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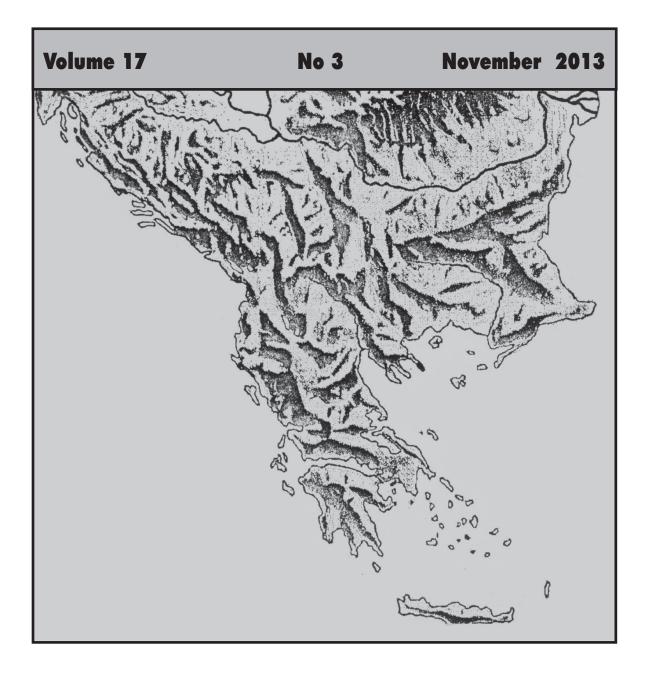
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## Forthcoming Meeting

### 19<sup>th</sup> Congress of the Balkan Stomatological Society April 24-27, 2014, Belgrade, Serbia

BALKAN STOMATOLOGICAL SOCIETY & SERBIAN DENTAL SOCIETY

Invite you cordially to Belgrade on the occasion of the 19<sup>th</sup> Congress of the Balkan Stomatological Society April 24-27, 2014

The Congress will take place at the "SAVA CENTAR", the Congress centre in Belgrade

President of the Congress	Prof. Dragoslav Stamenković
President of the Organizing Committee	Prof. Obrad Zelić
President of the Scientific Committee	Prof. Dejan Marković

Dear Colleagues,

On behalf of the Balkan Stomatological Society and the Serbian Dental Society, we cordially invite you to the 19<sup>th</sup> Congress of the BaSS in Belgrade. You will be able to attend an interesting scientific programme with well known and respect speakers, who will present contemporary achievements in different fields of dentistry.

We are sure you'll enjoy high quality lectures, stimulating discussions, cutting-edge research and interesting presentations, which will lead us professionally enriched into the future. Apart from the scientific aspect of the congress, we wish you to experience unforgettable pleasure and traditional hospitality of Belgrade, as well as to strengthen the existing friendships and create a lot of new.

We shall be glad if you accept our invitation to attend the 19<sup>th</sup> Congress of the BaSS, which will be held in the capitol of Serbia. Wishing this Congress to be both informative and enjoyable, the organizers and the city of Belgrade welcome you with pleasure and friendliness.

We look forward to seeing you in Belgrade.

**Prof. Dragoslav Stamenković** President of the 19<sup>th</sup> BaSS Congress

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## **Complications of Oral Piercing**

#### SUMMARY

Over the last decade, piercing of the tongue, lip or cheeks has grown in popularity, especially among adolescents and young adults. Oral piercing usually involves the lips, cheeks, tongue or uvula, with the tongue as the most commonly pierced. It is possible for people with jewellery in the intraoral and perioral regions to experience problems, such as pain, infection at the site of the piercing, transmission of systemic infections, endocarditis, oedema, airway problems, aspiration of the jewellery, allergy, bleeding, nerve damage, cracking of teeth and restorations, trauma of the gingiva or mucosa, and Ludwig's angina, as well as changes in speech, mastication and swallowing, or stimulation of salivary flow. With the increased number of patients with pierced intra- and peri-oral sites, dentists should be prepared to address issues, such as potential damage to the teeth and gingiva, and risk of oral infection that could arise as a result of piercing. As general knowledge about this is poor, patients should be educated regarding the dangers that may follow piercing of the oral cavity.

Keywords: Oral Piercings; Complications

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#### LITERATURE REVIEW (LR) Balk J Stom, 2013; 17:117-121

#### Introduction

Body piercing is a form of body art or modification, and as a cultural practice, dates back to antiquity. Piercings have been found on preserved bodies of people who lived between 4,000 and 5,000 years ago<sup>1</sup>. It has been practiced by many tribal societies, particularly in Africa, Asia, and South America, as far back as can be traced and has involved a variety of materials, including wood, metal, pottery and ivory<sup>1,2</sup>. Anthropologists describe piercing as a way for an individual to identify with a specific group, to denote one's financial or marital status or even as a method of beautifying the body. In some parts of the world, body and oral piercing may be part of religious beliefs<sup>1-7</sup>.

Currently, in western societies, piercing is growing in popularity, particularly among adolescents and young adults, who view it as denoting marginality, beauty, or group identity<sup>1-8</sup>. Various body parts are preferred for this type of adornment; most commonly the ears, nostrils, eyebrows, navel, and tongue. In the body areas of concern to the dentist, the most frequently punctured body parts are the tongue and lips, but other areas may also be used for piercing, such as the cheek, uvula, and lingual frenum<sup>1,7,9</sup>. The data show that the tongue (5.6%) is the most commonly pierced site, followed by lips (1.5%). On the basis of 3 studies, the prevalence of cheek piercings was 0.1%. Less common locations were the lingual fraenulum, the dorso-lateral tongue and the uvula<sup>1,10-12</sup>. According to the existing literature, uvula piercing is rare because of the inherent difficulties in performing the piercing, as well as the risk of nausea, throat irritation and/ or dysphagia<sup>1,10</sup>.

Piercing jewellery is predominantly made of metal, usually stainless steel, gold, niobium, titanium, or metal alloy<sup>13</sup>. Recently, however, synthetic materials like Teflon and nylon or plastic have also been used<sup>14</sup>. The shape and size of the piercing are determined by the body part to be pierced and personal preferences. The most common type of jewellery used in the tongue is the barbell, which consists of a curved or straight metallic stem like a needle with a sphere attached to each end. A common modification of this is the labret, where 1 of the "spheres" is replaced by a smooth flat disc. A third type of piercing is a ring with 1 or 2 spheres on each end. Labrets, with the flat end on the mucosal side of the lip, as well as rings and barbells, are used for lip piercing<sup>5,7,9,15</sup>.

Piercing procedures are usually performed by unlicensed, non-medical people, often self-trained, who have little knowledge of local anatomy, medical conditions, sterilization, prevention of complications, or emergency procedures<sup>7,9,16-18</sup>. Simple precautions such as using an aseptic technique for puncturing could reduce the occurrence of complications. Anaesthesia is rarely used. Pain is the most common immediate complication<sup>12,16,19-21</sup>.

Oral piercing is linked to a series of local and systemic risks and complications, some of which are both immediate (related to the immediate moment of puncturing and lasting through the first postoperative day) and chronic, because of long-term display (Tab. 1)<sup>5,7,9,22</sup>.

 	 associated	 P	

Complications during piercing procedures	Primary postoperative complications	Secondary postoperative complications
Pain	Swelling, oedema, inflammation	Mucosal injury, tissue alteration
Bleeding, haemorrhage	Allergic reactions	Damage to the periodontium
Nerve damage	Localized and systemic infections	Dental trauma
Infectious complications	Increased salivary flow	Aspiration and ingestion of the jewellery
	Ludwig's angina	Problems during medical/dental care procedures
	Thrombophlebitis	Generation of galvanic currents

The purpose of this article is to present a brief review of the literature on potential complications of oral piercings and to highlight the need for dentists to inform patients of the associated risks.

## Complications during piercing procedures

*Pain*: Pain is the most profound and immediate consequence, and results from the lack of any kind of anesthesia during the piercing procedure<sup>12,16,19-21</sup>.

*Haemorrhage*: During the piercing process, blood vessels may be torn and vascular nerves damaged. Major haemorrhage is not a frequent complication, nevertheless it is of great concern, especially in tongue piercing, due to the high vascularity of this organ and the implications for medically compromised patients<sup>9,23,24</sup>. One study reports

a case of significant loss of blood from haemorrhage following tongue piercing, which resulted in hypotensive collapse<sup>23</sup>. Prolonged bleeding and hematomas have also been reported following lip piercings. Although major haemorrhage is rare, controllable bleeding usually results<sup>5,9</sup>.

*Nerve Damage and Paraesthesia*: Piercing sites are innervated with sensory or motor fibres, and special care must be taken to avoid causing damage or paraesthesia. The tongue is typically pierced in the midline and just anterior to the lingual frenum<sup>20,25</sup>. As a result, injury to the lingual frenum is the most common complication during the piercing procedure, sometimes resulting in impaired mastication, deglutition, or speech<sup>5,25</sup>.

*Infectious Complications*: As piercing remains largely unregulated, and is often performed without adequate cross-infection protection and hygiene measures, it has been identified as a possible vector for transmission of blood-borne viruses (HIV, hepatitis B, C, D, and G, herpes simplex, Epstein-Barr), tetanus, syphilis or tuberculosis<sup>18,21,26,27</sup>.

#### Complications Immediately Following Piercing (Primary Post-Operative Complications)

*Swelling, Oedema and Inflammation*: Painful ulceration is a common primary postoperative complication of piercing, reported by almost half of recipients, followed by inflammation, involving around 9% of cases<sup>5,18,28-30</sup>. Inflammation usually occurs between 6 and 8 hours after piercing, reaching a peak on day 3 or 4. Inflammation can sometimes persist for weeks<sup>5,30</sup>. Swelling and inflammation may cause problems, such as dysphonia, dysphagia, interference with mastication or swallowing, respiratory difficulties or even asphyxia<sup>5,7,18,20,28-30</sup>.

*Allergic Reactions*: The most widely reported allergic reaction to piercing is contact dermatitis produced by nickel, chromium or nickel-cobalt<sup>5,9,16,25,27,31</sup>. The European Union has issued a directive to limit the amount of nickel in all products that are in direct contact with human tissue, with a limit of 0.05% for the nickel used in oral/perioral piercing jewellery. It also recommends that gold used for this purpose should be at least 14-18K<sup>29</sup>. Some of the materials inserted may also cause anaphylactic reactions<sup>7,9,18</sup>.

**Increased Salivary Flow**: Increased salivary flow is a less common complication and tends to disappear with time<sup>7,9</sup>.

*Localized Infections*: Because piercing invades the subcutaneous tissues, it has an inherently high potential for causing infectious complications. During piercing procedures, infection control standards, which include the

use of disposable gloves, sterile or disposable instruments and sterilized jewellery, are not always followed. Thus, oral piercing customers are at high risk of developing localized infections<sup>16,32,33</sup>. The accumulation of dental biofilm and calculus at pierced sites may aggravate the development of these infections<sup>24</sup>.

Systemic Infections: The invasion of subcutaneous tissues, disruption of mucosal integrity and placing of a foreign body in the wound involved in oral piercing also have implications for systemic infections. The wound originating from the insertion of the jewellery can allow numerous different microorganisms that normally inhabit the oral cavity to enter the bloodstream and cause metastatic infections in other organs, including vital organs such as the heart<sup>5,7,9,33,34</sup>. There is a particular risk of this after tongue piercing through the lingual veins that drain into the internal jugular vein. Infective endocarditis may be caused by metastatic oral bacteria. The subsequent infection of endocardium typically affects valves with congenital or acquired dysfunction (birth valve defect, damaged heart valve, new heart valve after surgery, history of endocarditis)<sup>7,9,34</sup>. Another life threatening complication which can result is the development of a cerebral abscess<sup>9,35,36</sup>.

*Ludwig's Angina*: A severe complication of tongue piercing is acute glossitis, which can lead to Ludwig's angina<sup>12,25,32,36</sup>. Ludwig's angina is a cellulitis, or connective tissue infection, of the floor of the mouth. It may cause stridor or difficulty in breathing and is potentially life threatening<sup>9,36-38</sup>.

*Thrombophlebitis*: A case of thrombophlebitis associated with pneumonitis after tongue piercing has been reported<sup>36</sup>.

#### Long-Term Complications (Secondary Postoperative Complications)

Mucosal Injury and Tissue Alteration: Among late complications, traumatic injuries to the mucosal surfaces at the piercing site have been documented<sup>5,7,9,30</sup>. These include enlargement of the piercing hole<sup>39</sup>, chemical burns associated with excessive aftercare<sup>40</sup>, sarcoid-like foreign-body reactions<sup>41</sup>, granulomas and scar tissue formation. Oral piercings have also been linked to the formation of reactive hypertrophic tissues<sup>5,7,9</sup> and keloid scarring<sup>5,7,9,42,43</sup>. Tissue overgrowth can also be caused by continuous movement of the jewellery in the pierced tissue. In most cases of tissue proliferation, surgical interventions are not necessary, because healing occurs after removal of the jewellery. However, the insertion wound can become covered with epithelium, complicating the removal of the jewellery<sup>9,29,30</sup>. The swelling and excessive tissue growth reaction can cause the jewellery

to be incorporated into the oral tissues. Lingual piercings that become embedded in the ventral<sup>20</sup> or dorsal<sup>3,32</sup> surface of the tongue have been reported. By contrast, long-stemmed jewellery moves inside the piercing location and traumatizes the surrounding tissues more easily, in addition to favouring the build-up of plaque and calculus<sup>25,39</sup>.

Effects on Periodontal Tissues: Microbiological analyses of oral piercing sites have shown that jewellery can serve as a reservoir for periodonto-pathogenic bacteria<sup>1,7</sup>. In addition, in the long run, the friction caused by oral piercings can cause gingival recession, loss of periodontal attachment, tooth mobility and tooth loss<sup>7,16,44</sup>. All these complications are influenced by the location and size of the piercing object, as well as the duration of wear<sup>14,20,25</sup>. Gingival recession has been especially correlated with lip piercing and commonly occurs on the labial aspect of the lower central incisors<sup>8,20,25,31,45-49</sup>. The use of tongue jewellery was found to be strongly associated with the occurrence and severity of gingival recession in the mandibular anterior lingual region<sup>20,25,45-50</sup>. The consequences of piercings on the gingival margins should not be overlooked as severe attachment loss can develop, even when gingival recession is minimal, and it is therefore critical that patients with oral piercings routinely undergo comprehensive periodontal assessment<sup>20,25</sup>.

Damage to the Dentition: One of the late adverse effects of oral piercing is traumatic injury to the teeth such as chipping, fracturing of teeth and restorations and pulpal damage. The lesions are usually limited to enamel and dentin but the pulp may also be involved<sup>5,7,9,17,44</sup>. Tongue piercings are the main reported cause of damage to the dentition<sup>4,7,9,20,25,51-53</sup>. A possible reason for the damage to teeth is that the beaded jewellery may become trapped between the teeth during speaking, mastication and/or intentional interposition. The range of lengths of the jewellery allows a varying degree of mobility for the devices and therefore could contribute to the degree or severity of the observed complication. Dental trauma is more common where longer jewellery is used<sup>4,9,18,44</sup>. It has been reported that switching to shorter jewellery reduces damage to teeth. A positive correlation between the duration of wear and the occurrence of dental hard tissue damage has been demonstrated<sup>7,20,25,53</sup>. However, physical damage to the dentition may occur even within the first year of use of the device<sup>20</sup>. Jewellery with soft rubber ends and acrylic screw caps are considered less likely to cause tooth chipping than those with metal ends<sup>9</sup>.

*Aspiration and Ingestion*: the potential risk of aspiration or inhalation of parts of the jewellery when piercings are not fastened securely should not be overlooked.

Complications Concerning Medical and Dental Care Procedures: The presence of oral jewellery may cause problems during medical procedures such as intubation and administration of anaesthetics<sup>54</sup>, radiographic examination<sup>54-56</sup>, and teeth bleaching<sup>54</sup>.

*Production of Galvanic Currents*: Galvanic currents between jewellery and metallic dental restorations can be generated<sup>19,25</sup>.

#### **Practical Implications**

Dental care professionals can play an active role in providing information to those who are planning to obtain oral and/or peri-oral piercings, and helping patients make informed decisions. As general knowledge on this subject is poor, patients should be educated regarding the dangers that may follow piercing of the oral cavity. If a patient presents with an oral and/or peri-oral piercing, a dental care professional should examine the device and the surrounding tissues for possible short- and long-term complications on the patient's general and/or oral health. With the increased number of patients with pierced intraand peri-oral sites, dentists should be prepared to address issues, such as potential damage to the teeth and gingiva, and risk of oral infection that could arise as a result of piercing<sup>1,5,7,9,25</sup>. Additionally, it is recommended that questions regarding piercings be included in medical questionnaires<sup>1,9,56</sup>.

