglass; 24hrs vsbioglass and 5mins vsbioglass. the results were not significant in the following i.e., 5min vs 24hrs; and 5mins vs control.

Graph 4: - Mean valueof Hardness at 100 microns, 50 microns, 40 microns and 30 microns among different groups



DISCUSSION

Enamel erosion is predominantly a surface phenomenon with a centripetal bulk substance loss combined with a small partly demineralized surface layer with decreased microhardness.[83]

There is evidence that the prevalence of erosion in children who drank fruit drinks is rapidly increasing. (Lussi A).[84] Measurement of erosion was confined to primary and permanent maxillary incisors; 19.9% of the children exhibited dental erosion. From this total, 61.8% of the lesions were found in the primary and 38.2% in the permanent dentition.This is an alarming issue for the clinical practitioners as erosion leads to irreversible loss of dental hard tissues. [85]

Dental erosion is a relatively recent dental health risk factor brought on by today's lifestyle. The regular interaction of the tooth surface with acids causes demineralization of the tooth.Extrinsic factors are related to frequent consumption of acidic foodstuffs or beverages and exposure to acidic contaminants in the working environment.[86] The extrinsic component is becoming increasingly important as the use of acid drinks such as soft drinks, sports drinks, and fruit juices increases in modern society.[87]

Human saliva is an important natural factor that protects against erosive demineralisation. Apart from the activity of human saliva in diluting, clearing, neutralizing and buffering acids, it also reduces demineralization and enhances the remineralization process. The most effective ions present in human saliva that play a role in this protection are calcium, phosphate .During erosion there is significant fall in the percentage of calcium and phosphate ions. In Saliva calcium and phosphate levels interact as common ions to the minerals in enamel and dentin, causing a slower mineral dissolution rate.[85] Therefore, finding ways to prevent or minimize this condition is very important and is an area of the present research.

Remineralizing agents like Flouride have been used to prevent enamel or dentin loss due to demineralization.

Arnold, in 1957, was the first author to mention the post-eruptive effect of fluoride in the drinking water and the ability of topical fluoride to reduce the incidence of caries.(Allan I et al,Azarpazooh et al,Brunton pa et al)[88]

The first set of theories concerning the mechanism of action of fluoride was based exclusively on its pre-eruptive effect. The mechanism by which fluoride increases caries resistance may arise from both systemic and topical applications of fluoride and can be broadly grouped as follows - increased enamel resistance, increased rate of maturation, remineralization of incipient caries, interference with micro-organisms and improved tooth morphology.(Cross KJ et al)[89-93]

For many years Flouride has been considered to be the gold standard in remineralising agents, the application of NaF varnish was effective in reducing enamel erosion for 30 min of acid exposure, but the protective effect declined thereafter(Magalhaes et al).[94,95] As the anti-erosive effect of conventional fluorides requires a very intensive fluoridation regime it is difficult for patients to follow that(Ganss et al)[96].According to Amaechi et al Flouride was able to improve the micromechanical properties but to a very less extent, The viability of cells was altered when NaF was applied to unattached gingiva.[97]

Oral mucosal fibroblasts are cytotoxic to NaF. According to the study done by Hume et AL,Jeng et al On human oral mucosal fibroblasts, the pathobiological effects of NaF were studied. Protein synthesis, cellular ATP level, and functional mitochondrial activities were all decreased in a dose-dependent manner when cells were exposed to NaF for 2 hours.[98]

To overcome the toxicity which has been reported in studies done by hume et al and jeng et al.[98] researchers have tried to replace flouride with bioglass as it has properties of biocompatibility and its ability to avoid immune reaction and fibrous encapsulation.

