EFFECTIVENESS OF NANOPARTICLE BASED ACIDULATED PHOSPHATE FLUORIDE (APF) GEL ON SURFACE ENAMEL FLUORIDE UPTAKE – AN INTERVENTIONAL STUDY

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MASTER OF DENTAL SURGERY

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And

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INTRODUCTION

Over 50 decades, fluorides have been the cornerstone in the prevention of dental caries, and have proven benefits on both topical and systemic supplementation [1,2]. The term 'topically applied fluoride' refers to those delivery systems which provide fluoride to exposed surfaces of the dentition, at elevated concentrations, for a local protective effect and are therefore not intended for ingestion [3,4,5]. Considering the quantity and post eruptive benefits, the use of topical fluorides has outweighed the systemic supplementation, as they provide contact with highest possible concentrations that could confer caries inhibitory effect for deciduous and permanent teeth in the post eruption period [6,7,8,9]. The current trend of evidence based practice also support this fact wherein a panel from the American Dental Association in 2007, discussed the available literature on the pros and cons of professionally applied topical fluorides (PATF) [10]. The recommendations for the use of topical fluorides and conclusions of the panel include:

- Fluoride gel is effective in preventing caries in school-aged children
- There are considerable data on caries reduction for professionally applied topical fluoride gel treatments of four minutes or more. In contrast, there is laboratory, but no clinical equivalency, data on the effectiveness of one-minute fluoride gel applications.

- Four-minute fluoride foam applications, every six months, are effective in caries prevention in the primary dentition and newly erupted permanent first molars.
- There is insufficient evidence to address whether or not there is a difference in the efficacy of NaF versus APF gels.

The aforementioned evidence substantiates the efficacy of the Acidulated Phosphate Fluoride (APF gel) as a gold standard among Professionally Applied Topical Fluoride (PATF) agents, which is cost effective and has high patient acceptance [11-14]. Numerous longitudinal trials among different population have reported a considerable decrease in the percentage fraction of dental caries using APF gel in both deciduous and permanent dentition [11,12].

Prospective randomized trial assessing bi-annual application of thixotropic APF gels has reported an average of 35% reduction in the annual caries increment among 11 to 14 year old children [15]. Also another trial using bi-annual APF foam has showed a decrease of 24.2% in the increment of dental caries in primary teeth[16]. Although, there is a lack of similar evidence among Indian population, a study by Agarwal N and Pushpanjali K et al in 2011 showed the potential of APF gel in reducing the occurrence of incipient enamel lesion among 9 to 16 year old school children belonging to the high risk group [17].

A Cochrane meta regression on the use of PATF with reference to APF gels has reported moderate to low level of evidence in the mean caries increment in permanent teeth with the value ranging from 0.2 to 11.5 in the control group compared to only 0.27 in the APF group, during a period of 3 years [11]. While the same when assessed for primary dentition ranged from 1.8 to 5.1 in the control group compared to 0.52 in the APF group[12]. However, the prevented fraction of caries was estimated to be 28% (95%CI 19% to 36%) and 20% (95% CI 1% to 38%) for the permanent and primary dentition respectively[14].

Despite the proven effectiveness of topical fluoride gels in caries prevention there still remains a lacunae between the quantity of fluoride supplemented and the quantity taken up by the enamel surface which is in turn responsible for the prevention of demineralization [18-20].

In order to overcome this, alternative strategies have been tried to improve the fluoride uptake like altering the temperature of the topical fluoride agent, surface treatment of the enamel with phosphoric acid, prophylaxis of teeth before application, combining chelating metallic ions and laser irradiation of the enamel surface [3,4,7,8].

Among all, heating of the topical fluoride solutions have shown to have very minimal effect on the surface enamel uptake and cannot be applied to other form like gels and foams[4,21]. A prospective trial by Johnson WD, et al; concluded that fluoride application before/ after prophylaxis had no significant effect on caries

increment[22]. When it comes to surface treatment, the most commonly used agent was 37% phosphoric acid or currently with the use of Nd-YAG or diode laser [23]. Chand BR, et al; in 2015 reported that a combination of laser irradiation and APF gel used was effective in reducing demineralization compared with APF gel when used alone [24].

Similar results measured in terms of caries reduction, demineralization, reduction in Streptococcus mutans count have also been reported with the use of metallic ions which have chelating properties, in combination with APF gels[25]. The most prominent among those are silver amines and theobromine which have shown commendable results in terms of reduction in salivary *S.mutans* count[26]. Although numerous improvements have been made, economic level implications of these when applied on a community wide basis is questionable.

Technological advancements have been applied to the field of medical and dental sciences time and again to improve the quality of service that is delivered. Currently this is implemented with the application of nanoscience which works on particles of dimension one hundredth of a micro meter[27]. The term 'nanotechnology' is derived from the Greek word 'nanos' meaning 'dwarf' and was first described by Dr.Richard P Feyman in 1959[28]. The use of nanotechnology has improved the methods of diagnosing and treatment in the medical field.

Applying similar strategies in dentistry, the term nanodentistry was developed to refer to as the science and technology of diagnosing, treating and preventing oral and dental diseases, relieving pain and improving dental health using nano-structured material[29]. This is mainly done through 2 approaches: top-down approach and bottom up approach. The former refers to the breakdown of larger molecules to small dimensions whereas the latter is the combining of small particles to form bigger molecules[28,29].

The advances in nanotechnology have made a paradigm shift in all the areas of dentistry from prevention to restorative and surgical materials. In diagnosis, there is the development of nano-cantelivers, nanowires and nanopores which act as sensors of DNA, pathogens and carcinogens[29]. The antimicrobial property of certain metallic ions like silver has been exploited at nano level for better drug delivery in the form of nanocapsules, scaffolds, quantum dots [30].

Other applications include use of nanotite in implantology, nano graft materials in periodontology and nanoionomers, bioceramics for restorative purpose[29,30]. However, in preventive dentistry it has been largely restricted to the alteration of the oral biofilm by addition of metallic nanoparticle to existing fluoride varnishes, remineralizing agents like CPP- ACP or other preventive and restorative materials like dentin bonding agents, composites and cements[30].

Keeping these in consideration, one other possible way, which is still unexplored in improving the enamel uptake of fluoride from APF gels, is altering the particle

size of the active component which is sodium fluoride (NaF). Also many authors have suggested that the improvement in fluoride therapy should be such that the formulation results in increased concentrations of permanently bound fluoride.

The active ingredient of APF gel, sodium fluoride compound has an octahedral lattice of 462 pm with each particle measuring approximately 99.47 μm in diameter. Despite the years of proven efficacy, literature evidence shows that, over 90% of this fluoride is freely available as calcium fluoride which is quiet rapidly dissolved as it is the one that is interacting with the saliva[4,5]. Only a small fraction remains in a permanently bound form of fluorapatite within the enamel crystals. Hence, a consistent amount of fluoride is not available on enamel surface for remineralization. Thus, further improvements on these topical fluorides are suggested such that they improve the fluoride retention on the enamel surface.

Numerous in vitro and in vivo studies using APF gel have reported an immediate increase in fluoride uptake following application which gradually decreased with increasing depth or time of measurement[31,32]. A mean increase of 1500 ppm to 3500 ppm has been reported by authors previously in both deciduous and permanent dentitions [31]. An indirect method of this surface enamel fluoride uptake was through measuring the surface microhardness of the extracted tooth samples. An average increase of 40- 60% in the hardness values, 24 hours following the application of APF gel has been demonstrated from in vitro, in situ and ex vivo models by researchers across the globe in both deciduous and permanent

dentitions [33,34]. Our previous study on extracted teeth also showed similar results at 24 hour evaluation, which gradually decreased during the one month observation period[35].

However, the lack of ability of these models to simulate oral conditions implies the need for more in vivo studies to assess the fluoride uptake from topical gels by the enamel surface. The novelty of this study is that it uses a top down approach where the particle size of the active compound of APF gel i.e, sodium fluoride is reduced to nano size. Hence, it was hypothesized that the reduced particle size of sodium fluoride and incorporation in freshly prepared APF gel will alter the fluoride uptake compared to the conventional APF gel, which may in turn affect the fluoride retention on the enamel surface.

HYPOTHESIS

RESEARCH QUESTION:

Is the surface enamel fluoride uptake from nanoparticles based APF gel same as that from conventional APF gel (16 Oz Pascal Corp.; Strawberry Flavour) ?

RESEARCH HYPOTHESIS:

There is a significant difference in the surface enamel fluoride uptake from nanoparticles based APF gel and conventional APF gel (16 Oz Pascal Corp.; Strawberry Flavour).

NULL HYPOTHESIS:

There is no difference in the surface enamel fluoride uptake from nanoparticles based APF gel and conventional APF gel (16 Oz Pascal Corp.; Strawberry Flavour).

AIM AND OBJECTIVES

Aim:

To assess the effectiveness of nanoparticle based acidulated phosphate fluoride (APF) gel on surface enamel fluoride uptake.

Objectives:

1. To assess the surface enamel fluoride uptake in the tooth pre-operatively using acid etch biopsy method given by Brunn et al 1975 [36].
2. To assess the change in the surface enamel fluoride uptake after the application of nanoparticle based APF gel after a period of 24hrs and 30 days using acid etch biopsy method given by Brunn et al 1975 [36].
3. To assess the change in the surface enamel fluoride uptake after the application of conventional APF gel after a period of 24hrs and 30 days using acid etch biopsy method given by Brunn et al 1975 [36].
4. To compare the difference in surface enamel fluoride uptake between nanoparticles based APF and conventional APF.
5. To determine the depth of penetration of nanoparticles based APF and conventional APF from the extracted teeth surface and compare the same with the help of scanning electron microscope (SEM).

REVIEW OF LITERATURE

Candeli A et al in 1967 [37] used small enamel fragments from deciduous molars of 13 children of 7 to 8 years age, living in low fluoride areas (drinking water with 0.04 ppm), no prior treatment with fluoride and surfaces of extracted some permanent mottled teeth were assessed in two sets of experiments respectively. Experiment 1 showed a fluoride concentration of 0.0028 to 0.019 g in 30 days of treatment with fluoridated dentifrice. A reduction of upto 79% was observed in 15 days after the withdrawal of the intervention. The latter experiment revealed that mottled teeth showed higher fluoride than the deciduous teeth and that this concentration varied between each tooth.

Brudevold F, McCann HG and Gron P in 1968 [38] assessed fluoride levels of intact 1-2 μ outer enamel layers of anterior teeth of a group of military personnel. They found that the mean concentrations varied from 400 to 2500 ppm. A second biopsy from the same surfaces showed lesser concentration of fluoride and thus confirmed a gradient distribution of fluoride in the enamel.

Caslavska V et al., in **1971** [39] assessed in vitro and in vivo response of the human enamel to topical application of ammonium fluoride using extracted teeth and among 7^{th} and 8^{th} grade school children respectively. Solutions of sodium and ammonium fluoride of 0.62 M concentration with application time of 3 minutes were used in both the cases. From the in vitro study they concluded that

ammonium fluoride showed a greater deposition than sodium fluoride at lower pH of 4.4. A total of 4 groups with or without pre-treatment using phosphoric acid were assessed in the in vivo part. Enamel biopsies were taken from maxillary central incisors. The results of the study showed that etching improved the fluoride uptake by enamel surface and the concentration of fluoride deposited, decreased from surface to inward. Also they found that acidic pH of the topical solution increased the uptake while the calcium fluoride from ammonium fluoride was better bound than those from the sodium fluoride.

Larsen MJ, Kold M and von der Fehr FR in 1972 [40] determined a mean fluoride content of 1000 to 6000 ppm in 0.2 μ thickness of enamel from single tooth surfaces of 207 children of 7 to 16 years residing in high and low fluoride areas. When 2 successive enamel layers were biopsied from 33 children, they found the concentration to vary from 2000 to 5800 ppm in first layer to a range of 1500 to 4800 ppm in the second layer.

Munksgaard EC and Bruun C in 1973 [41] estimated the amount of fluoride in superficial enamel layers of human teeth through gas chromatography. The laboratory phase was performed on 30 extracted premolars of 10-12 year old children stored 4 to 5 years ago rinsed with 0.2% sodium fluoride (NaF) mouthrinse. Clinical phase was performed on 15 permolars in 6 children of 10-11 years age. It was found that the variations in fluoride content between the teeth

samples were greater than within the tooth variations. Among these the fluoride treated teeth showed greater variations than the controls.

Stearns RI in 1973 [42] assessed the incorporation of fluoride on enamel surface from non-fluoridated prophylactic pastes and APF gel on 6 participants of 19 to 47 years of age. The enamel biopsies were done in 4 phases on the maxillary, mandibular canines and second premolars. The 1st was a baseline biopsy before treatment, followed by 2nd biopsy one hour after professional treatment with non-fluoridated pastes. The 3rd set of assessment was done 3 hour after application of APF gel and the final assessment was at 7 to 14 days after topical application. The final assessment alone was done on lateral incisors and 1st premolars. On the whole the fluoride treated enamel surfaces showed increased fluoride than the non-fluoridated surfaces.

Bruun C, Munksgaard EC and Stoltze K in 1975 [43] determined fluoride uptake by enamel surface through 235 enamel biopsies from maxillary first premolars of 40 school children of 12 to 13 years after fortnightly rinsing with 0.2% NaF mouth rinse. The authors reported an uptake of 3800 ppm, 2400 ppm and 2000 ppm in the first 3 successive layers of enamel at a depth of 0.6,1.0 and 1.1 μm respectively. An average decrease of 1800 ppm within this depth interval was reported.

Caslavska V, Moreno EC and Brudevold F in 1975 [44] studied the effects of calcium fluoride deposition from in vitro exposure of human enamel to APF, ammonium fluoride solutions and a synthetic mixture of fluorapatite with 1 M potassium hydroxide (KOH). The in vitro evaluation showed that there was a rapid dissolution of calcium and fluoride from the calcium fluoride (CaF_2) depressed solution of the apatites and this system of equilibrium was governed by the solubility products of CaF_2 and calcium hydroxide.

