EVALUATION THE AMOUNT OF FLOURID RELEASED FROM PIT AND FISSURE SEALANT MATERIAL (IN VITRO-STUDY)

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Declaration

I declare that this study is an individual work in which there was no unethical behavior during all the stages from planning the thesis until its writing and all the information in this thesis was obtained according to academic and ethical rules. I declare that I have referenced all the interpretations not obtained in this study and that these sources are listed in the list of sources, there is no violation samples or working during this study and the writing of thesis.

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I am deeply thankful and grateful to ***ALLAH*** *by the grace of whom only this work was possible.*

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Dedication

I dedicate this thesis to my family and friends for their great support.

Special feeling of gratitude to my father and deceased mother, who had taught me to work hard for the things that I aspire to achieve.

I would like to dedicate my work to my husband who offered unwavering encouragement and support.

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Abbreviation	**Meaning**
AI	Adequate Intakes
Al_2O_3	Aluminum Oxide
ASPA	Alumino Silicate Polyacrylate Cement
CaF_2	Calcium fluoride
CaO_4	Calcium Oxide
ESPE	European Society For Pediatric Endocrinology
F	Fluoride
F-PRG	Fully Reacted Glass Particles
HEMA	Hydroxy Ethyl Methacrylate
ISE	Ion Selective Electrode
GIC	Conventional glass ionomer cement
MID	Minimal Invasive Dentistry techniques
NIR	Near-infrared spectroscopy
pH	Is a scale used to how acidic specify or basic a water-based solution
Ppm	Parts Per Million
PPB	Parts per billion
PRGS	Pre-Reacted Glass Ionomer Particles
RMGIC	Resin Modified Glass Ionomer Cements
SiO_2	Silicon Dioxide
S-PRG	Surface Reacted Particles
SPADNS	Solution, For Fluoride, APHA At Spectrum Chemical
TISAB	Total Ionic Strength Adjustment Buffer
UL	Upper Intake Levels
UV	Ultra-Violate Rays
XRD	X-ray Diffraction Spectroscopy
Zr-SPADNS	Zirconium Indicator For The Determination Of Fluoride

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Abstract

Background: Pit and fissure caries are the most prevalence of dental caries in permanent and primary posterior teeth. Application of sealant containing fluoride is a preventive approach providing a physical barrier against dental caries.

Aim: This study aimed to evaluate the amount of fluoride release from two available pit and fissure sealant materials (Transeal- F, (B) for Conseal- F.),

Materials and Method: Eighty specimens were prepared from two tested materials were immersion in artificial saliva for 1, 7, 14, 21 and 28 days. The recharge of the sealant materials were evaluated after 14 days in mouthwash (potassium nitrate fluoride) and toothpaste (Sensodyne pro-enamel). The prepared specimens were kept in 20ml artificial saliva and incubate at 37^0C. The fluoride released was measured using spectrophotometry method. The results were statistically analyzed by using (one-way ANOVA) and T-testes.

Results: The quantity of fluoride released was higher in 1st day for both samples .The Transeal-F group showed slightly higher fluoride release than

Conseal-F. In 7th day the fluoride released from Conseal-F group was higher than that of 1st day while, Transeal-F showed lower fluoride released. At 28th day the amount of fluoride released in both groups was lowest value. In recharging analysis the Transeal-F samples showed slightly higher fluoride release than that of Conseal-F samples in both mouthwash and toothpaste, there was no significant difference between two recharging medium. The less amount of fluoride released after recharging found in day fourteen in comparison to that of first and seven day. The recharging ability in current study seems to be not effective, since it showed continues decrease of fluoride amount compared with that of day 28.

Conclusion: The ability of restorative materials for recharging by fluoride found to be depending on the material composition, the incidence of fluoride exposure, the kind and concentration of fluoride agent used.

1. Introduction

Dental caries is one of the most common oral diseases. If the dental caries is left untreated, it will lead to the early loss of dentition in both children and adults[1,2]. With the introduction of fluoride and better dental hygienic measures, a decline in caries incidence has been observed in developing countries. This decline is restricted to coronal caries. The incidence of root caries in the adult population has increased due to gingival recession[3].

Recurrent caries other than bulk fracture is one of the main reasons for replacement of a restoration when restoring a decayed tooth, a more surgical approach of removing the entire infected as well as sometimes affected structure which is followed by placement of a suitable material had traditionally been performed. For dentists, conservation and prevention of tooth structure from caries attack has taken a new direction and emphasis has been placed on become a desirable goal.

The traditional method of “Extension for Prevention” by G.V Black has been replaced by Minimal Invasive Dentistry techniques (MID). One approach in MID is the a traumatic restorative treatment (ART) which was developed for countries for which conventional methods are not practical. In addition to several other measures, many researches have been undertaken to develop a restorative material that to fulfill the functional and aesthetic demands as well be able to remineralise the surrounding tooth structure.[4]

Dental caries is a transmissible disease caused by the bacterial fermentation of carbohydrates, producing acids which cause dissolution of the dental hard tissues[4]. There are several pathological factors involved in the dissolution or demineralization of tooth structure. These pathological factors include cariogenic bacteria, substrate (carbohydrates) and salivary dysfunction[5].

Nature has provided numerous protective factors to balance these pathological factors. The disease only leads to cavitation when there is an imbalance between the pathological and protective factors; the process of dental caries is a combination of biological, chemical and physical actions. Oral cavity has a diverse microbial ecology and all the hard surfaces in the mouth are susceptible to microbial attachment. The initial attachment of early colonizers, later followed by secondary colonizer subsequently leads to the formation of biofilm on the tooth surface. The metabolically active biofilms ferment carbohydrates and produce organic acids as a by product[5]. The bacteria have to be acidogenic (able to produce acids) and acidouric (able to survive `in acidic environment) to be considered as pathogenic[6.]

Although many bacteria are present, mutans streptococci and lactobacilli are considered as the chief pathogens of dental caries[5,6]. This postulation is debatable, since these organisms are rather indicative of the environmental condition than being considered as the causative factors[7]. However, another researcher reported that there was no direct association of caries with these species, as caries can also occur in their absence and there could be no sign of caries in the presence of mutans streptococci[8] .

Saliva directly and indirectly helps in maintaining oral homeostasis and the integrity of tooth structure[9]. It acts as a vehicle and carries many protective factors that are essential to reverse the process of demineralization and re-deposits the lost minerals which called remineralisation process. These factors are calcium, phosphate and fluoride which are required for the reformation of the acid attacked crystal structure. It also contains acid buffering components and antibacterial agents[8].

It is worth mentioning that saliva is not always in direct contact with the tooth surface and an interface is usually present which is in the form of biofilm or the plaque. The acids produced as the by product of carbohydrate metabolism tend to bring a shift in the resting pH of the biofilm and it decreases from 7.0 to 5.5, which is the critical pH of hydroxyapatite, $Ca_5(PO4)_3OH_2$ The critical pH occurs when the overlying fluid is just saturated with respect to the hydroxyapatite crystals. Further decrease in pH causes the dissolution of crystals and induces demineralization[6].

The H^+ ions attack the crystal lattice and form complexes with PO_4^- and OH^- thus, making the fluid undersaturated and act as a driving force for more ions to leach out[10]. Although the structure and chemical composition of enamel do affect the kinetics of demineralize at the normal physiological levels of calcium, phosphate and fluoride are higher in the overlying plaque than saliva[9]. After the acid attack, plaque fluid becomes under saturated with respect to hydroxyapatite and a subsurface lesion forms. The surface layers, however, remain intact as the fluid remains supersaturated with respect to fluorohydroxyapatite[10].

The supersaturated fluid allows the process of reprecipitation on partially damaged crystals. The reprecipitation also occludes the possible ingress of ions in the body of the lesion and leaves an intact surface with a subsurface lesion, clinically diagnosed as ‘white spot’ lesion[6,11] .Therefore, the low and constant supply of the calcium, phosphate and fluoride ions are required for effective remineralisation to take place. Ion diffusion was the normal physiological level of calcium, phosphate and fluoride, were found to be higher in the overlying plaque than saliva[9], considered as the rate-limiting step[12].

The fissure sealants have a significant role in prevention of pit and fissure caries, and studies on the fluoride released from different types of fissure sealant materials are required. Therefore, the aim of present study is to assess the fluoride release/uptake capacities of two fissure sealant materials. The null hypothesis of the current study is that there is no significant difference in fluoride release and uptake capacity between the tested fissure sealant materials.

2. Literature review

2 .1 Fluoride used in medicine

Fluoride is added to public drinking water to prevent tooth decay. Children who do not drink fluorinated public water because their homes use water from a private well often they take fluoride tablets to prevent tooth decay. Fluoride is added to toothpaste and mouthwashes so it can be applied directly on the teeth to prevent tooth decay. Fluoride is also taken by mouth for treating weakened bones (osteoporosis) and for preventing bone loss in people with rheumatoid arthritis and Crohn's disease.[13]

Fluoride used in treating osteoporosis (bone loss) taken by mouth continuously or cyclically (three months on, one month off) might increase bone mineral density, which is an indicator of bone strength. Fluoride seems to work better for improving bone strength in older women when combined with hormone replacement therapy. However, it is not clear whether taking fluoride actually reduces the chance of weak bones breaking.[13]

2.1.1Safty of fluoride

Fluoride is safe for most people in the amounts added to public water supplies used in toothpastes and mouthwashes, or when it applied by dentists on the dental clinic. Low doses (up to 20 mg per day of elemental fluoride) of supplemental fluoride taken by mouth appear to be safe for most people. Higher doses are unsafe and can weaken bones and ligaments and cause muscle weakness and nervous system problems.

High doses of fluoride consumption by children before eruption of their permanent teeth can cause tooth discoloration. Toothpaste and fluoride rinses should not be swallowed routinely, particularly by children. It is recommended that children under six years of age use only a pea-sized toothpaste containing fluoride. Sodium fluoride contains 45% elemental fluoride, while monofluorophosphate contains 19% elemental fluoride[14] .

2.1.2 Precautions and warnings of fluoride

The fluoride seems to be safe during pregnancy and breast-feeding when taken in doses below the tolerable upper intake level (UL) of 10mg per day of elemental fluoride and when applied directly to the teeth in toothpastes and mouthwashes. On other hands higher doses are unsafe and can weaken bones and ligaments, and cause muscle weakness and nervous system problems[13].

2.1.3 Dosing considerations for fluoride

The following doses have been studied in scientific research to prevent tooth decay: in the US, fluoride is added to city water to a concentration of 0.7 to 1.2 parts per million (ppm). To prevent dental caries in areas where the fluoride level in drinking water is less than 0.3ppm (such as in well water), children 6 months to 3 years should receive a fluoride supplement of 0.25 mg per day; children 3 to 6 years, 0.5 mg per day; and children 6 to 16 years 1 mg per day. For children living in areas where the fluoride level is 0.3 to 0.6 ppm, children 3 to 6 years should receive 0.25 mg per day, and children 6 to 16 years, 0.5 mg per day. No supplement is needed in areas where the fluoride in drinking water exceeds 0.6 ppm. For treating weak bones (osteoporosis); 15 to 20 mg per day of elemental fluoride was recommended[13].

The daily Adequate Intakes (AI) for elemental fluoride from all sources including drinking water as following; from infants birth to 6 months 0.01 mg, from 7months to 12 months 0.5 mg, from 1year to 3 years 0.7 mg, from 4years to 8 years 1 mg, from 9 years to13 years 2 mg; from14years to18 years, 3 mg; men 19 years and older 4 mg female from

14 years up including those who are pregnant or breast feeding 3 mg per day.