Bioactive glass (Bioglass®) was invented by Dr. Larry Hench in1960s. It works as a biomimetic mineralizer, mimicking the body's mineralizing characteristics while also altering cell signalling, assisting in tissue structure and function repair. (Karlinsey RL et al)[99]

Bioactive glass has a remineralizing and strengthening effect on human hard tissue and is beneficial for the treatment of acid-caused enamel erosion by releasing ions able to form a mineral matrix equivalent to that of hydroxyapatite.[100]

According to researchers Bakry and amaechi Bioglass proved to be the least toxic remineralising agent ,and stated that bioglass had better penetration in the subsurface lesions compared to other remineralising agents.They also came to the conclusion that bioglass improved the micromechanical properties of enamel till a depth of 50 micrometre .[101,102]

To our best knowledge Flouride and bioglass haven't been directly evaluated on the basis of their remineralisation efficiency and their subsurface penetration.So in this present study a comparative evaluation was done between fluoride and bioglass on the above parameters

In present study permanent maxillary and mandibular molars were opted for investigation procedure as these teeth have more mesiodistal dimensions of the buccal surface in comparison to other teeth,wider dimension of the buccal surface of the molar helped in carrying out the erosion test in this study.

Teeth were stored in 10%Formalin solution as done in earlier studies.According to the study done by Nawrocka et al storing the extracted tooth in a 10%Formalin solution remain biologically safe with unaffected mechanical properties. The chemical composition remained unchanged reflected the condition of the tooth as observed in the natural environment of the oral cavity.

In this research Water-cooled silicon carbide discs were used to flatten the sectioned surfaces of the samples.;alluminium oxide sandpaper for decreasing granulations.

It is important to determine the original surface features of teeth, especially when testing the possible interactions and alterations that materials and treatments may produce. Some techniques, especially grinding, are required surface preparation for in vitro experiments. Since enamel has an irregular surface, it is routine for researchers to improve and optimize tests by adopting different surface treatment protocols such as grinding and polishing from well-established methodologies that are utilized in metallography and in dentistry (Finke et al.)[103]

Therefore in this study sectioned surface of teeth were grinded. as they enhance the microstructures of interest with or without specific acid treatments .

There is a leap up in the prevalence and severity of dental erosion in the last few decades due to changes in the food habits which led to the intake of high calorie and low pH foods/beverages. Most of the carbonated beverages and fruit juices have a pH below 3.5 and scientific studies had shown that enamel dissolution occurs below pH 4 leading to irreversible/irreparable damage.[104,105] Long term exposure to the acidic pH leads to the dissolution of enamel and causes erosive lesions. Therefore in the present study Pure orange juice with pH 3.5 was used to create erosion on enamel surface

Changyoon Lee et al in their study selected four kinds of orange juice to induce erosion on extracted teeth, The results of this study showed that the average pH of orange juices was 3.5 which was acidic but the differences were in small quantity for each group of teeth, however, the result was statistically so significant that he came to the conclusion that orange juices can cause enamel erosion in relatively short period of time.[106]

Kitchens M et al also used orange juice on enamel in their research to create erosive lesions and came to a conclusion that all beverages having pH at 3.5 or below will cause significant long term enamel erosion.[104]

In the current study a Magnetic stirrer was used at a speed of 120 RPM when the specimens were immersed in orange juice. It was utilised to create an artificial environment in which the process of in vivo tooth erosion on human enamel could be replicated.

In similar type of study Barac r et al. assessed the erosive potential of soft drinks on extracted teeth,where they used a magnetic stirrer to create an oral environment which simulates the process of erosion further.[107]

The indentation depths employed in our investigation were based on a number of studies and other published data showing that indenting degraded enamel near its surface can induce cracks in the enamel and damage the specimens being evaluated. (Honorio HM et al,Magalhaes et al)[108]

Vickers microhardness test was used in the present study to check the hardness of the specimens after the application of Bioglass paste and Flouride gel.

Microhardness tests have several advantages, including their low cost, extensive research experience with the equipment, and the ability to combine them with abrasive surface loss studies.[109] The indentation boundaries are not clearly defined in heavily degraded dental substrates, resulting in measurements that are erroneous or impossible to make. Hardness assessments of the residual surface cannot quantify the decline in the surface of advanced erosive lesions. Another restriction is that surface hardness tests may not be representative when fluoride is deposited.

In his study, Fosse proposed a method for evaluating microhardness in vivo with an indenter he created. With a Vicker's diamond, indentations were made with a steady force on the natural outer enamel surface, followed by perfect duplicates of the indented enamel surfaces.

