BALKAN JOURNAL OF STOMATOLOGY

Official publication of the **BALKAN STOMATOLOGICAL SOCIETY**

Volume 17 **No 2** **July 2013**

ISSN 1107 - 1141

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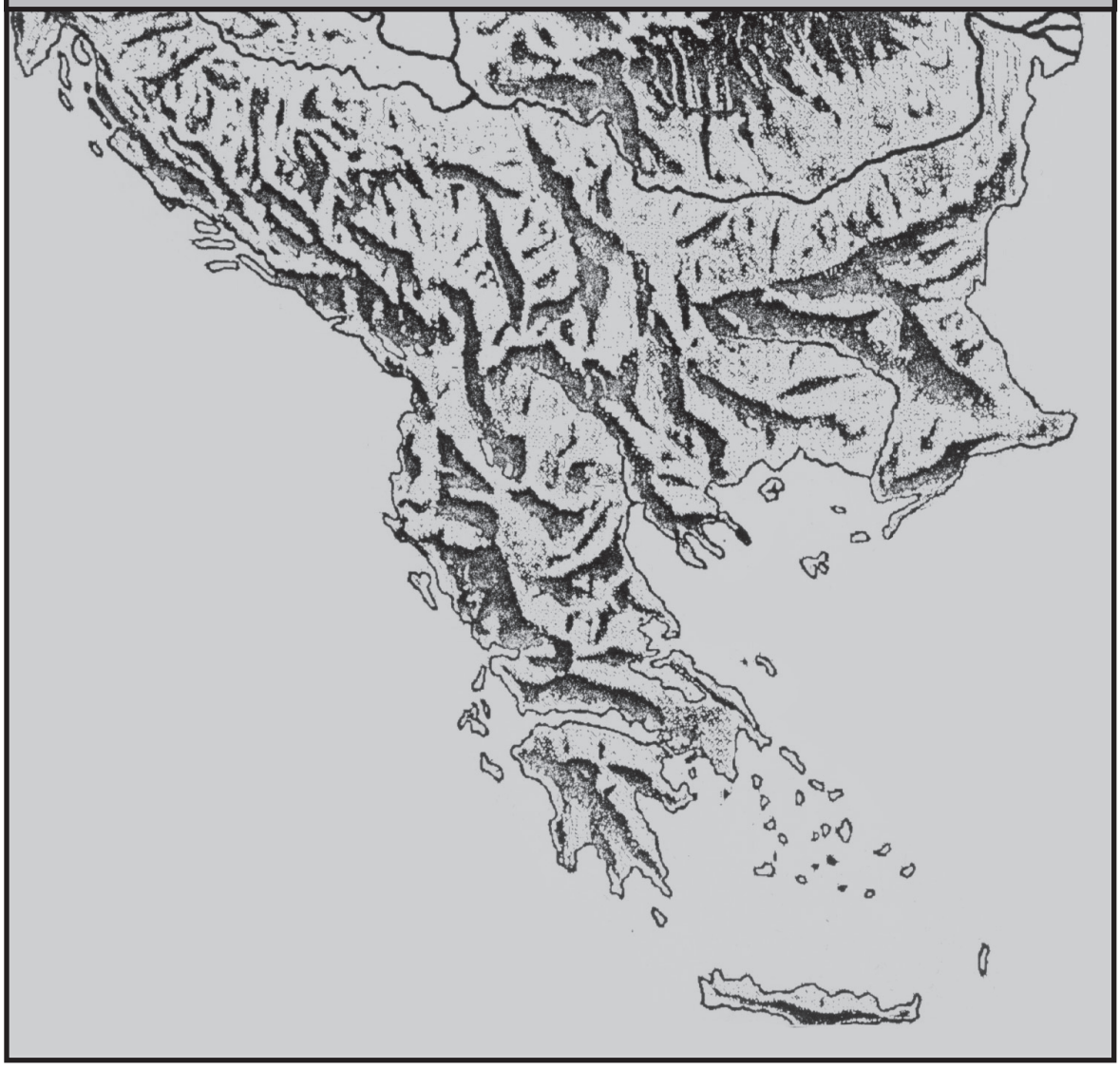

ISSN 1107 - 1141

BALKAN JOURNAL OF STOMATOLOGY

BaSS

ISSN 1107 - 1141

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BALKAN JOURNAL OF STOMATOLOGY

BaSS

ISSN 1107 - 1141

VOLUME 17 **NUMBER 2** **JULY 2013** **PAGES 57-112**

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BALKAN JOURNAL OF STOMATOLOGY

BaSS

ISSN 1107 - 1141

Influence of Hyaluronic Acid in Periodontal Tissue Regeneration

SUMMARY

Hyaluronic acid is a high molecular weight polysaccharide - glycosaminoglycan, which plays a vital role in the functioning of extracellular matrices, including those of mineralized and non-mineralized periodontal tissues. Hyaluronic acid is also important because of its numerous actions in the mechanisms associated with inflammation and the wound healing process.

Hyaluronic acid has been identified in all periodontal tissues in varying quantities, being more prominent in the non-mineralized tissues, such as gingiva and periodontal ligament, compared to mineralized tissues, such as the cement and alveolar bone. Preliminary evidence suggests that hyaluronic acid is a very promising candidate as a mediator of periodontal tissue regeneration and periodontal disease treatment, by promoting a rapid remission of symptoms, not only to the marginal gingiva, but also to the deeper seated periodontal tissues. However, further researches for the therapeutic effects of hyaluronic acid in periodontal disease are essential for realization of true benefits of hyaluronic administration in periodontal tissue regeneration.

Keywords: Hyaluronic Acid; Gingival Inflammation; Periodontal Disease; Periodontal Reparation

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LITERATURE REVIEW (LR)
Balk J Stom, 2013; 17:61-64

Increasing advances in our knowledge of the mechanism of inflammation and healing process associated with periodontal disease indicated the possible significance of the extracellular matrix components as promoters of periodontal tissue regeneration and healing. Numerous evidence emphasizes the role of hyaluronic acid as a possible candidate in hastening periodontal tissues regeneration.

Structure of Hyaluronic Acid

Hyaluronic acid is a high molecular weight polysaccharide with a molecular weight of 10,000 to 10,000 000 Daltons; this polymer is composed of repeated disaccharide units of N-acetyl glucosamine and D-glucuronic acid and belongs to a family of glycosaminoglycanes, with chemical formula ($C_{14}H_2O$ $NNaO_{11}$) n N-acetyl-D-glucosamine D-glucuronic acid.

Physiology of Hyaluronic Acid

Hyaluronic acid is non sulphurous component, which plays a vital function in the structure and function of the extracellular matrix of several tissues: vitreous corpus, synovial fluid, umbilical cord, synovial joints, skin - where in the presence of 55% is a major component of the basal epidermis and in the mucosa of the oral cavity, including the one in mineralized and non-mineralized periodontal tissues.

Hyaluronic acid has been identified in all periodontal tissues in a different quantity, more present in non

mineralized tissues - gingiva and periodontal ligament, compared to mineralized - cement and alveolar bone[1].

As a result of the high level of hyaluronic acid in the blood serum, it is constantly present in the gingival fluid as a serum factor in large quantities[2,3].

Physiology of Hyaluronic Acid in Gingival Tissue

Endogenous hyaluronic acid is a natural biological substance, which is a major component of the matrix of connective tissue, especially the gingiva. Its interaction with other proteoglycans and collagen gives stability and elasticity of the extracellular matrix of connective tissue. Hyaluronic acid binds to various proteins and water molecules through hydrogen bonds, forming viscous macro aggregate whose primary function is to regulate hydration of the tissues and allows the flow of substances in the interstitial space.

Hyaluronic acid is able to absorb water 50 times more than its normal dry weight. This makes the tissue matrix highly compact and increases exchange and diffusion of small molecules, but also acts as a barrier to diffusion of macromolecules and other invasive substances. When hyaluronic acid binds to cell receptors that are presented only on active defense cells, it acts as a regulator of migration and cellular defense mechanisms that are particularly important in wound healing and tissue repair. Hyaluronic acid probably binds to CD44, heparin-type proteoglycan containing sulphate that is specific for epithelial cells of epithelial-mesenchymal border and regulating reactions between cells and the extracellular matrix, especially their binding with hyaluronic acid. This same type of receptor is involved in the interaction between gingival fibroblasts and T and B lymphocytes, and can speed up the gingival immune response in the presence of pathogenic bacterial flora. Its production rises by bacterial endotoxin stimulation performed on fibroblasts[4,5].

Hyaluronic Acid and Periodontal Disease

Periodontal tissue represents a unique complex where gingival epithelium as non-mineralized and other mineralized tissues formed union at cement-enamel junction (CEJ)[6]. Maintaining the integrity of the union is essential in providing an effective barrier against microbial invasion and preventing the destruction in the deeper periodontal tissues, such as periodontal ligament, cement and alveolar bone from bacterial toxins, enzymes, etc. Structural integrity of the union has been lost by the chronic inflammation associated with periodontal disease in which such developments have harmful effects on the components of the extracellular matrix of the deeper periodontal tissues, including collagen, proteoglycans and hyaluronic acid. Clinical studies indicate that hyaluronic acid in chronic inflamed gingival tissue undergoes extensive degradation to low molecular products, which reduces hyaluronic function, whereas related sulphurized glycosaminoglycans, as hondroitin4-sulfate and dermatan sulfate, remain relatively intact[7,8]. Primarily responsible for degradation of hyaluronic acid in these cases are thought to be bacterial enzymes - hyaluronidases[9].

The growing number of evidence also suggests additional role of cellular reactive oxygen species, as superoxide radicals (O_2-) and hydroxyl radicals (OH), obtained during hyaluronic destruction in periodontal disease[10-12].

Hyaluronic acid and Periodontal Regeneration

Hyaluronic acid has more structural and physiological functions in tissues, including extracellular and cellular interactions, interactions with "growth" factors and regulation of osmotic pressure and tissue lubrication, which helps in maintaining the structural and homeostatic integrity of tissues[13]. Hyaluronic acid is a key component of chronic injuries during wound healing processes among mineralized and non-mineralized periodontal tissues, namely in the processes of inflammation, granulation tissue formation and remodelling of the epithelium[14]. Diseased tissue in the early stage of reparation is rich in hyaluronic acid[15,16] with the origin of the extracellular matrix cells (fibroblasts and keratinocytes in the gingiva and periodontal membrane, cementoblasts in cement and osteoblasts in alveolar bone) in the inflamed areas, or derived from vascular blood supply in affected site[1,8,17,18].

Hyaluronic acid has multiple roles in the initial inflammatory stages, such as providing a structural framework, through interaction of hyaluronic acid with fibrin plug, which modulates infiltration of inflammatory cells from extracellular matrix of the host. Hyaluronic acid also induces the production of a series of polypeptide molecules (pro-inflammatory cytokines) from fibroblasts, keratinocytes, cementoblasts and osteoblasts[1,18], which promotes the inflammatory response and consequently stimulates hyaluronic synthesis by endothelial cells of blood vessels[19]. Hyaluronic acid continues to be involved in the activation of inflammatory cells, such as polymorphonuclear leukocytes and macrophage function, including their migration and adherence at the site of injury, phagocytosis and destruction of microbial pathogens[20-22], in order to affect the colonization and

proliferation of anaerobic pathogenic bacteria in the gingival sulci and surrounding periodontal tissue. With somewhat contradictory role, hyaluronic acid can regulate the inflammatory response through removal of reactive oxygen species[8,22-24] that are released by inflammatory cells, which may contribute to stabilization of granulation tissue matrix. Furthermore, hyaluronic acid may indirectly act on the development of inflammation and granulation tissue stabilization, preventing the release of enzymes - proteases of the inflamed cells that break down extracellular matrix proteins as healing progresses[25].

Acid content of hyaluronic acid in non-mineralized tissues, where are chronic changes, increases during subsequent formation of granulation tissue and restoring the epithelium[26-27], which is due to the increased hyaluronic synthesis of fibroblasts and keratinocytes[6]. In mineralized periodontal tissues such as alveolar bone, the phase of granulation tissue is gradually replaced by mineralized callus[18]. During these stages, hyaluronic acid participates in multiple cellular functions, such as promoting the migration of cells from the extracellular matrix in the matrix of the injury, cell proliferation and granulation tissue organization. These developments allow reattachment of basal layer of gingival epithelium to the basal lamina and full maturation of mineralized tissues, resulting in reformation of the union of the tooth surface. In later granulation stage, hyaluronic synthesis stops and the existing hyaluronic acid is depolymerised by host enzymes hyaluronidase, which results in the formation of low molecular compounds and alteration of the granulation tissue composition. This indicated that low molecular hyaluronic fragments formed after subsequent hyaluronidases activity promote formation of blood vessels (angiogenesis) in the lesion, although the precise mechanism of action is still unknown[28-30].

Exogenous Application of Hyaluronic Acid

Participation of hyaluronic acid in control mechanisms of tissue regeneration was an advantage to be used as an exogenous agent with more functional role in the treatment of chronic inflammatory changes. As a consequence of its non-toxicity, biocompatibility and numerous biochemical and physico-chemical features, topic and systemic application of exogenous hyaluronic acid offers beneficial effects in modulation and acceleration of the host response through mechanisms described in numerous medical fields. In systemic administration, hyaluronic acid is distributed in plasma with a half-life of 10 minutes and is metabolized in the liver. After local application plasma concentrations are very low, thus allowing optimal presence of the drug at the site where have to act[31-33]. Studies in mice and rats showed no acute toxic effects or chronic and reproductive effects at doses up to 200mg/kg.

Indications for application of hyaluronic acid in dentistry are numerous:

1. restoration, healing and gingival tissue regeneration as an integral element in the treatment of gingivitis;
2. addition in periodontal treatment;
3. the treatment of stomatitis;
4. eating irritations and lesions on the gingiva and oral mucous membranes (such as aphthae);
5. irritations caused by dentures, fixed or mobile, or during oral surgery procedures;
6. maintenance of the gingiva when dental implants are placed.

Hyaluronic acid is a natural and safe physiologically important substance that can be used by children during the second dentition, pregnant women and the elderly.

Conclusion

Conducted and published clinical studies have shown good results and a high degree of tolerance and acceptability by patients, which is an indicator of clinical value of hyaluronic acid in the treatment and handling gingival disease[34].

It is evident that it has a more functional role in the treatment of chronic changes, including those that occur during periodontal disease. Preliminary evidence suggests that hyaluronic acid is a promising candidate as a mediator of periodontal tissue regeneration and treatment through promoting rapid remission of symptoms, not only in the area of the marginal gingiva, but in deeper periodontal tissues[35-37]. However, further investigations for therapeutic effects of hyaluronic acid application in periodontal disease are essential for evaluating real benefits of its application and full realization of periodontal tissue regeneration.

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BALKAN JOURNAL OF STOMATOLOGY

BaSS

ISSN 1107 - 1141

Candida Albicans and *Staphylococcus Aureus* in Obturators Used for Rehabilitation of Maxillary Defects

SUMMARY

Objective: *The purpose of the study was to evaluate* Candida albicans *and* Staphylococcus aureus *colonization in the maxillary defect, internal surface of prosthesis, nasal cavity and saliva of patients with oronasal obturator prosthesis (OP).*

Method: *18 (12 male and 6 female, mean age 52.6 years), with oronasal OP, already attending the routine control. Microbiological analysis was performed using conventional culturing methods.*

Results: *None of the patients were suffering from any subjective complains, while 9 of them (50%) had diffuse erythema of the defect area. The patients' mean duration time of prosthesis wearing was 3.8 years (between 1-14 years). 13 patients (72.2%) have been wearing their prosthesis 24 hours per day.* C. albicans *were detected in 10 (55.5%), 11 (61.1%) and 11 (61.1%) of the samples from the maxillary defect area, OP and saliva, respectively.* S. aureus *were detected from 8 nasal cavity samples (44.4%), simultaneously the saliva samples of these patients were also positive for* S. aureus*, except 1. Both* C. albicans *and* S. aureus *were detected in 4 (22.2%) of the saliva samples.*

Conclusion: *We suggested that the patients with oronasal OP must be educated for cleaning their prosthesis and they also should be checked microbiologically semi-annually.*

Keywords: Obturator prosthesis; *Candida albicans; Staphylococcus aureus*

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ORIGINAL PAPER (OP)
Balk J Stom, 2013; 17:65-69

Introduction

Maxillary defects are result of the congenital malformation, trauma, and surgical treatment of benign and malignant tumours. Postsurgical maxillary defects predispose the patient to hyper nasal speech, fluid leakage into the nasal cavity, impaired masticator function, and in some patients, various degrees of cosmetic deformity[2]. The oral disabilities are minimized or eliminated almost immediately with obturation. The primary aim for treatment of maxillary defects with obturator prostheses (OP) is closure of the defect area and separation of the oral cavity from the sinus and nasal cavities[14,22]. Therefore, OP restore the functions of mastication, deglutition and speech and provide the satisfactory appearance.

Most commonly used materials for fabrication of OP is polymethyl methacrylate. The primary disadvantage of polymethyl methacrylate is that microorganisms find excellent conditions for growth on the surface[18]. Pores, cracks, and structural defects formed by the release of gases during the polymerization process offer microorganisms the opportunity to initially adhere to the surface of the denture base material and, subsequently, penetrate into the denture and persist in the interior of the OP[11].

The composition of microbial flora of denture plaque resembles that of dental plaque, but with an increased number of *Candida* species[19]. Most manifestations of oral candidiasis are in fact associated with the formation of *Candida* biofilms on surfaces of prostheses. Candidial

colonization and subsequent biofilm formation on denture materials may lead to stomatitis[25].

Candida albicans is the most common opportunistic fungal pathogen in the oral cavity[27]. The incidence of intraoral *Candida* species varies from 20% to 50% in a healthy edentulous population[24] and up to 75% in a population wearing dentures[32]. Multiple factors may predispose to oral candidial infections, such as malignancies[4], dentures[5], smoking[1], broad-spectrum antibiotics[29], dietary factors[10], immunosuppression[21] and xerostomia[28].

Staphylococcus aureus is frequently isolated from healthy individuals and causes a variety of diseases in humans. The external nares are almost certainly the main reservoir of *S. aureus*[12,15], but little is known about its presence in the oral cavity. Although it is considered to be transiently resident in the oral cavity[8,23,31], these may be continuously provided from the nasal cavity. Nasal bacteria may be transmitted through an oronasal fistula to the oral cavity, and it may be able to survive in the oral environment in patients with cleft lip and palate[35].

The purpose of the study is to evaluate *C. albicans* and *S. aureus* colonization in the maxillary defect, internal surface of prosthesis, nasal cavity and saliva of patients with oronasal obturator prosthesis because of the possible cross-contamination.

Material and Methods

Patient Selection

18 patients (12 male and 6 female, mean age 52.6 years) already attending the routine control at Istanbul University, Faculty of Dentistry, Department of Maxillofacial Prosthodontics, were selected in this study. The patients had been using OP who underwent maxillectomy due to some reasons, such as malignancy or had cleft palate. Age, gender and medical history of all patients were recorded. Patients were questioned about smoking habits and about their OP for duration and cleaning periods. Intraoral examinations were performed for oral mucosal lesion presence. Exclusion criteria included acute infections, antimicrobial therapy within the previous 4 weeks, insufficient oral hygiene, diabetes mellitus, leucopenia, viral infection, and the abuse of analgesics or antipsychotic drugs. Because no intervention was undertaken or drug administered, only informed consent of the patients was obtained. According to the Helsinki declaration, a witness assisted the patients before signing the informed consent form.

Microbiological Investigation

Microbiological examinations were performed at the Department of Microbiology. The presence of *C. albicans* in the maxillary defect, internal surface of the prosthesis and in the saliva and furthermore *S. aureus* in the nasal cavity and saliva were investigated.

Samples from the nasal cavity, maxillary defect and internal surfaces of OP were taken by a sterile cotton swabs and transferred into a vessel with 1 ml saline solution and mixed 20 sec for homogenization by means of a Vortex mixer.

Paraffin stimulated saliva samples were taken for 5 minutes. Aliquots of 0.1 ml samples were 10-fold serially diluted and plated onto Mannitol Salt Agar (Acumedia Manufacturers Inc, Baltimore, Maryland) for staphylococci and onto Sabouroud Dextrose Agar (Oxoid Ltd, Basingstone, UK) for yeasts and incubated aerobically at 37°C for 48 hours.