Mellberg JR and co-workers in 1976 [45] determined the mean thickness of biopsy specimens on the enamel surfaces of permanent human maxillary incisors and primary bovine incisors using foam-silicone laminate cylinders. They found the thickness to vary from 1.92 to 2.3 μ for the foam laminates while the corresponding values for felt cylinder were 2.37 to 2.62 μ, showing equivalence in both the procedures in the amount of enamel removed.

Retief DH, Navia Jm and Lopez H in 1977 [46] studied the microanalysis of fluoride estimation in rat molars following twice daily topical application of sodium fluoride solutions of concentration1000μg F/ml and 2000μg/ml was. The average depth of biopsy was found to 6.3±0.2μm and 3.1±0.1μm for 1.0 M and 0.5 M perchloric acid solutions. Average fluoride uptake was found to be 573±38 ppm, 776±48 ppm for 1000μg F and 2000μ F solutions respectively.

Mellberg JR, Nicholson CR, Franchi GJ, Englander HR and Mosle GW in 1977 [47] determined uptake and retention of fluoride acquired from topical application of 1.2% APF gel of pH 3.2 on 1389 Caucasian children of average age 11 years. The first phase was done on children born in 1959 who received a 15 minute application of APF gel for 25 consecutive days. The 2nd phase was on children born in 1960 who received 10 minutes application for 10 consecutive days and the 3rd phase was on children born in 1961 10 minute applications for 5 days. The exfoliated deciduous teeth on analysis showed a decrease in fluoride from 2 to 10 months period after which the fluoride was considered to be permanently bound.

Shern RJ, Discroll WS, Korts DC in 1977 [48] determined fluoride uptake and caries increment from administration APF tablets were assessed among 611 children of mean age 9.1 years. Totally there were 3 groups: group A (placebo- no intervention), group B (APF tablet containing 1mg fluoride) and group C (2 APF tablets at an interval of 3 hours). The found that the mean biopsy depth ranged from 2.12 to 2.18 μm and the mean fluoride concentration ranged from 1633 ppm to 1688 ppm. However, this increase in values did not show any significance in the 30 months evaluation.

Caslavska V and Gron P in 1981 [49] assessed fluoride deposition on enamel surface after the application of sodium, potassium or ammonium fluoride. In their

in vitro study, 0.8 M sodium fluoride (NaF), 1.5 M potassium fluoride (KF) or 1.5 M ammonium fluoride (NH_4F) were applied in acidic or neutral pH on extracted tooth samples. Fluoride deposition was assessed from 8 consecutive enamel layers by acid etch biopsy after an incubation period of 24 hours at 100% humidity in 37°C and the application time were 5 minutes, 1 hour, 6 hours and 24 hours. They concluded that 5 minutes application of KF with orthophosphate and acidic NaF deposited most firmly bound fluoride at a depth of 20μm and fluoride penetration increased with the increase in application time, highest concentration seen at 6 hours.

Gron P and Caslavska V in 1981 [50] studied in in-vitro settings the fluoride deposition in enamel from 5 minute application of monofluorophosphate solution. They found that overall penetration depth was only 5μm with neutral sodium monofluorophosphate solution while greater penetration was seen at acidic pH of 3.2. Also neutral ammonium monofluorophosphate showed better fluoride deposition than neutral sodium monofluorophosphate.

Caslavska V, Gron P, Stern D, Skobe Z in 1982 [51] analysed chemical and morphological aspects of fluoride acquisition on enamel surface in in-vitro conditions, following the topical application of ammonium fluoride and ammonium monofluorophosphate. The application times were 5 minutes and 6 hours. The authors found that greater pe 13 on of >20μ and increased

fluorapatite formation was seen after 6 hour application of neutral ammonium fluoride solution. This group also showed extensive enamel dissolution and precipitation reaction with calcium fluoride formation on microscopic examination.

Gron P and Caslavska V in 1983 [52] assessed the fluoride uptake with and without the addition of surfactants by under laboratory conditions. This was assessed after the application of potassium fluoride for an incubation period of 24 and 96 hours. It was found that fluoridedeposition was greater in the presence of surfactants in 24 hour period whereas no such differences were observed at 96 hours.

Dijkman AG, de Boer P, Arends J in 1983 [53] longitudinally studied the fluoride in human enamel for a period of 3 months after topical application of APF gel, Duraphat and Fluor protector. The study was performed on 12 patients who wore treated enamel specimens and controls for 3 time periods of 1, 4 and 12 weeks. On analysis, the APF and the Duraphat group showed complete loss of calcium fluoride (CaF_2) during the study period while the amount of CaF_2 was noticeable in the Fluor protector group. However, no specific enrichment of the sound enamel in the control group was found.

Wei SHY and Connor Jr CW in 1983 [54] assessed the fluoride uptake and retention in maxillary and mandibular incisors of 70 children, following the

application of 3 topical fluoride gels namely APF gel, thixotropic fluoride gel and a pluronic gel containing 1% NaF. A uniform 4 minute application was carried out and the assessments were carried out at baseline, 24 hours, 7 days and 28 days. The results obtained were that 0.72 to 13.6 µm with a mean of 3.03±1.56µm. Over all, the mean fluoride uptake was higher in mandibular incisors compared to their maxillary counterparts with mean values of 5120±6036 ppm and 3839±2141 ppm respectively. It was concluded that fluoride content increased one day following the topical application and gradually returned to the baseline levels by the end of 28 days. There was no significant difference observed between the 3 different agents used, with respect to uptake and retention of fluoride on the enamel surface.

Ögaard B and colleagues in 1983 [55] determined uptake and retention of alkali soluble and insoluble fluoride on sound enamel after using 0.05% and 0.2% sodium fluoride (NaF) mouth rinse was evaluated on 20 orthodontic patients of 10 to 14 years age and indicated for bilateral extraction of premolars in at least one arch. One tooth used as control in each participant was extracted prior to intervention. Ten of them were given 0.05% solution and the remaining 0.2% solution. The authors concluded that a significant portion of the fluoride deposited was in the 1st layer of enamel and majority of this belonged to alkali soluble fraction.

Tyler JE and Poole DFG in 1984 [56] conducted an in vitro study on 14 freshly extracted caries free premolars after topical application of 1% ammonium fluoride solution. The samples were sectioned and one half was subjected to 1% ammonium fluoride solution for 2 minutes. Both the halves were then subjected to cariogenic challenge in 0.04 % w/v solution of synthetic hydroxyapatite in 0.06M lactic acid was adjusted to pH 4.5 with sodium hydroxide and one treated, one untreated specimen were removed at the interval of 25 days for a period of 120 days. It was found that caries like lesion was formed in both the groups, although the mean depth of penetration was only 19±3 μm in the treated group compared to 149±34 μm in the controls.

Caslavska V, Gron P, Ahern JM in 1987 [57] compared the fluoride acquisition by enamel from solutions and self gelling preparations. Neutral sodium fluoride solution of 0.65 M concentration was used with addition of 1% cetylpyridinium chloride, 1% sodium lauryl sulphate or 20% tetraethoxysilane for self gelling of the solution. It was observed that the self gelling systems delivered increased fluoride than the neutral solution which resulted in increased amount of firmly bound fluoride on the enamel surface.

Caslavska V, Duschner H in 1991 [58] assessed among 2 populations the amount of calcium fluoride in enamel biopsies at two different times of 6 weeks and 18 months after 3 minute application of 0.62 M sodium fluoride and ammonium

fluoride solution with 1 minute etching using 0.05 M phosphoric acid. The first group consisted of 47 11 to 13 year old children studied for 6 weeks, belonging to an area having fluoridated water supply. The second group had 58 children of 9 to 12 years from a non- fluoridated area who received 3 semi annual applications of the topical solution or placebo. The biopsies were taken from upper right maxillary central incisors and on the whole ammonium fluoride solution showed better results.

Whitford GM, Adair SM, Hanes CM, Perdue EC and Russell CM in 1995 [59] conducted a crossover trial among 46 child dental patients to study the difference in fluoride uptake from 4 minute application of APF gel and foam. The children were of 8 to 12 years age and the enamel biopsy was carried out on maxillary central incisors. The net increase in enamel fluoride in both the groups was 3925±290 ppm and 3905±413 ppm respectively which was not significant. The authors concluded that the difference in fluoride uptake from gel and foam at both 15 minutes and 16 days did not show any significance. Also the salivary fluoride levels were found to relatively higher with gel compared to foams during the study period.

Waszkiel D, Opalko K, Lagocka R and Chlubek D in 2004 [60] determined magnesium and fluoride content of teeth with erosions was on 14 teeth with and without enamel erosions among a group of 18 to 30 year old subjects who had age and gender matched controls. The enamel biopsies were taken from the labial

surfaces of upper and lower anterior teeth, below or above the eroded areas respectively. A total of 1 upper central incisor, 5 upper canines, 2 upper premolars, 3 lower canines and 3 lower premolars were studied in each group. The mean depth of biopsy for the 2 biopsy layers was 1.10±0.40µm, 1.29±0.43µm for the study group while it was 0.93±0.22µm, 1.01±0.25 respectively for the control group. The corresponding fluoride concentrations were 33.49±6.12 mmol/kg, 16.76±4.88 mmol/kg and 55.77±8.21 mmol/kg, 43.50±7.98 mmol/kg respectively in the study and control group.

Villena RS, Tenuta ALM, Cury JA in 2004 [61] studied the effect of APF gel application time on enamel demineralization and fluoride uptake in in-situ conditions. The crossover blind study was carried out in 3 phases for 28 days among 15 volunteers who were subjected to 3 groups control (not treated), pre-treated with APF gel for 1 minute and 4 minutes. All of them wore palatal appliances containing 4 enamel blocks which was left for plaque accumulation and kept in 10% sucrose solution 3 times a day. It was found that APF gel significantly reduced enamel demineralization, fluoride formation and retention irrespective of the application time.

Malekafzali B and Tadayon N in 2006 [62] conducted an in vitro randomized control trial testing the fluoride uptake of deciduous teeth after application of 2 paediatric dentifrices (Aqua fresh and Pooneh) on 20 sound canines. The duration

of exposure was 24 hours at 37°C. The former showed an uptake of 4098.44 and 3755.25 ppm in the first, both consecutive layers while the corresponding values for the latter were 2420.51 ppm and 2242.73 ppm. Also it was estimated that the enamel thickness was less for Aqua fresh group.

Pai N, McIntyre J, Tadic N and Lapardis C in 2007 [63] compared fluoride uptake from APF gel (1.23%), NaF gel (9000ppm), fluoride mouthrinse (1000ppm), fluoridated dentifrice (1000ppm), 20% stannous fluoride solution, 40% silver fluoride solution, titanium fluoride solution (30 and 30400 ppm) and ammonium fluoride solution (38 and 38000 ppm) in in-vitro conditions. It was found that the uptake of fluoride was proportional to the amount of fluoride in the topical agent. Additionally, it was found that, uptake from APF gel was relatively higher than NaF solution and the prior etching or prolonged application time did not confer any additional benefit to APF gel.

MATERIALS AND METHODS

Study Design : An intervention study

Study Setting : Ragas Dental College and Hospital, Uthandi
Hubert Enviro Care Solutions Private Ltd.(HECS).,Vadapalani

Study Duration : 6 months (From May to November 2018)

Study Population : Patients reporting for orthodontic correction and are indicated for extraction of premolar

ETHICAL CLEARANCE:

A detailed protocol of the study was prepared and submitted to the Institutional Review Board of Ragas Dental College and Hospital, Chennai. The intervention study was started after obtaining ethical clearance from the Institutional Review board (Annexure I) and registering in the Clinical Trials Registry – India (CTRI) hosted at the ICMR's National Institute of Medical Statistics (http://nims-icmr.nic.in) (Annexure II) (Reference no: REF/2018/05/019794; Trial registration no. CTRI/2018/05/013848). A bilingual written informed consent was obtained from the participants of age above 18 years and parental consent was obtained for participants of age 15 to 18 years.

PERMISSION FROM AUTHORITIES:

Permission to conduct the study was obtained from the

1. Principal, Ragas Dental College and Hospital, Chennai
2. Head of the Department, Department of Orthodontics, Ragas Dental College and Hospital (Annexure III)
3. Project Head, Hubert Enviro Care Solution Private Limited, Vadapalani, Chennai (Annexure IV)

4. Informed consent (bilingual) was obtained from the adult study participants of age above 18 years and parental consent was obtained for participants of age 15 to 18 year in the vernacular language (Tamil) and English. (Annexure – V and VI)

STUDY DESIGN:

This interventional study was designed to determine the effectiveness of nanoparticle based acidulated phosphate fluoride (APF) gel on surface enamel fluoride uptake. This was a split mouth active controlled, phase II interventional study where the nanoparticle based APF gel (prepared according to the method suggested by Brudevold et al 1965)[64] was applied to the right half of the patients' mouth and the conventional APF gel (16 Oz Pascal Corp.; Strawberry Flavour) was applied to the left half of the mouth. Fluoride uptake of enamel was assessed by acid etch biopsy using 0.5M perchloric acid according to the method suggested by Brunn et al 1975 [36] at time intervals of baseline, 24 hours and 30 days after intervention. The study was conducted over a period of 6 months, from May to November 2018.

ELIBILITY CRITERIA:

INCLUSION CRITERIA:

1. Participants who reported to the Department of Orthodontics for correction of mal-alignment. Participants between age 15-30 years who gave a written consent to participate in this study for adults above 18 years and parental consent obtained for participants of age 15 to 18 years.
2. Participants who were indicated for extraction of premolars bilaterally in at least one arch for orthodontic correction due to any one of the following reasons:
    a. Tooth size – arch length discrepancy: Discrepancy between tooth material and arch length is more than 5mm
    b. Class II division 1 arch relation on Skeletal I base with mild mandibular crowding
    c. Correction of bimaxillary protrusion
    d. Mild class III arch relationship with mild crowding in maxillary arch
    e. Ectopic eruption or presence of impacted canines
3. Participants who were permanent residents of Chennai where the optimal fluoride levels in the drinking water is restricted to 1 ppm were only included.

