BALKAN JOURNAL OF STOMATOLOGY

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Guest Editorial

The Establishment of the BaSS

Balkan Stomatological Society was established in March 1996, during a Balkan Dental Congress organized by the Dental Association of Thessaloniki. The Council members of this professional Association, under the presidency of K. Hatzipanagiotou, had worked out the idea of creating an organization of Balkan dentists in order to promote professional and scientific collaboration. So, long before inviting the leaders of the Dental Profession to participate to this Congress, a delegation of the Dental Association of Thessaloniki had visited Belgrade and Sofia and had some discussion with the leaders of the profession in these 2 towns. The response they received to their proposals was more than encouraging and this was enough to put their idea into action.

At that time, I was serving as dean of the Dental School of Aristotle University, when K. Hatzipanagiotou and G. Tsiogas visited me in my office. They asked for my support and collaboration to the Congress and offered me the presidency of the Organizing Committee. I accepted gladly, as I found the idea fascinating. With similar way the idea was received by all the younger colleagues who manned the Organizing Committee.

Invitations to the Congress were sent to all the professional leaders in the Balkans. These were addressed to University Dental Faculties and Departments, and to Dental Associations, where they existed as in most of the previously socialist countries professional organizations did not exist. All the members of the organizing committee worked hard and enthusiastically, overwhelmed by the spirit of re-establishing the Balkan collaboration, which had been discontinued since the end of the 2nd World War and the creation of the "iron curtain".

The responses to the invitations were positive from almost all. The Congress was held on 28-31 of March 1996, and it was attended by dentists from Albania, Bulgaria, Cyprus, Romania, Turkey, Yugoslavia, and of course by many Greeks. During that Congress, in the corridors of the Congress Hall and in 2 invitational meetings, I had the opportunity to acquaintance with colleagues from all these countries. All colleagues were glad meeting each other and working together as neighbours and friends for the first time in their professional life. We all shared the same feelings.

I recall that the first day we had lunch in a teacher's room of the students club of Aristotle University. There we met, and each one of the participants introduced himself to all the others, to begin a communication before getting into discussion. Prof. D. Djukanovic (Belgrade) put forward an important argument. He said: "Most of us in this room know the scientific work being done in Germany, France and USA, but we don't know what are being done in any of our neighbour countries". We all agreed that it was so indeed, and the next prevailing thought was to work together to cover the lacking information.

So, some hours later, in an invitational meeting, we all agreed to establish a scientific and professional organization, proposed to be called Balkan Stomatological Society. The term "stomatological" was preferred to the term "dental" not only because it was extensively used in the Balkans, but mainly because it refers to the whole mouth and it is not restricted only to teeth. The Society should be a non political and non governmental organization, based on individual membership. However, there were not similarities in the existing professional organizations. An aroused question, whether Cyprus should participate or not was answered positively, according to the fact that Cypriots were either Greeks or Turks. Another important decision was the one concerning the headquarters town. Thessaloniki and Istanbul were proposed. All, except the Turks, voted for Thessaloniki and

so it was decided the Headquarters to be in Thessaloniki and consequently the secretariat and the treasury to be manned by Thessalonicians.

A provisional council was formed in order to prepare a constitution for the Society. I was given the responsibility to be the president of that council. Although all the members of the provisional council were invited to propose Bylaws, it was my job to collect and put them in some order, before delivering them for consideration by the members of the provisional council.

Figure. Most of the members of the first BaSS Council, photographed during a
Council meeting in Thessaloniki on the 29th of November 1997.

So, having settled the basic principles for future BaSS, Prof. Nuri Yazicioglu, on behalf of the Turkish Dental Association, invited all the participating members and expressed his wish to continue the discussion for a formal constitution of the Society in Ankara, on the venue of the Turkish Dental Congress in 20-21 June 1996. The invitation was gladly received by all. At that time, besides the official meeting, we had separate talks with many, listening to one another's ideas. In one discussion I had with Prof. Ljubomir Todorovic, he mention to me the importance of publishing a Journal and his thought to start immediately with this. My first reaction was that it was very difficult task. I started mentioning the financial, linguistic, and practical problems that we had to be faced. His enthusiasm was persuading and finally I promised him to help, if he was undertaking the responsibility of this publication. We discussed some more details and we decided to propose to the council the idea of incorporating this ambition within the main aims of the Society. So, it happened and by his enormous efforts, during the following decade and until today, he proved how much right he was for the importance of the Journal to the dentistry in the Balkans. The discussions continued for a second meeting the following day, where the idea for the Journal was put forward. At the end of the second meeting, together with saying "goodbye" to each other, we all wished for a successful continuation of our plans in the Ankara meeting.

The Ankara meeting was held in hotel Hilton, with the traditional Turkish hospitality, and was attended by representatives from all the interested countries. In 2 days various propositions about the articles of the Bylaws were discussed, and final agreements were achieved unanimously by all present. In that meeting Marko Vulovic was appointed President of the 2nd Balkan Dental Congress to be held in Belgrade, and Ljuba Todorovic was appointed editor in chief of the Balkan Journal of Stomatology (BJS).

On November 23 of the same year (1996), another meeting of the provisional council was held in Thessaloniki. The Constitution, as it had been agreed in the Ankara meeting, was approved there, and signed by all representatives present. Later, the constitution was confirmed unanimously at the First General Assembly of the Balkan Stomatological Society, held in Belgrade in the 3rd of April 1997, and subsequently it was registered in the City Court of Thessaloniki. The Constitution has the signatures of the following members of the Provisional Council: D. Karakasis (president), D. Beloica (vice president), N. Atanassov (vice president), D. Iakovidis (secretary general), K. Hatzipanagiotou (specific secretary), H. Baylas (honor, treasurer), R. Quafmolla, P. Kongo and V. Guzhuna (for Albania), N. Sharkov (for Bulgaria), D.

Veleski and L. Gugucevski (for FYROM), G. Tsiogas, S. Chrisafis and K. Louloudiadis (for Greece), A. Creanga and A. Bechir (for Romania), N. Yazicioglu and D. Temucin (for Turkey), M. Vulovic, L. Todorovic and J. Vojinovic (for Yugoslavia) and G. Pantelas (for Cyprus). In the same meeting, many other details were discussed and decided. The emblem of the Society designed by Alexandras Kolokotronis and the logotype "BaSS", brought forward by Nikolai Sharkov, were both accepted. Concerning the 2nd Congress, it was finalized to be held in Belgrade under the presidency of D. Beloica. During 2nd Congress the first two General Assemblies of the new Society were arranged, one for the approval of the Constitution and the second for the election of a first official Council. A meeting of the Deans of the Balkan Dental Schools was also arranged, while Prof. LTodorovic was given approval on all his propositions about the cover page of the Journal, the instructions to authors and every other detail.

The 2nd Congress in Belgrade was a great success, both scientifically and socially. As it has been mentioned, in Belgrade we had elections for the first Council of the BaSS. This was proposed by the provisional council and approved by the General Assembly, according to the constitution. I was honoured to serve as the first president from 1997 to 1999, Dragan Beloica was the president from 1999 to 2001, Nuri Yazicioglu from 2001 to 2003, Nikola Atanasov from 2003 to 2005, Andrei Iliescu from 2005 to 2007, to be succeeded from 2007 by Marko Vulovic. The Belgrade Congress set the high standards for the superb series of Congresses, that were taking place regularly every year in spring time. During the past decade, and up to now, they were:

2nd   Congress - Belgrade
        (April 1997, president D. Beloica);

3rd   Congress - Sofia
        (April 1998, president N. Atanassov);

4th   Congress - Istanbul
        (March 1999, president N. Yacizioglu);

5th   Congress - Thessaloniki
        (March 2000, president D. Iakovidis - G.Tsiogas);

6th   Congress - Bucharest
        (May 2001, president A. Iliescu);

7th   Congress - Kusadasi
        (March 2002, president N. Arpak);

8th   Congress - Tirana
        (May 2003, president R. Qafmolla);

9th   Congress - Ohrid
        (May 2004, president M. Carcev);

10th   Congress - Belgrade
        (May 2005, president M. Vulovic);

11th   Congress - Sarajevo
        (May 2006, president H. Sulejmanagic);

12th   Congress - Istanbul
        (April 2007, president H. Bostanci).

This continuous series of successful Balkan Dental Congresses, together with the increasing importance of publication of the BJS (Balkan Journal of Stomatology), have built up a high reputation for our Society. These achievements have been based on very hard work offered by too many members during all these years. Without this voluntary offer, nothing would have been achieved. Thus, the honour and pride of the existence of the BaSS is shared by all its members.

Unfortunately, political problems still exist in the Balkans, and occasionally they appear threatening. The Council of BaSS, during the last decade, has overcome all the political crises in a manner characteristic of the willing of the majority of dentists, which is to work together into a peaceful South East Europe.

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Pit and Fissure Sealants: Types, Effectiveness, Retention, and Fluoride Release: A Literature Review

SUMMARY

Sealing occlusal pits and fissures in teeth is a common and highly effective preventive method. The main purpose of sealing the pits and fissures is to prevent plaque microflora and food debris accumulation in the fissures where saliva cannot reach and clean the debris, re-mineralise initial lesions, and buffer the acid produced by cariogenic bacteria. Resin-based sealants, as well as glass ionomer materials, are used for pit and fissure sealing. The resin-based sealants require the use of acid for preparation of the enamel surface of the teeth, which is then rinsed and dried before the sealant material is applied. The success of this procedure depends on good isolation of the teeth and prevention of any contamination of the etched enamel surface by saliva or water. Tooth isolation may be achieved by the use of cotton rolls or rubber dam. Additionally it has been suggested that the benefit provided by protecting pits and fissures is based on good retention and the integrity of the sealant material. However, since the retention of the sealant is not permanent, this physical effect could be enhanced if the material simultaneously released fluoride. The durability of fluoride containing sealants would now appear to be comparable to conventional resin sealants. However, further long-term clinical trials are necessary to determine the clinical longevity of sealant retention is not adversely affected by the presence of incorporated fluoride. Also the clinical importance of fluoride in sealants in terms of caries prevention remains to be shown.

Key words: Pit Sealants; Fissure Sealants

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Introduction

Dental caries is a disease that continues to affect the majority of people. Dental caries is a bacterially based disease that progresses when acid, produced by bacterial action on dietary fermentable carbohydrates, diffuses into the tooth and dissolves the mineral (demineralisation). Pathological factors including acidogenic bacteria (*Mutans Streptococci* and *Lactobacilli*), salivary dysfunction, and dietary carbohydrates are related to caries progression[1]. In addition caries is mainly a disease of pits and fissures[2]. Manton and Messer[3] reported that pit and fissure caries nowadays represent a greater proportion of coronal lesions than interproximal lesions. Thus there is a major need to protect the occlusal surface of teeth from the caries process. According to Williams[4], a fissure sealant is "a substance that is placed in the pit and fissure pattern of the teeth such that it prevents the ingress of plaque, bacteria and carbohydrate and in so doing prevents the onset of caries at those sites".

In order to intensify the caries protective benefits of sealants, several kinds of fluoride sealants have been developed over the years. 2 methods of fluoride incorporation are used; fluoride is added to unpolimerised resin in the form of a soluble fluoride salt, or an organic fluoride compound is chemically bound to the resin[5].

In this literature review, the early techniques used to prevent occlusal caries are discussed briefly and the history of fissure sealants is reviewed. The rationale of pit and fissure sealants used in caries prevention is analysed and the literature is reviewed regarding all the different types of sealants, their effectiveness in reduction of occlusal caries and the factors affecting sealant retention on pits and fissures of posterior teeth. Reference is made on sealant innovations: combination of their action with

fluoride action in order to constantly release fluoride to the oral environment. The literature is reviewed regarding all the kinds of fluoride containing fissure sealants.

History of Modern Pit and Fissure Sealants

The high caries susceptibility of the pit and fissure surfaces of posterior teeth has been recognized for many years and a number of techniques have been proposed in order to prevent occlusal caries (Tab. 1). None of these

attempts were successful until 1955, when Buonocore reported the use of acid to etch the enamel surface prior to the application of acrylic resin[10].

3 different kinds of plastics have been used as occlusal sealants: cyanoacrylates, polyurethanes and bisphenol A-glycidyl methylacrylate (Bis-GMA).

The first extensive clinical study of adhesive sealing using an acid etchant was that of Cueto and Buonocore[11] who employed methyl-2-cyano-acrylate monomer with filler to seal pits and fissures of permanent molars and premolars. This technique was soon proved unsatisfactory because the cyanoacrylates disintegrated after a slightly longer time[12].

Table 1. Techniques used for prevention of occlusal carries

Study	Technique
Wilson (1895) [6]	Placement of dental cement in pits and fissures to prevent caries
Hyatt (1923)[7]	Insertion of small restorations in deep pits and fissures before carious lesions had the opportunity to develop: "prophylactic odontomy".
Bödecker (1929)[8]	Deep fissures could be broadened with a large round bur to make the occlusal areas more self-cleansing: "fissure eradication".
Ast et al (1950)[9]	Attempted either to seal or to make the fissures more resistant to caries with the use of topically applied zinc chloride and potassium ferrocyanide and the use of ammoniacal silver nitrate; they have also included the use of copper amalgam packed into the fissures
Buonocore (1955)[10]	Use of acid to etch the enamel surface prior to the application of acrylic resin

The polyurethanes proved to be too soft and totally disintegrated in the mouth after 2 to 3 months[13]. 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[14].

Dimethacrylates represent the reaction product of bisphenol A and glycidyl methacrylate (Bis-GMA), which is considered by its originator to be a hybrid between a methacrylate and an epoxy resin[15]. The most commercial sealants today are Bis-GMA[16]. They were first produced as a potential dental material by Bowen in 1962, although the first fissure sealant based on Bis-GMA was introduced to the profession in 1971 under the trade name Nuva-seal[14]. The initially claimed high retention rates with this ultraviolet photoactive material[17] were revised downwards when the same sealant was looked at over 5 years[18]. Commercially available sealants differ in whether they are free of inert fillers or are semi-filled, and whether they are clear, tinted, or opaque. A principal difference is the manner in which polymerization is initiated. The first marketed sealants, called first-generation sealants, were activated with an ultraviolet light source and they are no longer used. Second-generation sealants are auto-polymerizing and set upon mixing with a chemical

catalyst accelerator system. The third-generation sealants are photo-initiated with visible light[19].

Rationale for the Use of Pit and Fissure Sealants

Tooth surfaces with pits and fissures are particularly vulnerable to caries development[3]. Ripa[19] observed that although the occlusal surfaces represented only 12.5% of the total surfaces of the permanent dentition, they accounted for almost 50% of the caries in school children. 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[19-21]. Resin sealants are the most widely used and also have the greatest evidence of effectiveness[22]. The effectiveness of fissure sealants carried out in fluoridated and non-fluoridated areas, as part of public health measures and in private clinics, has been proved beyond doubt[19]. Brown et al[23] and Kaste et al[24] showed that in fluoridated communities over 90% of dental caries occurred in occlusal and buccal-lingual surfaces and represented, almost exclusively, pit and fissure caries, while from 1987 to 1991, interproximal caries was

reduced by 25%, whereas pit and fissure caries decreased by 18%. The reason why fluoride is less effective in preventing caries in fissured surfaces may be related to the total depth of enamel on smooth surfaces compared with that underlying the fissure. The base of an occlusal fissure can be close to or within the underlying dentine, consequently lateral spread of the lesion along the enamel-dentine interface results in an increased rate of progression of the lesion, and therefore fluoride has relatively little time to increase demineralisation. On the contrary, fluoride ions have enough time to positively affect the demineralisation process in a smooth proximal surface, where the thickness of enamel is approximately 1mm[25,26].

Different Types of Pit and Fissure Sealants

Once pit and fissure sealants were judged to be caries preventive as long as they remained adherent to the teeth; the initial evaluation of sealant effectiveness by clinical trials comparing sealant treated and non-treated teeth was considered unethical. Clinical retention and longevity became the measure of sealant success[19].

First and Second Generation Pit and Fissure Sealants

Ripa[27] in 1985 reviewed the results of more than 60 studies on the effectiveness of first-generation (ultraviolet-initiated) and second-generation (chemical-initiated) sealants. The sealants were evaluated from 1 to 7 years after placement. He concluded that second-generation sealants provided superior retention and caries protection than first generation sealants, especially as the time increased between initial treatment and follow-up observation. Several studies reported the effectiveness of second generation sealants (Tab. 2). As a result of the better performance of chemically polymerized sealants (due to the change in the diluent in the Bis-GMA system from methyl methacylate to glycol dimethacrylate), and the increasing criticism for the use of ultra-violet light, first-generation sealants are no longer marketed[27].

Table 2. Studies for the effectiveness of second generation sealants

Study	Longevity of the study	Retention of sealants
Wendt and Koch (1988)[28]	10 years	94% partial and complete retention
Romcke et al (1990)[29]	10 years	41% complete retention
Simonsen (1987)[30]	10 years	8% partial retention 57% complete retention
Simonsen (1991)[31]	15 years	28% complete retention

Third Generation Pit and Fissure Sealants

Since third- and second-generation sealants compete with each other in the market place, clinical comparison of sealant types is fundamental for clinicians to make an informed selection. Ripa[19] reviewed numerous studies that have been carried out, comparing the retention between third and first and/or second generation sealants. The mean results indicate that the performance level for chemical initiated sealants and visible light photo-initiated sealants are similar within an observation period of up to 5 years. However, in 3 comparison studies of longer duration, greater longevity was reported for the chemically cured pit and fissure sealants[32-34].

Filled and Unfilled/Clear, Opaque and Tinted Pit and Fissure Sealants

The addition of filler particles to the sealant appears to have little effect on clinical results[35]. Filled and unfilled sealants penetrated the fissures equally well[36,37], demonstrated no difference in microleakage[38] and had similar retention rates[39-41].

Pit and fissure sealants are available as clear, opaque or tinted. No product demonstrated a superior retention rate, but the tinted and opaque sealants have the advantage of even better appreciation by the patient, and evaluation by the dentist at subsequent recalls[35]. Rock et al[42] found significant differences in the accuracy with which 3 dentists identified a clear and an opaque fissure sealant.

During the mid-1990's safety concerns were expressed regarding leaching of bisphenol-A (BPA) and bisphenol-A dimethacrylate (BPA-DMA) from sealants, and a possible oestrogenic effect. It is known that incomplete conversion of BPA during the setting reaction may allow this non-reacted monomer to be released into the oral environment[43]. Nathanson et al[44] analyzed 7 pit and fissure sealants and provided reassuring evidence regarding the safety of these materials. Soderholm and Mariotti[45] considered the dosages and routes of administration and the modest response of oestrogen-sensitive target organs, and concluded that the short-term risk of oestrogenic effects from treatments using bisphenol A-based resins is insignificant. Fung et al[46] showed that BPA released orally from a dental sealant may not be absorbed or may be present in non-detectable amounts in the systemic circulation.

Glass Ionomer Cement (GIC) Pit and Fissure Sealants

The use of GIC as a pit and fissure sealant was introduced more than 25 years ago[47,48]. Studies of the use of GIC's as a fissure sealant indicate significantly lower retention rates than resin-based pit and fissure sealants[49-51]. An interesting finding in the studies by Williams and Winter[52] and by Shimokobe et al[53] was that glass ionomer

sealants seemed to exert a cariostatic effect after they had disappeared macroscopically. As retention of glass ionomer sealants is less dependent on good moisture control, this material has been suggested as an alternative to resins for sealing primary teeth[54]. Overbo and Raadal[55], comparing the extent of microleakage that occurred in GIC pit and fissure sealants and a diluted composite fissure sealant, concluded that extensive leakage occurred in the GIC throughout the material, and at the margin of the cement and the enamel. Birkenfeld and Schulman[56] concluded that etching prior to application of GIC enhances the bonding to fissure enamel. Therefore, although GIC's with their ability to release fluoride and adhere to enamel were initially worthy of consideration[57], clinical trials related to their effectiveness discouraged their use as pit and fissure sealants[35]. The use of GIC has been suggested for erupting teeth, where isolation from saliva is a problem[58].

Effectiveness of Pit and Fissure Sealants

Manton and Messer[3], in their review article in 1995, stated that sealant effectiveness can be evaluated by 4 measures: a) the per cent effectiveness, which compares the caries experience of sealed and unsealed teeth; b) the per cent retention, which reflects the number of sealants needing replacement, assuming a failed application requires replacement; c) the per cent sealed teeth/surfaces which become carious and/or restored; and d) the rate at which sealants require reapplication. Sealant effectiveness was measured initially by half mouth trials, but as the efficacy became established this approach became unethical and investigators changed to comparative studies of different sealant products[59].

Caries Prevention with Pit and Fissure Sealants

The ability of pit and fissure sealants to inhibit caries was first reported by Cueto and Buonocore[11], when they claimed an almost 100% reduction in caries over 1 year with the use of an acid etching technique. Romcke et al[29] reported a 10-year observation of more than 8000 sealants; complete sealant retention, without need for resealing, was 58-63% for 7 to 9 years and 41% at 10 years. They reported sealant success (freedom from caries) of 96% for the first year and 85% after 8-10 years (Tab. 3). Wendt and Koch[28] followed for 1-10 years 758 sealed surfaces, and the resulting examination showed 80% total sealant retention after 8 years. Another 16% of the surfaces were judged as partially retained. After 10 years only 6% of the sealed occlusal surfaces showed caries and restorations. Simonsen[31] conducted the longest clinical study to date on sealant retention and effectiveness. In children who received a single application of a white-coloured auto-cured sealant in 1976, 74% of the pit and fissure surfaces of permanent first molars were non-carious 15 years later. Chestnutt et al[60] reported on more than 7000 sealants after 4 years and 57% of the sealed tooth surfaces remained fully sealed with 18% scored as deficient or failed and 24% completely missing. 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 baseline became carious. Wendt et al[61] reported 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. In a different study the same authors, reported that 74% of first permanent molars that had been sealed were caries free after 15 years[62].

Table 3. Pit and fissure sealants and caries prevention

Study	Longevity of the study	Percentage of sealed teeth without caries
Cueto and Buonocore (1967)[11]	1 year	100%
Romcke et al (1990)[29]	1 year	96%
	8-10 years	85%
Wendt and Koch (1988)[28]	10 years	94%
Simonsen (1991)[31]	15 years	74%
Wendt et al (2001)[61]	15 years	95% second permanent molars
	20 years	87% first permanent molars
Wendt et al (2001)[62]	15 years	74% first permanent molars

Factors Important for Retention

The retention and caries-preventive effects of pit and fissure sealants have been well documented for the past 20 years[27]. There is good evidence that teeth sealed very early after eruption require more frequent re-application of the sealant, than teeth sealed later[63,64]. 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[11,60]. The application procedure for a conventional sealant involves the placement of etching material, a waiting

time, rinsing, and drying, followed by the application of the sealant and the exposure to the curing light. Thus, there are many time consuming steps involved, increasing the risk of saliva contamination during the procedure. Contamination by saliva after etching may have deleterious effects on bonding[65]. Consequent partial loss of material and/or micro-leakage and gaps may result in the formation of secondary caries around the sealed fissure. The annual incidence of caries development in sealed teeth is estimated to be approximately 2-4%[66]. The following parameters are important for fissure sealant retention: method of prophylaxis before sealant application, moisture control, use of etching gel or liquid, etching time, washing and drying times, and fissure sealant application itself[47,48,67,68].

Surface Cleaning

The need and method for cleaning the tooth surface prior to sealant placement are controversial. Usually, acid etching alone is sufficient for surface cleaning[69]. This is attested by the fact that 2 of the most cited and most effective sealant longevity studies by Simonsen[30,31] were accomplished without use of a prior prophylaxis. The use of prophy-pastes, especially those with fluoride, has been discouraged[69]. Garcia-Godoy and Gwinnett[70] and Garcia-Godoy and Medlock[71] showed in studies with SEM that pumice particles become lodged in the fissures and are not removed after rinsing with a stream of water. Additionally, treatment with fluoride before etching has been proposed to strengthen the enamel by reducing its solubility[72]. 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[73,74]. 2 clinical trials revealed similar retention rates between cleaning the debris of fissures with a prophy-brush and pumice or gently running a probe[75] and toothpaste[76], respectively.

Air polishing of the occlusal surface with special devices has been suggested[77,78]. *In vitro* studies with air polishing of the occlusal surface before acid etching demonstrated greater penetration[79], a greater number of resin tags for micromechanical retention[80], and higher bond strengths[81] than fissures cleaned with rotary instrumentation and pumice.