#### Conclusions

Oral piercing has gained wide acceptance in western societies, mainly among young people.

Although complications from the use of oral piercing may involve simple, self-limiting local changes, direct and indirect damage to both soft and hard oral tissues, there is always the possibility of potentially fatal problems.

Dentists need to play an active role in educating patients about the dangers of oral piercing before the patients indulge in this body art.

Patients who have oral piercings should be regularly examined and taught about the possible short- and longterm complications they might face.

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## Effects of Repeated Firings on Colour of Leucite and Lithium Di-Silicate Ceramics\*

#### SUMMARY

**Purpose:** To evaluate the effects of repeated firings on colour of leucite and lithium di-silicate ceramics.

Materials and method: 10 disc specimens (15.5mm × 2.1mm) for each pressable all-ceramic (Empress 2, Finesse, Cergo, Wegold) were prepared. The colour data of all specimens after 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> firing periods were expressed in CIE L\*a\*b\* system [L(light-dark), a(red-green), b(yellow-blue),  $\Delta E$  values] by using CM-2600d Spectrophotometer and Spectra-Magic 3.1 PC Software. 1-way analysis of variance, Tukey's test and Newman Keul's test were used for statistical analysis. The results were determined at significance level P<.05.

**Results:** There were significant differences in  $L^*$  and  $a^*$  values of Empress 2 at all firing periods (P<.0001). The L\*,  $a^*$ ,  $b^*$  values of Finesse were only affected at 7<sup>th</sup> firing period (P<.001). The L\* and b\* values of Cergo (P<.001) and  $a^*$  and  $b^*$  values of Evopress were found statistically significant (P<.0001, P<.01). The colour change value of Empress 2 was between 1.6 ( $\Delta$ E1-3) and 4 ( $\Delta$ E1-7) that can be perceived by eyes ( $\Delta$ E>1).

**Conclusions:** The study results showed that the colour of lithium di-silicate ceramic Empress 2 was affected by repeated firings more than the others.

Keywords: Colour Change; CIELab System; Pressable Ceramics; Repeated Firings

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#### Introduction

Providing anterior aesthetics in dental restorations is an important aspect. Ceramics have a long history of usage for this purpose. If used in appropriate situations, ceramics are aesthetic, functional and biocompatible materials<sup>1</sup>. The aesthetic success of ceramic restorations depends on several factors, such as surface characteristics<sup>2</sup>, thickness<sup>3</sup>, shape, colour<sup>4</sup> and core material<sup>2</sup>. Dentin is considered to be the primary source of colour for teeth<sup>5</sup>. The core materials that are used for strengthening the restoration may also be considered to be a source of colour for a crown restoration. Metal core structures have been successfully used for strengthening the ceramic for many years<sup>6</sup>. However, it then became necessary to develop more translucent and aestheticallypleasing ceramic materials to eliminate the light transmission problem<sup>7</sup>. Thus, metal-free all-ceramic restorations were produced and were accepted because of their high aesthetic quality<sup>8</sup>.

All-ceramic systems can be classified according to the laboratory processing procedure (pressable, slip-casting, milling, or sintering) and the chemical composition (feldspar: high leucite and low leucite; glass ceramic: mica, leucite, and lithium disilicate; core reinforced: alumina, spinel injection moulded, magnesia, and zirconia)<sup>6,9</sup>. Pressable glass ceramics are among the most popular dental restorative systems due to several factors: ease of fabrication, occlusal accuracy,

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better marginal integrity, translucency, good mechanical properties, net-shaped forming by pressing, and decreased porosity<sup>10,11</sup>.

A pressable all-ceramic restoration would be fired several times to produce a natural appearance by correcting its form and colour, but the effects of repeated firings on colour of a ceramic material is a big challenge. Several studies have investigated the effects of temperature or firing conditions on various dental porcelain systems. Claus<sup>12</sup> reported that the firing cycle, temperature, rate of temperature increase, holding time, and cooling time affect the distribution of the sintering, glass, and crystal phases in the microstructure of the porcelain. In addition, there have been numerous studies and reports on the effects of temperature or firing techniques on ceramic systems<sup>13-16</sup>.

Colour assessment is a complex psychological and physiological process subject to variables. Variations in perception of colour changes are possible from numerous uncontrolled factors<sup>17,18</sup>. The use of colorimetric measurements provides exact colour values by CIE  $L^*a^*b^*$  colour order system. The system was developed in 1978 by the Commission Internationale de I'Eclairage (International Commission on Illumination)<sup>19-21</sup>. CIE  $L^*a^*b^*$  defines colour in 3 dimensions: L\* represents brightness-darkness, a\* represents redness-greenness, b\* represents yellowness-blueness of the object. The colour change or difference is stated by  $\Delta E^{22}$ . The perception level of  $\Delta E$  changes from 1 to 3.7 in different studies, but, most of the studies reported that colour change ( $\Delta E$ ) under 1 unit cannot be perceived by eyes<sup>23-25</sup>.

The **aim** of this study was to investigate colour changes in lithium di-silicate and leucite ceramic materials after repeated firings. The null hypothesis was that both the colour values of lithium di-silicate and leucite ceramic materials would be affected by repeated firings and  $\Delta E$  would be greater than 1 for all specimens.

#### **Materials and Method**

Table 1 shows the ceramic materials selected for this study. A lithium di-silicate ceramic (Empress 2®, Ivoclar Vivadent, Schaan, Liechtenstein) and 3 different leucite reinforced materials (Finesse®, Ceramco, NJ, USA), (Cergo®, Degussa Dental, Dusseldorf, Germany), (Evopress®, Wegold Edelmetalle, Wendelstein, Germany) were tested. 10 disc specimens were prepared for each group according to their manufacturers' instructions.

Table 1. The materials tested in the study

Materials	Туре	Manufacturer	Batch number
Empress 2	Lithium di-silicate pressable all-ceramic	Ivoclar Vivadent, Schaan, Liechtenstein	S61194
Finesse	Leucite reinforced pressable all-ceramic	Ceramco, NJ, USA	311200
Cergo	Leucite reinforced pressable all-ceramic	Degussa Dental GmbH, Hanau, Germany	0032/2
Evopress	Leucite reinforced pressable all-ceramic	Wegold Edelmetalle, Wendelstein, Germany	41001

Prefabricated wax discs (Ivoclar Vivadent, Schaan, Liechtenstein) with 15.5 mm radius and 2.1 mm thickness were sprued and invested by using each material's own investment material. Then, the specimens were pressed according to their manufacturer's pressing program. After pressing, the investment moulds were taken from the furnace and allowed to air-cool. The investment material around the discs was removed by using an airborne particle abrasion unit (Toptec-Bego, Bremen, Germany) with 50 µm glass beads at a pressure of 4 to 2 bars. A diamond disc bur (Horico, Berlin, Germany) was used for separating the sprues from the discs. Both surfaces of the specimens were serially wet-ground with 220, 320, 500, 600, and 800 grade silicon carbide papers mounted on a surface grinder and polisher machine (Buehler Metaserv

Grinder-Polisher, Buehler UK Ltd., United Kingdom). The final size of the disc specimens were 15.5 mm radius and 2 mm thickness after the surface treatment.

After surface finishing and polishing, each specimen was placed in its own furnace for the first firing program. After cooling, the colour of each specimen was measured by using a spectrophotometer (CM-2600d, Konica Minolta Optics Inc, Tokyo, Japan) and Spectra-Magic 3.1 PC Software (Konica Minolta Optics Inc, Japan). Before colour measurement, the calibration of the spectrophotometer was performed according to the manufacturer's instructions. 3 measurements were made at 1 surface of the disc, and the average reading was calculated for each specimen. Instrument was recalibrated after measurement of each group (n=10). This procedure

was repeated after 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> firing periods. All disc specimens in each group were evaluated in CIELAB system [L(light-dark), a(red-green), b(yellow-blue),  $\Delta E$  values] after each firing period. The CIELAB system is a uniform 3-dimensional colour order system and changes in any 3 coordinates can be perceived as visually similar. Total colour changes were calculated with the use of the following equation (*Knispel G.* Factors affecting the process of color matching restorative materials to natural teeth. *Quintessence Int*, 1991; 22:525-531):

 $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ 

The L\* coordinate of a specimen is the value of the lightness-darkness. The greater the L\* is, the lighter the specimen. The a\* coordinate is the chroma along the red-green axis. A positive a\* relates to the amount of redness, and a negative a\* relates to greenness. The b\* coordinate is the chroma along yellow-blue axis, which means, a positive b\* relates the amount of yellowness, and, a negative b\* relates the amount of blueness of the specimen.  $\Delta L^*$ ,  $\Delta a^*$  and  $\Delta b^*$  are the differences in the CIE colour-space parameters of the 2 measurements.

The statistical analysis in this study was performed using the Graphpad Prism V3 packet program (GraphPad Software Inc, CA, USA). In addition to calculation of the mean value and the standard deviation, a 1-way analysis of variance was used to compare the repeated measurements for the groups. To compare the colour values of the materials, Tukey multiple comparison test was used. The colour changes after each firing period were analyzed with Newman-Keuls multiple comparison test (P= .05).

#### **Results**

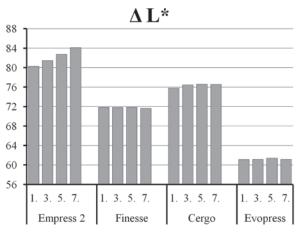
The mean values and repeated measures ANOVA results of L\*, a\* and b\* results are listed in table 2. There were significant differences of Empress 2® material's L\* and a\* values in all firing periods (P< .0001). The L\*, a\*, b\* values of Finesse® were only affected by repeated firings significantly at 7<sup>th</sup> firing period (P< .001). The L\* and b\* values of Cergo® were affected by repeated firings significantly (P< .001). However, the other values were not affected after the 3<sup>rd</sup> firing and a\* value at any firing period. The a\* and b\* values of Evopress® were found statistically significant (P< .0001, P< .01). The others were not affected.

Table 2. Mean values and repeated measures ANOVA results for each material tested

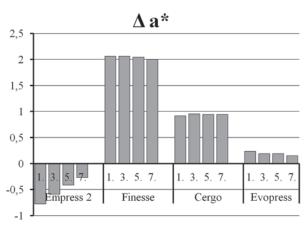
		Number of Firings				
Property	Material	1	3	5	7	Sig.
L*	Empress 2	80.26±0.4	81.48±0.28	82.69±0.36	84.09±0.32	< .0001
	Finesse	71.86±0.16	71.80±0.15	71.85±0.15	71.64±0.17	< .0001
	Cergo	75.79±0.98	76.47±0,5	76.58±0.33	76.53±0.38	< .0001
	Evopress	61.12±0.57	61.15±0.25	61.39±0.34	61.16±0.33	> .05
a*	Empress 2	-0.78±0.11	-0.59±0.18	-0.41±0.21	-0.27±0.2	< .0001
	Finesse	2.06±0.07	2.06±0.06	2.05±0.07	2±0,07	< .0001
	Cergo	0.92±0.06	0.96±0.07	0.94±0,05	0.94±0.05	> .05
	Evopress	0.24±0.15	0.19±0.13	0.19±0.15	0.15±0.14	< .0001
b*	Empress 2	10.74±0.52	11.67±0.6	11.82±0.62	11.74±0.63	< .0001
	Finesse	11.29±0.11	11.27±0.13	11.28±0.13	11.11±0.12	< .0001
	Cergo	12.64±0.46	12.94±0.3	13.06±0.37	12.98±0.28	< .0001
	Evopress	10,68±0.23	10.64±0.24	10.7±0.16	10.51±0.27	< .01

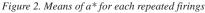
Figures 1, 2 and 3 graphically show changes in colour values of each material after repeated firings. The  $\Delta E$  values of Finesse®, Cergo® and Evopress® were

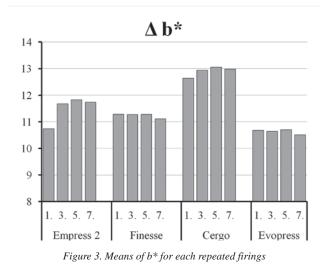
found to be lower than 1 ( $\Delta E < 1$ ). However, Empress 2<sup>®</sup> material's colour change results were between 1.6 and 4 (Tab. 3).



*Figure 1. Means of L\* for each repeated firings* 







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Table 3. The colour changes ( $\Delta E$  values) of each ceramicmaterial between all firing periods

ΔΕ	Empress 2	Finesse	Cergo	Evopress
ΔE <sub>3-1</sub>	1.58±0.38	0.1±0.05	0.8±0.59	0.33±0.25
$\Delta E_{5-3}$	1.25±0.4	$0.09 \pm 0.04$	0.27±0.2	0.3±0.15
$\Delta E_{7-5}$	1.41±0.21	0.28±0.07	0.2±0.12	0.35±0.11
$\Delta E_{5-1}$	2.70±0.63	0.07±0.03	0.96±0.75	0.43±0.32
$\Delta E_{7-3}$	2.64±0.31	0.26±0.06	0.21±0.16	0.29±0.17
ΔE <sub>7-1</sub>	4.01±0.51	0.31±0.08	0.87±0.68	0.43±0.32

#### **Discussion**

This *in vitro* study measured the changes in colour of pressable all-ceramic materials after repeated firings. Within the limitations of this study, the results support the hypothesis regarding the colour effect of repeated firings of pressable all-ceramic materials. All of the materials' colour values were affected by repeated firings; however, the colour change ( $\Delta E$ ) results were not greater than 1 for all the investigated materials. The colour changes may only be perceivable for lithium di-silicate ceramic Empress 2<sup>®</sup>, which had  $\Delta E$  values between 1.6 and 4.

Ceramics have many uses in restorative dentistry because they have so many aesthetic and physical advantages<sup>1,5</sup>. In particular, all-ceramic systems are preferred because of their light-transmitting features<sup>4,5</sup>. In this study, the most commonly-used pressable all-ceramic materials were selected to be tested.

In most studies, firing periods were limited between once to 9 times<sup>6,17,23-25</sup>. The first firing serves to eliminate micro-cracks and release the stresses associated with grinding and polishing procedures, as recommended by the manufacturers<sup>7</sup>. The second and third firings are considered to be necessary steps for producing the restoration using the staining or layering technique. The fourth and subsequent firings are necessary when shape and colour corrections are needed. After the third firing, an all-ceramic restoration is ready for installation in the mouth by the dentist. The additional 4 firings were assumed to be necessary only if the dentist needs further shape and colour corrections.

In most of the studies, the wax specimens were produced with handmade moulds. However, in this study, prefabricated and pre-sprued wax discs (Ivoclar Vivadent, Schaan, Liechtenstein) were used to eliminate dimensional errors between specimens. It is known that leucite ceramics exhibit an increase in leucite content after repeated firings<sup>26,27</sup> and the size of lithium di-silicate crystals in lithium di-silicate content ceramics was found to increase after repressing<sup>28</sup>. Although these kinds of changes in the microstructure of leucite and lithium di-silicate content ceramics were reported, in this study the colour change values of ceramics could be perceived by eyes only for lithium di-silicate ceramic. Further investigations on effects of repeated firings to the colour of different core and layering materials should be planned.

#### Conclusion

The study results showed that the L\*, a\* and b\* values of all materials were affected by repeated firings, but not statistically significant for all. The colour change value of Empress 2® was between 1.6 ( $\Delta$ E1-3) and 4 ( $\Delta$ E1-7) that can be perceived by eyes ( $\Delta$ E>1). Colour change of Empress 2® after repeated firings may be perceived by eyes. The reason may be in its lithium di-silicate content, which is different from other materials tested in the study.

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## The Influence of Re-Polymerization on the Microhardness of Commercial Acrylic Teeth

#### SUMMARY

Re-polymerization of removable prosthesis and acrylic teeth may affect their hardness, which is the basic factor affecting successful longterm function of removable prosthesis. The aim of this work was to evaluate commercial acrylic teeth microhardness after the standard polymerization process for construction of complete and removable partial dentures, and to compare it with that of the as-supplied teeth.

Vivodent Orthotyp and Vita Vitapan premolars were subjected to re-polymerization in water bath at 90°C for 12 h in the presence or absence of the acrylic base resin. 12 slice-cut specimens of 1 mm thickness from each tooth type, including as-supplied and re-polymerized teeth, were tested for microhardness with an Anton Paar microhardness tester. ANOVA and Dunnett tests were used to evaluate the level of significance between the microhardness values of all groups of acrylic teeth.