EXCLUSION CRITERIA:

1. Participants who had systemic disorders and/or who were under medications which would affect their oral health.
2. Participants who were indicated for orthodontic correction without extraction of premolar teeth, extraction any teeth other than premolars or any other method of correction of teeth.
3. Participants having any form of developmental defects, enamel defects like hypomineralization, hypoplasia, dental caries present or any form of restoration or preventive procedure except oral prophylaxis previously done on the premolars.
4. Participants who had, or developed any acute dental problems during the study period which required emergency care.

SAMPLE SIZE ESTIMATION:

The sample size for each group was calculated using the G power statistical software. The mean fluoride uptake by enamel surface of permanent teeth after APF gel application has been measured only in few studies. Of these, two studies by Bruun C et al (1975) and Whitford GM et al (1995) [36,59] were done using acid etch biopsy of enamel to assess fluoride concentration. Also, the test agent used in the present study is a modification of the existing formulation of APF gel thereby limiting the availability of literature evidence except for the

previous in vitro study done comparing the same agents on extracted tooth samples[35]. Though only surface microhardness was the only parameter assessed, the mean values of the same have been taken into consideration for sample size estimation as microhardness is assumed to be proportional to the amount of fluoride uptake. The power of the study was kept at 80%, with an alpha error of 0.5 and the estimates were calculated to 95 % confidence interval (Annexure VII).

Sample size was calculated using the formula:

$$n = \frac{Z^2_{1-\alpha/2}\,[2s_p^2]}{d^2}$$

For d:

$$d = |\mu_z| / s_z$$

$$s_p^2 = \frac{s_1^2 + s_2^2}{2}$$

Where ,

s_1 : Standard deviation in the first group

s_2 : Standard deviation in the second group

s_p : pooled standard deviation

d : precision

μ_z : mean difference between the sample

s_z : difference between the sample standard deviation

α : Significance level (0.5)

$1-\beta$: Power (0.80)

Substituting the above values for sample size calculation:

$$n = (1.96)^2 \times 2\,(33.7)^2/(20)^2$$

$$= 22$$

We obtained a sample size of 22. This was a phase II split mouth clinical trial designed on a special group of population, namely patients reporting for orthodontic correction and indicated for bilateral extraction of premolars in at least one arch. In order to account for loss to follow up, any change of treatment modality during the course of the study and based on the availability, a total of 30 subjects were included in the study.

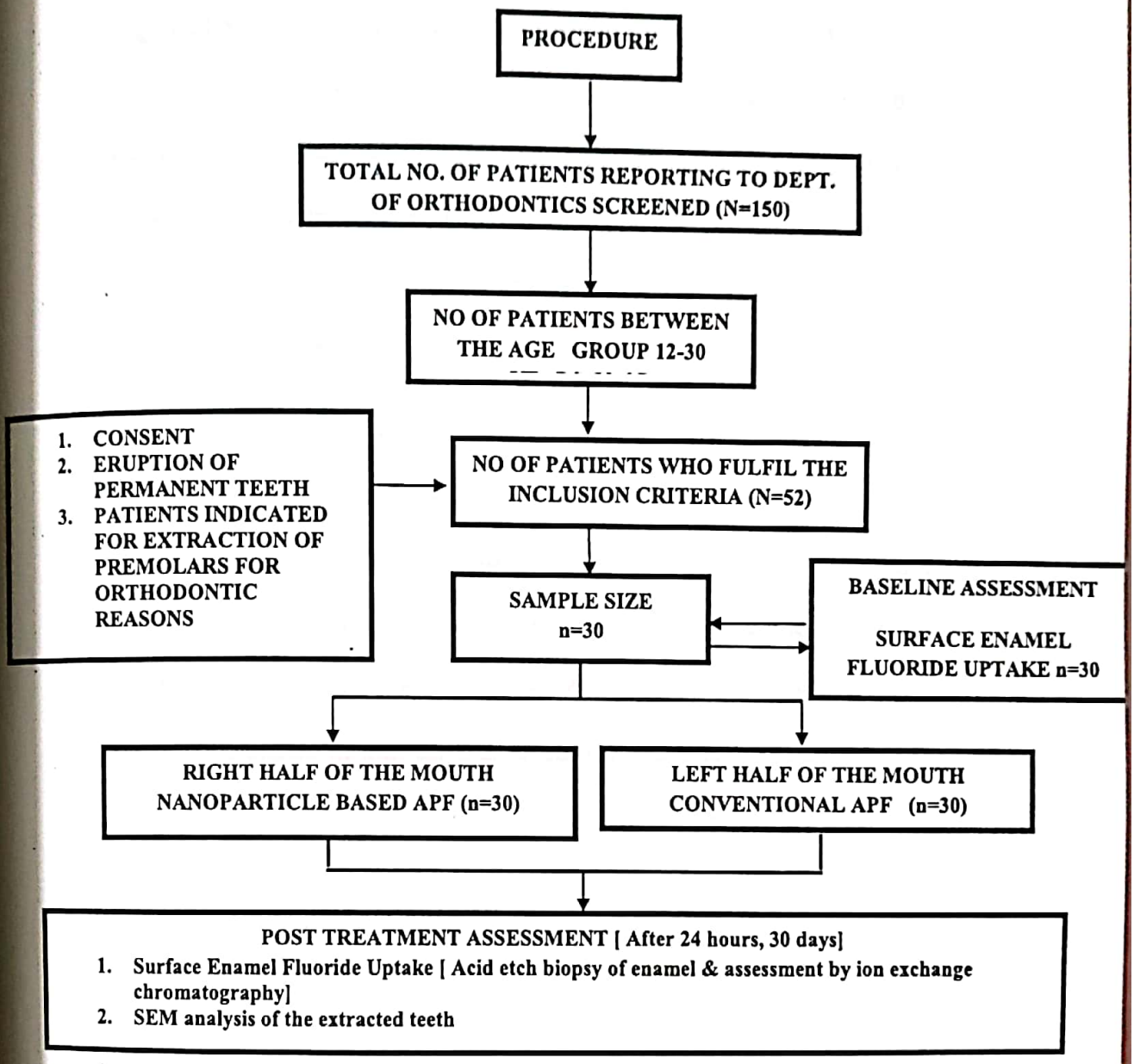
RECRUITMENT OF THE STUDY SUBJECTS:

The study subjects were recruited from the patients reporting for orthodontic correction to the Department of Orthodontics, Ragas Dental College and Hospital, Chennai. All the patients reporting to the Department of Orthodontics, between the age of 15-30 years, fulfilling the inclusion criteria, adults who gave a written consent to participate and parents of children who gave consent to participate were included in the study.

This was a split-mouth interventional trial wherein every subject received a single application of both the prepared nanoparticle based APF gel and the active control agent of conventional APF gel. A total of 30 participants were included in the study. The nanoparticle based APF gel was applied to the right half of each

participant's mouth and the conventional APF (16 Oz Pascal Corp.; Strawberry Flavour) was applied to the left half.

FLOWCHART ILLUSTRATING THE METHODOLOGY OF THE STUDY



PREPARATION AND CHARACTERIZATION OF SODIUM FLUORIDE NANOPARTICLE: (Photograph 1and 2)

The preparation of sodium fluoride nanoparticles and further the nanoparticle based APF gel was done in Avanz Bio Private Ltd, Tambaram. Sodium fluoride (NaF) nanoparticles were synthesized by co-precipitation method as suggested by Pandurangappa C et al (2010) and Bala W.A et al (2017) [65,66]. 0.02 M ammonium fluoride (NH_4F) was added into the aqueous solution of 0.01 M sodium chloride (NaCl) under vigorous stirring. Stirring process was continued for 2 hours until the transparent solution turned into a white opaque suspension indicating the formation of NaF nanoparticles. The obtained suspension was set aside for 24 hours without any disturbance.

The settled precipitate was then filtered and washed with ethanol thrice to remove any residual chloride and ammonium ions. NaF nanoparticles obtained were air dried and stored in vacuum. The characteristics of the particles formed were confirmed with the help of scanning electron microscope (SEM) at Central Workshop Division, College of Engineering, Anna University, Guindy, Chennai. The size of the sodium fluoride particles ranged between 20nm to 80nm.

PREPARATION OF NANOPARTICLE BASED APF GEL: (Photograph 3)

Acidulated Phosphate Fluoride gel was synthesized by adding a gelling agent to NaF nanoparticle based solution. The solution was prepared by dissolving 20 grams of NaF nanoparticles in 1 litre of 0.1M phosphoric acid and to this 50% hydrofluoric acid was added such that pH is adjusted to 3.0 and fluoride concentration was maintained at 1.23%. Further hydroxyethyl cellulose was added to the solution under constant stirring on a mechanical vibrator to obtain APF gel. The gel was stored in an air tight container under refrigerated conditions till further use.

CALIBRATION OF THE EXAMINER:

The investigator was adequately trained and calibrated to take acid etch biopsy of surface enamel of teeth as suggested by Bruun et al in 1975 [36], using perchloric acid and micropipette, in the Department of Public Health Dentistry, Ragas Dental College and Hospital, Chennai, under the supervision of Guide and the Head of the Department. A single calibrated investigator performed the acid etch biopsy in order to avoid the variations in the amount of biopsy sample collected from the study participants.

DATA COLLECTION:

BASELINE EXAMINATION AND ASSESSMENT: (Photograph 4,5,6 & 7)

A type III clinical examination was carried out in the dental chair at the Department of Public Health Dentistry to ensure the oral hygiene is good and there are no clinical visible active carious lesions. The patient was kept in semi-supine position. The premolars indicated for bilateral extraction at a later date were used for fluoride assessment. The buccal surface of the premolar was air dried and isolated using a cheek retractor and cotton roll. Teflon tape of 1cm x 1cm with a hole punched in its center measuring 2mm in diameter was cut and placed on the buccal surface of the tooth. It was placed such that the punched hole was in the mesiobuccal half of the underlying tooth. The enamel biopsy was done by placing 4.0 μL of 0.5M perchloric acid on the enamel surface demarcated by the hole in the teflon tape. The acid was dispensed using an adjustable volume pipettor set at 4.0μL and nonwettable disposable plastic tips. As the tape and enamel surface were not wettable, the acid drop was confined to the exposed area and was in the shape of a hemisphere. After 5 seconds, the

acid was removed by drawing it back into the plastic tip and was placed in a plastic container prefilled with 1ml of 0.5 M perchloric acid and 4.0μL of 0.5M NaOH. This procedure was repeated for the second and third time to get sufficient

quantity of enamel dissolved in the acid. The biopsy was first performed on the right premolar followed by the left premolar. The tooth surface was then dried with cotton pellet. The time taken for clinical examination and biopsy of enamel surface of 2 teeth for each participant was 10- 15 minutes. The collected samples in the plastic container were then sealed with parafilm tape. The armamentarium used for the examination and baseline assessment was as follows:

1. Plane mouth mirror
2. Dental explorer
3. Tweezer
4. Scissors
5. Cotton
6. Calibrated micropipette (4µL) :1 no.
7. Disposable plastic tips : (2 x 30)x3 = 180 (Baseline, 24 hours, 30 days)
8. Teflon tape
9. Rubber dam punch
10. Sterile collection containers(5ml) : (2 x 30)x3 = 180 (Baseline, 24 hours, 30 days)
11. Zip lock pouches (small size) : (2 x 30)x3 = 180 (Baseline, 24 hours, 30 days)
12. Parafilm tape: 1 roll

ASSESSMENT OF FLUORIDE CONTENT: (Photograph 8 and 9)

The fluoride estimation was done using ion chromatography method at Hubert Enviro Care Solutions Private Limited, Vadapalani. The collected samples were transported in sealed individual pouches within a span of 10 days to the laboratory. Each sample was neutralized by adding 1N NaOH drop by drop. One milliliter of the neutralized solution was then made up to 10ml with distilled water. From this stock solution 1ml was taken for analysis. The biopsy solution was analyzed for F^- and phosphate ions. The mass of enamel biopsied was calculated based on the assumption that enamel contains 17.5 % phosphate by weight (Figure 1). The depth of the biopsy was calculated based on the assumptions: (i) density of enamel is 2.95g/cm^3; (ii) the geometry of the biopsied site was a cylinder. The same procedure was repeated in the subsequent analysis of biopsy samples after 24 hours and 30 days.