In recent years, a 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[82]. Several studies have been conducted to compare sealants placed on laser- or acid-conditioned enamel. In 1996, a split mouth clinical trial was undertaken to compare the retention of fissure sealants placed using both methods that found that, after

a mean follow-up period of 14.5 months, the retention rate for CO_2 laser conditioning was greater than that for acid etching (97.9% versus 94.6%, respectively), although this difference was not statistically significant[83]. In the *in vitro* study, do Rego and de Araujo[84] compared the effect of different surface preparations on the micro-leakage of pit and fissure sealants, and found that Nd:YAG laser irradiation with an energy level of 120 mJ per pulse and an energy density of 1.4 Jcm[-2] did not decrease the micro-leakage degree when using a fluoride resin-filled sealant and resin-modified GIC as pit and fissure sealants. It has been shown 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[85].

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[69].

Isolation

Adequate isolation is the most critical aspect of sealant application[69]. Salivary contamination during or after acid etching allows rapid precipitation of glycoproteins onto the surface, greatly decreasing bond strength[61,62,86,87]. Silverstone et al[88] and Tandon et al[89] suggested that even a one second exposure to saliva can lead to the formation of a protein layer resistant to 30 seconds of vigorous irrigation, and they agreed that it would be necessary to repeat the etching procedure to ensure adequate bonding of a resin material.

In general, 2 methods of isolation from salivary contamination are used: rubber dam or cotton roll isolation. Several clinical studies have demonstrated that rubber dam isolation and cotton roll isolation provide comparable retention rates[90,91]. In the longest published comparison study, Lygidakis et al[90] found that after 4 years of application the complete retention rate was 81% for sealants placed using cotton roll isolation and 91% for sealants placed using rubber dam isolation. Rubber dam isolation is ideal but may not be feasible in certain circumstances. Clinical studies using Vac-Ejector moisture control, another alternative to the rubber dam, concluded that sealant retention is comparable to that with sealant placed under rubber dam or cotton roll isolation[92,93]. Interestingly, reports indicate that applying a halogenated bonding agent (Scotchbond®) after acid etching significantly increased the bond strength of sealant to saliva-contaminated enamel, and also to uncontaminated enamel[94,95].

It has been shown that sealants, placed soon after tooth eruption, are far more likely to need replacement. Additionally, tooth position in the mouth appears to be an important determinant for adequate isolation[63,96]. Many

of the resin trials included premolar teeth, and sealant retention has been found to be superior for the more anteriorly placed teeth[17,97,98]. Sealants have been recorded as being more effectively retained on lower teeth than on upper teeth[99,100]. The cooperation of the patient, the skill of the operator[19], and the presence or absence of a dental assistant[101], altogether are important factors affecting sealant retention.

Etchants and Conditioners

The goal of etching is to produce an uncontaminated, dry, frosted surface[3]. Acids, such as phosphoric, maleic, nitric, or citric acid, are used 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. Etching implies the dissolution of the substrate, whereas conditioning involves cleaning, structural alteration, and increasing the adhesiveness of the substrate[102]. Resin-based fissure sealants are usually placed after cleansing and orthophosphoric acid etching of the fissure enamel[103].

Orthophosphoric acid.   The most frequently used is orthophosphoric acid, provided that 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[35]. Orthophosphoric acid 36% is available as both a liquid solution and a gel. Numerous studies *in vitro*[104-107], found similar penetration of enamel, while *in vivo* studies[108] showed that gel etchant was as effective as the liquid form. The clinical disadvantage lies in the doubling of the rinsing time required with the gel form[33]. However, many clinicians prefer to use a gel because it is easily applied and controlled and because of its colour, easy to tell where it has been applied[34].

Variation in time during which the tooth enamel is exposed to the etching solution is more important. Several laboratory studies involving permanent teeth have shown resin-to-enamel bond strengths after 15-seconds to be comparable to those after 30- and 60-seconds etches[107,109,110]. Clinical studies comparing the same etching times (20 and 60 seconds) resulted in no statistically significant differences in retention rates[111,112]. Laboratory studies indicate that it may be more difficult to gain adequate retention by etching the enamel of primary teeth[113,114], but clinical studies[112] suggest it may not be necessary to increase the etching time when sealing primary molars. Redford et al[115] in the *in vitro* study showed that the etch depth increases between 60-120 seconds, but there was no corresponding increase in bond strengths. More recently, Duggal et al[116] showed 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.

After etching, the tooth is irrigated vigorously with both air and water for 30 seconds and then dried with uncontaminated compressed air for 15 seconds[3]. It has been suggested washing for 60 seconds if an etchant in solution is used and 90 seconds when a gel etchant has been applied. Compressed air is checked for contamination by directing the flow onto paper or a clean mirror surface; contaminants will appear as droplets of water or oil[117]. According to Waggoner and Siegal[35], 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 its surface and give a chalky, frosted appearance.

Maleic acid.   Combining acidic conditioners and resin primers began several years ago with the development of self-etching primers, such as those provided with Scotchbond 2® (2.5% maleic acid in 55% HEMA/water - 3M Dental Products), Syntac® (4% maleic acid in 25% TEGMA/water - Vivadent) and recently NRC® (maleic acid in itaconic acid and water - Dentsply). These primers are acidic enough to demineralise 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[102,118].

Fluoride and Pit and Fissure Sealants

Ripa[21], in his review article, stated that as fluoride becomes more ubiquitous in the UK, the difference in caries activity between smooth and pit- and fissure-surfaces becomes more pronounced and dental caries is becoming primarily a disease of the pits and fissures. Pit and fissure sealants were established as the only clinical regimen available for preventing occlusal caries[31]. In an effort to enhance the caries protective benefits of sealants, several kinds of fluoride fissure sealants have been developed over the years[119].

The addition of fluoride to pit and fissure sealants was considered more than 25 years ago[16,120-122] but were not found to reduce caries incidence perhaps because they were poorly retained on the tooth surface. Efforts to combine the 2 continue today[123,124]. According to Kadoma et al[125] the properties a fluoride containing sealant should have in order to replace a conventional one are listed in the table 4.

Better or at least comparable retention rates with the conventional sealant

Constant fluoride release for a prolonged period of time

Function as a reservoir of fluoride ion for enamel and to promote fluorapatite formation in enamel

Methods of Fluoride Incorporation in Pit and Fissure Sealants

Fluoride is incorporated into resins in 1 of 2 ways; the first utilizes a soluble fluoride salt which, after application, dissolves releasing fluoride ions, possibly compromising the integrity of the resin[19]. This method has been questioned, because fluoride release resulting from the dissolution of a soluble salt might weaken the sealant *in situ* and thereby might reduce its usefulness as a preventive agent[126]. The other system uses an organic fluoride that is subsequently released by an exchange with other ions in the system[19,127]. In this method (anion exchange systems), fluoride constitutes only a small amount of the total structure, and is replaced rather than lost. Thus, there should not be any significant decrease in the strength of the sealant[126].

Soluble Fluoride Salts Added to Unpolymerized Resins

Lee et al[120] were the first to formulate a polyurethane fluoride-containing sealant material that would release fluoride on the enamel surface for an extended period of 24h - 30 days. They concluded that Na_2PO_3F added to polyurethane reduced enamel acid solubility, increased fluoride uptake in enamel and released fluoride up to 1 month.

Swartz et al[122] conducted an *in vitro* study to test the feasibility of imparting anti-cariogenic properties by adding 2-5% NaF to BIS-GMA resin pit and fissure sealants. The findings revealed a reduction of enamel acid solubility and an increased enamel fluoride uptake. The physical properties of the resins remained the same. However, the greatest amount of fluoride was released during the first day or two, after which the amount rapidly diminished.

Based on the previous study, el-Mehdawi et al[128] studied, *in vitro*, the fluoride release of an ultraviolet fissure sealant (Nuva-seal) throughout a 3-week period by adding several concentrations of NaF to the sealant. They concluded that Nuva-Seal decreased fluoride release over the 3-week study period, while the quantity of fluoride ions increased when the concentration of the fluoride salt in the sealant increased.

In 1990, a commercially available sealant with fluoride was marketed that purportedly released fluoride. This product (FluoroShield) was a visible light-cured resin containing 2% NaF and 50% by weight inorganic filler[129]. Cooley et al[129] compared in their *in vitro* study, FluoroShield with a fluoride sealant (Helioseal). They found no significant difference between the 2 sealants in ability to penetrate fissures, but FluoroShield was found to have more leakage. All specimens of the FluoroShield released fluoride over the 7-day period; there was a 'burst effect' in which larger amounts of fluoride were released on the first and the second day, and then the release tapered off. Jensen et al[130] in the *in vitro* study, compared the size and depth of artificial caries lesions when using FluroShield or its non-fluoride containing analogue, PrismaShield. Lesion depth was found to be over 3-times greater in specimens that contained the conventional sealant compared with specimens that contained the fluoride-releasing sealant.

Hicks and Flaitz[119], in another *in vitro* study, compared the effects of FluroShield, PrismaShield and Ketac-Fil (GIC material) on initiation and progression of caries-like lesions around class V restorations. They concluded that FluroShield and Ketac-Fil showed less lesions than PrismaShield.

Park et al[38] compared FluroShield, PrismaShield and Delton pit and fissure sealants to each other through shear bond strength, scanning electron microscopy and microleakage. They concluded that the shear bond strength in FluroShield and PrismaShield was significantly higher than in Delton, better adaptation to the etched enamel with FluroShield and PrismaShield than with Delton, and no significant difference in microleakage among the 3 pit and fissure sealants.

Loyola Rodriguez and Garcia-Godoy[123] estimated the antibacterial activity and the fluoride release, of FluroShield, Helioseal and a new fluoride containing sealant Teethmate F. Only Teethmate F showed inhibition activity against all strains of *Mutans Streptococci* tested; there was no significant difference in the inhibition between strains of *S. Mutans* and *S. Sorbinus*. Teethmate exhibited higher fluoride release than FluroShield during the 7-day study period. During 2 days after setting, these materials showed their highest concentration of fluoride release, which decreased to approximately 50% (below 0.1 PPM F⁻) at 7 days. Rock et al[124] came to similar results regarding fluoride release, *in vitro*, from FluroShield in comparison to a GIC material Baseline. They also found 70% complete retention of FluroShield in first permanent molars, *in vivo*, after a 3-year follow-up.

In another clinical study, Jensen et al[130] evaluated the retention and salivary fluoride release of FluroShield compared to its non-fluoride analogue PrismaShield. There was no significant difference in retention between the 2 sealants at 6 and at 12 months. However, fluoride release was significantly increased when compared to the

baseline values, only at the 30 min post-sealant sampling interval. Rock et al[124] found 70% complete retention of FluroShield applied to contralateral caries-free first permanent molars in 86 children aged 7-8 years, after a 3-year follow-up. Do-Rego and de Araujo[131] found that 91.35% of FluroShield and 93.14% of Delton Plus sealants were intact after 2 years of follow-up.

Lygidakis and Oulis[132] evaluated the retention rate and the caries increment differences between FluroShield and Delton. The sealants were applied in a half-mouth design to all 4 caries-free first permanent molars of 112 children aged 7-8 years. At a 4-year follow-up, the complete retention for FluroShield was 76.5% and for Delton 88.8% - the difference being statistically significant.

Morphis and Toumba[133] evaluated the retention rates of 3 different sealants: a conventional sealant Delton, its recently marketed fluoride-containing analogue Delton Plus, and an experimental fluoride-containing sealant, which was prepared by adding fluoride-glass powder to Delton. The sealants were applied to 104 permanent molars in children aged 6-16 years, in a randomized way. Results showed no significant difference in retention among the 3 sealants after a 1-year follow-up.

Organic Fluoride Compounds Chemically Bound to the Resin (Anion Exchange System)

Instead of incorporating fluoride into an inert sealant material, ion exchanging resins were developed[134,125]. These resins have relatively high fluoride content and exchange fluorine ions from the sealant materials for hydroxyl and chloride ions in the oral environment. Inhibition of caries formation and re-mineralization of enamel caries have been shown to occur *in vitro* and *in vivo*. A significant level of fluoride is taken up by the sealed enamel. Both superficial and deep enamel layers incorporate the released fluoride, with fluoride levels of 3500 ppm and 1700 ppm reported for enamel biopsy depths of 10 μm and 60 μm, respectively, while the fluoride levels were 650 ppm and 200 ppm for the same enamel biopsy depths in contra-lateral control teeth[134]. Research of the anion exchange system-sealant is in progress but, to date, no commercial product is available[5].

Conclusions

Pits and fissures are recognised as highly susceptible to caries and least benefit by systemic or topical fluoride. Sealants do prevent caries[59] and are cost-effective[112]. Mertz-Fairhurst[59] reported in 1984 that at the end of 10 years 78% of those first permanent molars with a single application of sealant placed in pits and fissures were caries free compared with the unsealed matched pairs which had a caries free rate of 31.3%.

Fluorides also work in more than one way. They reduce enamel solubility and stimulate re-mineralization, actually reversing the course of caries during its early stages[126]. For these reasons fluoride has been incorporated into pit and fissure sealants. The rationale is that the sealants act as reservoirs from which the added fluoride is gradually released into the oral cavity[127]. It is essential that the effective levels of fluoride release are maintained for long periods of time, preferably at a constant rate, for at least 6 months since these materials are always subjected to leaching by saliva[135].

Despite the fact that no anti-caries clinical studies have been reported[21], *in vitro* studies indicate that a fluoride releasing sealant substantially reduces the amount of enamel demineralization adjacent to it[130]. However, the main problem with the existing fluoride releasing sealants is that they give no lasting effects on salivary fluoride concentration levels[124, 129, 130].

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Fluorine Content of Drinking Water in Relation to the Geological-Petrographical Formations From FYROM

SUMMARY

The aim of the study was to determine the association between different concentrations of the fluoride ion in drinking water and some geological variables in FYROM, by using information from Institute for Geological and Mineral studies. From May 2003 to May 2004 we studied the fluoride concentration in the sources of drinking water in 92 localities. Measurements of F-concentration were performed using a special ion-Analyser Model EA 920 produced by ORION and a special F-electrode. For the chemical analysis 10% TISAB-Aluminon (Total Ionic Strength Adjusted Buffer) was used.

Starting of the 68 settlements of the republic, 9 were found to have naturally fluoridated drinking water. Highest concentrations were found in 3 thermal baths (Katlanovo, Bansko and Negorci). Optimal fluorine contents were found in the tap water from Gratsko, Kolesino and Stip, and suboptimal in the southern region of the country (Balinci, Marvinci, Brajkovci, Murtino and Pirava) mainly, with the exception of Kocani, which is situated in the eastern part of the country. As a total, 80.300 people are gaining benefit from the naturally fluoridated water. The water from lake Dojran contained high 5,6 ppm F natural fluoride concentration. The lake is situated in the southern region of the country.

Geological-petrographical characteristics of the terrain can help in identifying areas with optimal or high concentrations of the fluorine ion in the drinking water, so the volcanic rocks as well as the geothermal fluids might be considered to be key factors that lead to unusually high concentrations of fluorine within water.

Keywords: Natural Fluoridated Water; Geology

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Introduction

Fluorine contents in drinking water samples are affected by factors such as availability and solubility of fluorine-containing minerals, rock's or soil's porosity through which the water passes, residence time, temperature, pH and the presence of other elements, e.g. calcium, aluminium and iron, which may complex with fluorine[1]. Water is the major source of consuming fluorine for people[8]. There is no water which does not contain fluorine at all, but there are waters with various fluorine contents, depending on a whole series of factors that have mostly geological origin[1,2]. Being familiar with the fluorine content of the drinking water for each area is especially important datum for the dentist. In many countries, separate maps of naturally fluoridated drinking water have been made[7,10].

There are 150 minerals which contain fluorine, although the most important are as follows: fluorite (CaF_2; 49%F), fluor-apatite ($Ca_{10}F_2$ $(PO_4)_6$; 3.4% F), cryolite (Na_3AlF_6; 54%F) and etc[5]. Fluorine distribution is the most intensively expressed within acid magmatic rocks (granites, granodiorites etc)[1,5]. The fluorine contents of within magmatic rocks are as follows: ultrabasic 100 ppm, basic 400 ppm, intermediate 500 ppm, acid rocks 735 ppmF. Fluorine distribution inside sedimentary rocks is as follows: sandstone 270 ppmF, carbonate 330 ppm, clay 740ppm, Shales 740ppm[1,5].

The main purpose of this paper was to determine the relation between different fluorine ion contents in drinking

water and some geological-petrographical variables in the FYROM, using information from Geological and Mineral Survey Institute, Skopje.

Material and Method

From May 2003 to May 2004 we studied the fluorine contents in the sources of drinking water for 92 localities (tap water from urban and rural communities, dug wells, thermal baths, natural springs and water from 3 lakes). The collecting method and storing the water samples were predetermined. Plastic (polyethylene) bottles were used, because of the reaction of fluorine with the glass and they were washed out with the water sample. Collected bottles were stored in a cool place until the start of fluorine measurement. Time between collection and measurement was no longer than 2 months.

The appropriate data, e.g. the kind of water sources (surface water, drilled or natural spring), were taken from the local records onsite. The measurements of F-contents were performed at the University of Thessaloniki, department of Preventive Dentistry, Periodontology and Implant Biology, using a special ion-Analyser Model EA 920 equipment produced by ORION, and a special F-electrode. For the chemical analysis 10% TISAB-Aluminon (Total Ionic Strength Adjusted Buffer) was used. The electrode was adjusted against standard F-solutions (0.1 to 1 ppm, and 1.0 to 10 ppm F).

Information was collected from the local authorities, Geological and Mineral Survey Institute, the Republic Institute for Health Protection, as well as the State statistical Institute of the FYROM.

Results and Discussion

On the basis of the obtained results of each drinking water sample, the cities have been classified into 5 categories (Tab. 1).

Table 1. Summary statistics of determined F values in drinking water samples in the FYROM

	>1.1 ppmF	0.7-1.0 ppmF	0.4-0.6ppmF	0.2-0.3 ppmF	<0.2 ppmF
No of samples	3	3	6	8	51
Minimum	1.8	0.75	0.45	0.20	0.021
Maximum	3.4	0.86	0.59	0.28	0.19
Mediana	2.6	0.86	0.48	0.24	0.10
Mean	2.6	0.823	0.495	0.24	0.109
SD	0.80	0.064	0.048	0.031	0.043
No of inhabitants		46.700	33.600	50.600	1.050.000

Waters with high F-contents (> 1.1 ppm);
Waters with ideal F-contents (0.7 - 1.0 ppm);
Waters with suboptimal F-contents (0.4 - 0.6 ppm);
Waters with insufficient F-contents (0.2 - 0.3 ppm);
Waters with a lack of F (< 0.2 ppm).

The examination revealed as follows: a) 3 thermal baths with fluorine containing waters above the optimal concentrations (1.5 - 5.3 ppm F); b) 3 settlements with optimal F-concentration (0.7 - 1.2 ppm F) with 46.700 inhabitants; c) 6 settlements with suboptimal F-content (0.4 - 0.65 ppm F) beneficial to 33.600 inhabitants; d) 8 settlements with insufficient F-concentration (0.2 - 0.3) with 50 600 inhabitants; e) the remaining 51 communities (including city of Skopje, with population of approximately 1 million) with water containing only traces of F (< 0.3 ppm F).

The territory of the FYROM is characterised by a very complex geological-petrographical composition. According to the geotectonic structure of the terrain, as well as general evolution of the same, from east to west, in the territory of the FYROM 4 structural facial zones can be distinguished: Serbo-Macedonian Mass, Vardar zone, Pelagonian-horst-anticlinorium, and the Western-Macedonian zone[6]. Different types of rocks are represented from the oldest to the youngest geological formations. The tectonic structure of the terrain, especially the neotectonics, is influencing formation of the thermal, thermomineral and mineral basins of the aquifer water[4,6]. These waters are mainly found in direct relation with the tectonically active faults. The largest number of them is found in the area of tectonically very unstable Vardar zone[6].

According to the recent examinations given in this paper, the water from the Dojran lake contains 5.6 ppm F.

Dojran Lake is of tectonic-volcanic genesis. The lake is a natural rarity and unique in the region and its surrounding. It has been located on the main tectonic regional structure that represents a border line between the Rodop mass and the Vardar zone[9]. The special geological conditions that lead to high concentrations of fluorine within water are connected to the volcanic activity, acid rocks very poor with calcium and fluorine abundant, which along with high temperatures leads to release of fluorine from the rocks or fluids after eruptive processes, and hydration within water bodies[1,10].

According to the geologic formations through which the water drains, using the geological map of our country (Fig. 3), we grouped the samples (Tab. 2) of water into waters that drain through volcanic rocks, granites schists, basites and carbonates (marble, limestone). So,

the drinking waters originated from carbonate faces (limestone, marbles, etc) show lowest fluorine contents (0.096 ppm in average). The drinking waters originating from mafic rocks show a little bit higher value (0.129 ppm in average), the schists much higher (0.249 ppm in average), the granites much higher (0.533 ppm in average), and the volcanic rocks show highest fluorine contents (2.2 ppm in average).

According to the achieved results, the fluorine contents in the drinking water from the FYROM can be quite well compared with the geological-petrographic composition. In the contributed figure 1 a corellation between the average fluorine values in the water samples and the geological formations through which water drain can be seen. The results are depicted on a chart of FYR of Macedonia (Fig. 2).

Table 2. Summary statistics of measured F values in drinking water samples that originate from different groups of rocks (geological formations)

	Volcanic rocks	Granites	Schists	Mafic rocks	Carbonates
Nor of samples	3	12	13	4	39
Minimum	0.26	0.071	0.11	0.09	0.021
Maximum	5.6	1.8	0.86	0.19	0.23
Mean	2.2	0.533	0.249	0.129	0.096
Median	0.75	0.48	0.20	0.113	0.098
SD	2.95	0.449	0.210	0.043	0.048

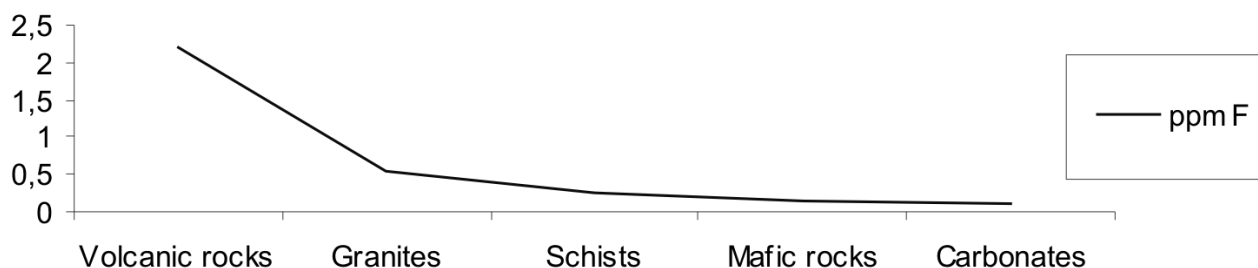*Fig. 1. Correlation between the average contents of F in the water samples and the geological formations from which the water originate*

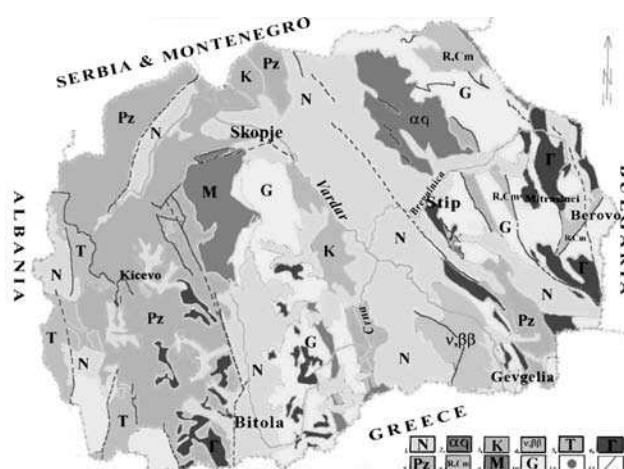*Figure 2. Map of naturally fluoridated drinking waters in the FYROM*

Figure 3. Geological map of the FYROM N-Neogen, αq-volcanic rocks, K-Creta, vßß-gabbros and diabases, T-Triassic, G-granites, Pz-Paleosoic, RCm-Reef Cambrian, M-marbles and G-gneisses

Conclusions

Starting of the 68 settlements of the republic, 9 were found to have naturally fluoridated drinking water. The highest concentrations were found in three thermal baths (Katlanovo, Bansko and Negorci); optimal fluorine contents were found in the tap water from Gratsko, Kolesino and Stip and suboptimal mainly in the southern region of the country (Balinci, Marvinci, Brajkovci, Murtino and Pirava), with the exception of Kocani, which is situated in the eastern part of the country.