The daily upper intake levels (UL) for fluoride, at higher level which will not cause harmful effect was 0.7mg for infants birth to 6 months, 0.9 mg for infants 7months to 12 months, where the 1.3 mg for children 1 year to3 years, and the 2.2mg for children 4years to 8 years. While, 10mg of fluoride daily was recommended for a child older than 8 years, adults, pregnant and breast feeding women.[13]

2.1.4 Fluoride role in demineralisation and remineralisation

Dental hard tissues mainly composed of inorganic compound closely resembles calcium hydroxyapatite $Ca_{10} (PO4)_6 (OH)_2$ which has a defined structure. Although the biological apatites resembles the pure calcium hydroxyapatites but still differs in stoichiometry, composition and morphology. Dental apatite is essentially a carbonated apatite, their imperfect crystalline structure allows substitution of many ions and thus changes the solubility product of the apatite.[15]

The inclusion of carbonate and magnesium induces instability. The presence of fluoride improves the crystallinty of the structure.[16] Fluoride competes for hydroxyl ions in hydroxyapatite structure and can either form fluoroapatite or fluorohydroxyapatite, the latter is more likely to form in human enamel. The resulting F-replaced hydroxyapatite has a lower solubility product which is due to its high charge density and its symmetry, more over it reduces the lattice energy and stabilizes the crystal structure.[12]

X-ray diffraction spectroscopy (XRD) has shown that inclusion of fluoride or other trace metals in carbonated apatite resulted in a much better crystalline structure than pure carbonated apatite.[17]

The pre-eruption absorption of fluoride from the tissue fluids and the post eruption inclusion of fluoride from saliva contribute to a higher amount of fluoride in the superficial layer of enamel than the deeper layers.[12]

The presence of fluoride in the solution surrounding the crystals has been found to be more effective in inhibiting demineralization as it travels along with acid and is absorbed on the crystal surface and prevents dissolution of crystals.[4,-6] This process is rather associated with decrease in demineralization than remineralisation as the structure formed is different than the one being replaced.[18]

Fluoride has a very integral role in maintaining the balance between demineralization and remineralisation. After the source i.e. carbohydrates is depleted and saliva neutralizes the acids, the pH of the plaque is restored back to the resting pH. The deficient crystals act as nucleates and attract calcium and phosphate and along with fluoride forms fluoro-hydroxyapatite, which is less susceptible to acid attack compared to carbonatedhydroxyapatite.[4,18]

Thus for remineralisation to take place the presence of calcium, phosphate and fluoride is essential.[19] The shift from demineralization to remineralisation is possible only if the overlying biofilm fluid or the saliva becomes supersaturated with respect to hydroxyapatite. In some studies, a constant low supply of fluoride is recommended for effective remineralisation.[6,15]

Conversely a high clinical dosage of fluoride was favoured as the postulation is that the mineral gain in artificial lesions was found to be dose dependent and likelihood of fluoride surrounding the crystals increases.[20]

2.1.5 Antibacterial properties of fluoride

Numerous studies have established the antimicrobial activity of fluorides.[21-,22-23] However, its anticariogenic property still remains debatable since most of the studies supporting the arguments were performed. Fluoride works in two main ways first inhibiting a wide variety of enzymes second enhancing the proton permeability of cell membranes by forming hydrofluoric acid (HF) which discharges pH across the membrane, and causes acidification of cytoplasm and inhibition of glycolytic enzymes.[24]

Secondary caries has been identified as the one of the major reasons for replacing existing restorations.[25] The formation of bacterial biofilms on all the hard surfaces of the mouth is inevitable. Therefore the need of preventing or minimizing the formation of cariogenic biofilm is also one of the requirements of an ideal restorative material. Several studies have suggested the antibacterial activity of fluoride releasing materials.[21,-23]

It has been postulated that GIC either inhibits the bacterial growth or prevents adherence by an initial outburst of fluoride release and initial low pH of the cement.[26] The high fluoride content of plaque covering ionomeric material was considered responsible for the reduction of enamel demineralisation by interfering with the bacterial metabolism.[27]

The antibacterial property was mainly contributed by the fluoride release, although in a study in the complementary role of other ions has also been highlighted.[23] The percentage of S.Mutans collected from the overlying plaque of restorations from a group of children was found to be more extensive for composites and amalgam than glass ionomer cements[28]. A high fluoride uptake in the enamel and low mutans count on GIC restorations were observed in an in situ study.[29]

The antibacterial activity of GIC is debatable as many studies did not support the antibacterial aspect of GIC.[30,31] One of the studies suggested the action of fluoride to be insignificant in reducing or inhibiting the bacterial growth as the biofilm growth was found to be more dominant on the surfaces of GIC compared with other materials. The antibacterial effect of GIC needs further elucidation. So far the studies have just been able to determine the short term antibacterial potential of GIC and the responsible factors could most likely be the acidity of the initial set or the initial outburst of fluoride release. However, clinically long term antibacterial effect of GIC is desirable.[32]

2.2. Fluoride and restorative materials

The oral cavity acts as a reservoir for fluoride and to maintain a cariostatic environment, a constant supply of topical fluoride is vital, due to the therapeutic effect of fluoride, many oral health care products have been introduced in the market incorporating fluoride as their major constituent.[33]

Restorative dentistry is not exception, the idea of restoring a tooth with added caries prevention has lead to the inclusion of fluoride into dental restoratives either as part of the chemistry or as additive. Fluoride was first used as the main constituent of the glass component of dental silicate cements. However, due to poor physical and mechanical properties of this material was later replaced by glass ionomer cements[33].

The beneficial aspects of glass ionomer are well recognized. It chemically adheres to tooth structure and releases and uptakes fluoride on a continuous basis. Inferior mechanical strength is the main drawback of GlCs and to broaden its application, several modifications have been developed. In some of these materials, the parent compound and chemistry have remained the same, with some modifications which resulted in the resin modified glass ionomer cement, polyacid modified composites and giomers. Attempts have also been made to incorporate fluoride in composites and amalgam. However, fluoride release from these materials found to be decreased with time. GICs are believed to possess the recharge capability affording the long term protection against cariogenic attacks.[33]

2.3 Glass ionomer containing restorative materials

2.3.1 Glass ionomer cements

Glass ionomer (GIC) was discovered to overcome the drawbacks of silicate cements. Wilson and Kent 1972 altered the Al_2O_3/SiO_2 ratio in silicate glass and developed the material which was initially named as aluminosilicate polyacrylate cement (ASPA).[34]

GIC was defined by Crowley et al.,2017[35] as an acid-based cement formed by reacting a polycarboxylate. such as poly acrylic acid or acrylic/maleic acid copolymer with an ion-leachable acid degradable glass of the generic form $SiO_2–Al_2O_3–XF_2$ X where being any bivalent cation in the presence of water to produce a cross linked hydrogel matrix in which the glass-filler phase is embedded.[35]

2.3.2 Composition and setting chemistry of GIC

Since its advent, glass ionomer cement has undergone many changes. However, the basic chemistry has remained the same. The cement basically consists of ion leachable glass particles and poly alkenoic acid and the two components react in the presence of water to yield set cement. The glass formulations which have been widely studied are SiO_2-Al_2O_3-CaO_4 and SiO_2 Al_2O_3-CaF_2.[36]

The glass particles are prepared by fusing alumina, silica, metal oxides and metal fluorides at a very high temperature usually ranging from 1200-1550C^0. To give cement its radiopacity, barium, lanthanum and strontium are also added. The molten mixture is shocked cooled and are grounded to fine particles, the size of which varies according to the clinical usage of the cement.[36]

Fluorine and phosphates are added to the glass composition as they tend to reduce the melting temperatures and enable the material to have better working/setting characteristics. Fluoride acts as a flux and facilitates the breaking of the glass network to make the acid attack easier[37]. Clinically, fluoride lowers the reflactive index, allowing for more aesthetics which are useful for anterior restorations and also provides anti-cariogenicity to the material.[37]

Polyacrylic acid is another essential component of glass ionomer cements. Initially 45% polyacrylic acids were used but were soon discarded due to early gelation and a reduced shelf life. Several variations of polyacrylic acids either as homopolymers and/or its co-polymers like itaconic acid, maleic acid, di- or tri carboxylic acid were introduced to overcome the problems of gelation.[38]

Water is an indispensible component of glass ionomer cement. The acid-base reaction requires an aqueous medium for the initiation of the setting process. Water breaks the internal hydrogen bonding for acidic carboxylic groups and facilitates their reaction with glass particles.[39] Tartaric acid is also added to the cement formulation as a rate controlling additive. Being a stronger acid, it reacts with the glass particles and forms stable metal ion complexes which allow an increase in the working time and a reduction in the setting time.[38]

The setting of glass ionomer cement is initiated as soon as the acid reacts with basic glass particles in the presence of water leading to the formation of polysalts. However, the reaction is not as simple and it can be divided into three stages. The first stage involves dissolution in which the protons from acid react with the outer surface of glass particles. This causes the leaching of many non-network and network forming ions which are mainly Ca^{+2} and Al^{+3}. Tartaric acid at this stage reacts with glass and prevents the premature formation of Ca-acrlyate salts thus prolonging the working time. The preferential sites for acid attack are usually the(Ca) rich ones as they are believed to be more basic in nature.[36]

Dissolution is followed by gelation. This initial setting takes place due to weak ionic cross linking between the carboxyl groups and released (Ca^{+2})and(Al^{+3}) ions, which also contributes to the viscoelastic behaviour of the freshly set material. In the last phase of hardening, the formation of Al-polyacrylates replaces Ca-acrylate salts and enables the material to acquire strength and rigidity. The material gains its final strength after 24-48 hours which may continue for several months.[38]

2.3.3 Resin modified glass ionomer

Resin modified glass ionomer cements (RMGIC) were developed to control the early moisture sensitivity of conventional glass ionomer cements. Resin modified materials share the chemistry between conventional glass ionomer cements and composites as the material is modified by resin and at the same time it retains the characteristics of GIC.[40] It contains a resin component from composite resin and ion leachable glass from GIC to optimize the useful properties of the two materials. RMGICs have been able to overcome the problem of moisture sensitivity and are believed to have better aesthetics and strength than conventional GICs. RMGICs also share the fluoride release/uptake and chemical adhesion characteristics of conventional GICs. However, resin addition makes it prone to polymerization shrinkage.[38]

2.3.4. Composition and setting chemistry of RMGIC

The basic components are similar to conventional glass ionomer i.e. fluoroaluminosilicate glass, polyacrylic acid and water. However, it contains an additional resin component, 2-Hydroxyethyl Methacrylate (HEMA). Different methods have been employed for the production of RMGICs. The interpenetrating network of resin matrix with GIC matrix is achieved just by simply adding up the two components along with the photoinitiators.[41] In the other method, polyacid is modified partially by attaching a polymerizable group.[42] RMGICs are light activated materials, whilst still retaining the acid base reaction which remains an integral part of its setting chemistry.[43]

The light activated polymerization predominates the setting mechanism of RMGICs, where as the acid-base reaction starts after 4 minutes of mixing.[44] The acid base reaction starts as soon the material is mixed or being sensitive to ambient light, the polymerization can be initiated with dental operating lights and this explains the short working time of these materials.[40]

2.3.5 Polyacid modified composites / Compomer

The word “Compomer” was derived from composites and glass ionomer, since its chemistry shares a close proximity with composites. Hence, “polyacid modified composites” is a more suitable term for these materials. The material is available as a single paste system as the mixing time has been eliminated and requires a primer for its bonding. The material primarily contains all the components of composite resin. In addition, it contains dimethyacrylate monomer with carboxylic groups and strontium.[42]

2.3.6 Giomer

Giomer is a relatively newer material in field of tooth coloured materials which contains a unique component of pre-reacted glass ionomer particles (PRG). This material is a hybrid of glass ionomer and composite resin. In this technology the glass particles are reacted with polyacid in an aqueous medium to produce siliceous hydrogel, which is freeze dried, ground milled and silanized to produce PRGs.[45]

The resin matrix consists of hydroxyl ethyl methacrylate (HEMA) and urethane dimethyl acrylate (UEDMA). The polyacid is completely eliminated as the particles have already been reacted. Hence, water sorption is not crucial for the initiation of an acid base reaction. Giomers are further divided based on fully pre reacted glass particles (F-PRG) and surface pre reacted particles (S-PRG).[46]

2.4 History of pit and fissure sealants

Many efforts were carried out to prevent caries at deep pits and fissures, such as enamel fissure eradication (fissurotomy) by making the fissures more wide which not allow the food debris accumulation and become self-cleansable. The ammoniacal silver nitrate also was used to treat the deep pits and fissures. However, none of these methods were had a great success. Hyatt in 1923 introduced a new method by prepare the deep pit and fissures as cavity class-I which then filled with prophylactic restoration, this technique continues as a treatment option in such case until the 1970.[47]