The amount of fluoride taken up by the enamel surface was computed using the formula:

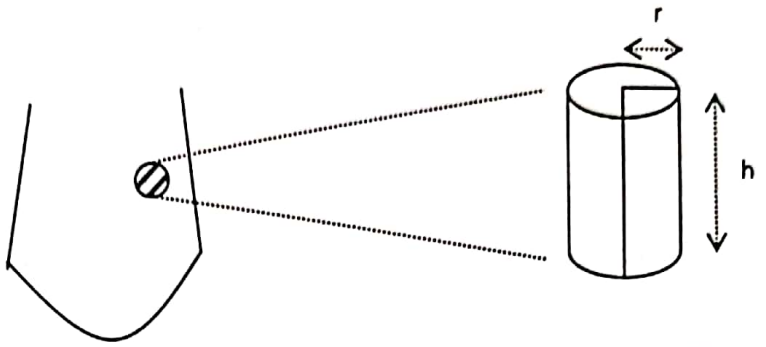
Mass of enamel biopsied = Mass of PO_4 biopsied/ 0.175

Depth of biopsy = Mass of enamel biopsied ÷[(Density of enamel)x(Biopsy surface area)]

$$= \frac{(Praw - 0.37) * 3 * 1000}{3.14 * depth * 0.175 * 2950/1000}$$

F^- content in ppm= $\frac{(Fraw-0.1)*1000000}{3.14*depth*2950/1000}$

Figure 1: Schematic diagram representing acid etch biopsy of enamel surface of a tooth



Tooth surface showing area of biopsy | **3-Dimensional view of the area of biopsy**

We know that,
Volume of a cylinder (V)=$\pi r^2 h$ [Volume of the enamel biopsied]
Density of enamel=mass of enamel÷ volume of enamel biopsied
$= 2.95 g/cm^3$

Since, we know phosphate forms 17.5% by weight of enamel

Also we have,
Mass of enamel biopsied (m) = Mass of PO_4 biopsied/ 0.175
Thus , $2.95=m/V$
$h=m/2.95*\pi r^2$

Depth of biopsy (h) = Mass of enamel biopsied ÷[(Density of enamel) (Biopsy surface area)]

$$= \frac{(Praw-0.37)*3*1000}{3.14*depth*0.175*2950/1000}$$

Total amount of Fluoride (in ppm)= mass of fluoride/ volume

$$= \frac{(Fraw-0.1)*1000000}{3.14*depth*2950/1000}$$

NOTE:
The formulas for the estimation of depth and fluoride concentration are according to the modifications presented by Stearns RL et al in 1972 and Sokoloff P in 1975 [67,68]

The raw scores of Phosphate and Fluoride were 0.37µg/ml and 0.1µg/ml respectively as assessed from the average of 3 blank samples

CALIBRATION OF ION CHROMATOGRAPHY DEVICE:

(Photograph 19)

Fluoride estimation was done using 850 Professional IC – AnCat – MCS (2.850.3030). The instrument works on the principle of reversible electrostatic interaction of ions with the separation matrix, (i.e.,) the separation occurs by reversible exchange of ions between the ion exchange resin and the ions present in the solution. The ions are then classified accordingly as strong or weak cations or anions respectively. For cations natural resin columns like zeolite or clay are used while for anions dolomite columns are used. In our present study, the fluoride (F^-) and phosphate ion (PO_4^-) concentrations of the test sample were determined after making a 1:1000 dilution with distilled water using dolomite column. Prior to analysis, all standards and samples were buffered by the addition of an appropriate volume of 1N NaOH solution to adjust the pH (6.6 to 7.0) and ionic strength of the standards and samples to the same values. This was done to avoid erosion of the resin column of the chromatography device. A pre-test of distilled water, two standard solutions containing 0.5ppm, 1ppm of sodium fluoride respectively and the acid-base solution used for enamel biopsy and 3 blank samples were done to check for any errors in estimation and the least

detectable limit was set at 0.1µg/ml, 0.37µg/ml for fluoride and phosphate ions respectively.

APPLICATION OF GEL: (Photographs 10 to 14)

Each subject received a single application of both the nanoparticle based APF gel and the conventional APF gel. Each subject was positioned to sit straight in the dental chair. The nanoparticle based APF was applied to the right half of the mouth containing teeth in the first and fourth quadrant while the teeth on the left half of the mouth received conventional APF gel. At a time only one gel was applied in both the arches using foam trays of appropriate size. Firstly the nanoparticle based gel was applied during which the premolar teeth on the opposite quadrant were covered with teflon tape to avoid cross-over effect from the tray. Followed by this a similar procedure to cover the premolars on the right side was done during the application of conventional gel. A time span of 30 minutes was maintained between the application on the right and left halves of the mouth. During each application, the subjects were asked to tilt their head forward by 45° to prevent accidental ingestion of fluoride and a high volume suction was used to aspirate the excess fluoride during each application. The armamentarium used for fluoride application, was as follows:

1. Mouth mirror

2. Dental explorer
3. Tweezer
4. Cheek retractor
5. Suction tips
6. Foam tray
7. Ice cream sticks

After application of both the gels, the participants were instructed not to drink or eat anything and expectorate the excess gel that was present for the next 30 minutes. All the participants were advised to brush two times a day using fluoridated dentifrice and take a non cariogenic diet by avoiding sticky carbohydrates.

DATA COLLECTION AFTER 24 HOURS AND 30 DAYS:

(Photograph 15 to18)

Similar to baseline assessment, procedure for acid etch biopsy of enamel was performed on the buccal or palatal/ lingual surface of the respective premolars on either side. In order to avoid re etching of the same biopsy site again, after 24 hours the biopsy was done on the distal half of the buccal surface of the premolars and after 30 days the biopsy was done on the palatal/ lingual surface of the corresponding tooth. Also after each biopsy the corresponding test or control APF gel was applied with the help of microbrush on the biopsied area for 4

minutes (nanoparticle based APF gel on the right side and conventional APF on the left side).

EXTRACTION OF TEETH:

The extraction of the premolars on either side was done atraumatically under aseptic conditions at 2 intervals of time using closed method with teeth on one side being extracted at a time. The extraction was carried out within 30 to 60 days post the final evaluation. The extracted teeth were collected separately in two different containers prefilled with 5ml formalin for 24 hours. After this two teeth were randomly selected from each group, washed under running water and air dried. The root portion of the teeth were sectioned and removed and the crown was sliced longitudinally with the help of carborundum disc on a slow speed hand piece. The sectioned portion was mounted on circular resin base of 2cm diameter, made of poly methyl methacrylate. The mounted specimens were observed under scanning electron microscope.

SCANNING ELECTRON MICROSCOPE (SEM) ANALYSIS:

The premolars were later collected from the study participants after extraction and stored in formalin in two separate containers each representing our test and control group. Two teeth from each container were mounted on a polymethyl methacrylate resin base and subjected to SEM analysis of the surface after coating

with palladium. The SEM analysis was done at Central Workshop Division, College of Engineering, Guindy, Chennai.

STATISTICAL ANALYSIS:

The following procedures were carried out:-

1. Data compilation and presentation
2. Statistical analysis

1. Data compilation and presentation:

Data obtained were compiled systematically in Microsoft Excel spreadsheet (Annexure VIII). The dataset was subdivided and distributed meaningfully and presented as graphs and tables.

2. Statistical analysis:

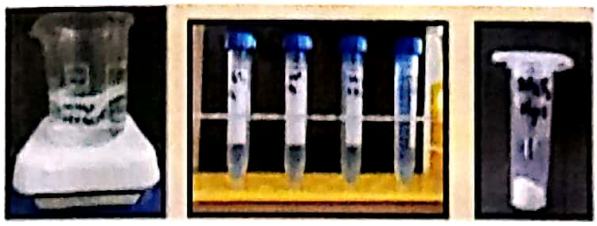
Statistical analyses were performed using Statistical package for Social Sciences software (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). Normality of the data was assessed using Kolmogorov-Smirnov test and Shapiro-Wilk numerical test and it was found that all variables were normally distributed. Due to the unequal difference in between the 3 time points of estimation, interaction effect was assessed using generalized estimating equation (GEE). Depending

upon the significance, appropriate parametric statistical tests were chosen p value of < 0.05 was considered to be significant.

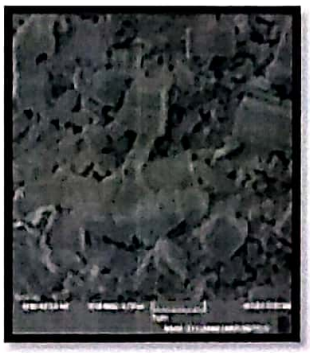
Students' unpaired t- test was used to compare the mean fluoride uptake between the nanoparticle based gel group and conventional APF gel group at baseline, 24 hours and 30 days. Paired t- test was used to compare the mean differences within each group at the same time intervals.

PHOTOGRAPHS

PHOTOGRAPH 1: PREPARATION OF SODIUM FLUORIDE NANOPARTICLES

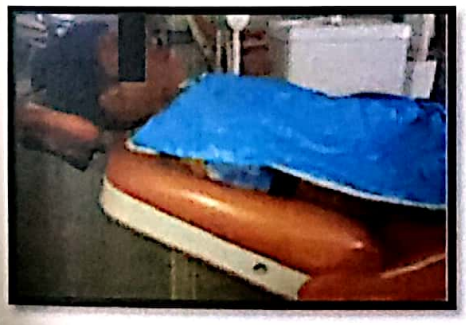


PHOTOGRAPH 2: CHARACTERIZATION OF SODIUM FLUORIDE NANOPARTICLES

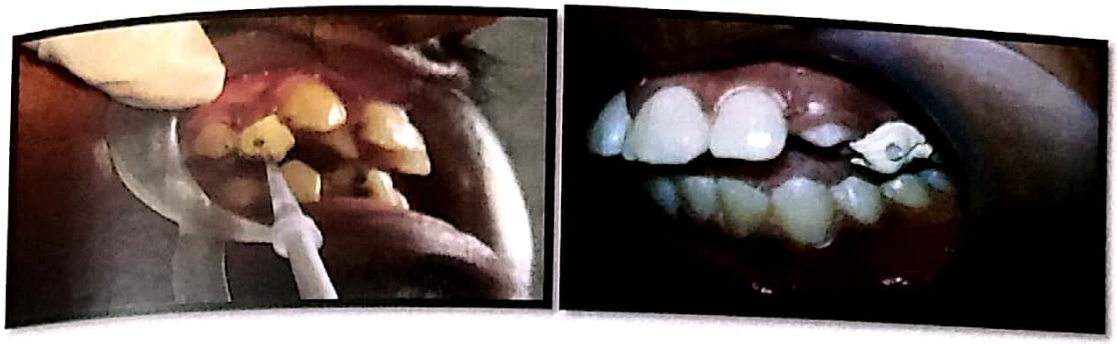


PHOTOGRAPH 3: PREPARED NANOPARTICLE BASED APF GEL IN SEALED CONTAINER

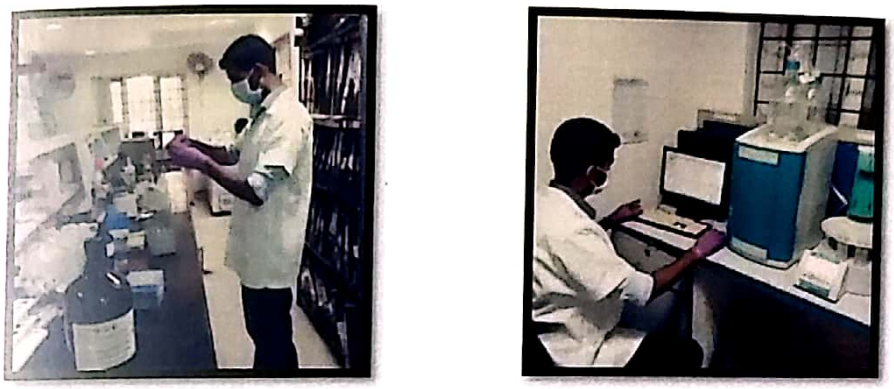
PHOTOGRAPHS 4 & 5 : BASELINE EXAMINATION & PLACEMENT OF TEFLON MEMBRANE



PHOTOGRAPH 6 & 7: ACID ETCH BIOPSY OF ENAMEL SURFACE USING 0.5 M PERCHLORIC ACID SOLUTION



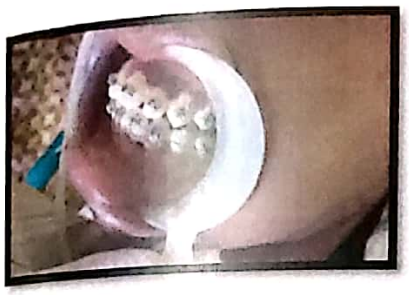
PHOTOGRAHS 8 & 9: DILUTION AND ANALYSIS OF THE BIOPSY SAMPLES

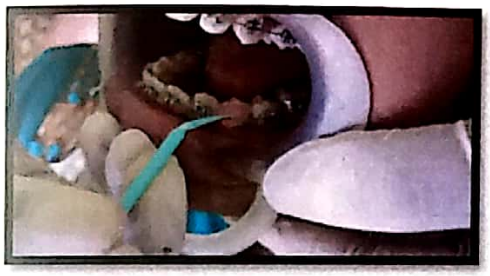


PHOTOGRAPHS 10, 11 & 12: APPLICATION OF NANOPARTICLE BASED APF GEL ON THE RIGHT HALF OF MOUTH

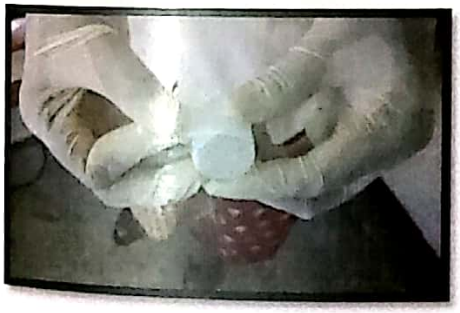
PHOTOGRAPH 13 & 14: APPLICATION OF CONVENTIONAL APF GEL ON THE LEFT HALF OF MOUTH

PHOTOGRAPHS 15, 16 &17: APPLICATION OF THE CORRESPONDING INTERVENTION GEL IN THE BIOPSIED AREA USING MICROBRUSH

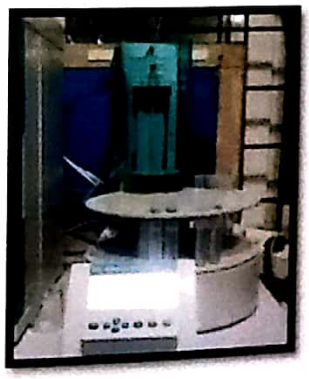




PHOTOGRAPH 18: SEALING OF BIOPSIED SAMPLE



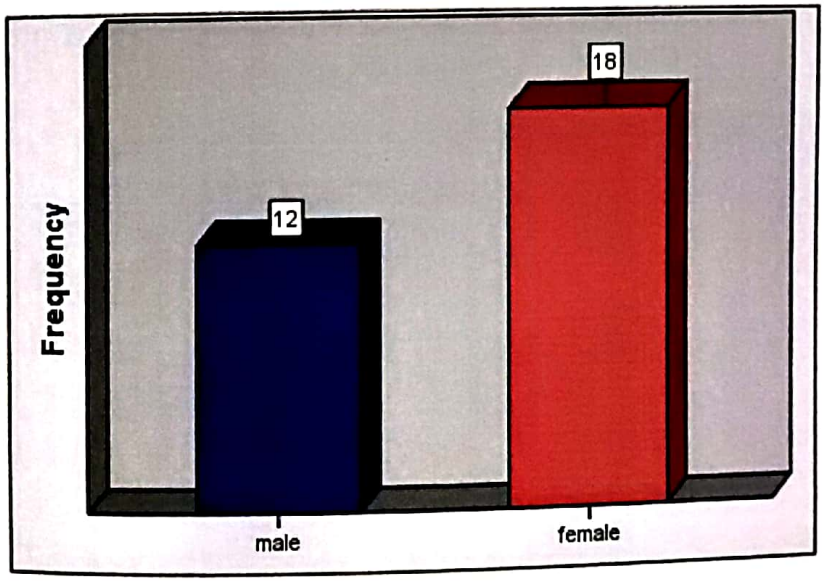
PHOTOGRAPH 19: METROHM 850 IC APPARATUS



RESULTS

The present study was conducted to compare the effectiveness of nanoparticle based APF gel and conventional APF gel on the surface enamel fluoride uptake. This was a split mouth interventional trial done among 30 subjects with the right half of the participants' mouth being subjected to nanoparticle based APF gel and the left half to conventional APF gel. The study was conducted over a period of 6 months.