As a total, 80.300 inhabitants are gaining benefit from the naturally fluoridated water. Geological-petrographical characteristics of the terrain can help identify areas with optimal or high concentrations of the fluorine ion in the drinking water, so the volcanic rocks, as well as the geothermal fluids, might be considered to be key factors that lead to unusually high concentration of fluorine within water.

Most of the children population in the FYROM during the period of their teeth formation drink water with very low concentration of fluorine, which is insufficient for prevention of dental caries.

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Analysis of Buffer Value of Bicarbonate In Saliva

SUMMARY

Saliva has a buffer capacity which neutralizes acids in the mouth. The buffer capacity of human saliva is regulated by 3 buffer systems: the carbonic acid/bicarbonate system, the phosphate system, and the proteins. In non-stimulated saliva, the concentration of inorganic phosphate is rather high, while the concentration of carbonic acid/bicarbonate system is low. Carbonic acid/bicarbonate system is the most important buffer in stimulated saliva due to its higher concentration. The aim of this study was to determine salivary bicarbonates levels in patients with different degree of caries activity. We examined 60 children of both sexes, 16 years of age, which were divided in 2 groups according to the condition of the teeth, i.e. the DMFT - index: the first group consisted of 30 examinees with very low caries index (0-3), and the second group consisted of 30 examinees with high value degree of caries (>10). Concentration of salivary bicarbonates was determined with the enzyme method of continuous measuring ("Cobas Mira" - Roche Diagnostic Systems), within different periods: 5, 30, 60 and 120 min. after consuming the meal, as well as before consuming the meals - basic values.

The results refer to close connection between the concentrations of the salivary bicarbonates with the occurrence of dental caries. The concentration of the salivary bicarbonates were remarkably higher (p < 0.01) in examinees with lower DMFT- index, compared with the examinees with higher values of DMFT. This refers to the basic values as well as to the values of the bicarbonates in stimulated saliva. The obtained results confirm the importance of the buffer capacity role of the salivary bicarbonates within the oral media and may serve as parameters for determining the caries risk; according to that, we can plan and take appropriate caries-preventive measures.

Keywords: Saliva; Salivary Bicarbonates; Dental Caries

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Introduction

General term "saliva" refers to the fluid that surrounds all oral hard and soft tissues. This oral fluid (that is, whole saliva) represents a mixture of individual fluids and components derived from several sources. Major and minor salivary glands make the bulk contribution to whole saliva, with minor contributions from non-glandular sources, such as crevicular fluid, oral microorganisms, host-derived cell, and cellular constituents, as well as diet-related components[1,2,11].

The fluids secreted by the parotid, submandibular, sublingual, and minor salivary glands have been shown to differ considerably from each other, to be complex in composition, and to be affected by (1) type, intensity, and duration of stimulation, (2) time of day, (3) diet, (4) age, (5) a variety of diseases, and many pharmacologic agents.

During the day 0.5 - 1.0 litre per day saliva is produced. Whole saliva is about 99% water and contains a mixture of inorganic ions, including calcium, phosphate, sodium, potassium, chlorine, bicarbonate and magnesium, together with some minor ionic components, including fluoride. Apart from these inorganic components, pooled saliva also contains very wide range of organic molecules. Some of these are simple proteins, such as the enzyme albumin, together with free amino acids. However, the bulk of the organic component is made up of a group of complex glycoprotein, the mucins[13,15,16].

Salivary secretion is an important factor for oral health, accomplishing mechanical cleansing and protective

functions through various physiological and biochemical mechanisms[3,9,18].

Theoretically, saliva can affect caries in four general ways:
- mechanical cleansing, resulting in less accumulation of plaque;
- reducing enamel solubility by means of calcium, phosphate and fluoride;
- buffering and neutralizing the acid produced by cariogenic organisms or introduced directly through diet;
- by anti-bacterial activity.

Buffer Systems

Solutions containing both weak acids and their salts are referred to as buffer solutions. These solutions have the capacity of resisting changes of pH when either acids or alkalis are added to them.

Maintaining of buffer capacity of the acid - base balance is one of the most important protective functions of the saliva. The buffer capacity of human saliva is regulated by 3 buffer systems - the carbonic acid/bicarbonate system, the phosphate system, and the proteins. The carbonic acid/bicarbonate system is the most important one in saliva, but only at high flow rates. Its concentration varies from less than 1 mmol/l in non-stimulated parotid saliva to almost 60 mmol/l at very high flow rates. Thus, in non-stimulated saliva, the level of bicarbonate ions is too low to be an effective buffer[7,8].

Several studies have show that bicarbonate is one of the salivary components that potentially modifies the formation of caries by changing the environmental pH and possibly the virulence of bacteria that cause decay. Tanzer et al[20,21] tasted the efficacy of a sodium bicarbonate based dental power and paste with the addition of fluoride on dental caries and on *Streptococcus sobrinus* or *Streptococcus mutans* recoveries in rats. These authors observed that the caries reductions in these studies ranged from 42 to 50% in the rats treated with bicarbonate dentifrices when compared with rats treated with water[4,10,12,14,20,21].

The **aim** of this study was to determine salivary bicarbonates and urea levels in the patients with different degree of caries activity.
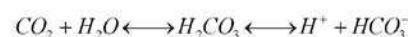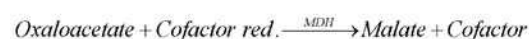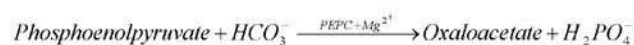
Material and Method

60 children (30 males and 30 females), 16 years old, with same diet habits, in good health except dental caries, took place in our examination. According to their DMFT-index, they were divided in 2 groups: first group consisted of 30 examinees with very low caries index (0-3), and second group consisted of 30 examinees with higher value degree of caries (>10).

The concentration of salivary bicarbonates wa determined within different periods: 5, 30, 60 and 120 min.

after consuming the meal, as well as before consuming the meals - basic values.

For the collection of non-stimulated saliva, the patient was seated comfortably, with their eyes open, in a standard dental chair. The child sat with their head bent forward and after an initial swallow spat out into a graduated tube approximately every 30s for 5 min. The samples were taken in sterile calibrated bottles (specially intended for this purpose). The collection volume was about 5 ml. The saliva was kept at 4^0C and transported to the laboratory within 30 minute, centrifuged for 30 minute and the supernatant part was analyzed. HCO^{3-} concentration was determined by enzymatic colorimetric method using a commercial kit from GmbH Diagnostic. For enzymatic test phosphoenolpyruvate carboxylase (PEPC) and a stable NADH analogue were used[17], utilizing the principle:

$$Phosphoenolpyruvate + HCO_3^- \xrightarrow{PEPC+Mg^{2+}} Oxaloacetate + H_2PO_4^-$$

$$Oxaloacetate + Cofactor\ red. \xrightarrow{MDH} Malate + Cofactor$$

$$CO_2 + H_2O \longleftrightarrow H_2CO_3 \longleftrightarrow H^+ + HCO_3^-$$

The decrease of reduced cofactor concentration was measured at 405 or 415 nm and it was proportional to the concentration of total carbon dioxide in the sample.

For statistical evaluation, a 1-way analysis of variance (ANOVA) was initially used to see if there was a significant difference between 2 groups; the Student "t" test was used to compare the DMFT and concentration of HCO^{3-} between 2 groups.

Results

Table 1 shows the basic values of concentration of salivary bicarbonates in both groups. There was a significant difference ($p < 0.01$) in bicarbonate concentration between first and second group.

Table 1. Basic values of concentration of

salivary bicarbonates (mmol/l)

Group	$\overline{x}$	SD	SE	min	max
I	7.94	0.8700	0.1588	6.70	9.80
II	2.48	0.7993	0.1459	1.00	3.90

t = 25.298; df = 58; p < 0.01

Values of the salivary bicarbonate in 5 min period after consuming the meal are illustrated in table 2. The concentration of the salivary bicarbonate in first group was 6.76 ± 1.3402 (SE 0.2447), and 4.66 ± 0.9409 (SE 0.1718)

in the second group. The results display high statistically significant difference (p < 0.01) between both groups.

Table 2. Values of salivary bicarbonates in

5 min period after consuming the meal (mmol/l)

Group	$\overline{X}$	SD	SE	min	max
I	6.76	1.3402	0.2447	3.90	9.10
II	4.66	0.9409	0.1718	2.70	5.90

t = 7.046; df = 58; p < 0.01

After 30 min of consuming the meal, concentration of the salivary bicarbonate in first group was 5.94 ± 1.996 (SE 0.2190), and 3.74 ± 1.0539 (SE 0.1924) in the second group (Tab. 3). The results display statistically significant difference (p < 0.01) in bicarbonate concentration between first and second group.

In table 4 the values of the salivary bicarbonate concentration after 60 min and 120 min of consuming the meal are illustrated, and it's very clearly that the values are lower than the values after 5 min of consuming the meal; however, there is still some difference between both groups.

Table 3. Values of salivary bicarbonates in 30 min period after consuming the meal (mmol/l)

Group	$\overline{X}$	SD	SE	min	max
I	5.94	1.1996	0.2190	3.00	7.90
II	3.74	1.0539	0.1924	1.50	5.30

t = 7.546; df = 58; p < 0.01

Table 4. Values of salivary bicarbonates in 60 min and 120 min period after consuming the meal (mmol/l)

Time	Group	$\overline{X}$ ± SD	t	df	p
Bicarbonates - 60 min	I	4.85 ± 0.9580	6.926	58	p > 0.01
	II	3.08 ± 1.0206			
Bicarbonates - 120 min	I	4.51 ± 0.9011	6.439	58	p > 0.01
	II	2.94 ± 0.9856			

Discussion

Dental caries is a multifactorial disease, which has been afflicting people throughout ages. An important factor which influences the development of dental caries is saliva. There are also studies showing the effect of diet on saliva secretion and caries development. Saliva provides one of the principal defence mechanisms in the mouth and is know to be important in the pathogenesis of dental caries. Saliva also helps acids quickly to clear away food debris from the mouth and to buffer the organic acids that are produced by the bacteria.

Saliva's protective role is very important to maintain a neutral pH in plaque and in the oral cavity. Its ability to perform this function can largely be attributed to bicarbonates and to a lesser extent to phosphate, as well as other factors. The chief salivary buffer is the carbonic acid/bicarbonate system, while phosphates and proteins play a minor role. The bicarbonate ions, possibly other salivary components, are important in the buffering capacity of this oral fluid and their neutralization of dietary acids will help to determine the pH at the tooth surface after eating.

When saliva secretion is stimulated, the increased rate of flow through the ducts means that there is little time for the ducts to re-absorb sodium and chloride, and the fluid resembles the isotonic primary secretion. Another changer the secretion of bicarbonate ions, which means the composition of saliva, now, is very different from the resting secretion. This bicarbonate raises the pH of the saliva, and greatly enhances its buffering power. The saliva is now more effective in neutralizing and buffering foods, and acids arising in plaque from the fermentation of carbohydrate[5,6,19].

The results obtained in this study refer to the close connection between the concentrations of the salivary bicarbonates with the occurrence of dental caries. The concentration of the bicarbonates were remarkably higher (p < 0.01) in examinees with lower DMFT index, compared to the examinees with higher values of DMFT. This refers to the basic values as well as to the values of the bicarbonates in saliva within different periods from the moment/time of mechanical stimulation (having a meal).

The obtained results confirmed the importance of the buffer capacity role of salivary bicarbonates within the oral media and its responsibility for rapid neutralization of the acid.

Conclusions

Saliva has an important role in maintaining oral health. Saliva accomplishes its mechanical cleaning and protective functions through various physical and biochemical mechanisms. Saliva has a buffer capacity which neutralizes acids in the mouth.

The carbonic acid/bicarbonate system is the most important buffer in stimulated saliva due to its higher concentration. The values of the bicarbonates in saliva may serve as parameters for determining the caries risk patients and, according to that, we can plan and take appropriate caries-preventive measures.

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Fluoride Contents in Teas and Investigation of Children's Tea Consumption in Relation to Socioeconomic Status

SUMMARY

The aim of the study was to determine the fluoride content in teas and investigate the consumption frequency of tea by children with special reference to socioeconomic status. Tea infusions for herbal teas (n=6) and black tea - Camellia sinensis (n=7) were prepared according to the manufacture's instructions. 9 samples were prepared by inserting the tea bag to hot water and 4 kinds of tea were brewed for 2 hours. The fluoride contents of the infusions were measured by using ion specific fluoride electrode. Questionnaires were filled for 120 children from low and 80 children from high socioeconomic status. The amount of herbal and black tea consumed by children were recorded.

The findings of the study revealed that the fluoride contents of herbal teas were ranging among 0.12 to 0.17ppm. Fluoride levels of black tea increased by the brewing time and were measured between 0.62ppm to 1.17ppm. Questionnaire findings showed that children from low socioeconomic status consume black tea more frequently but, in general, children do not drink tea regularly. Although children do not prefer highly to drink black tea, the effect of high fluoride content of tea after brewing on dental caries and dental fluorosis should be evaluated by further studies.

Keywords: Fluoride; Black Tea; Herbal Tea

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Introduction

Tea is an infusion of dried leaves of the plant *Camellia sinensis* and is consumed as a very popular drink all around the world. Dried tea is produced each year mainly in India, China, Sri Lanka, Turkey, Russia and Japan[1]. Teas are classified into 3 major types according to the manufacturing process. These are non-fermented green tea, the semi-oxidized oolong tea and the fermented black tea. The manufacturing process can affect the properties of various teas. Green tea is the richest in the antioxidant constituents of pharmacological interest[2].

Many studies have shown antimicrobial activity and oral health benefits of green tea or oolong tea[3-7]. Tea polyphenols, oxidized polyphenols called tannins, antioxidant nutrients such as carotenoids, tocopherols, ascorbic acids and fluoride have been accepted as important components of tea on dental health[1,2,8].

Fluoride significantly reduces caries risk. Studies showed that children in communities with fluoridated water had fewer cavities than the children living in communities with insufficient fluoride in early sixties[9,10]. In developed countries, risk of dental fluorosis lead researchers to re-evaluate the benefits of systemic fluoride[11,12]. Epidemiological evidence showed that ingestion of high concentrations of fluoride could cause severe fluorosis. With widespread usage of fluoride toothpaste and other fluoride sources such as processed foods and beverages, greater fluorosis risk prompted the investigators to suggest various educational efforts and controls of extraneous sources of fluoride[9-12]. High levels of fluoride in tea may have anti-caries potential, but the role on dental fluorosis should be taken into account especially for young consumers.

Studies have focused primarily on black tea since 80% of the tea consumed is this type, especially in Europe and North America[1,2]. However, there is a wide variety of tea and herbal tea available in the market, with no data on fluoride content. Therefore, the purpose of this study was to determine fluoride concentrations in black and herbal teas available in Turkey, and investigate the

children's tea preferences and frequency with reference to socioeconomic status (SES).

Materials and Methods

Preparation of Tea Infusions

A total of 13 commercial herbal and black tea samples were used (Tab. 1). 2 samples from each tea brand were purchased from the market and prepared. All the samples were prepared in plastic containers with lids.

Tea samples that were presented as tea bags were prepared by keeping a bag in 100ml boiled distilled water (100 °C) for 5 minutes. The infant tea, presented as a brewing bag, was prepared by adding 100 ml distilled water at 80°C and brewed in boiling tank for 2 hours. Dried tea leave samples were weighed and 1g of tealeaves was brewed in 100 ml of distilled water at 80°C for 2 hours in a boiling tank.

Table 1. Tea samples and their preparation techniques

Tea Type	Preparation
Herbal teas	
Apple	Tea bag
Linden	Tea bag
Daisy	Tea bag
Rosehip	Tea bag
Children's tea*	Tea bag
Infant tea**	Brewing bag
Black teas	
Lipton Yellow Label	Tea bag
Stassen Pure Ceylon Tea	Tea bag
Lipton (Strawberry)	Tea bag
Lipton Earl Grey (Bergami)	Tea bag
Lipton Yellow Label	Tea leaves
Caykur Rize	Tea leaves
Tomurcuk (Bergami)	Tea leaves

* Content of children's tea: Fennel, anise, root of licorine plant, peppermint leaves, yellow daisy flower

** Content of infant tea: Daisy, peppermint, anise)

Measurement of Fluoride Content

Fluoride contents were measured by using a fluoride ion selective electrode (96-09 BN Orion Ionplus fluoride) attached to a digital pH-meter (Jenco 671P). The fluoride ion selective electrode was calibrated by standard solutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} M NaF at the start of the measuring and repeated every 2 hours. Equal amounts of TISAB II buffer solution was added to the samples during fluoride measurements. The measured fluoride content was in milivolt, so a computer programme was used to change milivolt readings to ppm values.

2 ml of tea was taken from samples prepared by tea bags straight after the preparation and fluoride content was measured. This was repeated 3 times for each tea sample. Mean of 3 measurements was recorded.

Fluoride contents of the brewed samples were measured in 5 minutes, 10 minutes, 15 minutes, 30 minutes, 1 hour and 2 hour intervals. 2 samples for each time interval was taken and mean of both measurements were recorded.

Questionnaire

A questionnaire consisting 10 questions on SES and tea drinking frequency was applied to 200 children at the age of 8-9 years. All children were living in Izmir, with fluoride concentration of 0.4ppm in drinking water. Children were categorized as none, medium (1-3 cups/day) and heavy (>4 cups/day) drinkers according to their tea consumption frequency per day. The findings were evaluated statistically by χ^2 test.

Results

Fluoride contents of teas

Fluoride contents that were measured after 5 minutes for teas prepared by tea bags are presented in table 2. Fluoride contents of teas prepared by brewing for 2 hours are presented in table 3.

Table 2. Fluoride contents of teas prepared by tea bags (ppm)

Herbal teas	Fluoride content (ppm) ± SE
Children's tea	0.12 ± 0.003
Linden	0.12 ± 0.006
Daisy	0.12 ± 0.005
Rosehip	0.14 ± 0.005
Apple tea	0.17 ± 0.003
Black teas	
Lipton yellow label	0.32 ± 0.006
Lipton – Strawberry flavour	0.92 ± 0.008
Stassen Pure Ceylon Tea	1.09 ± 0.002
Lipton Earl Grey	1.27 ± 0.005

Table 3. Fluoride contents of teas during brewing (ppm)

TEA	5′	10′	15′	30′	60′	120′
Infant tea	0.07	0.07	0.08	0.09	0.07	0.06
Lipton	0.83	0.87	1.01	1.06	1.01	1.01
Caykur Rize	0.62	0.68	0.75	0.83	0.79	0.79
Tomurcuk	0.83	0.92	1.01	1.06	1.06	1.17

Questionnaire findings

Questionnaire findings revealed that 120 children were from low SES whereas 80 children were from high SES. The children who preferred to drink black tea were higher in low SESs (Fig. 1). Herbal tea drinking frequency was higher in high SES (Fig. 2). There was a statistically significant difference among SES and frequent black tea consumption (p<0.01). It was recorded that all children drink all kinds of tea with sugar.
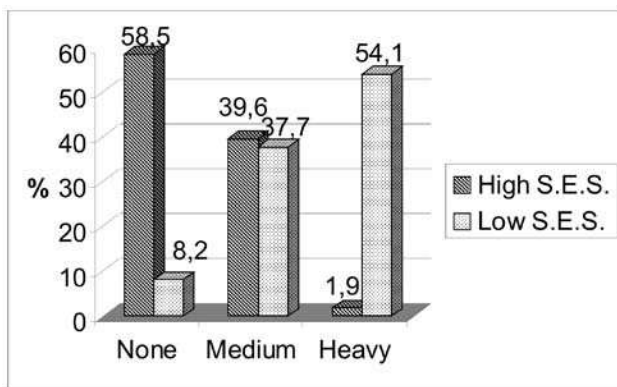

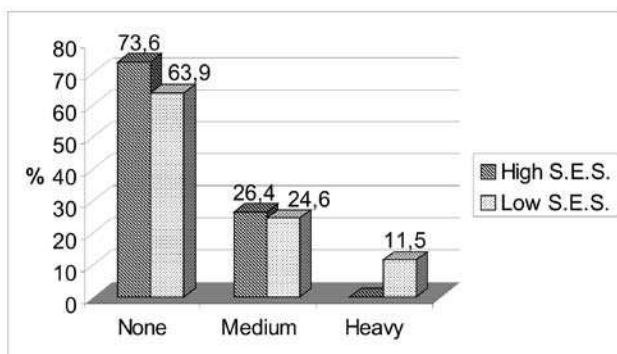*Figure 1. Black tea drinking frequency among children (%)*

Figure 2. Herbal tea drinking frequency among children (%)

Discussion

There are several studies on diet, nutrition or frequency of food consumption and dental caries that are showing the hazardous effect of sugar[13]. Diet may contain anti-cariogenic potential, as well as cariogenic effect. One of such snack drink is tea - in one hand there is a beneficial effect of fluoride, but with sugar content it is a cariogenic challenge.

However, the evidence of beneficial reduction in caries by systemic fluoride should be considered together with the increased prevalence of dental fluorosis[9-11]. Franco et al[14] recently reported that daily fluoride intake of young children was above the upper estimated threshold of 0.07 mg/kg/day. The 2 major sources of systemic ingested fluoride in the study by Franco et al[14] were fluoridated salt and dentifrices. Systemic review of water fluoridation also reported increased prevalence of dental fluorosis and focused on reconsidering the sources of high fluoride[10].

Tea is comparatively cheap and is readily available drink for consuming enjoyably. Fluoride is accumulated mainly in tea leaves and increased with age of the leaf. Lu et al[15] reported that fluoride could be regarded as a qualitatively important element in tea and that it could be used as a quality estimation of the product. Fluoride concentrations in tea infusions of green, oolong or black tea ranged from 0.6 to 1.9 mg/l, whereas brick tea liquors contain 4.8 to 7.3 mg/l[16]. The high fluoride contents in brick teas were due to the use of old leaves[16,17]. Chan and Koh[18] reported that de-caffeinated tea had higher fluoride content. In the present study, the fluoride amount released by brewing increased by time especially in half an hour and higher concentrations were measured with scented teas (bergami). On the other hand, similar to our findings, Hayacibara et al[19] also reported that fluoride levels in herbal teas were very low.

Simpson et al[20] demonstrated that tea can provide an effective vehicle for fluoride delivery to the oral cavity and this may lead to local topical as well as the systemic effects. Jamel et al[21] reported that beneficial effects of consuming tea due to its high fluoride content on dental caries were outweighed by the impact of the sugar levels in the tea consumed. In our study, it was found that all the children drink all kinds of tea with sugar.

Ramsey et al[22] evaluated the effect of tea drinking on dental caries in 12 years old children for 2 years and reported that children who drank 4-4.9 cups of tea had 1.5 more increase in the DMF-S index.

Duckworth[23] reported that tea consumption was showing an increase by age and that the children at the age of 7 were not usually drinking tea. However, Malde et al[24] reported that most children in rural areas in Ethiopia had been introduced to tea before the age of 12 months. Therefore, it seems that there is a cultural difference among tea consumption among young children and, similar to our findings, it is reported that children from lower SES were drinking tea more frequently[25].

It is clear that there is a high fluoride concentration in black tea infusions and not a clear relationship is found among beneficial effects of this on dental caries or the effect on dental fluorosis. Therefore, there is a great need for further studies to evaluate the role of black tea drinking, especially for young children, as a preventive agent or a factor in dental fluorosis.

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Release of Fluoride from Glass-Ionomer-Lined Amalgam Restorations in De-Ionized Water and Artificial Saliva

SUMMARY

Purpose: The present study evaluated the amount of fluoride released from glass ionomer lined amalgam restorations in de-ionized water and artificial saliva.