The typical colonies of the saliva samples were enumerated and calculated as cfu/ ml. *C. albicans* isolates were identified by the germ tube test; while *S. aureus* isolates were identified by DNase test performed using DNase test agar (Difco Lab, Detroit MI 48232-7058, USA).

Methicillin resistance was detected with disc diffusion method on Mueller Hinton agar (Merck KgaA, 64271, Darmstadt, Germany) plates with 4% NaCl using 1µg oxacillin discs (Oxoid Ltd) according to Clinical and Laboratory Standards Institute (CLSI).

Results

Characteristics of patients were shown in table 1. The patients had not any other systemic disease except their tumour. The reasons of wearing prosthesis were various malignant tumours in the maxilla (83.3%), cleft palate (11.1%) and arterio-venous malformation (5.5%). 7 patients (38.8%) were treated with radiotherapy (RT) and 4 patients (22.2%) with chemotherapy (CT). 4 patients (20%) were smoking. The patients' mean duration of wearing their prosthesis was 3.8 years (between 1-14 years). 13 patients (72.2%) have been wearing their prosthesis 24 hours per day. The mean cleaning frequency was 2.11 times per day (between 1-3 times).

C. albicans were detected in 10 (55.5%), 11 (61.1%) and 11 (61.1%) of the samples from the maxillary defect area, prosthesis and saliva, respectively. *S. aureus* were detected from eight nasal cavity samples (44.4%), simultaneously the saliva samples of these patients were also positive for *S. aureus*, except 1. All *S. aureus* strains were MSSA. None of the patients were suffering from any subjective complains, while 9 of them (50%) had diffuse erythema in the defect area. Both *C. albicans* and *S. aureus* were detected together in 4 (22.2%) of the saliva samples.

Table 1: Characteristics of patients with used obturator

Patient	age	gender	pathology	RT	CT	Smoking (pieces/ per day)	Duration (year)	Duration (hour/per day)	Cleaning period (times/per day)	*C.albicans*			*S.aureus*		Lesion	Treatment*
										defect	obturator	Saliva (cfu/ml)	nasal	Saliva (cfu/ml)		
1	60	m	Epidermid ca	+	+	10	14	24	3	+	+	$2x10^3$	+	$1x10^4$	+	1
2	50	m	Epidermid ca	-	-	-	3	24	1	+	+	$1.4x10^3$	-	-	-	2
3	48	f	Cleft palate	-	-	-	1	24	2	+	+	$1x10^4$	-	-	-	2
4	50	m	Cleft palate	-	-	-	3	24	2	+	+	$1x10^4$	+	$1x10^4$	+	1
5	65	m	Epidermid ca	+	-	20	16	24	2	+	+	$1x10^4$	+	$1x10^5$	+	1
6	56	m	Epidermid ca	-	-	-	4	16	3	+	+	$2x10^4$	-	-	+	1
7	59	m	Adenocarcinoma	-	-	-	1	24	1	+	+	$1x10^4$	-	-	+	1
8	40	m	Epidermid ca	-	-	-	7	24	3	-	-	-	-	-	+	1
9	37	f	Epidermid ca	-	-	-	2	24	1	-	-	-	-	-	+	1
10	31	m	Chondrosarkoma	+	+	-	4	24	2	-	-	-	-	-	-	2
11	60	f	Epidermid ca	+	+	-	3	24	2	-	-	-	+	$6x10^5$	+	1
12	41	f	Arteriovenous malformation	-	-	-	3	14	1	+	+	$4x10^2$	-	-	-	2
13	51	m	Beningn tumor	-	-	1	1.5	24	3	-	-	-	-	-	-	2
14	60	m	Epidermid ca	+	-	6-7	1	24	3	+	+	$1x10^4$	-	-	-	2
15	75	f	Epidermid ca	+	-	-	5	24	3	+	+	$3x10^3$	-	-	+	1
16	57	m	Cleft palate	-	-	-	1	24	2	-	+	$2x10^3$	+	$8x10^2$	-	2
17	67	m	Epidermid ca	+	+	-	6	16	2	-	-	-	+	-	+	1
18	48	f	Epidermid ca	-	-	-	2	24	3	-	-	-	+	$4x10^5$	-	2

RT:radiotherapy, CT: chemotherapy,

***1**: Itraconazole capsules 100 mg daily and 0.12 %chlorhexidine gluconate and 0.15%benzidamin HCl mouth wash and meticulous cleaning during the 15 days

2: 0.12 %chlorhexidine gluconate and 0.15%benzidamin HCl mouth wash and meticulous cleaning during the 15 days

Discussion

Most patients with acquired maxillary surgical defects can be restored to close to normal function and appearance. Oral candidiasis is a frequent oral lesion in those patients using dental prostheses. Most manifestations of candidiasis are in fact associated with the formation of *Candida* biofilms on surfaces of prostheses [36]. Biofilms in denture plaque represent a protective reservoir for oral microbes[7]. Candidial colonization and subsequent biofilm formation on denture materials are important in the development of denture stomatitis[20].

At least 70% individuals with clinical signs of denture stomatitis exhibit fungal growth, and this condition most likely arises from yeast colonization of the oral mucosa, combined with bacterial colonization[23]. In our study, it has been observed in 10 (55.5%), 11 (61.1%) and 11 (61.1%) the samples from the maxillary defect area, prosthesis and saliva, respectively. This confirms that *C. albicans* may easily colonize at OP because of the relation between sinus and nasal cavities and oral mucosa. An unfavourable situation for prosthetic rehabilitation by the OP occurs when the size of a defect is so large that it overwhelms the remaining structures that stabilize prosthesis over the defect. Instability of the obturator results in air and fluid leakage through the nasal cavity and thereby compromises function[17,30,37].

Tuna et al[34] found *S. aureus* colonization in 53.1% and 40.6% of the children with oronasal fistula in the saliva and nasal samples, respectively and they showed that bacterial transmission was proven for large oronasal

fistulas and a correlation was found with *S. aureus* counts in the saliva of children with cleft lip and palate. In our study, *S. aureus* was detected from 44.4% of the nasal samples; simultaneously, the saliva samples of these patients were also positive for *S. aureus*, except 1.

In our study, 4 (36.4%) of the 11 *C. albicans* positive saliva samples were also positive for *S. aureus*. Yeast may play a synergistic pathogenetic role with opportunistic bacterial pathogens in oral mucosal infections[9]. *C. albicans* and *S. aureus* are among the leading pathogens and are often co-isolated from sites of infection. Some investigations demonstrated the presence of synergistic interactions between these species as they co-exist in a biofilm and even on denture surfaces[6,13,33]. The *C. albicans* and *S. aureus* biofilms are more resistant to antimicrobial treatment than planktonic cells, individually[16,26]. So it is important to brush the biofilms on dentures, while the denture cleaning may play a role in reduction of such microorganisms.

In conclusion, *C. albicans* and *S. aureus* may easily colonize OP due to the relation between nasal and oral cavities. Our results suggest that the patients with OP prosthesis must be educated for cleaning their prosthesis and they also should be checked microbiologically semi-annually.

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BALKAN JOURNAL OF STOMATOLOGY

BaSS

ISSN 1107 - 1141

Efficacy of a Mouth Spray on Denture Microorganisms: An *In Vitro* Pilot Study

SUMMARY

Complete dentures are contaminated by microorganisms, which can be a source of infection, such as denture stomatitis. The aim of this study was to test the efficacy of mouth spray against specific test bacteria and fungi, and to consider its potential.

3 bacteria and 1 fungus representing a broad microbial spectrum with a relevance of oral bacteria were used in 3 laboratory tests, including European suspension test, AOAC germicidal spray products test and in vitro denture disinfection test. The results of 3 laboratory tests showed that CloSYSII proved to be almost 100% effective for all microorganisms tested in this study. It can be concluded that the use of mouth sprays is efficient and easy way for disinfecting complete dentures.

Keywords: Complete Dentures; Microorganisms; Candida albicans

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ORIGINAL PAPER (OP)
Balk J Stom, 2013; 17:70-74

Introduction

The presence of microbial film on the tissue surface of maxillary dentures is an important etiologic factor in denture stomatitis[1-4]. Denture base acrylic resin is easily colonized by oral endogenous bacteria and *Candida spp*, and possibly by extra-oral species, such as *Staphylococcus spp* or members of enterobacteriaceae[5-7]. This microbial reservoir can be responsible for denture-related stomatitis, especially in geriatric patients. Oral and denture hygiene of elderly individuals is extremely poor and denture cleaning is a common problem[8,9]. Proper routine cleaning of dentures is necessary to prevent denture stomatitis and maintain healthy supporting tissues. Effective plaque removal requires a degree of manual dexterity that is often lacking; especially among elderly patients[8]. The use of chemical denture cleaning agents produces more effective results, especially in geriatric patients and in people who have problems with wearing dentures[8,10]. A variety of experimental approaches have been tested in attempt to examine the efficacy of denture cleaning agents[6,11-13]. The general impression is that available chemical cleansers are effective on denture microorganisms[6,11,14]. However, some studies showed that not all of the disinfectants are effective on the most important microorganism for dentures - *Candida albicans*[15].

Effervescent tablets (alkaline peroxide types, enzyme types) are the most used denture cleaning agents[11,13,16-18]. The main disadvantage of these effervescent tablets is that dentures need to be kept in the glass of water a certain period of time, which causing a pleasant view. Therefore it appears from the literature that there is no chemical denture cleaning material which is practical and effective in a short period of time. CloSYSII oral spray is the first pocket/purse size chlorine dioxide based oral spray on the market. The advantage of spray application is that it is quick and easy to apply and delivers a clean fresh portion of the solution each time it is used, whereas with a soaking regime the solution rapidly becomes contaminated, needs frequent replacement and can easily be knocked over. Therefore the aim of this study was to investigate *in vitro* and *in vivo* an effect of CloSYSII on denture microorganisms.

Material and Methods

The solution, CloSYSII (Portola Plaza Dental Group, CA, USA) consists of chlorine dioxide (6%). Its disinfectant properties were evaluated against a range of pathogenic bacteria, such as *Candida spp*, *S. aureus*,

S. mutans, *Neisseria* and *S. β-hemolyticus*. Previous studies[19] showed that analysis of swab samples taken from healthy complete denture wearers showed they contained considerable amounts of *α-hemolytic streptococcus*, *Neisseria*, *β-hemolytic streptococcus* and *C. albicans*. The same microorganisms were used in this study because they represent a broad spectrum of antimicrobial activity and they can colonize oral mucosa and be of potential risk for oral infections[19,20]. These microorganisms were purchased as a stock culture (KUKENS Study group, Department of Microbiology, University of Istanbul, Istanbul, Turkey).

European Suspension Test

For each test organism 10 ml of CloSYSII solution, supplemented with 0.5% bovine serum albumin to simulate "dirty conditions", was inoculated with 0.1 ml inoculums suspension to give approximately 1-5×10^7 cfu/ml[20]. Immediately after inoculation (Time 0) and then at intervals of 3, 5, 15, 30 and 60 min, 1 ml sample was transferred into 9 ml European standard test inactivator fluid to neutralize the antimicrobial action of the solution. Serial decimal dilutions were made with buffered peptone water and duplicate 1 ml portions used to prepare TSA (Tryptic Soy Agar) or SDA (Saubauraud Dextrose Agar) pour plates as appropriate. An inoculum control count was carried out by inoculating 10 ml sterile distilled water and sampling at Time 0. All plates were incubated at 37+/-2°C for 18-24 h and the numbers of bacterial and fungal colonies counted; by reference to the Time 0 count, the log reduction and percentage mortality of each test organism was calculated at each time point. The initial inoculum concentration for each test organism was 10^6 viable cells/ml, and the detection limit for recovery of viable cells was 10 ml.

AOAC Germicidal Spray Products Test

This test was to determine the efficacy of CloSYSII as a spray disinfectant. 7 replicate glass slides were inoculated for each organism. The initial inoculum concentration for each organism was 10^6 viable cells/ml. Glass slides were inoculated with 0.01 ml suspension of each organism by spreading over an area of 1 square inch. The slides were allowed to dry, sprayed with CloSYSII (10 times and 5 cm away; 1 spray=150 micl), and then allowed to stand at room temperature until the CloSYSII had evaporated (about 45min). They were transferred, using flame sterilized forceps, to screw cap glass containers each containing 20 ml subculture broth (nutrient broth for the bacteria and glucose/peptone broth for the fungi) and the containers shaken on a flat bed shaker for 2 min; since the primary subculture broths remained clear after 30 min of shaking, subculture into secondary broths was not required. The containers were then incubated at 37+/-2°C for 48 h for all strains except the trichophyton, which was incubated at 25-30°C for 7 days. After incubation the containers were examined for the presence of growth as judged by turbidity in the medium and scored as positive (+) for growth and negative (-) for no growth. Killing of the test organisms in 10 out of 10 of the treated slides was considered as giving presumptive evidence of disinfecting action[20].

In Vitro Denture Disinfection Test

6 acrylic resin samples (1cm^2) were obtained using polymetyl-methacrilate acrylic resin (Meliodent, Bayer Dental ltd, Germany) and immersed in isopropyl alcohol for 1h. They were placed in sterile laminar airflow cabined to allow the alcohol to evaporate and then immersed in sterile distilled water and rinsed by shaking for 1 min. Each denture piece was placed in empty sterile Petri dish and inoculated with 0.1 ml of a mixed suspension of 3 bacteria, each at a concentration of 1.5x10^6 cfu/0.1 ml, together with 0.5% bovine serum albumin. The inoculated denture pieces were then pre-incubated at 20°C for 4h. 3 dentures were treated with the solutions (9 sprays to wet the denture thoroughly) and 3 dentures acted as untreated control. The treated dentures were incubated app. 20°C for 2h. They were then all placed in separate sterile glass bottles containing glass beads and 100 ml of sterile recovery medium (Tryptone Soya Broth containing European Standard Test inactivator constituents), shaken vigorously for 1 min and then sample withdrawn to determine the numbers of surviving bacteria. Spread plates counts were preformed in duplicate TSA using 0.1 aliquots from serial decimal dilutions and after incubation at 37+/-2°C for 18-24 h[20].

Results

The results of European suspension test, AOAC germicidal spray products test and *in vitro* denture disinfection test are shown in tables 1-3.

European suspension test - the solution achieved 100% mortality of all 4 bacteria and *C. albicans* within 3 min of exposure. Since the initial inoculum concentration for each test organism was greater than 10^6 viable cells/ml and the detection limit for recovery of viable cells was 10 per ml, this represents a 5 log kill against each organism (Table 1).

AOAC Germicidal Spray Products test - the solution achieved 100% mortality of all 5 microorganisms tested. Since the initial inoculum concentration for each test organism was greater than 10^6 viable cells/ml, this represents a 6 log kill in each case (Table 2).

In vitro denture disinfection test - the solution achieved 100% mortality of the mixed bacterial changes inoculum in 2 of 3 replicated denture pieces tested, and 99% mortality in the third after exposure of 2h. These results represent a 5 log kill for the first 2 replicates and a 4 log kill for the third (Table 3).

*Table 1. The efficacy of CloSYSII in the European Suspension Test**

Time (min)	α-hem. Str.		β-hem-Str.		Neisseria		Candida albicans	
	Count (cfu/ml)	mortal (%)	Count (cfu/ml)	mortal (%)	Count (cfu/ml)	mortal (%)	Count (cfu/ml)	mortal (%)
SDW at 0	1.75		1.25		1.05		1.71	
0	15	99.99	<10	100	<10	100	<10	100
3	<10	100	<10	100	<10	100	<10	100
5	<10	100	<10	100	<10	100	<10	100
10	<10	100	<10	100	<10	100	<10	100
15	<10	100	<10	100	<10	100	<10	100
30	<10	100	<10	100	<10	100	<10	100
60	<10	100	<10	100	<10	100	<10	100

* All counts are expressed as $\times 10^6$

Table 2. The efficacy of CloSYSII in the AOAC Germicidal Spray Products test.

Replicate slide	A-hem. strep		β-hem strep		Neisseria		C.albicans	
	Cont	Test	Cont	Test	Cont	Test	Cont	Test
1	+		+		+		+	
2	+		+		+		+	
3		-		-		-		-
4		-		-		-		-
5		-		-		-		-
6		-		-		-		-
7		-		-		-		-
8		-		-		-		-
9		-		-		-		-
10		-		-		-		-
11		-		-		-		-
12		-		-		-		-

Table 3. The efficacy CloSYSII in the in vitro denture disinfection tests

Replicate	Bacterial count (cfu/ml)		Mortality (%)
	Control	Denture	
1	6.8 x 105	77	99.9
2	7.00 x 105	0	100
3	6.25 x 105	0	100

Discussion

Schou et al[9] showed that only 60% of elderly patients who were living in shelters had complete dentures that were not found to be clean. They showed that these elderly patients did not have a habit of cleaning dentures, and their reason for not cleaning was that they would have had to expend effort. They were difficult to stimulate to clean their dentures, and the investigators were not successful in increasing the percentage of clean dentures.

It is well accepted that chemical disinfectants have some advantages over mechanical cleaning, such as effective disinfection and ease of use[10,15,21]. However, some studies showed that not all of the disinfectants are effective on *Candida albicans* at dentures[15]. The alternative and economically more acceptable solution would decontaminate dentures and the results of this study showed that CloSYSII can produce such desired effects: it has highly effective antimicrobial disinfection properties against 4 test pathogenic microorganisms that represent a broad of spectrum with relevance of oral bacteria under a range of *in vitro* conditions. It is possible that the development of biofilm *in vivo* on the denture may reduce the effectiveness of CloSYSII; therefore, further *in vivo* studies were planned to investigate efficacy of the chemical cleansers. The *in vitro* part of the study could show different results from the *in vivo* study because of the variation in soaking temperature, time, and variation of the operators[22]. In this *in vitro* study, after even 3 minutes, the effect of CloSYSII was seen; but in *in vivo* study, the same effect could be seen different times. Therefore, further studies should focus on both the *in vivo* and *in vitro* studies, to explain the noted variations.

There have been few studies using CloSYSII oral spray as a denture cleanser in the literature. The effectiveness of topical chlorine dioxide (0.8%) in the management of chronic atrophic candidiasis was demonstrated by Mohammad et al[23]; they stated that ClO_2 provided a safe and clinically effective option in the management of chronic atrophic candidiasis. The results of this study show that CloSYSII can produce such adesired effect: it has highly effective antimicrobial disinfection properties against 5 test pathogenic microorganisms that represent a broad spectrum with relevance to oral bacteria under a range of *in vitro* conditions.

The significant reduction in the number of *C. albicans* in this study suggests that the use of mouth spray is a suitable method for cleaning dentures. Further studies are needed to determine if daily use of mouth spray can reduce the high prevalence of denture stomatitis patients.

Conclusion

Within the limitations of these experiments, it was found that CloSYSII mouth spray is easy to use and effectively influence denture microorganisms; therefore, it can be used as a denture disinfectant.