Both Vivodent and Vita acrylic polymer teeth exhibited an either constant or enhanced microhardness value after polymerization in the presence of acrylic resin base material. This is important in order to preserve hardness and abrasion resistance of acrylic polymer teeth after the conventional polymerization procedure for constructing artificial dentures.

Keywords: Acrylic Teeth; Microhardness; Polymerization; Re-Polymerization; Acrylic Resin Base Material

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#### Introduction

Artificial teeth are produced from acrylic resin, porcelain or composite products and can be exploited for complete and partial denture construction. The mostly utilized artificial teeth for dental prosthesis are the acrylic resin polymer teeth, since their consistency insures the advantages of acrylic resins<sup>1,2</sup>. These comprise acceptable appearance, convenient handling, high toughness and compatibility with the acrylic base resin materials, offering to acrylic resin polymer teeth the lead for dental prosthesis applications<sup>1-3</sup>. Furthermore, the low density of acrylic teeth does not increase considerably the weight of the denture.

The composition of acrylic resin polymer teeth is, basically, poly-methyl-methacrylate (PMMA) beads and colour pigments in a cross-linked polymer matrix.

Many manufacturers supply their acrylic teeth with compolymer or highly cross-linked resin polymers in order to increase their resistance to fracture, abrasion and wear<sup>1-</sup> <sup>5</sup>. During the construction of acrylic resin polymer teeth, by the moulding technique, the monomer of the crosslinked polymer matrix can penetrate into the beads and the ridge lap area of teeth may be softened<sup>4,6-8</sup>. Although acrylic polymer teeth are supplied polymerized, they should undergo re-polymerization during the conventional process for composing artificial dentures. The presence of the same monomer in the mass of the acrylic resin base material, throughout the standard procedure for the construction of dentures, can act as plasticizer<sup>2,4</sup>, affecting appreciably the ridge lap area of acrylic teeth and soften them to some extent, in order to be chemically bonded to the base material<sup>1,2,6</sup>. This could affect the whole mass of acrylic teeth, since the monomer belongs to the organic solvents1-4,6,7.

Mastication<sup>7-9</sup> occurs on the surface of acrylic teeth<sup>2,3,9</sup>, consequently these must be hard enough to resist the abrasive forces and crazing that develop inside the mouth<sup>1-3</sup>. In addition, de-bonding of teeth from the complete or partial dentures should be avoided<sup>7-9</sup>. Hardness is an intrinsic physical property of a material, indicating its resistance to plastic deformation<sup>10-13</sup>. Although the microhardness values of acrylic polymer teeth are, generally, known within the range of 180-200 MPa<sup>1-3</sup>, their hardness after polymerization for producing complete or partial dentures is much less studied.

This study **aims** to evaluate the influence of the standard polymerization process on the microhardness of acrylic teeth, as compared to the microhardness of the as-supplied teeth. In addition, the role of the monomer of the acrylic base resin released during re-polymerization is investigated by comparing microhardness of acrylic teeth subsequent to polymerization in the presence or absence of the acrylic denture base material.

#### **Material and Methods**

Vivodent (IVOCLAR-Schaan, Liechtenstein) Orthotyp (VO) and Vita (VITA-Zahnfbrik H. Rauter GmbH, Germany) Vitapan (VV) acrylic polymer teeth were tested. 3 groups of each type of teeth were formed as follows:

- 1<sup>st</sup> group: As-supplied teeth (VOS-VVS).
- 2<sup>nd</sup> group: Teeth were, initially, set in moulds and inserted in boiling water for 10 min. The moulds were separated and rinsed with soap and boiling water in order to eliminate the presence of wax on the teeth. Then acrylic base resin in dough stage was inserted into the moulds and boiled in water bath for 12 h in 90°C for complete polymerization (VOP-VVP). The acrylic base resin used in this study was the Paladon 65 Kulzer.
- 3<sup>rd</sup> group: Teeth were boiled in gypsum moulds for 12 h in 90°C, without the presence of acrylic base material (VOB-VVB). This group would verify the influence of the monomer of the acrylic base resin on the microhardness of teeth after re-polymerization.

Acrylic teeth of all groups were cut in cross-section slices with a diamond wafer blade, in a Buhler cutting machine, under a load of 0.4 kg at 300 rpm. The load and rotation speed of the cutting were kept as low as possible in order to produce the smallest damage to the teeth slices. 4 1.5 mm thick sliced specimens were cut from each tooth. Internal cross-section slices of teeth were selected as specimens for microhardness measurements since the effect of polymerization on the whole mass of teeth was investigated. Subsequent to cutting, specimens were mechanically thinned, on both sides, using silicon carbide grinding papers of 1200 and 2000 grade and a final mechanical polish was accomplished using 10 µm and 0.3 µm alumina (a-Al2O3) pastes. A final thickness of 1 mm for each slice was reached prior to microhardness testing. Mechanical grinding and polishing were necessary for elimination of cutting damages and production of smooth surfaces for microhardness testing. Microhardness values of all 3 groups of specimens were evaluated using an Anton Paar MHT-10 microhardness tester loaded on a Zeiss "Axiolab A" optical microscope. After the indenter came to rest, indentation prints were projected to a monitor through a CCD camera attached to the microscope. The diagonals of the indentation prints were then determined using image-processing software. Due to the difference in structural characteristics of 2 types of teeth, both Knoop and Vickers indenters were used for microhardness measurements. The Knoop microhardness values were calculated from the following expression:

$$H_{\rm K} = 14,229 \ {\rm P/d^2},$$
 (1)

where P is the loading force in gf (1 gf  $\cong 10^{-2}$  N), d is the longer diagonal of the indentation print in  $\mu$ m and H<sub>K</sub> is the Knoop microhardness value. Correspondingly, the Vickers microhardness values were calculated from the following expression:

$$H_V = 1,854 \text{ P/d}^2,$$
 (2)

where P is the same with previous case, d is the mean value of the indentation diagonals in  $\mu$ m and H<sub>V</sub> is the Vickers microhardness value in kgf/mm<sup>2</sup>. In the literature, both methods are considered to produce equivalent microhardness values for the same indentation loads; consequently they can be used concurrently.

#### Results

Anova and Dunnett tests (P<0.005) indicated a significant difference between all groups of Vita Vitapan acrylic teeth. The same tests indicated significant difference between VOS(1) and VOB(3) at Vivodent orthotyp acrylic teeth, while no significant difference between VOP(2), VOS(1) and VOB(3) at a p value of 0.005. These are in agreement with the calculated values of standard deviations listed in tables 1 and 2.

Applying equation (1), Knoop microhardness measurements performed under a loading force of 0.3 N on the Vivodent Orthotyp teeth resulted in the following: a) The as-supplied teeth (VOS) exhibited a mean microhardness value of 196.81.1 MPa, which is within the range expected for acrylic polymer resin teeth; b) VOS teeth after re-polymerization with the acrylic base (VOP) presented, virtually, identical mean microhardness value with the VOS teeth, i.e. equal to 197.8 $\pm$ 5.2 MPa; c) VOS teeth polymerized without the presence of the acrylic base resin (VOB) showed a slightly increased mean microhardness value of 204.5 $\pm$ 3.9 MPa, which was not significant. All 10 measurements for each group of Vivodent teeth are depicted in table 1.

Vivodent Orthotyp	Knoop m	Knoop microhardness HK (MPa)									Mean values (MPa)
VOS	194.6	198.6	196.7	196.7	197.8	197.8	195.9	196.7	196.7	196.7	196.8±1.1
VOP	199.5	199.5	205.9	195.9	196.7	205.9	194.3	189.9	193.3	196.7	197.8±5.2
VOB	200.3	209.7	200.3	202.2	202.2	202.2	208.0	209.7	208.0	202.5	204.5±3.9

Table 1. Knoop microhardness measurements and mean microhardness values of the Vivodent acrylic teeth

Table 2. Knoop microhardness measurements and mean microhardness values of the Vita acrylic teeth

Vita Vitapan Knoop microhardness HK (MPa)								Mean values (MPa)			
VVS	228.8	229.8	237.8	236.7	226.4	240.3	219.9	224.4	230.8	225.4	230±6.5
VVP	251.6	259.2	267.6	245.1	271.9	258.0	247.8	265.0	279.5	252.8	259.9±11.1
VVB	285.5	296.7	288.1	284.9	295.2	279.5	311.7	282.9	295.2	281.5	290.1±9.8

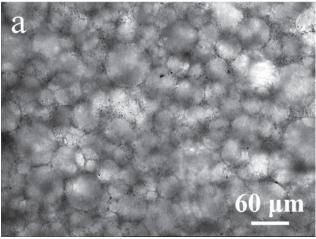
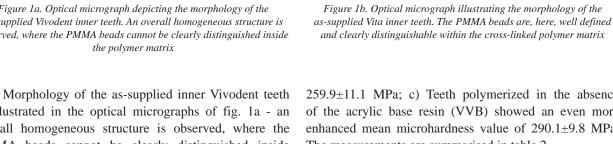


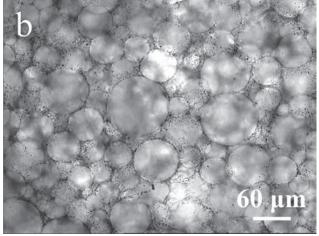
Figure 1a. Optical micrograph depicting the morphology of the as-supplied Vivodent inner teeth. An overall homogeneous structure is observed, where the PMMA beads cannot be clearly distinguished inside the polymer matrix



is illustrated in the optical micrographs of fig. 1a - an overall homogeneous structure is observed, where the PMMA beads cannot be clearly distinguished inside the polymer matrix. The homogeneity of the structural characteristics of Vivodent teeth is verified from the low values of the standard deviations in the mean microhardness values.

The corresponding Knoop microhardness measurements for the Vita Vitapan teeth can be summarised in the following: a) the as-supplied teeth (VVS) presented a mean microhardness value of 230±6.5 MPa, appreciably higher than VOS; b) Teeth polymerized in the presence of the acrylic base (VVP) exhibited an unpredictably high mean microhardness value of 259.9±11.1 MPa; c) Teeth polymerized in the absence of the acrylic base resin (VVB) showed an even more enhanced mean microhardness value of 290.1±9.8 MPa. The measurements are summarised in table 2.

Morphology of the as-supplied Vita teeth is illustrated in figure 1b, where the difference with the previous case is evident. The PMMA beads are, here, well defined and clearly distinguishable from the cross-linked polymer matrix. All groups of Vita teeth exhibited unpredictably high mean microhardness values, which are linked, however, with rather high standard deviations. These deviations in the microhardness values suggest anisotropy of the overall sliced teeth surface concerning microhardness. This anisotropy most likely arises from hardness differences between the PMMA



beads and the cross-linked polymer matrix. Since the Knoop indenter produces a rhombic print that has a long diagonal, it is difficult to isolate the indentation inside a PMMA bead or within the matrix to detect differences in microhardness values. Thus, Knoop indentations resulted in the average microhardness value of the beads and the matrix. Alternatively, a Vickers indenter was used for these particular indentations, since it produces a square print, which can be imprinted inside a bead. In order to have a small imprinted area, Vickers microhardness measurements were performed under 0.1 N and 0.3 N loads. Both loads belong to the plateau area of the Indentation Size Effect (ISE) curve<sup>10-12</sup> that were recorded for Vita teeth (Fig. 2), and thus the Vickers hardness values calculated are considered to be highly plausible.

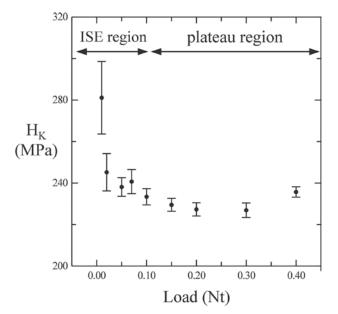


Figure 2. Schematic illustration of Vickers microhardness (Hv) as a function of the applied load, where the ISE and the Plateau areas are depicted for the VVS beads. The error bars represent the mean standard deviations

Vickers measurements performed on the PMMA beads of Vita teeth revealed the following: a) VVS beads present a mean microhardness value of only  $198\pm3.9$  MPa, whereas the corresponding matrix value is of the order of 440 MPa; b) VVP beads exhibit a mean microhardness of 205.5±8.4 MPa, which is practically equal to that of the VOS beads, and the corresponding matrix value is of the order of 470 MPa; c) VVB beads exhibit the highest mean microhardness that is of the order of 236.9±6.1 MPa and the corresponding matrix value is of the order of 490 MPa. Variation in the mean microhardness value of the cross-linked polymer matrix is mainly caused from the heterogeneity of its structural elements. In addition, the microhardness value of the matrix is difficult to be precisely determined, since the

areas between beads are small for clear indentation prints. The substantial difference between the microhardness values of the PMMA beads and the matrix is undoubtedly illustrated in figure 3, where 2 Vickers indentation prints, performed under the same loading force on a PMMA bead and on the matrix of a VVP tooth are shown. Since the particle and the matrix areas have a different contrast, the percentage of the total area they cover can be easily calculated with digital image processing. For the application of image processing, 10 sections of 25 mm<sup>2</sup> were used to assess the corresponding areas mean values. The area covered with matrix is estimated to be the 20% of the total area. Taking this into account, a good approximation of the average microhardness value (H<sub>Vav</sub>) can be resolved by adding the relative microhardness "weight" of each component as follows:

$$I_{Vav} = W_b x H_{Vb} + W_m x H_{Vm}$$
, (3)

where  $W_b$  and  $W_m$  are the relative "weights" of the PMMA beads and the matrix respectively, and  $H_{Vb}$ ,  $H_{Vm}$  the corresponding Vickers hardness values. Applying equation (3), we find the following average microhardness values for Vita teeth: i) In VVS  $H_{Vav} = 246.4$  MPa; ii) in VVP  $H_{Vav} = 258.4$  MPa; and iii) in VVB  $H_{Vav} = 287.5$  MPa.

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These are, practically, equivalent with the microhardness values obtained from the Knoop measurements.

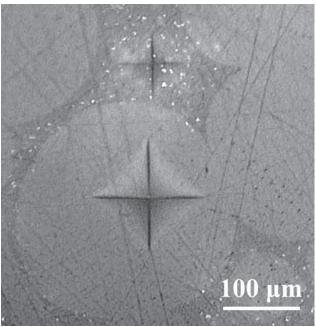


Figure 3. Vickers indentation prints, performed under the same loading force, on a PMMA bead and on the matrix of a VVP tooth. The difference in diagonals of the indentation prints is indicative of the difference in microhardness

#### Discussion

2 vital parameters that influence the quality of microhardness measurements are the loading force of the indenter and the duration of the indentation. The indentation load should be selected to be high enough, so the measurements will not be influenced from the Indentation Size Effect (ISE), which is responsible for the apparent enhanced microhardness values obtained with indentations belonging to the low-load region<sup>10,12,14,15</sup>. Furthermore, the indentation duration should be long enough to produce plastic deformation but simultaneously, short enough to eliminate the possibility of undesirable external vibrations. For Knoop measurements we have used already published data<sup>16</sup>, whereas for Vickers measurements loads belonging to the plateau of figure 2 were utilized.

Vivodent acrylic polymer teeth presented a homogeneous morphology of the inner teeth, exhibiting an almost constant microhardness regardless re-polymerization with or without the presence of acrylic resin base material. Due to the lack of apparent boundaries between the PMMA beads and the polymer matrix, it seems that there is no influence of the monomer on the hardness of the material. This is verified from the fact that microhardness values of teeth boiled in the absence or presence of acrylic resin were practically equal.

Conversely, Vita acrylic resin polymer teeth exhibited an enhanced microhardness value after re-polymerization in the presence of acrylic resin base material. Furthermore, their hardness increased even more when boiled without the presence of acrylic resin. Since the morphology of the inner Vita teeth is heterogeneous, showing clear boundaries between the PMMA beads and the polymer matrix, microhardness was measured separately for the 2 components. The results revealed an increase in the microhardness value of the PMMA beads after the conventional procedure for constructing artificial dentures, while matrix microhardness remained, practically, constant. In the absence of the acrylic resin base the enhancement of the PMMA beads microhardness is noticeably higher. This allows us to deduce that monomer of the acrylic resin base during re-polymerization penetrates into the boundaries between the 2 components and softens the PMMA beads<sup>6</sup>. This softening, however, is by no means important given that microhardness of PMMA beads after re-polymerization in the presence of the acrylic resin base is considerably higher than that of the as-supplied teeth.