Materials and Methods: 40 human extracted molars were divided into 5 groups of 8 teeth each. Class V cavities (2x2x6mm) were prepared at the facial and lingual surfaces of the teeth and restored as follows: Group 1 with Dispersalloy amalgam; Groups 2 and 4: Same as group 1 except that 1mm of photo-curing glass-ionomer liner Vitrebond was placed at the axial wall before amalgam insertion; Groups 3 and 5: Same as group 1 except that 1mm of the traditional glass-ionomer liner GC Lining Cement was placed at the axial wall before amalgam insertion. The teeth of Groups 1,2 and 3 were placed in plastic tubes with 4ml of fresh fluoride-free de-ionized water, whereas the teeth of Groups 4 and 5 were placed in plastic tubes with 4ml of artificial saliva. The samples were subjected to hydrothermal cycling (300x, 5°/55°C, 4C/min). At weekly intervals, each tooth was removed from its aqueous medium and transferred to another vial containing de-ionized water or artificial saliva. Fluoride release was measured 5 times at weekly intervals with a fluoride-ion selective electrode.

Results: At 1 week and 4 weeks Vitrabond released significantly more fluoride than GC Lining Cement (p<0.05). Glass-ionomer lined amalgam restorations released significantly less fluoride in artificial saliva than in de-ionized water (p<0.05).

Significance: The availability of fluoride ions around the margins of the glass-ionomer lined amalgam restorations may reduce the development of secondary caries.

Keywords: Fluoride Release; Glass Ionomer Liners

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Introduction

The presence of a gap at the amalgam tooth interface permits the microleakage of oral fluids and bacteria into the interface and therefore may result in secondary caries development and pulpal irritation[1]. Microleakage is defined as the clinically undetectable passage of bacteria and fluids between cavity walls and restorative materials. The loss of marginal integrity provides potential pathways for re-infection, as cariogenic bacteria can penetrate into the underlying dentin through these defects[2]. Secondary caries formation around existing restorations is considered as the primary reason for replacement of amalgam and composite resin restoration[3,4]. The ability of a restored cavity to resist microleakage and secondary caries attack is the major determinant factor for the success or failure of a restoration.

The considerably lower incidence of secondary caries associated with glass-ionomer cements and fluoride-containing amalgam compared to amalgam and composite restorations has been explained by the release of fluoride from the filling materials[5,6].

Traditional or conventional glass-ionomer liners are chemically set materials. They have been used extensively because of their ability to bond to dentine[7]. The ability to release fluoride has also been considered a unique

advantage of these materials. However, manipulation variables and technique sensitivity associated with the 2-stage chemical setting of conventional glass-ionomer liners have been recognized as major disadvantage of these materials.

Visible-light cured glass ionomer liners have been introduced to overcome these disadvantages. These materials provide longer working and controlled setting times, rapid development of strength and lower sensitivity to environmental moisture changes[7]. Light-cured glass-ionomers are designed for use in the composite/glass-ionomer sandwich technique, as first suggested for chemically set glass-ionomers[7], where they should act as protective coating for dentine.

The adhesive properties, compressive strength, radiopacity and fluoride release of glass-ionomers have prompted some investigators to recommend them as bases for amalgam restorations[7-9]. It is believed that in order to be effective in reducing recurrent caries, the glass-ionomer liners should release fluoride at the margins of the amalgam restorations.

The **aim** of this *in vitro* study was to evaluate the amount of fluoride released from glass-ionomer lined amalgam restorations in de-ionized water and artificial saliva and the interfacial micro-morphology of these restorations. The null hypothesis tested was that de-ionized water and artificial saliva create similar F-releasing patterns from amalgam restorations lined with 2 commercially available glass-ionomer liners and that there is a gap between amalgam and dentin and between the liners and underlying dentin.

Materials and Methods

40 extracted human molars, free of caries and other defects, that had been stored in 10% neutral formalin were selected and randomly assigned to 5 groups. The teeth were not allowed to dry during any stage of the experiment. Before use, the teeth were washed in tap water to elute the formalin fixative, and were then cleaned with periodontal curettes and aqueous slurry of pumice using a handpiece and rubber cup. They were then rinsed with de-ionized fluoride free water (<0.02ppm), stored in plastic scintillation tubes with fluoride-free water and tested for fluoride release prior to any subsequent experimental manipulation. For each tooth, 2 class V cavity preparations were made at both buccal and lingual surfaces, located at the enamel region. The approximate dimensions of the cavities formed were: 6mm mesio-distally, 2mm occluso-gingivally and 2mm in depth. A #330L; pear-shaped carbide bur (SS White Burs Inc, Lakewood NJ, USA) attached to an air turbine handpiece with copious water coolant was used to prepare the cavities. The bur was always held at a right angle to the tooth surface to produce

a cavo-surface angle close to 90°. The cavity margins were finished with a flat fissure bur No 170 (SS White Burs, USA) using a slow-speed handpiece. After rinsing with water, the cavities were dried with compressed air. The burs were replaced after preparing up to 5 cavities. All the cavities were prepared by the principal author to ensure standardization in cavity preparation. The cavities were treated with the test materials (Tab. 1) in accordance with manufacturers' instructions. The 5 treatment groups used in this study are listed in table 2.

Table 1: The restorative materials used in the study

Materials	Manufacturer	Batch number
Vitrebond	3M, ESPE Dental Products, St. Paul, MN, USA	7512L 3EN
GC Lining Cement	GC Int, Tokyo, Japan	280641
Dispersalloy	Dentsply Caulk, Dentsply Inter. Milford, USA	040322 04329 E

Table 2: The experimental groups and the treatments tested

Group	Filling and cavity lining material	Treatment
1	Amalgam (without liner)	De-ionized water
2	Amalgam + Vitrebond	De-ionized water
3	Amalgam + GC Lining Cement	De-ionized water
4	Amalgam + Vitrebond	Artificial saliva
5	Amalgam + GC Lining Cement	Artificial saliva

Group 1 cavities were restored with the amalgam. Amalgam was inserted with an amalgam-carrier and condensed by hand instruments. Cavities of groups 2 and 4 were lined with Vitrebond and light cured under standard irradiation intensity of 750mW/cm^2 for 30s using a halogen bulb unit (Elipar Highlight, ESPE, Germany). The liner was extended 1mm short of the margins and was placed at the axial wall. After lining, the cavities were restored with amalgam as previously described. For groups 3 and 5 specimens, cavity preparations were lined with the GC Lining Cement. The material was extended 1mm short of the margins and was placed at the axial wall. 4 minutes after placing the liner the cavities were restored with amalgam as previously described. The thickness of the liner for both products was approximately 1mm. The thickness was controlled by measuring the depth of the cavities before and after liner application.

All restored teeth were stored in a humid environment for 24h before finishing and polishing the restorations with a bristle brush and aqueous slurry of pumice. The

teeth were then subjected to hydrothermal cycling for 300 cycles between 5ºC and 55ºC, with a dwell time of 15 s.

Fluoride Release Measurements

Teeth of groups 1, 3 and 5 were placed in plastic tubes with 4ml of fresh fluoride-free de-ionized water, and teeth of groups 2 and 4 were placed in plastic tubes with 4ml of fluoride-free artificial saliva. The composition of the artificial saliva employed in this study is shown in table 3[10]. All teeth were incubated at a constant temperature of 37±0.5ºC during the entire experimental period.

Table 3. Composition of artificial saliva

NaCl	0.400g
KCl	0.400g
$CaCl_2 \cdot H_2O$	0.795g
$NaH_2PO_4 \cdot H_2O$	0.69 g
$Na_2S \cdot 9H_2O$	0.005g
Distilled water qs	1000ml
pH	5.525

Fusayama et al[10]

The first measurement of fluoride concentration in each solution was carried out 1 week after polishing of the restorations. From each container 4ml of liquid was taken and 0.5ml of TISAB (total ionic strength adjustment buffer solution - Merck, Darmstadt, Germany) was added to it. Following equilibration of the solution, the fluoride ion concentration was measured in duplicate by a fluoride-ion specific ion electrode (Orion Research Inc, Cambridge MA, USA) calibrated using standard solutions of 0.1, 1, 10, 50 and 100 mg/l fluoride. Recalibrations were performed every 10 measurements with the standard solutions 1 and 10.

The teeth were then rinsed with 5ml of de-ionized water and immersed in new containers with 4ml of de-ionized water or artificial saliva and again placed in the incubator. The same procedure was repeated every week for 5 weeks. The amount of fluoride released from the four tested groups (mean ± SD) was expressed in mg/l.

Data were analysed by 2-way ANOVA and t-test. ANOVA was performed in the context of General Linear Models[11,12] using the SPSS v. 12 packet. A level of significance of 0.05 was selected in all cases.

SEM Evaluation

This part of the study used scanning electron microscopy (SEM) to investigate features of the tooth-restoration interfaces. 2 specimens of each group were used to evaluate the presence or absence of marginal gaps along the entire tooth-restoration interface. The teeth were sectioned in a bucco-lingual plane with a hard tissue microtome with water cooling (ISOMET, Buehler Ltd, Lake Bluff IL, USA). The sections were polished with medium and fine polishing discs (Soflex discs, 3M, ESPE, St Paul, USA) under continuous water spray. To remove the smear layer the sections were slightly etched with 35% phosphoric acid for 3-5s, rinsed with water for 20s and briefly dried.

The sections were then replicated by taking impressions of the sectioned surfaces with a vinyl polysiloxane material (President light body, Coltene, Altstätten, Switzerland). After 24h the impressions were poured with a slow-setting epoxy resin (Glycidether 100, SERVA Electrophoresis GmbH, Heidelberg, Germany) and allowed to cure for 5 days. The replicas were mounted on stubs, sputter coated with carbon and examined under a scanning electron microscope (SEM, JSM –840, JEOL Ltd, Tokyo, Japan) at 19KV accelerating voltage under high vacuum.

Results

Figure 1 and table 4 show the fluoride release rates obtained from the groups tested. The fluoride release was gradually decreasing by time in all experimental groups, except for group 1 (controls - amalgam, no liner, de-water), where it remained stable throughout the 5 weekly intervals.

Table 4. Mean fluoride release (ppm) and SD at weekly intervals from glass- ionomer lined amalgam restorations treated in de-ionized water or in artificial saliva

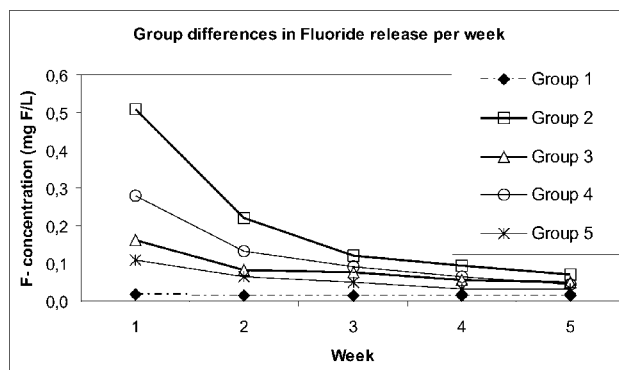| Time	Group	Mean	SD
	1	0.017	0.002
	2	0.510	0.232
Week 1	3	0.161	0.085
	4	0.280	0.102
	5	0.110	0.052
	1	0.015	0.004
	2	0.221	0.112
Week 2	3	0.084	0.042
	4	0.133	0.083
	5	0.064	0.038
	1	0.014	0.005
	2	0.121	0.064
Week 3	3	0.075	0.034
	4	0.092	0.056
	5	0.050	0.032
	1	0.015	0.003
	2	0.094	0.051
Week 4	3	0.055	0.034
	4	0.065	0.044
	5	0.033	0.012
	1	0.016	0.003
	2	0.070	0.045
Week 5	3	0.051	0.030
	4	0.043	0.024
	5	0.031	0.013

Figure 1. Fluoride released at weekly intervals from glass-ionomer lined amalgam restorations treated in de-ionized water or in artificial saliva. Group 1: amalgam in de-ionized water; Group 2: Vitrebond + amalgam in de-ionized water; Group 3: GC Lining Cement + amalgam in de-ionized water; Group 4: Vitrebond + amalgam in artificial saliva; Group 5: GC Lining Cement + amalgam in artificial saliva

Although the fluoride release was steadily decreasing from the 1st to the 5th week in all groups, the reduction was not statistically different (p<0.05) for the control (group 1) and the GC Lining Cement groups (groups 3 and 5). The only differences observed were for Vitrebond (groups 2 and 4).
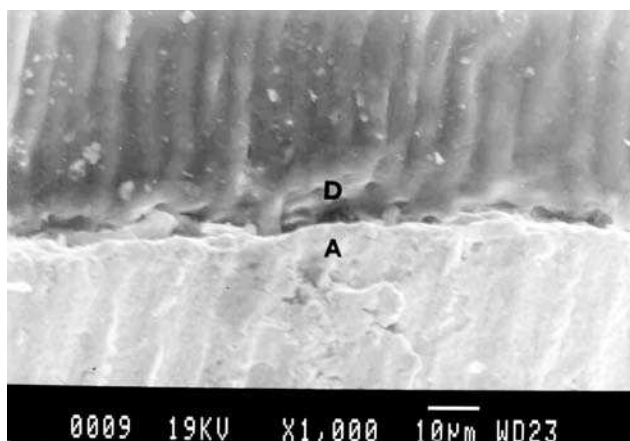

Figure 2. Gap formation between amalgam and dentine of gingival wall
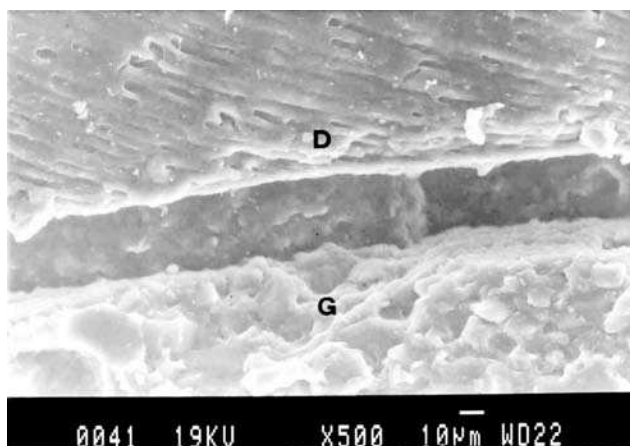

Figure 3. Gap formation between Vitrebond and dentine of gingival wall

In group 2 there were differences between the 1st and 2nd weekly intervals, as both differed from all the next intervals. In group 4 there were statistically significant differences between the 1st and all the rest weekly intervals. The 2nd weekly interval differed statistically significantly from all except from the 3rd one, whereas the 3rd weekly interval differed only from the 1st.

Vitrabond released more fluoride in de-ionized water (group 2) than GC Lining Cement in de-ionized water (group 3) at weeks 1 and 2 (p<0.05). Vitrabond released more fluoride in de-ionized water (group 2) than Vitrabond in artificial saliva (group 5) at first week p<0.05. Vitrabond released more fluoride in artificial saliva (group 4) than GC Lining Cement in artificial saliva (group 5) at first week (p<0.05). GC Lining Cement (group 3) released more fluoride in de-ionized water than in artificial saliva (group 5) but the difference was not statistically significant.

SEM images (Figs. 2-4) show that none of the replicas examined demonstrated a hermetic seal between the restoration and dentine. The gaps between amalgam and dentine ranged from 3μm to 15 μm, between Vitrebond and dentine ranged from 10μm to 40μm and between GC Lining Cement and dentin from 0 to 12μm.
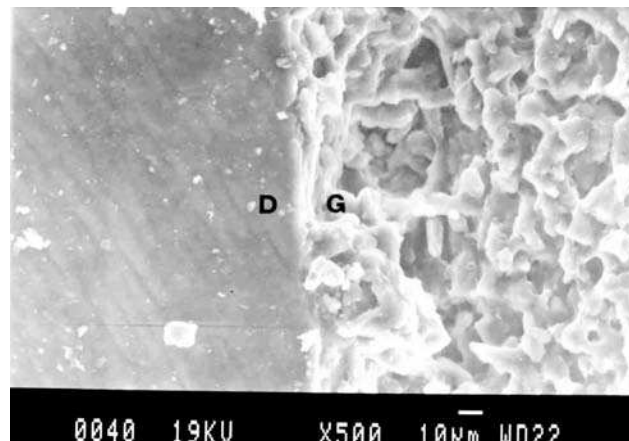
Figure 4. Gap formation and areas with good adaptation adjacent to each other between GC Lining Cement and dentine of axial wall

Discussion

The results of the present *in vitro* study indicated that amalgam restoration lined with 2 commercially available glass-ionomer liners release more fluoride in de-ionized water than in artificial saliva. The results also indicated that there is a gap between amalgam and dentin and between the liners and underlying dentin.

The availability of fluoride to an aqueous medium from glass-ionomer lined amalgam restorations has been demonstrated[8,13] and a reduction of the development of lesions *in vitro* around glass-

ionomer lined amalgams as compared to unlined non fluoride-containing amalgam fillings has also been reported[14,15].

The inhibiting effect on the development of experimental cavity wall lesions around glass-ionomer lined amalgam fillings reported in these studies may be due to fluoride present along the fluid phase of the tooth interface. The glass-ionomer liners release fluoride[16,17] and fluoride uptake, into enamel and dentine from glass-ionomer cements has been reported[18,19]. Depending on the amount of enamel and dentin fluoride uptake, a cariostatic effect of the glass ionomer should be expected.

The results from all 4 groups in this study showed measurable amounts of fluoride released to the distilled water and artificial saliva media, indicating the availability of fluoride ion around the margins of the restorations. The 2 groups in de-ionized water and artificial saliva had the same qualitative fluoride release pattern during the 5 experimental weeks. The concentration of fluoride released was higher during the first period, declined sharply on second week, then gradually diminished to a nearly constant level for each material. These results are in agreement with previous studies[20-23]. This pattern suggests, according to Tay and Braden[24] and Verbeeck et al[22] that the elution of fluoride occurs as 2 different processes. The first process is characterized by an initial burst of fluoride release from the surface, after which the elution is markedly reduced. The first process is accompanied by a second bulk diffusion process, in which small amounts of fluoride continue to be released into the surrounding medium for a long period of time[24].

In this study, at one week, Vitrebond released significantly less fluoride in artificial saliva than in de-ionized water. At 2, 3, 4 and 5 weeks there was no significant difference in fluoride release of Vitrebond and GC Lining Cement in artificial saliva and de-ionized water, but a trend was apparent towards a reduced fluoride release in artificial saliva.

Glockman et al[25] showed that glass-ionomer cements released more fluoride in water than in artificial saliva. A wide variety of methods have been used to study fluoride release from dental materials containing fluoride. In most of these tests, a sample of a set material was suspended in water and, in some tests, artificial saliva was used[25,26]. El-Mallakh and Sarkar[26] showed that the values of fluoride release in water and in artificial saliva were consistently different, the lowest values noted in the second medium, caused by the presence of cations and anions in artificial saliva, with an ionic effect on the solubility.

The results of SEM analysis showed clearly that substantial gaps were formed between amalgam and dentine and between glass-ionomer liners and the underlying dentine. The gaps between amalgam and dentine ranged from 3μm to 15μm, between Vitrebond and dentine ranged from 10μm to 40μm and between GC Lining Cement and dentine from 0 to 12μm. This explains the fluoride release from the glass-ionomer lined amalgam restorations. This also confirms other studies showing that amalgam restorations have an interface gap present along their periphery[1] which allows microleakage leading sometimes to recurrent caries[2,27]. In time, corrosion products of the amalgam would fill the interface between amalgam and enamel or amalgam and dentine and the tooth would be protected from the decalcifying action of caries-producing bacteria.

Corrosion products of the amalgam could also affect the amount of fluoride released to the media from glass ionomer-lined amalgam restorations by forming a barrier at the interfacial gap.

Thus continuous small amounts of fluoride in the fluid phase surrounding the teeth may inhibit demineralization and enhance remineralization[28,29] and affect microbial activity[30]. From a clinical point of view the results from this study imply that glass-ionomer lined amalgam restorations may act as an intra-oral source for the controlled slow release of fluoride at sites at risk for recurrent caries for the first 5 weeks of placement.

Conclusions

Measurable amounts of fluoride released to the de-ionized water and artificial saliva media indicate the availability of fluoride ion around the margins of amalgam restorations lined with glass-ionomer liners for the 5 weeks experimental period. The fluoride release was steadily decreasing from the first to the fifth week in all the groups.

At weeks 1 and 2, Vitrebond released significantly more fluoride than GC Cement in de-ionized water (p<0.05) and at week 1 more fluoride in artificial saliva (p<0.05). At week 1 Vitrebond released significantly less fluoride in artificial saliva than in de-ionized water (p<0.05).

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Fluoride Release from Polyacid-Modified Composites (Compomers) in Artificial Saliva and Lactic Acid

SUMMARY

The slow release of fluoride from restorative materials has been clinically important because of its anticariogenicity. The aim of this study was to assess the fluoride release from compomers in lactic acid and artificial saliva at different period of times. 42 specimens (n= 7 per group) in disc forms (7 mm diameter, 2 mm thickness) from 3 different compomers (Compoglass F, Dyract AP, Glasiosite) were placed in artificial saliva (pH = 7.0) and lactic acid (pH = 4.0). The amount of the fluoride in the solutions was measured at 1st, 7th, 14th, 21th and 28th day by means of the fluoride ion selective electrode. The fluoride amount was calculated by concentration (ppm).

The 3-way Analysis of Variance (ANOVA) and the Multiple Comparison Tests (Duncan) indicated that the relative amount of fluoride release was dependent on both the material and the storage medium. Significant differences were also found between the different types (P<0.01). A time dependent increase in the fluoride content was observed for all the compomers in both media. For all the tested materials, the fluoride release was higher in the artificial saliva (P<0.01). The amount of fluoride release was the most from Compoglass F (80.7- 45.2 ppm), followed by Dyract AP (58.2 - 14.7 ppm) and Glasiosite (19.2-12.2 ppm) at 28th days, in both artificial saliva and lactic acid, respectively. The least amount of fluoride release was observed at the first day ranging between 3.5 - 6.7 ppm in artificial saliva, and 2.2 - 6.5 ppm in lactic acid. Fluoride release was evident for all the compomers, but the rate of release varied considerably between the materials.

Keywords: Compomers; Artificial Saliva; Lactic Acid; Flouride Release

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Introduction

New restorative material, polyacid-modified composites or compomers have been developed[1,2]. These materials adhere to dentin and enamel, have a stable matrix structure, release fluoride, and reduce microleakage. These materials are a composite resin containing fluoride releasable filler. Compomer contains a light activated polymerizable dimethacrylate monomer and one containing carboxylic acid group[2,3]. To determine which material has optimal fluoride release for caries resistance, the relative concentrations and the duration of fluoride release should be examined among materials. Many factors affect fluoride release. There are several studies for the fluoride release from compomers. The use of different experimental condition in the respective studies also affects the results such as the manipulation of the material, powder-liquid ratio, the way of mixing, different amount of exposed area for the specimens or the nature of the storage medium. Lactic acid and artificial saliva were often used for the dissolution in experiments, and most of the studies have been conducted *in vitro*. Therefore, the actual results in clinical conditions could only be speculated. The acid is most likely to exist in the oral environment and relevant to caries initation[4-8].

This *in vitro* study evaluated a short time fluoride release from 3 commercial compomers into artificial saliva and lactic acid, in an effort to simulate clinical conditions.

Material and Methods

3 different compomers, namely Compoglass F, Dyract AP, and Glasiosite were selected for this study (Tab. 1).

42 specimens for 2 different testing media (artificial saliva and lactic acid, n = 7 per group) were prepared in disc forms (7 mm diameter and 2 mm thickness), according to the manufacturers' instruction. The specimens were light cured both from the bottom and the top of the mold for 20 seconds, which made 40 seconds totally.   After their polymerization, they were removed from the teflon molds and placed in individual plastic tubes containing 2 ml of de-ionized water and incubated for 24 hours at 37 °C.

Table 1. The materials and the manufacturers of the products

Materials	Chemical Composition
Compoglass F Ivoclar, Vivadent, Münich, Germany	4 EDMA/TEGDMA BisPMA, Photo initiators, Ba-Al-fluorosilicate-glass, Stabilizer Ion-leachable glass, Yb trifluoride
Dyract AP Dentsply, DeTrey, Konstanz, Germany	UDMA polymerizable resins, TCB resin, St-Al-Na-P-fluorosilicate-glass, Strontium fluoride, Photo initiators, Stabilizers
Glasiosite Voco,Cuxhaven, Germany	Bis-GMA/TEGDMA, Diurethan-dimethacrylate Ion-leachable glass

Before each fluoride concentration measurement, the calibration curve was obtained. The artificial saliva was prepared according to Karantakis et al[9]. Each specimen was placed separately in plastic tubes containing 10 ml artificial saliva and 10 ml lactic acid. All specimens were stored at 37 °C during the time of each measurement.