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BALKAN JOURNAL OF STOMATOLOGY

BaSS

ISSN 1107 - 1141

Effect of Different Light Curing Systems on Surface Hardness of Composite Resins

SUMMARY

Aim: *The purpose of this study was to evaluate the effect of different light curing systems on surface hardness of composites.*

Materials and Methods: *Composite samples (2 mm thick, 6 mm in diameter, n=10) were prepared in a teflon mould using different light curing systems. Group 1: Hybrid composite samples Filtek Z250 (3M ESPE, St. Paul MN, USA) were polymerized with halogen light source (PolyLUX II, KaVo, Germany) for 20 seconds. Group 2: Hybrid composite Filtek Z250 samples were polymerized with halogen light source for 20 seconds, then additional polymerization was performed in Colténe D.I.-500 oven. Group 3: Composite samples Filtek Z250 were polymerized with LED light source (Elipar FreeLight 2, 3M ESPE, St. Paul MN, USA) for 20 seconds. Group 4: Composite samples Filtek Z250 were polymerized with LED light source for 20 seconds, then additional polymerization was performed in Colténe D.I.-500 oven. Group 5: Tescera indirect composite samples were polymerized in Tescera ATL (Bisco, Inc. Schaumburg, IL, USA). The hardness test was performed using a digital microhardness tester (Buehler, Lake Bluff, Illinois, USA) with load of 500 g and dwell time of 15 seconds. The hardness was measured from the top and the bottom of the composite discs. Data were analyzed by using Student t-test, 1-Way ANOVA and Tukey's tests ($p<0.05$).*

Results: *The mean values and standard deviations were as follows: Group 1 (top = 66.50 ± 1.28; bottom = 64.81 ± 1.45); Group 2 (top = 68.06 ± 1.76; bottom = 66.71 ± 2.27); Group 3 (top = 69.80 ± 0.97; bottom = 67.01 ± 2.16); Group 4 (top = 69.85 ± 0.92; bottom = 68.05 ± 0.81); Group 5 (top = 71.05 ± 1.46; bottom = 71.33 ± 1.08).*

Conclusion: *Tescera ATL system exhibited the highest microhardness values. The group in which halogen lamp and additional polymerization was used, showed significantly higher hardness values than the group in which only halogen lamp was used. However, additional polymerization did not affect the values when LED systems were used.*

Keywords: Hybrid Composite; Polymerization, techniques; Microhardness

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ORIGINAL PAPER (OP)
Balk J Stom, 2013; 17:75-78

Introduction

Composite materials have changed over the past decade. The new developments, along with an increase in patient aesthetic awareness, have led many practitioners to use composites to restore posterior teeth. But, inadequate polymerization can cause some clinical problems such as discoloration, pulpal irritation, postoperative sensitivity or failure of the restoration[1].

The degree of conversion of a resin material may affect physical properties, such as compressive strength, wear and hardness[2]. Highly polymerized composites characterized by increased cross-link density and low residual monomer have been shown to exhibit greater

wear resistance, hardness and flexural strength[3-6]. Hardness, which is defined as the resistance of a material to indentation, cutting, stratching or abrasion, can be used as one indicator for the completeness of polymerization since the hardness of a polymer is directly related to its degree of cure[7].

Many different light curing systems have been demonstrated in response to dramatic rise in the use of composite restorations over the past few years. Most of these systems purpose at decreasing polymerization shrinkage or reducing curing time[8-11]. There are various technologies used for curing lights, which range from conventional halogen bulbs to more expensive systems using lasers, plasma arc and LEDs (light-emitting diodes). Nowadays, halogen-based light curing units are the most widely used light curing units in dentistry[12]. LEDs hold several advantages over halogen-based units, including having longer lifetimes of several thousand hours, converting electricity to light more efficiently, producing less heat, not requiring filters and resistance to shock and vibration[13]. Rather than a hot filament, as used in halogen lamps, LED uses junctions of doped semiconductor to generate blue light[14]. LEDs operate around 470 nm, which falls conveniently within the camphorquinone absorption spectrum[15-17]. Blue LEDs present spectral purity for highly efficient curing of dental resins. Moreover, LEDs have an effective lifetime of more than 10.000 hours and do not present significant degradation of light emission over time[17].

An adequate curing of resin composites may influence the mechanical properties and clinical optimization of these materials. Microhardness is a typical parameter for indicating the degree of polymerization of resin composites. However, adequate surface hardness does not ensure proper polymerization throughout the restoration. Therefore, hardness analysis must also be performed on the bottom surface of the samples, since insufficient polymerization of this area may increase the risk of bulk and marginal fracture[18].

There is a relationship between polymerization shrinkage and microhardness of resin composite. Less polymerization shrinkage causes higher microhardness levels. Recently, in order to reduce polymerization shrinkage, soft-start and low-intensity curing systems have been utilized in restorative dentistry[10,11,19-23].

A positive correlation has been established between composite hardness and inorganic filler content[24,25]. Curing light irradiance exposure duration and composite light transmission are variables significantly affecting hardness and conversion profiles with sample depth[26,27] .

Bottom to top hardness ratios ranging from 0.80-0.90 have been used as criteria for adequate conversion at a specific sample depth[28] .

Developments in the organic matrices and a better polymerization increase their mechanical and physical properties[29]. Oxygen inhibits the polymerization of resins by reacting with free radicals so that they are not available to induce the polymerization reaction[7,30-32]. This inhibition can be significant. It is hypothesized that air mixed into the composite during packing may not only increase the porosity, but also inhibits polymerization of composite resins, both of which can lead to a reduction in the composite's hardness[30].

The purpose of this study was to evaluate the effect of different light curing systems on surface hardness of composites.

Material and Methods

A hybrid composite - Filtek Z250 (3MESPE, St. Paul MN, USA) was used in this study. Test specimens, 2 mm in thickness and 6 mm in diameter, were prepared. Specimens were randomly divided into 5 groups (n=10). *Group 1*: Hybrid composite samples - Filtek Z250 (3M ESPE, St. Paul MN, USA) were polymerized with halogen light source (PolyLUX II, KaVo, Germany) for 20 seconds; *Group 2*: Hybrid composite (Filtek Z250) samples were polymerized with halogen light source for 20 seconds, then additional polymerization was performed in Colténe D.I.-500 oven; *Group 3*: Composite samples (Filtek Z250) were polymerized with LED light source (Elipar FreeLight 2, 3M ESPE, St. Paul MN, USA) for 20 seconds; *Group 4*: Composite samples (Filtek Z250) were polymerized with LED light source for 20 seconds, then additional polymerization was performed in Colténe D.I.-500 oven; *Group 5*: Tescera indirect composite samples were polymerized in Tescera ATL (Bisco, Inc. Schaumburg, IL, USA).

The hardness test was performed using a digital microhardness tester (Buehler, Lake Bluff, Illinois, USA) - load: 500 g; dwell time: 15 seconds). All samples were measured from the top and the bottom of the composite discs.

Statisticall analysis was performed using Student t-test, 1-Way ANOVA and Tukey's tests ($p<0.05$).

Results

When compared with the top and bottom surface roughness values, Groups 1 and 3 showed statistically significant difference. However, there was no statistically significant difference between the top and the bottom roughness values of Groups 2, 4 and 5 (Tab. 1). The highest surface roughness value was observed in Group 5 and the lowest value was observed in Group 1.

Table 1. The mean values and standard deviations (S.D)

Groups	Mean and S.D. (Top Surface)	Mean and S.D. (Bottom Surface)
Group 1	66.50 ± 1.28	64.81 ± 1.45
Group 2	68.06 ± 1.76	66.71 ± 2.27
Group 3	69.80 ± 0.97	67.01 ± 2.16
Group 4	69.85 ± 0.92	68.05 ± 0.81
Group 5	71.05 ± 1.46	71.33 ± 1.08

There was a significant difference between Groups 1-3, 1-4, 1-5, 2-3, 2-4 and 2-5. No significant difference was observed between Groups 1-2, 3-4, 3-5 and 4-5.

Discussion

One of the most significant factors affecting the longevity of restorative materials is their surface hardness and subsequent resistance to abrasive forces. While it is expected that all materials posses sufficient resistance in the dynamic oral environment in terms of surface hardness, this criteria is specifically important for the restorative materials in the posterior region. Therefore; in the present study, "Filtek Z 250", a microhybrid composite was used in the posterior region, and "Tescera" an indirect restorative material was selected as a control material. This study evaluated the effect of additional heat application to 2 different systems in which only light curing is accomplished. Tescera, which requires both light and heat curing application was therefore taken as a control group.

The results indicated that samples polymerised with halogen light source reached significantly higher surface hardness values when an additional heat polymerization was used. On the other hand, surface hardness values were not affected by additional heat polymerization when LED light application was used (Tab. 1). This shows that LED light source is more effective compared to halogen light system and minimizes the amount of residuel monomers, resulting in a highly qualified polymerization level. The high-quality polymerization capacity of LED system has also been reported by other researchers[17,33-35].

However, it seems more appropriate to make this comment for the top surfaces of the samples rather than the base ones. Because the light penetration to the base surfaces is less in light-polymerised samples, the hardness values show a drop in these regions for all groups in this investigation (Groups 1-4). In previous studies, the same results were obtained[36,37]. In case a halogen lamp is used, the difference between top and base surface hardness values in the group with no heat application is approximately the same with the group with heat application. On the other hand, in the LED group, the difference between top and base hardness values in the heat applied group is less than the group where no heat application was used. As a result, even though, LED provides an effective polymerization on surfaces it directly penetrates; if an additional heat polymerization has been used, it is evident that it is unable to reach the base surfaces efficiently.

Within limitations of this study, it can be concluded that in LED systems with direct light exposure, high surface hardness values were obtained as if additional heat has been applied. However, the inclusion of additional heat polymerization to the procedure can still be recommended to enhance the mechanical and surface properties of the bottom of composite restorations and render this area comparable to the top.

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BALKAN JOURNAL OF STOMATOLOGY

BaSS

ISSN 1107 - 1141

SEM Comparison of Acid-Etching, ER,CR:YSGG Laser and Combined Treatment on Dentin Surfaces

SUMMARY

The aim of this study was to evaluate the SEM morphological changes related to phosphoric acid, Er,Cr:YSGG laser applications and combined treatment on dentin surfaces. 12 dentin discs, 3 mm thick, were prepared with a diamond bur from the middle of human molars. The samples were divided in 3 groups: in the first group, only phosphoric acid was applied to dentin surfaces; in the second group, dentin surfaces were treated only by Er,Cr:YSGG laser; in the third group, the combination of laser and phosphoric acid applications were applied. 37% phosphoric acid was applied to dentin discs for 15 seconds. Laser irradiation was performed on dentin discs at 4W for 20 seconds with 90% air and 75% water. The effects of these applications to dentin surfaces were evaluated by using SEM with the magnifications x2000 and x3000.

Acid etching (the first subgroup) was effective for removal of the smear layer. After laser application, rough dentinal surface was seen. In the third group, acid etching after laser ablation caused rough and irregular surface, with craters and grooves. The applications of Er,Cr:YSGG laser and acid etching combination on dentin surfaces created more irregular surfaces than the application of only acid etching or only laser treatment.

Keywords: SEM; Acid-etching; Er,Cr:YSGG laser; Dentin Surface

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ORIGINAL PAPER (OP)
Balk J Stom, 2013; 17:79-86

Introduction

The idea of using phosphoric acid on dental surfaces was first introduced by Buonocore[1] and this idea has become a usual clinical procedure to increase the bond strength between the composite resin and etched enamel surfaces. The success obtained with enamel etching inspired its use on dentin surfaces also.

Several etching agents that are currently in use remove the smear layer, open the orifices of the dentinal tubules to varying degrees and demineralise dentin in depth. However, the degree of surface etching and demineralization of surfaces depend on the type of acid, the etching time and concentration of the etchant[2-7].

Although acid etching is routinely used to roughen dental surfaces prior using bonding agents, today a newer method, Laser Systems, has also been investigated. Irradiation of dental surfaces with laser device was discussed whether to be an alternative to acid etching of enamel and dentin surfaces[8-17]. Among the most promising laser systems that are used for etching treatment in dentistry, is the family of erbium lasers. Erbium:YAG (Er:YAG) and Erbium,Chromium:Yttrium Scandium Gallium Garnet (Er,Cr:YSGG) laser were studied for etching virtue[9,13,14,18-20] .

As an alternative, some researchers have explored the use of acid etching after Er:YAG or Er,Cr:YSGG laser application on dental hard tissues, to improve bonding of restorative materials[4-12,18,21-26]. Er,Cr:YSGG laser, Millenium Hydrokinetic System (HKS), is the last system of the erbium family. There are several studies about this new system[9,18,19,25,27,28]. The aim of this study was to evaluate the SEM morphological changes on dentin surfaces related to phosphoric acid, Er,Cr:YSGG laser applications and the combination of acid etching after Er,Cr:YSGG laser treatment.

Materials and Methods

12 freshly extracted intact human third molars were stored at room temperature in distilled water for 1 month to prevent them from drying out. A high-speed dental drill with a torpedo tapered diamond bur (ISO NO: 806 314 298534 023-KOMET) was used to form 3 mm thick dentin discs. Standard smear layer was prepared on dentin samples using 600 grit SiC disks for 1 minute.

The study was planned in 2 main groups, which were divided in 3 subgroups related to the applications on dentin samples (Tab. 1). In the first group, dentin samples were only etched by 37% phosphoric acid gel (Vivadent, Liechtenstein) for 15 seconds, then washed with a water air spray for 30 seconds and dried. In the second group, only Er,Cr:YSGG laser (Millennium Hydrokinetic-Biolase Technology, Inc., San Clemente, USA) irradiation was performed on dentin discs at 4W for 20 seconds with 90% air and 75% water. In the third group, the combination of Er,Cr:YSGG laser and phosphoric acid applications were applied on dentin surfaces.

Table 1. Summary of the treated main groups and subgroups

		Group A	Group B
		Treatment with diamond bur on a high-speed dental drill	Treatment with diamond bur and sand paper (600 grit)
Subgroups	I.	Application of 37% phosporic acid gel for 15 seconds .	
	II.	Irradiation of Er, Cr: YSGG laser at 4 W for 20 seconds.	
	III.	Combination of 37 % phosporic acid gel application after irradiation of Er,Cr: YSGG Laser	

Er,Cr:YSGG laser operates at a wavelength of 2.78μm, pulse duration of 140-150 μm with a repetition rate (frequency) of 20 Hz. Average power output can vary from 0 to 6.0 W and pulse energy can vary from 0-300 mJ depending on the tissues to be cut. Laser energy was delivered through a fiberoptic system to a Z6 type with a diameter of 600 μm and with a 6 mm standard length (TIP-Z6 600 μm, 6mm; No: 6000201). The tip was bathed in an adjustable air-water spray during cutting. In order to simulate clinical conditions as closely as possible, the laser beam was directed manually, without the use of a fixed support. The Millennium system removes tissues in non-contact mode, so it was required to maintain a fiberoptic tip to the treatment tissue distance of 0.5 to 3.0mm while moving handpiece parallel to the tissue surface. According to the manufacturer directions, handpiece was removed gently and slowly in a circular, brushing or in and out motion to remove the desired materials. Dental hard tissues were lased at a 90 degree angle to the previously flattened surface areas of approximately 5x5mm.

After etching and laser treatments, all of the specimens were kept wet.

In every group, 3 dentin specimens were examined separately. The effects of the applications on dentin surfaces were evaluated by using SEM with the magnifications x2000 and x3000. Prior placement into SEM (JEOL JXA-840 A, Electron Probe Microanalyzer-JAPAN), the samples were immediately vacuum dried and sputter-coated (Edwards Sputter Coater S150 B- ENGLAND) for 180 seconds with gold. SEM observations were carried out at accelerated voltage 20 kV with the 25 mm working distance. SEM findings were scored blind to evaluate the effect of the applications on the smear layer. Scores from 0 to 4 were rated by 3 separate assessors and the median score was used to produce a ranking order for the smear removal agents and laser applications tested. The scoring system was based on that of Brannström et al[29]:

0 = Surface completely covered with thin smear layer, no tubules visible;

1 = Surface covered with thin smear layer but orifices of tubules visible, and occasionally open;

2 = Smear layer partly removed - orifices of most tubules open or partially open;

3 = Smear layer mainly removed, most tubules completely open;

4 = Smear layer completely removed; peritubular dentin removed, resulting in increased size of tubular orifices.

Results

SEM examinations of dentin surfaces showed that differences in surface appearances related to 3 surface treatments were present among the dentin surfaces of 3 groups:

In group A I, acid etching was effective for removal of the smear layer and enlarging the orifices of dentinal tubules. The inter-tubular dentinal surface had a fibrillar texture and dentinal tubules were partially filled with odontoblastic processes. These findings were compatible with Score 4 (Figs. 1 and 2). The surface appearances of group B I were similar to group A I, with the same score (Figs. 3 and 4).

In group A II, laser application revealed the exposed orifices of tubules composed of melted material. Resolidification of the dentinal smear layer caused a sponge-like appearance on the surface. Smear layer was partly removed, orifices of most tubules were opened. So it can be reported that this group was Score 2 (Fig. 5 and 6) and the findings of group B II was similar to group A II (Figs. 7 and 8).

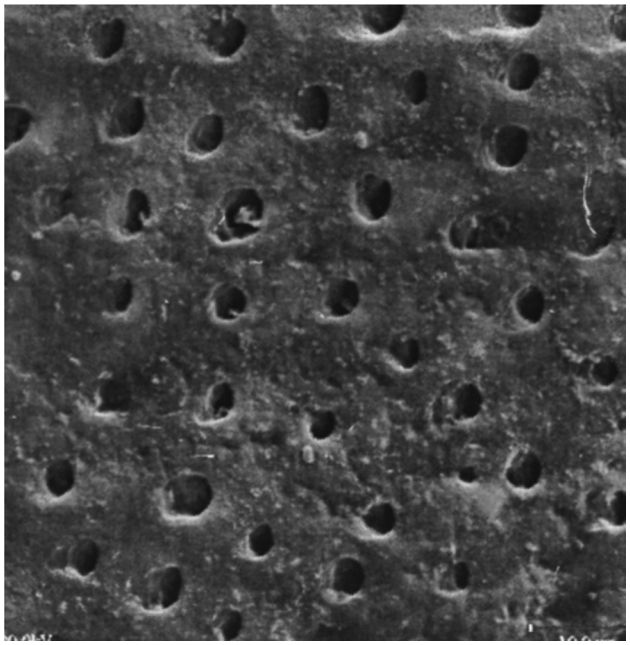

Figure 1. SEM photograph of dentin surface prepared with diamond bur and etched with 37% phosphoric acid for 15 seconds (original magnification x 2000)

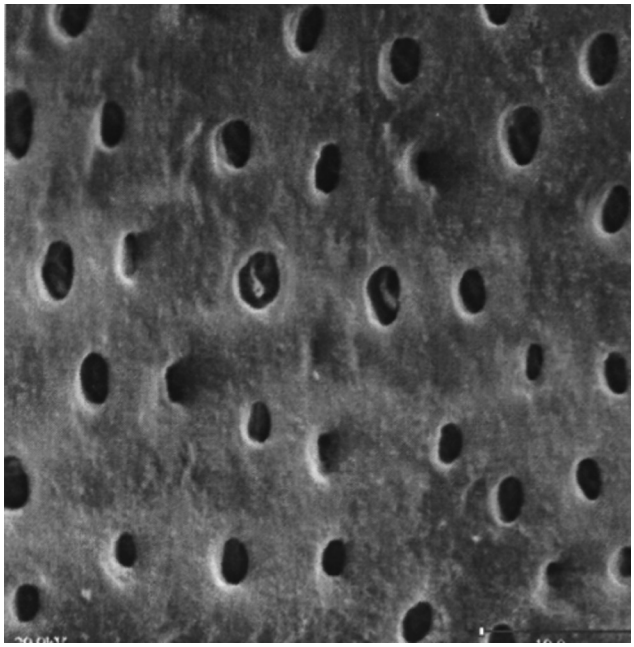

Figure 2. SEM photograph of dentin surface prepared with diamond bur and etched with 37% phosphoric acid for 15 seconds (original magnification x 3000)

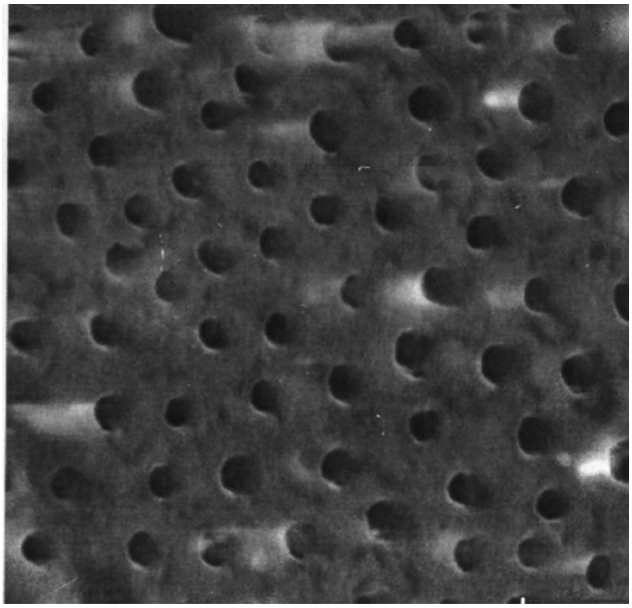

Figure 3. SEM photograph of dentin surface prepared with diamond bur and sand paper and etched with 37% phosphoric acid for 15 seconds (original magnification x 2000)