In 1955, Buonocore introduced a new technique of bonding of acrylic resin to previously etched dental enamel. The technique was used 85% phosphoric acid for 30s, as a tool to increase the adhesion of self-curing methyl methacrylate resin materials to dental enamel. This study was actually the beginning of a revolution in dental clinical practice but this method was not widely used until 1970.[47] Buonocore made additional advances technique in pit and fissure sealant, displayed the successful use of BIS-GMA resin with the use of ultraviolet light cure.[48]

2.5 Pit and Fissure Sealant Materials:

Sealants are classified into three sealant materials. The predominant types of sealant materials in the market at present are resin-based sealants and glass ionomer cement-based sealants.[49] Three types of polymeric materials have been used as pit and fissure sealants such as cyanoacrylates, polyurethanes and bisphenol-A glycidyl methylacrylate (Bis-GMA). The first clinical adhesive sealing using acid etching was methy l-2-cyano-acrylate monomer with filler to seal pits and fissures of posterior teeth. This technique was soon proved unsatisfactory because the cyanoacrylates disintegrated after a slightly longer time.[50]

The polyurethanes proved to be too soft and totally disintegrated in the mouth after 2 to 3 months. Despite this problem, their use was continued for some time – not as a sealant but as a vehicle with which to apply fluoride to the teeth.[51] Dimethacrylates is the reaction product of bisphenol A and glycidyl methacrylate (Bis-GMA), which is a hybrid between a methacrylate and an epoxy resin. The most commercial sealants today are Bis-GMA. They were first created by Bowen in 1962, although the first fissure sealant based on Bis-GMA was introduced to the profession in 1971 under the trade name Nuvaseal.[51]

Commercially available sealants may are free of inert fillers or are semi-filled, and whether they are clear, colored, or opaque. The main different between these types of sealants is in their initiation of polymerization and source used. The first marketed sealants, called first-generation sealants, were activated with an ultraviolet light source and they are not market today. The second generation sealants are self curing material and set upon mixing with a chemical catalyst accelerator system. The third generation sealants are photo-initiated with visible light.[50,52]

2.6 Ratio of pit and fissure sealants to tooth surface

The caries can be developed in smooth surfaces of tooth even with use of pits and fissures sealants. Although the occlusal surfaces represented only 12.5 % of the total surfaces of the permanent dentition, they found to be the most surfaces of caries incidence in 50 % of the caries in school children.[52] This can be explained by the fact that enamel forming pits and fissures do not receive the same level of caries protection from fluoride as smooth surface enamel. Resin sealants are the most widely used preventive materials and also have great evidence of effectiveness.[53]

The efficiency of fissure sealants depends on fluoridated and non-fluoridated areas, as part of public health measures. Studies by Brown et al.,1996[54] and Kaste et al.,1996[55] showed that in fluoridated communities over 90% of dental caries was pit and fissure caries, interproximal caries was reduced by 25 %, whereas pit and fissure caries decreased by 18 % only. They found that the fluoride was less effective in pit and fissure caries due to total depth of enamel on smooth surfaces compared with that in underlying the fissure. The base of an occlusal fissure can be close to or within the underlying dentine, therefore the progress of lesion is higher along the enamel-dentine interface, while fluoride takes time to increase demineralization rate to compensate enamel lose. On the other hand, fluoride ions have enough time to positively affect the demineralization process in a smooth proximal surface, where the thickness of enamel is approximately 1mm.[50]

2.7 Types of pit and fissure Sealants

Pit and fissure sealants were recommended to be caries preventive as long as they remained adherent to the teeth surfaces, the initial evaluation of sealant effectiveness by clinical trials comparing sealant treated and non-treated teeth was considered unethical. Clinical retention and prolonged existence became the measure of sealant success for prevent tooth surfaces caries.[47]

2.7.1 Generations of pit and fissure sealants

2.7.1.1 Resin based sealants

There are four generations from Resin-based sealants (RBS), determined by the method of polymerization:

- The first generation of RBS was polymerized by the action of ultraviolet rays on the initiators in the material that initiate polymerization currently this type is no longer used, an example of first generation is Nuva-Seal® (LD. Caulk Co.: Milford, DE, USA), a resin-based sealant polymerized by an ultraviolet light source. [47]
- The second generation was the auto-polymerizing resin-based sealants (ARBS) or chemically-cured sealants; tertiary amine (the

activator) is added to one component and mixed with another component. The reaction between these two components produces free radicals that initiate the polymerization of the resin sealant material. This type currently replaced by the third generation.[47]

- Third generations are the visible light activates by photoinitiators that are present in the sealant material and are sensitive to visible light in the wavelength region of around 470 nm (blue region). On comparing this visible light polymerizing to its previous generation, the autopolymerizing resin-based sealant, sets in a shorter time, from 10 to 20 seconds, compared to the 1 to 2 minutes setting time of autopolymerizing resin-based sealants. The working time is longer and the material does not set until exposure to the polymerizing light. Through the elimination of the mixing step, fewer air bubbles are incorporated with the sealant application.[47]
- The fourth generation is the fluoride-releasing resin-based sealants. Fluoride resin-based sealant is the product resulting from adding fluoride-releasing particles to light activated sealant in an attempt to inhibit caries.[47]

2.7.1.2 Glass ionomer cement (GIC) as pit and fissure sealants:

The use of GIC as a pit and fissure sealant was introduced more than 30 years ago. GICs have been recommended as an alternative to resins for sealing primary teeth.[56] Study by Yamamoto *et al.*,2003 [57] evaluating of use the GICs as a fissure sealant indicate a significant lower retention rates than resin-based pit and fissure sealants.

Overbo and Raadal 1990[58], comparing the degree of microleakage that occurred in GIC pit and fissure sealants and a diluted composite fissure sealant, concluded that wide leakage occurred in the GIC throughout the material, and at the margin of the cement and the enamel.

Birkenfeld and Schulman 1999[59] concluded that etching prior to application of GIC enhances the bonding to fissure enamel. Although GICS with their ability to release fluoride and adhere to enamel were initially worthy of consideration[60], clinical trials related to their effectiveness discouraged their use as pit and fissure sealants.[61] The use of GIC has been suggested for erupting teeth, where isolation from saliva is a problem.[62]

2.8 Caries prevention with pit and fissure sealants

The pit and fissure sealants found to be inhibiting the dental caries. Romcke et al, 1990 [63] evaluated more than 8000 sealed surfaces, were 58-63 % for 7 to 9 years and 41 % at 10 years. They reported that of 96 % for the first year free from caries and 85 % after 8-10 years free of caries.

Simonsen, 1991[64] conducted a follow-up clinical study on sealant retention and effectiveness in children who received a single application of a white-coloured self-cured sealant for 15 years, 74% of the pit and fissure surfaces of permanent first molars were free of caries.

Chestnutt *et al.*,1994 [65] studied more than 7000 sealants surfaces follow-up for 4 years were 57% of the sealed tooth surfaces remained fully sealed with 18% scored as deficient or failed and 24% completely missed. While, a total of 23% of the surfaces originally scored as deficient at baseline were scored as carious compared with 21% of surfaces not sealed. Only 14.4% of the sound/sealed surfaces at base line became carious.

Wendt *et al.,2001*[66] conducted along study on first and second permanent molars. Were reported that 95% complete or partial retention without caries in second permanent molars after 15 years and 87% complete or partial retention without caries in first permanent molars after 20 years.

2.9 Factors effect on retention of sealants:

The retention and caries-preventive effects of pit and fissure sealants have been well documented in the literatures. There is good evidence that teeth sealed very early after eruption require more frequent re-application of the sealant, than teeth sealed later.[67,68] Therefore, sealant placement may be delayed until the teeth are fully erupted, unless high caries activity is present. Sealant placement even in the absence of regular follow-up is beneficial.[65]

Conventional sealant procedure including many steps including; etching, rinsing, and drying, followed by the application of the sealant and the exposure to the curing light. These steps are time consuming and increasing the risk of saliva contamination. The contamination after etching may have harmful effects on bonding and retention mechanisms.[69] The partial loss of material and/or micro-leakage lead to gap formation may result in the formation of secondary caries around the sealed fissure. The incidence of caries in sealed teeth found to be approximately 2-4 %.[70] There are many important factors for fissure sealant retention such as method of prophylaxis before sealant application, moisture control, use of etching gel or liquid, etching time, washing and drying times, as well as fissure sealant application it self.[71]

2.9.1 Surface cleaning

The cleaning step of tooth surface prior to sealant placement is debated in the literature. Usually, acid etching alone is sufficient for surface cleaning.[70] This is proved by Simonsen,[64] the most effective sealant longevity studies were accomplished without use of a prior prophylaxis. Moreover, treatment with fluoride before etching has been proposed to strengthen the enamel by reducing its solubility. However, no significant differences were observed in bond strengths in vitro following the use of non-fluoridated or fluoridated pastes, a pumice slurry or water and bristle brush.[71] The use of prophy-pastes, especially those with fluoride, has been dejected were pumice particles become lodged in the fissures and were not removed after rinsing with a stream of water.[70]

Two clinical trials revealed similar retention rates between cleaning the debris of fissures with a prophy brush and pumice or gently running a probe and toothpaste, respectively. Air polishing of the occlusal surface with special devices has been suggested.[72] In vitro studies with air polishing of the occlusal surface before acid etching demonstrated greater penetration, a greater number of resin tags for micromechanical retention, and higher bond strengths than fissures cleaned with rotary instrumentation and pumice.[72]

New technique for caries removal and cavity preparation has been introduced, i.e. laser irradiation. Lasers with a wide range of characteristics are available today and are being used in several fields of dentistry. Laser energy is absorbed by the dental enamel, promoting superficial modification, which may have clinical significance.[73-74]

Do Rego and De Araujo1999[75] evaluated the microleakage scores of two sealants a fluoride resin-filled sealant and resin-modified GIC as pit and fissure sealants, on different surface preparations. The author reported that occlusal surfaces treated exclusively by a very short pulsed Er:YAG laser (120 mJ at a frequency of 4 Hz under air-water spray for 30 s) showed poorer marginal sealing than those treated by acid etching alone. Whatever the cleaning preferences, either by acid etching or other methods, all heavy stains, deposits, and debris should be removed from the occlusal surface before applying the sealant.

2.9.2 Tooth surface isolation

Adequate isolation of tooth surface during application of sealant is the most critical aspect of sealant application.[70] Saliva contamination during or after acid etching allows rapid precipitation of glycoproteins onto prepared surface, significantly decreasing bond strength.[76]

Silverstone et al.,1985[77] and Tandon et al.,1989[78] suggested that even one second exposure to saliva can lead to the formation of protein layer resistant to 30 seconds of vigorous irrigation, they reported that it was necessary to repeat the etching procedure to ensure adequate bonding of a resin material. There are two isolation methods to prevent saliva contamination; rubber dam or cotton roll isolation. The rubber dam isolation and cotton roll isolation provide comparable retention rates.

Lygidakis et al.,1994[79] found that after 4 years of application the complete retention rate was 91% for sealants placed using rubber dam isolation and 81% for sealants placed using cotton roll isolation. Rubber dam isolation found to be ideal but may not be feasible in certain circumstances.

Foreman and Matis1992[80] conducted that clinical study using Vac-Ejector moisture control, another alternative isolation method, and they concluded that sealant retention was comparable to that with sealant placed under rubber dam or cotton roll isolation.