Measurements were made at the intervals of 1th, 7th, 14th, 21th, 28th days. Measurements were repeated 3 times and the concentration values were averaged. Data were analysed by using a calibration curve. Before each measurement, 5ml artificial saliva was taken from the plastic tube and then 5 ml fresh artificial saliva was added in this plastic tube for the previous storage solution.

In order to measure the fluoride concentration, 5 ml of the artificial saliva was mixed with 14 ml distilled water and 1 ml TISAB solution (Orion Research Inc, 940911) and fluoride ion-specific electrode (combination electrode Fluoride 960900; Orion Research Inc) was used to read the fluoride content of the solution in parts per million (ppm). To measure fluoride release of compomer materials into the lactic acid (pH= 4; 10^{-3}M) similar protocol was conducted as for artificial saliva.

The data were analyzed by using 3-way Analysis of Variance (ANOVA) and Multiple Comparison Test (DUNCAN).

Results

The mean fluoride release values and standard deviations of each compomer materials were shown in tables 2 and 3.

Table 2. The mean fluoride release values and standard deviation of each compomers in artificial saliva

Materials	1st day	7th day	14th day	21st day	28th day
Compoglass F	4.7± 0.2 (A)e	26.7± 0.6 (A)d	58.5± 0.8 (A)c	75.1± 0.7 (A)b	80.7± 0.8 (A)a
Dyract-AP	6.7± 0.3 (A)e	28.5± 0.8 (A)d	48.7± 1.3 (B)c	56.2± 0.7 (B)b	58.2± 0.7 (B)a
Glasiosite	3.5± 0.3 (A)e	8.7± 0.5 (B)d	12.7± 0.5 (C)c	17.7±0.5 (C)b	19.7± 0.5 (C)a

Table 3. The mean fluoride release values and standard deviations of each compomer in lactic acid

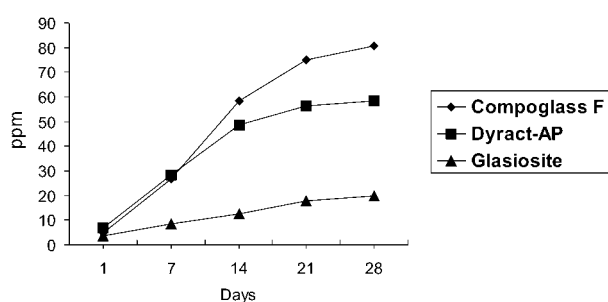| Materials	1st day	7th day	14th day	21st day	28th day
Compoglass F	6.5± 0.3 (A)e	22.7± 0.3 (A)d	32.3± 0.5 (A)c	41.2± 1.2 (A)b	45.2± 0.2 (A)a
Dyract-AP	2.7± 0.2 (B)d	7.3± 0.3 (B)c	12.7± 0.8 (B)b	12.8± 0.3 (B)b	14.7± 0.3 (B)a
Glasiosite	2.2± 0.3 (B)d	3.5± 0.3 (C)d	7.2± 0.3 (C)c	10.2± 0.3 (B)b	12.2± 0.2 (B)a

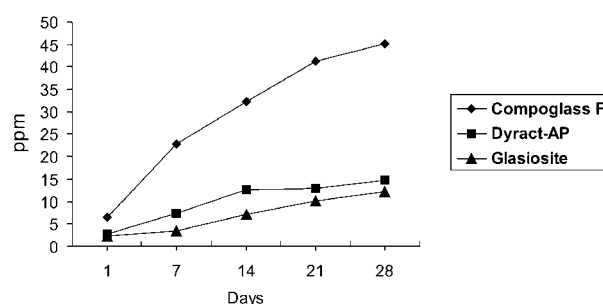*Figure 1. Fluoride release from 3 different of compomers in artificial saliva*

Figure 2. Fluoride release from 3 different compomers in lactic acid

Significant differences in fluoride were found among the 3 different compomers in both artificial saliva and lactic acid (P<0.01). For all the tested materials, fluoride release was significantly higher in the artificial saliva than in the lactic acid (Figs. 1 and 2). All brands of compomers released increasing amounts of fluoride as a function of time, but the rate of release varied considerably among the materials.

The amount of fluoride release in descending order was the most from Compoglass (80.7 - 45.2 ppm), followed by Dyract-AP (58.2 - 14.7 ppm) and Glasiosite (19.2 - 12.2 ppm) at the end of 28 days, in both artificial saliva and lactic acid, respectively. The least amount of fluoride release was observed at the first day, ranging between 3.5 - 6.7 ppm in artificial saliva and 2.2 - 6.5 ppm in lactic acid. Fluoride release was evident for all the selected compomers. The least fluoride release was found with Glasiosite in lactic acid.

Discussion

Several investigations have been performed on fluoride release from various dental restorative materials, including resin composites, glass-ionomer cements, and compomers[10-11]. In these studies, fluoride release was evaluated using various experimental designs and storage media. It is generally accepted that fluoride should be released slowly, through a diffusion process, without leading to the deterioration of physical properties of the material. Sales et al[12] reported that the fluoride, aluminium and strontium ions from compomers were released much more in the lactate buffer (pH 4.1) than in distilled water. The pattern of fluoride release from the materials was similar, peaking with in the first few days after being placed in the storage solutions. The pH of the environment affected the fluoride release differently among the materials.

All the compomer materials evaluated in this study demonstrated low fluoride release initially at the first day, but the amount increased at 7th and 14th days. The fluoride release then proceeded with a slow increase at 21th and 28th days of observation periods. The rate of the release remained relatively constant after 21th day. Even though all the materials tested demonstrated similar dissolution patterns during our examination period, the amount of fluoride release from the different compomers varied from one to another at various time intervals. This depended not only on the concentration of fluoride, but on whether it could diffuse out from within the material. This finding is in accordance with Attin et al[13], where maximum fluoride release from compomers was detected within the 1st day after setting, followed by a decrease in the rate after a few days. Vermeesch et al[14] observed that the fluoridated resin composites released fluoride in small amounts, it was approximately 10 times less than compomers during the first day.

In order to understand the differences between the materials, it is important to note that they all contain fluoride in their glass filler particles. Fluoride release depends not only on the concentration of fluoride, but also on diffusion from the material. The difference between the fluoride release mechanism in glass-ionomer cements and compomers at short immersion periods may be due to the loss of bonding property of fluoride in compomers. Therefore, after polymerization a less amount of fluoride containing glass fillers will be exposed to the storage medium.

The relatively low fluoride leaching into the artificial saliva is important because many leaching studies use water as the medium[15-18].

It was also observed in this study that fluoride release from the compomer materials was dependent on the storage medium as statistically significant differences were observed in the fluoride release amount between artificial saliva and lactic acid. Furthermore the amounts of fluoride ions released from compomers, through the 21th day were lower than the reported values for glass-ionomer cements.

The setting mechanism of the compomers was entirely a free-radical polymerization that was proposed to be a relatively slow reaction. Once the monomer of compomers were polymerized and exposed to saliva, the acid groups caused the resin to take up the moisture, thereby activating the acid-base reaction between the acidic functional groups and the basic glass filler[19-21].

Forsten[22] have shown that the compomers release fluoride less than conventional and light-polymerized glass-ionomer cements being exposed to storage medium.

Conclusions

1. Significant differences in fluoride release were found among the 3 different compomers in both artificial saliva and lactic acid.
2. For all the tested compomers, fluoride release was significantly higher in artificial saliva than in lactic acid.
3. The pattern of fluoride release was similar for all of the examined materials.
4. The pH of the environment strongly affected the fluoride release from the materials.

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Incidence of Voids in Packable *versus* Conventional Posterior Composite Resins: An *In Vitro* Study

SUMMARY

Aim. *Study aimed to determine effects of flowable composites as liner on marginal and internal voids in MOD composite restorations with different gingival levels.*

Methods. *45 molars were prepared for MOD cavities. Finish line was prepared 1 mm apical to mesial, and 1 mm coronal to the cemento-enamel junction on distal. Teeth were restored with: Solitaire; Solitaire + Revolution; Surefil; Surefil + Dyract Flow; Alert; Alert + Flow-it; Amelogen (control); Amalgam (negative control); and Prodigy Condensable. Then resin embedded and sectioned specimens were observed under stereomicroscope to determine the number and size (mm) of voids at margins and within material. Data was analyzed by Kruskal-Wallis analysis of variance and Wilcoxon Signed Ranks Test (α = .05).*

Results. *According to number of voids, there was no significant difference at margins (P>0.05,) but Alert showed significant differences with Solitaire, Amalgam and Solitaire + Revolution at occlusal material (P<0.05) and Solitaire at distal material. According to size, Alert showed differences with Solitaire, Amalgam, Surefil, Surefil + Dyract Flow, also between Aler + Flow-it, and Solitaire at total material. Mesial and distal comparisons were significant in Amalgam (P = 0.042) at material for number, and in Amelogen (P = 0.042) at margin for size of the voids.*

Conclusion. *The number and size of voids of packables did not show difference at restoration margins, within material. Different gingival levels and flowable usage did not make difference among packables. Usage of flowable with packable in a MOD resin restoration at different gingival levels did not achieve reduction in the number and size of voids at the margins and internally.*

Keywords: Void, packable, flowable, MOD, gingival level

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Introduction

Posterior resin-based composites have become an important part of restorative process. In larger Class II preparations, it may be more difficult to obtain proper contour and achieve adequate proximal contact with conventional composite than with amalgam. To improve ease of manipulation, the ideal resin-based composite should have a viscosity stiff enough to facilitate placement without adhering to the condensing instrument[1-4]. Continuous development of composite restorative materials has lead to the development of packable composites. These materials have a higher, modified filler content and, as a result a stiffer consistency than conventional resin composites, they have been described "condensable" [5]. In addition, it was also reported by manufacturer that these materials could be manipulated as amalgam clinically, condensed as amalgam and have physical properties that are similar to amalgam[6]. Therefore, packables can be described for this amalgam alternative material[7-10].

There are difficulties in placing conventional composites incrementally into the proximal box of Class II restorations. Any gap between the layers may lead to a definitive restoration that has a compromised integrity, either at the margins or within the bulk of the material[11].

Voids and porosities appear to have a negative effect on physical properties of the material[12,13].

Microleakage may result from many factors, such as the extent of marginal gap or polymerization shrinkage of materials used. Microleakage *via* the tooth restoration interface may lead to marginal stain, post-operative sensitivity, recurrent caries and possibly pulpal problems[14,15]. Gap formation is especially prevalent if gingival margins are located apical to the cementoenamel junction (CEJ) in dentin[16,17].

Amalgam insertion techniques used with packable composites can produce acceptable interproximal contacts[18]. Because of the claims for high depth of polymerization and low polymerization shrinkage of packable composites, a bulk-fill technique may be possible[19]. However, several studies reported that bulk placement causes insufficient polymerization, resulting in microleakage[20-23]. The stiffness and flow characteristics of packable composites may result in voids in the completed restoration[24]. Because of this risk, some manufacturers recommend that a flowable composite be injected initially, thus lining the internal surfaces of the preparation to a thickness ranging from 0.5 to 1.0 mm. Flowable composites exhibit favorable wetting properties and as a result adapt intimately to dentin and enamel surfaces of preparation, better than packable composites[25]. They also possess a relatively low elastic modulus, which theoretically could benefit the polymerization of packable composites[25,26]. As hypothesized by Moon[27], as the overlying packable composite undergoes polymerization contraction, the adjacent flowable composite can stretch or elongate, thus, acting as a stress breaker. Additionally, flowable composite in a packable restoration decreased microleakage at the gingival margin and thus improved the integrity of Class II restorations[28-30]. Besides, some *in vitro* studies have reported a reduction in microleakage but an increase in the presence of internal voids in Class I and II flowable composite fillings when compared to hybrid composite restorations[31,32]. However, 2 different studies by Chuang et al[33,34] showed that flowable composite reduced the voids in the interface and within the restoration, but didn't improve microleakage. They reported that there was no significant correlation between number of voids and microleakage as well. Another *in vitro* study by Malmström et al[35] was also unable to demonstrate reduced microleakage in Class II composite restorations with flowable.

The **aim** of this study was to compare the number and size of voids, present at the margins and internally, in packable composites (with/without flowable resins) to conventional composite in Class II restoration. The materials were placed according to the manufacturer's recommendation in bulk or by means of an incremental insertion technique with and without lining the preparation with a flowable composite. For this purpose, packable composites of different brands were used for the restoration of MOD cavities.

Materials and Methods

45 recently extracted sound human molar teeth disinfected in 10% buffered formalin solution, without incipient decay or cracks, were used for the study. The teeth were scaled and cleaned with slurry of pumice and tap water to remove any contamination. 5 teeth were selected and assigned to 9 groups. The teeth of each group were placed in a block made from pink wax to simulate interproximal gingival area (Cavex Set Up Modelling Wax; Cavex, Holland) and molar plastic teeth were placed to each side of the block. Then teeth were embedded into arch shaped stone blocks from apical thirds for each group. 1 operator prepared all the MOD cavities using a tungsten carbide bur (269; Brassler, USA) in a high-speed handpiece with water spray. The bucco-lingual width measured 4 mm and the pulpal depth was 2 mm. The proximal boxes of the preparations were 1 mm apical to CEJ on mesial surface and 1 mm coronal to CEJ on distal aspect (Fig. 1). The mesial gingival margins were located on dentin/cementum, while the distal margins were located solely on enamel. Digital compass and a ruler were used to standardize the all cavity preparations. The boxes were formed at a 90-degree angle to the cavo-surface. All specimens were polished with pumice powder and rinsed with tap water after preparation. A matrix system (Hawe SuperMat; Hawe-Neos Dental, Switzerland) was used and 2 wooden wedges (Hawe-Neos Dental, Switzerland) were inserted at the buccal and lingual sides to tightly seal the matrix-cavity margin.

4 packable composites (Solitaire, SureFil, Alert, and Prodigy Condensable), a hybrid composite (Amelogen as positive control), and amalgam (as negative control) were selected as experimental materials. All specimens were
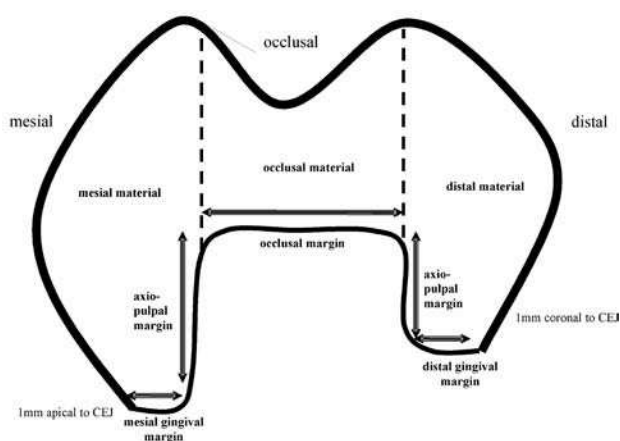

Figure 1. Representative cross section of MOD restoration.
Total material= mesial material + occlusal material + distal material
Mesial margin= axio-pulpal margin + mesial gingival margin
Distal margin= axio-pulpal margin + distal gingival margin
Total margin= mesial margin + occlusal margin + distal margin
Bulk material= margin total + material total

restored according to table 1. Use of the materials and application techniques provided by the manufacturers were carefully followed. Table 2 lists a summary of the material tested, including, type, composition, filler content and manufacturer information. The restorations were finished with a scalpel and fine diamond burs (# 0290; Denstply Maillefer, Switzerland) then polished with paper disks (Sof-Lex, 3M Co, USA). After finishing and polishing, the experimental teeth were removed from the wax block and sectioned in a mesio-distal direction along the long axis using a low speed diamond saw (Isomet 1000 Precision Saw, Buehler, USA) and continuous water cooling. It was possible to obtain 3 sections per tooth. Each section was immersed in 0.5% basic fuchsine dye for 24 hours for better dye penetration into the porosities. All sections were rinsed in tap water, and examined for internal voids using a stereomicroscope (Leica Microsystems Ltd. Business Unit SM, Switzerland) at x48 magnification (x102.81 magnification on screen).

Table 1. Groups planned for the study

Group 1	PQ1+ Solitaire
Group 2	PQ1+Revolution+ Solitaire
Group 3	Prime & Bond NT + Surefil
Group 4	Prime & Bond NT + Dyract Flow compomer resin + Surefil
Group 5	Bond One + Alert
Group 6	Bond One + Flow-it + Alert
Group 7	PQ1+ Amelogen
Group 8	Amalgam
Group 9	PQ1+ Prodigy Condensable

Table 2. The used materials

Material/ Manufacturer	Instructions for use	Type	Composition	Particle Size	Weight	Volume
PQ1 (Ultradent, South Jourdan, Utah)	etch with %35 acid gel 15s, rinse and lightly dry. Apply PQ1 with moderate pressure into the surface for 15s. Gently air thin and light polymerization for 20s.	Single component Dentin bonding system	HEMA, %40 filled with barium borosilicates, fluoride, % 8 ethanol			
Prime & Bond NT (Dentsply/ Caulk, Milford, De)	etch with %34 Tooth conditioner gel 15s, rinse and lightly dry. Apply Prime & Bond NT for 20s. Gently air thin for 5s and light polymerization for 20s	Single component Dentin bonding system	PENTA, urethane modified Bis-GMA, acetone, cetylamine hydrofluoride, nonfiller			
Bond One (Jeneric/ Pentron, Wallingford, Conn)	etch with %35 acid gel 15s, rinse and lightly dry. Apply Bond 1 into the cavity for 20s. Gently air thin for 10s and light polymerization for 10s.	Single component Dentin bonding system				
Revolution (Kerr, Orange, CA, USA)	Apply one or two drops of flowable composite to the internal surfaces light polymerization for 40s.	Flowable resin	Barium glass, synthetic silica	1 μm	62% 55%	46%
Dyract Flow (Dentsply/Caulk)	Apply one or two drops of flowable composite, avoid excessive pooling and remove it by air blowing. Light polymerization for 20s.	Flowable compomer				
Flow it (Jeneric/ Pentron)	Apply one or two drops of flowable composite to the internal surfaces light polymerization for 20s.	Flowable resin	Barium barosilicate glass	1,5 μm 1 μm	70.5%	54%
Solitaire (Heraeus Kulzer, Armonk, NY, USA)	Apply the resin in bulk	Packable composite resin	Polyglass monomers	2-20 μm	76%	45%, 66%
SureFil (Dentsply/ Caulk)	Apply the resin in bulk	Packable composite	Barium fluoroaluminoborosilicate glass, SiO2, nonfiller; Bis-GMA, TEG-DMA	0.8 μm	77%, 82%	58%, 66%
Alert (Jeneric/ Pentron)	Apply the resin in bulk	Packable composite	Ba-B-Al-Silicate, SiO2, Ethoxylated Bis-GMA	0.7 μm	80%, 84%	62%, 70%
Amelogen (Ultradent)	Apply the resin incrementally (maximum 2 mm)	Hybrid composite	Bis-GMA	0.7 μm	72%	60%
Prodigy condensable (Kerr)	Apply the resin in bulk	Packable composite	Barium fluorosilicate; BIS-GMA	Prodigy fillers	80%	62%
Amalgam (Novalloy, President Dental, München, Germany)	Condense and burnish after the initial setting	Gamma II- free	Ag 45 %, Cu 24%, Sn 31%			

The assessment of voids was performed in 6 different areas of the restorations; the first 3 within the material (mesial, occlusal and distal material) and the second 3 at the margins (mesial, occlusal and distal margins), as shown in figure 1. According to figure 1, bulk material refers to mesial + occlusal + distal material; total margins refers mesial + occlusal + distal margin. For all sections and groups, the number of voids was recorded by measuring the longest part of the voids were obtained for size measurements (Leica Q Win V3 Digital Image Processing and Analysis Software); then the mean values were achieved for the margins and internal material. The data was tabulated for statistical analysis.

Statistical analyses can be grouped into 2 steps. Kruskal-Wallis test was used to determine the difference between 9 groups, in terms of number and size of voids at the margins and internal material. For the differences that were significant between groups, a Post Hoc test, Dunnett C was performed. In the second step, differences between mesial and distal, in each 9 groups were determined by using Wilcoxon matched paired sign test for number and size (P = .05).

Results

The mean and median values of number and size of voids at the margins and materials were illustrated in tables 3 and 4. Table 5 shows the comparison of groups according to their number of voids at the margins and materials. Post Hoc test results showed no significant difference between the groups at the mesial, occlusal and distal margins (P>0.05).

Table 3. Number of voids

Group (n = 5)	Margin (mean ± SD, median)				Material (mean ± SD, median)				Bulk Material (mar.total+ mat.total) (mean ± SD, median)
	mesial	occlusal	distal	margin total	mesial	occlusal	distal	material total	
Solitaire	2.2±1.9	1.0±0.7	0.8±0.5	4.0±1.7	6.2±2.2	3.6±2.6	2.4±1.1	12.2±4.13	16.2±9.52
	3	1	1	5	6	3	2	15	19
Solitaire+ Revolution	1.0±1.3	0.6±0.49	0.4±0.49	2.0±1.41	11.6±3.7	6.8±3.76	9.0±5.97	27.4±8.11	29.4±9.63
	-	1	-	2	12	5	7	33	34
Surefil	1.2±1.2	1.8±0.7	1.0±0.6	4.0±1.1	6.2±2.0	8.4±4.6	10.4±5.2	25.0±3.9	29±7.14
	1	2	1	4	6	8	8	24	28
Surefil+ Dyract Flow	1.2±0.85	2.6±1.3	1.4±0.9	5.2±1.3	9.4±7.1	10.2±4.6	10.4±6.3	30.0±14.2	35.2±14.6
	1	2	2	5	6	8	9	35	42
Alert	1.6±1.2	3.6±2.4	1.6±1.2	6.8±2.5	26.4±8.5	28.2±6.5	18.6±5.5	73.2±12.4	80±11.45
	1	2	1	8	23	28	19	72	80
Alert+ Flow-it	0.8±1.6	0.8±0.75	1.2±1.6	2.8±2.32	23.2±6.24	23.4±8.55	21.8±9.06	68.4±20.18	71.2±21.23
	-	1	-	4	26	24	16	77	80
Amelogen	1.6±2.0	5.2±2.3	2.8±1.2	9.6±4.0	11.4±6.7	13.4±3.4	8.8±4.2	33.6±11.5	43.2±9.36
	-	6	3	11	11	12	11	32	40
Amalgam	2.0±0.63	0.4±0.8	0.4±0.49	2.8±0.75	7.2±3.19	3.4±1.62	3.6±1.74	14.2±4.58	17±5.48
	2	-	-	3	5	3	3	12	16
Prodigy Condensable	1.2±1.2	2.4±2.4	2.4±2.1	6.0±5.2	14.8±7.5	12.4±3.3	6.0±1.7	33.2±7.8	39.2±12.87
	1	2	2	5	20	14	6	33	34

Table 4. The size of voids (mm)

Group (n = 5)	Margin (mean ± SD, median)				Material (mean ± SD, median)				Bulk Material (mar.total + mat. total) (mean ± SD, median)
	mesial	occlusal	distal	margin total	mesial	occlusal	distal	material total	
Solitaire	0.89±1.26 0.58	0.06±0.09 -	0.09±0.08 0.15	1.18±1.25 0.73	0.85±0.67 1.05	0.59±0.64 0.40	0.35±0.34 0.58	1.78±1.04 2.03	2.96±2.14 2.75
Solitaire+ Revolution	0.11±0.15 -	0.05±0.04 -	0.05±0.07 -	0.20±0.17 -	1.96±1.24 4.15	1.01±0.64 2.05	1.42±1.11 0.50	4.39±1.89 6.70	4.59±1.88 6.70
Surefil	0.17±0.23 0.20	0.31±0.29 0.10	0.11±0.09 0.08	0.59±0.24 0.38	1.07±1.14 0.35	1.15±0.83 0.45	0.68±0.58 0.68	2.89±2.35 1.48	3.48±2.44 2.00
Surefil+ Dyract Flow	0.16±0.10 0.25	0.71±0.76 0.20	0.16±0.14 0.20	1.03±0.77 0.65	1.09±0.85 0.15	1.34±0.48 0.60	1.22±0.74 0.53	3.65±1.58 1.28	4.67±1.69 1.93
Alert	0.80±0.99 0.65	0.64±0.74 0.18	0.27±0.30 0.60	1.70±1.60 1.43	3.76±1.63 3.08	3.10±2.02 4.60	2.10±0.84 1.15	8.90±0.90 8.83	10.65±2.08 10.25
Alert+ Flow-it	0.11±0.25 -	0.25±0.28 0.45	0.19±0.31 0.23	0.55±0.57 0.68	3.47±1.13 5.03	3.37±1.23 3.15	2.47±1.33 4.53	9.30±2.71 12.70	9.85±2.67 2.00
Amelogen	0.17±0.24 0.30	1.20±0.80 0.28	1.25±0.88 1.65	2.61±1.23 2.23	1.64±0.97 2.05	1.87±0.75 1.85	1.33±0.89 1.90	4.84±2.17 5.80	7.59±1.75 8.03
Amalgam	1.11±1.00 1.70	0.38±0.84 -	0.35±0.49 -	1.84±1.09 1.70	1.87±1.01 0.40	0.52±0.35 0.08	0.73±0.52 1.50	3.12±0.86 1.98	4.95±1.08 2.00
Prodigy Condensable	0.16±0.21 0.08	0.75±0.70 0.15	0.58±0.76 0.30	1.48±1.21 0.53	2.46±1.74 2.78	2.54±1.86 1.60	1.22±0.81 1.08	6.22±3.37 5.45	7.69±4.18 5.98

Table 5. Comparison of results of voids' number

		Chi - Square	P	Post hoc Test Results
Margin	mesial	4.390	0.820	
	occlusal	23.596	0.003	
	distal	13.748	0.089	
	total	17.478	0.025	
Material	mesial	22.864	0.004	
	occlusal	31.436	0.000	Solitaire & Alert Solitaire+Revolution & Alert Alert & Amalgam
	distal	25.743	0.001	Solitaire & Alert
	total	32.499	0.000	Solitaire & Alert+Flow-it Solitaire & Alert Solitaire+Revolution& Alert Alert & Amalgam Alert & Prodigy Alert & Surefil Alert+Flow-it & Amalgam
Margin + Material	mesial	2.906	0.940	
	occlusal	20.954	0.007	Amalgam & Amelogen
	distal	11.498	0.175	
	total	32.877	0.000	Solitaire & Alert Solitaire+Revolution& Alert Surefil & Alert Surefil+Dyract Flow & Alert Amelogen & Alert Amalgam & Alert Amalgam& Alert+Flow-it Amalgam& Amelogen

For the overall comparison Kruskal-Wallis analysis of variance test and Post hoc analysis were carried out.
Only statistically significant results are summarized in the table. Significant level P<0.05

While there was no significant difference at the mesial, Alert showed significant differences with Solitaire, Amalgam and Solitaire + Revolution groups at the occlusal material (P<0.05). At distal material, the only difference was between Solitaire and Alert. At the total material, Alert and Solitaire, Alert and Solitaire + Revolution, Alert and Surefil, Alert and Amalgam, Alert and Prodigy, Alert + Flow-it, and Solitaire, Alert + Flow-it and Amalgam showed significant differences (P<0.05).