#### Conclusions

The influence of the standard polymerization process on microhardness of acrylic teeth was evaluated and compared to the microhardness of the as-supplied teeth. The role of the monomer of the acrylic base resin released during re-polymerization was also investigated by comparing the microhardness of acrylic teeth subsequent to polymerization in the presence or absence of the acrylic denture base material.

Both Vivodent and Vita acrylic polymer teeth exhibited an either constant or enhanced microhardness value after re-polymerization in the presence of acrylic resin base material. This is crucial in order to avoid softness, crazing and preserve abrasion resistance of acrylic polymer teeth after the conventional re-polymerization procedure for constructing artificial dentures.

Softening of the PMMA beads in Vita teeth during polymerization seems to be directly related to the diffusion of the monomer, present in the mass of the acrylic base resin, within the boundaries between PMMA beads and the polymer matrix. This does not, however, influence significantly the overall microhardness of the teeth that remains higher than the corresponding of the as-supplied teeth. Due to their homogeneous morphology Vivodent teeth are not influenced by the presence of the monomer.

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## Evaluation of 2 Different Types of Extra-Coronal Frictional Attachments with Plastic Matrices in Contemporary Dental Prosthodontics

#### SUMMARY

Nowadays, due to fast dental progress, planning of partial dentures is continuously improving, permitting application of contemporary and modern retention systems. Because of the presence of many different dental attachments on the market, the decision which one to use for each particular case is a big challenge for the dentist. The purpose of this paper is to examine the advantages and disadvantages of extra-coronal frictional attachments with plastic matrices and to present a critical review for 2 types of retention systems.

We used 2 types of extra-coronal frictional attachments with plastic matrices: the AcryLock attachments and the Vario-Soft 3 attachments. The results confirm that AcryLock and Vario-Soft 3 represent similar systems. They insure good retention, with an additional option for dosed retention by using different plastic matrices depending on each particular case. Although these attachments with plastic parts are not newest innovation, they have a big value due to their characteristics - durability, affordable price and possibility for easy and simple maintenance, offering a chance to replace the plastic part if necessary. They insure high quality and long lasting dental prosthodontic appliances that can be used in many different situations.

Keywords: Extra-Coronal Attachments; AcryLock; Vario-Soft 3

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#### Introduction

Nowadays, planning complex fixed-removable constructions is very common. But, due to fast dental progress, planning of partial dentures is continuously improving, permitting application of contemporary and modern retention systems<sup>1</sup>. The need to discover an alternative solution for inferior dental clasps that would satisfied functionality and aesthetics, led to rapid development of attachments. Today, the attachments represent an optimal biological, functional and aesthetic solution for retention. If correctly planned, designed and constructed, the attachments, as elements for direct retention, allow the denture to resist forces that tend to move it out of position, limiting the transfer of damaging forces on the abutment teeth and supportive tissues, simultaneously making proper aesthetic effect of "invisibility" of their structural elements<sup>2</sup>. Because of presence many different dental attachments on the market, the decision which one to be used for each particular case is a big challenge for the dentist.

Selection of the attachment must be made as a result of studious assessment of the oral conditions<sup>3</sup>. In patient treatment, when planning complex fixed-removable constructions, successful selection of an attachment depends on many factors, such as available space, vertical dimension, condition of remaining teeth (their length, periodontal condition)<sup>4</sup>. Another very important factor is the patient, his demands, behaviour, habits and his prior experience with dentures (if any), as well as needs for aesthetics, comfort and psychological profile<sup>2</sup>.

The retention systems with plastic matrices are present for a relatively short period of time on our market. Because of the growing trend for their application, there was a need for studying their characteristics. The **purpose** of this paper is to examine the advantages and disadvantages of extra-coronal frictional attachments with plastic matrices and to present a critical review for 2 types of retention systems.

#### **Material and Method**

We used 2 types of extra-coronal frictional attachments with plastic matrices: the AcryLock and the Vario-Soft 3 attachments.

AcryLock is a plastic rod attachment, composed of a stress-breaker, patrix and plastic matrix. The patrix is placed extra-coronal. It can be placed separately or coupled up to a double groove stress-breaker called IS (Integrated Stress-breaker). The stress-breaker is placed on the proximal surface of the wax model of the abutment crown. It provides sufficient distance of the patrix from the interdental papilla. The stress-breaker is then coupled with the patrix, which is placed on the stress-breaker in respect to the position and shape of the alveolar ridge. The stress-breaker and the patrix are made of plastic, which volatilizes completely during the heating up of the muffle. The whole construction, the wax crown with the plastic stress-breaker and the patrix, is than casted from stable alloys. The plastic patrix which volatilizes completely is oversized with 0.04 mm in order to get its proper size after processing and polishing of the metal<sup>5</sup>. The matrix of the AcryLock attachment is made of hard plastic. It is inserted into the matrix-housing of the metal partial denture, using a special instrument for that purpose. The matrices are available in 3 different dimensions to adjust friction (regulation of the retentive force) - the green matrix allows normal friction, the yellow one allows medium, and the red matrix causes high friction. As a result, the retentive force of the attachment can be adjusted appropriately<sup>5</sup>. Should the need arise; the change of the matrix in the metal matrix-housing is performed very fast and simple. Based on shaping of the matrix with a retention point, changing of the friction inserts is very simple without spending a lot of time for shortening and fitting in. This extends the durability of the prosthetic construction as a whole (Figs. 1-4). Besides Microdent, similar systems are produced by Heraus, Interdent etc...<sup>6</sup>



Figure 1. Preview of the fixed and removable part of the construction (occlusal view)



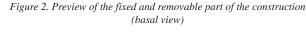




Figure 3. The complete complex construction



Figure 4. The patient with the prosthetic construction

Vario-soft 3 is an attachment designed and manufactured by Bredent. This attachment is made of plastic patrix, which burns without residue and is casted in 1 part with the rest of the construction on the abutment teeth. After producing metal construction, duplicating matrix that is included in the kit is placed on the patrix and the rest of the model is prepared for duplicating. On the model obtained from fireproof investment material, a matrix-housing made of wax is placed on the matrix. The wax matrix-housing is included in the kit. Then, wax moulding can be continued. After manufacturing



Figure 5. Preview of the fixed and removable part of the construction

the denture skeleton from cast alloys, a plastic matrix is placed in the casing using an appropriate instrument set. This system provides 6 levels of retention. The 3 basic matrices are: red matrix - provides great friction, yellow matrix - allows normal friction and green matrix-reduced friction. Besides the basic, there are particularly soft matrices made of soft plastic that can compensate for small divergences and minor processing imperfections (Figs. 5-8). The softer matrices are marked with brighter colours: light red - with greater friction, light yellow normal friction and light green - the slightest friction<sup>7</sup>.



Figure 6. The complete complex construction (basal view)



Figure 7. The complete complex construction (occlusal view)



Figure 8. The patient with the prosthetic construction

Except customary Vario-Soft 3 attachments, there are the Vario-Soft 3 sv attachments that contain integrated shear distributor. The shear distributor provides protection of periodontal structures of the remaining teeth, making the milling of the crown unnecessary. This results in a better aesthetic effect, reduces costs and reduces time required for processing of the construction. In cases of limited space, the Vario-Soft 3 attachments are available with reduced dimensions as Vario-Soft 3 mini and Vario-Soft 3 mini sv. In Vario-Soft 3 mini sv attachment, its

prefabricated component is only 3.5 mm wide and 4 mm long, and includes patrix and shear distributor<sup>7</sup>.

We used 2 types of attachments, AcryLock and Vario-Soft 3 for partial edentulous patients with shortened dental arches. We followed clinical procedures for manufacturing the complex fixed-removable prosthetic solutions retained by AcryLock and Vario-Soft 3 systems, with preparation of the remaining natural dentition. First, we made the tooth crowns containing the patrix of the attachment. After trying the fixed structure in the patient's mouth, we continued with the procedures of making removable part of the prosthetic construction - cast denture that contains the plastic matrix. We monitored our patients with the complex fixed-removable prosthesis for 4 years through regular check-ups every 3 months.

#### **Results and Discussion**

In all the patients we had accomplished adequate functional and aesthetic values with our prosthodontics treatment. Within monitoring our patients for 4 years, we noticed that condition of the remaining natural teeth did not change, which verifies the preventive effect that the dentures with AcryLock and Vario-Soft 3 attachments have on oral tissues.

The results confirm that AcryLock and Vario-Soft 3 represent similar systems. They insure good retention, with an additional option for dosed retention by using different plastic matrices, depending on each particular case. Although these attachments with plastic parts are not newest innovation, they have a big value due to their characteristics - durability, affordable price and possibility for easy and simple maintenance, offering a chance to replace the plastic part if necessary. They insure high quality and long lasting prosthodontic appliances that can be used in many different situations. Besides, the AcryLock system provides 3 levels of retention - normal (green matrix), medium (yellow matrix) and high (red matrix) and the Vario-Soft 3 systems, moreover the above-mentioned 3 possible retention levels, provide additional 3 (6 in total) levels of retention. Thus, the Vario-Soft 3 systems provide particularly soft matrices made of soft plastic that can compensate for small divergences and minor processing imperfections.

While locating the extra-coronal attachments, depending of the attachment distance from the vertical axis of the abutment tooth, the possible detrimental forces should be neutralized. To prevent overloading, it is recommended that at least 2 abutment teeth should be connected in 1 block with milled surfaces (double abutments). So planned construction will help denture stabilization and correct distribution of the load. The forces that are harmful to the remaining dentition and periodontal ligament are significantly reduced by reciprocal arm and the precise path of insertion of the denture. The milled surface must have a minimum height of 2.5 to 4 mm. The reciprocal arm, the shoulders and the interlock contribute to the prevention of negative impacts of forces. The chamfered margin provides hygienic advantages over butt or square shoulders. The cervical milled ledges should be positioned approximately 1 mm above the gingival margin. If the abutment crown is naturally short, it is recommended to provide a ledge with

a square edge. Occlusal shoulders can be made at  $90^{\circ}$  right angels or can be chamfered. Shoulders positioned at right angles to the path of insertion are best suited for the transmission of loads; however chamfered shoulders are easier to produce. The Interlock must be positioned  $180^{\circ}$  opposite to the attachment<sup>8</sup>.

The Vario-Soft 3 sv systems include integrated share distributors. The shear distributor provides protection of periodontal structures of the remaining teeth, making the milling of the crown unnecessary. This results in a better aesthetic effect, reduces costs and reduces time required for processing of the construction. In addition, this simplifies the technical procedures necessary for denture manufacturing.

In terms of longevity and maintenance of constructions that are retained with attachments with plastic matrices, unlike most metal attachments, which are adjustable (they can improve their power of retention with simple activation), the non-metal (plastic) attachments need to be replaced (as a whole or only a certain element)<sup>9</sup>. If significant decline in value of retention of the attachment occurs as a result of wear and plastic deformation of the matrix, it is necessary to replace it with new one (new matrix). The procedure of changing the plastic matrix is carried out very simply and quickly. It requires no additional laboratory procedures. The old matrix is removed from its metal housing in the denture, and is replaced with new one using a special instrument. This extends durability of the prosthetic construction as a whole. Moreover, the same metal housing of the denture can hold all the plastic matrices which have different retention capability. This makes the adjustment of the prosthetic construction to the current oral conditions possible. For example, reducing or increasing retentive force of the attachment is made possible by placing matrices that allow more or less friction if the circumstances require so<sup>10</sup>.

Among our patients, during the regular checkups every 3 months, a need to change the plastic matrices occurred several times, but the denture behaved quite normal afterwards - we managed to re-establish the desired retention value of the attachment.

It should be noted that the wear of metal patrix when using frictional attachments with plastic matrices is considerably smaller compared to frictional attachments made entirely of metal. This is due to the greater softness of plastics compared to metal<sup>11</sup>. Thus, it is considered that if regularly controlled and maintained, dentures with plastic attachments have a potential for greater longevity compared to dentures with metal attachments. Moreover, economic viability of the attachments with plastic matrices should not be neglected. Namely, due to its simple structural design and materials of manufacturing, the plastic attachments have relatively low price, making them accessible to a wider circle of patients.

#### Conclusion

Although these attachments with plastic parts are not newest innovation, they have a big value due to their characteristics - durability, affordable price and possibility for easy and simple maintenance, offering a chance to replace the plastic part if necessary. They insure good retention, with an additional option for dosed retention by using different plastic matrices depending on each particular case. Thus, they insure high quality and a long lasting dental prosthodontics that can be used in many different situations.

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## Importance of Proper Oral Hygiene in Patients Undergoing Treatment with Fixed Orthodontic Appliances

#### SUMMARY

**Objective:** The aim of this study was to evaluate the importance of proper oral hygiene in patients undergoing treatment with fixed orthodontic appliances.

Materials and Methods: Clinical examinations encompassed 40 patients with diagnosed malocclusion - and it started before the orthodontic treatment. Subjects were divided in 2 groups (20 subjects in each group). The first group was treated with dental cream GC Tooth Mousse, and the second group with Fluorogal-solution containing low concentration of fluoride - 0.05%F). Control group comprised 20 patients. OHI-index was registered in all subjects (60) before and at the end of orthodontic treatment, using the simplified method of Greene-Vermillion (OHI-S).

**Results:** Improvement of oral hygiene was detected in the group where preventive treatment with Fluorogal was implemented (statistically significant difference between medium values of the OHI-S index before and after the orthodontic treatment), which was not the case with control group. The subjects treated with dental cream (GC Tooth Moussne) at the end of the orthodontic treatment had decreased OHI-S (1.49) in comparison to the beginning of the treatment, where the average monthly value of the index was 1.55 (however, the difference was not statistically significant).

**Conclusions:** The habit to maintain oral hygiene regularly is very important for maintaining gingival health throughout the orthodontic treatment and after it is completed. A high level of oral hygiene should be achieved before, during and after any orthodontic treatment in order to prevent side effects on periodontal tissues.

Keywords: Oral Hygiene; Fixed Orthodontic Appliances; Periodontal Tissue

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#### Introduction

Orthodontic treatment has a preventive effect against periodontal disease and caries because it facilitates establishing functional occlusion and makes all tooth areas accessible to oral hygiene. Numerous studies have shown that orthodontic patients are in high risk of developing periodontal disease and caries due to longterm orthodontic treatment. Presence and position of fixed orthodontic appliance create poor conditions for maintaining oral hygiene<sup>1</sup>. The region of the tooth surface around the brackets is prone to adhesion of oral bacteria and subsequent biofilm formation. Oral biofilm, or "dental plaque", is difficult to be removed and regular brushing is often insufficient to remove plaque from retention sites, such as the vulnerable brackets-adhesive-enamel junction and the sensitive region between brackets and the gingiva. Moreover, orthodontic appliances severely hamper the efficacy of tooth brushing, reduce the self-clearance by saliva<sup>2</sup>, change the composition of the oral flora<sup>3</sup>, and increase the amount of oral biofilm formed<sup>4</sup>, colonization of oral surfaces by cariogenic<sup>5</sup> and periodontopathogenic bacteria<sup>6</sup>. These factors strongly complicate orthodontic treatment and illustrate that the need for oral biofilm

control is even greater during orthodontic treatment than usually.

Plaque accumulation is promoted by physical constitution of different parts of the fixed appliance, but there are some other factors that have a great impact on plaque accumulation. In the oral cavity all of the tooth surfaces are exposed and rapidly covered by salivary proteins causing different effects (interactions between material, pellicle and bacteria). As part of fixed appliances, orthodontic bands can cause gingival inflammation<sup>7</sup>. Plaque accumulates particularly beneath bands from which some cement has been washed out adjacent to adhesive retention elements<sup>8</sup>. Plaque is found predominantly cervical to brackets under the arch wires.

Maintaining oral hygiene during orthodontic treatment will help in maintaining good gingival health, which reflects in final orthodontic treatment outcome. However, the level of gingival health knowledge among orthodontic patients is not adequate. Poor maintenance of oral hygiene is due to either lack of knowledge or negligence by patients themselves. Patients are not given proper instructions, or they may not comply properly with the instructions.

Administration of topical agents containing fluoride or casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), maintenance of oral hygiene, and dietary control have been suggested as mechanisms to control formation of enamel lesions during fixedappliance treatment<sup>9</sup>. Fluoride ions in plaque immediately promote remineralisation by formation of flourapatite<sup>10</sup>. In addition, fluoride application can promote remineralisation of previously demineralised enamel in cases where adequate amounts of calcium and phosphate ions are available<sup>11</sup>. Fluoride and CPP-ACP applications are accepted approaches for remineralising the previously demineralised enamel.

The **aim** of this study was to evaluate the importance of proper oral hygiene in patients undergoing treatment with fixed orthodontic appliances.