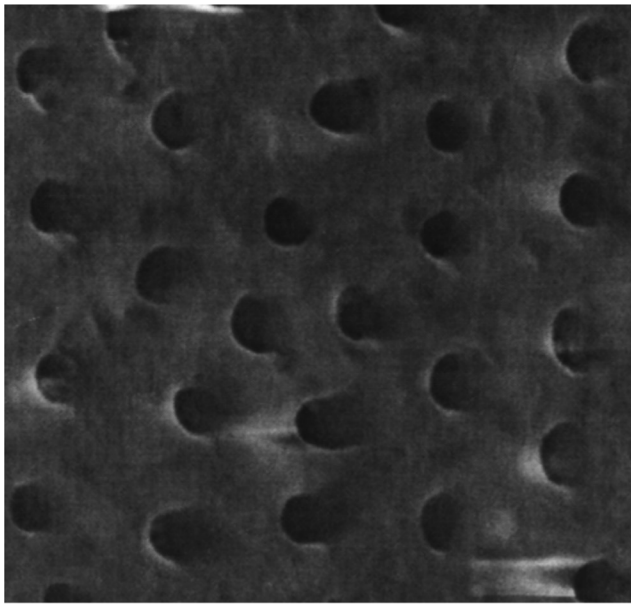

Figure 4. SEM photograph of dentin surface prepared with diamond bur and sand paper and etched with 37% phosphoric acid for 15 seconds (original magnification x 3000)

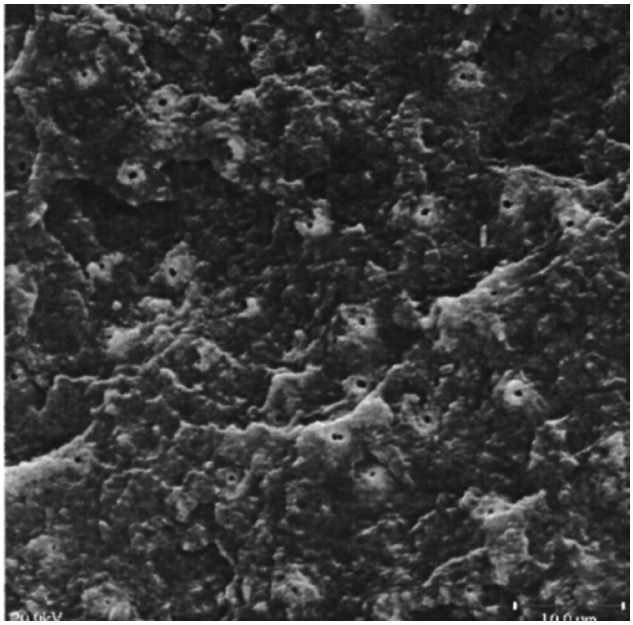

Figure 5. SEM photograph of dentin surface prepared with diamond bur and laser prepared dentin with Er,Cr:YSGG laser at 4W for 20 secods (original magnification x 2000)

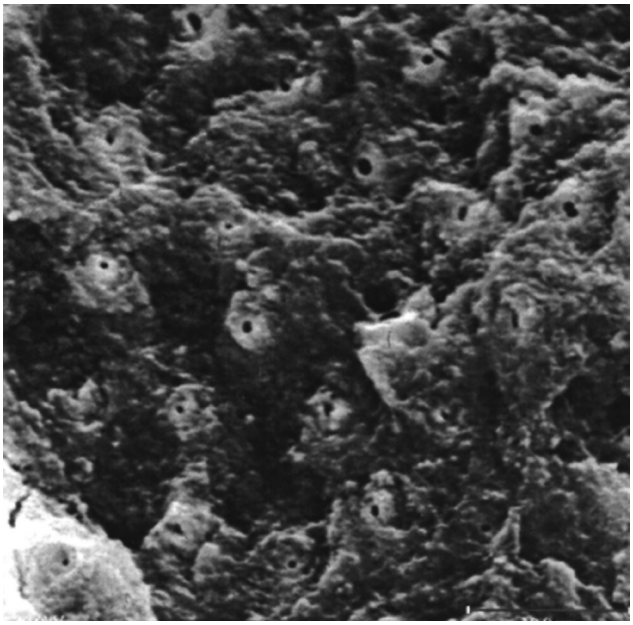

Figure 6. SEM photograph of dentin surface prepared with diamond bur and laser prepared dentin with Er,Cr:YSGG laser at 4W for 20 secods (original magnification x 3000)

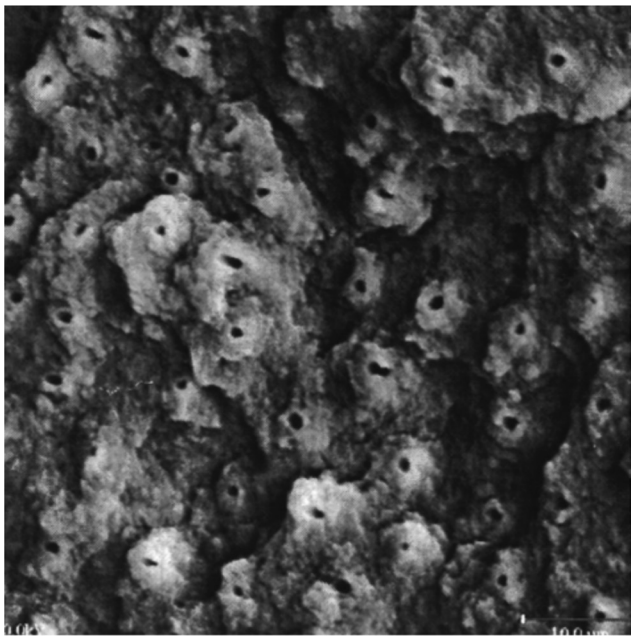

Figure 7. SEM photograph of dentin surface prepared with diamond bur and sand paper and laser prepared dentin with Er,Cr:YSGG laser at 4W for 20 secods (original magnification x 2000)

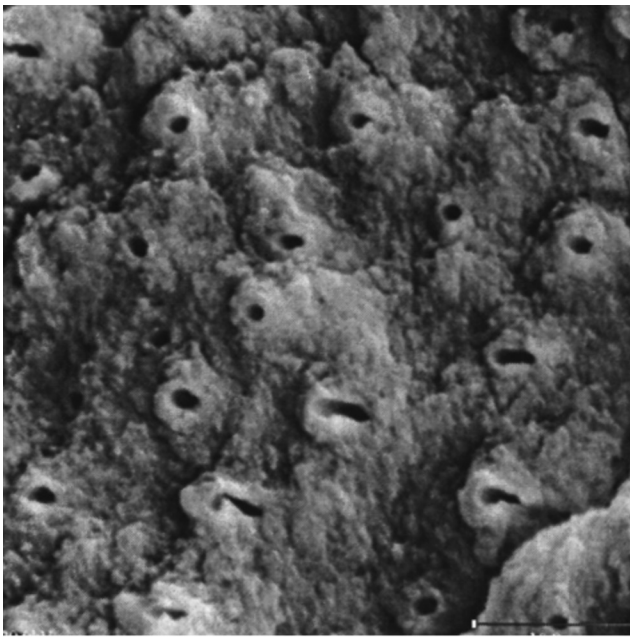

Figure 8. SEM photograph of dentin surface prepared with diamond bur and sand paper and laser prepared dentin with Er,Cr:YSGG laser at 4W for 20 secods (original magnification x 3000)

In group A III, acid etching of laser treated samples appeared to reveal dentin tubules. The intertubular dentinal surface appeared to be more regular than in group A II (only laser application). Smear layer was mainly removed, orifices of the tubules were completely opened and widths of the orifices were more than in group A II. In addition to this, peritubular dentin areas were more degraded by both laser application and acid etching than with laser application only (Figs. 9 and 10). The appearance of the dentin surface of group B III was similar to group A III (Figs. 11 and 12). Micro-cracks and valleys were observed between peritubular and intertubular dentin areas. Appearance of the junction of peritubular and intertubular dentin were different in this group. These findings showed that the score of this group was Score 3.

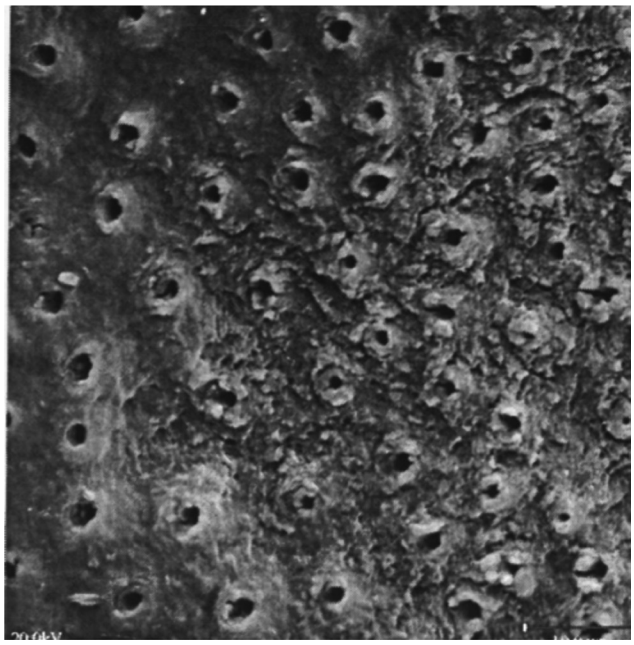

Figure 9. SEM photograph of dentin surface prepared with diamond bur and etched with phosphoric acid after Er,Cr:YSGG laser irradiation (original magnification x 2000)

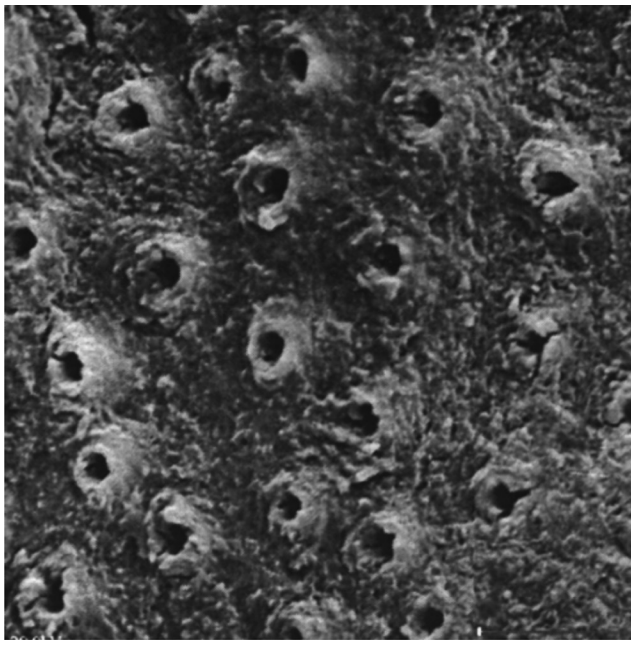

Figure 10. SEM photograph of dentin surface prepared with diamond bur and etched with phosphoric acid after Er,Cr:YSGG laser irradiation (original magnification x 3000)

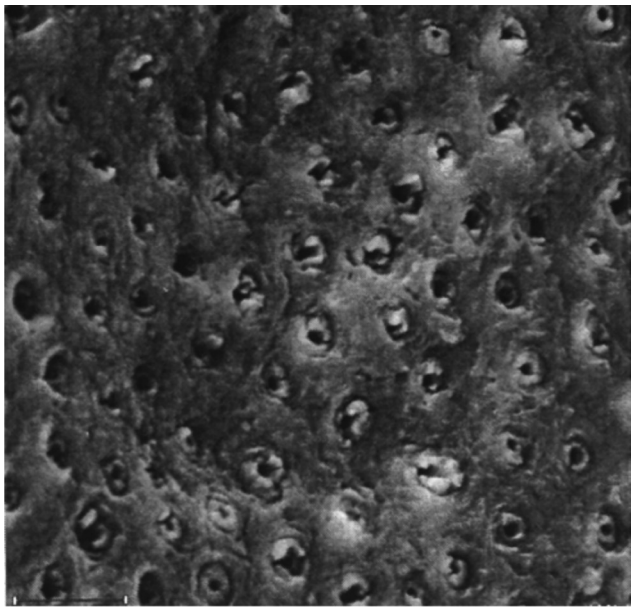

Figure 11. SEM photograph of dentin surface prepared with diamond bur and sand paper and etched with phosphoric acid after Er,Cr:YSGG laser irradiation (original magnification x 2000)

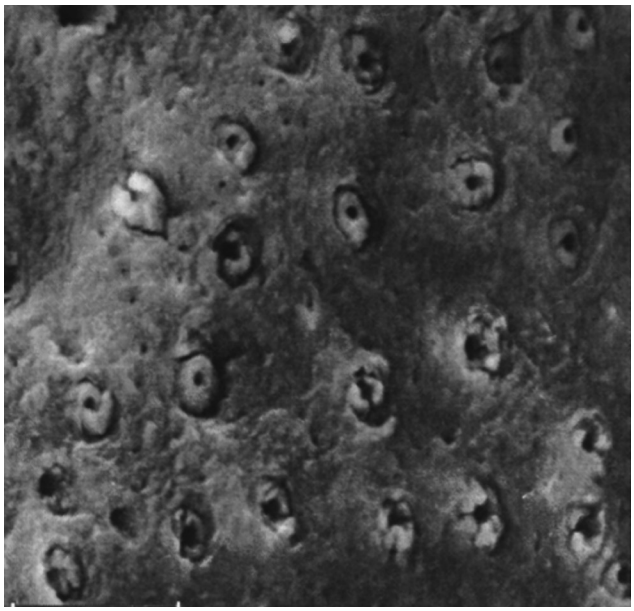

Figure 12. SEM photograph of dentin surface prepared with diamond bur and sand paper and etched with phosphoric acid after Er,Cr:YSGG laser irradiation (original magnification x 3000)

Discussion

Simultaneous conditioning of the hard dental tissues appears to be a recent innovation. Many studies have demonstrated that after cavity preparation, a smear layer is deposited on the dentin surface and this layer is removed from the dentin surfaces with different type, concentration and etching time of acid solutions[2-4]. In previous studies, most currently used phosphoric acid has been proven in concentations from 20% up 50% with application times varying from 15 seconds to 5 minutes[1,2,4]. Recently, ideal results were achieved with 37% phosphoric acid applied for a period of 15 to 30 seconds on dentin surfaces[23,30,31]. For that reason, the application time of 15 seconds and the concentration of 37% phosphoric acid was used in this study.

Although acid etching is accepted application for adhesion of composite materials to enamel and dentin, today, new innovative method, such as using laser systems, has been suggested for creating retentive areas on dental surfaces[10,23,30,32]. Furthermore, laser etching of dental surfaces has been reported to yield a fractured surface and open dentinal tubules, both apparently ideal for adhesion[33-36]. So, etching dentin surfaces by using laser system was added to this study.

In addition to currently used laser types in dentistry (Nd:YAG, excimer, argon, carbon dioxide and Er:YAG laser), Er,Cr:YSGG laser has been used in the last period. But, Er,Cr:YSGG Millennium Hydrokinetic System (HKS) is the last system of erbium family used especially for surface roughening or etching of the dental hard tissues[27,37,38]. Because of the inadequate studies about the etching virtue of this HKS, Er,Cr:YSGG laser was chosen in this study.

Within the last decade, the potential for laser irradiation to produce dental surface conditioning has been studied using several laser systems, such as Er:YAG laser with the combination of phosphoric acid[10,11,22,30]. Since the combination of acid etching and Er:YAG laser is still being used in previous studies[39-41] and, moreover, the inadequate studies exist using Er,Cr:YSGG laser system in combination with acid-etching, this application was also added to this study.

In order to reflect clinical conditions effectively, diamond bur was used to flatten dentin surfaces. As for comparison with other studies[22,30,31,42], diamond bur and sand paper (600 grit) were applied to dentin surfaces.

Application of 37% phosphoric acid for 15 seconds to dentin surface removed the smear layer and opened the dentinal tubules. The results of this morphological study revealed similarities described earlier[3,42,43]. Analyzing dentin conditioning agents, acid solutions have been considered as chemical agents. These agents improve dentin surface chemically. As a result of this interaction, a smooth and homogenous appearances of intertubular dentin areas were determined with the help of compressed air-water.

In this study, SEM photographs of laser prepared dentin showed a sponge-like appearance on the surface. This can be explained by micro-explosions at the tissue surface, resulting from the sudden boiling of water within the tissue (thermo-mechanical ablation). Also, these physical changes might be indicators of the melting and re-crystallization process of dentin surface reported by Stabholz et al and Dankner et al[44,45].

The difference between appearance of the etched dentin surface and laser prepared dentin surface was observed in this study. This appearance can be the result of the different conditioning technique that was used in this study. Less homogenous and less regular surface aroused from the union of different craters in the laser prepared dentin surface. The finding of this study show similarities to Sazak et al[8].

Although acid-etching was effective for removal of the smear layer, less degraded dentin surface was obtained by using the combination of laser and acid application[46]. In addition to this, smear layer was mainly removed. In previous studies, acid-etching with 37% phosphoric acid after Er:YAG laser irradiation ensured a complete surface conditioning[23,27,30] . In our opinion, the difference between this and previous studies could be related to the application time, type and concentration of the acid and, also, type and application time of the laser device. However, it is still being discussed whether severe removal of the smear layer with excessive enlarged orifices of tubules (Score 4) is better than mainly removal of the smear layer with mildly opened tubule orifices (Score 3) for ideal hybridization.

Removal of the smear layer in laser prepared and etched dentin surface was more than the laser prepared dentin surface only. In laser prepared and etched dentin surface, both physical and chemical interaction was held. By physical interaction related to laser application, irregular and heterogeneous surface was obtained as a result of the melted material. Thus, in this type of surface, partly removal of the smear layer was observed. In the combination type of the laser and acid application, this irregular surface was dissolved by acid etching. So, the surface that was irradiated previously, was chemically induced additionally. As a result of this, score of the laser prepared and etched dentin surface appearance was higher than with only laser prepared dentin surface appearance.

Conclusion

Instead of only laser application, laser and acid application was found to prepare a better surface to obtain a successful adhesion.

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BALKAN JOURNAL OF STOMATOLOGY

BaSS

ISSN 1107 - 1141

Remineralisation of Enamel Subsurface Lesions with Casein Phosphopeptide - Amorphous Calcium Phosphate in Patients with Fixed Orthodontic Appliances

SUMMARY

One of the most common problems in everyday dental practice is the occurrence of dental caries. The easiest way to deal with this problem is its prevention. A lot of research has been done to find a material that would help to prevent the occurrence of dental caries, which means to stop tooth demineralization (loss of minerals from the tooth structure) and replace it with the process of remineralisation (reincorporating minerals in dental tissue).

In this review article we will present the remineralisation potential of casein phosphopeptide - amorphous calcium phosphate (CPP-ACP) in clinical studies. We considered all articles that were available through the browser of Pubmed Central. After analyzing the results obtained from these studies, we concluded that casein phosphopeptide - amorphous calcium phosphate has significant remineralisation effect when used in patients with fixed orthodontic appliances.

Keywords: Dental Remineralisation; Casein Phosphopeptide; Amorphous Calcium Phosphate

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LITERATURE REVIEW (LR)
Balk J Stom, 2013; 17:81-91

Introduction

Dental caries is defined as localized destruction of tooth hard tissue with acids, produced during fermentation of carbohydrates, by bacteria in dental plaque[1,2]. Scientific advances in restorative dentistry, new materials and techniques used, as well as understanding of its pathogenesis, have led us to more efficient preservation of oral health[3-5]. Main efforts are focused on reducing the risk of caries in patients by preventing its occurrence[6,7]. Particular role in this has the correct approach of the dentist with the patient, patient's motivation to maintain oral hygiene and regular dental checkups.