The diagonal of each depression was measured using a light microscope on the replicated indentations.[110]

Gabriella Strnad in her study evaluated the effect of microhardness using Vickers microhardness test on specimens which were erosion induced.[111]

Comparisons of the individual groups (Fluoride exposed for 5 minutes, Fluoride exposed for 24 hours, Bioglass and Control) was made for four different hardness levels (100 microns, 50 microns, 40 microns and 30 microns)which is mentioned in Table No 3.5,7,9 respectively. The importance of the present study could be attributed to pointing out of the variability of hardness data and the mineral content probed at various time intervals to identify the most effective treatment alternative. Comparison in between the groups showed statistically significant differences ($p<0.0001$) between each group(Table no 4,6,8,10).

Group 1which consists of teeth with enamel erosion (Control)had hardness levels (214.70 at 100 microns, 210.50 at 50 microns, 184.40 at 40 microns and 173.30 at 30 microns)which was minimal amongst the tested groups(Table 9) .In a similar type of study Bakry et al compared the microhardness of teeth with enamel erosion with the teeth remineralised with different remineralising agents and observed minimal microhardness of eroded enamel without treated remineralising agents.[102] This result was similar to the present study . This could be attributed to the absence of any adjunct re-mineralizing material.

In Group 1, EDX mean values of Ca & P was 17 &77. Kodaka *et al* found a moderate correlation between the Vicker hardness and Ca & P concentration in enamel. They indicated that VHN values, Ca and P percentage significantly decreased in the order of outer, middle and inner enamel sites.

In the present study EDX analysis in group 1 showed mean Ca and P percentage (15 & 70) (Graph 1),as no remineralising agents were applied.In a similar study done by Bakry et al EDX analysis revealed similar percentage of Ca & P in the group where enamel specimens were eroded but not treated with any remineralising agents.[102]

Group 2(fluoride 5 mins exposure) had hardness levels of (241.60 at 100 microns, 191.40 at 50 microns, 197.10 at 40 microns and 181.10 at 30 microns).(Table no 3,5,7,9)

Fluoride ions replace hydroxide ions in the hydroxyapatite crystal structure of teeth when fluoride products are applied to the enamel surface. (Cury JA, Tenuta LM)[112] Because

fluorapatite has a lower solubility than hydroxyapatite, it has a stronger acid resistance than hydroxyapatite. The hardness of the teeth is increased by larger binding forces between fluoride and apatite crystals..(Lata S et al,Cury JA, Lee YE et al,)[49,112,113]

A small quantity of high density fluorides remains in touch with the teeth for a long time as a result of the administration of these fluoride gels, and then penetrates the tooth structure to form bonds. (Seppa L).[114] Fluoride gels, remain in contact with teeth for a shorter period of time, resulting in the creation of bonds in the enamel's superficial layers. (Cho MJ, Lee HL)[115]

According to the study done by Seon Mi Byeon et al The amount of fluoride released at each time point following fluoride application decreased rapidly from the first to the second day. Diffusion from a high-fluoride-content enamel surface is thought to be responsible for the initial burst of fluoride ion release after topical fluoride treatment.[116]

EDX analysis of group 2 revealed mean values of Ca & P to be 18 & 80.Parvathy et al compared the calcium phosphate ratios using EDX in their study and the mean values of Ca & P were similar to this study.(Graph 1)

Group 3(Fluoride application for 24 hours)hardness levels values were (325.90 at 100 microns, 297.00 at 50 microns, 280.40 at 40 microns and 193.80 at 30 microns). (Table no 3,5,7,9)

Increased calcium, phosphate, and fluoride ion concentrations on the tooth surface, according to Reynolds et al, would drive diffusion into the enamel, resulting in higher activities of the ions in the subsurface lesion fluid, and higher levels of remineralization and fluoride incorporation into the mineral phase.[46]

Application of fluoride for 24 h slightly improved the sub-surface remineralization capacity of fluoride at 40 mm due to the long exposure time to the fluoride.The exposure time explains the higher microhardness in this group compared to Group 1 and group 2. This could be because of the availability of the compound locally for an extended time period which increased the absorption of Fluoride by the enamel surface.