Figure 2: Distribution based on sex of the study population



The mean age of the study population was 22.27±4.5 years. Table 1 gives the frequency distribution of the study population based on gender. Figure 2 gives graphical representation of the gender distribution of the study group. Overall there were 12 males (40%) and 18 females (60%) in the study group.

Since the assessment of fluoride uptake and biopsy depth was done at 3 time points of varying interval a multivariate analysis using GEE controlling for the covariates namely group (nanoparticle based APF, conventional APF gel group) and time of evaluation (baseline, 24 hours and 30 days) was done to compare the overall mean values of the 2 groups.

Table 1: Distribution of study population based on Sex

Gender	Frequency	Percent
Male	12	40.0
Female	18	60.0
Total	30	100.0

Both individual and combined effect of covariates on the fluoride uptake and biopsy depth as independent variables are given in tables 2 and 3. On the whole, fluoride uptake and depth of biopsy showed significant difference when controlled for the covariates individually and simultaneously with p value <0.001.

Table 2: GEE with fluoride uptake as independent variable controlling for group, time and simultaneously for group and time

Parameter	B	Std. Error	95% Wald CI		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	p-value
Group	1874.929	144.6119	1591.495	2158.363	168.098	1	<0.001*
Time	117.174	20.7108	76.582	157.767	32.009	1	<0.001*
Group * Time	-89.124	12.5363	-113.695	-64.554	50.542	1	<0.001*
(Scale)	5498595.4						

Dependent Variable: Fluoride uptake
Model: Group, Time, Group * Time
*$p \leq 0.05$ indicates significance

Table 3: GEE with depth of biopsy as independent variable controlling for group, time and simultaneously for group and time

Parameter	B	Std. Error	95% Wald CI		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	p-value
Group	42.405	1.4835	39.497	45.312	817.039	1	<0.001*
Time	2.495	.0313	2.434	2.556	6372.593	1	<0.001*
Group * Time	-1.509	.0569	-1.621	-1.397	702.610	1	<0.001*
(Scale)	313.365						

Dependent Variable: Biopsy depth
Model: Group, Time, Group * Time
*$p \leq 0.05$ indicates significance

Individual difference between the groups at the baseline, 24 hours and 30 days was further assessed. Table 4 represents the distribution of mean fluoride uptake at the baseline, 24 hours and 30days. There was no significant difference observed between the right and the left side at the baseline and after 30 days ($p \geq 0.05$). However the mean surface enamel fluoride uptake showed a significant difference after 24 hours ($p=0.046$).

Intragroup comparison showed that there was an average decrease from 5495.25±2171.12 to about 2492.64 ± 1491.49 ppm in nanoparticle based APF gel group and from 4317.86±2294.33 to 1944.25 ± 1087.67 ppm in the conventional group between the 24 hours and 30 days evaluation. The mean fluoride uptake showed significant differences on pairwise comparison of all 3 time points.

Figure 3 gives the graphical representation of the mean levels of surface enamel fluoride at baseline, 24 hours and 30 days. In both the groups the mean level of surface enamel fluoride increased at the 24 hour evaluation which gradually decreased over a period of 30 days.

Table 4: Mean difference in Fluoride uptake between nanoparticle based APF gel and conventional APF gel (both between the groups and within group comparison)

TIME OF TESTING	NANO-BASED GEL (n=30)	CONVENTIONAL APF GEL (n=30)	p VALUE
BASELINE	1487.69±826.8$^{\dagger\Phi}$	1509.39±844.83^{ac}	0.920
AT 24 hrs	5495.25±2171.12$^{\dagger\beta}$	4317.86±2294.33^{ab}	0.046*
AFTER 30 DAYS	2492.64 ± 1491.49 $^{\beta\Phi}$	1944.25 ± 1087.67^{bc}	0.109

*p value ≤0.05 indicates significance

†,β,Φ indicates pair wise comparison within Nanobased APF gel group at baseline, 24hrs and 30 days with significance at the level of p<0.001

a,b,c indicates pair wise comparison within Conventional APF gel group at baseline, 24hrs and 30 days with significance at the level of p < 0.01

FIGURE 3: MEAN SURFACE ENAMEL FLUORIDE UPTAKE AT BASELINE, 24 HOURS AND 30 DAYS

Table 5 represents the distribution of mean depth of enamel biopsy at the baseline, 24 hours and 30days. There was no significant difference observed between the nanoparticle based group and the conventional APF gel group at the baseline and 24 hours ($p \geq 0.05$). However the mean depth of biopsy between the 2 groups showed a significant difference at the 30 days evaluation ($p=0.046$).

Intragroup comparison group showed that there was an average significant decrease in the biopsy depth from 71.15 ± 3.18µm at the baseline to about 69.33 ± 2.11µm in nanoparticle based APF gel group during the 30 days of evaluation ($p \leq 0.05$). In the conventional group the corresponding values were 70.92 ± 2.95µm and 70.45± 2.15 µm respectively. However, in the conventional group the

decrease in the depth of biopsy was significant only between baseline and 24 hours.

Table 5: Mean difference in the depth of enamel biopsy between nanoparticle based APF gel and conventional APF gel (both between the groups and within group comparison)

TIME OF TESTING	NANO-BASED GEL (n=30)	CONVENTIONAL APF GEL (n=30)	p VALUE
BASELINE	$71.15 \pm 3.18^{\dagger\Phi}$	70.92 ± 2.95^{a}	0.767
AT 24 hrs	$70.18 \pm 2.92^{\dagger\beta}$	70.31 ± 2.93^{a}	0.858
AFTER 30 DAYS	$69.33 \pm 2.11^{\beta\Phi}$	70.45 ± 2.15	0.046*

*p value ≤0.05 indicates significance

†,β,Φ indicates pair wise comparison within Nanobased APF gel group at baseline, 24hrs and 30 days with significance at p=0.00,0.027 and 0.00 respectively

a indicates comparison of conventional APF gel group at baseline and 24 hours significance at the level of p=0.002

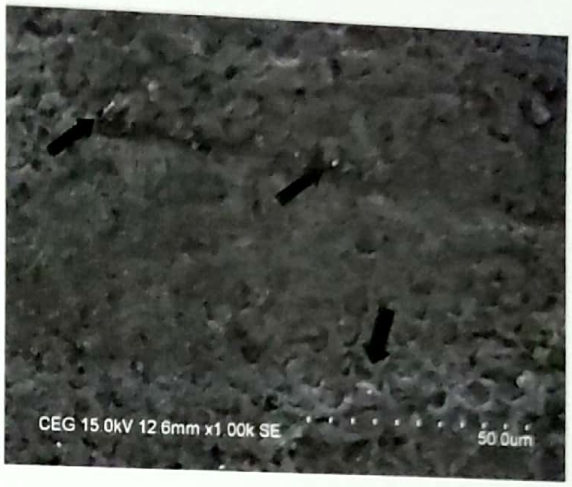
Figure 4 gives the graphical representation of the biopsy depth of the enamel at baseline, 24 hours and 30 days. Both the groups showed a decrease in the biopsy depth of enamel; however the nanoparticle based APF gel showed a greater decrease to 69.33± 2.11µm at the end of 30 days, compared to the 70.45±2.15µm in the conventional APF gel group.

FIGURE 4: MEAN BIOPSY DEPTH AT BASELINE, 24 HOURS AND 30 DAYS

Scanning electron microscope analysis of the extracted teeth samples showed calcium fluoride like precipitates deposited on the tooth surface of samples from both the groups. However, the prismatic nature of the enamel surface was better retained in the nanoparticle based APF gel group compared to the conventional group which was similar to an etched surface (Figures 5 to 12).

Figures 5 to 8 : Scanning electron microscope images of tooth surface from nanoparticle based APF gel group at 500x, 1000x, 2000x and 5000x magnifications

[5]



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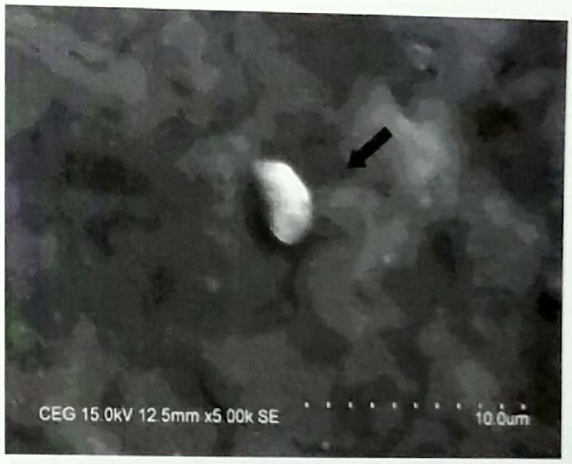
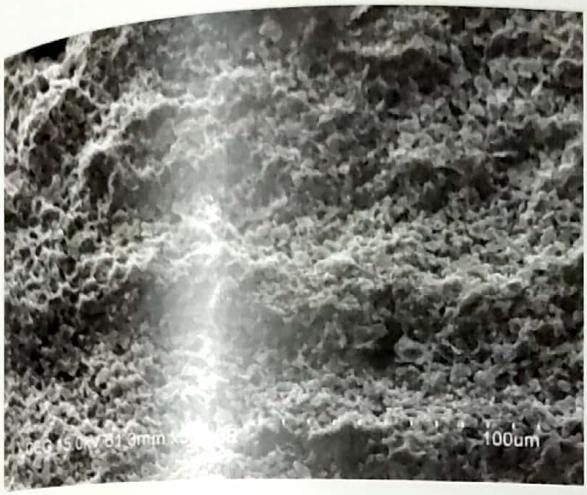


[8]

➡ Represent Calcium Fluoride like precipitates on the tooth surface

Figures 9 to 12: Scanning electron microscope images of tooth surface from conventional based APF gel group at 500x, 1000x, 2000x and 5000x magnifications



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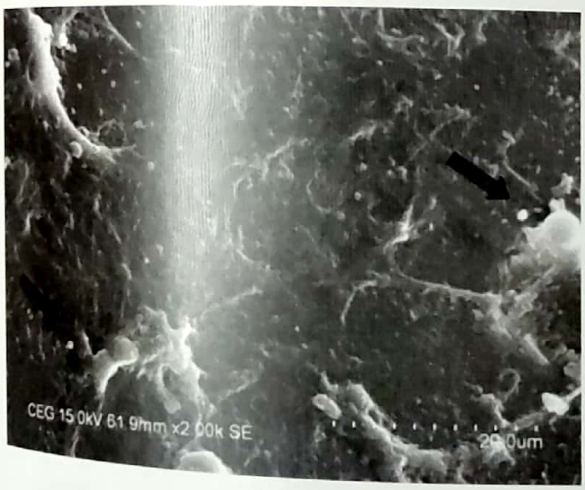


[11]

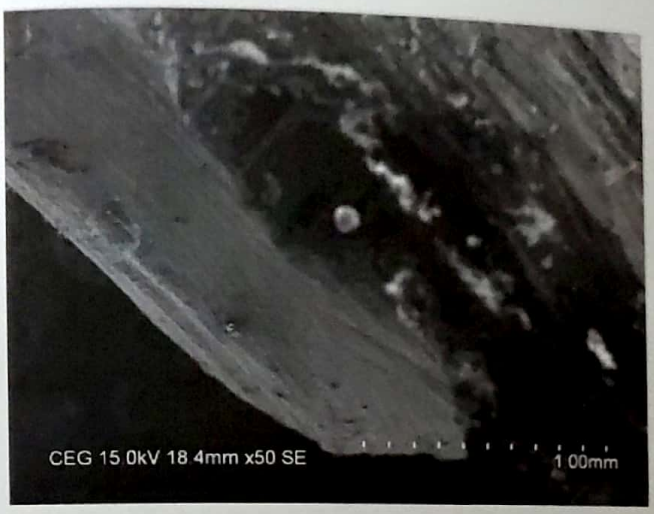
[12]

➘ Represent Calcium Fluoride like precipitates on the tooth surface

Examination of the longitudinal section of the extracted tooth samples under SEM showed the presence of a glossy layer above the enamel surface, similar to the fluorapatite layer described by Oliveira M et al in 2007 in the nanoparticle based APF gel group (Figure 13). However, no similar layer was seen on the sample from the conventional APF gel group (Figure 14).

Figure 13: Longitudinal section of tooth sample from nanoparticle based APF gel group showing the presence of varying width of fluorapatite layer

Figure 14: Longitudinal section of tooth sample from conventional APF gel group not showing the presence of fluorapatite layer



DISCUSSION

The present study was conducted to determine the effectiveness of nanoparticle based Acidulated Phosphate Fluoride (APF) gel on the human enamel surface. This was assessed in terms of surface enamel fluoride uptake and depth of penetration in the enamel surface during acid etch biopsy at the different time points of measurement namely baseline, 24 hours and 30 days. A split mouth design on a healthy group of subjects undergoing orthodontic correction was chosen to be appropriate as it was experimentally the next step after the successful in- vitro evaluation of the surface microhardness of the same agents. This would also reduce the influence of the other related factors like dental caries, plaque/ calculus in affecting the outcomes as good oral hygiene is mandatorily ensured before the commencement of any orthodontic procedure. The subjects were considered to belong to a special group, which included patients indicated for orthodontic correction with the extraction of premolars bilaterally in at least one arch which allowed us to examine the extracted teeth towards the end of the study.