#### **Materials and Methods**

The study included clinical examination and statistical processing of the data. Clinical examination encompassed 40 patients with diagnosed malocclusion, and it started before the orthodontic treatment. Subjects were divided in 2 groups (20 subjects in each group). The first group was treated with dental cream GC Tooth Mousse, and the second group with Fluorogal - solution with a low concentration of fluoride (0.05%F). Control group comprised 20 patients.

OHI-index was registered in all subjects (60) before and at the and of the orthodontic treatment, using the simplified method of Greene-Vermillion (OHI-S)<sup>12</sup>, where index values are within the 0 to 3: index 0 - absence of sediments; index 1 - presence of a third layer on the surface of the tooth crown; index 2 - presence of a number of deposits of a third, and less than  $\frac{2}{3}$  of the crown surface; index 3 - presence of a number of deposits of the  $\frac{2}{3}$  of the crown surface.

Using the simplified method for determination of the oral hygiene index (OHI-S), only 6 areas have been assessed, which are representative sample for the entire dentition: vestibular area in upper first molars, top cover right central incisor and lower left central incisor; oral surface of the first molars. The obtained values were added together, and the score was divided with the number of the examined teeth.

For preventive treatment of the teeth in clinical conditions, the following tools were used: (1) a tool with a rich mineral composition (GC Tooth Mousse), from which we expected mineralization effect, and enrichment of the surrounding enamel brackets (clinical), or the enamel tooth crown; (2) a tool which also contains fluoride exempt (Fluorogal); (3) material for bonding the brackets which contained the fluorine GC Fuji Ortho <sup>TM</sup> LC, or did not contain fluoride - Dentaurum (Orthodontic Bonding System).

#### Results

Student's t-test for dependent samples in subjects treated with fluoride solution (Fluorogal) showed a statistically significant difference between medium values of the OHI-S index before and after orthodontic treatment. The differences were not statistically significant between the group treated with preventive dental cream (GC Tooth Mousse), and in the control group (Tab. 1).

Table 1. The values of the OHI-S index in subjects of all 3 groups

groups*	OHI-S	$\overline{\mathbf{X}}$	SD	t	р	
1.	before treatment	1.55	0.48	1.087	0.2905	
	after treatment	1.49	0.46	1.087		
	before treatment	1.71	0.45	5.940	0.0000101	
2.	after treatment	1.43	0.47	5.849	0.000012†	
2	before treatment 1.67 0.56		0 60 4	0.5016		
3.	after treatment	1.75	0.44	- 0.684	0.5016	

\* - 1. group: brackets bonded with Fuji OrthoTM LC and treatment with GC Tooth Mousse

2. group: brackets bonded with Fuji OrthoTM LC and treatment with Fluorogal

3. group: brackets bonded with Fuji OrthoTM LC (control group) † statistically significant differences The analysis of variance (Tab. 2; Fig. 1) showed no statistically significant difference between the groups in relation to the OHI-S index by the treatment (F=0.486; p=0.6176). Tukey's HSD (honestly significant difference) test showed differences (not statistically significant), among medium values in the OHI-S index in the examined groups before the treatment (Tab. 3).

Table 2. Values of OHI-S index before treatment in I, II and III group

Group	x	SD	Ν
1.	1.55	0.48	20
2.	1.71	0.45	20
3.	1.67	0.56	20

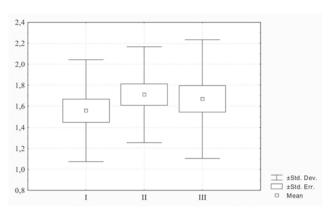


Figure 1. Values of OHI-S index before treatment in subjects of all groups

Table 3. Difference between values of OHI-S index beforetreatment in all groups

groups	р
1. and 2.	0.6089
1. and 3.	0.7684
2. and 3.	0.9634

\*Tukey (HSD) test

The analysis of variance (Tab. 4; Fig. 2) showed no statistically significant difference between the groups in relation to the OHI-S index by the treatment (F=2.744, p=0.0727). Tukey's HSD test showed differences (not statistically significant), among medium values in the OHI-S index in the examined groups after the treatment (Tab. 5).

Table 4.	Values of	<sup>e</sup> OHI-S index	in subjects	after the	e treatment

groups	X	SD	N
1.	1.49	0.46	20
2.	1.43	0.47	20
3.	1.75	0.44	20

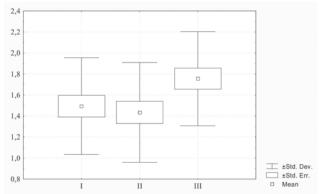


Figure 2. Values of OHI-S index in subjects after the treatment

 Table 5. Difference between values of OHI-S index in subjects
 after the treatment

0.9127
0.1806
0.0795

\* Tukey (HSD) test

#### Discussion

Orthodontic treatment has preventive effect against periodontal disease and caries because it facilitates establishing functional occlusion and makes all tooth areas accessible to oral hygiene. Numerous studies have shown that orthodontic patients are in high risk of developing periodontal disease and caries because orthodontic treatment lasts for long time. Presence and position of fixed orthodontic appliances gives poor conditions for maintaining oral hygiene.

Biofilm is often seen as a complex structure which is prone to a number of external factors. They can both alter its structure and the formation process. Wearing of removable and fixed orthodontic appliances is listed among these factors. Biofilm has a tendency to accumulate on retentive areas of springs, clasps and acrylic base plates<sup>13</sup>. According to Jordan et al<sup>14</sup> the increase in number of bacteria belonging to *Streptococci mutants* and *Lactobacilli* species, as well as the alteration of oral microbiota is observed during orthodontic treatment with removable appliances. Furthermore, according to Mitchell<sup>15</sup>, fixed orthodontic appliances also pose threat to both patients and clinicians by increasing the risk of biofilm formation. It has been demonstrated that in the majority of orthodontic patients biofilm is present resulting in enamel decalcification around orthodontic brackets.

Evaluation of oral hygiene index (OHI) can be useful in biofilm diagnostics. Importance of oral hygiene in orthodontic patients is always intensified to prevent any further periodontal disease. In the absence of oral hygiene maintenance, plaque accumulation on orthodontic appliance components is paving way to destruction of periodontal tissues<sup>16</sup>. Positive effects of orthodontic treatment may be compromised if adequate and regular oral hygiene is not maintained. Efficient plaque control consists of developing several effective methods and instruments<sup>17,18</sup> for plaque removal, materials and methods for improving the resistance of teeth and oral tissues to caries and gingivitis, as well as instructions for oral hygiene maintenance<sup>19,20</sup>. The role of oral hygiene in the genesis of caries was illustrated. However, remains a question on what and how much is the participation of oral hygiene in the development of these diseases.

Oral hygiene is a significant factor for oral and dental health. Mathiesen et al<sup>21</sup>, analyzing association among oral hygiene (OHI-index), DMFT-index and index of gingival inflammation in 14-year-old children confirmed the inverse relationship to the appearance of caries and oral hygiene. The positive effects of oral hygiene instructions for patients with fixed orthodontic appliances have been recognized<sup>22</sup>, and significant improvement of the OHI-S index was observed in our study as well; this is in accordance with the results by Al-Jewair et al<sup>23</sup>, who reported good OHI compliance in 73% of patients. Our research confirms the significance of cleaning the oral cavity. Improvement of oral hygiene was detected in the group where preventive treatment with Fluorogal was implemented (statistically significant difference between medium values of the OHI-S index before and after orthodontic treatment), which was not the case with the control group. This finding might be a result of explanation in the way of oral hygiene maintenance (adequate and not adequate oral hygiene). The subjects treated with dental cream (GC Tooth Mousse) at the end of orthodontic treatment had a decreased oral hygiene index (1.49) in comparison to the beginning of the treatment, where the average monthly value of the index of oral hygiene was 1.55 (however, the difference was not statistically significant).

Improvement in OHI-S index can be explained by the precise instructions in oral hygiene measures during each check up and the resolving of crowding during the first 12 weeks of the orthodontic treatment, but it can also be attributable to the Hawthorne effect (patients' awareness of being examined and evaluated)<sup>24</sup>.

#### Conclusions

Before commencing the treatment, patients should be informed about the increased risk of developing caries and periodontal disease and the necessity for ultimate and regular oral hygiene in order to reduce this risk to the minimum. The habit to maintain oral hygiene regularly is very important for maintaining gingival health throughout the treatment and after it is completed. A high level of oral hygiene should be achieved before, during and after any orthodontic treatment in order to prevent any side effects on periodontal tissues.

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## Analysis of Dental Calcifications According to the Structure

#### SUMMARY

The aim of the study was to inspect the structure of dental calcifications by histopathological analysis. The research was made on 40 pulps of the extracted teeth and 60 extirpated pulps of teeth with endodontic diagnosis of chronic pulpitis.

According to the method of light microscopy, and by using standard differential histo-chemical colouring, 3 morphological images were gained: (1) calcifications with morphological features similar to the dentin structure; (2) calcifications with lamellar concentric structure, and (3) calcifications with granulated fine granular structure. The first group of calcifications showed greater affinity to eosin i.e. get coloured in intense red, unlike the other 2 groups of calcifications, implying that there is a greater quantity of organic matrix in them. The second group of nodules was similar in size to the previous ones. These calcification were spherical in shape, and more intensively coloured with haematoxylin i.e. they showed more intensive basophilic behaviour. In the third group, according to the shape, calcifications were spherical, oval or irregular. These 2 types of calcifications had pretty similar structure, with amorphous and uniformed zones of encrustations, up to zones with fine granulated material.

Keywords: Dental Calcifications; Structural Changes

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#### Introduction

Denticles more often occur in molars than in premolars and incisives<sup>1,6</sup>. Greater percentage of denticles occurs in maxillary than in mandibular teeth, with the exception of incisives, which have more denticle occurrence in the mandible<sup>1</sup>. Denticles more often occur in males than in females, both in the maxilla and the mandible<sup>3</sup>. Denticles more often occur in teeth with caries i.e. in the restored teeth than in the non-restored teeth<sup>1,16</sup>.

Regarding the size, calcifications show a wide range of variations<sup>11</sup>. The findings show values smaller than 1 micron, up to 1cm measured per sample, with continuous areas of calcifications which fill in almost the whole pulp, in a longitudinal direction. The transverse section is within the limits of 20 to 200 microns, whereas the longitudinal section is up to 500 microns.

According to shape, 2 groups are identified. The first group consists of calcifications of oval shape, which have a degree of bending similar to circle or spherical objects; these calcifications are nodular. The second group of calcifications consists of calcifications which are of irregular shape, corner-like, except the bigger ones, which are relatively elongated. The spherical calcifications show tendency of grouping according to their number, with average value of 3 calcifications per volume of one pulp. Irregular calcifications do not show tendency of grouping. Perceived in longitudinal direction, with ordinary segmentation of the pulp in 3 thirds, calcifications are localized in each third as well as in the transition areas among them. Spherical calcifications occur more often in the middle third, whereas irregular calcifications do not show any predilection. Regarding the transverse measuring of the pulp, parts of spherical calcifications are closer to the lateral sides of the pulp i.e. to the surface

of the pulp. Irregular calcifications are situated more centrally, as well as across the whole width of the pulp.

Calcifications of the dental pulp can be dentine and non-dentine. Dentine calcifications are spherical, nodular, solitary and more numerous, they contain greater quantity of organic matrix, occur more at the younger age and have hamartoma's aspect. Non-dentine calcifications can be spherical in nodular manner, irregular at shape, and can also represent diffused dotted encrustations. They contain smaller quantity of organic matrix, occur more at the middle and older age, and have inflammatory dystrophic background. In teeth with periodontitis, they appear in the coronary and radicular areas of the pulp, although they can occur as abundant dystrophic calcifications<sup>1,2</sup>.

#### **Materials and Methods**

Material for histological examination was provided with endodontic extirpation and vertical section after tooth extraction; the material consisted of 40 extirpated vital pulps and 60 extirpated pulps of teeth with endodontic diagnosis of chronic pulpitis. Teeth were being cured in endodontic manner up to their final obturation.

Distribution of teeth sampled for examination is presented in tables 1 and 2.

For the purpose of histological processing, various methods and procedures were used, such as: fixation, decalcification, tissue processing, provision of paraffin sections, standard colouring, differential colouring, microscopy and morphological analysis with photographies.

Jaw	Left teeth										Right teeth						
	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8	
Maxilla	1	2	2	2	2			1	1		1	1	1	2	1		
Mandible		2	2		2	1		2	2	1	1		1	3	4	2	

Table 1. Distribution of vital pulps

			Tabl	e 2. Di	stributi	on of e.	xtirpate	ed pulp	s from a	teeth w	ith chro	onic pul	pitis				
Jaw 8	Left teeth									Right teeth							
	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8		
Maxilla		2	2	3	2	4	1	2	8	4	2		2	3			
Mandible	2	1	3	2		2	3	2	5	2		2			1		

#### Results

Regarding the structure of calcifications, 3 morphological images were identified: (1) Calcifications with morphological features similar to the structure of dentin, (2) Calcifications with lamellar concentric structure, and (3) Calcifications with granulated fine granular structure.

(1) Calcifications with morphological features similar to the structure of dentin showed greater affinity to eosin i.e. get coloured in intense red, unlike the other 2 groups of calcifications, which further implies that there is a greater quantity of organic matrix in them. Within the scope of visibility with the light microscopy, fine tubular-trabecular structures were identified, with some radial layout. The periphery of the spherules i.e. the periphery of the pulp calculi, was coloured more lightly in the middle area and created a morphological image similar to crown, with different width and longitudinally different radial projections to inwards and outwards. This part of the spherule was suitable to dentin with smaller quantity of calcium, the one being known as predentin. The middle part of the spherules had more affinity to bond haematoxylin and eosin. This affinity for colours suggests greater presence of both organic matrix and greater quantity of calcium salts. The analogy towards the normal structure of dentin and its morphogenesis during the formation implies that this zone is a zone of calcified mature dentin. In some of the samples, on areas where sections were made, junction zones of interconnection between the dentin mass of the spherule and the basic mass of the peri-pulped dentin were noticed, representing another parameter of identical histogenesis of both the tooth dentine and these spherical, more or less calcified, structures (Fig. 1).

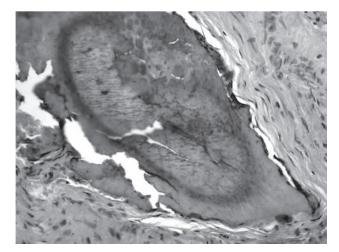


Figure 1. HE colouring (magnify. 10x20) - formation of solitary pulp stone with composite elements in the structure (towards the periphery with greater dentin content, towards the middle area with transformation of morphology of non-dentin calculus, resulting most probably from the abundant deposit of calcium in the organic nidus). Hyalinised pulp in the surrounding area

(2) Calcifications with lamellar concentric structure were spherical. They were similar in size as the previous ones. These calcifications were more intensively coloured with haematoxylin i.e. they showed more intensive basophilia unlike the denticles. The depositions of calcium salts were rough, with no presence of fine trabecular tubular structures. In search of associative morphological comparisons, the section of these calculi to a significant extent reminded of the tree circles. The lamellar structure and concentricity of the discolorations of the transverse sections of these calculi imply that there was organic smell, as initial nidus, with timely protracted circular, organically interpolated encrustation with calcium salts. These morphological changes, known as false denticles, fall under the wider group of dystrophic calcifications and as being such, from terminological perspective, they probably deserve to be referred to with another name (Figs. 2-4).

(3) Calcifications with granulated fine granular structure, according to their shape, were spherical and oval, or irregular (Figs. 5 and 6). These calcifications had pretty similar structure to previous, presenting from zones of encrustations which were amorphous and uniformed, up to zones with fine granulated material. Common for all the calcifications that belong to this group was the intensive colouring with haematoxylin i.e. the basophilic colouring, which implies that there is the greatest presence of calcium salts in them when compared to the previous 2 groups. The organic matrix is reduced, so that after the decalcification phase it became transparent and in some places appeared to be missing (where empty cracked and lacunar spaces were formed). This type of uniformed calcification shows that there is a continuous dynamics in calcium depositing.