Reynolds et al. found that the in situ single application of sodium fluoride products resulted in a higher rate of remineralization than the combination application of NaF solution with other products.[46]

The positive findings achieved by applying fluoride for 24 hours should be viewed with caution because there are numerous clinical problems to consider regarding the safety of utilising fluoride for 24 hours using the aforementioned technique. A previous study found that applying NaF to gingival tissues for 1 hour reduced cellular protein and DNA production significantly. The viability of cells was also reduced by the administration of 1.2 percent NaF to unattached gingiva. Furthermore, in vitro, NaF can be harmful to oral mucosal fibroblasts by inhibiting protein synthesis, mitochondrial activity, and cellular ATP depletion. (Hume et al ,Jeng et al).[98]

Sharon Vincent and Abi M. Thomas in their study compared the fluoride concentration at different time intervals and concluded that fluoride at 24 hours peaked with 1000 ppm concentration.[117] The reason for this phenomenon is due to the binding of fuoride to intraoral reservoirs and its subsequent release into saliva over time.

M. Heijnsbroek in their study concluded that Fluoride bioavailability in plaque increased afer 30 min and was back to baseline afer 6 hours.[118]

EDX analysis of group 3 revealed mean values of Ca & p (19&82). Talai et al reported a positive response IN EDX analysis with fluoride gel applications. Maximum calcium uptake was noted in fluoride gel ($P < 0.001$) and 10% nHA ($P < 0.001$) groups. Maximum phosphorus uptake was noted in 10% nHA ($P < 0.001$) followed by fluoride gel ($P < 0.001$) and 5% nHA ($P < 0.001$) groups but the difference among the afore-mentioned three groups was not significant ($P = 0.437$).[119]

Group 4(Bioglass) hardness levels measured (331.50 at 100 microns, 311.10 at 50 microns, 293.10 at 40 microns and 235.80 at 30 microns).

At depths of 30 mm, 40 mm, and 50 mm, 100 mm, bioglass paste was able to greatly improve the micromechanical characteristics of enamel. Since 45S5 bioglass takes at least 2 hours to complete its bioactive cycle[38], it entails the release of calcium and phosphate ions from its network, its profound remineralization capacity can be linked to its bioactivity features. (Qi YP et al)[120]

In a study done by Bakry et al The bioglass paste applied was able to form an “interaction layer” which was formed from a calcium phosphate-rich layer free of soluble

silanol compounds, on eroded enamel surfaces within 24h.After 14 days of storage, the layer showed resilience to erosion and converted into hydroxyapatite crystals in the remineralizing solution.; these results suggested the possible use of the current technique for treatment of incipient enamel eroded lesions.[102]

Bioactive glass has a larger surface area and a quicker dissolution rate, resulting in a faster generation of apatite. They've also been found to improve the mechanical characteristics of enamel and generate biomimetic nano-structuration that improves cell adhesion. (Vichery, C.; Nedelec, J.M)[121]

When compared to topical fluoride and CPP-ACP therapy, Taha et al. found that bioactive glasses were more successful at inducing remineralization. They came to the conclusion that bioactive glasses improved enamel remineralization.[67]

Bioglass 45S5 has a couple of problems, including the potential for gaps to grow between the material and the host tissues due to its quick breakdown rate. (Damen, J.J.; Ten Cate, J.M.).[122] Porosity deficiency should be attributed not only to the composition, but also to the technique used and the degree of particle aggregation. (Salonen, J.I et al).[123] A Bioglass® 45S5 may also produce cytotoxicity as a result of a high rise in pH caused by high Na+ and Ca2+ leakage, as well as delayed hydroxyapatite production. (Ali, S.; Farooq, I.; Iqbal, K).[124] Due to poor mechanical qualities, such as being overly fragile, the glass composition may not be suitable for the creation of porous scaffolds. (Chen Q et al, . Zachariasen, W.H)[125]