The emergence of the subsurface tooth enamel lesions in patients with fixed orthodontic appliances is a common problem in dental practice[8]. The use of orthophosphoric acid as a dental etching material, in order to get better connection between the tooth surface and orthodontic rings and brackets, makes the structure of the enamel porous and sensitive to internal and external factors[9]. Apart of this, maintaining oral hygiene is difficult in patients with fixed orthodontic appliances; this is the reason for accumulating food debris on the tooth surface[10]. Moreover, with this condition, a lot of bacteria are accumulated also. Acids produced by bacteria demineralise the tooth hard tissue[11]. These initial demineralised parts of the tooth are called white spots. They have white chalky colour and indicate an area of demineralization of the enamel. This is the earliest sign of new carious lesion. According to Gorelick et al[12], their incidence in such patients reaches 49.6%. White spots have the potential to develop for a period of 4 weeks after the placement of these appliances[13]. Without acting, these spots furthermore will possibly turn into a cavitation[14,15]. Before the cavity forms the process is reversible, but once a cavity forms the lost tooth structure cannot be regenerated. Therefore, efforts are made to prevent this process and replace it with

the process of remineralisation[16]. Remineralisation of teeth is a process in which minerals, such as fluoride, calcium and phosphate ions, already lost in the process of demineralization of the tooth itself, are returned in place. This process strengthens the structure of the enamel[17].

Nowadays people are searching for new products, which would prevent or to some point reduce the process of demineralisation[18]. Pharmaceutical companies are constantly in rush to provide new materials that will be easily accepted by the masses, simple to use and their results will represent a major step towards improvement of oral health.

Listing the literature we can find a lot of information about the anti-cariogenic effect of the dairy products[19]. This effect is due to multiple-phosphoseryl, which contains sequences of casein. By enzymatic reaction these sequences can turn into casein phosphopeptide. Casein phosphopeptide has great ability to stabilize casein phosphate, as a solution of amorphous calcium phosphate complex. Anti-cariogenic mechanism of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is due to localising of amorphous calcium phosphate on the tooth surface, wherefrom free active ions of calcium and phosphate are released and incorporated into the tooth enamel[27]. Following this, it prevents demineralisation and increases the process of remineralisation of the tooth structure[20,21].

Casein phosphopeptide - amorphous calcium phosphate (CPP-ACP) is a natural protein derived from milk. Lately, it has great use in the prevention of the occurrence of dental caries[27]. Therefore, its application and role in remineralisation process of dental structures is subject of many scientific studies. Clinical studies are taken to provide what are the benefits of its use, what are possible ways of application, when is the best time to start using it and over what period, as well as which patients could benefit from it.

In this article, we will briefly try to summarize the results and conclusions obtained from multiple clinical trials performed on this issue.

Method of Search

Searching the literature through Pubmed Central browser, we found approximately 15 papers where clinical trials related to this issue were performed. The purpose of these studies was to prove the remineralisation effect of CPP-ACP ions on the initials subsurface enamel lesions. These studies were done *in vitro*. Electron microscope was used as a method of inspection of the treated teeth.

The conclusions of all the papers we found highlight the positive effect of CPP-ACP in the process of remineralisation.

Discussion

Calcium and phosphate are essential components of enamel and dentin. They form insoluble complexes but, in the presence of CPP, they become soluble and biologically available. These CPP-ACP complexes applied on tooth surface by chewing gums, mouth rinses, tooth pastes or applied by using a spray, are able to adhere to dental biofilm and enamel hydroxyapatite crystals. Thus, bioactive calcium and phosphate ions are formed in the biofilm. Also, CPP-ACP complex serves as a reservoir of bioactive calcium and phosphate ions. The oral environment becomes supersaturated, which enables an uninterrupted supply of these ions in places that are previously demineralised[22]. This is proved by the fact that significantly higher levels of calcium and phosphate are found in biofilms, as well as lower levels of demineralisation of dental tubules and enamel surfaces, in patients previously treated with CPP-ACP based products.

Numerous clinical studies prove the anti-cariogenic effect of CPP-ACP, as well as in laboratory conditions, animal and human *in situ* experiments[20]. This examination usually is made by using scanning electron microscope (SEM) with energy dispersive X-ray analysis (Fig. 1). SEM provides detailed high resolution images of the sample by rastering a focussed electron beam across the surface. An Energy Dispersive X-Ray Analyser (EDX or EDA) is also used to provide elemental identification and quantitative compositional information. This allows us to estimate quantitatively the amount of minerals present at the tooth sample. Based on the results taken from the analysis we can calculate the amount of minerals loss during the process of demineralisation, or amount of minerals incorporated by the process of remineralisation. From the microscopic images, noted below, we can easily see zones of remineralisation, as thickened hyper-calcified lines around the porous spots of the enamel structure (Fig. 2)[23].

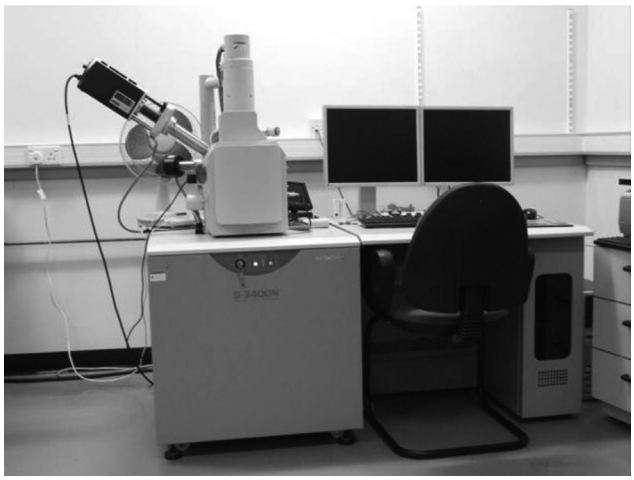

Figure 1. Scanning electron microscope that uses energy dispersed X-rays

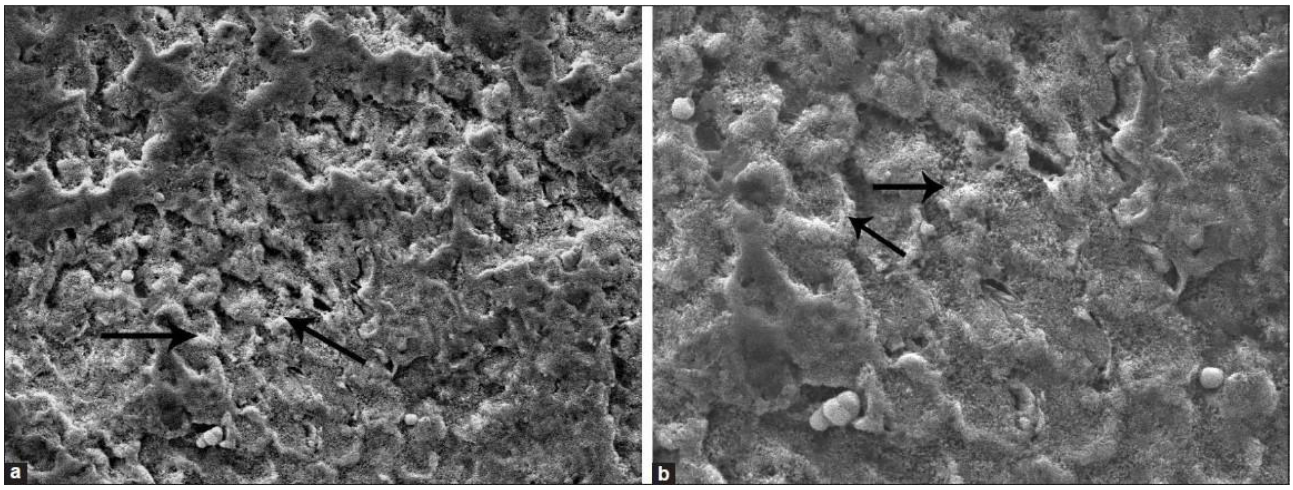

Figure 2. CPP-ACP treated inter-prismatic enamel surface. (a) Porous spots and zones of remineralisation around them, 1000x (b) Thickened hyper-calcified areas around enamel prisms, 2000x

Couple of clinical studies confirm the increased remineralisation effect of CPP-ACP by using chewing gum[20Error: Reference source not found], tooth paste[24] or gel based products[25]. Last one is especially important in patients with fixed orthodontic appliances, as they are high risk subjects for occurrence of enamel defects[26].

Tooth paste rich with CPP-ACP complexes, applied on the tooth surface with an initial carious lesion, can prevent tooth demineralisation process and increase remineralisation. They also speed up the fluoride activation process[27].

According to the results of several studies conducted in order to determine the remineralisation effect of CPP-ACP right after etching of the tooth surface with orthophosphoric acid, it has been proven that there is a significant difference in structure of the enamel in patients treated with CPP-ACP and those who are not[28]. So, we can conclude that the use of CPP-ACP significantly reduces the risk of caries after the micro-abrasion has been made.

As clinical practitioners, we are witnesses on great presence of white spots (initial subsurface enamel lesions) in patients while wearing fixed orthodontic appliances. These sub-structural changes are visible with a naked eye, due to the change of the colour of tooth enamel and they are confirmed by electron microscopy. White spots are created because of the reduced ability to maintain oral hygiene. Therefore, lot of bacteria deposits are present on tooth surface, with their acids damaging the hard dental tissue. So, preventing the development of white spots into the carious lesion is the main goal in patients with fixed orthodontic appliances. Daily application of CPP-ACP, regress the process of demineralisation and supports the dental tissue with new calcium and phosphate ions on the place of already lost ones[29].

The positive effect of CPP-ACP makes it applicable to various ranges of patients:

- Patients with mineral disbalance in oral cavity;
- Patients with high risk of caries;
- Patients with xerostomia;
- High dental sensitivity;
- After professional teeth cleaning and curettage;
- Before, during and after teeth whitening;
- During the entire orthodontic treatment;
- In patients with dental erosion or recession of the gingiva;
- At sufficiently formed early lesions;
- Lesions of the white spots;
- Stimulating the process of remineralisation;
- For patients with diabetes or HIV;
- Patients who are treated by radiotherapy or chemotherapy.

There are many methods of application and they are quite simple. For example, special individual template can be made for this purpose in which we put paste that contains CPP-ACP. Then, we can apply it by using dental micro-brush, interproximal brush, dental sponge stick, or simply put the required amount of paste on a finger and abundantly coat the tooth surface.

One of the studies that we process in this review article indicates the dependence of dose and duration of use of CPP-ACP products in remineralisation process. So, it is noted that the process of remineralisation reaches its maximum effect after 35 days of its use, if it is applied twice a day for a period of 3 minutes (Figs. 3 and 4)[30].

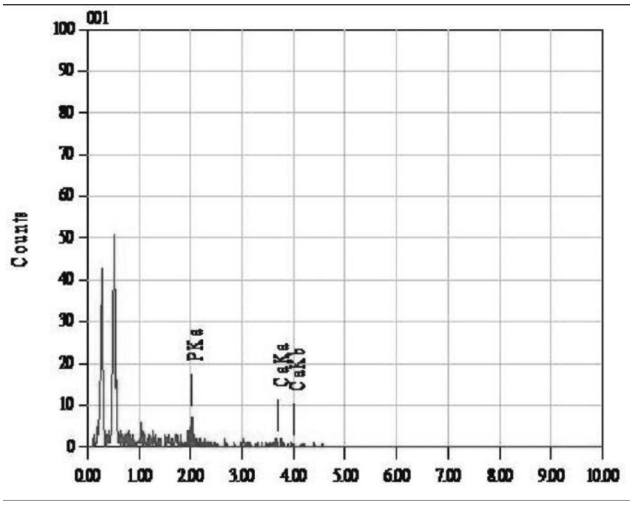

Figure 3. Analysis of demineralised enamel sample by Energy Dispersive X-ray

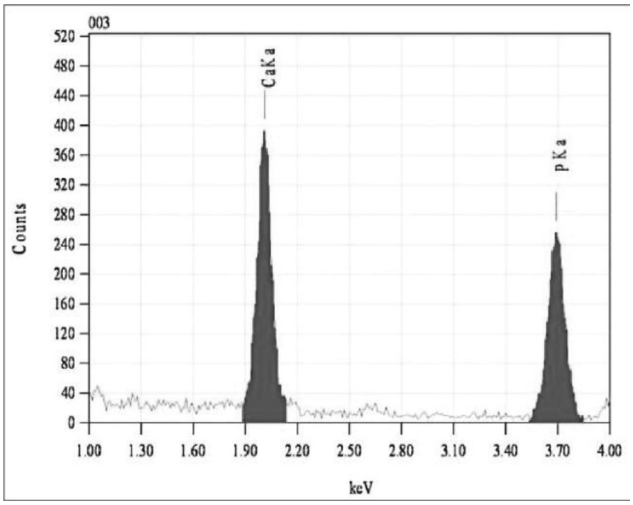

Figure 4. Analysis of enamel sample by Energy Dispersive X-ray after 35 days of use of 10% CPP-ACP paste - GC Tooth Mousse

Beside the use of CPP-ACP as a material for remineralisation of the tooth surface in dental practice, also other fluoride based materials are used for this occasion, such as fluoride rinses and fluoride pastes. However, it is proven that the use of fluoride rinsing solutions has significantly lesser effect than the use of CPP-ACP ions - GC Tooth Mousse[31].

Based on the results obtained, we can conclude that CPP-ACP complexes have a positive effect in dental practice. Therefore they should be included in treatment of patients with fixed orthodontic appliances, such as risk group that has a predisposition for the occurrence of enamel defects.

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BALKAN JOURNAL OF STOMATOLOGY

BaSS

ISSN 1107 - 1141

Relationship between Lower Incisors Crowding, Incisor Position and Craniofacial Morphology

SUMMARY

The aim of this study was to evaluate correlation between incisor mandibular crowding and vertical craniofacial configuration or sagittal lower incisor position. The investigation was made on mandibular dental casts and lateral cephalometric radiographs of 100 children at the age of 9-12 years. Evaluation of the degree of mandibular incisor crowding was made on mandibular dental casts, as a difference between the arch length of the anterior segment minus the combined mesio-distal tooth widths of the mandibular front teeth. On all cephalograms, 16 parameters of vertical craniofacial configuration and 9 parameters of lower incisor position were measured. Arithmetic mean values, standard deviations, and ranges were computed for all data. Further, correlation coefficients were calculated between anterior crowding and all 25 cephalometric parameters. The mean degree of crowding was 2.2 mm with a range of 1.0 to 6.6 mm. The values of all cephalometric parameters were close to well accepted published norms. The correlation coefficients between lower incisor crowding and all cephalometric parameters varied from r = 0.01 to r = 0.21.

According to this study there were no correlations between mandibular anterior crowding and vertical craniofacial configuration or sagittal lower incisor position.

Keywords: Lower Incisor Crowding; Dentofacial Morphology; Vertical Skeletal Morphology; Lower Incisor Position

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ORIGINAL PAPER (OP)
Balk J Stom, 2013; 17:92-100

Introduction

Dental crowding can be defined as a disparity in the relationship between tooth size and jaw size[10]. Conditions which may predispose the dental crowding are excessively large teeth, excessively small bony bases of the jaws, a combination of large teeth and small jaws[22], and the shape and function of the oral musculature[4,22]. Nance described dental crowding as the difference between the space needed in the dental arch and the space available in that arch - that is the space discrepancy[10]. Thus, crowding can be described as an expression of an altered tooth/ tissue ratio or as dentoalveolar disproportion[4,7].

Today with the contemporary man, the number of primary crowding cases is increasing compared to other orthodontic irregularities.

Space problems in the mandibular anterior segment are the most frequent form of malocclusion[5]. Mandibular anterior crowding is identified as the discrepancy between mesio-distal toothy widths of 4 permanent incisors and the available space in the alveolar process. However, incisor crowding is not merely a tooth-arch size discrepancy but a discrepancy among many variables[26]. Anterior crowding seems, however to be more than a problem of tooth size. The space problems are not only associated with variations within the dentition itself, but may even be related to the general craniofacial pattern of the individual and variations of the direction of the growth in the mandibular condyles[20].

Several factors can be assumed to affect the development and the severity of mandibular crowding, such as direction of mandibular growth[3], early loss of deciduous molars[23], mesio-distal tooth and arch

dimensiones[10], the oral and perioral musculature[26], and incisor and molar inclination[26].

Lower anterior crowding is a relevant topic to explore because it has an impact on prognosis, application of treatment methods, and retention. The **aim** of this study was to evaluate correlation between incisor mandibular crowding and vertical craniofacial configuration or sagittal lower incisor position, through determination of:

1. The degree of lower incisor crowding;
2. Vertical skeletal parameters as indicators of facial growth directions;
3. Parameters that locate mandibular incisor position;
4. Correlation between the degree of lower incisor crowding and the vertical skeletal dimension;
5. Correlation between the degree of lower incisor crowding and lower sagittal incisor position.

Material and Method

Our investigation was made on mandibular dental casts and lateral cephalometric radiographs of 100 children at the age of 9-12 years. Evaluation of the degree of mandibular incisor crowding was made as a difference between the arch length of the anterior segment minus the combined mesio-distal tooth widths of the mandibular front teeth.

The arch length of the anterior segment (arch circumference), defined as the distance between the distal contact points of contralateral deciduous canines, was determined by use of a transparent arcumeter (Fig. 1). Mesio-distal tooth widths (generally at the anatomical contact point) of the lower incisors and deciduous canines were measured with sliding calipers to the nearest 0.1 mm.

All cephalometric radiographs were obtained under identical conditions and taken with the patient's teeth in centric occlusion. All necessary landmarks were located and the resulting reference lines were drawn .The most labial inclined incisor was traced so that the incisal edge and apex of the template coincided with these structures in the cephalogram. All angular measurements were performed with a protractor, with interpolation to 0.5^0. All linear measurements were performed with an enlarged scale ruler that allowed measurements to 0.5 mm.

Arithmetic mean values, standard deviations, and ranges were computed for all data. To evaluate the relationship between measured cephalometric findings and anterior mandibular crowding, a correlation analysis was performed.

In the cephalometric radiographic analysis, angles and distances were measured that generally accepted as indicators of facial growth directions and lower incisor position. There were grouped into: (1) variables that measure skeletal configuration and (2) variables that locate mandibular incisor position.

Vertical Skeletal Variables

1. Vertical skeletal variables by Downs (Fig. 2)

- FH/MP;
- FH/Y axis;
- FH/NPg.

Figure 1. Schematic representation of transparent arcumeter positioned on lower arch to measure incisor crowding

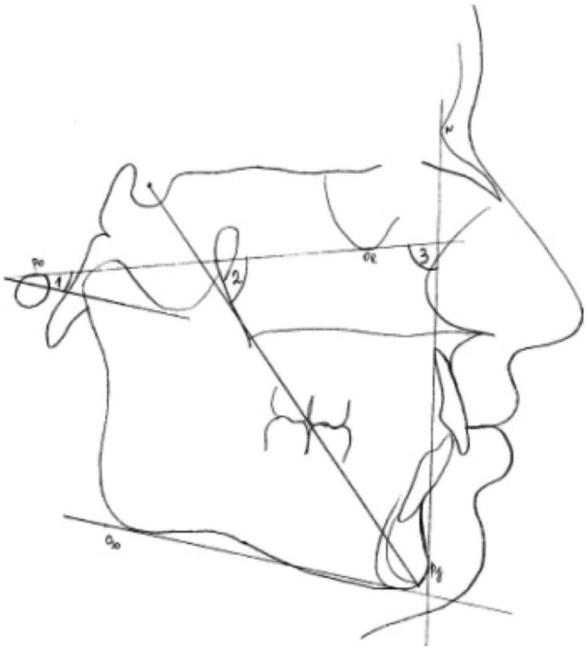

Figure 2. Vertical skeletal variables by Downs

2. Vertical skeletal variables by Hasund (Fig. 3)

- N-Sna/Sna-Me - facial height index - the proportion of upper (1) to lower (1a) anterior facial height;
- N-S-Ar-Go-Me - sum of angle N-S-Ar (2), S-Ar-Go (2a) and Ar-Go-Me (2b).