Figures 2 and 3 showed huge amount of voids in the sections. When we consider margin and material together, the significant differences were between Alert and Solitaire, Solitaire + Revolution, Surefil, Surefil + Dyract Flow, Amelogen, Amalgam groups and also between Amalgam and Alert + Flow-it, Amelogen (P<0.05). Figures 4 and 5 showed the microscopic view of an amalgam specimen.
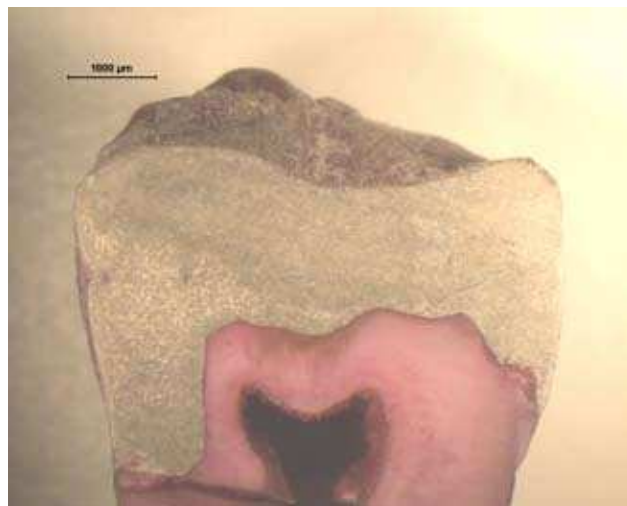

Figure 4. Image of an Amalgam specimen (x9.45)

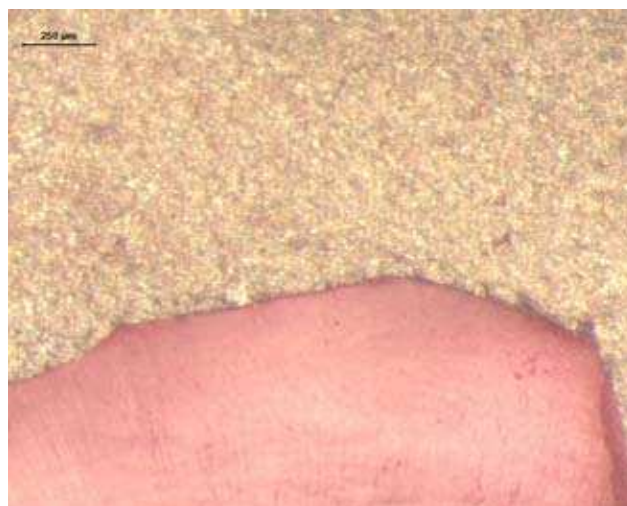*Figure 2. Image of a specimen from Alert group (x9.45)*

Figure 5. Same specimen in figure 4 at higher magnification at occlusal (x48)
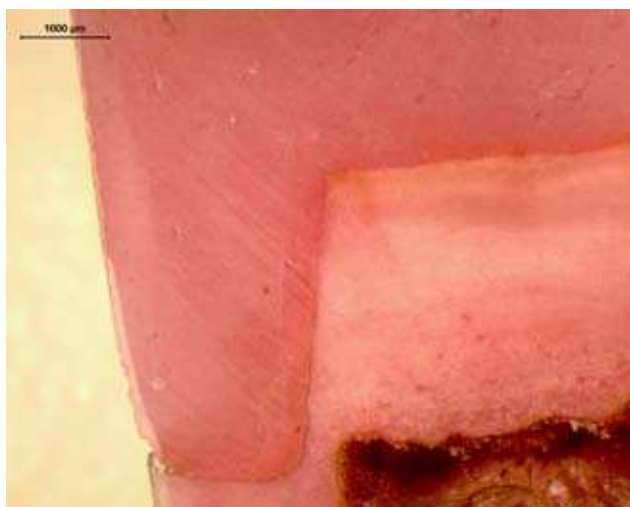

Figure 3. Image of a specimen from Alert + Flow-it group (x15)

Table 6 shows the comparison of groups according to their size of voids at the margins and materials. While there was no significant difference between the groups at the margins, Alert showed differences with Solitaire, Amalgam, Surefil, Surefil + Dyract Flow groups and also between Alert + Flow-it and Solitaire at the total material. Figures 6 and 7 show large voids at the margin and material from Alert group. When we consider margin and material together, the significant differences were between Alert and Solitaire, Alert and Amalgam.

Table 6. Comparison of results of voids' size

		Chi - Square	P	Post hoc Test Results
Margin	mesial	12.370	0.135	
	occlusal	17.965	0.021	
	distal	15.170	0.056	
	total	21.155	0.007	
Material	mesial	19.279	0.013	
	occlusal	22.908	0.003	
	distal	17.267	0.027	
	total	28.044	0.000	Solitaire & Alert+Flow-it Solitaire & Alert Surefil & Alert Surefil+Dyract Flow & Alert Alert & Amalgam
Margin + Material	mesial	8.791	0.360	
	occlusal	20.626	0.008	Solitaire & Amelogen
	distal	13.883	0.085	
	total	28.226	0.000	Solitaire & Alert Amalgam & Alert

For the overall comparison Kruskal-Wallis analysis of variance test and Post hoc analysis were carried out. Only statistically significant results are summarized in the table. Significant level $P<0.05$

Figure 6. A large void at the mesial material of a specimen from Alert group (x30)

Figure 7. The mesial margin of a specimen from Alert group (x37.5)

Table 7 shows the mesial and distal comparison according to the number and size of the voids. A specimen from Solitaire + Revolution group showing similar views for mesial and distal margins and materials were in figures 8-10. There was no significant difference between mesial and distal at the margins, but was significant at the material in Amalgam (P=0.042) according to the number, as shown in figure 11. According to the size of the voids, the only difference was at the margins in Amelogen (P=0.042).

Table 7. Comparison of voids at mesial and distal sides

| | Number | | | | Size | | | |
| | margin | | material | | margin | | material | |
Group	Difference (d-m)	P	Difference (d-m)	P	Difference (d-m)	P	Difference (d-m)	P
Solitaire	-1.604[a]	0.109	-1.826[a]	0.068	-1.604[a]	0.109	-1.461[a]	0.144
Solitaire+ Revolution	-0.816[a]	0.414	-0.405[a]	0.686	-1.069[a]	0.285	-0.405[a]	0.686
Surefil	-0.272[a]	0.785	-1.461[b]	0.144	0.000[c]	1.000	-1.214[a]	0.225
Surefil+ Dyract Flow	-0.272[b]	0.785	-0.365[b]	0.715	-0.687[a]	0.492	-0.135[b]	0.893
Alert	0.000[c]	1.000	-1.214[a]	0.225	-0.674[a]	0.500	-1.483[b]	0.138
Alert+ Flow-it	-0.272[b]	0.785	-0.000[c]	1.000	-0.535[b]	0.593	-1.753[a]	0.080
Amelogen	-1.604[b]	0.109	-0.674[a]	0.500	-2.032[b]	0.042*	-0.944[a]	0.345
Amalgam	-1.841[a]	0.066	-2.032[a]	0.042*	-1.483[a]	0.138	-1.483[a]	0.138
Prodigy Condensable	-1.225[b]	0.221	-1.753[a]	0.080	-1.753[b]	0.080	-1.214[a]	0.225

Wilcoxon Signed Ranks Test

a : Based on positive ranks

b : Based on negative ranks

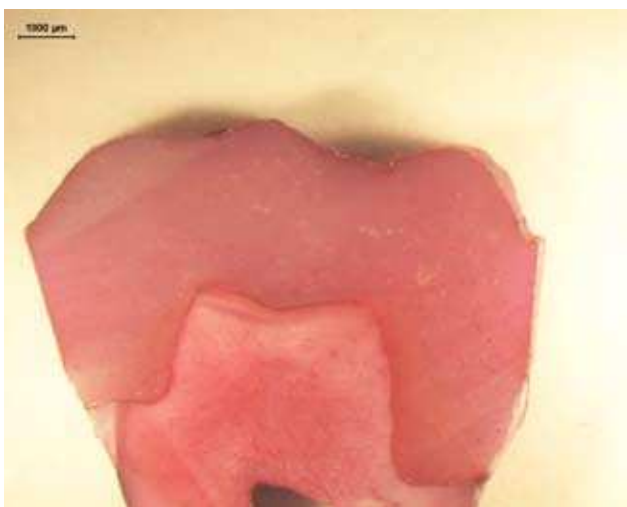c : The sum of negative ranks equals the sum of positive ranks

* : Significant (P<0.05)

Figure 8. Image of a specimen from Solitaire group (x9.45)
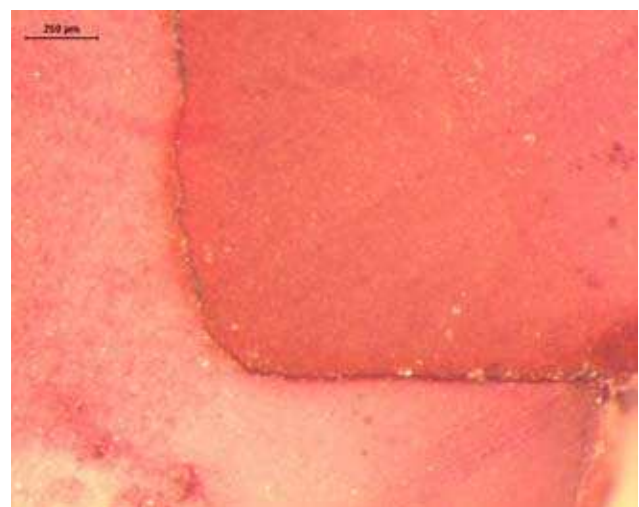

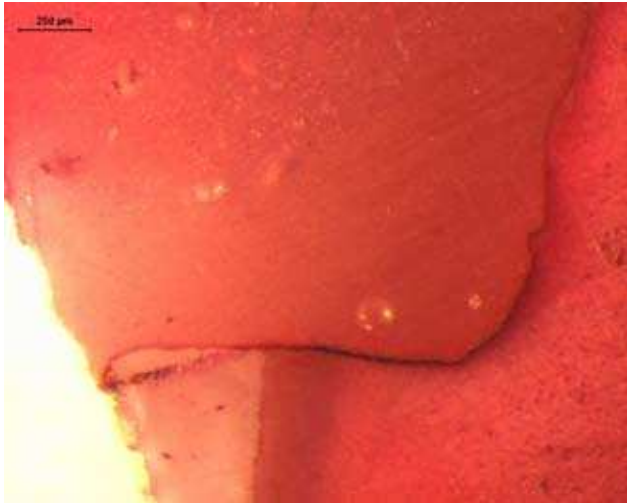*Figure 9. Mesial view of the same specimen in figure 8 (x48)*

Figure 10. Distal view of the some specimen in figure 8 (x48)

Figure 11. Image of an Amalgam specimen (x12)

Discussion

Improved handling characteristics developed for packable composite materials have made them more suitable for posterior applications compared to conventional composites. For the gingival proximal area of posterior teeth, where isolation is difficult and access and visibility are compromised, the technique sensitivity of composite materials is more likely to put this type of restoration at risk[1]. To improve the quality of the restoration, the preparation should be filled without voids and porosities. It has been recognized that voids at the cervical margins are an undesirable complication in composite restorations[2]. Marginal gaps between the preparation walls and the restoration, and voids on the surface or within the restorative material, can cause microleakage, discoloration, post-operative sensitivity, and secondary caries[14-17]. Porosity on the external surface of the restoration will result in surface roughness and may lead to stain[11]. Opdam et al[12] reported that syringable composites result in a better restoration with less voids compared to a packing technique with a highly viscous composite. Similarly Fano et al[13] reported that the highest amount of porosities were found in a highly viscous resin composite. Kelsey et al[8] reported that the mechanical properties revealed significant differences among high-performance packable and conventional hybrid composites. Studies reporting on bulk placement showed a decrease in depth of polymerization, greater microleakage and inferior degree of polymerization at cervical thirds of composite specimen restorations[20-22]. On the contrary, packable composites used in this study, did not showed any differences with incrementally placed hybrid composites both in number and size of voids. Similar to this, among packable groups there was no difference at the margins according to the number and size. However, inside material,

Alert showed significant differences with some groups both for size and number. As seen in tables 3 and 4, groups with Alert, which had individual micro-glass fibres, showed the highest number and size at the bulk material (Figs. 2 and 6). Leevailoj et al[2] reported that Alert was the stiffest material, while showing the most microleakage at the gingival margins.

The use of a combination of flowable and packable composites is an accepted concept[25]. As reported in some studies, flowable composite when used as a liner underneath a packable composite, demonstrated improved resistance to microleakage on enamel and dentin margins and was consistent with fewer voids[28-30]. However, Chuang et al[33] showed no significant difference in the marginal microleakage between with/without flowable composite linings. Leevailoj et al[2] reported that in Class II preparations, flowable composites reduced, but did not eliminate, microleakage of the tested packable and microhybrid resin composites at gingival margins apical to the CEJ. Chuang et al[34] showed a reduction in the presence of internal restoration voids when using flowable composites as a lining material for composite restorations. The incidence of internal voids was significantly reduced at both the restoration's interface and within its mass. This design of proximal cavity extension difference in this study and usage of flowable liner could conceivably have an effect on the voids in the restoration. The same study reported that no correlation exited between the number of voids and marginal microleakage[34]. In the present study, the use of flowable composites did not show any differences in number and size of the voids.

In the present study, to compare the different gingival levels, proximal margins were prepared 1 mm coronal and apical to the CEJ. In the literature there was a study comparing different gingival levels for microleakage but not for voids[35]. In the present study, there was no

statistically significant difference in number and size of voids in different gingival levels for packable composites at the margin and material. Besides, Amelogen showed difference for size (mm) of the voids at the margin. The reason for this might be related with incremental placement technique leading to more pores between layers during placement and lower inorganic content in hybrids (Figs. 12 and 13).
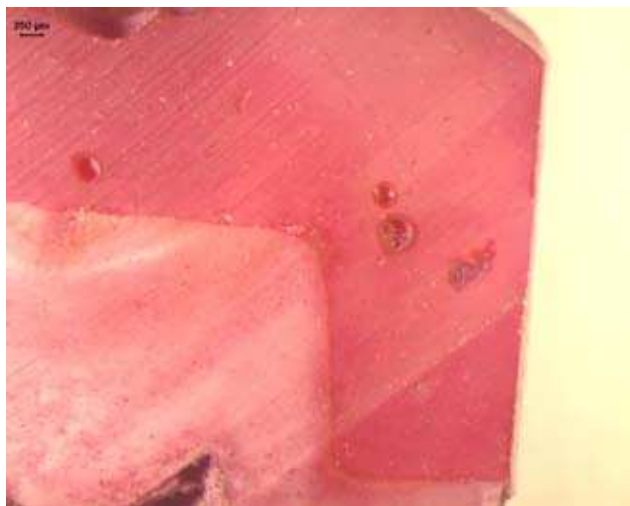
As a conclusion, the number and size of voids of the studied packables did not show any difference at the restoration margins and within the material. Also, different gingival levels and flowable usage did not make any difference among packables.

Figure 12. Image of a specimen from Amelogen group (x15)

Figure 13. Mesial image of a specimen from Amelogen group (x30)

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Fracture Resistance of Endodontically Treated Teeth Restored with Fibre or Cast Posts

SUMMARY

   The purpose of this study was to determine the fracture resistance of 4 post and core systems. 40 extracted maxillary canines (for orthodontic reason) were used for this study. The samples were divided into 4 groups, which were: Group (1) Cast post luted with Rely X ARC; Group (2) Cast post cemented with zinc phosphate; Group (3) Fibre post luted with Rely X + Filtek Z-250 as core material; Group (4) Fibre post luted with Rely X + Vitremer Core Build Up as core material. The post cores were loaded (N) to fracture by a universal testing machine and data were analyzed (One-way ANOVA). The obtained fracture resistance results were as follows: Gr 1 (2103.50 N ± 185.75) > Gr 2 (1494.80N ± 164.04) > Gr 3 (1004.90 N± 108.72) > Gr4 (739.40 N ± 96.93). Vertical root fractures were observed in the cast post-core groups. Hybrid composite cores in group 3 showed cohesive failures, whereas resin modified glass ionomer cores in group 4 showed a failure of adhesive nature from dentin.

   Keywords: Adhesive Resin Cement; Fibre Post; Cast Post

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Introduction

   Fracture of coronal part of the tooth is a commonly observed situation in dentistry. After endodontic treatment of the fractured tooth, an application of post may be necessary due to the insufficient remaining hard tissue[1,2]. Various types of post and core systems have been introduced for the management of this kinds of teeth[3-7]. Posts can either be individual or prefabricated such as steel, carbon fibre[2], quartz fibre[8] ceramic, zirconia, titanium[9] or fibre ribbond[10]. Recently introduced core build-up materials are tooth-coloured resin based materials instead of amalgam[5-7,11]. Tooth coloured post and core restorations luted with resin based cements are preferred for the restoration of non-vital anterior teeth, because of the increasing aesthetic demand of the patients and use of metal-free fixed prosthodontics. The improvement in adhesive systems, composites and resin cements led to evaluate aesthetic posts and core materials[12-16]. Recently, the effects of post-core systems and ferrule on fracture strength of aesthetic posts is an important research subject[17,18].

   The purpose of this study was to determine the fracture resistance of fibre post systems (with hybrid resin composite or modified glass ionomer cement cores) in comparison to conventional - zinc phosphate cemented or adhesive luted (Rely X ARC) cast posts, when the coronal portion of the tooth is lost.

Material and Methods

   In this study 40 extracted maxillary unerupted canines (for orthodontic reason) were used. The coronal portions were removed from cemento-enamel junction (no ferrule) with a diamond bur (Acurata, Germany). Root canal preparations were performed with Hero 642 nickel-titanium rotary instruments (Micro Mega, Hero 642, Besancon, France). The root canals were filled with Maillefer-Thermafil (Dentsply, Maillefer, Switzerland) assorted anterior kit according to the system described by Walcott et al[19]. After the root canal treatment, post space was prepared. A warm plugger was placed into the canal for removing the filling material from the cervical portion of the root. Reamer drills (LARGO Peeso Reamer, Dentsply, Maillefer, Switzerland) were serially (2, 3, 4 and 5) used for shaping the root canal. Post holes were prepared until the 2/3rd lengths of the root canals. The remaining tissue surrounding the post hole was minimally 1.5 mm in mesio-

distal and 2 mm in labio-lingual direction. Impressions for cast post restorations were made with a low shrinkage modelling resin (Pattern Resin LS, GC, Japan). The teeth were randomly divided into 6 groups of 10 (Tab. 1):

Group 1. Cast posts were fixed with Rely X ARC (3M ESPE, St. Paul, USA), according to the manufacturer's instructions;

Group 2. Cast posts were cemented with zinc phosphate without any dentin pre-treatment;

Group 3. Quartz fibre posts (AEstheti-Plus, Full Aesthetic Composipost, RTD, France) were luted with

Rely X ARC. Composite resin (Filtek Z-250, 3M ESPE, St. Paul, USA) cores were build up using a performed polyester matrix after etching and applying 3M Single Bond Adhesive (3M Single Bond Adhesive, 3M ESPE, St. Paul, USA) to the surrounding dentin and post;

Group 4. Fibre posts were luted with Rely X ARC. Vitremer core build up material (3M ESPE, St. Paul, USA) were build up using a performed polyester matrix according to the manufacture's instructions as core material.

Table 1. Experimental design

	Etching	Bonding agent	Luting Cement	Post material	Core material
Group 1	35 % phosphoric acid, 15 seconds	Single Bond Adhesive (x2)	RelyX ARC	Cast post-core	
Group 2	-	-	zinc phosphate	Cast post-core	
Group 3	35% phosphoric acid,15 seconds	Single Bond Adhesive (x2)	RelyX ARC	Fibre post	Filtek Z-250
Group 4	35% phosphoric acid,15 seconds	Single Bond Adhesive (x2)	RelyX ARC	Fibre post	Vitremer CBU

35% phosphoric acid in groups 1, 3 and 4 was used for 15 seconds, to etch the root canal dentin. 3M Single bond (3M ESPE, St. Paul, USA) was used in groups 1, 3 and 4 and polymerized with a translucent wedge (10 seconds), because of the depth of the root canal. A second polymerization was applied from the orifice of the root canal (10 seconds).

In group 3 and 4, quartz fibre posts (AEstheti-Plus, Full Aesthetic Composipost, RTD, France) were used. This composiposts were parallel sided and smooth in configuration.

The cores of each group were prepared in a standard height of 6 mm. The teeth were immersed in condensation

silicon (Oran Wash, Zhermack, Italy) and mounted parallel in acrylic resin blocks. The condensation silicon represents an artificial periodontal ligament. The cemento-enamel junctions of the teeth were 2 mm over the acrylic block surface.

After storage in distilled water for 24 hours (37°C), the restored teeth were loaded to fracture in a universal testing machine (Adamel Lhomary DY 30, France) with gradually increasing forces at a 45° angle to the long axes of the roots. Mode of failure from all specimens was determined. Data were analyzed with the One-way ANOVA tests (Tab. 2).