Improvement of the surface micro hardness of the enamel tissue was noted post application of the compounds. And at 100 microns the difference between the groups was most comparable.(Table no 4) Another study reported that After treatment with bioactive glass, the recovery rate of microhardness on demineralized enamel surface was 28.8%.Thus, it could be inferred that the Bioglass performed better than any other compounds for re-mineralization and improved hardness of the enamel surface.[126]

The Ca/P ratio was higher in the bioactive glass-treated area than in other sections that were not covered with bioactive glass particles, according to energy-dispersive X-ray spectroscopy (EDX) analysis.In the present study significant differences can be noted between group 1(Control)and group 4(Bioglass) where calcium uptake increased by 7-

9%.Phosphorus uptake was also increased in group 4 by 5% when compared to other groups .(Graph no 1)

In a similar study done by Bakry et al they came to a conclusion that Bioglass had the highest Ca/P ratio compared to other remineralising agents used in the study.[102]

The Scanning Electron Microscope was utilised in this study to highlight the rough enamel surface caused by enamel grinding and erosion. SEM micrographs were utilised because they produce a distinctive three-dimensional image that is beneficial for analysing the sample's surface structure. The effect of superficially produced precipitates resulting from mineral breakdown by various agents have been investigated using this method. Differentially acting acids, fluoride's anti-erosive capability, and the re-mineralization and re-hardening capacity of diverse agents in eroded enamel are among them.[127]

According to researches The scanning electron microscope (SEM) is one of the most commonly utilised equipment for examining ultramicroscopic surface modifications associated with erosion on both enamel and dentine. It is possible to observe very high-resolution photographs of a sample surface with a size of less than 1nm. After gold sputtering, SEM examinations can be performed on both polished and unpolished native surfaces, which is highly repeatable and simulates conditions at the tooth surface in vivo.[109]

Study done by Ratthapong on scanning electron microscope (Original magnification x 7500)characterization of erosive enamel in human teeth showed the significance of the surface characteristics of the eroded enamel when viewed under such powerful magnification.[127]

In the present study Qualitative assessment was carried out using SEM analysis.

In the control group where nothing was applied, The disorganised demineralized enamel had lost its structural properties.The demineralized enamel showed areas of focal holes or microcavities and a prismatic pattern of destruction with a honeycomb appearance, which is a peculiar characteristics of carious enamel the depressions appeared wedge-shaped. Detailed examination disclosed globular crystals which appeared less well packed, resulting in varying degrees of intercrystalline spaces. The prism sheath regions were widened. . The crystals in the more affected areas showed enlarged intercrystalline spaces.(Fig no 32)

In Group 2 Shallow depressions and fine porosities were observed where fluoride was applied for 5mins. the porosities were evident on the enamel surface and faint lines of mineralization could be seen in and around the porosities. Areas of mineralized deposits in the form of spherical globules agglomerates which were formed of calcium fluoride were discernible and seen profusely scattered along the porous defects.((Fig no 33)

Group 3, where Flouride was applied and left for 24 hours, revealed a rough enamel surface with prominent abrasion lines due to enamel grinding. the entire enamel surface was covered with surface reaction product, spherical globular agglomerates of fluoride were observed in the reaction product layers. The spherical globules appeared to coalesce and form a surface layer microstructure. The large clumps of reaction products on the outer surface of the coating were often seen to consist of this microstructure. (Fig no 34)

In group 4 where bioglass was applied showed complete coverage of the enamel surface with crystalline structures.It showed a nearly smooth surface, with complete obtrusion of inter-rod spaces in some fields. The rods appeared as they were fused together with some globules deposited on the surface, relatively no evidence of porosities or irregularities. (Fig no 35)

CONCLUSION

Within the limitation of the present study it can be concluded that-

1. Bioglass paste was able to significantly improve the micromechanical properties of eroded enamel at all indented depths examined.

2. EDX Analysis applied in this research showed that bioglass paste significantly improved the Ca/P percentage after remineralisation..

3.Visualisation under SEM demonstrated significant qualitative changes in surface texture of eroded enamel.After remineralisation the specimens on which Bioglass was applied showed complete coverage of the enamel surface with crystalline structures.