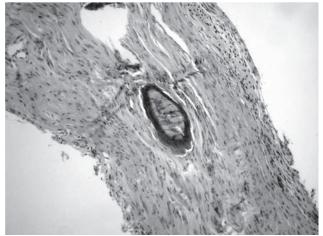


Figure 2. HE colouring (magnify. 10x10) formation of solitary partial decalcified pulp stone - the structure being partially lamellar partially compact. In the surrounding a hyalinised pulp stroma is present with reduction of the vascular compartment

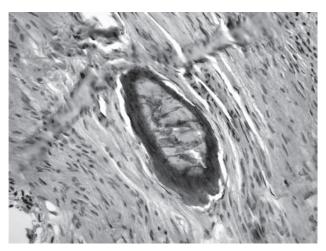


Figure 3. The same material with greater zoom - absence of odontoblasts and dentin tubules

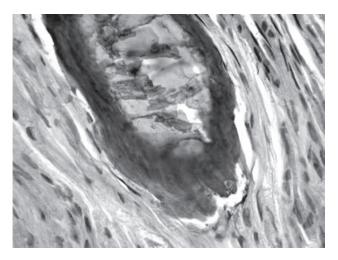


Figure 4. The same material with greater zoom - visible details of the pulp stone and surrounding stroma of the pulp

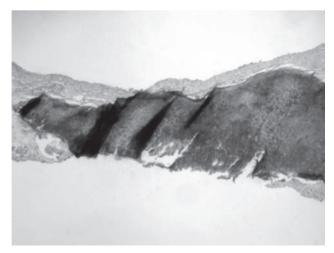


Figure 5. HE colouring (magnify. 10x4) - formation of dental calcification, denticle with fine granulated structure, the size of which occupies the pulp almost across all its width and along its length. Visible congested blood vessels



Figure 6. HE colouring (magnify. 10 x 20) - greater zoom than the one shown in the figure 6 (formation of dental calcification with fine granular structure)

## Discussion

As a basis for discussion is the finding that the dental calcifications represent a separate model of pathological calcification, fitting into overall pathological classification, although with different structure. Literature is rich with descriptions of dental calcifications. The greatest attention is paid to the significance of denticles<sup>4,6,7,15,19,21,22</sup>. The influence of the tablet fluoridation on primary teeth is often analyzed<sup>8,9</sup>, but studies on the structure of dental calcifications are scarce<sup>10,18</sup>, which leaves possibility to try to define it in a more accessible manner, and to clarify this dental entity. Moss-Salejtin and Hendricks-Klyvert described 2 types of calcifications - pulp stones and diffused calcifications<sup>16</sup>, dividing them, accordance to their structure, into real and false denticles<sup>17</sup>. According to these authors, there exists another histological division i.e. denticles that have central gap which is filled with epithelial remains, wrapped up peripherally with odontoblasts, and pulp stones wrapped up with compact degenerative masses of calcified tissues.

Both from clinical and pathological aspect, it is impossible to avoid the question concerning appropriateness and clarity of terminology that is being actually used. It seems that there is a significant degree of overlapping terms, which impedes notion of dental calcifications and mutual communication. The most frequently used terms such as real denticles, false denticles and dystrophic calcifications, contribute to confusion as do not provide sufficiently precise answer of whether these calculi are calcifications. What remains untold in huge number of descriptions is the structure of real denticles, which further leaves open the question about what they really are?

Structural features of dental calcification, described in our study, showed a morphological picture similar to that of the dentin structure - lamellar, concentric and with fine granular structure. The presented structural features were similar to dentin with smaller quantity of calcium, i.e. predentin, with increased presence of calcium salts in the medium area, which unambiguously shows that there is identical histogenesis of both the dentin and the spherical more or less calcified dental calcifications (Fig. 4). This structure of calculi, i.e. calcifications with morphological features of dentin structure, implies that a term "denticle" should be used, although they are also known as "real denticles".

Histopathological findings of calcification with lamellar and concentric structure (Figs. 1-3) show that there is a rough deposition of calcium salts, absence of fine trabecular-tubular structures, otherwise present in the dental calcification, presence of organic matrix initially, as well as protracted interpolated encrustation with calcium salts. These morphological features allow us to use the term "false denticles", as a part of the wider group of dystrophic calcifications. Therefore this could represent a direction towards a more concrete etiological conditioning of this pathological entity.

In our findings, the structure of fine granulated calcifications (Figs. 5 and 6) consists of the biggest presence of calcium salts and maximum reduced organic matrix. With reference to the previous 2 groups, this can represent a proof of the time-dimension regarding the dynamics of calcium depositing due to chronic etiological provocation of traumatic or infective origin.

As a separate subgroup in this category also fall the fine granulated multifocal and confluent calcifications along the longitudinal axis of the pulp, dissociated of bundles of hyalinised connecting tissue, rich in collagen. They also get intensively coloured with haematoxylin which implies that there is a prevalence of calcium salts compared to the organic matrix.

Being engaged in comparing denticles in the pulp of human teeth and those in cow's teeth, Kodaka et  $al^{12}$ emphasize that denticles in the pulp of human teeth contain biological apatite as well as organically dependent and amorphous minerals. According to them, denticles in the pulp of cow's teeth in their medium area contain granulated structures, so called "nidi", which could be thrombi or necrotic blood with erythrocytes. The authors draw a conclusion that such "calcospherulites" in the cow dental pulp are similar to the "spherulitic" dental stones in humans. The human's "nidi" could be present in different parts of the human organism<sup>13</sup>. In the SEM research of various calcified formations of the pulp, Le May and Kaqueler<sup>14</sup> described the presence of resorptive zones at the surface. The authors gave special review on observations of the fractures in the sense of the presence of non-typical organization in places where mineralized masses are compact and homogenous and concentric architecture around the initial central core and linear orientation along the pulp axis, with a display of mineralized fibres and blood vessels<sup>14</sup>. Dard et al<sup>5</sup> assume that there must be extremely important the role of cytoskeleton in the process of imbibing calcification.

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# The Importance of Oral Mucosa and Minor Salivary Glands Biopsy for Diagnosing Chronic Graft-Versus-Host Disease. A Clinical Study of 188 Cases

#### SUMMARY

The biopsy examination of oral mucosa and minor salivary glands has been proposed as a valuable screening test for the diagnosis of chronic graft-versus-host disease (cGVHD). In this light, the purpose of our study was to illustrate the importance of biopsy in the early diagnosis of cGVHD. The study included 188 patients from 2003 to 2009, who had undergone allogenic bone marrow transplantation. The patients' ages ranged from 8 to 51 years. The distribution of the blood diseases was as follows: AA - 15; ALL - 46; AML - 70; CML - 19; MDS - 13; and NHL - 25. Approximately 3 months after the haematopoietic stem cell transplantation (HSCT), the patients were examined for the expression of cGVHD. A clinical diagnosis of "normal mucosa" was made for all the patients. The biopsy specimens were taken from the anterior left buccal mucosa, and from the minor salivary glands of the lower lip.

Of the 188 patients with no obvious clinical features of cGVHD, 108 patients (57.44%) showed positive expression of cGVHD in the histological examination, whereas 80 patients (42.53%) showed negative expression of cGVHD. The statistical analysis revealed a higher occurrence of cGVHD (p=0.041) in younger patients of those with AML and CML, whereas among patients with ALL and NHL, the older ones were diagnosed more frequently with cGVHD (p=0.04).

Keywords: Graft-Versus-Host Disease; Oral Mucosa; Minor Salivary Glands

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## Introduction

The growing success of allogenic haematopoietic stem cell transplantation (HSCT) in the treatment of malignant haematological diseases has contributed to a steady increase in its use<sup>1-5</sup>. In HSCT, the primary disease is eradicated according to the established protocols, by use of chemotherapy or radiation therapy, and then the bone marrow is replaced by harvested donor haematopoietic stem cells<sup>6</sup>.

Graft-versus-host disease (GVHD) is one of the most important complications of HSCT and is a leading cause of morbidity and mortality in HSCT patients. GVHD develops when the transplanted donor immune cells react and try to destroy the host tissues<sup>5</sup>. The pathophysiology of GVHD consists of 3 stages: stage I is characterized by tissue damage caused to the recipient; stage II is related to the activation of the donor T-cells which recognize the host antigens as foreign and attack target organs; and, finally, stage III involves the release of cellular and inflammatory factors, such as cytokines, endotoxins, and other bacterial products<sup>6,7</sup>.

Depending on the time of disease onset, GVHD is divided into acute GVHD (aGVHD), in which clinical features appear within 100 days after bone marrow transplantation, and chronic GVHD (cGVHD), if clinical features occur later<sup>5,6,8</sup>. Nowadays, with the advances in HSCT, criteria for acute and chronic GVHD have changed, and the current belief is that the distinction between aGVHD and cGVHD is made on the basis of clinical features rather than the time of symptoms onset after transplantation<sup>5,6</sup>. Clinical picture in acute GVHD comprises an erythematous rash, diarrhea, and/or liver involvement. Chronic GVHD is a distinct syndrome which can affect every major organ system, but most commonly involves skin, oral, vaginal and conjunctival mucosa, salivary and lacrimal glands, as well as the liver<sup>6,9</sup>.

One of the main barriers to the conduct of effective clinical research in cGVHD has been the absence of standardized criteria for the diagnosis and staging of the disease, as well as the response to therapy. The National Institutes of Health (NIH) Consensus Development Project on Criteria for clinical trials in cGVHD has recently proposed standardized criteria for the diagnosis of cGVHD. The working group recommended that the diagnosis of cGVHD should require at least 1 diagnostic manifestation of cGVHD, or at least 1 distinctive manifestation (Tab. 1), with the diagnosis being confirmed by a pertinent biopsy, laboratory tests, or radiology in other organs<sup>10</sup>. Because the NIH gave the most recent system of clinical criteria for cGVHD, few studies using it have been published in the English literature.

Table 1. Criteria of	CGVHD with e	emphasis on l	lesions in ora	l mucosa

Diagnostic (sufficient to establish the diagnosis of cGVHD	Distinctive (seen in cGVHD, but insufficient alone to establish a diagnosis of cGVHD	Common (seen with both aGVHD and cGVHD
Lichen-type features Hyperkeratotic plaques Restriction of mouth openings from sclerosis.	Xerostomia Mucocele Mucosal atrophy Pseudomembranes Ulcers	Gingivitis Mucositis Erythema Pain
A.H. Filipovich et al 2005		

Even though cGVHD can affect every organ system, the most commonly involved are skin and oral mucosa<sup>6,11</sup>. Oral involvement occurs in 80% or more of cGVHD patients, and the typical clinical features include lichenoid changes (white reticulation and/or plaques), ulcerations (yellow-to-white pseudomembranes), mucosal atrophy, salivary gland dysfunction (xerostomia, hyposalivation), and restricted oral opening. Mucosal lesions are similar to those encountered in oral lichen planus, salivary gland infiltrates mimic those found in Sjögren syndrome, while fibrosis and restricted oral range of motion suggest

scleroderma. Common clinical oral signs of both aGVHD and cGVHD include mucositis, gingivitis, oral erythema, and pain<sup>5,10-12</sup>. Although xerostomia is a commonly reported complaint in cGVHD, criteria for evaluation of the prevalence and characteristics of salivary gland involvement have not been well-defined in the literature because complaints of oral dryness are regarded as "oral" or "mouth" involvement, whereas pathologic changes in the minor salivary glands encountered in cGVHD are

seen as a continuation of oral mucosal lesions found in the disease. It is also remarkable that, in recent NIH reports, criteria for salivary and mucosal involvement were grouped together as "oral involvement"<sup>10,13-15</sup>.

Oral clinical examination and the biopsy of oral mucosa and minor salivary glands have been proposed as valuable screening tests for the diagnosis of cGVHD about 3 months following transplantation, due to the high incidence of oral mucosa involvement, and high predictive value of the examination (nearly 100%)<sup>8</sup>. It is believed that an oral cGVHD lesion may be the only observed indicator of cGVHD, systemic cGVHD being defined by that.

The purpose of this study was to provide more knowledge on cGVHD, particularly when the disease occurs in patients with no clinical features in the oral cavity and other systems (skin, liver, gastrointestinal tract), showing the importance of biopsy of the oral mucosa and minor salivary glands for early diagnosis of the disease.

## **Materials and Methods**

In the period between January 2003 and December 2009, a total of 835 patients were referred to our clinic at the "G. Papanikolaou" Hospital in Thessaloniki by the Haematological Clinic for diagnosing cGVHD by use of biopsy of oral mucosa and minor salivary glands. All these patients had undergone allogenic HSCT for haematological malignancies. From this large number of patients, we excluded those with obvious oral clinical findings (lichenoid lesions, erythema, xerostomia, etc). From the remaining patients, we selected a group of 188 patients who showed normal oral mucosa and no involvement of other systems (skin, liver or gastrointestinal tract). This final group of patients was studied for cGVHD under similar clinical parameters. No

discrimination was made about the patients' nationality, social status, gender or age.

There were 110 males and 78 females. The patients' ages ranged from 8 to 51 years. The distribution of the blood disease was as follows: aplastic anaemia (AA) - 15; acute lymphocytic leukaemia (ALL) - 46; acute myeloid leukaemia (AML) - 70; chronic myeloid leukaemia (CML) - 19; myelodysplastic syndrome (MDS) - 13; and non Hodgkin lymphoma (NHL) - 25. All the patients had undergone allogenic bone marrow transplantation (BMT).

About 3 months (100 days) after the HSCT, the patients were examined for the presence of oral mucosal lesions or gingival diseases. A clinical diagnosis of "normal mucosa" was made for all the patients based on their medical history and a thorough clinical examination. As "normal" we regarded mucosa that had no oral lesions (lichenoid hyperkeratotic lesions), no infection (gingivitis, mucositis, erythema) or xerostomia, no signs of hyposalivation (salivary flow rate at 0.2 ml/ min or 1ml/5min unstimulated), no minor salivary gland mucoceles, oral mucosa atrophy, oral pseudomembranes or ulcers. None of the 188 patients showed any underlying infection, any oral malignancies and received only prophylactic treatment (no other drugs as steroids etc). Other systems, such as the skin, liver, and gastrointestinal tract, were not involved.

The diagnosis of cGVHD was established by biopsy of the oral mucosa and minor salivary glands. Biopsies were performed approximately 100 days after the BMT, under local anesthesia. 1 biopsy was taken from 2 sites in all 188 patients - the oral mucosa (anterior left buccal mucosa) and the minor salivary glands (lower lip). Histological specimens were fixed in formalin and embedded in paraffin. H & E stained sections were evaluated by a pathologist with expertise in cGVHD, who was blinded to the clinical evaluation results. In the specimens of oral mucosa a presence of epithelial atrophy with apoptotic bodies, hydropic degeneration of basal cells, interface mucositis, or subepithelial lymphocyte infiltration of the connective tissue was evaluated. Also, in the specimens of minor salivary glands a presence of diffuse/periductal lymphocyte infiltrate, atrophy or destruction of acini, ductal dilatation or fibrosis was examined.

#### Statistical Analysis

Data were analyzed with the SPSS program for Windows (version 10.0, Chicago, IL, USA). The Kolmogorov-Smirnov and the Shapiro-Wilk tests were used to check normality of continuous variables. Results are presented as mean  $\pm$  standard deviation (SD) for parametric variables and median (range) for nonparametric variables. The ANOVA, Kruskall-Wallis and Mann-Whitney tests were used to calculate the significance of differences. Qualitative data were also examined by use of the  $\chi^2$  or Fisher exact test. The age variable was used either quantitatively or qualitatively. Statistical significance was defined as  $p \le 0.05$ .

### Results

The mean age of the investigated patients was 31.78 years (SD = 10.86 years). Of the 188 patients with no obvious oral clinical features of cGVHD, 108 patients (57.44%) showed positive expression of cGVHD, whereas 80 patients (42.53%) showed negative expression of cGVHD on histological examination (Tab. 2).

Table 2. Expression of cGVHD

Blood disease	cGVHD (+)	cGVHD (-)
AA (Aplastic anaemia)	6	9
ALL (Acute lymphocytic leukaemia)	24	22
AML (Acute myeloid leukaemia)	44	26
CML (Chronic myeloid leukaemia)	14	5
MDS (Myelodysplastic syndrome)	6	7
NHL (Non Hodgkin lymphoma)	14	11
	108	80

All the histological findings represented minimal histological criteria of cGVHD in this study with different degree of expression. The most frequent findings in oral mucosa were localized or generalized epithelial changes consisting of lichenoid interface inflammation, hydropic degeneration of basal cells, or interspersed areas of atrophy with apoptotic bodies. In the connective tissue, variable amounts of perivascular inflammation and lymhocytic infiltration could be found (Fig. 1). Minor salivary glands revealed periductal infiltration with lymphocytes, atrophy of salivary gland lobules and periglandular fibrosis (Fig. 2).

The distribution of the patients according to blood disease and with regard to gender and age is presented in Table 3. Table 4 presents all statistically significant correlations between the examined parameters (blood disease, age, cGVHD). No significant overall statistical correlation was observed between gender and blood disease (df=5; p=0.166), gender and cGVHD (df=1; p=0.295), age and gender (f=0.077; p=0.782), and age and cGVHD (f=0.757; p=0.385).