The use of fluorides has been considered the gold standard in the prevention of dental caries since its discovery in 1931[3]. However, despite the topical and systemic delivery methods which are in practice today, lacunae still exists in terms of better retention and availability of the fluoride on the tooth surface for effective prevention against the dynamic changes in the oral cavity. Literature is

evident in explaining the sustained protection offered by systemic supplementation of fluoride, which has been predominantly found effective when administered during the growth and maturation phase of enamel[1,6]. Thus topical fluoride preparations evolved as additional means to deliver substantially higher concentrations of fluoride to improve the quality of the enamel surface[4]. This posteruptive fluoride acquisition by enamel is mainly through topical

solutions, gels, prophylactic pastes, mouthrinses, dentifrices and fluoride containing beverages. Amongst these, Acidulated Phosphate Fluoride (APF) gel has been time tested for its cariostatic action due the following properties[69]:

a. large concentrations (upto 1,23,000 ppm) of fluoride that is made available,
b. different modes of delivery like gels, foams and solution making it convenient for at-home and in-office use,
c. duration of application (4 minute application bi-annually) which minimises the recall period and,
d. lack of staining properties which are usually encountered with fluoride varnish applications.

These topical fluorides are mainly available in two major chemical forms for use in dentistry, those being inorganic fluoride and organic fluoride[2,3]. The anti-cariogenic action of topical fluorides depends on the solubility of the fluoride containing compound and its adhesion to the tooth surface. Extensive evidence

exists comparing the effectiveness of various topical inorganic fluoride (sodium fluoride, calcium fluoride, stannous fluoride and sodium mono-fluoro phosphate) therapies in the prevention of dental caries in both primary and permanent dentition. It is also an established fact that the anticariogenic properties of inorganic fluorides are predominantly due to decrease in the enamel solubility, enhanced remineralization and anti-enzymatic property by formation of fluorapatite crystals, improving the crystal growth, stabilizing the crystal structure, affecting the enzymatic glycolytic pathway, sugar transport and intracellular pH homeostasis of bacteria respectively[4,70].

Similar evaluation of organic fluorides by Muhleman et al in 1957 found that organic fluorides also showed similar properties of reducing the enamel solubility in in-vitro conditions [71].

The superiority of organic fluoride in preventing dental decay compared to inorganic fluoride was demonstrated by Muhlman in 1967 [72]. He also observed that amine fluoride had a pronounced affinity towards enamel, thus raising the quantity of fluoride in the enamel in addition to its anti-enzyme effect on the microbial activity of dental plaque.

He also concluded that even in low concentration amino fluorides produced the most powerful enrichment in fluoride by its unique amphilic nature with hydrophilic and hydrophobic parts within one molecule which when used in higher concentrations was found to be toxic with not much effect on the enamel

surface. The preventive action of organic fluoride was attributed to its chemical structure with fluoride on one side and the organic fraction on the other, thus arresting the formation of dental plaque, as a result of the tension-active properties.

However when analysed in terms of bound and free fluoride which are essential in determining the anticariogenic properties, inorganic fluorides perform better by providing high concentrations of free fluoride [31]. Amongst these, APF which contains sodium fluoride mainly acts through the formation of calcium fluoride precipitates and fluorapatite by reacting with calcium present in the enamel crystals. The higher concentration and low pH of APF, results in the formation of large quantities of calcium fluoride (CaF_2) and dicalcium phosphate dihydrate (DCPD). The acidic pH further favours the uptake of fluoride ions by enamel surface, by slight dissolution of the enamel minerals in the superficial layers. This results in a transient phase in the period initially after topical application where there are high levels of fluoride seen which accounts for almost 90% of the fluoride incorporated on the tooth surface. As time elapses, this CaF_2 dissolves rapidly in the oral environment leaving behind only a small fraction of firmly bound fluoride in the form of fluorapatite. Hence, an effective topical fluoride regimen would be the one which results in a relatively higher concentration of permanently bound fluoride on the enamel surface.

The presence of this free or available fluoride is evaluated in terms of fluoride uptake from the tooth surface, and is restricted to the first few layers of enamel[3,4]. Fluoride uptake from various topical and systemic fluoride agents have been assessed since 1960s and studies comparing the mean uptake of different products also have been reported. Enamel surface uptake of fluoride from APF application has been tested in both deciduous and permanent teeth.

The loosely bound and firmly bound fluoride after topical treatment with APF gel was evaluated by Sieck B et al., (1990) and Takagi S et al., (1992)[73,74]. The 4 minute APF application resulted in 27.2μg, 31.5μg of firmly bound fluoride respectively and 184 ppm, 230 μg/ cm^2 of loosely bound fluoride respectively. However, both the above studies were done on human enamel specimens in laboratory conditions. The corresponding comparison with our present study could not be done as our primary objective was to determine only the fluoride uptake and not to differentiate between free or bound fluoride.

The effective mode of delivery of the APF was assessed by Whitford GM et al.,(1995)[59] who compared the enamel fluoride uptake from APF gel and foam preparations after 4 minute application. The authors found that uptake at 15 minutes, 16 days showed similar results in both the groups, concluding that both the preparations were equivalent in their effectiveness. Various modifications like altering the temperature, pH, mode of delivery, time of application, prior etching

of the surface have been tried previously to enhance the fluoride uptake and retention by the enamel surface.

Time dependent fluoride acquisition from APF gel were tested in laboratory and in-vivo conditions by Mellberg JR et al in 1977, Wei SHY and co-workers in 1988, Nishiyoka Y et al.(1995), and Calvo AFB et al.,(2012)[47,54,75,76]. The authors concluded that the fluoride concentrations in the enamel was maintained high during the treatment periods and sustained for an average of 2 to 10 months after which the levels stabilized at 1600 ppm. They found that fluoride uptake significantly increased with increase in the contact with topical agent and recommended professional application time of 4 minutes for APF gel which was adhered to in our present study. However, there is also evidence that the amount of calcium fluoride and fluorapatite formed showed no difference with respect to application time of APF gel in both deciduous and permanent dentitions.

The choice of professional prophylaxis prior to APF application was demonstrated in in-vitro by Ripa LW (1984), Beijella MFTB, et al.,(1985), [77,78] while in clinical conditions was reported by Houpt M, et al., (1983), Katz RV et al.,(1984) and Rippa LW, et al., (1984) [79-81]. From both the laboratory and clinical investigations the authors demonstrated that the presence of unclean teeth did not affect fluoride uptake or the caries inhibitory properties of fluoride. Also it was found that the time and labour intensity required per patient was drastically reduced in the absence of prophylaxis procedure. This was effectively found in

our study, where the maximum time taken for APF gel application and the enamel biopsy together was less than 10 minutes for each half of the mouth, with an interval of 30 minutes in between, after which the patient was asked to rinse thoroughly. This ensured that cross over effect of one agent over the other is minimised.

The uptake and retention of fluoride on the enamel surface had been assessed through a variety of methods like acid etch biopsy of enamel, use of surface electrodes and dielectrophoresis. Of all these, many modifications have been tried for the acid biopsy technique by Brudevold F etal., (1968), Candeli A et al., (1967), Bruun C et al., (1975), Caslavska et al., (1981), Mellberg JR et al.,(1976) [36,37,38,45,49] predominantly differing in terms of with/ without the use of burs (silicon carbide, felt burs, impregnated burs) or the type and concentration of acid used (hydrochloric, perchloric acid). In our present study, the field biopsy technique as suggested by Bruun C et al., (1975)[36] was used as it did not involve any invasive procedure and was performed only on specific demarcated area and not the whole teeth.

Applications of laser and cold atmospheric plasma tried in the recent past in combination with APF gel have also showed promising results[82]. The CO_2 and Nd-YAG were the common types of lasers which showed better uptake when used along with regular professional application of APF gel by Zancope BR, et al., (2016) and Chand BR et al., (2015) respectively[83,24]. The application of cold

atmospheric plasma (CAP) was tried by Kim YM et al.,(2017) in in-vitro conditions[82]. The four week evaluation showed that the combination of APF and CAP showed better fluoride retention and resistance to demineralization almost till the end compared to APF alone.

In line with these advancements of technological application in healthcare a commendable stride has been made with the introduction of nanoscience where in molecules are manipulated at $1/100^{th}$ of a micrometer for their probable usefulness and efficiency. In dentistry, nanotechnology has been applied from diagnosis to treatment and improved drug delivery. From the perspective of preventive dentistry, the landmark inventions include the introduction of nanohydroxyapatite, nanoparticles of organic and inorganic compounds for sustained fluoride delivery, for preventing early enamel lesions and secondary caries[83,84]. Souza BM,etal., in 2015 in his cross over, randomized, double blinded in-situ trial assessed the effect of combination of nanohydroxyapatite crystals with sodium fluoride (NaF) on dentin remineralization[84]. They found that the combined use of nanoparticles improved fluoride delivery into early dentinal lesions and prevented demineralization. Similar experiments were carried out by Cheng L etal., (2014) who coated nanopolymeric fibers with NaF which could be used for restorative purposes[85]. They found that, the fluoride coated particles enhanced the mechanical properties of the restorative material and further reduced the chances of secondary caries by sustained release of fluoride. Improved

antibacterial activity of fluoride coated organic and inorganic particles have also been demonstrated in laboratory conditions by Fan Y et al., (2013) and Wassel et al., (2017) [86,87].

A recent experiment by Nguyen S et al., (2017) found that use of sodium fluoride with chitosan nanoparticles by means of ionic gelation ensured continuous delivery of fluoride especially in acidic environment and gave promising results in dental delivery systems in protection against dental caries[88]. Till date no modification of the particle size of sodium fluoride (NaF), the principal component has been tried to improve fluoride fixation in the enamel surface. Our present study showed an overall increase in the fluoride uptake in both groups following APF gel application. However, the average increase in the nanoparticle based APF group was higher than the control at 24 hour evaluation which gradually returned close to baseline at the end of 30 days in both the groups. This was similar to the mean fluoride concentration of 6520±407 ppm, 15 minutes after gel application as found by Whitford GM et al., (1995)[59].

On comparing the biopsy depth at different time points, our study showed a minimal but gradual significant decrease from 71.15 ± 3.18 μm to 69.33 ± 2.11μm in the nanoparticle based APF group whereas, similar estimation in the conventional group was not significant. Clinical estimation of the depth of enamel biopsy done by Whitford GM et al.,(1995) also showed an insignificant decrease

in APF gel although the values ranged between 2.92 and 2.63µm. However, the above estimations were done on maxillary incisors of children of 8 to 12 years which is different from our study population. Dijkman AG et al., in 1983 [53] made similar observations in extracted teeth samples in in-situ condition wherein the biopsy depth for APF was found to be around 2.3 to 9.1µ in the 1 to 5 layers of enamel over an etching period of 120 seconds. This could also be not considered equivalent as the assessments were done on extracted teeth samples mounted on prosthetic appliances which was worn by 12 volunteers.

In vitro experiment on 528 enamel specimens to assess uptake and retention of APF, thixotropic and pluoronic gel as determined by Wei SHY et al.,(1983)[54] showed an average presence of 6929± 6091ppm, 5913± 10,135 ppm and 3566 ±1463ppm of fluoride respectively immediately at 24 hours, 7 and 28 days evaluation. The mean biopsy depth in this study was found to be 3.02±1.19 µ for the APF group.

A cumulative etching depth of 54µm after an etching period of 20 minutes was determined in the study by Pai N et al., (2007)[63]. This is different in that the etching was done with 0.1M HCl acid whereas it was 0.5M perchloric acid in our study.

Further analysis of the extracted teeth revealed deposition of calcium fluoride like precipitates on the surfaces of specimens from both the groups. This was similar

to the investigations by Nelson N et al., (1983,1984) and Oliviera M et al., (2007) [89-91]. However, the surfaces of the specimen in the nanoparticle based APF group showed finer characteristics in terms of absence of loss of prismatic structure which was not established in the conventional group. This was similar to the findings reported by Nishioka Y et al., (1995) who said topical application of APF results in loss of minerals on the tooth surface due to acidic pH and creates lacunae [75].

On the whole there is very little evidence comparing the uptake and retention of fluoride from APF gels in clinical conditions of which none of them are from the recent past. The earliest evidence of assessing minerals in the tooth surface dated back to 1968 when Brudevold F and colleagues [38] performed the procedure on military personnel to demonstrate the usefulness of this method. Subsequent applications of this acid etch biopsy was predominantly to determine the normal fluoride uptake of human enamel (both deciduous and permanent dentitions) of people exposed and unexposed to community water fluoridation, the gradient variations between different teeth and different sites of the same tooth and further the uptake from different topical fluoride agents.

However with respect to topical agents, literature corresponds mostly to in-vitro or in-situ estimations with little clinical evidence, thus preventing the possibility of direct comparison to the current results.

STRENGTHS

1. To the knowledge of the authors this was the first attempt to modify the particulate size of the sodium fluoride, the principal component of APF in order to enhance fluoride fixation in enamel.
2. The use of split mouth design favoured in simultaneous evaluation of 2 different interventions.
3. Ethical clearance was obtained from Institutional Review Board. The trial was registered in Clinical Trial Registry – India (CTRI)
4. This was a phase 2 trial done after prior evaluation of the new agent in in-vitro conditions.
5. Clinical evaluation was supported by microscopic evaluation of the extracted teeth at the end of study.

LIMITATIONS

1. Differences in the fluoride levels on varied areas of the tooth surface and between maxillary and mandibular teeth were not taken into account.
2. No attempt was taken to randomize the participants.
3. The effect of fluoride from other topical agents like dentifrices, mouthrinses, food and beverages were not considered.