Table 2. One-way Anova analysis

One Factor				
Source	DF	Sum Squares	Mean Square	t-test
Among groups	3	10798320	3599440	235
Within groups	36	550787	15300	P=0.0001
Total	39	113449107		

Results

The obtained results showed that statistically differences existed among all groups. Cast posts cemented with Rely X ARC (group1) required the greatest amount of force to fracture and it was significantly higher than the other groups (p<0.05). Group 4 exhibited the lowest

fracture resistance. Fracture strength results were as follows: group 1 (2103.50 N ± 185.75) > group 2 (1494.80N ± 164.04) > group 3 (1004.90 N± 108.72) > group 4 (739.40 N ± 96.93) (Tab. 3). The statistical differences between the groups were given in table 4.

Failure modes were determined for all specimens. In group 1 and 2 root fractures were observed. A cohesive

type of failure was determined in group 3, while the failure was of an adhesive nature in groups 4. Displacement of the posts was not observed in any group with cast or fibre posts.

Table 3. Fracture strength values and standard deviations for each group

Group	Treatment	Mean Value (N)	SD (N)
1	Cast posts luted with Rely X ARC	2103.50[a]	185.75
2	Cast posts cemented with zinc phosphate	1494.80 [b]	164.04
3	Fibre post with Filtek Z-250 core	1004.90 [c]	108.72
4	Fibre post with Vitremer CBU core	739.40 [d]	96.93

Different superscript letters mean statistically significant difference (p<0.05)

Table 4. Statistical analysis among groups

Groups	P value
Gr1-Gr2	p=0.0001
Gr1-Gr3	p=0.0001
Gr1-Gr4	p=0.0001
Gr2-Gr3	p=0.0001
Gr2-Gr4	p=0.0001
Gr3-Gr4	p=0.0001

Discussion

After endodontic treatment, adhesive-luting cements, prefabricated post systems and light cured core restorative materials are preferred due to the reduction the chair time. Fracture resistance of endodontically treated teeth restored with aesthetic post-core systems was evaluated with *in vitro* designed researches[8,15]. The results of the present study showed that statistical differences exist among all groups. The cast post-core restorations luted with adhesive resin cement exhibited the highest fracture resistance, which was also reported in other studies[15,20]. But the results, which were obtained in other groups (2, 3, and 4) also exceeded the biting forces, which has been reported as 60 lb for anterior teeth (1 lb = 0.4535 kilogram)[7]. All the groups are acceptable for clinical treatments. The obtained results for fibre post-core restorations are similar to the results of Akkayan and Gulmez[21].

It is reported that the size of the root will have a great impact on the fracture resistance[3]. This study examined the fracture strength of fibre post-core systems in comparison with conventional cast post-cores using unerupted canine teeth. The differences of size and shape of the teeth can influence the fracture resistance and may be the explanation for standard deviations of this study. The standard deviation in our study groups might be also due to the artificial periodontal ligament. The thin layer of condensation silicone simulated the periodontal ligament, acrylic resin the alveoli and blocks the bony sockets, according to Simirai et al[10]. By embedding the roots not directly into the acrylic resin blocks, external reinforcement of the root structure by the rigid acrylic resin material was avoided[10].

Investigations focused on cementation showed various results related to zinc phosphate and resin based cements. Nissan et al[12] reported that Flexi-Flow (reinforced composite resin cement) significantly increased the retention of post systems compared with zinc phosphate. In the present study cast post restorations luted with Rely X ARC (group 1) fractured in higher values in comparison to zinc phosphate luted posts. Adhesive cements, which bond not only to tooth structure but also to various types of posts, have the potential to create a highly retentive intracoronal restoration without the disadvantage of creating undue stress to the remaining root structure[14].

Many studies investigated the physical properties of core materials[11,12,14,22-24]. Authors showed that glass ionomer materials' failure rate is the highest[4,7,11,25]. In the present study, Vitremer CBU restorations showed weaker physical properties compared to the hybrid composite, Filtek Z-250. Post core restorations with Vitremer CBU showed the lowest fracture resistance (group 4). The disadvantage was the brittleness and poor adhesion characteristic of resin modified glass ionomer.

Fracture modes for post core restorations were also described in the literature. Martinez et al[2] reported that cast posts and cores typically showed fracture of the tooth, albeit in response loads that rarely occurs *in vivo*. Fracture in the root was also observed in our cast post groups (groups 1 and 2), but the fracture resistance data are very high and may occur in the anterior region. Teeth restored with fibre posts showed an adhesive failure with Vitremer CBU material and cohesive failure with hybrid composite O'Keefe et al[13] determined that higher bond strengths resulted in a higher percentage of cohesive failures.

The easy application of prefabricated post restorations is important from the viewpoint of the clinician. The fibre post provide retention for the core material and can be placed immediately after endodontically treatment. A clinical advantage is that root fractures are not observed. A disadvantage may be that the luting procedures have high technical sensitivity. The success of the luting procedures depends on the dentin conditioning, ie. concentration of the etching agent and

application time, the dryness or moisture content of the root canal, individuals' dentinal configuration, age of the patient, generation of the bonding system, irrigation solution, the reaction of the endodontic filling materials with dentin, the presence of the smear layer and the characteristics of the irrigation solutions.

The clinical performance of post restorations depends on multiple factors, such as remaining hard tissue, core materials, fixed prosthodontics and individual criteria, occlusal forces or habits. Direction and speed of force (shear) and fatigue behaviour are also influencing factors on clinical performance.

The results presented in this study were obtained from restorations without any prosthodontics abutment. Clinically, the final restoration might enhance the fracture strength. Future studies should aim the evaluation of direct post core restorations with prosthodontics abutment and a clinical follow-up study is definitely required for making conclusions about oral conditions.

Conclusions

It was concluded that cast post-core and fibre post-core restorations could be acceptable clinically for endodontically treated teeth with a limited remaining hard tissue. Adhesive luting of the cast posts exhibited higher fracture resistance in comparison to zinc phosphate cemented cast post-core restorations. Fibre post restorations with Filtek Z-250 composite cores showed higher fracture resistance than fibre post restorations with Vitremer CBU.

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Evaluation of the Effect of Different Ligature System On Microbial Attack

SUMMARY

The objective of this study was to investigate the effect of elastomeric and stainless steel ligatures on the microbiology of local dental plaque. Clinical reports have shown that patients who receive orthodontic treatment are more susceptible to enamel white spot formation. Metallic orthodontic brackets have also been found to inflict ecologic changes in the oral environment, such as decreased pH and increased plaque accumulation. Changes manifested in the oral flora included elevated Streptococcus mutans and Lactobacilli colonization and imposing a potential risk for enamel decalcification. The subjects were 40 patients at the beginning of their treatment with fixed orthodontic appliances. Orthodontic brackets were bonded to the buccal surface of the test teeth with a non-fluoridated adhesive and than arch wires were fixed by elastomers and stainless steel ligatures at the different time in same patients.

There were no significant differences in account of S. mutans and Lactobacilli after the use of metallic ligature (p>0.05); elastomeric ligatures increased these level significantly (p<0.05). There was a significant difference between these groups (p<0.05).

Keywords: Elastomeric Ligature; Microorganisms; S. Mutans; Lactobacilli

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Introduction

In orthodontics, white spots and decalcification are attributed to prolonged accumulation and retention of bacterial plaque on the enamel surface adjacent to the attachments[9,12]. Demineralization of enamel has been reported to occur around orthodontic brackets after only 1 month[9]. Ligature ties represent new retentive areas around the brackets, so their role in caries formation is very important. Formation, origin and shape of the ligatures affect the oral microflora balance differently[4].

Metallic orthodontic bracket ligatures have been found to cause ecological changes in the oral environment, such as decreased pH, elevated *Streptococcus mutans* colonization, and increased plaque accumulation, which adversely affect orthodontic patients who are susceptible to enamel white spot formation[2,8]. Recently, the biophysical properties and chemical constituents of orthodontic bracket pellicles were reported by Eliades et al[5]. However, no information is available on the molecular identification of adsorbed salivary pellicles on orthodontic materials, including brackets, and this limits our understanding of the mechanism of initial microbial adherence to the surfaces of orthodontic materials[6].

The advantages of elastomeric ligatures are that they can be applied quickly, are comfortable to the patient, and are available in a variety of colours. Disadvantages are that the dentition and soft tissues may be adversely affected by microbial accumulation on the tooth surfaces adjacent to brackets ligated with elastomeric ligatures, arch wires may not completely seat during torque or rotational corrections, and binding may occur with sliding mechanics.

Plaque is a major etiological factor in the development of dental caries. The control of plaque is fundamental in the control of caries and periodontitis. It has been shown that placing a fixed orthodontic appliance leads to both an increase in the levels[1] and a change in the composition of dental plaque[7]. Sakamaki and Bahn[10] showed an increase in the lactobacillus index and the salivary lactobacillus counts after the placement of orthodontic bands. Corbett et al[3] and Scheie et al[11] demonstrated an increase in the level

of *S. mutans* in the plaque surrounding an orthodontic appliance, and suggested that placing an orthodontic appliance leads to the creation of new retentive areas favouring the local growth of this organism.

Fixed orthodontic appliance treatment significantly increases the risk of white spot lesions and enamel decalcification[2,12]. Enamel decalcification is caused by an imbalance between demineralising and demineralising of enamel, and the resultant white spot lesion is considered to be a precursor of enamel caries[12].

Materials and Methods

The subjects of this study were 40 children undergoing orthodontic treatment at the Department of Orthodontics, Faculty of Dentistry, Dicle University, with fixed orthodontic appliances in both jaws. Exclusion criteria included the use of oral antimicrobials or antibiotics within the past 3 months, the presence of prosthodontic appliances, or significant systemic disease. We advised them to brush their teeth and the appliances 4 times every day during this study period.

The CRT Bacteria Test (Vivadent Ets, Lichtenstein) was used to determine the *S. mutans* and *Lactobacilli* counts in saliva by means of selective culture media (Fig. 1).

Figure 1: The production kit of S. Mutans and Lactobacilli

At visit 1, the fixed appliance brackets and bands were placed. Stainless steel ligature ties were used to fix the arch wires. The patients were given standard fluoridated toothpaste (Colgate - Palmolive company, UK). Conventional non-fluoridated elastomers were placed on the remaining teeth.

Visit 2 was 4 weeks later. At this first adjustment appointment, metallic ligatures on the teeth were aseptically removed, placed in separate containers with a pre-reduced transport medium and coded. These were taken to the laboratory within 10 minutes. The appliance was adjusted, it was advised patients to brush their tooth and non-fluoridated elastomers were placed on all teeth to allow for a washout any mouth rinse period of at least 4 weeks.

At visit 3, the appliance was adjusted and the conventional elastomers were removed from teeth surface. These were taken to the laboratory.

In the laboratory, the agar carrier was removed from the test vial, and a $NaHCO_3$- tablet was placed at the bottom of the vial. The protective foils were removed carefully from the agar surface. Using transporters, agar surfaces were wetted with ligatures and excess was allowed to drip off. The agar carrier was placed back into the vial, which was closed tightly. The vials were incubated at 37°C for 48 hours. After that all of the samples were evaluated as product company directions by its scale. Findings of 10^5 CFU or more of lactobacilli and mutans streptococci per ml saliva indicated a high caries risk.

For statistical evaluation of the differences in the levels of the *S. mutans* and *Lactobacilli*, Wilcoxon Singned Ranks test was used.

Results

In the *S. mutans* evaluation group, there wasn't any significant difference between 1 and 2 visit samples (p = 0.655); differences were found in comparison of 1-3 visit and 2-3 visit (Tab. 1). In the *Lactobacilli* group, there wasn't any significant difference between 1 and 2 visit samples (p = 0.265). However, comparing 1-3 visit, and 2-3 visit we found significant differences (Tab. 2).

Table 1. Comparison of different ligatures' effect to S. Mutans level

GROUP	p	Significance
A-B	0.002	**
B-C	0.655	n.s.
A-C	0.007	**

A: Elastic Ligature
B: Stainless Steel Ligature
C: Initial Treatment
n.s.: not significant (p>0.05)
** p< 0.01

Table 2. Comparison of different ligatures' effect to Lactobacilli level

GROUP	p	Significance
A-B	0.025	*
B-C	0.206	n.s.
A-C	0.007	*

A: Elastic Lıgature
B: Stanless Steel Ligature
C: Initial Treatment
n.s.: Not significant (p>0.05)
* p< 0.05

There was no significant difference in account of *S. mutans* and *Lactobacilli* after the use of metallic ligatures (p > 0.05); elastomeric ligatures increased this account significantly (p < 0.05). Moreover, there was a significant difference between *S. mutans* and *Lactobacilli* groups (p < 0.05).

Discussion

This study has shown that, after a clinically relevant time in the mouth, there were significant differences in percentage of *S. mutans* and *Lactobacilli* counts in plaque obtained from elastomeric ligatures compared with stainless steel ligature. This study also provides valuable information for understanding bacterial colonization on the surfaces of orthodontic brackets ligatures and for investigating means to interfere with the adherence of pathogenic bacteria to the pellicle of orthodontic ligatures.

Forsberg et al[6] found that most patients had a higher bacterial count on teeth ligated with conventional elastomers than on teeth ligated with steel ligatures. In the present study, it was noticed that, clinically, there was a marked deterioration in the physical properties of elastomers in the mouth; they were considerably swollen compared with the conventional elastomers after 4 weeks, and several were missing when the patient returned. Besides, there wasn't any deterioration and deformation in the stainless steel ligature group.

Eliades et al[5] suggested that the presence of different materials intraorally, such as elastomers and metals (arch wires and bands), and exposure of adhesive resin margins, will presumably increase plaque accumulation on the appliances.

Wearing orthodontic appliances has been found to induce specific changes, such as a lower pH, increased plaque accumulation, and elevated *S. mutans* and *Lactobacilli* colonization, all of which increase orthodontic patients' susceptibility to enamel demineralization. Knowledge about the relationship between the bracket ligatures and oral bacteria will provide the basis for

preventing the adhesion of pathogenic microorganisms around the bracket surface. This study showed that various microorganism adhered selectively to the orthodontic materials. The selective adherence was due to differences in the bracket ligatures.

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Central (Endosteal) Osteoma of the Maxilla: Report of a Case

SUMMARY

Osteomas of the jaws are well-differentiated bone lesions, affecting more frequently the mandible than the maxilla. They are classified in 2 groups, central and peripheral, although the existence of central osteoma is debated. They usually remain asymptomatic, except when they take large dimensions or produce functional disturbances.

This paper describes a rare case of central osteoma in a 74-year-old man. The lesion was presented as an asymptomatic ulcer, dens-like protuberance, which was located on the residual alveolar ridge of the left maxilla, with no other clinical symptoms. The 3 years follow-up after complete surgical excision showed no sign of recurrence.

Keywords: Osteoma, central; Maxilla

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Introduction

Osteomas are benign, well differentiated bone lesions, which are found almost exclusively in the flat bones of the skull, in paranasal sinuses, and more rarely in extra-skeletal soft tissues[1,4,9,17]. Their location in the jaws is rare, and the maxilla is less frequently affected than the mandible[4,11,18,19].

Osteomas usually remain asymptomatic for a long period of time. However, when they take on large dimensions, they might produce disfigurement of the face, or functional disturbances such as difficulties in mastication and swallowing, or vision and balance problems due to their vicinity to the carotid sinus or to the internal carotid artery[7,14,16,18]. They are seldom associated with pain[8,15,23].

Osteomas are classified according to their location in 2 main groups, central (endosteal) and peripheral (subperiosteal), although severe doubts have been raised as to whether a central osteoma is a real entity[4,9,15,20]. Up until now, we have found only 1 fairly well documented case of endosteal osteoma in the English literature[17]. In this paper, an extremely rare case of a central osteoma of the maxilla is presented. We also discuss the pathogenesis, the clinical and radiological features, and the pathology of such lesions.

Case Report

A 74-year-old man was referred by his dental practitioner to the Department of Maxillofacial Surgery of the Aristotle University of Thessaloniki for evaluation and treatment of an asymptomatic ulcer, dens-like protuberance of the posterior alveolar ridge of the left maxilla (Fig. 1). The lesion had appeared 2 weeks previously, with no other clinical symptoms. The patient had been through a full mouth restoration with full dentures 6 months earlier. The oral mucosa was normal, with a slight bony prominence in the area of the lesion and a small ulcer located on the affected area, with no other intraoral findings.

Figure 1. Intraoral appearance of the lesion. A small ulcer at the area of the residual crestal ridge can be seen

The radiographic imaging showed a round, well defined, high-density radiopaque mass in the left maxilla, measuring 20x30 mm, without any obvious correlation with the left sinus (Fig. 2). Physical and laboratory examinations were within normal limits. The patient's medical history was free of gastrointestinal symptoms or skeletal abnormalities, and the possibility of Gardner's syndrome was excluded.
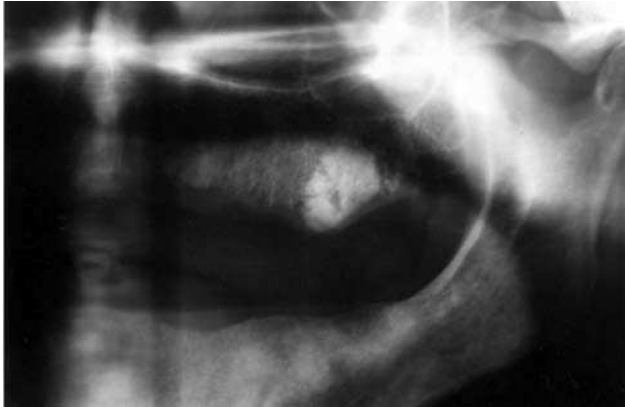

Figure 2. Panoramic radiograph of the patient, showing the lesion in the body of the left part of the maxilla

The clinical diagnosis was "odontogenic tumor". A decision was made for a total removal of the lesion in order to achieve a complete histology. Under local anesthesia and through a labial mucoperiosteal flap, the mass was exposed and revealed (Fig. 3). It is noteworthy that the lesion was much harder than the surrounding healthy bone, without any clear distinguishing border between them. The upper part of the lesion was firmly attached to the surrounding bone and we used a small round burr and a straight elevator to remove it (Fig. 4). So, in a manner of speaking, the mass was not encapsulated. After removal of the lesion, some bone particles from the surrounding tissues were also removed. (Fig. 5).

Figure 3. Exposure of the osteoma

Figure 4. Removal of the osteoma by exerting light pressure with a lever
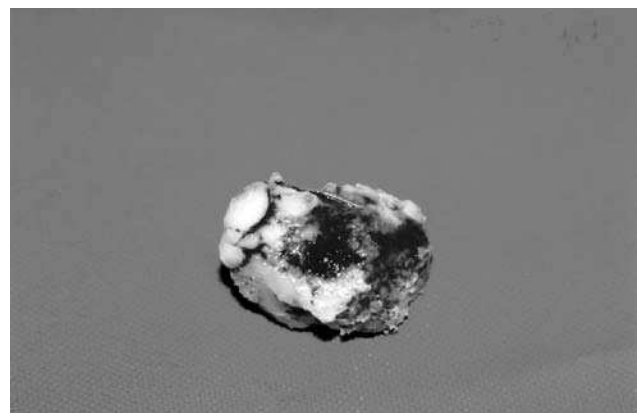

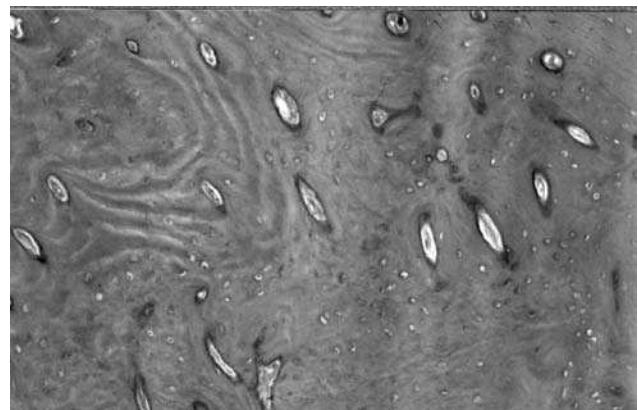*Figure 5. The surgical specimen from its inner site*

Figure 6. Photomicrograph of the lesion. Compact bone containing few very small spaces with thin vessels
(HE stain, original magnification x400)

Microscopically, the excised mass was mainly composed by well-differentiated dense compact bone containing few very small spaces with thin vessels (Fig. 6). Taking into consideration the other diagnostic parameters, a diagnosis of central osteoma of the maxilla was made. There has been no recurrence during a follow-up of 3 years (Figs. 7 and 8).

Figure 7. Intraoral appearance of the patient 3 years after the operation
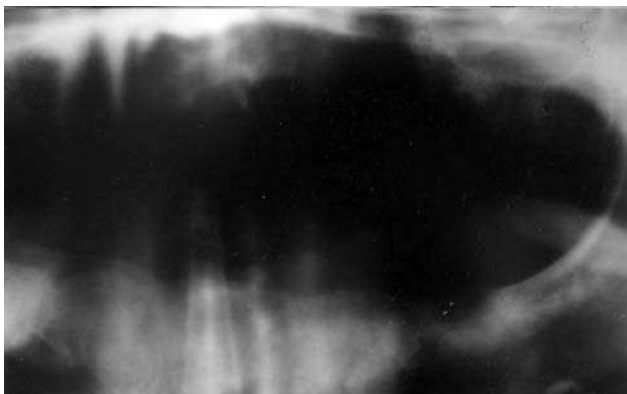

Figure 8. Panoramic radiograph showing the absence of the osteoma in the maxillary region

Discussion

Osteomas of the jaws are benign neoplasms consisting of well-differentiated compact or cancellous bone, characterized by continuous osseous growth[18,21,22]. They are generally found in the skull and facial jaw bones, and are classified as peripheral or central[21].

Peripheral osteomas are considered to arise from periosteum. The site most frequently affected by peripheral osteomas is the frontal sinus, followed by the ethmoidal and maxillary sinuses[19,24]. Peripheral osteomas have also been described in various locations of the skull, such as the pterygoid plates[10] and the temporal bone[2]. Peripheral osteomas are usually located on various sites of the mandible[3,6,11,22], while the maxilla is less frequently affected[,5,19]. Trauma or infection has been suggested as a possible etiologic factor in the formation of these lesions. Trauma is considered to play an important role since many osteomas are encountered on the lower border or the buccal aspect of the mandible, a location which is more vulnerable to trauma than the lingual aspect[6,11,12].

Central osteoma is considered to arise from endosteum. However, great controversy surrounds the existence of this pathologic entity[17]. Indeed, central osteoma, as a sound pathological entity, has been questioned up until now, since many reported cases have been reevaluated and reclassified. Osteomas of the facial skeleton, associated with skeletal abnormalities and gastrointestinal symptoms, should reinforce the possibility of Gardner's syndrome[12,24]. The dental abnormalities of such patients also include supernumerary and impacted teeth, odontomas and dentigerous cysts. The most frequent sites for osteomas associated with Gardner's syndrome are the external surface of the skull, the paranasal sinuses and the mandible[13].

Although peripheral osteoma is now an acceptable and classified lesion, the classification of central osteoma as a discrete lesion remains equivocal, as many cases of central osteoma proved to be other pathologic entities, such as cementoma, fibrous dysplasia or focal sclerosing osteomyelitis[17].

In our case, the radiographic and surgical findings along with histological features and the patient's history strongly suggest for the diagnosis of central osteoma of the maxilla. It is clear from the panoramic X-ray that the lesion was entirely developed into the body of the maxilla, without having any relation to the ipsilateral maxillary sinus. We also could conclude that the slight intraoral prominence of the lesion from the neighboring healthy maxillary bone was created gradually, as a result of the pressure exerted by the patient's denture. This pressure could also cause the small ulcer of the mucosa, observed over the osteoma.

Histological examination revealed a well demarcated lesion from the surrounding trabecular bone, which consisted of dense compact bone. There were neither odontogenic epithelial remnants, nor any cement or cement-like findings. There was also no evidence of active or previous inflammation. Another point favoring the diagnosis of central osteoma was the absence of previous trauma or infection at the affected site.

In contrast to another published case of central osteoma, where severe pain was the main clinical symptom, our patient was free of pain and other related symptoms. Correlating the above mentioned findings, we conclude that the lesion we removed was an osteoma of the maxilla with central location.