Therefore, it can be suggested that Bioglass may aid in treating the sub-surface incipient enamel erosive lesions more effectively when compared to Flouride.However further in-vivo studies comparing Bioglass to other remineralising agents are suggested for better clinical correlation.

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APPENDICES

ANNEXURE-I

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES (FACULTY OF BBD UNIVERSITY), LUCKNOW

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled **"A Comparative Evaluation of Microhardness and Surface Texture of Bioglass Paste and Topical Flouride on Eroded Enamel: An *In Vitro* Study."** submitted by **Dr Dibyajit Sur** Post graduate student from the **Department of Conservative Dentistry and Endodontics** as part of MDS Curriculum for the academic year 2018-2021 with the accompanying proforma was reviewed by the Institutional Research Committee present on **26th November 2018** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.

Prof. Vandana A Pant
Co-Chairperson

Prof. B. Rajkumar
Chairperson

ANNEXURE-II

Babu Banarasi Das University
Babu Banarasi Das College of Dental Sciences,
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the VII[th] Institutional Ethics Sub-Committee

IEC Code: 24 **BBDCODS/01/2019**

Title of the Project: A Comparative Evaluation of Microhardness and Surface Texture of Bioglass Paste and Topical Flouride on Eroded Enamel: An *In Vitro* Study.

Principal Investigator: Dr. Dibyajit Sur **Department:** Conservative Dentistry & Endodontics

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: New, MDS Project Protocol

Dear Dr. Dibyajit Sur,

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 10[th] January 2019.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry, BBDCODS, Lucknow
2.	Dr. Amrit Tandan Member	Prof. & Head, Department of Prosthodontics and Crown & Bridge, BBDCODS, Lucknow
3.	Dr. Rana Pratap Maurya Member	Reader, Department of Orthodontics & Dentofacial Orthopedics, BBDCODS, Lucknow
4.	Dr. Sumalatha M.N. Member	Reader, Department of Oral Medicine & Radiology, BBDCODS, Lucknow

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.
The comments were communicated to PI thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Forwarded by:

Lakshmi Bala 22/01/19
Member-Secretary
(Dr. Lakshmi Bala) Ethic Committee
Member-Secretary BBD College of Dental Sciences
IEC BBD University
Faizabad Road, Lucknow-226028

(Dr. B. Rajkumar)
PRINCIPAL Principal
Babu Banarasi Das College of Dental Sciences BBDCODS
(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow-226028

ANNEXURE III

DATA SHEET

	Hardness at 30 mm	Hardness at 40 mm	Hardness at 50mm	Hardness at 100mm
Group 1	175	178	195	211
	152	190	213	205
	185	180	224	206
	170	175	226	209
	182	186	200	210
	178	188	202	215
	165	190	205	220
	186	171	209	228
	185	192	199	219
	155	194	232	224

	Hardness at 30 mm	Hardness at 40 mm	Hardness at 50mm	Hardness at 100mm
Group 2	167	192	245	244
	176	198	220	230
	201	201	218	241
	188	223	209	235
	195	221	201	246
	196	208	198	255
	165	176	203	235
	171	186	205	258
	172	180	215	232
	180	186	200	240

	Hardness at 30 mm	Hardness at 40 mm	Hardness at 50mm	Hardness at 100mm
Group 3	210	265	300	336
	204	280	306	330
	205	300	295	335
	199	302	306	315
	201	286	302	320
	181	265	300	320
	186	274	300	328
	196	285	298	322
	176	282	285	338
	180	265	278	315

	Hardness at 30 mm	Hardness at 40 mm	Hardness at 50mm	Hardness at 100mm
Group 4	276	285	320	336
	245	290	316	340
	220	300	305	340
	213	302	306	325
	210	295	316	320
	256	285	310	330
	234	294	300	339
	243	300	310	342
	241	292	315	328
	220	288	313	315

	Ca(wt percentage)	P(wt percentage)
EDX analysis of group 1	78.34	15.65
	78.43	16.79
	79.81	16.01
	78.21	14.32
	78.34	15.01
	78.39	14.01
	77.98	14.65
	78.87	14.56
	78.43	14.67
	78.21	14.98