Another study indicated that the applying a halogenated bonding agent after acid etching significantly increased the bond strensgth of sealant to saliva contaminated enamel and also to uncontaminated enamel.[80] Furthermore, it was found that sealants placed soon after tooth eruption is far more likely to need replacement. Also tooth position in the mouth appears to be an important factor for adequate isolation.[81] Sealant retention had been found to be superior for the more anteriorly placed teeth. Sealants have been recorded as being more effectively retained on lower teeth than on upper teeth.[50] The cooperation of the patient, the skill of the operator, and the presence or absence of a dental assistant, altogether are important factors affecting sealant retention.[82]

2.9.3 Etchants and Conditioners:

The purpose of using etching is to produce an uncontaminated and clean frosted surface. Many acids were used such as phosphoric, maleic, nitric, or citric acid, with commercial dentine adhesive systems for partial or total removal of the smear layer and superficial demineralisation of the underlying dentine. Such liquids or gels are termed etchants and may also be called conditioners by some dental manufacturers.[50]

Etching shows the dissolution of the substrate, whereas conditioning involves cleaning, structural alteration, and increasing the adhesiveness of the substrate. Resin based fissure sealants are usually placed after cleansing and orthophosphoric acid etching of the fissure enamel.[83]

Orthophosphoric acid is the most regularly acid used, its concentration lies between 30 and 50% by weight, small variations in the concentration do not appear to affect the quality of the etched surface.[61]

Orthophosphoric acid 36% is available in two form liquid solution and a gel.[84] it found similar penetration rate to enamel in gel or liquid form. Another study showed that gel etchant was as effective as the liquid form. However, the gel form require double rinsing time than liquid form.[85] Many clinicians prefer to use gel form because it is easily to apply and control where their colour, easy to detect where it has been applied.[86]

Exposed time during etching is very important. In-vitro study using permanent teeth showed that resin-to-enamel bond strengths after 15-seconds was comparable to those after 30 and 60 seconds etching time.[87]

Duggal et al1997[88] found no significant difference in retention of pit and fissure sealants after 1 year follow-up on second primary and first permanent molars when 15, 30, 45 or 60 seconds etching times were used. It has been suggested that washing for 60 seconds if an etchant in solution is used and 90 seconds when a gel etchant has been applied.[50] Exact washing and drying times are not as important as ensuring that both the washing and drying of the tooth are thorough enough to remove all of the etchant from the surface and give a chalky, frosted appearance.[61]

Maleic acid included in resin primers used with the development of self-etching primers. These primers are acidic enough to demineralize the smear layer and the very top of the intact underlying dentine. As they etch, they also infiltrate the exposed collagen with hydrophilic monomers, which then copolymerize with the subsequently placed adhesive resin. These primed surfaces are not rinsed with water, leaving solubilised mineral to re-precipitate within the diffusion channels created by the acid primers.[89]

2.10 Fluoride and Pit and Fissure Sealants:

The difference in caries activity between smooth and pit and fissure surfaces becomes more noticeable and the dental caries is becoming mainly a disease of the pits and fissures. Pit and fissure sealants are recognized as the clinical materials for preventing occlusal caries.[66] To increase the effectiveness of sealants against dental caries the fluoride was included in sealants materials and several kinds of fluoride fissure sealants have been developed over the years.[89] The addition of fluoride to pit and fissure sealants was considered more than 30 years ago[90] But were not found to reduce caries incidence this may be due to poorly retained on the tooth surface.

2.10.1 Methods of addition fluoride into pit and fissure sealants:

Fluoride is added into sealants using two ways; the first utilizes a soluble fluoride salt which, after application, dissolve the released fluoride ions, this may effect on the resin properties[52]. The fluoride release may result in the dissolution of a soluble salt which weaken the sealant in situ and thereby might reduce its usefulness as a preventive agent.

The second method uses an organic fluoride through which fluoride ions are released by an ion exchange mechanism. In this method fluoride release will be only at a small amount from the total structure, and is replaced rather than lost. Therefore it will not deteriorate the sealant properties[50].

2.11 Methods of assessing fluoride release:

Fluoride, either from environmental or biological samples can be detected by numerous methods. The extensive methods employed in fluoride detection are beyond the scope of this literature review, only the techniques used in dentistry for the detection of liquid samples will be discussed.

Fluoride in biological samples exist as inorganic form which can be further divided into ionic (uncomplexed fluoride) and non-ionic (complexed fluoride) forms. Fluoride is usually present in biological fluids at trace levels and care should be taken to use a technique which offers lower detection limits and sensitivity for precision and accuracy.[91]

The techniques used in dentistry included potentiometry (Ion Selective Electrode) and chromatography (Ion and Gas chromatography).
The potentiometric method or ion selective electrode (ISE) is widely used in dentistry for fluoride detection.[92] It consists of a probe or electrode which is selective for each ion to be analyzed, a meter and a buffer (Total Ionic Strength Adjustment Buffer, TISAB). The buffer is to be mixed with the samples and its function is to decomplex the ions, provide a constant background and balanced pH. ISE offers many advantages over other methods, including low cost, ease of use and accuracy. The minimum detection limit of ISE is 0.02 ppm.

Ion chromatography (IC) is another method used in dentistry for fluoride detection.[93-94] This method is expensive, time consuming and technique sensitive. However, it offers lower detection limits, better accuracy and precision. IC enables the measurement of fluoride at ultra trace levels i.e. ppb whereas ISE's minimum detection limits is at ppm level. IC also allows the detection of free fluoride whilst ISE enables the detection of total amount of fluoride i.e. complex and uncomplex due to the interaction of TISAB.[93]

2.11.1 Spectrophotometer analysis:

Spectrophotometer is an instrument that determines how much light of a particular colour is absorbed by a liquid sample. The more there is a coloured substance in the solution, the more light will be absorbed (i.e. the less light passes through the solution). After measuring how much light is absorbed by a series of solutions containing known concentrations of the coloured substance, you can draw a graph of this data and use it to calculate the concentration of that substance in an unknown sample from a measurement of how much light it absorbs.

2.11.1.1 Spectrophotometer SPANDS method:

This method relies on the fact that when fluoride reacts with certain zirconium dyes, a colourless complex of an ion and a dye is formed. The complex tends to bleach the dye which therefore becomes progressively lighter as the fluoride concentration increases.[95]a white light from a bulb (source) is focused into a narrow beam by passing it through a thin slit.

A prism is used to split the beam of white light into its component colours, in the same way that water droplets can split sunlight into its component colours to make a rainbow. Different colours of light have a different wavelength. A second thin slit, just after the prism, can be moved from side to side to select just one colour of light to pass through the sample. The light passes through a container with the liquid sample inside (usually the light passes through 1cm thickness of the liquid). A light detector measures amount of the light is transmitted through the sample, and compares this with how much light was emitted by the source.

The difference between these values gives a measure of how much light was absorbed by the sample. Which in turn gives an indication of fluoride content in the sample. This method is more accurate but is rare used in dentistry[96]. This method will be new method used for determine of fluoride content in restorative materials due to the fact that it is easy to use and less cost than other methods have been used before.

2.12 Recharge of pit and fissure sealants by Fluoride

The recharging capability of glass ionomer cements was first identified by Walls.[97] Since then several studies have shown the recharge potential of glass ionomer based materials i.e. the capability to absorb fluoride from its surrounding and re-release it. Glass ionomer protects the tooth mainly due to its fluoride releasing property. In order to have a long term protection against cariogenic challenge these materials should posses a constant fluoride release mechanism.

The fluoride release from glass ionomer based materials tends to taper off after a certain time period. Since the optimum amount of fluoride required for its protective action is yet to be determined[97]The need for constant recharge becomes mandatory to resist ariogenic challenges. It has also been suggested that material selection for high risk patients should be based on the fluoride release/uptake.[98]

During orthodontic treatment, white spot lesions are commonly encountered around brackets. Glass ionomer based materials are widely used as a bonding cement to prevent early caries attack in such patients due to their fluoride release and uptake property.[99]

The oral cavity is regularly exposed to fluoride in the form of mouthwashes, dentifrices, and drinking water. In addition, the clinical sources of fluoride include the fluoridated gels and varnishes. These dentifrices and clinical fluoride applications contains high amount of fluoride which can act as the recharge sources for the GIC based materials.

Several studies have been conducted to prove and enhance the recharge capability of these materials; using either commercially available products or laboratory prepared solutions containing the amount of fluoride present in commercial products.[100-101]

Research objective

This study aimed to evaluate the amount of fluoride ions release from two commercially available pit and fissure sealant materials immersion into simulated body fluid and incubate at ± 37^0C. The collected solutions were measured for fluoride content using special indicator of zirconium-SPADNS method based on the effect of fluoride ion on the colour system (Zr-SPADNS), read by spectrophotometric method. The tested materials were divided into two groups.

Group1: Transeal F, light cured fluoride releasing materials (opaque white sealant).

Group2: Conseal F, light cured fluoride releasing materials.

The specific objectives were as following:

1. Evaluation the fluoride ions release in interval day at 1st, 7th, 14th, 21st and 28th day.
2. Evaluation of recharging procedure of fluoride by application of homecare regime (toothpaste and mouthwash) at interval day 1st , 7th and 14th day.

3. Material and Methods

3.1 Materials

Two groups of pit and fissure sealants materials were evaluated in this study; **Group1:**Transeal F, light cured fluoride releasing materials (opaque white sealant) manufactured by NEXOBIO –Korea. (Figure3.1A)

Group2: Conseal F, light cured fluoride releasing materials manufactured by SDI limited-Australia. (Figure3.1B).

Figure 3.1: A. Transeal F, light cured fluoride releasing materials
B. Conseal F, light cured fluoride releasing materials

3.2 Method

3.2.1 Sample preparation

The specimens were prepared using stainless steels mould (Figure3.2). The mould were sandwiched between transparent matrix strip and glass slab[102] (Figure3.3). Eighty specimens measuring 5 mm diameter and 2mm thickness were prepared from two tested materials (n = 80).

Samples were came into contraye (serang) which packed into the mould until overfilled after that put 100 g loading over the top glass slab to extrude the excess material (Figure 3.4), then exposed each sample by light cure for 10 secends (.Figure3.5) .as recommended by the manufactures.

3.2. 2 Fluoride release evaluation

A total of eighty specimens were prepared from (GI) Transeal- F, and (GII**)** Conseal- F (n=40 for each group), then sub divided into five samples (n=5) for each tested period. The amount of fluoride released was measured during 1st ,7th ,14th ,21st and 28th days. The prepared specimens were kept in sterile plastic containers contained 20ml of artificial saliva (simulated artificial saliva- Ringer's solution manufactured by Gulf Inject LLC –Dubai UA) and incubated at 37° C to simulate mouth temperature using incubator (Figures 3.6 and 3. 7). (According to method quoted from Basso et al.,2001[103]). Solutions at the end of day1, day7, day14, day 21 and day28 were collected and fluoride release was measured as following;

Measuring of fluoride release into artificial saliva using special indicator (SPANDS Reagent for Fluoride Release USA) (Figure3.8). The zirconium-SPADNS method for the determination of fluoride based on the effect of fluoride ion on the colour system (Zr-SPADNS), were adapted to the continuous flow spectrophotometry. Fluoride analysis kindly carried out at analytical laboratory at Butraba water distillation plant - Tukara city – Libya.

A manifold consisting of two lines was used. The decrease in the absorbance of Zr-SPADNS reagent at 590 nm is linear with fluoride concentration in the range of 0.00-3.5 ppm. The method has a detection limit of 0.02 ppm fluoride. the precision was about 1% relative standard deviation. The method was applied for the determination of fluoride in natural water samples (Dilution coefficient = sample size +dilution size divided by sample size). Dilution of the extract by distilled water 1:10, the amounts of fluoride leached in the water were determined by spectrophotometer (Figure3.9). A calibration curve was produced with the values of the known standards from which the values of test samples were calculated. samples analysis for each specimens was done three times and the mean value was taken to obtain accurate results.

3.2.3 Recharging of the fluoride (fluoride Re-release)

The method of evaluation the amount of fluoride release after recharged was quoted from Bansal,2015[104] as following; After 28thday initial fluoride release analysis, the samples of each group were divided into three subgroups (SG) (five samples each). Subgroup-A (SG-A), control group no topical fluoride application. Subgroup-B (SG-B), the samples were exposed to mouth wash (potassium nitrate fluoride, Sensi Kin- Figure 3.10), the samples were immersed in the solution for 4 minutes and washed with copious of distal water for 10 sec and dried on absorbent paper). SubgroupC, the samples were hand brushed with fluoride toothpaste 1450 ppm (Sensodyne pro-enamel-France. Figure 3.11). The toothpaste applied for four minutes and then wiped clean with absorbent paper and rinsed for 10 sec using distal water and dried. Each sample after fluoride application was suspended in plastic bottles containing 20 ml of artificial saliva and incubated at 37°C through period of 1st, 7th and 14th day. The collected data were tabulated then the mean and standard deviation values were estimated for each tested group.