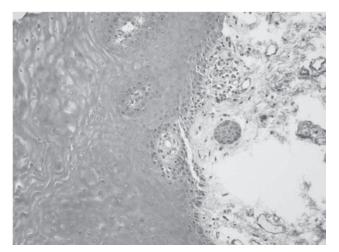


Figure 1a. Normal histology of oral mucosa (HEx200)

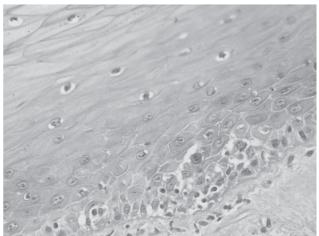


Figure 1b. Chronic GVHD of oral mucosa - lymphoplasmatic infiltration of the upper lamina propria with focal invasion of basal epithelial layers, and single apoptotic body (HEx400)

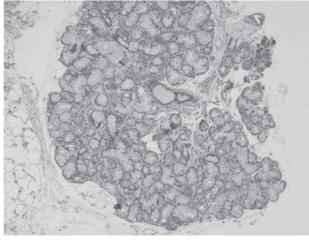


Figure 2a. Normal histology of minor salivary gland (HEx200)



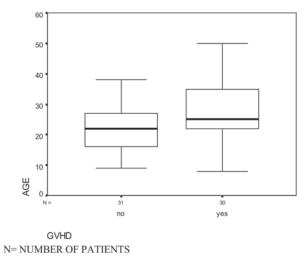
Figure 2b. Minor salivary gland tissue shows acinar atrophy and destruction, as well as an extensive periductal fibrosis (HEx200)

Age							
Disease	Ν	% of total	M/F	Mean	SD	Min	Max
AA	15	8	8/7	19,40	7,44	10	35
ALL	46	24.5	25/21	27,46	10,09	8	50
AML	70	37.2	38/32	34,86	9,33	8	51
CML	19	10.1	11/8	34,84	10,13	16	51
MDS	13	6.9	7/6	42,62	7,16	31	51
NHL	25	13.3	21/4	30,56	10,57	16	51
Total	188	100	188	31,78	10,86	8	51

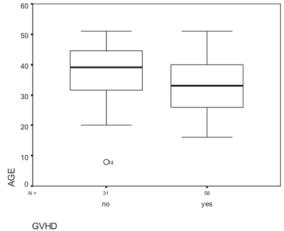
## Table 3. Distribution of patients according to blood disease, gender and age

			р			
			GVHD	disease	(Y/O)	Diagnosis
AA	ALL	Age	0,04	0,007	Younger	AA
	AML	Age	ns	<0,001	Younger	AA
	CML	Age	ns	<0,001	Younger	AA
	MDS	Age	ns	<0,001	Younger	AA
	NHL	Age	ns	0,001	Younger	AA
ALL	AML	Age	ns	<0,001	Younger	ALL
	CML	Age	ns	0,01	Younger	ALL
	MDS	Age	ns	<0,001	Younger	ALL
	NHL	Gender	0,046	0,018		
AML	CML	Age	0,041	ns		
	MDS	Age	ns	0,006	Younger	AML
	NHL	Gender	ns	0,009		
CML	MDS	Age	ns	0,024	Younger	CML
MDS	NHL	Age	ns	0,001	Oldest	MDS

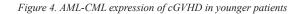
Table 4. Statistical correlations between examined parameters (blood disease, age, cGVHD)











Among patients with AA and ALL (Fig. 3), the older patients were diagnosed more frequently with cGVHD (p=0.04). Regarding patients with AML and CML (Fig. 4), the younger developed higher rates of cGVHD (p=0.041). Among patients with ALL and NHL, the male patients were diagnosed more often with cGVHD (p=0.046).

## Discussion

Chronic GVHD remains the most significant longterm challenge after allogenic HSCT. It is estimated that 40% to 70% of transplanted patients surviving the initial transplantation will eventually develop cGVHD that requires long-term treatment<sup>5,16</sup>. Therefore, earlier and more precise diagnosis is important both for the timely application of an effective therapeutic schema, as well as for a successful long-term follow-up of the malignant disease. As mentioned earlier, clinical features of oral mucosa and minor salivary glands have been noted to reflect the development of cGVHD better than any other affected organs. Minor salivary glands can be affected by cGVHD more frequently than oral mucosa, probably due to the higher tissue expression of histocompatibility antigens by salivary tissues and the accessibility of glands to pathogenic lymphocytes<sup>5,8</sup>.

As mentioned in the literature, histological features of oral cGVHD resemble those of oral lichen planus to a certain degree, but the inflammatory response in cGVHD is usually not as intense as in lichen planus<sup>10</sup>. Minimal histological criteria for oral mucosal cGVHD are localized and extensive epithelial changes (lichenoid interface inflammation, exocytosis and apoptosis), whereas the connective tissue is characterized by variable amounts of perivascular inflammation and lymphocytic infiltration<sup>5,8,17-19</sup>. Concerning minor salivary glands, histological criteria characteristic for cGVHD are lymphocytic infiltration of salivary gland ducts, individual ductal epithelial cell necrosis (apoptosis) and destruction of acinar tissues with periductal fibrosis<sup>8,19</sup>. Obviously, the results of our study are in compliance with those in the literature.

The biopsy as a means of confirming clinical diagnosis of current cGVHD is recommended in the cases when an alternative diagnosis is possible, when there are no diagnostic clinical features of cGVHD, or when only internal organs exhibit clinical signs of current cGVHD. In all these implications and also when infections with atypical clinical features are present, a biopsy is essential to establish a correct diagnosis of cGVHD<sup>20</sup>. Though controversial, the evaluation of biopsy specimens is also a challenging issue in distinguishing the current disease from the past disease. Dense fibrosis and acinar destruction most probably reflect past disease, while acinar and periductal inflammation most probably reflects current cGVHD. As is believed, such histological implications relate to patients who have undergone more than one transplantations<sup>5,7,8</sup>.

Several clinical studies have reported that oral lesions are common in patients with cGVHD, which is estimated to occur in 45% to 83% of the patients<sup>21,22</sup>. Flowers et al<sup>1</sup> noted that oral mucosa was the second most common affected site in those patients who developed cGVHD. Mohty et al<sup>23</sup>, in their study of 101 patients, showed that 51% of the surviving patients developed oral cGVHD during the first 3 years after transplantation. Similar 3-year cumulative incidence of cGVHD with oral lesions was found in the cohort of 126 patients monitored by Flowers et al<sup>1</sup> with just under 50% of the patients developing oral cGVHD during the first 3 years. Treister

et  $al^{21}$  reported oral features in 49 children who had been transplanted for a variety of benign and malignant diseases.

Approximately 90% of the patients had a history of any cGVHD, and about 50% were found to have oral cGVHD (erythema, atrophic glossitis, superficial mucoceles, etc.). Hiroki et al<sup>24</sup> examined 14 patients who received allogenic BMT. 10 of 14 patients were diagnosed as having cGVHD in skin, liver, and other organs. The cGVHD patients had also objective evidence of oral involvement (xerostomia, lichenoid lesions). The conclusion of their study was that oral examination, including biopsy of oral mucosa, is useful for the diagnosis of cGVHD. The same authors<sup>25</sup>, 2 years later, examined 37 patients who had undergone an allogenic BMT and compared oral findings with systemic involvement of cGVHD. The results of their study suggested that a systematic oral examination, especially pathologic examination of the labial salivary gland and buccal mucosa, is useful in evaluating the status of cGVHD. Resende et al<sup>26</sup> selected 60 patients with diagnosed systemic cGVHD and studied the relationship between systemic cGVHD and the oral lesions. Although their results concerning the accuracy of oral cGVHD tests was low for the diagnosis of cGVHD, the conclusion of their study was that the presence of oral symptoms and histopathological manifestations in the salivary glands have good properties for the diagnosis of cGVHD.

Although there are clinical studies on the appearance of cGVHD in transplanted patients with obvious clinical features in the oral cavity, there are no clinical studies about the appearance of cGVHD in transplanted patients with no oral clinical features or oral lesions. Demarosi et al<sup>6</sup> studied the cGVHD in 13 patients who had been transplanted for haematological malignancies. Biopsy specimens were taken from 4 patients with clinical manifestations of oral cGVHD and from 9 patients with normal oral mucosa. Histological cGVHD changes were detected in each one of the 4 patients (100%) with clinical manifestations of oral cGVHD and in 6 of the 9 patients (66.6%) with apparently healthy oral mucosa. The same authors mentioned that the number of patients was insufficient for a definite diagnosis of oral cGVHD with no oral clinical features. Nevertheless, a longer followup period in patients showing histological changes of cGVHD with no clinical features may be useful for the further development of the disease.

In our study, we studied 188 patients without any oral clinical feature of cGVHD, with 108 of these patients having been found positive for cGVHD. Even though these histological features in the oral mucosa without corresponding clinical symptoms may be considered insufficient for a definite diagnosis of cGVHD, this status, which is recognized by some authors as subclinical cGVHD, may be the only highly predictive index of the presence of cGVHD, or a sign of future possible outbreak of the disease. The investigated parameters revealed significant correlations between the blood disease (AA-ALL and AML-CML), the age of the patients, and the expression of cGVHD. The conclusions are analyzed in figure 1 and figure 2, and it is obvious that older patients with AA and ALL were more frequently diagnosed with positive expression of cGVHD, while among patients with AML and CML, younger ones were diagnosed with positive expression of cGVHD more frequently. It has been reported that the incidence of cGVHD in patients who survived after HSCT is as follows: 13% in patients younger than 10 years, 28% in patients aged 10 to 19 years, and over 40% in patients older than 20 years. However, no correlation is made between the blood disease and the age of the patients<sup>27</sup>.

Pathophysiology of cGVHD remains indefinite and progress in the development of effective therapeutic and preventive schemata proceeds very slowly. Moreover, there are close clinical and histopathological similarities between cGVHD and autoimmune disorders. Therefore, progress in cGVHD research may be beneficial not only to HSCT patients, but also to larger number of patients<sup>5</sup>.

Biopsy of oral mucosa and minor salivary glands is an easy surgical procedure and we believe, according to the results of our study, that it can contribute significantly to the diagnosis of cGVHD, even when oral clinical features are absent. The histological findings of the biopsy examination are evaluated by haematologists who monitor the development of the disease in the course of time. Some of these patients, even without clinical evidence, will develop cGVHD, to which a better therapeutic approach will be possible thanks to early diagnosis. This is what constitutes the importance of the biopsy of oral mucosa and minor salivary glands, and it is why it has been established in literature as a diagnostic criterion.

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## Prophylactic Effects of Chlorine Compounds on Recurrent Aphthous Ulceration

#### SUMMARY

**Objective**: Purpose of the study was to evaluate the effects of 2 different chlorine compounds on recurrent aphthous ulcerations (RAU).

**Method**: The study was performed on 30 RAU patients divided into 2 groups. None of these patients had aphthous ulcer at presentation. In the first group 0.2% chlorhexidine gluconate and in the latter 0.1% Chloramin-T mouthwashes were applied. The follow-up period was 3 months and cytological smear examination of the buccal mucosa was done before and at the end of the study.

**Results**: Aphthous ulcer formation did not occur clinically during this study in both groups. In cytological examination no statistically significant difference could be demonstrated in maturation index to examine the keratinisation and oral flora between 2 groups (p>0.05).

**Conclusion**: We have observed the same effect of both compounds concerning prophylaxis of RAU; however, by cytological examination, we could not prove keratinisation effect of chlorine compounds. Further studies must be carried out to evaluate mechanisms of chlorine compounds activity in the prophylaxis of RAU.

Keywords: Recurrent Aphthous Ulceration, prophylaxis; Chlorhexidine Gluconate Mouthwash

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## Introduction

Recurrent aphthous ulceration (RAU) or recurrent aphthous stomatitis is the most common oral mucosal disease known to human beings. RAU is divided into 3 varieties: minor recurrent aphthous stomatitis, major recurrent aphthous stomatitis and herpetiform ulcers<sup>10,18,21</sup>. The most common presentation is minor recurrent aphthous stomatitis: recurrent, round, clearly defined, small, painful ulcers that heal in 10 to 14 days without scarring<sup>10,18,21</sup>. Lesions are larger in major recurrent aphthous stomatitis (greater than 5mm), and can last for 6 weeks or more, and frequently scarring<sup>20</sup>. The third variety of recurrent aphthous stomatitis is herpetiform ulcer, which presents as multiple small clusters of pinpoint ulcers that can fuse together to form large irregular ulcers and last 7 to 10 days<sup>10,18,20,21</sup>. There have been numerous proposed etiologic mechanisms for RAU, including trauma<sup>20</sup>; autoimmune disease such as cyclic neutropenia<sup>17</sup>, Behçet's disease<sup>20</sup>; deficiencies in iron, folic acid and vitamins B1, B2, B6, B12<sup>14</sup>; genetic basis<sup>10</sup>; microbial factors; gastrointestinal dysfunction<sup>10, 18, 21</sup>. Some studies have found a correlation between stress and RAU<sup>10,16</sup>; however, a more recent investigation revealed no association between stress in psychological life and RAU<sup>10,13, 20</sup>. RAU may be more common in HIV-infected patients because it has been suggested that RAU represents a local breakdown in immunoregulation, a condition that could be amplified by HIV disease<sup>12</sup>. Despite much clinical and research attention, as mentioned above the causes remain poorly understood.

Immunologic studies clearly demonstrated that ulcers of RAU represent a cell-mediated immunologic dysfunction in which infiltrating T-lymphocytes play a primary role<sup>15</sup>.

There is no specific treatment for RAU, and management strategies depend on the symptoms, duration, severities and associated systemic conditions. Management of RAU includes the use of analgesics, antimicrobials, and immunomodulatory drugs<sup>1,4-</sup> <sup>9,11,20</sup>. However, ulcers of RAU heal in 10 to 14 days spontaneously.

The purpose of this study was to evaluate and compare the cytological and clinical effects of 2 different chlorine compounds, which are not harmful to the tissues on patients with recurrent aphthous ulceration whatever the etiologic factor could be.

## **Material and Method**

#### **Clinical Method**

A total of 30 RAU patients between 20 and 60 years of age, with a history of minor aphthous ulceration, but with an ulcer-free period not exceeding 3 weeks and nonsmoker, entered the study. Each individual was asked to stop any other ulcer therapy, if applicable, at least 3 weeks before entering the study. None of these patients had aphthous ulcer at presentation. Patients were divided into 2 groups of 15 patients.

In the first group 5 ml mouthwashes with 0.1% Chloramin-T granules were applied twice a day for 1 minute during 3 months. Patients were advised not to drink and eat anything at least 1 hour after this application.

In the second group 0.2% Chlorhexidine gluconate (Orohex<sup>®</sup>, Bilim) mouthwash was applied in the same protocol with the first group.

### Cytological Method

Cytological smear examination of the buccal mucosa was done before and at the end of the study. The PAP smear was performed.

Examination of keratinisation was evaluated according to the maturation, which is a method of examination in the vaginal cytological smear. It was modified to the maturation index for mouth according to the following criteria<sup>3</sup>:

0. Non-keratinised surface cells: Intermediate maturity may be slightly flattened, with somewhat irregular cytoplasmic morphology and some degree of anuclear contraction. Para-basal and immature prickle cells are spherical or cuboidal and have a centrally placed nucleus with even distribution of chromatin;

1. Keratinised surface cells: Mature or cornified cells are more flattened and irregular in appearance and have small, pyknotic nuclei or are anucleated.

Examination of polymorph nuclear leukocyte (PNL) accumulation was evaluated according to the following criteria:

- 0. Nil
- 1. Minimal
- 2. Moderate
- 3. Dense

#### Statistical Analysis

Statistical analysis was done using Graph Pad Prism V.3 program. Qualitative data were analyzed by employing  $\chi$ -square parametric test. The results were accepted as significant at the p<0.05 level.

## Results

A total of 30 patients (age range 22-60, mean age 33.2 years) entered the study, of whom 12 were male and 18 were female. Aphthous ulcer formation did not occur clinically during this study in both groups.

In cytological examination no statistically significant differences ( $\chi^2$ : 2.226; p=0.329) could be demonstrated in maturation index when keratinisation was examined (Tabs. 1 and 2; Figs. 1 and 2).