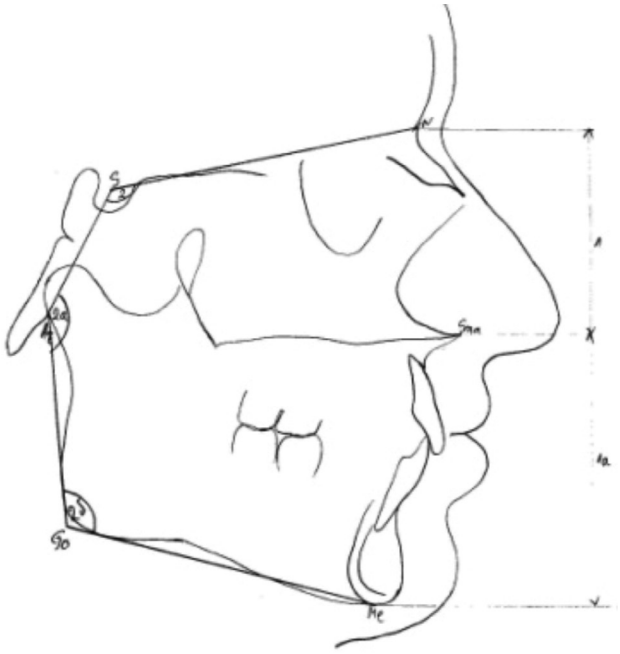

Figure 3. Vertical skeletal variables by Hasund

3. Vertical skeletal variables by Jarabak (Fig. 4)

- S-Go/N-Me- ratio - the proportion of posterior (1) to anterior (1a) facial height;
- Ar-Go-Me (2) - gonial angle - divided into upper Ar-Go-N (2b) and lower N-Go-Me(2a) components.

Figure 4. Vertical skeletal variables by Jarabak

4. Vertical skeletal variables by Schwarz (Fig. 5)

- OcP/MP;
- Sna-Snp/MP - base angle.

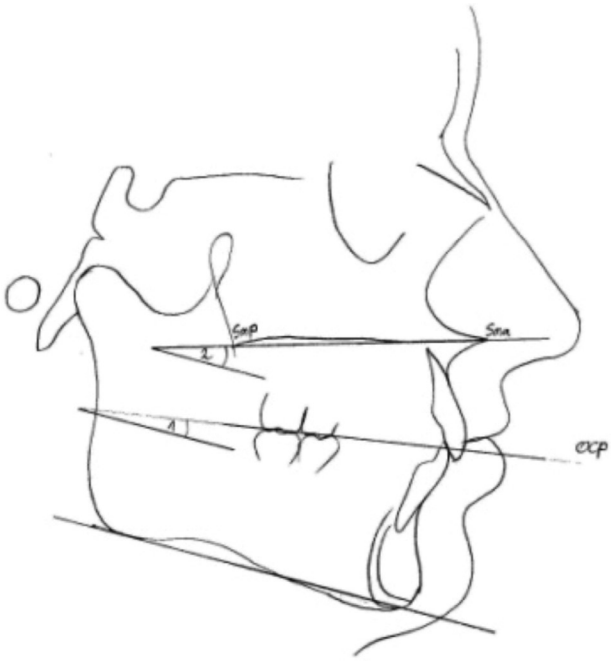

Figure 5. Vertical skeletal variables by Schwarz

5. Vertical skeletal variables by Steiner (Fig. 6)

- SN/Go-Gn.

Figure 6. Vertical skeletal variables by Steiner

6. Vertical skeletal variables by Ricketts (Fig. 7)

- Sna-Xi-Spm - lower facial height;
- N-Ba/Pt-Gn - facial axis.

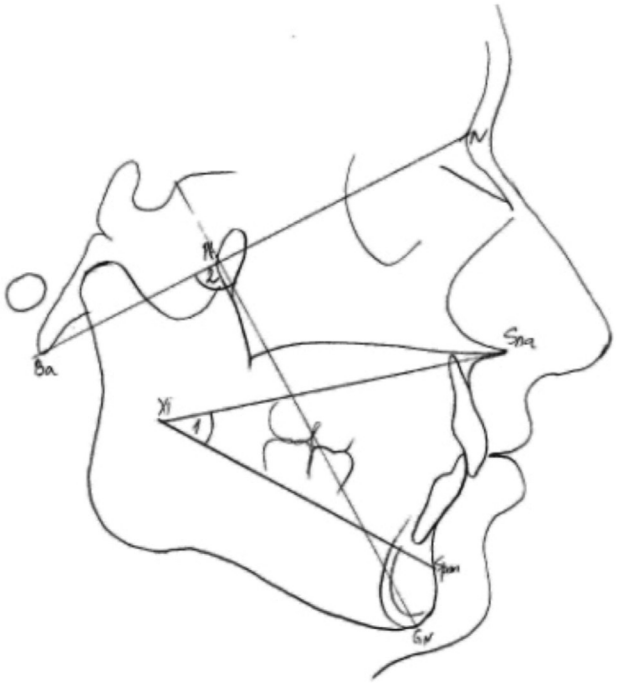

Figure 7. Vertical skeletal variables by Ricketts

Variables that Locate Mandibular Incisor Position

1. Variables by Holdaway (Fig. 8)

- Ii-NB/NB-Pg - ratio Ii to NB(a) and NB to Pg(b) = "Hold-1".

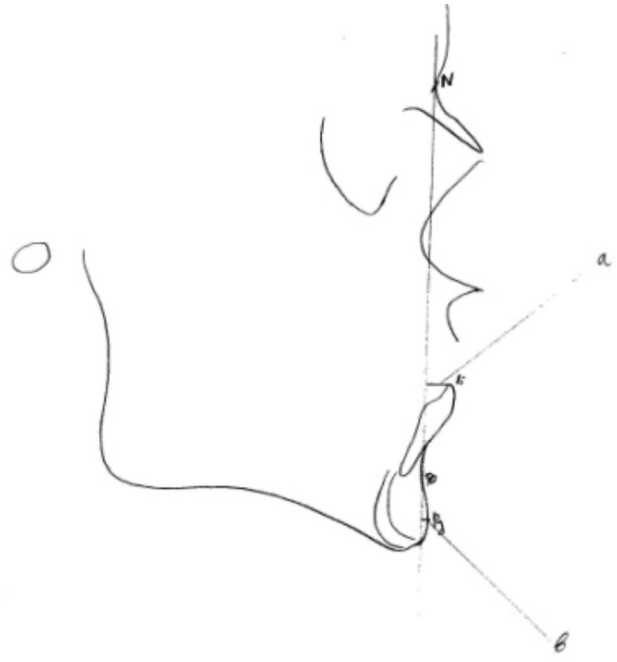

Figure 8. Variables by Holdaway

2. Variables by Schwaninger (Fig. 9)

- "Hold-2"- 2 lines are constructed parallel to NB; one line passes through the incisal edge, the other through the Ii apex (1).

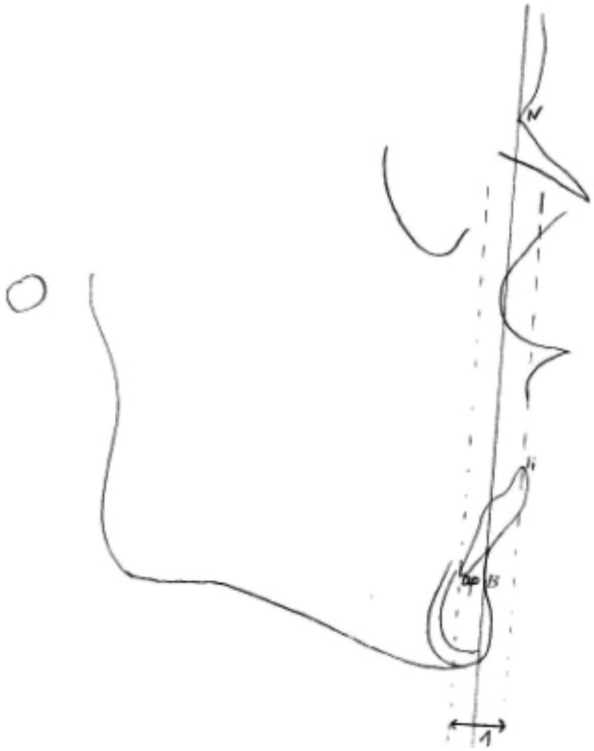

Figure 9. Variables by Schwaninger

3. Variables by Jarabak (Fig. 10)

- Ii-NPg - distance from Ii to NPg (1).

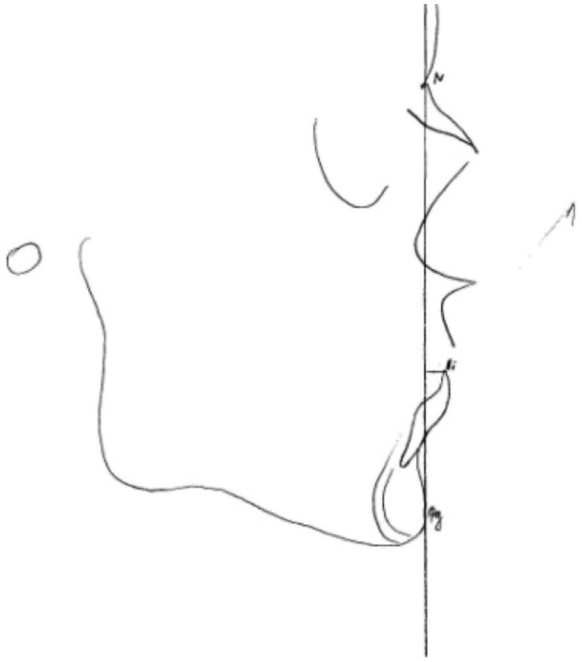

Figure 10. Variables by Jarabak

4. Variables by Ricketts (Fig. 11)
- Ii/APg - taken as the angle (1);
- Ii-APg - taken as the distance (2).

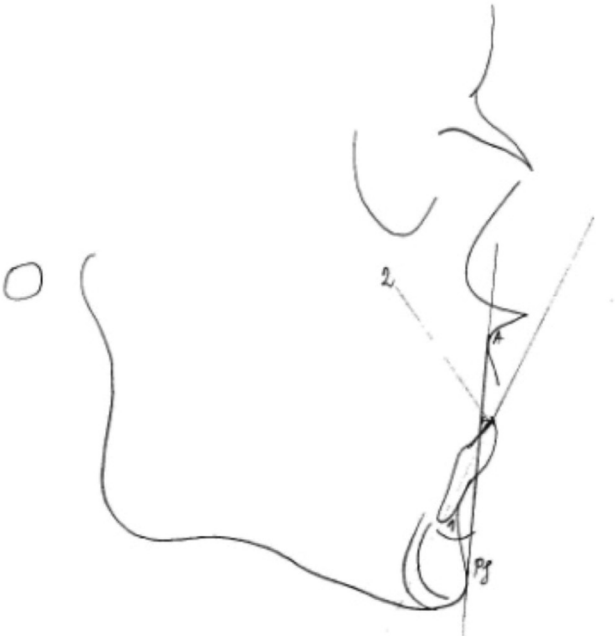

Figure 11. Variables by Ricketts

5. Variables by Steiner (Fig. 12)
- Ii/NB - taken as the angle (1);
- Ii-NB - taken as the distance (2).

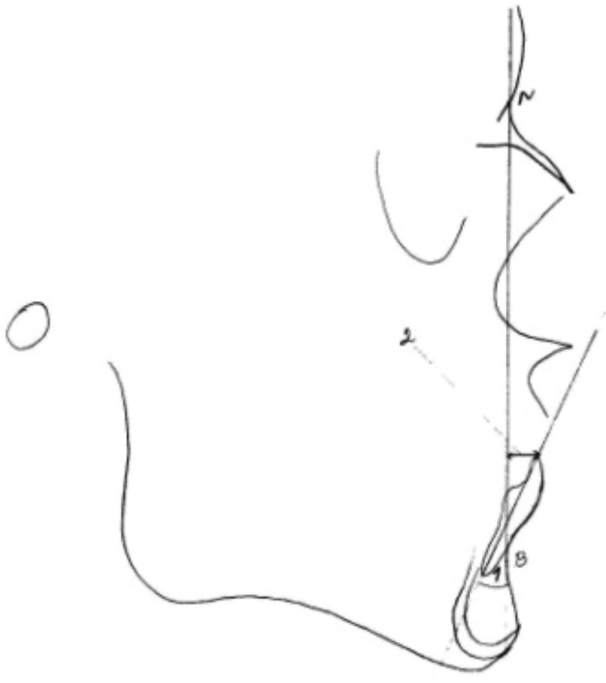

Figure 12. Variables by Steiner

6. Variables by Tweed (Fig. 13)
- Ii/MP (1);
- Ii-FH (2).

(Ii = inferior incisor = most labially inclined lower incisor)

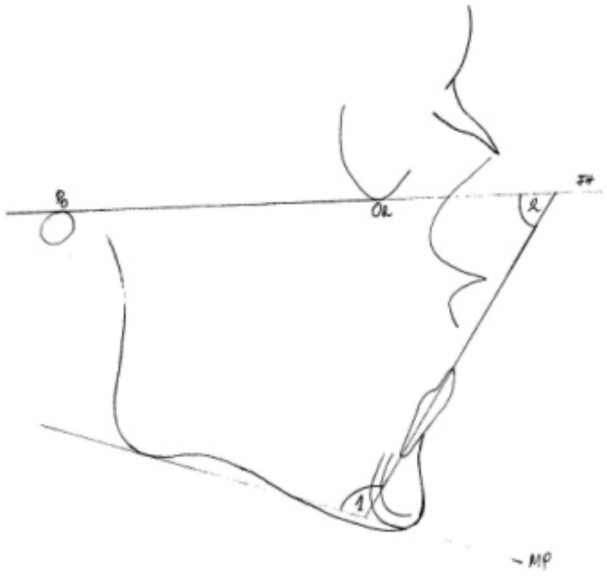

Figure 13. Variables by Tweed

Results and Discussion

Lower incisor crowding is seen in malocclusions that are primarily dental in nature and those with atypical skeletal patterns[9].

Degree of Crowding

The sample distribution by severity for each sex is illustrated in table 1. The table presents 3 arbitrary groupings of lower incisor crowding. These were labelled as minimal (1.0-2.0 mm), moderate (2.1-4.0 mm) and severe (4.1 mm and grater). Measurements of casts provided a mean degree of crowding of 2.2 mm, with a range extending from1.0 to 6.6 mm. No sex differences were evident .The table demonstrates that the most of samples fell within the minimal and moderate groups. This is a consequence of non-selection. Primary crowding as well as spacing (both an expression of tooth/jaw size discrepancy) are freely fluctuating characteristics with a continuous quantitative distribution, ranging from severe spacing, over balanced space conditions, to extreme crowding[16]. Although selected by their existing crowding, these patients still seem to be representative of a Caucasian population since all their cephalometric parameters were well within the accepted range. The few existing differences could be readily explained by minor variations in the roentgenographic method, as well as by

varying definitions of the "ideal", patient's age, and ethnic backgrounds in the reference studies[12].

Table 1. Distribution of crowding according to severity (mm) and gender

Degree of crowding	Boys	Girls	Total
1.0 - 2.0 mm	37	16	53
2.1 - 4.0 mm	19	22	41
4.1 mm and >	1	5	6
	57	43	100

The mean age of our patients was 125 months (10.3 years), the minimal age was 105 months (8.7 years), and the maximal was 145 months (12.0 years). Primarily, the relatively high mean age of our patients is surprising since eruption of lateral incisors is normally finished at about 8.5 years. The fact that these patients were much older on average might be due to the existing lack of space which, according to Witt at al[19], delayed eruption of the permanent incisors.

Vertical Skeletal Measurements

Table 2 illustrates the average values, standard deviations, and ranges of all angular and linear vertical skeletal measurements. The values of this study are similar to standard values reported in the literature. Skeletal differences were found in angles depending on FH (FH/MP, facial angle and Y-axis). These variances might be a result of the differences in technique of tracing and radiographic equipment.

Table 2. Vertical skeletal measurements

Variables	X	SD	Min	Max
N-S-Ar	122.8	6.29	111	136
S-Ar-Go	145.1	7.09	129	164
Ar-Go-Me	125	6.30	113	135
Ar-Go-N	54.3	4.39	45	65
N-Go-Me	74.4	4.79	65	83
N-S-Ar-Go-Me	393	16.53	354	421
S-Go/N-Me	63.3	5.09	54	75
N-Pg/FH	79.8	3.29	71	86
Y oska	63.7	3.40	56	72
FH/MP	32.7	5.09	22	41
N-Ba/Pt-Gn	88.7	3.43	80	96
Sna-Xi-Spm	46.7	4.69	37	57
Sna-Snp/MP	28.7	5.69	17	40
OcP/MP	16	4.18	7	26
SN/Go-Gn	35	5.80	23	45
N-Sna/Sna-Me	83.6	8.09	69	103

Lower Incisor Position

Table 3 illustrates the findings in this sample for measurements related to lower incisor position. As in vertical skeletal measurements, these values were similar to standard values reported in the literature. Noticeable difference was recorded only for dental parameter Ii-FH (again depending on FH). Ii-FH was lower in the study's patients than Tweed's mean value. This variance may be related to Tweed's ideal of what was considered an optimal facial pattern; the optimal facial pattern is viewed now by many critical clinicians as an excessively upright lower incisor angulation. At the same time, the difference in incisor angulation from the mean value is reflected in the "Hold-1" and "Hold-2" measurements. Finally, a further explanation for differences between our data and those of the pertinent literature could be a consequence of this sample's lower mean age. Under all these premises, the apparent conformity of cephalometric evaluation indicated that our patients could be considered as representative of a normal population and also that age differences may be of minor importance.

Table 3. Lower incisor position measurements

Variables	X	SD	Min	Max
Ii/FH	54.7	6.89	42	72
Ii/MP	92.8	6.09	80	105
Ii/A-Pg	22.2	4.79	12	32
Ii/NB	25.2	5.20	13	36
Ii-A-Pg	0.3	2.29	-4	5
Ii-N-Pg	2.2	3.16	-4	8
Ii-NB	4.4	2.09	0	8
Ii-NB/NB-Pg	3.4	2.61	-5	8
"Hold-2"	11.8	2.59	6	17

Correlation Analysis

In table 4 correlation coefficients (r) between the degree of incisor crowding and the vertical dimensions are shown. The findings for correlation coefficients were between 0.01 and 0.21, which indicated the absence of relationship between lower anterior crowding and any of the vertical skeletal dimensions.

A second correlation analysis, which was performed between the degree of incisor crowding and lower incisor position, is shown in table 5. The findings for correlation coefficients were between 0.01 and 0.20, which indicated the absence of relationship between lower anterior crowding and lower incisor position.

Table 4. Correlation coefficients (r) between lower incisor crowding and vertical skeletal measurements

Variables	r
N-S-Ar	-0.08
S-Ar-Go	-0.02
Ar-Go-Me	-0.01
Ar-Go-N	-0.04
N-Go-Me	-0.06
N-S-Ar-Go-Me	-0.03
S-Go/N-Me	0.12
N-Pg/FH	-0.21*
Y oska	0.02
FH/MP	0.11
N-Ba/Pt-Gn	-0.15
Sna-Xi-Spm	0.10
Sna-Snp/MP	0.14
OcP/MP	-0.06
SN/Go-Gn	0.12
N-Sna/Sna-Me	-0.03

Table 5. Correlation coefficients (r) between lower incisor crowding and lower incisor position

Variables	r
Ii/FH	-0.13
Ii/MP	-0.12
Ii/A-Pg	0.01
Ii/NB	0.14
Ii-A-Pg	0.07
Ii-N-Pg	-0.06
Ii-NB	-0.20*
Ii-NB/NB-Pg	0.11
"Hold-2"	0.03

It was concluded that there were no definite linear or nonlinear relationships between lower anterior crowding and the selected, separately measured, radiographic variables.

It is hardly surprising that all correlation coefficients for skull configuration and incisor inclination were of similarly low value since many of the evaluated parameters depend on identical or at least on similar reference point or lines. This creates the typical problem of multicollinearity. In the context of multicollinearity, one open point to criticism is that only NS-GoGn was measured, but also the sum angle, which is simply the sum of NS-GoGn+360^{0} [12]. Results are compatible with those of Miethke[18,19], who also did not find correlations between lower incisor crowding and either skeletal morphology or lower incisor position. Howe et al[10] and Sinclair and Little[28] also found no clinically significant association between various mandibular parameters and incisor crowding.