Figure 3.2: Stainless steel mould

Figure3.3: The mould filled with material

Figure3.4:put of 100g load over top glass slap until complete setting of material

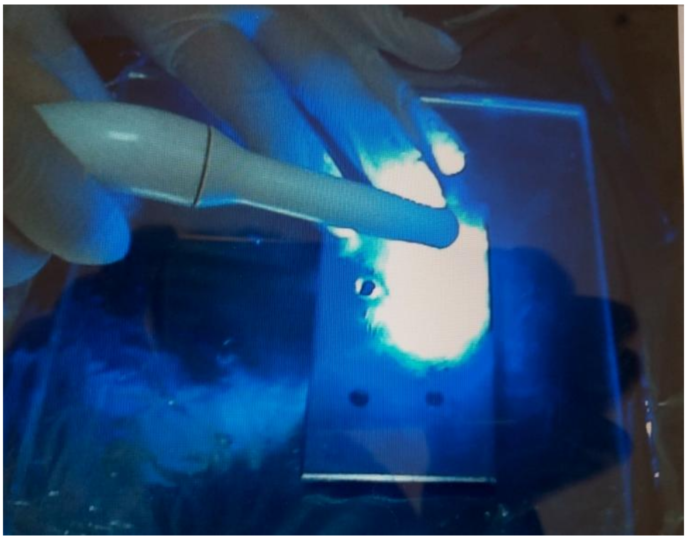

Figure 3.5: curing the sample in the mould

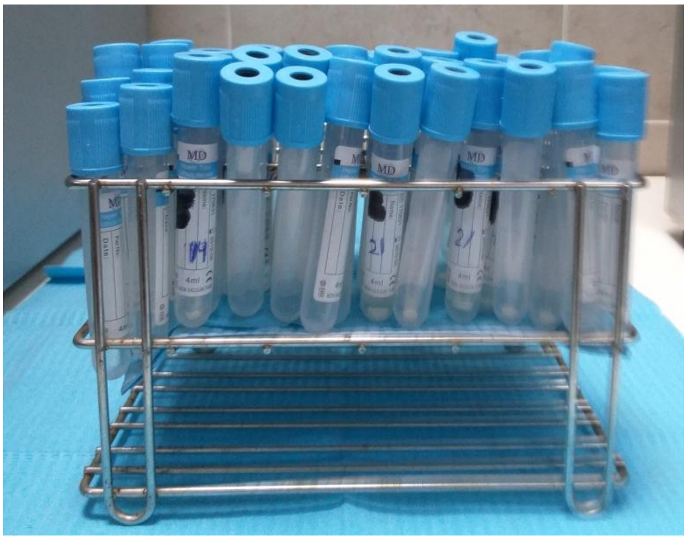

Figure 3.6: Samples kept in plastic container filled with artificial saliva.

Figure 3.7: Samples inside the incubator

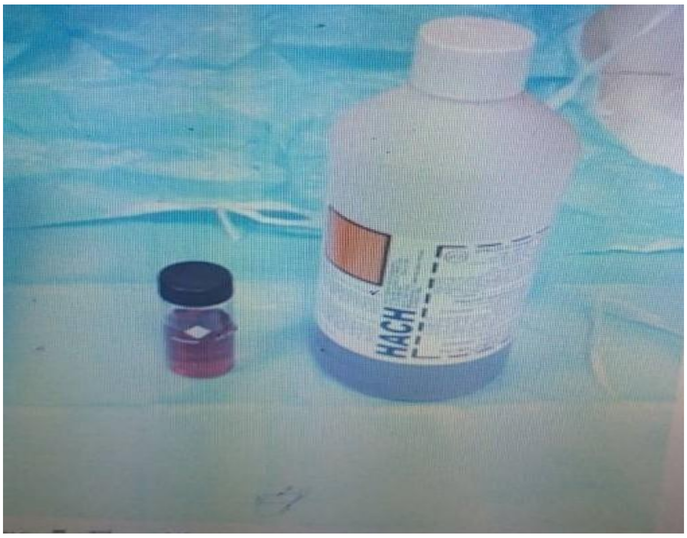

Figure 3.8:Fluoride indicator SPANDS Reagent for Fluoride release USA.

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Figure 3.9:Spectrophotometer apparatus .

Figure 3.10:MouthwashNaF0.3

Figure 3.11:ToothpasteNaF1450
SensodynePro-Enamel

4. Results

4.1Evaluation of Fluoride released

The results of fluoride release from pit and fissure sealant materials (Transeal-F and Conseal-F) at first day are shown in Table 4.1 and Fig 4.1. The Transeal-F group shows slightly higher fluoride release than Conseal-F ,statistically analyses observe significant difference between two groups (*P*<0.008). Table 4.2, shows the amount of fluoride released at 7th day. The fluoride released from Conseal-F group was higher than that of 1st day, while,Transeal-F showed lower fluoride released. In comparison between two groups at seven day of immersion the amount of fluoride released was different from first day where the Conseal-F group showed higher amount of fluoride (1.226 ppm) compared to Transeal-F group (0.618 ppm)(Fig.4.2), statistically there was significant variance between two groups (*P*< 0.038).

Table 4.1: The amount of fluoride release at 1st day of immersion.

Material	Ppm	P –value
Transeal-F	0.774 ± 0.14	0.008
Conseal-F	0.522 ± 0.08	

Table 4.2: The amount of fluoride release at 7th day of immersion.

Material	Ppm	P –value
Transeal F	0.618 ± 0.32	0.038
Conseal F	1.226 ± 0.44	

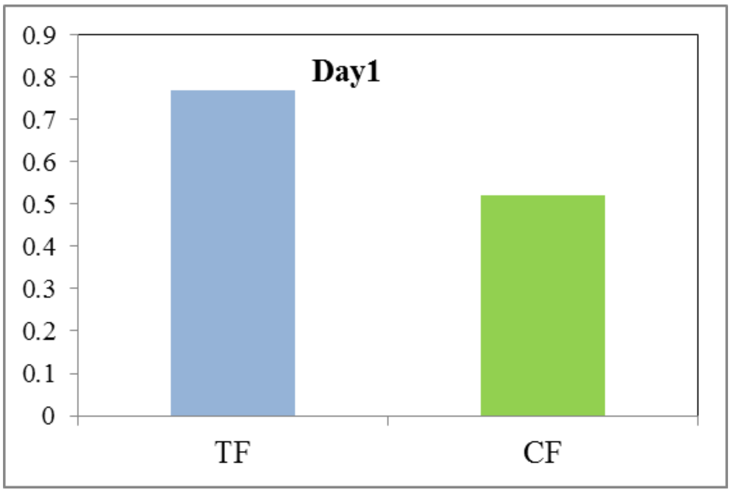

Figure 4.1: the mount of fluoride released at 1st day.

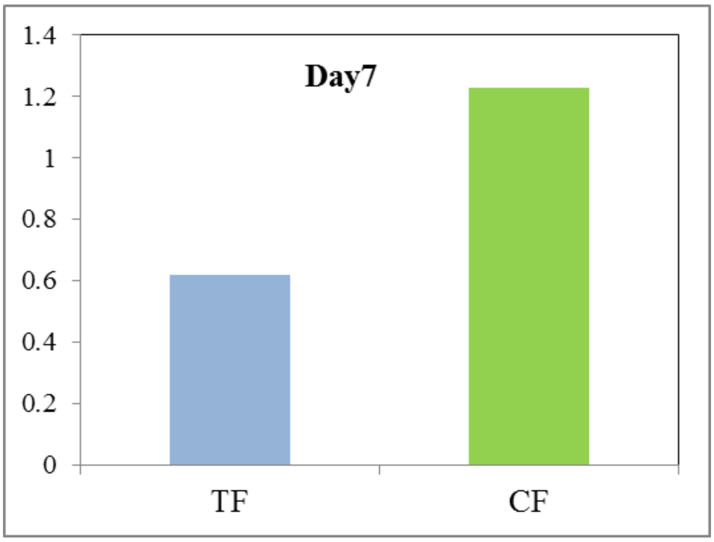

Figure 4.2: the mount of fluoride released at 7thday.

Table 4.3 and Fig .4.3 they show the amount of fluoride released at 14th day, the Transeal-F group showed slightly higher fluoride release (0.888 ppm) compared to Conseal-F. ,Transeal-F group (0.512 ppm), where found to be significant difference between two groups ($P < 0.012$). In comparison with seven day, the fluoride released from Transeal-F groups was higher than that of 7th day, while Conseal-F showed lower fluoride released.

Table 4.4 and Fig 4.4 they show the amount of fluoride released at 21st day. It can be seen that there was absence of differences between two tested groups ($P < 0.065$). In contrast with day fourteen, the Conseal-F group showed slightly higher value (1.766 ppm) than that of Transeal-F group (1.032 ppm). However, the amounts of fluoride released from both groups were higher than 1st, 7th and 14th days of immersion.

The fluoride released at day 28th display in Table 4.5and Fig 4.5, the Conseal-F group showed slightly higher fluoride release (0.888 ppm) compared to. ,Transeal-F group (0.512 ppm), where found to be significant difference between two groups ($P < 0.012$).

In comparison with seven day, the fluoride released from Transeal-F groups was higher than that of 7th day while Conseal-F showed lower fluoride released wed slightly higher value (0.332 ppm) than that of Transeal-F group (0.174 ppm). However, there was no significant difference between two tested groups ($P < 0.547$). It can be seen that the amount of fluoride released at 28th day was the lower observed amount in both groups at all days of immersion.

The amounts of fluoride released from Transeal-F group and Conseal-F group from day 1 to day 28 are illustrated in Table 4.6 and

Fig4.6. It can be seen that the fluoride released from Transeal-F group shows slightly decrease at day seven then increase gradually from day fourteen to day twenty one then clear decrease at day twenty eight. The higher amount of fluoride released was at 21st day (1.032 ppm) while day 28 depicted the lower amount of fluoride released (0.174 ppm). While, Conseal-F group shows amounts of fluoride released from day 1 to day 28found to be increased at day seven then decreased at day fourteen then increased at day twenty one, finally decrease at day twenty eight. Same as Transeal-F group the higher amount of fluoride released was at 21st day (1.766 ppm) while day 28 depicted the lower amount of fluoride released (0.332).

Table 4.3: The amount of fluoride release at 14th day of immersion

Material	ppm	P –value
Transeal-F	0.888± 0.21	0.012
Conseal-F	0.512± 0.15	

Table 4.4: The amount of fluoride release at 21th day of immersion.

Material	Ppm	P –value
Transeal-F	1.032± .33	0.065
Conseal-F	1.766± .70	

Table 4.5: The amount of fluoride release at 28th day of immersion.

Material		P –value
Transeal-F	0.174 ± .08	0.547
Conseal-F	0332 ± 0.56	

Table 4. 6: The amount of fluoride released from day 1 to day 28 of immersion in ppm.

Material	Day 1	Day 7	Day 14	Day21	Day28
Transeal-F	0.774 ±0.14	0.618 ±0.32	.888 ±0.21	1.032 ± 0.33	0.174 ± 0.08
Conseal-F	0.522 ±0.08	1.226 ±0.44	0.512 ± 0.15	1.766 ± 0.70	0.332 ± 0.56

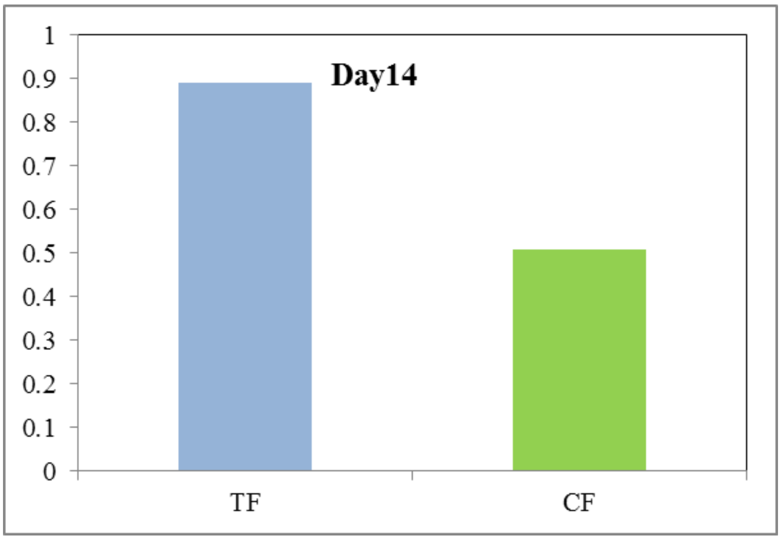

Figure 4.3: the mount of fluoride released at 14^{tht} day.