		Chlorhexidine n=15	Chloramin-T n=15	р	$\chi^2$ test
	Non-keratinised cells	3	2		
D.C		20%	13.3%	> 0.05	0.24
Before	Keratinised cells	12	13	ns*	0.24
		80%	86.7%		
	Non-keratinised cells	11	7		
After	Keratinised cells	73.3%	46.7%	> 0.05	
		4	8	ns*	2.22
		26.7%	53.3%		
				p=0.329	2.226

Table 1. Keratinisation during the treatment in both groups

\* - not significant

Keratinisation	Patient	groups	
	Chlorhexidine n=15	Chloramin-T n=15	Total
No changes in the keratinised cells	4	8	12
	13.3%	26.7%	40.0%
Keratinised cells differentiated to non-keratinised cells	8	5	13
	26.7%	16.7%	43.3%
No change in the	3	2	5
non-keratinised cells	10.0%	6.7%	16.7%
TOTAL	15	15	30
	50%	50%	100%

Table 2. Cross-tabulation of keratinisation variation during the treatment

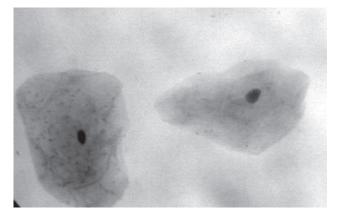


Figure 1. Mature non-keratinised cells before the treatment (PAP; x400)

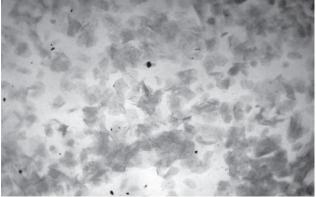


Figure 2. Anucleated mature keratinised cells before the treatment (PAP, x100)

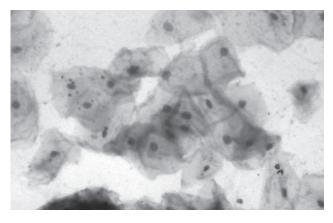


Figure 3. Immature keratinised cells after the treatment (PAP; x400)



Figure 4. Intermediate mature non-keratinised cells after the treatment (PAP; x100)

Variations in keratinisation in both groups are described in table 3. Keratinised cells differentiated to non-keratinised cells after the treatment in 8 patients of the first group and 5 patients of the second group (Fig. 3 and 4). No statistically significant difference

( $\chi^2$ : 9.714; p=0.286) could be demonstrated in polymorphonuclear leukocyte (PNL) accumulation in both groups (Tab. 3).

Hif/pseudohif was encountered in 3 patients before the therapy. At the end of the therapy all were cured (Fig. 3).

TREATMENT	PNL Accumulation	Chlorhexidine n=15	Chloramin-T n=15	р	χ2 test
	Nil	2	2		
DEFODE	Minimal	6	11	> 0.05	4.261
BEFORE	Moderate	3	1	ns*	
	Dense	4	1		
AFTER	Nil	7	7	> 0.05	0.41
	Minimal	7	6		
	Moderate	1	2	ns*	0.41
	Dense	0	0		
				p=0.286	9.714

Table 3. Table of PNL accumulation during the treatment in both groups

\* - not significant

## **Discussion**

Recurrent aphthous ulcers are common, painful lesions of the oral mucosa. There is no specific treatment for RAU. Although ulcers of RAU heal in 10 to 14 days spontaneously, antimicrobial rinses have some clinical efficacy for the treatment of RAU<sup>1</sup>.

Chlorine containing mouthwashes have been available for a number of years. There have been relatively few studies of the antimicrobial properties of this mouthwash. Several studies have reported that mouthwashes with chlorine compounds reduce the number of ulcer days, increase ulcer-free days and interval between bouts of ulceration<sup>1,4,6,10,19</sup>. The role of chlorine compounds in prophylaxis of RAU may be due to the antibacterial effect of these agents<sup>1,19,20</sup>. In this study cytological examination demonstrated that there are no statistically significant differences in PNL accumulation in both groups. But we have observed that PNL accumulation diminished after application of these chlorine compounds.

Chloramin-T, which is a swimming pool disinfectant, has been used as a mouthwash in the prophylaxis of RAU since many years in our clinic. Chlorhexidine gluconate, which is also a chlorine compound, was evaluated and compared with Chloramin-T in this study. We observed the same effect of both compounds in the prophylaxis of RAU. Aphthous ulcer formation did not occur clinically during this study in both groups.

We assumed that the effect of chlorine compounds on patients with RAU was increased keratinisation of oral mucosa. But by cytological buccal mucosal smear examination, keratinisation was not observed - it was decreased in 43.3% of patients.

On the other hand, several studies reported that cigarette smoking prevents aphthous ulcers by causing

increased keratinisation of oral mucosa<sup>1,2</sup>. Grady et al<sup>8</sup> reported that smokeless tobacco also prevents aphthous stomatitis. 1456 professional baseball players were examined, about half of whom were smokeless tobacco users. Usage of smokeless tobacco significantly reduced the risk of aphthous ulcers among healthy young men. They suggested that smokeless tobacco may protect aphthous ulcers by the increasing of keratinisation<sup>8</sup>. As a result, the antibacterial effect of chlorine compounds in the prophylaxis and treatment of RAU is open to discussion.

In this study, we have seen that chlorine compounds are effective in prophylaxis of RAU without increasing keratinisation. On the other hand, several studies have shown that smoking and smokeless tobacco can prevent RAU by increasing the keratinisation. In conclusion the effects of chlorine compounds mouthwashes in prophylaxis of RAU needs further investigation.

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## Immediate Loading of Dental Implants Using Flapless Technique with Electric Welding

#### SUMMARY

**Purpose:** to illustrate the implant-prosthetic rehabilitation using immediate loading under flapless technique. This is carried out by creating an immobility state of implants, which means a maximum tolerance of about  $150 \,\mu\text{m}$  movement during the healing phase.

Material and Methods: In order to create the stability of implants and realization of immediate load of coalesced implants with a titanium rod through intraoral welding. In this way, we realized a structure with large surface on which occlusal forces operate without causing any unwanted movement of implants. This approach, in indicated situation, favours integration of fixed implants in bone, defining the success in distant time of the implant-prosthetic rehabilitation.

**Results and Conclusions:** The presented method offers significant advantages with biomechanical functional unit that is achieved, because surgical proceeding is minimally invasive, a fracture of implant composition is avoided, bone is subjected to overload and it is possible to achieve a real and immediate loading in the same session of the implantation.

Keywords: Implant; Flapless Implantation; Electric Welding; Immediate Implant Loading

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## Introduction

Flapless technique of inserting the implants with immediate loading is done according to individual patient conditions, as well as anatomical and functional situation, which adhered to consistently. Contrary to the humorous phrase that says "*Huge cutting, great surgeon*", modern surgery has laid constant demands to minimize invasion, using increasingly advanced techniques<sup>1,2,4</sup>. Clinically, it is necessary to precisely estimate the area without teeth by detailed clinical and radiological assessment. From biomechanical view as well, it is obligatory to regulate mechanisms of transmission of load in the osseo-implant system.

Today, most of the prosthetic-implant rehabilitation provides positioning of 1 or more implants, leaving a "quiet" period to favour osseointegration and then applying the prosthetic device. Nowadays, technological evolution allows using the immediate load systems that provide to the patient a real possibility of completing the intervention of implant positioning by applying temporary crowns, which may be subjected to occlusal load immediately after surgery, allowing the patient to chew immediately. In this report, prosthetic-implant rehabilitation under flapless technique with immediate loading is illustrated.

## **Material and Methods**

Protocol of prosthetic-implant intervention predicted 3 stages:

**1. Stage - Pre-Surgery**: Firstly, clinical condition of the patient should be evaluated, concerning general condition of health and local condition of the chewing apparatus, particularly the area without teeth. Then we cross into radiological assessment, which finalizes planning of insertion and positioning of implant in respect structures nearby the implant site (jaws environment, the inferior alveolar nerve, maxillary sinus etc...). Studying of models and diagnostic wax allows planning of

prosthetic temporary devices, which latter realizes also the permanent ones. The treatment plan should be discussed with the patient, as well as the timing of intervention.

**2. Stage - Surgery**: Firstly, local anaesthesia should be provided in the area of interest (we use 4% articaine with adrenaline) and instruments for implantation prepared. We use the flapless technique for implantation of monophasic Mono-Mac implants with extensive and dense spirals (Facchini®, Italy). The strong side of this procedure lies in the welding of a titanium rod through a welding machine with Argon flow (ImplaMed Srl, Cremona - by means of which an intraoral electric welding of the rod of titanium is reached over the implants (Fig. 1).



Figure 1. ImplaMed Srl - Cremona

After having united the implants, we prepare them with special millers of titanium until we make possible adapting and aligning a temporary bridge with resin. After that we disinfect oral environment and relevant advice, and prescribe antibiotic.

**3. Stage - Post-Surgery:** A permanent prosthetic rehabilitation is afforded, first controlling the balance and stability of occlusal closure through temporary teeth (composite, resin), and continuing finally with the permanent prosthetic devices (bridge, ceramic crown or zircon).

## 2 Cases Report

We report 2 clinical cases achieved in the Prosthetic-Implant Department at the "ALDENT" Medical University. These patients were of good health and had no general or local contraindications for tooth implantation, presenting different toothless areas.

#### The First Clinical Case

A female patient, aged 50 years, appeared with a partial posterior toothless area and a frontal part with dental elements compromised by a periodontal disease (Fig. 2). The further treatment planning for this patient predicted rehabilitation of the posterior area of the 4<sup>th</sup> quadrant. For this purpose, using flapless technique (Fig. 3), 6 monophasic implants with large and dense spirals (the Mono-Mac model - Fig. 4) were used and united with a 1.5 mm titanium rod through the process of sincrystallisation with Argon flow (Fig. 5).



Figure 2. Patient's OPT (a) and the intraoral view (b)



Figure 3. The flapless technique for the 4 implants



Figure 4. A Mono-Mac implant with dense spirals



Figure 5. Argon flow used to unit a 1.5 mm titanium rod through the process of sin-crystallisation

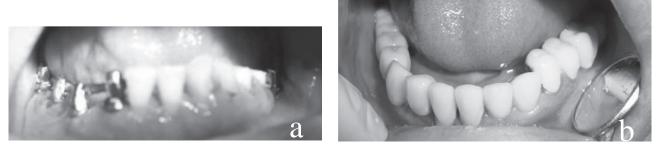


Figure 6. The preparation of implants (a) and the bridges in ceramic (b)



Figure 7. Patient's OPT done after surgery with the welding rod

After this, we prepared the implants for the temporary device in resin (Fig. 6a). The same protocol we activated also for the permanent implant-prosthetic rehabilitation of the lower posterior-lateral sector, in the 3-rd and 4-th quadrants (Fig. 6b). After surgery, the final OPT showed the result (Fig. 7).

#### The Second Clinical Case

A male patient, aged 55, presented a total area without teeth in the upper jaw that belonged to the 1<sup>st</sup> and the 2<sup>nd</sup> quadrant, as a result of a serious periodontal disease that caused big functional problems to the chewing apparatus. Preliminary extraction of teeth was done, and rehabilitation plan predicted intervention initially on the upper jaw. 8 implants with dense spirals,

type Mon-Mac were installed in the same session and united by means of the titanium rod, by diameter 1.5 mm through intraoral welding with the process of sincrystallisation with Argon flow (Fig. 8). We prepared the implant, adopted the temporary devices using wax bite and done with resin base (Fig. 9), which were re-based with auto polymerizing resin after the adjustment with the implants; therefore, the immediate reinstatement of functions of the chewing apparatus was achieved, as well as the aesthetic.

At the same patient, the intervention was undertaken in the lower jaw after a week, in the  $3^{rd}$  and  $4^{th}$  quadrant posteriorly. Here we positioned according to flapless technique 5 Mono-Mac implants with dense and large spirals (Figs. 10 and 11). Panoramic radiography after surgery allowed observing the position of implants (Fig. 12).

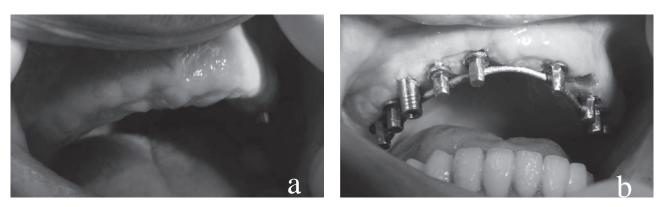


Figure 8. 8 implants with dense spirals, type Mono-Mac and their union by means of the titanium rod, by diameter 1.5 mm through intraoral welding with the process of sin-crystallisation with Argon flow



Figure 9. A provisory prosthetic by resin base



Figure 10. The pre-surgery OPT (a), 3 Mono-Mac implants (b) and after the electric welding auto-polymerizing resin was placed over them at the 3<sup>rd</sup> quadrant

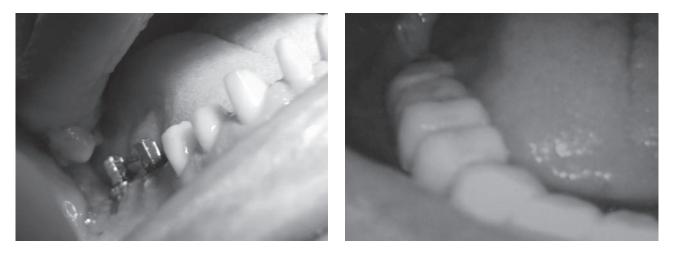


Figure 11. 2 implants at the 4<sup>th</sup> quadrant and auto-polymerizing resin placed over them

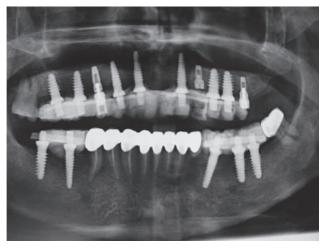


Figure 12. The patient's OPT done after surgery

## **Discussion**

Dental implants implanted by flapless technique could be immediately loaded due to several reasons among which the most important are primary stability and immobility of implants.

Primary stability is addressed to osseointegration and is dependent on numerous factors, such as the area of implantation, its design and mechanical use, chemicalphysical characteristics and implant surface<sup>26,27</sup>. The authors agree that the lack of stability of implant leads to failure of implantation and cannot be considered for application of loads. Considering bone quality, it seems that it influences the long term success, although such a role, in reality, is not well defined<sup>26,27</sup>. This factor may be exceeded according to the procedure that we applied to joining of implants. This method based on the welding of a titanium rod has probably allowed achieving stabilization of implants by exceeding the quality of bone; however, the target of positioning the implants bi-cortically needs to be considered<sup>7-9</sup>.

Immobility of implants is necessary after implantation in the osseous site if it is intended for immediate load<sup>17-23</sup>. In recent years, many authors have illustrated methods used for the immediate implementation of load, providing corresponding stabilization protocols. However, "biomechanical" terms are scarcely analyzed.

It seems that bone needs incentive to keep the shape and density, even in the presence of teeth. When an implant is inserted, there is a destruction of the osseous trabecular structure and change of functional incentives<sup>12,13,15,16</sup>. By modifying the intensity and direction of forces, the structure of bone starts to change. If the pressure forces increase, formation of a new bone tissue may be noticed, while the reducing of pressure forces corresponds to the formation of an osteoid tissue, which after application of a mechanical stimulus is transformed into a bone tissue. Therefore implantation techniques should allow realization of a "biologicalfunctional" system, a system that is in equilibrium with facing chemical and biomechanical structures. It seems that this biological-functional system predicts the use of monophasic implants with dense and large spirals, like Mono-Mac with macro-retentive features, which have adequate stability when inserted bi-cortically. They are inserted under the flapless technique without osseous destruction, and do not exert any bone loss<sup>3,5,6</sup>. The strong side of this whole system consists of titanium rods, welded in intraoral way by means of a sin-crystallisation. The process consists of forming a crystallized net, where the atoms of the 2 metals join.

The welding machine allows a weld inside the mouth with a constant intensity, in a saturated atmosphere with Argon, which avoids any reaction with the surrounding environmental oxygen. There is no risk for patients, because during the welding phase, pliers is automatically disconnected from electrical net and also the heat produced is distributed through the electrodes, for the reasons that copper has greater thermal conductivity. Even more, as titanium is a very stable against charges, the released forces become a physiological stimulus to the osseous remodelling<sup>14,28,29</sup>.

## Conclusions

By merging implants with intraoral welding a single biomechanical functional unit is created, which gives mutual support to the prosthetic device and distribution of forces uniformly at a wide surface. Flapless technique is minimally invasive and with excellent post-surgery results. Fast functional recovery gives additional comfort to the patient. Good visibility during surgery due to the lack of major bleeding results in precise preparation of the implant site.

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