On the other hand, Berg[1] evaluated crowding of dental arches longitudinally from 6 to 12 years of age and reported a significant negative correlation between S-N and lower facial length dimensions at the age of 6 years. He also found that, when compared with normal subjects, children with crowding were characterized by significantly lower mean values for mandibular length.

Sakuda et al[24] reported a significant correlation between an increase in lower incisor crowding and high mandibular plane angles, short mandibular body lengths, great upper face height, and small vertical dimensions in the upper posterior segments.

A correlation between lower anterior crowding and growth vector of the mandible was cited by Fisk[6]. He believed that a prediction of arch form, final tooth position, and the available space is possible only if the relationship between the skeletal growth pattern and development of the dentition is known.

However, Lundstrom[14] found no correlation among arch dimension changes in the developmental stage, changes in the incisor position within the mandible, and direction of mandibular growth.

The results of this study were contrary to Sanin and Savara[26], who observed that between the ages of 8 and 14, in cases with lower incisor crowding, an increase in lingual crown tipping (alteration of tooth position) occurred.

Leighton and Hunter[12], found that crowded mandibular dentitions exist in a morphologically distinct supporting structure, which has a downward growth direction and relatively deficient amount of growth. They investigated the relationship between lower anterior crowding/spacing on one hand and the shape and growth direction of the face and the mandible on the other. The results of their longitudinal study showed that patient with severe crowding (that is tooth size-jaw discrepancy of > 4.0 mm) had a steeper mandibular plane, greater SNA-SNP to MP angle, shorter mandibular body, shorter posterior facial height, less mandibular size increase between the age of 9 and 14, and a more clockwise (downward and backward) growth pattern of the mandible. The same conclusions could be drawn from illustrations of Bjork's implant studies[3].

To interpret our results, one most consider that this was a cross-sectional study, while Leighton and Hunter[12], and Sanin and Savara[26] examined a longitudinal series. In addition, differences in technique from those of the aforementioned authors and the more sophisticated and intensive statistical analysis applied to the accepted standards reported in the literature.

The results do not exclude the occurrence of lower incisor crowding, vertical skeletal excess, and lingually inclined lower incisor position in individual instances. Such a set of variables may be present but not necessarily interrelated. In the cross-sectional sample, there was no statistical interaction among these variables. Considering that 53% of our patients showed only minimal crowding (1.00 to 2.00 mm), which could be regarded as physiologic, the negative results of correlation analysis is hardly surprising. If one considers "minimal" crowding to be a typical physiologic variation of this particular stage of dental development, one would not expect any specific vertical skeletal morphology associated with "minimal" crowding. A statistically significant difference in vertical skeletal morphology and lower incisor position was not found between the "minimal" group and the "moderate" and "severe" groups. Thus, the difference in severity of lower incisor crowding could not be considered as the source of contradictory results between this study and previously cited literature. The correlation analysis itself had inherent limitations since several variables derived from the same or closely related reference points and lines. It is possible that measurement errors obscured a weakly existing correlation.

Conclusion

The results of this study reinforce the concept that lower incisor crowding is a local, independent, genetically determined discrepancy between tooth width and size of supporting bone. It should be accepted as fact that lower incisor crowding manifests itself in different skeletal morphologies, independent of lower incisor position. Treatment of lower incisor crowding without a foreknowledge of its cause must therefore be performed symptomatically, singly, or in combination with enlargement of the apical base by reduction of the number of teeth (extraction) or reduction of individual mesial-distal tooth widths (interproximal stripping).

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BALKAN JOURNAL OF STOMATOLOGY

BaSS

ISSN 1107 - 1141

Tissue Damage after Inadvertent Citric Acid Extrusion during Root Canal Treatment: Report of a Case

SUMMARY

A female patient was seen for an emergency visit after accidental citric acid (40%) injection into soft tissues during root canal irrigation. The patient was experiencing pain, swelling in the right mandible, paraesthesia of the right lower lip and regional necrosis of the buccal mucosa. She was reassured and given analgesics and antibiotics. Follow-up visits were scheduled to monitor the case. When symptoms of swelling and pain resolved, completion of endodontic treatment was decided. A perforating defect was considered responsible for the citric acid extrusion. 6 months after the accident, complete rebound of sensation was noted without any symptoms from the affected area.

Keywords: Citric Acid; Mucosal Necrosis; Paraesthesia; Root Canal Treatment

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CASE REPORT (CR)
Balk J Stom, 2013; 17:101-106

Introduction

Citric acid is a commonly used chelating agent in root canal treatment, offering removal of the smear layer[10,23,29], dentine wall cleaning and root canal disinfection[27,36,39]. Its efficacy is associated with the dissolution of Ca^{++} ions from dentine due to the formation of constants of complexes between citric acid and calcium[7]. It is used at different concentrations, ranging from 1% to 50%[10,11,13,15]. Higher concentrations (25-50%)[11,15,18,28], as well as accumulated application times[33], increase its efficacy. The maximum effectiveness is achieved at pH 1.2[17]. On the contrary, application of citric acid at neutral pH values is not effective for Ca^{++} ions removal[9].

Many studies have investigated the effectiveness of citric acid for smear layer removal[9,10,23,30,33]. However, its cytotoxic and tissue damaging effects have been poorly investigated. The available data on the topic is contradictory. Some studies[1,4] have shown that although citric acid is less cytotoxic compared to other chelating agents used in endodontics, such as EDTA and EGTA[1,24,32], it has short- and long-term damaging effects on cell cultures *in vitro*. The detrimental effect on vital cells is associated with its acidic pH[21]. Chan et al[4] investigated morphological alterations associated with the citric acid cytotoxic and cytostatic effects on cultured dental pulp cells[4]. The exposure of cells to pure 1% citric acid (pH = 2.26) for 60 sec caused immediate cellular death. Citric acid (15%) placed on murine macrophage cultures reduced macrophage viability by 50-70% within 24 hours[1]. Concentration and time of application are contributing factors to the cytotoxic behaviour of citric acid[4]. Other studies have demonstrated that the citric acid solution is non-cytotoxic to vital fibroblasts in cultures[24,32].

Citric acid can also potentially cause a decalcifying action on periapical bone and affect inflammatory and neuro-immune regulation when extruded into peri-radicular tissues[1].

A comprehensive literature review revealed that there are no available studies describing tissue damage after inadvertent citric acid extrusion into soft tissues during root canal treatment. The objective of this case report was to describe clinical signs, symptoms and management of a case of chemical injury caused by accidental citric acid injection into soft tissues during root canal treatment.

Case Report

A 46-year-old Caucasian female with non-contributory medical history was referred by her private

dentist to the Faculty of Dental Medicine (Plovdiv, Bulgaria) for assessment, consultation and treatment of progressively intense facial pain and swelling in the posterior region of her right mandible. The referring dentist informed us that root canal treatment of the tooth #46 had been initiated 3 hours earlier, following the diagnosis of chronic apical periodontitis. On questioning, the patient reported acute pain during the procedure and swelling in the right mandible some minutes later.

On extraoral examination, there was swelling in the right mandible (Fig. 1) and paraesthesia of her right lower lip. In particular, the patient reported tickling, tingling and numbness. There was no external appearance of ecchymosis or haematoma. The intraoral examination revealed a regional buccal mucosa necrosis adjacent to the tooth #46 (Fig. 2) and remnants of a class II (MOD) amalgam filling, as well as temporary cement used to seal the access cavity preparation on tooth #46. Radiographic examination revealed a periapical lesion around the mesial root of tooth #46 (Fig. 3a). On request for further details and after personal contact with the referring dentist, it was concluded that accidental injection of 40% citric acid (Cerkamed, Poland) into soft tissues had occurred during irrigation of the root canals.

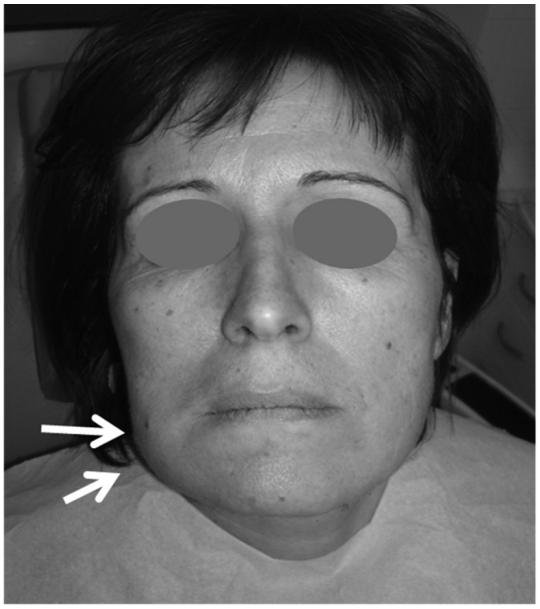

Figure 1. Mild swelling in the right mandible region

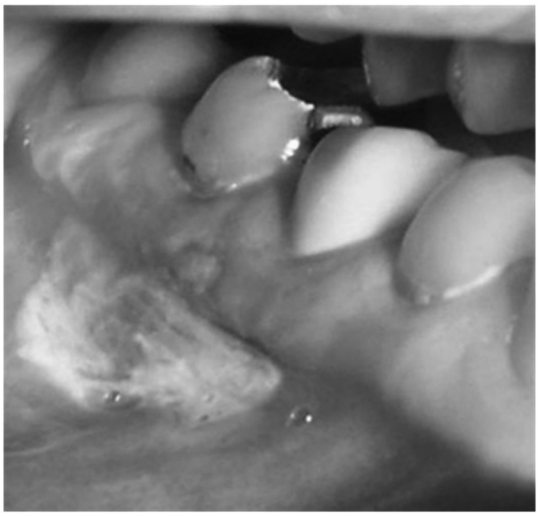

Figure 2. An area of soft tissue necrosis surrounded by well-vascularized tissue near the periapical area of tooth #46

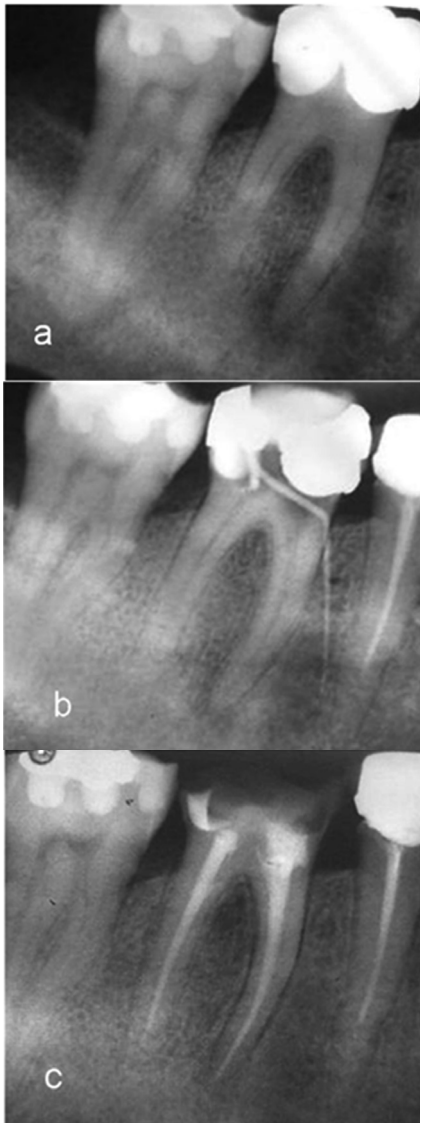

Figure 3. Periapical radiographs of tooth #46. a. Preoperative radiograph showing a periapical lesion around the mesial root of tooth #46; b. Radiograph with a gutta-percha point inside the perforating defect. The rubber dam was secured in place by ligatures of dental floss and wedges, so as to prevent the interference of the metallic dental clamp to the radiographic image; c. Postoperative radiograph taken immediately after root canal obturation

A complete explanation of the situation was provided to the patient. The initial treatment plan included administration of an oral analgesic (Diclofenac 50mg tid pc for 3 days), as well as an antibiotic (Amoxicillin 250 mg q8h for 7 days) for pain and infection control, and continuous monitoring of the case. Additionally, oral rinsing with Chlorhexidine gluconate 0.12% (Peridex, Periocard) was prescribed. Cold compresses applied for the first 4-6 hours, followed by warm saline soaks were recommended to reduce swelling. The patient was seen on day 2 and the swelling was reduced. A part of the necrotic tissue was spontaneously detached 5 days after the incident (Fig. 4). After a week, the swelling had disappeared and the motor function of the facial nerve remained intact. 3 weeks later, oral mucosa completely healed (Fig. 5), but there was residual paraesthesia of her right lower lip.

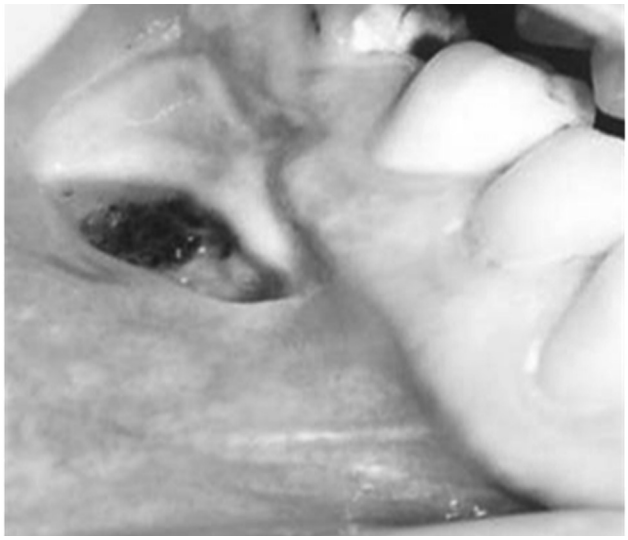

Figure 4. 5 days after the accident, necrotic tissue was evident after spontaneous detachment of the most severely affected mucosa

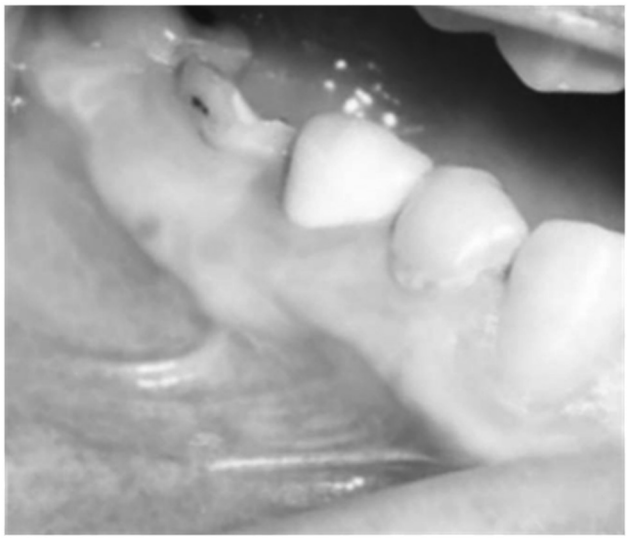

Figure 5. Complete healing of the oral mucosa 2 weeks after the accident

Completion of the root canal treatment on tooth #46 was recommended. Following rubber dam isolation and removal of the amalgam remnants and temporary cement, the pulp chamber floor was explored with the aid of magnification under operating microscope (Kaps International, USA). The examination revealed the presence of a perforation next to the mesial axial walls of the pulp chamber. Its presence was clinically confirmed with the aid of apex locator (Root ZX Morita, CA, USA) and radiographically following the insertion of a gutta-percha point into the perforating defect (Fig. 3b). Bleeding was arrested by clamping the area with a cotton pellet and the perforation was immediately sealed with resin-based glass-ionomer cement (Vitremer, 3M/ESPE, USA). Root canal instrumentation was completed with Pro Taper rotary files (Dentsply Maillefer, Switzerland) according to manufacturer's instructions under copious irrigation between successive instruments with 2.5% w/w NaOCl using 5-ml disposable plastic syringes and 30-gauge needle tips (Endo EZ; Ultradent Products Inc., UT, USA). The tip was placed passively into each canal, as far as 3 mm from the apical foramen, without binding. The mesial canals were enlarged to size F2 and the distal to size F3 to their full working lengths. A final flash with 17% EDTA at the end of the chemo-mechanical preparation was performed. The root canal system was dried with sterile paper points (Roeko Dental Products, Langenau, Germany) and chemically pure $Ca(OH)_2$ mixed with saline was placed in the canal with a #40 lentulo paste carrier (Antaeos; Vereinigte Dentalwerke & Co.). The access cavity was temporarily sealed (Cavit; Espe, Seefeld, Germany) and the patient was scheduled for the final appointment after 1 week. The 1-week period was uneventful. Only residual paraesthesia was observed at the final appointment. The intra-canal medicament was removed by means of instrumentation and copious irrigation and canals were obturated with gutta-percha and I-Root SP bio-ceramic sealer (IBC, Canada) using the lateral condensation technique. The tooth was sealed temporarily with resin-based glass ionomer cement (Fig. 3c). The patient was referred back to her private dentist for tooth restoration and scheduled for follow-up examinations on a 15-day basis to monitor the progress of paraesthesia.

The patient did not comply with the scheduled appointments. She re-appeared 6 months later with no symptoms from the affected area, but tooth remained un-restored. Clinical examination displayed rehabilitation of sensation in the areas of innervation of mental nerve branches. Upon questioning, the patient informed us that the paraesthesia symptoms were improving slowly and a total rebound of sensation was evident nearly 4 months after the completion of the root canal treatment. Radiographic examination revealed a reduction in size of the periapical lesion, despite the lack of permanent restoration (Fig. 6).

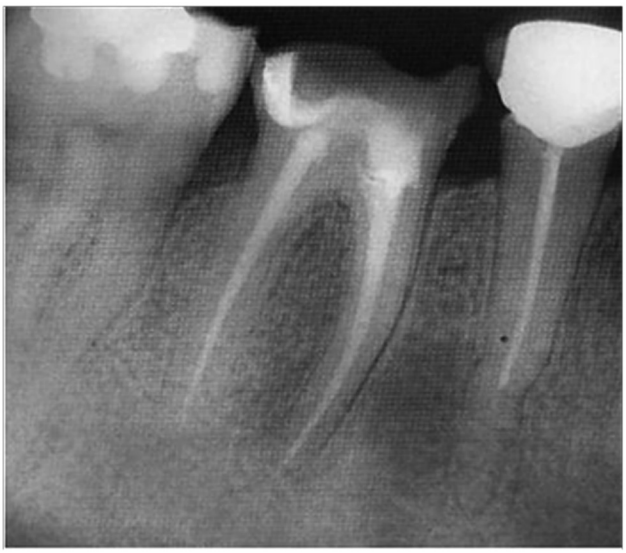

Figure 6. Follow-up radiograph 6 months after the completion of root canal treatment

Discussion

Although root canal irrigants and chelating agents are essential elements of root canal treatment, offering disinfection, debris and smear layer removal[10,23,26,29], they are cytotoxic in contact with vital tissues[1,4,16]. It is well-known that a small amount of irrigant may be extruded into periapical tissues during root canal instrumentation, irrespective of the preparation technique and type of the utilized instruments[19]. A questionnaire survey among diplomats of the American Board of Endodontics indicated that only 58% of the responders had never experienced NaOCl accident, while the remaining 42% had at least one NaOCl accident during their practice[20].

The clinical signs and symptoms following periapical extrusion of alkaline solutions, such as NaOCl, have been widely described in many previously published case reports[2,8,14,22,25,31]. However, this is the first case report in which the effects of inadvertent acid extrusion into soft tissues during root canal irrigation are presented.

The injection of acid solutions into soft tissues causes coagulation necrosis *via* intracellular dehydration and protein coagulation, limiting the spread of the chemical in the tissues[12,38]. On the contrary, alkaline agents, such as NaOCl, penetrate local tissues rapidly and deeply, causing liquefaction and necrosis, which facilitates the spread of the chemical[34,35].