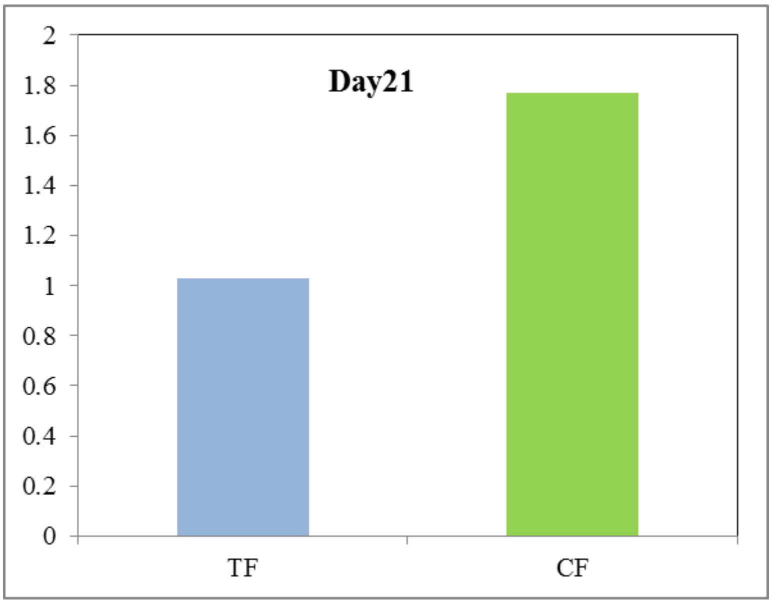

Figure 4.4: the mount of fluoride released at 21st day.

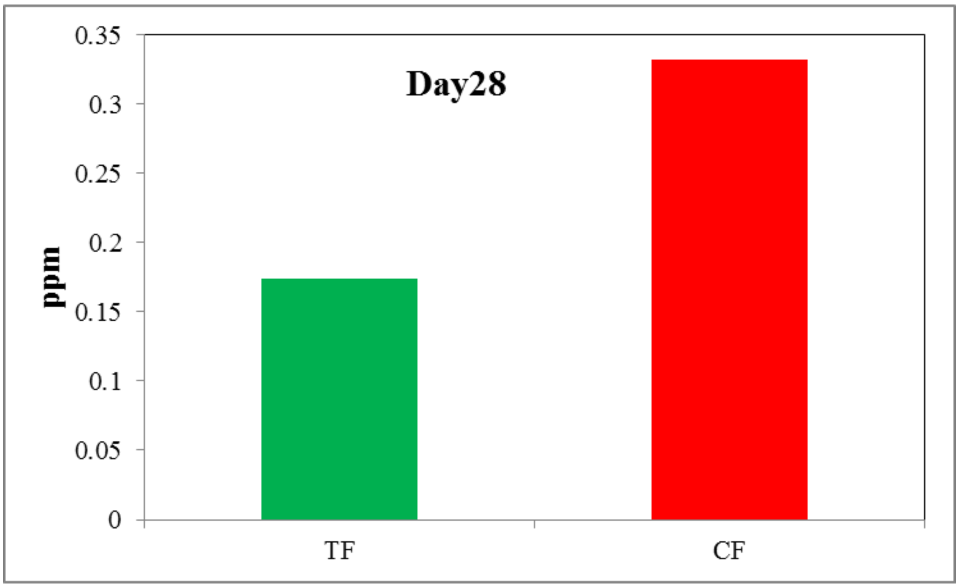

Figure 4.5: the mount of fluoride released at 28th day

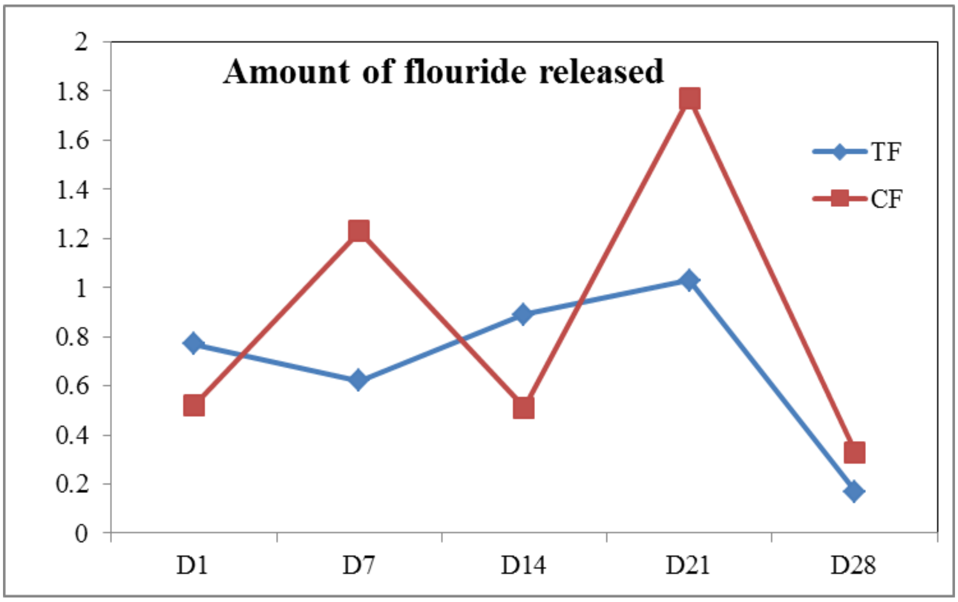

Figure4.6: The amount of fluoride release from day1 to day 28

4.2 Recharging of pit and fissure sealant materials with home care fluoride containing materials

A. Application of mouthwash

The sum of fluoride release after application of home care fluoride containing materials (mouthwash) were collected, tabulated and presented as mean and standard division from day 1 to day 14. The results of recharging at first day using mouthwash are presented in Table 4.7 and Fig 4.7. It can be seen that Transeal-Fgroups showed slightly higher fluoride release (1.15 ppm) than that of Conseal-F (1.14 ppm). However, there was no significant difference between two groups ($P < 0.920$).

Table 4.8 and Fig 4.8 show the amount of fluoride released after recharging by mouthwash at 7th day, the result was differed from first day where Conseal-Fgroups were slightly higher (2.50 ppm) than Transeal-F groups (1.27 ppm) with significant difference between them ($P < 0.014$).

Table 4.9 and Fig 4.9 show the released fluoride after recharging by mouthwash at 14 days of immersion. The amount of fluoride released of Transeal-Fat day fourteen after immersion in mouthwash was slightly higher fluoride release (0.86 ppm) than that of Conseal-F (0.68 ppm). However, there was no significant difference between two groups ($P < 0.060$).

Table 4.10 and Fig 4.10 presented the amount of collected fluoride during recharging by mouthwash from day1 to day 14. It can be seen that the fluoride release increase from day1 to day7 then decrease in day 14 for both tested groups.

Table 4.7: The amount of fluoride after rechargingby mouthwash at 1st day of immersion

Material	Ppm	P –value
Transeal-F	1.5± 0.21	0.920
Conseal-F	1.14 ± 0.15	

Table 4.8: The amount of fluoride after recharging by mouthwash at 7st day of immersion

Material	Ppm	P –value
Transeal-F	1.27± 0.50	0.014
Conseal-F	2.50± 0.88	

Table 4.9: The amount of fluoride after recharging by mouthwash at 14st day of immersion

Material	Ppm	P –value
Transeal-F	0.86 ± 0.11	0.060
Conseal-F	0.68 ± 0.15	

Table 4. 10: The amount of fluoride released after recharging by mouthwash from day 1 to day 28 of immersion.

Material	Day 1	Day 7	Day 14
Transeal-F	1.5 ±0.21	1.27 ±0.50	0.86 ±0.11
Conseal-F	1.14 ± 0.15	2.50 ±0.88	0.68 ±0.15

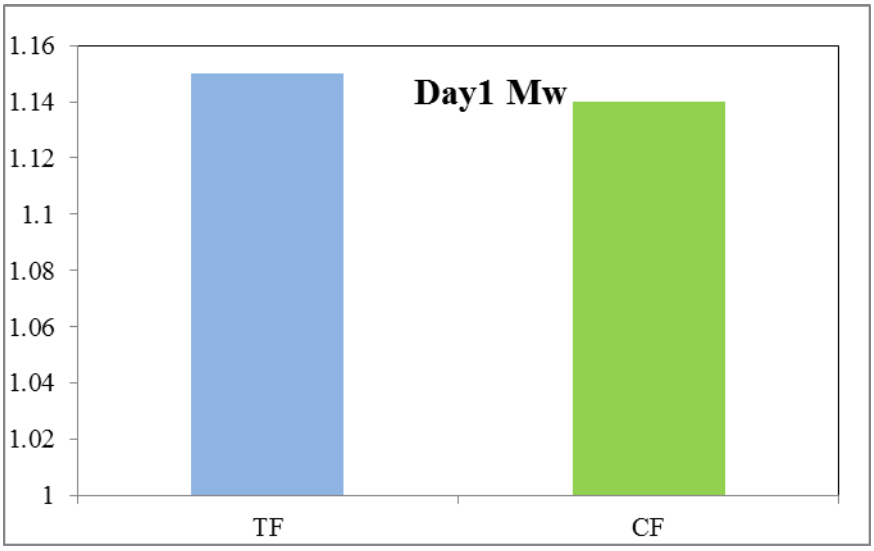

Figure 4.7: the mount of fluoride released after recharging by mouthwash at 1st day.

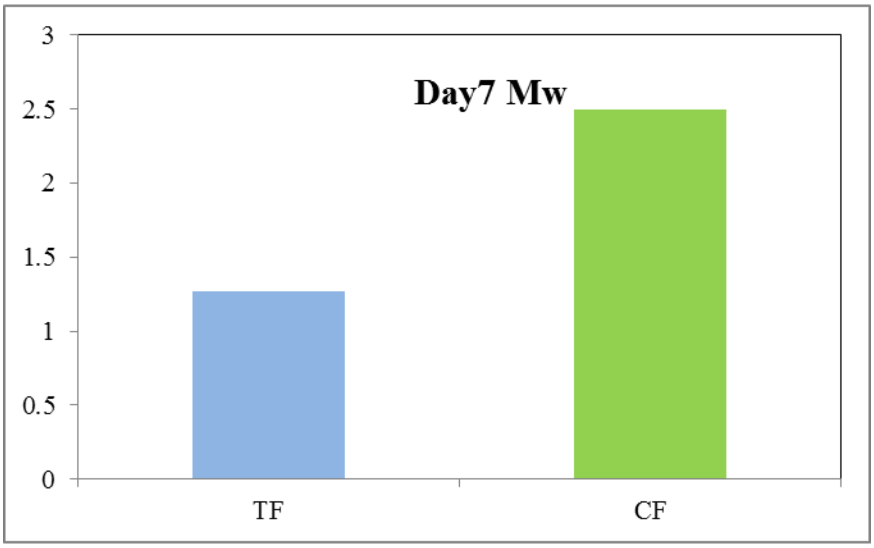

Figure 4.8: the mount of fluoride released after recharging by mouthwash at 7st day.

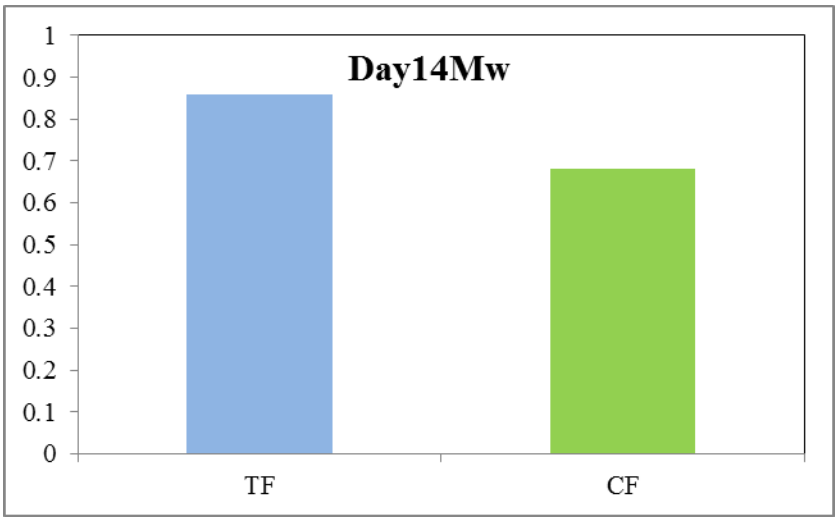

Figure 4.9: the mount of fluoride released after recharging by mouthwash at 14thday.