SUMMARY

The present split mouth intervention study was conducted to compare the effectiveness of nanoparticle based APF gel with the conventional APF gel on surface enamel fluoride uptake. The study was conducted over a period of six months on patients undergoing orthodontic treatment and indicated for bilateral extraction of premolars on at least one of the arch. Each participant received one application of 4 minutes duration of both the intervention: Right half of the mouth received nanoparticle based APF gel, and Left half of the mouth received conventional APF gel (16 Oz Pascal Corp.; Strawberry Flavour). A total of 30 participants were included in the study. All the participants gave written consent to participate in the study. Ethical approval was obtained from the Institutional Review Board of Ragas Dental College and Hospital, Chennai and prior permission was obtained from the Head of the Department, Department of Orthodontics, Ragas Dental College and Hospital. The trial was also registered in the Clinical Trials Registry of India (CTRI). Oral examination and prophylaxis were done prior to the commencement of the study as all the subjects were patients undergoing orthodontic treatment. Acid etch enamel biopsy was taken for all the study subjects preoperatively and post-operatively, bilaterally on the buccal and palatal/lingual surface of maxillary or mandibular premolars indicated for extraction, using 1µl of 0.5M perchloric acid according to the method

suggested by Brunn et al in 1975. The surface enamel fluoride uptake and biopsy depth were estimated at 3 intervals of time namely baseline, 24hrs and 30 days. After the extraction of premolars scanning electron microscope (SEM) analysis was done to determine the surface characteristics of enamel in both the nanoparticles based APF and conventional APF gel group. The data was collected and analyzed using SPSS software and results were generated.

The study results showed:

1. The nanoparticle based APF gel showed a significant increase in group at 24 hour evaluation with a mean value of 5495.25±2171.12 ppm.
2. A similar comparison of biopsy depth showed a significant decrease in the nanoparticle based APF group, with a mean value of 69.33 ± 2.11 μ at the 30 day evaluation.

Multivariate analysis controlling for the covariates time of evaluation and group showed that both fluoride uptake and depth of biopsy significantly contributed to the change in uptake when controlled independently as well as simultaneously with p value < 0.001.

The study concluded that the nanoparticle based APF gel showed a comparatively better uptake than the conventional APF gel in the 24 hour evaluation which was relatively maintained at a numerically higher level in the 30 days evaluation, even though not statistically significant. Better surface properties were also seen in terms of decrease in biopsy depth (resistance to acid etch during biopsy) and

through scanning electron microscope images in the nanoparticle based gel group. Further evaluation of the new agent is proposed in terms of any alteration in the application time or frequency of application with respect to the current APF regimen for large scale application in preventive dentistry.

CONCLUSION

The highest level of literature evidence in terms of systematic review and meta analysis exists for APF gel to be used as the most commonly recommended professionally applied topical fluoride agent which is cost effective, has acceptable formulation, and has shown significant reduction of caries in both deciduous and permanent dentitions. However, saturation has been achieved in terms of the percentage reduction in dental caries despite the increasing quantities of fluoride supplementation through various forms.

The incorporation of nanotechnology has been proven effective in various disciplines of science including dentistry from drug delivery to improved techniques of diagnosis and patient care. The present study highlights the effective application of nanotechnology in preventive dentistry by modifying the particle size of sodium fluoride compound in APF gel. The results of the study showed a significant increase in both fluoride uptake and decrease in mean depth of biopsy in the nano-based gel group at the 24 hour evaluation. Further evaluation of this nanoparticle based gel in terms of alteration in duration of application; frequency of application should be done to substantiate its long term effectiveness.

RECOMMENDATIONS

1. The clinical effectiveness of the nanoparticle based APF gel should be evaluated in terms of proportion of caries fraction prevented among both children and adult population using longitudinal trials before recommending it as a part of regular preventive regimen.
2. Studies to determine the maximum reduction in particle size of sodium fluoride for effective fluoride uptake and caries reduction should be carried out.
3. Evaluation of the new preventive regimen in terms of the reduction in application time and frequency of applications required should also be done in comparison with the existing formulation of APF gel.
4. Further, new methods to improve the fluoride uptake by addition of metallic ions/ coupling agents which chelate to the calcium in the tooth surface should also be tried for improving the concentrations of surface enamel fluoride.

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ANNEXURE – I

RAGAS DENTAL COLLEGE & HOSPITAL
(Unit of Ragas Educational Society)
Recognized by the Dental Council of India, New Delhi
Affiliated to The Tamilnadu Dr. M.G.R. Medical University, Chennai
2/102, East Coast Road, Uthandi, Chennai - 600 119. INDIA
Tele : (044) 24530002, 24530003 - 06. Principal (Dir) 24530001 Fax : (044) 24530009

TO WHOMSOEVER IT MAY CONCERN

Date: 29.12.2017
Place: Chennai

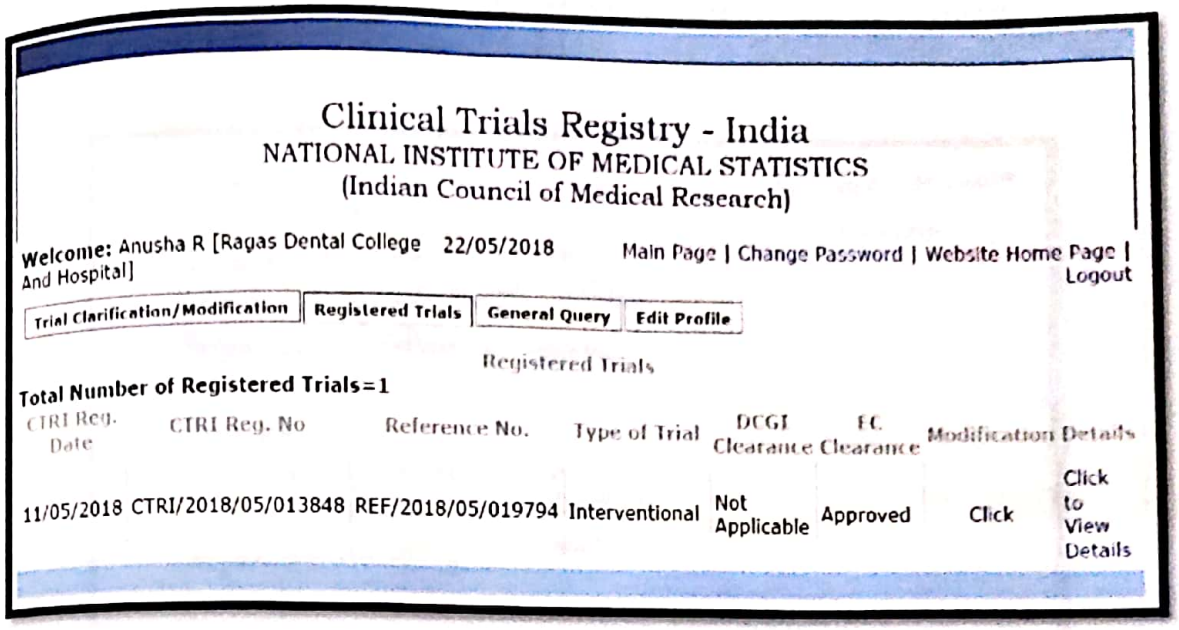
From
The Institutional Review Board,
Ragas Dental College and Hospital,
Uthandi,
Chennai – 600 119.

The study topic titled **"Effectiveness of nanoparticle based acidulated phosphate fluoride (APF) gel on surface enamel fluoride uptake"** submitted by **Dr. ANUSHA.R.**, has been approved by the Institutional Review Board of Ragas Dental College and Hospital.

Dr. N.S. Azhagarasan M.D.S.,
Member secretary,
Institution Ethics Board,
Ragas Dental College & Hospital
Uthandi, Chennai – 600 119.

RAGAS DENTAL COLLEGE Chennai

ANNEXURE – II



Clinical Trials Registry - India
NATIONAL INSTITUTE OF MEDICAL STATISTICS
(Indian Council of Medical Research)

Welcome: Anusha R [Ragas Dental College And Hospital] 22/05/2018 Main Page | Change Password | Website Home Page | Logout

Trial Clarification/Modification | Registered Trials | General Query | Edit Profile

Registered Trials

Total Number of Registered Trials=1

CTRI Reg. Date	CTRI Reg. No	Reference No.	Type of Trial	DCGI Clearance	EC Clearance	Modification Details
11/05/2018	CTRI/2018/05/013848	REF/2018/05/019794	Interventional	Not Applicable	Approved	Click Click to View Details

ANNEXURE – III

From
Dr. Anusha. R
II year Post graduate student
Department of Public Health Dentistry
Ragas Dental College and Hospital

December 21, 2017
Chennai

Through
The Head of the Department
Department of Public Health Dentistry
Ragas Dental College and Hospital

To
The Head of the Department
Department of Orthodontics
Ragas Dental College and Hospital

Respected Sir,

Subject: Req:. Permission for carrying out thesis work

This is to bring to your kind attention that myself, Anusha R (II year post graduate) from Department of Public Health Dentistry will be doing my main dissertation titled, "Topical effect of nanoparticle based APF gel compared to conventional APF gel on surface enamel fluoride uptake: an interventional study". As apart of my inclusion, I will be carrying out my research on patients undergoing orthodontic treatment (especially indicated for extraction). Hence, I request you to kindly permit me for the same.

Thanking you

Yours sincerely
R. Anusha

21/12/17

ANNEXURE - IV

HECS

Hubert Enviro Care Systems (P) Ltd.

(An Associate of Hubert Stavoren B.V., Holland)

Date: 04.06.2018

TO WHOMSOEVER IT MAY CONCERN

This is to certify that, **Dr. Anusha R**, Post Graduate student, Department of Public Health Dentistry, Ragas Dental College and Hospital, Chennai has done Fluoride estimation using **Ion Chromatography** method for her dissertation entitled "**EFFECTIVENESS OF NANOPARTICLE ACIDULATED PHOSPHATE FLUORIDE (APF) GEL ON SURFACE ENAMEL FLUORIDE UPTAKE**" in our laboratory.

She has successfully completed her study over a period of 6 months.

HUBERT ENVIRO CARE SYSTEMS (P) LTD. CHENNAI 600 083

Authorized Signatory
Laboratory Manager

#18, 92nd Street, Ashok Nagar, Chennai - 600 083. Ph: 42985555 Fax: 42985500
E-mail : marketing@hecs.in Website : www.hecs.in

MOEF Recognized, ISO / IEC 17025 / NABL accredited laboratory
ISO 9001:2008, ISO 14001:2004, OHSAS 18001:2007 certified company

NABET

ANNEXURE - V

INFORMED CONSENT FORM

TITLE:

EFFECTIVENESS OF NANOPARTICLE BASED ACIDULATED PHOSPHATE FLUORIDE (APF) GEL ON SURFACE ENAMEL FLUORIDE UPTAKE – AN INTERVENTIONAL STUDY

UNDERTAKING BY THE INVESTIGATOR:

Your consent for the above study is sought. We undertake to maintain complete confidentiality regarding the information and assessment obtained from you during the study. If you have any doubts regarding the study, please feel free to clarify the same. The investigator's name and contact number is given below:

Dr.Anusha.R, Mob no- 9952031886.

PARENT'S CONSENT

I _____, P/O, _____, residing at _____

_____ do hereby solemnly state as follows.

I am the respondent herein; as such I am aware of the facts stated here under.

I was informed and explained about the pros and cons of the study and the health education provided to my son/daughter in the _____ language known to me.

I give my consent after knowing the full consequences of the study.

I have given voluntary consent for including my child in the study without any individual pressure or duress.

I have also been informed about the purpose and procedures of the study that is to be conducted on my Son/Daughter. I understand that if I give my consent for the study, I will have to provide the necessary details required for the study and co-operate.

I _____ give my consent for my son/daughter to be a part of this investigation.

Signature of the investigator.

Signature of the Parent.

Date:

Place:

Signature of the Witness.

PARTICIPANT'S CONSENT

I_____,residingat_____

_, do hereby solemnly and state as follows.

I am the deponent herein; as such I am aware of the facts stated here under.

I was informed and explained about the pros and cons of the study, the intervention that will be provided to me.I was explained that there would not be any invasive procedure done and any form of discomfort would be avoided to me during the study in the _____ language known to me.

I give my consent after knowing the full consequences of the study.

I have given voluntary consent for including me in the study without any individual pressure or duress.

I have also been informed about the purpose and procedures of the study that is to be conducted on me. I understand that if I give my consent for the study, I will have to provide the necessary details required for the study and co-operate to the assessment that will be made during the study.

I _____ give my consent to be a part of this investigation.

Signature of the investigator. Signature of the Participant.

Date:

Place: Signature of the Witness

ANNEXURE - VI

ஒப்புதல் கோரும் படிவம்

தலைப்பு:

மேற்பரப்பு பற்சிப்பி ஃப்ளோரைடு உட்செலுத்துதல் மீது நானோபர்டிக்கள் அடிப்படையிலான பாஸ்பேட் ஃப்ளோரைடு ஜெல்லின் செயல்திறன் - ஒரு தலையீடு ஆய்வு

ஆய்வாளரின் பொறுப்பு:

மேற்கூறப்பட்ட ஆய்விற்குத் தங்களின் ஒப்புதலைக் கோருகிறோம். இந்த ஆய்வின் போது தங்களிடமிருந்து பெறப்பட்ட தகவல்களையும் அதன் மீதான முடிவுகளையும் பற்றி முழுமையான ரகசியத்தைக் காப்போம் என்று உறுதி கூறுகிறோம். இந்த ஆய்வு பற்றி தங்களுக்கு ஏதேனும் சந்தேகம் இருந்தால் தயவுசெய்து அது பற்றி சுதந்திரமாகக் கேட்டுத் தெளிந்துகொள்ள விழைகிறோம். ஆய்வாளரின் பெயரும் தொலைபேசி எண்ணும் கீழே தரப்பட்டுள்ளன:

டாக்டர். அனுஷா. ரா., அலைபேசி எண்:9952031886

தகவலாளியின் ஒப்புதல் படிவம்

நான்----------,----------

---------- என்ற முகவரியில் இருந்து கீழ்க்கண்டபடி உறுதியுடன் தெரிவித்துக் கொள்வது:

இங்கு நான் தகவலாளி, கீழ்க்கொடுத்திருக்கும் தகவல்களைப் பற்றித் தெரிந்துவைத்திருக்கிறேன்.