	Ca(wt percentage)	P(wt percentage)
EDX analysis of group 2	78.98	17.32
	80.21	16.79
	80.54	17.01
	80.49	14.32
	79.98	16.01
	80.21	17.89
	80.02	15.65
	80.34	17.69
	80.22	15.67
	80.44	16.98

	Ca(wt percentage)	P(wt percentage)
EDX analysis of group 2	81.51	18.28
	81.49	18.24
	81.5	18.22
	81.45	16.24
	81.48	18.26
	81.46	18.22
	81.44	18.23
	81.47	18.25
	81.48	18.26
	81.48	18.27

	Ca(wt percentage)	P(wt percentage)
EDX analysis of group 4	25.43	81.54
	25.4	81.4
	25.41	81.52
	28.22	81.49
	25.39	81.44
	26.23	81.87
	26.78	81.43
	24.34	80.31
	27.34	81.23
	26.83	80.98

ANNEXURE IV

Formula used in the analysis

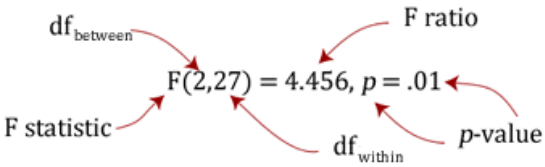
ONE-WAY ANALYSIS OF VARIANCE (ANOVA)

The one-way analysis of variance (ANOVA) is used to determine whether there are any statistically significant differences between the means of three or more independent (unrelated) groups. The one-way ANOVA compares the means between the groups you are interested in and determines whether any of those means are statistically significantly different from each other. Specifically, it tests the null hypothesis:

$$H_0: \mu_1 = \mu_2 = \mu_3 = \cdots = \mu_k$$

where μ = group mean and k = number of groups. If, however, the one-way ANOVA returns a statistically significant result, we accept the alternative hypothesis (H_A), which is that there are at least two group means that are statistically significantly different from each other.

The general form of writing the result of a one-way ANOVA is as follows:



where df = degrees of freedom.

TURKEY'S HSD TEST

The Tukey HSD ("honestly significant difference" or "honest significant difference") test is a statistical tool used to determine if the relationship between two sets of data is statistically significant – that is, whether there's a strong chance that an observed numerical change in one value is causally related to an observed change in another value. In other words, the Tukey test is a way to test an experimental hypothesis.

The Tukey test is invoked when you need to determine if the interaction among three or more variables is mutually statistically significant, which unfortunately is not simply a sum or product of the individual levels of significance.

The results have been put forward with the help of tables, graphs and pie charts.

PIE CHART

A pie chart is a circular statistical graphic, which is divided into slices to illustrate numerical proportion. In a pie chart, the arc length of each slice, is proportional to the quantity it represents.

BAR GRAPH

A bar chart or bar graph is a chart or graph that presents categorical data with rectangular bars with heights or lengths proportional to the values that they represent. The bars can be plotted vertically or horizontally. A vertical bar chart is sometimes called a column chart.

LINE GRAPH

A line graph is a type of chart used to show information that changes over time. We plot line graphs using several points connected by straight lines. We also call it a line chart. The line graph comprises of two axes known as ‘x’ axis and ‘y’ axis.

SOFTWARE

SPSS (Statistical Package for Social Sciences) Version 25.0 (IBM Corporation, Chicago, USA)

ANNEXURE V

URKUND

Urkund Analysis Result

Analysed Document:	plagairism check.docx (D110176525)
Submitted:	7/5/2021 10:31:00 PM
Submitted By:	1180322005@bbdu.ac.in
Significance:	9 %

Sources included in the report:

https://www.mdpi.com/1422-0067/20/23/5960/pdf
https://www.researchgate.net/publication/348676706_Uses_of_sodium_fluoride_varnish_in_dental_practice
http://www.ijohmr.com/upload/Fluoridated%20Varnishes.pdf
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http://sphinxsai.com/2014/PTVOL6/PT%3D11(487-493)AJ14.pdf

Instances where selected sources appear:

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