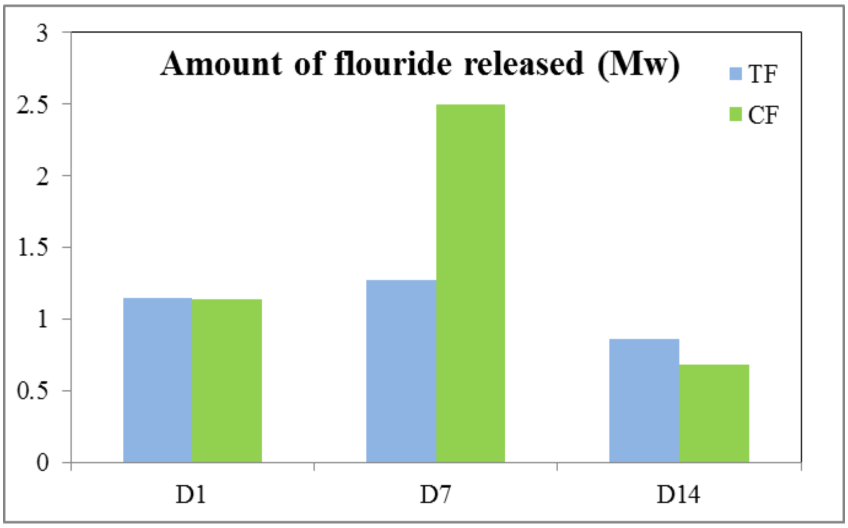

Figure 4.10: the mount of fluoride released after recharging by mouthwash from1-14days.

B. Application of toothpaste

The result of recharging procedure at first day using toothpaste is presented in Table 4.11 and Fig 4.11. It can be seen that Transeal-F groups showed slightly higher fluoride release (1.44ppm) than that of Conseal-F (1.21ppm). However, there was no significant difference between two groups ($P < 0.050$).

Table 4.12 and Fig 4.12 show the amount of fluoride released after recharging by toothpaste at 7th day, the result of Transeal-F groups showed slightly higher fluoride release (1.41 ppm) than that of Conseal-F (1.31 ppm). Same as first day there was no significant difference between two groups ($P<0.345$).

Table 4.13 and Fig 4.13 show the released fluoride after recharging by toothpaste at 14 days of immersion. The amount of fluoride released of Transeal-F at day fourteen was slightly higher (0.86 ppm) than that of Conseal-F (0.53 ppm). There was significant difference between two groups ($P < 0.015$).

Table 4.14 and Fig 4.14display the amount of collected fluoride during recharging by toothpaste from day 1 to day 14. It can be seen that the fluoride release increase from day1 to day 7 then decrease in day 14 for both tested groups.

Table 4.11: The amount of fluoride after recharging by toothpaste at 1st day of immersion

Material	Ppm	P –value
Transeal-F	1.44 ± 0.15	0.050
Conseal-F	1.21 ± 0.16	

Table 4.12: The amount of fluoride after recharging by toothpaste at 7st day of immersion

Material	Ppm	P –value
Transeal-F	1.42 ± 0.14	0.345
Conseal-F	1.31 ± 0.19	

Table 4.13: The amount of fluoride after recharging by toothpaste at 14st day of immersion

Material	Ppm	P –value
Transeal-F	0.86 ± 0.23	0.015
Conseal-F	0.53 ± 0.04	

Table 4. 14: The amount of fluoride released after recharging by toothpaste from day 1 to day 28 of immersion.

Material	Day 1	Day 7	Day 14
Transeal-F	1.44 ± 0.15	1.42 ± 0.14	0.86 ± 0.23
Conseal-F	1.21 ± 0.16	1.31 ± 0.19	0.53 ± 0.04

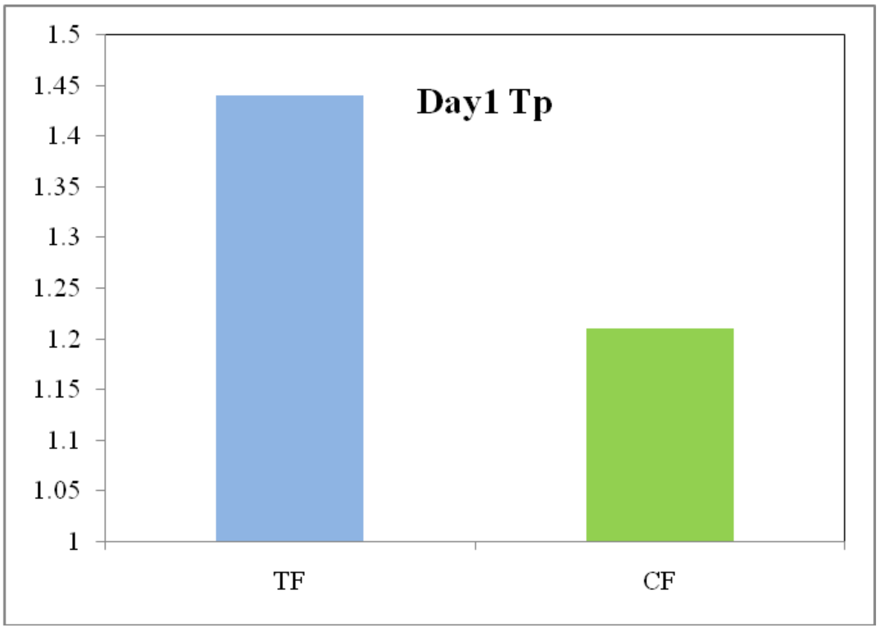

Figure 4.11: the mount of fluoride released after recharging by toothpaste at 1st day.

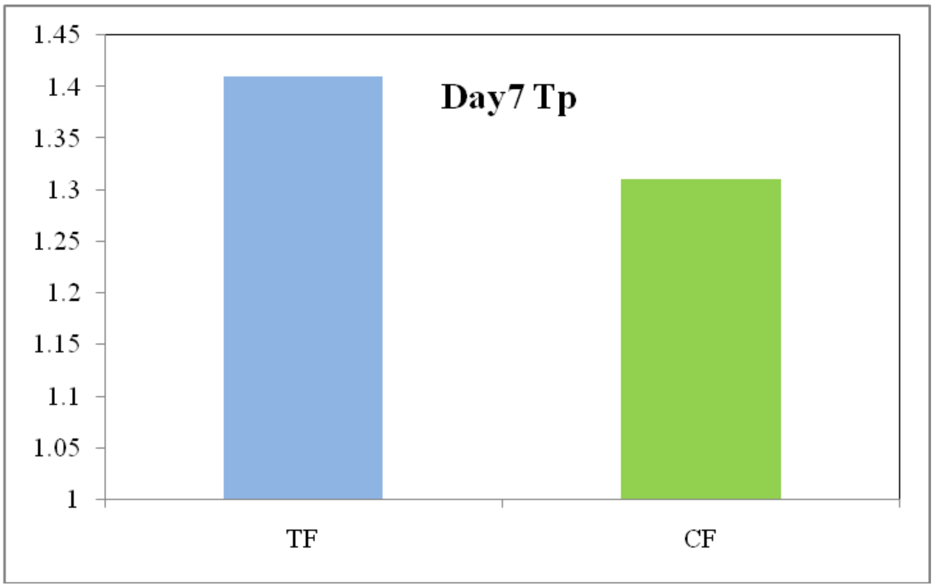

Figure 4.12: the mount of fluoride released after recharging by toothpaste7st day.

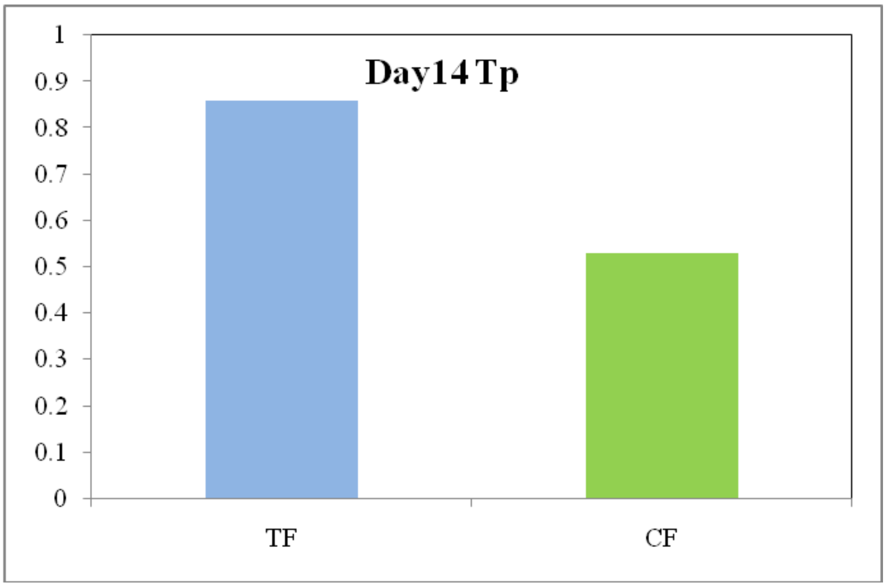

Figure 4.13: the mount of fluoride released after recharging by toothpaste at 14th day.

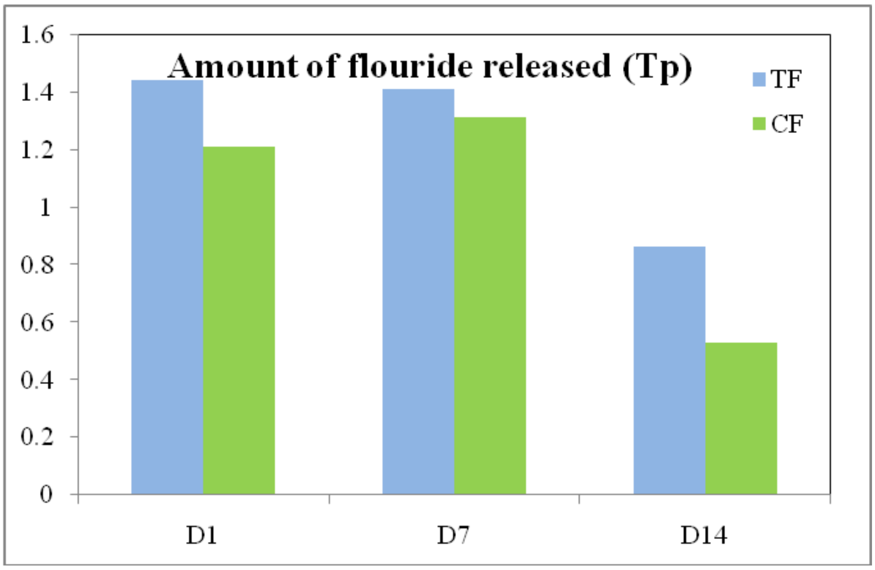

Figure 4.14: the mount of fluoride released after recharging by toothpaste from1-14days.

5. Discussion

The current study was conceded to determine the amount of fluoride leached during twenty eight days from two pit and fissure sealant materials. The amount of re-released of fluoride after recharging with home care fluoride content materials over a period of next fourteen days after last day of fluoride release evaluation was also evaluated.

The light cure pit and fissure sealant materials were selected due to the fact that they commonly used as sealant and preventive materials with highly fluoride released and are available in local market, as well easy to use and inexpensive when compared to other types of restorative materials. Pit-and-fissure sealants and topical fluoride are the primary preventive treatment for dental caries and are widely used in public dental programs[105]

The fluoride released and their content into restorative and preventive materials should be higher without altering the physical and mechanical properties as well not produce dangerous degradation of the filling material. An early high value of fluoride released in the surrounding area will reduce the viability of bacteria. Consequently it reduced dental caries by inducing remineralization of enamel and dentin by fluoride released[103] .

When the filling materials release a strong initial fluoride, the content of the voids or spaces left between cavity walls and filling will be saturated with fluoride. Therefore, the activity of the bacteria is reduced and the negative bacterial effects such as secondary caries will be reduced .Consequently, the importance of the filling material used to be able to release a strong initial of fluoride. To secure the effect of the constant release it is therefore desirable to use a fluoride-releasing material that can be ‘recharged’ from external fluoride sources.