இந்த ஆய்வின் நல்லவை தீயவை பற்றியும் இதன் முடிவுகள் பற்றியும் எனக்கு விளக்கமாகச் சொல்லப்பட்டிருக்கிறது. இந்த ஆய்வின் போது எந்த விதமான துளையிடும் செயல்முறையும் செய்யப்படமாட்டாது என்றும் எந்த ஓர் அசௌகரியமும் எனக்கு நேராது எனவும் எனக்குத் தெரிந்த ----------மொழியில் விளக்கப்பட்டுள்ளது.

இந்த ஆய்வின் முழுமையான விளைவை அறிந்தே நான் என் ஒப்புதலைத் தருகிறேன். வலுக்கட்டாயமாகவோ தனிநபரின் நெருக்கடி காரணமாகவோ அல்லாமல் நான் சுயமாக என்னை இந்த ஆய்வில் சேர்ப்பதற்கு ஒப்புதலைத் தருகிறேன்.

என் மீது நடத்தப்படும் இந்த ஆய்வின் நோக்கத்தையும் செயல்முறையையும் பற்றி எனக்குத் தெரிவிக்கப்பட்டிருக்கிறது. இந்த ஆய்விற்கு ஒப்புதலைத் தருவதினால் ஆய்விற்குத் தேவையான முழுமையான தகவல்களை நான் சொல்ல சொல்லவேண்டும் என்பதையும் இந்த ஆய்வின் போது ஏற்படும் முடிவிற்கு நான் ஒத்துழைக்கவேண்டும் என்பதையும் நான் அறிந்திருக்கிறேன்.

நான் ---------- இந்த ஆய்வில் பங்கேற்க ஒப்புதலைத் தருகிறேன்.
(அ)

நான் ---------- என் மகன் / மகள் இந்த ஆய்வில் பங்கேற்க ஒப்புதலைத் தருகிறேன்.

ஆய்வாளரின் கையொப்பம தகவலாளியின் கையொப்பம்

இடம்: சாட்சியின் கையொப்பம்

ANNEXURE – VII

Wednesday, January 04, 2017 --

t tests - Means: Difference between two dependent means (matched pairs)

Analysis: A priori: Compute required sample size

Input:	Tail(s)	= Two
	Effect size dz	= 0.6264201
	α err prob	= 0.05
	Power (1-β err prob)	= 0.80
Output:	Noncentrality parameter δ	=3.0042053
	Critical t	= 2.0738731
	Df	= 22
	Total sample size	= 23
	Actual power	= 0.8188239

The required sample size was 22.

A 20% loss to follow up in addition included 22+ 8, a total of 30 subjects were included in the study.

ANNEXURE – VIII

SNo	group id	BASELINE ASSESSMENT					AFTER 24 HOURS					AFTER 30 DAYS				
		F raw	Phos raw	mass of enamel biopsied	Depth (μ)	Fconc (ppm)	F raw	Phos raw	mass of enamel biopsied	Depth (μ)	Fconc (ppm)	F raw	Phos raw	mass of enamel biopsied	Depth (μ)	Fconc (ppm)
1	1	1.87	38.16	218.0571429	70.32	2717.054	5.65	37.12	212.1142857	68.39175	8760.675	2.793	37.24	212.8	68.61507	4237.065
2	1	1.563	39.45	225.428	72.72788	2171.659	4.62	39.1	223.4285714	72.07653	6770.066	1.57	37.88	216.4571429	69.80611	2273.381
3	1	0.853	37.15	212.2857143	68.44758	1187.641	4.1	37.22	212.6857143	68.57785	6296.866	2.23	37.91	216.6285714	69.86194	3291.45
4	1	1.933	43.25	247.1428571	79.79968	2479.76	4.14	42.18	241.0285714	77.80841	5605.355	2.05	39.41	225.2	72.65344	2897.522
5	1	1.47	38.6	220.5714286	71.14603	2078.826	5.53	38.75	221.4285714	71.42518	8207.234	4.54	37.85	216.2857143	69.75028	6872.034
6	1	1.87	41.24	235.6571429	76.05907	2512.295	6.42	41.14	235.0857143	75.87297	8992.456	3.862	39.82	227.5428571	73.41645	5531.892
7	1	1.42	38.65	220.8571429	71.23908	2000.341	5.645	38.15	218	70.30858	8514.155	2.906	39.45	225.4285714	72.72788	4165.192
8	1	0.582	37.12	212.1142857	68.39175	760.837	1.65	37.02	211.5428571	68.20565	2453.351	0.673	37.35	213.4285714	68.81978	898.855
9	1	0.583	38.6	220.5714286	71.14603	732.9001	1.06	37.95	216.8571429	69.93638	1481.891	0.64	37.58	214.7428571	69.24781	841.8525
10	1	0.85	36.9	210.8571429	67.98233	1191.005	3.74	36.75	210	67.70318	5804.177	1.71	36.6	209.1428571	67.42403	2577.861
11	1	0.92	37.24	212.8	68.61507	1290.157	3.2	37.14	212.2285714	68.42897	4890.689	1.58	36.89	210.8	67.96372	2350.893
12	1	1.55	37.88	216.4571429	69.80611	2242.451	4.48	37.65	215.1428571	69.37808	6815.538	1.77	38.65	220.8571429	71.23908	2530.734
13	1	1.33	38.91	222.3428571	71.72294	1851.379	3.722	38.47	219.8285714	70.9041	5514.745	1.854	37.12	212.1142857	68.39175	2768.689
14	1	0.917	39.21	224.0571429	72.28124	1220.239	1.945	38.88	222.1714286	71.66711	2779.232	0.915	38.6	220.5714286	71.14603	1236.674
15	1	1.73	37.15	212.2857143	68.44758	2570.856	4.21	37.08	211.8857143	68.31731	6494.704	2.56	36.9	210.8571429	67.98233	3906.496
16	1	1.31	43.25	247.1428571	79.79968	1636.939	2.58	41.15	235.1428571	75.89158	3527.82	1.89	39.24	224.2285714	72.33707	2671.409
17	1	1.17	38.6	220.5714286	71.14603	1623.609	2.85	37.08	211.8857143	68.31731	4345.605	0.57	38.15	218	70.30858	721.6687
18	1	0.504	41.24	235.6571429	76.05907	573.4277	0.986	41.12	234.9714286	75.83575	1261.27	0.57	37.89	216.5142857	69.82472	726.6696
19	1	0.412	38.65	220.8571429	71.23908	472.8078	3.27	38.16	218.0571429	70.32719	4866.137	1.188	38.21	218.3428571	70.42024	1667.937
20	1	1.063	37.12	212.1142857	68.39175	1520.096	4.28	36.95	211.1428571	68.07538	6628.794	1.875	36.16	206.6285714	66.60519	2876.992
21	1	0.655	38.16	218.0571429	70.32719	851.9577	3.265	37.7	215.4285714	69.47113	4918.33	1.96	36.85	210.5714286	67.88928	2957.74
22	1	0.46	39.45	225.4285714	72.72788	534.3796	2.98	38.25	218.5714286	70.49468	4410.466	0.74	38.6	220.5714286	71.14603	971.1305
23	1	0.38	37.15	212.2857143	68.44758	441.6195	2.232	36.48	208.4571429	67.20071	3425.008	1.22	35.24	201.3714286	64.89307	1863.237
24	1	0.463	36.9	210.8571429	67.98233	576.4463	1.76	36.18	206.7428571	66.64241	2689.092	0.488	36.65	209.4285714	67.51708	620.3923

25	1	0.683	37.24	212.8	68.61507	917.2704	3.65	37.15	212.2857143	68.44758	5599.104	0.612	37.12	212.1142857	68.39175	808.192
26	1	0.37	37.88	216.4571429	69.80611	417.5598	3.2	36.88	210.7428571	67.94511	4925.517	1.147	37.65	215.1428571	69.37808	1629.194
27	1	1.1	38.91	222.3428571	71.72294	1505.186	4.12	38.65	220.8571429	71.23908	6091.946	1.72	37.12	212.1142857	68.39175	2557.17
28	1	1.132	39.41	225.2	72.65344	1533.458	5.63	38.85	222	71.61128	8336.658	2.14	38.6	220.5714286	71.14603	3095.479
29	1	0.9	37.85	216.2857143	69.75028	1238.204	3.64	37.65	215.1428571	69.37808	5508.449	0.985	36.9	210.8571429	67.98233	1405.386
30	1	2.48	36.89	210.8	67.96372	3780.49	5.537	35.64	203.6571429	65.63747	8942.435	2.4	35.24	201.3714286	64.89307	3826.29
31	2	1.75	38.26	218.6285714	70.51329	2526.162	4.375	37.84	216.2285714	69.73167	6618.421	1.57	37.89	216.5142857	69.82472	2272.775
32	2	1.53	40.15	229.4285714	74.03058	2085.322	4.1	39.65	226.5714286	73.10008	5907.319	1.126	38.65	220.8571429	71.23908	1554.81
33	2	0.853	37.05	211.7142857	68.26148	1190.879	3.43	37.05	211.7142857	68.26148	5266.436	0.85	37.12	212.1142857	68.39175	1183.875
34	2	1.983	41.25	235.7142857	76.07768	2672.03	4.14	41.78	238.7428571	77.06401	5659.5	2.12	41.6	237.7142857	76.72903	2842.104
35	2	1.47	37.16	212.3428571	68.46619	2160.194	4.12	36.98	211.3142857	68.13121	6369.836	2.6	36.9	210.8571429	67.98233	3970.016
36	2	1.97	39.87	227.8285714	73.5095	2746.29	5.847	39.65	226.5714286	73.10008	8487.341	3.52	39.14	223.6571429	72.15097	5117.198
37	2	1.452	38.65	220.8571429	71.23908	2048.834	4.373	38.15	218	70.30858	6561.043	1.98	38.15	218	70.30858	2886.675
38	2	0.58	37.12	212.1142857	68.39175	757.68	0.985	38.1	217.7142857	70.21553	1360.688	0.645	37.89	216.5142857	69.82472	842.6275
39	2	0.61	38.16	218.0571429	70.32719	782.88	0.854	38.12	217.8285714	70.25275	1158.661	0.514	38.21	218.3428571	70.42024	634.6747
40	2	0.75	37.28	213.0285714	68.68951	1021.577	2.127	36.98	211.3142857	68.13121	3211.855	0.78	38.16	218.0571429	70.32719	1043.84
41	2	0.97	36.84	210.5142857	67.87067	1383.839	1.037	36.87	210.6857143	67.9265	1489.185	0.96	38.25	218.5714286	70.49468	1317.014
42	2	1.65	37.16	212.3428571	68.46619	2444.015	5.07	36.9	210.8571429	67.98233	7892.392	1.565	37.6	214.8571429	69.28503	2282.688
43	2	1.43	39.12	223.5428571	72.11375	1991.049	2.49	38.67	220.9714286	71.2763	3619.937	1.647	39.24	224.2285714	72.33707	2308.754
44	2	1.12	39.12	223.5428571	72.11375	1526.97	0.926	39.05	223.1428571	71.98348	1238.784	0.885	38.65	220.8571429	71.23908	1189.596
45	2	1.652	37.08	211.8857143	68.31731	2452.501	3.46	37.06	211.7714286	68.28009	5312.433	1.875	37.12	212.1142857	68.39175	2801.838
46	2	1.35	43.25	247.1428571	79.79968	1691.053	0.854	42.65	243.7142857	78.68308	1034.519	1.168	41.63	237.8857143	76.78486	1501.564
47	2	1.27	39.18	223.8857143	72.22541	1748.816	2.42	38.68	221.0285714	71.29491	3512.997	1.12	37.12	212.1142857	68.39175	1610.07
48	2	0.504	41.3	236	76.17073	572.5871	0.75	41.24	235.6571429	76.05907	922.5941	0.54	38.6	220.5714286	71.14603	667.6523
49	2	0.422	39.45	225.4285714	72.72788	477.9729	1.78	38.76	221.4857143	71.44379	2538.593	1.25	38.69	221.0857143	71.31352	1740.902
50	2	1.063	38.85	222	71.61128	1451.754	2.78	37.28	213.0285714	68.68951	4212.042	1.764	37.24	212.8	68.61507	2618.075
51	2	0.642	37.7	215.4285714	69.47113	842.2543	3.26	37.4	213.7142857	68.91283	4950.343	1.98	37.24	212.8	68.61507	2957.922

52	2	0.46	39.4	225.1428571	72.63483	535.0642	1.895	38.78	221.6	71.48101	2710.954	0.654	37.88	216.4571429	69.80611	856.7708
53	2	0.387	36.85	210.5714286	67.88928	456.3825	2.05	36.7	209.7142857	67.61013	3113.66	1.152	38.91	222.3428571	71.72294	1583.456
54	2	0.46	37.1	212	68.35453	568.5694	1.132	36.89	210.8	67.96372	1639.271	0.387	36.41	208.0571429	67.07044	461.9543
55	2	0.653	36.48	208.4571429	67.20071	888.3817	2.95	36.42	208.1142857	67.08905	4586.079	0.72	37.85	216.2857143	69.75028	959.6084
56	2	0.37	38.12	217.8285714	70.25275	414.9051	2.95	37.56	214.6285714	69.21059	4445.5	1.038	37.82	216.1142857	69.69445	1452.958
57	2	0.97	38.3	218.8571429	70.58773	1330.572	3.89	38.1	217.7142857	70.21553	5827.125	1.65	38.45	219.7142857	70.86688	2361.221
58	2	1.132	39.41	225.2	72.65344	1533.458	5.26	37.72	215.5428571	69.50835	8014.216	1.58	37.35	213.4285714	68.81978	2321.65
59	2	0.875	37.85	216.2857143	69.75028	1199.511	2.93	37.8	216	69.65723	4385.999	0.965	38.58	220.4571429	71.10881	1313.231
60	2	2.48	36.89	210.8	67.96372	3780.49	4.68	35.85	204.8571429	66.02828	7488.31	2.52	38.6	220.5714286	71.14603	3672.087

ANNEXURE – IX

URKUND

Urkund Analysis Result

Analysed Document:	thesis-final.pdf (D46136878)
Submitted:	12/20/2018 6:10:00 PM
Submitted By:	anu1705@gmail.com
Significance:	7 %

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