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Kaposi's Sarcoma of an Intra-Parotid Lymph Node in a HIV-Negative Patient

SUMMARY

   Background. *Kaposi's sarcoma (KS) as one of the defining tumours of AIDS, was described as multiple slowly progressing pigmented skin plagues and as vaso-formative lesion in microscopic finding. Several forms of the disease have been suggested, such as mucocutaneous and lymph nodal. KS is rarely seen in the major salivary glands. Furthermore, KS of parotid tissue or intra-parotid lymph node is extremely rare in HIV-negative patients.*

   Case Report. *We report a case of a right parotid mass as an early sign of KS infection in a 57-year-old patient. The problems related to the diagno-sis, the management strategy of such a rare condition and prognosis are also discussed. Complete surgical excision is suggested, followed by adjuvant radiotherapy and management of any other suspicious lesions confirmed by clinical and histo-pathological examination.*

   Conclusions. *KS is a rare tumour of the parotid gland but practitioners need to be reminded of rare cases in their differential diagnosis.*

   Keywords: Kaposi's Sarcoma; Intra-Parotid Lymph Node; HIV Infection

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Introduction

   Kaposi's sarcoma (KS), a cutaneous malignancy of lymphatic endothelial cells, was originally described by Moritz Kaposi in 1872[1]. Since his original description, 4 new forms of the disease have been suggested[2]:
   sporadic;
   transplantation associated;
   endemic African;
   epidemic, acquired immunodeficiency syndrome (AIDS)-related.

   The sporadic or classical KS lesions usually are slowly progressive, involving the skin around the angles, the legs, the hands and arms to a lesser extend and frequently the lymph nodes draining those areas. The course of the disease is generally indolent, and the patients survive an average of 10-15 years. The transplantation associated or iatrogenic KS form is found among allograft recipients with fatal course, but spontaneous regression may be observed if immunosuppression is removed[1, 2]. The endemic form is occurred in African adult males and children. Extra-cutaneous involvement in the endemic form is usually associated with an extremely poor prognosis[2,5]. The epidemic KS is found among patients

with acquired immunodeficiency syndrome (AIDS) and has experienced a remarkably increased prevalence[3]. KS is one of the defining tumours of AIDS, and is rarely seen in the major salivary glands[5-8]. However, KS of parotid tissue or intra-parotid lymph node is extremely rare in a non-immunocompromised and HIV-negative patient.

   We present a case of a right parotid mass as an early sign of KS, in an HIV-negative patient. The problems related to the diagnosis, the management strategy of such a rare condition, and prognosis are also discussed.

Case Report

   A 57-year-old Caucasian male was referred for evaluation of a painless mass on the right parotid gland. The mass was firm, non tender and smooth on palpation. No palpable cervical lymph nodes were found, and the evaluation of parotid gland function didn't indicate any diminishing of salivary flow. The mass was painless for almost 2 years, and only recently (the last 6 months) increasing in size.

The ultra-sonography scan of the right parotid gland showed a solid mass measured 1-2 cm, while the left parotid gland was clinically healthy (Fig. 1). On MRI scan, the mass was solid, well defined, and located in the superficial lobe of the parotid gland, 1.2 x 1.4 cm in dimensions (Fig. 2). The facial nerve was intact and functional, and the differential diagnosis included almost all benign lesions of the parotid gland. The patient was serologically negative for HIV antibodies, whereas chest film showed no other evidence or any metastasis of the disease.
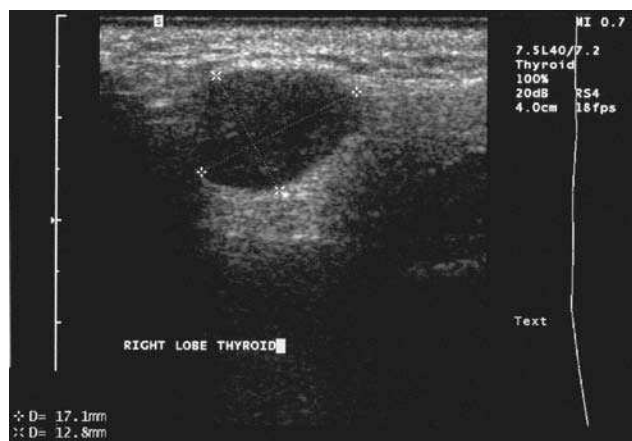
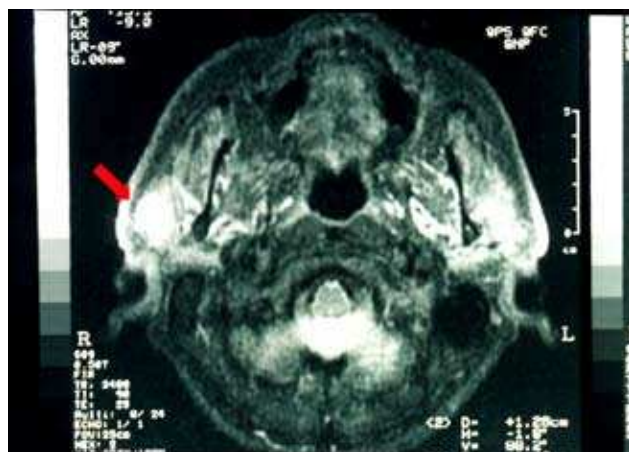*Figure 1. Sonography of right parotid gland with lesion*

Figure 2. MRI scan that shows a solid lesion on the right parotid gland (arrow)

Taking into consideration the radiological and clinical evaluation, an adequate parotidectomy was performed. The mass was excised completely and the remaining parotid tissue was clinically normal (Fig. 3).

The histological examination of the lesion was indicative of KS. The macroscopic findings revealed that the tumour was a well circumscribed grey-brown mass and was located within the substance of the parotid gland. The histology of the tumour was similar to the plague-

tumour stage KS of cutaneous lesions. It was well defined, surrounded by fibrous tissue varying in thickness, with anastomotic branches of spindle shaped tumour cells in vaso-formative pattern. Lymphocytes and plasmacytes were found within the spindle cells, whereas numbers of lymphocytes and 1-2 lymph nodules were identified in the periphery, resembling a lymph mode occupied by the tissue described above. Extravasated erythrocytes were abundant within the slit-like vascular spaces. Characteristic eosinophilic globules, PAS positive, were found occasionally (Figs. 4 and 5). The immunohistochemical analysis revealed spindle cells reacted with factor VIII-related antigen, CD 31, CD 34 and vimentin, confirming the vascular origin of the tumour. The microscopic findings and immunohistochemical results confirmed the diagnosis of KS.

In addition, HIV antibodies were negative in the follow-up evaluation, whereas serum positivity for HHV-8 antibodies was tested by the ELISA method.

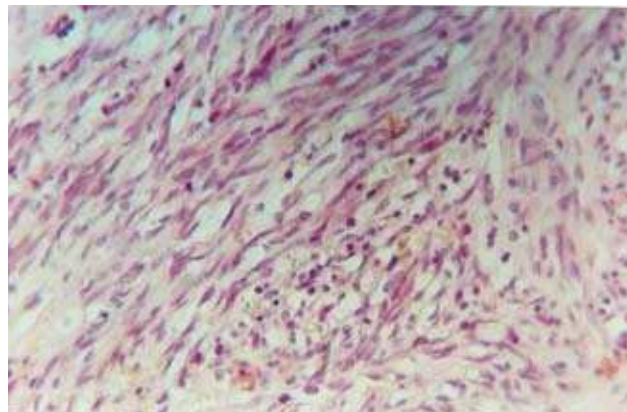*Figure 3. The excision of the intra-parotid tumour*

Figure 4. Branches of spindle shaped cells in vaso-formative pattern (H.E. x 400)
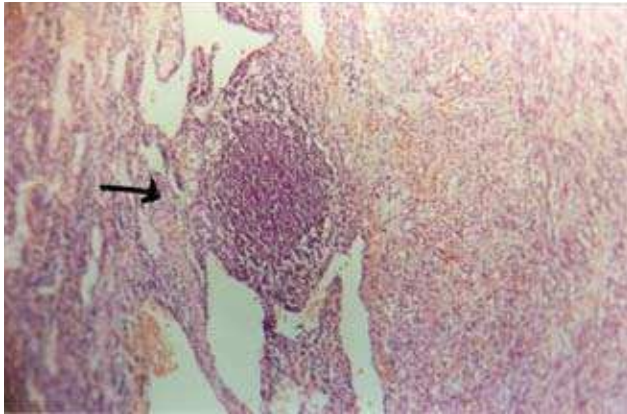
Figure 5. Residual elements of lymphadenoid tissue below the tumour follicle (H.E. x 100)

Adjuvant radiotherapy of 2000 cGy was administered 1 month postoperatively, and one year after treatment the patient was alive and in good general health, with no evidence of the disease, and serologically negative to HIV antibodies.

Discussion

KS is considered to be a virus-associated multifocal neoplasm. It develops with multiple reddish purple maculae in the skin, many of which evolve into plaques and finally subcutaneous nodules[2].

There are a number of AIDS-defining diseases including malignancies, of which KS is one of the more specific. Therefore, KS is likely to be included in the differential diagnosis of a variety of head and neck, and more specifically, salivary gland presentations of HIV infected patients. Although lymph node involvement may occur in all 4 clinical forms, and sometimes can precede the development of skin lesions or may even occur in their absence, it is more frequently seen in the AIDS-related form[10,11].

KS-associated herpes virus (KSHV) is believed to play an etiologic role in the development of KS in patients, either with or without evidence of HIV infection. In 1994 Chang et al[4] discovered a previously unknown KS-associated herpes virus, the human herpes virus type 8 (HHV-8), in virtually every KS lesion examined. KSHV now is believed to be the primary cause of all types of KS. HHV-8 also is believed to be transmitted sexually and to precede the development of KS. Additional studies have shown that antibodies to HHV-8 are present in approximately 90% of patients with KS[5,12].

In the present case, there was no evidence of HIV positive antigens, postoperatively and in the follow-up, but HHV-8 antigens were positive of infection, while histopathologic and immunohistochemical examination

confirmed the diagnosis of KS. A thorough dermatological examination showed no other evidence of the disease. In addition, chest and upper and lower abdomen MRI revealed no other evidence of KS, so the parotid lesion in our patient could be considered as a primary KS of the parotid gland (Figs. 6 and 7).

Figure 6. Chest MRI negative of any lesion and lymph nodes

Figure 7. Abdominal MRI free of lesions and lymph nodes

The treatment of KS is a stage and clinical form depended procedure. Surgical resection and adjuvant radiotherapy may be proved adequate in most of the

cases. There have been reports for advanced disease or un-resectable lesions to be treated with radiation therapy alone or with concomitant chemotherapy which includes vincristine, bleomycin, etoposide or vinblastine[9]. Our patient received adjuvant radiotherapy of 2000 cGy, 1 month postoperatively. In the 6 and 12 month follow-up, the patient has been free of the disease.

KS is rarely related with poor prognosis. Instead, patients with KS in the epidemic AIDS form, usually succumb to infectious compilations of AIDS[6]. Therefore, most HIV-positive patients affected by KS have a poor prognosis, and an infectious disease specialist is decisive for the initiation of specific therapy. This includes highly active antiretroviral therapy (HAART) and prophylactic antibiotic administration[7,8].

In conclusion, although KS is a rare tumour of the parotid gland, especially in HIV-negative patients, practitioners need to be reminded of such cases in their differential diagnosis.

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The Value of Identification Marking on Dentures*

SUMMARY

Since there is a large variation in the oral status of populations all around the World, the need for removable dentures will continue for the next decades. Denture marking can play an important social and legal role. There are 2 methods for denture marking: the surface method and the inclusion method. The purpose of this article is to present some cases of denture marking with various techniques from both methods. Some of them are easy to make, having their advantages and disadvantages. Marking by the inclusion method is more persistent, but the research for new marking materials continues. There is an obvious need for an international consensus about denture marking for clinical and forensic purposes.

Keywords: Denture Marking, methods; Human Identification; Forensic Odontology

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Introduction

In today's complicated and fast paced life, it often becomes difficult to identify deceased individuals. People may die in accidental disasters in trains, airplanes or buses, or in natural disasters such as floods and earthquakes. When these disasters occur, the bodies are often found decomposed, fragmented or burned. Persons who die as a result of these causes are often found decomposed and/ or skeletonized. With facial features and fingerprint pads often missing, the principal method of identification is through dental means.

Denture marking is a well-accepted mean of identifying both dentures and persons. It facilitates the identification of a patient in cases of unconsciousness, loss of memory and for forensic purposes (post-mortem identification) during war and civil unrest, crime cases, natural and mass disasters. It is also useful in geriatric institutions, hospitals and dental laboratories[1,2]. Since the oral status of population varies in different countries and the wearing of full dentures will continue for the next decades, the denture marking can play an important social and legal role[3]. The material from which a denture has been made, the type of the teeth and the standard of workmanship may help in identification[4]. Dentures are not

always marked. In European legislation, denture marking exists only in Sweden and Iceland[5,6].

There are 2 main methods in marking the dentures. In the surface marking method, the marks are located on 1 of the denture's surface. In the inclusion method, the marks are enclosed in the denture. The mark should be placed in a part of the denture without affecting the resistance of the denture, it will not be visible when the patient wears them, and it will be relatively protected in case of a fire. Therefore, the posterior regions of the lingual flange and palate are recommended[2].

The purpose of this article is to present some cases of denture marking, including marking by using metal materials. The dentures presented are 3 removable complete maxillary dentures, 1 removable partial maxillary denture and 2 removable partial mandibular dentures.

Case Reports of Different Marking Methods

Surface Method

Scribing or engraving the denture: This is the simplest way of marking dentures. In this technique 2 letters were engraved with a small round dental bur on the fitting surface of the maxillary complete denture, which resulted in countersunk letters (Fig. 1). The first letter is the initial letter of the name and the second letter is the initial letter of

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the surname. In this case, the letters KX are present on the fitting surface of the maxillary complete denture. The denture of Fig.1 belongs to a 70-year-old man.

Figure 1. A removable complete maxillary denture. The initials of the owner are engraved (surface method)

Marking with embossed letters: In this technique, embossed letters are made by scratching or engraving on the model before processing (Fig. 2a). The maxillary complete denture of Fig. 2a belongs to a 65-year-old man. His initial letters were written on the buccal surface of the disto-buccal flange. This technique can be also used in partial dentures, as shown in figure 2b.
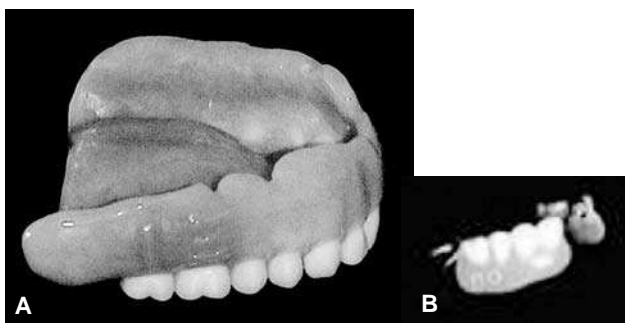

Figure 2 - a, b. A removable complete maxillary and a removable partial mandibular denture. The initials of the owner are written in relief (surface method)

Writing on the denture surface: In this technique, the tissue-fitting surface of the finished denture is temporarily marked with a fibre-tip pen or a sharp graphite pencil and covered with a clear varnish, like Vocopal Varnish (Voco Cuxhaven, Germany). The mark is better protected against abrasion by layers of varnish. The technique is as following: A small area of the surface of the denture is roughened, removing the polish with fine sandpaper. Then the patient's full name or initials or a special number are written on the denture surface, covered by at least 2 thin coats of varnish. Varnish may be prepared by dissolving 5 g of acrylic resin polymer in 20 ml of chloroform. A clear solution, easy to apply, with long life is produced, that has excellent resistance to abrasion, cleaning and disinfecting agents, and does not affect the strength of the denture or

induce surface crazing[2,7,8]. The first coat should be dried, before applying the other coats[2]. In our cases, in figure 3a, the identification mark, a special number 223, appears posterio-laterally on the fitting surface of the maxillary complete denture, which belongs to a 65-year-old man. In figure 3b, the patient's initial letter of the name and the surname was written with a felt marker on the buccal surface of the disto-buccal flange of the removable partial maxillary denture, which belongs to a 70-year-old man.
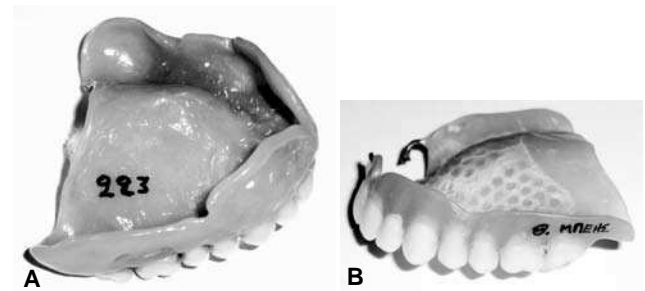

Figure - 3 a, b. Two removable complete maxillary dentures.
a. An identification number is written on the tissue-fitting surface of the finished denture;
b. Patient's initial letter of the name and the surname is written in felt marker on the buccal surface of the disto-buccal flange (surface method)

Inclusion Method

The removable partial mandibular denture seen in figure 4 belongs to 80-year-old man of Greek origin, living in Sweden. It was marked, according to the Swedish model of marking dentures, with a stainless steel metal band, the Swedish ID-Band. First, the denture was disinfected, cleaned and dried. Then a shallow recess for the metal band was prepared with a round bur on a hand piece in the denture base in the desired location, to a length 6 mm longer than the identification band. The preparation was 3 mm deeper than the thickness of the metal band.

Figure 4. A removable partial mandibular denture marked with a metal band in Sweden (inclusion method)

The metal band was placed in the lingual flange of the partial mandibular denture and contained a letter (S) and a 10-figure number. The letter S stands for Sweden. The first 6 digits are the patient's date of birth, date month year with zero as a prefix to numbers smaller than 10[9].

The next 3 digits are the birth number and the last digit indicates the sex. It is even for females and odd for males. The personal identification number contained in the metallic band of the case shown in figure 4 is S-260614-6788 (S=Sweden, 26=year of birth, 06=month of birth, 14=day of birth, 678=birth number, 8=control digit) all of which were not less than 1.5 mm high. This personal identification number of the patient appears also in the identification card, the passport, the hospital card, the unemployment card, etc.

A small amount of clear acrylic resin (Hygienic Dental Mfg. Co, Akron, Ohio USA) was placed on the bottom of the prepared recess. Then the metal band was placed on the recess and examined for proper fit. The band was covered with clear acrylic resin, trimmed and finished in the usual manner. After polishing, it was checked if the personal identification number was clearly readable.

Discussion

In large scale disasters, associated with fire, the damage caused by heat could make medico-legal identification of human remains difficult. Therefore, the role of forensic odontology can be crucial. As teeth, restorations and dental prostheses are quite resistant to high temperatures, they could be used as aids in the identification process[10]. The absence of some or all of the teeth is a common situation in older age groups. In that case, the presence or absence of dentures could aid the identification. In some cases, it is essential to demonstrate that the denture had been worn by the victim and was not discarded at the scene by someone else[4,11].

Denture marking or labelling is not a new concept in either prosthetic or forensic odontology, and forensic odontologists have proposed its routine international practice for many years. In 1835, the burnt body of the Countess of Salisbury was identified by her golden dentures and this was the first known case of identification by dentures[9]. In early 1920s, the idea of marking dentures was mentioned for the first time[6]. In 1972, at the Congress held in Mexico, the F.D.I. (Federation Dentaire International) proposed the marking of the dentures "recommending to all member associations to introduce denture marking in their respective countries". In some countries the marking of dentures is regulated by legislation, but in other countries it is the dentist's or the patient's decision[6]. The results of a survey by Alexander et al[12], aiming to determine the extent of the practice of denture marking in South Australia, indicated that no practitioner marked dentures routinely. The reasons for not marking dentures were cost, lack of awareness of standards and recommendations, and a belief that it was of little importance.

The standard requirements for denture markers as outlined by the British Council on Prosthetic Services and Dental laboratory Relations are the following[6]:
- The strength of the prosthesis must not be jeopardised;
- It must be easy and inexpensive to apply;
- The identification system must be efficient;
- The marking must be visible and durable;
- The identification must withstand humidity and fire;
- The identification mark should be aesthetically acceptable;
- The identification mark should be biologically inert (when incorporated into the denture).

In addition, the marking should be permanent and resistant to everyday cleansing, and withstand the cleansing and disinfecting agents[2].

Over the years, 2 methods of denture marking have been proposed: the surface marking method and the inclusion method. The surface method is easy to apply and relatively inexpensive. Skilled personnel are not necessary, but they wear off very easily and should be reapplied. The inclusion method is permanent and provides a more predictable result, but it could weaken the structure and create porosity. It is more expensive and is usually made by trained personnel in dental laboratories, or it can be done in a dental office with relatively basic lab equipment[2,7,8].

There is another surface marking technique in which the initials of the name and the surname of the patient are scratched with a sharp instrument (or with a dental bur) on the master cast. Mirror writing should be used. This technique produces embossed lettering on the fitting surface of the denture. This technique is not really recommended, since a carcinoma was reported close to a mark made in this way[9].

The inclusion method can be divided in 2 categories: a) inclusion method using non-metal materials like finely woven nylon tape, onion skin paper, etc and b) inclusion method using metal materials-markers. These materials (non metal or metal) can be incorporated into the denture at the packing stage. During the final closure of the flask and the processing of the denture, there is a possibility of dislocation, wrinkling or tear, thus reducing their value as identification markers. The other variation is to incorporate the metal or non-metal marker after the finishing of the denture, making a small cavity[2].

The Swedish ID-Band (SDI AB, Sweden) has become the international standard. It is a stainless steel metal band. Research has shown that ID-Band is not resistant to very high temperatures[3,6]. Olsson et al[5] tested 3 different types of steel bands (Jasch, Remanit, ID-band) exposed to temperature levels of $1100^{\circ}C$, $1200^{\circ}C$ and $1300^{\circ}C$. At $1100^{\circ}C$ only the ID-band and the Jasch band were readable, but none of them at $1200^{\circ}C$ and $1300^{\circ}C$. Thomas et al[13] tested ID-Band, Ho-Band (stainless steel matrix) and Titanium foil at $700^{\circ}C$ and $900^{\circ}C$. The performance of

ID-Band and Ho-Band was similar, meaning that Ho-Band could be used as a cheaper alternative.

Since there is no international consensus regarding the marking materials, the need for new more persistent materials is obvious. There are many proposals about the use of microchips for marking dentures. They have small size, they could include a lot of information (full mane of the patient, sex, country of origin, ID number, etc). The data can be detected with the aid of a reading device. Their disadvantage is the high cost of manufacture and data incorporation. At the same time they arise a number of ethical dilemmas[14,15].

Legislation for denture marking exists only in Sweden and Iceland. In 1986, the recommendation issued by the National Board of Health and Welfare of Sweden stated that "the patients shall always be offered denture marking and be informed about the benefit. Denture marking is not permitted if the patient refuses it"[6]. All dentures made in the Dental School of the University of Iceland are marked. However, Stenberg and Borrman[16] showed in a study that only about 35% of the full dentures in Sweden were ID-marked. In the USA, denture marking is mandatory in 21 states, while in New York State denture marking is performed only after request of the patient. Several states impose the obligation to mark dentures on long-term care facilities and denture marking is compulsory for the Army[6]. In the United Kingdom, denture marking is not compulsory. In Australia, the nursing homes require that the dentures of their residents should be "discretely labelled"[12]. In Greece, there is no legislation for marking dentures. It's the dentist's decision to present the benefits of denture marking to the patients and ask for their consent. The dentures of the cases presented in this article were marked after the written consent of the patients[17-20].

Andersen et al[21] have estimated that in Nordic countries, if denture marking was used generally, the contribution to the establishment of identity by forensic odontology in cases of fire would be increased by about 10%. Dentures can survive surprisingly well in fire provided they are not directly exposed to the flames[4]. At the same time, carefully taken and well-protected dental records are essential. Since there is no international consensus, international collaboration is needed to solve the issue of denture marking for clinical and forensic purposes[6].

Conclusions

Denture marking is not a new concept. There are 2 methods, the surface method and the inclusion method. Each method can be applied using various techniques. Some of them are easy to apply, having their advantages and disadvantages. Marking by the inclusion method is more persistent, but the need for new marking materials exists. Microchips could be an alternative solution. Accurate dental records should be taken and kept carefully for a long time. The need of an international consensus about denture marking for clinical and forensic purposes is obvious.

The author's suggestion is that dental associations of the Balkan countries and similar organisations should seriously consider bringing the issue to the attention of governments and populations, so that quality assurance programmes also involve the issue of denture marking for clinical and forensic purposes.

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