The evaluation of leached fluoride is depend on the amount of ingredients included in the solution which effect in equilibrium between gain and leach mechanism. According to a study performed on the influence of storage media on fluoride release by using of artificial saliva, it was reported that the filling materials released more fluoride into artificial saliva compared to another storage media.[106] As well this method simulating the oral condition in which the filling materials will be in direct contact with it therefore, artificial saliva where chosen to be storage media for existing study.

Fluoride is regularly found in biological fluids at small amount and attention should be taken to apply technique which provides a low detection limits and sensitivity for precision and accurate estimation.

The method that has been employed in current study to evaluate the content of fluoride released using spectrophotometry, due to fact related to its viability and ease of handling. The spectrophotometer device analysis is a firm, precise and economically priced handheld fixed wave length spectrophotometer.[.107]

Universally spectrophotometer used in life-sciences, environmental investigation, food, drinks and chemical industry to determine the concentrations of ingredients in aqueous samples, however using the spectrophotometer in analysis the fluoride content in dentistry is rear. Therefore the current study used spectrophotometer to evaluate the amount of fluoride leached from pit and fissure samples thought to give precise and actual fluoride content.

In current study Conseal-F and Transeal-F samples showed different fluoride release curves. In the first days, the Conseal-F samples were released less fluoride than that of the Transeal-F samples. This finding was in agreement with Vermeerschet et al.,2001[108] Most studies had showed significantly varying results of fluoride released from different restorative materials[109]. Due to different methodology followed, the size of the example used, storing media and frequency of change of storage media, quantity of media used to evaluate the level of fluoride, all of these variables attributed to give different results published in the literatures.

When comparing the amount of early fluoride leached in tested groups, it can was noted that the Transeal-F produced slightly higher amount of fluoride than Conseal-F samples. This order can be clarified by the extent to which a glass ionomer matrix layer surrounds the glass filler in the set material and the composition of each tested material[94].

This finding was in agreement with several studies which reported that the amount of fluoride released depends on the concentration of fluoride and the composition of tested materials.[110,111]

The results of fluoride release from pit and fissure sealant materials (Transeal-F and Conseal-F) at first day indicated that Transeal-F group showed slightly higher fluoride release than Conseal-F, statistically analyses observe significant difference between two groups ($P < 0.008$).At 7th day the fluoride released from Conseal-F group was higher than that of 1st day, while, Transeal-F showed lower fluoride released. In comparison between two groups at seven day of immersion the amount of fluoride released was different from first day where the Conseal-F group showed higher amount of fluoride (1.226 ppm) compared to Transeal-F group (0.618 ppm statistically there was a significant variance between two groups ($P < 0.038$).

The amount of fluoride released at 14th day from Transeal-F group showed slightly higher fluoride release (0.888 ppm) compared to Conseal-F

(0.512ppm), where found to be significantly difference between tested groups (*P*< 0.012). In comparison with seven day, the fluoride released from Transeal-F groups was higher than that of 7th day, while Conseal-F showed lower fluoride released.

Although tested materials had comparatively high fluoride release in the first day this rapidly decreased in 7thday and slightly increase in 14th then decrease until reach 28thday. The reason for rapid fall in fluoride leached is likely because to early burst of fluoride leached from the glass particles as they dissolve in acid during setting reaction during the first day[104]

This initial high level of fluoride release has been referred to in a previous study as the "burst effect". On the first day the released of fluoride is due to superficial rinsing effect and during the subsequent days released is attributed to its ability to diffuse through material pores and fractures.[112]

In the present investigation, samples were stored in artificial saliva, in previous studies where showed that the fluoride release into artificial saliva is lower than into deionized water. The amount of fluoride release in deionized water could be different from the one found in the oral cavity, because saliva is a constantly changing medium, with respect of temperature, pH, protein content and many other factors.[113]

The lowest fluoride releases during the whole experimentation period, these results are explained by the different composition of the tested materials. The finding in the present study in agreement with recent study on glass ionomer based sealants which found that glass ionomer sealants released significantly more fluoride than resin modified glass ionomer or resin composite sealants.[113]

In GlCs, there is an acid base reaction resulting in the leaching of Ca_2^+, Al_3^+ and F^- ions to form a polysalt matrix. This may be responsible for the short term elution process. Patterns of fluoride released from sealant materials are showed higher release during the first week, with the most rapid release occurring in the first days, followed by a considerably slow but continuous fluoride release for the next four weeks. This is in agreement with previous studies.[114]

The low level of fluoride released may be due to the little quantity of fluoride incorporated in tested materials as fillers, as well the low solubility of tested materials in water, as reported previously[110]. A material can only leach ions from the mass have be penetrated by the water. The degree of water penetration can be determined by diffusion of water in to material or by surface dissolution of the matrix.

The initial high level of fluoride ions release seen in the first day is called the "burst effect" of fluoride due to rapid release of fluoride from the glass particles as they set.[114]The initial superficial rinsing effect also may be responsible for the initial high level of fluoride release[111]. Later fluoride releases becomes slower and this is because of the gradual dissolution of glass into the hydrogel matrix.[115-116]

Resin based sealant found to be released much less fluoride than that of GIC. This is mainly because of an absence of any acid-base reaction which was seen in glass ionomer based sealant. Glass ionomer matrix phase is not present in resin-based sealant. Glass ionomer used as a sealant has the property of fluoride release which is responsible for anticariogenic action. Glass ionomer based sealants showed a higher fluoride release than resin based sealants.[117] However, Glass ionomer has poor mechanical properties which are inferior to resin based sealants.[118-119] Resin-based sealants have high retention rates but are difficult to use in moist environment .The dissolution of inorganic fluorides is responsible for a release of fluoride from these sealants.[120]

The fluoride ion release mechanism is a multi factorial process affected by intrinsic and extrinsic factors, such as material solubility, composition, powder/liquid ratio, surface area of the specimen, and the environment.[121] The amount of fluoride release, which prevents demineralization and promotes remineralization is not known exactly. In a previous study, the amount of released fluoride was reported to be between 0.02 and 0.06 ppm.[122] Where as in the current study showed higher amount of fluoride than this limit. According to the results of current study, resin-based sealant materials produced a fluoride release about 0.774 to 1.032 ppm for Transeal-F samples while Conseal-F samples released about 0.522 to 1.766 ppm from the first day up to day twenty first. The amounts of fluoride release decreased with time and remained at about 0.174 ppm for Transeal-F and about 0.332 ppm for Conseal-F sample after 28 days which is still in recommend range of released fluoride from sealant materials However, it is preferable to use materials with the high and prolonged fluoride ion release, since the fluoride ion presence in oral cavity reduces the enamel solubility.[123] The combination of sealant and topical fluoride application has shown enhanced anticariogenic properties due to recharge ability of fluoride-releasing fissure sealants.[124]

Sodium Fluoride was used to recharge specimens because it is commonly used in mouthwashes and toothpaste. Since toothpaste and mouthwashes can be used on regular basis they can serve as an effective recharging agent to prevent secondary caries.[115] In current study, during the first day following application of fluoride regime the concentrations of fluoride released from sealant materials were found to be slightly higher than that of 28day of immersion. However, the amount of fluoride starts to decrease until day fourteen.

As reported by Koga et al .,2004[125] the conventional resin based sealant had less ability of recharging where resin based sealants released fluoride after recharge in small amount compared to glass ionomer based sealant, this was in agreement with current study were the tested group showed less ability of recharging after fluoride application.

Shimazu et al .,2011[120], concluded that resin-based sealant containing surface pre-reacted glass ionomer (S-PRG) filler had greater recharge capacity than conventional sealants which is in accordance with current study. A lot of factors may affect the recharge capability like the penetrability of the material, the surface energy of the material and composition of the material. Greater the permeability of the material greater is the ability of the material to absorb and re-release fluoride[115].

6. Conclusion

The current study evaluates the fluoride leached from two preventive materials (pit and fissure sealant) (Transeal-F and Conseal-F light cured fluoride releasing materials) .Artificial saliva was used as release medium to determine the amount of fluoride content. Spectrophotometer was used to assess the fluoride released from the tested samples.

The quantity of fluoride released was higher in 1stday for both samples .The Transeal-F group showed slightly higher fluoride release than Conseal-F. In the seventh day the fluoride released from Conseal-F group was higher than that of 1st day while, Transeal-F showed lower fluoride released. In comparison between two groups at seven day of immersion the amount of fluoride released was different from first day where the Conseal-F group showed higher amount of fluoride compared to Transeal-F group. This may be due to burst effect which found to be high at first day then reduced gradually.

Transeal-F group showed slightly higher fluoride release compared to Conseal-F group after 14th day, the amounts of fluoride released from both groups at 21st day were higher than 1st, 7th and 14th days of immersion. In contrast with day fourteen, the Conseal-F group showed slightly higher value than that of Transeal-F group, at day 28ththe Conseal-F group showed slightly higher value than that of Transeal-F group. However, there was no significant difference between two tested groups. It can be seen that the amount of fluoride released at 28th day was the lower observed amount in both groups at all days of immersion.

In recharging investigation the Transeal-F samples showed slightly higher fluoride release than that of Conseal-F samples in both mouthwash and toothpaste although there was no significant difference between two recharging medium. the recharging at 7th day the Conseal-f samples showed slightly higher fluoride release than that of Transeal-f samples. However, the released amount was more than that of first day. Also there was no significant difference between both tested groups in both recharging medium . Less amount of fluoride released after recharging found in day fourteen in comparison to that of first and seven day. Nevertheless, there was a significant difference between mouthwash and toothpaste in Conseal-f and Transeal-f samples.

The recharging ability in current study seemed to be not effective, since it showed continue decrease of fluoride amount compared with day 28 of fluoride released which due to the ineffectiveness of the protocol used for recharging of the restorative materials was not effective. The ability of restorative materials for recharging by fluoride found to be depending on the material composition, the incidence of fluoride exposure, the kind and concentration of fluoride agent used.

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تقييم كمية الفلوريد المرتشح من مواد الشقوق والوهدات –دراسة معملية

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الملخص

مقدمة: مواد الشقوق والوهدات من اكثر المواد فاعلية في منع التسوس في الاسنان اللبنية والدائمة وذلك لإحتوائها على الفلوريد. وهذة المواد تعمل كحاجز فزيائي لمنع التسوس.

الهدف من الدراسة: وتهدف هذه الدراسة إلى تحديد كمية الفلوريد المرتشح من مادتين متوفرات تجارياً. وقد استخدمت مادتين في الدراسة الحالية (ترانسيل.ف, وكنسيل.ف).

طرق العمل: ثمانون عينة لجميع المواد المختبرة. تم غمر العينات في20 مل من اللعاب الصناعي عند درجة $37C^0$. تم قياس كمية الفلوريد المنبعثة خلال اليوم الأول و السابع و الرابع عشر والحادي والعشرين و الثامن والعشرين باستخدام مؤشر محدد ومقياس الطيف الضوئي. بعد اليوم الثامن والعشرين تم قياس قدرة إعادة الشحن بغمرها في محلول المضمضة (فلوريد بوتاسيوم نترات)، المجموعة الاخري تعرضت لـ 1450 جزء في المليون من معجون الأسنان (سنسوداين برو إينمل) خلال الفترة الأولى و السابعة و الرابعة عشرة. النتائج تم تحليلها إحصائيا باستخدام البرنامج الاحصائي (أحادي الاتجاه ANOVA) و T–testes.

النتائج: كمية الفلوريد الأعلى التي تم إطلاقها في كلتا المادتين كانت في اليوم الأول ، ولكن العينة ترانسيل.ف كانت أعلى قليلاً من عينات كنسيل.ف وانخفضت بشكل مستمر حتى اليوم الثامن والعشرين ، لم تكن هناك فروقات معنوية بين معجون الاسنان ومحلول المضمضة ولم تكن

إجراءات إعادة الشحن فعالة في الدراسة الحالية حيث كان هناك إنخفاض مستمر في كمية الفلوريد مقارنة باليوم الثامن والعشرون.

الخاتمة: قابلية مواد التعويضات لإعادة الشحن تعتمد على تركيب المادة وكمية التعرض للفروريد ونوع وتركيز وسيط الفلوريد المستخدم.

تقييم كمية الفلوريد المرتشح من مواد الشقوق والوهدات – دراسة معملية

قدمت من

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قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير
في خواص مواد الاسنان

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كلية طب وجراحة الفم والاسنان

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