NaOCl is the irrigant of choice in root canal treatment as it has the unique capacity to dissolve necrotic tissue and the organic components of smear layer[41]. Demineralising agents, such as citric acid, have been recommended as adjuvants in root canal therapy. In this case, only citric acid was utilized for root canal irrigation by the treating dentist. Although this is not recommended, the exclusive use of citric acid for irrigation prevented any further severe complications which might have been caused by NaOCl injection into soft tissues through the perforating defect[2,8,14,22,25,31].

In this case, a perforation of the mesial pulp chamber wall allowed for the injection of citric acid into soft tissues. The early recognition and seal of the perforation prior to root canal instrumentation would have prevented the accident. In a systematic review and critical analysis of published data upon irrigant extrusion and identification of causing factors, affecting or predisposing to irrigant extrusion during root canal irrigation in human mature permanent teeth, perforations were identified among the most frequently cited factors[13].

The cytotoxic behaviour of citric acid was enhanced[4] due to utilization of a high concentration (40%). The effectiveness of citric acid is a function of its concentration[11,15,18,28], but so is toxicity. This should be considered in clinical practice in order to select a citric acid solution with a concentration that can achieve maximum efficacy with minimum risk of tissue damage in cases of extrusion into soft tissues.

Citric acid has been also used in periodontology to promote periodontal healing and regeneration, as its demineralising effect can favourably affect migration, attachment and orientation of periodontal ligament cells to the diseased cementum and dentin[5,40]. The lack of toxic effects in these cases could be attributed to the low amount and concentration of citric acid coming in contact with vital tissues as compared to the corresponding amount and concentration of the solution in case of its accidental injection into soft tissues.

The clinical signs and symptoms following citric acid extrusion were alarming and their management was urgent. No specific treatment can reverse the deleterious effects caused by citric acid. The usage of a weak alkali to chemically neutralize the area is contraindicated, as this may stimulate an exothermic reaction, exacerbating tissue injury. The treatment of choice is supportive/palliative and targets to pain relief, control of swelling and prevention of secondary infection[12,38]. Reassurance should be provided to the patient for the symptoms from the involved area. For the immediate pain relief, a nerve block with a local anaesthetic should be considered. Adequate pain control can be achieved by administration of analgesics. Non-steroidal anti-inflammatory drugs and aspirin should be avoided in the acute stage to prevent the risk of interstitial haemorrhage in the soft tissues[12,38]. Because of the possible spread of infection, prophylactic antibiotic therapy is considered necessary[12,38]. Light corticosteroid and antihistamine therapy should be suggested in selected cases. Cold compresses should be used for the first 4-6 hours to minimize swelling in the affected area[6]. Thereafter, warm compression should be preferred to shorten the clearing up time of ecchymosis

by increasing circulation of the involved area[12,38]. Oral rinsing with normal saline can also improve the circulation to the affected intraoral tissues[12,38]. In cases of neurological damage, the patient should be referred to medical or dental specialists, who have experience in nerve assessment and repair, for follow-up and possible treatment[37].

The extrusion of citric acid into soft tissues is an extremely rare complication of root canal treatment and, to our knowledge, it has never been described before. In such a case, it is crucial to monitor closely the patient and provide appropriate medical care. The described case did not require hospitalization and surgical intervention of the affected area. No permanent damage to tissues occurred.

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BALKAN JOURNAL OF STOMATOLOGY

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ISSN 1107 - 1141

Acute Pain of the Trigeminal Nerve Due to Amalgam in the Mandibular Canal: A Case Report

SUMMARY

This is a rare case of amalgam allocation in an alveolar wound of an extracted third molar. During the extraction of a mandibular third molar to a 36-year-old woman, the adjacent mandibular second molar was broken. The dentist decided to put an amalgam filling to the mandibular second molar directly after the extraction and during the same appointment. The patient soon experienced an acute continuous pain of the whole facial region innervated from the trigeminal nerve. A panoramic radiograph of the patient after 3 days showed a large piece of amalgam near or inside the mandibular canal.

Amalgam was removed from the mandibular canal surgically and, as a result, acute pain disappeared during the postoperative days. Restoration with amalgam should be avoided during a post-extraction time. Amalgam can produce toxic effects to the mandibular nerve, being transferred to the whole trigeminal nerve as well.

Keywords: Acute Pain; Amalgam; Mandibular Canal; Trigeminal Nerve

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CASE REPORT (CR)
Balk J Stom, 2013; 17:107-109

Introduction

Despite numerous discussions about the toxicity of amalgam, it is still one of the most commonly used materials for dental restoration. The accidental implantation of amalgam into the mucosal connective tissue usually results in a permanent gray-black pigmentation, commonly referred to as amalgam tattoo. In most cases, the oral amalgam tattoo is an easily recognizable entity that usually does not require histological verification[8] and treatment if the pigmentation is cosmetically acceptable[7]. However, in some cases, if the amalgam particles are large, they should be removed to prevent a further foreign-body reaction as a therapy against fever and pain. Such complications are reported very rarely [9].

This is a rare case of amalgam allocation in an alveolar wound of an extracted third molar. This case report has an educational value and it is presented in order to warn the necessity of avoidance such a traumatic injury in the future.

Case Report

A 36-year-old woman was referred for a regular dental examination to a private practice. The general practitioner decided to extract the tooth #38. During the extraction, the neighbouring tooth #37 was broken. The general practitioner decided to make an amalgam filling to the tooth #37 during the same appointment, and directly after the extraction. As it was shown later this was a catastrophic decision.

Directly after the anesthesia was gone, the patient complained for acute pain, which extended to the whole region of the trigeminal nerve. The patient referred again to the same general practitioner and it was decided that teeth #36 and #37 had to be treated endodontically as it was thought that pain originated from pulp inflammation of the above mentioned teeth. During pulp extirpation and root canal preparation 2 root canal instruments were broken. The severe acute pain persisted, extending to the entire hemisphere of the trigeminal nerve. At that time a panoramic radiograph was made (Fig. 1), which showed that a large piece of amalgam was allocated near

or in contact with the mandibular canal and perhaps the mandibular nerve.

The patient was treated after that by an oral-maxillofacial surgeon, who removed the piece of amalgam and a piece of the orifice of the mandibular canal that was needed in order the mandibular canal to be evacuated from the oedema that was concentrated in the region. The whole surgical approach was done under local anesthesia. Antibiotics, anti-inflammatory drugs and corticosteroids were given in order to prevent inflammation.

A second panoramic radiograph was taken to confirm the total removal of amalgam (Fig. 2). A clinical re-examination after a month showed that the symptoms were gone and the wound was healed. Therefore the patient was referred to an endodontist to treat teeth #36 and #37. A clinical re-examination 6 months later showed that there were no symptoms and the wound healed.

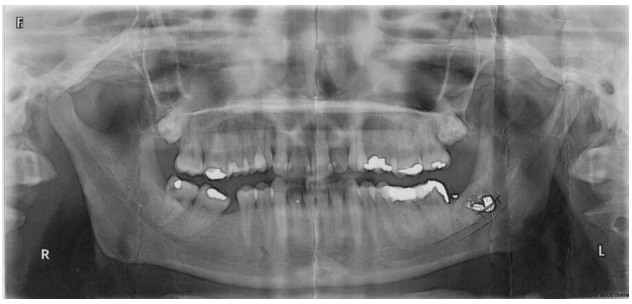

Figure 1. Panoramic radiograph showing a large piece of amalgam allocated near or in contact with the mandibular canal and mandibular nerve

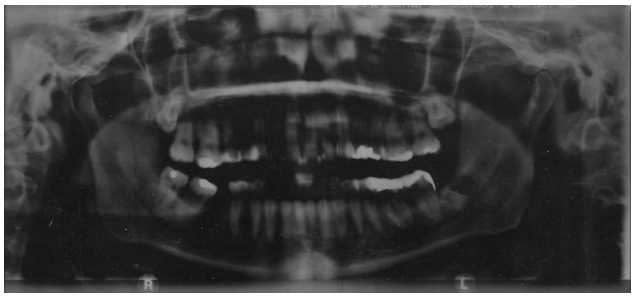

Figure 2. A second panoramic radiograph that confirms the total removal of amalgam

Discussion

It is well known that amalgam is still widely used as a restorative filling material. Amalgam consists mainly from mercury, zinc, silver, tin, copper etc. It is generally accepted that the release of mercury from amalgam can cause neurological problems[1]. The case we have described could be interpreted as a consequence of amalgam toxicity toward trigeminal nerve, probably due to mercury release. According to Lobner and Asrari[6], zinc that is an amalgams' constituent, demonstrates neurotoxicity as well.

In the above described case, it is not clear whether the acute pain that appeared in the whole region innervated by the trigeminal nerve was caused by mercury or by zinc. The level of release of the above mentioned mercury and zinc from amalgam in each case is not clear and therefore further investigation is suggested from Lobner and Asrari[6], in order to estimate the concentration of each of the metals released from amalgam. Moreover, in our case we assumed that the origin of the large piece of amalgam might be from the extracted (restored) 3rd molar or from the broken 2nd molar, because it was well-shaped (Fig. 1). So, in our case, the severe pain might be caused from the hard compression of the nerve trunk. However, the fact was that the patient had a hard time, the amount of amalgam was huge, it was in close proximity to the mandibular canal and mandibular nerve and a surgery was followed in order to control the patients' pain.

Different kind of foreign bodies have been described that were left during dental procedures, or found in the alveolar cavity of extracted teeth, especially third molars, that can cause a variety of pain. Amalgam has been described twice[3,5], eugenol-containing endodontic sealer extrusion[2] or bone wax[4].

Conclusively, restoration with amalgam has to be avoided during a post-extraction time. Amalgam can demonstrate toxic effects to the mandibular nerve, that can be transferred to the whole trigeminal nerve as well.

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BALKAN JOURNAL OF STOMATOLOGY

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ISSN 1107 - 1141

Odontogenic Pathologies of Dental Follicles in a Patient with Multiple Impacted Teeth: Are They Innocent?

SUMMARY

The presence of dental follicle (DF) adjacent to impacted teeth and the differences in the proliferation rates of oral epithelial cells occasionally lead to the development of cysts and tumours. The incidence of odontogenic cysts and tumours originating from this tissue are reported between 0.001% and 46%. The development of different odontogenic pathologies from DFs of multiple impacted teeth are rarely seen in the same patient. We report a 53-year-old edentulous female with a dentigerous cyst, keratocystic odontogenic tumour and odontogenic myxoma originating from DF of bilaterally impacted lower third molars and a lower canine.

Keywords: Dental Follicle; Dentigerous cyst; Keratocystic odontogenic tumour; Odontogenic Myxoma; Impacted Teeth, multiple

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CASE REPORT (CR)
Balk J Stom, 2013; 17:110-112

Introduction

Dental follicle is a component of a tooth germ, which is responsible to give rise of periodontal structures such as cementum, periodontal ligament and surrounding bone[4]. Under the influence of pathologic changes, however, dental follicles that possess reduced epithelium can proliferate into stratified squamous epithelium as far as to dental cysts[12].

The most common cysts, such as dentigerous cysts (DC), keratocystic odontogenic tumours (KCOT) and calcifying odontogenic cysts (COC)[2,9], and the possibility of tumours such as ameloblastoma, epidermoid carcinoma, and odontogenic carcinoma, arising from impacted molar teeth, have been stressed as an indication for prophylactic removal of impacted third molars[1,10]. Severe impaction of a mandibular third molar is reported to be a predisposing factor for cyst development[13], and most cystic changes were found in patients between 20 and 25 years. The authors concluded that age may be used as an indication for surgical removal of impacted lower third molars, as the risk of surgical morbidity also increases with the increasing age[1,6,10].

We report a 53-year-old female with a DC, KCOT and odontogenic myxoma originating from dental follicles (DF) of bilateral impacted lower third molars and a lower canine tooth.

Case Report

A 53-year-old edentulous woman was referred to our department with a complaint of pain and swelling in the left mandible. Extraoral examination showed no asymmetry in the face, but submandibular lymphadenopathy in the right side. On intraoral examination, the mucosa on the right edentulous area was hyperaemic and swelling was evident. On panoramic radiograph, bilaterally upper canines and left upper premolar were impacted in the maxilla, with no pathology. In the mandible, bilaterally third molars and right lower canine were impacted with follicular enlargements, which showed radiolucent lesions with radiopaque borders (Fig. 1).

Under local anaesthesia, all impacted teeth were extracted and follicles around the teeth were carefully removed for histopathological diagnosis. Histopathology confirmed as following: (1) dentigerous cyst in the region of the lower right impacted molar, which showed heavy chronic inflammatory cells infiltrate in the cystic wall (Fig. 2); (2) odontogenic myxoma in the region of the lower impacted canine, which showed stellate and spindle-shaped cells embedded in a richly myxoid extracellular matrix (Fig. 3); (3) keratocystic odontogenic tumour in the region of the left lower impacted tooth, which showed a thin, band-like lining of stratified squamous epithelium and corrugated keratinized lining (Fig. 4).

No recurrence was found after a 2-year follow up (Fig. 5).

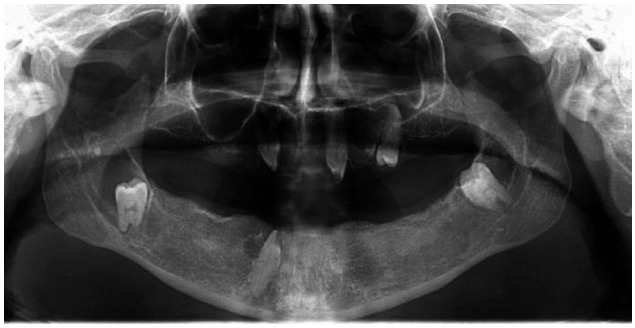

Figure 1. Panoramic radiograph showing multiple impacted teeth in both jaws and follicular enlargements around teeth in the mandible

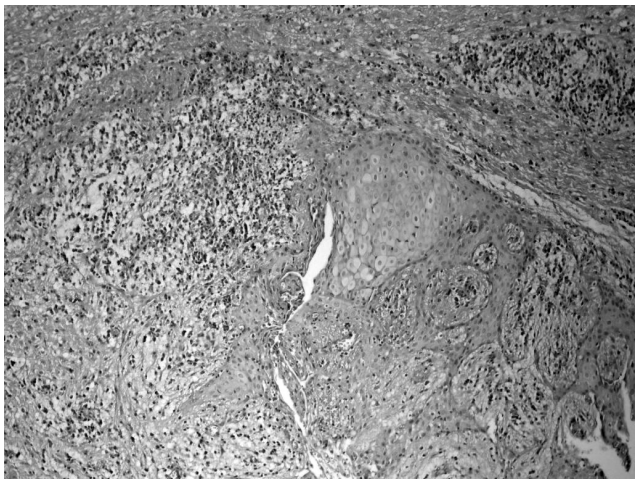

Figure 2. Infected dentigerous cyst showing heavy chronic inflammatory cells infiltrates in the cystic wall (HE x100)

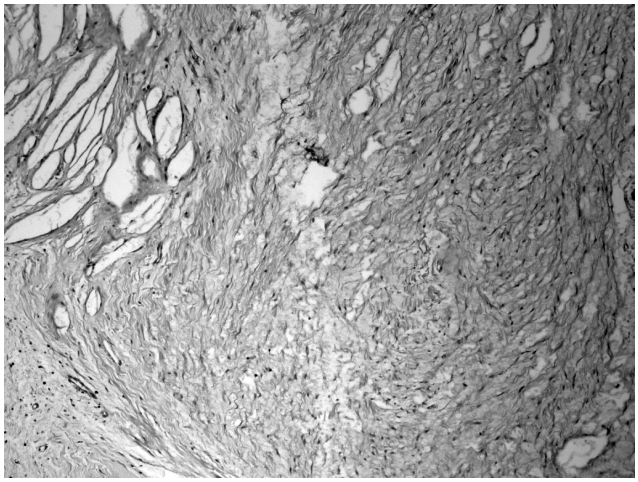

Figure 3. Odontogenic myxoma showing spindle and stellate cells in a mucoid background (HE x 100)

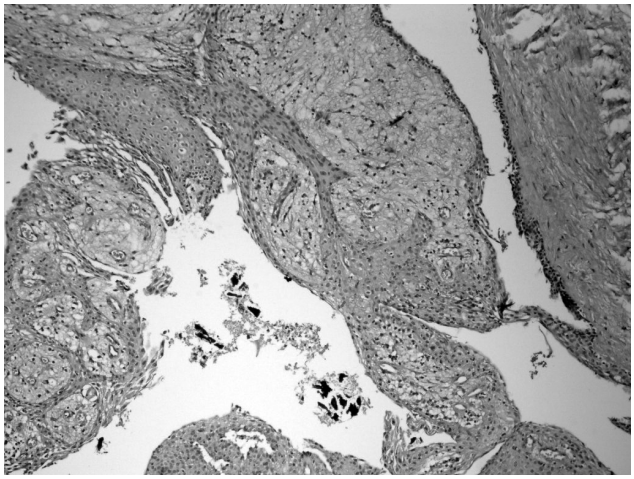

Figure 4. Keratocystic odontogenic tumour showing stratified squamous epithelium and a corrugated keratinized lining (HEx100)

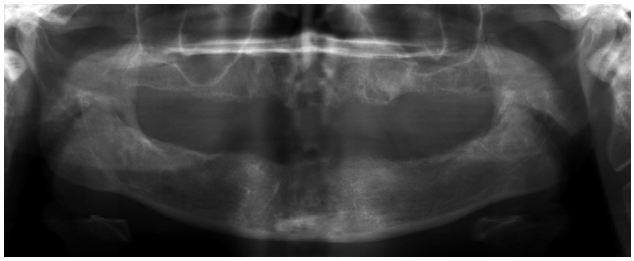

Figure 5. There was no pathology after surgery on the panoramic radiogram

Discussion

Impacted third molars are known to be associated with the risk of different disorders and complications. There are well-established indications for the removal of impacted third molars[1,3,6,7]. Prophylactic removal of asymptomatic unerupted or impacted third molars constitutes a large proportion of oral operations and the appropriateness of removal is still debated[3], although the highest frequency of infection is the reason why prophylactic extraction seems to be clinically justified, and that it should be done before the age of 20[6]. Studies on DFs of impacted teeth for defining the potential of pathological transformation showed more pathologies observed by using histological and immunohistochemical methods than that can be seen radiographically alone[1,2]. Histopathologically, the differentiation potential of DF of impacted teeth has been changed from squamous epithelium to various forms of metaplasia and degeneration. Mucous cells, ciliated cells, para and/or ortho-hyperkeratinization and formation of hyaline bodies have also been observed[10]. In the immunohistochemical studies, the cells of DF can be actively proliferating

and cysts and tumours can develop from these tissue cells. Therefore, the increase in proliferation rates of these cells may play a central role in the development of pathologies[2].

KCOT is a unique cystic lesion because of its locally aggressive behaviour, high recurrence rate and characteristic histological appearance. In 2005, WHO Working Group considered the para-keratinizing variant to be a cystic neoplasm and recommended the more descriptive term "keratocystic odontogenic tumour" (KCOT) based on its active epithelial proliferation, prostaglandin-induced bone resorption *via* interleukins (ILs) and tumour necrosis factors, and active collagenases in the fibrous cystic wall[7]. Odontogenic myxoma is thought to be derived from mesenchyme of a developing tooth or from the periodontal ligament; when occurs peri-coronal to the impacted tooth, it can also present as a cyst-like unilocular lesion. This fact points to a possible difficulty when trying to arrive at a proper diagnosis[11].

DCs, KCOTs and odontogenic myxomas in separate cases are well documented in the literature. However multiple cysts in patients without a syndrome is less common. Multiple cysts characterize certain syndromes, such as Maroteaux-Lawy syndrome, Hunter's syndrome (Mucopolysaccharidosis, type 6), cleidocranial dysplasia, basal cell nevus syndrome, Gardner's syndrome[5,8]. However, to the best of our knowledge, there are no reports that include DC, KCOT and odontogenic myxoma together in a non-syndrome patient. Multiple cysts and tumour that developed from DF of impacted teeth in the non-syndrome patient might be an essential point from the view of pathological differentiations of this tissue, and should be considered while discussions on prophylactic removal of impacted teeth.

Acknowledgments: We thank Prof. Sedat Çöloğlu for histological examinations and preparation of figures.

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